

# Effects of the essential oil constituent thymol and other neuroactive chemicals on flight motor activity and wing beat frequency in the blowfly *Phaenicia sericata*

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## Abstract

**BACKGROUND:** The effects were evaluated of the plant terpenoid thymol and eight other neuroactive compounds on flight muscle impulses (FMIs) and wing beat frequency (WBF) of tethered blowflies (*Phaenicia sericata* Meig.).

**RESULTS:** The electrical activity of the dorsolongitudinal flight muscles was closely linked to the WBF of control insects. Topically applied thymol inhibited WBF within 15–30 min and reduced FMI frequency. Octopamine and chlordimeform caused a similar, early-onset bursting pattern that decreased in amplitude with time. Desmethylchlordimeform blocked wing beating within 60 min and generated a profile of continuous but lower-frequency FMIs. Fipronil suppressed wing beating and induced a pattern of continuous, variable-frequency spiking that diminished gradually over 6 h. Cypermethrin- and rotenone-treated flies had initial strong FMIs that declined with time. In flies injected with GABA, the FMIs were generally unidirectional and frequency was reduced, as was seen with thymol.

**CONCLUSIONS:** Thymol readily penetrates the cuticle and interferes with flight muscle and central nervous function in the blowfly. The similarity of the action of thymol and GABA suggests that this terpenoid acts centrally in blowflies by mimicking or facilitating GABA action.

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**Keywords:** flight motor activity; wing beat; thymol; GABA

## 1 INTRODUCTION

In the search for new chemical agents to control pest populations, natural products such as plant extractives and essential oils hold substantial promise.<sup>1–3</sup> Thymol (Fig. 1) is a monoterpenoid found in *Thymus vulgaris* L. (Lamiaceae) and exhibits antibacterial,<sup>4,5</sup> antioxidant,<sup>6</sup> molluscicidal,<sup>7</sup> antifeedant<sup>8</sup> and insecticidal activity.<sup>1,8–10</sup> In wireworms, thymol causes initial hyperactivity followed by abdominal segment hyperextension, extended paralysis and then death.<sup>11</sup>

Priestley *et al.*<sup>12</sup> compared the GABA-modulating and GABA-mimetic activities of thymol on human GABA<sub>A</sub> and fruitfly (*Drosophila melanogaster* Meig.) homomeric RDL<sub>ac</sub> GABA receptors expressed in *Xenopus* oocytes. Thymol enhanced the GABA-dependent chloride currents in oocytes expressing various human GABA<sub>A</sub> receptor isoforms as well as the insect GABA receptor. Consistent with its action on mammalian GABA<sub>A</sub> receptors, thymol also potentiated the binding of [<sup>3</sup>H] *tert*-butyl bicycloorthobenzoate at this complex.<sup>13</sup> Likewise, ivermectin increases chloride ion permeability in invertebrate muscle and nervous tissue through positive modulation of GABA-gated and glutamate-gated chloride channels,<sup>14</sup> and paralytic effects have been reported in dipterans.<sup>15,16</sup> In contrast, fipronil blocks GABA-activated chloride influx<sup>17,18</sup> and glutamate-activated chloride currents in insect neurons,<sup>19</sup> causing

spontaneous electrical activity to increase in the central nervous system.<sup>20</sup>

Present understanding of the mode of action of terpenoids is incomplete,<sup>21</sup> but they may interfere with the octopaminergic system of insects.<sup>21,22</sup> In insects, octopamine functions as a neuromodulator, a neurotransmitter and a neurohormone.<sup>23–26</sup> Octopamine regulates and desensitizes sensory inputs, excites nerves and maintains rhythmic and more complex processes such as learning and memory. The octopamine receptor agonist chlordimeform (CDM) was used widely for control of insects and mites,<sup>27</sup> but has been withdrawn owing to potential carcinogenicity.<sup>28</sup> In arthropods, CDM causes loss of appetite and cessation of feeding,<sup>29–32</sup> hyperactivity and incoordination<sup>33</sup> and detachment and mortality of parasitic acarines.<sup>34</sup> When applied topically, CDM and its active metabolite *N*-desmethylchlordimeform (DMCDM) activate light output from the lantern of the firefly *Photinus pyralis* L.,<sup>35</sup> thus mimicking the

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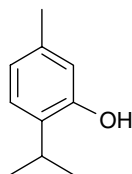


Figure 1. The structure of thymol.

endogenous agonist octopamine in this insect.<sup>36,37</sup> DMCDM also competes with mianserin (an octopamine antagonist) for binding sites in the cockroach nerve.<sup>38</sup> In contrast to CDM, rotenone causes paralysis in insects<sup>39–41</sup> and only weakly affects nerve conduction in insects.<sup>42</sup>

The dipteran flight motor system has provided considerable insight into the physiological actions of pyrethroids such as tetramethrin that elicit repetitive discharge activity and uncoupling of the flight motor pattern.<sup>43,44</sup> The present objective was to characterize the effects of thymol on flight muscle impulse (FMI) responses and wing beat frequency (WBF) in the blowfly and, by comparing it with neuroactive substances with known modes of action, gain insight into the mechanisms by which thymol may cause toxicity in insects. In this investigation, the effects of thymol were compared with those of octopamine, CDM, DMCDM, rotenone, cypermethrin, fipronil, ivermectin and GABA.

## 2 MATERIALS AND METHODS

### 2.1 Insects

A colony of blowflies (*Phaenicia sericata* Meig.) has been maintained at Simon Fraser University for over 20 years at 25 °C, 80% relative humidity and a 12:12 h light:dark cycle. Pupae were collected and placed in a separate cage and provided with sugar cubes and water *ad libitum*. Three- to five-day-old adult female blowflies were selected for electrophysiological studies. The food source was removed from experimental insects 4 h before experiments and replaced 3.5 h later to ensure that all insects had fed to repletion just before the experiments began.

### 2.2 Chemicals

Thymol, octopamine hydrochloride (OA), GABA, rotenone and dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich (St Louis, MO). Technical-grade chlordimeform (CDM), desmethylchlordimeform (DMCDM), ivermectin (IVM), cypermethrin and fipronil were from bona fide industrial sources. Cypermethrin [as a mixture of isomers (*RS*)- $\alpha$ -cyano-3-phenoxybenzyl (1*RS*)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate], rotenone, ivermectin, CDM, DMCDM and thymol were dissolved and administered in DMSO. OA and GABA were dissolved and administered in distilled water. Control insects were treated with DMSO or water.

### 2.3 Dosing and data recording

Blowflies were briefly anesthetized using carbon dioxide. Copper recording electrodes (50  $\mu$ m) were inserted into the left and right dorsolongitudinal muscles (DLM5) from the anterior of the pterothorax, and the common reference ground electrode was inserted into the left and dorsolongitudinal muscle 4 using micromanipulators. Muscle insertions were located by referencing

their locations to prominent dorsal setae.<sup>43</sup> Once positioned correctly, the electrodes themselves were sufficient to hold the blowflies and allow flight to occur. Electrical activity from the flight muscles was fed into a differential preamplifier with an input impedance of 100 M $\Omega$ , an amplification factor of 100 and a frequency response of 10 kHz. Output was recorded with a NI USB-6008 data logger (National Instruments, Vaudreuil-Dorion, Quebec, Canada). At the same time, acoustic signals (WBF) from wing beats were recorded with a miniature microphone (Realistic 33–1052) connected to an amplifier speaker (Archer Model 277–1008 B, Taiwan), and the signal was recorded by the same data logger. Ten minutes after recovering from carbon dioxide anesthesia, 50  $\mu$ g of thymol, CDM, DMCDM, rotenone, cypermethrin, IVM or fipronil were topically applied to the tip of the abdomen, or 50  $\mu$ g of octopamine or GABA were injected into the abdomen using a microsyringe. Relatively high doses of the neuroactive standards were chosen to bring on the various compound-related effects quickly. The LD<sub>50</sub> (24 h) of thymol was determined previously to be 63  $\mu$ g fly<sup>-1</sup> (95% confidence limits 49.2–80.5) (unpublished data). Control insects were topically treated or injected with 5  $\mu$ L of DMSO or water. Recordings began immediately.

