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Journal of Neuroscience Methods 84 (1998) 115–119

JOURNAL OF NEUROSCIENCE METHODS

A system for the delivery of general anesthetics and other volatile agents to the fruit-fly *Drosophila melanogaster*

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Received 11 February 1998; received in revised form 4 June 1998; accepted 9 June 1998

Abstract

The system described here provides a simple method of delivering anesthetic vapor to the fruit-fly *Drosophila melanogaster*. This system delivers known concentrations of volatile anesthetic vapor obtained from liquid anesthetics in a continuous gas stream of pure humidified air. It controls for evaporation, and absorption of volatile agents, whilst allowing for extracellular electrophysiological recordings. Recordings were made from the fly's escape muscles, the jump tergotrochanter muscle (TTM) and the flight dorsal longitudinal muscle (DLM). The system minimizes the quantity of anesthetic used, making the use of more expensive and more conventional anesthetics cost effective and practicable. It also permits monitoring the fly's movements during anesthesia.

Keywords: Microelectrode; Halothane; *Drosophila*; Electrophysiology; Microfilm; Giant fiber system

1. Introduction

The mechanism by which general anesthetics cause their typical effect, namely loss of consciousness and insensitivity to pain, has eluded scientists up till the present day. Investigations of the effects of general anesthetics have been pursued at many levels, from specific individual neurons in vitro, to whole animal preparations. Most recently, studies in model organisms like the fruit fly have been added to this spectrum. For such studies, controlled experiments must be performed in which the concentration of anesthetic applied to the preparation is accurately determined. Our laboratory is using the electrically induced escape response (ER) mechanism of the fruit-fly *Drosophila melanogaster* to study the actions of general anesthetics (Lin and Nash, 1996).

It is a well known fact that when startled, the fruit-fly *D. melanogaster* jumps into the air and flies away (Levin and Tracey, 1973; Levine, 1974; Tanouye and Wyman, 1980; Wyman et al., 1984). This flight behavior otherwise known as the 'escape response' has been shown to be elicited experimentally by an abrupt light-off stimulus and by the application of an electrical stimulation across the brain. The electrophysiological characteristics of the ER have been well characterized and well documented (Wyman et al., 1984; Trimarchi and Schneiderman, 1993, 1995a,b,c). In essence, it is principally mediated by the giant fiber systems (GFs), a group of interneurones that connect the visual input from the head of the fly to the thoracic muscles responsible for the ER (Tanouye and Wyman, 1980, 1983; King and Wyman, 1980; King and Tanouye, 1983).

Electrophysiological studies of the escape response require that stimulating electrodes be placed in the fly's compound eyes and recording electrodes in the left or right jump tergotrochanter (TTM) and flight dorsal longitudinal (DLM) muscles located in the thorax. In

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0165-0270/98/$ - see front matter
PII S0165-0270(98)00105-8

