ARTICLE
Inexpensive Methods for Live Imaging of Central Pattern Generator Activity in the *Drosophila* Larval Locomotor System

Jonathan R. H. Booth*[1], Varun Sane*[3], Malte C. Gather[1,2] and Stefan R. Pulver[3]
*[1]SUPA, School of Physics and Astronomy, University of St Andrews, St Andrews, United Kingdom; [2]Centre for Nanobiophotonics, Department of Chemistry, University of Cologne, Cologne, Germany; [3]School of Psychology and Neuroscience, University of St Andrews, St Andrews, United Kingdom. *These authors contributed equally to this work.*

Central pattern generators (CPGs) are neural networks that produce rhythmic motor activity in the absence of sensory input. CPGs produce 'fictive' behaviours *in vitro* which parallel activity seen in intact animals. CPG networks have been identified in a wide variety of model organisms and have been shown to be critical for generating rhythmic behaviours such as swimming, walking, chewing and breathing. Work with CPG preparations has led to fundamental advances in neuroscience; however, most CPG preparations involve intensive dissections and require sophisticated electrophysiology equipment, making export to teaching laboratories problematic. Here we present an integrated approach for bringing the study of locomotor CPGs in *Drosophila* larvae into teaching laboratories. First, we present freely available genetic constructs that enable educators to express genetically encoded calcium indicators in cells of interest in the larval central nervous system. Next, we describe how to isolate the larval central nervous system and prepare it for live imaging. We then show how to modify standard compound microscopes to enable fluorescent imaging using 3D printed materials and inexpensive optical components. Finally, we show how to use the free image analysis programme ImageJ and freely available features in the signal analysis programme DataView to analyse rhythmic CPG activity in the larval CNS. Comparison of results to those obtained on research equipment shows that signal-to-noise levels are comparable and core features of larval CPG activity can be observed. Overall, this work shows the viability of exporting live imaging experiments to low cost environments and paves the way for new teaching laboratory exercises revolving around optical imaging of CPG activity.

Key words: motor systems; teaching equipment; calcium imaging; GcamP; Drosophila; central nervous system; larval locomotion; open source; epifluorescence

Central pattern generators (CPGs) are neural networks that produce patterns of motor activity in the absence of sensory input. 'Fictive' behaviours produced by CPG preparations *in vitro* retain key timing and phasing features of motor activity in behaving animals. Evidence for the existence of CPGs was first discovered in mammals by Thomas Graham Brown (1914) in the early 20th century; this work was largely overlooked until researchers in the 1960s uncovered evidence for CPG networks in crustaceans (Hughes and Wiersma, 1960) and insects (Wilson, 1961). In subsequent years, CPG networks have been found to underlie rhythmic behaviours such as swimming (Pearce and Friesen, 1984; Grillner and Wallen, 1985), walking (Brown, 1914; Smith and Feldman, 1987; Dimitrijevic et al., 1998), crawling (Lemon et al., 2015; Pulver et al., 2015) and flying (Wilson, 1961); as well as other non-locomotor behaviours such as heartbeat (Stent et al., 1979), feeding (Marder and Calabrese, 1996; Marder and Bucher, 2001; Schoofs et al., 2010), singing (Wild, 2004), and copulation (Wagenaar et al., 2010) (Figure 1). CPG networks typically receive sensory feedback either directly or indirectly; however, the degree to which this feedback influences and modulates CPG activity varies across model systems. Additionally, CPGs across all species are subject to neuromodulation from either intrinsic or extrinsic sources. From this body of work, a consensus view has emerged, namely that CPG networks provide core rhythms in motor networks which are then sculpted by sensory feedback and neuromodulation to generate adaptive behaviours.

CPG networks have traditionally been studied by physically isolating sections of nervous system, placing resulting preparations in physiological saline, then performing electrophysiological recordings on identified neurons and nerves. In many systems, it can take tens of minutes or even hours of intricate dissection work to get CPG preparations on an electrophysiology set-up. Furthermore, recording CPG activity typically requires recording voltage on multiple channels simultaneously, which in turn, requires expensive extracellular and intracellular amplifiers, along with associated hardware and software. The combination of tough dissections coupled with a need for expensive electrophysiology equipment has made it difficult to export many preparations to teaching laboratories. This is unfortunate, as the study of CPG networks could provide an excellent introduction to signal processing and analysis of rhythmic activity, as well as principles of neural circuit operation and neuromodulation.

Researchers working with genetically tractable invertebrate model organisms such as *Drosophila,* have

