Antistatic Foam as a Shocking Surface for Behavioral Studies with Honey Bees (Hymenoptera: Apidae) and American Cockroaches (Orthoptera: Blattelidae)[1]

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Abstract The application of electric shock is useful in the study of the learning process in vertebrates and invertebrates. In most behavioral studies, electric current is delivered with a shock grid. When applying electric current to a terrestrial invertebrate the use of shock grids is problematic. An alternative used for many years is the "resistive-sheet," an asphalt and graphite mixture painted on a nonconductive surface such as plastic. The resistive sheet has several limitations, including difficulty in applying the mixture uniformly and uneven distribution of current. This article advocates replacing the resistive sheet with antistatic foam. Antistatic foam is used to prevent damage to integrated circuits and circuit boards due to static electrical charge. The foam is easily manipulated, standardized, and with the addition of two wires delivers consistent current across its area. The efficacy of antistatic foam as a shocking surface was examined in two experiments. In the first, escape behavior in honey bees, *Apis mellifera* L., was examined by placing two groups of bees (n = 26) in a two compartment shuttle box. One group received 25 V AC shock that terminated when the bee crossed into the opposing compartment. A control group did not receive any shock. Results showed that bees in the shock group crossed into the opposing chamber significantly more often than the no-shock group ($t = 6.796$, df = 24, $P < .001$). In the second experiment, a contraction response was tested in a group (n = 10) of American cockroaches, *Periplaneta americana* L. Each roach was individually placed in the apparatus and received 1 min no shock (baseline), 1 min (1 s 50 V AC every 10 s) shock, and 1 min baseline. Roaches contracted significantly more often in the shock condition than in either of the baseline conditions ($F = 10.049$, df = 2,18, $P = .001$). These results demonstrate that antistatic foam can be used to create a shocking surface suitable for investigating behavior in insects.

Key Words Shocking surface, resistive sheet, honey bees, *Apis mellifera* L., cockroaches, *Periplaneta americana* L., insect learning, insect behavior

The use of aversive stimuli, such as electric shock, contributes to the understanding of the learning process in both vertebrates and invertebrates (Mackintosh 1983, Smith and Abramson 2003). Aversive stimuli not only reveal aspects of the learning process, but are often the only viable alternative to motivate some invertebrates to

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perform in learning experiments (Abramson 1994, Bitterman 1966). The ability of cockroaches, for example, to survive without food and water for 30 d led Longo (1964) to use electric shock in his studies of maze learning in roaches.

The most widely used method of applying electrical current to non-aquatic invertebrates has been some variation of what is called the "resistive-sheet." It is an asphalt and graphite mixture painted on a nonconductive surface such as plastic (Longo et al. 1961) and was originally developed for studies of cockroach learning (Longo 1964). More recently, the resistive sheet has been used to study the effect of pre-exposing roaches to shock prior to performing an escape task. Roaches pre-exposed to inescapable shock perform poorly when given a subsequent chance to escape shock (Brown et al. 1999). Pre-exposing honey bees, *Apis mellifera* L., to electric shock prior to learning an avoidance task was also found to retard learning (Abramson and Bitterman 1986).

When attempting to use the resistive sheet in this laboratory, several limitations were encountered. The mixture used to create the resistive sheet, for example, is difficult to standardize. Unfortunately, when the asphalt-graphite mixture has been used in published experiments, the exact proportions of the ingredients were not reported. Moreover, the asphalt-graphite mixture frequently chips and is not easily replaced. The mixture is also difficult to uniformly apply to a surface. And, traditional shock grids have been found to be impractical when cockroach excrement short circuits the grid (Longo et al. 1961). These problems are eliminated with the use of antistatic foam.

Longo et al. (1961) noted an additional problem with the resistive sheet in that an animal will receive varying amounts of current depending on where it stands on a resistive sheet. They solved the orientation problem by the addition of a "choke" system or by the use of 8 transformers. Both systems produced a rotating electric field and were conceptually similar to repeatedly changing the polarity of shock grids (Dinsmoor 1966). These problems with the resistive sheet can be eliminated with the use of antistatic foam.

