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Influence of general anesthetics on a specific neural pathway in *Drosophila melanogaster*

(electrophysiology/synaptic transmission/halothane)

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ABSTRACT **The neural pathway that governs an escape response of *Drosophila* to sudden changes in light intensity can be artificially induced by electrical stimulation of the brain and monitored by electrical recording from the effector muscles. We have refined previous work in this system to permit reliable ascertainment of two kinds of response: (*i*) a short-latency response that follows from direct excitation of a giant fiber neuron in the interior of the fly brain and (*ii*) a long-latency response in which electrical stimulation triggers neurons in the optic ganglia that ultimately impinge on the giant fiber neuron. The general anesthetic halothane is reported here to have very different potencies in inhibiting these two responses. The long-latency response is obliterated at concentrations similar to those that cause gross behavioral effects in adult flies, whereas the short-latency response is only partially inhibited at doses that are 10-fold higher. Three other volatile anesthetic agents show a similar pattern. Thus, as in higher organisms, the *Drosophila* nervous system is differentiated into components of high and low sensitivity to general anesthetics. Moreover, this work shows that one of the sensitive components of the nervous system lies in the optic lobe and is readily assayed by its effect on downstream systems; it should provide a focus for exploring the effects of genetic alteration of anesthetic sensitivity.**

General anesthetics like halothane are simple, metabolically inert molecules that produce profound changes in the functioning of the nervous system. From the clinical perspective, the most important of these changes are those that lead to the loss of both voluntary and involuntary responses to painful stimuli and to amnesia for the events of the perioperative period. During clinical anesthesia, there are alterations in a large number of physiological and biochemical parameters of neural function (1). Many of these are also seen when general anesthetics are given to simpler organisms or to isolated preparations from the nervous systems of these organisms (2). An important goal for research on the mechanism of general anesthesia is to identify which of these anesthesia-induced changes is most directly responsible for the desired clinical effects.

One approach to this problem exploits genetic alterations of model organisms. In guinea pigs, mice, worms, and fruit flies, genetic variants have been isolated that alter the sensitivity of the animal to general anesthetics (ref. 3 and references therein). Typically, the assay for such studies is a gross behavior: maintenance of an upright posture, reestablishment of upright posture after perturbation, maintenance of normal motion, or purposeful movement in response to a noxious stimulus. While such assays may recapitulate features of the clinical state in humans, they also reflect very complex, usually unknown, neural circuits. As a result, it has been difficult to know how directly or indirectly a particular genetic change influences the anesthetic sensitivity of the assayed response. But, if genetic studies are to help in identifying the relevant targets of anesthesia, one needs to concentrate on those particular genetic changes that influence anesthetic response relatively directly, i.e., by altering a critical anesthetic target for the assayed behavior or at least a cell that contains such a target. One way to narrow the focus of genetic research is to replace gross behavioral assays by simpler measures of neural function. This strategy, however, is subject to the danger of oversimplification. At concentrations not much higher than those used clinically, general anesthetics can depress many cellular functions, including those not relevant to the clinical state (4). What is needed is an assay in a genetically malleable organism with a favorable balance of simplicity and sensitivity. In this work, we show that the neural pathway involved in the visual escape response of *Drosophila* has the desired combination and thus is an excellent tool for exploring the genetics of the anesthetic response.

MATERIALS AND METHODS

Fly Stocks and Drugs. Fruit flies from a stock of Canton-S (C-S), a standard wild-type strain, were raised on cornmeal/molasses/agar medium at room temperature (≈22°C) and transferred to fresh medium every 2–3 weeks. Flies that emerged from pupae were collected over a 48-hr period; males were selected under brief exposure to carbon dioxide, transferred to fresh food, and studied 2–8 days later.

The anesthetics used in this work (halothane, methoxyflurane, enflurane, and desflurane) were obtained as described (5) except that desflurane was purchased from Ohmeda PPD (Liberty Park, NJ). These agents are all volatile liquids; they were vaporized as described (6) and diluted with compressed air that had been humidified by passage through a cylinder of water to produce the desired concentration.

Electrophysiological Assay. Approximately 20 male flies were tapped from a food vial into a 10-cc disposable syringe. Following a design suggested to us by R. Wyman (Yale University, New Haven, CT), the surface of the experimental table contained a small hole (≈1 mm diameter) through which was applied a gentle vacuum. Individual flies were permitted to walk on top of the hole in the table from the tip of the syringe. With a fine forceps, the fly was maneuvered over this hole into a proper position for electrophysiological recording, i.e., with most of the legs splayed out so that suction held the thorax snugly against the table.