Firing rates (impulse s<sup>-1</sup>) were calculated by counting the number of action potentials that occurred after the application of the test compound. The mean responses and their standard errors were determined, and amplitudes were calculated with their standard errors using LABVIEW 8.5 (National Instruments, Quebec, Canada). Each compound was tested on a minimum of three blowflies with very similar results, and representative traces are displayed in the figures. The tethering apparatus did not restrict flight, and control flies could be induced to fly at any time throughout the experiment by touching the wings or tarsi. As described in the results, many treated flies lost the ability to fly.

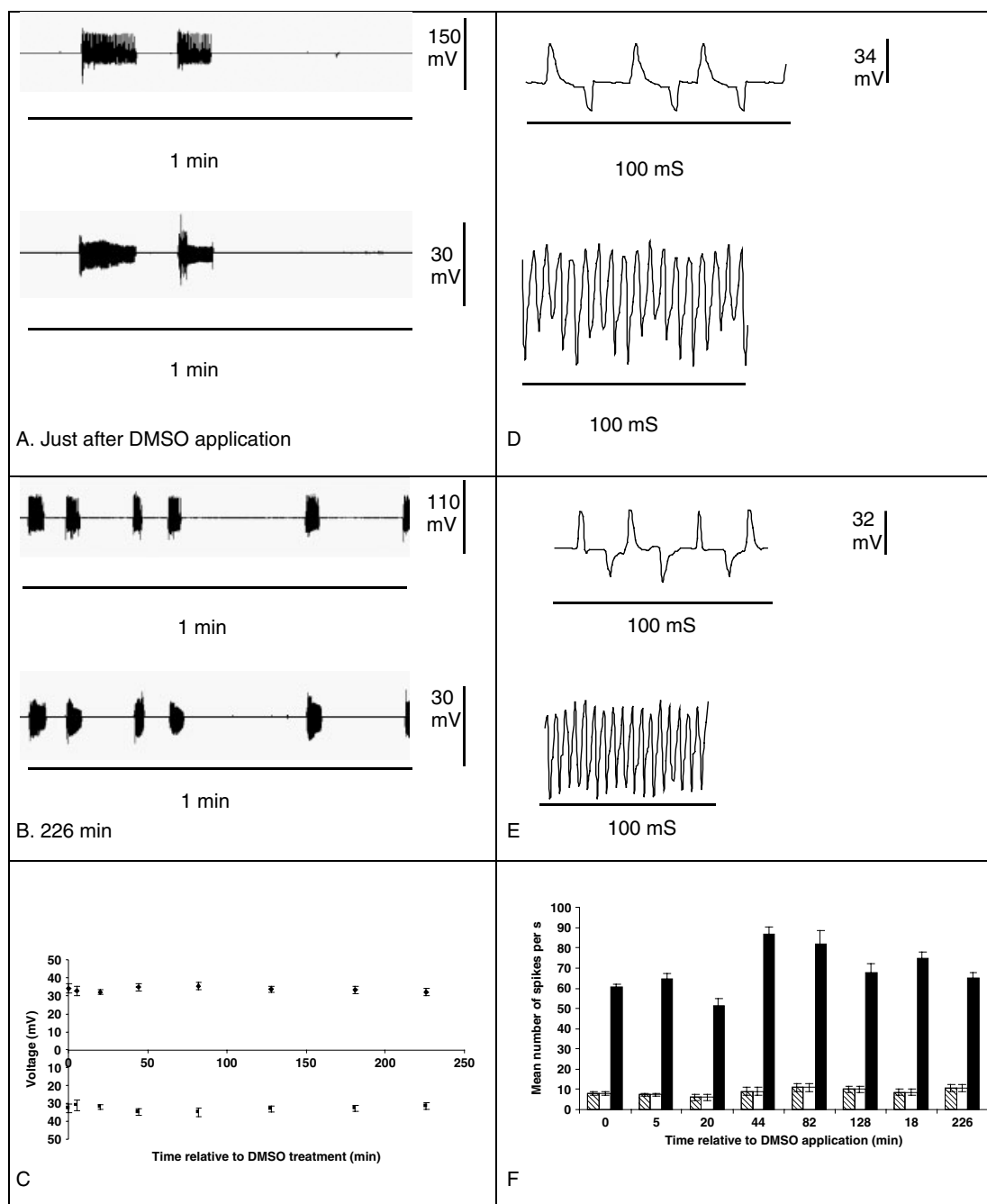
## 3 RESULTS

### 3.1 Baseline wing beat and dorsolongitudinal muscle electrical activity

The control FMIs from dorsolongitudinal muscles were typically bidirectional with asynchronous spikes presenting as positive and negative amplitude deflections (Fig. 2). This normal pattern of activity is consistent with other reports.<sup>44</sup> During flight, wing beating was always coupled closely to FMIs, with the FMI:WBF ratio averaging 1:9  $\pm$  1 during the 4 h recording period. Under the experimental conditions, control blowflies (water or DMSO treated) flew for extended periods, but not continuously.

### 3.2 Effects of thymol

The effects of topically applied thymol (50  $\mu$ g) on FMIs and the resulting WBF are shown in Fig. 3. The initial (first 15 min) FMIs of dorsolongitudinal muscles were similar to the control signals (Fig. 3A), after which a transient train of lower-frequency FMIs appeared (Fig. 3F). By 30 min, wings were motionless, but sporadic bursts of FMIs with mostly unidirectional spikes were observed over the next 3 h (Figs 3B and F). At 4 h, FMIs recovered to a more normal pattern (Figs 3C and F), but wings remained motionless (Fig. 3F). Amplitude changes are shown in Fig. 3C. The FMI:WBF ratio was 1:8  $\pm$  1.5. Treated flies groomed extensively after application of thymol, and slight tremors (spontaneous oscillation



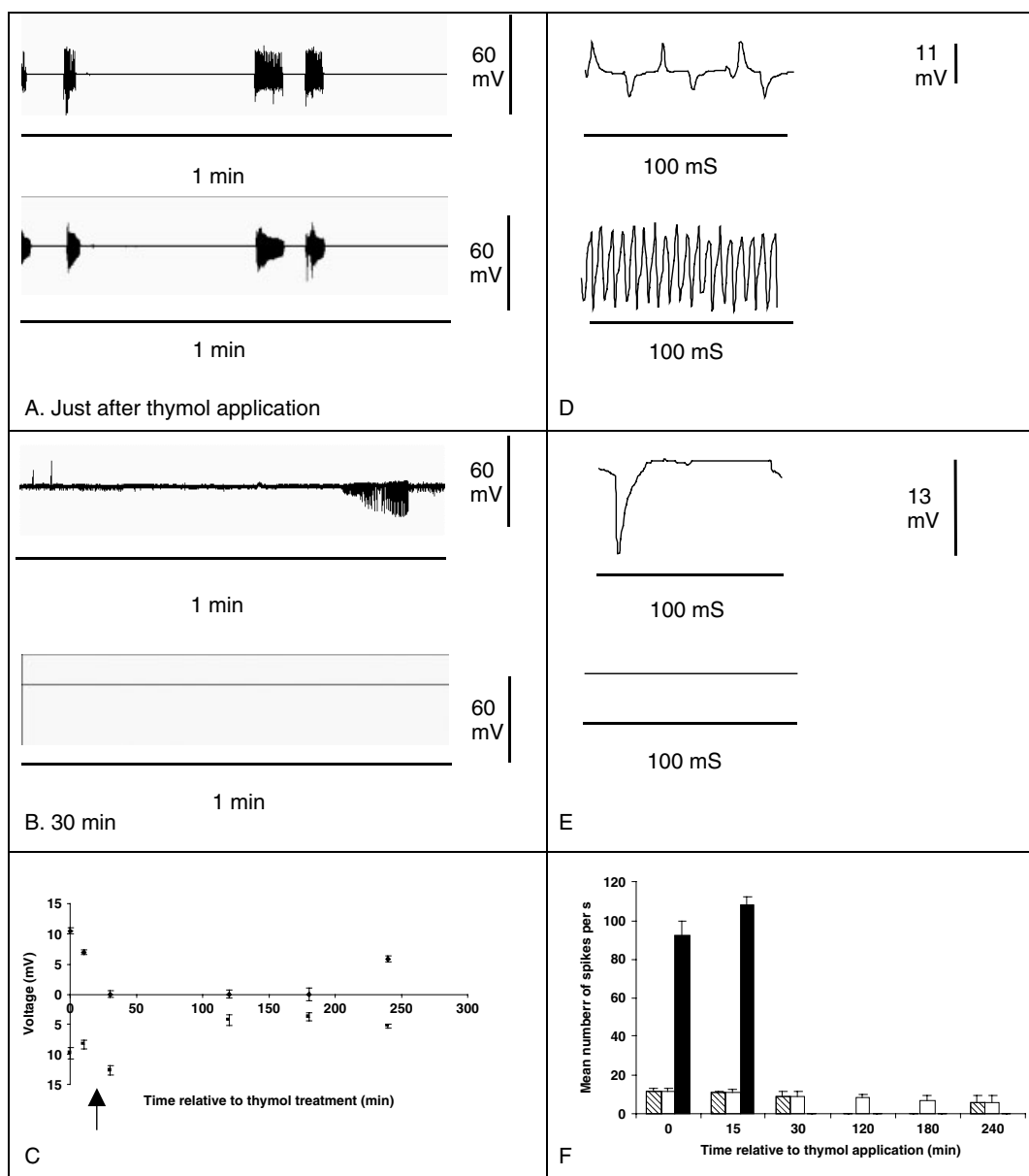
**Figure 2.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of control (DMSO-treated) blowflies. A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 226 min post-application respectively. C: changes in amplitude of the right (◆) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of DMSO on FMIs and WBF frequency over the 226 min recording period. Hatched bar: mean ( $\pm$  SE) number of spikes per second of the right DLM; white bar: mean ( $\pm$  SE) number of spikes per second of the left DLM; black bar represents WBF frequency.