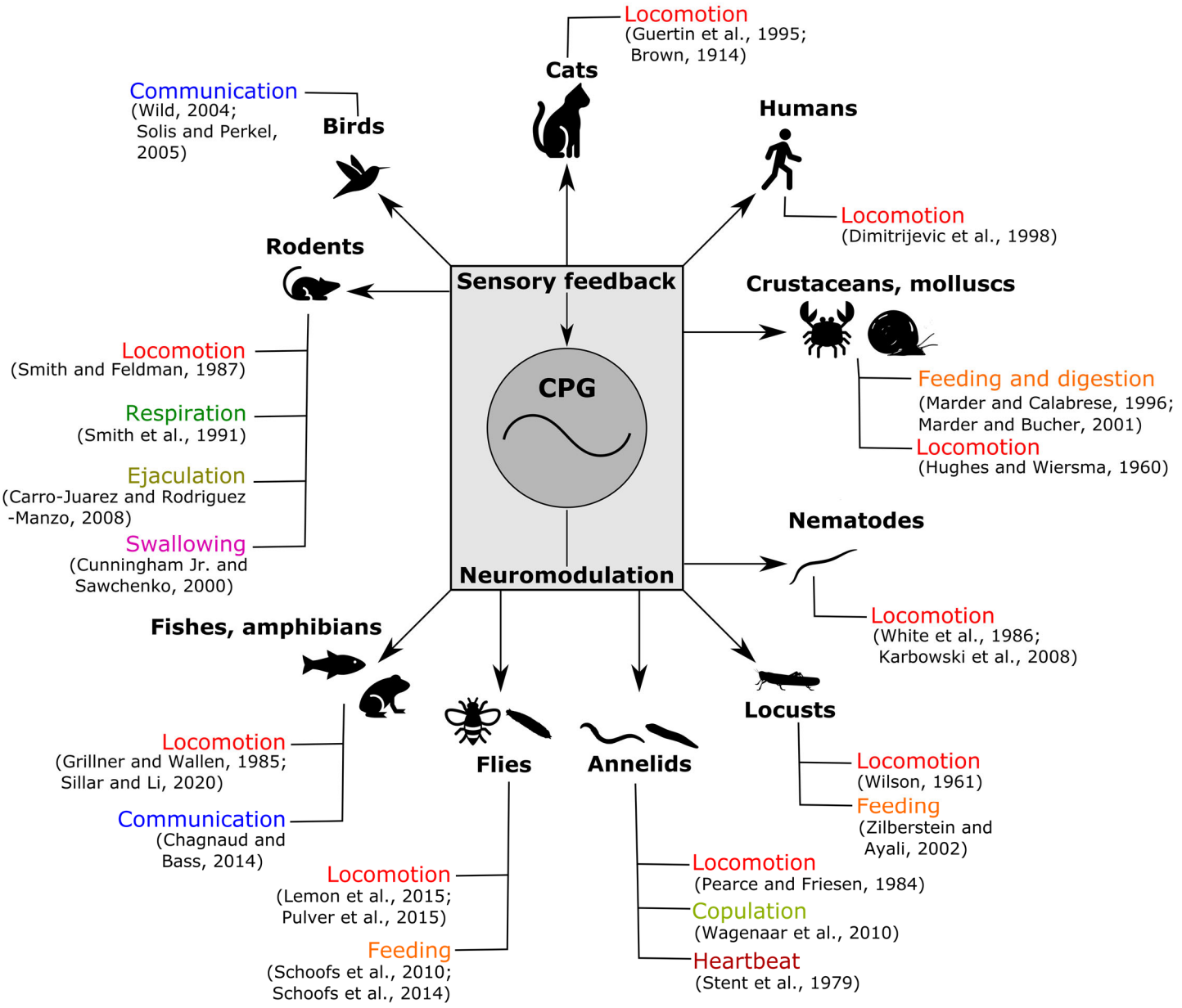


Figure 1. CPG activity is sculpted by sensory feedback and neuromodulation to produce rhythmic behaviours across a wide range of vertebrate and invertebrate species.

used genetic tools to demonstrate the operation of CPG networks. For example, researchers have used the GAL4-UAS system (Brand and Perrimon, 1993) to express toxins that conditionally inhibit synaptic release specifically in the peripheral nervous system, and then observed resulting motor behaviours (Suster and Bate, 2002; Hughes and Thomas, 2007; Mendes et al., 2013). More recently, *in vitro* preparations have been developed in *Drosophila* larvae that allow optical recording of activity using genetically encoded calcium indicators. Importantly, the same standards used to validate the presence of CPG networks in established preparations have also been applied to larval *in vitro* preparations (Pulver et al., 2015). The development of preparations that enable imaging of CPG activity in genetically tractable organisms opens up attractive possibilities for educators and can complement recent work aimed at making optogenetics accessible for educators (Pulver et al., 2011, Titlow et al., 2015).

Live imaging of neural activity using genetically encoded fluorescent calcium indicators has become commonplace in many neuroscience research laboratories (Reviewed in Lin and Schnitzer, 2016). Calcium imaging has traditionally been a costly enterprise, involving the use of laser light sources, costly microscope bodies and high-priced cameras. Recent advances in LED technology have led to the development of high intensity light sources suitable for fluorescent imaging. In parallel, advances in CMOS camera technology has led to development of relatively inexpensive, but highly sensitive cameras. Finally, advances in 3D printing technologies, coupled with

