Mild Cold Exposure Increases Survival in Rats with Medial Preoptic Lesions

Abstract. *High mortality rates in rats with large medial preoptic lesions discourage their use in studies of brain function. However, virtually all such animals (six out of seven) survived indefinitely if kept at an ambient temperature of 15°C for 2 hours before and 10 to 12 hours after the lesions were made. Although these rats appeared otherwise healthy, they could not maintain normal body temperatures in short-term cold tests. In contrast, five of the nine rats kept at 25°C died within 10 hours after the operation, and three more died within 5 days. Rats kept at 25°C had a much higher incidence of cardiac arrhythmias than did rats kept at 15°C, which may be responsible for their higher mortality rates.*

The medial preoptic area appears to be essential for normal thermoregulation in many mammalian species. After receiving large bilateral medial preoptic lesions, rats can no longer maintain normal body temperatures when tested in thermally extreme environments (*1–4*). It has been difficult to study this phenomenon because electrolytic or radio-frequency lesions of the preoptic area large enough to produce thermoregulatory deficits result in mortality rates as high as 45 to 80 percent (*2–8*). Death is usually attributed to pulmonary edema, severe hyperthermia, or hyperactivity (*3–5, 7*). We now report that mortality rates can be dramatically decreased by a simple change in the immediate pre- and postoperative environment.

Ten female (295 to 316 g) and 11 male (255 to 430 g) rats (*9*) were anesthetized and were permanently implanted with (i) bilateral electrodes in the medial preoptic area, (ii) thermistors to measure brain and tail temperatures (the latter our index of vasomotor tone), and (iii) leads to measure the electromyogram (EMG) and heart rate (*10*). Tests were conducted in a Plexiglas cylinder (30 cm high, 16.5 cm in diameter) modified to record oxygen consumption (*11*) and housed inside a temperature-controlled chamber. A socket attached to a swivel joint at the top of the cylinder and plugged into the connector on the rat's skull allowed the rat relatively free movement inside the cage. All leads went to a polygraph (Grass model 8).

After at least 4 days of recovery from surgery, the rats were adapted individually to the experimental apparatus for 2 to 2½ hours at an ambient temperature (T_a) of 25°C. On the day the lesion was made (5 to 17 days after surgery) a rat was placed in the cylinder at a T_a of 25°C (N = 13) or 15°C (N = 8). After 2 to 3 hours of baseline recording, the rat was briefly removed from the chamber and wrapped in an elastic bandage; preoptic lesions were made with 2 mA of anodal d-c current for 10 seconds. The rat was returned to the chamber, and measures were continued for 10 to 12 hours or until it died. Surviving rats were returned to the home colony. Body weights and rectal temperatures were measured daily, and palatable foods were given to aphagic animals.

Surviving rats were tested for thermoregulatory deficits in the cold 2 to 3 days after the lesion was made and every 2 to 4 weeks after that. The T_a was gradually lowered from 25° to 5°C over a period of 60 to 75 minutes. Data were recorded for 1 to 2½ additional hours or until the subject's brain temperature fell to 32°C, at which time it was removed from the cold. Only data from rats with substantial cold-defense deficits will be discussed here.

After postoperative periods from 2 weeks to several months, surviving rats were given an overdose of Equithesin and perfused with 0.9 percent saline and 10 percent Formalin. The brains were frozen and serially sectioned. The outline of each lesion was traced on sections taken from the atlas of König and Klippel (*12*) and the extent of the lesions was determined from these drawings.

Table 1. Mean (± standard error) baseline values, mean peak values after the lesion was made, and mean differences for brain and tail temperatures, oxygen consumption, and heart rate for rats kept at T_a of 25° or 15°C. Statistical comparisons (measures at 15°C versus measures at 25°C) were made with two-tailed t-tests.

Measure	Brain temperature (°C)	O_2 consumption (ml/kg-hour)	Heart rate (beat/min)
	T_a = 25°C		
Baseline	36.7 ± 0.2	1328 ± 86	360 ± 12
Peak	41.0 ± 0.2	3033 ± 185	631 ± 18
Change	4.3 ± 0.2	1738 ± 187	273 ± 19
	T_a = 15°C		
Baseline	37.5 ± 0.2*	1734 ± 73*	436 ± 22*
Peak	41.1 ± 0.3	3166 ± 157	596 ± 20
Change	3.6 ± 0.3	1432 ± 105	160 ± 20*

*$P < .01$.

