Two-Rods Method for Simultaneous Measurement of Mandibular and Thoracic Leg Movements of the Cabbage Armyworm, *Mamestra brassicae* (Lepidoptera: Noctuidae)[1]

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INTRODUCTION

A measurement of the mandibular movements of phytophagous insect larvae is often useful in biological, pharmacological, or toxicological studies. A device, capable of continuously recording mandibular activity following treatment with drugs, repellants, or feeding stimulants, can be particularly useful in feeding studies. In a previous paper, we described one such method for measuring the mandibular movements of the cabbage armyworm, *Mamestra brassicae*, and we presented data showing how the apparatus could be used to monitor the effects of chlordimeform hydrochloride on the cabbage armyworm (SHIMIZU et al., 1980).

Here we describe another system (the two-rods method) that can provide a continuous and simultaneous recording of mandible and leg movements of the cabbage armyworm. From a determination of the synchrony of mandible and leg movement, we offer a possible explanation regarding the neural processes affected by the application of chlordimeform hydrochloride.

MATERIALS AND METHODS

Insect. Last instar larvae (5-day-old) of the cabbage armyworm (*Mamestra brassicae*), reared on an artificial diet (SHIMIZU and YAGI, 1983) at 25°C under a long-day photoperiod, were used in the present study.

Ligature. The subjects were ligated with four threads located between the head and thorax (i.e., between the suboesophageal ganglion and first thoracic ganglion), between the mesothorax and metathorax, between the metathrorax and abdomen, and just anterior to the anal proleg (Fig. 1). The free ends of the ligatures were used to secure the animals, venter-side up, to a small wooden block. Because ligatures prevent the movement of hemolymph (JANDA, 1936), they can be used to create localized sites for the application of toxins. In this case, the ligatures allowed the toxin to be applied locally to the head and the thorax.

Apparatus. The apparatus for measuring mandibular and foreleg movements is shown in Fig. 1. Two clips were attached to the subject, one to a mandible and one to a foreleg, so that movements of the mandible and leg would activate the two rods of two isotonic transducers (TD-112S, Nihon Koden

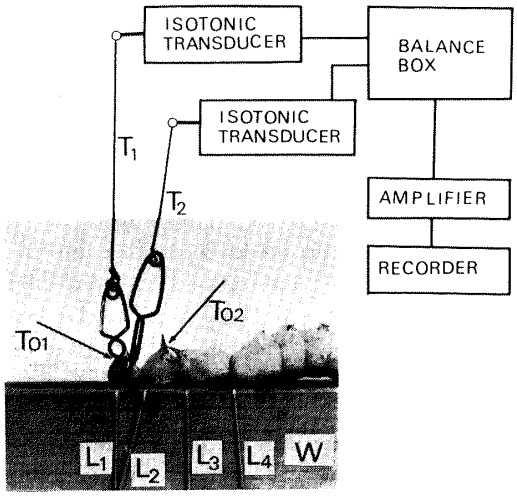

Fig. 1. A schematic illustration of the apparatus used to monitor the mandibular and thoracic leg activities of a restrained insect larva. Each movement of the subject's mandible and leg resulted in movement of the rod of the isotonic transducer. Key: W = wooden block; L_1 to L_4 = location of ligature; T_1 = thread for measuring mandibular activity; T_2 = thread for measuring thoracic leg activity; To_1 (with arrow) = topical application on the mouthparts; To_2 (with arrow) = topical application on the thoracic sternum.

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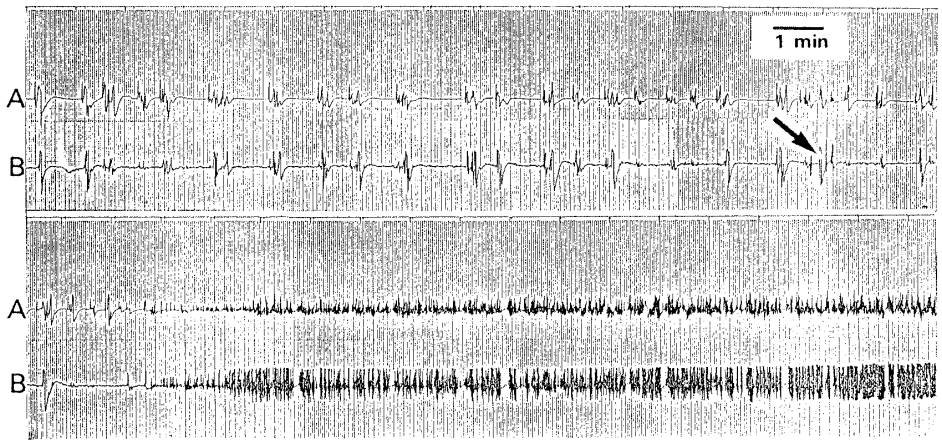

Fig. 2. Simultaneous recordings of mandibular (B) and foreleg (A) activities before and after treatment with a solution of chlordimeform hydrochloride. Spontaneous and synchronous mandibular and foreleg movements are illustrated to the left of the arrow, which indicates the time of the application of a 1,000 ppm solution to the animal's mouthparts. Six minutes after the application, synchronized continuous and repetitive bursts of mandibular and leg movements were observed. Increments in the time scale represent one minute.

