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Author(s) T.M. Rauch, D.I. Welch and L. Gallego

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TERRY M. RAUCH, Ph.D.
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Director, Health & Performance Division

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HYPERTHERMIA IMPAIRS RETENTION

OF AN OVERTRAINED SPATIAL TASK

IN THE MORRIS WATER MAZE

T.M. Rauch, D.I. Welch and L. Gallego

U.S. Army Research Institute of Environmental Medicine

Natick, MA 01760

Address communications to: Dr. Terry M. Rauch, U.S. Army Research Institute of Environmental Medicine, Kansas Street, Natick, MA 01760

ABSTRACT

Fifteen rats were trained to learn the location of a spatially fixed platform hidden in a Morris water maze (40 ± 2° C). Then retention of the spatial task was assessed immediately after raising core body temperature (T_c) to 42°, 40° or 37° C (the normothermic control). The hyperthermic treatment order was counterbalanced according to a Latin-square design. Hyperthermia at 42° C T_c significantly impaired the retention of spatial performance. Hyperthermic animals were cooled to normothermia (T_c = 37° C) and spatial performance tested again. Recooling resulted in a complete recovery of spatial performance. These results demonstrate that hyperthermia-induced amnesia can be obtained on an overtrained spatial mapping strategy and recooling initiates recovery of spatial performance.

Key Terms: Hyperthermia, thermoregulation, spatial performance.

Running heading: Hyperthermia Impairs Spatial Performance

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

INTRODUCTION

There is clear evidence that hypothermia impairs memory in animal paradigms involving the retention of one-trial passive-avoidance tasks (see review by Riccio and Richardson, 1984). In contrast, much less is known about the effects of hyperthermia on memory. Several studies have shown that hyperthermia administered after a conditioning trial produced retrograde amnesia for the retention of a one-trial passive-avoidance task in rats (Mactutus, Ferek, & Riccio, 1980; Misanin, Vonheyn, Bartelt, Bouldin & Hinderlighter, 1979). Ahlers and Riccio (1987) reported that increasing the core body temperature (T_c) of rats 3-4° C above normothermia prior to one-trial passive-avoidance training produced a significant anterograde amnesia for 24 hr retention. In the same study, when T_c was elevated only 1-2° above normothermia there was no impairment in retention. However, can hyperthermia-induced amnesia for one-trial passive-avoidance tasks be generalized to a visually cue dependent, overtrained, spatial mapping strategy?

The Morris water maze (Morris, 1981) requires a rat to learn the location of a spatially fixed platform hidden in a water maze. Accurate navigation is rewarded by escape from the water onto the platform. Recent studies have demonstrated the dependence of spatial navigation on 'extramaze' visual cues and on normal hippocampal function (Morris, 1981; Morris, Garrud, Rawlings and O'Keefe, 1982; O'Keefe and Nadel, 1979). Cholinergic systems in the forebrain i.e., the septum, hippocampus and septo-hippocampal pathway are well documented (Fibiger, 1982) and central blockade of cholinergic synapses produces an inability to utilize spatial mapping strategies during the acquisition of a place navigation task (Hagan, Tweedie and Morris, 1986). Recently, several studies (Hagan et al., 1986; Rauch, Welch and Gallego, in press) have also shown cholinergic blockade to impair the retention of a previously acquired spatial task.

To date, no studies have examined the role of T_c in the onset of, and recovery from, hyperthermic-induced amnesia on a 'reference memory' task such as place navigation. The purpose of the present study was twofold. First, to determine if retention of an overtrained spatial task would be affected by hyperthermia. Secondly, if hyperthermia induces amnesia on retention of a spatial task, does recooling initiate recovery of spatial performance.

MATERIALS AND METHODS

Subjects. Fifteen experimentally naive adult male Charles River CD strain rats, with a mean age of 140 days (300-500g) at the time of testing served as subjects. All rats were housed individually in hanging wire mesh cages with ad-lib access to food and water. Behavioral testing was conducted between 0900 and 1500 hours.