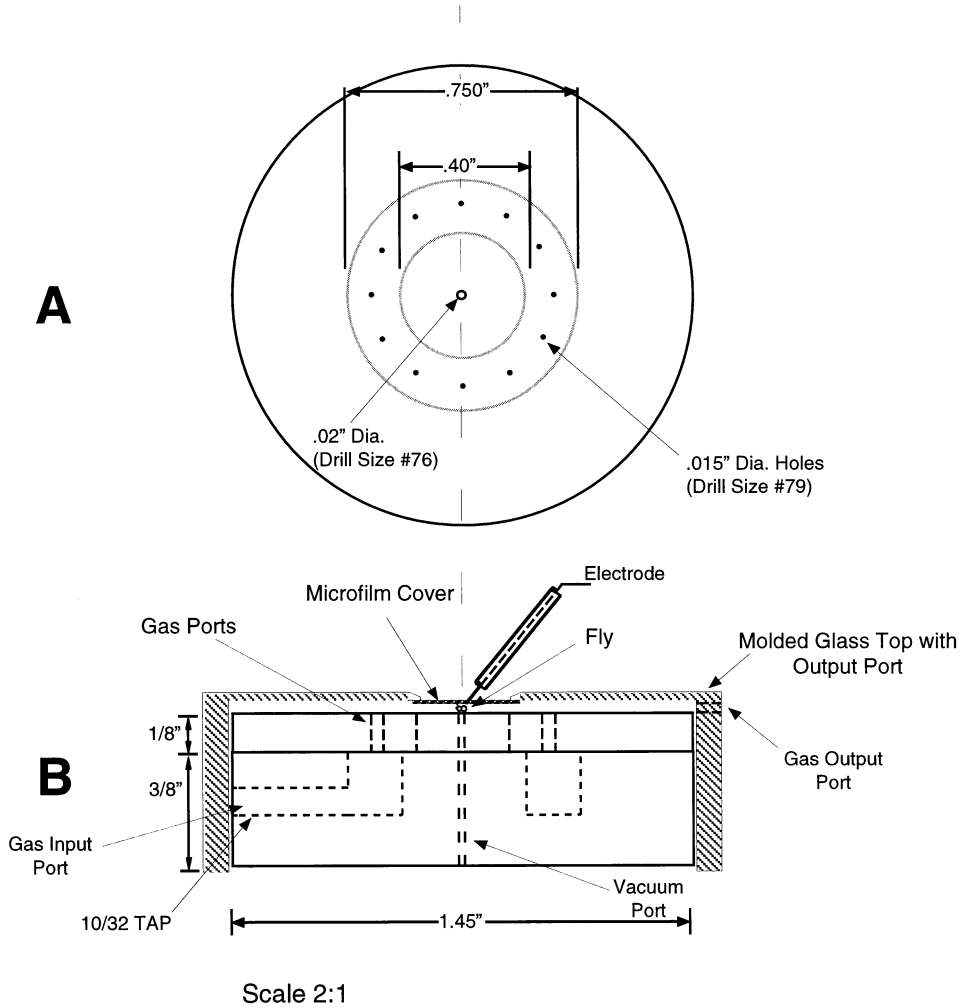

Fig. 1. Fly anesthesia chamber. (A) The base of the chamber showing the concentric outlets that allow for uniform distribution of gas around the fly held down by suction over the hole. (B) The glass chamber covering the base. The ensemble of glass chamber and base forms a tight seal that prevents the escape of gas into the air. Through the microfilm cover, microelectrodes penetrate to be inserted on the fly body.

anesthetic experiments, preparations are placed in air-tight compartments to prevent evaporation of volatile anesthetics into the environment and to protect the experimenter. For such electrophysiological experiments, previous researchers used a large box to cover all the microelectrode holders and the fly in a confined space (Lin and Nash, 1996). The drawbacks of this kind of setup are two-fold. First, the cumbersome nature of the box (35 cm × 35 cm × 38 cm), which must be held up by a pulley system to allow for fly impalement, and let down to cover the preparation once electrodes are in place. Secondly, the volume of this box requires the need for huge quantities of gas and anesthetic agents (6–7 l/min gas flow) for the system to reach equilibrium (Lin and Nash, 1996). This makes it impracticable to investigate research compounds available in limited supply. It is also difficult to observe the behavior of the fly during the application of an anesthetic agent. Here, we have developed a novel and simple system which bypasses these problems and allows the delivery of volatile anesthetics to the fly, either in an electrophysiological setting or otherwise. The system also allows the unobstructed viewing of a fly even during exposure to a given concentration of anesthetic, permitting repeated observation of gross responses. We also describe the methods employed for its calibration and its performance with reference to the preparation used in our experiments.

2. Description of apparatus

The basic apparatus consists of a Plexiglas block (1.45 in diameter by 0.54 in high containing a hole 0.02 in (drill size #76) in diameter, that is rigidly attached to the center of an experimental table through which a hole, approximately 1 mm in diameter had been made (Fig. 1). The hole in the Plexiglas base is carefully aligned with the 1 mm hole on the table. These holes are attached to a vacuum in order to provide gentle

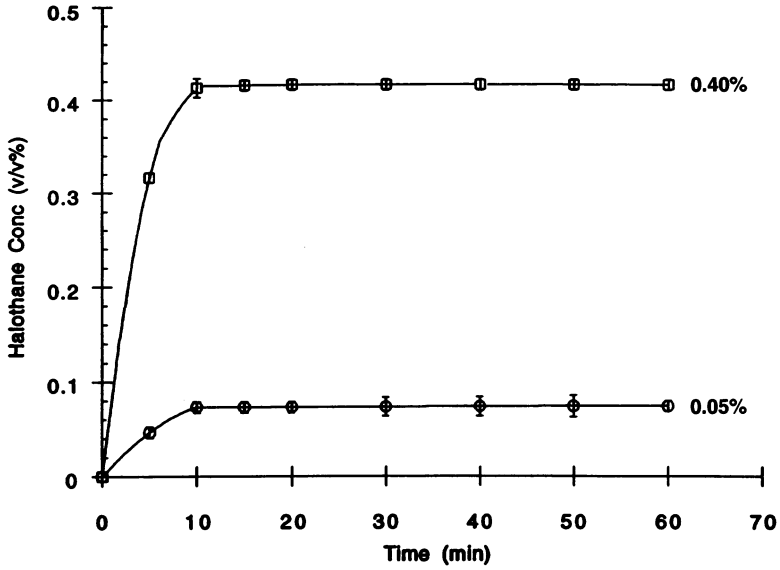

Fig. 2. Graph to show the equilibration times for halothane (0.05 and 0.40%) from 0 to 70 min. Equilibration is achieved within 10 min. Bar represents standard deviations.

