TOLERANCE TO HEAT STRESS

ASSOCIATIVE TOLERANCE TO REPEATED HEAT STRESS

BY

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Abstract

Learning processes have been demonstrated to play an integral role in drug tolerance. More recently, researchers have found that associative mechanisms also play an important role in the adaptation to cold exposure (Riccio, MacArdy & Kissinger, 1991). The present study investigated the effect of contextual stimuli on temperature response to repeated heat stress. Rats receiving repeated heat exposures (56°C, 10 min, 6 trials) demonstrated adaptation to the heat as measured by a decrease in hyperthermia. The tolerance to the heat stress was not disrupted by changing the contextual cues associated with the heat. These findings demonstrate tolerance to repeated heat stress but do not provide evidence of associative learning in this adaptation. Future experiments should assess the question of thermoregulation and associative processes using highly discriminant conditioning environments.

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The devotion and love from my husband Charles has inspired me personnally, socially, and academically to try to be the best that I can be, and I love him for this ...

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Associative Tolerance to Repeated Heat Stress

Thermoregulation is a common and essential phenomenon in animals. The temperature of the body is held in dynamic equilibrium as a function of environmental and metabolic influences. Even a small temperature imbalance can impose an enormous strain on the physiology of the body (Guyton, 1986). Thus, it makes adaptive sense that body temperature be tightly regulated to prevent any such deviations.

Temperature is thought to be controlled, in part, by thermosensitive neurons in the preoptic area of the hypothalamus. The firing rates of these neurons vary as a function of their temperature and bring about neural and behavioural mechanisms to counteract thermal imbalances (Satinoff, 1980). The neurophysiological mechanisms of thermoregulation have been studied quite extensively. In contrast, there have been few investigations into the adaptation to repeated thermal stress, and of learning processes involved in thermoregulation.

Houk (1988) suggests that there are three major control strategies that the body uses to effect homeotasis: negative feedback, feedforward, and adaptive control. Negative feedback is a control mechanism which generates physiological and behavioural functions by comparing an intrinsic reference signal (e.g., the set point) to actual conditions (e.g., the internal temperature) as monitored by a comparator (e.g., the preoptic area of the hypothalamus).

Operant thermoregulatory behaviour is an example of the effector mechanism of this negative feedback loop. Carlisle (1969) showed that after lesions to the preoptic area of the hypothalamus, animals were unable to regulate their body temperatures autonomically in either warm or cool environments. However, lesioned rats were still able to bar press to turn on a heat lamp to prevent severe hypothermia, suggesting that the damage did not impair motivated instrumental responding. Satinoff and Henderson (1977) suggest that reflexive and operant responses are examples of negative feedback effector mechanisms of thermoregulation, and that these mechanisms are functionally and anatomically separate (for a review see Satinoff and Henderson, 1977). Moreover, thermoregulatory research indicates that there may be more than a single comparator, with multiple inputs and outputs (Satinoff, 1978).

Biological homeostatic systems are more complicated than the feedback model can predict. For example, this model cannot explain the neurobehavioural response to an "external" disturbance that elicits a deviation. Temperature control systems may be sensitive not only to internal temperature, but also to the outside predictors of internal temperature disturbance. A feedforward control system can initiate neurobehavioural responses in anticipation to an impending disturbance. This control system generates commands without using continuous negative feedback and its inputs specify the goals of the overall control process. In contrast, the

adaptive controller alters the components (i.e., the feedback and/or feedforward mechanisms) of a control system as opposed to producing immediate changes in output.

Houk (1988) used the motor control of limb movements as an example of adaptive actions of feedforward controllers. That is, "once a feedforward mechanism for the generation of motor commands has been implemented, a variety of simple cues can be used to trigger the movements... Via the mechanism of associative conditioning, a great variety of sensory events can be used to trigger an equal variety of movements selected from the animal's repertoire" (Houk, 1988, p.105). Since the study of "the mechanisms of associative conditioning" is by definition the study of Pavlovian conditioning, it is suggested that Pavlovian processes function as a feedforward mechanism (Siegel, 1991). The following discussion delineates how Pavlovian/feedforward processes play an important role in homeostatic maintenance.

A Pavlovian Perspective

Investigations into the physiological properties of tolerance have helped elucidate many fundamental regulatory mechanisms. Although physiological properties have helped to explain homeostatic phenomena, there is cumulating evidence emphasizing the importance of learned associative processes for the development of tolerance (Siegel, 1989).

According to Pavlov (1927), living organisms respond both reflexively

and in anticipation to stimuli. In the classical Pavlovian conditioning paradigm, an association is usually formed between two stimuli whereby one stimulus is predictive of the occurrence of the other. Generally speaking, the second stimulus is termed the unconditional stimulus (UCS) as a result of eliciting physiological activity unconditionally. The first stimulus signals the presentation of the unconditional stimulus, but elicits little activity prior to its pairing with the UCS, and thus is termed the conditional stimulus (CS). Pavlov demonstrated that through association, the CS becomes capable of eliciting a new conditional response (CR) contingent upon its pairing with the unconditional stimulus.

Pharmacological conditioning has been greatly influenced by Pavlov's theory of conditional responding. It is believed that an 'association' builds between the systemic effects of a drug and the stimulus array surrounding the drug administration. According to the traditional theory, the CR is a replica of the UCR. More recent research has suggested that the CR depends on the nature and mechanism of the pharmacological effect (Eikelboom & Stewart, 1982; Siegel, 1989). It has been shown that for many drugs, the CR is an anticipatory compensation for the drug effect itself. A decreased response to a drug, over the course of successive administrations in a consistent environmental context, is defined as tolerance. Therefore, as suggested by Siegel (1991), "feedforward processes (drug-compensatory conditional responses) augment feedback processes (drug-compensatory unconditional

responses) in the control of tolerance" (p.408). There is ample behavioural evidence for this phenomenon in pharmacological conditioning (for a review see Siegel, 1989).

As is the case with associative exposure to drugs, stress research has shown that repeated exposure to various stressors results in an adaptation of the effects of stress on the organism (for a review see Burchfield, 1979). The following Pavlovian framework, and the concomitant feedforward homeostatic mechanism, can perhaps effectively explain the stress response over time: 1) when an organism encounters a stressor, it responds physiologically through sympathetic arousal and associates this change with predictive cues in the environment, 2) after repeated exposure to the same stressor with the same predictive cues, the arousal decreases due to an anticipatory compensatory response, 3) if the predictive cues are present but the stressor is not, a compensatory response is singly present, and 4) if the predictive cues are changed and the organism is exposed to the stressor, an autonomic response will be present in comparable levels to that incurred by intial exposure to the stress. Burchfield (1979) suggests that an evolutionary account should selectively favor organisms which are predisposed to learn cues predictive of stress. It is hypothesized that adaptation to a repeated thermal stress consists of a compensatory response of the physiological mechanism, and that these responses are a result of learning predictive cues.

Evidence for Thermoregulatory Conditioning and Associative Tolerance

Most studies examining thermoregulatory adaptation in rats have used cold-water swimming or cold-water restraint as a repeated-intermittent stressor, and have measured stress through a deviation in rectal temperature. As a thermic response to the cold (i.e., the first trial), rats exhibit a hypothermic condition. After many trials, rats seem to adapt to the cold water by either no longer exhibiting hypothermia or by exhibiting hypothermia to a lesser extent than in the initial trials [Bodnar, Kelly, Spiaggia & Glusman (1978); Hamm, Knisely & Lyons (1990); Hjeresen, Loebel & Woods (1982); Kokkinidis (1986); Riccio & Campbell (1966); Riccio et al. (1991)].

Kokkinidis (1986) examined the possiblity that associated stimuli may be involved in the adaptation to repeated-intermittent stress. More specifically, he manipulated contextual cues associated with a cold and warm water swim in order to assess their importance in the development of tolerance to the cold. In a first experiment, the thermic effects of repeated daily exposure of mice to cold swim (10°C for 3 min) were evaluated. Temperatures were taken rectally pre- and post-swim. By the fifteenth test session, tolerance to the hypothermic effects of the cold-swim were evident in the adapted group versus its appropriate control groups. In subsequent experiments, a distinctive environment was used to separate contextual cues associated with the cold and warm water swims. The distinctive environment consisted of an aluminum chamber and auditory white noise. The colony

room and a glass cylinder cage served as the alternate environment. Two groups of mice received the cold water swim (10°C for 3 min), and warm water swim (30°C for 3 min) for 24 days on an alternating schedule (i.e., a discrimination paradigm). The distinctive environment was counterbalanced across each temperature condition; all animals were subjected to 12 days of cold and warm water swim and differed only in the context cues associated with the condition. On the test day, both groups were exposed to the cold swim in the distinctive environment. Body temperatures were recorded immediately after and 15 min following exposure to the stressor. Results indicated that the degree of tolerance on the test day was comparable in both groups of mice exposed to the stressor, irrespective of the contextual cues associated with the cold swim. The absence of situational specificity in the development of tolerance suggests that the cues associated with the cold water swim were not critical in the adaptation to the thermic effects of the stress. The author suggests that learned adaptation to stress depends on a number of variables (e.g., type of cues) and that further work is needed in identifying the necessary conditions in which associative factors may play a role in the development of tolerance to repeated exposure to stress (Kokkinidis, 1986).

Recently, Riccio et al. (1991) examined the role of associative mechanisms in adaptation to repeated cold water exposure. Two groups of rats were exposed to two different contextual environments differing in size,

brightness, odor and sounds. The duration of exposure was determined for each animal based on the time required to decrease each animal's body temperature to 21.5°C on the first day of exposure to the cold (M=10 min). Thus, animals were exposed to cold water (4°C), by partial immersion, for a constant duration (determined from day 1) for 1 trial per day for 6 days in counterbalanced contexts. Half of the subjects were exposed to the cold in one context and half were exposed to the cold in the another context. After 6 days of conditioning, the two groups were significantly less hypothermic than on day 1 of conditioning (i.e., tolerance was observed). On day 7, half of the subjects (group 1) were exposed to the cold in the counterbalanced context. Results indicated that these animals were significantly hypothermic compared to animals (group 2) immersed in the same conditioning context. On day 8, group 1 was returned to the original conditioning context and tolerance was reinstated. In contrast, on day 8, group 2 was tested in the counterbalanced context and was significantly hypothermic compared to animals (group 1) immersed in the same conditioning context.

