© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb225151. doi:10.1242/jeb.225151

RESEARCH ARTICLE

Carbon dioxide-induced bioluminescence increase in *Arachnocampa* larvae

Hamish Richard Charlton and David John Merritt*

ABSTRACT

Arachnocampa larvae utilise bioluminescence to lure small arthropod prey into their web-like silk snares. The luciferin–luciferase light-producing reaction occurs in a specialised light organ composed of Malpighian tubule cells in association with a tracheal mass. The accepted model for bioluminescence regulation is that light is actively repressed during the non-glowing period and released when glowing through the night. The model is based upon foregoing observations that carbon dioxide (CO_2) – a commonly used insect anaesthetic – produces elevated light output in whole, live larvae as well as isolated light organs. Alternative anaesthetics were reported to have a similar light-releasing effect. We set out to test this model in *Arachnocampa flava* larvae by exposing them to a range of anaesthetics and gas mixtures. The anaesthetics isoflurane, ethyl acetate and diethyl ether did not produce high bioluminescence responses in the same way as CO_2. Ligation and dissection experiments localised the CO_2 response to the light organ rather than it being a response to general anaesthesia. Exposure to hypoxia through the introduction of nitrogen gas combined with CO_2 exposures highlighted that continuity between the longitudinal tracheal trunks and the light organ tracheal mass is necessary for recovery of the CO_2-induced light response. The physiological basis of the CO_2-induced bioluminescence increase remains unresolved, but is most likely related to access of oxygen to the photocytes. The results suggest that the repression model for bioluminescence control can be rejected. An alternative is proposed based on neural upregulation modulating bioluminescence intensity.

KEY WORDS: Glow-worm, Anaesthesia, Fungus gnat, Light organ, Photocyte

INTRODUCTION

Bioluminescence, the emission of visible light by a living organism as a result of a chemical reaction, occurs in a remarkable diversity of organisms spanning terrestrial and marine environments (Wilson and Hastings, 1998). Among arthropods, bioluminescence has been observed in crustaceans, insects and myriapods, with functions including sexual communication, aposematic signalling and prey attraction. In all bioluminescent arthropods, light is produced as the result of the luciferin–luciferase chemical reaction (Viviani, 2002). Luciferase enzymes catalyse the oxygenation of luciferins to produce electrically excited compounds and photons of visible light (Kahlke and Umbers, 2016).

School of Biological Sciences, The University of Queensland, Brisbane, QLD 4072, Australia.

*Author for correspondence (d.merritt@uq.edu.au)

D.J.M., 0000-0002-8573-7508

Received 15 March 2020; Accepted 25 June 2020

The best-known bioluminescent insects are the fireflies (Order Coleoptera: Family Lampyridae) and the members of the genus *Arachnocampa* (Order Diptera: Family Keroplatidae) (Branham and Wenzel, 2001; Meyer-Rochow, 2007). Among these insects, significant differences in bioluminescence production, utilisation and regulation have been observed (Lloyd, 1966; Meyer-Rochow and Waldvogel, 1979; Meyer-Rochow, 2007). Adult lampyrid beetles emit light in controlled, periodic, patterned flashes to detect and communicate with potential mates (Copeland and Lloyd, 1983; Lloyd, 1966). Lampyrid larvae release a steady glow, believed to be used aposematically, correlating with distastefulness (De Cock and Matthysen, 1999). *Arachnocampa* larvae are predators that produce light continuously throughout the night to lure arthropods into web-like silk snares (Broadley and Stringer, 2001, 2009; Mills et al., 2016). The light-producing organs in *Arachnocampa* and fireflies are evolutionarily independent and morphologically distinct, so bioluminescence production and regulation are expected to differ (Viviani et al., 2002). In addition, some other members of Keroplatidae emit light, but they do so through a different organ system than *Arachnocampa* via specialised cells located in the anterior or posterior segments of the larva (Bassot, 1978; Osawa et al., 2014; Falaschi et al., 2019).

The genus *Arachnocampa* is composed of nine species endemic to Australia and New Zealand (Baker, 2010; Baker et al., 2008; Meyer-Rochow, 2007). The larvae inhabit cool, dark places including rainforest embankments and the inside of wet caves (Berry et al., 2017; Merritt et al., 2012; Meyer-Rochow, 2007). The lifespan of an adult *Arachnocampa* is very short, 2–6 days, and the larval state lasts for many months (Baker and Merritt, 2003; Willis et al., 2011). The larvae are relatively immobile and construct snares consisting of mucous-dotted silk lines that hang downward from mucous tubes anchored to a rocky or earthen substrate (Baker and Merritt, 2003; Broadley and Stringer, 2001; Mills et al., 2016; Willis et al., 2011). The species used in the present study, *Arachnocampa flava*, is endemic to southeast Queensland.

Light is produced by a posterior light organ (LO), composed of the modified, large-diameter distal cells of the Malpighian tubules in association with a tracheal mass (Fig. 1) (Green, 1979; Wheeler and Williams, 1915). The photocytes have a dense cytoplasm with synaptic contacts on the cells of the LO containing dense-core vesicles that are indicative of neurosecretory regulation (Green, 1979). A single nerve runs from the terminal abdominal ganglion (TAG), separating into neural processes that innervate the LO (Gatenby, 1959; Rigby and Merritt, 2011). The lateral and ventral surfaces of the LO are covered by a mass of tracheoles, taking on a silvery appearance visible through the cuticle (Green, 1979; Rigby and Merritt, 2011). The tracheal layer is closely associated with the photocytes (Green, 1979), suggesting that access to oxygen is a critical factor in bioluminescence output, just as it is in fireflies (Ghiradella and Schmidt, 2004); however, the firefly LO is evolutionarily derived from a different tissue, believed to be fat body (Amaral et al., 2017).

Journal of Experimental Biology

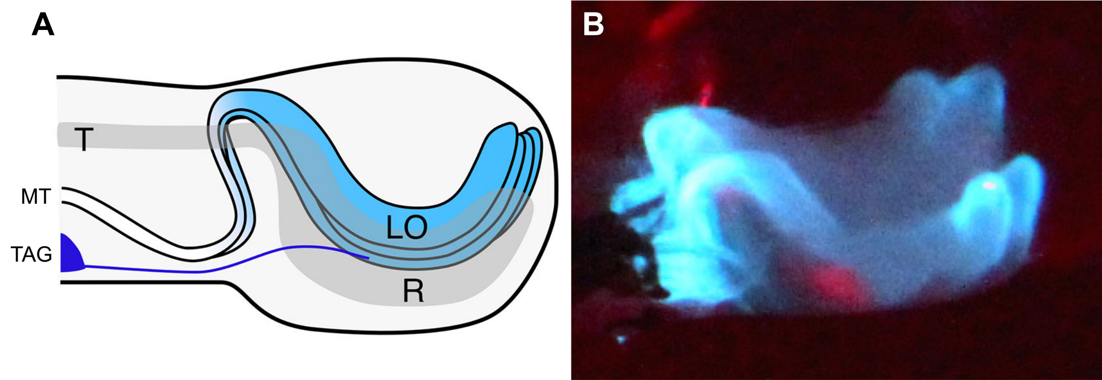

Fig. 1. The *Arachnocampa* light organ. Diagram (A) and macro photograph (B) of a larval light organ (LO) showing the bioluminescent region made up of cells of the Malpighian tubules (MT). The longitudinal tracheal trunks (T) connect with the reflector mass (R) adjacent to the LO cells. Dorsal is up; however, note that larvae in their snares lie in a ventral-up position. TAG, terminal abdominal ganglion.

