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Light-Weight Portable Electroantennography Device as a Future Field-Based Tool for Applied Chemical Ecology

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Abstract

Portable electroantennograms (pEAG) can further our understanding of odor plume dynamics and complement laboratory-based electroantennogram tools. pEAG's can help to address important questions such as the influence of plume structure on insect behavior, the active space of semiochemical-baited traps, and the impact of biotic and abiotic factors on this active space. Challenges associated with pEAGs include their miniaturization and sensitivity, confounding environmental odors, and processing of data. Here, we describe a pEAG built with modern engineering hardware and techniques that is portable in being both light in weight (516 g) and smaller (12 × 12 × 8 cm, volume 1152 cm^3) than earlier models. It is able to incorporate insects of a range of sizes (4 to 30 mm antennal length), has wireless communication (communication range of 600 m urban, 10 km line of sight), a stand-alone power supply, and uses both antennae of the test insect. We report normalized antennal responses from *Epiphyas postvittana* in a dose response experiment where our pEAG compared favorably with traditional laboratory EAG equipment for this species. Dose-response comparisons between *E. postvittana*, *Agrotis ipsilon*, and *Lymantria dispar dispar* showed mean detection limits from a pheromone source dose of 100, 100, and 1 ng, respectively, for our pEAG. This pEAG should allow future real-time analysis of EAG responses in the field in research on how insects interact with odor plumes and the factors that influence the active space of semiochemical-baited traps.

Keywords Electroantennogram · Electrophysiology · Biosecurity · Biosensor

Key Message

- Field-based electroantennography has been limited due to a lack of available equipment.
- We report a new portable device for recording electroantennograms (pEAG) that is highly portable, simple to use, can accommodate different sized insects, and permits real-time data processing with wireless communication.
- Sensitivity is demonstrated for three species of moth, showing detection of pheromone source concentrations as low as 1 ng.
- This promising tool will facilitate future work characterizing how insects interact with odor plumes and the factors that influence the active space of semiochemical-baited traps. Such knowledge can improve the design of traps for biosecurity surveillance and pest control.

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Introduction

Electroantennography (EAG) was first developed by Schneider (1957) as an electrophysiological technique to measure the composite neural activity of an insect antenna. Applying this technique to questions of chemical ecology requires the amplification of the neural activity of the antennal chemoreceptors (Thorpe et al. 2006) and has become a standard laboratory technique that is applied widely to both fundamental and applied studies of insect olfaction (Baker and Haynes 1989; Baker and Roelofs 1976; Boeckh et al. 1965; Moorhouse et al. 1969; Roelofs and Comeau 1971; Schneider 1957; Schott et al. 2013). Electrophysiologists have refined the technique and can now use a variety of antennal forms (Olsson and Hansson 2013), and can even use it on aquatic animals (Machon et al. 2016), to detect volatiles such as pheromones released from sources at concentrations as low as picograms under highly controlled laboratory conditions (Glatz and Bailey-Hill 2011).

In the 60 years since its development the basic principles of an EAG have changed little. To date, the majority of electroantennography has been laboratory based, using specialized, laboratory equipment that is not readily adapted to field applications due to the size of the instrumentation and the requirement for mains power. Nevertheless, several researchers have developed more portable electroantennogram (pEAG) equipment to study olfactory responses under field conditions.

Baker and Haynes (1989) produced the first pEAG using a whole-body preparation of *Grapholita molesta* to detect changes in pheromone plume concentrations at 30 m from an artificial source of pheromone. Shortly after this, a research team based at the Universitat Kaiserslautern (Koch 1990; Milli and De Kramer 1989; Sauer et al. 1992) tested a pEAG that used excised antennae to measure field concentrations of pheromone released from dispensers containing the synthetic pheromone of *Lobesia botrana*. Rumbo et al. (1995) then developed a dual antennal live insect preparation pEAG. The first commercially available pEAG was developed by Van der Pers and Minks (1998) and was used to study aerial concentrations of pheromone during mating disruption (Thorpe et al. 2006, 2007) and mechanisms of host location in tsetse flies (Voskamp et al. 1998). Van der Pers and Minks (1993) also developed a portable single sensillum recording device that was used to test pheromone release and single receptor-neuron responses in the field (Valeur et al. 1999, 2000). Details of these early pEAGs are given in Table 1.

