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An Olfactory Shuttle Box and Runway for Insects

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A runway and shuttle box are described for the study of aversive conditioning in insects. In both situations responses are recorded automatically and olfactory stimuli are presented automatically. The technique used to evacuate olfactory stimuli is tested with five carpenter ants. Results indicate that the technique appears viable. Application of the shuttle box to the problem of insect pest control is discussed.

Interest in insect learning as a subject of psychological research has been renewed during the past decade (Corning, Dyal, & Willows, 1973). Concomitant with this interest is the development of objective apparatus patterned after those used with vertebrates. These apparatus include runways for roaches (Longo, 1970), automated free operant-discrete trial apparatus for honey bees (Sigurdson, 1981a, 1981b) and choice situations for blowflies (Platt, Holliday, & Drudge, 1981). Automated aversive conditioning apparatus for ants (Abramson, Collier, & Marcucella, 1977; Morgan, 1981) and houseflies (Leeming & Little, 1977) are also available.

The apparatus here described is a modularly constructed shuttle box and runway capable of utilizing a variety of olfactory stimuli in conventional learning paradigms. The basic modular unit of this apparatus is the shuttle box. A shuttle box, patterned after the situation developed by Warner (1932), requires an organism to move from one compartment to another to escape or avoid aversive stimulation. This device has been successfully adapted for use with such diverse organisms as dogs (Solomon & Wynne, 1953), rats (Brush & Knaff, 1959), fish (Horner, Longo, & Bitterman, 1961), and houseflies (Leeming & Little, 1977). A precursor of the shuttle box had been used in the early 1930's (Hoagland, 1931) in the investigation of temperature on escape behavior in ants.

Shuttle Box Construction

The application of olfactory stimuli in a shuttle box requires that odors are directed to the appropriate location, and requires the elimination of unprogrammed interactions between qualitatively different odors. A shuttle box which incorporates these features is diagrammed in Figure 1. The shuttle box

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is constructed from a clear Plexiglas tube cut longitudinally into two pieces — an upper portion (7.5 cm x 3.7 cm x 2.4 cm) and a lower portion (7.5 cm x 3.7 cm x 2.4 cm) respectively. Construction in the upper portion consists of creating shuttle compartments, mounting response detection devices, and placing ventilation holes. The lower portion is divided into two separate but equal odor compartments that deal specifically with the application of olfactory stimuli.

The upper and lower portions are separated from each other by a fine mesh screen (1 mm thick), which serves as the floor of the shuttle compartments. The ends of both portions are drilled, tapped, and are used to connect other modules. They are also used to mount Plexiglas plates (3.2 cm x 3.2 cm x 3.2 cm) that keep the subjects within the apparatus and provide a medium on which to project stimuli. Stimulus projectors are mounted on the end plates to provide the experimenter with a variety of line orientations and colors which can be used as stimuli.

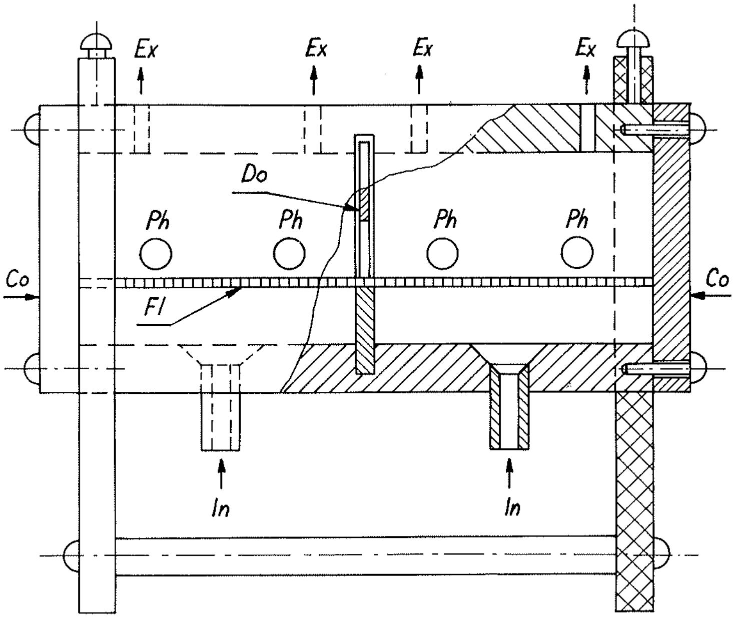

Figure 1: Lateral view of the olfactory shuttle box (Co: Plexiglas end plate, Ph: photocells, Do: sliding door, Ex: ventilation holes, In: odor input hole).