Using micromanipulators (Narishige, Tokyo), a stimulating electrode was placed in each of the compound eyes. Similarly, a ground electrode was placed in the abdomen and three recording electrodes were inserted through the dorsal cuticle: one into the fly's left tergotrochanteral muscle (TTM) and one each into a left and right dorsolongitudinal muscles (DLM).

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Abbreviations: TTM, tergotrochanteral muscle; DLM, dorsolongitudinal muscle; VER, visual escape response; S-L, short-latency; I-L, intermediate-latency; L-L, long-latency.

All electrodes were made from uninsulated tungsten wire (A-M Systems, Everett, WA) that was sharpened electrolytically; placement of recording electrodes was based on published stereotaxic maps (7, 8).

Stimulating voltage pulses, square waves of 0.1 msec duration, were generated with an electrical stimulator equipped with a optoisolator unit (A-M Systems model 2100). An AC amplifier (A-M Systems model 1700) was used to boost the signal from the recording electrodes 100-fold. As described (7, 9), these electrodes serve as pseudointracellular monitors of potentials from the impaled muscles. Both stimulating and recording voltages were monitored with a digital oscilloscope (Tektronix model TDS 420). Permanent records of the oscilloscope traces could either be printed immediately or processed by a pulse code modulator/digital recorder [Instrutech (Mineola, NY) model CRC VR-100B] for storage and subsequent playback on a VCR tape recorder.

After electrode placement, single stimuli of increasing voltage were applied at intervals of 5 sec until a threshold was reached for a muscle response, typically at a stimulating voltage of 2–5 V. Thereafter, the stimulating voltage was varied to establish a reliable protocol for eliciting responses of different latencies from the particular fly under study. Measurement of these latencies, taken from the end of the stimulating square wave to the beginning of the evoked response in the muscle, was made directly from the storage screen of the oscilloscope or from the printed output. Unless otherwise noted, mean values for latency are presented ± sem.

The frequency characteristic of electrically evoked responses was tested with trains of 10 evenly spaced stimuli. For the long-latency (L-L) response, a single train was used and the number of responses was recorded as the interval between stimuli was varied. For measurements of the short-latency (S-L) response, we determined the following frequency (FF50), the stimulation frequency (in Hz) at which a set of three stimulus trains, each train separated from its neighbor by 5 sec, produces a total of 15 responses (10). The refractory period of the S-L response, measured using a double-stimulus paradigm as the shortest interstimulus interval consistent with a double response, was also determined.

Measurement of the Effect of Anesthetics. After control experiments without anesthetic had established parameters of the various evoked responses, the fly and the electrodes were covered by an air-tight box (35 cm × 35 cm × 38 cm). Gas flow through this box was then set at 6–7 liters/min. A low flow of humidified air or anesthetic was also applied to the fly from below. The concentration of anesthetic gas within the box was measured by removal of 250-μl samples with a gas-tight syringe followed by gas chromatography as described (5). Anesthetics were applied to the fly for at least 45 min before testing for their effect on electrophysiology. For at least one concentration of each anesthetic, we showed that the effect was unchanged when the period before testing was lengthened to 120 min, indicating that our standard equilibration protocol was adequate.

Anatomical Location of Stimulating Electrodes. Histological sections of the head were examined for the imprint of the electrodes on brain tissue as follows. After electrophysiological recording, stimulating electrodes were gently withdrawn and the fly was immediately put in a cold (−20°C) vial. After storage at −70°C, the fly was mounted on a cutting disk with OCT embedding compound (Tissue Tek, Miles) and was transferred to the chamber of a cryostat (Leica Jung Frigocut model 2800E). The whole fly head was cut into 20-μm horizontal sections and the frozen slices were thaw-mounted onto gelatin-coated microscope slides. Slides were stained with 0.2% cresyl violet solution, washed with Dulbecco's PBS buffer, and then dried with increasing concentrations of ethanol. The slides were examined by conventional microscopy and photographs were taken of representative examples at ×600 magnification.

