Brief communication

Modulations in the light of the firefly

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Continuous light could be produced from the firefly by making it inhale vapours of ethyl acetate. Here we perform such a *control* experiment on the Indian species of the firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae), and analyse the light in the microsecond time scale. The amplitude of the continuous train of triangular pulses is apparently altered in accordance with the instantaneous values of a hypothetical signal, which exhibits pulse amplitude modulation (PAM). In addition to sampling in amplitude, this scheme apparently provides sampling in time, representing pulse width modulation (PWM). A Fourier transform spectrum of this waveform shows the 'carrier' frequency and the accompanying 'side bands'.

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1. Introduction

Bioluminescence is the process by which living organisms convert chemical energy into light. Light-emitting organisms in nature include fireflies, bacteria, fungi, crustaceans, mollusks, fishes and insects. In most bioluminescence systems, light results from the oxidation of an organic substrate, a luciferin, catalysed by an enzyme called a luciferase. The reaction is called chemiluminescence reaction. The system is a cold one. There is no intermediate stage involving heat: energy in the form of light is produced directly from the chemical reaction. That is where the importance of chemiluminescence lies. Chemiluminescent glow products never heat up; these are not sources of ignition, and are not flammable.

By far the most efficient example of a bioluminescent system discovered to date is the firefly. Its efficiency is about 90%, meaning about 90 photons of light are produced for each 100 molecules of the reactant. Compared with the dozens of light-producing chemical reactions, most of which are only about 1% efficient, this is an astonishing efficiency.

Fireflies belong to the glowworm family Lampyridae of which there are more than 2000 species throughout the world, especially in the tropics. The firefly species used in the present study is the Indian one *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae).

Quite a few scientific investigations have been carried out on the flashing of the firefly. The duration of a single flash has been reported to vary from about 70 ms (Branham and Greenfield 1996) to a few hundred milliseconds (Buck *et al*. 1963; Lloyd 1973; Barry *et al.* 1979; Saikia *et al.* 2001; Gohain Barua *et al.* 2009) up to a couple of seconds (Gohain Barua *et al.* 2012). Influences on flashing by calcium (Carlson 2004), nitric oxide vapours (Trimmer *et al.* 2001), gating of oxygen to light-emitting cells (Timmins *et al.* 2001), geographic locations (Ohba 2004), temperature variations (Iguchi 2010), static and pulsed magnetic fields (Gohain Barua *et al.* 2012; Iwasaka *et al.* 2011) are some of the aspects investigated in recent times. It has been shown that pulses emitted by the Indian species of the firefly *Luciola praeusta* are very short, lasting a couple of microsecond only (Gohain Barua *et al.* 2009), and that these pulses are manifestations of an oscillating chemical reaction (Gohain Barua and Rajbongshi 2010), like the Belousov-Zhabotinsky (BZ) reaction. This implies that changes in the light-producing chemiluminesence reaction occur at radio frequencies.

2. Materials and methods

Experimental details of the present study have been given elsewhere (Gohain Barua and Rajbongshi 2010). Briefly, an

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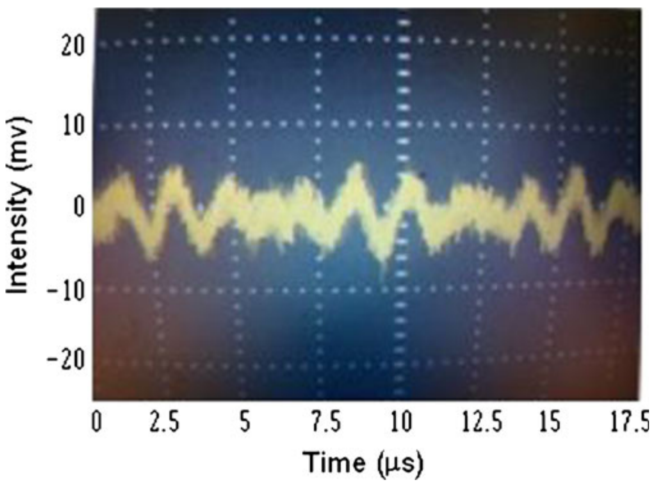

Figure 1. Continuous train of microsecond-duration triangular pulses recorded from an ethyl acetate-affected firefly. A set of three (and a half) pulses is a mirror image of the preceding set of three (and a half) pulses – the resultant of an oscillating chemical reaction.

ethyl acetate-affected continuous light-emitting firefly was placed on the window glass of the photomultiplier tube PHOTONIX XP 2050, whose bias voltage was set at 1.7 V. After amplification by this PMT and then pre-amplification by a preamplifier circuit, a Tektronix TDS 2022 DSO generated the waveform. Sony Cyber-shot DSC-H7S camera was used to record those events from the oscilloscope screen. A total of 10 specimens were used in the experiment. The average temperature in the laboratory during the experiments was 28°C. Origin 6.0 was used to plot the Fourier power spectrum.