The regulation and production of light by *Arachnocampa* larvae is less well known than that of fireflies (Ghiradella and Schmidt, 2004; Lloyd, 1966; Timmins et al., 2001; Trimmer et al., 2001). Prior to the present study, the prevailing model for bioluminescence regulation in *Arachnocampa* was that bioluminescence is actively repressed when larvae are not glowing, such as under daylight or when disturbed, and that the repression is released under darkness (Gatenby, 1959; Rigby and Merritt, 2011). *Arachnocampa* larvae brighten substantially when exposed to a vibration stimulus (Mills et al., 2016); vibration of whole larvae in containment produced a 7- to 10-fold increase in bioluminescence. To incorporate this neurally based brightening, Mills et al. (2016) proposed a two-part regulatory system: (1) the bioluminescence-inhibiting system originally proposed by Gatenby (1959) that prevents bioluminescence when larvae are exposed to daylight or natural light, and (2) an acute vibration response mediated via signals from the central nervous system, which is followed by a return to pre-stimulus levels. Evidence for the repression component came from ligation and gas exposure experiments. Ligating larvae behind the terminal abdominal ganglion anterior to the LO caused the LO to emit light (Gatenby, 1959), and isolated LOs with neural connections removed emitted low levels of light (Rigby and Merritt, 2011), interpreted as being due to the release of inhibition. In *A. richardsae*, the anaesthetics CO_2, ether and chloroform caused light release in whole larvae while methanol and ethyl acetate were ineffective (Lee, 1976). Isolated LOs of *A. flava* released low-intensity light when removed from the body and then emitted very bright light when CO_2 was introduced (Rigby and Merritt, 2011).

In fireflies, the current understanding is that neurally regulated oxygen-gating is involved in the regulation of flash bioluminescence. Oxygen transfer to light-producing peroxisomes is interrupted by the mitochondria consuming oxygen between episodes of bioluminescence (Timmins et al., 2001; Trimmer et al., 2001). In contrast to adult fireflies, *Arachnocampa* larvae slowly modulate light emission, suggesting that the systems will differ (Mills et al., 2016). A common feature is that both fireflies and *Arachnocampa* possess a rich tracheal supply to the LO and both require ATP and oxygen for light release (Kahlke and Umbers, 2016; Mills et al., 2016; Timmins et al., 2001). It appears the access to oxygen is a vital factor for light production in both the coleopteran and dipteran bioluminescence systems.

Here, we explore the physiological and morphological mechanisms of bioluminescence regulation by exposing *A. flava* larvae to a range of anaesthetic, atmospheric and vibration stimuli and examining the consequent responses. This study complements other studies of the *Arachnocampa* luciferin–luciferase system, which are revealing similarities with coleopteran systems – the luciferases belong to the same family of enzymes (Sharpe et al., 2015; Silva et al., 2015) – as well as differences – the *Arachnocampa* luciferin is novel, being different from any described to date (Watkins et al., 2018). Given that bioluminescence is emitted in long bouts and comes under slow neural control, the bioluminescence regulatory mechanisms of *Arachnocampa* are of significant interest when compared with the well-known adult firefly system.

MATERIALS AND METHODS

Experimental animals: collection and maintenance

Arachnocampa flava Harrison 1966 larvae were collected from Springbrook National Park, Queensland, Australia, in accordance with a Department of Environment and Science permit (PTU18-001356). Larvae (~2–2.5 cm length), probably corresponding to the fourth or fifth instar stages, were collected. Larvae were individually housed in halved, inverted plastic containers (7×7 cm height×diameter) with clay pressed into the upturned base, and fronted with transparent plastic. The containers were kept in glass aquaria filled with ~1 cm of water and sealed with a glass lid and plastic wrap to ensure high humidity. The aquaria were placed inside a temperature-controlled (24±1°C) room under 12 h:12 h light:dark conditions with artificial light produced by a 12 V DC white LED lamp controlled by a timer. Larvae were acclimatised to the conditions for a minimum of 1 week before being utilised in experiments. Larvae that pupated during or immediately following the experiment were not used in analysis. Once per week during the photophase of a non-treatment day (at ~13:00 h), larvae were fed three CO_2-anaesthetised *Drosophila melanogaster* adults by securing the fruit flies to the silk lines of *A. flava*, taking care to minimise damage to the lines. All experiments were carried out during the day, when larvae would not normally be glowing.

Measuring bioluminescence

Larval bioluminescence was imaged using a SLR camera (Canon EOS 1000D), using identical exposure time, lens and distance between the lens and larva for all experiments. Camera settings were 25 s exposure, F5.6, ISO 640. A Pclix XT intervalometer camera controller was used to define intervals between exposures. The application, ImageJ (Version 1.52a), was used to analyse the light output from the individual larvae using the method described by Mills et al. (2016). Briefly, the greyscale images were imported into ImageJ, then a threshold value (maximum: 250, minimum: 30, where 0 is white and 256 is black) was applied to distinguish the

Journal of Experimental Biology (2020) 223, jeb225151. doi:10.1242/jeb.225151

image of the glowing LO region from the background and the intensity of the pixels comprising the glow was summed. As the intensity of isolated pixels is a non-SI unit, the bioluminescence output is referred to in terms of relative light units.

Light output of live larvae

To investigate the effect of CO_2, live larvae were pinned to a wax dish, taking care not to damage any internal structures. They were then placed under the camera in a transparent, airtight plastic container (400 ml) with gas ingress and egress connectors and imaging initiated. The pre-treatment bioluminescence output was recorded once per minute for 15 min, then larvae were exposed to the treatment gas for 1 min at a rate of 10 litres min^{-1}. The inflowing CO_2 was humidified by bubbling through water. Imaging continued for 15 min after treatment.

Light output in ligated larvae

To investigate light emission when there was no direct connection between the brain and LO, larvae were ligated with a silk thread at two different locations – (1) behind the head or (2) just anterior to the LO – and the LO-containing region separated by cutting anterior to the ligation (Fig. 2B). The LO section was then placed on a wax dish (head ligation) or in an excavated glass block under saline (2.6 mmol l^{-1} KCl, 1.8 mmol l^{-1} $CaCl_2$, 150 mmol l^{-1} NaCl and 11 mmol l^{-1} sucrose) (Lozano et al., 2001) and exposed to CO_2 using the chamber described above with a 15-min pre-treatment recording period followed by a 1-min CO_2 exposure, followed by 15 min of recording.

Light output of isolated light organs

To isolate the LO, each larva was pinned to a wax dish and a cut was made along the dorsal midline to expose the internal organs. The LO, visible as a small cell mass associated with the silvery-white tracheal reflector (Fig. 3), was removed from the body and placed in saline. For each application, a baseline bioluminescence output was established over 15 min. The saline was then replaced with CO_2-saturated saline and imaged for another 15 min. CO_2 saturation was achieved by bubbling the gas through the saline beforehand via an aquarium aerator stone for 60 s. As a control, the isolated LOs of five larvae were treated with acidified saline at the same pH as the CO_2-saturated saline (pH 5.3). As an alternative means of introducing CO_2, the gas was introduced for 1 min into the airspace above an excavated block holding isolated LOs under saline.