suction to hold the fly in place during experiments. The hole in the base is made even smaller by sealing it off with filter paper and then punching another hole with a 14-mm gauge needle to allow only a small area of the midsection of the fly's thorax to be snugly held by suction. A gas stream of either pure air or a mixture of a given concentration of anesthetic and air is introduced through an inlet connected to the block. A set of 12 tiny holes, 0.015 in (drill size # 79) in diameter, located at the periphery of the block, and surrounding the fly, allow this stream to be delivered uniformly around the fly. At the beginning of an experiment, a fly is sucked up into a fire-polished glass tube from a pool of flies previously presorted in CO_2 48 h prior to experiments. The fly is then allowed to walk down the fire-polished glass tube onto the hole on the block. With the suction from a vacuum on, the fly is held down as it walks onto the hole. Using a pair of fine forceps to gently hold a wing, and during brief interruptions of the vacuum, the fly is properly positioned so that suction is applied to the ventral thorax. During this step, great care is taken so as not to damage the fly. Once the fly is properly positioned on the block, a glass cover (1.56 in diameter and 0.62 in high) is placed over the block. A 0.37-in opening in the middle of the glass chamber has been previously covered with a transparent microfilm (0.5×10^{-4} mm thick), that is secured with glue (Pronto™, CA9 Instant Adhesive). See section below on details of how to make the microfilm. The microfilm serves to prevent the escape of air or anesthetic from the preparation, and has two characteristics that make it useful for our purpose. First, its very thin and penetrable nature allows for easy microelectrode (tungsten wire, 0.005 in) piercing and placement at designated points on the fly. Secondly its transparency allows for unobstructed viewing of the fly during experiments. The glass cover tightly fits over the block, and rests on a cushioned rubber, making the whole ensemble an air-tight chamber. Clamps on both sides of the chamber allow us to rigidly stabilize the ensemble thus causing no disturbance during sampling. A sampling port on the glass cover allows us to withdraw 250 μl samples of anesthetic from the preparation with a gas-tight syringe (Hamilton, 1825RN) for analysis on the gas chromatography (Shimadzu, GC-9A) equipped with a Rtx-50 capillary column (Restek Corp Bellefonte, PA). Once the electrodes have been properly positioned on the fly, the suction is turned off so that the fly is not unnecessarily disturbed during experiments, and also to eliminate whatever variations the suction may contribute to the various anesthetic concentrations used. One side of the glass covering contains a gas outlet which is connected to a vacuum, and this permits waste gas from the experimental chamber to be expelled.

2.1. Making the microfilm

The film is specially prepared in the laboratory using 100 parts nitrate dope (Aero Gloss; Pactra), 30 parts n-amyl acetate (Sigma), 30 parts methyl isobutyl ketone (Sigma), 25 parts acetone (Fluke), plus 2.0 mg of cellulose acetate butyrate (Sigma; Fantham, 1979). These different proportions are mixed together in a glass container, and properly homogenized. The film is then prepared by pouring the solution onto the surface of clean water at room temperature in a shallow tank

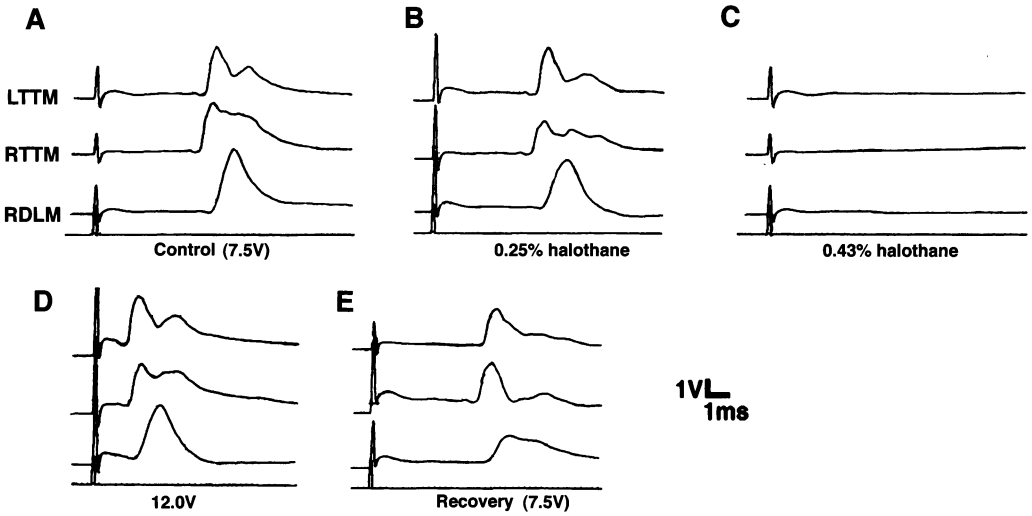

Fig. 3. Electrophysiological recording of muscle activities of the TTM, and the DLM of a Canton-S (Wuzburg) fly. (A) Normal long-latency response. (B) Response maintained in 0.25% halothane. (C) The long-latency response is completely abolished in 0.43% halothane. (D) Short-latency response is elicited only when the voltage is increased to 12.0 V and above. (E) Normal long-latency response is restored 45–50 min after washing in pure air.