These results provide clear evidence for associative processes in adaptation to a repeated cold stress, such that tolerance to cold was disrupted by altering the context in which the animal had been conditioned to the cold. As recognized by the authors, it would have been interesting to observe whether an anticipatory compensatory response would have occurred had the rats been exposed to the conditional cues (CS) without the cold-

stress (UCS) in a second test trial.

Heat Tolerance

There is considerably less literature which examines the role of associative processes to repeated heat stress. This is perhaps due to the procedural difficulties encountered in this line of research. For example, precise calibrations and temperature recordings are essential considering that rats are very sensitive to this type of stressor; the body temperature range for rat survival lies approximately between 0°C and 45°C with an average body temperature of 37°C. Therefore, the average allowance of thermic change is greater in response to cold than to heat stress (Heller, Crawshaw & Hammel, 1978). In regards to heat stress, researchers must take care in selecting appropriate temperature settings and exposure time for the animals.

Only one study has examined associative processes of heat stress (Bermant, Reeves, Levinson & Justesen, 1979). The authors assessed classical conditioning of microwave and tail-shock induced hyperthermia in rats. The researchers hypothesized that conditional response would be in the same direction as the unconditional response (i.e., a hyperthermic response) in both treatment groups. Rats were presented with a 525-Hz auditory signal as the conditional stimulus for 30 seconds. Four groups of animals (n=3) received either a tone-only, tail-shock, 10-s microwave radiation or 30-s microwave radiation with the simultaneous presence of the CS. Phases of the experiment included habituation of the CS for 30 trials, conditioning for

200 trials and extinction for 100 trials. Unfortunately, attrition left the experimenters with only two rats in each of the radiation groups. Therefore, even though they report a modest conditional response in a hyperthermic direction (i.e., an increase in colonic temperature), the data they present seem inconclusive. It is evident by the attrition rate that the dose of heat/radiation given was lethal. Futhermore, it has been demonstrated that animals which receive pre-conditioning exposures to the conditional stimuli are usually slower to learn the conditional response than animals for which the CS is novel (Lubow & Moore, 1959). Thus, the pre-exposure to the conditional stimulus (i.e., auditory noise) in the above experimental paradigm may have interfered with the subsequent learning of an association with the unconditional stimulus (i.e., the heat effect). Moreover, the simultaneous presentation of the CS with the UCS may have weakened the association between the two stimuli by omitting the predictiveness of the conditional stimulus with the unconditional stimulus.

A second study examined the role of heat intolerance in human males by measures of plasma cortisol levels and core temperatures (Follenius, Brandenberger, Oyono, Candas, 1982). The main objective of the experiment was to determine plasma cortisol levels and body temperatures in relation to different heat environments, and to assess corresponding subjective reports of discomfort. One group of four men was exposed to four randomnized experimental sessions. These consisted of a control session and three

different lengths and strengths of heat exposure. The second group of three men were exposed to the same condition for five successive daily exposures: 43°C, 165 minutes. Results indicated that males repeatedly subjected to the same condition (i.e., the second group) demonstrated lower plasma cortisol levels and lower body temperatures at the end of exposure to the last session. Moreover, the subjects reported less discomfort in proportion to the number of trials to which they were exposed. The experimenters attribute part of the latter observations to physiological tolerance; that is, an increase in perspiration rate (Davies, Harrison, Cochrane, Edwards, & Gibson (1981) cited in Follenius et al., 1982). Although the authors were not explicitly examining the phenomenon of associative heat tolerance, their data suggest that humans may be able to cope with heat stress more efficiently with repeated associations to environmental cues.

Experimental Hypothesis

The pattern of attenuated corticosterone/cortisol response and attenuated body temperature response in reaction to repeated stress can be attributed to tolerance. Moreover, as presented, there is evidence which demonstrates that part of cold tolerance can be ascribed to a Pavlovian conditioning process. Based on this associative process, it is hypothesized that an anticipatory compensatory response occurs in response to cues contingently associated with the thermic stimuli, and that this accounts for the tolerance observed. More specifically, the theory predicts that tolerance to

repeated stress consists of lowered overall physiological responding over conditioning trials, and that these responses are effective by learning predictive cues. If the development of heat tolerance is solely a result of physiological adjustments to maintain homeostasis, then a change in contextual cues should have little bearing on tolerance. However, from an associative perspective, changes in contextual stimuli should alter the conditional stimuli controlling tolerance and thus result in impaired responding. The purpose of the present experiments was to investigate the effect of contextual stimuli on temperature response to repeated heat stress.

Experiment 1

The significance of the conditional stimulus to the unconditional stimulus has been shown to govern the rate of classical conditioning; that is, some CSs are more relevant to certain USs. In a classic experiment, Garcia and Koelling (1966) showed that rats conditioned with sickness learned a stronger aversion to taste than to audiovisual cues. On the other hand, rats conditioned with shock demonstrated a stronger aversion for audiovisual cues than taste cues. The CS/US relevance effect has since been demonstrated in many experiments. However, it is not known what makes a CS relevant to a US. Garcia, Hankins & Rusiniak (1974) suggest that "evolution has designed this species (rats), and many others, to cope with foods that produce illness, but has left them relatively helpless to deal with places that produce illness, even when the agent producing the illness can be detected" (p.825). They suggest that internal cues (e.g., tastes) may be more easily associated with interoceptive stimuli (e.g., illness), and that external cues (e.g., audivisual) may be more easily associated with exteroceptive stimuli (e.g., shock).

When an animal is given a spatial choice between two identified areas, one opened to radiation, and the other guarded from radiation, it is apt to repeatedly enter the exposed area, even to a fatal dose. On the other hand, after a single dose of radiation, a rat will demonstrate tendencies to

alter its diet in order to cope with the radiation effects. It will avoid nutrients consumed prior to, and during the exposure to the radiation (Garcia, Kimeldorf & Hunt, 1961). It is hypothesized that a taste (flavour-olfactory) cue, which is usually referred to as an "internal" cue because of its ingestive properties, may be more apt to contingently prompt the animal to heat stress, since heat stress results in internal physiological adjustments. Therefore, the use of taste cues may enhance the associative process, and thus enhance tolerance to the heat stress. Moreover, the associability of the taste to the heat may be assessed by direct measurement of the ingested solution. Analogous to the taste aversion experiments, the animal may demonstrate an aversion to the taste associated with the heat.

One study has looked at whether rats learn to associate cues with heat and whether heat exerts its associative effects in the internal or the external milieu (Green, Hart & Hagen, 1981). In experiment 1, subjects were given a saccharin solution and then immediately exposed to a) a hot environment (95°F) for a three hour period (aversion group) b) shifted from a hot (95°F) to normal (75°F) environment for a three hour period (preference group) c) left in the thermal environment where they had been living (control group). The animals were given five conditioning trials and then tested for conditioned taste aversions and preferences. No conditioned taste aversions or preferences were found in a two-bottle test with water versus a saccharin solution. The authors suggest that the failure of the taste cue to act as

conditional stimuli may have been a result of the non-noxious thermal levels used. In a second experiment, the researchers utilized a more heat-susceptible strain of rats. Moreover, an almond-flavoured water was implemented as a cue. It was reasoned that the strong flavour and odor of almond flavoured water may make it more associable to heat. A heat susceptible test (given before conditioning to confirm the greater sensitivity of the strain) resulted in the deaths of half of the subjects and they again found no taste aversion in the remaining rats. In a third experiment, the experimenters looked at whether locomotion may be a more sensitive indicator of the effects of heat. Two experimental groups were given five exposures to heat in one or the other side (black or white) of a shuttle box, whereas the control group was given five exposures to heat in a plastic cage. Results indicated that the rats tended to stay out of a shuttle box compartment that had been previously associated with heat. The rats learned to move away from an environment where heat had been encountered suggesting that the physiological consequences of heat stress may be viewed as belonging to the external milieu, and therefore can be effectively associated with a physical environment. In contrast, neither taste cues of experiment 1 or 2 were effective in reducing ingestion when paired with heat. However, since the flavoured solution was the only liquid consumed per conditioning day, it may have served as a reinforcer to the dehydrated rat; thus confounding its associability to the rat.

The present experiment evaluated the thermic effects of repeated exposure to heat stress in a discrimination design, whereby all animals were exposed to cued-heat and cued-no-heat conditions. The cues were taste (coffee vs. vinegar), light (regular vs. strobe), and spatial orientation of the baseline box (left vs. right) to the experimental chamber. It was reasoned that the use of taste and non-taste cues would make both internal and external modalities available for association with the heat.

If associative factors mediate thermoregulatory responding to heat stress, then animals may learn to respond to the context of the thermic conditions. More specifically, animals may learn to respond to conditions through association with feedforward cues. Through this association, animals may develop tolerance in the cued-heat condition, i.e., exhibit less hyperthermia over repeated trials. When presented with cues to the no-heat condition and then exposed to the heat, it is hypothesized that animals will respond hyperthermically in comparison to their own cued-heat responses. By analogy to other taste aversion experiments, associability of the tastes to the thermic conditions can be assessed through a flavour preference test. More specifically, it is hypothesized that the rats may demonstrate an aversion to the taste paired with the heat stress.

Method

Subjects

Seventeen adult male Sprague-Dawley rats (250-300g) were obtained from Charles River breeding farms in Quebec, Canada. The rats were housed individually in hanging wire-mesh cages in a colony room maintained on a 12/12 dark/light cycle. The rats were handled and weighed for two minutes each day for a week prior to the surgical implant of thermic transmitters (see below for details). After a week of recovery, the animals were put on a 18 hour water deprivation schedule for 11 days. Food was available ad libitum throughout the experiment except in the experimental chamber. The temperature in the colony room was maintained at 23 degrees Centigrade.

Surgery and the Biotelemetry System

Body temperatures were recorded with an analog to digital data acquisiton system (Dataquest III, 1988 Data Sciences). A biotelemetry transmitter (VM-FH Model) was surgically implanted into the abdominal cavity of the rat. The transmitter (or minimitter) consits of two thermistors and a battery operated transmitter which emits electronic pulses. The rate of pulses is proportional to the surrounding temperature. Pulses from the minimitters are collected through receivers (RA-1000) and are sent to a computer (Tandy 3000) via a consolidation matrix (BCM-100). The dataquest software calculates temperature from the inter-pulse interval, and the thermistors are

accurate to .1 degree Centigrade. The dataquest system allows for objective, non-obtrusive measurements, without handling and or probing. Handling has been shown to be a critical variable in temperature measurement (Eikelboom, 1986).

