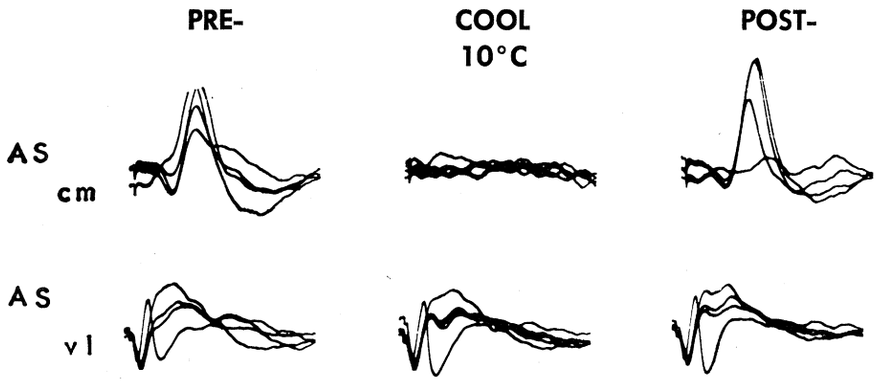

Fig. 2. Superimposed oscilloscope recordings showing the differential effects of cryogenic blockade of the inferior thalamic peduncle (ITP) on recruiting (top) and augmenting (bottom) responses. These recordings were all taken from the same locally anesthetized cat preparation immobilized by gallamine triethiodide (Flaxedil). Recruiting responses elicited by stimulation of the animal's left nucleus centrum medianum (*cm*) were recorded contralaterally on the right anterior sigmoid gyrus (*AS*) and are shown in the upper row of traces. Augmenting responses elicited by stimulation of the right nucleus ventralis lateralis (*vl*) were recorded at the same site as the recruiting responses and are shown in the lower row of traces. The effect of reversible cryogenic blockade is shown by the responses during precooling (*PRE-*); cooling of ITP (*COOL 10°C*), 3 minutes after the onset of cooling; and postcooling (*POST-*), 3 minutes after the cessation of cooling. Calibrations: 200 μv and 20 msec = 5 mm.

duncle (ITP), which is located just medial to the internal capsule in the rostral forebrain area, completely abolishes recruiting responses but does not affect augmenting responses (*2*) mediated by fibers in the adjacent part of the internal capsule (*10*) which are less than 3 mm lateral to the ITP. Thus, the blocking effects of subcortical cooling are limited to tissue within a few millimeters of the cryoprobe tip when its temperature is 10°C. When the temperature of the cryoprobe tip was lowered to 0°C, augmenting responses were partially reduced in amplitude, suggesting that cooling had begun to spread to the internal capsule.

These physiological results, which are in agreement with the results of other investigators, based upon temperature measurements within the brain, show that subcortical cooling produces limited blockade of neural tissue restricted to the vicinity of the cryoprobe. By adjustment of the temperature of the cryoprobe tip, the volumetric size of the blocked tissue can be regulated. With 0°C cooling, which produces completely reversible effects, the region of functional blockade tends to be a spheroid of about 3 mm radius around the cryoprobe tip.

In freely moving animals with chronically implanted electrodes and cryoprobes, the effects of cooling upon behavioral and electrophysiological responses have been studied concurrently (*2*). Blockade of the thalamo-orbitocortical system by cooling at ITP not only blocked the electrocortical activity mediated by this system but it also disrupted learned behavioral tasks; cessation of cooling of ITP restored electrocortical and behavioral activities to precooling levels within approximately 3 minutes.

Thus the applicability and effectiveness of this cryogenic cooling system recommends it for a variety of studies where reversible functional blockade of neural structures and pathways is desired in either acute, or unrestrained, chronic animal preparations.

