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Illinois. An automated system for stimu-
lating several flies individually in
studies of the proboscis extension reflex.

The proboscis extension reflex in *Drosophila melanogaster* (Vargo & Hirsch 1982b; Vaysse & Medioni 1973) has been used for studies of conditioning (Medioni et al. 1978; Medioni & Vaysse 1975) and of central excitation (Vargo & Hirsch 1982a).

In Medioni's research one fly is stimulated automatically. It walks on a rotating, vertically positioned kymograph drum carrying, on the surface, felt pads soaked with appropriate solutions to stimulate the fly's tarsal chemoreceptors. A limitation to this arrangement is that only one animal can be tested at a time, making tedious the collection of large data sets for genetic studies.

The Vargo & Hirsch (1982a, 1982b) study employed a technique whereby the stimulus solutions were delivered to the animals by hand. While this method was sufficient to demonstrate the existence of the central excitatory state, it caused concern because (a) experimenter fatigue could produce uncontrolled variations in the timing and manner of stimulus presentation and (b) differences in technique among experiments might affect results. Because of these concerns about the manual technique, we modified the Medioni apparatus to permit testing several flies together, thereby circumventing the problems of sample size and inter-experimenter reliability.

The equipment consists of a vertically positioned, electrically powered kymograph connected in series with a rheostat (used for adjusting rotational drum speed), a stereomicroscope mounted on an adjustable arm (for moving the microscope over an array of mounted flies), and an 18 inch fluorescent lamp (15 W) placed approximately 30 cm above the flies to illuminate the animals. The kymograph drum is made by bolting together from the inside two 16 cm diameter X 15.5 cm drums and spray painting them white (Figure 1).

Our major modification of the Medioni apparatus has been to use long stimulus strips (Whatman #3 filter paper) instead of felt pads and to place these strips obliquely across the drum. With oblique placement of the stimulus strips, flies positioned vertically along the side of the drum, one above another, encounter the stimulus strips at different times, allowing the experimenter time to observe the proboscis extension by each, one after another. The length of time that an individual fly walks over a strip (length of stimulus presentation) is dependent on (a) width of the strip, (b) drum rotational speed, and (c) the angle the stimulus strip is placed along the drum. Therefore, depending on the requirements of the paradigm to be employed, many combinations of these parameters may be used.

The stimulus strips (approximately 1 mm in height) are saturated with solutions held in 15 ml polyethylene reservoirs placed on top of the drum. Solutions are fed to the strips through capillary tubes, whose tip diameter controls the rate of flow. The saturated strips adhere to the drum surface and require no other means of attachment. The stimulus strips act as wicks and prevent solutions from spilling over onto other sections of the drum. However, if two strips need to be contiguous, leaking could occur. To prevent leaking, a rubber band is cut open, stretched along the boundary between the two strips, and secured around sheet metal screws at the ends of the drum. The stimulus strips, positioned on each side of the rubber band, are now nearly contiguous. To test the effectiveness of the barrier to leaching, the strip placed below the barrier was given distilled water, while the one above received dyed water. After one hour of observation, no dye was found on the adjacent undyed strip. Finally, a drip pan (19 cm in diameter with a 6 cm hole in the center) is placed under the drum to catch the fluid run-off.

Drosophila are mounted in micropipet tips (Vargo & Hirsch 1982a, 1982c). Wooden dowels (1/16 in diameter x 6 in) are inserted into the larger opposite ends of the micropipet tips. The dowels are secured in modeling clay molded around a support stand positioned next to the kymograph. The clay provides the flexibility in three dimensions to adjust the pitch, yaw, and extension of a pipet tip so that each fly is positioned as desired. Flies are positioned so that when their prothoracic legs are fully extended, the ends of their legs are approximately 1 mm above the drum surface. As the drum rotates and the strips approach each animal, the flies extend their legs and walk over the strips, thus stimulating the chemoreceptors on the tarsi.

