Associative and Nonassociative Mechanisms in the Development of Tolerance to the Thermic and Cardiovascular Effects of Ethanol

by

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Tolerance (in the drug field) is usually defined as a decrease in the response to a drug due to repeated exposures to it (Goldstein, Aronow & Kalman, 1974). Two mechanisms of drug tolerance discussed by Kalant, LeBlanc and Gibbins (1971) are dispositional (also termed physiological) and functional (also termed pharmacological). Interpretations of tolerance that involve dispositional mechanisms explain reduced responsivity to a drug by appealing to changes in the pharmacokinetic properties of the drug caused by repeated administration (e.g., changes in rates of distribution, metabolism, excretion, or protein binding of the drug). The decreased response is ascribed to a reduction in drug concentration at active drug sites or target tissues. Interpretations of drug tolerance based on functional mechanisms appeal to changes in the pharmacodynamic properties of the site of drug action after repeated drug administration (e.g., decreased affinity of a membrane receptor for a drug or an increase in resistance to nonreceptor-mediated membrane disruption). The reduced drug response is not due to changes in drug concentration at the active site.

There have been reports in the literature substantiating both functional and dispositional interpretations of changes in drug responsivity induced by previous drug administrations. An example of functional tolerance is that cell membranes of animals made tolerant to ethanol (tolerance was operationally defined by the amount of ethanol the animal had received) were found to exhibit an increased resistance to the disruptive or fluidizing effects of ethanol as compared to membranes of nontolerant animals (Chin & Goldstein, 1976; Rottenburg,

Waring & Rubin, 1984; Rubin & Rottenberg, 1982). In addition, Chin and Goldstein (1976) measured the extent of tolerance to a behavioral effect of ethanol, noting that none of the drug-experienced subjects lost their righting reflex at blood ethanol levels that would be hypnotic for drug-naive animals. Melchior and Tabakoff (1981a) provided an example of dispositional tolerance, in that brain levels of ethanol were decreased in animals exhibiting ethanol tolerance. Dispositional tolerance has relatively little effect on the peak intensity of drug action and does not usually result in more than a three-fold decrease in sensitivity (Jaffe, 1981). It has also been observed with narcotic analgesics that differences between tolerant and non-tolerant subjects in absorption, distribution, metabolism or excretion are not usually sufficient to account for differences in analgesia levels or disruption of task performance induced by the narcotic (see Hug, 1972).

Explanations of tolerance that appeal to functional or dispositional mechanisms require repeated drug administration as the only condition necessary for tolerance to develop but this has not been shown to be a sufficient condition in all cases. Recently, associations between environmental cues and concurrent drug states have been found to increase the rate of development and magnitude of drug tolerance, leading to the general conclusion that tolerance may be due, in part, to learning. However, the importance of learned components in tolerance development and their interaction with more traditional determinants of tolerance, such as frequency of dosing, is still unclear. It is possible that learning is the major mechanism by which

tolerance develops under normal dosing schedules. For example, Mansfield and Cunningham (1980) found no evidence of tolerance in animals with previous ethanol exposure (as compared to ethanol-naive animals) when ethanol was injected in an environment where it was unexpected, implying that tolerance does not occur unless drug presentations are signalled. On the other hand, LeBlanc and colleagues (e.g., LeBlanc, Gibbins & Kalant, 1973) consistently found ethanol tolerance to be present when associative factors were supposedly absent, suggesting that conditioning is only one process by which tolerance can develop.

Some nonassociative behavioral states, such as stress, appear to affect drug tolerance and have even been thought by some to account wholely for examples of conditioned tolerance. Studies directly assessing the effects of nonassociative variables on drug tolerance will be discussed in the presentation of Experiment 2.

The different hypotheses concerning the determinants of drug tolerance are not necessarily mutually exclusive but may merely deal with the same phenomena in different terms. Usually, the effects or interactions of associative and nonassociative behavioral mechanisms of tolerance are tested separately from dispositional and functional mechanisms of drug tolerance. This obscures the nature of the relations among these different mechanisms under various conditions. A procedure used in some behavioral studies of tolerance to control for the involvement of dispositional tolerance equates all subjects for drug exposure. An analysis of blood or brain ethanol levels may reveal differences in distribution, absorption or clearance of the drug which

are correlated with the occurrence of tolerance in some animals and not others.

Melchior and Tabakoff (1981a) found that brain levels of ethanol were significantly decreased in animals tested in the presence of cues previously paired with ethanol administration. Blood levels of ethanol after the last injection showed that these animals demonstrated a much larger volume of drug distribution than other animals. They suggested that tolerance to ethanol produced in a conditioning paradigm may be due to altered peripheral distribution of ethanol although they never specifically postulated in what ways the distribution was altered (i.e., where ethanol was distributed after tolerance had developed). These findings have not yet been replicated by others nor have these methods been used to study changes in ethanol distribution ocurring throughout drug administration. Of course, assessment of brain levels requires decapitation, while blood sampling is usually a painful procedure and many experimenters are unwilling to present a painful stimulus contiguous with ethanol intoxication. Interpretations of results from experiments designed to distinguish between associative and nonassociative behavioral effects on tolerance (which are discussed below) may be easily complicated by the presence of such procedures.

Behavioral Tolerance

Factors other than drug dosing schedule and similar pharmacological manipulations have been shown to affect the development of tolerance to a variety of drug classes in a number of response systems. When tolerance has been found to be accelerated by the formation of associations between environmental stimuli and drug

injections (classical conditioning) or by the presence of certain reinforcement schedules during drug intoxication (instrumental conditioning), then it has been described as behavioral tolerance . For example, these kinds of variables have been shown to affect the tolerance which occurs to the analgesic effects of morphine (Sherman, 1979; Siegel, 1975, 1976, 1977, 1978), to the discoordinating (Chen, 1968; LeBlanc, Gibbins & Kalant, 1973) and hypothermic effects (Mansfield & Cunningham, 1980) of ethanol, and to the depressant effects of tetrahydrocannabinol (Carder & Olson, 1973) and amphetamine (Brown, 1965) on food-reinforced barpressing. Some of these earlier studies, however, did not equate experimental and control groups for handling and injection cues or practice of the response to which tolerance was measured, thereby allowing explanations other than those involving conditioned tolerance (e.g., involvement of stress or state-dependent learning). However, since more recent studies have attempted systematically to eliminate these confounding influences, the hypothesis that past experience or conditioning can affect the development of tolerance has become more accepted by the scientific community.