Apparatus

Four plexiglass boxes (28cm X 22cm X 15cm) with removable grid floors and trays served as the experimental chambers. A dual control (heat and fan) hairdryer (Europa, model # 14037), modified to be thermostatically controlled, was attached to each chamber and served as the heat and ventilation source. The experimental chambers were thermically regulated to maintain a temperature of 56°C during the heat conditions. The temperature in the experimental chamber was approximately 27°C during the no-heat conditions. Separate clear plastic boxes (35cm X 30cm X 15cm) covered with a metal grid served as individualized holding and recovery chambers. Drinking solutions were presented in graduated cylinders, thus allowing the volume ingested of each solution to be measured.

Transmitter Calibration and Surgical Implantation

The minimitter was fitted with a battery and encased in a plastic capsule. The capsule was then coated with melted paraffin (80°C, Du Pont de Nemours & Co.). Silk thread was tied around each sealed minimitter and the capsule was then coated again. The minimitter was then calibrated in a temperature-controlled water bath (Polytemp Model 730 Immersion Circulator).

Five frequency values were recorded for each transmitter at 35 and 39 degrees Centigrade using the Dataquest Software. Mean values were calculated for each transmitter and those temperatures were used as calibration values. The transmitters were then soaked in alcohol prior to implantation.

Subjects were anaesthetized with sodium pentobarbitol (65 mg/kg). Their abdomen was shaved and disinfected. A four centimeter incision was made in the abdomen and the outer tissue layer was pushed back. Another incision, approximately 3 cm, was made in the peritoneum. The transmitter was tied to the inner peritoneal wall using the silk thread tied to the transmitter. The peritoneum was sutured with cat gut, the outer skin layer closed with wound clips, and each animal was given a .3 ml injection of an antibiotic (Derepam).

Contextual Cues

On heat condition days, group 1 was given a cider vinegar (Heinz,.3%) -saccharin (.05%) (Vin-Sac) solution to drink in the holding box to the right of the experimental chamber and was exposed to regular lighting. On the no-heat days, the same group was given coffee (Sanka decaf.,.1%) (Coff), left side placement and exposed to a strobe light (Grass PS2 photo stimulator) turned on to maximal intensity with a frequencey of 4 cycles per second. The cues were counterbalanced such that group 2 had coffee, left placement and strobe light signaling heat, and vin-sac, right placement, and

regular lighting signaling no heat. The experimental chambers were counterbalanced within the experimental room such that half of the animals received heat and half received no heat to the contextual cues every other day. Due to the limitation of only four experimental chambers, several experimental sessions were formed: an early session (7:30 am) and a later session (9:00 am), which were counterbalanced for both groups. Subjects were placed in individualized baseline and recovery boxes which were different for heat and no-heat sessions. The same experimental chamber was used for heat and no-heat conditions for each subject and was carefully cleaned after each cycle (i.e., after two conditioning trials). All other conditions were held constant.

Procedure

Subjects. Sixteen animals had minimitters implanted in their abdominal cavities. One animal died as a result of the anaesthetic. After a week of recovery, all subjects had functional minimitters and were put on an 18-hour deprivation schedule for 11 days prior to experimentation. During this time, the animals were twice transferred to the experimental room to confirm the functionality of the minimitters and to habituate the animals to the procedure of the experiment. Seven minimitters failed over the course of the experiment. However, these animals were still included in order to assess their drinking data and to retain stimulus consistency from one conditioning trial to the next within the experimental room.

Design. The design was a within-subjects, discriminant paradigm, whereby all subjects were exposed to both the heat and the no-heat conditions alternately every 48 hours. Subjects were tranferred from the colony room to the experimental room in their home cages via a cart. They were then placed in the holding boxes and given the appropriate solution to drink. The solutions were removed after 30 minutes, and the temperature control of the experimental boxes were turned on. Baseline measurements were taken for 10 minutes following which the subjects were transferred to the experimental chamber for another 10 minutes. This exposure time was chosen based on Riccio et al.'s (1991) study of conditioning to cold stress. Two minutes after the placement into the chamber, the lighting was turned off and the group was left in red light. The animals were then placed into the recovery box for 19 minutes in order to record post-condition temperatures. Body temperatures were recorded every minute with the dataquest system throughout the 75 minute session except for one minute between transferring the animals between the holding box and the experimental chamber, and three minutes between transferring the subjects from the experimental chamber to the recovery box. The animals were then returned to their home cages in the colony room. Experimentation took place during the animal's active (i.e., dark) cycle. Animals received their water bottle 75 minutes after a session. The bottles were removed after three hours to complete the 18-hour deprivation schedule.

Experimental Schedule. All rats experienced six heat and six no-heat conditioning trials (i.e., six cycles) and were then exposed to a tolerance test. The test trial consisted of placing the subject in a cued no-heat condition, then exposing the subject to the heat. After the test trial, the animals received one more cycle, following which they were given a two-bottle preference test in their home cage. Both the Vin-Sac and Coff solutions were presented in the home cage at the time that the subject would typically get a solution in the experimental room. One solution was presented at a time before both of the cylinders were inserted. Flavour presentations were counterbalanced within the groups. Table 1 presents the order of the experimental schedule.

Table 1. Schedule for experiment 1.

Procedure	Days
Handling	8
Surgery/recovery	7
Water deprivation	11
Conditioning	24
Test trial	4
Conditioning	4
Preference test	1

Results

Temperature data were transferred to a spreadsheet program (Lotus 1-2-3, version 2.2) and the occasional missing data were replaced with temperature values linearly interpolated from surrounding data points. Only the data of the subjects with working minimitters are reported (N=9) (see Appendix A).

Conditioning

Figure 1 illustrates the means of the maximum body temperatures (+/- S.E.M.) of animals (N=9) exposed to the heat and the no-heat conditions (minutes 36-52) during cycles one and six.

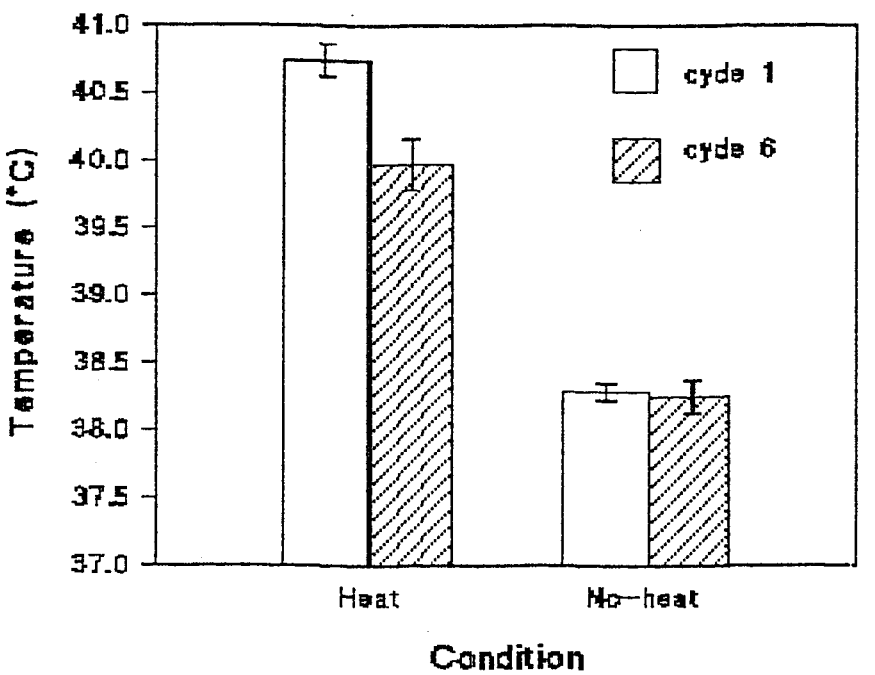

Figure 1. Means of maximum body temperatures (+/- S.E.M.) during cycles 1 and 6 in heat and no-heat conditions.

A mixed analysis of variance, Group (1,2) X Cycle (1,6) X Condition (heat, no-heat), of maximum body temperature yielded a Group X Condition interaction, $F(1,7)=18.57$, $p<.01$, and a Cycle X Condition interaction, $F(1,7)=8.65$, $p<.05$. Newman-Keuls multiple comparisons (alpha=.05) for the above interactions revealed significantly higher temperatures of subjects in group 2 in response to the heat condition, suggesting that the contextual cues may have affected temperature differently in the two groups to the heat condition. However, in both groups, body temperatures of Cycle 6 were significantly lower than in Cycle 1 in the heat condition but not in the no-heat condition.

It was hypothesized that an anticipatory hypothermic response elicited through feedforward cues may occur during the baseline of the cued-heat condition after repeated trials. Analysis of minimum baseline temperature (minutes 25-35) was done in order to assess this anticipatory response. A mixed analysis of variance, Group (1,2) X Cycle (1,6) X Condition (heat, no-heat), on minimum baseline temperature revealed only a significant interaction between groups and conditions, $F(1,7)=22.91$, $p<.01$. Newman-Keuls post-hoc analyses indicated that minimum baseline temperatures were significantly higher in groups exposed to coff-strobe cues as opposed to vin-regular-lighting cues, again suggesting that the cues may have affected temperature differently in the two groups.

Figures 2 to 5 illustrate the pattern of temperature response during cycles 1 and 6 for groups 1 (n=5) and 2 (n=4) in both the heat and the no-heat conditions for minutes 26 through 66 of the experimental trial. The figures show a tolerant response over cycles for the heat condition, but no difference in response over cycles in the no-heat condition.

