J. exp. Biol. (1981), 92, 165–172
With 8 figures
Printed in Great Britain

NEURAL CONTROL OF THE MALE *PHOTURIS VERSICOLOR* FIREFLY FLASH

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(*Recieved* 28 *July* 1980)

SUMMARY

1. Continuous electrical stimulation of the ventral nerve cord or the lantern of the decapitated male *Photuris versicolor* firefly over a wide range of stimulus frequencies can produce a flash that is multi-peaked, like the courtship flash of this species. The central nervous system does not shape these stimulated compound flashes because they can be induced in deganglionated posterior lantern segments.

2. The stimulated compound flashes show a fixed oscillatory character with peak frequencies independent of stimulation frequency. They can be generated by individual lantern areas. Compared with the peaks of courtship flashes the peaks of stimulated flashes show higher frequency, significantly lower temperature coefficients $(Q)_{10}$, and incomplete extinction.

3. *P. lucicrescens* males produce a courtship flash that has a single peak and their lanterns respond to continuous stimulation with an unstructured glow.

INTRODUCTION

The species specific flash of the male *Photuris versicolor* firefly has three or more peaks, usually of declining intensity, which appear as a series of twinkles to the naked eye (Barber, 1951) (Fig. 1A). The *Photuris lucicrescens* male produces a brilliant, single-peaked flash (Fig. 1B). In the genus *Photinus*, the flashes represent a signal in a courtship dialogue between male and female which is unique for each species (Lloyd, 1966). No comparable study has been made on *Photuris* fireflies, probably because unmated females are difficult to find.

In *Photuris* fireflies the flash is initiated by a complex neural burst from the brain which travels down the ventral nerve cord and activates the lantern (Case & Buck, 1963). These neural bursts can be monitored by extracellular electrodes in the photogenic tissue and probably represent brain activity modified by neural integration in the lantern ganglia (Fig. 2). The flash begins about 85 ms after the neural burst reaches the lantern (Buck & Case, 1961) (Fig. 2) which suggests some further complexity in the nerve-effector coupling process. It is possible that the complex male flash, characteristic of each species, is generated by a neural programme in the firefly brain. Although they found some obvious correlation between gross volley structure and gross flash form, Case & Buck (1963) concluded that no close relationship existed between volley structure and flash contour. This suggests that the peripheral

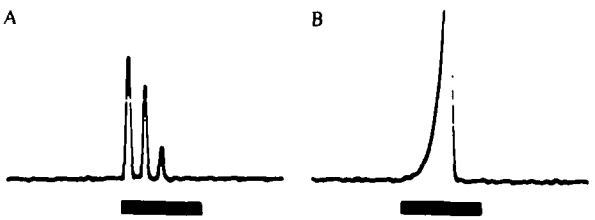

Fig. 1. Photomultiplier recordings of courtship flashes of *Photuris* male fireflies. A. Twinkling, multi-peaked courtship flash of *P. versicolor*. B. Crescendo courtship flash of *P. lucicrescens*. Recording intensities are not equivalent; *P. lucicrescens* flash is much brighter than *P. versicolor* flash and the peak is not shown. Calibration, 1 s.

Fig. 2. Lantern neural potentials which trigger courtship flash of *P. versicolor* male. Photomultiplier output (upper trace). Action potential volleys recorded from anterior lantern segment (middle trace) and posterior lantern segment (lower trace). Calibration, 100 ms.

lantern structures including lantern ganglia, motor nerves, end-organ complexes and photocytes (Kluss, 1958; Smith, 1963) do not respond passively to this neural input from the brain.

In this paper, the role of the peripheral lantern structures in the control of the male flash is investigated in *P. versicolor and P. lucicrescens*.

MATERIALS AND METHODS

Male *P. versicolor* fireflies were captured during the summer on Long Island and maintained in containers for short periods prior to use. Male *P. lucicrescens* were obtained from larvae collected in the late spring from Princeton, New Jersey. They were pupated in the laboratory and identified by their characteristic crescendo flash and light body colour (see p. 172).

The species-specific courtship flashes were recorded from spontaneously flashing males using a photomultiplier, the output of which was stored on a four-channel HP Instrumentation FM tape recorder. Recordings were photographed on an oscilloscope with a Grass C_4 camera. Stimulated flashes were recorded from decapitated males immobilized ventral side up. The lantern was directly stimulated by a pair of copper wire electrodes (80 μm diam.) penetrating the photogenic tissue through two small holes in the central cuticle overlying the lantern. The ventral nerve cord was stimulated with a suction electrode on the abdominal connective. Stimuli were delivered from an American Electronics Laboratory stimulator. Flashes were recorded as above.