JAMES E. SKINNER
DONALD B. LINDSLEY
Departments of Psychology and
Physiology, University of
California, Los Angeles 90024

References and Notes

1. M. Dondey, L. Rey, J. Le Beau, *Rev. Neurol.* **103**, 220 (1960); L. Weil, J. Chanteur, M. Dondey, *Compt. Rend.* **252**, 2947 (1961); V. H. Mark, J. C. Chato, F. G. Eastman, S. Aronow, F. R. Ervin, *Science* **134**, 1520 (1961); M. Dondey, D. Albe-Fessard, J. Le Beau, *Electroencephalog. Clin. Neurophysiol.* **14**, 758 (1962); I. S. Cooper, F. Grissman, R. Johnston, *St. Barnabas Hosp. Med. Bull.* **1**, 11 (1962); D. G. Stuart, L. H. Ott, F. C. Cheshire, *Electroencephalog. Clin. Neurophysiol.* **14**, 132 (1962); R. Byck and P. Dirlick, *Science* **139**, 1216 (1964); F. Proctor and H. Landsbergen, *Electroencephalog. Clin. Neurophysiol.* **18**, 91 (1965).
2. J. E. Skinner and D. B. Lindsley, *Brain Res.* **6**, 95 (1967).
3. I. S. Cooper, *St. Barnabas Hosp. Med. Bull.* **1**, 1 (1962).
4. EutecRod Co. solder and flux No. 157.
5. Adams Intramedic polyethylene tubing PE 100 (0.85 mm inside diameter and 1.50 mm outside diameter) and PE 50 (0.58 mm I.D. and 0.95 O.D.).
6. P. Dirlick and R. Byck, *Rev. Sci. Instr.* **33**, 1472 (1962).
7. Burr-Brown Co. model 1505B or Data Device Corp. model D-16.
8. Data Device Corp. Picopower Comparator Relay model PPC-1.
9. M. Dondey, D. Albe-Fessard, J. Le Beau, *Electroencephalog. Clin. Neurophysiol.* **14**, 758 (1962); R. Byck and P. Dirlick, *Science* **139**, 1216 (1964).
10. K. Mechelse, *J. Hirnforsch.* **5**, 408 (1962).
11. This research was supported by U.S. Navy contract Nonr-4756, NASA grant NGL 05-007-049, and the Mental Health Training Program (PHS grant MH-6415). We acknowledge with appreciation the helpfulness of Dr. R. Byck, Albert Einstein College of Medicine, New York, whose laboratory we visited in December 1965.

10 June 1968

Alarm Pheromone in the Earthworm Lumbricus terrestris

Abstract. *Noxious stimulation of the earthworm* Lumbricus terrestris *elicits secretion of a mucus that is aversive to other members of the species, as well as to the stimulated animal when it is encountered later. This alarm pheromone is not readily soluble in water and retains its aversive properties for at least several months if not disturbed. Its influence may be responsible for some features of the data on instrumental learning in earthworms.*

The earthworm *Lumbricus terrestris* secretes a mucus apparently from segments over the full length of its body. It has been suggested that this mucus acts as a lubricant when the animal moves through its burrow and, by binding soil particles together, prevents the burrow walls from collapsing. It also has been noted that the mucus probably acts "as a buffer system outside the body since it is secreted in large amounts when the animal is immersed in a noxious stimulant such as acid" (*1*, p. 46).

We have observed that handling, pinching, and severing the worm, as well as stimulating it with electric shock, results in a copious secretion of mucus. Furthermore, we have found that, unlike the effect of the mucus secreted by an undisturbed worm, the mucus produced in response to such noxious stimulation is highly aversive to other members of the species. Thus, the substance has the properties of an alarm pheromone (*2*).

The present report describes two experiments that demonstrate the aversive properties of the mucus secreted in response to electric shock. In both experiments, worms were exposed to Plexiglas plates (7.5 by 7.5 cm) that had been treated in three different ways: (i) plates on which mucus had been

secreted by a worm repeatedly stimulated with electric shock (the shock plates); (ii) those on which worms had been allowed to crawl without being exposed to electric shock (the nonshock plates); and (iii) clean plates on which no worm had crawled (the control plates). Two days before their use in an experiment, all plates were cleaned by scrubbing them with surgical gauze under hot tap water and then rubbing them dry. They were kept at room temperature [72° to 75°F (22° to 24°C)] under a dust cover after they were cleaned.