Apparatus. The rats were trained and tested in a Morris water maze. The water maze consisted of a circular black fiberglass pool (134 cm in diameter X 50 cm in height) and filled to a depth of 30 cm with water (40 $\pm$ 2° C). The water was rendered opaque with blue food coloring. A circular stainless steel escape platform, 10 cm in diameter at the top and base, was painted black. The escape platform was 1 cm below the surface of the water and always positioned in the center of the southeast quadrant of the pool. Two start positions were located on the perimeter of the pool: one in the north, the other in the west, at approximately a 45° angle equidistant from the escape platform. The maze was illuminated by overhead lights and the testing room contained numerous extramaze cues.

Spatial Learning Procedure. Prior to the first week of training, rats were handled each day for approximately three minutes. One day before training the rat was placed in the pool with no escape platform and allowed to swim for 90 s. The rats were then returned to their home cages. The animals were trained ten trials per day for six days and five trials on the seventh day for a total of 65 trials. The start position (north *vs* west) was randomized for each rat, across trials, but the submerged escape platform was always in the center of the southeast quadrant. A trial started when the rat was immersed in water and held with his head facing the wall of the pool at one of the two starting locations. Each animal was allowed 120 s to escape onto the platform. If the animal failed to escape within this time it was guided to the platform. After the animal escaped onto the platform or was guided to the platform, it remained there for 15 s before being removed and placed in the home cage. Escape latency was recorded for each trial. All rats were trained to asymptotic performance prior to the hyperthermia treatments.

Hyperthermia treatments consisted of restraint and immersion in a warm water bath. Therefore, to control for any confounding due to restraint, one-half of all training trials for each rat were randomly designated and preceded by restraint and immersion up to the neck in a 36° C water bath for 14 minutes. Restraint and immersion in this temperature water bath allows the animal to maintain a normothermic T_c of 37° C.

Hyperthermia and Spatial Retention Procedure. Hyperthermia treatment consisted of restraining the rat in an acrylic rat restrainer and immersion up to the neck in a water bath (Lauda, RC 20). T_c was monitored with a Yellow Springs series 400 temperature probe, inserted 1.5 cm in the rectum, and a Beckman 450 Digital Thermometer. On treatment days the animals were restrained and immersed in the water bath (42° C) for 10-14 min, until a T_c of 40° or 42° C was obtained. The control treatment consisted of restraint and immersion in the water bath at 36° C for 14 min. T_c was recorded every two minutes for all treatment conditions until the criterion T_c was achieved.

Each animal was treated and assessed on measures of spatial retention on test days 1, 3 and 5. Each animal received five 'normothermic' trials in the water maze on days 2 and 4. The hyperthermia treatment order was counterbalanced according to a Latin-square design. After hyperthermic treatment the animal was immediately tested (i.e., heated trial) in the Morris water maze (40 ± 2° C). Then each animal was returned to its home cage and allowed to thermoregulate for approximately 30 min to a T_c of 37° C. T_c was monitored every 2 min during recooling. Then all animals were tested again in the water maze (40 ± 2° C) (i.e., cooled trial) during which all animals were normothermic at the time of testing.

Spatial retention was assessed by measuring escape latency, heading error, swim speed and swim distance for each treatment and trial. The animals' swims were videotaped with a Sony videorecorder placed above the center of the maze. A videotape of each swim was played to trace swimming paths onto a paper map. Swimming paths for each rat were also recorded on a map of the maze by an experimenter seated by the pool's edge. Maps traced from the videotape were placed on a translucent digitizing tablet, 44 x 44cm (Altek Corp. Model No. R-22) and a cursor with a magnetic search coil was used to trace movement and position. The movement and position of the animal was digitized by an AC 40 DKF controller (Alteck Corp.) which then computed the swim distance (cm). Swim speed was computed from swim distance and escape latency and expressed as cm/sec. Heading error was calculated by modifying the procedure described by Whishaw and Tomie (1987). After each animal reached asymptotic performance an 18-cm-wide path from the start point to the center of the platform was designated an error-free alley. If the animal swam outside the alley over the initial 12 cm of the swim it received a maximum of one heading error.

