THE ROLE OF ETHANOL-INDUCED HYPOTHERMIA IN ETHANOL'S HEDONIC EFFECTS IN GENETICALLY HETEROGENEOUS MICE

by

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ABSTRACT

The mechanisms underlying drug-taking behavior have been the topic of much research in recent years. It is generally agreed that the positive motivational effects of drugs of abuse play an extremely important role in the maintenance of drug-taking behavior. Drugs may also have negative motivational effects that can be important in determining the frequency and amounts of drug intake. Recent research in rats has suggested that the aversive motivational effects of ethanol may be related to its thermal effects. Similar studies using HOT and COLD mice did not fully support this hypothesis.

The experiments in this thesis were designed to test the hypothesis that the motivational effects of ethanol are related to its thermal effects in genetically heterogeneous mice. Specifically, the hypothesis states that ethanol-induced hypothermia contributes to the aversive properties of ethanol. In these experiments, as in previous studies with rats, the magnitude of ethanol-induced hypothermia was altered by manipulating ambient temperature. Two conditioning paradigms, taste and place conditioning, were used to assess ethanol's motivational effects. In all experiments, an ambient temperature of 34° C was used to attenuate ethanol-induced hypothermia, and an ambient temperature of 10° C was used in an attempt to enhance hypothermia.

Experiments 1-3 examined the effects of altered hypothermia on ethanol-induced conditioned taste aversion (ethanol dose = 2.5 or 2.25 g/kg) to a saline solution (Experiments 1 and 2) or a saccharin solution (Experiment 3). According to predictions based on the temperature hypothesis, attenuation of hypothermia should attenuate the taste aversion, and enhancement of hypothermia should lead to enhancement of the aversion. In all experiments, ethanol hypothermia was altered by exposure to altered temperatures, and a robust conditioned taste aversion was found. However, alteration of ambient temperature had no effect on the conditioned taste aversion. This finding does not coincide with previous studies in either rats or HOT and COLD mice.

Experiment 4 used the place conditioning paradigm to assess ethanol's motivational effects. In mice, place conditioning with ethanol generally results in a conditioned place preference. Thus, according to the hypothesis that ethanol's thermal effects contribute to its aversive properties, attenuation of hypothermia should decrease these aversive properties and increase the magnitude of place preference. As in Experiments 1-3, exposure to the warm ambient temperature (T_a) greatly diminished ethanol-hypothermia; however, no enhancement of hypothermia was seen. Exposure to either increased or decreased T_a during conditioning or testing eliminated the conditioned place preference seen in mice exposed only to a normal ambient temperature of 21° C. This finding does not support the temperature

hypothesis. The data are explained as a combination of two stimulus effects, associative overshadowing and generalization decrement.

In general, the present studies do not offer additional support for the temperature hypothesis of ethanol's motivational effects. However, given the limited nature of the current experiments, further studies are warranted, especially in the area of taste conditioning.

INTRODUCTION

"Positive reinforcement has been considered the major reason for maintaining drug-taking behavior. . ." (cf. Tabakoff & Hoffman, 1988, p. 29). In operant terms, a positive reinforcer is defined as a stimulus (e.g., a drug) which, when its presentation is made contingent on a specific response, will increase the likelihood of that response (Skinner, 1953). Positive reinforcement in humans is often associated with the subjective sensation of pleasure (especially in drug use) and it is natural to assume that animals experience similar sensations (Liebman, 1989). However, an operant definition of reinforcement does not include such constructs as pleasure or motivation. According to Liebman (1989), an alternative to the operant definition of reinforcement uses approach and withdrawal behaviors (e.g., consummatory responses, running speed, preference tests, bar-pressing) to define such psychological constructs as motivation and incentive. In this way, stimuli that produce approach behaviors are considered hedonically positive while those that elicit withdrawal behaviors are considered hedonically negative. This definition allows for the assimilation of motivation and pleasure into the idea of 'reinforcement'. The term 'reward' may be used to differentiate the concept of reinforcement that includes motivational and hedonic effects from strict operant reinforcement.

Although it is generally agreed that drugs of abuse have hedonically positive effects and that these effects are important for the initiation and

maintenance of drug-taking behavior, most drugs also have negative hedonic properties. These aversive properties may be important for the termination of drug-taking episodes or otherwise limiting drug intake. Additionally, aversive drug effects may be involved in determining the frequency of drug-taking behavior.

Assessment of Ethanol's Hedonic Effects

In the last 30 years several experimental techniques have been developed to assess the rewarding or motivational effects of abused drugs (see Bozarth, 1987 for a general review). These techniques include 'direct' measures of reinforcement such as self-administration as well as 'indirect' measures such as conditioning paradigms, which will be the focus of the present discussion. The decision to use one technique over another may depend not only on characteristics of the methods themselves (i.e., necessity of simple vs complex apparatus or of complicated, delicate surgery) but also on the characteristics of the drug to be studied. For example, cocaine and morphine readily support i.v. self-administration in monkeys (Deneau, Yanagita, & Seevers, 1969; Thompson & Schuster, 1964) and rats (Collins, Weeks, Cooper, Good, & Russell, 1984). However, while monkeys have been reported to self-administer ethanol intravenously (e.g., Altshuler, Phillips, & Feinhandler, 1980), i.v. self-administration of ethanol in rats has not consistently been demonstrated (e.g., compare Collins et al. 1984 and DeNoble, Mele, & Porter, 1985 with Smith & Davis, 1974 and Lyness & Smith, 1992).

Additionally, oral self-administration of ethanol has high face validity as it is the route of administration commonly used by humans, although it is not without problems. For example, because of the aversive taste of 'straight' ethanol, the low concentrations often used can make it difficult for animals to administer a pharmacologically relevant dose. A review by Samson, Pfeffer, and Tolliver (1988) discusses several procedures used to induce oral self-administration of ethanol in rats.

Although self-administration procedures provide 'direct' assessment of the reinforcing properties of drugs, there are advantages to using 'indirect' procedures. For example, extensive training with the drug is not necessary as in many self-administration procedures, allowing examination of drug effects in drug-naive animals. In addition, conditioning paradigms allow for assessment of both positive and negative hedonic drug effects, and since testing is often drug-free, possible motoric effects of drug treatment do not pose a problem. Finally, 'indirect' paradigms do not typically involve the difficult surgeries necessary for i.v. or intracranial self-administration.

Place Conditioning

Conditioning paradigms offer an alternative to self-administration procedures. One conditioning procedure that has been used to assess the hedonic properties of drugs is the place conditioning paradigm (Carr, Fibiger & Phillips, 1989; Swerdlow, Gilbert & Koob, 1989; van der Kooy, 1987). The central tenet of place conditioning is that rewarding stimuli will elicit

approach and contact behaviors whereas aversive stimuli will elicit withdrawal responses (Carr, Fibiger & Phillips, 1989). In a typical place conditioning procedure, administration of a drug and the resulting pharmacological effects are paired with one distinct environment while drug vehicle administration is paired with another distinct environment (Cunningham, Hallett, Niehus, Hunter, Nouth & Risinger, 1991; Mucha & Iversen, 1984). With repeated pairings, an association is made between the internal effects of the drug and distinct external cues. Via Pavlovian conditioning processes, these external cues assume the hedonic properties of the drug effect. After repeated exposures, the animal is given a choice between the two environments in a drug-free test. If the animal chooses to spend more time in the drug-paired environment, a conditioned place preference is demonstrated, and it is inferred that the drug effects were rewarding. Alternatively, a place aversion is demonstrated if the animal spends more time in the vehicle-paired environment, and it is concluded that the drug effects are aversive. Many variations of the place conditioning procedure have been developed with visual, tactile and/or olfactory cues serving as the distinct environmental stimuli (for recent reviews see Carr, Fibiger & Phillips, 1989 and Swerdlow, Gilbert & Koob, 1989).

One advantage to using the place conditioning paradigm with ethanol is that palatability is not an issue since the drug is typically injected. In addition, treatments aimed at affecting ethanol reward may be used during

either the acquisition or testing phase, allowing examination of the processes underlying the initial reward as well as expression of a conditioned preference. Finally, since testing may be done drug-free, possible motor effects of ethanol or other agents do not impact the expression of the conditioned preference.

The place preference paradigm has only recently become widely used as a measure of assessing the motivational properties of a drug. It was first reported by Olds and Milner (1954) who observed that rats preferred the area of the experimental apparatus that was associated with brain stimulation. Conditioned place preferences have since been shown to occur with many drugs abused by man, including amphetamine (Reicher & Holman, 1977), cocaine (Spyraki, Fibiger, & Phillips, 1982), morphine (Mucha & Iversen, 1984) and heroin (Bozarth & Wise, 1981). Place conditioning in rats with ethanol, however, has generally resulted in a conditioned place aversion rather than preference (Cunningham, 1979, 1981; Stewart & Grupp, 1986; van der Kooy, O'Shaughnessy, Mucha, & Kalant, 1983), although place preference has occasionally been reported (Black, Albiniak, Davis, & Schumpert, 1973; Bozarth, 1990; Reid, Hunter, Beaman, & Hubbell, 1985).

In contrast to the place aversion typically seen in rats, several inbred and selectively bred lines of mice, as well as genetically heterogeneous mice, have recently been shown to exhibit a robust conditioned place preference for tactile cues paired with ethanol (e.g., Cunningham et al., 1991; Cunningham,

Niehus, Malott, & Prather, 1992; Cunningham, Niehus, & Noble, 1993; Risinger, Dickinson & Cunningham, 1992). Why mice show a conditioned place preference for cues paired with ethanol while rats show an aversion is not known. A study by Cunningham et al. (1993) demonstrating preference in mice and aversion in rats with identical place conditioning procedures indicates that procedural differences are not the source of the discrepancy.

Cunningham et al. (1993) suggested several possible explanations for the differences in place conditioning between rats and mice, one of which centered on ethanol's ability to induce locomotor activity in most mice while suppressing it in most rats. These activity data, in conjunction with the preference data, support the psychomotor stimulant theory (Wise & Bozarth, 1987) in that the species showing increased activation also demonstrated preference. Alternatively, Cunningham et al. (1993) also suggested that perhaps mice become tolerant to ethanol's aversive effects (or sensitize to its reinforcing effects) more rapidly than do rats, such that with repeated conditioning trials a species difference is seen in place preference. It has also been suggested that ethanol has biphasic hedonic effects that change from positive to negative over time, i.e., an opponent process occurs (Solomon, 1977; Risinger & Cunningham, 1992). It could then be speculated that this change occurs more quickly in rats than in mice, so that within a conditioning session rats would be experiencing the negative hedonic effects of ethanol, while in mice these effects would still be positive, resulting in

place aversion and place preference, respectively. While the underpinnings of the species difference in ethanol place conditioning are not known, it appears that the positive hedonic effects of ethanol in the mouse may be assessed with this paradigm.

Taste Conditioning

The taste conditioning paradigm is generally presumed to measure the negative hedonic properties of drugs, although other interpretations have been offered (e.g., Hunt & Amit, 1987). In a typical taste conditioning experiment, drug administration (or other treatment believed to be noxious) occurs immediately after the presentation of a distinctively flavored substance (cf. Goudie, 1987). After the effects of the drug or other treatment have worn off, the taste is again presented and consumption of the substance is measured. A decrease in consumption of the distinctive taste substance is taken to indicate the formation of a conditioned taste aversion (CTA). Many agents have been shown to produce taste aversion, including emetic agents such as lithium chloride and X-irradiation (Garcia & Koelling, 1966) as well as drugs abused by man and self-administered by animals, including ethanol, morphine, heroin, amphetamine and cocaine (see Hunt & Amit, 1987 for a review).

The apparent paradox of conditioned taste aversion to drugs found to be rewarding in self-adminstration and place conditioning procedures cannot be easily resolved. Pharmacological manipulations that block the positively

reinforcing properties of many drugs also have been shown to diminish the CTA-inducing properties of these drugs (Hunt, Switzman & Amit, 1985; Sklar & Amit, 1977). Based on these results, it has been postulated that the neural mechanisms underlying both the positive and aversive hedonic properties of drugs may be the same or functionally similar (Hunt & Amit, 1987).

It has also been suggested that the CTA induced by self-administered drugs is qualitatively different from that induced by emetic agents such as lithium chloride (Hunt & Amit, 1987; Parker, 1988). Using the taste reactivity (TR) test, Parker and colleagues have demonstrated that orofacial and somatic responses to taste CSs paired with lithium chloride differ from responses to those CSs paired with self-administered drugs (e.g., Parker, 1993). While neither CS elicits an 'ingestive' response such as tongue protrusion, only the taste paired with lithium has the capability of producing a 'rejection' response such as chin rubbing. Thus, it has been suggested that reinforcing drugs do not produce an aversion to a paired taste (as measured by the TR test) although they do invoke an avoidance of that taste (Parker, 1982; Parker, 1988; Parker, 1993; Parker & Carvell, 1986). The mechanism of this avoidance, however, is still not understood.

The apparent paradox of CTA experiments may also be explained using Solomon's opponent-process theory (Solomon, 1977). According to opponent-process theory, an event produces a certain affective response that is followed by an affective response in the opposite direction. For example, if an injection

of ethanol produces a euphoric reaction, dysphoria should follow at some later time, i.e., ethanol should produce biphasic hedonic effects. Indeed, it has been suggested that the hedonic effects of ethanol are positive during the rising phase of the blood ethanol concentration curve and negative during the falling limb of this curve (Reid et al., 1985). Studies by Risinger and Cunningham (1992) and Cunningham and Prather (1992) demonstrated that the magnitude of ethanol-induced conditioned place preference is strongest when conditioning occurs shortly after ethanol injection, lending further support to this contention. With this in mind, it is possible that paradigms such as self-administration assess the initial positive effects of a given drug, whereas taste aversion paradigms measure the second phase, which may be hedonically negative, resulting in the paradox at hand. Because of bidirectional place conditioning results, it appears that place conditioning may be able to address both the positive and negative affective reactions.

If it is assumed that drugs have both positive and negative hedonic effects, stimulus associability differences or cue-to-consequence learning may also be used to explain the paradoxical finding of taste aversions to drugs of abuse. Specifically, it may be that an internal cue such as taste is more readily paired with the aversive or illness-producing effects of a drug while the positive effects are more easily associated with external cues such as a sound, a light, or a tactile cue.

Mechanisms of Ethanol's Hedonic Effects

That humans engage in the behavior of self-administering drugs such as alcohol, cocaine and heroin suggests that such drugs are rewarding. The mechanism of these hedonic effects has been the focus of much research in the past 20-30 years. In this time, it has been determined that some abused drugs bind to endogenous receptors (e.g., morphine, heroin) or block reuptake of neurotransmitters (e.g., cocaine) that presumably initiate neural cascades resulting in the sensation of pleasure. However, the mechanism of ethanol reward has not yet been determined.

The neurotransmitter hypothesis

Unlike many other psychoactive drugs, ethanol does not bind specifically to an endogenous membrane-bound receptor or uptake site. It has been suggested, however, that ethanol reward is mediated via the dopamine system (Pfeffer & Samson, 1986; Wise & Rompre, 1989). In addition, other neurotransmitters such as serotonin (Murphy, Waller, Gatto, McBride, Lumeng & Li, 1988; Naranjo, Sellers & Lawrin, 1986), norepinephrine (Amit & Brown, 1982), GABA (Sudzak, Glowa, Crawley, Schwartz, Skolnick & Paul, 1986) and the opioids (Froehlich, Harts, Lumeng & Li, 1990; Reid & Hunter, 1984) have also been implicated in ethanol's motivational effects. However, pharmacological agonist and antagonist studies have failed to consistently link any single neurotransmitter system to ethanol's motivational and/or behavioral effects (Koob, 1992).

The membrane fluidization hypothesis

It is widely recognized that ethanol, as well as other alcohols, has a fluidizing effect on brain membranes composed of a lipid bilayer (Chin & Goldstein, 1977; Harris & Schroeder, 1981). It has been suggested that this membrane fluidization may be responsible for several of ethanol's behavioral actions, including anesthesia (Seeman, 1972) and depression (Syapin, Chen, Finn, & Alkana, 1988). Given that endogenous receptors for the major neurotransmitters are embedded in the lipid bilayer of the neuronal membrane, any disruptions of the membrane could impact more than one neurotransmitter system. This could potentially explain the apparent involvement of several neurotransmitter systems in ethanol's effects, including its motivational effects.

The psychomotor stimulant theory

Wise and Bozarth (1987) have suggested that the mechanisms by which stimulant drugs induce locomotor activity are homologous to those mechanisms responsible for the rewarding properties of these drugs. While ethanol is often classified as a sedative-hypnotic, it has been shown to stimulate locomotor activity in some strains of mice (e.g., Frye & Breese, 1981; Strömbom & Liedman, 1982). According to psychomotor stimulant theory, treatment that affects ethanol-induced activation should also affect ethanol reward. However, this prediction was not supported by recent studies demonstrating that treatment with the dopamine antagonist haloperidol or

the GABA inverse agonist RO 15-4513 during ethanol place conditioning decreased ethanol-induced activity although neither compound affected ethanol-induced conditioned place preference (Risinger et al., 1992; Risinger, Malott, Riley & Cunningham, 1992).

Studies of ethanol's motivational effects in mice selectively bred for differences in ethanol-induced locomotor activity (FAST and SLOW mice) have demonstrated mixed support for the psychomotor stimulant theory (Risinger, Malott, Prather, Niehus & Cunningham, in press). Specifically, FAST mice, which show increased activity to ethanol, drank more ethanol than SLOW mice in both one and two-bottle drinking studies. In addition, conditioned taste aversion to a saccharin solution paired with ethanol injection developed more readily in SLOW mice than in FAST mice. These findings support the psychomotor stimulant theory in that mice showing greater activity drank more ethanol and were more resistant to the aversive effects of an ethanol injection. However, in place conditioning, both the FAST and SLOW lines developed a significant ethanol-induced conditioned place preference. As the psychomotor stimulant theory would predict that FAST mice should show greater ethanol place preference, this finding does not support Wise and Bozarth's theory.

