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DEVELOPMENT OF AN ANIMAL MODEL OF HUMAN NON-FREEZING COLD INJURY: CHANGES IN THERMAL SENSITIVITY FOLLOWING COLD EXPOSURE

S. T. Ahlers
J. R. Thomas
K. F. Van Orden
J. F. Schrot
M. P. McAndrew

Naval Medical Research
and Development Command
Bethesda, Maryland 20889-5055

Department of the Navy
Naval Medical Command
Washington, DC 20372-5210

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LARRY W. LAUGHLIN
CAPT, MC, USN

Commanding Officer
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Non-freezing cold injury (NFCI) is a debilitating injury that results from damage to peripheral tissues exposed to cold temperatures for a prolonged period of time. NFCI continues to be a major operational hazard for personnel who must perform in cold environments. Despite considerable research, the mechanisms underlying NFCI have remained elusive. The objective of the research described in this report represents the initial efforts to develop an animal model that adequately reflects the symptomatology observed in human NFCI. A salient feature in the post cold exposure manifestation of NFCI in humans is that, following an initial period of sensation loss in the affected limb, an increased, often permanent, thermal sensitivity develops. This is one of the more debilitating aspects of NFCI since personnel are unable to tolerate even minor alterations in temperature of the hands or feet and are thus greatly restricted from performing in inclement weather conditions. In the present study, thermal sensitivity was measured in the tail of adult rats before and after the tail and a portion of the hind flank were exposed to cold (1-4°C) or warm (28°C) water for one to nine hours. Results indicated that prolonged exposure of the rat to cold water for six to nine hours produced initial anesthesia followed by heightened sensitivity to remove the tail from a warm

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19) (50°C) stimulus; exposure to cold water for less than six hours did not affect thermal sensitivity. These data indicate substantial progress towards the development of an animal model that displays NFCI-type pathology analogous to the condition observed in humans.

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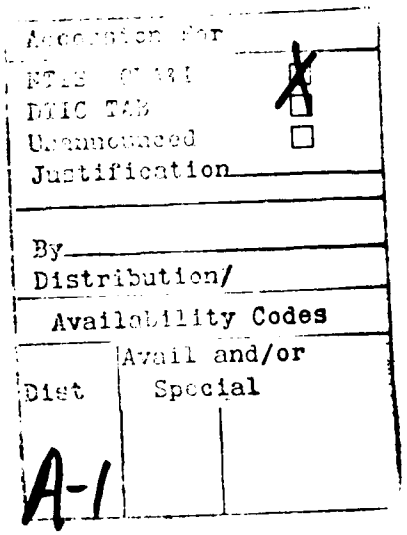

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These experiments were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory animal resources, National Research Council, DHHS Publication (NIH) 86-23-1985.

INTRODUCTION

Non-freezing cold injury (NFCI), a unique syndrome resulting from damage to peripheral tissues exposed to cold temperatures, remains a major threat to Navy and Marine operations carried out in cold weather (1). NFCI is an injury that does not involve freezing of tissues, which distinguishes it pathologically and clinically from cold-induced frostbite injury (2, 3).

Historically, NFCI has been a potential threat to military operations carried out in cold weather at least since the Crimean War. Recently, NFCI was a major source of disability among ground forces in the Falkland conflict, accounting for twenty percent of all British casualties (4). Because NFCI is liable to continue as an important medical problem in any extended military cold weather operation, it has become increasingly important to elucidate the mechanisms that underlie this debilitating condition.

NFCI usually involves rather distinct phases in the pathogenesis (2, 3, 5). Initially, individuals exhibit poor circulation and lack of sensation in the affected limbs that continue for some time after rewarming, often inducing total immobility of the individual. However, after the initial period of anesthesia, a gradual but permanent sensitivity to thermal stimuli frequently occurs. In fact, increased thermal sensitivity is one of the more salient features of NFCI since affected personnel often become intolerant of even minor temperature alterations in the hands or feet, thus, greatly impeding performance in subsequent inclement weather operations. Although several studies have shown injury to peripheral nerves in animals with prolonged cold exposure (6, 7, 8), it is not clear how these changes relate to the underlying mechanisms of NFCI observed in humans.

