Levels of leukocyte arylsulfatase B (*6*) and acid phosphatase (*8*) were determined as previously described. The mean value (± standard error) of arylsulfatase B activity in 15 controls was 90 ± 13.6 nmole of nitrocatechol released per hour per milligram of protein as compared with 94 ± 14.9 nmole in the six patients. Similarly, the mean value of acid phosphatase activity among the controls was 74 ± 6.9 nmole of *p*-nitrophenol released per minute per milligram of protein, whereas in the preparations from patients the mean was 79 ± 9.9 nmole.

It is appropriate to consider the relation of arylsulfatase A with the accumulated product in MLD, that is, sulfatide. Studies by Mehl and Jatzkewitz (*9*) suggest that arylsulfatase A is the heat-labile, nondialyzable factor in their preparation, capable of hydrolyzing cerebroside sulfate (sulfatide). Sulfatide may then be the natural substrate of arylsulfatase A, as recently reported (*10*), and nitrocatechol sulfate represents the artificial substrate that was used in the earlier characterization of this class of enzymes.

Arylsulfatase activity has been detected principally in the granulocyte series and only to a slight degree in lymphocytes. It is not detectable in red blood cells or platelets (*5*). In suspected cases it is therefore essential to determine the white cell and differential counts in peripheral blood to control for low activity that might vary with the white-cell population. In the patients examined in this study, the number and distribution of white cells were within the normal range. Therefore, the decrease in arylsulfatase A activity in the leukocytes of patients with MLD is indicative of an enzymatic insufficiency in this condition. Leukocyte preparations from the parents of three patients with MLD were also tested. The values obtained for arylsulfatase A and B did not differ significantly from those that were obtained in the control population.

This assay should provide a simple and reliable method for the diagnosis of MLD. From these studies, however, it is not possible to delineate heterozygous carriers of this abnormality.

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9 AUGUST 1968

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Reversible Cryogenic Blockade of Neural Function in the Brain of Unrestrained Animals

Abstract. *Reversible cryogenic blockade of neural systems in the brain may be accomplished by local cooling. A small cryoprobe is described which may be implanted in the brain of an unrestrained, behaving animal. Cooling may be restricted to the tip of the cryoprobe and the temperature of the tip and the shaft may be regulated and monitored independently by miniature thermocouples and appropriate control and indicator devices. Electrophysiological results are presented which show that the cryogenic blocking effect may be localized to within a few millimeters of the tip of the cryoprobe and that the size of the region blocked is proportional to the tip temperature. The system described has been shown to be effective in both electrophysiological and behavioral studies.*

An important experimental approach to the study of the brain and its role in behavior has been to destroy parts of it and observe deficits, malfunctions, or aberrant behavior. Destruction of brain tissue has been accomplished by ablation, thermocoagulation, high-energy ionizing radiation, focused ultrasonics, and local application of chemical and radioactive substances. However, lack of functional reversibility poses a serious constraint upon behavioral study.

Among methods capable of reversibility are (i) use of local anesthetics, (ii) production of spreading depression by KCl, (iii) polarization by direct current, and (iv) application of local pressure, heating, or cooling. Each of these methods has disadvantages when used to block localized subcortical structures, and especially so in freely moving animals. There may be difficulties in application, control of degree and duration of effectiveness, and reversibility. Restricted local cooling of brain tissue appears to be the most precisely controllable and completely reversible method available (*1*). Functional blockade of electrophysiological and behavioral activities has been demonstrated in cats by cooling to 10°C (*2*); complete recovery of function occurs even after cooling to −10°C and without evident pathologic damage (*3*).

Cryogenic methods previously used for reversibly blocking restricted subcortical structures have presented technological and application problems in chronic, behaving, animal preparations. These, in part, have been resolved by developments reported here: (i) provision to localize cooling at the tip by heating the shaft of the probe, (ii) reduction of probe size, and (iii) use of flexible, light-weight connections to the freely moving animal.