of appendages or other body parts) began around 20 min. None of the treated flies died within 4 h.

### 3.3 Effects of octopamine

The effects of 50  $\mu$ g octopamine (OA) on FMIs and accompanying WBF after injection are shown in Fig. 4. An abnormal pattern of activity started approximately 10 min after treatment, and bursts of FMIs became shorter and higher in frequency over the next 3 h (Figs 4B and F). WBF remained robustly linked to FMIs until

186 min (Fig. 4F), when FMI amplitude was reduced dramatically and the wing beating stopped after 260 min (Figs 4C and F). The amplitude of the FMIs typically increased fourfold at 10 min of OA application and then declined to almost zero (Fig. 4C). Similarly, the mean number of impulses per second increased at 10 min, and this frequency was generally maintained until the end of the experiment (Fig. 4F). Following OA application, the FMI : WBF ratio was  $1 : 7 \pm 2$ . Blowflies treated with OA groomed their abdomen and wings at the beginning of the experiment, but extended



**Figure 3.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies treated topically with thymol (50 µg). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 30 min post-application respectively. C: changes in amplitude of the right (◆) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of thymol on FMIs and WBF frequency over the 240 min recording period. Hatched bar: mean (± SE) number of spikes per second of the right DLM; white bar: mean (± SE) number of spikes per second of the left DLM; black bar represents WBF frequency. In panel C the arrow indicates the time point at which tremors were first observed.

their probosces during the later stages. No flies died during the experimental period.

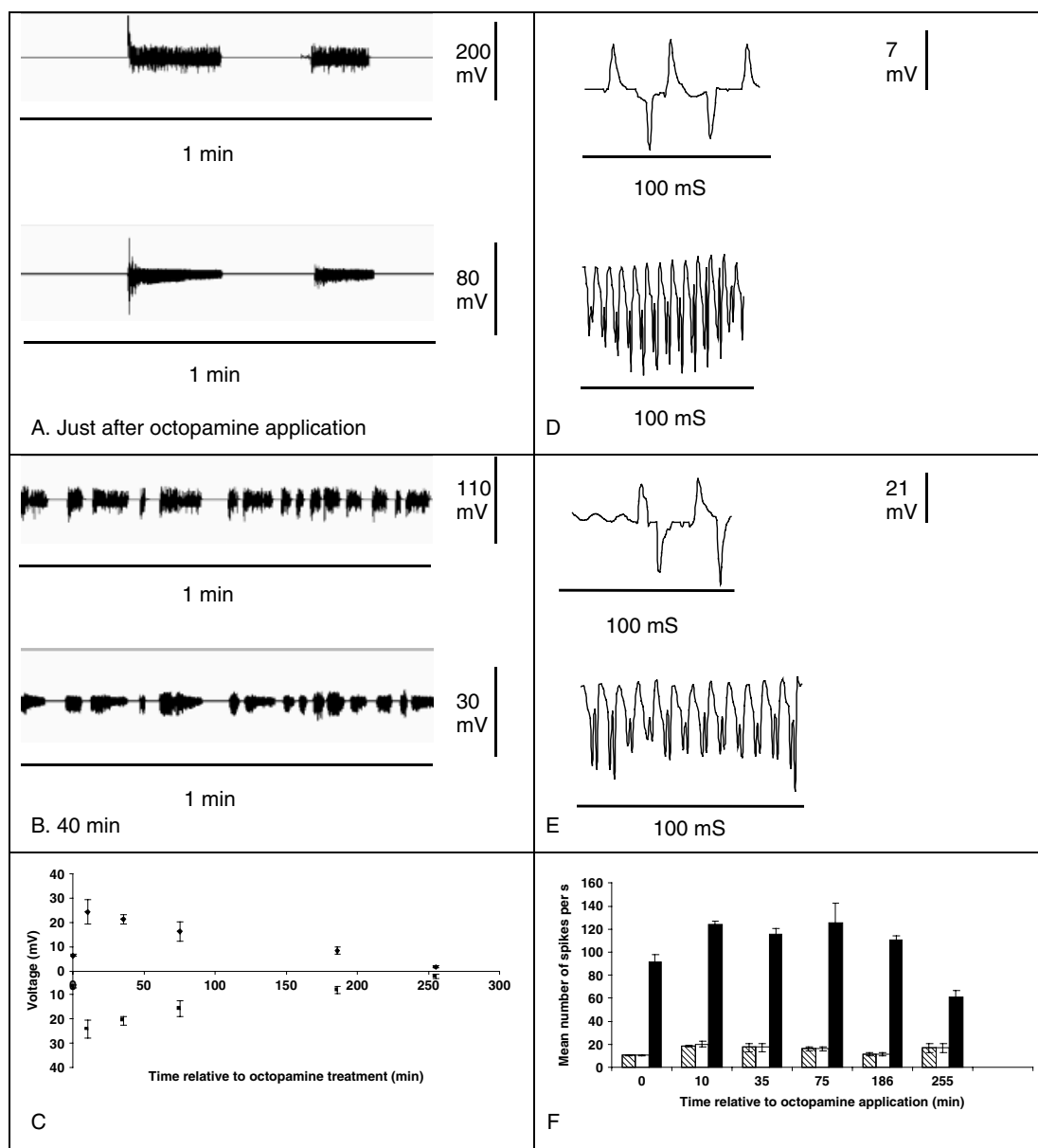
### 3.4 Effects of chlordimeform (CDM)

Normal FMIs and WBF were observed immediately after dosing blowflies with 50 µg CDM (Fig. 5A). At 10 min the bursts of FMIs were shorter in duration, and wing beating ceased by 30 min (Fig. 5F). Between 30 and 90 min, a weaker pattern of discontinuous FMI activity occurred. The mean frequency of spikes increased immediately after treatment (Fig. 5F). By 150 min after treatment, FMI activity had become continuous (Fig. 5B), but the amplitude declined gradually to zero by 300 min (Fig. 5C). The FMI:WBF ratio averaged 1:4±1 after CDM treatment. CDM-treated

blowflies showed increased grooming, tremors and paralysis, and all died.