Photographs of flashes were produced by videotaping the stimulated lantern on a Sony Videocorder, using a Sony videocamera fitted with a MicroNikor lens. Individual frames of the flash were photographed from the Sony video monitor with a Grass C_4 oscilloscope camera.

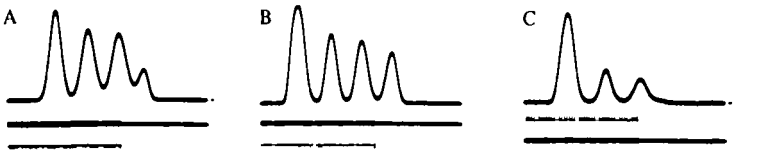

Fig. 3. Flashes produced by continuous electrical stimulation of a decapitated *P. versicolor* male. A. Stimulation of ventral nerve cord at level of third abdominal ganglion (stimulus frequency, 140 Hz). B. Stimulation of ganglionated, posterior lantern segment (stimulus frequency, 170 Hz). C. Stimulation of deganglionated, posterior lantern segment (stimulus frequency, 185 Hz). In this and all subsequent oscilloscope photographs, photomultiplier recording is on upper trace and electrical stimulation is on lower trace. Except where noted, stimulus pulse duration equals 1 ms, stimulus train duration equals 550 ms and frame width equals 1 s.

RESULTS

1. *Effect of stimulation of ganglionated and deganglionated lantern of male* P. versicolor

P. versicolor males normally produce simple, single spontaneous flashes when immobilized during external nerve recording. These flashes are triggered by single neural bursts composed of 1 ms impulses at about 150 Hz frequency (Case & Buck, 1963) (Fig. 2). Stimulation of the ventral nerve cord with a suction electrode at the level of the third abdominal ganglion with a 500 ms continuous burst of 1 ms impulses at about 200 Hz frequency results in a peaked flash similar to the conspecific flash (Fig. 3A). Direct stimulation of the lantern with electrodes positioned on either side of the anterior (6th abdominal) or posterior (7th abdominal) lantern segment will also produce a peaked flash (Fig. 3B).

The posterior lantern segment was deganglionated by removal from the animal, since the last abdominal ganglion resides in the anterior lantern segment (Hanson, 1962). The isolated segment could be stimulated to produce a compound flash (Fig. 3C).

2. *Effect of stimulus parameters on luminiscence*

The male *P. versicolor* lantern responds to stimulation frequencies over a very wide range with a compound, multi-peaked flash. Between frequencies of 25 and 500 Hz multi-peaked flashes can be elicited with direct lantern stimulation. Above and below these frequencies only very small responses can be obtained (Fig. 4). Each preparation responds to a particular frequency range, and this can vary depending on whether nerve cord or direct lantern stimulation is used. The peak interval of the compound flashes is independent of stimulus frequency (Fig. 4), whether the stimulus is supplied through the nerve cord or directly to the ganglionated or deganglionated lantern. The peak interval varies considerably between animals, however, averaging 213·7 ms ± 37·4 S.D. in 6 animals tested at 21 °C.

Stimulus-burst duration affects the number of peaks per flash. A stimulus duration of 550 ms induces a three- or four-peaked flash (Fig. 3A, B and C), while shorter bursts result in fewer peaks and longer bursts induce more peaks of rapidly declining intensity. Stimulation of up to 10 s results in an initial bout of peaks rapidly approaching extinction, followed by random, low-level flashes (Fig. 5).