RESULTS

The Visual Escape Response (VER). A sudden decrease in light intensity is apparently perceived by the nervous system of many insect species as a sign of danger: a shift from light to darkness triggers a response in which key motor elements involved in escape are activated (11). While this escape or startle behavior can be observed grossly, the response can be more conveniently quantified by electrical recording from the relevant effectors such as the jumping (TTM) and flight (DLM) muscles. In *Drosophila melanogaster*, the appearance of characteristic potentials that signify activation of these muscles often follows a lights-off stimulus (12–14). More than 20 years ago, it was shown that electrical stimulation of the brain provides an even more reliable way to elicit this motor response (12). This and subsequent work (9) distinguished two variants of the response that occur with differing intensities of brain stimulation:

(*i*) At high stimulating voltages, spikes are recorded in the effector muscles ≈1 msec after stimulation, defining a S-L response. Work by Wyman and colleagues (9, 14) have established that this response follows when the electrical stimulus triggers a neuron in the brain of the fly that gives rise to a descending giant axon. Conduction down this giant fiber conveys the impulse to the thorax. Here, the giant fiber neuron makes electrical synapses with the motorneuron of the TTM and an interneuron (PSI), which in turn makes a chemical (acetylcholine) synapse (15) with the motorneuron of the DLM. Activation of the motorneurons presumably releases the transmitter glutamate to produce the characteristic spikes in the TTM and the DLM.

(*ii*) When stimulating voltages lower than those used to elicit the S-L response are used, the same set of muscle potentials appears but only after a longer delay (12). This response appears to use the same giant fiber, interneuron, and motorneuron elements as that for the S-L pathway (9). The lower stimulating voltage is thus deduced to be insufficient to trigger the giant fiber neuron directly. Instead, the electrical stimulus serves to activate neurons in the brain that indirectly innervate the giant fiber; the increment in latency relative to that of the S-L response presumably reflects the circuitry of these neural elements in the brain.

Parameters of Three Distinct Responses to Electrical Stimulation of the VER. Although the classical work divided responses to brain stimulation into two classes, individual nonshort responses actually have a wide range of latencies. Not all of these responses can be observed regularly but we find that three types of response are sufficiently reproducible to constitute a distinct experimental category, each identified by a characteristic latency. These are called L-L, intermediate-latency (I-L), and S-L responses; typical data from five individual flies are shown in Table 1. All three kinds of response appear to be the result of giant fiber conduction. In each case, all the muscles assayed from a single fly (two DLMs and one TTM) respond in concert to the stimulus, indicating that they are innervated through a common element. Moreover, in each case there is always a brief delay (≈0.4 msec) between DLM and TTM recordings, a hallmark of giant fiber transmission that reflects its innervation of the motorneurons of the DLM and TTM by disynaptic and monosynaptic pathways, respectively.

When recorded from the DLM, the mean latency for the S-L response is 1.43 ± 0.02 msec (n = 18). This response is the result of supramaximal stimulation, i.e., there is no change in latency with further increase of stimulating voltage. The S-L response has a following frequency (FF50) of ≈200 Hz for the DLMs and ≈250 Hz for the TTM. The refractory period is

Table 1. Latencies of different responses to electrical stimulation

	L-L		I-L		S-L	
Fly no.	DLM	TTM	DLM	TTM	DLM	TTM
1	4.35 ± 0.05	3.95 ± 0.05	2.23 ± 0.06	1.77 ± 0.12	1.47 ± 0.03	1.03 ± 0.03
2			2.35 ± 0.00	1.95 ± 0.00	1.40 ± 0.00	1.02 ± 0.03
3	4.00 ± 0.17	3.60 ± 0.17	2.17 ± 0.03	1.77 ± 0.06	1.42 ± 0.03	1.02 ± 0.03
4	4.05 ± 0.13	3.70 ± 0.17			1.43 ± 0.03	1.05 ± 0.00
5	4.52 ± 0.25	4.13 ± 0.23			1.37 ± 0.03	1.02 ± 0.03

For each of five C-S flies, measurements are shown for the left TTM and one DLM. Each entry is the average ± SD (in msec) of the latency recorded in three consecutive determinations at a given stimulus intensity. The latencies fall into three distinct categories: S-L, I-L, and L-L responses. Entries left blank represent cases in which the particular response could not be obtained.

between 5 and 7 msec for the DLMs and between 3 and 5 msec for the TTM. These properties are similar to previous observations on the S-L response (9, 10). We conclude that the S-L pathway that we observe is the result of direct electrical stimulation of the giant fiber neuron.