RESULTS

Spatial Training

Repeated measures analysis of variance was performed on escape latencies over trials during the training phase of the study. Throughout training all animals rapidly learned to swim to the hidden escape platform. Over 65 trials the mean escape latency declined from 90 to 5 seconds. All rats achieved asymptotic performance in 30 trials. There was a significant main effect for training trials on escape latency, F (64,896) = 14.17, $p <$ .001. There was no significant effect of restraint on escape latency over trials.

Effect of Hyperthermia and Recooling on Spatial Retention

Spatial performance was analyzed using a repeated measures analysis of variance. The two factors in the analysis were hyperthermia (3 levels: 40, 42° and 37° C T_a) and trials (2 levels: heated and cooled). The analysis of variance on escape latencies revealed significant main effects for hyperthermia, F (2,28) = 13.79, $p < .001$ and trials, F (1,14) = 13.17, $p < .001$, and a significant hyperthermia x trials interaction, F (2,28) = 18.49, $p < .001$. Post hoc Tukey ($p < .05$) comparisions, on the heated trial, showed that escape latencies for rats heated to 42° C $T_a$wwere significantly greater than rats heated to 40° C T_a and the normothermic controls. However, after recooling performance recovered completely and there were no significant differences in escape latencies.

There were significant main effects for hyperthermia on swim distance, F (2,28) = 9.03, $p < .001$ and trials, F (1,14) = 8.72, $p < .01$ and a significant hyperthermia x trials interaction, F (2,28) = 11.43, $p < .001$. Tukey analysis, of the heated trial, showed the mean swim distance for the T_a = 42° C treatment to be significantly greater than the T_a = 40° C and the normothermic control. Performance recovered and there were no significant differences in swim distance found on the cooled trial. Escape latencies and swim distances for each treatment and trial are shown in Figures 1.

The analysis of heading errors showed significant a main effect for hyperthermia, F (2,28) = 7.58, $p < .002$ and a significant hyperthermia x trials interaction, F (2,28) = 24.33, $p < .001$, but no significant main effect for trials. Tukey follow-up analysis showed that rats heated to 40° and 42° C T_a made significantly more heading errors than the normothermic controls. Yet, performance recovered when T_a returned to normal and there were no differences in heading errors during the cooled trial.

The analysis of swim speed (cm/sec) showed a significant main effect for hyperthermia, $\underline{F}$ (2,28) = 3.71, $\underline{p} < .05$ and a significant hyperthermia x trials interaction, $\underline{F}$ (2,28) = 15.84, $\underline{p} < .001$, but no main effect for trials, $\underline{F}$ (1,14) = 4.38, $\underline{p} < .055$. Tukey follow-up analyses showed tha rats heated to 42° C T_{c} swam significantly slower than those heated to 40° C T_{c} and the normothermic controls. Swim speeds recovered when hyperthermic animals cooled to normothermia. The results of the heading error and swim speed measures are shown in Figure 2.

Figure 3 shows the swim patterns of one animal heated to 42°, 40° C T_{c} and the control 37° C T_{c}. Both levels of hyperthermia impaired spatial performance. The swim patterns of hyperthermic animals were often haphazard, frequently looping around the perimeter of the pool, much like a naive rat when first learning the maze. There was no significant difference between clockwise and counter-clockwise generated loops among the hyperthermic rats exhibiting this behavior.

DISCUSSION

The present study shows that severe hyperthermia impairs the retention of a reference memory task in overtrained rats. Therefore, our results show that hyperthermia-induced amensia can also be obtained on a task which uses a spatial mapping strategy for acquisition and retention. Our findings are consistent with previous studies reporting hyperthermic-induced impairments in the retention of a one trial passive avoidance task (Misanin et al., 1979; Mactutus et al., 1980; Ahlers and Riccio, 1987). Moreover, the hyperthermia-induced amnesia for spatial retention is reversible; animals allowed to cool to normothermia showed a strong recovery of spatial performance. The hyperthermia-induced amnesia for spatial performance is also important considering the results of a recent study in our laboratory (manuscript in preparation) showing the amnesic effects of hypothermia on spatial performance.