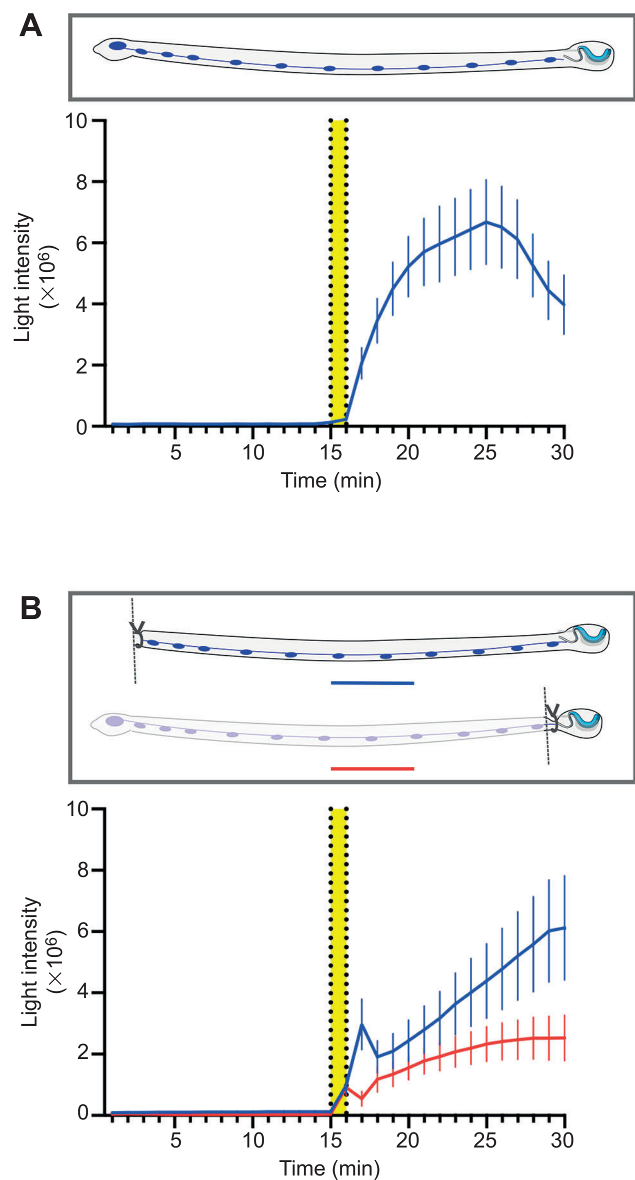

Fig. 2. The bioluminescence output following exposure to CO_2 in the airspace. (A) Live *A. flava* larvae (*n*=8, mean±s.e.m.). (B) Ligated, head-removed larvae (*n*=13, blue) and ligated LO-isolated larvae (*n*=12, red) (means±s.e.m.). Yellow bar indicates period of gas exposure. Insets depict the type of treatment applied to the larvae before exposure to CO_2.

Light output in semi-intact dissected larvae

An apparatus was designed to allow introduction of gas to exclusively the head region or the LO-containing region of a partly dissected larva (Fig. 4). Larvae were pinned and dissected along the dorsal midline on a wax dish. A plastic cylinder (5 mm diameter) was then placed over either the LO or the head region containing the brain, and an air-tight barrier was formed using petroleum jelly to isolate the cylinder from the rest of the larva. A glass cover-slip was then placed over the top of the cylinder. A gas inlet and outlet port were drilled into the side of the cylinder. This approach allowed sequential directed introduction of CO_2 into the airspace either above the LO or above the brain.

After dissection and mounting of the cylinder, each larva was imaged for 15 min (1 frame min^{-1}) and CO_2 was introduced into the cylinder airspace for 1 min (10 litres min^{-1}). The larva was then imaged for a further 25 min and the CO_2 cylinder was moved to the alternative target area (either LO or brain) not exposed in the initial phase. CO_2 was introduced at the second location and imaged for 15 min.

To apply CO_2 in solution, larvae (*n*=5 for each treatment) were pinned and dissected on a wax dish to reveal the LO and internal organs, as described above. Before any saline was applied, a petroleum jelly barrier was constructed across the mid-body so that a solution applied to either segment would not contact or flow through to the other, then both the anterior and posterior regions were immersed in a drop of saline. The larvae were imaged for 15 min, and then CO_2-suffused saline was placed on either the LO segment or the brain segment and imaged for a further 15 min. Standard saline was used to flush away the CO_2-suffused saline before imaging for a further 25 min. CO_2-suffused saline was then placed on whichever target site was not exposed in the initial phase of the experiment and imaged for a further 15 min.

Effect of hypoxia on light output

To investigate the effect of hypoxia, the isolated LOs in saline were exposed to humidified N_2 in the airspace above the LOs for 1 min. After 15 min, CO_2 was introduced for 1 min at a rate of

Journal of Experimental Biology

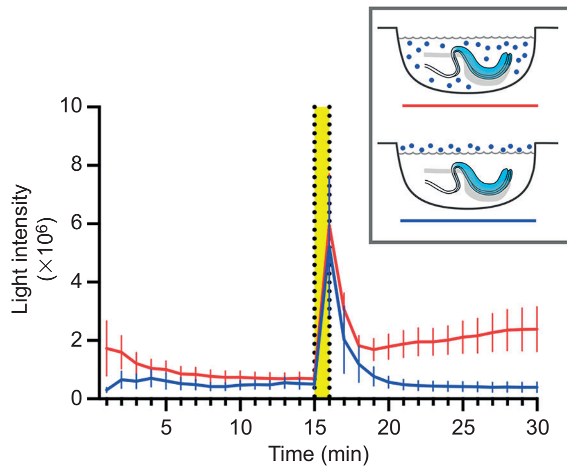

Fig. 3. The bioluminescence outputs of isolated *A. flava* LOs in saline. Exposure to CO_2-saturated saline (n=10, red) and the introduction of CO_2 into the airspace (n=11, blue) (means±s.e.m.). Yellow bar indicates CO_2-diffused saline application or introduction of CO_2. Inset is a diagram of the experimental conditions.

10 litres min^{-1}. In a modified approach, isolated LOs in saline were exposed to N_2 for 1 min followed immediately by CO_2 for 1 min, both at a rate of 10 litres min^{-1}. After 15 min, the CO_2 exposure was repeated. This treatment was also applied to ligated, head-removed sections of larvae. Hypoxia recovery was further explored by exposing either isolated LOs (n=9) or ligated, head-removed sections of larvae to N_2 for 1 min at a rate of 10 litres min^{-1}, followed by a recovery time of either 5, 10 or 15 min before being exposed to CO_2 at the same rate and duration. The imaging set-up and pre- and post-exposure periods were the same as for CO_2 exposures described above.

Alternative anaesthetics

To investigate bioluminescence output when the LO was exposed to alternative anaesthetics, isolated LOs in saline were exposed to ethyl acetate or diethyl ether (n=4 for each treatment). Approximately 50 ml of either ethyl acetate or diethyl ether was placed in a 500 ml glass conical flask and vapour was allowed to accumulate for 15 min. To expose the LO, air was pumped through this bottle into the LO chamber for 1 min at a rate of 10 litres min^{-1} with pre- and post-exposure imaging periods of 15 min each. An isoflurane vaporiser (Model V300PS) was used to expose the larvae to 4% isoflurane at the same rate and duration as the other anaesthetics.

Vibration response

Larvae were placed in a humidified aquarium before the onset of darkness. Two 10 mm diameter vibrating AD1201 haptic motors (180–200 Hz) were attached to the sides of the aquarium with duct tape and operated using a programmed Raspberry Pi 3 (model B V1.2). The set-up and stimulus frequency were similar to that used previously to assess the response of larvae to vibration at insect wing-beat frequency (Mills et al., 2016). Light output of the larvae was recorded at 1-min intervals through the night using a SLR camera (Canon EOS 1000D) under time-lapse control, viewing the larvae from below the aquarium. The vibrating haptic motors were activated in 15 s pulses at a range of inter-pulse intervals throughout the scotophase. Treatment nights were interspersed between control nights with no vibration stimuli, using the same larvae. Light intensity of individual larvae was determined using ImageJ as described above. In this series of experiments, the light units are not comparable to those from all other experiments owing to the different location of the camera. The light output was displayed as the mean intensity of the larvae in the aquarium. The same larvae were used in multiple treatments; however, data from larvae that pupated during or within 3 days of an experiment were removed.