Worms obtained from a local supplier of bait were group-housed in waxed cardboard containers on boiled newsprint bedding and kept in a darkened refrigerator at 40° to 45°F (4° to 7°C), except where otherwise noted. The day before an experiment, animals weighing from 3.5 to 7.5 g were removed from the group containers and transferred individually to covered 3-oz. (88-ml) polyethylene cups. Those to be used as subjects were returned to the refrigerator with some bedding material in their cups and were not disturbed again until the experiment the following day. The others, to be used for tracking the plates, were cleaned of all visible bedding material by allowing them to crawl over a sheet of clean wet newsprint before being slid into clean polyethylene cups without bedding. An effort was made to disturb these animals as little as possible in the process of cleaning, and they were left in the refrigerator for at least 1 hour before being used to track the plates.

A plate randomly selected for the shock condition was positioned within a frame, and a worm, also selected at random from those cleaned beforehand, was transferred to the plate by slowly inverting the cup. A plastic cylinder, 10.5 cm in diameter and 10 cm high, was then positioned around the plate to confine the worm, and the inverted cup was removed. For the next 3 minutes the animal was given a shock of approximately 1-second duration every 5 seconds by touching it simultaneously with a pair of electrodes connected to an Applegate stimulator set for a shock intensity of 0.15 ma. At the end of the 3 minutes the cylinder was lifted and the worm was allowed to crawl off the plate. In each case, worms receiving the shock left a viscous deposit of mucus that turned white when it dried on the plate.

Plates and corresponding worms randomly assigned to the nonshock condition were treated in exactly the same way as those assigned to the shock condition, except that the worms were never shocked or touched with electrodes. Control plates were left undisturbed until the day of the experiment. In experiment 1, 12 subjects randomly assigned to each condition were transferred from the polyethylene cups to 1-oz. paper cups 1 to 5 hours before testing. For each trial, a paper cup containing a worm was placed upright in a holder below which one of the stimulus plates was positioned. Surrounding the stimulus plate was a large clean sheet of waxed freezer paper. The cup-holder was attached to a long rod that passed through a small hole in an opaque screen. An experimenter sitting behind the screen slowly inverted the cup by rotating the rod and caused the worm to slide about 2.5 cm onto the plate. The worms were never touched during the trials, they were exposed to the treatments in a randomized sequence, and the experimenter inverting the cups was kept blind with regard to the sequence. Illumination came from a 7-watt red bulb 1 m above the stimulus plate. The latency from the worm's first contact with the plate to the movement of its entire body from the plate to the waxed paper was recorded by a second experimenter.

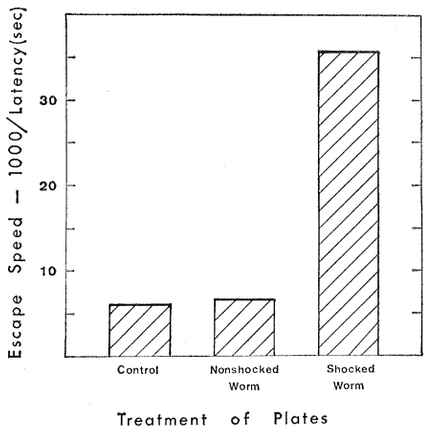

Fig. 1. Mean escape speed as a function of treatment of the stimulus plates.

The escape latencies were transformed into escape speeds [1000/latency (in seconds)] in order to normalize the distributions and equate variances for an analysis of variance. The analysis yielded an F-ratio of 8.22 (d.f. 2/33; $P < .005$). The mean escape speeds for the three groups are shown in Fig. 1. Worms escaped significantly faster from the plates on which other worms had been shocked than from clean plates or from plates on which worms had been allowed to crawl without shock. Escape speeds for the latter two conditions did not differ significantly.