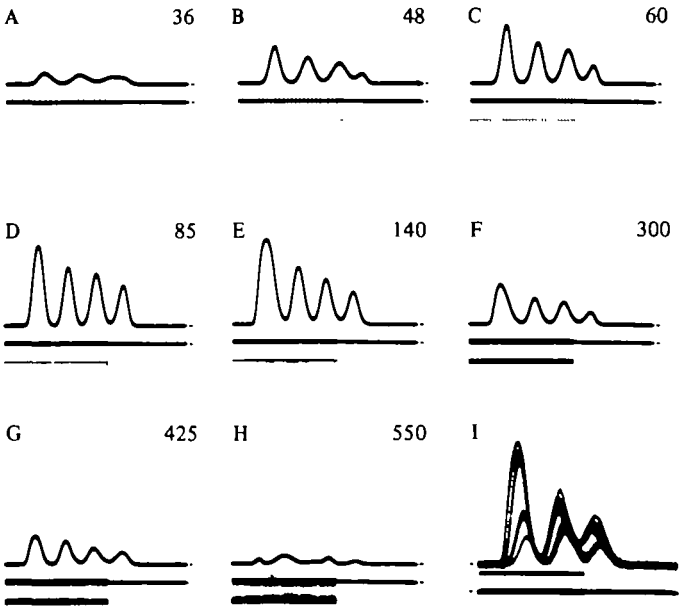

Fig. 4. Effect of stimulus frequency on flash produced by 550 ms, stimulation of ganglionated, anterior lantern segment of male *P. versicolor*. In frames A–H, stimulation frequencies in Hz are shown in the upper right of each frame. Frame I. Fifteen superimposed flashes produced by five stimuli each at 240 Hz, 300 Hz and 350 Hz.

Fig. 5. Effect of 10 s continuous stimulation of ganglionated, anterior lantern segment of male *P. versicolor*. Stimulus frequency, 350 Hz. Calibration, 2 s.

3. *Effect of temperature on the peak intervals of spontaneous and stimulated flashes*

The interval between peaks is more influenced by temperature in spontaneous than in stimulated flashes. Fig. 6 shows the effect of temperature on the peak frequency of the first two flash peaks measured in a male which spontaneously flashed at temperatures between 15 and 25 °C. The male was then decapitated and the peak frequency of its stimulated flashes was measured across the same temperature range. The regression lines differed significantly ($F_{[1,83]} = 6{\cdot}25$; $P = 0{\cdot}0144$), indicating temperature coefficients (Q_{10}) of 2·09 and 1·48 respectively. At the same temperature, spontaneous flashes had lower peak frequencies than stimulated flashes.

Four spontaneously flashing males, each of which was tested at a different temperature, showed a Q_{10} of 2·24 for peak frequency and their regression line did not differ significantly from that of the spontaneously flashing male in Fig. 6 ($F_{[1,91]} = 0{\cdot}163$; $P = 0{\cdot}6874$).

Six decapitated males stimulated between 15 and 25 °C showed a Q_{10} range of 1·37 to 1·59 for peak frequency and these slopes were apparently heterogeneous

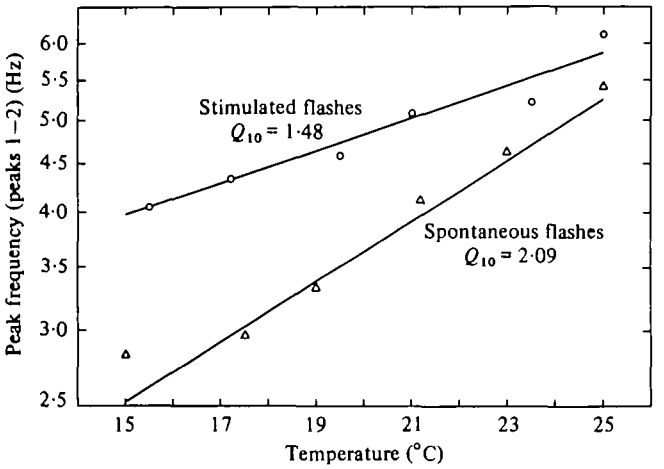

Fig. 6. Effect of temperature on the frequency of the first two peaks of the spontaneous, courtship flash (triangles, n = 46), and the stimulated flash (circles, n = 41) of the male *P. versicolor*.

($F_{[15,358]} = 2{\cdot}255$; $P. = 0{\cdot}0485$). Still, the Q_{10} of their stimulated flashes was very significantly different from that produced by the spontaneously flashing male in Fig. 6 ($F_{[1,401]} = 40{\cdot}286; P \ll 0{\cdot}001$).

4. *Luminescence dynamics of* P. versicolor *lantern during stimulation*

Single areas of the lantern would produce rapid light pulses in response to a continuous stimulus. Fig. 7 shows the response of different areas of the lantern to a 500 ms burst of 60 V shocks delivered to the posterior edge of the 5th abdominal ganglion. The anterior lantern segment produced three bright flash peaks while the posterior lantern segment also flashed less intensely. Stimulation could result in different areas flashing in an unsynchronized fashion.