Therefore, the aim of the present research is to develop an animal model that characterizes the distinctive stages observed in humans; an animal model of NFCI can be used to test treatment regimens for the prevention of NFCI. The rat tail model was employed since evidence suggests that this appendage does in fact develop nerve pathology after prolonged cold exposure (6, 7, 8, 9). Basically, the experimental procedures entailed placement of an

unanesthetized rat into a cylindrical container so that its tail and a portion of the flank were immersed into cold (1-4°C) or warm (28°C) water. Rats were given varying amounts of exposure to these conditions, ranging from one to nine hours. Before and after cold water exposure, thermal sensitivity of the rat tail was measured by placing the rat tail in warm (50°C) water and measuring the latency to flick the tail.

METHODS

Subjects:

Approximately 40 adult male Long Evans rats obtained from Charles River Laboratories (Wilmington, MA) served as subjects. Animals were housed in standard hanging metal cages with free access to food and water.

Apparatus:

For measurement of thermal sensitivity, rats were restrained in an adjustable restraint device (Braintree Scientific) measuring 17 cm in length. This device accommodated rats of varying weights ranging from 250-400 grams. While each rat was restrained in this device, its entire tail protruded through a 2 x 3 cm opening. During the test procedure the restraint device was placed on a 30° upward angle so that the tail rested on an adjacent platform next to the restraint device. The platform covered an 8 x 8 cm opening of a circulating water bath (Forma Scientific) in which the water was kept at a constant temperature of 50°C. During the test procedure the platform was removed temporarily by the experimenter to allow the tail to drop into the water. The latency to remove the tail from the warm water was then recorded over ten trials using a hand held timer.

During cold exposure, rats were placed into a cylindrical restraint device 22 cm in length with a variable inside diameter to accommodate rats weighing from 250-400 grams. This device had a 2 x 3 cm opening on one end through which the tail protruded. A 20 cm metal rod extended from the top of the cylindrical restraint device. It was clamped to a horizontal rod that extended 20-30 cm above the surface of the water contained in a circulating water bath (Forma Scientific). During exposure to warm (28°C) or cold (1-4°C)

water the rat held in the restraint device was in a vertical orientation to the surface of the water; this restraint device configuration could be adjusted vertically to allow the experimenter to adjust how much of the tail and a portion of the flank were exposed.

Procedure:

In the initial stages of the experiment, rats were habituated to the restraint device used for thermal sensitivity measurements. Following a three week period in which the duration of restraint exposure was gradually increased, animals were tested for thermal sensitivity to a warm water stimulus. Thermal sensitivity measurements were obtained twice per week and were continued until stable performance was achieved. Rats were then given a single exposure to varying durations to cold (1-4°C) or warm (28°C) water. Only the tail was exposed in the initial stages of the research. However, because exposure of the tail alone did not produce any systematic effect on thermal sensitivity of the tail, subsequent animals received exposure of the entire tail and a portion of the hindquarters. This modification of the procedure systematically produces a pattern of thermal sensitivity changes that are similar to those observed to occur in human NFCI.

Thermal Sensitivity:

Once animals were habituated to the restraint procedure, thermal sensitivity was measured by placing the rat tail in warm (50°C) water and measuring the latency to flick the tail out of the warm water. Each subject received ten tail flick tests per session, two test sessions per week. At least one minute separated each of the 10 test trials. The time from when the tail dropped into the water until the rat flicked the tail was recorded using a hand held timer; the tail flick latency was recorded to the nearest hundredth of a second. Rats typically flicked their tails out of the warm water after three or four seconds of exposure. If the rat did not remove its tail before eight seconds, the experimenter physically removed the tail and scored the tail flick latency of eight seconds for that trial.