Severe hyperthermia (42° C T_a) produced the greatest escape latencies, swim distances, heading errors and the slowest swim speed. Animals heated to 42° C T_a were observed to be lethargic, yet, were good swimmers, exhibiting forepaw inhibition, although their swim speed was significantly slower. However, the fact that both levels of hyperthermia resulted in an overwelming number of heading errors is sufficient evidence that the impairment in spatial performance is not the result of a simple defict in swimming.

Interpretation of the hyperthermia-induced impairment in memory is at best fraught with problems. Nevertheless, we suggest that the impairment in spatial performance may be due to a retrieval deficit. Rats use three strategies to solve spatial navigation problems (O'Keefe and Nadel, 1978). First, a place or spatial-mapping strategy is used when the relational properties of distal cues are used to locate a hidden platform. Second, a cue strategy is used when navigation to the hidden platform is guided by approaching a dominant cue. Lastly, a praxis strategy is used when navigation to the hidden platform is based on a sequence of movements, such as turn right and then turn left. Rats normally develop a map of the pool based on distal 'extramaze' cues and they use a spatial-mapping strategy to direct some portion of their swims (Morris et al., 1982). However, will mapping information acquired in the normothermic state be retrieved in the hyperthermic state to solve spatial problems?

The processing and retrieval of distal extramaze cues may be impaired by a hyperthermic effect on the brain similiar to the effects of central cholinergic blockade on spatial performance (Hagan et al., 1986; Rauch et al., 1988). In fact, a 'central anticholinergic syndrome' bears a striking resemblance to heat illness (Hubbard, Matthew, Durkot and Francesconi, 1987). Hubbard et. al. (1987) suggests that central anticholinergic mechanisms could be operative in the pathophysiology of heatstroke which may be related to a 'functional central hypocholinergic state'. Moreover, it is interesting to note that anticholinergic agents, with potent antimuscarinic properties, not only predispose to heat stroke (Hubbard et al., 1987) but also impair memory. Possibly, hyperthermic rats may have difficulty processing the relational properties of distal cues; an effect demonstrated by central cholinergic blockade and necessary for accurate place navigation (Whishaw and Tomie, 1987). However, we can not exclude a hyperthermic effect on attentional or motivational factors which may have influenced spatial performance.

To summarize, our data demonstrates that hyperthermia-induced amnesia severly impairs the retention of an overtrained spatial mapping strategy. The impairment in spatial retention is reversed by recooling to normothermia. The physiologic basis of the hyperthermia-induced disruption in memory is problematic. We suggest that the hyperthermia-induced amnesia of spatial memory may be related to a functional central hypocholinergic state of heat illness (Hubbard et al., 1987). Such cholinergic dyfunction may disrupt the processing of distal visual cues which rats use in place navigation and which is dependent on normal hippocampal and visual function.