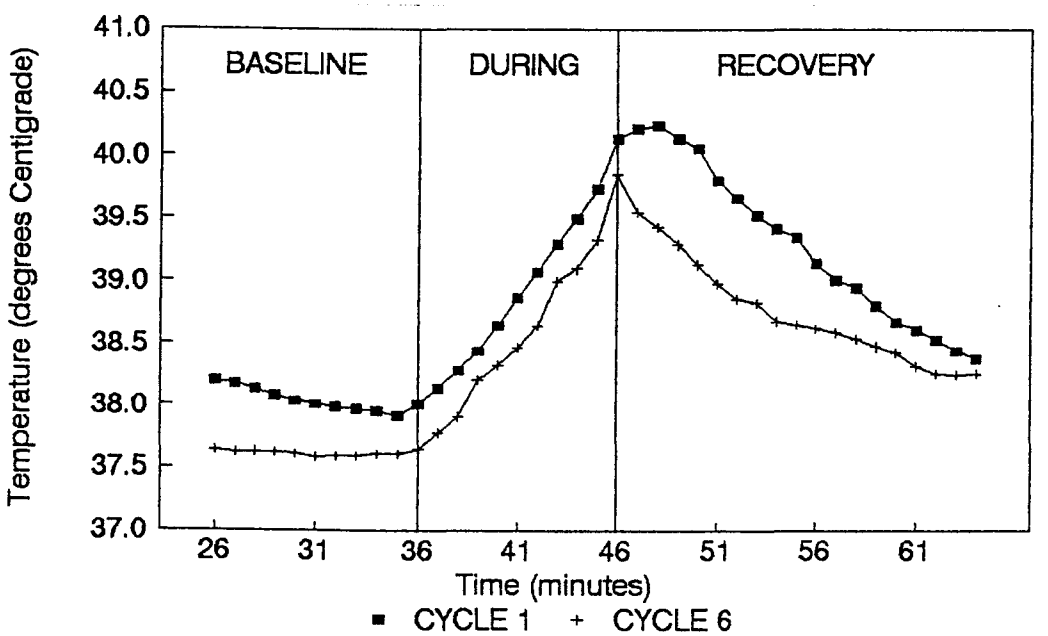

Figure 2. Group 1 (n=5) mean body temperatures as a function of time periods during the heat session of cycles 1 and 6.

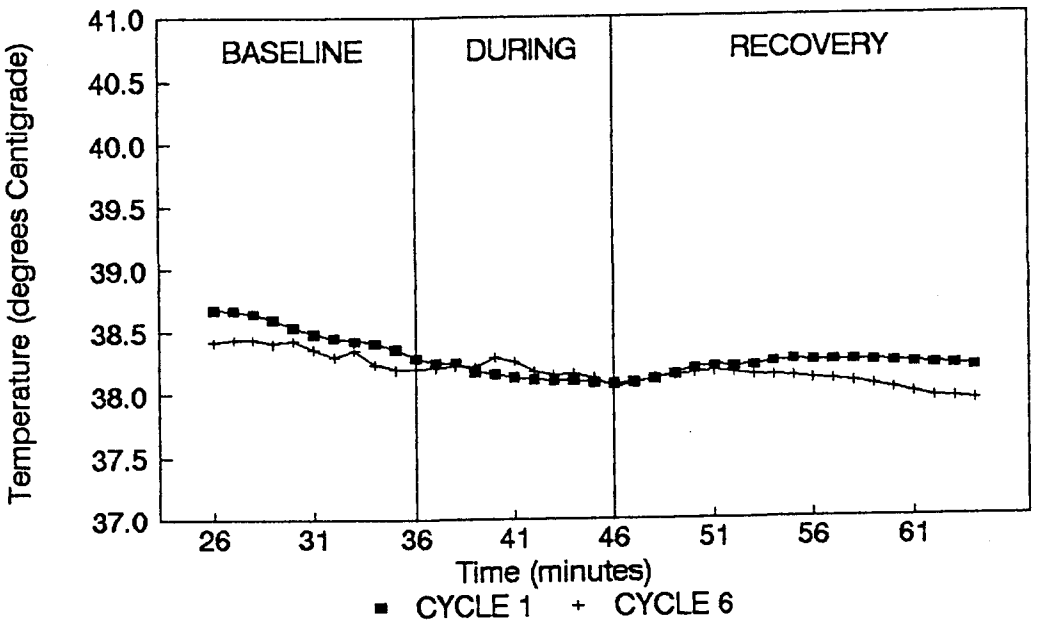

Figure 3. Group 1 (n=5) mean body temperatures as a function of time periods during the no-heat session of cycles 1 and 6.

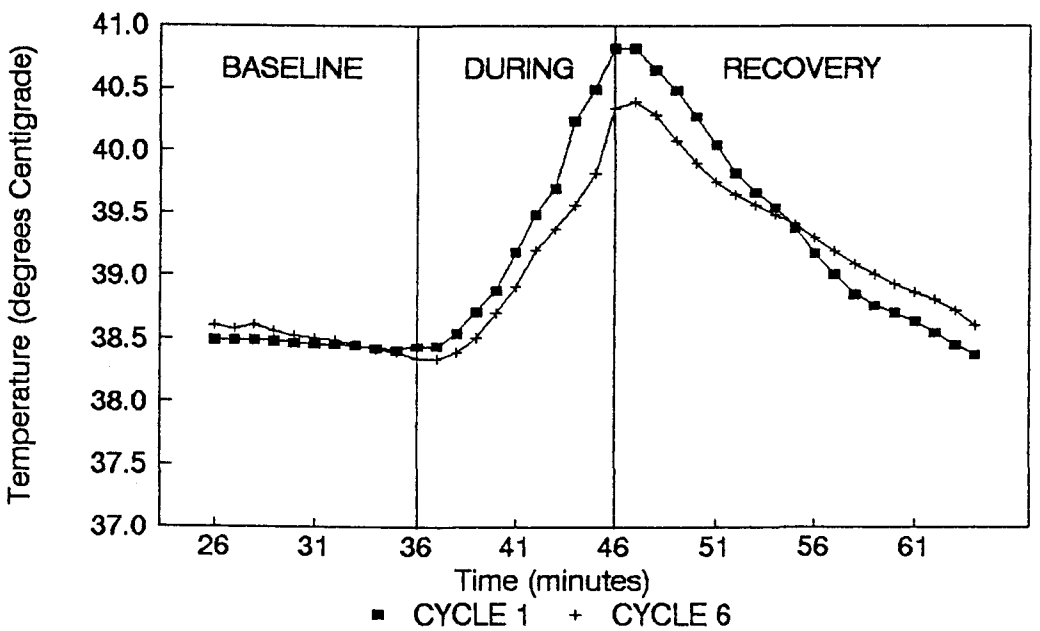

Figure 4. Group 2 (n=4) mean body temperatures as a function of time during the heat session of cycles 1 and 6.

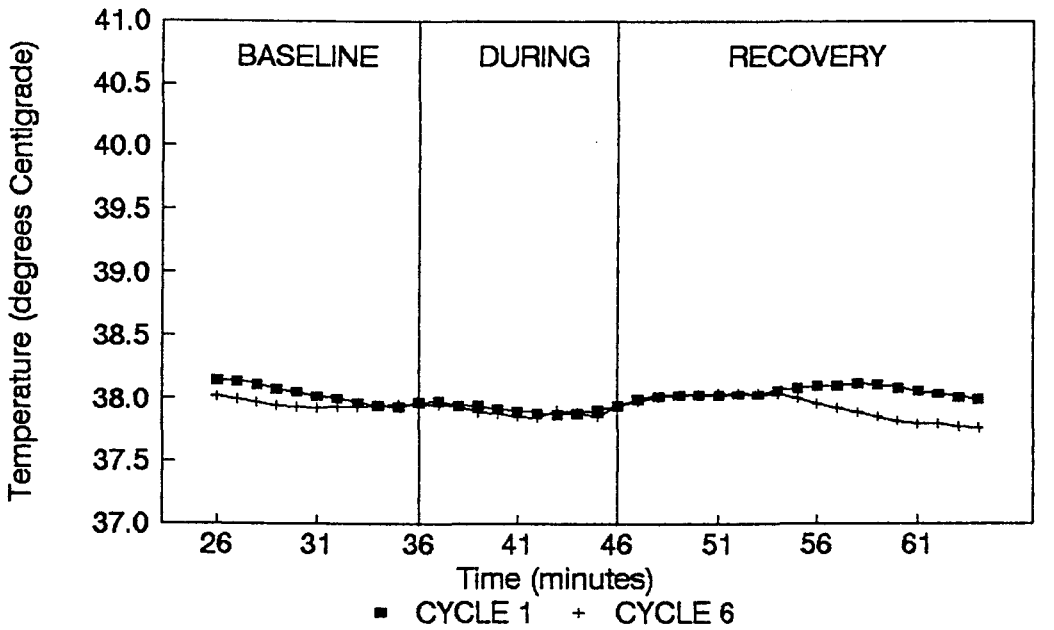

Figure 5. Group 2 (n=4) mean body temperatures as a function of time periods during the no-heat session of cycles 1 and 6.

Test Trial

Figure 6 illustrates the means of maximum body temperatures (+/- S.E.M.) for groups 1 (n=5) and 2 (n=4) in the heat conditons during Cycle 1, Cycle 6, and the Test trial (i.e., cued no-heat, then given heat).

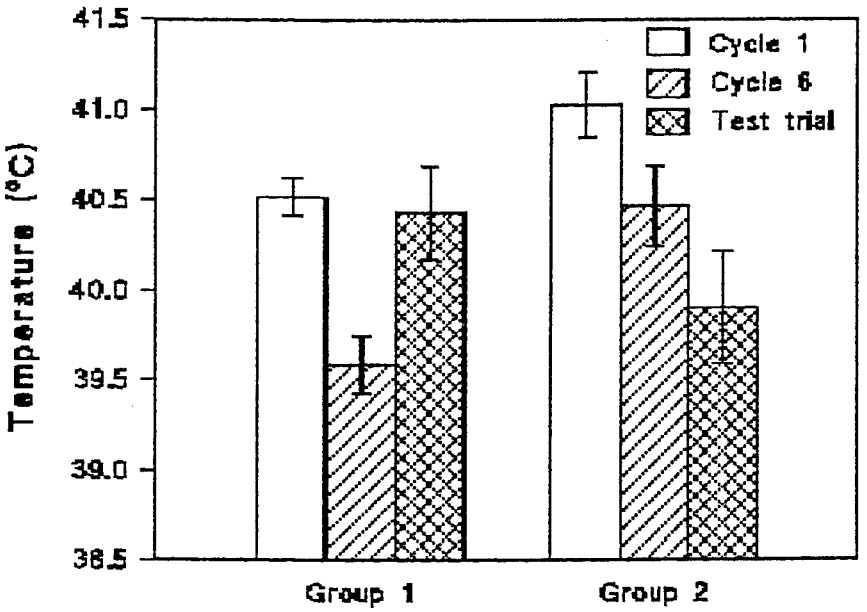

Figure 6. Means of maximum body temperatures (+/- S.E.M.) during cycles 1, 6, and test trial of groups 1 (n=5) and 2 (n=4).