Antistatic foam is widely used to prevent a static electrical charge from damaging integrated circuits and circuit boards. The foam is commercially available in sheets or rolls varying in thickness from 3 to 7 mm, which are available in assorted colors and are readily obtainable from a host of suppliers. The foam is standardized and is easily manipulated and replaced.

We demonstrate herein the usefulness of the foam in two experiments. In the first, honey bees were confined to a shuttle box and escape behavior investigated. In the second, the contraction response of American cockroaches, (*Periplaneta americana* L.), in response to shock was measured.

Materials and Methods

Insects. For Experiment 1, European worker honey bees, *A. mellifera,* were selected at random as they departed a laboratory hive between 0630 and 0730 h. They were tested approximately 24 h later. Bees collected in this way display a mixture of different behavioral specializations that require either departure from the hive (i.e., foragers or nest cleaning bees) or remaining near the entrance (i.e., guards). They were captured individually in glass vials and taken to the laboratory where the vials were placed in an ice water bath. No attempt was made to determine the age of the subjects.

When the bees became inactive to permit handling, they were removed from the vials and secured in individual restraining harnesses constructed from 0.38-caliber shells with a small strip of duct tape placed between the head and thorax and attached to the sides of the harness. Once harnessed, subjects were fed a 1.8 M sucrose solution until satiated. They remained overnight and were used in testing the next morning.

For Experiment 2, American cockroaches, *P. americana,* were obtained from Ward's Natural Science (Rochester, NY) and housed in 37.85 L glass containers. Two days prior to the experiment, roaches between 2.5 and 3 cm in length were housed individually in plastic containers. They were given unlimited access to food and water. On the day of the experiment, they were placed individually in the testing apparatus. No attempt was made to determine the sex of the subjects. All subjects were tested during the light phase of a 12:12 h light/dark cycle.

Apparatus. In both experiments, a rectangular-shaped section of antistatic foam (3 mm thick × 27 cm long × 5 cm wide) was used. Two parallel 18-gauge wires were embedded in the underside (i.e., the smooth side) of the foam. The shock source was a variable transformer. The voltage level was adjusted for each of the species so that an escape or contraction response was detected.

In Experiment 1, the foam was placed inside of a two-compartment shuttle box (Abramson et al. 1982) the dimensions of which were identical to the foam (27 cm long × 5 cm wide). Each compartment measured 13.5 cm × 5 cm. The bees escaped shock by moving from one compartment to another. To prevent the animals from climbing on the roof of the shuttle box, a false ceiling was placed in such a way that the bee always remained in contact with the foam.

In Experiment 2, cockroaches were placed in an identical apparatus, but only one chamber was utilized. The dependent variable was contraction. Contraction was used rather than shuttling in order to determine if excrement would short circuit the foam. We also investigated the contraction response as a dependent variable in anticipation of performing Pavlovian conditioning studies in harnessed cockroaches.

Prolonged electrical stimulation can cause sustained involuntary muscle contractions. Pilot testing was done with voltages ranging from 5 to 100 V AC to determine at what voltage roaches respond to shock without eliciting involuntary muscle contractions (Holt 2002). Most roaches did not respond to less than 35 V AC, while at levels of more than 80 V AC, many showed an involuntary muscle contraction lasting for the duration of the shock. Roaches showing such a contraction appeared to be "frozen" in place and their legs contracted and released when shock was applied. In order to avoid the sustained muscle contraction, a shock voltage was selected that allowed the roaches to move. Roaches utilized in pilot testing were not used in the experiment.

Procedures. In Experiment 1, 26 honey bees were captured and harnessed during summer and fall of 2002 and divided into experimental (n = 13) and control groups (n = 13). On the morning of training, an individual harnessed bee was un-harnessed and placed into the shuttle box. Once in the shuttle box, the bee received a 5 min adaptation period, after which the first trial was presented. For bees in the experimental group, a trial began with the onset of a 25 V AC shock and terminated when either 90-s elapsed or the bee escaped into the opposite compartment. Following termination of shock, a 60-s intertrial interval was imposed. At the end of this interval the next shock trial began. The performance of the experimental group was compared to a control group treated the same way as the experimental subjects, but no shock was given. The dependent variable was the number of times the animals crossed into the opposite chamber.