With proper electrode placement (see below), the L-L response is as reproducible as the S-L response. For the DLMs of different flies, the mean latency is 4.34 ± 0.06 msec (n = 18). For individual flies, this L-L response could be obtained reliably when tested at intervals over the course of several hours; during this time, the latency remained at a constant duration. In a fly displaying the L-L response, a S-L response could always be elicited by raising the stimulating voltage sufficiently. Thereafter, one could usually shift the response reversibly by suitable alteration of the stimulating voltage. A typical trace showing the two responses from the same fly is shown in Fig. 1. (In most experiments, we avoided unnecessary alternation between S-L and L-L responses to minimize the exposure of the fly to high voltages.) In addition to the latency, the other characteristic feature of the L-L response is the FF50; in contrast to the S-L response, the value for the L-L response is only about 1 to 1.5 Hz.

As mentioned above, the ability to achieve a L-L response is conditional. We first noted that, while virtually every preparation yielded a S-L response, some preparations did not yield a L-L response despite careful attempts to vary the stimulating voltage gradually. Later, we came to suspect that the difference depended on the way the stimulating electrodes were placed. To determine the location of these electrodes, we did the following exercise. After electrophysiological monitoring of the VER, stimulating electrodes were removed from the compound eyes and frozen sections were made of the entire head. We compared 41 flies from which we had obtained a L-L response (to avoid possible tissue damage from increased voltage, no attempt was made in these flies to record a S-L response) with 35 flies that failed to give a L-L response and showed a S-L response. As a control, we sectioned 30 flies that had not been pierced by stimulating electrodes. In these controls, several brain structures could be readily identified (e.g., retina, lamina, medulla, lobula, and central body); none of these showed a hole or track. In contrast, 17 of 41 L-L flies and 20 of 35 S-L flies had clearly defined electrode tracks. Fig. 2 shows typical sections from both L-L and S-L brains. All 20 of the traceable S-L flies had tracks that penetrated through the retina into the lamina of the optic lobe. In contrast, in all 17 of the traceable L-L flies, the electrode tracks left the pigment layer of the retina intact. Although the track of permanent damage may not be a perfect indicator of the location of the stimulating electrode or of the cells it fires, it appears that the L-L response depends on stimulation of neurons in the first optic ganglion.

Several flies had a response to electrical stimulation that was characterized by a latency intermediate between that for the S-L and L-L responses. As shown in Table 1, this response could not be elicited in all flies, even those that displayed a L-L

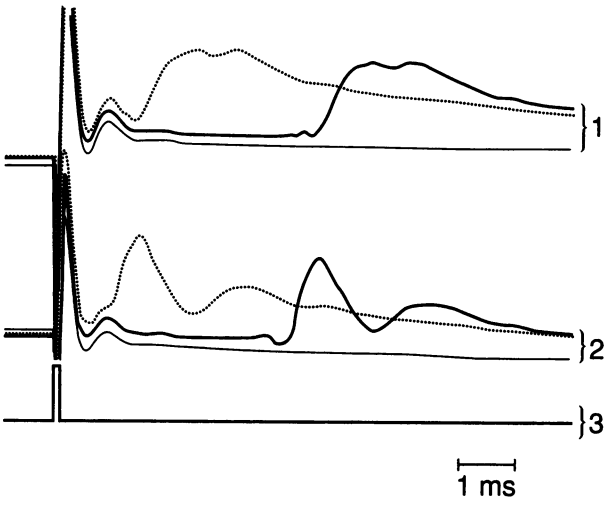

FIG. 1. Typical recordings of the L-L and S-L response. The trace of channel 3 marks the timing of the stimulating voltage, which is applied to the brain through electrodes implanted in the compound eyes of the fly. The traces of channels 1 and 2 show the potentials recorded from tungsten wire electrodes inserted through the cuticle of a C-S fly into one of the DLM and TTM muscles, respectively. In both cases, the potential is measured relative to a ground electrode inserted into fly's abdomen (see text for details). For each channel, three traces from the same muscle are shown. These include the S-L (dotted line) and L-L (thick solid line) responses to high- and low-voltage stimulation, respectively, as well as a control record (thin solid line) of a subthreshold stimulus.