The cryoprobe is constructed of 24 and 18 gauge (0.55 and 1.2 mm), stainless steel, hypodermic-needle tubing soldered together (*4*) to form a concentric, double cannula system (Fig. 1C). A thin, insulated, low-resistance heater-wire (0.025 mm in diameter) is wrapped around the outer cannula except for 2 mm at the tip. Alcohol (95 percent ethanol or methanol), cooled in a dry-ice and alcohol bath, is circulated by pressure through the probe, and direct current is passed through the heater-wire warming the shaft to body temperature while the tip remains cold. The degree of cooling, determined by the flow rate of the coolant through the cryoprobe, is controlled by the pressure in the steel reservoir tank (Fig. 1D). This in turn is determined by a variable pressure regulator (0 to 400 psi) (0 to 27.2 atm) attached to a nitrogen gas pressure source. The cold alcohol is carried to and away from the probe in

the animal by thin (0.85 mm inside diameter) polyethylene tubes (*5*) which remain flexible at low temperatures. A high-pressure fitting for the input coolant hose (f_w, Fig. 1C) can be made by wrapping 28 gauge (0.31 mm) stainless steel wire around a piece of polyethylene tubing (0.55 mm inside diameter) after it is slipped over a piece of 24 gauge (0.55 mm) inner cannula tubing (*6*).

Microthermocouples (0.12 mm in diameter), whose copper-constantan junctions are cemented on the tip and shaft of the cryoprobe, are used for monitoring temperatures during adjustments in the alcohol flow rate and heater current. Once these adjustments are made, the temperatures remain relatively stable.

For temperature measurements at the monitoring junctions on the probe the constantan portion of each junction is connected by constantan wire to the constantan portion of the reference junctions. The two reference junctions are located in the frontal sinus. The copper portion of each reference and monitoring junction can therefore be connected by copper wire to the recording apparatus, eliminating the necessity of copper and constantan connecting wire and special connectors. The small voltage generated between the copper wire of the reference junction and the copper wire of the monitoring junction in the case of each thermocouple is approximately a linear function (40 μv/ °C) of the temperature difference between the monitoring and reference junctions. Thus the temperature of the monitor junction can be determined when its respective reference junction is at a known temperature. The reference junctions for the shaft and tip thermocouples are implanted in the animal's frontal sinus and cemented to the skull with dental acrylic, thus serving as a body-temperature reference.

The small thermocouple voltages generated can be amplified with an economical d-c operational amplifier (*7*) and read on a meter (Fig. 1B) or recorded. For safeguarding the animal a comparator relay system (*8*) continuously monitors the shaft temperature and shuts off the heater current (Fig. 1A) if the shaft temperature should rise above body temperature, which could happen if the alcohol coolant hoses accidentally became detached.

Selected cooling of portions of the cryoprobe shaft can be accomplished by distributing separate heater coils along the probe. In this way different levels along the length of the probe can be heated or cooled, selectively, as desired.

Previous studies have shown a restricted temperature gradient around a cold cryoprobe tip inserted in a brain at 37°C or in agar-agar medium (*9*). Restricted localization of cooling is demonstrated in Fig. 2 which shows that a cryoprobe with a 10°C tip and 37°C shaft implanted in a thalamocortical fiber tract in a cat's brain completely blocks the cortical responses mediated by that system, but does not affect conduction in another fiber tract only a few millimeters away. Cryogenic blockade of the inferior thalamic pe-