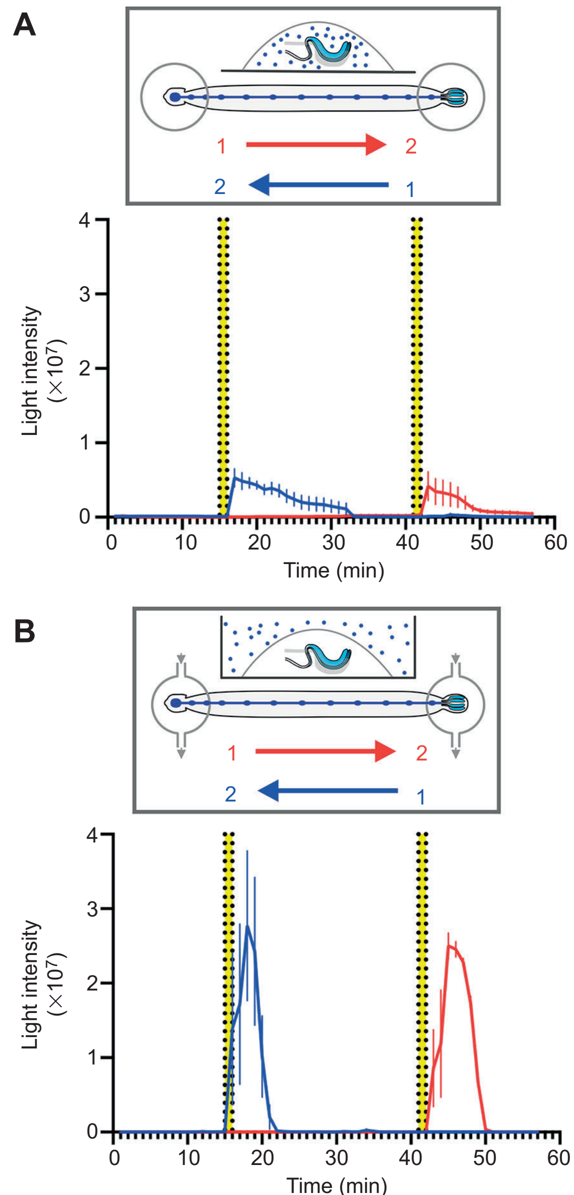

Fig. 4. The bioluminescence outputs of semi-intact *A. flava* larvae with CO_2 exposure alternating between the head and LO regions. (A) Dorsally dissected larvae were exposed to CO_2-saturated saline (yellow bars) in the order (1) the brain followed by the LO (n=5, red) or (2) the LO followed by the brain (n=5, blue). (B) Dissected larvae exposed to CO_2 in the airspace above the head or LO region depicted using the same colour codes as A (n=5, for each treatment order) (means±s.e.m.). Insets are diagrams of the experimental conditions.

Statistical analysis

Data from physiological experiments were analysed using a paired t-test, comparing the total mean light output for 15 min pre- and post-exposure to stimuli. Further, a paired t-test was performed to compare light output in paired trials on the same larvae, as this test compares two different methods of treatments, where the treatment is applied to the same subject. The test determined

Journal of Experimental Biology (2020) 223, jeb225151. doi:10.1242/jeb.225151

whether the two methods of treatment produced results with significant difference.

RESULTS

CO_2 response

The bioluminescence output of live larvae (*n*=8) increased when exposed to CO_2 in the airspace (Fig. 2A). Very low levels of light were emitted by the larvae before treatment, but this output did not register on the scale that shows the subsequent response to CO_2. On exposure, the larval light intensity increased over the next 10 min and started to decline after reaching a mean peak light intensity of 6.6×10^6 units.

Ligated, head-removed larvae and ligated, LO-isolated larvae (*n*=13, 12, respectively) displayed increases in bioluminescence intensity when exposed to CO_2 in the airspace (Fig. 2B). Both treatments showed an initial increase in bioluminescence followed by a brief decrease and then a steady increase in bioluminescence, culminating at a mean peak light intensity of approximately 6.1×10^6 units (ligated, head-removed) and 2.5×10^6 units (ligated, LO-isolated). In both, the bioluminescence level remained elevated after the 15-min recording period.

When LOs were removed by dissection, they were isolated from the larval body and all contact with nerves and main tracheal trunks was removed. After this treatment, the LOs released light at relatively low levels through the pre-exposure period (mean of 3.9×10^5 units). Exposure to CO_2-saturated saline (*n*=10) or CO_2 in the airspace (*n*=11) produced an immediate increase in bioluminescence intensity within 1–2 min (Fig. 3). The CO_2-saturated saline treatment produced a peak bioluminescence level of 5.9×10^6 units, which exponentially declined over the next 15 min but remained above the pre-stimulus levels of bioluminescence. The CO_2–airspace treatment showed a lower peak of 5.2×10^6 units and returned to pre-stimulus bioluminescence levels after approximately 7 min.

Localisation of the CO_2 effect

The high light emission response to CO_2 was localised by sequentially exposing the LO and the brain to CO_2 using two different approaches: (1) applying CO_2-saturated saline to either region or (2) exposing the airspace above either region. When CO_2-saturated saline was applied to the LO first (*n*=5) (Fig. 4A), bioluminescence production increased greatly and no obvious increase occurred when the treatment was applied to the brain. Similarly, when the CO_2-saturated saline was applied to the brain first and then the LO (*n*=5), bioluminescence intensity increased when the treatment was directed to the LO. The sequential application of CO_2 to the airspace above the brain and the LO (*n*=5 for each treatment) (Fig. 4B) produced similar results: exposure to the brain region produced no bioluminescence response while exposure to the LO produced a large response. Overall, the bioluminescence responses recorded through exposure to CO_2-saturated saline (Fig. 4A) produced less light than those exposed to CO_2 in the airspace (Fig. 4B). Airspace exposure produced a shorter-lasting light output than saline exposure.

To determine whether the application of CO_2 was indirectly triggering acute bioluminescence response by altering either the internal pH of *A. flava* or the pH of the saline, an acidified saline of pH 5.26, identical to that of CO_2-saturated saline, was applied to isolated LOs (*n*=4). No bioluminescence responses were recorded after exposure (data not shown).

Combining hypoxia and CO_2 exposure

To determine the effect of hypoxia on bioluminescence production, isolated LOs (*n*=3) were exposed to N_2 for 1 min, immediately followed by the introduction of CO_2 for the same period. No bioluminescence responses were detected (data not shown). When a 15-min recovery period was allowed, and a second CO_2 treatment was applied, the isolated LOs (*n*=3) produced no bioluminescence responses (data not shown). However, when this same treatment series was applied to ligated, head-removed larvae (*n*=3) (Fig. 5), a low response was elicited after the first CO_2 exposure and a much higher bioluminescence level was recorded following the second CO_2 exposure. As an additional control, introduction of N_2 into the airspace above isolated LOs produced no bioluminescence (*n*=3, data not shown).

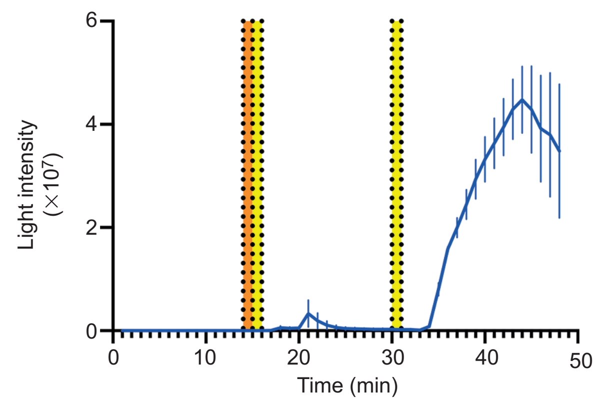

Fig. 5. The bioluminescence output of ligated, head-removed *A. flava* larvae in response to N_2 exposure. Larvae were exposed to N_2 (orange bar) immediately followed by CO_2 exposure (yellow bar), which was repeated after a 15-min recovery period (yellow bar) (*n*=3). Data are shown as means±s.e.m.