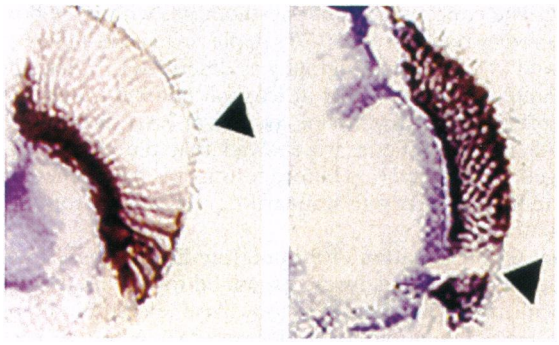

FIG. 2. Placement of stimulating electrodes associated with the L-L response. Shown at the left and right, respectively, are cresyl violet-stained horizontal sections of heads of flies from which the L-L response could be and could not be elicited. In both sections, the track made by one stimulating electrode can be clearly seen (arrowheads). Only one half of the sectioned brain is shown; typically, a track from the other electrode can be seen in a different section from the same head. Note that, although the electrodes routinely penetrate into the retinal layer, penetration through the pigment layer that underlies the retina into the lamina (optical lobe) distinguishes the fly from which a L-L response could not be obtained.

response. The I-L response was always generated by stimulating voltages that were lower than those needed for the S-L response and, where measurable, higher than those needed to elicit the L-L response. Moreover, even when the I-L response was obtained in one preparation, it often proved to be unstable and could not be elicited after a period of rest. While it persisted within a given fly, the length of the latency of the I-L response was constant (e.g., see Table 1) and the variation between flies was unremarkable; for the DLMs, the mean latency is 2.23 ± 0.04 msec (n = 18). We note that this value is similar to what has been described as a L-L response (9, 16). However, like us, other investigators have reported responses with latencies of 4 to 5 msec (12, 13). We believe that different placement of the stimulating electrodes underlies the differing outcomes. In our experiment, the following frequency for the I-L response varied from 30 to 100 Hz. The variability of the I-L response precluded a detailed study of the optimal electrode placement. However, the fact that, when present, this response had a latency and stimulating threshold intermediate between those of the S-L and L-L responses strongly suggests that the I-L response reflects electrical stimulation of a neural element that lies between the first optic ganglion and the giant fiber neuron.

The Effect of General Anesthetics on the Electrically Induced VER. The results described above permit us to use responses with different latencies and following frequencies to assess different segments of a hierarchical neural pathway responsible for the VER. To determine the effect of general anesthetics on these segments, we adopted the following paradigm. Individual flies were impaled with stimulating and recording electrodes without recourse to anesthetic. Electrical stimulation was gradually increased until a reproducible L-L response was ascertained. In agreement with the above description of the frequency characteristic for this response, Fig. 3 shows that the number of responses to a train of 10 stimuli is constant when the frequency of stimulation (the reciprocal of the interstimulus interval) is less than 0.5 Hz. In control experiments, this L-L response persisted with little or no change in threshold or following frequency when the fly was exposed to a flow of air for 2 to 3 hr. To assess the influence of anesthetics, instead of air the fly was exposed to a constant dose of a volatile agent and, after a suitable period of equilibration (usually 60 min), the search for a stimulation threshold was repeated. When concentrations of halothane between 0.4 and 2.0% volume were used, the L-L response could not be obtained. However, by increasing the stimulating voltage, some response could always be elicited. This was never the L-L response; in some cases, an I-L response was evoked and in all cases a S-L response could be obtained. When halothane was replaced by air, the L-L response recovered over a similar time span as used for drug exposure. When concentrations of halothane between 0 and 0.4% volume were used for the initial exposure, some L-L responses could be elicited. It is important to note that, although these responses were elicited at reduced efficiency, their latency was indistinguishable from that seen in the absence of anesthetic. The degree of inhibition by halothane is dose-dependent but frequency-independent at stimulating frequencies below 0.5 Hz (Fig. 3). We therefore routinely used an interstimulus interval of 2 sec to evaluate a complete dose-response curve. As shown in Fig. 4, the curve is very steep and is characterized by an EC_{50}, the dose that gives a 50% reduction in the number of L-L responses, of 0.142 ± 0.002% volume. Using the same approach, we evaluated the effect of three other volatile anesthetics on the electrically evoked VER. These proved to be similar to halothane in that exposure preferentially eliminated the L-L response, whereas the S-L response (and sometimes I-L response) could be obtained by increasing the stimulating voltage. Moreover, the L-L response was restored when the atmosphere of the flies was cleared of these agents. The dose-response curve for these anesthetics is also steep (Fig. 4). As measured by the EC_{50} value, their potency covers a broad range: 0.245 ± 0.005% volume for methoxyflurane, 0.710 ± 0.009% volume for enflurane, and 2.864 ± 0.001% volume for desflurane. In the *Discussion*, we consider how well these values correlate with other measures and predictors of potency.