### 3.5 Effects of desmethylchlordimeform (DMCDM)

The initial discontinuous trains of FMIs and wing beat after dosing blowflies with 50 µg DMCDM were similar to those of controls (Fig. 6A). At 10 min there were intense continuous FMIs of increased amplitude, and WBF remained tightly linked to this activity (Figs 6C and F). Brief reversion to atypical discontinuous FMIs at 60 min preceded a final phase of continuous firing which declined to zero amplitude by 190 min (Fig. 6C). No wing beats were observed after 60 min (Fig. 6F). FMI frequency increased within the first hour and then declined (Fig. 6F).



**Figure 4.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies injected with octopamine (OA; 50 µg). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 40 min post-application respectively. C: changes in amplitude of the right (◆) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of OA on FMIs and WBF frequency over the 255 min recording period. Hatched bar: mean (± SE) number of spikes per second of the right DLM; white bar: mean (± SE) number of spikes per second of the left DLM; black bar represents WBF frequency.

DMCDM-treated flies had an FMI:WBF ratio of 1 : 5 ± 2. DMCDM symptoms were similar to those of CDM, including complete mortality.

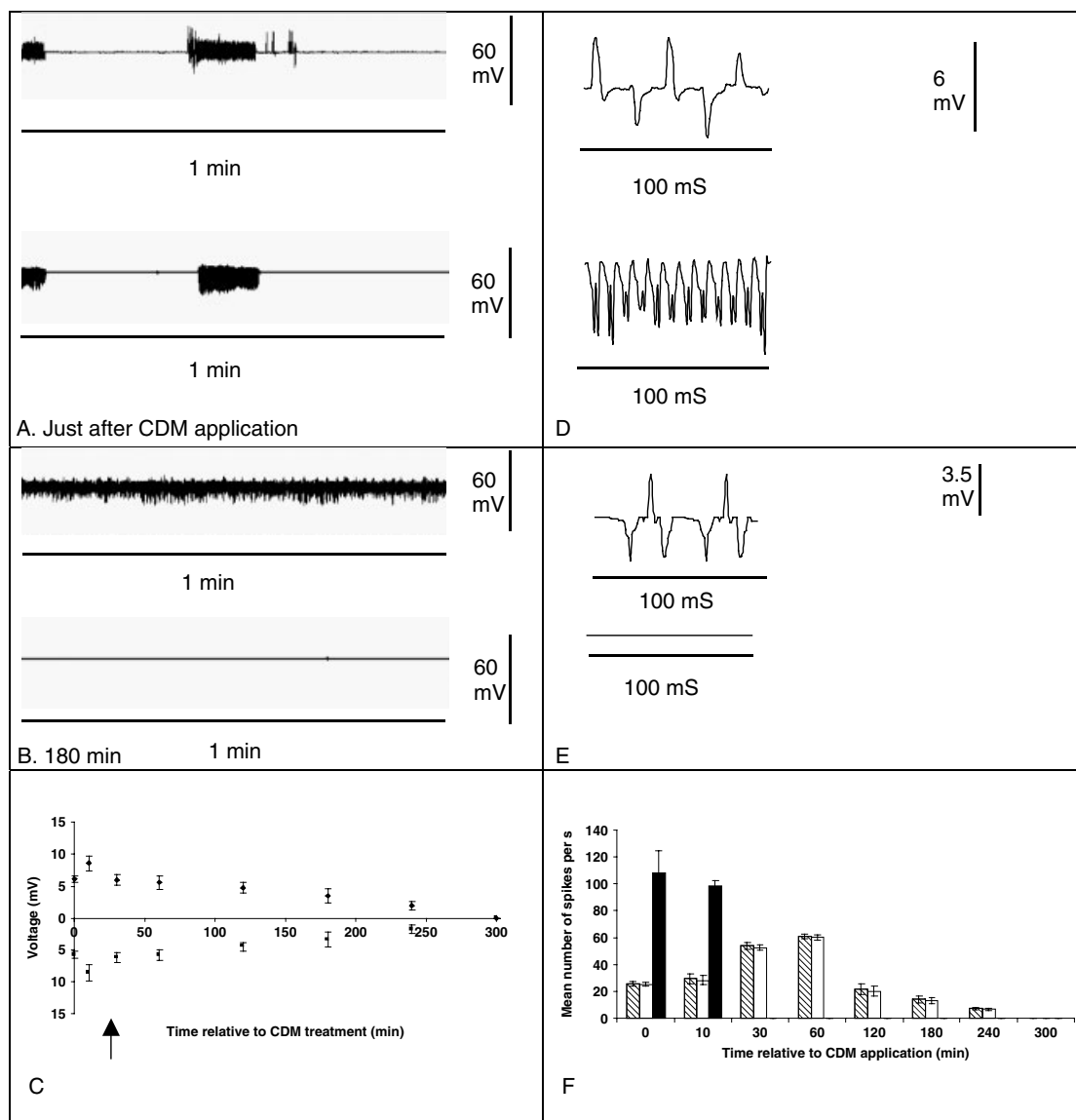
### 3.6 Effects of rotenone

The initial discontinuous trains of FMIs and wing beating immediately after treatment with 50 µg rotenone (Fig. 7A) were similar to those of control insects. At 24 min after treatment, sporadic bursts of FMIs of increased amplitude occurred, and WBF remained tightly linked to this activity (Figs 7C and F). After 24 min, the wings stopped beating (Fig. 7F). At this time the amplitude of the FMIs dropped below the initial amplitude and continued to drop over the duration of the experiment (Fig. 7C).

In parallel to amplitude reduction, the absolute frequency of both wing beat and FMIs dropped (Fig. 7F). At 154 min, continuous weak FMIs appeared and continued for a few minutes (Figs 7C and F). Rotenone gave an FMI:WBF ratio of 1 : 7 ± 1. Rotenone-treated blowflies groomed initially then became paralyzed and died.

### 3.7 Effects of cypermethrin

The initial trains of FMIs and wing beating immediately after application of 50 µg cypermethrin (Fig. 8A) closely paralleled those of controls. At 5 min, however, there were intense, multiple FMIs of high amplitude, and WBF remained tightly linked to this activity (Figs 8C and F). Intense high-frequency FMIs were still



**Figure 5.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies treated topically with chlordimeform (CDM; 50 µg). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 180 min post-application respectively. C: changes in amplitude of the right (♦) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of CDM on FMIs and WBF frequency over the 300 min recording period. Hatched bar: mean (± SE) number of spikes per second of the right DLM; white bar: mean (± SE) number of spikes per second of the left DLM; black bar represents WBF frequency. In panel C the arrow indicates the time point at which tremors were first observed.

present 44 min after treatment (Fig. 8F). However, 5 min after application, the amplitude of the FMIs fell below their initial level and did not return to pretreatment levels (Fig. 8C). At 82 min, FMIs and WBF became uncoupled (Figs 8B, C and F). FMI frequency declined markedly, and by 226 min both the frequency and the amplitude of the FMIs had fallen to zero (Figs 8C and F). No wing beating was observed after 44 min. Cypermethrin produced an FMI:WBF ratio of 1:6 ± 1. Cypermethrin caused extensive grooming, tremors and convulsions, and at 226 min all flies were dead.