With the above technique, individual flies positioned along the drum can be observed sequentially as the stimulus strips approach each fly. So far we have used this apparatus to simulate in *Drosophila* the paradigms for central excitatory state (Vargo & Hirsch 1982a, 1982b) and a modification of the excitatory classical conditioning paradigms used with *Phormia regina* (Hirsch & McCauley 1977; McGuire & Hirsch 1977; Nelson 1971).

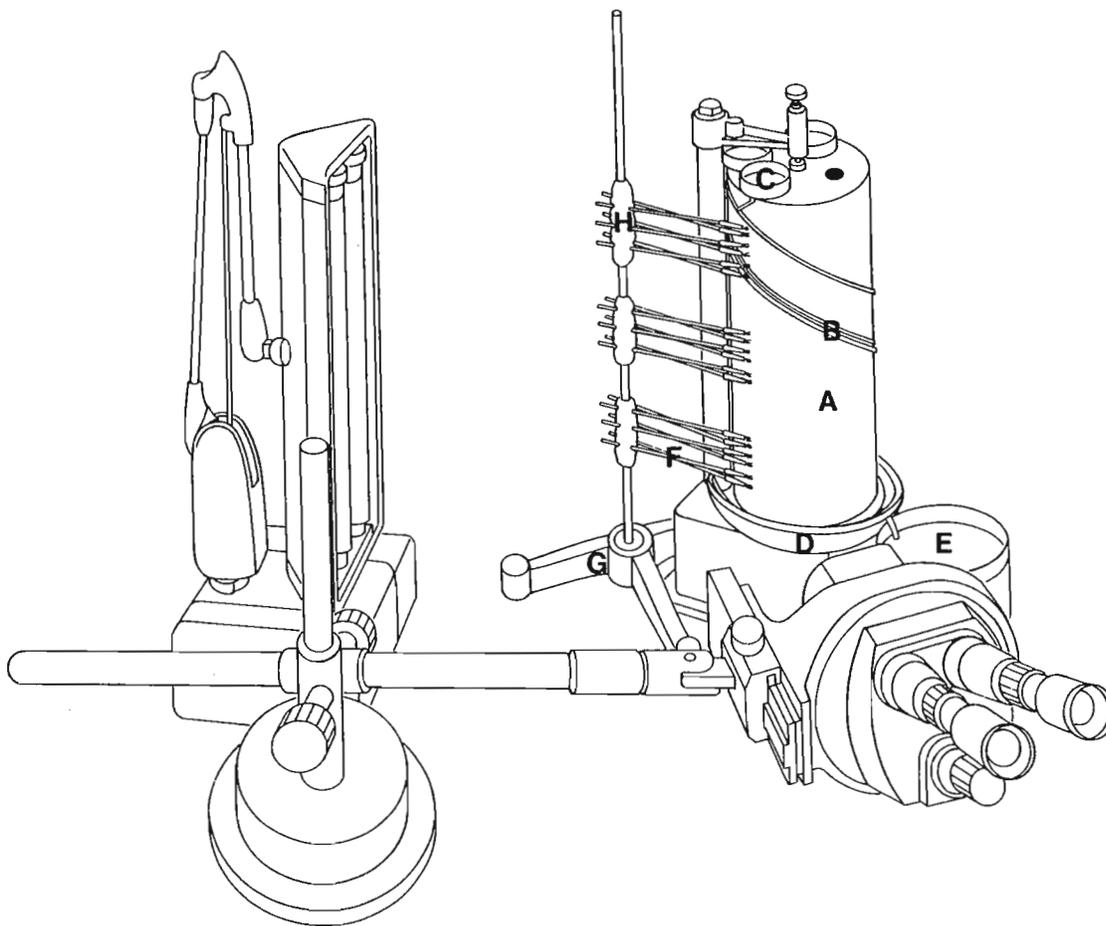


Figure 1. Basic equipment and their relative positions during a test. The kymograph is shown with the 2 drums (A) bolted together, stimulus strips placed obliquely across the drum (B), fluid reservoirs (C) placed on top of the drums, and the drip pan (D) which empties into a larger container (E). The dowel rods (F) are attached to the support stand (G) with clay (H). On the ends of the dowel rods are pipet tips containing the flies. The fluorescent light and stereomicroscope are also shown.

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