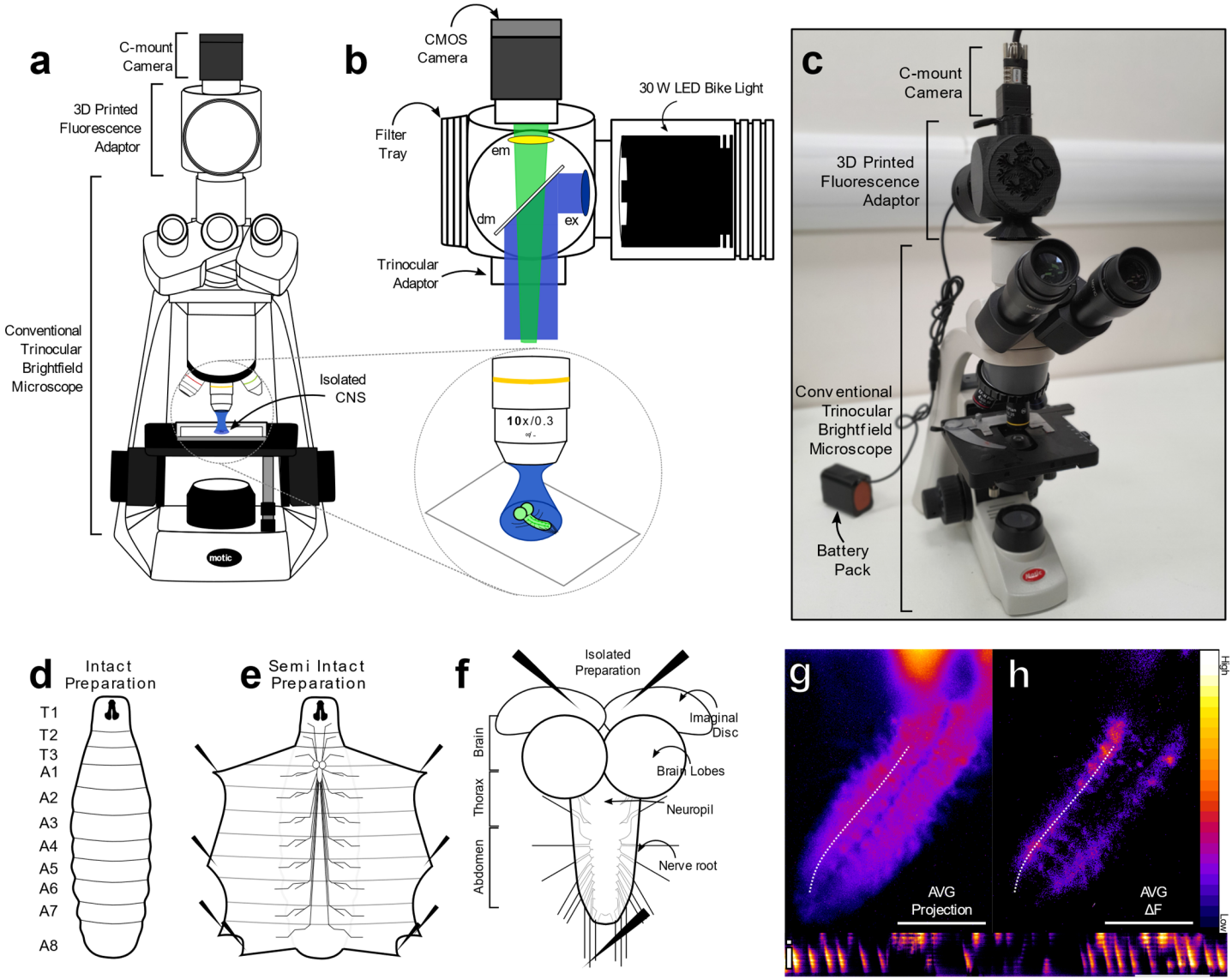


Figure 2. Conversion of a trinocular compound microscopes into an epifluorescence live imaging system. *a)* A simple episcopic illuminator module was designed and 3D printed allowing it to fit to the top of any trinocular brightfield microscope. *b)* Customized module based on work by Stewart and Giannini (2016), allowing for a simple customizable filter cube which spectrally filters and redirects light from a 30W bicycle light onto the sample through the objective. *c)* Photograph of a working setup mounted on a standard teaching microscope. *d)* Wandering third instar larvae expressing GCAMP6S in all neurons were selected. *e)* Animals were then carefully dissected, pinned and all visceral organs were removed, leaving behind the central nervous system. *f)* The larval CNS was then isolated from the body and pinned by the imaginal discs and posterior nerve roots. *g)* Snapshot of CNS expressing GCAMP6S in all neurons. scalebar = 100 µm *h)* Subtracting the average background allowed for imaging of rhythmically active neurons exclusively; scalebar = 100 µm. *i)* Fictive locomotor patterns can be seen via a kymograph by showing the change of fluorescent activity across a line defined in x-y space over time (line shown in g,h) scale bar = 10 seconds.

the evolution of 'DIY' and 'open source' scientific communities have made it relatively easy for scientists to design and build microscope components. This sea change in science has enabled researchers, educators, and lay people to design and build their own fluorescent microscope systems using discarded transmitted light microscopes (Peidle, et al., 2009; Stewart and Gianni, 2016). In addition, researchers have also developed ways to build fluorescent imaging systems from scratch using inexpensive components (Tristan-Landin et al., 2019; Maia Chagas et al., 2017; Grier et al., 2018; Ryan et al., 2020). In parallel, free and/or open source software has increasingly become available for use by researchers and educators. Although excellent progress has been made in developing hardware solutions for fluorescence imaging, there are relatively few publications that present integrated start-to-finish solutions for performing specific calcium imaging experiments in teaching laboratories (Maia Chagas et al., 2017).

Recent developments in *Drosophila* motor systems research, coupled with the development of inexpensive fluorescent imaging hardware and software presents a

