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1 Antennal responses to volatiles related to host location in the ambrosia beetle *Platypus*
2 *quercivorus* (Murayama)

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ABSTRACT

The ambrosia beetle *Platypus quercivorus* (Murayama) is a vector of Japanese oak wilt, a fungal pathogen that causes mass mortality of Fagaceae trees. The male locates and lands on the host tree and releases an aggregation pheromone that attracts both males and females. Thus, aggregation pheromones and host tree volatiles are important chemicals for host location. However, the antennal responses to these volatiles have not yet been examined. To obtain physiological property of *P. quercivorus* antennae, we electrophysiologically recorded the antennal responses of this beetle to candidate volatiles, including isoprene and other leaf volatiles (1-hexanol, 2-ethyl-1-hexanol, trans-2-hexenal, decanal, and benzaldehyde) of the host trees, as well as quercivorol (aggregation pheromone) and ethanol (widely used for trapping this insect in the field). Electroantennograms (EAGs) exhibited clear dose-dependent responses to the tested volatiles, but antennae did not respond to decanal. Sexual differences in responses were not observed for almost all volatiles. However, the EAGs suggested that the quercivorol and trans-2-hexenal sensitivity of males was higher than that of females. Ethanol elicited a large EAG amplitude in both sexes, but the sensitivity of males or females to ethanol was lower than that to the other volatiles. EAGs showed a very weak response to isoprene only in males, although isoprene is considered a possible attractant for host location. Our study will facilitate to understand the mechanism of the host location ecologically and neurophysiologically.

KEYWORDS

Ambrosia beetle, Electroantennogram, Olfaction, Host location, Aggregation pheromone.

ABBREVIATIONS

EAG: electroantennogram

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INTRODUCTION

Antennae are the major olfactory sensory organs for insect olfaction and play important roles in various behaviors, such as mating, food seeking, and finding suitable habitats (Martin et al. 2011; Hansson and Stensmyr 2011). On the surface of the antenna there are various types of sensilla which contain dendrites of olfactory sensory neurons (Watanabe et al. 2018; Biswas et al. 2023; Liu et al. 2021; Wang et al. 2023). Once an odorant binds to an olfactory receptor on the membrane of an olfactory sensory neuron, an electric signal is generated, which is then transmitted to the primary olfactory center, the antennal lobe (Clyne et al. 1997; Stensmyr et al. 2003; Ammagarahalli and Gemenio 2015; Tateishi et al. 2020). After information processing in the primary olfactory center, the higher-order center in the brain integrates the processed information from the primary olfactory center, creating motor signals as an output from the brain to the motor centers governing the muscles (Okada et al. 2003). Thus, decision-making occurs in the brain.

Insect olfaction is important for the detection of a suitable host, and it also applies to the ambrosia beetle *Platypus quercivorus* (Murayama), whose female is known as a vector of the fungal pathogen, *Raffaelea quercivora* Kubono et Shin-Ito that causes Japanese oak wilt disease and mass mortality of Fagaceae trees (Kubono and Ito 2002). Since the 1980s, this disease has been expanding throughout Japan and has become a serious problem (Kobayashi and Ueda 2005). The male first locates a host tree, bores into the bark, and then releases an aggregation pheromone that attracts both males and females. Consequently, the host tree is mass attacked by conspecifics (Ueda and Kobayashi 2001), and eventually dies. In addition to aggregation pheromones, volatiles from host trees can be key to finding a host.

Two chemicals are known to be involved in the olfactory response of *P. quercivorus*: quercivorol and ethanol. The aggregation pheromone of *P. quercivorus* has been identified as a monoterpene alcohol ((1*S*, 4*R*)-p-menth-2-en-1-ol, quercivorol) (Kashiwagi et al. 2006), and its effectiveness has been confirmed in the field (Tokoro et al. 2007). Ethanol is emitted from trees suffering from abiotic and biotic stresses, and many bark and ambrosia beetle species, which attack stressed and weakened trees, are attracted to ethanol (Kelsey and Joseph 2003; Ranger et al. 2013). In the case of *P. quercivorus*, a synergistic effect of ethanol, which enhances the attractiveness of quercivorol, has been confirmed (Tokoro et al. 2014), although ethanol alone was not effective in attracting beetles in the field (Iidzuka et al. 2016).

In addition to aggregation pheromones and ethanol, volatiles emitted from host trees have been suggested to be involved in the host detection process in *P. quercivorus*. Based on our previous work (Yamasaki et al. 2014, 2023, 2024), we are proposing that the beetles approach from the top

of the host tree by detecting host leaf volatiles. The detection of host leaf volatiles by the beetle was confirmed in the laboratory using an olfactometer; *P. quercivorus* was attracted to and avoided volatiles from seedling host and non-host trees, respectively (Pham et al. 2020). Additionally, *P. quercivorus* was attracted to volatiles from fresh leaves, although it was repelled by volatiles from the dried leaves of its hosts, suggesting a preference for healthy hosts over declining or withering hosts (Pham et al. 2019). A gas chromatography-mass spectrometry (GC-MS) analysis from four host tree species and two non-host tree species revealed that many kinds of chemicals emitted from host trees were overlapped with those from non-host tree although the amount was different among tree species (Pham et al. 2020). For example, whereas 2-ethyl-1-hexanol was abundant (dominant) in both seedling host and non-host trees, decanal was emitted from both seedling host and non-host tree but poor (minor).

These results raise an important question relating to host selection: how *P. quercivorus* nervous system process volatile information for host selection. Although the antenna is the first organ to detect volatiles, it has been unclear how their antennae respond to possible candidate chemicals. Is the strength in the antennal responses to the aggregation pheromone similar with that to other chemicals? Is the sensitivity for the pheromone higher than for other chemicals? How about sexual differences? Are there any differences in response strength and/or sensitivity between for dominant and minor compounds? The host selection should be executed in a complex manner via a decision making in the brain. Thus, the antennal response property is important for understanding a host selection mechanism from the neurophysiological side.

There are several candidate volatile compounds for *P. quercivorus* host selection. Because beetles attack broadleaved trees, green leaf volatiles (GLV) are the likely candidates. Our GC-MS analysis detected 14 and 45 compounds from seedlings (Pham et al. 2020) and leaves (Pham et al. 2025) of host and non-host, respectively. Among the various compounds listed as GLV, we selected test chemicals as a first step based on the emission amount from trees: rich or poor, from seedlings or leaves, common compound among tree species or not. Consequently, we selected four chemicals in this study (Table 1). 2-Ethyl-1-hexanol is one of dominant components of volatiles in the host and non-host seedlings of *P. quercivorus* (Pham et al. 2020). Trans-2-hexenal is emitted from dried leaves of the host of *P. quercivorus* (Pham et al. 2025). In addition to GLV, decanal was detected in seedlings of host and non-host species tested (Pham et al., 2020), although its peak area identified in GC-MC was lower than 2-ethyl-1-hexanol. Benzaldehyde is emitted from the dried leaves of the host and non-host species (Pham et al. 2025) and is a major component of the essential oil extracted from the dried leaves of *Quercus dentata* (Kameoka et al. 1983).