The temperature hypothesis

Recently, it has been suggested that ethanol's thermal effects may be important in mediating its motivational effects (Cunningham, Hawks &

Niehus, 1988; Cunningham & Niehus, 1989; Cunningham, Niehus & Bachtold, 1992; Hunt, Spear & Spear, 1991). In these studies, the preference or aversion for ethanol-paired stimuli appeared to be related to the degree of hypothermia induced by ethanol. Specifically, the temperature hypothesis posits that a positive correlation exists between ethanol-induced hypothermia and ethanol's aversive effects. In other words, the greater the ethanol-induced hypothermia, the more aversive the motivational effects of the drug are.

The thermal effects of ethanol are not static, but they may be altered by the organism's history of drug exposure or by changing the environment. For example, the magnitude of hypothermia caused by a given dose of ethanol can be increased by lowering ambient temperature (T_a) (Lomax & Lee, 1982; Malcolm & Alkana, 1981). Alternatively, raising T_a can prevent ethanol-induced hypothermia (Freund, 1973; Grieve & Littleton, 1979; Alkana, Bejanian, Syapin & Finn, 1987), and may even produce hyperthermia if raised high enough (Malcolm & Alkana, 1981; Myers, 1981).

Previous studies - rats. If ethanol's thermal effects are related to its hedonic effects, alterations of the thermal effects may lead to changes in the hedonic effects as well. Indeed, previous studies (Cunningham & Niehus, 1989; Cunningham, Hawks & Niehus, 1988; Cunningham, Niehus & Bachtold, 1992; Hunt, Spear & Spear, 1991) have shown that ethanol-induced place aversion and taste aversion in rats can be reduced if the hypothermia

that normally follows an injection of ethanol is blocked by exposure to high T_a. Additionally, rats will drink more ethanol if its effects are experienced at high T_a than if its effects are experienced at normal ambient temperature (Cunningham & Niehus, 1989). These findings led to the hypothesis that ethanol's aversive properties are related to the hypothermia produced by ethanol.

Cunningham et al. (1988) first tested the temperature hypothesis using a taste conditioning paradigm that paired saccharin consumption with ethanol injection (1.5 g/kg) in two groups of rats. One group of subjects experienced the effects of ethanol in a warm (32° C) room while the other group remained at normal T_a (21° C). Both groups developed a conditioned taste aversion to the saccharin solution. However, the group that experienced ethanol's effects at the warm T_a showed less aversion than did animals kept at a normal ambient. Body temperatures taken on the first conditioning trial indicated that ethanol produced hypothermia in rats at normal room temperature while no hypothermia was seen in the warm group. This pattern of results is consistent with the temperature hypothesis.

To further examine the relationship between ethanol's aversive and thermal effects, Cunningham and Niehus (1989) used a differential flavor conditioning procedure in two groups of rats. All subjects received access to one of two flavored ethanol solutions each day. For each group, one flavor was paired with placement in a warm (32° C) environment (where

hypothermia would not occur) and the other flavor was paired with placement in a room maintained at normal T_a. Two-bottle choice tests were conducted three times, after six, 12 or 18 pairs of conditioning trials. As conditioning progressed, subjects drank more of the ethanol solution that was paired with the warm T_a. Additionally, choice tests showed that they preferred the flavor that had been paired with the warm environment, i.e., when no hypothermia occurred.

Cunningham et al. (1992) further tested the hypothesis that ethanol-induced hypothermia contributes to the negative hedonic properties of ethanol by including exposure to a high and a low T_a in a taste conditioning paradigm. Whereas the temperature hypothesis predicts that both hypothermia and conditioned taste aversion should be diminished by exposure to a high T_a, a low ambient would be expected to increase the degree of hypothermia and the magnitude of the taste aversion. In addition, this series of experiments tested the generality of the temperature hypothesis by examining the effects of altered T_a on conditioned taste aversions induced by morphine and lithium chloride. Finally, a control experiment was also included to determine the possible direct effects of altered T_a on saccharin consumption.

Six groups of rats underwent a taste conditioning procedure that paired consumption of saccharin with injection of ethanol (1.2 or 1.8 g/kg), morphine sulphate (6 or 25 mg/kg), lithium chloride (0.3 or 0.6 mEq/kg), or

saline and exposure to high (32° C) or low (5 or 10° C) T_a. The findings confirmed those of previous experiments in that ethanol-induced taste aversion and hypothermia were attenuated by exposure to a warm T_a but only at the high ethanol dose. As predicted, the low T_a facilitated the development of ethanol-induced taste aversion and enhanced hypothermia, but only at the lower dose. Altered T_a did not affect the taste aversion produced by morphine or lithium chloride, suggesting that this effect may be specific to ethanol. Finally, it was demonstrated that with no drug treatment, alterations in T_a alone did not result in a decrease in saccharin consumption.

To further generalize the temperature hypothesis, Cunningham and Niehus (1993) used a conditioning paradigm that did not involve ingestive behavior, i.e., place conditioning. A series of three experiments paired the effects of ethanol (1.2 or 1.8 g/kg) or lithium chloride with a distinct tactile stimulus, while pairing a different stimulus with vehicle injection. As in the previously described studies, alterations in T_a (32° and 5° C) were used to manipulate the degree of hypothermia.

Activity data collected during the place conditioning study indicated that ethanol generally decreased activity relative to saline at each T_a and this difference was most apparent at the warm T_a. Across saline and ethanol groups, animals exposed to the warm T_a had higher activity than those at the normal or low ambient temperatures.

The pattern of results from the place conditioning studies was taken as additional support for the temperature hypothesis. Specifically, at both doses of ethanol, exposure to the warm T_a attenuated both ethanol hypothermia and the degree of conditioned place aversion. Facilitation of the conditioned aversion was not seen in subjects exposed to the low T_a. Cunningham and Niehus (1993) suggested that a floor effect was responsible, i.e., the conditioned aversion was maximal after four trials, so no effect of low T_a could be seen. Additionally, it was suggested that because exposure to low T_a did not enhance ethanol hypothermia, it did not affect conditioned place aversion.

Additional evidence for the temperature hypothesis comes from discrete-trial operant studies in which rats were trained to bar-press for a sweetened 10% ethanol solution (Cunningham, in press). On some trials, access to the bar (and thus ethanol) was signalled by an external stimulus, and, on some trials access to ethanol was unsignalled. As a result of this training, the signal came to elicit a conditioned hyperthermia. Intake of ethanol was higher on signalled trials than on unsignalled trials, and it was suggested that the conditioned hyperthermia produced by the signal attenuated the hypothermic response to the ethanol consumed, thereby decreasing its aversive properties (increasing its reinforcing properties).

Previous studies - mice. Another method of testing the hypothesis that ethanol's thermal effects play a role in its hedonic effects has used lines of

mice or rats selectively bred for sensitivity or insensitivity to the thermal effects of ethanol. Such lines of mice, called COLD and HOT, respectively, have been successfully bred by John Crabbe and colleagues at the Portland VA Medical Center (Crabbe, Kosobud, Tam, Young & Deutsch, 1987). As part of the selective breeding project, each line has been maintained in duplicate form, known as Replicates 1 and 2.

According to the theory that ethanol-induced hypothermia is aversive, as suggested by previous studies in rats, HOT mice should show less conditioned taste aversion than COLD mice because they experience less ethanol-induced hypothermia. HOT mice should also drink more ethanol than COLD mice. In contrast to the place aversion seen in rats, ethanol has been shown to produce a conditioned place preference in mice (e.g., Cunningham et al., 1991, Risinger et al., 1992). To the extent that a greater degree of place preference indicates less aversion, the theory predicts that HOT mice should show greater ethanol-induced place preference.

A series of experiments by Cunningham et al. (1991) used oral consumption of ethanol, conditioned taste aversion and place conditioning to assess the hedonic effects of ethanol in HOT and COLD mice. Since these lines show differential hypothermic responses to ethanol, manipulations of T_a were not used.

As in the rat place conditioning studies, HOT and COLD mice were exposed to a Pavlovian conditioning procedure that paired ethanol injection

(2.25 g/kg) with a distinct tactile stimulus and saline injection with a different stimulus. In contrast to the place aversion for ethanol-paired cues typically seen in rats, this procedure produced a conditioned place preference in HOT mice. However, COLD mice showed no evidence of place conditioning. To the extent that a decrease in ethanol's aversive properties could be manifested as an increase in its rewarding properties, results of this study support the temperature hypothesis derived from rat data.

In contrast to the rat study, ethanol produced an increase in locomotor activity in both HOT and COLD mice with greater activation in HOT mice. Interestingly, COLD mice sensitized to ethanol's activating effect over trials while HOT mice showed no change.

In the taste conditioning study, 1 h of access to saccharin was paired with injection of ethanol (2.25 g/kg) every other day. On days between conditioning trials, mice were given 2 h access to water. Intake of saccharin decreased over trials in both lines, indicating development of a conditioned taste aversion. However, the magnitude of the aversion was greater in HOT mice than in COLD mice, i.e., subjects experiencing greater ethanol-induced hypothermia showed less aversion for a taste paired with ethanol. This finding contradicts predictions based on the temperature hypothesis.

In the ethanol drinking study, a forced-exposure procedure was used to ensure consumption of ethanol. Specifically, on alternating days a single drinking tube containing ethanol or tap water was available 22.5 h per day.

Ethanol concentration increased from 1% to 12% over the course of the experiment. In general, HOT mice drank less ethanol than did COLD mice, although the difference was statistically significant only in mice from Replication 1. As with results from the taste conditioning study, this outcome is not consistent with predictions based on rat drinking studies.

In sum, studies with rats where the thermal effects of ethanol were altered by manipulating T_a appear to support the hypothesis that ethanol's aversive properties are related to its thermal effects. In selectively bred mice that experience different degrees of ethanol hypothermia because of genotype, only the place conditioning paradigm produced data consistent with this hypothesis.

Rationale for Current Studies

Cunningham et al. (1991) suggested several reasons for the apparent discrepancies between rats and mice in the taste conditioning and ethanol drinking studies. For example, these discrepancies could be caused by general species differences in the mechanisms mediating ethanol's hedonic effects. Alternatively, it was suggested that perhaps the results of the rat studies were caused by the effects of high T_a on a nonthermal response to ethanol (e.g., ataxia) rather than differences in ethanol-induced hypothermia. Another possibility is that the process of genetic selection resulted in chance fixation of other alleles unrelated to the thermal response to ethanol, but which influence other behaviors, e.g., taste conditioning.

Aside from the use of two different species, a major difference between the previous rat and mouse experiments is the manner in which the degree of hypothermia was manipulated. The experience of ethanol-induced hypothermia in the rats was altered by exposure to various ambient temperatures. However, HOT and COLD mice differ in the experience of hypothermia because of genetic influences so no additional manipulations were used. This difference in methodology could have contributed to the discrepant findings of the previous studies. Accordingly, changing the species and employing the same technique to alter ethanol hypothermia may help resolve the discrepancy.

The current experiments were designed to test the hypothesis that ethanol's thermal effects play a role in its hedonic effects in genetically heterogeneous mice. In these experiments, the experience of ethanol-induced hypothermia was altered by changing T_a with no genetic manipulations involved. In this regard, these experiments are similar to the previous studies done with rats. In order to make comparisons between the present studies and previous studies more amenable, two of the same behavioral tasks, taste and place conditioning, were used to assess ethanol's hedonic effects.

Predictions for Current Studies

If the disparity between previous rat and mouse studies is indeed caused by the different methods of modifying ethanol-induced hypothermia,

the pattern of results for the current studies should resemble that of the rat studies. Specifically, mice exposed to a warm T_a should show attenuated ethanol-induced hypothermia and conditioned taste aversion. Changes in taste aversion may be manifest either in absolute magnitude or in rate of development. Mice in the low T_a group may show enhanced hypothermia and taste aversion. This group of mice should also show decreased conditioned place preference compared to mice exposed to the warm and normal T_as. According to the temperature hypothesis, it is possible that exposure to a high T_a during place conditioning could enhance the rewarding effects of ethanol and thereby enhance place preference relative to mice conditioned at normal temperatures.

The outcome of the present studies should resemble the pattern seen with the HOT and COLD mice if species differences alone are responsible for the observed discrepancy. The prediction for place conditioning remains the same; mice not experiencing hypothermia (similar to HOT mice) should demonstrate greater conditioned place preference. However, taste aversion would be predicted to be greater or perhaps develop more rapidly in these mice than in those experiencing ethanol's effects in the low or normal T_a (similar to COLD mice). Cunningham et al. (1992) suggested that perhaps the hypothermia experienced by COLD mice somehow served to reduce or "protect" these mice from the aversive effects of ethanol. If this is the case for mice in general, genetically heterogeneous mice should show the same result.

Since mice have typically been shown to exhibit locomotor activation with low to moderate doses of ethanol (e.g., Frye & Breese, 1981; Phillips & Dudek, 1991), activity data in the current place conditioning study are expected to mimic those found with HOT and COLD mice in that ethanol should increase locomotor activity in all groups, regardless of T_a. Based on results from Cunningham et al. (1991), it would also be predicted that mice at the warm T_a should show higher ethanol-induced activity initially but that mice at the low T_a should sensitize over trials.

GENERAL METHOD

Subjects

Subjects in all experiments were genetically heterogeneous naive male mice (8 - 10 weeks old) obtained from the Portland VA Medical Center. Mice used were from one of two genetic control lines, WSC and CON. The WSC, withdrawal seizure control, line has been maintained in two replicates (WSC-1 and WSC-2) in an ongoing selection study for severity of ethanol withdrawal severity (Crabbe, Kosobud, & Young, 1983). The CON line has also been maintained in two replicate lines (CON-1 and CON-2) as part of the selective breeding projects for HOT and COLD (Crabbe et al., 1987) and FAST and SLOW (Crabbe, Young, Deutsch, Tam & Kosobud, 1987) mice. Both WSC and CON lines are non-selected and originated from HS/Ibg stock, an eight-way cross of inbred strains (McClearn, Wilson, & Meredith, 1970).

In the taste conditioning experiments mice were housed individually in hanging stainless-steel cages (24 x 18 x 18 cm) with wire mesh fronts and bottoms. In the place conditioning experiments, mice were housed in groups of two to four in polycarbonate cages (27.9 x 9.5 x 12.7 cm) with corn cob bedding. The colony room was maintained at 22 $\pm$ 1° C and all experiments occurred in the light phase of a 12-h light-dark cycle (lights on at 0700). Lab chow was available at all times in the home cage in all experiments and in the experimental cages in the taste conditioning experiments. Water was available ad lib in the home cage in the place conditioning experiments. Fluid access in the taste conditioning experiments was restricted as described below.

Surgical procedure

Prior to conditioning trials in each experiment, mice were fully anesthetized with halothane gas and a biotelemetry device for detecting body temperature (Model X-M, Mini-Mitter Co., Sunriver, OR) was implanted in the peritoneal cavity. After induction of anesthesia with a mixture of oxygen and 4% halothane gas, the abdomen was shaved and anesthesia was maintained for the remainder of the procedure at 2.5-3% halothane. A 2-cm incision was made in the skin and muscle layers just to the left of midline. The Mini-Mitter was placed in the peritoneal cavity and sutured to the muscle wall. Muscle and skin were then sewn closed with three to five sutures each with 5-0 suture silk. Animals were then removed from

anesthesia, ear-punched for identification and placed in a recovery chamber which consisted of a shoebox with corn cob bedding on a heating pad. All animals appeared fully recovered from anesthesia and were returned to the home cage within 10 to 20 min. Animals were given 2 days recovery for the first taste conditioning experiment and 4-5 days for all other experiments.

Apparatus

Three Rheem Model CEC-50 refrigerated incubators were used to manipulate ambient temperature in all experiments. These chambers are equipped with ventilating fans and allow for temperature control between 0° and 40° C. In all experiments, the warm T_a was 34 ± 1° C, the normal temperature was 21 ± 1° C, and the cold T_a was maintained at 10 ± 1° C. These temperatures were determined by a pilot study where 35° C was shown to block the hypothermia normally produced by 1.5, 2.25 and 3.0 g/kg ethanol. The cold T_a of 10° C was shown to enhance the hypothermia produced by 2.25 g/kg ethanol.

The X-M Mini-Mitter consists of two thermistors and a battery-powered transmitter encased in a small, nontoxic waterproof capsule and coated with Parafin/Elvax to prevent corrosion by body fluids. The device emits an AM signal at a rate proportional to the surrounding temperature. Prior to implantation, each unit was calibrated in a water bath at 34, 37 and 40° C, and the interval between each signal was used to create calibration parameters for each Mini-Mitter. This system allows temperature resolution of 0.1° C.

In all experiments, telemetry receivers (Mini-Mitter Model RA-1010) were positioned under each cage or place conditioning chamber in the refrigerated incubators to detect signals from the Mini-Mitters. These receivers were connected to an Apple II computer by a custom-built interface which transforms the radio signals to short square-wave pulses. The intervals between pulses were timed (10 msec resolution), averaged over 5-min bins by computer and converted to body temperature based on the previously determined calibration parameters. Specifics of the hardware and software for the biotelemetry system have been described elsewhere (Cunningham & Peris, 1983).

Analysis of Temperature Data

Collection of telemetry data can be subject to two kinds of signal error, radio interference and missing signals. Radio interference from electronic equipment in the laboratory environment results in 'noise' that can be misinterpreted as a Mini-Mitter signal. Missing signals result when an intersignal interval is longer than pre-set limits or differs dramatically from the previous intersignal interval. These criteria, the absolute value of the intersignal interval and the relative change in interval duration from one signal to the next, determine whether a given signal was included in the sample period. In these studies, the absolute limits for intersignal interval were set at 200 - 600 msec, based on Mini-Mitter characteristics. This corresponds to body temperatures from approximately 30° C to 43° C. The

acceptable level of relative change was set at 50 msec. If both criteria were not met for a given signal, that signal was not included in calculating the average intersignal interval for the 5 min sample period. For data analysis, sample values (temperatures) were not used if the percentage of good signals in the sample period was less than 30% or if the relative change was more than 50 msec and the body temperature was unlikely. For discarded samples, the mean of the two adjacent periods was substituted for the rejected value. If 50% or more of the sample periods were unreliable for any given subject, that subject's data were not included for analysis on that day.