Construction of shuttle and odor compartments. The upper and lower portions are divided into two smaller compartments by a groove. The groove in the upper portion guides a sliding solenoid-operated door. Twenty gauge aluminum is placed in the corresponding groove of the lower portion. Olfactory stimuli enter the lower portion through a 7 mm diameter hole located in the center of each odor compartment. Elbow tubes, placed within each hole, provide a method of introducing odors.

In many experimental designs it is desirable for shuttle compartments to serve a dual function. For example, in experiments utilizing a two-way shuttle box procedure, aversive stimuli are presented in either compartment. This function can be performed in the present apparatus with the aide of *Y* tubes that conduct various odors and/or compressed air. In the present shuttle box odors are removed from the shuttle compartments by compressed air. The compressed air forces previously applied stimuli through a series of ventilation holes located in the ceiling (an experimental test of the validity of this technique is presented in the application section of this paper).

Monitoring the subject's position. To record automatically the location of a subject within the shuttle compartments, a series of four photo-emitters (G.E. led 55cf) and four photo-detectors (G.E. L14 g3) are placed in the base of the upper portion. The position of the subject in the single unit shuttle box is monitored by the inner pair of photocells, located 5 mm on either side of the center guide. When multi-modular shuttle boxes are used the outer pair of photocells are activated. A response is defined in both situations as an interruption in the beam farthest away from the subject at any given time.

An interesting Pavlovian technique applicable to a variety of subjects such as fish (Horner, Longo, & Bitterman, 1960) and monkeys (Harris, 1943) is general activity conditioning. Such conditioning involves the training of gross motor behavior rather than localized activity such as limb flexion or eye blink. To obtain a measure of general activity in the shuttle box, simply activate all four pairs of photocells.

In the current version of the shuttle box the inner pair of photocells are located 5 mm on either side of the center guide. This distance is satisfactory for large carpenter ants and honey bees. If smaller insects are used it is possible for the subject to bypass the photocells completely. This can easily be remedied by constructing additional photocell placements.

Constructing the housing. The completed shuttle box is slid into the housing shown in Figure 2. The housing consists of a solenoid enclosed between two rectangular pieces of clear Plexiglas (each piece measures 14 cm x 7.5 cm x .5 cm) spaced 6.5 cm apart. A 4 cm diameter hole in each piece allows the shuttle box to be slid into the housing, while set screws, located appropriately, secure its position. The solenoid (Guardian Electric TP6x12-

C24) is centered within the housing so that its armature, when activited, produces a smooth movement through the guide in the upper portion.

Utilizing a housing has several advantages over the more traditional method of attaching peripheral equipment directly to the shuttle box structure. For example, all heads from the emitters and detectors can be soldered onto a 16 pin IC socket. This socket, mounted in front, provides easy access to control equipment. The housing also provides a convenient method of interconnecting modular units.

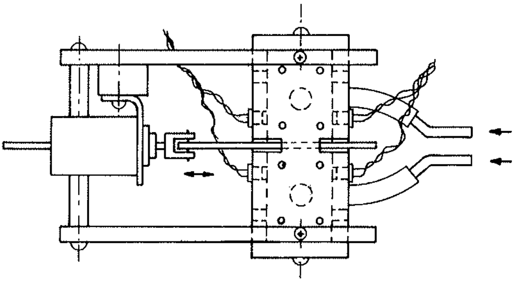

Figure 2: Overview of the olfactory shuttle box placed within a housing. The housing contains a solenoid used to operate the sliding door.

Runway construction. The most widely used situation developed for the study of vertebrate and invertebrate learning is the runway. The response reinforced in the runway is locomotion. The measure of performance is time.