Furthermore, isoprene should be considered a candidate volatile for *P. quercivorus* host selection. The main target species of *P. quercivorus* are *Q. crispula* and *Q. serrata* (Kobayashi and Ueda 2005), both emitting isoprene from their leaves (Tani and Kawawata 2008). These two *Quercus* are the only species which emit isoprene among several dominant tree species in Japan (Matsunaga et al. 2012), therefore, it could be advantageous for the beetle to utilize isoprene to discriminate between host and non-host trees. The possibility that isoprene plays a role in the host detection process of *P. quercivorus* is further supported by (1) the restricted occurrence of beetle mislanding on native non-hosts, non-emitter of isoprene, in a close vicinity of hosts (Yamasaki et al. 2024), and (2) the frequent mislanding of beetles on introduced non-hosts that emit isoprene irrespective of the absence of surrounding hosts (Yamasaki et al., personal communication).

As the electroantennogram (EAG) shows the responses of most, if not all, olfactory sensory neurons to a stimulant, EAG recording has been often used, as a first step, for investigating antennal recognition of chemicals (Whitehead 1986; Krishnan et al. 1999; Germinara et al. 2017; Balakrishnan et al. 2017; Gao et al. 2019) and ecological insights related to host volatiles (Kendra et al. 2011; Romero et al. 2022; Park et al. 2001; Blažytė-Čereškienė et al. 2016). In order to probe the characteristics of the olfactory reception of *P. quercivorus* antennae as an olfactory sensor, which leads to a neural mechanism on the host location, we made EAG recordings for six possible candidate volatiles related to host location as well as the aggregation pheromone.

METHODS AND MATERIALS

Animals.

Both adult male and female *P. quercivorus* were collected from dead *Q. serrata* trees attacked by beetles in 2021 and 2022. Individuals that emerged in 2022 and 2023 were kept in a plastic tube with a cotton ball (about 1 cm in diameter) soaked by water in the dark until the experiments with both females and males. Sex was examined under a microscope to determine whether an individual had mycangia on the dorsal thorax (female) or not (male). In most experiments, 1-3 days old beetles were used.

Stimulus Apparatus.

The basic experimental setup was modified from that of Nishino et al. (2003) (Fig. 1A). Air current was generated from an air pump (N86KN.18, KNF Neuberger, Freiburg, Germany) and cleaned by passing it through a cotton filter, charcoal filter, and water. The air was bifurcated after filtration. One of the tubes (0.5 L/min background constant air flow) was connected directly to the output part,

made by a 1000 μ L micropipette tip, of the stimulation apparatus. The outlet was 0.5-1 cm away from the insect. The other tube (0.5 L/min) was further bifurcated into two tubes. One was for an odor or a control stimulus, and the other was for the compensated airflow. These two tubes converged to the output of the stimulation apparatus via a solenoid valve (Takasago, Nagoya, Japan). For odor simulation, a 1 cm x 1 cm filter paper (No1, Advantech, Tokyo, Japan) soaked by 10 μ L of stimulant was put into a cartridge (70 mm in length and 4 mm in inner-diameter) that was between the solenoid valve and output part of the stimulation apparatus. Each cartridge was used separately only for one concentration of one odorant. A tube (i.d. = 10.5 cm) was placed behind the insects to exhaust air.

Electrophysiology.

A beetle was immobilized on ice and put into a plastic tube made by a 200- μ L micropipette tip. The distal end of the pipette tip was cut so that the diameter of the tube tip was slightly larger than the pronotum. The entire abdomen was inserted into the tube, and the head and part of the thorax (in most cases, the anterior half) protruded from the tube tip. The insects were fixed to the tube using dental wax (yellow impression wax, GC, Tokyo, Japan). The heads and forelegs were immobilized using dental wax (color wax, Shofu, Kyoto, Japan). The tube inserted into the animal was fixed on a plastic stage such that the right antenna was up. Then, a small hole (120-150 μ m in diameter) was made with a fine tungsten needle near the dorsal rim of the right compound eye. A 100- μ m Ag/AgCl wire was inserted into the head through the hole as an indifferent electrode. A glass electrode (ca 100- μ m in the inner diameter) filled with 0.17 M NaCl was used as a recording electrode (about 2 M Ω). The recording electrode was placed on the distal end of the right antenna and gently pushed toward the head capsule to immobilize the antenna. The recording electrode was connected to a differential amplifier (MEG-5200, Nihon Kohden, Tokyo, Japan). Through the amplifier, the electric signal was amplified (x 1000) with a low (0.08 Hz) and high (30 or 100 Hz) cut filter and a hum filter (60 Hz). The amplified signal was stored on a PC via a noise eliminator (HUMBUG, Digitimer, Hertfordshire, UK) with a stimulus waveform at a sampling frequency of 2 kHz, using an A/D converter (POWER 1401, CED, UK) and Spike2 (CED, Cambridge, UK).

Stimulation.

The test volatiles were chosen based on the results of previous studies regarding *P. quercivorus* and their host tree species as described above, 2-ethyl-1-hexanol ($\geq 99.6\%$, 838051-500ML, Sigma-Aldrich, Tokyo, Japan), benzaldehyde ($\geq 98.0\%$, 025-12206, Wako, Osaka, Japan), decanal ($\geq 90.0\%$,

045-21532, Wako, Osaka, Japan), ethanol (Sigma-Aldrich, Tokyo, Japan), isoprene ($\geq 99.0\%$, I0160, TCI, Tokyo, Japan), quercivorol (Sankei Chemical Co. LTD, Kagoshima, Japan), and trans-2-hexenal ($\geq 95.0\%$, 07144-62, Nacalai, Kyoto, Japan). Additionally, 1-hexanol ($> 98.0\%$, 18013-32, Nacalai, Kyoto, Japan) was used for standardizing because our preliminary work found that this chemical elicit large antennal responses. Indeed, the antennae of other coleopteran species that attack *Quercus* trees respond to this chemical (Fürstenau et al. 2012). Ethanol was diluted by distilled water and the other volatiles were diluted by mineral oil (Sigma-Aldrich, St. Louis, MO, USA) to 10^{-4} , 10^{-3} , 10^{-2} , 10^{-1} , 10^0 , 10^1 , and 10^2 $\mu\text{g}/\mu\text{L}$. Odor was applied for 1 s (Fig. 1B). Diluted and undiluted volatiles (10 μL) were applied in an ascending manner with an interval of at least 30 s (25% quartile, median, and 75% quartiles of the interval distribution of all stimulations were 38.2, 41.1, and 45.3 s, respectively). In addition to the odor stimuli, mineral oil, distilled water, and air (blank: filter paper alone) were used as control stimuli. As ethanol and isoprene are highly volatile, they were prepared immediately before stimulation. For all stimulants, we capped the cartridge immediately after an application of stimulant onto the filter paper in the cartridge and never opened until use (at least 5 min usually, 10 min or longer). Usually, it was shorter than 10 seconds after opening the cap and before installing the cartridge into the stimulus apparatus. In addition, even after installing the cartridge, all valves were closed. Thus stimulant molecules cannot leave outside of the cartridge but they may be filled in the cartridge.