Eight of the nine rats kept at a T_a of 25°C died, five between 2 and 10 hours and three 3 to 5 days after the lesions were made. The latter rats were adipsic and aphagic, refusing even highly palatable foods. They were akinetic and hypothermic (rectal temperatures ranging from 33° to 36°C). The brain temperature of one rat (MPO 19) tested in the cold dropped from 37.6° to 32°C in the 75 minutes required to lower T_a from 20° to 5°C. The remaining rat, which lived until it was perfused at 110 days, had continuing severe deficits in the cold.

All but one of the seven rats kept at a T_a of 15°C lived until they were perfused 41 to 150 days later. (One rat was perfused at 2 weeks, when its body weight had dropped precipitously and it could not right itself.) Typically these rats began eating wet mash and drinking water within a day after the lesions were made. They were hyperthermic, with rectal temperatures ranging from 39° to 41°C. These rats had marked cold-defense deficits with brain temperatures falling to 32°C within 60 minutes after the chamber temperature reached 5°C. Cold tests performed at irregular intervals showed that some rats had major thermoregulatory deficits in the cold as long as 4 months after the lesions were made, while others partially recovered their thermoregulatory ability. For example, the brain temperature of one rat tested 2 days after the lesion was made fell 9°C, to 32°C, in 2 hours. Two weeks later it dropped 6.1°C in 3½ hours, and 10 weeks later it dropped 5.8°C in 4 hours. In general, all deficits were similar to those reported for animals with lesions made under anesthesia: extended hyperthermia at T_a of 25°C and, in the cold, the gradual return of shivering and nonshivering thermogenesis (*3*).

All rats in both groups became equally hyperactive, hyperthermic, and tachycardic and had equally high metabolic rates (Table 1). The major differences between the groups were that the rats kept at a T_a of 25°C exhibited a variety of cardiac arrhythmias and marked periods of vasodilatation that lasted 1½ to 3½ hours while brain temperature was at its highest levels. Only one rat kept at a T_a of 15°C had a relatively brief period of skipped beats; the others showed no signs of any arrhythmias besides tachycardia. All of these rats were relatively vasoconstricted, with tail temperatures remaining at or below their baselines throughout the 10- to 12-hour testing period. We will discuss each symptom with respect to the groups as a whole and in

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detail for representative rats (Fig. 1, A and B).

All rats in both groups had equally high heart rates after the lesions. Rats kept at 25°C exhibited long periods of various cardiac arrhythmias. The electrocardiogram (EKG) of MPO 19 (Fig. 1A) is typical. Heart rate increased gradually from 420 to 600 beats per minute with a short-lived peak at 780 beats per minute. Skipped beats were seen throughout this tachycardic period (Fig. 2B). Five hours after the lesion was made the EKG was characterized by sinoatrial block with an echo rhythm (Fig. 2C) and skipped, irregular beats. Heart rate dropped even further, to 150 beats per minute, before returning to baseline levels. The EKG then showed a pattern of inverted t waves characteristic of myocardial infarction (Fig. 2D). The heart rate of MPO 24 (Fig. 1B) similarly rose from 492 to 624 beats per minute, but it remained high throughout the session. Rat MPO 24 showed no signs of arrhythmias other than tachycardia, nor did five of the six other rats kept at 15°C.