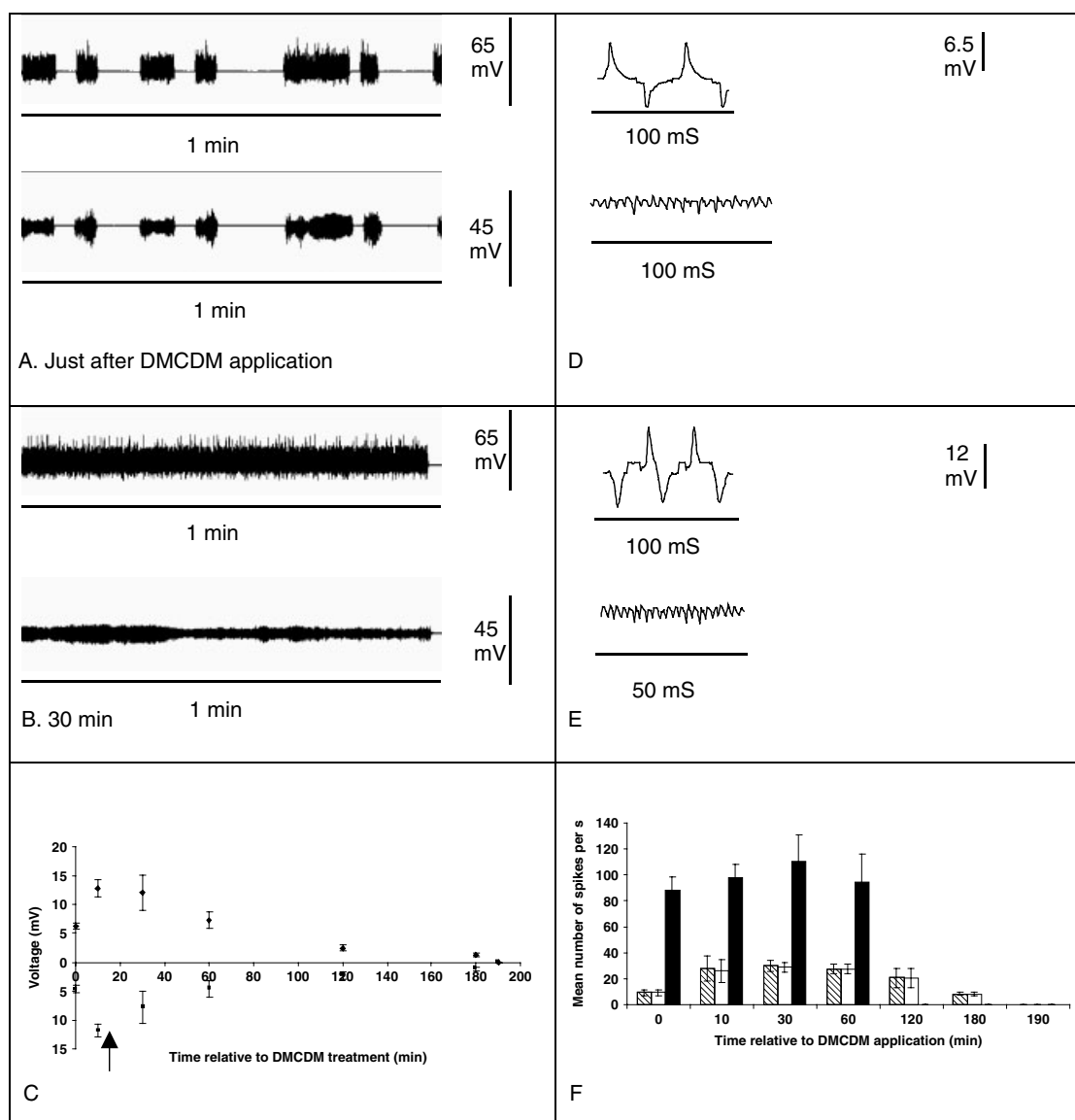
### 3.8 Effects of fipronil

The effects of fipronil are shown in Fig. 9. Soon after treatment, WBF became less regular, and after 30 min the insects could not fly (Figs 9B and F). Between 10 min and 2 h after dosing,

a pattern of initially variable-frequency FMIs that subsequently became continuous was observed (Figs 9B and F). The amplitude of FMIs was maximal at 2 h (Fig. 9C), but by 6.5 h the electrical activity had almost entirely ceased (Figs 9C and F) and all flies died. Fipronil produced an FMI:WBF ratio of 1:4 ± 0.3. Fipronil-treated flies showed grooming and convulsions before death.

### 3.9 Effects of ivermectin

Fifteen minutes after application of ivermectin, the amplitude and the frequency of FMIs increased slightly and then declined below initial levels (Figs 10C and F). Sporadic trains of FMIs appeared after 47 min and became more infrequent at 162 min. The FMI:WBF ratio was 1:8 ± 1. Wing beats were closely linked to FMIs until about 47 min, when wing beating



**Figure 6.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies treated topically with desmethylchloridimeform (DMCDM; 50 µg). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 30 min post-application respectively. C: changes in amplitude of the right (◆) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of DMCDM on FMIs and WBF frequency over the 190 min recording period. Hatched bar: mean (± SE) number of spikes per second of the right DLM; white bar: mean (± SE) number of spikes per second of the left DLM; black bar represents WBF frequency. In panel C the arrow indicates the time point at which tremors were first observed.

ceased (Fig. 10F). By 162 min, FMIs became almost unidirectional (Fig. 10B). This effect became more pronounced as time progressed (Fig. 10C). Ivermectin-treated flies showed increased grooming and paralysis but no mortality over the timecourse of the experiment.

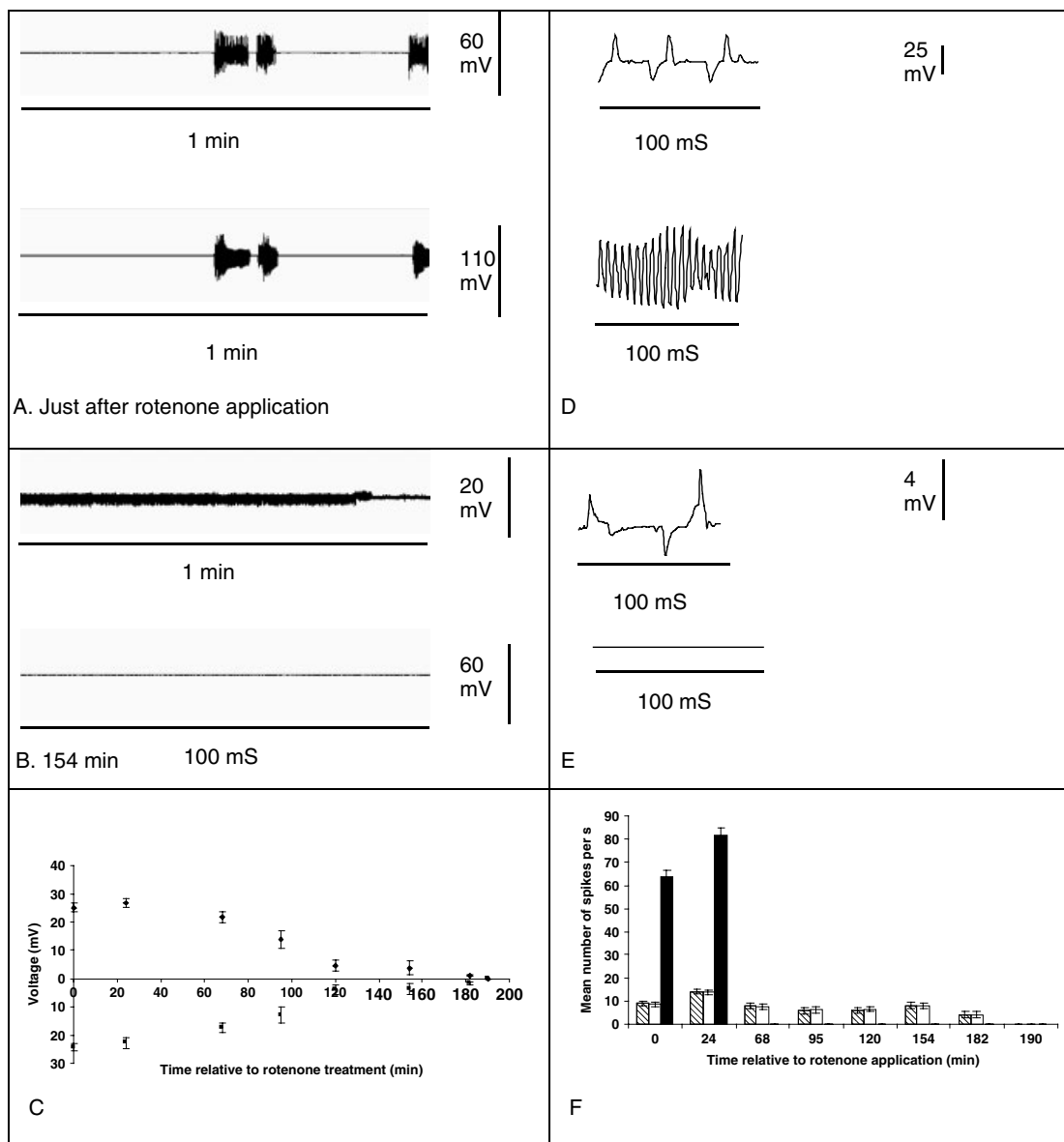
### 3.10 Effects of GABA

GABA-treated flies showed patterns of activity most similar to those of thymol-treated flies. Typical effects of GABA on the FMIs and wing beat pattern are shown in Fig. 11. Wing beats were closely linked to the FMIs for about 10 min, but then wing movement ceased (Fig. 11F). By 47 min, the FMI became unidirectional (exclusively negative deflections), and this pattern continued until 162 min (Figs 11B and C). The frequency of the FMIs dropped steadily from 47 min after treatment to the end of the

experiment (Fig. 11F). Following GABA application, the FMI:WBF ratio was  $1:5 \pm 1$ . GABA treatment triggered grooming activity but no mortality.