A Groups (1 & 2) by Cycles/Test (1, 6 & Test) mixed analysis of variance on maximum body temperature revealed a significant interaction between the two factors, $F(2,14)=6.32$, $p<.05$. Because the previous analyses indicated that the two sets of cues were affecting the dependent measure (i.e., temperature) differently, within comparisons of the test trial and cycles 1 and 6 were not analyzed. However, between subject analyses of groups 1 and 2 indicated that the tolerance to the heat was not disrupted by changing the context in which the rat experienced the heat. This suggests that unexpected heat affected body temperature similarly to expected heat.

Taste Preference

A mixed analysis of variance, Groups (1,2) X Cycles (1,6) X Conditions (heat, no-heat), on volume of solution consumed showed no significant main effects or interactions ($p>.05$) indicating that animals in both

groups drank similar amounts of solutions across cycles during both heat and no-heat conditions.

Preference Test. A mixed analysis of variance (Groups X Flavours) on the volume consumed for the two-bottle preference test showed no interaction between the groups and the volume of flavour consumed ($p>.05$). Moreover, there was no main effect for the mean volume consumed between groups ($p>.05$). However, a significant difference between flavours consumed was revealed ($F(1,7)=5.76$, $p<.05$) indicating that the vin-sac solution was preferred to the coffee solution overall ($M=10.67$, S.E.M.=1.40; $M=5.0$, S.E.M.=.93, respectively). Figure 7 illustrates the volume of coffee and vinegar solutions consumed for groups 1 and 2 in the two-bottle preference

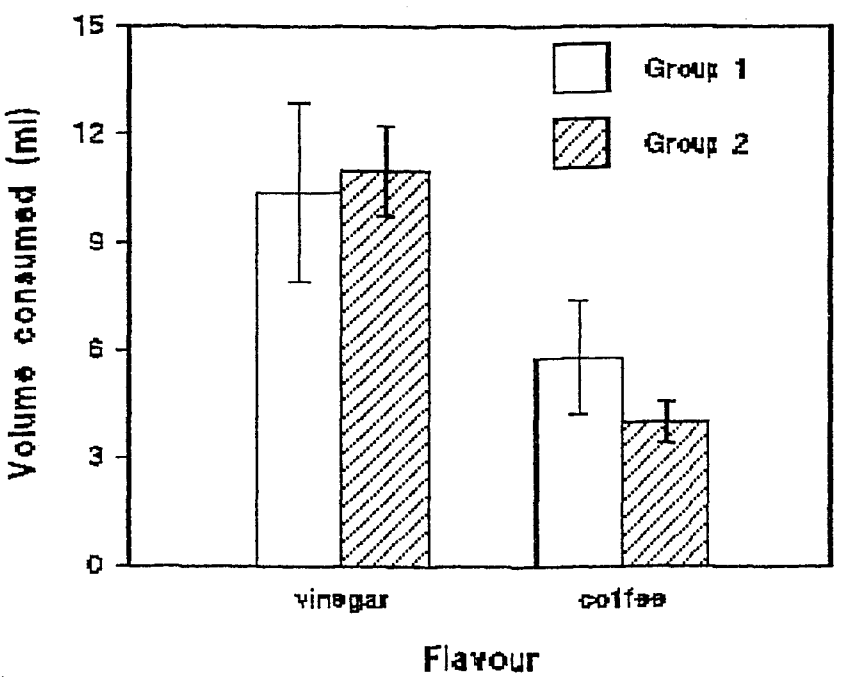

Figure 7. Mean (+/- S.E.M.) volumes of solutions consumed in a two-bottle preference test for groups 1 ($n=5$) and 2 ($n=4$).

Discussion

Both groups demonstrated tolerance to repeated heat stress such that the subjects' temperatures were lower on the last conditioning day as compared to the first exposure to the heat. This finding is similar to the research literature which shows thermic tolerance to repeated cold stress [Bodnar et al. (1978); Hamm et al. (1990); Hjeresen & al. (1982); Kokkinidis (1986); Riccio & Campbell (1966); Riccio et al. (1991)]. However, there was no evidence of associative processes in this adaptation; animals still responded with reduced hyperthermia during the test trial (cued no-heat, then exposed to heat) as compared to the first conditioning trial (re: between group comparisons). Moreover, there was no significant anticipatory response to the heat stress, although some animals did demonstrate a decrease in body temperature in anticipation to the heat stress.

The difference in baseline temperatures to the two contextual conditions seemed puzzling. Initially, it was thought that the decaffenated coffee solution may have contained some chemical that was causing higher temperature responding. However, when exposed to coffee and dark versus strobe and water, animals showed no differences in baseline temperatures - both groups were hyperthermic. Moreover, no baseline differences were found between animals run in the dark and animals run in the strobe light. Finally, a dark versus light baseline session indicated that animals exposed to the dark in their active cycle are hyperthermic as compared to animals

exposed to the light in their active cycle. Thus, the baseline differences between contextual conditions were probably due to the lighting and not the solutions ingested. Further investigation would have to assess associative tolerance with cues that elicit equal temperature reponses.

No preference was found for the taste associated with the no-heat condition. Unfortunately, the results indicated an overwhelming preference for the vinegar-saccharin solution in the two-bottle preference test, which may have masked any associations. Future investigations should utilize tastes of equal palatibility such that masking or overshadowing cannot occur. Recent pilot data have shown that the consumptions of unsweetened cherry (.05%) and grape (.05%) Koolaids with added saccharin (.05%) to be not-significantly different in a two-bottle preference test in animals naive to the tastes.

The rats in experiment 1 were water deprived. Since hydration is an important variable in thermoregulation, it may be wiser to implement a design such that the animals are not water deprived at all. Cunningham and Hallett (1991) examined the effects of a taste cue on thermic tolerance to ethanol. They manually restrained the animal and then infused approximately 1 milliliter of flavour into its mouth. Unfortunately, handling is an obvious problem to the above procedure. Other experimenters have trained their animals to drink at a particular time by pre-exposing the animals to a saccharin solution (Gowan, S., Tordoff, M. & Weingarten, H., 1991). Training the animals to drink requires approximately one week; the same amount of

time it would require animals to learn a deprivation schedule.

Discriminant paradigms require highly distinctive contextual environments. In the following experiment, a tactile cue replaced the lighting cue. The baseline box was modified such that a grid floor and clear sides were contingent upon a thermic condition, and a regular and opaque sides were to be contingent upon the counterbalanced thermic condition. The inclusion of a tactile cue was used to enhance the possiblity of an associative mediation to discriminatory tolerance.

Experiment 2

The present experiment was a replication of the latter with minor alterations. First, the animals were not water deprived. They were trained to drink a solution at a specific time. Since hydration is an important variable in thermoregulation, it was felt that this procedure would eliminate any potential confound related to dehydration. Furthermore, the animals were trained to drink in the experimental room which allowed them to habituate to the non-discriminant environment.

Second, cherry and grape Koolaid flavours were used as taste cues since pilot data (described earlier) have demonstrated equal palatability of the two flavours.

Third, researchers have found conditioned place avoidance to heat such that rats tend to stay out of a shuttle box compartment previously associated with heat (Green et al., 1980). This suggests that the physiological consequences of heat might be viewed as belonging to the "external milieu". Thus, a tactile cue (i.e., grid-clear box vs. no-grid-opaque box) was added as a potential exteroceptive cue as a replacement of the lighting cue which was found to interact with the dependent variable (i.e., body temperature).

This experiment was carried out in replication, with eight rats run in each replication. The research question and hypothesis remained the same

from the previous experiment: if associative factors are mediating thermoregulatory responding to heat stress, then the animals may learn to respond discriminately to the context of each conditioning session.

Method

Subjects

Sixteen adult male Sprague-Dawley rats (250-300g) were obtained from Charles River breeding farms in Quebec, Canada. The rats were housed individually in hanging wire-mesh cages in a colony room maintained on a 12/12 dark/light cycle. Food was available ad libitum throughout the experiment except in the experimental room. The temperature in the colony room was maintained at 23 degrees Centigrade.

Apparatus

The experimental chambers (described previously) were thermically regulated to maintain a temperature of 56°C during the heat condition. Clear and opaque plastic boxes were used as baseline and recovery boxes. An opaque plexiglass grid with a grid size of 1cm X 1cm fitted but was also removable from the bottom of the boxes. All other equipment was identical to the previous experiment.

Surgery and the Biotelemetry System

As described in experiment 1.

Contextual Cues

On heat condition days, group 1 was given a cherry Koolaid [non-sweetened (.05%) with added saccharin (.05%)] solution to drink in the grided-clear holding box. On the no-heat condition days, the same group was given a grape [non-sweetened (.05%) with added saccharin(.05%)] in the no-grid-opaque holding box. The cues were counterbalanced such that group 2 had a grape flavour and non-grided-opaque holding box signaling heat, and a cherry flavour and clear-grided holding box signaling no heat. The experimental chambers were counterbalanced within the experimental room such that half of the animals received heat and the other half received no heat in a particular conditioning session. Unlike the previous experiment, the holding box served as both the baseline and recovery box and was counterbalanced for placement beside the experimental chamber (i.e., two were to the left, and two were to the right of the experimental chamber). The same experimental chamber was used for heat and no-heat conditions for each subject and it was carefully cleaned after each cycle. All other conditions were held constant.

Procedure

Baseline training. Water bottles were removed five minutes prior to the animal's dark cycle. After 15 minutes, the animals were transferred to the experimental room in their home cages and were placed in holding boxes (counterbalanced for texture over time). They were given a .05% saccharin

solution to drink for 30 minutes after which were returned to their home cages in the colony room. After eight days of training, all the animals were reliably drinking the saccharin solution.

Pre-exposure. On day 9, the animals were exposed to both the cherry and grape flavoured solutions in the experimental room. The logic behind pre-exposing the animals to the taste cues was to be certain that the animals would not be neophobic to the taste cue on the first conditioning trial. The animals were then put back on one more baseline training day prior to surgery.