In all experiments, analysis of body temperature data includes only subjects who contributed data to both the first and last trials. Temperature data from the first trial were vital for assessing the initial hypothermic response while differences between the first and last trials were necessary to evaluate the development of tolerance to the hypothermic effects of ethanol. Since different subjects had reliable data on different trials, inclusion of all trials in data analysis would have resulted in the loss of many more subjects than the loss incurred by only including those on the first and last trials. Group sizes are noted in figure captions. The alpha level was set at $p \leq .05$ for all analyses.

TASTE CONDITIONING

Experiments 1 - 3 were designed to test the hypothesis that blockade of ethanol-induced hypothermia by exposure to a warm T_a would decrease the

magnitude of ethanol-induced taste aversion in genetically heterogeneous mice and that enhancement of ethanol hypothermia would lead to an increase in the CTA. In all these experiments, consumption of the flavored solution (NaCl or saccharin) took place at normal T_a to rule out direct effects of T_a on drinking. Access to the flavored solution was temporally paired with injection of ethanol. After injection, animals were placed in temperature chambers so the effects of ethanol would be experienced at an altered T_a chosen to block or enhance ethanol-hypothermia.

Experiment 1

The first taste conditioning experiment consisted of two phases, conditioning and extinction. The ethanol dose was 2.5 g/kg and a 0.15% NaCl solution was the flavor used.

Method

Subjects

The subjects were 36 male HS mice (8 - 10 weeks old) with a mean weight of 25.7 g at the beginning of fluid deprivation (range 22.9 - 30.9 g). At the end of the experiment the mean body weight was 25.8 g (range 20.8 - 30.1). Animals were housed as described under General Methods in two racks and each rack constituted one squad of animals (18 mice per squad). Food was available ad lib in the home cage and in the experimental cage but fluid was restricted as described below.

Apparatus

Fluid consumption took place in the home cage in a laboratory room maintained at normal T_a (Room N). Fluids were presented at room temperature in 25-ml graduated glass cylinders (0.2 ml resolution) fitted with curved stainless steel drinking spouts inserted through the fronts of the cages. Six identical cages were placed on 1/4" Plexiglas rails on top of telemetry receivers in each of the three refrigerated incubators. A sheet of Plexiglas with ventilation holes was used as a lid for each cage.

Procedure

Subjects were acclimated to individual housing in the colony for 10 days. All mice then received 2-h access to tap water each day between approximately 1000 - 1200 h for 4-5 days prior to Mini-Mitter surgery (2 days in the colony room and 2-3 days in Room N). Mice were given ad lib tap water for 24 h after surgery and were then placed on 22 h fluid restriction for 2 additional days (one day each, Room N and colony room).

An overview of the experimental procedure for animals in Squad 1 is contained in Table 1. This procedure was staggered by 24 h for Squad 2 with the exception that Squad 2 received one additional day of water deprivation prior to surgery. Two days after surgery all subjects were assigned to one of three T_a groups for taste conditioning. Six mice from each squad were assigned to each T_a group for a total of 12 mice per group.

Table 1 Taste Conditioning Procedure - Experiment 1
N=36 (18/squad), all Ss implanted with Mini-Mitters

Day 1-4:	2 h water access [2 days in colony, 2 days in lab room at normal T_a (Room N)]
Day 5:	Mini-Mitter surgery and 24 h ad lib water
Day 6:	water bottles removed at 1800 h
Day 7:	2 h water in Room N
Day 8:	2 h water in colony
Day 9:	Conditioning trial (C1) * 1 h access to NaCl in Room N * ethanol inj (2.5 g/kg) * placed in cage in warm (W), normal (N), or cold (C) refrigerated incubator * 5 h later returned to home cage and colony room * 30 min access to tap water in colony room
Day 10:	2 h water in colony (off-day)
Day 11-14:	Repeat Days 9 & 10 twice (Trials C2 - C3)
Day 15:	Extinction trial (E1) * 1 h access to NaCl in Room N * 5 h later returned to home cage and colony room * 30 min access to tap water in colony room
Day 16:	2 h water access in colony
Day 17:	Extinction trial (E2)
Day 18:	2 h NaCl access in colony -- 30 min water approx. 1800 h
Day 19-24:	Repeat Day 17 & 18 three times (Trials E3 - E5)

Subject assignments were made based on body weight and water consumption after surgery and were balanced as much as possible for cage position within the rack.

For taste conditioning, mice were weighed in the colony room, transported in the cage rack to Room N at approximately 0900 h and allowed to sit for approximately 1 h to allow body temperature to return to normal. Subjects then received 1 h access to a room temperature 1.1% (w/v) NaCl solution (0.2 M) in Room N. The order in which drinking tubes were put on the cages was staggered with the Warm Group (W) getting fluid first, followed 5 min later by the Normal Group (N) and the Cold Group (C) 5 min after that. This group order was changed each conditioning day. After 1 h, tubes for each T_a group were read and removed (in the same order they went on) and subjects were transported to the incubator room, given an intraperitoneal injection of 2.5 g/kg ethanol (20% v/v in physiological saline, with an injection volume of 12.6 ml/kg) and placed into a cage in the appropriate incubator. All injections were completed in 6 to 7 min. After a 5 h conditioning session, mice were returned to the home cage in Room N and left alone for at least 1 h to dissociate the conditioning trial from the trip back to the colony room. In the colony room subjects received 30 min access to tap water to avoid dehydration (between 1700-1800 h). This entire procedure constituted a CS+ day. On off-days (the days after conditioning trials), mice were given 2 h access to tap water in the colony room during the normal

drinking period.

After three conditioning trials, the procedure was changed to an extinction procedure because of nearly complete suppression of NaCl drinking. This procedure was identical to that on conditioning trials up to the point where drinking tubes were removed. After removal of the tubes, subjects remained undisturbed in Room N until between 1700 - 1800 h when they were returned to the colony room and given 30 min access to tap water. After the first extinction trial (E1), mice remained in the colony room and received 2 h of tap water. However, to facilitate extinction, the fluid on off-days was changed to NaCl on E2 and ensuing trials. On these days, mice were given an additional 30 min of access to tap water between 1700 - 1800 h to prevent dehydration. Five extinction trials were run for each squad. Because of experimenter oversight, intake data from the 30 min post-trial and off-day drinking sessions for E5 were not collected for Squad 2, therefore analyses of these data only include the first four extinction trials.

Results

For all taste conditioning experiments, the amount of fluid consumed was calculated by taking the difference between the initial reading and the final reading of the graduated cylinder drinking tube. For each drinking period, an additional tube was placed on an empty cage and the difference between initial and final readings was subtracted from each subject's intake to control for evaporation and spillage. All consumption data were analyzed by

two factor Analysis of Variance (ANOVA) with T_a Group as the between-group factor and Trials as the within-group factor. Conditioning trials were analyzed separately from extinction trials. However, since the first extinction trial was identical to conditioning trials until removal of drinking tubes, it was also considered the last conditioning trial. Accordingly, NaCl consumption data from this trial were included in analyses of both trial types. Significant main effects of Group were analyzed by Tukey's HSD test. Only animals who contributed consumption data to all trials in any given phase were included in analysis of that phase.

The consumption data for three mice (two Group C, one Group W) were discarded from analyses of the conditioning trials. One Group C mouse died from a bad injection on the third conditioning trial (C3), the other Group C mouse had a slightly leaky drinking tube on C2; however, data from this mouse were included in extinction trials. One Group W mouse became sick after C1 and died, probably from a bad injection. Thus, the number of subjects in Groups W, N, and C were 11, 12, and 10, respectively for analysis of NaCl intake on conditioning trials, and 11, 12, and 11 for analysis of post-session water intake and off-day water intake. Group sizes differed for these analyses because NaCl intakes on E1 were included as conditioning data but post-session data were not. For extinction trial analysis, one Group N mouse was sick on E5; accordingly, intake data for this mouse were excluded from analysis of the extinction phase. Therefore, all groups had 11 subjects for

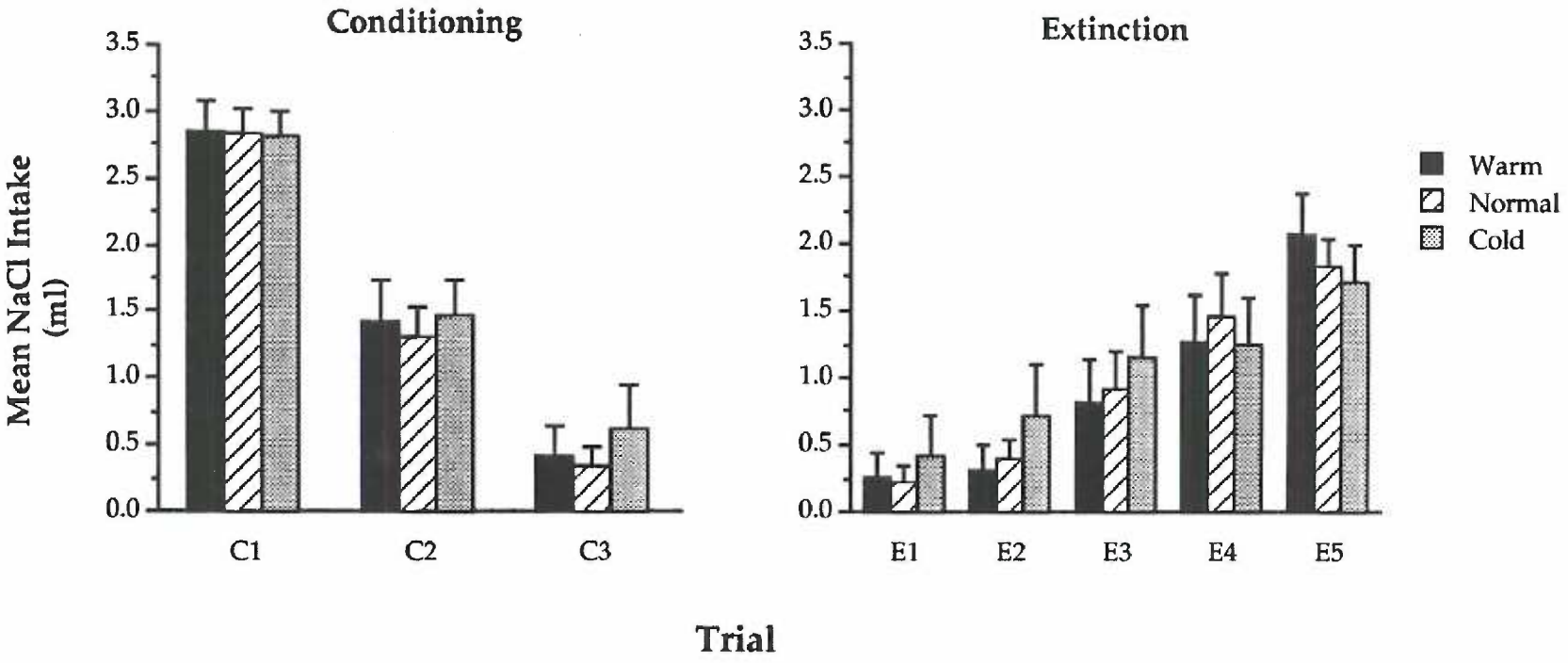
extinction trial analysis.

Body temperature data were collected for the entire 5 h session, but since ethanol's effects occurred primarily during the first hour, only the first 60 min were analyzed. A two-way ANOVA with T_a Group as the between-group factor and Trials as the within-group factor was applied to the data from the first and last conditioning trials (C1 and C3). To verify that manipulation of T_a had an effect on ethanol-induced hypothermia, follow-up one-way ANOVAs (T_a Group) were conducted on data from C1 and C3. Follow-up pairwise comparisons of group means were made using Tukey's HSD test. Since tolerance may develop to ethanol's thermic effects with repeated exposure, significant effects involving Trials were analyzed by separate one-way ANOVAs (Trials) within each T_a group.

Intake Data

The left panel of Figure 1 shows the mean NaCl intake for the three T_a groups on each of the conditioning trials. The amount consumed decreased across trials, indicating the development of a conditioned taste aversion. One pairing of ethanol and NaCl resulted in 50% suppression of drinking, and three trials produced 80% suppression of drinking. Mean NaCl intake collapsed over T_a Group on C1, C2 and C3 was 2.82, 1.41 and 0.47 ml, respectively. No differences in either the extent of the aversion or its rate of development were seen between the T_a groups.

Figure 1. Mean NaCl intake (ml ± SEM) for the warm (W), normal (N), and cold (C) T_a groups during conditioning (left panel) and extinction (right panel) in Experiment 1. The dose of ethanol was 2.5 g/kg. N = 11 for all groups during both phases.


Experiment 1
Conditioning
Extinction
Mean NaCl Intake (ml)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
C1
C2
C3
E1
E2
E3
E4
E5
Warm
Normal
Cold
Trial

A two-way ANOVA (T_a Group x Trials) supported these observations and showed only a main effect of Trials, $F(3,90) = 145.78$, $p < .001$, with no significant interaction or main effect of T_a Group. The right panel of Figure 1 depicts the mean NaCl intake for the three T_a groups on extinction trials. Consumption increased across trials, indicating that the learned aversion was extinguished. Extinction occurred at nearly the same rate in all groups. These observations were supported by a two-way ANOVA (T_a Group x Trials) which revealed a main effect of Trials, $F(4,120) = 32.76$, $p < .001$. Neither the main effect of T_a Group nor the interaction was significant.

Table 2 shows the mean 30 min post-trial intake of each T_a group during the conditioning and extinction phases. Water intake in the 30 min post-trial drinking session increased over conditioning trials as NaCl consumption on conditioning trials decreased, with no group differences. Water intake after extinction trials did not change over time, even though NaCl intake rose somewhat during these trials. Two-way ANOVAs (T_a Group x Trials) supported these observations, revealing a main effect of Trials,$F(2,62) = 48.93$, $p < .001$ during conditioning, and no significant effects during extinction.

The mean water intakes for off-days during conditioning are also shown in Table 2. Water intake on off-days did not change significantly across conditioning trials. However, mean intake for the T_a groups was different.

Table 2

Mean Fluid Intake (ml ± SEM) During 30 min Post-trial and 2 h Off-day Drinking Sessions in Experiment 1. N = 11/group.

	Group W		Group N		Group C	
Trial	Post-trial	Off-day	Post-trial	Off-day	Post-trial	Off-day
C1	.095 ± .130	2.67 ± .199	1.05 ± .123	3.12 ± .212	1.33 ± .133	3.46 ± .191
C2	1.44 ± .114	3.07 ± .211	1.35 ± .144	3.35 ± .167	1.20 ± .121	3.64 ± .271
C3	2.22 ± .172	2.86 ± .140	1.98 ± .178	3.02 ± .177	2.16 ± .242	3.26 ± .373
E1	2.64 ± .154	3.20 ± .101	2.40 ± .207	3.63 ± .157	2.60 ± .214	3.84 ± .192
E2	2.56 ± .248	**1.13 ± .416**	2.50 ± .185	**1.27 ± .439**	2.64 ± .157	**0.91 ± .462**
E3	2.09 ± .163	**2.00 ± .492**	2.37 ± .192	**2.42 ± .448**	2.44 ± .152	**1.71 ± .532**
E4	2.31 ± .136	**2.84 ± .500**	2.20 ± .187	**2.73 ± .364**	2.60 ± .249	**3.27 ± .546**

Note. Values in boldface are NaCl consumption, all others are tap water.

W = warm, N = normal, C = cold

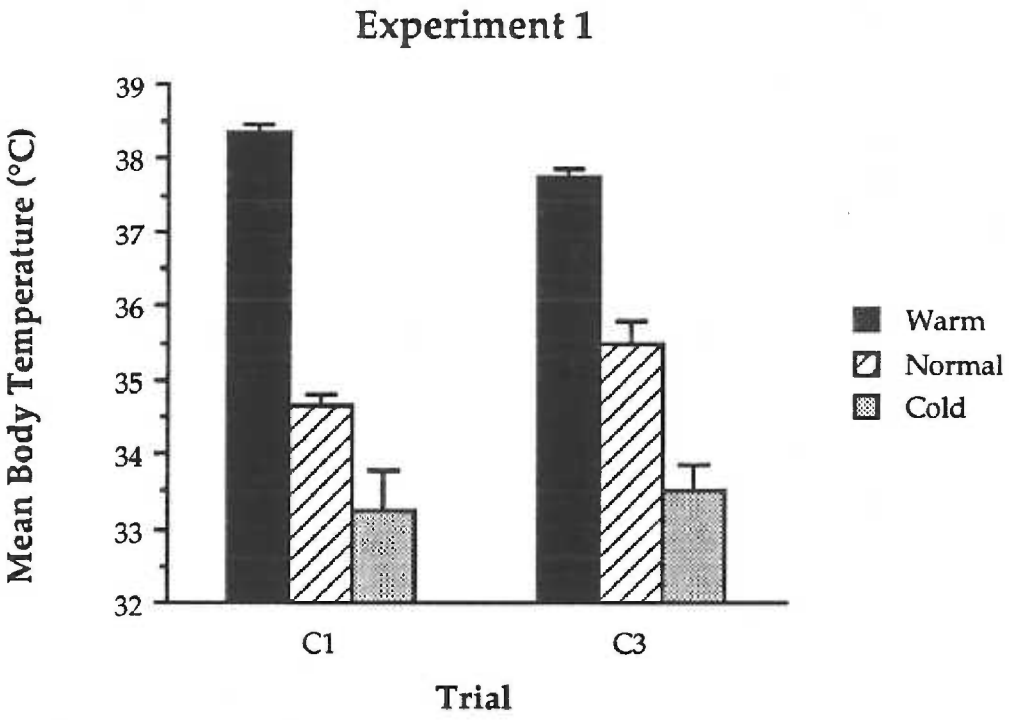
A two-way ANOVA (T_a Group x Trials) revealed a significant main effect of T_a Group, $F(2,31) = 3.48$, $p = .042$. However, pairwise comparisons of group means collapsed over trials did not reveal any significant differences between T_a Groups ($p > .05$, Tukey's HSD). Mean off-day water intakes for each T_a Group were 2.87, 3.16, and 3.45 ml, respectively for Groups W, N, and C.

Finally, Table 2 also shows the mean off-day intake of each T_a group during extinction. Consumption on off-days during extinction decreased dramatically when NaCl was substituted for water but increased nearly to initial levels as the aversive response extinguished. No effects of T_a group were seen. These observations were supported by two-way ANOVA (T_a Group x Trials) of all four trials which revealed a significant effect of Trials, $F(3,93) = 45.47$, $p < .001$. Additional analyses of those off-days where NaCl was the fluid also revealed a significant main effect of Trials, $F(2,62) = 54.47$, $p < .001$.