Constraints to the wide-spread development and implementation of these pEAG devices as research tools included their size and weight (e.g. van der Pers & Mink's unit, size 26 × 15 × 8 cm, weight 4 kg), the requirement for a stationary/stable platform (Baker and Haynes 1989), the overall fragility and size of the relatively cumbersome equipment, and how to process and interpret data (Suckling and Karg 2001). These pEAGs needed expert operators (Milli et al. 1997) and specific skills to transcribe data (Thorpe et al. 2007).

Excising antennae from the insect body may increase the sensitivity of the EAG, as it removes background noise from other neural activity, e.g., muscle movement. The increase in signal to noise ratio does come at a cost as the antenna, and therefore the signal, degrades over time, whereas a whole body preparation can remain stable for a working day or longer (Martinez et al. 2014; Merlin et al. 2007). Park and Baker (2002) expanded on earlier approaches using excised antennae (Milli and De Kramer 1989; Sauer et al. 1992; Van der Pers and Minks 1998) by developing a multi-antenna EAG. This was later developed into a quadprobe pEAG (Park et al. 2002) that connected four excised antennae in series to achieve a 10-fold increase in sensitivity and an 8-fold increase in sensor longevity. Myrick et al. (2009) and Martinez et al. (2014) independently developed whole-body pEAG preparations that provide greater temporal recording stability than that of excised antenna that degrade over minutes to 1 hr.

To date, pEAGs have been used to quantify *in situ* pheromone concentrations during mating disruption trials in greenhouses (Van der Pers and Minks 1998), open field (Bengtsson et al. 1994; Färbert et al. 1997; Koch et al. 2002, 2009), vineyards (Karg and Sauer 1995, 1997; Milli and De Kramer 1989; Sauer and Karg 1998; Sauer et al. 1992; Suckling et al. 2012), orchards (Karg and Suckling 1997; Karg et al. 1994, 1997; Koch and Witzgall 2001; Milli et al. 1997; Rumbo et al. 1995; Suckling et al. 2007; Suckling and Angerilli 1996; Suckling and Karg 1997; Suckling et al. 1994, 1996, 1999), and forests (Thorpe et al. 2006, 2007); assess plant damage (Schütz et al. 1999, 2000), and evaluate the impact of pheromone plume structure on male moth flight behavior (Baker and Haynes 1989; Hetling et al. 2003; Myrick and Baker 2011; Myrick et al. 2008, 2009; Park and Baker 2002; Park et al. 2002; Valeur et al. 1999; Van der Pers and Minks 1993, 1997; Voskamp et al. 1998).

More recently, pEAGs have been integrated with 2-dimensional ground-based robots to create insect biosensors to study insect orientation (Kuwana et al. 1995, 1999; Martinez et al. 2014; Nagasawa et al. 1999; Ortiz 2006). Martinez et al. (2014) include an automated algorithm that allows the robot to track a plume to its source by mimicking the behavior used by flying male insects to locate calling females. Tracking algorithms, reviewed by Chen and Huang (2019), provide a mathematical construct of the behavior of insects in flight (Frayle-Pérez et al. 2017) and can potentially be used to direct robotic movement to an odorant point source without an operator by mimicking insect behavioral reactions (e.g., Martinez et al. 2014).

Despite these developments, a pEAG tool that is both easy to use and can be used to test the response of a wide range of species is still lacking. For research and commercial use, a

Table 1 Comparison of portable EAG systems using information available in the published literature

Reference	Baker and Haynes 1989	Sauer et al. 1992, (revised Milli et al. 1997)	Rumbo et al. 1995	Van der Pers and Minks 1998	Martinez et al. 2014	This paper
Insect antenna	Whole insect	Excised	Whole insect	Excised	Whole insect	Whole insect
Size and Volume	Unknown	Unknown	Unknown	26 × 15 × 8 cm; 3120 cm^3	19 × 13 × 13 cm; 3211 cm^3	12 × 12 × 8 cm; 1152 cm^3
Weight	Unknown	Unknown	Unknown	4 kg	1 kg	516 g
Electrodes	Silver	Silver	Silver	Silver	Silver	Tungsten
Internal Airflow (L min^{-1})	2.5	0.84	14	0.5 m/s	NA	1
Power supply	Unknown	Mains or 12 V 9.5 Ah NiCd accumulator with DC-DC convertors.	Unknown	Rechargeable batteries, unknown lifespan	Battery operated, 8 h lifespan	Rechargeable LiPo batteries, 5 h life span
Communication	Unknown	Direct	Direct	Direct	Wireless	Wireless
Data	5–25 mm/s	unknown	100 Hz	unknown	1 kHz	1 kHz
Data collection	portable chart recorder	portable chart recorder and on-board tape recorder	direct connection to PC	On-board tape recorder	Data file onto remote computer	On-board computer with SD card
Data visibility	portable chart recorder	portable chart recorder	computer screen	portable LCD oscilloscope	Laptop – Qt-C++ GUI	Laptop – Python GUI