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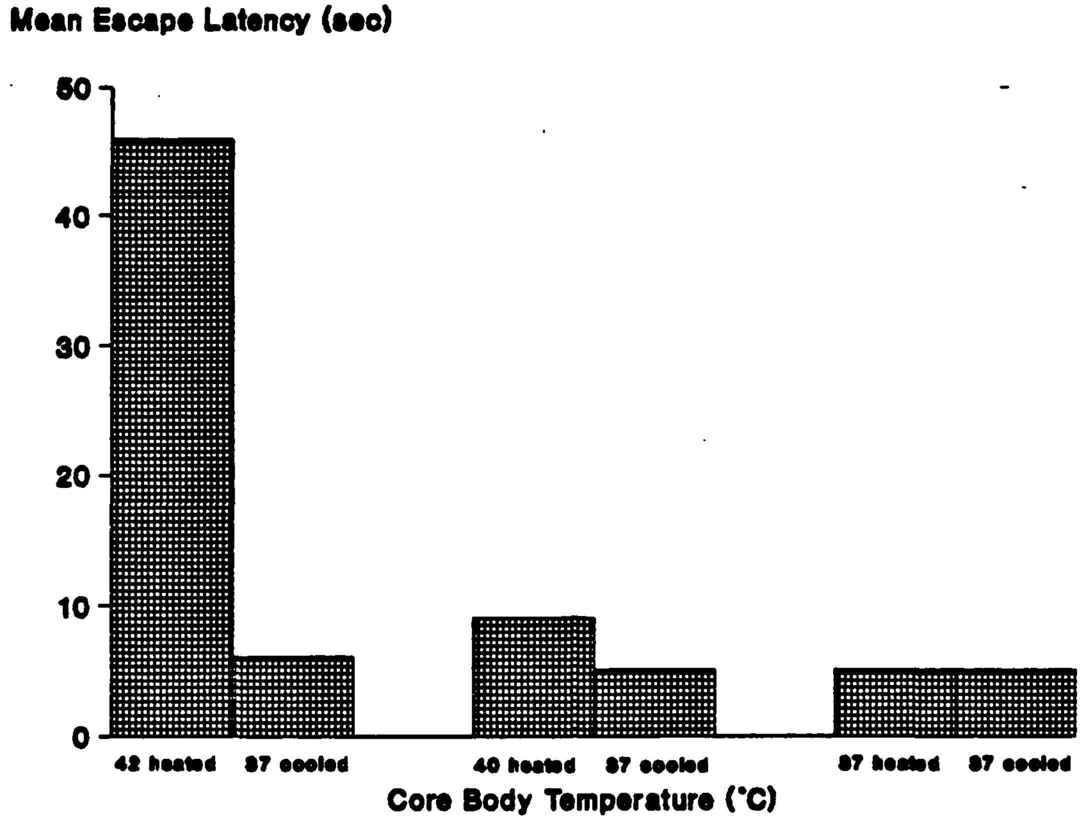