Basically, two procedures have been used that result in behavioral tolerance: instrumental conditioning and classical conditioning. Experiments employing these procedures have attempted to determine the contributions of associative variables to the development of tolerance.

Instrumental Conditioning Models

Schuster (1978) put forth a general hypothesis concerning the

development of behavioral tolerance which he supported with studies of the effect of different reinforcement schedules on tolerance to the depressant action of d-amphetamine on operant behavior in rats. Behavioral tolerance developed in those aspects of the organism's behavioral repertoire where the drug disrupted the organism's ability to meet requirements for reinforcement. Conversely, where the actions of the drug enhanced, or did not affect, the organism's behavior in meeting reinforcement requirements, the development of behavioral tolerance was neither to be expected nor was it seen.

An example of the development of behavioral tolerance to the debilitating effect of ethanol was offered by Chen (1968). He tested the effects of ethanol on rats trained to run a circular maze for food reinforcement. Half the rats received ethanol before daily training trials (Behavioral group) and the other half received ethanol after daily training (Physiological group). An initial injection of ethanol before testing increased the number of errors made in the maze but as daily injections before testing continued, the error rate of these intoxicated animals decreased. On the last day of the experiment, the performance of the Behavioral group during intoxication was compared to that of the Physiological group (which was being tested for the first time while intoxicated). Performance of the Behavioral group on this test trial was significantly better than that of the Physiological group in terms of the number of errors committed and the number of correct trials completed even though both groups had been equally exposed to ethanol and to practice prior to this test.

A comparison of performance during the first maze run while

intoxicated (and also the first ethanol exposure) of the Behavioral group and the first maze run while intoxicated (but fourth ethanol exposure) of the Physiological group revealed no differences in performance. Chen concluded from this finding that previous ethanol injections did not lead to tolerance unless animals could practice the different instrumental contingencies imposed during intoxication. However, for this comparison, the two groups were not equated in terms of unintoxicated practice on the maze, handling, and injection procedures. A better test for the presence of tolerance in the Physiological group would have been to compare the performance of these animals on their first intoxicated session with that of animals who had received an equal amount of practice in the maze, but saline injections instead of ethanol. The initial effects of ethanol on performance in the maze could be assessed by comparing ethanol-naive animals and ethanol-experienced animals when both had equal practice (while unintoxicated) in the maze and equal amounts of handling and injections before the test.

Chen (1979) also tested the effects of differing degrees of reward on tolerance development by giving pre-trained groups either no reinforcement, one food-pellet reinforcement or 10 min access to food reinforcement after the rats had completed the behavioral response while intoxicated for the first time. Tolerance was as low in animals receiving no reward while intoxicated as in a group receiving saline before the test trial and ethanol afterwards (these animals were also allowed 10 min access to reinforcement). Tolerance was not expected to develop in the latter group but would be expected in the former group

if ethanol exposure during response performance was sufficient. Instead, tolerance was greater in animals allowed access to reinforcement. These findings support Schuster's (1978) general hypothesis that tolerance develops when a reinforced behavior is disrupted by intoxication.

Subsequent experiments by LeBlanc, Gibbins and Kalant (1973, 1975), and LeBlanc, Kalant and Gibbins (1976) addressed the confounding aspects of Chen's studies by including saline injections to equate groups for exposure to handling and injection cues. The experiments also addressed the possibility that instrumental contingencies involving aversive reinforcement (e.g., shock avoidance training) might affect ethanol tolerance. In these studies, rats required to perform a motor coordination task (shock escape/avoidance) while under the influence of ethanol (2.2 g/kg) developed significant tolerance compared to rats receiving ethanol after each session.

In most of the studies by LeBlanc and colleagues, tolerance developed in the physiological groups (which infrequently received practice when intoxicated) but at a slower rate than in the behavioral groups. The findings of LeBlanc and colleagues would seem to require modification of Schuster's general hypothesis that a drug-induced disruption of a reinforced behavior is necessary for tolerance development. LeBlanc et al. (1975) termed the effects of intoxicated practice on tolerance "behaviorally augmented tolerance" and believed the mechanism by which it developed was not different in nature from the tolerance that developed in physiological control groups. They stated that repeated administration of ethanol was sufficient to lead

to tolerance and that practice of a response while intoxicated merely accelerated the rate of tolerance development while not affecting its asymptotic level.

LeBlanc et al. (1976) supported the hypothesis that behaviorally augmented tolerance and tolerance produced by mere drug exposure are products of a similar mechanism. The degree of tolerance produced by behavioral methods in intoxicated animals could not be increased by daily gastric lavage of a large dose (6.0 g/kg) of ethanol (a procedure presumed to increase physiological tolerance). They concluded that if behavioral tolerance and physiological tolerance developed through different mechanisms, then the effects of these procedures on the degree of tolerance should be additive. Since these effects were not additive, they proposed that general neuronal adaptive changes are responsible for both "types" of tolerance and that these changes are influenced by the functional demand imposed upon the central nervous system during periods of tissue saturation by the drug. Practice of the response while intoxicated simply increases this functional demand, thereby accelerating the development of drug tolerance. They did not address the question of whether behaviorally augmented tolerance might be ascribed to classical conditioning to environmental cues.

Mansfield, Benedict and Woods (1983) addressed the question of response specificity in apparent instances of learned tolerance by recording both a physiological (body temperature) and a behavioral (motor coordination) measure of tolerance to ethanol. The development of tolerance to the discoordinating effect of ethanol was seen only in rats receiving daily injections before practice of the response. No

tolerance developed in rats receiving ethanol after training or before every fourth day of training (this last group of animals received ethanol injections after training on the other three days).

Tolerance to the hypothermic effects of ethanol (measured every fourth day) developed equally in all animals regardless of activity levels or stimulation during tolerance training. The augmented development of tolerance to the motor effects of ethanol in the group receiving the most intoxicated practice but the absence of increased tolerance to the hypothermic effect of ethanol in this group suggests that tolerance is not a generalized state of neuronal adaptation as suggested by LeBlanc et al. (1975). Instead, it appears to reflect the acquisition of fairly specific responses by the organism.

It is possible that tolerance developed in the physiological control groups of LeBlanc's studies because these animals were intoxicated during practice on test days occurring intermittently throughout the experiment (e.g., every fourth day in LeBlanc et al., 1973). When LeBlanc et al. (1976) compared tolerance in six groups of rats that practiced a shock-avoidance task while intoxicated every 1, 2, 3, 4, 6, or 8 days, tolerance was found to increase in a graded fashion as a function of the amount of intoxicated practice. All groups were equated for ethanol exposure (with post-practice injections) and for handling and injection exposure (with saline injections).