pEAG must be robust, simple to use, easily and quickly deployed, and flexible to accommodate insects of a variety of shapes and sizes. Here we report on a pEAG that meets these requirements and attempts to address the features that have limited the use of pEAGs as a tool in the past. We describe the key components of our pEAG that is a portable, light weight system with wireless data communication. We present a comparative study of EAG responses from the pEAG and a laboratory EAG using *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), and a comparison of pEAG recordings from three species, *E. postvittana*, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), and *Lymantria dispar dispar* (Linnaeus) (Lepidoptera: Erebidae).

Methods and Materials

Insects

Adult *E. postvittana*, *A. ipsilon* and *L. dispar dispar* were used for electrophysiological testing in three different countries. All insects used for dose response assays were virgin male moths between 2 and 4 d old. Individual rearing conditions of moths varied as described below.

Recordings of *E. postvittana* were conducted at Scion (Christchurch, New Zealand) using laboratory-reared males from wild caught breeding pairs. Larvae were reared on artificial diet (Singh 1983), pupae were sexed, and males and females kept separately. This supply of pupae was supplemented from the long-term *E. postvittana* colony at Plant and Food Research facility in Mt. Albert (Auckland, New Zealand). The Scion colony was reared in a Conviron® incubator (ThermoFisher Scientific, Auckland, New Zealand) at 20 °C and the pupal stage held in a Conviron at 10 °C, 55–60% RH, L:D 0:24 hr for 2–7 days. Newly emerged adults were placed in rearing cages at 18 °C in a temperature controlled room with an ambient L:D cycle, and provided with honey water prior to EAG recordings.

Recordings of *A. ipsilon* were conducted at INRAE (Versailles, France) using laboratory-reared males. *Agrotis ipsilon* larvae were fed on an artificial diet (Poitout and Bues 1974) in individual cups until pupation. Pupae were sexed, and males and females kept separately at 22 °C, 50–60% RH and L:D 16:8 hr. Newly emerged adults were moved to rearing cages and held at 22 °C, 50–60% RH and L:D 16:8 hr and provided sugar water prior to recordings.

Recordings of *L. dispar dispar* were conducted at the Great Lakes Forestry Centre (Sault Ste. Marie, Canada) using laboratory-reared adult males. Larvae were mass reared (8 to a container) on artificial diet. Pupae were separated by sex and kept until emergence at 20 °C, 80% RH and L:D 16:8 hr. Pupae were transferred to a Conviron® at 22 °C, 75% RH and L:D 16:8 hr. Adults were housed in the same

Conviron® room as the pupae, as described above, contained individually in 60 ml clear plastic cups with ventilated lids until they were used for pEAG recordings.

Test Compounds

All compounds used were > 97% pure. Compounds were diluted in decadic steps in hexane and stored at −18 °C until needed.

For *E. postvittana* a two-component pheromone mixture of (*E*)-11-tetradecenyl acetate (*E*11–14:Ac) and (*E*,*E*)-9,11-tetradecadienyl acetate (*E*9,*E*11–14:Ac) (Pherobank, The Netherlands) in 20:1 ratio (Bellas et al. 1983) was used at doses of 1 to 10,000 ng of *E*11–14:Ac and *E*9,*E*11–14:Ac. For *A. ipsilon* a single-component pheromone (*Z*)-7-dodecenyl acetate (*Z*7–12:Ac) (Pherobank, The Netherlands) was used (Hill et al. 1979) at doses of 0.001 to 1000 ng. for *L. dispar dispar* a single-component pheromone of (+)-disparlure, (7*R*, 8*S*)-cis-7,8-epoxy-2-methyloctadecane, (Shin-Etsu Chemical Co., Ltd., Japan) was used (Bierl et al. 1970) at doses of 0.001 to 1000 ng.