Baseline training. The animals were put back on their baseline drinking schedule three days after surgery for seven more days. At this time, baseline temperature data were recorded in order to ascertain the functionality of the minimitters.

Design. The design was a within-subjects, discriminant paradigm, whereby all the subjects were exposed to both the heat and the no-heat conditions alternately every 48 hours. Two groups of four animals were formed (re: replicated over time) and within each group, cues were counterbalanced such that half of the subjects received heat and the other half received no heat to the same set of cues in the experimental room.

As in the baseline training, one group of four animals had their water bottles removed five minutes prior to their active cycle and fifteen minutes prior to their transfer from the colony room to the experimental room. They

were then placed in the appropriate holding boxes and given the appropriate solution to drink (the same procedure as in the baseline training). The solutions were removed after 30 minutes, and the experimental boxes were turned on. Baseline measurements were taken for 10 minutes following which the subjects were transferred to the experimental chamber for another 10 minutes. The animals were then placed back into the holding box for 19 minutes in order to record post-condition temperatures. Body temperatures were recorded every minute throughout the experimental session with the Dataquest system. The animals were then returned to the colony room in their home cages and the water bottles were reinstated. The group of animals that were not run on a conditioning day remained in the colony room.

Experimental Schedule. All rats experienced six heat and six no-heat conditioning trials (i.e., six cycles) and were then exposed to a tolerance test. The test trial consisted of placing the subject in a cued no-heat condition, then exposing the subject to heat. After the test trial, the animals were put back on the conditioning schedule for two more cycles, following which they were given a two-bottle preference test in their home cage. Both the grape and cherry solutions were presented in the home cage at the time that the subject would typically get a solution in the experimental room. One solution was presented at a time before inserting both of the cylinders for 30 minutes. Flavour presentations were counterbalanced within each group. The volume of each solution consumed was recorded. Table 2 summarizes

the order of procedures.

Table 2. Schedule for experiment 2.

Procedure	Days
Handling	2
Drinking schedule	8
Pre-exposure	1
Drinking schedule	1
Surgery/recovery	3
Drinking schedule	7
Conditioning	24
Test trial	4
Conditioning	8
Preference test	1

Results

Temperature data were transferred to a spreadsheet program (Lotus 1-2-3, version 2.2) and missing data were replaced with temperature values linearly interpolated from surrounding data points. Since no differences between replications were observed, all data were collapsed across time replications. Only the data of the subjects with functional minimitters are reported and one subject was discarded for statistical analyses because of questionably accurate temperature responses over time (see Appendix B).

Conditioning

Figure 8 illustrates the means of the maximum body temperatures (+/- S.E.M.) of animals (n=13) exposed to the heat and the no-heat conditions

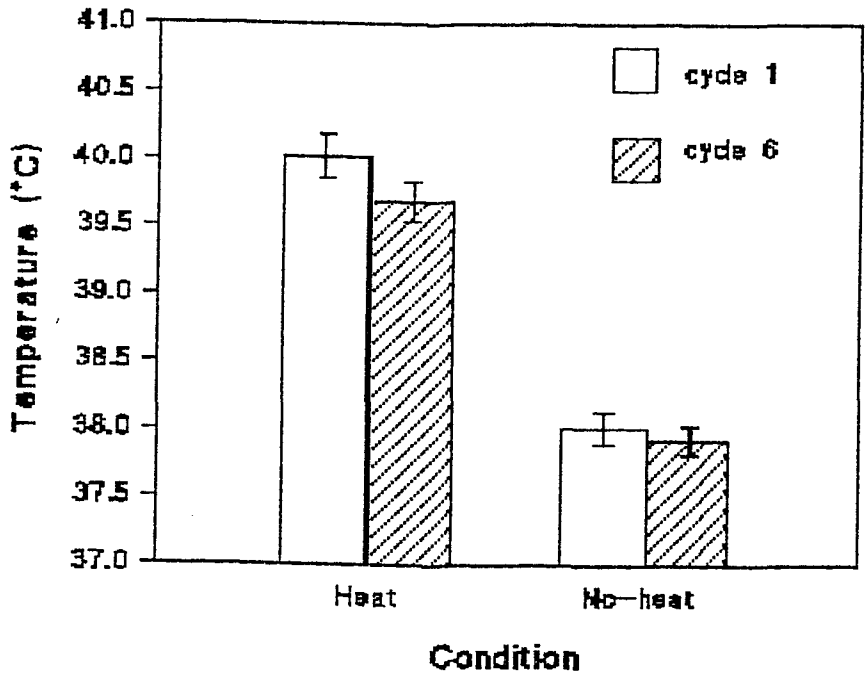

Figure 8. Means of maximum body temperatures (+/- S.E.M.) during cycles 1 and 6 in heat and no-heat conditions.

A mixed analysis of variance [(Group(1,2) X Cycle (1,6) X Condition (heat, no-heat)] yielded a Group X Condition interaction, F(1,11)=49.77, p<.0001, and a Cycle X Condition interaction, F(1,11)=4.54, p<.057), of maximum body temperatures. Newman-Keuls multiple comparisons (alpha=.05) for the above interactions revealed significatly higher temperatures in group 1 in response to the heat condition suggesting that, similar to experiment 1, the contextual cues may have affected temperature differently

differently in the two groups. However, within both groups, body temperatures of Cycle 6 were significantly lower than in Cycle 1 in the heat condition but not in the no-heat condition.

Similar to experiment 1, a mixed analysis of variance [Group (1,2) X Cycle (1,6) X Condition (heat, no-heat)] of minimum baseline temperature yielded a significant interaction between groups and conditions, $F(1,11)=51.69$, $p<.01$. Post-hoc analyses indicated that minimum baseline temperatures were significantly higher in groups exposed to the cherry-grid cues as opposed to the grape-no-grid cues, suggesting that the cues may have differentially affected body temperatures. Although there was no Cycle by Condition interaction ($p>.05$), certain animals had decreased baseline body temperatures over conditioning cycles in anticipation to impending heat stress (see Appendix B).

Figures 9 to 12 illustrate the pattern of temperature response during cycles 1 and 6 in groups 1 ($n=6$) and 2 ($n=7$) in both the heat and the no-heat conditions for minutes 26 through 66 of the experimental trial. The figures show a tolerant response over cycles in the heat condition, but no difference over cycles in the no-heat condition.

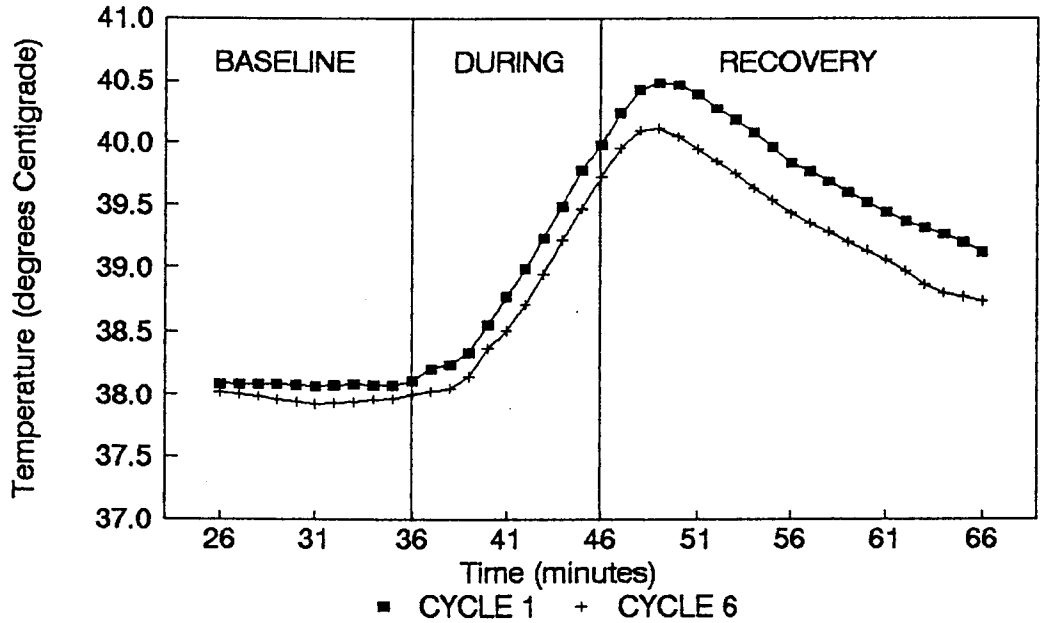

Figure 9. Group 1 (n=6) mean body temperatures as a function of time periods during the heat condition of cycles 1 and 6.

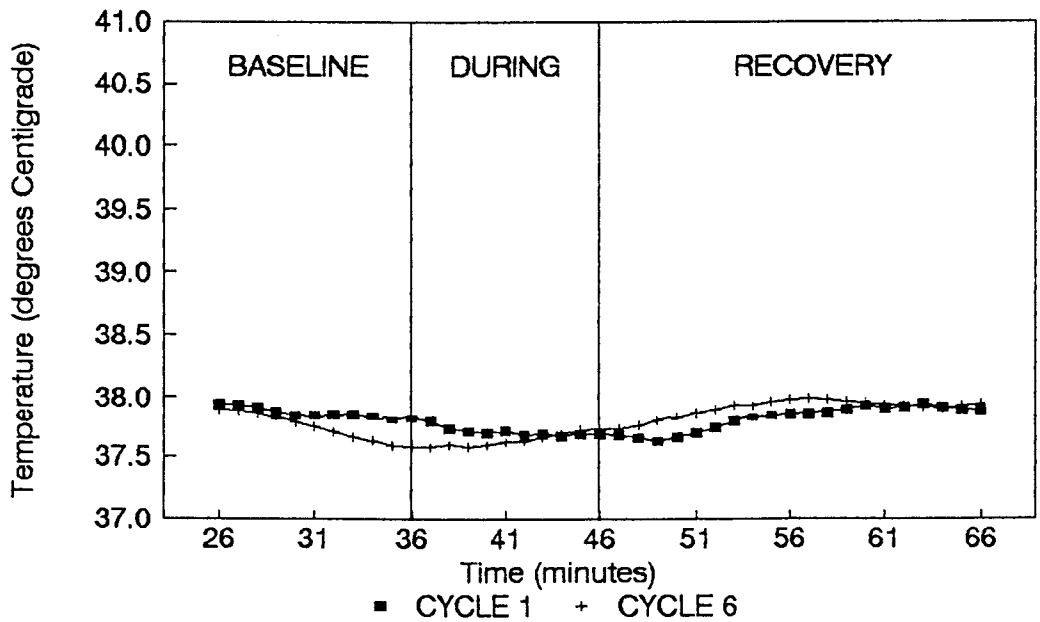

Figure 10. Group 1 (n=6) mean body temperatures as a function of time periods during the no-heat session of cycles 1 and 6.