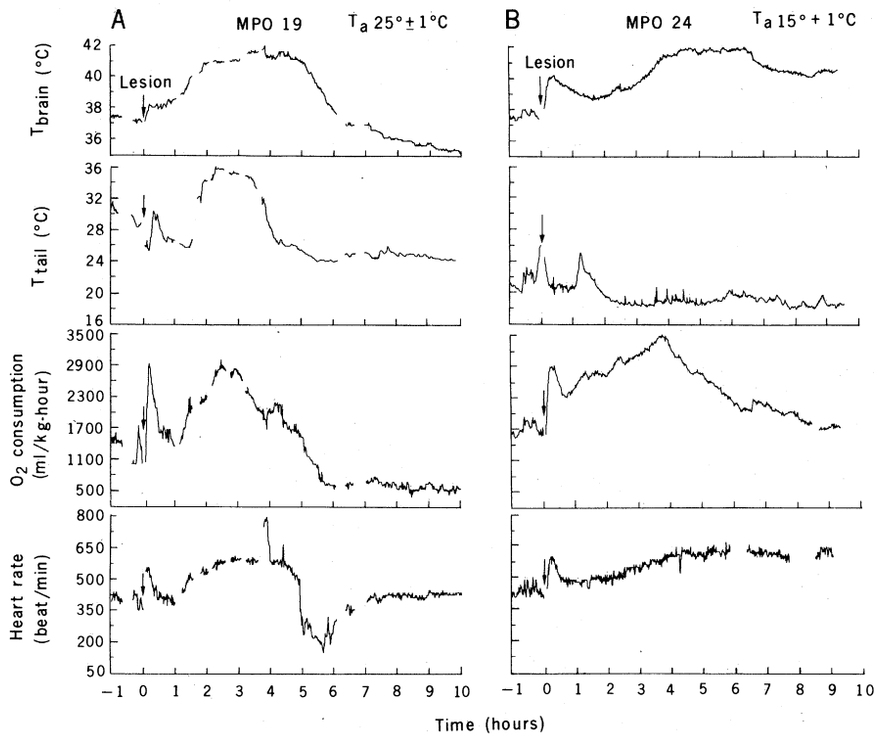

Fig. 1. Computer-drawn records of brain and tail temperatures, oxygen consumption, and heart rate for rats kept at 25°C (A) or 15°C (B). Peaks seen in tail temperature, oxygen consumption, and heart rate immediately after the lesions were made are produced by handling alone since they were also seen in rats without lesions that were simply removed from the cage. Note the long period of tail vasodilatation and the brief period of extreme tachycardia in MPO 19.

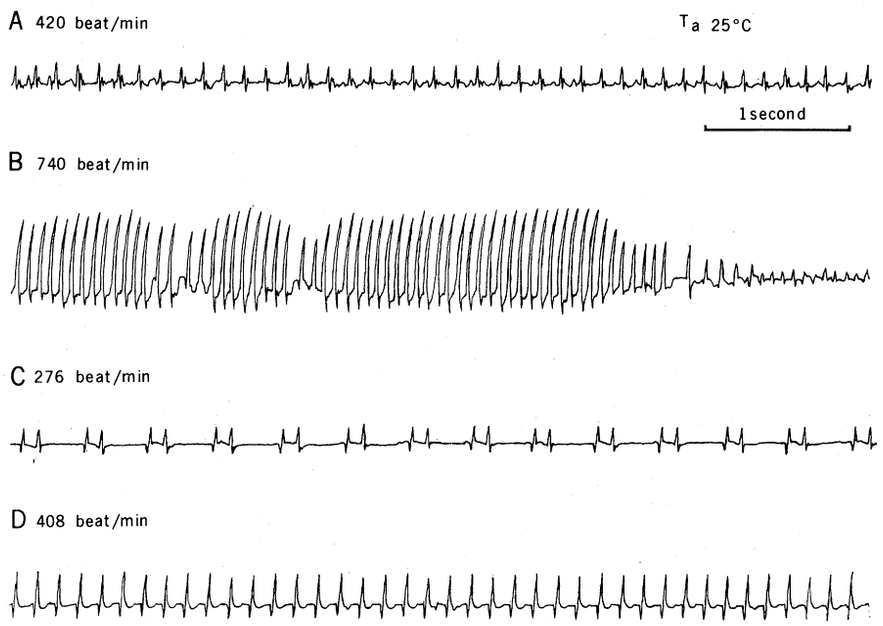

Fig. 2. Electrocardiogram records from rat MPO 19. (A) Normal EKG recorded 15 minutes before the lesion was made. (B) Ventricular tachycardia at 235 minutes. (C) Sinoatrial block with echo rhythm at 304 minutes. (D) Inverted t wave at 426 minutes.

