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A simple method for monitoring the respiratory rhythm in intact insects and assessing the neurotoxicity of insecticides

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Abstract

A simple, non-invasive method, for the monitoring of the respiratory rhythm of insects was developed. The insect was restrained and a force displacement transducer attached to one abdominal segment of the dorsal region was used to monitor the respiratory rhythm. Although the method was applied to four species of insects, the main study focused on the yellow mealworm beetle, *Tenebrio molitor*, due to the simplicity of the recorded respiratory rhythm. In this case, the recorded rhythmic activity represents the synchronised contraction of the dorsal abdominal muscles, driven by the respiratory central pattern generator (CPG). This non-invasive method allows prolonged and stable recording of the respiratory rhythm. The records showed a decrease in the amplitude and the duration in the rhythmic contractions to about 25 and 31% of the initial values, respectively, in the first 7 h of continuous recording. The respiratory rhythm was strongly influenced by the presence of the CO_2, creating the CO_2-reflex. In the beginning of the application of CO_2, there was total inhibition of the respiratory rhythm and then there was a gradual increase in the tension developed by the dorsal abdominal muscles. Using this recording method, it was possible to quantify the function of the respiratory CPG, measuring the amplitude of the respiratory contractions, under normal conditions and in the presence of insecticides. The insecticides imidacloprid and deltamethrin of certain quantities were diluted in the appropriate solvent to make 50, 100 and 500 ng per insect, and were applied to the cuticle, topical application, of the immobilized insect, while recordings of the respiratory rhythm were made continuously.

Keywords: Insects; Respiratory rhythm; In vivo; Imidacloprid; Deltamethrin; Assessment; Toxicity

1. Introduction

Respiration in insects is one of the basic behaviors in the rhythmical functions of the body, providing an adequate supply of oxygen to the tissues through the tracheal system. Depending on the insect body size respiratory mechanisms range from passive gas exchange by diffusion to active convection in the tracheal system, mainly mediated by coordinated motor activity [1]. This motor activity depends on patterned neural control, which in insects is generated by a CPG that is supposed to be located somewhere in the nerve cord, in the central nervous system (CNS) of insects. In the locust, the respiratory CPG is located in the metathoracic ganglion [2]. The neural patterns from this respiratory CPG exhibit a wide range of bursting frequencies. They depend mainly on behavioral or metabolic conditions influencing the insect. The respiratory CPG in insects is a network—of partly known interneurons [3]—that provides the rhythms to the motor neurons which supply the respiratory muscles. Backed by the basic rhythmicity of this CPG, metabolic, hormonal, sensory, neural and behavioral influences can modify the rhythmic output and many of these aspects were studied, mainly with intracellular recordings from the respiratory interneurons [3–8]. Finally, the effects of insecticides on the output of the respiratory CPG from an isolated nervous system of mammalians (deltamethrin) [9] and insects (imidacloprid) [10] were investigated. In these in vitro studies the distortion of the abdomen caused by dissection and the replacement of hemolymph with saline has provoked unnatural afferent feedback from the basic rhythm genera-

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tor neurons, mainly resulting in stressed respiration, while the vitality of the isolated preparation was very short, with a maximum of 1 h.

The purpose of this work is: first, to develop a simple non-invasive method to monitor the respiratory motor pattern, the respiratory contractions, from a restrained but intact insect for a relatively long period of time. These recordings could be considered as an indication of the proper functioning of the respiratory CPG and of the extent of the CNS in the insect; second, to investigate how neurotoxic compounds, like the insecticides imidacloprid and deltamethrin, when applied to the surface of the cuticle, topical application, can penetrate the exoskeleton and modify the output of the respiratory CPG.

2. Materials and methods

The study of the respiratory rhythm concentrated on the yellow mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae). Experiments were performed on adult insects of either sex, reared in laboratory colonies, fed on bran, potatoes and fruit under a controlled temperature of 26 ± 2 °C and with a photoperiod of 16L-8D. The insect was fixed, with the ventral part of the thorax and the abdomen (Fig. 1B) on a small platform of non-toxic plasticine in the center of a Petri dish, using a mixture of wax and colophony (Gum resin, natural resin from Fluka, Hanover, Germany). Care was taken to leave most of the spiracles open. Then a micropin was attached to the probe of an isometric force displacement transducer (Grass 103, Grass Company, USA) and was gently hooked onto the thin cuticular membrane covering the dorsal surface of the abdomen, at the region of the second abdominal segment (for the location of the fdt see Fig. 1B). The insect was left to recover for over 1 h. The analog signal of the transducer was digitized at 256 samples/s (A/D converter, KPCI-3102, Keithley Instruments Inc., USA) and stored in a computer using the TestPoint program. The same method was used to monitor the respiratory movements of the abdominal segment of an immobilized honey bee, *Apis mellifera macedonica*, the bush-cricket *Decticus albifrons*, and the hornet, *Vespa orientalis*. In these species, the abdomen construction differs from *T. molitor*, since the abdominal segments are divided into distinct tergites and sternites. In this case, the probe of the transducer was always attached to the edge of the 2nd or 3rd tergite.