Body Temperature Data

Figure 2 shows the mean body temperatures for the three T_a groups in the first 60 min of C1 and C3. Mean body temperatures on each trial are presented in Appendix A. In general, exposure to a warm T_a attenuated ethanol-induced hypothermia while exposure to a cold T_a enhanced hypothermia on both trials.

Figure 2. Mean body temperature (°C ± SEM) of the warm (W), normal (N), and cold (C) T_a groups during the first 60 min of conditioning trials C1 and C3. Group sizes are 10, 9 and 9 for Groups W, N and C, respectively. Only animals with reliable data on C1 and C3 are included.


Experiment 1
Mean Body Temperature (°C)
39
38
37
36
35
34
33
32
C1
C3
Trial
Warm
Normal
Cold

A two-way ANOVA (T_a Group x Trials) revealed a significant T_a Group x Trials interaction, $F(2,25) = 5.32$, $p = .012$, as well as a significant effect of T_a Group, $F(2,25) = 88.22$, $p < .001$. To follow-up the interaction, mean body temperatures were compared for each group using a one-way ANOVA with Trials as the repeated factor. These analyses revealed a main effect of Trials in Group N, $F(1,25) = 6.51$, $p = .016$. Body temperatures in this group were higher on C3, indicating the development of tolerance to the thermal effects of ethanol. Temperatures in Groups W and C did not change significantly over trials.

Follow-up ANOVAs on C1 and C3 revealed a significant effect of T_a Group on each trial, $Fs(2,25) = 66.88$ and 61.85, $ps < .001$, respectively. Pairwise comparisons showed that mean body temperature in Group W was significantly higher than in both the N and C Groups and that the mean temperature of Group C was significantly lower than in Group N on both trials (all $ps < .05$, Tukey's HSD).

Discussion

Experiment 1 demonstrated that a robust conditioned taste aversion can be produced by pairing NaCl flavor with a 2.5 g/kg dose of ethanol. Exposure to a warm T_a after injection blocked the hypothermia that this dose of ethanol produced at normal T_a. In addition, the hypothermia was enhanced by exposure to a cold T_a. However, alteration of ethanol hypothermia apparently had no effect on the development of taste aversion

in this experiment. It is possible that this lack of effect could be caused by the rapid development of a very strong taste aversion, i.e., any effects of T_a might have been masked by the rapid development and strength of the conditioned response.

Experiment 2

To examine the possibility that rapid development of a robust taste aversion precluded any effects of altered T_a, some procedural changes were made for Experiment 2. The dose of ethanol was decreased slightly from 2.5 g/kg to 2.25 g/kg. The reduction in dose was expected to produce a somewhat weaker aversion. In addition, a CS pre-exposure trial was added, i.e., mice received NaCl once (with no injection) prior to conditioning. CS pre-exposure has been shown to retard the development of learned responses (Lubow & Moore, 1959), and it was hoped that slowing the development of the aversion would allow any effects of altered T_a to be seen.

Method

Subjects

Subjects were 36 naive male HS mice, divided into 2 squads of 18 mice each and housed as described in General Methods. The mean weight at the beginning of fluid deprivation was 29.4 g (range 25.3 - 32.9 g). At the end of the experiment, mean body weight was 26.6 g (range 20.7 - 32.0 g).

Apparatus

All equipment and laboratory rooms for this experiment were identical to those used in Experiment 1.

Procedure

Table 3 gives an overview of the experimental procedure for Squad 1. Squad 2 underwent the identical procedure staggered by 24 h. Procedures used were the same as in Experiment 1 with the following exceptions. After 12-13 days of acclimation to individual housing in the colony mice were implanted with Mini-Mitters. Mice were given 24 h ad lib water after surgery and were then given 2 h access to tap water each day for 4 days (1 day in colony room, 2 days in Room N, 1 day in colony room). The following day, subjects received 1 h access to NaCl in Room N during the drinking period (CS pre-exposure). Mice were assigned to one of the T_a groups based on NaCl consumption and body weight. Group assignments were balanced as much as possible across cage position within the rack. Each T_a group comprised 6 animals from each squad, giving a total of 12 mice per group.

After five conditioning trials, an extinction procedure identical to that in Experiment 1 was used, with one exception. The off-day fluid was water until after E3, when it was changed to NaCl for E4 and E5.

Results

As in Experiment 1, consumption data for Squads 1 and 2 were combined for analysis.

Table 3 Taste conditioning procedure - Experiment 2
N=36 (18/squad), all Ss implanted with Mini-Mitters

Day 1:	Mini-Mitter surgery
Day 2:	water bottles removed at 1800 h
Day 3-6:	2 h water access [1 day in colony, 2 days in lab room at normal T_a (Room N) , 1 day in colony]
Day 7:	CS pre-exposure * 1 h access to NaCl in Room N * 5 h later returned to home cage and colony room * 30 min access to tap water in colony room
Day 8:	2 h water access in colony
Day 9:	Conditioning trial (C1) * 1 h access to NaCl in Room N * ethanol inj (2.25 g/kg) * placed in cage in refrigerated incubator (W, N, C) * 5 h later returned to home cage and colony room * 30 min access to tap water in colony room
Day 10:	2 h water access in colony
Day 11-18:	Repeat Day 6 & 7 (trials C2 - C5)
Day 19:	Extinction trial (E1) * 1 h access to NaCl in Room N * 5 h later returned to home cage and colony room * 30 min access to tap water in colony room
Day 20:	2 h water access in colony
Day 21-22:	Repeat Day 19 & 20 (trial E2)
Day 23:	Extinction trial (E3)
Day 24:	2 h NaCl access in colony -- 30 min water approx. 1800 h
Day 25-28:	Repeat Day 23 & 24 twice (E4 & E5)

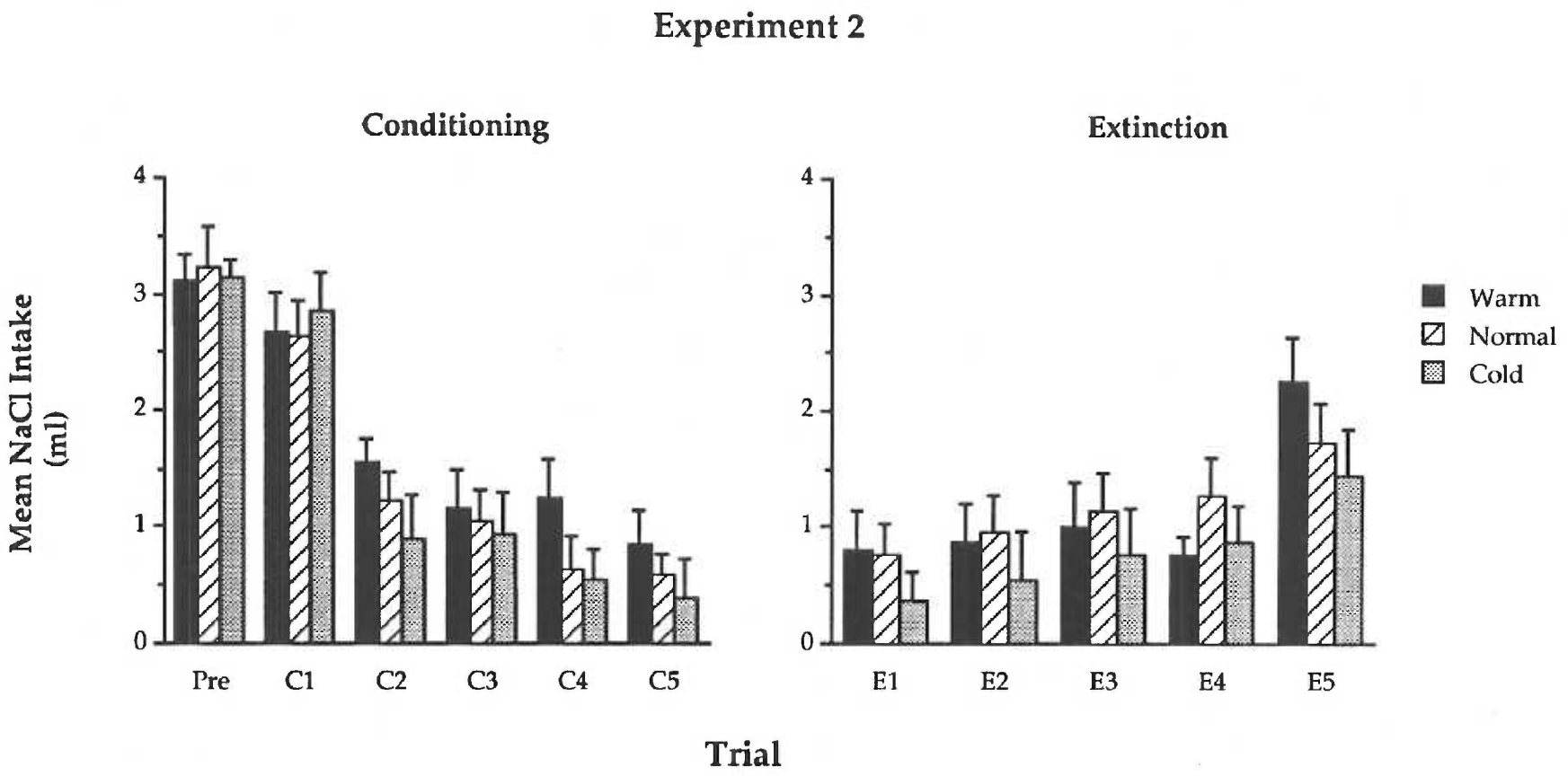
Over the course of conditioning, three mice from Group W, two mice from Group C, and one mouse from Group N either died or were euthanized because of illness. No additional subjects were lost during extinction. Therefore, group sizes (W, N, C) were 9, 11, and 10 for analysis of conditioning and extinction trials. Data from the first extinction trial (E1) were again included in analyses of NaCl intake for both conditioning and extinction phases. Body temperature data were collected and analyzed as in Experiment 1 with fewer data lost because of interference. The number of mice contributing data to analyses is included in figure captions.

Intake data

The left panel of Figure 3 depicts the mean NaCl intake for the three T_a groups during pre-exposure and on all conditioning trials. As in Experiment 1, consumption decreased approximately 50% after C1 (from 2.7 on C1 to 1.2 ml on C2). However, drinking decreased more slowly on ensuing trials, indicating that the aversion did not develop as rapidly as in Experiment 1. Mean NaCl intake on trials C3, C4 and C5 was 1.0, 0.8 and 0.6 ml, respectively. No significant differences were seen between groups. A two-way ANOVA (T_a Group x Trials) yielded only a significant main effect of Trials, $F(6,162) = 60.84$, $p < .001$.

NaCl intake for the three T_a groups during the extinction phase is also shown in Figure 3 (right panel). Consumption increased over trials as extinction of the aversion occurred, but there were no group differences.

Figure 3. Mean NaCl intake (ml ± SEM) for the warm (W), normal (N), and cold (C) T_a groups during the CS pre-exposure trial and conditioning trials (left panel) and extinction (right panel) in Experiment 2. Ethanol dose was 2.25 g/kg. Group sizes on all trials were 9, 11, and 10 for Groups W, N and C, respectively.


Experiment 2
Conditioning
Extinction
Mean NaCl Intake (ml)
0
1
2
3
4
Pre
C1
C2
C3
C4
C5
E1
E2
E3
E4
E5
Warm
Normal
Cold
Trial

A two-way ANOVA showed a significant main effect of Trials, $F(4,108) = 16.09$, $p < .001$, with no significant effects involving T_a Group. Mean comparisons using Tukey's HSD test showed that NaCl intake on the last extinction trial (E5) was significantly higher than in trials E1 through E4 ($p < .05$). No other significant differences were seen.

Analysis of water intake during the 30 min post-trial drinking session for both conditioning and extinction trials by two-way ANOVAs (T_a Group x Trials) yielded only a significant main effect of Trials in both phases, $F(5,135) = 15.49$, $p < .001$ and $F(4,108) = 5.95$, $p < .001$, respectively. Mean 30 min intake values are given in Table 4.

Mean fluid intakes on off-days are also presented in Table 4. Fluid intake on off-days increased slightly during conditioning then decreased during extinction when NaCl was substituted for water. NaCl intake increased over successive extinction trials. A two-way ANOVA (T_a Group x Trials) revealed a significant main effect of Trials during the conditioning phase, $F(5,135) = 5.67$, $p < .001$. Mean water intake values on off-days of trials C0, C1, C2, C3, C4, and C5 were 3.6, 3.0, 3.1, 3.6, 3.8, and 3.4 ml, respectively. Mean comparisons revealed that intake on C4 was higher than that on C1 or C2, and that intake on C3 and C0 was higher than on C1 (Tukey's, $p < .05$). The same analysis on all extinction trials yielded a significant main effect of Trials, $F(4,108) = 13.48$, $p < .001$.

Table 4

Mean Fluid Intake (ml ± SEM) During 30 min Post-trial and 2 h Off-day Drinking Sessions in Experiment 2. N = 9/Group W; 11/Group N; 10/Group C.

	Group W		Group N		Group C	
Trial	Post-trial	Off-day	Post-trial	Off-day	Post-trial	Off-day
C0[a]	1.43 ± .142	3.72 ± .223	1.32 ± .145	3.43 ± .049	1.55 ± .081	3.68 ± .245
C1	1.09 ± .234	2.74 ± .219	1.07 ± .162	3.35 ± .173	1.27 ± .156	2.82 ± .150
C2	1.26 ± .197	2.83 ± .269	1.54 ± .118	3.09 ± .214	1.54 ± .124	3.40 ± .211
C3	1.60 ± .108	3.22 ± .319	1.84 ± .096	3.46 ± .300	2.01 ± .126	3.94 ± .222
C4	1.71 ± .137	3.54 ± .190	1.80 ± .145	3.89 ± .311	1.99 ± .116	4.02 ± .268
C5	1.71 ± .203	3.29 ± .271	1.90 ± .179	3.21 ± .330	2.19 ± .152	3.65 ± .268
E1	2.36 ± .181	3.66 ± .342	2.42 ± .131	4.01 ± .276	2.53 ± .151	4.36 ± .230
E2	2.38 ± .227	4.01 ± .255	2.70 ± .194	4.24 ± .270	2.70 ± .139	3.83 ± .336
E3	2.66 ± .226	**2.30 ± .745**	2.51 ± .283	**2.26 ± .574**	2.46 ± .196	**1.68 ± .556**
E4	1.69 ± .221	**3.91 ± .619**	1.88 ± .144	**3.27 ± .574**	2.29 ± .140	**2.78 ± .599**
E5	2.14 ± .293	**3.99 ± .696**	2.56 ± .147	**3.76 ± .513**	2.48 ± .253	**3.37 ± .473**

Note. Values in boldface are NaCl consumption, all others are tap water.

[a]Trial C0 is the CS pre-exposure trial.

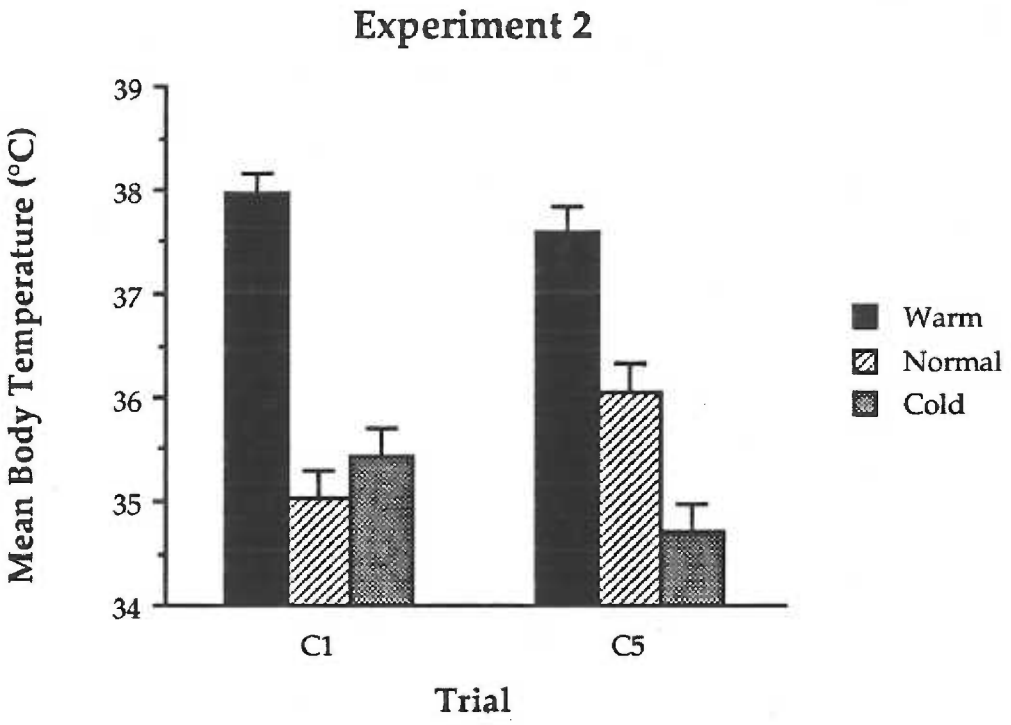
W = warm, N = normal, C = cold

Additional analysis of trials E3 - E5 (where fluid was NaCl) also revealed a significant main effect of Trials, $F(2,54) = 19.31$, $p < .001$. No significant effects involving T_a Group were seen. Mean fluid consumption for off-days on trials E1, E2, E3, E4, and E5 were 4.0, 4.0, 2.1, 3.3 and 3.7 ml, respectively. Fluid intake on E3 was significantly lower than that on all other extinction trials (Tukey's, $p < .05$).