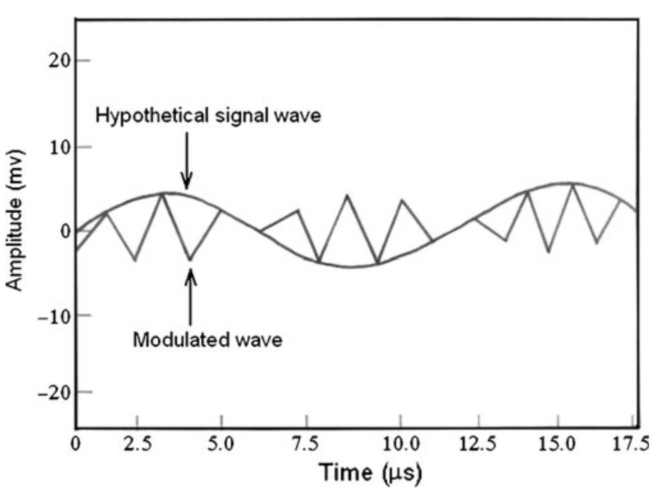

Figure 2. Smoothened waveform along with a hypothetical signal. Both amplitude and duration appear to be modulated according to the 'command' signal, exhibiting pulse amplitude modulation (PAM) and pulse width modulation (PWM).

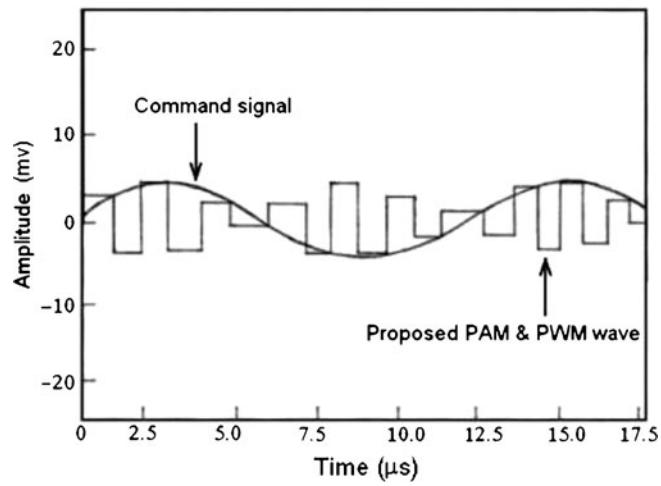

Figure 3. Proposed clock waveform produced by the firefly. It is speculated that the discrete rectangular waveform is 'integrated' in the light-producing organ, producing a continuous train of triangular pulses.

3. Results and discussion

Continuous train of pulses inherent in the 'dc' light from such an anaesthetized firefly appears in the DSO screen as shown in figure 1. While pulses emitted by a normal flashing firefly were quite noisy (Gohain Barua *et al.* 2009), making an analysis difficult, those coming out from the ethyl acetate-affected firefly are quite ordered and noise-free to a large

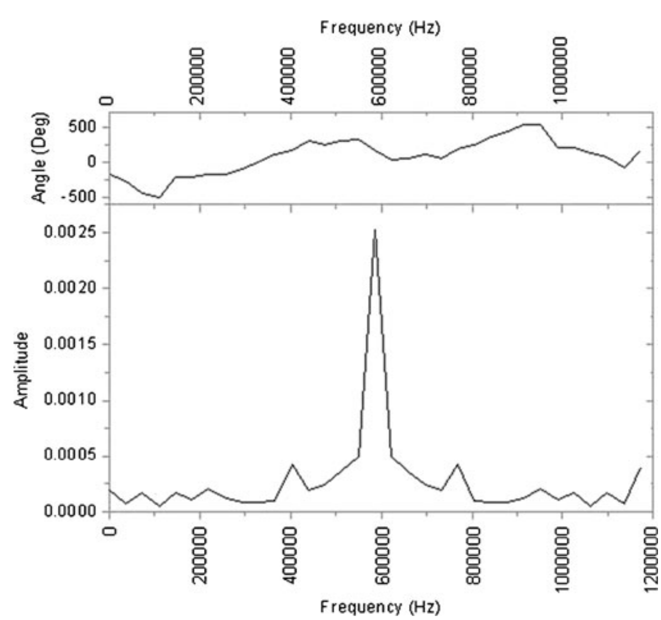

Figure 4. Fourier power transform of the emission data. The peak frequency is at 0.586 MHz, and upper and lower side band frequencies are at 0.769 MHz and 0.403 MHz, respectively.