Electroantennography

Dose Response Assays

A 1 μl aliquot of each pheromone concentration was applied to sections of filter paper (15 × 10 mm) and left in a fume-hood for 30 sec to allow hexane to evaporate and the filter paper was then placed into an individual glass Pasteur pipette (1.1 mm internal diameter (ID) tip, 5.85 mm ID body, 145 mm length; Thermofisher Scientific NZ Ltd., New Zealand) or piece of Tygon laboratory tubing (4.7 mm ID, 7.9 mm OD, 50 mm long; Saint-Gobain Plastics, France) to act as pheromone holders. The control stimulus for all dose response assays was hexane (1 μl).

For EAG recordings a stimulus file was used to control the data acquisition and delivery of a 200 ms pheromone stimulus. In the laboratory the stimulus files were controlled via LabView (National Instruments, USA) whereas a custom Python (Python Software Foundation, USA) script controlled recordings in the pEAG. A single puff of pheromone was applied through the pheromone holder (glass pipette or Tygon tubing) with puffs of air containing the control (hexane) applied before and after each dose to monitor the base response of the insect to the additional airflow from the internal stimulus system. Pheromone doses were applied sequentially in ascending order of concentration at 1-min intervals.

Laboratory EAG

Insects were anesthetized with CO_2 and restrained in a 200 μl pipette tip (Axygen Scientific, USA) using cotton wool such that the upper thorax, head and antenna were exposed at the narrow end of the pipette tip. The restrained insect was mounted within range of the EAG stage for placement of the electrodes. All manipulations were conducted using a stereomicroscope (M205C; Leica, Germany). Electrodes were made from 99.95% pure 0.3 mm tungsten wire (Goodfellow Cambridge Ltd., United Kingdom) and mounted into tungsten probe holders (Ockenfels Syntech, Germany). The reference electrode was sharpened with a tungsten etching device (Ockenfels Syntech, Germany), dipped in a small quantity of electroconductive gel (SIGNAGEL®, Parker Laboratories Inc., USA), and then inserted by micromanipulator (UM-3C; Narishige, Japan) into the base of the head. The unetched recording electrode was brought in contact with the cut tip of an antenna with electroconductive gel after the last antennal segment was excised with microscissors.

EAG signals were amplified (×100) and filtered (0.1 Hz - 30 Hz band pass) using an EX1 amplifier with a 4002 head stage (Dagan, Minneapolis, USA), and digitized at 2 kHz by a 16-bit acquisition board (NI9215 coupled to a cDAQ-9174; National Instruments, USA) using LabView (National Instruments, USA) for a graphical user interface and visualization. All EAG preparations and experiments were conducted within a Faraday cage. The laboratory room temperature during all EAG recordings was controlled at 21 ± 1 °C and the climate control fan was turned off during recording.

Stimulus was applied to the EAG preparation by an odor delivery system that pumped humidified and charcoal filtered (Restek, Refillable Hydrocarbon trap #22012) air through twin flowmeters (Cole-Parmer Valved Acrylic Flowmeter, 50 mm Scale for Air, 0.4–5 Litre min^{-1} and 0.04–0.5 Litre min^{-1}) that controlled a constant base flow of air (1 Litre min^{-1}) and the stimulus air stream (0.1 Litre min^{-1}). The base airflow was pumped through Norgren plastic tubing with an internal diameter of 6.5 mm. The stimulus airflow was pumped through Tygon tubing (1.5 mm ID tubing, Saint Gobain Tygon S3 E-3603 Non-DEHP). The stimulus airflow was controlled by a 3-way solenoid valve (LHDA 1233215 H; Lee Company, Japan) and a custom switch that interfaced with LabView via an analog output module (NI9264, National Instruments, USA) coupled with the cDAQ-9174. The stimulus could be programmed and activated via LabView to deliver a 200 ms puff of pheromone (on filter paper) into the main air, which flowed to the insect through a glass mixing tube (200 mm, ID 7 mm) with a hole positioned 150 mm from the exit to deliver the stimulus.