In most cases, each odor was applied once. However, we applied odor stimulation twice or more when a recording was unreliable; for example, when the recording showed odor responses overlapped by an unpredictably large noise at a stimulation moment, this may be due to our preparation (the whole body of a living animal). Additional stimulation was also applied to confirm that the EAG was correctly recorded when it seemed to have been recorded incorrectly (extremely larger or smaller amplitude than usual). The volatiles used in the simulations were randomly applied. For the control stimuli, one or two (in most cases, two) blank stimuli, mineral oil, or water were applied after one odorant stimulation series was completed. As well as control stimuli, 10^3 μg 1-hexanol was repeatedly applied as a “standard” because responses to 10^3 μg 1-hexanol were sufficiently large and stable for normalizing all responses (see below). In most cases, the standard stimuli were presented before a new volatile stimulus series began. To exclude the possibility that the concentration of 1-hexanol as a standard stimulus reduces due to repeated application, fresh 10^3 μg 1-hexanol was used every 2 or 3 stimulations. As we used the whole body of a living animal to record EAG, recording ceased when the recording condition (S/N ratio) became too poor to continue. Therefore, not all insects received the complete set of stimuli.

Data Analysis.

The obtained EAG signals were smoothed using a simple moving average method. Each data point was obtained by averaging original data between 50 ms and 50 ms (201 data points in total). Baseline EAG activity was defined as the average EAG amplitude for 500 ms before stimulus onset (Fig. 1C). The response amplitude was defined as the difference between the baseline and minimum EAG values during stimulation. As EAGs varied due to the contact condition between the electrode and the antenna during recording, each EAG response was normalized by the amplitude of the response to the temporarily closest $10^3 \mu\text{g}$ 1-hexanol. This concentration of 1-hexanol produced a large amplitude in preliminary experiments (Fig. 2). Relative amplitudes to $10^3 \mu\text{g}$ 1-hexanol were obtained for all stimuli for each individual. Responses to $10^3 \mu\text{g}$ 1-hexanol as a standard stimulus were never mixed with response to $10^3 \mu\text{g}$ 1-hexanol in the test series, i.e., a response to $10^3 \mu\text{g}$ 1-hexanol in test series was normalized by response to the ‘standard stimulus ($10^3 \mu\text{g}$ 1-hexanol)’ similar to other concentrations and stimuli. Each odor stimulus was applied once; however, in some individuals, odors were applied more than once. When multiple stimulations were performed, the responses to the odor were obtained by averaging all relative responses to that odor. Finally, the relative responses to each stimulus were averaged among individuals. All relative EAGs are shown as a mean ($\pm$ SEM). The responses were compared with the 95% confidence intervals (CI) of the relative responses to water for ethanol and mineral oil for other volatiles.

Statistics.

Stimulant-dose (from 10^{-3} to $10^3 \mu\text{g}$) effect was assessed by repeated measures ANOVA with post-hoc test (Tukey-Kramer test) for each sex. Since not all individuals received a complete set of concentration series, statistical analyses of the concentration effect were performed using only individuals who received a complete set of stimulations (Table 2). The densities of the undiluted volatiles differed from each other. Therefore, we treated the undiluted volatiles separately from the dilution series. For undiluted stimulants, EAGs were assessed using the Wilcoxon signed rank test against responses to mineral oil for volatiles or distilled water for ethanol. Statistical analyses were performed using R v.4.3.2 (R Core Team, 2019) for the concentration effect and MATLAB (Math Works, Natwick, MA, USA) for undiluted stimulants. All statistical results are shown in Supplementary File.

RESULTS

General Property of EAG Responses in P. quercivorus.

EAGs showed dose-dependent responses to odor stimuli (Fig. 3). The responses to $10^3 \mu\text{g}$ 1-hexanol that was used as a standard were similar in an absolute amplitude between males and females ($1.12 \pm 0.23 \text{ mV}$ and $1.31 \pm 0.20 \text{ mV}$ for males and females, respectively, $\text{mean} \pm \text{SEM}$). In our preparation, control stimuli showed small relative amplitudes of EAGs irrespectively of control types (male: 0.284 ± 0.02 , 0.252 ± 0.02 , and 0.2301 ± 0.02 for mineral oil, water, and blank, respectively; female: 0.277 ± 0.01 , 0.222 ± 0.02 , and 0.266 ± 0.02 for mineral oil, water, and blank, respectively). In all volatiles, relative amplitudes were not larger than 95% CI for control stimulants when the volatile concentration was $10^0 \mu\text{g}$ or lower. Two exceptions were observed in the female responses to 10^{-3} and $10^{-2} \mu\text{g}$ benzaldehyde. They were above 95% CI. These were likely the result of experimental errors due to a small response amplitude because it is hard to consider that the lowest concentration produces a significant response, although the higher concentration (for example, 10^0 and $10^1 \mu\text{g}$) failed to evoke responses.

In our experiment, 1-hexanol induced the largest relative response among tested volatiles in both males and females (for example, $10^3 \mu\text{g}$: 1.04 ± 0.04 in males and 0.99 ± 0.05 in females; undiluted: 1.12 ± 0.05 in males and 1.11 ± 0.04 in females). Although quercivorol, 2-ethyl-1-hexanol, and trans-2-hexenal induced relatively large responses, relative amplitudes were between 0.65 and 0.84 in males and 0.59 and 0.77 in females even when undiluted volatiles were applied. On the other hand, responses to decanal were very weak ($10^3 \mu\text{g}$: 0.29 ± 0.03 in males and 0.28 ± 0.03 in females; undiluted: 0.37 ± 0.04 in males and 0.30 ± 0.04 in females) and found no significant response in either sex (for males, $p = 0.405$; $df = 6$; $F = 1.054$, and for females, $p = 0.804$; $df = 6$; $F = 0.502$, repeated measures ANOVA for dose-dependency, and for males, $p = 0.055$; $Z = -1.960$, and for female, $p = 0.203$; $Z = -1.362$, Wilcoxon signed-rank test for undiluted decanal). Although responses to benzaldehyde were weak, undiluted and $10^3 \mu\text{g}$ benzaldehyde were notable; however, the relative amplitude (relative amplitude = $0.41\text{--}0.54$) was not as large as quercivorol, 2-ethyl-1-hexanol, or trans-2-hexenal. Similar benzaldehyde-response characteristics were observed for ethanol. However, responses to undiluted ethanol are significant (relative amplitude: 0.98 ± 0.12 in males and 1.14 ± 0.16 in females), similar to the responses to high concentrations of 1-hexanol.