To investigate the ability of ligated, head-removed larvae to recover from hypoxia, the segments were exposed to N_2 for 1 min, followed by a recovery period of 5, 10 or 15 min (*n*=5 for each treatment) before exposure to CO_2 for 1 min. In all cases, light was released on exposure to the CO_2 pulse (Fig. 6). When the identical treatments were applied to isolated LOs, no light was emitted after the recovery periods.

Alternative anaesthetics

To determine whether the CO_2 response was due to an anaesthetic effect, the alternative anaesthetics isoflurane, ethyl acetate and

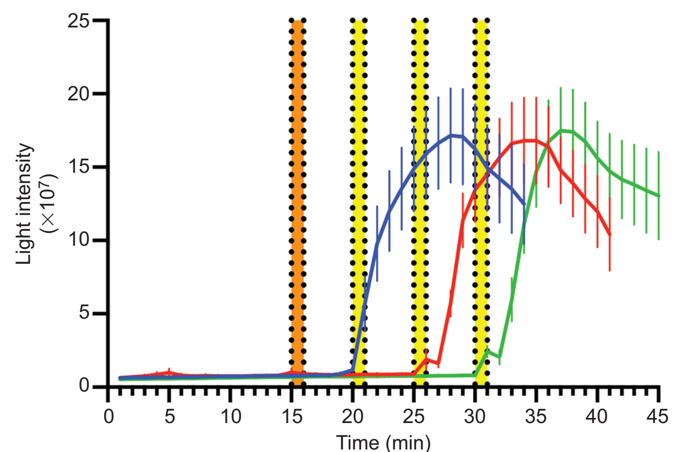

Fig. 6. The bioluminescence output of ligated, head-removed *A. flava* larvae in response to N_2 exposure followed by CO_2 exposure after delays. N_2 exposure (orange bar) was followed by CO_2 exposure after 5 min (*n*=5, blue), 10 min (*n*=5, red) or 15 min (*n*=5, green) (yellow bars). Data are shown as means±s.e.m.

Journal of Experimental Biology

Journal of Experimental Biology (2020) 223, jeb225151. doi:10.1242/jeb.225151

diethyl ether were applied to isolated LOs via airspace treatment (*n*=4 for each treatment) (Fig. 7). None of the treatments produced bioluminescence responses at levels comparable to that induced by CO_2. A small increase was noted in the ethyl acetate and diethyl ether treatments; however, it did not precisely coincide with exposure.

Vibration

Undisturbed larvae began bioluminescence shortly after the onset of darkness and reached peak light intensity approximately 1 h into the 12-h scotophase (Fig. 8). Following the peak, the bioluminescence intensity steadily declined over the next 11 h and did not completely cease until lights-on.

Exposure of larvae to vibration produced significant increases in bioluminescence output compared with the controls recorded during the preceding scotophase without vibration (paired *t*-test, $P<0.001$) (Fig. 9, Fig. S1). Larvae exposed to 1 min of vibration at frequencies of 1 pulse h^{-1}, 1 pulse per 30 min and 1 pulse per 15 min, with each pulse consisting of 15 s at 180–200 Hz, showed acutely elevated light responses to vibration stimulus (Fig. 9A–C, Fig. S1A–C). The bioluminescence outputs peaked following vibration and then exponentially returned to approach the pre-stimulus levels. It is apparent that the average amplitude of the acute vibration response decreased as the interval between pulses shortened. Acute responses followed by declines were not seen when treatments occurred less than 15 min apart (Fig. 9D–F, Fig. S1D–F). Rather, larvae displayed persistent, elevated levels of bioluminescence without the response decline seen when treatments were more widely spaced. Larvae vibrated at 1 pulse min^{-1} recorded the greatest bioluminescence increase.

When larvae (*n*=9) were exposed to vibration pulses at 1 pulse per 5 min for 3 h, the bioluminescence level was elevated and responses to the individual pulses were not apparent (Fig. 10). When vibration ceased between 3 h and 6 h 55 min after lights-off, bioluminescence returned to a lower baseline level and approached that recorded in the control treatment. When the pulse series was re-initiated, the larval light output immediately elevated and remained higher than in the rest period.

DISCUSSION

Elevated bioluminescence in response to CO_2

At the initiation of this study, it was believed that CO_2 causes a major release of light in *Arachnocampa* through its anaesthetic effect (Lee, 1976; Rigby and Merritt, 2011). In insects, CO_2 is a widely used anaesthetic that is believed to block synaptic transmission at the neuromuscular junction by influencing glutamate sensitivity (Badre et al., 2005). Diethyl ether and the halogenated ether isoflurane are commonly used to anaesthetize both vertebrates and invertebrates (Whalen et al., 2005; Barber et al., 2012; MacMillan et al., 2017), and ethyl acetate is another insect anaesthetic (Loru et al., 2010) with an unknown mode of action. Here, we found low to zero response to any anaesthetic other than CO_2, casting doubt on the concept of anaesthesia releasing neural repression of light output. Lee's (1976) experiments were performed on an unstated and possibly small sample size and responses to the different anaesthetics were not quantified, so it is possible that light levels were not strictly compared among treatments. It has been noted since that the light released upon separation of the LO from the body was very dim compared with that released under exposure of the LO to CO_2 (Rigby and Merritt, 2011). The present study confirms that observation, that CO_2 produces intense light production.

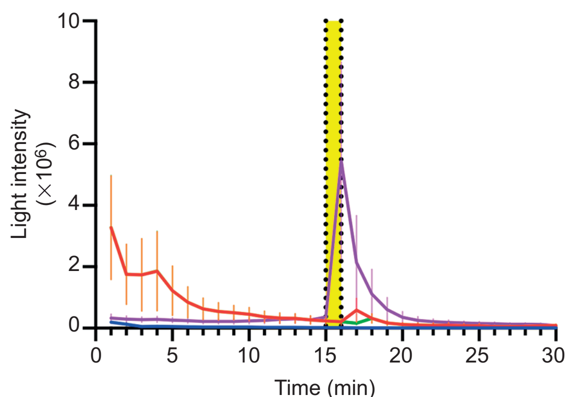

Fig. 7. The bioluminescence response of isolated *A. flava* light organs to anaesthetics in the airspace. Anaesthetics were ethyl acetate (red), diethyl ether (green), isoflurane (blue) (*n*=4 for each treatment) and CO_2 (purple; *n*=8). Data are shown as means±s.e.m.

To explore the location of the CO_2 effect, either the brain or the LO of whole, semi-dissected larvae (the body wall was opened up and pinned out under saline to reveal the internal organs) was exposed to either CO_2-saturated saline or CO_2 in the airspace. Using either method, bioluminescence responses were seen only when CO_2 was directed to the LO, indicating that its effect is initiated at the LO and that exposure of the brain has no direct effect on bioluminescence. The outcome also supports the above: that the CO_2-induced elevation of light is not due to a general anaesthetic effect acting on the brain. The findings cast doubt on the validity of models where neural signals from the brain repress bioluminescence and where CO_2 acts as a general anaesthetic. A simpler model is that the brain or possibly other components of the central nervous system such as the thoracic or abdominal ganglia actively elevate bioluminescence in response to neural stimuli (see below).

How then does CO_2 have such a strong effect on bioluminescence? A supply of air to the larva is essential for light emission, as shown by dimming of larvae under vacuum, and brightening of larvae when air was readmitted (Lee, 1976). A previous study has highlighted the intimate anatomical relationship between the LO and the tracheal mass (Green, 1979), indicating that the availability of oxygen is necessary for the oxidative bioluminescence reaction, just as it is in firefly larvae (Carlson, 1965; Hastings and Buck, 1956) and adults (Buck, 1948; Timmins et al., 2001). Here, we confirmed that hypoxia limits the CO_2-induced brightening. When CO_2 was introduced immediately following N_2 exposure or after recovery periods of 5–15 min, the isolated LOs showed no bioluminescence. When CO_2 was applied to ligated, head-removed larvae immediately after N_2, a low light output was recorded and a second CO_2 exposure 15 min later produced a significantly higher light output, indicating that the ligated section is capable of recovery from hypoxia. Using another approach, ligated head-removed larvae exposed to N_2 followed by CO_2 after a spaced delay (5–15 min) showed a recovery of the intense CO_2 response.