Rats at 15°C were relatively vasoconstricted both before and after the lesions were made. Tail temperatures tended to remain approximately within a 3°C range at 2° to 7°C above ambient temperature, although some rats, like MPO 24, had brief periods of vasodilatation. In contrast, rats kept at 25° typically exhibited strong vasodilatation within 5 to 90 minutes after the lesions were made. Tail temperature tended to be elevated for 90 to 210 minutes, to drop to baseline levels, and to remain there for the rest of the test period.

Analysis of EMG records and behavioral observations revealed that all rats at both T_a's became extremely hyperactive 15 minutes to 2 hours after the lesions were made. Activity consisted of extended bouts of grooming, rearing, and circling the cage. Activity levels increased until the rats were in constant motion, reached peak levels 2½ to 5 hours later, and then either gradually subsided or ceased abruptly.

In all rats, brain temperatures began to rise within 10 to 90 minutes after the lesions were made, often reaching 41.5°C (Table 1). Hyperthermias were accompanied by mean increases in oxygen consumption of 13 percent (T_a, 25°C) and 89 percent (T_a, 15°C). Neither peak brain temperatures nor oxygen consumption or their changes from before to after the lesions were made were significantly different between the two groups.

Lesions typically destroyed the entire medial preoptic nuclei between the anterior commissure and the optic chiasm. In many rats, there was also substantial though less than total damage to the anterior hypothalamic nuclei caudally and the diagonal bands of Broca rostrally. Lesion placements were indistinguishable between the two groups, although, generally, lesions of rats kept at a T_a of 25°C appeared smaller than those of rats kept at 15°C, presumably because most rats at 25°C died within 1 day, before the complete spread of tissue damage had occurred (*13*).

Our data support those of others reporting hyperactivity and hyperthermia

after medial preoptic lesions (*3, 7, 8, 14*). However, neither of these factors appears to be responsible for death, since there were no apparent differences in the degree of hyperthermia or hyperactivity between those rats that lived and those that died. Our data do not support the hypothesis that rats with preoptic lesions typically die of pulmonary edema. Autopsies and macroscopic inspection of lung tissue revealed signs of pulmonary edema only in one rat that died 2 hours after the lesion was made. This rat had the classical symptoms including pink frothy fluid exuding from its mouth and nostrils and enlarged lungs (*8, 15*). Although the lesion placement in this animal was indistinguishable from those of the others, lesions that produce pulmonary edema have generally been reported to lie more posteriorly than those produced here (*15*).

Medial preoptic lesions disrupt cardiovascular function, and moderate cold stress lessens this disruption. Although the mechanism of this protection is unclear, our results describe a simple method for keeping rats with large bilateral medial preoptic lesions alive and in good health while greatly impairing their ability to maintain normal body temperatures in the cold.

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9. Hooded rats from Blue Spruce Farms were housed in a colony room maintained at 25° ± 1°C on a 12:12 light-dark cycle, with free access to dry food (Wayne Lab Blox) and water.
10. Rats were injected with atropine sulfate (0.25 ml) and anesthetized with 3.0 to 4.0 ml of Equithesin per kilogram of body weight. Stainless steel insect pins (#00) insulated to within 0.5 mm of their tips were stereotaxically implanted 0.5 mm anterior to bregma, 0.75 mm lateral to the midsaggital suture, and 8.0 mm below the surface of the cortex (horizontal skull). A miniature thermistor (Veco 32A7) was attached to one of the electrodes 4 mm above its tip. Tail temperature was measured with a thermistor (Gulton L1547) implanted under the skin of the tail 5 cm from the base. The EMG and EKG were recorded from two loops of stainless steel wire sutured to the acromiotrapezius muscle (EMG) or to the sides of the rib cage (EKG). All leads were soldered to a connector (ITT Cannon, MDI-15SL1) cemented to the rat's skull.
11. Oxygen consumption was measured with an oxygen analyzer (Applied Electrochemistry, S-3A or Beckman OM-11). Values are reported at standard temperature and pressure.
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16. Supported by NSF grant BNS77-03151 and ONR grant N00014-77-C-0465 to E.S. We thank J. Schmidt and W. Seaman for performing the autopsies, N. Peshkin and L. McKeown for technical assistance, and P. Teitelbaum, E. Stricker, and P. Lomax for their criticisms of earlier versions of the manuscript.