Sexual Differences in the EAG-response Property.

Sexual differences were observed in responses to quercivorol, trans-2-hexenal, and isoprene. Male EAG responses to $10^1 \mu\text{g}$ quercivorol (relative amplitude = 0.40 ± 0.07) were larger than the upper 95% CI although the deviation is large compared to those for other concentrations of quercivorol.

On the other hand, the female response to $10^1 \mu\text{g}$ quericivorol (relative amplitude = 0.31 ± 0.03) fell within 95% CI of the control. These results suggest that male antennae are more sensitive to quericivorol than female antennae. The same trend is observed for trans-2-hexenal. The male response to $10^1 \mu\text{g}$ trans-2-hexenal (relative amplitude = 0.37 ± 0.04) was out of 95% CI but that of females was not (relative amplitude = 0.28 ± 0.04). Repeated measures ANOVA showed that the dose of isoprene was significantly effective in the male response amplitude ($p = 0.043$; $df = 6$; $F = 2.298$) but not in females ($p = 0.141$; $df = 6$; $F = 1.653$). The post-hoc test only found a significant response for a dose of $10^3 \mu\text{g}$ in males. However, EAGs showed no significant responses to undiluted isoprene from mineral oil in either sex ($p = 0.151$; $Z = -1.490$, and $p = 0.241$; $Z = 1.224$ for males and females, respectively, Wilcoxon signed-rank test).

DISCUSSION

We measured antennal responses to candidate compounds for host location using the EAG technique. Seven of the eight tested volatiles induced a dose-dependent EAG response. This dose dependency is consistent with that observed in other insects (e.g., Whitehead 1986; Gao et al. 2019; Zhang et al. 2000; Levi-Zada et al. 2020). In our study, the EAG showed a very weak response to isoprene in males, no response in females, and no response to decanal in males or females. The EAG technique is a useful approach for investigating antennal recognition of semiochemicals, such as kairomones and pheromones (Whitehead 1986; Balakrishnan et al. 2017; Nojima et al. 2003; Sevarika et al. 2022). However, there is a methodological limitation in EAG. For example, the failure to induce a clear EAG response does not directly imply that the animal does not sense this substance. Sensilla are not uniformly distributed throughout the antenna (Ali et al. 2016; Nishino et al. 2009; Nakanishi et al. 2009). Hence, it is possible that our recording electrode, covering the sensilla responsible for these ligands, prevented the antenna from responding. We also do not exclude the possibility of the spatially asymmetrical (dorsal and ventral) localization of the sensilla on the antenna, as in some other species of ambrosia beetle, *Typodendron lineatum* (Biswas et al. 2023). Although we tried to create a space for the contralateral side of the antenna when we pushed our electrode toward the head side for recording, we cannot exclude the possibility that stimulation might be weaker on this side than on the recording side, or that the responsible sensilla are localized dominantly on the head side. However, it is unlikely that the conditions of our preparation completely precluded responses. EAG recording can bring us a clue for understanding the host selection mechanism neurophysiologically and ecologically, but behavioral experiments are necessary for full understanding because EAG responses are not always linked to behavior. As well as a perceptual

ability of the beetle, we need further behavioral experiments to reveal whether each chemical is an attractant or a repellent.

Ethanol is often used as an attractant in insect traps (Byers 1992; Miller and Rabaglia 2009; Tokoro et al. 2007; Romero et al. 2022). In the present study, EAGs showed strong responses to undiluted ethanol (100% ethanol) and much weaker responses to 10^3 μ g ethanol (Fig. 3). Although the response amplitude to 10^3 μ g ethanol were significantly higher than that to distilled water, the sensitivity for ethanol appears very weak because other volatiles such as 2-ethyl-1-hexanol and trans-2-hexenal elicited clear response to even 10^2 μ g stimulants. 10^3 μ g ethanol is about 12.2% ethanol in water solution. EAG showed an obvious response to 50% or higher ethanol and 25% ethanol produced a response similar with 10^3 μ g ethanol (data not shown). Because 70% ethanol has been used in trap experiments for *P. quercivorus* (Tokoro et al. 2007), it is intriguing to clarify the lowest ethanol concentration for this beetle to respond and attract in the field.

Quercivorol (an aggregation pheromone) exhibited sexual differences in EAG response. Sexual differences in the responses to pheromones have been found in moth sex pheromones (De Cristofaro et al. 2004; Byers 2006) as well as some aggregation pheromones of bark beetles (Byers, 1983a,b, 1989). When 10^1 μ g quercivorol was applied, males showed a response higher than the 95% CI for the control, although the data deviation was large, whereas females did not, suggesting that males may be more sensitive than females. This may explain the ecological process of the mass attack, in which males, as pioneers, seek and locate a host tree and release the aggregation pheromone. In contrast, results of the gas chromatography-electroantennographic detector (GC-EAD) showed a possibility that female antennae have more sensitive sensillae to quercivorol than male antennae (Tokoro et al. 2007), suggesting that females are more sensitive to quercivorol than males. Because the odor exposure in the GC-EAD experiment is much longer than that in our experiment, their result might mean that male antennae might be habituated more rapidly than the female. If so, females would be able to select an optimal site without a decrease in a responsiveness to the pheromone during the prolonged exposure by males.

Sex differences were also observed in the response to isoprene. Repeated measures ANOVA showed that the male EAGs had a significant effect on the change in the dose of isoprene, although the EAG responses were very small in amplitude. While EAG amplitude for 10^3 μ g isoprene was out of 95% CI for mineral oil, no significant difference between responses to undiluted isoprene and those to mineral oil was found by Wilcoxon signed-rank test (Fig. 3). However, we cannot explain why undiluted isoprene failed to induce this response. This might be due to the large individual differences in responses to undiluted isoprene. Approximately 66.7% of the tested males showed

higher EAG amplitudes for undiluted isoprene than for mineral oils. On the other hand, except for decanal, to which the antenna did not respond, all individuals showed higher EAG for other undiluted stimulants (only one exceptional individual failed to respond to undiluted ethanol). As (1) statistical analysis indicated isoprene is effective and 10^3 μ g isoprene is out of 95% CI, (2) EAG responses to lower concentrations of isoprene fell within 95% CI of mineral oil, and (3) the response curve seems dose-dependent, we strongly believe that *P. quercivorus* antennae can detect isoprene electrophysiologically although the sensitivity might be low. Further field experiments such as trapping *P. quercivorus* would help reaching a conclusion on ecological effect of isoprene.