The present runway, diagrammed in Figure 3, is constructed from a .6 cm thick, 15.3 cm long, clear Plexiglas tube. It is similar in construction to the shuttle box. The Plexiglas tube is sliced longitudinally to form an upper portion (15.3 cm x 3.7 cm x 2.4 cm) and a lower portion (15.3 cm x 3.7 cm x 2.4 cm). Construction in the upper portion consists of mounting response detection devices and placing ventilation holes. Construction in the remaining portion consists of locating odor input holes. Both portions are separated from each other by a screen which serves as the floor of the runway. The ends of both portions are drilled and tapped, and are compatable with the individual shuttle box modules.

The position of the subject in the runway is monitored by two pairs of emitters and detectors located 5 mm and 10 mm from both ends of the tube. The placement of response detection devices in these locations in concert with those established in the shuttle box permit the recording of start box, alley, and goal box latencies.

Three olfactory input holes located in the lower portion are spaced every 3.8 cm. To prevent the interaction of olfactory stimuli when multiple modules are used, it is necessary to insert aluminum partitions in the lower portion.

The start and goal boxes are formed with individual shuttle box modules. Their size can be adjusted to 7.5 cm or 3.7 cm.

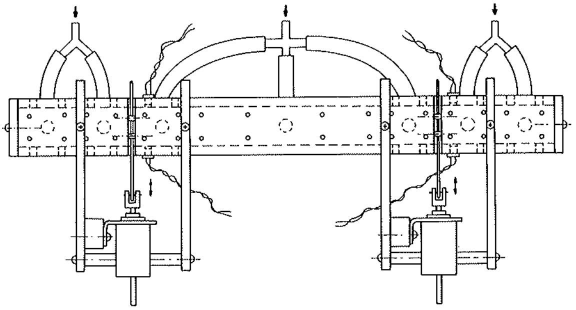

Figure 3: Overview of the modularly constructed olfactory runway. The start and goal boxes are created out of individual shuttle box modules. The sizes of the start and goal boxes can be manipulated by adjusting the location of the housing. Start and goal box latencies can be recorded by appropriately locating the photocells and the photoemitters.

Applications: Shuttle Box

Runways have become a standard apparatus for the study of invertebrate learning. Data obtained from this apparatus suggest, for instance, that harvester ants are sensitive to partial reinforcement schedules (Fleer & Wyres, 1963) as well as the pattern of reinforcement and nonreinforcement (Ramos, 1966). Shuttle boxes, however, have seldom been used in the study of insect learning. While several versions exist (Hoagland, 1931; Leeming & Little, 1977), they are not capable of using a wide range of stimuli; they are also rather crude in design.

The purpose of the experiment reported here is to obtain data on the efficiency of the evacuation technique used to eliminate odor after-effects. When odors are presented rapidly in an area as small as a shuttle box, olfactory residues may influence performance long after the stimulus has been terminated. Performance may be influenced, for instance, during the intertrial interval (ITI). To investigate this possibility, ITI responding was manipulated by varying the rate of peppermint odor presentation. It is assumed that the strength of potential residues will be greatest at shorter ITI values. It is also assumed that potential residues will be greatest in a descending series of ITI values.

Five carpenter ants (*Compontus p.*) were housed individually with food and water freely available. Each was tested in a shuttle box (7.5 cm x 3.7 cm x 2.4 cm) under escape procedures similar to that used by Keller (1940). An occurrence of a shuttle response in the presence of peppermint odor was followed by: (1) the termination of the odor, (2) initiation of an intertrial interval, (3) onset of compressed air, and (4) restoration of the peppermint odor at the end of the ITI. An occurrence of a shuttle response during the ITI was without experimental consequence. The session, conducted under general illumination, was terminated after 20 minutes. The rate of peppermint odor was manipulated by varying the length of the ITI. Four such intervals were used in descending order: 100 sec, 50 sec, 25 sec, 100 sec (return to baseline), and 12.5 sec. Subjects received 6 sessions under each condition.

Figure 4 shows the mean number of responses which terminated the odor, and the mean number of ITI responses for all sessions. As inspection of this figure indicates, rate of peppermint odor does not increase ITI responding ($F = 1.81$ with 4 and 16 df, $p < .05$). Though responding is not sensitive to changes in the intertrial interval, responses which terminated the peppermint stimulus are ($F = 8.63$ with 4 and 16 df, $p < .05$). The results indicate that odor after-effects do not significantly influence performance.