The restrained insect, *T. molitor*, was treated with the insecticides under investigation by applying 2 µl of the solvent, in which imidacloprid (or deltamethrin) had been diluted, to the dorsal surface of the 1st abdominal segment (for the location see the filled dot in Fig. 1B.). For imidacloprid (Fluka Pestanal, Hanover, Germany) the final concentration in the 2 µl of dimethyl sulphoxide (DMSO, Sigma, St. Louis, MO) was 0.49×10^{-4}, 0.98×10^{-4}, 0.195×10^{-3} and 0.98×10^{-3} M, which corresponds to 25, 50, 100, and 500 ng per insect, respectively. These quantities were chosen because, in honey bees, the LD_{50} for contact experiments, topical application, using imidacloprid was defined as being between

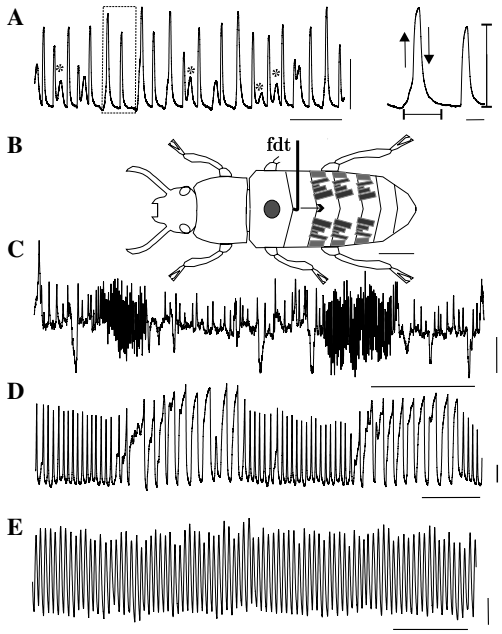

Fig. 1. (A) Rhythmic contraction of the dorsal abdominal muscles recorded from the cuticular membrane covering the dorsal region of the abdominal cavity in the beetle, *Tenebrio molitor*. Horizontal scale bar indicates duration in second: 19.50 and 1.95 for the expanded record. Vertical scale bar indicates force in Newton: 1.06 and 1.59 for the expanded record. The asterisks indicate additional respiratory contractions not related to the normal respiratory rhythm. (B) Diagrammatic representation of the anatomy of the abdominal muscles, called dorsal external posterior [14] and the position of the force displacement transducer (fdt) on the cuticle membrane. The dot in the diagram indicates the position where 2 µl of the compounds under investigation, imidacloprid and deltamethrin, were placed on the cuticle. The arrow indicates the direction of the force applied on the transducer during the respiratory contractions. Horizontal scale bar: 1.6 mm. (C–E) Recordings of the respiratory contractions from the dorsal region of the 2nd or 3rd abdominal segment of an immobilized honey bee *A. mellifera* (C), the bush-cricket *D. albifrons* (D), and the hornet, *V. orientalis* (E). In this case, the transducer was placed on the 2nd or 3rd abdominal segment. Vertical scale bars: 1.06 N (C–E). Horizontal scale bars: 19.50 s (C and D); 5.00 s for (E).

49 and 103 ng per bee [11]. Due to the presence of the solvent in the solution applied, control experiments were performed on insects treated with topical application of 2 µl DMSO.

For the application of deltamethrin at 50 or 100 ng per insect, 2 µl dose volumes were obtained as follows: 2.5 mg (or 5 mg) of deltamethrin (Fluka Pestanal, Hanover, Germany) were diluted in 2 ml of acetone and then in 98 ml of water; the final concentration of acetone being 2%. For local application, a drop of 2 µl (of the selected concentration) was always placed on the same point of the exoskeleton, the first abdominal segment (the dot in Fig. 1B), using a Hamilton syringe (Hamilton Company, Nevada, USA) and a stereoscope. The final doses of deltamethrin applied were 50 or 100 ng per insect to be compared with to the LD_{50} of 50 ng per insect reported in contact experiments using the honey bee [12].

In all four cases of exposure to the chemicals acetone, DMSO, imidacloprid and deltamethrin, the respiratory contractions of the immobilized insect were recorded