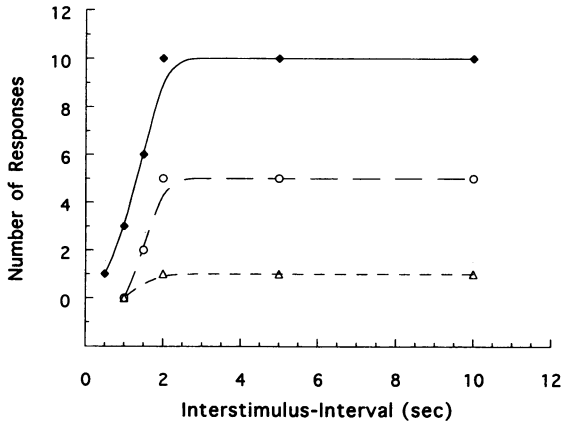

FIG. 3. Frequency characteristic of the L-L response in the presence and absence of halothane. The ordinate of each point gives the number of successful responses elicited by a train of 10 stimuli and the abscissa gives the temporal separation between each stimulus in the train. The three curves are the result of experiments with C-S flies tested in the absence of halothane (◆) or at concentrations of halothane of 0.14% volume (○) and 0.17% volume (△).

Because of the instability of the I-L response, we could not reliably assess the degree to which it was affected by concentrations of anesthetics higher than those that eliminated the L-L response. However, such a study was undertaken for the effect of halothane on the parameters of the S-L response: latency, following frequency, and refractory period (for details, see *Materials and Methods*). Briefly, latency and refractory period were unaffected by concentrations of halothane up to 2.5% volume. The following frequency of the S-L response was sensitive to halothane, but only to concentrations over 1.5% volume. As shown in Fig. 4, the dose response curve for this effect was much less steep than that for depression of the L-L response. Remarkably, following removal of high concentrations of halothane, the S-L response was restored. Thus, although halothane can induce reversible inhibition of the S-L pathway, this inhibition merely reduces the carrying capacity of the pathway and only occurs at 10 times the dose than that required to abolish the L-L response. Although we have not performed a similarly detailed study of the effect on the S-L response of the other agents described in Fig. 4, our preliminary data indicate that they are similarly ineffective. We discuss below the implications of these results for identifying the locus of an anesthetic-sensitive element in the brain of *Drosophila*.

DISCUSSION

In higher organisms, differential sensitivity of various parts of the nervous system is a hallmark of general anesthesia (1, 4). Indeed, anesthetics are useful clinical tools because the portions of the nervous system that control respiration and circulation are less sensitive to these agents than the neural elements involved in the perception of and response to painful stimuli. Differential sensitivity is revealed by electrophysiological recording techniques that show, for example, that a significant fraction of both endogenous and evoked electrical activity persists in the brain of patients during clinical anesthesia (17). The studies reported here show that the nervous