Wenger, Berlin and Woods (1980) used single tolerance-test sessions in an experiment that was otherwise similar to LeBlanc et al. (1973) in order to eliminate the possibility of learning occurring over

test days. They found that rats given ethanol each day after training did not become tolerant relative to saline controls whereas rats intoxicated before training were more tolerant than both these groups. Wenger, Tiffany, Bombardier, Nichols and Woods (1981) tested intoxicated animals either every day or every fourth day and compared their performance on a final ethanol test with Physiological and Saline control groups. Performance of rats that had been intoxicated every day during practice was not significantly different from that of rats that had performed intoxicated only every fourth day. However, performance of these two groups was significantly better than that of the two control groups. There was no difference between performance of rats in the two control groups, indicating that exposure to ethanol for 23 consecutive days with no intoxicated practice was not sufficient to induce tolerance. Blood alcohol concentrations were analyzed and were not different between the groups.

These studies support the hypothesis that the tolerance which LeBlanc and colleagues reported to be a consequence of mere exposure to ethanol was actually due to practice given the animals during tolerance acquisition. In addition, Wenger et al. (1981) stated that all types of tolerance, including learned tolerance, are mediated physiologically but suggested that tolerance to ethanol could also be defined behaviorally as learned, if it was the result of practice during intoxication.

In summary, the instrumental conditioning model of drug tolerance states that specific reinforcement contingencies requiring practice of the reinforced response while drugged in order to overcome a

drug-induced performance decrement are involved in the development of tolerance. In other words, the development of tolerance is indexed by a return to unintoxicated performance levels (and greater reward due to the better performance). This model cannot explain drug tolerance unless performance on an instrumental task is initially degraded by the drug. Although reinforcement levels were disrupted in the studies described here and in a number of others in the literature, there are cases in which the occurrence of disruption is less clear. For example, the hypothermic effect of ethanol could be explained as being due to disruption of temperature regulating behaviors thus causing discomfort to the animal. Tolerance might then be due to practice of a temperature regulating response that counteracts the acute debilitating effects and restores normal body temperature. Such an explanation forces the assumption that the inability to regulate body temperature is aversive. An analysis of behavioral tolerance employing a classical conditioning model will now be discussed which does not involve the use of instrumental reward contingencies.

Classical Conditioning Models

According to an interpretation of drug tolerance emphasizing Pavlovian (classical) conditioning principles, tolerance results from an association between environmental cues and the systemic effects of a drug (Siegel, Hinson, & Krank, 1978). The development of an association between the environmental conditioned stimulus (CS) and the pharmacological unconditioned stimulus (US) is usually appraised by giving a placebo such as saline in a place where the drug has been repeatedly administered. Such methods have been used to reveal CRs

whose directions are opposite to those of drug-induced URs, e.g., cardiodeceleration in anticipation of epinephrine-induced cardioacceleration (Subkov & Zilov, 1937), hyperalgesia in anticipation of morphine-induced analgesia (Siegel, 1975, 1976, 1977, 1978) and hyperthermia in anticipation of ethanol-induced hypothermia (Mansfield and Cunningham, 1980). The summation of the CR and UR is assumed to account for the decrement in responding to the drug, i.e., tolerance.

Siegel (1975) employed a classical conditioning paradigm during which paw lick latencies were measured on test days when rats were placed on a hot copper plate. Animals that received training trials consisting of morphine injections before placement on a cold plate performed similarly to animals previously exposed to morphine and trained on the hot plate. These findings imply that practice of the paw lick response while drugged was not as necessary as exposure to the environmental cues of the test while drugged. Both groups were more tolerant than animals receiving morphine in the home cage or saline before the hot-plate test. There was no difference in responding between groups that received morphine or saline if neither group received practice while drugged. The animals receiving the drug paired with the hot plate also exhibited a hyperalgesic conditioned response when placed on the hot plate after an injection of saline. The occurrence of a conditioned compensatory response would not be predicted by LeBlanc et al.'s (1976) general neuronal adaptation theory of behaviorally augmented tolerance.

Animals in the above study were not equated for exposure to the testing apparatus nor for handling and injection cues prior to testing.

Siegel (1976) controlled for these problems by exposing four groups of rats to two types of analgesia tests (hot plate and paw pinch) when the test devices were either functional or nonfunctional. Half of each group received morphine before exposure to the test apparatus and the other half received saline. After training, all rats received morphine before testing on both the functional hot plate and the functional paw pinch device.

Tolerance was greater, regardless of whether the apparatus was functional or nonfunctional during training, if analgesia was measured by the same procedure as during training. Tolerance was significantly decreased when the test for analgesia was different from the training test. For example, when analgesia was measured on the hot plate, animals previously receiving morphine paired with the hot or cold plate were more tolerant than those previously receiving morphine paired with either the functional or nonfunctional paw pincher. Analgesic levels of drug-treated groups tested on an apparatus different from that presented during training were equal to those of animals that had received saline injections throughout the experiment. This implies that previous drug exposure had no effect on tolerance unless it was preceded by signals of impending drug injection.

There were no differences in analgesic levels among any one of the saline groups, indicating that effects due to mere practice of the escape response or specific responses required by each test did not affect the results. Siegel, Hinson and Krank (1978) controlled for differences in practice effects between groups by not exposing any groups to practice on the hot plate until the final test, thereby

eliminating all possibilities that animals differed in amounts of practice. Siegel interpreted his findings as due to the classical conditioning of a compensatory response (CR) to the drug-induced unconditioned response (UR).

LaHoste, Olson, Olson and Kastin (1980) performed an experiment designed to bring the putative compensatory CR (proposed to attenuate morphine-induced analgesia) under a greater degree of stimulus control than had been previously demonstrated at that time. They found that when morphine administration was strictly paired with one set of environmental cues (CS+) and explicitly unpaired with another (CS-), analgesic tolerance was decreased when the cues were reversed. More specifically, after 11 morphine training trials, there was an increase in tailflick latencies under a heat lamp for drugged rats tested in the presence of the CS- compared to those tested in the presence of the CS+. However, in contrast to Siegel's findings, when a saline injection was given in the CS+ environment (where morphine was expected), there was no evidence of a hyperalgesic CR. They suggested that this absence was due to the presence of low control-group latencies, especially after 11 days of practice, that obscured further decreases in latency.