Body Temperature Data

The mean body temperatures in the first 60 min on the first and last conditioning trials for each T_a group are shown in Figure 4. Mean body temperatures for all conditioning trials are listed in Appendix A. As in Experiment 1, exposure to the warm T_a attenuated development of hypothermia. A two-way ANOVA (T_a Group x Trials) of temperatures on the first and last conditioning trials yielded a significant interaction between T_a Group and Trials, $F(2,25) = 8.71$, $p = .002$, and a significant main effect of T_a Group, $F(2,25) = 64.91$, $p < .001$. The presence of the interaction suggests group differences in development of tolerance or sensitization to ethanol's effects. To explore this possibility, mean body temperatures on C1 and C5 were compared for each group using one-way ANOVAs with Trials as the repeated factor. As in Experiment 1, a main effect of Trials was revealed in Group N, $F(1,25) = 11.96$, $p = .002$, with less hypothermia on C5 than C1, indicating the development of tolerance. A main effect of Trials was also found in the C Group, $F(1,25) = 5.31$, $p = .028$.

Figure 4. Mean body temperature (°C ± SEM) during the first 60 min of conditioning trials C1 and C5 of the warm (W), normal (N) and cold (C) T_a groups. Ethanol dose was 2.25 g/kg. Only animals who contributed data to both trials are included. N = 9, 10, and 9 for Groups W, N, and C, respectively.


Experiment 2
Mean Body Temperature (°C)
39
38
37
36
35
34
C1
C5
Trial
Warm
Normal
Cold

However, in this group, mean temperature was lower on C5 than on C1, which suggests that these animals became sensitized to ethanol's thermal effects.

Additional follow-up one-way ANOVAs yielded a significant effect of T_a Group on both trials,$F(2,25) = 44.27$, $p < .001$; C5, $F(2,25) = 39.77$, $p < .001$. Pairwise comparisons of group means showed that mice in Group W had higher temperatures than subjects in both N and C Groups on both trials (all $ps < .05$, Tukey's HSD). In contrast to Experiment 1, enhancement of ethanol hypothermia by the cold T_a was not present on C1, but was seen on C5 ($p < .05$, Tukey's).

Discussion

Although the CS pre-exposure and lower ethanol dose did slow the rate of acquisition of the taste aversion somewhat after the second conditioning trial, the results of Experiment 2 were similar to those of Experiment 1. The effects of altered T_a on body temperature were consistent with the first experiment in that hypothermia was attenuated in the warm T_a. Enhancement of hypothermia was seen in the cold T_a, but not on the first trial. However, altering the degree of ethanol-induced hypothermia did not significantly affect the development of the ethanol-induced conditioned taste aversion. Interestingly, not only was tolerance to ethanol's thermal effects seen in Group N, but subjects in Group C appeared to sensitize to these effects over trials.

Experiment 3

For Experiment 3, it was decided to switch the flavor of the solution from NaCl to saccharin. It was thought that the use of saccharin would further impede the development of the aversion since it is typically considered a preferred flavor (e.g., Lynch, 1986; Murray, Wells, Kohn, & Miller, 1953). Since the rate of acquisition of taste aversions is typically slower with saccharin than NaCl (Risinger, personal communication), CS pre-exposure was not used. The dose of ethanol was kept at 2.25 g/kg so that not all variables would be changed at once.

Method

Subjects

Subjects were 48 naive male HS mice, divided into two squads of 24 mice each and housed as described in General Methods. The mean weight at the beginning of fluid deprivation was 29.2 g (range 26.0 - 37.3 g). At the end of fluid deprivation (before the second two-bottle test) mean body weight was 25.6 g (range 20.8 - 29.8 g).

Apparatus

All equipment and laboratory rooms for this experiment were identical to those used in Experiment 1, with the addition of two test cages in each incubator. Because of apparatus limitations, body temperature data could not be collected from animals in these cages. Accordingly, the cages were placed on inoperative telemetry receivers but were identical to the other test cages in

all other respects.

Procedure

Procedures used were generally the same as in Experiment 1 with some exceptions. An overview of the experimental procedure is available in Table 5. One exception is the use of a two-bottle choice test (saccharin vs water) rather than an extinction procedure. The two-bottle test has been reported to be a more sensitive measure of taste aversions (cf. Goudie, 1987; Riley & Tuck, 1985) than one-bottle tests. This increased sensitivity is because in a one-bottle procedure, an animal's need for water may overcome any aversion for the flavored solution while concurrent presentation of water with the flavor in a two-bottle test eliminates this problem (cf. Goudie, 1987). In addition, if extraneous variables cause a change in fluid consumption at any given time, this should be reflected in water consumption as well as consumption of the test solution (Grill, Spector, Schwartz, Kaplan, & Flynn, 1987). A 0.15% (.007 M) sodium saccharin solution was used instead of the previous NaCl solution.

Mini-Mitter implantation occurred after 5 - 6 days of acclimation to individual housing in the colony. Because of apparatus limitations, body temperature data could only be collected from 18 mice at one time (six mice/incubator). Accordingly, a total of 36 mice were implanted with functional Mini-Mitters (two squads, 18/squad) while 12 were implanted with an empty Mini-Mitter capsule to equate the experience of surgery (6/squad).

Table 5 Taste conditioning procedure - Experiment 3
N=48 (24/squad) 36 Ss implanted with
Mini-Mitters, 12 with empty capsules

Day 1:	Mini-Mitter surgery
Day 2:	water bottles removed at 1800 hr
Day 3-7:	2 h water access [1 day in colony, 2 days in lab room at normal T_a (Room N), 1 day in colony]
Day 8:	Conditioning trial (C1) * 1 h access to saccharin in Room N * ethanol inj (2.25 g/kg) * placed in cage in refrigerated incubator (W, N, C) * 5 h later returned to home cage and colony room * 30 min access to tap water in colony room
Day 9:	2 h water access in colony (off-day)
Day 10-17:	Repeat Days 8 & 9 four times (trials C2 - C5)
Day 18:	1 h two-bottle Test 1, saccharin vs water
Day 19:	2 h water access
Day 20:	1 h two-bottle Test 2, saccharin vs water
Day 21:	assessment of 24 h intake on Test 2

Mice were given 24 h ad lib water after surgery and were then given 2 h access to tap water each day for 5 days (2 days in colony room, 2 days in Room N, 1 day in colony). Mice were assigned to one of the T_a groups based on water consumption over the two previous days and on body weight. Group assignments were balanced as much as possible across cage position within the rack. Each T_a group was comprised of 8 animals from each squad, giving a total of 16 mice per group (12 with Mini-Mitters/group).

After five conditioning trials, a two-bottle drinking test was conducted instead of the extinction procedures used in Experiments 1 and 2. Subjects were transported to Room N as in conditioning but received a bottle of saccharin and a bottle of tap water during the 1 h access period. Left vs right position of the two bottles was counterbalanced within T_a groups. After drinking bottles were removed, mice remained undisturbed in Room N until approximately 1700 h when they were returned to the colony room. All animals received 2 h access to tap water the following day.

A second two-bottle test was given 48 h after the first one to assess whether extinction had occurred. This second test was conducted in the colony room. The left/right positions of the bottles were reversed from those used on Test 1 and consumption was assessed after 1 h and after 24 h.

Results

Data for conditioning trials were analyzed as in the previous experiments. Two mice (one from Group N, one from Group C) were

euthanized because of illness and their data were excluded from analyses during conditioning and testing. An additional mouse from Group C received a bad injection after C5. Saccharin intakes were included for this mouse but the data were excluded from the 30 min drinking period and off-day analyses, as well as the two-bottle test. Accordingly, group sizes for saccharin intake during conditioning are 16, 15, and 15 for the W, N, and C Groups, respectively and 16, 15, and 14 for all other analyses.

Intake data

Figure 5 shows the mean saccharin intake for the three T_a groups on all conditioning trials. All groups developed a conditioned taste aversion although the magnitude of aversion was less than in previous experiments. As in Experiments 1 and 2, there were no significant differences between groups. A two-way ANOVA (T_a Group x Trials) yielded only a significant main effect of Trials, $F(4,172) = 58.28$, $p < .001$, but no other significant effects. Mean saccharin consumption on trials C1, C2, C3, C4, and C5 was 2.6, 2.0, 1.4, 1.1, and 1.1 ml, respectively. Pairwise comparisons revealed that intake decreased significantly after C1, C2 and C3 (Tukey's, $p < .05$).

Water intake during the 30 min post-trial drinking session increased across trials as morning consumption of saccharin decreased. Mean 30 min water intake values are presented in Table 6. A T_a Group x Trials ANOVA revealed significant main effects of T_a Group, $F(2,42) = 6.15$, $p < .001$, and Trials, $F(4,168) = 15.05$, $p < .001$.

Figure 5. Mean saccharin consumption (ml ± SEM) of Group W (n = 16), Group N (n = 15) and Group C (n = 15) during conditioning trials. The dose of ethanol was 2.25 g/kg.

Experiment 3

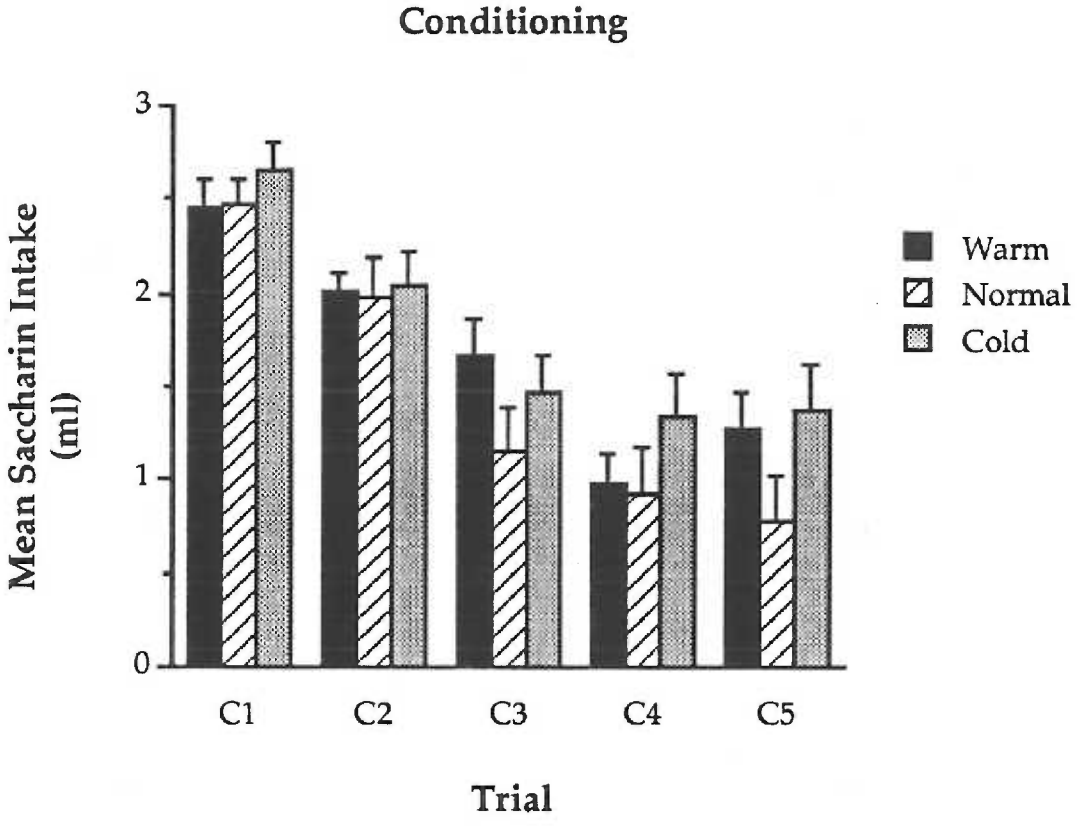


Table 6

Mean Water Intake (ml ± SEM) During 30 min Post-trial and 2 h Off-day Drinking Sessions in Experiment 3. N = 16/Group W; 15/Group N; 14/Group C.

	Group W		Group N		Group C	
Trial	Post-trial	Off-day	Post-trial	Off-day	Post-trial	Off-day
C1	1.08 ± .131	2.17 ± .166	1.07 ± .077	1.91 ± .181	1.22 ± .104	2.09 ± .152
C2	1.11 ± .087	2.08 ± .151	1.29 ± .121	1.96 ± .153	1.76 ± .132	2.31 ± .184
C3	1.49 ± .122	2.47 ± .309	1.48 ± .160	2.81 ± .145	1.78 ± .104	2.45 ± .268
C4	1.39 ± .108	2.56 ± .186	1.68 ± .118	2.84 ± .230	1.91 ± .162	3.11 ± .221
C5	1.54 ± .174	2.47 ± .139	1.83 ± .123	2.41 ± .158	1.91 ± .169	3.26 ± .253

W = warm, N = normal, C = cold

Pairwise comparisons of T_a Group means collapsed over trials did not reveal any significant differences between groups. Mean intake levels were 1.3, 1.5, and 1.7 ml for Groups W, N, and C, respectively. To follow-up the main effect of Trials, data were collapsed across T_a Group and mean intake values on each trial were compared with Tukey's HSD test. This analysis showed that intake on trials 3, 4, and 5 was higher than on trial 1 and that intake on trials 4 and 5 was higher than on trial 2 ($p < .05$).

Water intake on off-days in Group C increased somewhat over all conditioning trials, while intakes in Groups N and W appeared to level off after C3. A two-way ANOVA (T_a Group x Trials) revealed a significant main effect of Trials, $F(4,168) = 12.81$, $p < .001$, as well as a significant T_a Group x Trials interaction, $F(8,168) = 2.23$, $p = .027$. Follow-up analyses using one-way ANOVAs (Trials as repeated factor) yielded a significant main effect of Trials in Groups N and C, $F(4,168) = 6.77$,$p = .001$, and $F(4,168) = 8.43$, $p < .001$, respectively, but not in Group W. Table 6 contains mean off-day consumption data on all trials.

In two-bottle Tests 1 and 2, all groups preferred water to saccharin, indicating development of a strong taste aversion that did not extinguish rapidly. Mean fluid intake levels for both tests are presented in Table 7. Two-way ANOVAs with T_a Group as the between factor and Flavor as the within factor revealed a significant main effect of Flavor after 1 h on both tests, Test 1: $F(1,42) = 67.58$, $p < .001$, Test 2:$F(1,42) = 31.13$, $p < .001$.

Table 7

Mean Fluid Intake (ml ± SEM) During Two-Bottle Test in Experiment 3. N = 16/Group W; 15/Group N; 14/Group C.

	Group W		Group N		Group C	
	Saccharin	Water	Saccharin	Water	Saccharin	Water
Test 1 (1h)	0.25 ± .069	1.39 ± .166	0.29 ± .102	1.32 ± .269	0.32 ± .101	1.70 ± .191
Test 2 (1h)	0.80 ± .245	1.89 ± .248	0.56 ± .155	2.38 ± .179	0.78 ± .298	2.07 ± .237
(24h)	3.02 ± 1.44	9.11 ± 1.10	1.89 ± .712	9.99 ± .640	3.56 ± 1.51	9.70 ± 1.23

W = warm, N = normal, C = cold

After 24 h, a significant effect of Flavor was still present, $F(1,42) = 27.24$, $p < .001$, indicating that the aversion did not extinguish. There were no significant effects involving T_a Group.

Body Temperature Data

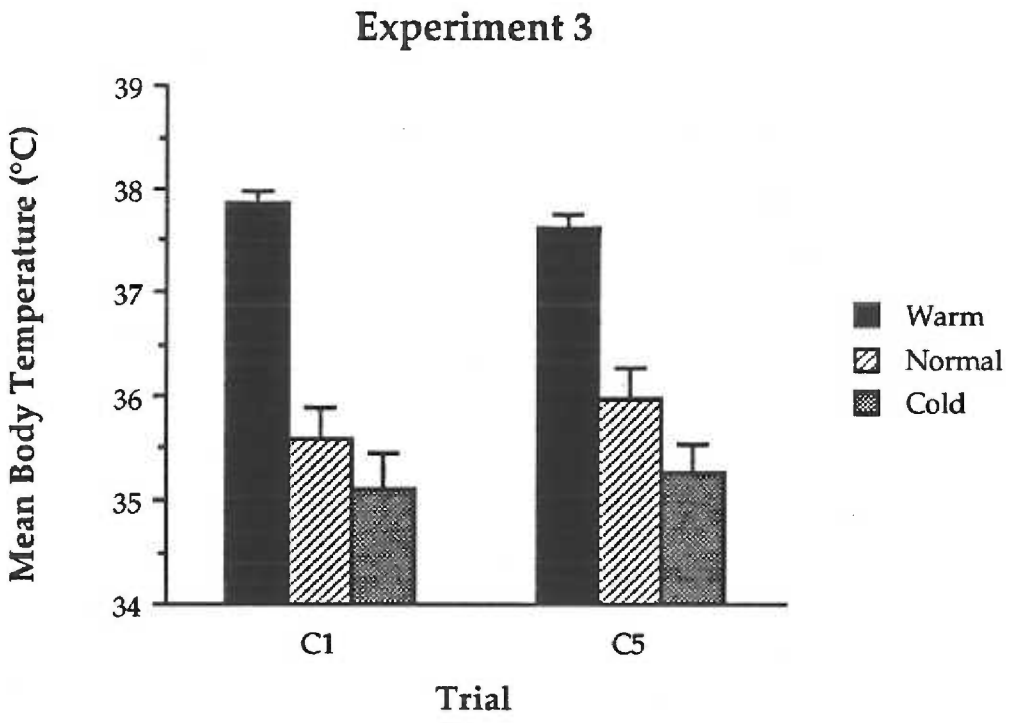
Figure 6 depicts the mean body temperatures for T_a groups on conditioning trials 1 and 5. As in Experiments 1 and 2, ethanol-induced hypothermia was diminished by exposure to a warm T_a. The overall two-way ANOVA yielded a significant effect of T_a Group, $F(2,27) = 32.62$, $p < .001$. Using pairwise comparisons, mean temperatures in Group W were shown to be higher than those in both the N and C Groups ($ps < .05$, Tukey's HSD). However, no evidence of enhancement of hypothermia was seen, i.e., Groups N and C did not differ ($ps > .05$, Tukey's HSD).

In contrast to the previous experiments, a two-way ANOVA (T_a Group x Trials) revealed no significant effects involving Trials, indicating that no tolerance or sensitization to ethanol's thermal effects developed. This is surprising in light of the tolerance seen in Group N in Experiments 1 and 2.

Discussion

The primary finding of Experiment 3 is in accord with that of Experiments 1 and 2. Specifically, manipulation of ethanol-induced hypothermia had no significant effect on conditioned taste aversion.