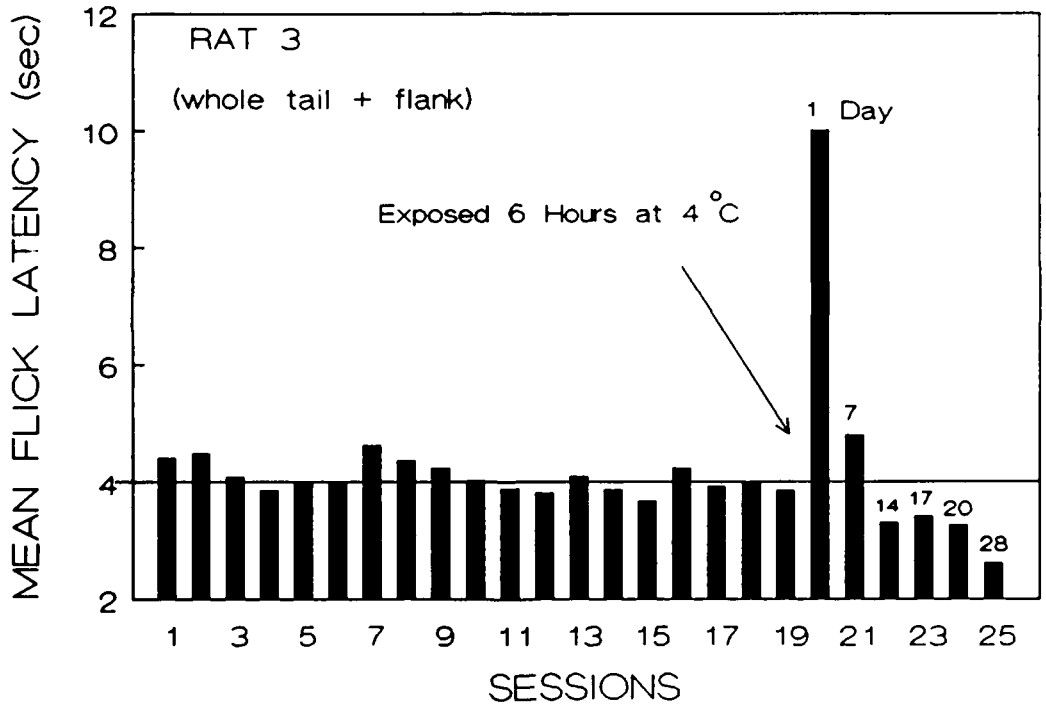
Cold Exposure:

The primary experimental procedure consisted of placing rats in a restraining device and immersing their tails and/or a portion of their hindquarters in cold water for varying durations. Rats were exposed to 1-4°C water for one, three, six, or nine hours. Control animals were given equivalent exposures to 28°C water for the same durations. Rats were constantly monitored by the experimenter during the exposure period.