Kogyo Co., Ltd.). With each movement of mandible and leg, the transducers transmitted a pulse to a balance box (JD-112S, Nihon Koden Kogyo Co., Ltd.), a bioelectric amplifier (AB-620 G, Nihon Koden Kogyo Co., Ltd.), and a recorder.

Preparation of chlordimeform. Chlordimeform hydrochloride was prepared at 1,000 ppm with distilled-water. One microliter of this solution was applied either to the mouthparts or to the thoracic sternum of the subjects.

RESULTS AND DISCUSSION

Figure 2 presents simultaneous recordings of mandible and foreleg movements before and after the application of chlordimeform to the mouthparts of the subject. Prior to the application of chlordimeform, the movements of the mandible and foreleg were closely synchronized, possibly indicating that both movements are controlled by the same motor neuron in the suboesophageal ganglion. It is known that the suboesophageal ganglion contains the motor centers for the mouthparts and exerts an excitatory influence on the locomotor coordinating system in the thoracic ganglion and that nerve impulses passing down a single motor nerve will trigger contraction in all the muscle fibers (Wigglesworth, 1972).

When chlordimeform was applied topically to the mouthparts, continuous and repetitive bursts of mandibular and foreleg movements were recorded simultaneously (Fig. 2). However, topical application of chlordimeform to the thoracic sternum did not produce any detectable burst of mandibular and foreleg movements for 30 min following the application (c.f. Shimizu et al., 1981 b).

Since the ligatures prevented the movement of chlordimeform beyond the region of application, the primary site of action of chlordimeform must be in the head. This is supported by the observation that brain removal also causes larvae to show continuous bursts of mandibular movements (Shimizu and Fukami, in preparation). Consequently, we suggest that the action site of chlordimeform may be the motor center of the suboesophageal ganglion, and that chlordimeform acts through the efferent motor neuron. This hypothesis could explain some of the pestistatic actions of chlordimeform previously reported by many investigators, especially, for example, the dropping action of the tick, *Boophilus microplus*, and of lepidopterous, *Manduca sexta*, species (Stone, 1974; Lund et al., 1979).

Based on the present experiment and on previous flight and wandering experiments (Shimizu and Fukami, 1981, 1983), we offer in Fig. 3 a hypothetical schematic diagram of the set of motor neurons believed to be responsible for the induced continuous and repetitive burst of mandibular and foreleg movements following the application

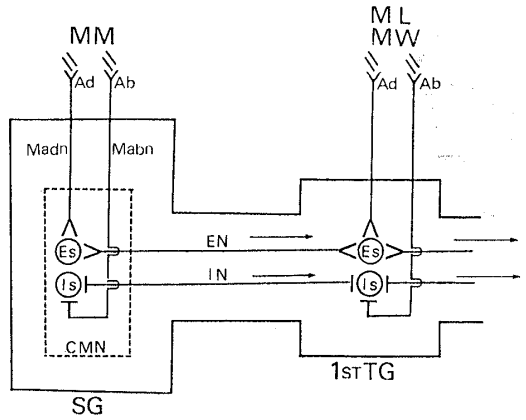

Fig. 3. Hypothetical representation of motor-neuron center responsible for the induced continuous burst of mandibular and foreleg movements observed following application of chlordimeform hydrochloride. Key: SG=suboesophageal ganglion; TG=thoracic ganglion; MM=mandibular movements; ML=movements of foreleg; MW=movements of wing; Ad and Ab=adductor and abductor muscles; Es=excitatory synapses; Is=inhibitory synapses; CMN =center of motor neurons; IN=inhibitory neuron; EN=excitatory neuron; Madn=mandibular adductor neuron; Mabn=mandibular abductor neuron; arrows=direction of impulses.

of chlordimeform hydrochloride. The motor-neuron center is present in the suboesophageal ganglion and signals from the suboesophageal ganglion are transmitted throguh the efferent nerves (inhibitory and excitatory neurons) to the thoracic peripheral nerve. Others (HIRAO et al., 1976; BERNAYS and CHAPMAN, 1974) have also suggested that the motor neuron is present in the suboesophageal ganglion.

In conclusion, the two-rods method can be used to provide a sensitive measure of the concurrent effects of various toxicants upon mandibular and thoracic leg activities. In the present experiment, the method detected synchronization between mandible and leg movements and allowed the development of hypotheses to explain the mechanism of toxic action of chlordimeform hydrochloride.

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