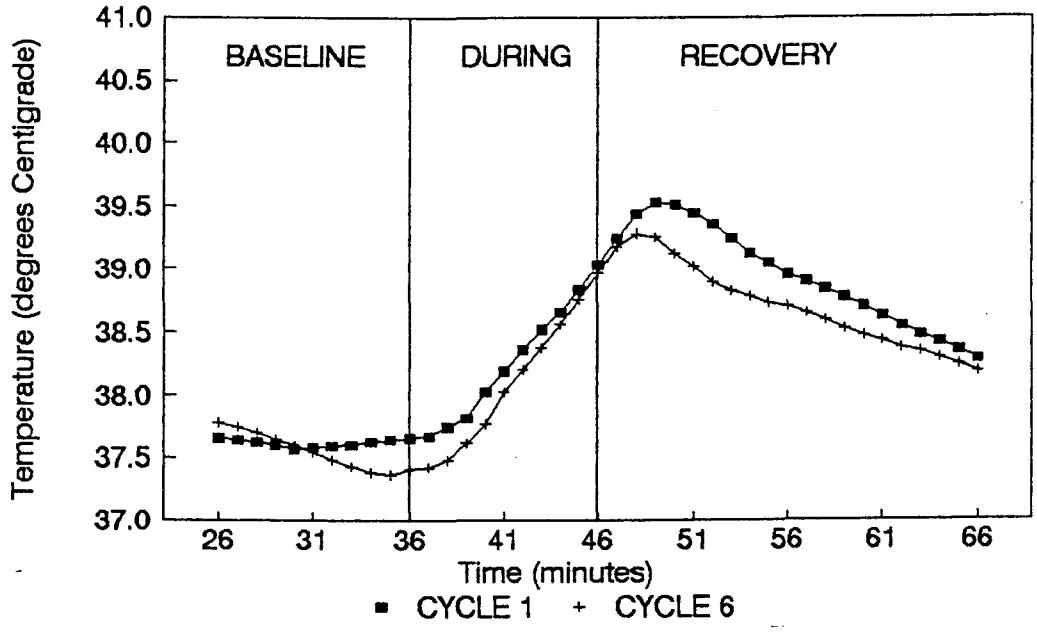

Figure 11. Group 2 (n=7) mean body temperatures as a function of time periods during the heat session of cycles 1 and 6.

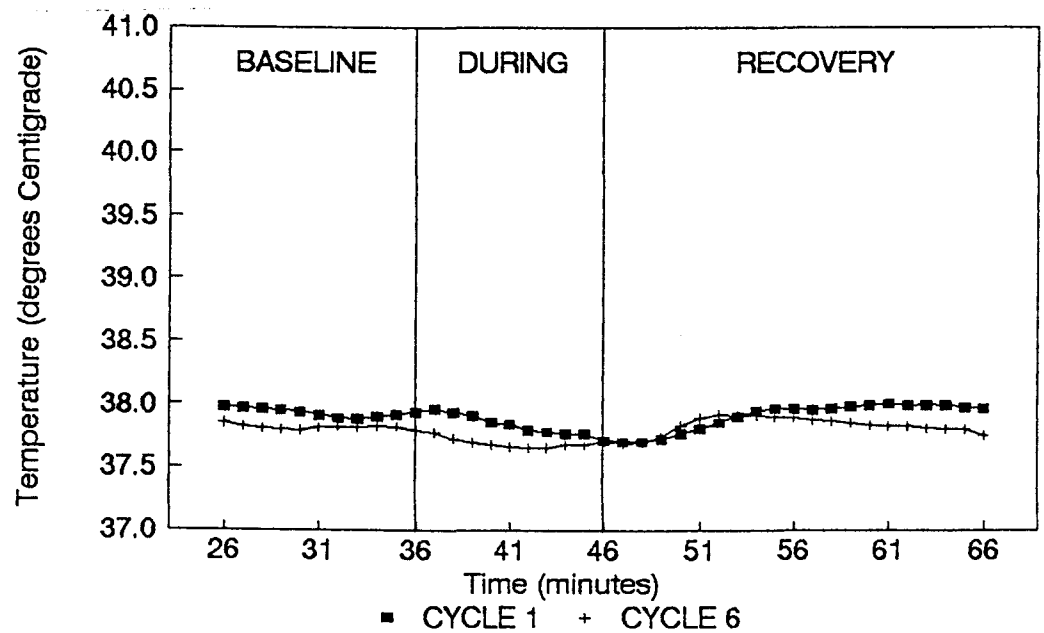

Figure 12. Group 2 (n=7) mean body temperatures as a function of time periods during the no-heat session of cycles 1 and 6.

Test trial

Figure 13 illustrates means of the maximum body temperatures for both groups in the heat condition of Cycle 1, Cycle 6 and the Test trial (i.e., cued no-heat, then given heat). If associative processes are mediating the tolerance observed then a change in contextual cues at testing may impair responding. A two-way mixed analysis of variance was performed on Groups (1 & 2) and Cycles/Test (1, 6 & test) for maxmimum body temperature response in the heat. Analyses indicated that both groups responded similarly to the cycles/test ($F(1,11)=3.69$, $p=.081$) but that group 1 had higher maximum body temperatures across all cycles/test than group 2 ($F(1,11)=9.69$, $p<.01$). A main effect for Cycles/Test (1,6 & test) was observed ($F(1,11)=7.05$, $p<.05$) and Newman-Keuls post-hoc analyses indicated that body temperatures of Cycle 1 differed significantly from Cycle 6 and Test ($p<.05$), but that maximum heat responses of Cycle 6 did not differ significantly from the Test trial ($p>.05$).

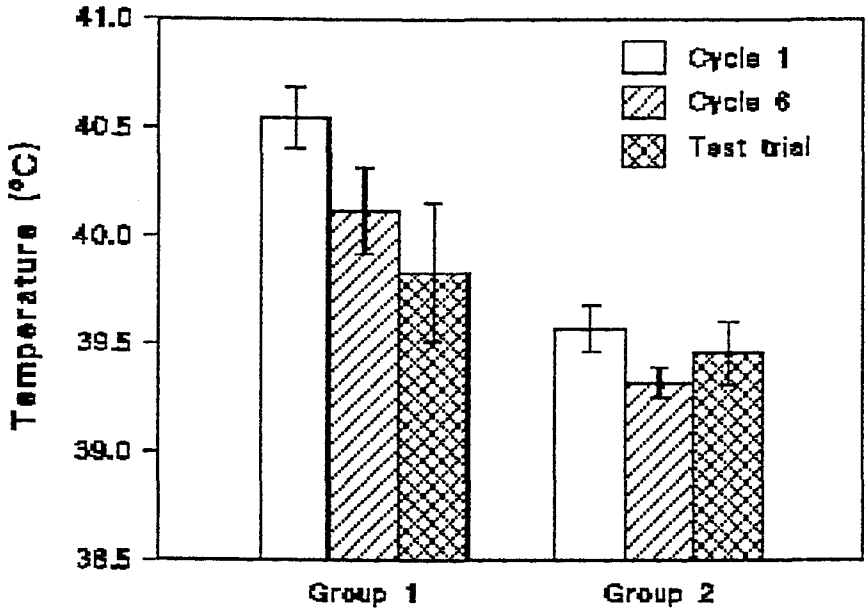

Figure 13. Means of maximum body temperatures (+/- S.E.M.) during cycles 1, 6, and test trial of groups 1 (n=6) and 2 (n=7).

Taste Preference

A mixed analysis of variance, Groups (1,2) X Cycles (1,6) X Conditions (heat, no-heat), of volume consumed showed a significant interaction between Groups and Conditions ($F(1,11)=8.45$, $p<.05$)). Animals consumed more of the cherry solution, which was associated with counterbalanced conditions across groups.

Preference Test. One of the subjects in group 1 knocked off a cylinder during the preference test, thus the data for that subject was not included. A mixed analysis of variance of (Groups X Flavours) on volume

consumed for the two-bottle preference test showed no interaction between the groups and the volume of flavours consumed ($p>.05$). There was no overall preference in flavour ($p>.05$), however group 2 consumed significantly more solution than group 1, ($F(1,10)=8.14$, $p<.05$). Figure 14 illustrates the volume of cherry and grape solution consumed for groups 1 ($n=5$) and 2 ($n=7$) in the two-bottle preference test.

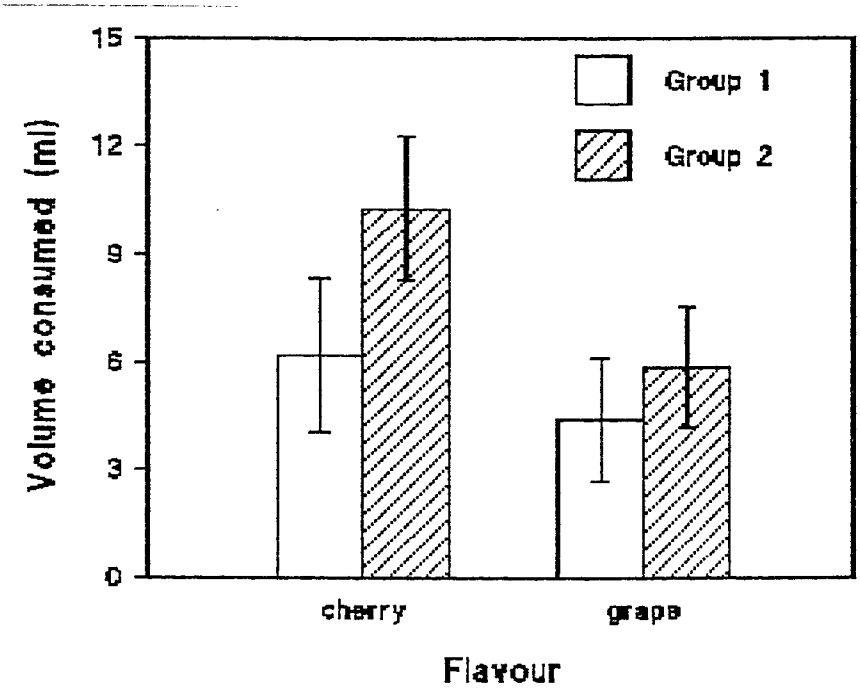

Figure 14. Mean (+/- S.E.M.) volumes of solutions consumed in a two-bottle preference test for groups 1 ($n=5$) and 2 ($n=7$).