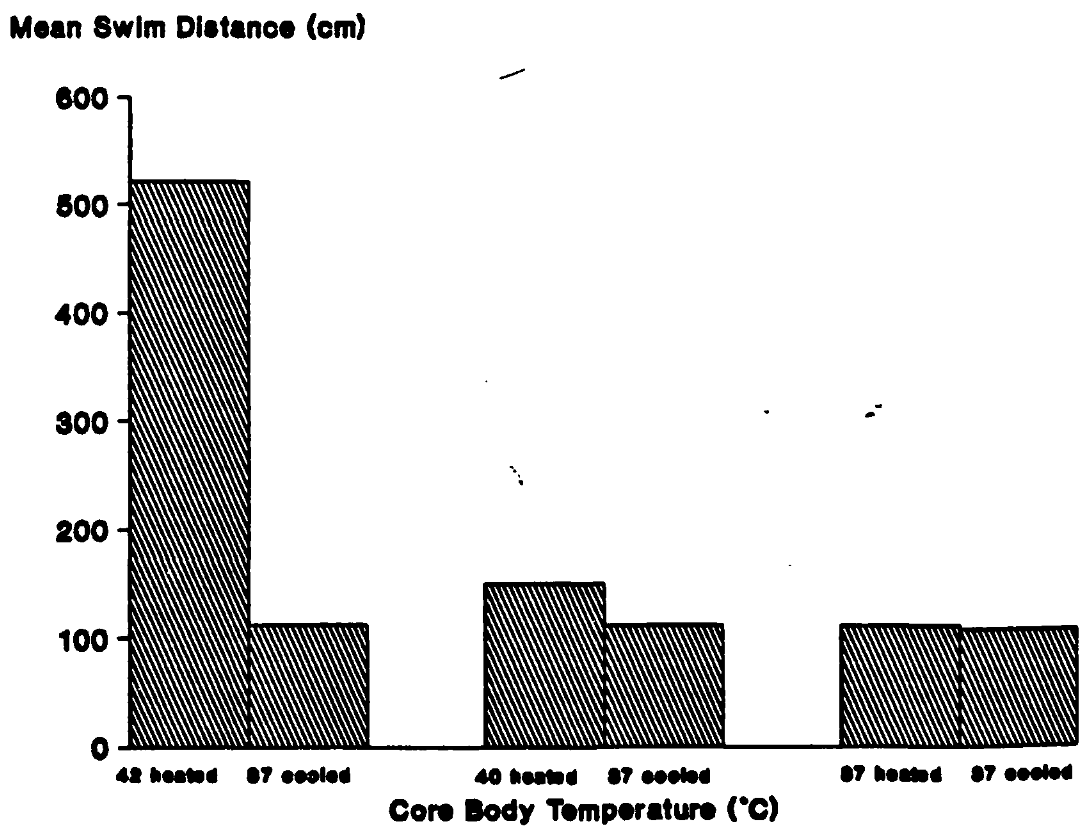
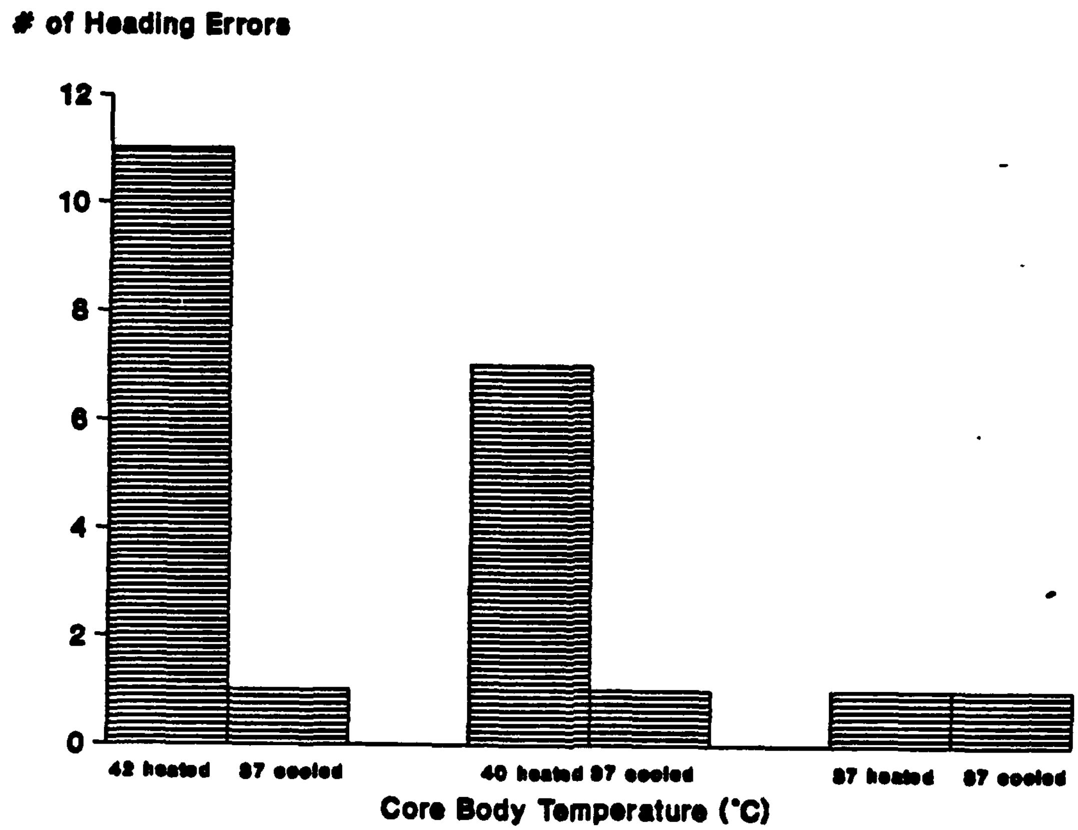