5. *Effect of stimulation of* P. lucicrescens *lantern*

Direct stimulation of the anterior lantern segment of the *P. versicolor* male with 100 ms bursts of 8 Hz frequency resulted in short flashes (Fig. 8A). Stimulation of the lantern of *P. lucicrescens* under similar conditions also produced short flashes (Fig. 8B). Direct stimulation of the *P. lucicrescens* lantern with a continuous stimulus of 1 s, however, did not produce a multi-peaked flash but resulted in a continuous glow (Fig. 8C).

DISCUSSION

Continuous electrical stimulation of the male *P. versicolor* lantern produced a flash with three peaks, as in the courtship flash (compare Figs. 1A and 3). These flashes appear to be controlled by different processes, on the basis of the following differences.

1. The temperature coefficients measured for peak frequency of the stimulated flashes are significantly lower than those of courtship flashes. The Q_{10} of stimulated flashes averages 1·45 while that for courtship flashes is slightly above 2·0 (Fig. 6).

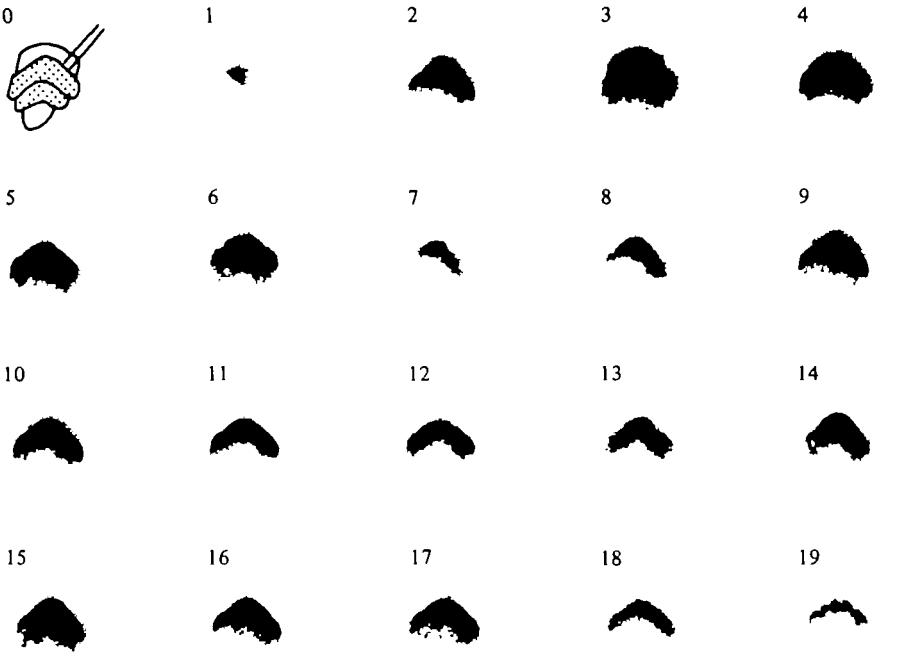

Fig. 7. Videophotographs of lantern of male *P. versicolor* during compound flash produced by 500 ms stimulation of left posterior edge of fifth abdominal segment. Frame 0 is a diagram of lantern showing position of stimulating electrodes. Stippled area shows luminous segments. Frame 1–19 are video-photographs of lantern showing three luminescent oscillations. Frame interval equals 33 ms.

Fig. 8. Comparison of effect of stimulation of anterior lantern segment on decapitated *P. versicolor* and *P. lucicrescens* male lanterns. A. Flashes produced by 100 ms bursts of 100 Hz stimuli on *P. versicolor* lantern (frame width, 1 s). B. Flashes produced by 100 ms bursts of 110 Hz stimuli on *P. lucicrescens* lantern (frame width, 2 s). C. Glow produced by 955 ms stimulation at 100 Hz on *P. lucicrescens* lantern (frame width, 2 s).

2. Light extinction between the peaks of stimulated flashes is less complete than in courtship flashes (compare Figs. 1 and 2 with 3 and 4).

3. The peak frequency of the stimulated flash is higher than that of the courtship flash (Fig. 6).

4. The peaks of stimulated flashes always decline in intensity whereas those of the courtship flash need not.

It therefore appears that the pattern generator for the courtship flashes resides in the brain while that for the stimulated flashes is located in the lantern itself. That the brain controls the courtship flash is further supported by the one to one correspondence between neural bursts recorded from the nerve cord with those recorded in the lantern (Case & Buck, 1963). The peak frequency of courtship flashes shou

low a Q_{10} approaching 2·0 if the pattern generator controlling the peaks was composed of a complex neural circuit. This was found to be the case.