We consider it most likely that recovery is due to the LO tracheal mass maintaining a connection with the main longitudinal tracheal trunks in ligated larvae, whereas that connection had been removed in isolated LOs. *Arachnocampa* larvae are apneustic (Ganguly, 1959), i.e. they have no spiracles, although the longitudinal tracheal trunks are air-filled. This respiratory strategy is common in aquatic or endoparasitic insect larvae (Keilin, 1944; Thorpe, 1932). Gas exchange is cutaneous – across the cuticle – which is probably aided in *Arachnocampa* by the fact that larvae have thin cuticle and dwell inside a mucous tube. In apneustic insects, air-filling is attributed to the cells lining the trachea having the ability to withdraw fluid from

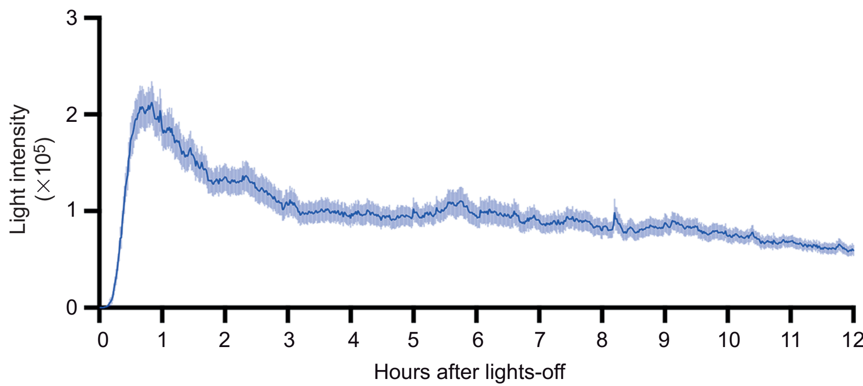

Fig. 8. Bioluminescence intensity of *A. flava* larvae through a night. The generalised bioluminescence output (mean±s.e.m.) derived from 108 *A. flava* larvae recorded in laboratory conditions under an artificial light cycle.

the tracheal lumen and replace it with air (Buck and Keister, 1955). In *Arachnocampa* larvae, the longitudinal tracheal trunks run directly into the tracheal mass associated with the photocytes (Green, 1979). We propose that the epithelial cells of the main paired longitudinal tracheal trunks modulate the oxygen content of the lumen through active transport. The recovery seen in ligated larvae is attributed to reoxygenation of the tracheal lumen during the recovery period and suggests that available oxygen is consumed during the CO_2-induced bioluminescence.

There have been no reports of similar acute bioluminescence outputs in larval or adult fireflies upon CO_2 exposure (Buck, 1948), so the response of *Arachnocampa* appears to be unique to its regulatory system. CO_2 is known to be a product of light production in the ATP-dependent light production system of fireflies (Plant et al., 1968), which is likely to also apply to *Arachnocampa* because of the related luciferase system (Lee, 1976). It could be that light production in the photocytes is modulated by both CO_2 and O_2 levels and that the light regulation mechanism is reliant on a balance

Fig. 9. Bioluminescence output of live *A. flava* larvae in response to spaced vibration. Larvae were exposed to a 15-s (180–200 Hz) vibration at a rate of: (A) 1 pulse h^{-1} (n=17), (B) 1 pulse per 30 min (n=17), (C) 1 pulse per 15 min (n=17), (D) 1 pulse per 12 min (n=7), (E) 1 pulse per 10 min (n=8) and (F) 1 pulse per min (n=9). The bioluminescence output of the same larvae recorded during the prior night's scotophase is shown in red. Lights-off occurred at 00:00 h. Data are shown as means±s.e.m.

Journal of Experimental Biology (2020) 223, jeb225151. doi:10.1242/jeb.225151

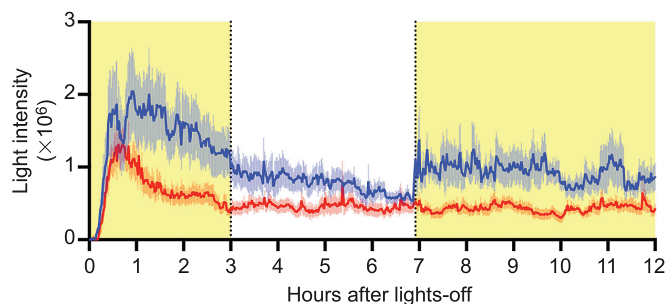

Fig. 10. Bioluminescence output (mean±s.e.m.) of nine live *A. flava* larvae in response to a 15-s 180–200 Hz vibration at a rate of 1 pulse per 5 min between 00:00 h and 03:00 h, and 06:55 h and 12:00 h (yellow shaded areas). Vibration responses were compared with the bioluminescence output of an untreated control (red) recorded the previous night. Lights-off occurred at 00:00 h.

between both gases. It is possible that high partial pressure of CO_2 triggers a runaway reaction owing to the supra-metabolic levels of CO_2 producing maximum light production in the LO cells. Such high and persistent light levels were also seen when larvae were fed prey items dosed with either phentolamine or octopamine, involving biogenic amines in bioluminescence regulation (Rigby and Merritt, 2011), but how neurotransmitters interact with gas regulation remains unknown.

Vibration response

Larvae are capable of upregulating the level of bioluminescence in situations where prey are detected in their web, during aggressive interactions with other larvae (Broadley and Stringer, 2009; Mills et al., 2016) and at the onset of rainfall (Merritt and Patterson, 2018). These responses are believed to be mediated through detection of vibration, and this stimulus–response system is readily manipulated in the laboratory (Mills et al., 2016). When whole larvae were glowing at an undisturbed level during the night, a vibration stimulus produced an acute increase in light output to 7–10 times the base level that slowly returned to pre-stimulus levels (Mills et al., 2016). The upregulation is likely mediated through the nervous system; however, it is not known whether the elevated light emission is attributable to an increased oxygen supply to the photocytes or to the availability of the light-producing reaction involving luciferin, luciferase or ATP. Here, we examined whether persistent stimulation would produce adaptation or effector fatigue. First, we tested whether light emission decreased with repeated vibration exposures. When larvae were exposed to 1 pulse per 15 min or less frequently, an acute response was recorded. When pulses were more closely spaced, light output remained persistently elevated above the control level. This appears to be due to a general elevated excitation level, because a 3-h gap in stimulus exposures resulted in a gradual decline in light output, approaching the control level, followed by a return to a higher level when stimuli were reinstated. We conclude that adaptation does not occur through a 12 h scotophase because the level of response to spaced pulses was consistent. Second, evidence of effector fatigue was seen: the more widely spaced pulses produced consistently higher peak responses. This could be due to reduced availability of light-producing metabolites as the stimuli become more closely spaced. Although the experimental observations presented here do not shed light on the mechanism by which vibration elevates light output, it is assumed to be via sensory receptors such as chordotonal organs found in the terminal papillae and along the body wall, the vibration response will be a useful readout for quantifying the effect of gas mixtures on the bioluminescence system.