Figure 6. Mean body temperature (°C ± SEM) during the first 60 min of conditioning trials C1 and C5 for the warm (W), normal (N), and cold (C) T_a groups. Ethanol dose was 2.25 g/kg. Only subjects with reliable data on both trials are included. Group sizes are 11, 10, and 9 for Groups W, N, and C, respectively.


Experiment 3
Mean Body Temperature (°C)
39
38
37
36
35
34
C1
C5
Trial
Warm
Normal
Cold

The T_a group differences seen in water intake after conditioning trials indicate that alterations in hypothermia may have had some effect, but the lack of effect on acquisition of taste aversion makes this finding difficult to interpret.

Surprisingly, no evidence of tolerance to ethanol's thermic effects in Group N mice was found in Experiment 3. This was possibly due to a smaller hypothermic response on the first conditioning trial compared to Experiment 2. In contrast to the first two experiments, no enhancement of hypothermia was seen in Group C on any trial.

Discussion - Taste Conditioning

The pattern of results found in Experiments 1 - 3 suggests that the thermal effects of ethanol in mice are not related to its aversive motivational properties as measured by taste conditioning. This conclusion follows from the finding that alteration of ethanol-induced hypothermia, either attenuation by exposure to a warm T_a or enhancement by exposure to a cold T_a, had no effect on the magnitude of the conditioned response or on the rate of extinction of the response.

An alternative interpretation of the failure of the low T_a to enhance taste aversion could be that exposure to 10° C did not consistently enhance ethanol-hypothermia. Accordingly, mice in Group C did not always have body temperatures that were significantly different than mice conditioned at 21° C, and, according to the temperature hypothesis, would not necessarily be

expected to show a differential conditioned aversion. However, this argument cannot explain the lack of effect of the warm T_a inasmuch as mice in Group W experienced less hypothermia than those in Group N, yet both groups showed equal taste aversion.

One could argue that external changes in temperature have a modulatory influence on the ethanol US in rats but not in mice, thus explaining the effects of warm T_a on rat taste conditioning and the lack of effect of external temperature changes on taste conditioning in mice. However, this does not seem likely given that changes in core body temperature, i.e., internal ethanol effects, were seen in mice in the present experiments.

Finally, it is possible that the appropriate parameters were not used to reveal the relationship between the thermal and motivational effects of ethanol in mice. For example, it may be that the procedures used to reduce the magnitude of the taste aversion (CS pre-exposure and use of saccharin) were not sufficient. Thus, the conditioned taste aversions may have been too robust to be influenced by manipulations in ethanol hypothermia. If this were the case, further studies using lower doses of ethanol or more extended pre-exposure to the flavor should demonstrate an effect of altered T_a.

The importance of parameters such as ethanol dose is underscored by the findings of Cunningham et al. (1988, 1992). Specifically, attenuation of taste aversion with a warm T_a was seen only with 1.8 g/kg ethanol, and

facilitation of taste aversion with exposure to a low T_a with 1.2 and 1.5 g/kg. This suggests that an effect of altered T_a may be seen in mice if a range of ethanol doses is used, especially doses lower than those used here.

PLACE CONDITIONING

Experiment 4

The place conditioning experiment was designed as a further test of the hypothesis that exposure to a warm T_a during conditioning would decrease the aversive motivational effects of ethanol (thereby increasing its positive effects) in genetically heterogeneous mice. A distinctive floor texture was paired with ethanol injection while a different floor type was paired with saline injection in three groups of mice. Each group underwent conditioning at a different T_a, chosen to prevent, enhance, or have no effect on ethanol-hypothermia. To determine the effects of T_a on non-drug testing, tests were conducted at a normal and altered T_a.

Method

The place conditioning experiment was conducted as four separate, identical experiments because of the limited number of mice available at one time. Inasmuch as all experimental procedures were identical, the four experiments were treated as one, with data combined for analysis. In successive experiments, attempts were made to keep group sizes as equal as possible. Unequal group sizes reflect unavoidable subject attrition.

Subjects

Subjects were 202 naive male HS mice, housed in groups of two to four as described in General Methods. The mean weight at the beginning of the experiment was 27.5 g (range 20.7 - 35.9 g).

Apparatus

The place conditioning apparatus consisted of 12 identical acrylic and aluminum boxes (30 x 15 x 15 cm). Six sets of infrared light sources and photodetectors were mounted opposite each other at 5-cm intervals on the sides of each box, 2.2 cm above the floor. Occlusion of the infrared beams was used to measure locomotor activity and to determine the animal's position (left vs right) in the box. Total activity counts and amount of time spent on each side of the box were recorded each minute by an Apple II computer (10 msec resolution). The floor of each box consisted of interchangeable halves of one of two distinct textures, 'grid' and 'hole'. Grid floors were 3.18-mm stainless-steel rods mounted 6.4-mm apart in acrylic rails, and hole floors were perforated 16-gauge stainless steel with 6.4-mm round holes on 9.5-mm staggered centers mounted on acrylic rails. The floors and the inside of the boxes were wiped with a damp sponge and the litter paper beneath the floors changed after each animal.

Four place conditioning boxes were positioned on telemetry receivers (previously described) and enclosed in each of the three refrigerated incubators. Ambient temperatures in the incubators were the same as in all

taste conditioning experiments, (i.e., 34° C, 21° C, and 10° C).

Procedure

Animals were implanted with Mini-Mitters as described above and were given 4 to 5 days of recovery prior to experimentation. Subjects were assigned to one of the three T_a groups with the restriction that all animals in any given cage belonged to the same group. The experiment consisted of three phases: one habituation session, eight conditioning sessions, and a test session, conducted on each of 10 consecutive days. Each day, animals were weighed in the colony room, and all cages were arranged on a small stainless-steel cart for transport to Room N. Subjects were allowed to sit in Room N for at least 1 h before each session to allow body temperature to return to normal before trials were started.

For place conditioning, mice were randomly assigned to either the Warm (W), Normal (N) or Cold (C) T_a treatment groups (n = 58, 87, and 57, Group W, N, and C, respectively). Within each of these experimental groups, mice were randomly assigned to one of two conditioning subgroups (n = 28-30/conditioning group in Groups W and C, and 41-45/conditioning group in Group N) and exposed to a Pavlovian differential conditioning procedure following a one-day habituation session. During the habituation session, animals received an IP injection of saline (12.6 ml/kg) before being placed in the conditioning box on a smooth, paper covered floor (the telemetry receiver). The habituation session was intended to reduce the stress and

novelty of the handling and injection procedure. On all conditioning trials, both halves of the floor were homogenous (either grid or hole), and mice had access to both sides of the apparatus. On alternate days, mice in the Grid+ subgroup received a 2.25 g/kg IP injection of ethanol (20% v/v in physiological saline, 12.6 ml/kg) prior to placement on the grid floor (CS+ days) and saline injection before placement on the hole floor (CS- days). Conversely, mice in the Grid- subgroup received saline paired with the grid floor and ethanol treatment prior to placement on the hole floor. The conditioning subgroups within each experimental group were matched for overall exposure to floor type and drug. The order of exposure to ethanol and saline was counterbalanced within groups and all subjects received four complete conditioning trials, each of which consisted of one CS+ day and one CS- day.

The preference test took place 24 h after the final conditioning session. All subjects received a saline injection immediately prior to placement in the apparatus for a 60 min session. Saline injections were given so that handling and injection cues and concomitant stress effects would be equivalent on conditioning and test days. The floor consisted of both grid and hole textures with left/right position counterbalanced within groups.

To examine the effects of the T_a cue on test performance, conditioning groups were subdivided and tested either at conditioning T_a or a different T_a with equal numbers of Grid+ and Grid- subjects tested at each T_a.

Conditioning and test conditions for each group are given in Table 8. On the test, Group W mice were tested at 34° or 21° C, and Group C mice were tested at 10° or 21° C. Group N was subdivided into three groups with one subgroup tested at each of the three temperatures.

This complicated testing regimen was used because of recent data suggesting that test temperature can affect expression of ethanol-induced place aversion. In recent place conditioning studies with rats, Cunningham and Niehus (1993) demonstrated that rats conditioned and tested in a warm T_a display less place aversion than those conditioned in a warm ambient but tested at normal ambient. This finding is possibly due to an increase in activity caused by the warm T_a on the test day (Cunningham & Niehus, 1993). An increase in general activity could negatively affect the expression of place conditioning in that the animal may be scurrying around too much to pay attention to tactile cues.

In addition to possible effects on activity, changing the T_a during testing could influence the conditioned response by altering the temperature cues. During conditioning, these cues may have become part of a complex CS along with the tactile floor stimulus and other environmental cues. Altering the environmental CS during the test could result in an incomplete expression of the CR, i.e., the conditioned preference (cf. Balsam, 1985; Pearce, 1987).

Table 8

Ambient Temperature Conditions for Each T_a Group During Conditioning and Testing

	Cond	Test
Group W	W	W
	W	N
Group N	N	W
	N	N
	N	C
Group C	C	C
	C	N

Results

Four animals were euthanized because of illness during conditioning trials (two each from Groups N and C). Two Group W animals were given wrong injections and data were excluded from C4 and preference tests. Data from two Group N mice were excluded from preference tests because of experimenter error. Resulting group sizes were 56, 83, and 55 for Groups W, N, and C, respectively, on the preference test. Activity and body temperature data were collapsed over sample periods for analysis.

Body temperature data were analyzed as in the taste conditioning experiments, i.e., only data from subjects with reliable data on both the first and last conditioning trials were used. As in the taste conditioning studies, analysis was conducted in this manner to assess the initial hypothermic response and any change in this response with repeated exposures. Analysis of activity data during conditioning trials included Trials as a within-group factor; thus, only data from animals contributing to all trials are included. Group sizes are included in figure captions.

Conditioning Trials

Activity data. The time course of activity on the first conditioning trial for all T_a groups is presented in Figure 7. In general, activity was highest at the beginning of the trial and decreased as the trial progressed. Figure 8 shows the mean activity for each T_a group on the first and last conditioning trials.

Figure 7. Mean minute by minute activity (± SEM) for the warm (W), normal (N) and cold (C) T_a groups on CS+ (ethanol) and CS- (saline) days of the first conditioning trial. Data are collapsed over conditioning group. Ethanol dose was 2.25 g/kg. Group sizes are 56, 85, and 55 for Groups W, N, and C, respective

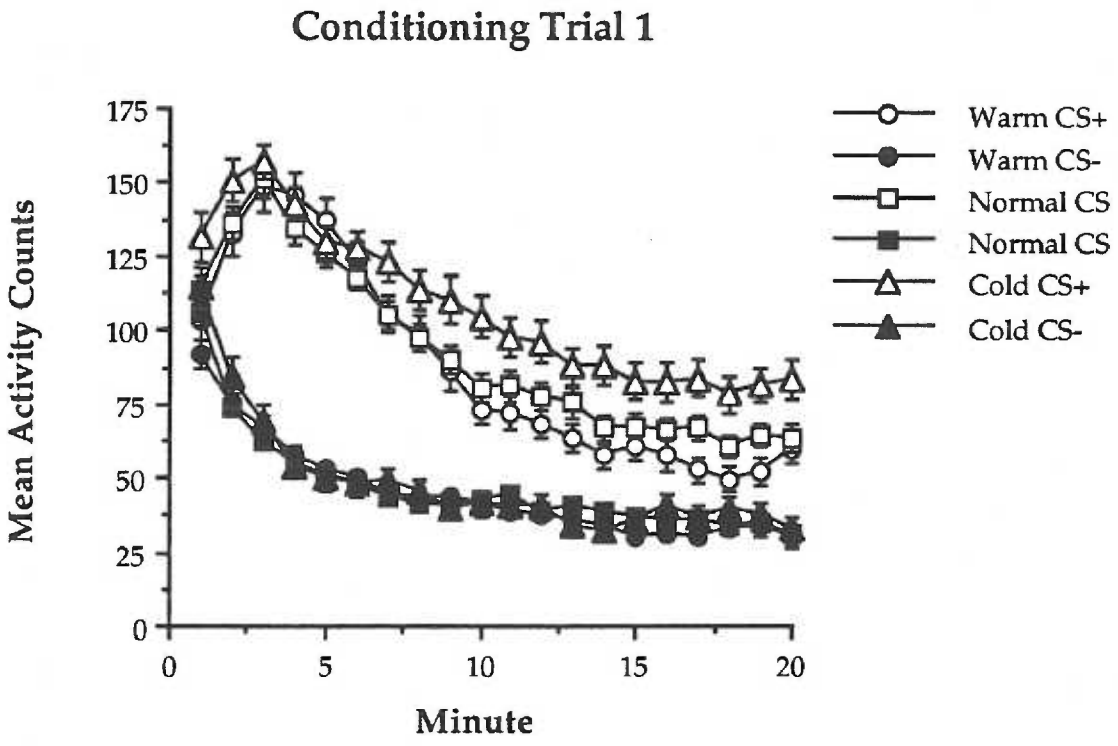
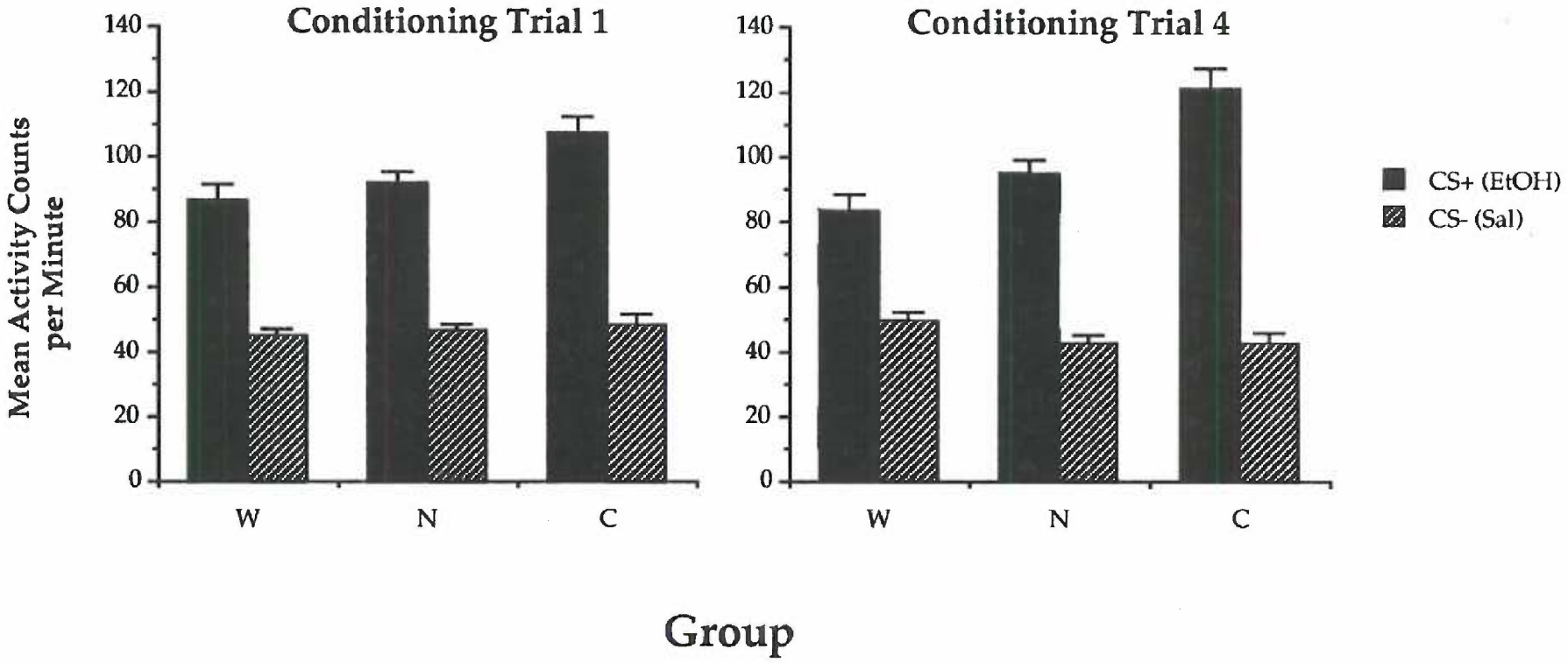

Conditioning Trial 1
Mean Activity Counts
175
150
125
100
75
50
25
0
0
5
10
15
20
Minute
Warm CS+
Warm CS-
Normal CS
Normal CS
Cold CS+
Cold CS-

Figure 8. Mean activity per minute (± SEM) on CS+ (ethanol) and CS- (saline) days of the first and last conditioning trials for the warm (W), normal (N) and cold (C) T_a groups. Data are collapsed over conditioning groups. Ethanol dose was 2.25 g/kg. Group sizes are 56, 85, and 55 for Groups W, N, and C, respectively.


Conditioning Trial 1
Conditioning Trial 4
Mean Activity Counts per Minute
140
120
100
80
60
40
20
0
W
N
C
CS+ (EtOH)
CS- (Sal)
Group

In general, activity on CS+ days was higher than on CS-days, indicating that ethanol increased locomotor activity. Additionally, activity under ethanol was highest in Group C on both trials. Activity in this group was higher on C4 than C1, suggesting that these animals became sensitized to ethanol's activating effects.

Activity data from all four conditioning trials were analyzed by a three-way ANOVA (T_a Group x Drug x Trials) which revealed a significant three-way interaction, $F(6,579) = 4.91$, $p < .001$, as well as a significant T_a Group x Drug interaction, $F(2,193) = 19.81$, $p < .001$, and a significant Drug x Trials interaction, $F(3,579) = 4.28$, $p = .006$. In addition, this analysis yielded significant main effects of T_a Group, $F(2,193) = 6.55$, $p = .002$, Drug, $F(1,193) = 570.80$, $p < .001$, and Trials, $F(3,579) = 5.55$, $p = .001$.