Portable Electroantennogram (pEAG)

The pEAG (Fig. 1) consists of two independent 0.3 mm tungsten signal electrodes (Goodfellow Cambridge Ltd.) that are attached to a customized preamplifier and amplifier. A sharpened 0.3 mm tungsten reference electrode (the same as

Fig. 1 Portable electroantennogram units showing an individual unit with the airflow tube removed (lower image) and a unit mounted for handheld detection. Insert, shows *Lymantria dispar dispar* connected to the dual signal electrodes and central reference electrode of the pEAG

laboratory reference electrode) is operated by a three-axis micromanipulator (US-3F; Narishige, Japan). Insects are anesthetized as in the laboratory treatment and held in a customized foam block for holding/restraining insects of various sizes. Both antennae are prepared for connection to independent signal electrodes as in the laboratory treatment. The insect is positioned on the pEAG stage such that air flowed over both antennae simultaneously.

A cover (18 mm ID) was placed over the insect stage and air drawn past the insect by a 25 mm axial fan (Sunon, Taiwan) mounted at the rear of the stage and operated at reduced voltage to ensure a continuous main airflow of 1 L min^{-1}. The stimulus airflow was pumped through Tygon tubing (1.5 mm ID tubing, Saint Gobain Tygon S3 E-3603 Non-DEHP) at 0.1 L min^{-1} to a 3-way solenoid valve (LHDA 1233215 H; Lee Company, Japan) controlled by a custom control board that incorporated a Teensy development board (Teensy 3.6, PRJC, USA). During periods of non-stimulus this airflow was vented to atmosphere and the insect was only exposed to the main airflow. During periods of stimulus it was diverted through Tygon tubing (1.5 mm ID) that contained a piece of filter paper loaded with pheromone into the constant airflow 45 mm in front of the insect. Stimulus airflow duration could be programmed and activated remotely, or manually by a button on the pEAG, and for this experiment it was set to deliver a 200 ms pheromone puff.

All pEAG preparations and experiments were conducted at a constant temperature within a Faraday cage, as in the laboratory treatment. Development of the pEAG technology has continued since the data presented here and a prototype metal cover is being tested as a replacement to the Faraday cage such that the pEAG can be used indoors in areas subject to electrical noise, e.g., as a GC-EAD where 50 Hz noise from other lab machinery may be present.

Signals from the insect were amplified (×100), low pass filtered 460 Hz and digitized (24-bit synchronous 4096 kHz Delta Sigma Analogue to Digital Converter, ADC). The ADC was configured for 2048 times oversampling, for an output sample frequency of 2 kHz. The EAG signals were downsampled to 1 kHz for acquisition to the SD card and decimated to 33 Hz for visualization over the wireless transceiver (Xbee Pro S3B; Xbee, USA). The dual channel head amplifier, main amplifier, and filters are a modification of Martinez et al. (2014) that were reconfigured to suit the selected ADC. Recordings were visualized using a custom Python application via the wireless transceiver that enabled remote real-time viewing of EAG data at distances of 600 m in urban environments and to a theoretical 10 km with clear line of sight. The custom Python application also controled the delivery of programmed stimulus puffs via an internal stimulus delivery system using a configuration file. Custom firmware was developed in C++ (ISO/IEC 14882:2017(E)) for the control board to enable communications with the ADC and SD card, the Python application (Python Software Foundation, USA), and to control the axial fan and pheromone stimulus pumps. The pEAG is powered by an autonomous, stand-alone power supply (main board power 1 × 1.1 Ah 11.1 V; head amplifier 2 × 0.12 Ah 7.4 V rechargeable lithium ion batteries), allowing for approximately 5 hr continuous operation per battery.

Analyses

EAG and pEAG recordings were sampled at 1 kHz and peaks were assessed using Clampfit 10.7.0 (Molecular Devices LLC, USA) with post filtering: low pass, type: Gaussian,

−3 dB cut off: 50 Hz. EAG and pEAG responses were normalized relative to the response to the hexane control stimulus (= 1.0). Mean normalized responses ± standard error were calculated in R version 3.4.1 (R Development Core Team 2018) using the package plyr (Wickham 2011).

Results

Electroantennogram responses recorded on the pEAG from all three test species, *E. postvittana*, *A. ipsilon* and *L. dispar dispar* are shown in Fig. 2. For *E. postvittana,* normalized laboratory EAG and pEAG responses to individual 200 msec pheromone puffs were visually detectable above background noise at doses as low as 100 ng (Fig. 3). At lower doses the antennal response was indistinguishable from that to the hexane control (= 1.0). Although the laboratory EAG elicited a numerically higher normalized mean response at 100 ng, the pEAG recorded higher values with lower variation at doses of 1000 and 10,000 ng (Fig. 3).