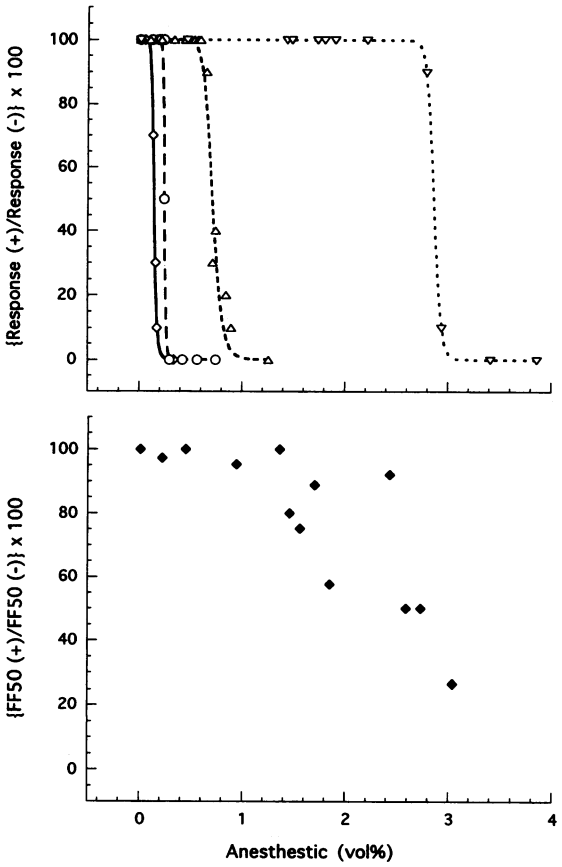

FIG. 4. Effect of anesthetic dosage on the L-L and S-L responses. (*Upper*) L-L response. The points give the number of successes with which 10 stimuli, each separated by a 2-sec interstimulus interval, elicit a motor response. Each value [Response (+)] is from a single fly and is presented relative to [Response (−)], the value determined before the administration of anesthetic; for the experiments illustrated here, this value was always 10. The different symbols show the effect on this ratio of different concentrations of halothane (◇), methoxyflurane (○), enflurane (△), and desflurane (▽). The dose response curve for each agent is fitted as described (5) to the formula:

$$R = 100 \cdot [1 - [D^s \div [D^s + EC_{50}{}^s]]]$$

where R is the response ratio, D is the anesthetic dose, s is a parameter that reflects the slope of the dose-response curve, and EC_{50} is the drug concentration that reduces the response ratio by half. (*Lower*) S-L response. The points (◆) relate a frequency characteristic of the S-L response to the concentration of halothane. Each value [FF50 (+)] is from a single fly and is presented relative to [FF50 (−)], the value determined for that fly before the administration of anesthetic; for the flies used here, this value ranged between 182 and 286 Hz.

system of *D. melanogaster* similarly comprises elements with high and low sensitivity to anesthetics.

Our laboratory has defined doses of the anesthetic halothane that render the fruit fly inert in a variety of behavioral tests (3, 5). At these concentrations, which are comparable to those used in clinical practice, some elements of the neuromuscular system of *Drosophila* must have ceased to function. However, we report here that the motor output portion of the visual escape response pathway is fully functional at these and even higher doses. This active portion includes several connected elements: conduction of an impulse along the giant fiber neuron that leads from the head to the thorax, transmission across electrical and chemical synapses that connect the giant fiber to motor neurons, and, finally, activation of neuromuscular junctions (14). Together, these elements contribute to the so-called S-L (≈1 msec) response to electrical stimulation of the brain. We find that this response can be recorded successfully at frequencies up to ≈200 Hz unless the halothane concentration is raised to greater than 1.5% volume, a concentration 10 times higher than that needed to interfere with postural control (5). Even at these high concentrations, the S-L response can be elicited, albeit at progressively lower frequencies. Our finding is reminiscent of an earlier report that reflex movements of the legs of the fruit fly in response to light are abolished only at concentrations of halothane much higher than that required to depress more complex optomotor responses of the head (18). It would be of interest to know if, at the high doses of halothane required to inhibit spontaneous movement of the nematode *Caenorhabditis elegans* (19, 20), other components of the worm's nervous system are still functional. In any case, the present results validate the use of *Drosophila* as a model system for clinical anesthesia by showing that, as in higher organisms, the nervous system of the fly is differentiated into high-sensitivity and low-sensitivity elements.

It is the high-sensitivity elements that one must use to study the neural response of *Drosophila* to clinical doses of anesthetics. Fortunately, one portion of the VER pathway contains at least one such element. This portion can be assayed when the electrical stimulation of the brain is insufficient to trigger the giant fiber neuron itself. Under these conditions, the response depends on innervation of some higher center, presumably one lying closer to the stimulating electrodes. Such stimulation produces a so-called L-L (≈4 msec) pathway, which we find to have a sensitivity to halothane like that displayed by *Drosophila* in behavioral assays of postural control (5). Although the data presented in this paper were collected with a single wild-type strain, Canton-S, we find that another wild-type strain, Oregon-R, has virtually the same parameters of the L-L response and, most importantly, virtually the same sensitivity to halothane. That the L-L pathway is generally more delicate than the S-L pathway is indicated by our finding that, in the absence of anesthetics, the carrying capacity of the former is only 1–2 impulses per second instead of 100–200 impulses per second seen with the latter. Confirmation of this view is provided by the report that a response with a latency of >2 msec is more sensitive to the combined effects of temperature and genetic manipulation of ionic current than is the S-L response (16).