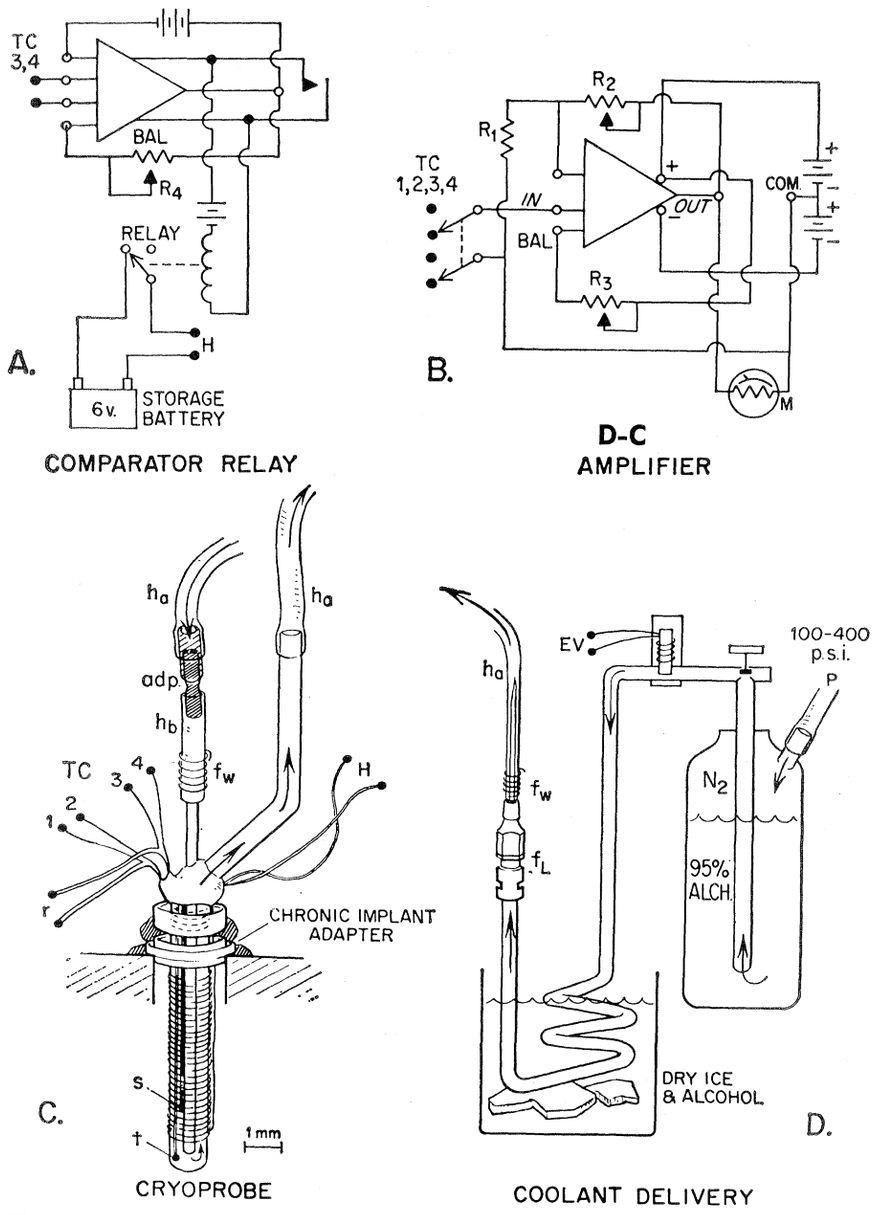

Fig. 1. Complete cryogenic system. (A) Comparator relay: *H*, heater connections. R_4, 25-turn, 50-kohm Trimpot balance resistor; *RELAY*, power relay with 4-amp contacts; *TC*, shaft thermocouple connections. (B) D-C amplifier: *M*, 2-kohm, 50 microammeter; R_1, 1 kohm; R_2, 25-turn, 100-kohm Trimpot [amplifier gain = $(R_1 + R_2)/R_1$]; R_3, 25-turn, 50-kohm Trimpot balance resistor. (C) Cryoprobe: *adp.*, adapter from 18 to 22 gauge, (O.D., 1.2 mm to 0.7 mm); f_w, wire fitting; *H*, heater-wire leads; h_a, polyethylene coolant hose, PE 100 (0.85 mm I.D., 1.50 mm O.D.); h_b, polyethylene coolant hose, PE 50 (0.58 mm I.D., 0.95 mm O.D.); *r*, reference junctions for thermocouples; *s*, shaft thermocouple junction; *t*, tip thermocouple junction; *TC*, thermocouple leads (all copper). (D) Coolant delivery: *EV*, electric valve; f_L, Luer-lock fitting; f_w, wire fitting; h_a, polyethylene coolant hose, PE 100; *P*, rubber pressure hose from a variable pressure regulator.

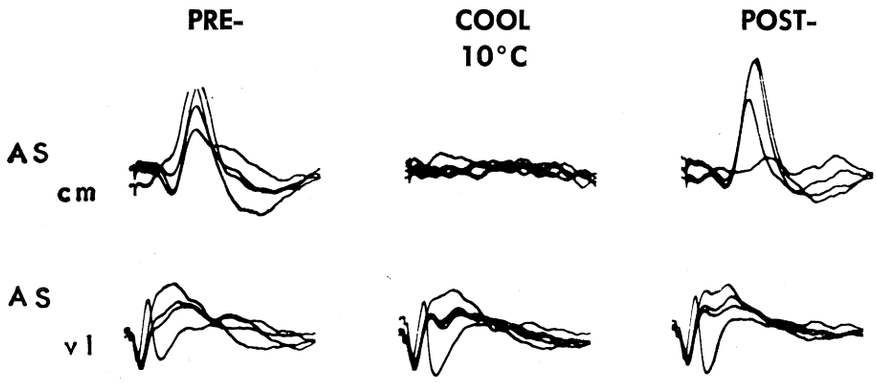

Fig. 2. Superimposed oscilloscope recordings showing the differential effects of cryogenic blockade of the inferior thalamic peduncle (ITP) on recruiting (top) and augmenting (bottom) responses. These recordings were all taken from the same locally anesthetized cat preparation immobilized by gallamine 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.

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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