In experiment 2, 24 worms were exposed in a two-choice situation to shock plates paired either with nonshock plates or with clean control plates. The apparatus was in the basic form of a T-maze. A transparent Plexiglas tube, 20 cm long and having an inner diameter of 1 cm, formed the stem. Both the floor and far wall of each end arm of the T consisted of a Plexiglas plate treated on the day before the experiment in the same way as those described for experiment 1. An opaque screen was positioned between the starting end of the stem and the stimulus plates. The experimenter introducing the worm into the stem was kept blind with regard to the arrangement of conditions on any trial. For 12 subjects the shock plates were paired with nonshock plates and for 12 subjects they were paired with control plates. For half the subjects in each case the shock plates were on the left and for the other half they were on the right. Illumination during experiment 2 came from fluorescent ceiling fixtures. Stimulus plates and stem sections were used for only one subject before they were scrubbed with gauze under hot water, dried, and allowed to return to room temperature.

To begin each trial, the anterior end of a worm was inserted into the stem by hand and, after the worm was released, a pair of flexible leads, 1.2 cm apart, was applied lightly to the dorsal surface of the worm about 2.5 cm from its tail. In order to provide a controlled stimulus for movement toward the choice point, a shock of 0.15 ma and 0.75-second duration was passed through the worm between these leads when the worm's prostomium reached a point 5 cm from the starting end of the stem. The experimenter holding the shock leads on the subjects was also kept blind with respect to the conditions of the trial. Two other experimenters observed the subject when it emerged from the tube and recorded which end arm it crossed. Crossing an end arm was defined by the worm's prostomium reaching beyond the far edge of the stimulus plates.

None of the 24 worms crossed the end arm on which a worm previously had been shocked. Fourteen subjects crossed the alternate end arm—six out of 12 when it consisted of nonshock plates and eight out of 12 when it con-

sisted of control plates. The remaining ten subjects either backed into the stem after emerging one or more times and remained there until the predetermined 3-minute cutoff time for a trial was reached or, after reaching the choice point, backed completely out of the apparatus through the starting end of the stem. In each of these ten instances the subject's prostomium had made contact at least once with a part of the shock plate. The probability that of the 14 subjects that did cross an end arm none would cross the shock side on the basis of chance is less than .001 (binomial test).

The surface on which the substance is secreted does not appear, within some limits at least, to be critical to its effectiveness. We have seen it act effectively on aluminum, stainless steel, paper, and soil, as well as on Plexiglas. However, the substance is most effective when dry, and thus when it is deposited on a dry surface. It is not readily soluble in cold water. A tracked surface immersed in cold water appears to lose little, if any, of its effectiveness after it has been allowed to dry. Also with regard to the persistence of its effects, we have found an undisturbed deposit of the substance apparently as potent more than 3 months after secretion as it was a few hours after secretion and even more potent than when still wet immediately after it had been secreted. This is in marked contrast to the relatively short effective duration of pheromones released by insects in the air or by fishes in water (*2*).

Effects of the alarm pheromone may be responsible for certain features of the data on instrumental learning in earthworms. Strong negative reactions to unidentified stimuli in a T-maze commonly have been observed when electric shock has been used to punish incorrect responses (*3*). Our findings suggest that these may be responses to deposits of the alarm pheromone left in the maze on previous trials and either spread or only partially removed by the procedures used for cleaning the apparatus (*4*). The effect that such deposits would have on choice data would depend upon the cleaning procedure used and upon whether shock was applied on the same side of the maze for all subjects or was varied; even if it was varied, it would depend upon whether the same subject received several trials in a row or was tested in rotation with other subjects. Different combinations of these conditions could lead to either rapid appearance, slow and irregular appearance, or no appearance of data that would be interpreted as evidence of learning.

In any case, our data show that without any opportunity for learning an earthworm can display a tendency to avoid an area where an aversive event previously has occurred. As a result, firm conclusions regarding whether earthworms can learn a maze response, or at the least, what the characteristics of the learning process are, should await a test under conditions in which the effects of the alarm pheromone unequivocally have been eliminated.