Siegel (1977) found that tolerance was greater in animals receiving three paired trials than in animals receiving nine unpaired trials, emphasizing the importance of learned associations in the development of tolerance. In addition, he attempted to draw as many similarities as possible between classically conditioned tolerance and other examples of Pavlovian conditioned responses. For example, he

demonstrated extinction, latent inhibition, and the retarding effect of partial reinforcement on acquisition.

Siegel et al. (1978) attempted to demonstrate conditioned inhibition by comparing tolerance in animals receiving explicitly unpaired presentations of cue and morphine with that of animals receiving saline. They expected to show retardation of the development of tolerance during subsequent conditioning trials but were unsuccessful. They believed they gave too few trials in which conditioned inhibition could develop. Later, Siegel, Hinson and Krank (1981) found evidence of conditioned inhibition after 15 daily drug sessions. A group receiving paired presentations of an environmental CS and a morphine US were more tolerant than animals receiving either only the CS, only the US, neither event or explicitly unpaired presentations of both the CS and the US. When three paired presentations were subsequently given to all animals, all groups except the explicitly unpaired group eventually reached an equivalent level of tolerance. The explicitly unpaired group remained less tolerant at the end of training.

In summary, the classical conditioning model can explain tolerance development in situations in which instrumental contingencies are not obviously present. This model requires only that the cues present during previous intoxication be present while tolerance is tested. The instrumental conditioning model is adequate when addressing changes in tolerance due to overt reinforcement contingencies, but cannot explain behavioral tolerance when such contingencies are not present.

Conditioned Tolerance to the Hypothermic Effect of Ethanol

Since tolerance to the hypothermic effects of ethanol was chosen for study in the present series of experiments, examples in the literature of this phenomenon will be reviewed in more detail. Specific emphasis is placed on designs that address associative effects on tolerance.

Lê, Poulos and Cappell (1979) measured conditioned tolerance to the hypothermic effect of ethanol using both a within-subject and a between-subject design. In the within-subject study, rats were injected on alternate days with 2.5 g/kg ethanol in a distinctive environment and with saline in the home cage. The magnitude of ethanol-induced hypothermia decreased over days, indicative of tolerance. After 20 days, rectal temperature was measured after ethanol injection in the home cage and hypothermia was found to be greater than that caused by the last ethanol injection in the distinctive environment. Two days later, the hypothermic effects of ethanol were again measured in the distinctive environment and tolerance was present to the same degree as before the injection in the home cage.

The between-group design by Lê et al. (1979) was similar to the within-group study in that animals initially received ethanol injections in a distinctive environment and saline injections in the home cage. This was followed by a tolerance test during which ethanol was given to one group of animals in the home cage and to the other group in the distinctive environment. They compared the

ethanol-induced hypothermia in these animals to that in groups that had received saline in both environments during training. The findings were similar to those in the first experiment (ethanol-induced hypothermia was decreased only in rats receiving cued ethanol), indicating that tolerance was greater in the presence of environmental cues predicting drug exposure. Neither design, however, took into account any different nonassociative effects of the two different environments on the temperature response to ethanol. This problem could be eliminated by using a discrimination design which counterbalances the environments that are paired with ethanol and saline in each group (see Cunningham, Crabbe & Rigter, in press).

Mansfield and Cunningham (1980) tested for conditioned tolerance to the hypothermic effects of ethanol by using a discrimination design that included two groups of animals: one received ethanol injections in a distinctive environment (Room A) and saline injections in a different environment (Room B) on alternate days while the other received saline in Room A and ethanol in Room B. Body temperature was monitored after injections during acquisition, during a subsequent tolerance test (injection of ethanol in both environments on different days) and during a conditioned response test (injection of saline in both environments on different days). Rats were found to be tolerant only in the presence of room cues previously paired with ethanol (regardless of whether this was Room A or B) and a hyperthermic CR was present when saline was given instead of ethanol in this environment. An extinction procedure designed to weaken tolerance mediated by classical conditioning was also found to be effective, in accordance with the

findings of Siegel (1977).

The findings of Mansfield and Cunningham were replicated by Crowell, Hinson and Siegel (1981) using a similar design but an increasing dose of ethanol (from 1.3 g/kg to 2.1 g/kg) over the 20 day training period. They also measured extinction of conditioned tolerance by comparing the effects of placebo injections in the paired environment with the effects of rest during the extinction phase. This procedure differs from the one used by Mansfield and Cunningham (1980) to test for extinction of conditioned tolerance. Instead, Mansfield and Cunningham (1980) compared placebo injections in the drug environment with placebo injections in the saline environment. The procedure used by Mansfield and Cunningham better equated groups for handling and injection cues over the extinction phase of the experiment.

Melchior and Tabakoff (1981b) found that a compensatory hyperthermic CR developed after repeated injections of 3.5 g/kg of ethanol. In this experiment, mice were injected twice daily in the home cage for four days during which body temperature was monitored. On the fifth day, some subjects were injected and their body temperature measured in the home cage while other subjects were injected and monitored in a novel environment. Body temperature dropped in both groups but to a lesser degree (about 0.5 ^{o}C) in the animals receiving ethanol in the home cage. Both of these groups exhibited less hypothermia than animals receiving saline during the training period and ethanol on the last day (regardless of whether temperature was measured in the home cage or novel environment). When

animals that previously received ethanol during training were injected with saline on the fifth day, a hyperthermic CR was exhibited (also regardless of whether testing took place in the home cage or novel environment). Thus, it appeared that tolerance was not conditioned exclusively to cues in the environment in which drug was administered. They concluded that features of the experimental procedure other than the environmental cues (e.g., weighing and injection cues) could have been used to some extent by the animals as cues for the body temperature response.

In summary, a number of different procedures have been used successfully to demonstrate conditioned tolerance to the hypothermic effects of ethanol. Most of these have also been able to demonstrate a conditioned compensatory response which may account for the diminished thermic response to a challenge dose of ethanol. A discrimination procedure similar to that used by Mansfield and Cunningham (1980) seems to be the least ambiguous design when one is interested in measuring the development of conditioned tolerance. This paradigm decreases the probability that injection and handling cues will be followed by drug administration, thus decreasing the predictive nature of these cues concerning subsequent drug events. This design also exposes groups of animals equally to the two test environments, thereby eliminating any possibility that tolerance in the test situation was affected by exposure to a novel environment.