In Experiment 2, 10 cockroaches (*P. americana* L.) were tested during the winter of 2002. The experimental design consisted of 1 min of baseline (no shock), 1 min of shock (1 s 50 V AC burst every 10 s), and 1 min of no shock (return to baseline). The dependent variable was contraction. Each cockroach was introduced into the shuttle box for a 10 min adaptation period. Following the adaptation period, the first phase of the experiment began.

Results and Discussion

Honey bee escape behavior was analyzed with an independent samples t-test. Results showed bees that received shock crossed into the opposing chamber significantly more times than their matched controls did in the same amount of time (t = 6.796, df = 24, $P < .001$ Fig. 1). Roach contraction behavior was analyzed with single factor within-subjects ANOVA, with shock as the single factor. Results show significant differences between shock and each baseline condition (F = 10.049, df = 2,18, P = .001). Roaches contracted significantly more often in the shock condition than in the no shock conditions (Fig. 2).

These results demonstrate that antistatic foam can be used to create a shocking surface suitable for investigating behavior in insects. Honey bees receiving shock shuttled to the opposite compartment more often than did their matched controls. Cockroaches also contracted more often during a shock condition than during either of two baseline conditions. These experiments were not designed to study learning *per se,* but to show that antistatic foam can be used to administer electrical stimulation. We have also used the foam successfully as a shocking surface for earthworms, *Lumbricus terrestris* L. (unpubl. data).

When using antistatic foam, we recommend embedding wires in the underside (i.e., smooth surface) to ensure equal voltage distribution throughout the shocking area. The initial data on substrates without embedded wires showed that current declined rapidly as the length of the antistatic foam increased. This creates a problem because animals will orientate to areas of low voltage. We also recommend that when securing electrodes from the shocking surface to the foam, one terminal should be

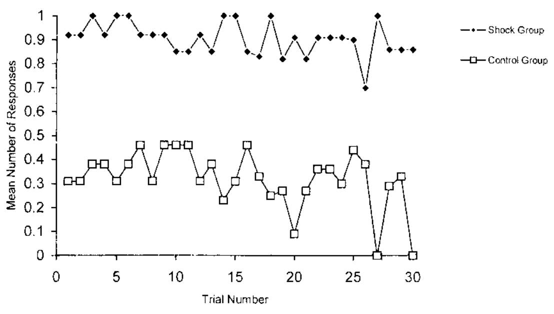

Fig. 1. Mean number of responses for 13 honey bees that received shock and for 13 matched controls over 30 trials.

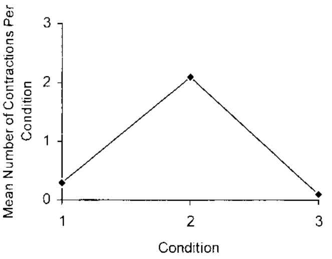

Fig. 2. Mean number of contractions for 10 cockroaches in three conditions: 60 s of baseline (no shock) followed by 60 s of shock (1 s burst every 10 s), and a 1 min return to baseline.

placed on each of the "short sides" (i.e., opposite ends) of the rectangle rather than side by side. Placing the terminals on the length of the rectangle provides a consistency in voltage across the length of the antistatic foam.

Standardization, ease of application, and consistency make antistatic foam superior to the resistive sheet. The antistatic foam substrate is easy to create and can be fashioned into shapes useful for many types of organisms, including the American cockroach (*P. americana*), *Paramecium caudatum* E., and honey bees (*Apis mellifera*) (Abramson and Bitterman 1986, Armus and Montgomery 2001, Longo et al. 1961). Traditional invertebrate learning apparatuses that can be fitted with antistatic foam include mazes, runways, and shuttle boxes (Abramson 1994).

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