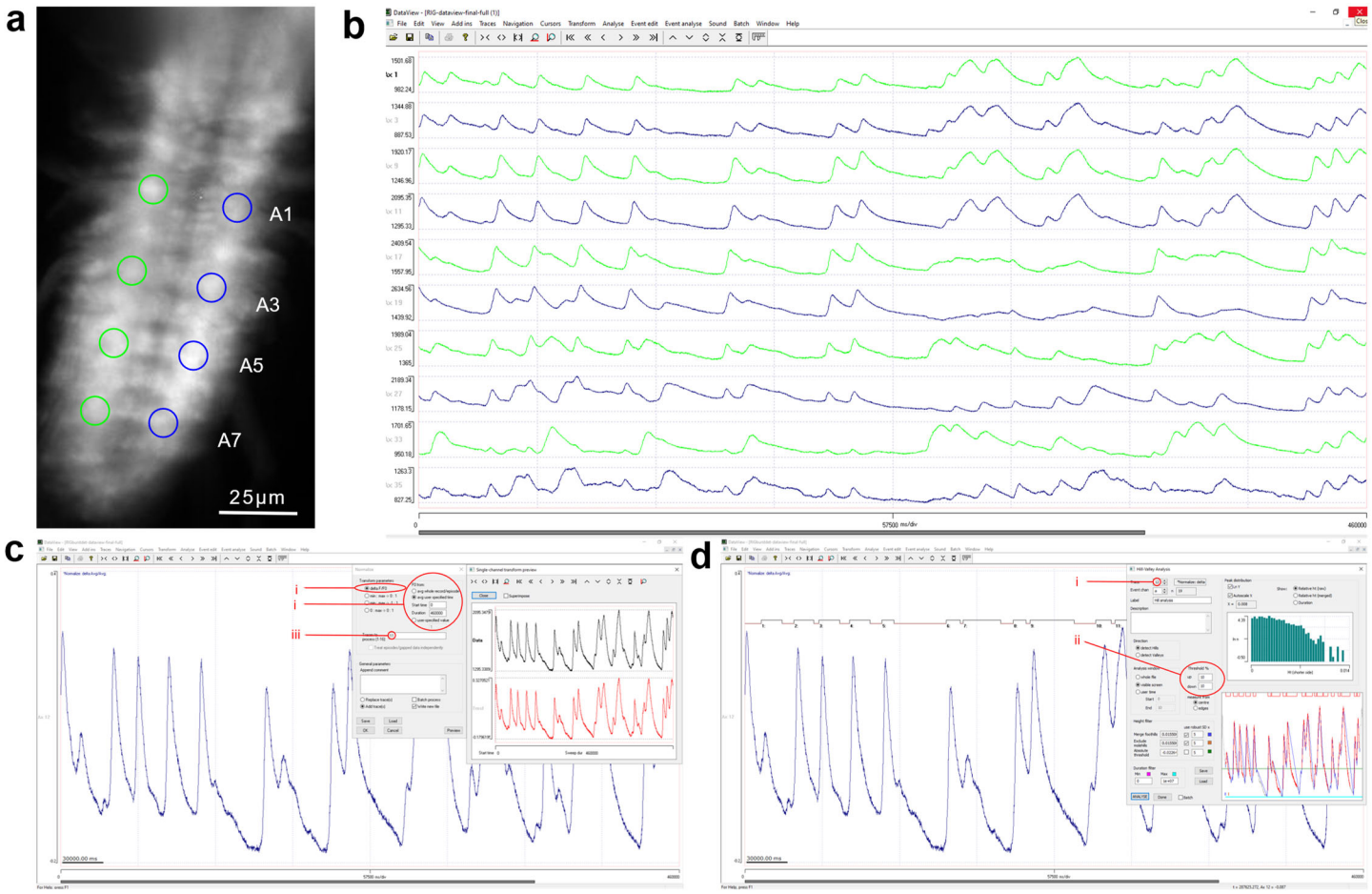


Figure 3. DataView GUI and analysis tools for analyzing calcium dynamics. *a)* Average projection of maximum intensity over time in a CNS expressing GamP6s. Circles indicate ROIs in which pixel intensity is averaged on every time step. *b)* Raw pixel intensities from ROIs visualized in DataView. *c)* dF/F calculations (baseline corrections) done for a selected trace and previewed as a new trace. i) Shows baseline correction function ii) Shows baseline F_0 measurement function iii) baseline normalization options *d)* Burst / peak detection for a given a trace (blue). i) Shows trace selection option. ii) Shows options for setting burst detection thresholds.

unique opportunity for neuroscience educators interested in teaching principles of CPG networks. Here we present an integrated solution for imaging and analyzing CPG activity in *Drosophila* larvae using freely available genetic constructs, relatively inexpensive imaging hardware, and freely available software. Comparison to research grade equipment reveals that this system costs approximately an order of magnitude less in price, but provides roughly equivalent performance. This inexpensive alternative for fluorescent imaging of neural activity provides opportunities for educators, as well as researchers in underserved communities to study principles of rhythmic motor systems.

MATERIALS AND METHODS

Animals

We used the GAL4-UAS system (Brand and Perrimon, 1993) to drive expression of calcium sensors in *Drosophila* larval neurons. For all experiments, we used 3rd instar feeding larvae expressing GcamP6s (Chen et al., 2013) panneuronally (20XUAS-IVS-GCcamP6S combined with the panneuronal driver 57C10-GAL4). Animals were raised on standard cornmeal-agar-yeast culturing media at 22-25°C**.** Fly line from available from Pulver laboratory upon request.

Light Source

The need for high intensity light sources amongst bicyclists has led to the development of inexpensive, compact, semi-collimated LED light sources. We used a bicycle light system incorporating a Cree XHP 70 light source (www.ledsupermall.com). To prolong experimental times, in some cases, we substituted a constant DC voltage source connected to mains power for the battery pack provided by the manufacturer.

3D Printing and Assembly

3D components were printed using an AnyCubic Mega i3 (AnyCubic; Shenzhen, China) Fused Deposition Modelling printer controlled via the open source slicing application, Ultimaker Cura (Ultimaker; Utrecht, Netherlands). Components were adapted from designs presented in Stewart and Giannini (2016) using the free modelling;

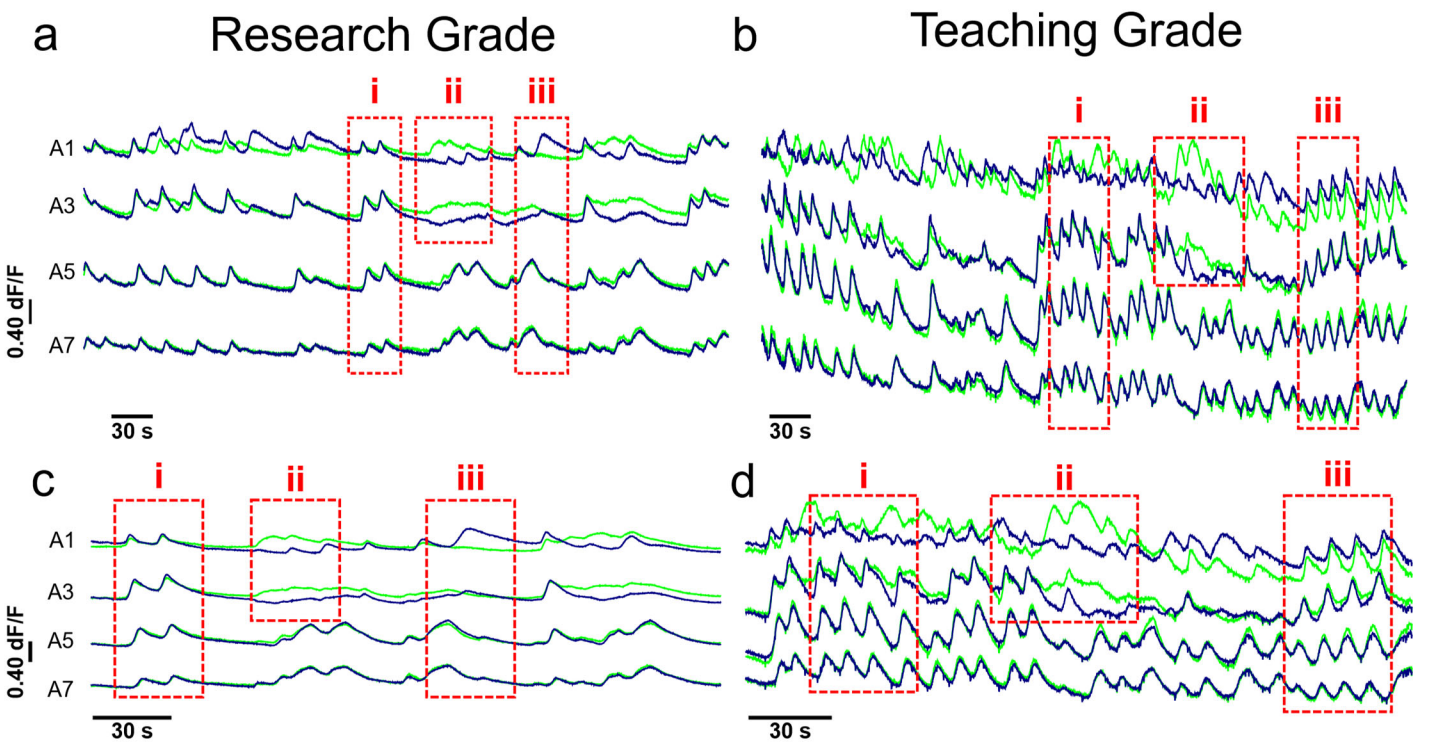


Figure 4. Comparison of data produced on research grade and teaching grade imaging systems. *a, b)* dF/F traces showing segmentally coordinated activity generated using research grade and teaching grade imaging systems, respectively. Green shows activity on left side, blue shows activity on right side; body segments shown at left. Highlighted areas denote different fictive motor patterns: i) Fictive backward waves, ii) fictive head sweeps and iii) fictive forward waves. *c, d)* Expanded time scale views of activity patterns shown in a, b. Examples shown are from two separate preparations.

software Autodesk TinkerCAD (Autodesk; California, USA) as well Blender (Blender Foundation Amsterdam, Netherlands). Design templates are available in the appendix.