## 4 DISCUSSION

The effects of the natural product thymol on flight motor-associated electrical activity and wing beat in live, tethered, adult female blowflies were examined. The electrophysiological results indicate effects on the central nervous system, the longitudinal flight muscles and neuromuscular junctions. This approach has proved useful for investigating the *in vivo* effects of insecticides and glutamate analogues in dipterans.<sup>43,44</sup> Monitoring WBF affords a convenient way of acquiring data pertinent to flight activity.<sup>45</sup> The pattern of disruption observed with thymol was



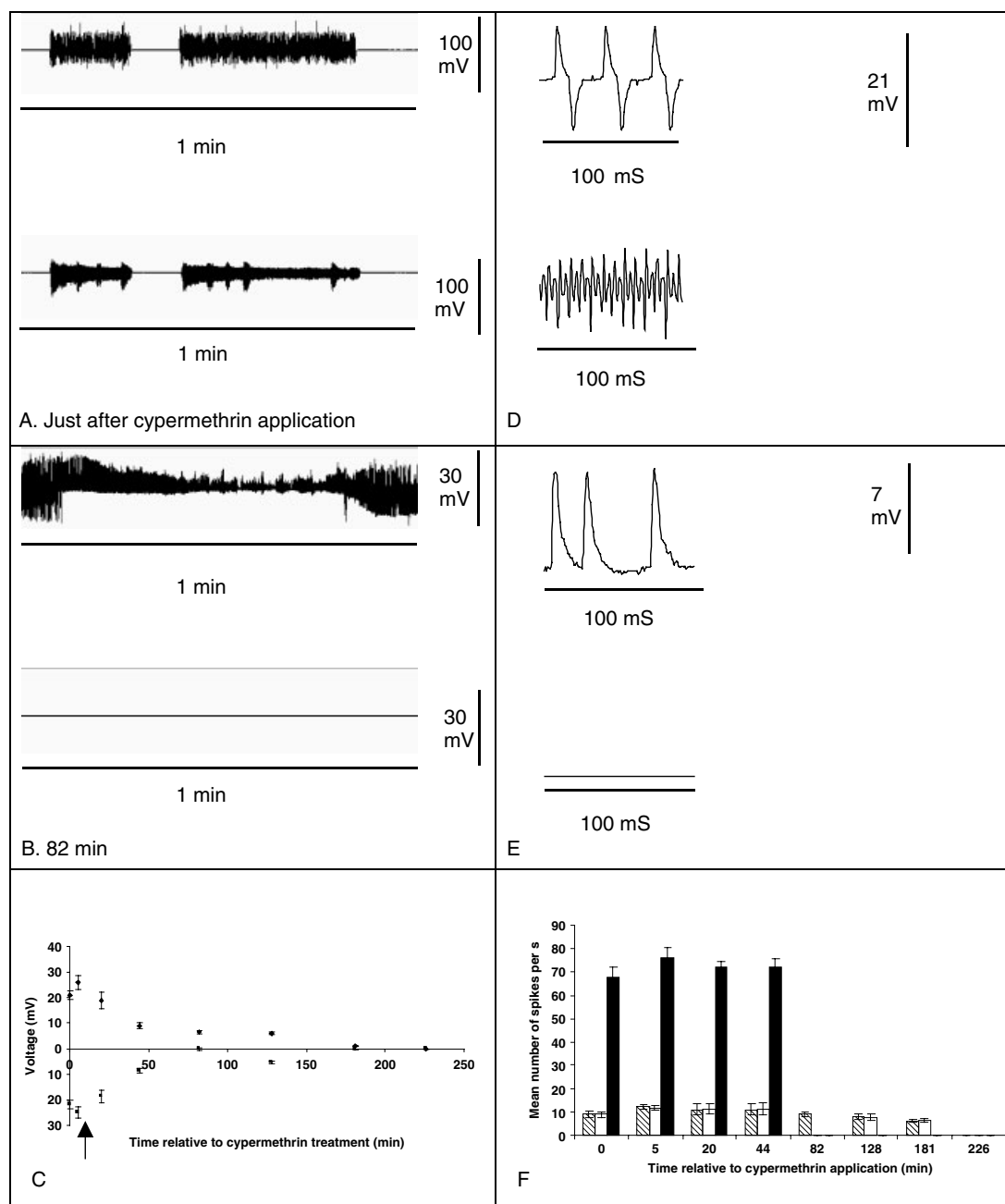
**Figure 7.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies treated topically with rotenone (50 µg). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 154 min post-application respectively. C: changes in amplitude of the right (◆) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of rotenone on FMIs and WBF frequency over the 190 min recording period. Hatched bar: mean (± SE) number of spikes per second of the right DLM; white bar: mean (± SE) number of spikes per second of the left DLM; black bar represents WBF frequency.

compared with those observed with neuroactive substances with known modes of action, to shed more light on how thymol interferes with central and peripheral flight pathways *in vivo*. These experiments can provide information on the ease with which thymol crosses the insect cuticle and gains access to tissues, identified in *in vitro* studies, to possess sites sensitive to the effects of thymol.

The first effect of thymol was a brief succession of lower-frequency spikes in the absence of wing beat that reverted to control-like activity. Within 30 min of topical application, wing beating was fully suppressed, and trains of FMIs with mostly unidirectional spikes were evident. These results clearly suggest that thymol efficiently penetrates the insect cuticle and accesses excitable tissues. Its speed of action, however, is

slower than those of the other topically applied insecticides; even slower than CDM which relies on bioactivation. Similar types of unidirectional FMI occurred in flies treated with ivermectin. The predominantly unidirectional pattern of spiking observed with thymol is more likely to arise through interference with flight motor control centrally rather than from a 'side-selective' neuromuscular block. Of all the neuroactive compounds examined, GABA showed the closest resemblance to thymol in causing inhibition of one component of bidirectional spiking. In insects, GABA is an important inhibitory neurotransmitter that plays a key role in the peripheral and central nervous system. Insect neurons exhibit transient hyperpolarizing responses following application of GABA.<sup>46</sup> GABA has been reported to block spontaneous spiking in corn borer ventral nerve cord,<sup>20</sup>