RESULTS

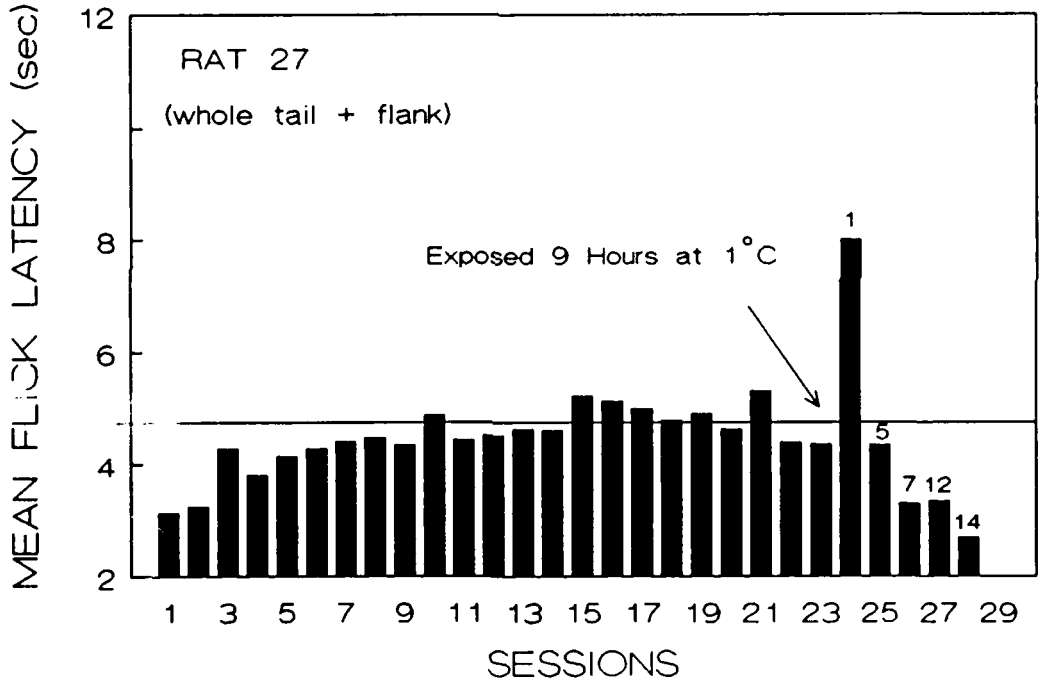
Alterations in thermal sensation occurred when rat tails were exposed to cold water for a relatively long period. In general, exposure durations of less than six hours did not produce any systematic change in thermal sensitivity in this preparation. Results from some of the animals in this study that developed NFCI-type pathology are described below. Although the pattern and magnitude of change for each animal varied somewhat, rats exhibited an initial loss of sensitivity in tail withdrawal. This is shown in figures 1-3 as a marked increase in tail withdrawal (flick) latency from the warm stimulus following cold exposure 24 hours (one day) after the animal had been exposed. On subsequent test sessions, rats showed an increased sensitivity to the warm stimulus, indicated by a decreased latency in tail withdrawal. The course of alterations in thermal sensitivity appears similar to those observed in humans with NFCI. Apart from the effects on the tail flick latency to remove the tail from the warm water stimulus, animals did not display any consistent outward pathology (e.g. lesions). Although the hind limbs were often partially exposed to the cold water, animals did not show impaired mobility of the limbs per se. Some animals, especially those that had received cold exposure durations of six or more hours, sometimes were observed to be lethargic following the exposure; however, this effect was not consistently observed in all subjects and lasted only a day. Similarly, the tail of some animals given relatively long exposures showed a transient pallor that sometimes lasted for two or three days. In contrast, nine hours exposure (or less) to 28°C had no effect on thermal sensitivity (Fig. 4).

FIGURE 1



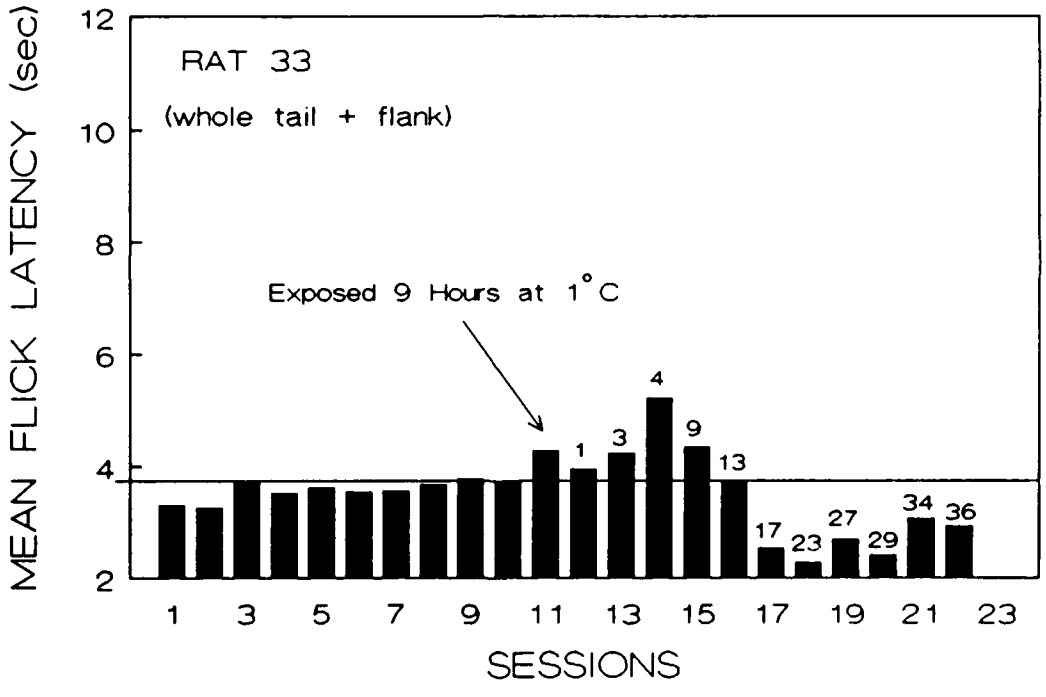
The latency to flick the tail from 50°C water is depicted. Each session represents the average of ten test trials. Tail flick latency data were measured initially until stable performance was achieved. In this rat, exposure to cold water (4°C) for six hours produced a deficit in thermal sensitivity one day later. Gradually, the impairment in sensitivity diminished and a relatively permanent hypersensitivity to the thermal stimulus developed. The number of days after the nominal cold exposure is indicated above each session.