Ethanol-Induced Effects on Heart Rate

The number of references in the literature on the cardiovascular effects of ethanol are few and can be classified by whether acute or

chronic administration of ethanol was given. Studies of chronic ethanol administration may indicate whether tolerance develops to the effects of ethanol in the cardiovascular system. Of these studies, only one has tested whether tolerance is affected by conditioning. The present series of experiments is concerned with whether tolerance occurs to the cardiovascular effects of ethanol and if so, whether it can be conditioned. Because studies addressing these points are few, both human and animal studies have been included.

Ethanol affects the cardiovascular system primarily by decreasing circulatory tone. This is accomplished by both a direct effect on arteriolar smooth muscle and by central nervous system regulation of adrenergic output (Ritchie, 1981). A direct effect on the heart is not evident but there are a number of compensatory reflexes in response to the decrease in blood pressure caused by ethanol. Unfortunately, these effects appear to vary widely.

Ethanol given to nonalcoholic humans generally accelerates heart rate. A number of studies have recorded cardioaccelerations occurring within 60 min after ethanol administration (Blomqvist, Saltin & Mitchell, 1970; Delgado, Fortuin & Ross, 1975; Giles, Cook, Sachitano & Iteld, 1982; Riff, Jain & Doyle, 1969; Timmis, Ramos, Gordon, Parikh & Gangadharan, 1974). A variety of ethanol doses were given orally in these studies (.7 g/kg - 1.15 g/kg, assuming subjects of 70 kg) and the range of blood alcohol concentrations varied from 85 to 156 mg/dl. None of the accelerations were greater than 10-15 beats per minute (bpm).

One instance of ethanol-induced cardiodeceleration was documented

by Gould, Reddy and Goswani (1973). They instructed 10 subjects to drink aproximately a .57 g/kg dose of ethanol (43.4 %, v/v) in 15 min and recorded a significant heart rate deceleration of about 6 bpm after 1 hr. No change in heart rate occurred after an equicaloric administration of glucose given to the same subjects on a different occasion. Blood alcohol levels were not measured.

The effect of ethanol on heart rate in animals varies widely depending on the species, the route of administration and the state of the animal during heart rate measurements. Ethanol infusion into the venous blood of dogs led to a decrease in heart rate in some studies (Friedman, 1981; Knott & Beard, 1967; Sulzer, 1924) and no significant change in others (James & Bear, 1967; Pachinger, Tillmans, Mao, Fauvel & Bing, 1973; Schmitthenner, Hafkenschiel, Forte, William & Riegel, 1958). All of the measurements in these studies were taken under sodium pentobarbital anesthesia while the chest was opened and animals were artificially respirated. It appeared that blood alcohol levels were greater by about 25-50 mg/dl in the studies reporting decelerations.

A study using chronically catheterized pregnant sheep found that both maternal and fetal heart rate increased after a dose of ethanol that raised blood levels to 140 mg/dl within 2 hrs (Cook, Abrams, Notelovitz & Frisinger, 1981). Ethanol raised heart rate in anesthetized Long-Evans rats (Walter & Laycock, 1982) and decreased heart rate in anesthetized Sprague-Dawley rats (Maines & Aldinger, 1967). The methods of these last two studies differed in that a dose of .395 g of ethanol (50 %, v/v) was infused in the first study after

rats were anesthetized with pentobarbital, while rats in the second study were allowed to drink a 25% solution ad libitum before anesthesia. Blood alcohol levels were not measured in either study.

Fitzgerald and Stainbrook (1978) found a dose-related biphasic effect of ethanol on heart rate. Heart rate of rats increased 25 bpm relative to baseline levels within 6 min after a 0.8 g/kg intraperitoneal injection of ethanol, reaching a 40 bpm peak acceleration after 12 min. On the other hand, a 2.4 g/kg dose increased heart rate about 15 bpm within 3-6 min after injection. After 9 min, heart rate was 20 bpm slower than both pre-injection heart rate and heart rate in a saline control group. Heart rate remained this low at least until the 15 min measurement period was over.

Crow (1968) measured the effect of infusion of 20 ml/kg of an 11.88% solution of ethanol in saline (equivalent to about 1.8 g/kg of ethanol) directly into the stomach (under light ether anesthesia) on the heart rate of four Sprague-Dawley rats. He found that higher cardiac rates tended to be associated with alcohol than with the ether control condition but that there was a large variation in the extent of overlap in the variance and range of the two conditions. Although Crow measured the effects of repeated administrations (ethanol was given six times over a 3-week period) he did not report any of the data from individual tests. Instead he reported a mean heart rate effect averaged over all six tests.

Tolerance to the cardiac effect of ethanol has not been directly studied in an animal model, but some studies have reported the effect of repeated administration of ethanol on heart rate. Maines and

Aldinger (1967) found that the 50% cardiodeceleration elicted by ethanol in rats decreased after 15 - 30 weeks of ethanol administrations. Wilkin, Cunningham and Fitzgerald (1982) recorded a biphasic heart rate response to ethanol (0.8 g/kg) in restrained rats used as a control in a drug conditioning experiment. These rats received unpaired presentations of ethanol and lithium while other groups received paired ethanol and lithium exposure. The response to ethanol alone began with a large deceleration but changed after 9-10 min to a smaller acceleration. After 10 ethanol injections, the deceleratory component was attenuated (resembling tolerance) but the acceleratory component was enhanced. It should be noted that the changes in the response to ethanol reported by Wilkin et al. (1982) may have been due to inhibitory conditioning from the ongoing drug conditioning study (ethanol exposure predicted the absence of lithium sickness) and not simply due to tolerance.

There is only one study reporting tolerance to the acceleratory effect of ethanol on heart rate (Dafters & Anderson, 1982). A discrimination paradigm was used to test whether tolerance was conditioned to environmental cues. Human subjects were given an oral dose of ethanol (0.47 g/kg, 10 % v/v solution in fruit juice) in a distinctive environment and placebo administrations of the juice alone in a second environment on different days. After 10 days of testing, all subjects received one session with ethanol administered in the placebo environment followed by one session with ethanol given in the ethanol environment. Tolerance to the tachycardia induced by ethanol was observed during training and this tolerance was reduced (i.e.,

tachycardia was increased) when ethanol was administered in an environment where it was not expected relative to tolerance displayed in an environment normally associated with the drug.