ROBERT H. RESSLER
ROBERT B. CIALDINI
MITCHELL L. GHOCA
SUZANNE M. KLEIST
Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee

References and Notes

1. M. S. Laverack, *The Physiology of Earthworms* (Pergamon, Oxford, 1963).
2. E. O. Wilson, *Science* **149**, 1064 (1965); W. Pfeiffer, *Experientia* **19**, 113 (1963).
3. A particularly graphic description of these negative responses to unidentified maze stimuli is given by J. S. Robinson [*J. Comp. Physiol. Psychol.* **46**, 262 (1953)]. See also R. M. Yerkes, *J. Anim. Behav.* **2**, 332 (1912); L. Heck, *Lotos* **68**, 168 (1920); H. Schmidt, Jr., *Science* **121**, 341 (1955); D. K. Zellner, *Physiol. Behav.* **1**, 151 (1966).
4. Procedures for cleaning apparatus, when reported, have included rinsing it with water, wiping it with dry cotton wool, changing a paper floor covering between trials (but walls and ceiling were not covered), and wiping the apparatus with a paper floor covering that had been used on the previous trial. Our observations of the relative insolubility of the alarm pheromone and its tendency to adhere to surfaces on which it has been deposited raise considerable doubt about whether any of the cleaning procedures previously described have successfully eliminated the substance from all parts of the apparatus.
5. This research was conducted while S.M.K. was an undergraduate research participant under NSF grant GY-2639.

27 May 1968

Discrimination of the Odor of Stressed Rats

Abstract. *Albino rats can reliably distinguish between the odors of stressed and unstressed rats. Five animals learned to interrupt an ongoing response when air from the cages of stressed rats was introduced into the test compartment, and to continue responding when air from unstressed rats was introduced. The discrimination does not seem to depend on recognition of odors of individual rats.*

Although alarm pheromones have been identified in a number of species (*1*), their presence in mammals is yet to be established. For a substance to serve in intraspecific communication, it must first be capable of discrimination from other similar odors; however, the fact that it is distinguishable in an experimental situation does not imply that it serves any function in the normal life of the organism. Our study was designed to determine if male albino rats will discriminate between air from the vicinities of stressed (S-air) and unstressed rats (U-air) by interrupting an ongoing bar press when S-air is presented.

Six 80-day-old Sprague-Dawley rats were trained to press a bar in an animal compartment (Lehigh Valley) whose normal air intake had been closed. Air (U.S.P. grade) passed at a measured rate of 600 ml/min from a cylinder, over an unstressed rat isolated in a Pyrex desiccator, and then through a delivery tube leading through the bottom of the animal compartment. Whatever might be common to the odor of all rats was thereby part of the compartment atmosphere; whatever differences test air samples introduce would thereby be accentuated. Test samples of air were introduced into the delivery tube from a 50-ml syringe (Fig. 1). A weight-driven device (not shown) emptied the syringe at a flow rate matching that of the air cylinder.

Stimulus air was sampled from the vicinity of animals in individual living cages from which food pellets and water bottles had been removed. Polyethylene sheet, secured by magnets to the sides and back of the cage, sheathed each cage to within 2 cm of the top. Any excrement remained on the sheeting just under the wire-mesh bottom of the cage. Thirty-millimeter samples of air were taken by insertion of a 9-cm needle to its full length through the sheeting into the center front of the cage immediately under the cage bottom. Three different syringes were used in mixed order for sampling U-air obtained from undisturbed rats, and another set of three for sampling S-air, drawn from the cage of a rat which had just received several 1-ma shocks with a probe to the flanks. Emission of two or three typical "alarm cries" (*2*) was the criterion of stress.

Animals for a given session were brought in their living cages from the colony housing room into the training room. To keep stressed and unstressed animals in equivalent locations in the room, we suspended all cages in the