Where is the anesthetic-sensitive element in the L-L pathway? Others have established that this pathway is activated when the electrical stimulus triggers a neural element that indirectly innervates the giant fiber neuron, which then uses the same circuitry to produce a muscle potential as that seen in the S-L pathway (9, 12). Since the latter is resistant to anesthetics, the sensitive component must lie upstream of the giant fiber. Our work has provided an important boundary for this question. We find that a response with a latency of ≈4 msec can only be obtained when stimulating electrodes are placed outside the optic lobe. This implies that the L-L pathway depends upon neural transmission through each of the optic ganglia: lamina, medulla, and the lobula complex. While the precise circuitry of the VER through these ganglia is not known, the morphology of the optic lobe has been well-studied (21) and one major input from them to the giant fiber neuron has been described (ref. 22 and references therein). An important goal for future research is to determine which one (or more) of these neural elements contributes to the anesthetic sensitivity of the pathway. Despite our ignorance of this issue, the present work localizes the search for an anesthetic-sensitive element to a few neural stations. The L-L VER thus provides an ideal substrate for distinguishing among mutations that influence anesthetic sensitivity (6, 23–25).

An important issue in anesthesia research is the uniformity of the mechanism of action of different volatile gases. While a traditional view held that all such agents influence identical targets in the same way, recent genetic and other studies suggest a more complex picture (3, 26–28). To provide a first

look at this question with the present assay, we studied three anesthetic agents whose chemical structure and physical properties are quite different from that of halothane. We find that, like halothane, these agents are more potent at inhibiting the L-L response than the S-L response. Thus, some portion of the visual system input to the giant fiber neuron contains a sensitive target to all four general anesthetics that we have tested. However, we do not know if the sensitive target for all these anesthetics is identical, i.e., the same molecular structure located in the same cell of the L-L pathway. Genetic studies, which are underway, should provide insight into this question but the following aspect of the existing data suggests that differences will be found. For halothane, enflurane, and desflurane, a similar proportionality constant relates the hydrophobicity of each compound to its potency as an anesthetic. In contrast, the proportionality constant for methoxyflurane appears to be distinct. This can be seen when the EC_{50} for each of these anesthetics, a measure of their potency, is multiplied by the relevant olive oil/gas partition coefficient (5), a measure of their hydrophobicity. The values of this product for halothane, enflurane, and desflurane are 0.47, 1.07, and 0.57, respectively, i.e., roughly equal to each other, but the comparable value for methoxyflurane is much higher, 4.3. Thus, when compared with the other agents, methoxyflurane is 4- to 10-fold less potent than predicted by its olive oil/gas partition coefficient. We have no reason to believe that the reduced potency reflects an unusual metabolism or distribution of this agent. Flies are equilibrated with a continuous supply of anesthetic so that any loss to metabolic processes (29) would be replaced by fresh gas. Furthermore, flies are preequilibrated with this anesthetic for over 1 hr before electrophysiological testing so that there should be ample time for equilibration of the gas with body fluids. If pharmacokinetics are not responsible for the unusual potency of methoxyflurane, perhaps this agent acts at a target whose hydrophobicity is different from the target of the other agents we have tested. Indeed, we note that in previous studies from this laboratory using behavioral assays, although the discrepancy is not as large as that seen in the present work, methoxyflurane exhibits a higher proportionality constant between potency and olive oil/gas partition coefficient than does halothane, enflurane, and desflurane (3, 5). Formally, these results can be described as showing that methoxyflurane deviates from the Meyer–Overton relationship between hydrophobicity and potency (1, 4). Although such a deviation may merely mean that a compound other than olive oil is the proper model for the hydrophobicity of the physiological target of all anesthetics, it is simpler to argue that different anesthetics have targets of differing hydrophobicity. We note that a similar deviation can be seen in data collected on anesthetic effects of methoxyflurane versus other volatile agents on the resting potential of spinal motor neurons of the frog (30). Perhaps focusing on a single neural element highlights a difference in anesthetic action that is obscured in complex whole-organism assays for anesthetic end points.

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