The effects of ethanol on heart rate in animals cannot be easily summarized. Even when one considers only those studies using rats, similar doses of ethanol were found to induce either accelerations or decelerations or both (a biphasic response), with the results seemingly uncorrelated with restraint or anesthesia levels. The conflicting nature of the reports of the effects of ethanol on heart rate were thought by Riff et al. (1969) to be due to the length of the period of observation and the choice of experimental subjects, with more changes being found in animals and alcoholic subjects. Although species-specific responses to ethanol are probably the greatest cause of variability among the results of these studies, the variability of the doses and the absence of data on blood alcohol concentrations in some studies increases the confusion concerning the effects of ethanol on heart rate. It appears that tolerance to the varied cardiac effects of ethanol might be expected to develop on the basis of some of the chronic studies depending on the direction of the change and the duration of ethanol dosing schedules employed. According to Dafters and Anderson (1982), one might also expect that tolerance (when it occurs in the cardiovascular system) might be conditionable. Whether their results can be generalized from human to animal models needs to be addressed.

Aim of this Project

This project was concerned with the development of tolerance to the cardiovascular and thermic effects of ethanol in the rat, both under conditions where drug administration could be anticipated and when it could not. The first experiment was a dose-response study using two commonly employed doses of ethanol. Heart rate and body temperature were measured for 2 hr after ethanol administration. A schedule of repeated injection allowed for the assessment of tolerance to the effects of ethanol on these physiological responses. In addition, the interaction of a nonassociative factor (i.e., stress) with conditioned anticipatory responses to ethanol and the resulting effect on tolerance development were studied in subsequent experiments.

EXPERIMENT 1

The first experiment was designed to examine the development of tolerance to the cardiovascular and thermic effects of ethanol in the rat. An experimental procedure was used such that exposure to handling and environmental cues was adequately controlled.

The absence of literature reporting systematic studies of ethanol on heart rate in both drug-naive and tolerant awake animals prompted the inclusion of heart-rate measurements during procedures designed to induce ethanol tolerance. If the biphasic effects of ethanol on heart rate found by Wilkin, Cunningham and Fitzgerald (1982) could be replicated, it would be of interest to observe whether tolerance would develop to one or both components of this effect. Because a great deal of research has been done on tolerance to the hypothermic effect of ethanol, this phenomenon was also chosen for study. This was in order to allow comparison of the present results

with a large body of existing data on the development of tolerance (both associative and nonassociative) to a physiological effect of ethanol in rats.

It is possible that tolerance develops and persists over a different time course in different physiological response systems, by adaptation at different sites of action. For example, tolerance develops to the sedative/depressant effects of ethanol but not to its activating effects (Masur & Boerngen, 1980; Tabakoff & Kiianmaa, 1982). Responses in different systems that have been classically conditioned using non-drug reinforcement have been found to extinguish at different rates (termed schizokinesis by Gantt, 1960). For example, Gantt (1960) found that classically conditioned heart rate responses could be evoked up to one year after training while conditioned motor responses had usually extinguished by this time. If conditioning is an important determinant of tolerance, then schizokinesis (which is a characteristic of conditioning) may also be a characterisic of tolerance, may affect the addictive process, and may be the basis of the resistance of addiction to therapeutic extinction, especially in a drug-taking situation where a variety of physiological responses are elicted and many neural centers are involved (Lynch, Fertziger, Teitelbaum, Cullen & Gantt, 1973). Alcoholics usually exhibit a high probability of relapse. This might be explained by the persistence of conditioned anticipatory responses to ethanol in one or more physiological systems after overt drug-taking behavior is extinguished.

Experiment 1 compared tolerance in animals receiving either one of two doses of ethanol or saline with heart rate and body temperature

being monitored. After nine exposures to the drug or saline, all animals underwent a test for tolerance and, subsequently, a placebo test. In the tolerance test, all rats received a high dose of ethanol. The development of tolerance was defined by diminished responding in animals that had received ethanol during the nine training days should have been diminished relative to that in animals that had received saline. The magnitude of tolerance is greater and develops faster when larger doses are given and greater URs are exhibited (LeBlanc, Kalant & Gibbins, 1969).

All rats received a saline injection during the placebo test. The placebo test provided an opportunity to determine the animal's reaction to cues provided by the test environment, by handling, and by injection in the absence of ethanol. Although the placebo test may reveal compensatory hyperthermic responses conditioned to the environment in which ethanol was administered, for example, it does not constitute a valid test for conditioned tolerance. Specifically, the appropriate control group for evaluating the associative influence of these cues on drug tolerance was not included in the first experiment. A group of animals receiving identical exposure to ethanol but given the placebo in an environment not previously paired with ethanol would have provided a better comparison for determining whether conditioned compensatory responses developed.

Thus, associative and nonassociative mechanisms of tolerance could not be differentiated on the basis of data from tolerance acquisition or the subsequent tests presented in Experiment 1. These factors were tested in the second experiment with the appropriate

controls. However, it was still possible to observe whether heart rate and temperature changes resembling the expected conditioned responses occurred. It might be expected that the magnitude of a compensatory CR would increase in proportion to the size of the ethanol dose administered during tolerance training. According to a classical conditioning model, such an increase would be needed to offset the larger magnitude UR induced by larger doses in order for tolerance to develop.

Method

Subjects

The subjects were 24 adult male albino rats (Holtzman Company, Madison, Wisconsin) which were 70 days old at the start of testing. They weighed an average of 400 g at that time and 370 g at the end of testing. The animals were housed in a temperature-controlled colony room with a normal 12 hr light/dark cycle (light onset was at 6:00 A.M.). They were maintained on a mild food-deprivation schedule with each rat receiving 20-25 g of food at the end of each day during testing. This procedure was adopted to reduce the chance of injection injury to the gastrointestinal system. Water was available to the animals ad lib throughout the experiment except during test sessions.

Surgical Preparation

Approximately two days before the start of the experiment, animals were anesthetized with halothane gas (loading dose = 7.5 % concentration in oxygen; maintenance dose = 1.7 % concentration in oxygen). Two heart-rate monitoring electrodes were implanted

subcutaneously and an automatic temperature monitoring device (Mini-Mitter Co., Inc.) was implanted in the intraperitoneal cavity, under antiseptic conditions.

Heart Rate Electrodes. Two 1-cm incisions were made through the skin, one dorsally, approximately 3 cm below and to the right of the base of the skull and the other, ventrally, approximately 1 cm rostral to the left foreleg. Connective tissue under the incisions was cleared with a sharp pair of scissors. Each electrode consisted of 36-cm of 32-gauge stainless steel suture wire which was loosely looped through the superficial muscle six times. The wire tails were twisted and then covered with polyethylene tubing (Intramedic, #PE100). Both electrode leads were run to and then through the dorsal incision, where they were looped once, close to the exit point. This loop was sutured to the superficial muscles.