Newly printed components were prepared for final use by removing printing supports using pliers and sanding down flat edges to minimize chances of components becoming stuck to one another. The Cree XHP70 lamphouse was then placed into the light source holder, which was friction fitted to the main body of the module. The light source was then aligned using 3 M6 hex screws and nuts. Filters were placed into their respective holders and the filter tray was then inserted into the main body. Appropriate excitation and emission filters (Comar Optics, Cambridge, UK) were placed within the housing together with a dichroic mirror (Thorlabs, Newton, NY, USA (See Appendix for parts list). Finally, a printed trinocular adapter was fitted to the bottom of the main body and an external c-mount threaded adapter ring was friction fitted to the top of the main housing. A XIMEA MQ013MG-E2 CMOS camera (Lamda Photo, Harpenden, UK) was threaded to the housing. The entire housing was then mounted onto the camera port of a Motic BA210 compound microscope (Motic Microscopy, Hong Kong, China) which was fitted with a 10x air objective and adjustable stage.

Experiments

Feeding 3rd instar larvae were pinned in a Sylgard®184 (DowSil; USA) lined dissection dish filled with physiological saline composed of (in mM) 135 NaCl, 5 KCl, 4 $MgCl_2 \cdot 6H_2O$, 2 $CaCl_2 \cdot 2H_2O$, 5 TES buffer, and 36 sucrose, pH 7.14 (Marley and Baines, 2011). A small lateral incision was then made posteriorly using fine dissection scissors, followed by a longitudinal cut from posterior to anterior. The cuticle of the animal was then pinned flat using insect pins. Trachea and internal organs were then removed with forceps. Nerve roots were then severed, and the central nervous system (CNS) was carefully removed by forceps using the nerve roots or eye imaginal discs. The nerve roots and imaginal discs were then pinned to the substrate using fine (0.01 mm diameter) tungsten wire (California Fine Wire; California, USA). The cuticle was then discarded, and physiological saline was exchanged before imaging.

Preparations were imaged using either a Motic BA210 microscope equipped with our epifluorescence module, or with an Olympus BX 51wi epifluorescence microscope (Olympus; Tokyo, Japan) equipped with a research grade camera (Retiga R1, Teledyne Photometrics, Tuscon, AZ) and light source (OptoLED, Cairn Research, Ltd, Kent, UK). Preparations were then imaged for ~ 7 minutes.

Image Acquisition and Analysis

Movies of calcium activity were recorded using the free open source image acquisition software μManager (Edelstein et al., 2014). Images were then processed

using ImageJ (Schneider et al., 2012). Fluorescence intensity levels in regions of interest (ROIs) were extracted in ImageJ, then exported to DataView (https://www.st-andrews.ac.uk/~wjh/dataview/) for visualization and baseline normalization and analysis. Peak amplitudes were measured in DataView and analyzed using scripts in python (www.python.org).

RESULTS AND DISCUSSION

Design and construction of fluorescence module

To facilitate the study of rhythmic motor systems in teaching labs, we designed and 3D printed a module that converts compound light microscopes into live imaging systems. Using relatively inexpensive components and a straightforward print design, we were able to assemble a module that produced an appropriate blue shifted wavelength for exciting green fluorescent protein (GFP) and/or GcamP. Incorporation of a dichroic mirror and emission filter allowed acquisition of green emitted light. Our design built directly on previous published designs (Peidle et al., 2009; Stewart and Giannini, 2016), but incorporated the use of a high powered LED light source designed for bicycles and the use of a modern CMOS camera that provided a balance between cost, sensitivity, resolution, and speed.

Maximizing light intensity delivered to preparations is critical for obtaining optimal performance in a fluorescent imaging system. Our system was able to deliver ~ 50 $\mu W/mm^2$ to preparations, compared to a maximum of 330 $\mu W/mm^2$ on a research set-up. We were able to boost light intensity of our module into the range of our research set up by simply replacing the bike light with a research-grade LED light source. This suggests that the quality of our optical components is high but that there is room for improvement in the design of our light source.

Our CMOS camera was able to acquire high quality snapshots of GcamP6s fluorescence in neurons in living larval CNSs with exposure times of ~100 ms. We were able to resolve neuropil regions (Figure 2G, H, top), confirming that this system is capable of acquiring high quality static fluorescent images. Visual inspection of movies acquired using similar exposure times (i.e., 10 frames/s) revealed that preparations produced all fictive motor programmes reported in previous research publications (i.e., forward waves, backward waves, bilateral asymmetries; Pulver et al., 2015).

Analysis in ImageJ and DataView

We initially visualized calcium dynamics using the kymograph plugin in ImageJ. This tool displays variation in fluorescence intensity along a line drawn through x-y space over time (Figure 2 G, H, bottom). Kymographs confirmed the presence of wave-like activity in all preparations (n = 3). We then defined regions of interest (ROI) in ImageJ and exported average intensity values from ROIs placed on multiple hemi-segments to DataView. DataView enabled visualization of raw fluorescence traces well as baseline normalization and detection and measurement of activity peaks (Figure 3). Signal-to-noise on our 'teaching-grade' system was comparable to that obtained on a 'research-grade' system (Figure 4). The amplitude of optical signals obtained on our teaching-grade system (mean dF/F = 0.225 +/- 0.218 S.E.M, n = 3) were also not substantially different from peak amplitudes obtained on a research set-up (mean dF/F = 0.253 +/- 0.034 S.E.M, n = 3). Critically, rhythmic waves of fictive forward and fictive backward locomotion were observable in our imaging system, as well as fictive head sweeps (Figure 4i, ii, iii, respectively). This means that educators and students can detect events and characterize different types of motor programmes regardless of variability in the amplitude of calcium signals across preparations. We used DataView to perform a relatively simple operation: detection and measurement of peak amplitudes. However, DataView provides a suite of additional options for signal processing and analysis of rhythmic activity. The software platform has been used extensively for analysis of CPG activity in both *Xenopus* tadpoles (Picton and Sillar, 2016) and mouse spinal cord (Acton et al., 2018). Students and educators can make use of these features in DataView to perform in depth analysis of cycle periods, duty cycles and/or inter-segmental phase lags.