or bath about five times the area of the required sheet. The solution is let to gradually run out of a 0.10-in (2.50 mm) hole from a glass pipette as it is drawn along close to the water surface. Film thickness is controlled by thinning the solution or changing the amount poured onto the water. After about 5–10 min, when the film should be fully polymerized and has stopped shrinking, it can be lifted from the water surface using a water moistened wooden frame laid gently on top of the film. The excess film outside the frame is gently folded over the edges of the frame, and lifted, starting from the far side of the tank to the other in one smooth action. After drying in air, the microfilm may be stored indefinitely. Excess film outside the frame can be simply torn away.

3. Results and conclusion

To determine the equilibration time of the chamber, anesthetic vapor was allowed to flow into the system at rates of between 2.0 and 3.0 ml/min for up to 70 min. During this period, 250 μl samples of anesthetic were withdrawn from the chamber and measured on the GC at different times from 5 min upwards. For each time point, the average of three withdrawals were used to calculate the anesthetic concentration following a given formula (Allada and Nash, 1993). To prepare specific anesthetic concentrations, a continuous flow of water vapor or anesthetic vapor was produced and maintained by bubbling compressed air through a fritted glass disc in an air-tight, 250 ml Pyrex glass bottle (Corning Inc.) containing water or liquid anesthetic. Using precaliberated glass flowmeters (size No. 0, GF-7060, Gilmont® Instruments), the flow rates of anesthetic and air can be adjusted to produce the desired anesthetic concentration. The resulting mixture of air saturated with water vapor and anesthetic vapor is allowed to flow into the anesthetic chamber. Fig. 2 represents the equilibration time for halothane. For this anesthetic, equilibration time within the chamber is 10 min, after which time its concentration in the chamber is steady for periods of more than 70 min. Thus once equilibration is attained, the concentration of anesthetic remained constant throughout the duration of an experiment. This shows that there are no significant leaks or absorption of anesthetic in the system. This condition also holds true when a fly is being monitored. Because of the very thin nature of the microfilm (0.5×10^{-4} mm thick), and very sharp microelectrode tips, the microfilm is penetrated without resistance. Although the electrodes make tiny holes on the film, the film does not crack, and the electrode plugs off the hole. While some leakage around the electrodes may be possible, observation through the microscope of the size of these holes in comparison with the size of the electrodes suggest that it must be very minimal. In fact, in control experiments, withdrawal of the microelectrodes following piercing of the film had no effect on measured anesthetic concentration.

In the absence of anesthetic, electrophysiological recording of muscle activities from the TTM and the DLM of fruit-flies placed in this system are comparable to those obtained in other laboratories as is shown in Fig. 3(A) (Wyman et al., 1984; Trimarchi and Schneiderman, 1993, 1995a,b,c). After ascertaining the presence of the long-latency response, the fly is equilibrated in a specific anesthetic concentration. In the fruit fly, we have shown that depending on the anesthetic concen-

tration, its effect on the percentage response was unchanged from 45 min to periods of up to 3–4 h. In these experiments, we have used 60 min as standard time for equilibration of a fly in anesthetic. The total time for a fly to attain equilibrium in halothane includes the time for the setup to equilibrate as determined above (10 min), plus 60 min. Thus after 70 min, the long-latency responses of both muscles in this animal are unaffected in 0.25% halothane (Fig. 3B), but is completely abolished in 0.43% halothane (Fig. 3C). However, a short-latency response is usually obtained by increasing the stimulating voltage up to 12.0 V (Fig. 3D). Complete recovery of these responses occurred after about 40–50 min of washing in pure air (Fig. 3E). This setup is therefore useful in experiments where there is the need to deliver anesthetics or other volatile agents in airtight environments to small animals or preparations.

Acknowledgements

The authors wish to thank Dr H.A. Nash for encouraging, discussing and proof-reading this manuscript. Our thanks to Brian Nicholson for all the help, discussion and valuable criticism at the early stages of this work. Thanks to Bill Dehn for constructing the glass chamber. Sincere thanks to Newlin Morgan and George Dold for working tirelessly in the construction of the electronics that go with the setup, and to Newlin Morgan for the accurate computer drawing of the setup.

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