Discussion

The results of the present experiment replicate the findings of experiment 1. Both groups demonstrated tolerance over heat conditioning days such that the animals' maximum temperatures were lower on the last conditioning trial as compared to the first; there was no difference in temperatures in the no-heat condition over cycles. Again, these results are similar to the tolerance observed to repeated cold stress. That is, over repeated exposure to a thermic stress, animals respond adaptively in the direction of homeostasis.

It was hypothesized that animals may learn to associate feedforward cues to impending heat stress, and then respond accordingly in an anticipatory hypothermic direction. Although this was not found to be a significant effect in the present experiments, it was interesting to note that certain animals clearly had decreased body temperatures in anticipation to heat stress. It is possible that some animals may have learned to discriminate more quickly than others, and that perhaps more animals may have discriminated with a greater number of conditioning trials.

Unfortunately, it seemed that the discriminant cues affected body temperature responses differently. Animals had higher body temperatures when exposed to the cherry-grid cues as opposed to the grape-no-grid cues suggesting that perhaps the grid itself may have acted as an additional

stressor. Between subject analyses indicated that tolerance to the heat stress was not disrupted by changing the context in which the rat experienced the heat stress. This is similar to Kokkinidis's (1986) results; a change in the context in which a rat experienced repeated cold did not disrupt adaptation. Thus, the environmental cues predictive of thermic stress in these experiments may not have been optimal for learning to occur. As mentioned earlier, it is still not known what makes stimuli relevant for conditioning. One important stimulus variable for classical conditioning is the novelty of the conditional stimulus. In order for neophobia not to occur on the first conditioning trial, the animals were pre-exposed to the flavours. It can be argued that this pre-exposure to the flavours may have resulted in latent inhibition; i.e., preconditioning exposures to the flavour may have slowed associative processes (Lubow & Moore, 1959). Animals in the present experiment received only six conditioning trials in this discrimination design. Animals may have learned to associate the cues with the conditions if given more conditioning trials.

Similar to experiment 1, there was no preference for the taste associated with the no-heat condition in the two-bottle preference test. Although taste cues have been extensively used in other conditioning paradigms as a way of assessing associative processes, the present experiments demonstrate that taste is either not readily associable to heat stimuli, or that the pre-exposure to the flavours led to latent inhibition, or that

the preference test is not an ideal procedure for assessing its associability.

The intensity of the conditional and unconditional stimuli are important factors in Pavlovian conditioning. In general, more intense stimuli elicit stronger associations (Kalat & Rozin, 1970). However, if either stimulus intensity is too high, conditioning may be disrupted. For example, there is evidence which suggests that hyperthermia may hinder memory/learning in rats (Misanin, Vonheyn, Bartelt, Boulden & Hinderliter, 1979). Results from this study indicated that hyperthermia produced severe amnesia (as measured by a one-trial avoidance task) and that there was a direct relationship between the severity of the hyperthermia and the degree of amnesia to the task. It is possible that the degree of hyperthermia in this study was too high for learning to occur. It is interesting to note that there was also a relationship between the degree of hyperthermia and tolerance in the present experiment such that group 2 (i.e., grape-no-grid), which demonstrated less overall hyperthermia than group 1 (i.e., cherry-grid), developed greater tolerance. This relationship was also observed in experiment 1.

As presented, it is hypothesized that organisms are predisposed to learn cues predictive of thermic stress through a feedforward homeostatic mechanism. Thus it was thought that the animals would become tolerant (i.e., less hyperthermic) over conditioning trials. The thermic response over conditioning trials to heat stress in the present study was indeed decreased, as measured by a decrease in body temperature. By analogy, the thermic

response to cold stress over conditioning trials in Riccio et al.'s (1991) study was also decreased, as measured by an increase in body temperature. Thus, the direction of the temperature response of an animal to a repeated thermic stressor is consistent with an evolutionary-homeostatic model of responding.

Future experiments examining the effects of contextual environments on tolerance to heat stress should include contextual environments that elicit equal temperature responses. Furthermore, the cues should be highly discriminant to the animals in many modalities (e.g., visual, tactile, olfactory/flavour, & spatial) in order to enhance the possibility of an associative mediation. For example, Riccio et al. (1991) found associative tolerance to repeated cold stress with highly discriminant thermic environments (i.e., separate rooms which differed in cues such as size, brightness, odor, and sounds). Again, it is not known what makes a CS relevant to a US. Perhaps the use of many modality cues may have enhanced associative processes in their experiment.

Observation and analysis of thermoregulatory behaviour (e.g., saliva spreading, grooming, locomotion) may be a good dependent measure in determining whether associative processes play a role in tolerance to thermic stress. Although thermoregulatory behaviours in the present experiments were not quantitatively monitored, animals were observed to groom and spread saliva in anticipation and in response to thermic stress. No study has

looked at thermoregulatory behaviour and conditioning to thermic stress. This is perhaps due to reliability difficulties of behavioural observations. However, the increasing popularity and decreasing cost of video equipment may aid in the facility and reliability of such a measure.

In summary, the present experiments demonstrate that tolerance to repeated heat stress does occur. However, tolerance to the heat stress was not disrupted by changes in predictive contexts to the heat, as an associative theory may predict. The carefully chosen contexts in this study were not sufficient in demonstrating an associative tolerance effect. Future experiments should assess the question of associative processes and thermoregulation using highly discriminant environments.

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Appendix A

Maximum body temperatures (°C) of minutes 36-52 during heat and no-heat conditions of cycles 1, 6 and test.

GROUP	CYCLE 1		CYCLE 6		TEST
	HEAT	NO-HEAT	HEAT	NO-HEAT	
1	40.6	38.3	40.0	38.4	40.8
	40.2	37.9	39.9	37.8	41.1
	40.3	38.3	39.2	38.1	39.9
	40.8	38.6	39.6	38.8	40.6
	40.6	38.6	39.3	38.8	39.8
2	40.8	38.1	40.9	37.7	39.1
	41.1	38.3	40.0	38.0	39.9
	41.5	38.2	40.2	38.2	40.0
	40.7	38.2	40.8	38.3	40.6

Minimum body temperatures (°C) of minutes 26-35 during heat and no-heat conditions of cycles 1, 6, and test.

GROUP	CYCLE 1		CYCLE 6	
	HEAT	NO-HEAT	HEAT	NO-HEAT
1	38.2	38.6	37.6	38.6
	38.3	38.6	38.4	38.8
	37.8	38.3	37.3	38.1
	37.3	37.8	36.7	38.7
	38.0	38.5	37.5	37.2
2	38.3	38.1	38.4	37.6
	38.3	37.8	38.1	37.7
	38.5	37.4	38.4	37.9
	38.4	38.2	38.7	38.3

Volumes (ml.) of solutions ingested in heat and no-heat conditions of cycles 1, 6 and preference test.

GROUP	CYCLE 1		CYCLE 6		PREFTEST	
	HEAT	NO-HEAT	HEAT	NO-HEAT	VIN	OF
1	4	-	7	13	11	4
	4	6	1	4	1	12
	4	3	4	3	11	5
	7	6	7	4	14	5
	3	7	8	13	15	3
2	7	4	8	4	8	5
	5	5	5	3	10	5
	5	5	5	14	13	3
	5	4	7	9	13	3

Appendix B

Maximum body temperatures (°C) of minutes 36-52 during heat and no-heat conditions of cycles 1, 6 and test.

GROUP	CYCLE 1		CYCLE 6		TEST
	HEAT	NO-HEAT	HEAT	NO-HEAT	
1	40.1	37.1	39.9	37.6	39.2
	40.8	38.2	40.8	38.1	40.1
	40.4	37.5	39.3	37.2	38.6
	40.9	38.6	40.3	37.9	40.9
	40.2	38.1	40.4	38.2	40.3
	40.8	38.1	40.1	38.5	39.8
2	39.4	38.3	39.1	37.9	38.9
	39.4	37.5	39.1	37.5	39.3
	39.7	38.6	39.5	38.5	39.5
	39.9	38.2	39.5	38.2	39.9
	39.3	38.0	39.2	37.7	40.0
	39.9	38.0	39.4	38.0	39.3
	39.3	38.0	39.4	37.8	39.3

Minimum body temperatures (°C) of minutes 26-35 during heat and no-heat conditions of cycles 1, 6 and test.

GROUP	CYCLE 1		CYCLE 6	
	HEAT	NO-HEAT	HEAT	NO-HEAT
1	37.7	37.2	37.6	37.5
	38.2	37.8	38.2	37.7
	37.4	37.2	37.5	36.7
	38.2	38.1	38.0	37.5
	38.2	37.8	37.9	37.9
	38.1	37.9	38.1	37.9
2	37.3	38.1	37.4	37.5
	37.0	37.3	37.4	37.4
	37.5	37.6	37.0	38.1
	37.6	37.6	37.1	37.6
	37.6	38.0	37.1	37.6
	37.6	38.0	37.9	37.7
	37.6	38.0	37.4	37.8

Volumes (ml.) of solutions ingested in heat and no-heat conditions of cycles 1, 6, and preference test.

GROUP	CYCLE 1		CYCLE 6		PREFTEST	
	HEAT	NO-HEAT	HEAT	NO-HEAT	CHERRY	[illegible]
1	5	4	8	6	6	8
	7	5	1	3	-	-
	4	3	7	5	10	3
	9	5	12	12	12	1
	9	10	8	7	1	9
	8	7	6	5	2	1
2	9	7	8	12	17	2
	4	4	2	3	5	8
	3	4	4	5	11	4
	12	13	12	14	11	4
	3	5	10	8	11	8
	9	10	14	10	2	14
	8	11	14	15	15	1