Obviously, image quality in these experiments depends on the quality of the optical components of the associated compound microscope. Our module can in theory be adapted to fit any available compound microscope with a camera port. While we did not systematically test our system on multiple types of compound microscopes, we did confirm that our module can work on other models of microscopes abandoned in cupboards and storerooms in various states of repair in our department (data not shown). We found that image quality also depended critically on maximizing light intensity delivered to each preparation. Periodic monitoring of light intensity delivered to preparations and adjustment of light source orientation was key to maintaining image quality and consistency over multiple experiments.

One obvious drawback of our system is the requirement for a compound microscope in the first place. Although low cost new and used compound light microscopes are available from a variety of vendors, this requirement does potentially limit access. While there have been multiple recent efforts describing how to build fluorescent microscopes from scratch using inexpensive components; to our knowledge, only Ryan et al. (2020) have confirmed feasibility of performing calcium imaging with a stand-alone system. Future work could be aimed at using the *Drosophila* larval preparation to systematically assess the suitability of inexpensive, stand-alone fluorescent imaging systems as these systems are pushed into classrooms and teaching laboratories.

Implementation in Teaching Laboratories

This system was designed and tested primarily by Ph.D. and Masters students in a designated 'maker space' at the University of St Andrews. In this active learning model, students design and build teaching tools for other students and, in the process, gain experience in computer aided design, engineering, neuroscience, and education. The Covid-19 pandemic has limited our ability to test the

robustness of our imaging system in actual practical courses. We have, however, implemented lab practicals in which students observe CPG imaging experiments and then conduct independent analyses of the resulting data sets. We have confirmed that undergraduates are capable of performing and analyzing these types of experiments as part of individual research projects. Allowing students to directly observe others conducting dissections is the best approach to teaching this particular dissection. Students typically find positioning fine tungsten pins around the isolated CNS to be the most challenging aspect of the dissection. Importantly, even simpler methods than those described here do exist for removing the larval CNS without the use of scissors by simply removing the larval mouth hooks as a single unit CNS with forceps (M. Zwart, personal communication). In addition to supporting student researchers, we have also successfully conducted annual lab practicals for classes of ~15 students (2015-2020) during which instructors and teaching assistants provide healthy preparations to students 'on demand'. A trained dissector can typically do a full dissection in 5-10 minutes, making it feasible to generate multiple preparations during a single laboratory session. Students appear highly motivated to work with live imaging data sets and have consistently been able to handle data sets produced and generate quality figures for assessment. Looking forward, we anticipate that data storage and handling will constitute a major practical barrier to implementing these experiments on a large scale in teaching laboratories. In general, development of appropriate data infrastructure is an important first step before implementing teaching exercises involving the hardware and software presented here.

Conclusions and Future Directions

Our work provides a blueprint for incorporating the practical study of CPG activity into the teaching of neuroscience. We show that CPG activity in larval *Drosophila* can be imaged and analyzed using inexpensive hardware and software. This provides educators with new options for teaching principles of motor systems. More broadly, this work presents a general purpose, inexpensive fluorescence imaging system and paves the way for use of optical activity indicators in both educational and low resource research environments.

REFERENCES

Acton D, Broadhead MJ, Miles GB (2018) Modulation of spinal motor networks by astrocyte-derived adenosine is dependent on D1-like dopamine receptor signaling. J Neurophysiol 120:998–1009.

Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118(2):401-15.

Brown TG (1914) On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. J Physiol 48:18–46.

Carro-Juárez M, Rodríguez-Manzo G (2008) The spinal pattern generator for ejaculation. Brain Res Rev 58:106–120.

Chagnaud BP, Bass AH (2014) Vocal Behavior and Vocal Central Pattern Generator Organization Diverge among Toadfishes. Brain Behav Evol 84:51–65.

Chen TW, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Schreiter ER, Kerr RA, Orger MB, Jayaraman V, Looger LL, Svoboda K, Kim DS (2013) Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature 499:295–300.

Cunningham ET, Sawchenko PE (2000) Dorsal medullary pathways subserving oromotor reflexes in the rat: Implications for the central neural control of swallowing. J Comp Neurol 417:448–466.

Dimitrijevic MR, Gerasimenko Y, Pinter MM (1998) Evidence for a spinal central pattern generator in humans. Ann N Y Acad Sci 860:360-76.

Edelstein AD, Tsuchida MA, Amodaj N, Pinkard H, Vale RD, Stuurman N (2014) Advanced methods of microscope control using μManager software. J Biol Methods 1:10.

Grier Z, Soddu MF, Kenyatta N, Odame SA, Sanders JI, Wright LP, Anselmi F (2018) A low-cost do-it-yourself microscope kit for hands-on science education. In: Optics Education and Outreach V (Gregory GG, ed), pp 19. Bellingham, WA: SPIE.

Grillner S, Wallen P (1985) Central Pattern Generators for Locomotion, with Special Reference to Vertebrates. Annu Rev Neurosci 8:233–261.

Guertin P, Angel MJ, Perreault MC, McCrea DA (1995) Ankle extensor group I afferents excite extensors throughout the hindlimb during fictive locomotion in the cat. J Physiol 487:197–209.

Hughes, CL, Thomas JB (2007) A sensory feedback circuit coordinates muscle activity in *Drosophila*. Molecular and Cellular Neuroscience, 35(2):383–396.

Hughes GM, Wiersma CAG (1960) The Co-ordination of Swimmeret Movements in the Crayfish, Procambarus Clarkii (Girard). J Exp Biol 37.

Karbowski J, Schindelman G, Cronin CJ, Seah A, Sternberg PW (2008) Systems level circuit model of C. elegans undulatory locomotion: Mathematical modeling and molecular genetics. J Comput Neurosci 24:253–276.

Lemon WC, Pulver SR, Höckendorf B, McDole K, Branson K, Freeman J, Keller PJ (2015) Whole-central nervous system functional imaging in larval *Drosophila*. Nat Commun 6:1–16.