Mean Escape Latency (sec)
50
40
30
20
10
0
42 heated
37 cooled
40 heated
37 cooled
37 heated
37 cooled
Core Body Temperature (°C)


Mean Swim Distance (cm)
600
500
400
300
200
100
0
42 heated
37 cooled
40 heated
37 cooled
37 heated
37 cooled
Core Body Temperature (°C)



# of Heading Errors
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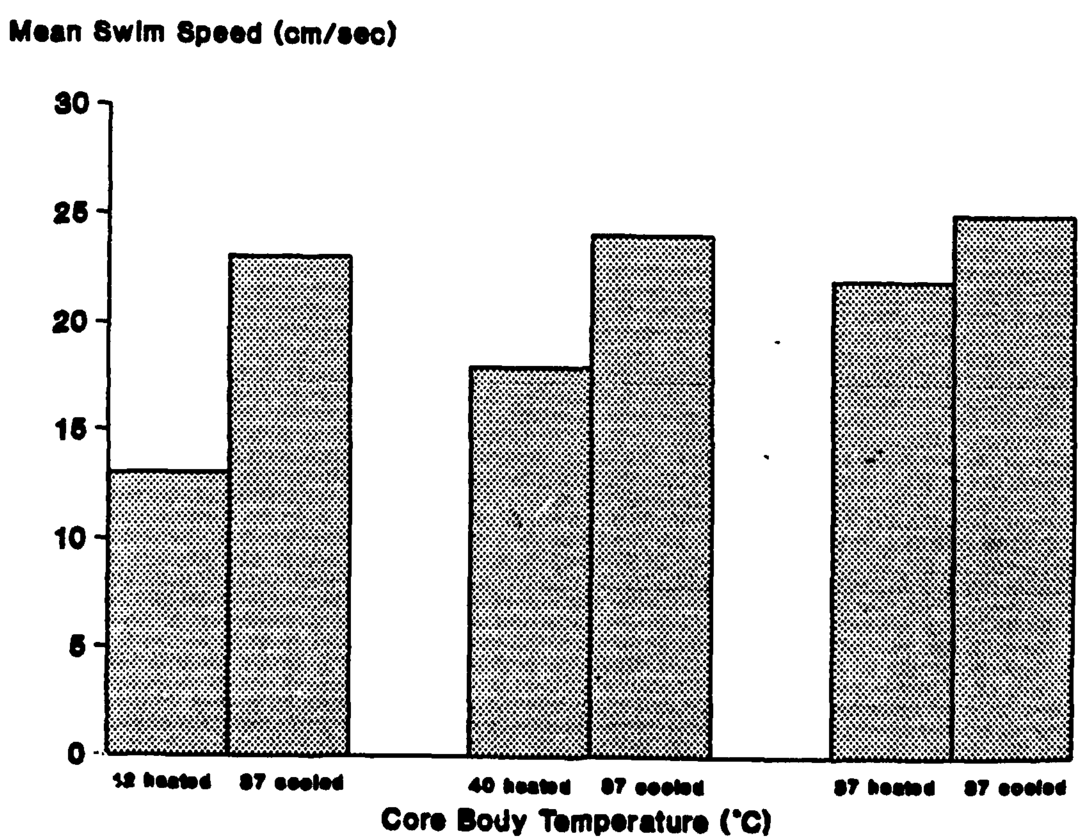
10
8
6
4
2
0
42 heated
37 cooled
40 heated
37 cooled
37 heated
37 cooled
Core Body Temperature (°C)



Mean Swim Speed (cm/sec)
30
25
20
15
10
5
0
42 heated
37 cooled
40 heated
37 cooled
37 heated
37 cooled
Core Body Temperature (°C)