Similar to isoprene, decanal did not elicit any electrophysiological response (Fig. 3). It is possible that *P. quercivorus* can respond to decanal behaviorally but EAG failed to catch the responses due to the complex odor environments in nature (numerous volatiles from hosts and non-hosts) compared to the single chemical stimulation at a time of EAG recordings. Some studies have shown that the EAG amplitude for a mixture of odors is not always a simple summation of the amplitudes of each component odor (Riffell et al. 2009; Hieu et al. 2014). If so, isoprene/decanal might elicit clearer responses of higher-order neurons, for example, antennal lobe projection neurons when blended with biologically significant components such as quercivorol. Such odor environment-dependent responses may explain the unexpected responses of EAG and behavior in the field.

Although responses to isoprene were very weak, the present EAG study does not contradict our scenario regarding the host finding (location), selection, and mass-attack of *P. quercivorus* described in our introduction. We speculate that *P. quercivorus* may use leaf volatiles to select the host tree when approaching or landing on a tree. However, under natural conditions, it is unknown how leaf volatiles are spatially distributed along concentration gradients from a host tree during flight of ambrosia beetles seeking a host tree in the forest. Although we tested eight volatiles as a first step, GC-MS detected 18 volatiles from host or non-host seedlings (Pham et al., 2020) and 45 volatiles from fresh or dried leaves of host or non-host (Pham et al., 2025). In addition, volatility differs among the natural volatiles. Therefore, different amounts of molecules hit the antenna, even if the concentration of the tested volatiles was accurately controlled. This means that the strength of stimulation differs even when the same concentration of stimulants is applied (Park et al. 2001). This may have led to a difference in the response strength depending on the volatiles. Taken together, measuring the volatility of chemicals and estimating the concentrations of candidate volatiles around host trees in forests will facilitate an understanding of the host selection mechanism of *P. quercivorus*. By combining the antennal physiological property such as our study with comprehensive volatiles

and detailed behavioral data obtained from well-designed experiments executed in the field as well as in the laboratory, kairomones will be specified and their roles on the host location will be revealed.

REFERENCES

Ali SAI, Diakite MM, Ali S, Wang MQ (2016) Effects of the antennal distribution pattern on the behavioral responses to *Tribolium castaneum* (Coleoptera: Tenebrionidae). Fla Entomol 99: 52-59.

Ammagarahalli B, Gemeno C (2015) Interference of plant volatiles on pheromone receptor neurons of male *Grapholita molesta* (Lepidoptera: Tortricidae) J Insect Physiol 81: 118-128.

Balakrishnan K, Hokighaus G, Weißbecker B, Schütz S. (2017) Electroantennographic responses of red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) to volatile organic compounds. J Appl Entomol 141: 477-486.

Biswas T, Yuvaraj JK, Hansson BS, Löfstedt C, Anderbrant O, Andersson MN (2023) Characterization of olfactory sensory neurons in the striped ambrosia beetle *Typodendron lineatum*. Front Physiol 14: 1155129. doi: 10.3389/fphys.2023.1155129.

Blažytė-Čereškienė L, Apšegaitė V, Radžiūtė S, Mozūraitis R, Būda V, Pečiulytė D (2016) Electrophysiological and behavioural responses of *Ips typographus* (L.) to trans-4-thujanol — a host tree volatile compound. Annal For Sci 73: 247-256.

Byers JA (1983a) Sex-specific responses to aggregation pheromone: Regulation of colonization density in the bark beetle *Ips paraconfusus*. J Chem Ecol 9: 129-142.

Byers JA (1983b) Bark beetle conversion of a plant compound to a sex-specific inhibitor of pheromone attraction. Science 220: 624-626.

Byers JA (1989) Chemical ecology of bark beetles. Experientia 45: 271-283.

Byers JA (1992) Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes. J Chem Ecol 18: 2385-2402.

420

421 Byers JA (2006) Pheromone component patterns of moth evolution revealed by computer analysis
 422 of the Pherolist. *J Anim Ecol* 75: 399-407.

423

424 Clyne P, Grant A, O'Connell R, Carlson J (1997) Odorant response of individual sensilla on the
 425 *Drosophila* antenna. *Invet Neurosci* 3: 127-135.

426

427 De Cristofaro A, Ioriatti C, Pasqualini E, Anfora G, Germinara GS, Villa M, Rotundo G (2004)
 428 Electrophysiological responses of *Cydia pomonella* to codlemone and pear ester ethyl (*E,Z*)2,4-
 429 decadienoate: Peripheral interactions in their perception and evidences for cells responding to both
 430 compounds. *Bullet Insectol* 57: 137-144.

431

432 Fürstenau B, Rosell G, Guerrero A, Quero C (2012) Electrophysiological and behavioral responses
 433 of the black-banded oak borer, *Coroebus florentinus*, to conspecific and host-plant volatiles. *J Chem*
 434 *Ecol* 38: 378-388.

435

436 Gao G, Dai L, Gao J, Wang J, Chen H (2019) Electroantennogram, behavioural responses, and field
 437 trapping of *Trypophloeus klimeschi* (Coleoptera: Curculionidae: Scolytinae) to eight host volatiles.
 438 *Can Entomol* 151: 236-250.

439

440 Germinara GS, Ganassi S, Pistillo MO, Di Domenico C, De Cristofaro A, Di Palma AM (2017)
 441 Antennal olfactory responses of adult meadow spittlebug, *Philaenus spumarius*, to volatile organic
 442 compounds (VOCs). *PLoS ONE* 12: e0190454.

443

444 Hansson BS, Stensmyr MC (2011) Evolution of insect olfaction. *Neuron* 72: 698-711.

445

446 Hieu TT, Jung JW, Kim SI, Ahn YJ, Kwon HW (2014) Behavioural and electroantennogram
 447 responses of the stable fly (*Stomoxys calcitrans* L.) to plant essential oils and their mixtures with
 448 attractants. *Pest Manag Sci* 70: 163-172.

449

450 Iidzuka H, Goto H, Yamasaki M, Osawa N (2016) Wood-boring beetles (Coleoptera: Scolytidae,
 451 Platypodidae) captured in ethanol-baited traps in a natural forest in Japan. *Appl Entomol Zool* 51:
 452 347-352.

453

454 Kameoka H, Tsujino H, Aso Y (1983) Constituents of essential oil of leaves of *Quercus dentata*
 455 Thunb. (In Japanese with English summary). Nippon Nôgeikagaku Kaishi 57 135-138.