Lin MZ, Schnitzer MJ (2016) Genetically encoded indicators of neuronal activity. Nat Neurosci 19:1142–1153.

Maia Chagas A, Prieto-Godino LL, Arrenberg AB, Baden T (2017) The €100 lab: A 3D-printable open-source platform for fluorescence microscopy, optogenetics, and accurate temperature control during behaviour of zebrafish, *Drosophila*, and Caenorhabditis elegans. PLOS Biol 15:e2002702.

Marder E, Bucher D (2001) Central pattern generators and the control of rhythmic movements. Curr Biol 11:R986–R996.

Marder E, Calabrese RL (1996) Principles of rhythmic motor pattern generation. Physiol Rev 76:687–717.

Marley R, Baines RA (2011) Increased persistent Na+ current contributes to seizure in the slamdance bang-sensitive *Drosophila* mutant. J Neurophysiol 106:18–29.

Mendes CS, Bartos I, Akay T, Márka S, Mann RS (2013) Quantification of gait parameters in freely walking wild type and sensory deprived *Drosophila melanogaster*. 8;2:e00231.

Pearce RA, Friesen WO (1984) Intersegmental coordination of leech swimming: comparison of in situ and isolated nerve cord activity with body wall movement. Brain Res 299(2):363-6.

Peidle J, Stokes C, Hart R, Franklin M, Newburgh R, Pahk J, Rueckner W, Samuel A, H Wolfs FL (2009) Inexpensive microscopy for introductory laboratory courses. Am J Phys 77(10):931–938.

Picton LD, Sillar KT (2016) Mechanisms underlying the endogenous dopaminergic inhibition of spinal locomotor circuit function in *Xenopus* tadpoles. Sci Rep 6(35749):1–15.

Pulver SR, Bayley TG, Taylor AL, Berni J, Bate M, Hedwig B (2015) Imaging fictive locomotor patterns in larval *Drosophila*. J Neurophysiol 114:2564–2577.

Pulver SR, Hornstein NJ, Land B, Johnson BR (2011) Optogenetics in the teaching laboratory: using Channelrhodopsin2 to study the neural basis of behavior and synaptic physiology in *Drosophila. Adv. Physiol. Ed.* 35(1):82-91.

Ryan J, Johnson BR, Deitcher D (2020) Building Your Own Neuroscience Equipment: A Precision Micromanipulator and an Epi-fluorescence Microscope for Calcium Imaging. J. Undergrad. Neurosci. Ed. 19(1):R11-R14.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675.

Schoofs A, Hückesfeld S, Schlegel P, Miroschnikow A, Peters M, Zeymer M, Spieß R, Chiang A-S, Pankratz MJ (2014) Selection of Motor Programs for Suppressing Food Intake and Inducing Locomotion in the *Drosophila* Brain Sengupta P, ed. PLoS Biol 12:e1001893.

Schoofs A, Niederegger S, van Ooyen A, Heinzel HG, Spieß R (2010) The brain can eat: Establishing the existence of a central pattern generator for feeding in third instar larvae of *Drosophila virilis* and *Drosophila melanogaster*. J Insect Physiol 56(7):695–705.

Sillar KT, Li W-C (2020) Neural control of swimming in hatchling *Xenopus* frog tadpoles. In: The Neural Control of Movement, Whelan PJ; Sharples SA, Ed. pp 153–174. Cambridge, MA: Academic Press.

Smith JC, Ellenberger HH, Ballanyi K, Richter DW, Feldman JL (1991) Pre-Bötzinger complex: A brainstem region that may generate respiratory rhythm in mammals. Science 254(5032):726–729.

Smith JC, Feldman JL (1987) In vitro brainstem-spinal cord preparations for study of motor systems for mammalian respiration and locomotion. J Neurosci Methods 21(2-4):321-33.

Solis MM, Perkel DJ (2005) Rhythmic activity in a forebrain vocal control nucleus in vitro. J Neurosci 25(11):2811-22.

Stent GS, Thompson WJ, Calabrese RL (1979) Neural control of heartbeat in the leech and in some other invertebrates. Physiol Rev 59(1):101-36.

Stewart C, Giannini J (2016) Inexpensive, open source epifluorescence microscopes. J Chem Educ 93:1310–1315.

Suster ML, Bate M (2002) Embryonic assembly of a central pattern generator without sensory input. Nature 416 (6877) 174–178.

Titlow JS, Johnson BR, Pulver SR. Light activated escape circuits: a behavior and neurophysiology lab module using *Drosophila* optogenetics (2015) J Undergrad Neuro Educ 13(3):A166-73.

Tristan-Landin SB, Gonzalez-Suarez AM, Jimenez-Valdes RJ, Garcia-Cordero JL (2019) Facile assembly of an affordable miniature multicolor fluorescence microscope made of 3D-printed parts enables detection of single cells PLoS One 14:e0215114.

Wagenaar DA, Hamilton MS, Huang T, Kristan WB, French KA (2010) A Hormone-Activated Central Pattern Generator for Courtship. Curr Biol 20(6):487-9.

White J, Southgate E, Thomson J, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos Trans R Soc London B, Biol Sci 314(1165):1-340.

Wild JM (2004) Functional Neuroanatomy of the Sensorimotor Control of Singing. Ann N Y Acad Sci 1016:438–462

Wilson DM (1961) The Central Nervous Control of Flight in a Locust. J Exp Biol 38:471–490.