FIGURE 2



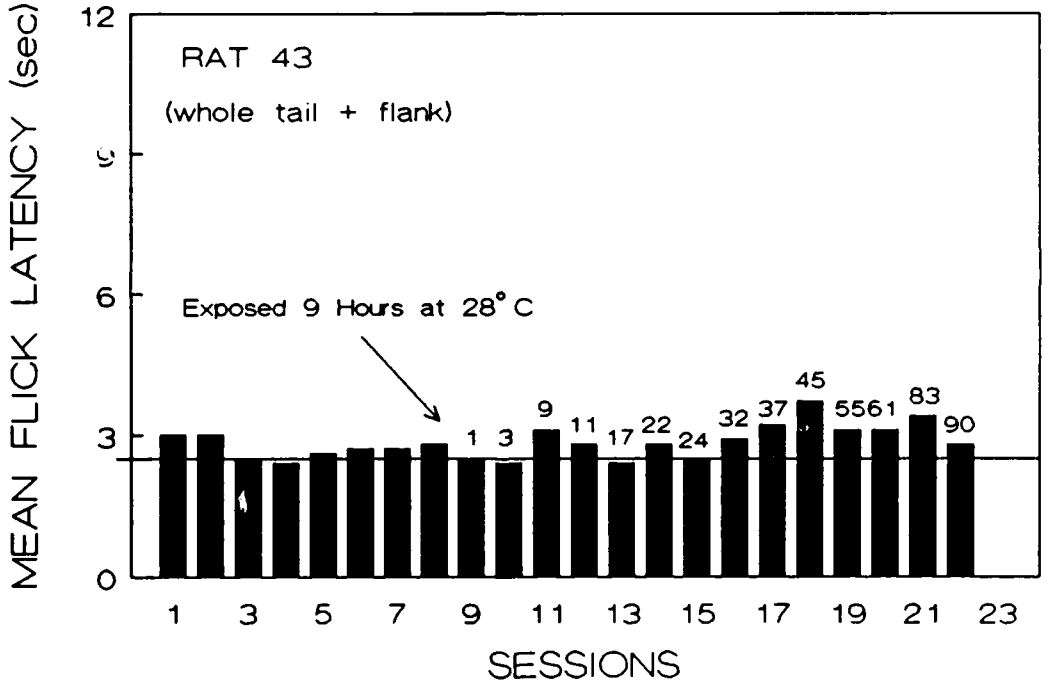
Exposure of the tail and flank to 1°C for nine hours produced a similar pattern. Exposure of the tail alone for a similar duration did not produce any systematic effect (data not shown).

FIGURE 3



In another subject exposed to cold water of 1°C for nine hours, th magnitude of the initial decrease in thermal sensitivity was less.

FIGURE 4



Exposure to 28°C for nine hours did not systematically affect the thermal sensitivity measure.