456

457 Kashiwagi T, Nakashima T, Tebayashi S, Kim CS (2006) Determination of the absolute
 458 configuration of quercivorol, (1*S*,4*R*)-*p*-menth-2-en-1-ol, an aggregation pheromone of the
 459 ambrosia beetle *Platypus quercivorus* (Coleoptera: Platypodidae). Biosci Biotechnol Biochem 70:
 460 2544-2546.

461

462 Kelsey RG, Joseph G (2003) Ethanol in ponderosa pine as an indicator of physiological injury from
 463 fire and its relationship to secondary beetles. Can J For Res 33: 870-884.

464

465 Kendra PE, Montgomery WS, Niogret J, Deyrup MA, Guillén L, Epsky ND (2011) *Xyleborus*
 466 *glabratus*, *X. affinis*, and *X. ferrugineus* (Coleoptera: Curculionidae: Scolytinae):
 467 Electroantennogram responses to host-based attractants and temporal patterns in host-seeking flight.
 468 Environ Entomol 41: 1597-1605.

469

470 Kobayashi M, Ueda A. (2005) Wilt disease of Fagaceae trees caused by *Platypus quercivorus*
 471 (Murayama) (Coleoptera: Platypodidae) and the associated fungus: Aim is to clarify the damage
 472 factors. J Jpn For Soc. 87,435–450 (in Japanese with English summary).

473

474 Krishnan B, Dryer SE, Hardin PE (1999) Circadian rhythms in olfactory responses of *Drosophila*
 475 *melanogaster*. Nature 400: 375-378.

476

477 Kubono T, Ito S. (2002) *Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese
 478 oak, and the ambrosia beetle (*Platypus quercivorus*). Mycoscience 43: 255–260.

479

480 Levi-Zada A, Levy A, Rempoulakis P, Fefer D, Steiner S, Gazit Y, Nestel D, Yuval B, Byers JA
 481 (2020) Diel rhythm of volatile emissions of males and females of the peach fruit fly *Bactrocera*
 482 *zonata*. J Insect Physiol 120:103970.

483

484 Liu YQ, Li J, Ban LP (2021) Morphology and distribution of antennal sensilla in three species of
 485 Thripidae (thysanoptera) infesting alfalfa *Medicago sativa*. Insects 21, 81.

486

487 Martin JP, Beyerlein A, Dacks AM, Reisenman CE, Riffell JA, Lei H, Hildebrand JG (2011) The
 488 neurobiology of insect olfaction: sensory processing in a comparative context. *Prog Neurobiol* 95:
 489 427-447.

490

491 Matsunaga S, Nakatsuka S, Chatani S, Mochizuki T, Tani A, Muller O (2012) Emission of high
 492 molecular weight biogenic volatile organic compounds and their inventory in Japan (in Japanese). *J*
 493 *Jpn Soc Atmos Environ* 47: A6-A8.

494

495 Miller DR, Rabaglia RJ (2009) Ethanol and (-)- α -pinene: attractant kairomones for bark and
 496 ambrosia beetles in the southeastern US. *J Chem Ecol* 35: 435-448.

497

498 Nakanishi A, Nishino H, Watanabe H, Yokohari F, Nishikawa M (2009) Sex-specific antennal
 499 sensory system in the ant *Camponotus japonicas*: structure and distribution of sensilla on the
 500 flagellum. *Cell Tissue Res* 338: 79-97.

501

502 Nojima S, Linn Jr. C, Morris B, Zhang A, Roelofs W (2003) Identification of host fruit volatiles
 503 from hawthorn (*Crataegus* spp.) attractive to hawthorn-origin *Rhagoletis pomonella* flies. *J Chem*
 504 *Ecol* 29: 321-336.

505

506 Nishino H, Yamashita S, Yamazaki Y, Nishikawa M, Yokohari F, Mizunami M (2003) Projection
 507 neurons originating from thermos- and hygro-sensory glomeruli in the antennal lobe of the cockroach.
 508 *J Comp Neurol* 455: 40-55.

509

510 Nishino H, Nishikawa M, Mizunami M, Yokohari F (2009) Functional and topographic segregation
 511 of glomeruli revealed by local staining of antennal sensory neurons in the honeybee *Apis mellifera*.
 512 *J Comp Neurol* 515:161-180.

513

514 Okada R, Sakura M, Mizunami M (2003) Distribution of dendrites of descending neurons and its
 515 implications for the basic organization of the cockroach brain. *J Comp Neurol* 458: 158-174.

516

- Park KC, Zhu J, Harris J, Ochieng SA, Baker TC (2001) Electroantennogram responses of a parasitic wasp, *Microplitis croceipes*, to host-related volatile and anthropogenic compounds. *Physiol Entomol* 26: 69-77.
- Pham DL, Ito Y, Okada R, Ikeno H, Isagi Y, Yamasaki M. (2019) Effects of leaf conditions and flight activity on the behavior of *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). *J Appl Entomol* 143:1000-1010. <https://doi.org/10.1111/jen.12671>.
- Pham DL, Ito Y, Okada R, Ikeno H, Kazama H, Mori N, Yamasaki M. (2020) Ambrosia beetles *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) use leaf volatiles in host selection. *Entomol Exp Appl* 168:928-939 doi: 10.1111/eea.12993.
- Pham DL, Ito Y, Yamasaki M (2025) Response of the oak ambrosia beetle *Platypus quercivorus* (Coleoptera: Platypodinae) to volatiles from fresh and dried leaves. *Arthropod-Plant Interact*, 19: 5.
- R Core Team (2019) A Language and Environment for Statistical Computing. R Foundation for statistical computing, Vienna, Austria.
- Ranger CM, Reding ME, Schultz PB, Oliver JB (2013) Influence of flood-stress on ambrosia beetle host-selection and implications for their management in a changing climate. *Agric For Entomol* 15: 56-64.
- Riffell JA, Lei H, Christensen TA, Hildebrand JG (2009) Characterization and coding of behaviorally significant odor mixtures. *Curr Biol* 19: 335-340.
- Romero P, Ibarra-Juárez LA, Carrillo D, Guerrero-Analco JA, Kendra PE, Kiel-Martínez AL, Guillén L. (2022) Electroantennographic responses of wild and laboratory-reared females of *Xyleborus affinis* eichhoff and *Xyleborus ferrugineus* (Fabricius) (Coleoptera: Curculionidae: Scolytinae) to ethanol and bark volatiles of three host-plant species. *Insects* 13: 655.
- Sevarika M, Rondoni G, Ganassi S, Marco Pisillo O, Salvatore Germinara G, De Cristofaro A, Romani R, Conti E (2022) Behavioural and electrophysiological responses of *Philaenus spumarius* to odours from conspecifics. *Sci Rep* 12: 8402.