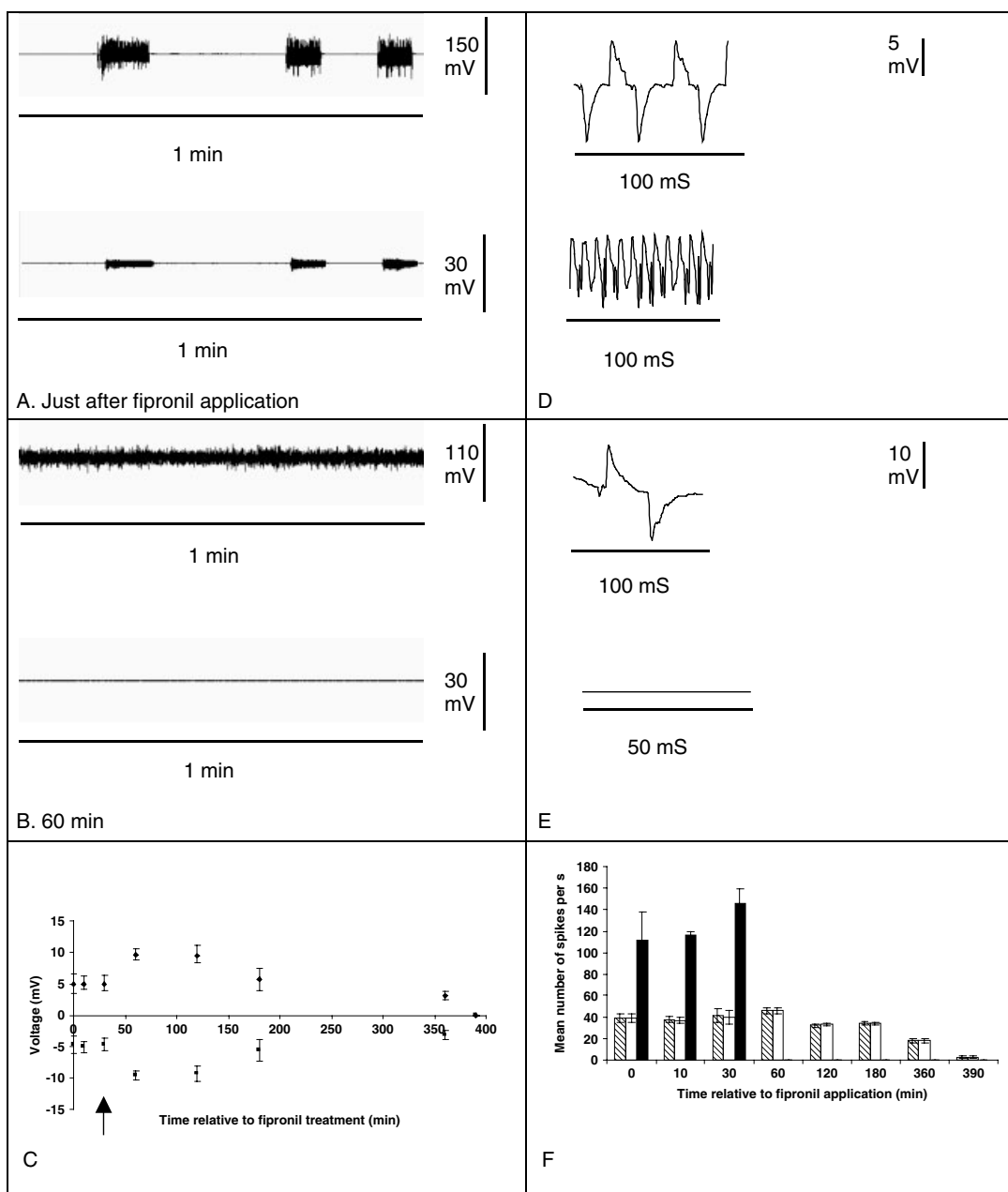




**Figure 8.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies treated topically with cypermethrin (50 µg). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 82 min post-application respectively. C: changes in amplitude of the right (◆) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of cypermethrin on FMIs and WBF frequency over the 226 min recording period. Hatched bar: mean (± SE) number of spikes per second of the right DLM; white bar: mean (± SE) number of spikes per second of the left DLM; black bar represents WBF frequency. In panel C the arrow indicates the time point at which tremors were first observed.

and ample evidence exists for its ability to penetrate into the CNS of insects at concentrations similar to those used in the present investigation.<sup>47,48</sup> The data obtained indicate that thymol acts on GABA-sensitive sites *in vivo*, either by mimicking or facilitating the effects of this inhibitory neurotransmitter. Such an *in vivo* action is consistent with a previous *in vitro* pharmacological study that reported a potentiation of GABA responses at insect GABA receptors by thymol.<sup>12</sup> It is important to note that, although the central action of thymol is predominantly GABA-like, the effects of this phytochemical on FMI amplitude

suggest an additional peripheral component to its action. The present data clearly distinguish the effects of thymol from those of the GABA receptor antagonist fipronil where continuous bidirectional spiking occurred. Although treatment with ivermectin also showed some activity similar to GABA, ivermectin acts on receptor-operated chloride channels, leading to long-lasting hyperpolarization or depolarization of the neuron or muscle cell, blocking function.<sup>49</sup> GABA-treated flies developed unidirectional impulses much earlier than did ivermectin-treated flies.

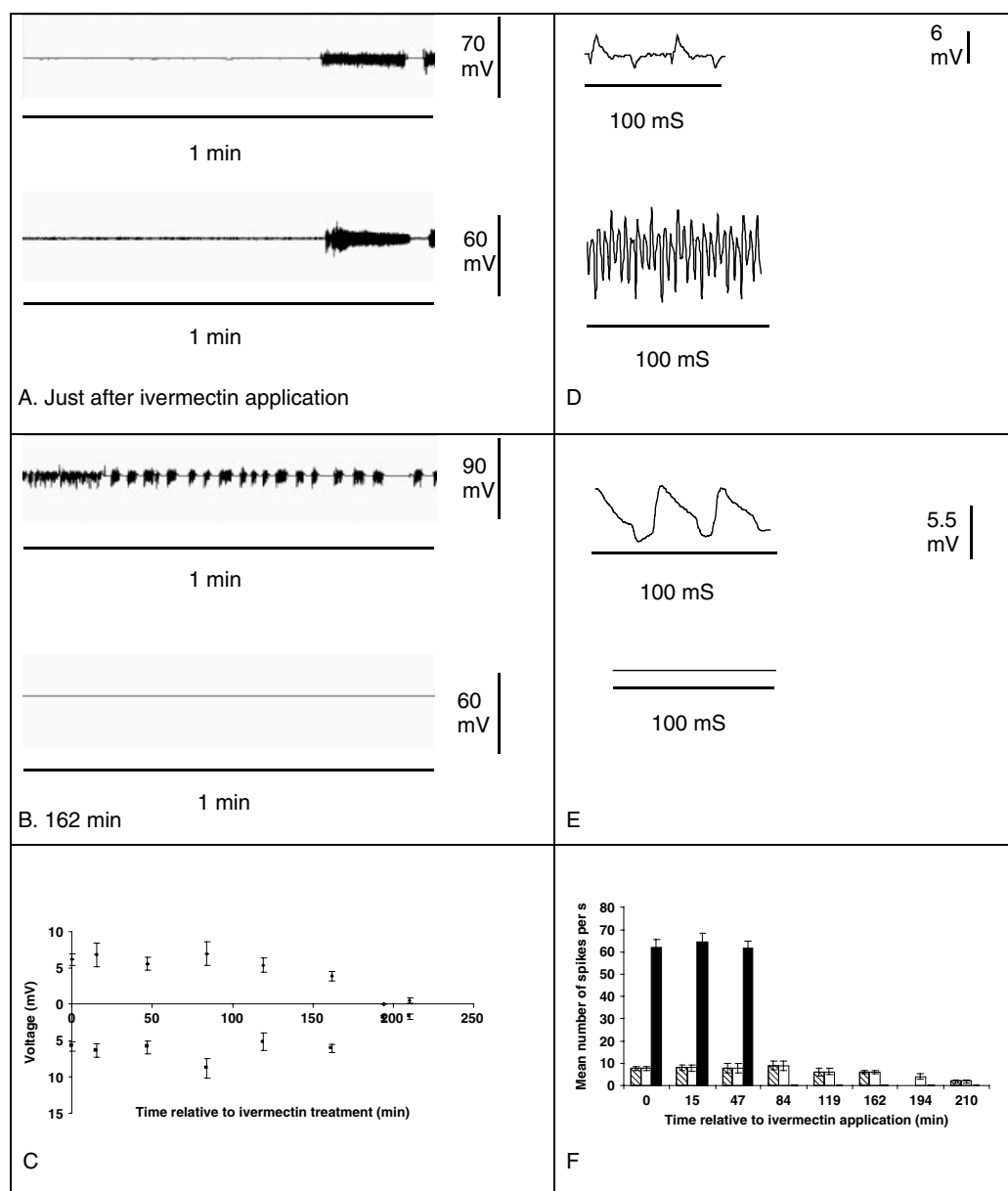


**Figure 9.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies treated topically with fipronil (50 µg). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 60 min post-application respectively. C: changes in amplitude of the right (◆) and left (■) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of fipronil on FMIs and WBF frequency over the 390 min recording period. Hatched bar: mean (± SE) number of spikes per second of the right DLM; white bar: mean (± SE) number of spikes per second of the left DLM; black bar represents WBF frequency. In panel C the arrow indicates the time point at which tremors were first observed.