26 December 1979

Retrograde Axonal Transport of Endogenous Proteins in Sciatic Nerve Demonstrated by Covalent Labeling in vivo

Abstract. *Extracellularly applied* N-*succinimidyl* [*2,3-*^{3}*H*]*propionate was used in vivo to covalently label intra-axonal proteins in the rat sciatic nerve. This technique permitted a unique view of axonal transport of proteins independent of biosynthesis. The proteins detected in slow anterograde transport (1 to 2 millimeters per day) correspond to cytoskeletal proteins described in previous reports. The slowly retrogradely transported component (3 to 6 millimeters per day) was composed primarily of a single protein with a molecular weight of 68,000.*

The movement of cytoplasm and intracellular particles in axons, referred to as axonal transport, is governed by elaborate mechanisms (*1*). The use of pulse-labeling (in which de novo synthesized proteins labeled by radioactive amino acids applied to the neuron perikarya are observed being transported intra-axonally) has provided a wealth of data about anterograde axonal transport (*1, 2*). This approach, however, cannot be applied to retrograde transport, because the distal axons and nerve terminals do not synthesize proteins.

The existence of retrograde transport has been established by direct microscopic visualization of intra-axonal particles (*3*), the use of exogenous macromolecular tracers (*4*), and ligation (*5, 6*). Attempts to use pulse-labeling in combination with the ligation approach has led to the hypothesis that the macromolecules are transported

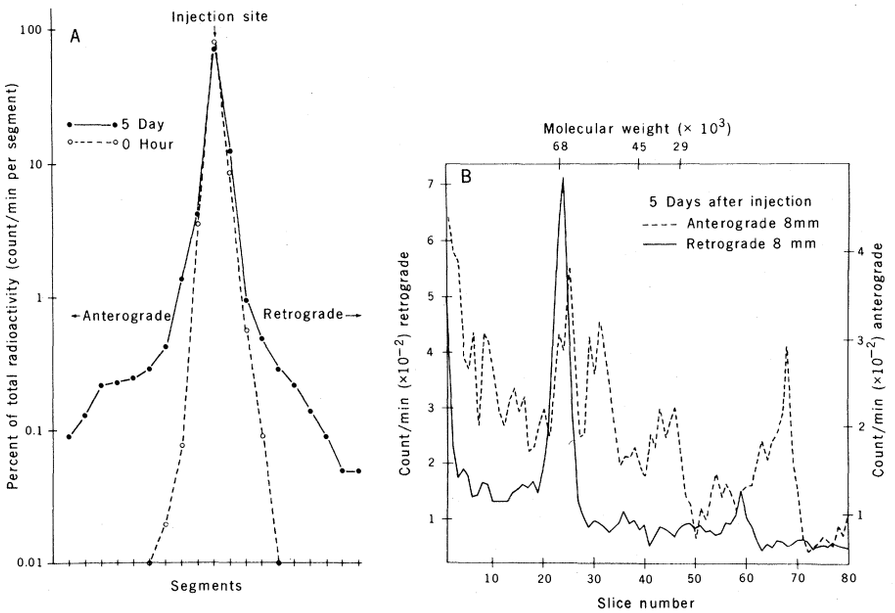

Fig. 1. Labeling of intra-axonal proteins that undergo axonal transport. (A) Distribution of TCA in 3-mm segments of nerve after injection of ^{3}H-*N*-SP into the sciatic nerve. Immediately after injection, virtually all the labeled protein was confined to the segment containing the injection site. At 5 days, significant labeled protein was found in segments greater than 20 mm proximal to and distal from the injection site. (B) Polyacrylamide gel electrophoresis of labeled proteins. Segments were homogenized in 0.1*N* HCl and, after TCA precipitation, were subjected to electrophoresis on 11 percent polyacrylamide gels in SDS. The gels were sliced into 1.5-mm slices, and the radioactivity of each slice was determined by liquid scintillation counting. The anterograde-transported proteins constitute a complex pattern including major peaks and complexes. In contrast, the retrograde transport shows only one major peak.

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