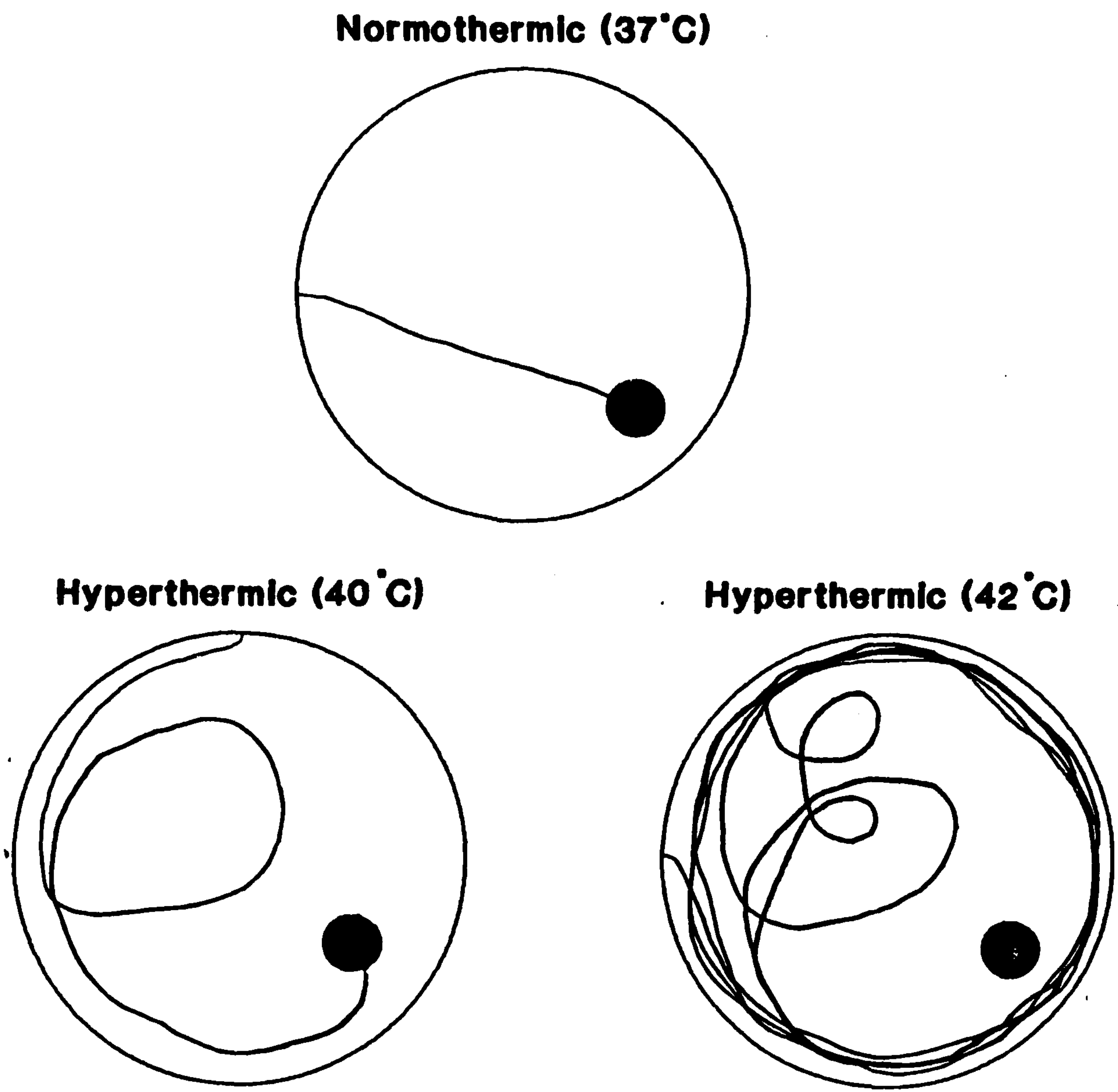

Normothermic (37°C)
Hyperthermic (40°C)
Hyperthermic (42°C)

Figure 1. Mean escape latencies and swim distances to reach the platform for hyperthermic and recooled rats.

Figure 2. The number of heading errors and mean swim speed to reach the platform for hyperthermic and recooled rats.

Figure 3. Swim patterns of one rat heated to a T_{c} of 42°, 40° C and normothermic control. Hyperthermia impaired spatial performance. Hyperthermic rats frequently looped around the perimeter of the pool much like a naive rat when first learning the maze.