The electrode wires were trimmed to about 3 cm from the exit point and then soldered to a two-pin plastic plug (3 cm x 0.75 cm x 1.5 cm). This plug was securely attached to an external saddle (Weeks, 1972) consisting of a foam rubber strap attached with Velcro strips to a foam cushion, stainless steel shim and collar. The saddle fit around the animal's upper chest and back, with the collar placed around the neck and the plug resting on the back.

Mini-Mitter. The Mini-Mitter is a small AM-band transmitter that sends out a signal pulse at a rate proportional to the temperature of the surrounding environment. Basically, it consists of two thermistors, powered by a hearing aid battery, enclosed in a non-toxic plastic case. This device allows detection of temperature changes as

small as 0.1 C. Two models were used; 14 were Model X-M (9 mm diameter x 16 mm length) and 10 were Model M (12 mm diameter x 16 mm length). Each device was coated with waterproof Parafin/Elvax to protect it from fluid corrosion and then individually calibrated in a temperature-controlled water bath. The Mini-Mitter was inserted through a 1.5-cm ventral midsaggital incision through both the skin and peritoneum wall about 5 cm below the diaphragm. All incisions were closed using 000 silk suture, and about 5 mg Furacin (0.2 % nitrofurazone, a topical anti-bacterial agent in a water-soluble base) was applied to the wounds.

Apparatus

The animals were tested in a clear plastic chamber (23 x 20.5 x 21 cm) the floor of which was covered with wood shavings placed inside a larger sound-attenuating chamber (50 x 52 x 45 cm). The ambient temperature of the test chambers measured immediately before each recording session averaged 25°C ($\pm$ 1.1°C) during all phases of the experiment. Two lengths of 18-gauge wire covered with stainless steel spring led from the two-pin plastic connector to a swivel (Cunningham, 1978) incorporated into the ceiling of the plastic chamber. After amplification, the heart rate signal was fed into a peak detector (Shimizu, 1978) and one-shot trigger which converted the R-wave of each pulse into a digital signal.

A modified transistor radio, set on the AM frequency band, was used to receive the signal broadcast from each Mini-Mitter. This signal was converted to a digital signal by passing it through a one-shot trigger (see Cunningham & Peris, 1983). A PDP8/F computer

calculated and recorded interpulse intervals (accurate to 20 msec) from both the Mini-Mitters and the heart rate electrodes.

Procedure

Following surgery, rats were placed in individual cages where they were left undisturbed for 48 hr. In order to habituate the animals to the injection procedure, they received a 0.5 ml injection of saline (i.p.) in the home cage three times daily on the day prior to the start of habituation and and on each day of the habituation phase.

All rats were given two apparatus habituation sessions, 48 hrs apart, and were tested in six squads of four rats each. Before each habituation session, a squad was transferred from the colony room to the test area and weighed about 10 min prior to placement in the test chamber where temperature and heart rate were recorded for 180 min. Following the recording period, the animals were transferred back to their home cages in the colony room, where they received their daily food ration. Animals were tested once every 48 hrs and rested on the alternate days. Test days for Squads 1, 2 and 3 were rest days for Squads 4, 5 and 6, and vice versa. Squads 1 and 4 started at about 7:00 A.M., Squads 2 and 5 started about 10:00 A.M. and Squads 3 and 6 started about 1:00 P.M.

After the habituation phase, the rats were distributed to three groups (n = 8/group): Group H, Group L and Group S. The only difference between the treatments of the three groups was that Group H received a high dose of ethanol, Group L received a low dose of ethanol and Group S received saline during tolerance acquisition training. These groups were matched for mean basal temperature, heart rate and

body weight measured during the habituation phase. Animals from each group were equally represented among the six squads.

The procedure used during the tolerance acquisition phase of the experiment was similar to that during habituation except that 60 min after placement in the test chamber, each rat was removed, injected, and then replaced in the chamber for the remaining 120 min of the session. Group H was injected (i.p.) with 2.0 g/kg ethanol, Group L with 1.0 g/kg ethanol and Group S with saline. Half of Group S received an injection volume equivalent to that of Group H (Subgroup S-hi) and the other half of Group S received the same volume as Group L (Subgroup S-lo). Ethanol was diluted to 17.8 % v/v concentration with saline. Thus, Group H and Subgroup S-hi received 15 ml/kg of ethanol or saline solution and Group L and Subgroup S-lo received 7.5 ml/kg of solution. All solutions were maintained and injected at room temperature (approximately 25°C). On days between injections, the rats were left undisturbed in their home cages.

After 9 injections and 9 rest days, two tests were administered at 48-hr intervals: a high-dose ethanol test and a placebo test. These tests followed the same procedure as described above except all animals received 2.0 g/kg ethanol, 60 min after placement in the chamber during the tolerance test; a volume of saline equal to that of injections given during tolerance acquisition was given during the placebo test.

Data Analysis

Each interpulse interval (IPI) from both the heart rate electrodes and the Mini-Mitters was recorded by a PDP8/F computer during each min

of the 3-hr session. All IPIs were ignored that were different by more than 20 msec from the previous IPI. In addition, all heart rate IPIs greater than 300 msec or less than 80 msec, were ignored as were temperature IPIs greater than 440 msec or less than 300 msec. Intervals outside these ranges were assumed to be noise or missing signals. Approximately, 5-15% of the heart rate IPIs and 15-25% of the temperature IPIs were discarded during acquisition and test phases.

The mean cardiac IPI recorded during each minute was translated into an average heart rate (bpm) and the mean IPI from the Mini-Mitters was translated into a mean body temperature using the calibration values obtained previously. If the total duration of accepted intervals for either measurement was less than 2 s, then all data obtained during that minute were discarded. Scores were averaged over 10-min periods. If data were discarded for a whole 10-min period, an average score was computed from adjacent periods and inserted in place of the discarded data for the statistical analyses. Out of 3544 scores used in all analyses of body temperature, 38 were inserted means (1%), while only 25 out of 4176 heart rate scores used were inserted means (0.6%). The degrees of freedom were properly adjusted according to the method of Linton and Gallo (1975).

Results

The data from three rats were discarded due to death: two animals from Group S (one died after surgery and one after the tolerance test) and one animal from Group H (death occurred after the first ethanol injection). Body temperature data were discarded from

five more rats at various times during acquisition due to loss of the Mini-Mitter signal.