Markedly different dose-response curves to 200 ms puffs of pheromone were observed with the pEAG for the three test species (Fig. 4). Significant responses were recorded from *L. dispar dispar* after 200 ms puffs of (+)-disparlure at doses as low as 0.1 ng. The inferred detection limit was between 0.01 and 0.1 ng. In comparison, the pEAG could only detect responses from *A. ipsilon* and *E. postvittana* at doses of 100 ng or greater. The response from *L. dispar dispar* peaked at 1 ng and then declined slightly thereafter, although at the higher doses the variability in normalized response increased (Fig. 4).

Discussion

Here we provide details of a portable electroantennogram (pEAG) built with modern engineering hardware and techniques, and demonstrate the ability to detect antennal responses with the same sensitivity as a laboratory EAG system. We have overcome several challenges that may have limited past adoption of pEAGs. It is lightweight, has a simple

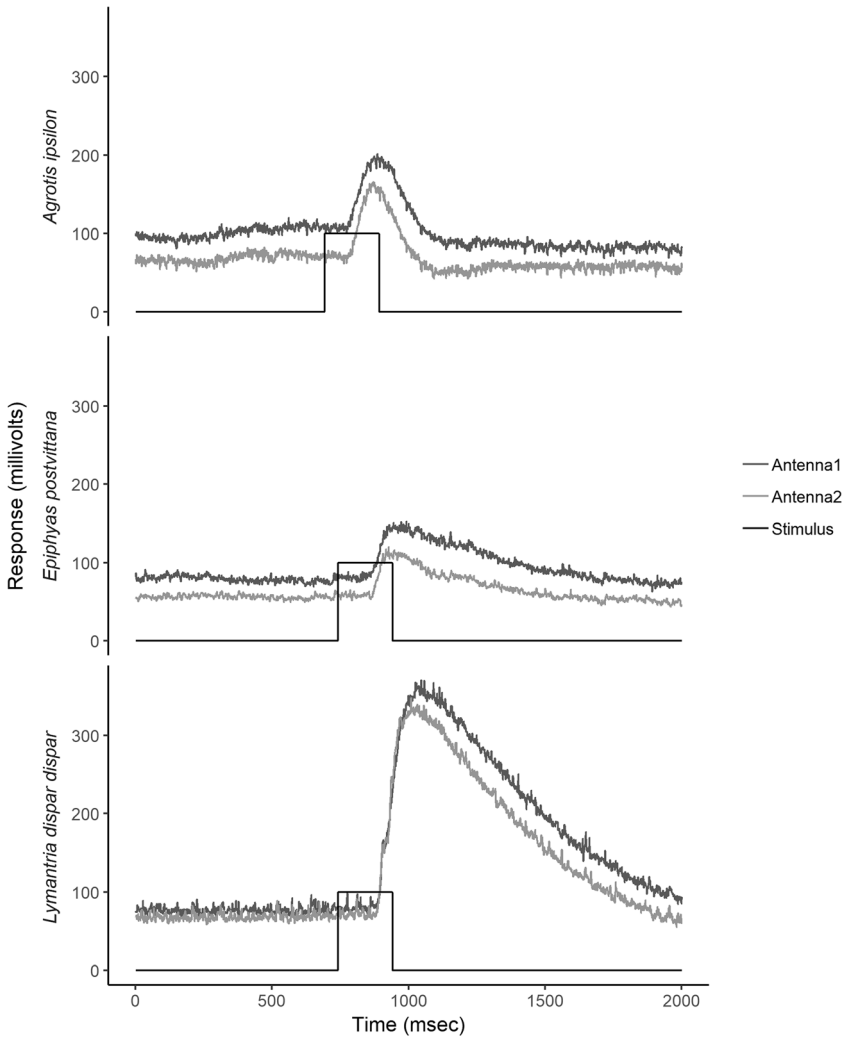

Fig. 2 Traces from portable electroantennograph of responses of *Epiphyas postvittana*, *Agrotis ipsilon*, and *Lymantria dispar dispar* to a 200 msec exposure to a 100 ng pheromone dose (response from antennae amplified × 100)