550

551 Stensmyr MC, Giordano E, Balloi A, Angioy AM, Hansson BS (2003) Novel natural ligands for
 552 *Drosophila* olfactory receptor neurones. J Exp Biol 206: 715-724.

553

554 Tani A, Kawawata Y (2008) Isoprene emission from the major native *Quercus* spp. in Japan. Atmos
 555 Environ 42: 4540-4550.

556

557 Tateishi K, Noshimura Y, Sakuma M, Yokohari F, Wawtanabe H (2020) Sensory neurons that
 558 respond to sex and aggregation pheromones in the nymphal cockroach. Sci Rep 10: 1995.

559

560 Tokoro M, Kobayashi M, Saito S, Kinuura H, Nakashima T, Shoda-Kagaya E, Kashiwagi T,
 561 Tebayashi S, Kim CS, Mori K (2007) Novel aggregation pheromone, (1*S*, 4*R*)-p-menth-2-en-1-ol, of
 562 the ambrosia beetle, *Platypus quercivorus* (Coleoptera: Platypodidae). Bull For For Prod Res Inst 6:
 563 49-57.

564

565 Tokoro M, Okada M, Saito S, Ohashi A, Kinuura H, Ino M, Yoshihama T (2014) Study of attractant
 566 to *Platypus quercivorus* (in Japanese). Forest Pests 63: 225-231.

567

568 Ueda A, Kobayashi M (2001) Aggregation of *Platypus quercivorus* (Murayama) (Coleoptera:
 569 Platypodidae) on oak logs bored by males of the species. J For Res 6: 173-179.

570

571 Wang W, He P, Liu T, Jing X, Zhang S (2023) Morphology and distribution of antennal sensilla on
 572 *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae and adults. Diversity 15: 992.

573

574 Watanabe H, Koike Y, Tateishi K, Domae M, Nishino H, Yokohari F (2018) Two types of sensory
 575 proliferation patterns underlie the formation of spatially tuned olfactory receptive fields in the
 576 cockroach *Periplaneta americana*. J Comp Neurol. 526: 2683-2705.

577

578 Whitehead AT (1986) Electroantennogram responses by mountain pine beetles, *Dendroctonus*
 579 *ponderosae* Hopkins, exposed to selected semiochemicals. J Chem Ecol 12: 1603-1621.

580

581 Yamasaki M, Ito Y, Ando M (2014) The effect of stem density on the probability of attack by the
 582 ambrosia beetle *Phatypus quercivorus* varies with spatial scale. Agric For Entomol 16: 54-62.

583
584 Yamasaki M, Tatsumi K, Ito Y (2023) Capture of the oak ambrosia beetle within the host tree canopy.
585 Can Entomol 155: E28.
586
587 Yamasaki M, Tatsumi K, Kobayashi T, Ito Y (2024) Wrong landing on a non-host occurs under the
588 canopy of a host: the presumed path of the ambrosia beetle *Platypus quercivorus* to approach its
589 host. Agric For Entomol 26: 135-147.
590
591 Zhang QH, Schlyter F, Birgersson G (2000) Bark volatiles from nonhost angiosperm trees of spruce
592 bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): chemical and electrophysiological
593 analysis. Chemoecology 10: 69-80.
594
595
596

Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceptualization: Ryuichi Okada, Yasuto Ito, Michimasa Yamasaki; Data curation: Ryuichi Okada; Formal Analysis: Ryuichi Okada; Investigation: Ryuichi Okada; Methodology: Ryuichi Okada; Funding acquisition: Yasuto Ito, Michimasa Yamasaki; Project administration: Ryuichi Okada, Yasuto Ito, Michimasa Yamasaki; Resources: Ryuichi Okada; Software: Ryuichi Okada; Resources : Yasuto Ito, Michimasa Yamasaki; Supervision: Ryuichi Okada, Yasuto Ito, Michimasa Yamasaki; Validation: Ryuichi Okada; Visualization: Ryuichi Okada; Writing-original draft: Ryuichi Okada, Yasuto Ito, Michimasa Yamasaki; Writing-review & editing: Ryuichi Okada, Yasuto Ito, Michimasa Yamasaki.

Data availability statements

Data will be made available on request.

FIGURE CAPTIONS

Fig. 1 Experimental set-up and analyses. A: The experimental set-up. Air was provided from the air pump via three bottles containing one of cotton, water, and active charcoal, in order (not shown for space). The air containing a volatile was applied by switching the solenoid valve (SV). Each insect was placed in front of the air tube exhaust. The recording electrode (rec. ele.) was put on the right antenna and the electric signal (EAG) was stored in a computer. B: The stimulation procedure. Volatiles were released in an ascending manner of concentration. Each stimulus was applied with the interval of at least 30 s. To confirm the response, volatiles were sometimes applied twice or more. In most cases, 1-hexanol was applied as a ‘standard’, and controls (mineral oil, blank, and water) were applied between ascending series of volatiles (Ctrl in the figure). C: Response amplitude and analysis. Actual EAG as shown by gray color and corresponding smoothed EAG is shown by black color superimposed on the actual EAG. After smoothing, the baseline was calculated by averaging EAGs for 500 ms before the onset of stimulus. Amplitude was obtained by subtracting the baseline from the minimum EAG, and then multiplying by -1. Abbreviations, CRO: cathode ray oscilloscope, HC: high cut filter, HDD: PC hard disk, LC: low cut filter, NE: noise eliminator, Stim: stimulator, U: undiluted

Fig. 2 An example of an EAG recording. Actual response to mineral oil (control) and 1-hexanol are shown. Black lines indicate stimulus timings. The numbers above the stimulus indicator represent dose (μg) of 1-hexanol. An arrow indicates an artifact depolarization. U: undiluted

Fig. 3 Relative EAGs. Black (male) and white (female) dots and bars indicate mean relative EAG responses. Error bars indicate SEMs. White horizontal lines indicate the mean relative amplitude of the response to control stimulus (mineral oil for volatiles or water for ethanol). Gray areas show 95% CIs for the control stimulus. Sample numbers for each stimulus have some degree of range because not all individual received the complete set of ascending series. Asterisks beside the sample number indicate significant effect for the dose of volatiles by repeated measures ANOVA. Different letters above the dots denote significant difference after the post-hoc test (Tukey-Kramer’s test). Asterisks above bars indicate significant difference from the control by Wilcoxon signed-rank test. U: undiluted. Black dot: male; open circle: female. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$

651 **Table 1.** Summary of four chemicals tested. Data are obtained from Pham et al., 2020 and 2025.