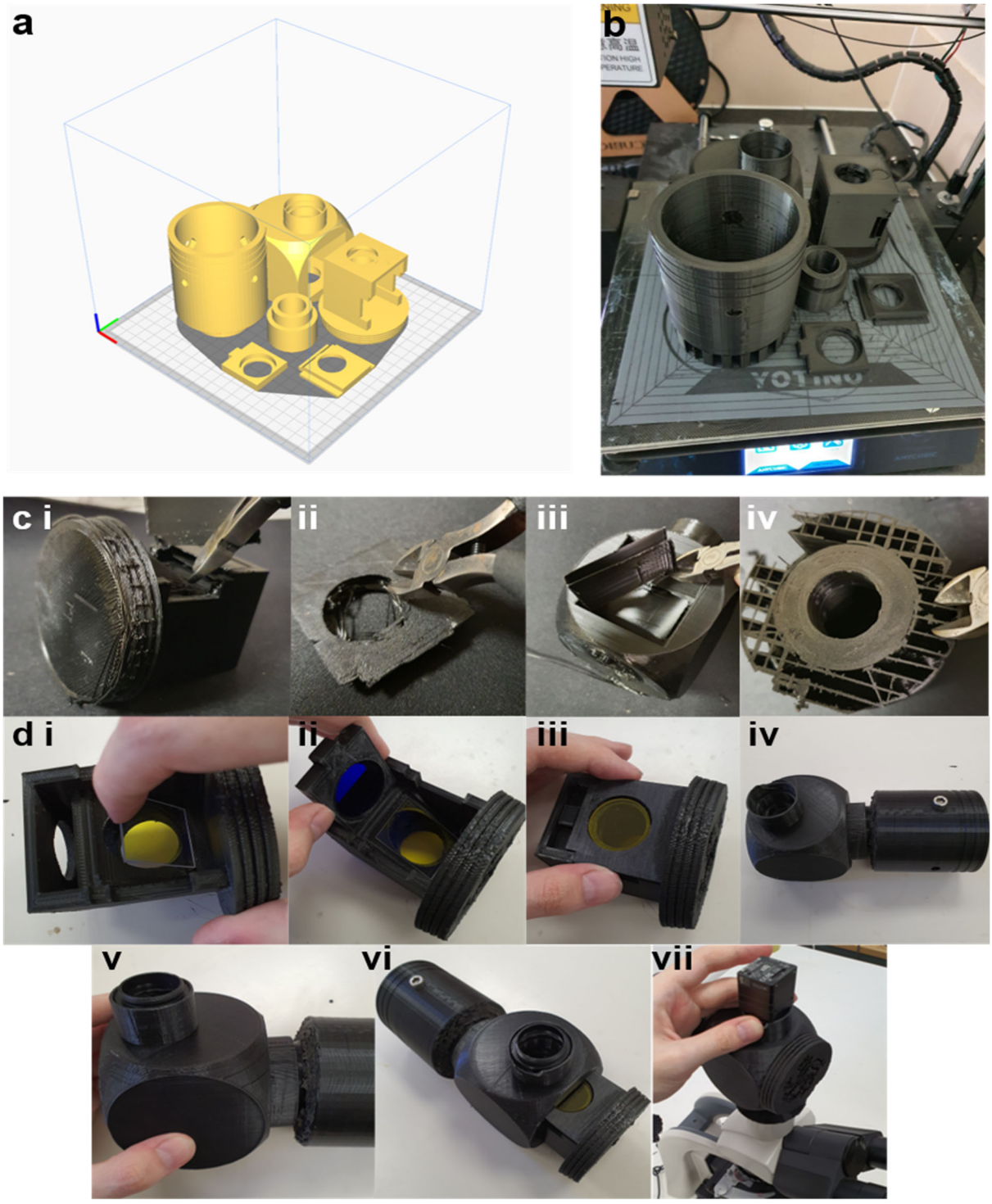
Zilberstein Y, Ayali A (2002) The role of the frontal ganglion in locust feeding and moulting related behaviours. J Exp Biol 205(18):2833-41..

Received September 23, 2020; accepted October 26, 2020.

This work was supported by EPSRC Doctoral Training grant EP/L505079/1) and a European Research Council Grant to MCG (640012) It was also supported by a British Council GREAT Scholarship (to VS) as well as grants from the St Andrews Learning and Teaching Initiative and the McCall-McBain Foundation to SRP. This work was also supported by the open source DIY communities as described throughout the paper. We would like to extend thanks to Dr. William J. Heitler for his work on DataView, Dr. Marcus Bischoff for technical advice and the University of St. Andrews MRes Neuroscience classes of 2018-2019 and 2019-2020 for their feedback and advice in the development of prototypes.

Address correspondence to: Stefan Pulver, School of Psychology and Neuroscience, University of St Andrews, St Andrews, United Kingdom. Email: sp96@st-andrews.ac.uk

APPENDIX



Appendix Figure 1. Printing and assembling 3D printed fluorescence module. *a)* 3D components laid out for a single print run in the slicing program Autodesk Cura. *b)* 3D components printing in a standard Fused Deposition Modelling printer. Note the rough Yotino adhesion mat on the buildplate (recommended for printing to prevent components from being knocked over mid printing). *c)* Supports are removed after printing manually from the filter tray (i), emissions filter holder (ii), main body (iii) and light source holder (iv), if there is any difficulty with this consider reducing support density. *d)* Assembly instructions: Carefully place the dichroic mirror into the filter tray (i), followed by the excitation filter (ii) and emissions filter (iii), then friction fit the C-mount adapter ring to the main body (iv) as well as the light source holder (v). After aligning the LED as much as possible, insert the filter tray (vi) and trinocular adapter (vii) then insert into microscope camera port with camera threaded (viii).

Component	Price (£)	Supplier (item code)
Excitation Filter	22.75	Comar Optics (445 GB 25)
Emission Filter	23.11	Comar Optics (515 GY 25)
Dichroic Mirror	177.74	Thorlabs (MD498)
PLA Filament	4.57	Amazon
C-mount adapter	3.69	Amazon
6000LM Rechargeable P70.2 LED Bike Headlight Front Rear Light 6400mAh battery	17.99	eBay
Subtotal (excluding camera):	**249.86**	**Per Module**

Appendix Table 1. Components list and cost analysis (excluding VAT) for 3D printed epifluorescence module. Note that availability and price of components may vary.

Component	Price ($)	Supplier (item code)
Excitation Filter	35.44	Thorlabs (FGB25)
Emission Filter	36.50	Edmund Optics (SCHOTT OG515)
Dichroic Mirror	236.98	Thorlabs (MD498)
PLA Filament	6.57	Amazon
C-mount adapter	11.99	Amazon
Rechargeable 8000LM XM-T6 LED Front Head Bicycle Bike Light Headlight MTB Lamp	23.99	eBay
Subtotal (excluding camera):	**351.47**	**Per Module**

Appendix Table 2. Alternative components list for US Suppliers and cost analysis in USD ($) excluding tax. Note that availability and price of components may vary.

Link to files for printing:

https://drive.google.com/file/d/11WLXopwN8U8gmHoHfEQikBWtBtCcQfqJ/view?usp=sharing

Please read the readme before progressing with the print.