Bioluminescence regulatory mechanism

Our findings suggest that the model of light production being suppressed under neural control when it is in the off state should be rejected owing to (1) the lack of a bioluminescence response when anaesthetics other than CO_2 were applied to the LO, and (2) the fact that CO_2 directly affects the LO. The alternative model we propose is that bioluminescence is activated under neural control during the glowing period and that a lack of neural activation produces the switch-off during the photophase. Although the models might not appear to be substantially different – both involve central nervous system integration of signals and ultimate control of light output – an active promotion of bioluminescence is much easier to reconcile with the vibration-induced elevation of bioluminescence than an inhibition mechanism because the latter calls upon bioluminescence being switched on by a loss of inhibition, but it also calls upon bioluminescence being increased by an activation mechanism such as detection of vibration. So the simpler control model is that excitatory neural signals initiate bioluminescence, maintain it at a steady state, and mediate the vibration-induced startle reflex. Octopamine is the prime candidate as the neurotransmitter (Rigby and Merritt, 2011). The time course of light elevation and reduction is much slower than comparable neutrally controlled bioluminescence such as that seen in fireflies. This suggests that the neural control acts on a second mechanism that works over a longer time course, with the most likely being the modulation of oxygen access to the photocytes. One possibility is a sphincter-based control system that modulates airflow between the longitudinal trachea and the tracheal mass, as mentioned by others (Gatenby and Ganguly, 1956; Rigby and Merritt, 2011) but not yet thoroughly investigated. Another is modulation of oxygen transfer across the junction between the tracheal mass and the photocytes, perhaps akin to the indirect way oxygen transfer between tracheal end cells and photocytes is modulated in adult fireflies (Timmins et al., 2001; Trimmer et al., 2001). At the ultrastructural level, the tracheolar units are very closely opposed to the basal lamina of the photocyte cells with many deep infoldings of the membrane (Green, 1979), all consistent with a functional association between the photocytes and the tracheal supply. There is no barrier of mitochondria between the photocyte cytoplasm and the air supply as seen in firefly LOs – the large mitochondria in *Arachnocampa* photocytes are distributed throughout the cytoplasm (Green, 1979) – however, the firefly arrangement appears to be specifically adapted to flash control. Further experiments exposing larvae to combinations of gas mixtures and excitatory/inhibitory stimuli should reveal more details of light regulation in *Arachnocampa*.

Acknowledgements

We thank Dr Simone Blomberg for statistical advice, Professor Bruno Van Swinderen and Michael Troup for use of isoflurane vaporiser equipment, and members of the Asgari Lab for help with pH measurement. We thank Rory Charlton for his invaluable assistance in designing and constructing the vibration apparatus.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: D.J.M.; Methodology: H.C., D.J.M.; Formal analysis: H.C.; Resources: D.J.M.; Writing - original draft: H.C.; Writing - review & editing: D.J.M.; Visualization: H.C.; Supervision: D.J.M.

Funding

The authors acknowledge financial assistance from the School of Biological Sciences, the University of Queensland.

Supplementary information

Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.225151.supplemental

References

Amaral, D. T., Silva, J. R. and Viviani, V. R. (2017). Transcriptomes from the photogenic and non-photogenetic tissues and life stages of the *Aspisoma lineatum* firefly (Coleoptera: Lampyridae): implications for the evolutionary origins of bioluminescence and its associated light organs. *Gene Rep.* **8**, 150-159. doi:10.1016/j.genrep.2017.07.004

Badre, N. H., Martin, M. E. and Cooper, R. L. (2005). The physiological and behavioral effects of carbon dioxide on *Drosophila melanogaster* larvae. *Comp. Biochem. Physiol. A* **140**, 363-376. doi:10.1016/j.cbpb.2005.01.019

Baker, C. H. (2010). A new subgenus and five new species of Australian glow-worms (Diptera: Keroplatidae: Arachnocampa). *Mem. Queensl. Mus.* **55**, 11-41.

Baker, C. H. and Merritt, D. J. (2003). Life cycle of an Australian glow-worm *Arachnocampa flava* Harrison (Diptera: Keroplatidae: Arachnocampinae). *Aust. Entomol.* **30**, 45-55.

Baker, C. H., Graham, G. C., Scott, K. D., Cameron, S. L., Yeates, D. K. and Merritt, D. J. (2008). Distribution and phylogenetic relationships of Australian glow-worms *Arachnocampa* (Diptera, Keroplatidae). *Mol. Phylogenet. Evol.* **48**, 506-514. doi:10.1016/j.ympev.2008.04.037

Barber, A. F., Liang, Q. and Covarrubias, M. (2012). Sevoflurane: a potent general anesthetic enhancer of kv channel activation. *Biophys. J.* **102**, 533a. doi:10.1016/j.bpj.2011.11.2914

Bassot, J. M. (1978). Les corps noirs, cellules geantes du diptere mycetophilide lumineux *Platyura fultoni* et leur secretion mitochondriale. *C. R. Seances Acad. Sci. III* **286**, 623-626.

Berry, S. E., Gilchrist, J. and Merritt, D. J. (2017). Homeostatic and circadian mechanisms of bioluminescence regulation differ between a forest and a facultative cave species of glowworm, *Arachnocampa*. *J. Insect Physiol.* **103**, 1-9. doi:10.1016/j.jinsphys.2017.09.005

Branham, M. A. and Wenzel, J. W. (2001). Evolution of bioluminescence in cantharoids (Coleoptera: Elateroidea). *Fla. Entomol.* **84**, 565-586. doi:10.2307/3496389

Broadley, R. A. and Stringer, I. A. N. (2001). Prey attraction by larvae of the New Zealand glowworm, *Arachnocampa luminosa* (Diptera: Mycetophilidae). *Invert. Biol.* **120**, 170-177. doi:10.1111/j.1744-7410.2001.tb00121.x

Broadley, R. A. and Stringer, I. A. N. (2019). Larval behaviour of the New Zealand glowworm, *Arachnocampa luminosa* (Diptera: Keroplatidae), in bush and caves. In *Bioluminescence in Focus - A Collection of Illuminating Essays* (ed. V. B. Meyer-Rochow), pp. 325-355. Signpost: Kerala.

Buck, J. B. (1948). The anatomy and physiology of the light organ in fireflies. *Ann NY Acad Sci* **49**, 397-485. doi:10.1111/j.1749-6632.1948.tb30944.x

Buck, J. and Keister, M. (1955). Further studies of gas-filling in the insect tracheal system. *J. Exp. Biol.* **32**, 681-691.

Carlson, A. D. (1965). Factors affecting firefly larval luminescence. *Biol. Bull.* **129**, 234-243. doi:10.2307/1539841

Copeland, J. and Lloyd, J. E. (1983). Male firefly mimicry. *Science* **221**, 484-485. doi:10.1126/science.221.4609.484

De Cock, R. and Matthysen, E. (1999). Aposematism and bioluminescence: experimental evidence from glow-worm larvae (Coleoptera: Lampyridae). *Evol. Ecol.* **13**, 619-639. doi:10.1023/A:1011090017949

Falaschi, R. L., Amaral, D. T., Santos, I., Domingos, A. H. R., Johnson, G. A., Martins, A. G. S., Viroomal, I. B., Pompéia, S. L., Mirza, J. D., Oliveira, A. G. et al. (2019). *Neoceroplatus betaryiensis* nov. sp.(Diptera: Keroplatidae) is the first record of a bioluminescent fungus-gnat in South America. *Sci. Rep.* **9**, 11291. doi:10.1038/s41598-019-47753-w

Ganguly, G. (1959). Notes on the histology and anatomy of the larva of *Bolitophila luminosa* of New Zealand. *J. R. Micr. Soc.* **79**, 137-154. doi:10.1111/j.1365-2818.1959.tb04463.x

Gatenby, J. B. (1959). Notes on the New Zealand glow-worm, *Bolitophila* (Arachnocampa) *luminosa*. *Trans. R. Soc. N. Z.* **87**, 291-314.