extent, and hence much easier to analyse. The pulses seen in figure 1 are clear manifestations of an oscillating chemical reaction, like the Belousov-Zhabotinsky (BZ) reaction. As oxygen is the immediate biochemical trigger for light production, it has been proposed (Gohain Barua and Rajbongshi 2010) that ethyl acetate inhibits the respiration of mitochondria, which densely pack the peripheral cytoplasm of photocytes (Trimmer *et al.* 2001) and thereby ensures a continuous oxygen supply to the luciferin-containing organelles (peroxisomes). Thus, the chemiluminescence reaction goes on uninterrupted while the firefly is in an unconscious state.

A smoothened waveform of the same, presented in figure 2, apparently represents pulse amplitude modulation (PAM), as a hypothetical 'command' signal is converted into a series of amplitude-modulated pulses. Every alternate half cycle of the hypothetical signal indicates a phase change of 180°, which point towards an oscillating chemical reaction. From a pure amplitude modulation point of view, the modulation index comes out as 0.5. The intervals at which the amplitude is modulated are a pointer to another scheme of modulation: the pulse width modulation (PWM).

The waveform is clearly triangular in shape. The approximately 100 ms duration flashes of this species of the firefly have also been reported to be triangular (Gohain Barua *et al.* 2012). Drawing an analogy from the production of triangular electronic pulses, we would like to propose the occurrence of an 'integration' of the light of the firefly in the light producing organ. It is well-known that charging and discharging of a capacitor through a suitable resistor produces a triangular waveform. Hence, we would like to propose the existence of a *passive component* in the firefly system whose action on photons is analogous to that of a condenser on electrons. With this hypothesis, the obvious corollary is that the rising half of the pulse indicates the ON state and the falling half of the pulse indicates the OFF state of the light production. As an illustration, for the minimum duration of 1.52 μs (figure 1) the ON and OFF times would be 0.76 μs. This implies that the continuous train of triangular pulses emitted by the firefly in the anaesthetized or unconscious condition actually represents emission of a discrete train of rectangular pulses in the light emitting organ of the firefly. In other words, the continuous light of the firefly possibly represents a rectangular clock waveform, sampled both in amplitude and time – manifesting both pulse amplitude modulation (PAM) and pulse width modulation (PWM). This hypothesis is graphically presented in figure 3. From the rate at which the widths of the pulses are modulated, the 'frequency modulation index' is determined as 0.7. These values are constants for all the specimens studied.

The Fourier transform of the waveform is presented in figure 4. In this figure, the carrier frequency is 0.586 MHz, and the side band frequencies are 0.769 MHz (USB) and 0.403 MHz (LSB). It is difficult to comment on the phase part of the spectrum from the figure.

It is tempting to speculate that the rectangular pulses of light coming out of peroxisomes get converted to triangular ones while coming out of photocytes. As the photocytes contain high concentrations of mitochondria, these could be responsible for giving triangular shapes to the pulses. As a matter of fact, electromagnetic wave propagation in filamentous mitochondria, shown by vertebrate cells, has been theoretically investigated by Thar and Kühl (2004) in a manner akin to that in the case of an optical fiber by considering a mitochondrion as 'core' and cytoplasm as 'cladding'. The distance between neighbouring mitochondria is often much less than the wavelength of visible light, and this would allow radiation propagating through a mitochondrion to cross the gap to the next mitochondrion where it can propagate further. This kind of crossing could possibly result in the capacitor-like action of mitochondria. Also, experiments with isolated mitochondria have demonstrated that external illumination influences their physiology significantly (Gordon and Surrey 1960; Kato *et al.* 1981; Passarella *et al.* 1984; Morimoto *et al.* 1994; Breitbart *et al.* 1996; Greco *et al.* 2001). Because of these characteristics, involvement of mitochondria in the formation of triangular pulses cannot be ruled out at the moment. Well-designed experiments on mitochondria, illuminated with ultra-short square pulses, would make this proposition conclusive. Similar time-resolved studies on the firefly luciferase–luciferin combination continuously triggered by oxygen should also be a future course of action to be undertaken definitely.

We conclude that the oscillating chemical reaction in the light-emitting organ of the firefly produces a pulse-modulated wave. It is interesting to note here that the PWM amplifiers are also 90% efficient, i.e. they deliver energy with 90% efficiency – the same efficiency at which fireflies produce the bioluminescent light!

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