The compound flashes produced by continuous stimulation of the central nerve cord of the lantern of decapitated males show a strong, fixed oscillatory character. The peak frequencies of the flashes are not affected by stimulus frequency (Fig. 4). The oscillatory generator appears to be controlled by less complex physicochemical processes than the courtship flash generator because of the lower Q_{10} (1·45) for peak frequency (Fig. 6). Individual areas of the lantern can produce the rapid luminescence oscillation (Fig. 7). Stimulation of the ventral nerve cord or the deganglionated lantern produces very similar effects (Fig. 3) which suggests that the oscillatory process is dependent on the lantern nerves as the final pathway of activation.

Although potentials recorded from nerve cord and from lantern show a direct correlation, it has not been proved that the potential bursts which trigger flashes, and which can be recorded from virtually any point on the lantern surface with gross extracellular electrodes, are actually action potentials of the lantern nerves. Case & Strause (1978) suggest that they may be epithelial spiking of tracheolar cells which have been implicated in transmitting excitation from the nerves to the photocyte membrane.

It is possible that continuous stimulation of the nerve cord or the lantern sets up an oscillatory pattern of electrical activity which results in multi-peaked flashes. It would be revealing to observe the activity in the lantern during continuous stimulation of the nerve cord but this has not been possible because the potentials are swamped by the stimulus artifact due to the short distances between stimulating and recording electrodes. The multi-peaked flash responses of the *P. versicolor* lantern produced by continuous nerve cord stimulation and direct lantern stimulation are so similar, however, that it is very likely that the same mechanism is being activated.

Case & Buck (1963) proposed three mechanisms to account for compound flashes. They were: (1) sub-peaks are due to synchronous repetitive firing of photocytes in response to repetitive volleys from the central nervous system; (2) sub-peaks represent responses of photocyte populations differing in latency and (3) sub-peaks reflect a conductional pattern leading to asynchronous excitation of different photocyte populations. Although mechanism (1) cannot explain the lantern response to continuous stimulation, unless the stimulation generates oscillatory neural bursts, mechanisms (2) and (3) are clearly possible. Although the lantern does not show characteristic surface flash compartmentalization which could account for the peaked nature of the flash (Fig. 7), a dorso-ventral stratification occurring throughout the depth of the photogenic tissue could produce the observed response. The characteristic declining intensity of the peaks, however, strongly points to a progressive loss of some component necessary to sustain the luminescence, and this component could be related to neural transmitter release or oxygen control. Although the intact lantern of the healthy *P. versicolor* firefly never glows, it can be made to do so by damage to the cuticle or injection of drugs such as octopamine or adrenaline. In these situations the normal lantern control mechanisms have obviously been by-passed, but it does show that the photocytes are capable of glowing continuously.

In spite of their differences, *P. versicolor* and *P. lucicrescens* lanterns can react very

similarly to short stimulus bursts and presumably to short neural bursts as w[illegible] (Fig. 8A and B). Also, both species produce short flashes and twinkles when irritated or, in the case of *P. lucicrescens*, during courtship (Barber, 1951). The species-specific courtship flashes, however, are strikingly different (Fig. 1A and B) and both species respond very differently to continuous stimulation (compare Figs. 3 and 8C). The lantern of *P. versicolor* is incapable of producing a slowly developing *P. lucicrescens*-type flash. Its lantern appears to be designed to produce a reasonable facsimile of its courtship flash even to a continuous neural burst from the brain. It is not known whether *P. versicolor* females would respond to this more rapidly pulsed flash, but observations by Zorn & Carlson (1978) suggest that the peak intervals in stimulated flashes are not too short to elicit responses from virgin females.

The observation that the lanterns of different species of *Photuris* fireflies differ in their responses to continuous stimulation raises the possibility that evolutionary changes in the lantern could initiate new flash patterns and thus lead to speciation.

The author wishes to thank Bruce Lum and John Gilbert for assistance with the experiments, R. Woodrow Setzer and Mindy Block for statistical analysis, and Thomas Christensen for help preparing the figures. Dr James Lloyd kindly confirmed our identification of *P. lucicrescens* male fireflies. This research was supported by NSF grant BNS 761832701.

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