A high FMI:WBF ratio indicates efficient coupling of motor output to the thoracic muscle units involved in flight activity. Thymol, ivermectin and OA produced an FMI:WBF ratio that was similar to their controls. However, the ratio was affected by other treatments. For example, flies treated with fipronil, CDM, DMCDM and GABA exhibited the lowest FMI:WBF ratios, while cypermethrin did not reduce the ratio as much. Based on these ratios, fipronil, CDM, DMCDM, GABA and to a lesser extent cypermethrin most likely affected the excitability of the indirect flight muscles.

An objective of the present research was to investigate whether octopamine-like activity occurred with thymol *in vivo*, as some

essential oil constituents are known to act at octopaminergic sites.<sup>21,41</sup> Because previous reports had suggested that formamidines were OA agonist,<sup>30,38,50,51</sup> CDM and DMCDM were included in this investigation. CDM, DMCDM and OA affected FMIs in a qualitatively similar manner. Bidirectional impulses occurred as discrete bursts of FMI activity, and spiking within bursts was always continuous. However, the time of onset, duration and intensity of effects differed among the compounds. Bursts with OA and CDM occurred earlier and were much more obvious than with DMCDM. Also, intense early- and late-phase continuous spiking were a consistent feature of DMCDM, whereas



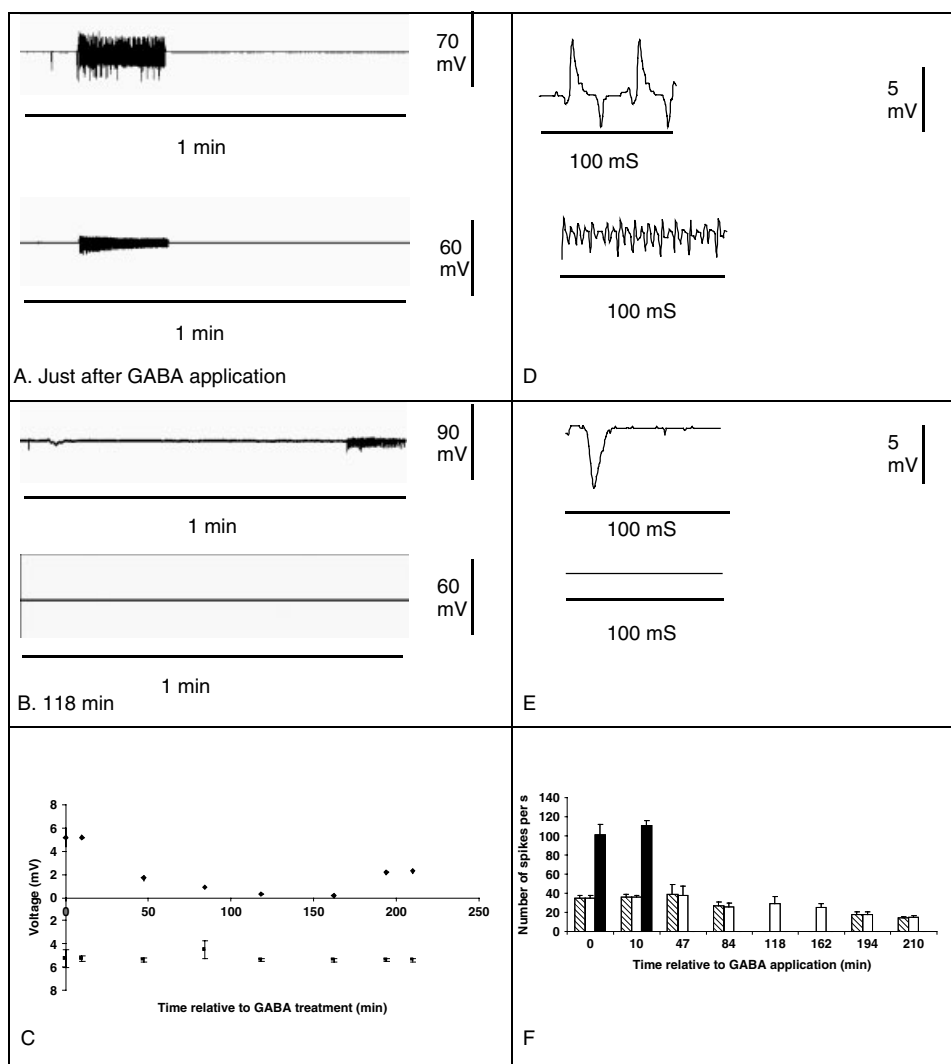
**Figure 10.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies treated topically with ivermectin (50  $\mu\text{g}$ ). A and B: temporal progression of FMIs (top panels) and WBF (lower panels) at 1 min and 162 min post-application respectively. C: changes in amplitude of the right ( $\blacklozenge$ ) and left ( $\blacksquare$ ) DLM FMIs over time. D and E: enlarged sections of FMIs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of ivermectin on FMIs and WBF frequency over the 210 min recording period. Hatched bar: mean ( $\pm$  SE) number of spikes per second of the right DLM; white bar: mean ( $\pm$  SE) number of spikes per second of the left DLM; black bar represents WBF frequency.

only the late-phase component was observed with CDM, which may be related to a requirement for bioactivation. Although late-phase multiple spiking was observed with OA, the FMIs were much smaller in amplitude. Nonetheless, the FMI patterns elicited by the formamidines are considerably different from those of thymol, and it is concluded that thymol does not act as an octopamine agonist on flight motor-specific pathways *in vivo*.

Voltage-dependent sodium channels represent major sites of pyrethroid action.<sup>52</sup> Pyrethroids delay the closing of sodium channels in neurons. All blowflies treated with cypermethrin showed symptoms of hyperactivity and convulsions, as described for other pyrethroids.<sup>43</sup> The activity patterns caused by cypermethrin were

different from those produced by GABA, ivermectin or thymol.<sup>43</sup> Intense FMIs were seen in blowflies treated with cypermethrin, which often lasted for over 30 min. Unidirectional spiking and reduced spike amplitude also occur with cypermethrin; however, the intense multiple and unidirectional spiking observed with this pyrethroid suggests a different action to that of thymol or GABA. In contrast, rotenone inhibits mitochondrial complex I, and the changes in electrical activity produced by rotenone were very different from those produced by GABA, ivermectin or thymol.

In summary, this investigation focused on the temporal progression of interference with FMIs and WBF after treatment of blowflies with the plant terpenoid thymol and various neuroactive



**Figure 11.** Typical electrophysiological responses recorded from the dorsolongitudinal muscles (DLM) of blowflies injected with GABA (50  $\mu$ g). A and B: temporal progression of FIMs (top panels) and WBF (lower panels) at 1 min and 118 min post-application respectively. C: changes in amplitude of the right ( $\blacklozenge$ ) and left ( $\blacksquare$ ) DLM FIMs over time. D and E: enlarged sections of FIMs (top panels) and WBF (lower panels) from A and B respectively. F: the effect of GABA on FIMs and WBF frequency over the 210 min recording period. Hatched bar: mean ( $\pm$  SE) number of spikes per second of the right DLM; white bar: mean ( $\pm$  SE) number of spikes per second of the left DLM; black bar represents WBF frequency.

substances. By comparing the action of a test phytochemical with those of various neuroactive compounds of known modes of action, this electrophysiological approach can shed light on how unknown substances might act on the insect nervous system *in vivo*. The data suggest that thymol may interfere with GABAergic control of the dipteran flight motor system *in vivo*, most likely through a central action. The effects of thymol should now be compared with those of other GABA receptor agonists, and the sensitivity of thymol's response to GABA receptor antagonists and compounds that enhance GABA function should be evaluated. This work re-emphasizes the utility of the flight motor system in helping to understand the *in vivo* actions of plant-derived compounds such as thymol, with potential applications in pest management.

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