Gatenby, J. B. and Ganguly, G. (1956). On a possible explanation of the sudden dousing of the light by the New Zealand glow-worm (*Arachnocampa luminosa*). *J. R. Micr. Soc.* **76**, 146-148. doi:10.1111/j.1365-2818.1956.tb00452.x

Ghiradella, H. and Schmidt, J. T. (2004). Fireflies at one hundred plus: a new look at flash control. *Integr. Comp. Biol.* **44**, 203-212. doi:10.1093/icb/44.3.203

Green, L. F. B. (1979). The fine structure of the light organ of the New Zealand glow-worm *Arachnocampa luminosa* (Diptera: Mycetophilidae). *Tissue Cell* **11**, 457-465. doi:10.1016/0040-8166(79)90056-9

Hastings, J. W. and Buck, J. (1956). The firefly pseudoflash in relation to photogenic control. *Biol. Bull.* **111**, 101-113. doi:10.2307/1539187

Kahlke, T. and Umbers, K. D. L. (2016). Bioluminescence. *Curr. Biol.* **26**, R313-R314. doi:10.1016/j.cub.2016.01.007

Keilin, D. (1944). Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. *Parasitol.* **36**, 1-66. doi:10.1017/S0031182000011975

Lee, J. (1976). Bioluminescence of the Australian glow-worm, *Arachnocampa richardsae harrison*. *Photochem. Photobiol.* **24**, 279-285. doi:10.1111/j.1751-1097.1976.tb06823.x

Lloyd, J. E. (1966). Signals and mating behavior in several fireflies (Coleoptera: Lampyridae). *The Coleopt. Bull.* **20**, 84-90.

Loru, L., Sassu, A., Fois, X. and Pantaleoni, R. A. (2010). Ethyl acetate: a possible alternative for anaesthetizing insects. *In Ann. Soc. entomol. Fr.* **46**, 422-424. doi:10.1080/00379271.2010.10697677

Lozano, V. E., Armengaud, C. and Gauthier, M. (2001). Memory impairment induced by cholinergic antagonists injected into the mushroom bodies of the honeybee. *J. Comp. Physiol. A* **187**, 249-254. doi:10.1007/s003590100196

MacMillan, H. A., Nørgård, M., MacLean, H. J., Overgaard, J. and Williams, C. J. A. (2017). A critical test of *Drosophila* anaesthetics: isoflurane and sevoflurane are benign alternatives to cold and CO_2. *J. Insect Physiol.* **101**, 97-106. doi:10.1016/j.jinsphys.2017.07.005

Merritt, D. J. and Patterson, R. (2018). Environmental influences on the bioluminescence display of the glow-worm, *Arachnocampa flava* (Diptera: Keroplatidae). *Aust. Entomol.* **57**, 107-117. doi:10.1111/aen.12274

Merritt, D. J., Rodgers, E. M., Amir, A. F. and Clarke, A. K. (2012). Same temporal niche, opposite rhythmicity: two closely related bioluminescent insects with opposite bioluminesce propensity rhythms. *Chronobiol. Int.* **29**, 1336-1344. doi:10.3109/07420528.2012.728549

Meyer-Rochow, V. B. (2007). Glowworms: a review of *Arachnocampa* spp. and kin. *Luminescence* **22**, 251-265. doi:10.1002/bio.955

Meyer-Rochow, V. B. and Waldvogel, H. (1979). Visual behaviour and the structure of dark and light-adapted larval and adult eyes of the New Zealand glowworm *Arachnocampa luminosa* (Mycetophilidae: Diptera). *J. Insect Physiol.* **25**, 601-607, 609-613. doi:10.1016/0022-1910(79)90076-3

Mills, R., Popple, J.-A., Veidt, M. and Merritt, D. J. (2016). Detection of light and vibration modulates bioluminescence intensity in the glowworm, *Arachnocampa flava*. *J. Comp. Physiol. A* **202**, 313-327. doi:10.1007/s00359-016-1077-0

Osawa, K., Sasaki, T. and Meyer-Rochow, V. B. (2014). New observations on the biology of *Keroplatus nipponicus* Okada 1938 (Diptera; Mycetophiloidea; Keroplatidae), a bioluminescent fungivorous insect. *Entomol. Heute* **26**, 139-149.

Plant, P. J., White, E. H. and McElroy, W. D. (1968). The decarboxylation of luciferin in firefly bioluminescence. *Biochem. Biophys. Res. Commun.* **31**, 98-103. doi:10.1016/0006-291X(68)90036-3

Rigby, L. M. and Merritt, D. J. (2011). Roles of biogenic amines in regulating bioluminescence in the Australian glowworm *Arachnocampa flava*. *J. Exp. Biol.* **214**, 3286-3293. doi:10.1242/jeb.060509

Sharpe, M. L., Dearden, P. K., Gimenez, G. and Krause, K. L. (2015). Comparative RNA seq analysis of the New Zealand glowworm *Arachnocampa luminosa* reveals bioluminescence-related genes. *BMC Genom.* **16**, 825. doi:10.1186/s12864-015-2006-2

Silva, J. R., Amaral, D. T., Hastings, J. W., Wilson, T. and Viviani, V. R. (2015). A transcriptional and proteomic survey of *Arachnocampaluminosa* (Diptera: Keroplatidae) lanterns gives insights into the origin of bioluminescence from the Malpighian tubules in Diptera. *Luminescence* **30**, 996-1003. doi:10.1002/bio.2850

Thorpe, W. H. (1932). Experiments upon respiration in the larvae of certain parasitic Hymenoptera. *Proc. R. Soc. Lond. B* **109**, 450-471. doi:10.1098/rspb.1932.0006

Timmins, G. S., Robb, F. J., Wilmot, C. M., Jackson, S. K. and Swartz, H. M. (2001). Firefly flashing is controlled by gating oxygen to light-emitting cells. *J. Exp. Biol.* **204**, 2795-2801.

Trimmer, B. A., Aprille, J. R., Dudzinski, D. M., Lagace, C. J., Lewis, S. M., Michel, T., Qazi, S. and Zayas, R. M. (2001). Nitric oxide and the control of firefly flashing. *Science* **292**, 2486-2488. doi:10.1126/science.1059833

Viviani, V. R. (2002). The origin, diversity, and structure function relationships of insect luciferases. *Cell. Mol. Life Sci.* **59**, 1833-1850. doi:10.1007/PL00012509

Viviani, V. R., Hastings, J. W. and Wilson, T. (2002). Two bioluminescent Diptera: the North American *Orfelia fultoni* and the Australian *Arachnocampa flava*. Similar niche, different bioluminescence systems. *Photochem. Photobiol.* **75**, 22-27. doi:10.1562/0031-8655(2002)075<0022:TBDTNA>2.0.CO;2

Watkins, O. C., Sharpe, M. L., Perry, N. B. and Krause, K. L. (2018). New Zealand glowworm (*Arachnocampa luminosa*) bioluminescence is produced by a firefly-like luciferase but an entirely new luciferin. *Scient. Rep.* **8**, 3278. doi:10.1038/s41598-018-21298-w

Whalen, F. X., Bacon, D. R. and Smith, H. M. (2005). Inhaled anesthetics: an historical overview. *Best Pract. Res. Clin. Anaesthesiol.* **19**, 323-330. doi:10.1016/j.bpa.2005.02.001

Wheeler, W. M. and Williams, F. X. (1915). The luminous organ of the New Zealand glow-worm. *Psyche* **22**, 36-43. doi:10.1155/1915/20893

Willis, R., White, C. and Merritt, D. (2011). Using light as a lure is an efficient predatory strategy in *Arachnocampa flava*, an Australian glowworm. *J. Comp. Physiol. B* **181**, 477-486.

Wilson, T. and Hastings, J. W. (1998). Bioluminescence. *Ann. Rev. Cell Dev. Biol.* **14**, 197-230. doi:10.1146/annurev.cellbio.14.1.197