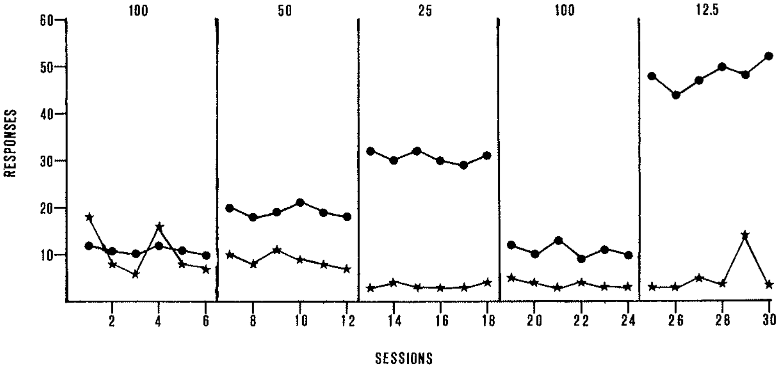

Figure 4: Mean number of intertrial interval responses (stars) and mean number of responses which terminates a peppermint odor (dots), plotted for all subjects across conditions. In condition 1, an occurence of a shuttle response in the presence of peppermint odor was followed by 100 seconds of peppermint free air; in condition 2—50 seconds; in condition 3—25 seconds; in condition 4—100 seconds; in condition 5—12.5 seconds.

The shuttle box appears to be a viable technique for the study of aversive conditioning in ants. Yet, one might question the importance of this technique. In these times of economic strife and diminishing grant support one can surely find better ways to expend energy. This rhetorical question can be satisfactorily answered by appealing to historical concerns of the comparative analysis of learning and the practical concerns of insect pest control.

Historically, the comparative analysis of learning has focused upon the construction of automatic data recording devices and objective control of structured environments. Comparative analysis has also been devoted to the extension and clarification of learning principles which can be generalized throughout the phylogenetic scale (Bitterman, 1975). Comparative analysis of avoidance learning suggests that vertebrates, such as rats and dogs, learn avoidance behavior instrumentally. Avoidance behavior in fish, however, appears to be governed solely by principles of classical conditioning.

Do ants and other invertebrates learn avoidance behavior? If so, is the process based on instrumental conditioning, classical conditioning, or a combination of the two? At present there are no clear answers to these questions. Avoidance experiments are described in the literature. However, they are typically poorly controlled demonstrations conducted without regard to parametric manipulations of variables known to influence avoidance performance.

The search for general laws of learning is interesting and necessary. The primary concern of the present apparatus design, however, is the application of avoidance paradigms to the problem of insect control. If insect pests exhibit learned avoidance behavior, perhaps the ability of some pests to escape erradication may also be learned. While it is beyond question that natural selection is the underlying mechanism of insecticide resistance (i.e., Grayson, 1951; Lindquist & Wilson, 1948; Melander, 1914); it can be argued that the typical method of insecticide testing fails to take into account the behavior of individual organisms. Screening methods vary from company to company, but usually potential insecticides are tested against insects of economic importance, both by spraying and by incorporating the chemical in the insects' food. The effectiveness of the chemical is measured as the LD_{50} value — the dose required to kill 50% of the population of test insects (Cremlyn, 1978). This method is clearly inadequate if avoidance behavior is to be measured. Chauvin (1969) has noted that the insecticide Dieldrin®, while effective in erradicating the fire ant under laboratory conditions, has failed miserably in the field. This is by no means the only example of insecticide failure. The typical response to such failures is to increase the dosage or the frequency of treatment until the chemical is no longer effective. A substitute is then found. The effect of such blatant ignorance has been poignantly chronicled by Rachel Carson (1962).

Laboratory experiments already suggest that avoidance learning may be responsible for the failure to control cockroaches (Ebeling, Wagner, & Rierson, 1966). It is also known that both harvester ants (Abramson, 1981) and carpenter ants (Martinsen & Kimmeldorf, 1972) are capable of passive avoidance learning. While the passive avoidance experiments represented a beginning to the objective analysis of avoidance behavior, no techniques were available for the detailed study of aversive conditioning The apparatus reported here will fill this void and provide an alternative approach to insect control, an alternative which includes behavioral as well as biochemical data.

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