To further explore the three-way interaction, two-way ANOVAs (T_a Group x Trials) were applied separately to the data from CS+ days (ethanol) and to data from CS- days (saline). Analysis of data from CS+ days revealed a significant T_a Group x Trials interaction, $F(6,579) = 2.84$, $p < .001$, and significant main effects of T_a Group, $F(2,193) = 13.87$, $p < .001$, and of Trials, $F(3,579) = 5.88$, $p < .001$. Follow-up one-way ANOVAs revealed a significant main effect of T_a Group on each of the four trials: C1, $F(2,193) = 5.88$, $p = .004$; C2, $F(2,193) = 9.16$, $p < .001$; C3, $F(2,193) = 14.68$, $p < .001$; C4, $F(2,193) = 13.49$, $p < .001$. Pairwise comparisons on CS+ days showed that activity in Group C was higher than in Groups N and W on C1, C3 and C4, and higher than Group W

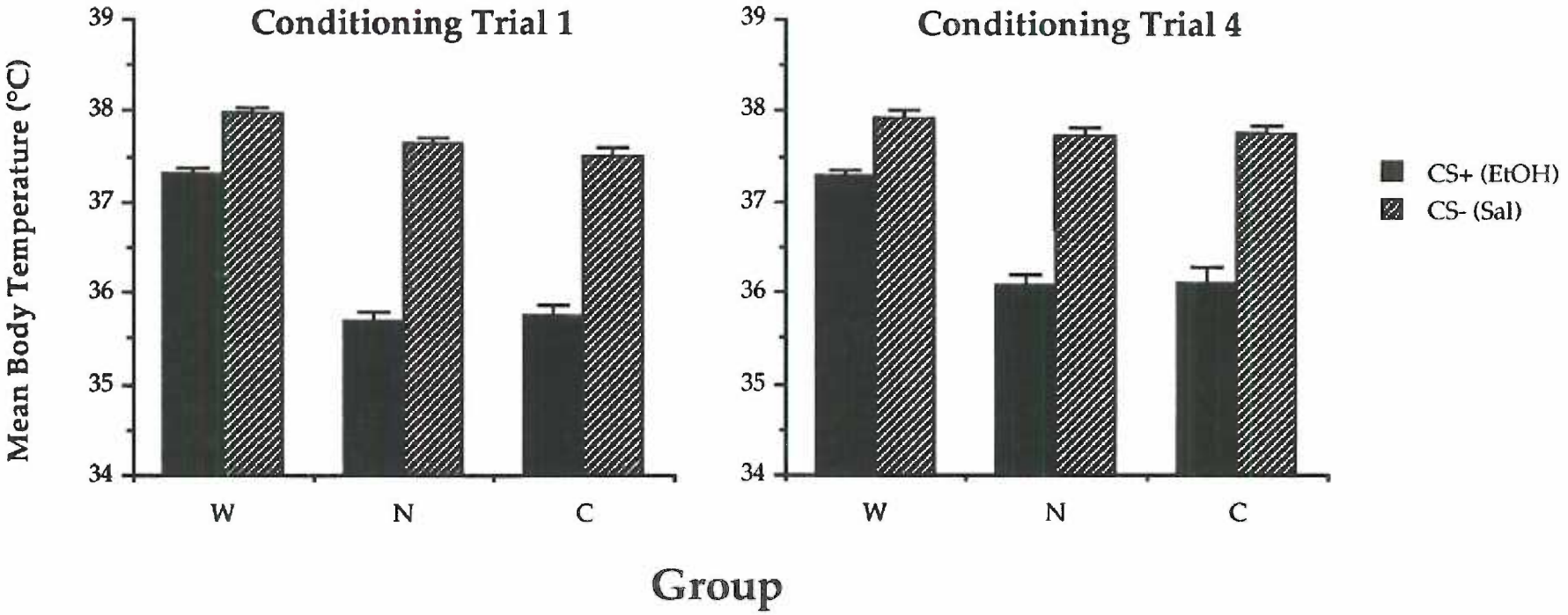
on C2 (all *ps*<.05, Tukey's HSD). Groups N and W did not differ from each other.

Follow-up one-way ANOVAs on each T_a Group revealed a significant effect of Trials only in Group C, $F(3,579) = 10.9$, $p < .001$, with higher activity on C4 than on C1 indicating the development of sensitization to the activating effects of ethanol.

The two-way follow-up ANOVA (T_a Group x Trials) applied to the data from CS- days also yielded a significant interaction, $F(6,579) = 3.27$, $p = .004$. However, analysis of each trial revealed no significant effects of T_a Group (all *Fs*<1.9). Additional follow-up analysis (one-way ANOVAs) of the interaction revealed an effect of Trials in Group W, $F(3,579) = 3.47$, $p = .016$, and in Group C, $F(3, 579) = 3.34$, $p = .019$, but not in Group N. Saline activity in Group W increased slightly over trials while that in Group C decreased slightly over trials, resulting in the significant interaction.

Body temperature data. Figure 9 shows the mean body temperature on CS+ and CS- days for each T_a group on the first and last conditioning trials (C1 and C4). Mean body temperatures for all subjects on all trials are presented in Appendix B. As in the taste conditioning experiments, exposure to 34° C reduced the magnitude of ethanol-induced hypothermia. Additionally, tolerance developed to the thermal effects of ethanol. However, differential tolerance between T_a Groups was not demonstrated.

Figure 9. Mean body temperature (°C ± SEM) on CS+ (ethanol) and CS- (saline) days on conditioning trials C1 and C4 for the warm (W), normal (N) and cold (C) T_a groups. Ethanol dose was 2.25 g/kg. Data are collapsed over conditioning group. Group sizes are 53, 81, and 47 for Groups W, N, and C, respectively.


Conditioning Trial 1
Conditioning Trial 4
Mean Body Temperature (°C)
39
38
37
36
35
34
W
N
C
Group
CS+ (EtOH)
CS- (Sal)

A three-way ANOVA (T_a Group x Drug x Trials) applied to temperature data from the first and last conditioning trials revealed a significant T_a Group x Drug interaction, $F(2,178) = 57.35$, $p < .001$, as well as significant interactions between T_a Group and Trials, $F(2,178) = 6.36$, $p = .003$, and between Drug and Trials, $F(1,178) = 4.87$, $p = .027$. As expected based on taste conditioning experiments, the overall ANOVA also yielded main effects of T_a Group, $F(2,178) = 62.94$, $p < .001$, Drug, $F(1,178) = 801.35$, $p < .001$, and Trials, $F(1,178) = 15.9$, $p < .001$.

As in the taste conditioning experiments, exposure to the warm T_a diminished ethanol-induced hypothermia as evidenced by the T_a Group x Drug interaction. This interaction was caused by the presence of a greater drug effect in Groups N and C than in Group W, i.e., hypothermia was greater in Groups N and C.

To follow-up the T_a Group x Trials interaction, data were collapsed across drug condition, and one-way ANOVAs (Trials) were applied to data from each T_a Group. A main effect of Trials was seen in Group N, $F(1,178) = 16.03$, $p < .001$ and in Group C, $F(1,178) = 13.87$, $p < .001$, but not in Group W ($F < 1$). In both groups, temperatures increased across trials.

The Drug x Trials interaction suggests the development of tolerance or sensitization. To explore this, data were collapsed across T_a Group and one-way ANOVAs (Trials) were run separately on data from CS+ days (ethanol) and CS- days (saline). A significant effect of Trials was found only for the

ethanol data, $F(1,178) = 16.40$, $p < .001$, not for the saline data ($F < 3.5$). Body temperatures on CS+ days increased from 36.3° on C1 to 36.5° C on C4, indicating tolerance to the hypothermic effects of ethanol. However, the lack of a significant three-way interaction indicates that the T_a Groups did not differ in the development of tolerance.

Preference Test

All data from the preference test, i.e., preference data, activity and body temperature data, were initially analyzed with time (first 30 min vs last 30 min) as a within-group factor. No effects of time were seen in the preference data. The effects of time seen in the activity and temperature data were sporadic and did not shed any light on the outcome of the preference test. Accordingly, data were collapsed over the 60 min test and are presented as such.

Preference data. For analysis of preference test data, planned comparisons between Grid+ and Grid- conditioning groups were made for each combination of T_a group and test temperature, i.e., Groups NN, NW, NC, WW, WN, CC, and CN. Each planned comparison consisted of a one-way ANOVA with Conditioning Group (Grid+ vs Grid-) as the between-group factor. The dependent variable was the number of seconds per minute spent on the grid floor. As stated above, no effects of time were found and data were collapsed over the full 60 min.

Place preference data from the three T_a groups are depicted in Figure 10. Data are given as seconds per minute spent on the grid floor by the conditioning subgroups during the test. The development of conditioned place preference is indicated by animals who had the grid floor paired with ethanol (Grid+) spending more time on the grid floor than those who had the grid floor paired with saline (Grid-) during conditioning. The magnitude of preference is determined by the difference between Grid+ and Grid- subgroups. Only animals conditioned and tested at the normal T_a (Group NN) showed a significant place preference. Exposure to altered T_a during either conditioning or testing attenuated the conditioned preference.

Application of a one-way ANOVA (Conditioning Group) to preference data from subjects conditioned and tested at 21° C (Group NN) revealed a significant main effect of Conditioning Group, $F(1,26) = 10.70$, $p = .003$. No significant effects were seen in any other analyses. Table 9 contains the results of all planned comparisons on the preference test.

Activity data. To analyze activity data from the preference test, T_a groups were divided into two overlapping subsets, and the data were analyzed using a two-way ANOVA (T_a Group x Test T_a). The first subset included data from animals conditioned and/or tested at 34° and 21° C, i.e. Groups WW, WN, NW and NN. The second subset included data from subjects conditioned and/or tested at 10° or 21° C, i.e. Groups CC, CN, NC and NN.

Figure 10. Mean seconds per minute (± SEM) spent on the grid floor by subjects in each conditioning subgroup of the warm (W), normal (N) and cold (C) T_a groups. Grid+ animals had the grid floor paired with ethanol (and the hole floor paired with saline) during conditioning and Grid- animals had the hole floor paired with ethanol (and the grid floor with saline) during conditioning. The first letter of each group name indicates conditioning temperature. The second letter of each group name designates test T_a. Numbers embedded in each bar indicate group size. For statistical analysis see Table 9.

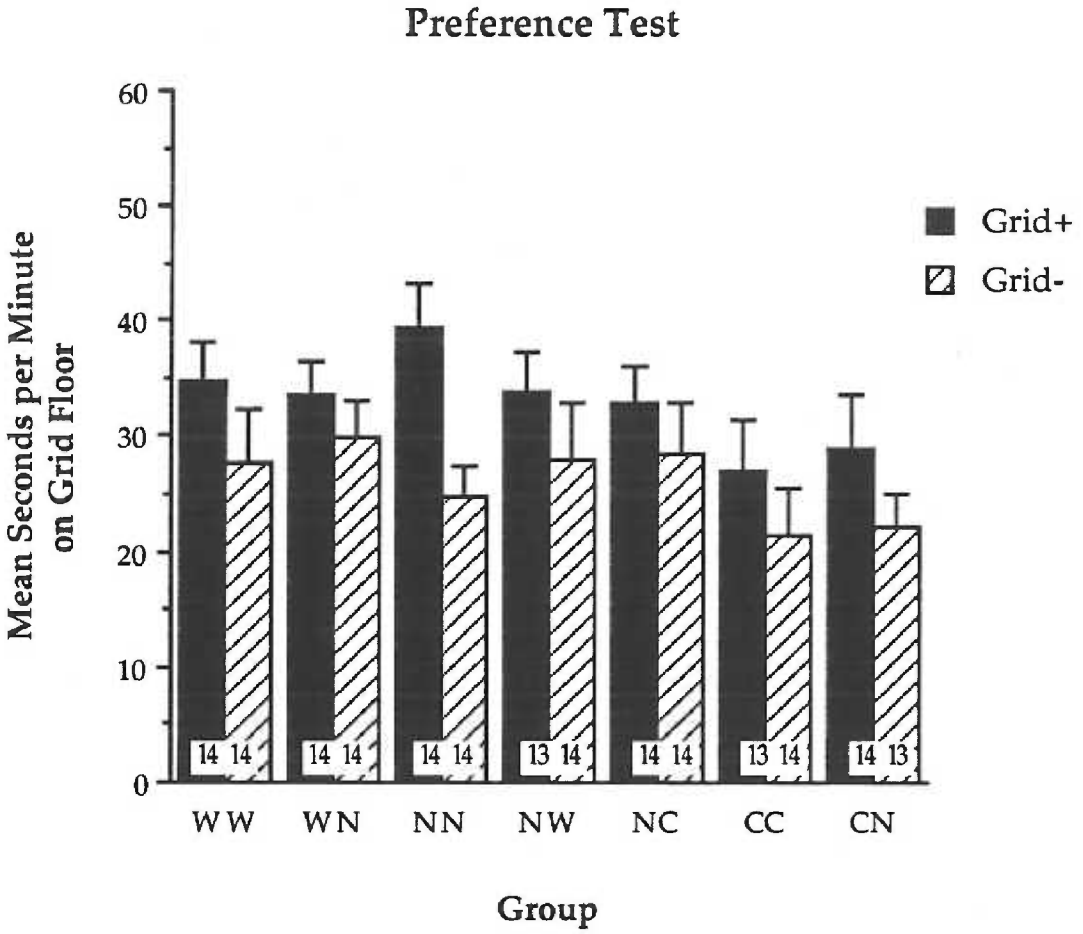

Preference Test
Mean Seconds per Minute on Grid Floor
60
50
40
30
20
10
0
Grid+
Grid-
14 14
14 14
14 14
13 14
14 14
13 14
14 13
WW
WN
NN
NW
NC
CC
CN
Group

Table 9

Results of Planned Comparisons Between Grid+ and Grid- Conditioning Groups For Each Ta Group at Each Test Ta.

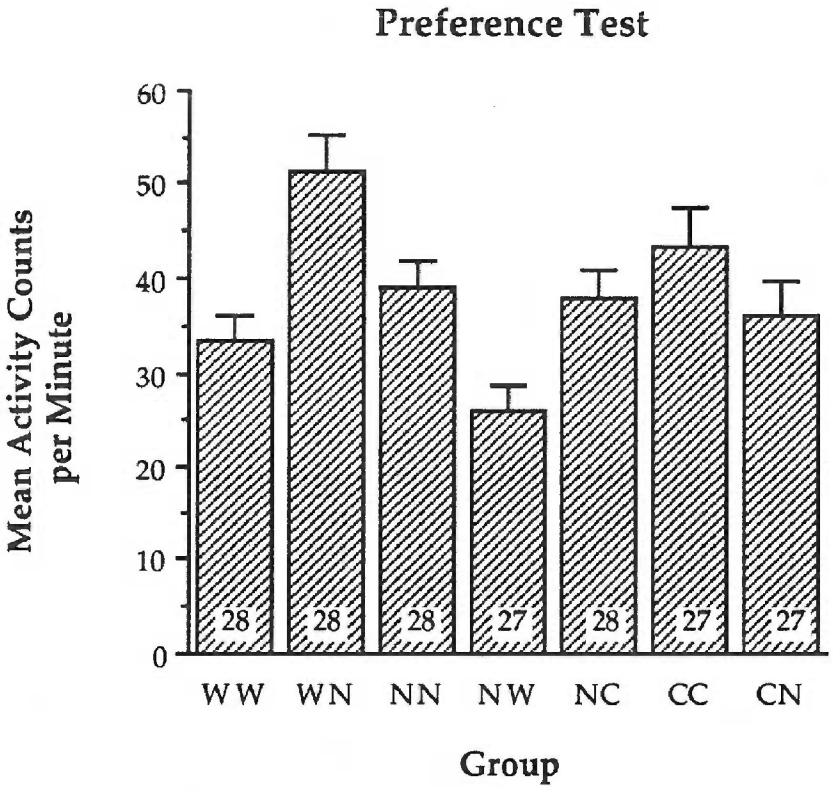
Group	df	*F*	*p*
WW	1,26	1.47	.237
WN	1,26	0.79	.381
NN	1,26	10.70	.003
NW	1,25	0.96	.336
NC	1,26	0.63	.435
CC	1,25	0.35	.558
CN	1,25	1.28	.269

Figure 11 depicts mean locomotor activity in the preference test for all groups. In general, for Groups WW, WN, NW and NN, testing at 34° C suppressed activity, but conditioning at 34° C resulted in higher activity on the test. No effects of testing or conditioning at 10° C were apparent. A two-way ANOVA (T_a Group x Test T_a) applied to the data from Groups WW, WN, NW and NN yielded significant main effects of T_a Group, $F(1,107) = 10.53$, $p = .002$ and Test T_a, $F(1,107) = 26.14$, $p < .001$, but no significant interaction.

The mean activity per minute of subjects conditioned at 34° C (Group W) was 42.4, compared to 32.5 for animals in Group N, regardless of T_a on the test, supporting the observation that exposure to the warm T_a during conditioning led to increased activity on the test. However, when collapsed across conditioning temperature, testing at the warm ambient decreased activity relative to animals tested at 21° C, as indicated by a mean activity of 29.7 counts per minute for Groups WW and NW and a mean of 45.2 counts per minute for subjects in Groups WN and NN.

A two-way ANOVA (T_a Group x Test T_a) applied to the data from Groups CC, CN, NC and NN revealed no significant effects, indicating that while the low T_a increased activity during conditioning it had no effect during the drug-free preference test.

Figure 11. Mean activity per minute (± SEM) during the preference test for subjects in Groups WW, WN, NN, NW, NC, CC, and CN. Data are collapsed across conditioning group. Group sizes are indicated by the number embedded in each set of bars.