	seedling		fresh leaf		dried leaf	
	host	non-host	host	non-host	host	non-host
behavior	attracted	avoid	attracted	attracted	avoid	avoid
2-ethyl-1-hexanol	+++	+++	-	-	-	-
trans-2-hexenal	-	-	-	-	++	++
decanal	+	+	+	+	+	+
benzaldehyde	-	-	-	-	+	+

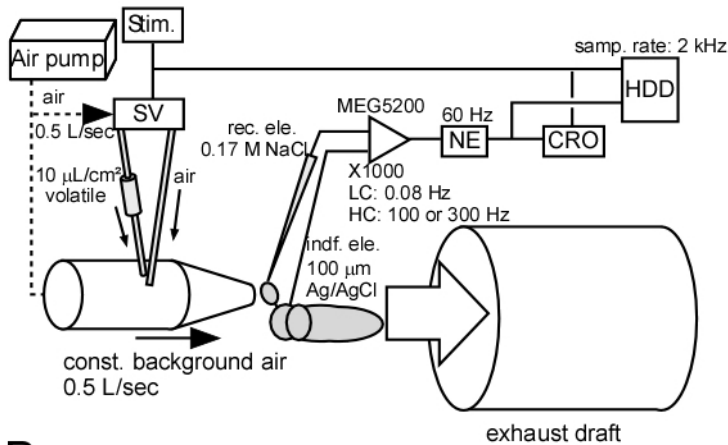
652 +++: rich, ++: moderate, +: poor, -: not detected or very low

653
654
655 **Table 2.** Number of individuals stimulated by a complete set of all concentration series and by
656 undiluted stimulants

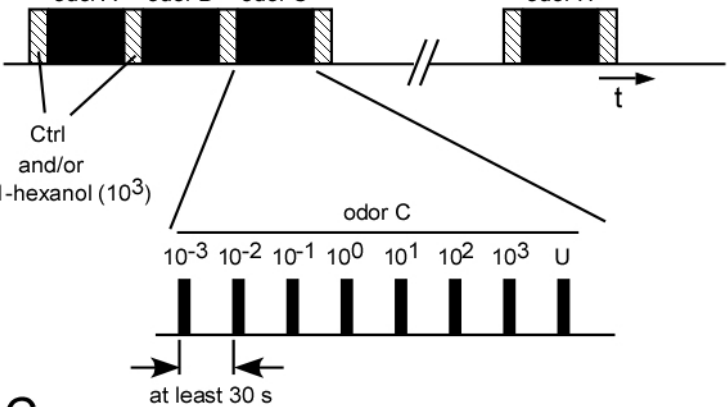
	ascending series		undiluted stimulant	
	male	female	male	female
1-hexanol	14	14	12	13
2-ethyl-1-hexanol	8	11	8	11
benzaldehyde	8	10	8	10
decanal	8	9	8	9
ethanol	8	9	8	9
isoprene	14	17	12	14
quercivorol	13	15	11	14
trans-2-hexenal	8	10	8	10

660 Fig.1

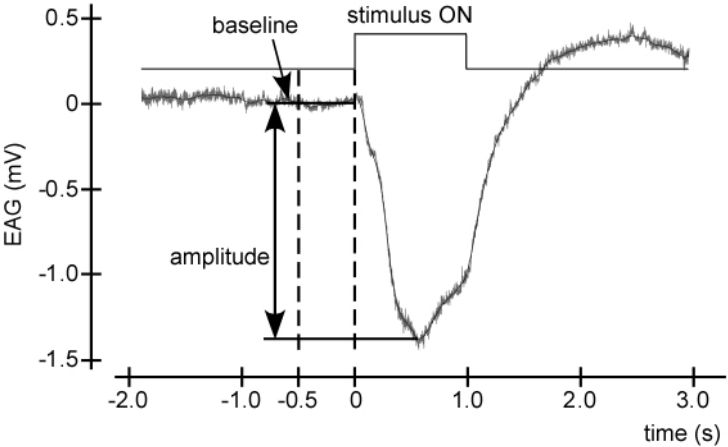
A



B



C



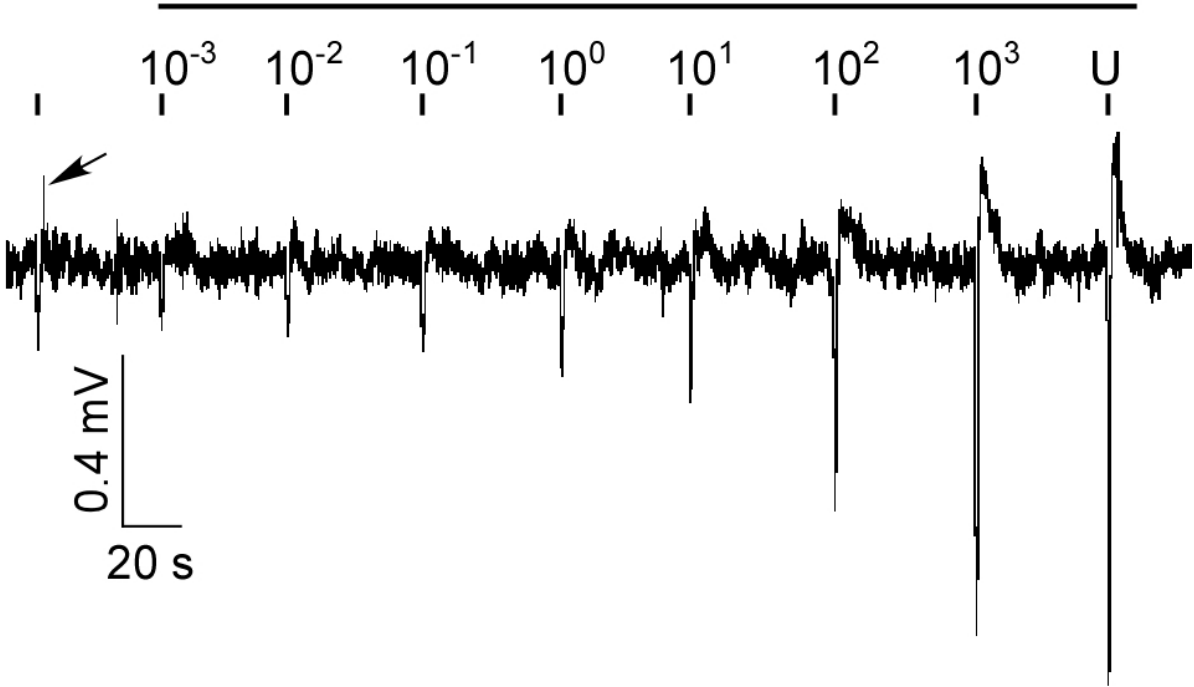
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662

663 Fig. 2

mineral oil

1-hexanol



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