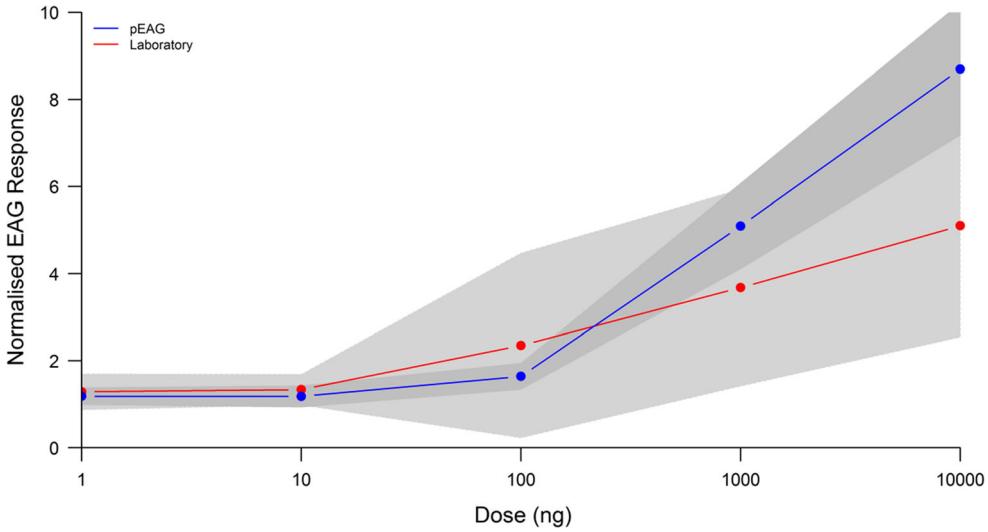

Fig. 3 Mean normalized EAG dose-response curves from *Epiphyas postvittana* in laboratory and mobile (pEAG) recordings systems stimulated with 200 ms pheromone puffs. Grey shading indicates 95% confidence intervals. For measurements with standard laboratory EAG equipment $N = 10$; for those with the pEAG $N = 13$ for all doses except 10 ng $N = 11$

mechanism for restraining a range of different sized insect species, and utilizes wireless communication. Our pEAG can be deployed by a non-specialist individual with minimal training which should also facilitate its usage in future field studies. The device could be mounted on an extendable pole or an unmanned aerial vehicle (UAV). Long-term we envisage a coupling like that of Martinez et al. (2014) where a pEAG biosensor can, in combination with appropriate search algorithms (Chen and Huang 2019), be used to track odor plumes to their source, i.e., an aerial biosensor.

Portable EAGs were first used to overcome the historical time delay in the detection of pheromone in air by other detection technologies, e.g., ion detectors, gas chromatography/mass spectrometry (Baker and Haynes 1989). Thorpe et al. (2007) used the pEAG described by Van der Pers and Minks (1998) to demonstrate in-field differences in pheromone detection by male gypsy moth in different mating disruption treatments. The response from the Van der Pers and Minks (1998) pEAG is expressed as a measure relative to the response elicited by an internal standard (hexyl acetate in the case of Thorpe et al. (2007)), hence we cannot compare our pEAG sensitivity to that of Thorpe et al. (2007). Previous laboratory electroantennography studies by Yamada et al. (1976) of the closely related *L. dispar* (Asian gypsy moth) showed responses to cis-(+) Disparlure at source doses of 100 ng. Here, our pEAG recorded responses from *L. dispar dispar* at a 0.1 ng dose. The extreme sensitivity of *L. dispar dispar* relative to the other species reported here is consistent with existing knowledge that pheromone communication in Lymantriinae can occur over longer distances relative to many other moths (Cardé 2016).

Extensive research has evaluated pEAGs in validating mating disruption of *E. postvittana* and the factors affecting its efficacy as a control tool (Rumbo et al. 1995; Suckling and