Preference Test
Mean Activity Counts per Minute
60
50
40
30
20
10
0
28
28
28
27
28
27
27
WW
WN
NN
NW
NC
CC
CN
Group

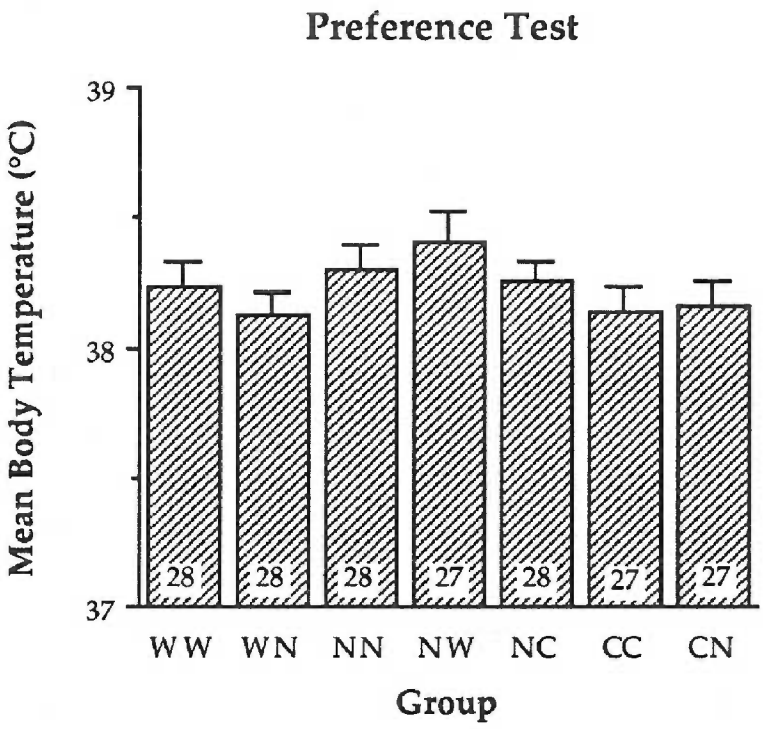
Body temperature data. Body temperature data from the preference test were analyzed in the same manner as activity data, i.e., T_a Groups were divided into two overlapping subsets and the data were analyzed using a two-way ANOVA (T_a Group x Test T_a). The first subset included data from animals conditioned and/or tested at 34° and 21° C, i.e. Groups WW, WN, NW and NN. The second subset included data from subjects conditioned and/or tested at 10° or 21° C, i.e. Groups CC, CN, NC and NN.

Temperature data from the preference test for Groups WW, WN, NW, NN, NC, CC and CN are plotted in Figure 12. Two-way ANOVAs (T_a Group x Test T_a) applied to each data set revealed no significant effects. This indicates that in the absence of ethanol, alterations in T_a on the test day had no effect on body temperature.

Discussion - Place Conditioning

During conditioning trials, ethanol produced a marked hypothermia which was attenuated by exposure to the warm T_a. Tolerance to ethanol-induced hypothermia was seen, but it did not develop differentially in the three T_a Groups. An increase in locomotor activity was also induced by ethanol during conditioning, with animals exposed to the cold T_a showing higher activity than those at the normal or warm T_a. In addition, sensitization to ethanol's activating effect occurred, but only in Group C. No effects of the low T_a were seen during the preference test.

Figure 12. Mean body temperature (°C ± SEM) during the preference test for Groups WW, WN, NW, NN, NC, CC, and CN. Data are collapsed across conditioning group. Numbers embedded in each bar indicate group size.


Preference Test
Mean Body Temperature (°C)
39
38
37
28
28
28
27
28
27
27
WW
WN
NN
NW
NC
CC
CN
Group

Interestingly, the warm T_a had no effect on activity during conditioning but tended to decrease activity on the preference test. Finally, subjects in Group W showed higher activity levels during the preference test than mice in Group N, regardless of test T_a.

Alteration of T_a affected conditioned place preference, but this effect did not appear to be caused by manipulation of ethanol-induced hypothermia. Specifically, hypothermia occurred only in mice conditioned at the cold T_a, yet neither these animals nor those conditioned at the warm T_a developed a conditioned place preference. In other words, a differential experience of hypothermia did not translate into differential expression of place preference. Interestingly, while mice conditioned at a normal T_a demonstrated a conditioned preference for tactile cues paired with ethanol, this preference was attenuated if the choice test was conducted at an altered T_a, (i.e., either 34° or 21° C).

Although the thermal effects of ethanol do not seem to explain the present findings, other ethanol effects, such as its locomotor activating effect, must be considered. In an amphetamine place conditioning study with rats, Swerdlow and Koob (1984) demonstrated that the development of place preference did not occur if animals were restrained during conditioning. The authors suggested that perhaps the positive properties of amphetamine are derived from the increase in activity it produces, which is consistent with the psychomotor stimulant theory of addiction (Wise & Bozarth, 1987).

However, Swerdlow and Koob (1984) couched this argument in terms of familiarity and postulated that restricted animals could not gain the increased familiarity with the drug-paired environment necessary for the development of preference. This analysis implies that a more familiar/less novel environment, such as one more thoroughly explored because of higher levels of activity, will be preferred. Application of this argument to the present data suggests that high activity levels on CS+ trials during conditioning, such as that produced by ethanol injection, could lead to an increased familiarity with the CS+ stimulus. This increased familiarity could then be translated to a greater preference for the CS+ cue on a choice test. However, while mice in Group C showed greater activity levels during conditioning than mice in Groups N and W (and should, therefore, show more preference), they showed less preference for the ethanol-paired cue than mice in Group N. Additionally, a lack of place preference was seen in both Groups C and W, although they did not experience equal levels of activity during conditioning. Thus, an explanation based on increased familiarity caused by increased activity does not seem to be applicable to the present results.

It has also been suggested (Carr, Phillips, & Fibiger, 1988; Parker, 1992) that animals prefer a somewhat novel stimulus to a more familiar stimulus in the CPP paradigm. Carr, Phillips, and Fibiger (1988) demonstrated that restraining animals results in preservation of environmental novelty which can in itself produce a place preference. The authors suggested that by

restraining animals in both the CS+ and CS- environments, Swerdlow and Koob (1984) effectively maintained the novelty of both stimuli, leading to a lack of preference for either compartment. According to this argument, i.e., that relative novelty is preferred, mice in Group C should not show a preference for the CS+ floor because increased activity during conditioning would decrease novelty. While this argument works to explain the results of Group C, it does not account for the attenuated place preference seen in Group W since activity in these animals during conditioning did not differ from subjects conditioned at a normal T_a.

The finding that mice in Group C sensitized to the locomotor activating effects of ethanol during conditioning trials is consistent with the finding of Cunningham et al. (1991) that COLD mice sensitized to this effect as well. However, in the present study, mice in Group C had higher ethanol activity than both other groups from the outset, while COLD mice were less active than HOT mice on the first conditioning trial (Cunningham et al., 1991). These results suggest that perhaps the experience of augmented hypothermia is important for the development of sensitization to the activating effects of ethanol in these mice. Greater levels of ethanol-induced activity may occur under these conditions because the mice are trying to offset hypothermia by generating heat.

The level of activity during preference testing may also have an impact on place preference (e.g., Vezina & Stewart, 1987; Cunningham & Niehus,

1993). For example, a general increase in activity may interfere with the expression of preference. That testing at the low T_a in the present study resulted in an increase in activity could explain the lack of place preference shown in subjects tested at 10° C, i.e., Groups NC and CC. Additionally, testing at 34° C resulted in a decrease in activity in mice from Groups WW and NW, neither of which showed place preference. It could be argued that perhaps ataxia produced by the warm T_a on the test interfered with the motor responses necessary for the expression of place preference in these groups of mice. However, Groups NC, CC, NW and WW were not the only groups that failed to demonstrate a place preference. Accordingly, activity differences during the preference test cannot fully explain the results obtained in the current study.

The present pattern of results may best be explained using two conditioning phenomena, associative overshadowing (to describe the differences between the three T_a groups) and generalization decrement (to explain the findings within Group N). In overshadowing, one element of a compound CS is strongly associated with the US to the extent that conditioning to other elements of the CS does not occur or is greatly attenuated (Mackintosh, 1976). In the case at hand, the cue of altered T_a (both high and low) may have been a very salient cue that overshadowed the tactile floor CS and prevented formation of an association between the floor cue and the effects of ethanol. In applying this explanation to the current data, it must

be assumed that manipulation of T_a in either direction during conditioning is a salient cue capable of overshadowing the tactile CS. Accordingly, only subjects exposed to normal temperature during conditioning should develop the CS-US association, i.e., subjects in Group N. However, a lack of place preference was seen in animals conditioned at a normal T_a and subsequently tested at an altered T_a, i.e., Groups NW and NC.

Changes in contextual cues have been shown to have an impact on conditioned responses, including effects on performance because of context controlled responses that are incompatible with the CR. In addition, changes in contextual cues may detrimentally affect retrieval of associations made in a different context (cf. Balsam, 1985; Riccio, Richardson, & Ebner, 1984). Decreased performance in the presence of altered contextual cues, i.e., an altered compound CS which includes context, may be considered generalization decrement in that the subject does not generalize the CR to the 'new' conditioned stimulus.

A generalization decrement analysis can be used to explain the preference data from Group N; subjects in Groups NW and NC did not show a place preference because they failed to generalize the preference response in the presence of the 'new' T_a cue, while subjects in Group NN were not exposed to a different T_a cue during testing. According to a generalization decrement explanation, place preference would be expected in mice from Groups WW and CC but not in Groups WN or CN. However, as stated

earlier, the lack of place preference in any subjects in Groups W and C may be attributed to associative overshadowing. Therefore, to explain the present pattern of results fully, both overshadowing and generalization decrement are necessary; overshadowing to explain data from Groups W and C, and generalization decrement to explain the results from Groups NW and NC.

An alternative interpretation of the present findings could be that alterations in T_a directly affect learning and memory processes. However, Cunningham and Niehus (1993) found place aversion in rats when conditioning occurred at a low T_a, i.e., learning occurred in an altered temperature. Additionally, Green, Hart, and Hagen (1981) demonstrated that a place aversion could be learned under conditions of toxic heat, indicating that learning was not impaired in a warm T_a. These data suggest that the present findings are not caused by a general impairment of learning by altered temperatures.

Another possible explanation for the lack of place conditioning seen in subjects conditioned at either the low or high T_a is that these altered temperatures affected ethanol metabolism. Previous studies of altered T_a on ethanol metabolism (e.g., Alkana, Boone, & Finn, 1985; Cunningham et al., 1992; Pohorecky & Rizek, 1981) have shown little or no effect of altered T_a. However, an increase in ethanol metabolism with increasing body temperature has been reported (Bejanian, Syapin, Finn, Jamieson, Jones, & Alkana, 1987; Romm & Collins, 1987). While an increase in metabolism in

Group W could have resulted in weaker place preference, both altered temperatures caused a decrement in place preference, forcing the conclusion that both warm and cold temperatures must have had the same effect on metabolism if this explanation is to be applicable. The most parsimonious explanation for the present outcome is one combining associative overshadowing and generalization decrement.

SUMMARY AND CONCLUSIONS

Overall, the results from the taste and place conditioning studies do not support the hypothesis that ethanol-induced hypothermia plays a role in mediating its aversive motivational effects in genetically heterogeneous mice. This hypothesis predicted a decrease in ethanol-induced conditioned taste aversion in mice exposed to a warm T_a and an enhancement of the aversion in mice exposed to a low T_a. To the extent that a decrease in the aversive hedonic effects of ethanol results in an increase in its positive hedonic effects, the hypothesis also predicted greater ethanol-induced conditioned place preference in Group W mice and attenuated preference in mice conditioned at a low T_a.

In general, pairing ethanol injection with distinctive flavors (NaCl and saccharin) resulted in conditioned taste aversions which were not affected by alterations of T_a. It is possible that by decreasing the magnitude of the taste aversion in future studies, an effect of ethanol hypothermia may be revealed. While altered T_a did have an effect on conditioned place

preference, both the high and low temperatures eliminated the conditioned preference. The results of the place conditioning study may best be described as an instance of overshadowing combined with generalization decrement. If this is so, considerable pre-exposure to the T_a cues prior to conditioning might eliminate contextual overshadowing, perhaps revealing a relationship between the thermal and motivational effects of ethanol (Lubow, 1973). Generalization decrement could be eliminated only by conducting the preference test at the conditioning T_a. However, testing at altered T_a can have potential effects on activity. Specifically, testing at the low T_a may increase locomotor activity to the extent that expression of any place preference is disrupted. Exposure to a high T_a may produce slight ataxia that could also interfere with the expression of place conditioning. Accordingly, eliminating the source of generalization decrement introduces additional problems.

Not only do the present results fail to lend further support to the temperature hypothesis, they do not help clarify the discrepancy between previous studies with rats vs those using HOT and COLD mice. Genetically heterogeneous mice did not demonstrate the same pattern of findings as either of these groups, indicating that neither species differences nor procedural differences are solely responsible for the observed discrepancy.

Although the present findings do not support the temperature hypothesis, they do suggest an alternative interpretation of previous findings. Specifically, associative overshadowing may be responsible for the

attenuation of ethanol-induced place aversion by exposure to a warm T_a found by Cunningham et al. (1992), i.e., the warm T_a may have acted as a salient stimulus that overshadowed the tactile CS and was associated with the ethanol US. Based on the present findings, the low T_a would also be expected to overshadow the floor cue and attenuate place conditioning in rats. For one component of a CS (e.g., the cold T_a) to overshadow another component (e.g., the tactile floor stimulus), the first component must be more salient than the second (Mackintosh, 1976). The fact that the cold T_a overshadowed that floor stimulus in mice indicates that cold is a salient stimulus to mice. The failure of the low T_a to do so in rats suggests that cold is not a significantly salient stimulus to rats. Given the difference in body mass between mice and rats, it may indeed be true that cold temperatures are quite salient to mice, and therefore have the ability to overshadow other stimuli.

The temperature hypothesis predicts enhancement of ethanol-induced place aversion in rats with exposure to a low T_a. However, enhancement of place aversion was not seen in Cunningham et al. (1992), nor has it been demonstrated in recent studies designed to explore this prediction (Cunningham & Gibson, unpublished data). These data, in conjunction with the alternative interpretation of overshadowing, suggest that the temperature hypothesis may not hold true for place conditioning.

Results from taste conditioning studies in rats are consistent with the temperature hypothesis in that taste aversion was attenuated with exposure

to a warm T_a and potentiated with exposure to a low T_a (Cunningham et al., 1988; 1992; Cunningham & Niehus, 1989). However, findings with HOT and COLD mice (Cunningham et al., 1991) are opposite those predicted by the temperature hypothesis, and the present findings with genetically heterogeneous mice neither support nor oppose the hypothesis. As discussed above, further taste conditioning studies appear necessary to either substantiate or refute the suggestion that ethanol's aversive effects are related to its thermic effects.

Although place conditioning data from HOT and COLD mice are consistent with the temperature hypothesis, taste conditioning data are not (Cunningham et al., 1991), i.e., HOT mice showed stronger place preference as well as stronger taste aversion. This pattern of results could be caused by mechanisms other than ethanol's thermal effects. For example, it may be that HOT mice perform better on conditioning tasks than COLD mice.

In summary, the current experiments were designed to test the hypothesis that ethanol's motivational effects are related to its thermal effects in genetically heterogeneous mice. Experiments 1 - 3 examined the effects of high and low T_a on ethanol-induced conditioned taste aversion. Experiment 4 looked at the effects of altered T_a on ethanol-induced conditioned place preference. The predictions of the temperature hypothesis were not borne out, suggesting that this hypothesis is not a highly generalizable explanation of the mechanisms underlying the motivational effects of ethanol. However,

the limited nature of the current studies (one type of mouse and only two procedures) preclude a complete dismissal of the hypothesis.

FUTURE DIRECTIONS

As stated above, the present findings suggest that the temperature hypothesis cannot be generalized to genetically heterogeneous mice. In addition, the explanations offered for the pattern of results in place conditioning (overshadowing and generalization decrement) may be used to explain previous findings in place conditioning studies with rats, eliminating the need for the temperature hypothesis. However, the hypothesis remains a valid tool for discussion of taste conditioning results in rats. Accordingly, additional taste conditioning studies in mice should be done with various doses of ethanol. As stated earlier, it is possible that the failure to find an effect of T_a on ethanol-induced taste aversion was simply a matter of inappropriate parameters. Additionally, other paradigms that assess the motivational properties of ethanol, such as oral self-administration, should be used in genetically heterogeneous mice before the temperature hypothesis is ruled out.

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Appendix A. Taste Conditioning.

Mean Body Temperature (°C ± SEM) On Each Conditioning Trial For All Groups

Expt. 1	Group W	Group N	Group C
C1	38.36 ± .111 (10)	34.65 ± .142 (10)	33.33 ± .451 (11)
C2	38.14 ± .257 (10)	35.46 ± .511 (9)	33.54 ± .441 (10)
C3	37.82 ± .120 (11)	35.33 ± .275 (11)	33.40 ± .333 (10)
Expt. 2			
C1	37.83 ± .177 (12)	35.20 ± .302 (11)	35.33 ± .233 (11)
C2	37.97 ± .135 (9)	36.16 ± .384 (10)	35.97 ± .290 (8)
C3 [a]	-----	-----	-----
C4	37.87 ± .219 (10)	36.02 ± .160 (11)	34.85 ± .422 (10)
C5	37.75 ± .186 (9)	36.09 ± .233 (11)	34.70 ± .247 (10)
Expt. 3			
C1	37.93 ± .107 (12)	35.60 ± .263 (12)	35.16 ± .330 (11)
C2	37.81 ± .104 (11)	35.79 ± .258 (12)	35.07 ± .340 (12)
C3	37.76 ± .080 (11)	36.46 ± .158 (8)	35.80 ± .265 (10)
C4	37.81 ± .126 (11)	35.90 ± .265 (10)	35.70 ± .238 (12)
C5	37.61 ± .145 (11)	35.97 ± .312 (10)	35.43 ± .302 (10)

[a] Rectal temperature data were collected on this trial

Appendix B. Place Conditioning.

Mean Body Temperature (°C + SEM) On CS+ (EtOH) and CS- (Saline) Days of Each Conditioning Trial For All Groups

		Group W	Group N	Group C
C1 :	EtOH	37.34 ± .062	35.93 ± .111	35.71 ± .105
	Sal	37.99 ± .066 (54)	37.42 ± .098 (83)	37.53 ± .075 (57)
C2 :	EtOH	37.24 ± .055	35.90 ± .081	36.01 ± .114
	Sal	37.94 ± .055 (57)	37.78 ± .051 (84)	37.85 ± .072 (52)
C3 :	EtOH	37.24 ± .064	35.95 ± .085	36.19 ± .140
	Sal	37.91 ± .060 (57)	37.78 ± .056 (84)	37.76 ± .079 (51)
C4 :	EtOH	37.28 ± .054	36.08 ± .090	36.13 ± .157
	Sal	37.94 ± .058 (56)	37.73 ± .069 (84)	37.78 ± .093 (49)