DISCUSSION

The data presented herein indicate substantial progress towards the development of an animal model that displays NFCI-type pathology analogous to the condition observed in humans. Although several studies have attempted to induce NFCI in animals, these studies have tended to focus only on demonstrable pathological changes in the exposed appendage. For example, Blackwood and Russell (10) observed substantial local swelling of the rat tail as well as damage to muscles and nerves with 48 hours of cold and wet exposure. After a two month period, the tail and hind limb region showed considerable damage to muscles and nerves. More recently, Nukada, Pollock, and Allpress (7); Peyronnard, Pedneault, and Aquayo (8); and Gilliatt and Kennett (6) have shown damage to peripheral nerve fibers in rat tails after exposing them to cold. In particular, these investigators observed demyelination (i.e. Wallerian degeneration) of peripheral myelinated nerves after prolonged exposure to wet, cold conditions for varying lengths of time. Unfortunately, although injury to large myelinated fibers suggests that cold may indeed impair neuronal function, it is less clear whether these observed changes relate to the different stages or reflect the pathology observed in humans. The clinical pathology of altered thermal sensitivity and impaired local blood flow in the affected limb suggests that damage to unmyelinated and small myelinated fibers may be a critical component that underlies the unique conditions observed in NFCI (3, 4); this condition has not previously been reliably produced in an animal model.

Our laboratory has recently examined somatosensory evoked potentials in rats where tail and flank have been exposed to cold. The data indicate that cold exposure produces substantial damage to nerve fibers (9). Interestingly, although preliminary, the findings suggest that the duration of cold exposure needed to produce significant amplitude changes in neural evoked potentials recorded from the tail, back, and somatosensory cortex from stimulation at the tail nerve fibers is much shorter than the exposure duration observed to affect thermal sensitivity (9). Because the evoked potential response

procedure is more likely to reflect stimulation of large myelinated nerves, whereas the thermal sensitivity measure is sensitive to unmyelinated C fibers, these data suggest that these distinct classes of neural fibers are differentially susceptible to cold-induced injury.

The clinical pathology of altered thermal sensitivity and impaired local blood flow in the affected limb suggests that damage to unmyelinated and small myelinated fibers may underlie the unique conditions observed in NFCI in addition to any direct effects of cold on large fibers. Histological analysis presently under way will examine gross morphological changes in muscle, blood vessels, and nerve fibers following varying lengths of cold exposure, with emphasis on subsequent regeneration of neural fibers post exposure in this model system. Also, more study is needed to show whether alteration in peripheral blood flow, another salient feature of NFCI in humans, is also impaired in the rat tail preparation.

REFERENCES

1. Hanson, H. E. and R. F. Goldman. Cold injury in man: A review of its etiology and discussion of its prediction, Milit. Med. 134 (1969), pp. 307-316.

2. Francis, T. J. R. and F. St. C. Golden. Non-freezing cold injury: The pathogenesis, J. Roy. Nav. Med. Serv. 71 (1985), pp. 3-8.

3. Montgomery, H. Experimental immersion foot: review of the physiopathology, Physiol. Rev. 34 (1954), pp. 127-137.

4. Francis, T. J. R. Non-freezing cold injury: A historical review, J. Roy. Nav. Med. Serv. 70 (1984), pp. 134-139.

5. Osborne, J. W and J. Cowen. Psychiatric factors in peripheral vasoneuropathy after chilling, Lancet. 2 (1945), pp. 204-206.

6. Gilliatt, R. W. and R. P. Kennett. Experimental non-freezing cold injury in the tibial nerve of the rabbit, J. Physiol. 64 (1987), pp. 134-137.

7. Nukada, H., M. Pollock, and S. Allpress. Experimental cold injury to peripheral nerve, Brain. 104 (1981), pp. 779-811.

8. Peyronnard, J. M., M. Pedneault, and A. J. Aguayo. Neuropathies due to cold: quantitative studies of structural changes in human and animal nerves, Neurol: Proc of the 11th World Congress of Neurology. (1977), pp. 308-329.

9. Van Orden, K. F., S. T. Ahlers, J. R. Thomas, J. Schrot, and D. Shurtleff. The development of evoked potential procedures for the assessment of non-freezing cold injury in the rat, NMRI 90-25 (May 1990).

10. Blackwood, W. and H. Russell. Experiments in the study of immersion foot, Edin. Med. J. 50 (1943), pp. 385-398.