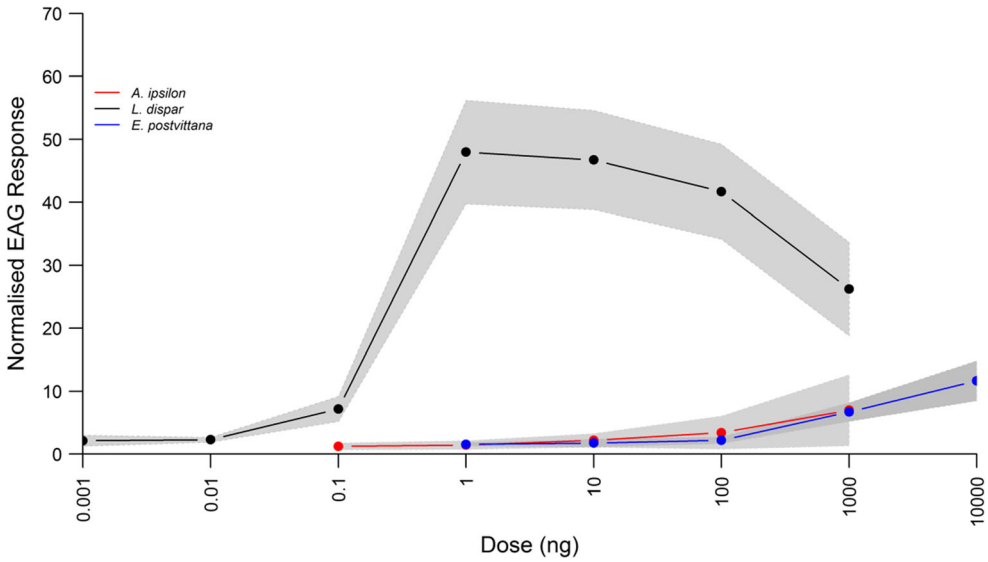

Fig. 4 Dose-response comparisons of mean normalized pEAG responses from three test species to 200 ms pheromone puffs. Grey shading indicates 95% confidence intervals. For *Epiphyas postvittana* $N = 13$ for all doses except 10 ng $N = 11$; for *Lymantria. dispar dispar* $N = 12$; for *Agrotis ipsilon* $N = 12$ at 0.1 ng, 1 ng and 1000 ng, and $N = 10$ at 10 ng and 100 ng doses

Angerilli 1996; Suckling et al. 1994). As with *L. dispar dispar* most of the available research does not present dose response information and therefore we cannot compare our results to these studies. Suckling et al. (1994) show typical EAG responses to 500 ms pulses of orchard air where pheromone traps with doses of 10 or 100 μl were applied. Suckling et al. (1994) also show a dose response curve from 1 to 100 μl, also reported by Karg et al. (1997). Although, differences among these studies complicate comparisons our detection threshold between 100 and 1000 ng for a 200 ms puff for live preparations, is at least as sensitive as previous excised antennal preparations.

Although we know the dose applied to the pheromone source in our experiments, we cannot infer the concentration of pheromone that was presented to the insect using our current method. Due to the different vapor pressures of various compounds they will be released at different rates from the source for a given temperature. Thus, comparisons between species is challenging and we cannot be certain of the precise detection limits of our pEAG.

Future field-based studies will use the pEAG to attempt to characterize the active space of semiochemical-baited traps, the impact of biotic (e.g., insect age) and abiotic (e.g., wind and temperature) factors on trap active space, and the influence that trap design has on the fine-scale structure of odor plumes and thus insect flight behavior and ultimately trap performance (Lewis and Macaulay 1976; Willis et al. 1994; Wyatt et al. 1993, 1997). In the longer-term, we intend to evaluate pEAG technology for its potential as an in-field location tool to detect pheromone sources (Myrick and Baker 2011), i.e., calling females, when the pheromone is unknown or unstable, and this will be of value in surveillance. There is also potential for the device to be incorporated into a GC-EAD. The small size of our pEAG provides flexibility and allows set up away from sensitive equipment and lab odors before connecting to the GC outlet. The real-time data transfer capabilities of this pEAG are a step towards these goals, however aligned technologies (wind sensors and tracking algorithms) are yet to be incorporated. We believe that combined with current (and future) technological advances pEAG devices like the one reported here will facilitate new discoveries in semiochemical plume dynamics and how insects respond behaviorally to such plumes by advancing the practice of electrophysiology.

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Authors' Contributions SP, JK, BO, and TS conceived and designed the research. PL and DM contributed to the design of the pEAG. BO and JK conducted experiments. PL and JA and their respective labs contributed to pEAG testing of *Agrotis ipsilon*, and *Lymantria dispar dispar* respectively. SP, JK, BO wrote the manuscript with all authors subsequently contributing to and approving the final manuscript.

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