Mean heart rate and temperature during each 10-min period of the 3-hr habituation sessions were used to assess the effects of handling. Three types of scores were derived from both heart rate and body temperature data to assess the effects of saline or ethanol injections during tolerance acquisition and test days: (1) Baseline scores measured during the first hour after placement in the chamber; (2) Post-injection scores measured during the 2 hr immediately following injection; and, (3) Change scores (post-injection minus last 10-min period of baseline) calculated for the 2 hr following injection.

Temperature data were discarded for the first sample period following all injections because of an artifactual decrease in temperature that occurred from the cold injection fluid surrounding the Mini-Mitter. In addition, because of a possibility that up to half of the Mini-Mitter units (Model M) may have exhibited drift of up to 2.0 C over the period of use, only change scores were reported for temperature. The nature of the drift is discussed in the analysis of baseline scores below. Information concerning the drift problem in Mini-Mitters used less than 30 days was not received from the manufacturer until after Experiment 1 had commenced.

In all three-way analyses of variance (ANOVAs) performed on the data, dose was used as a between-group variable and days and 10-min sample periods as within-group variables. In two-way ANOVAs for the data from the two tests, dose was used as a between-group variable and 10-min sample periods as a within-group variable. All p values less

than 0.05 were considered significant in this and all subsequent experiments.

Habituation

Heart Rate

Heart rate during the two habituation sessions is plotted in Figure 1 over days and sample periods (collapsed over groups). As can be seen from the graph, heart rate was initially elevated in all groups after placement in the chambers on both days. Heart rate then decreased about 70-100 bpm within the first 30 min. The final level of heart rate was lower on the second day.

A three-way ANOVA on these data revealed significant effects of Days ($F\{1,20\} = 24.99$) and Sample Periods ($F\{17,340\} = 62.71$). There were no significant main effects or interactions involving Dose (which at this time was a dummy variable).

Temperature

Temperature in the three groups during the two habituation sessions is shown in Figure 2 over sample periods (collapsed over days). Temperature was elevated at the start of the session on both days but tended to decrease after the first 20 min. A three-way ANOVA revealed a significant effect of Sample Periods ($F\{17,340\} = 14.36$) but no significant effects or interactions due to Dose or Days.

Summary: Habituation

Both temperature and heart rate were initially elevated due to handling and to placement in the recording chamber. This effect, however, was temporary, disappearing within 60 min. While average heart rate decreased from the first to the second habituation day,

Figure 1. Mean heart rate during the two habituation sessions. Data are collapsed across groups.

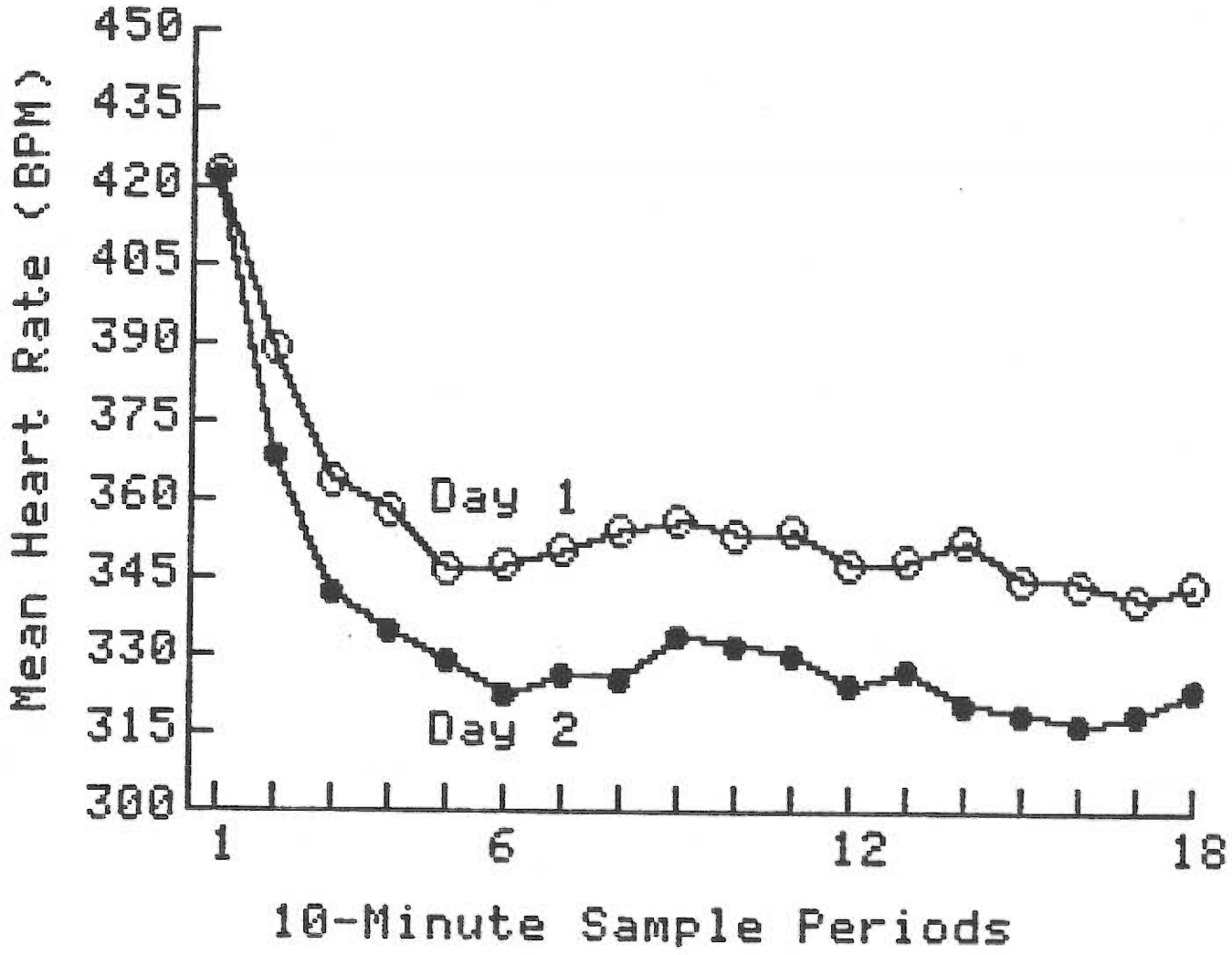
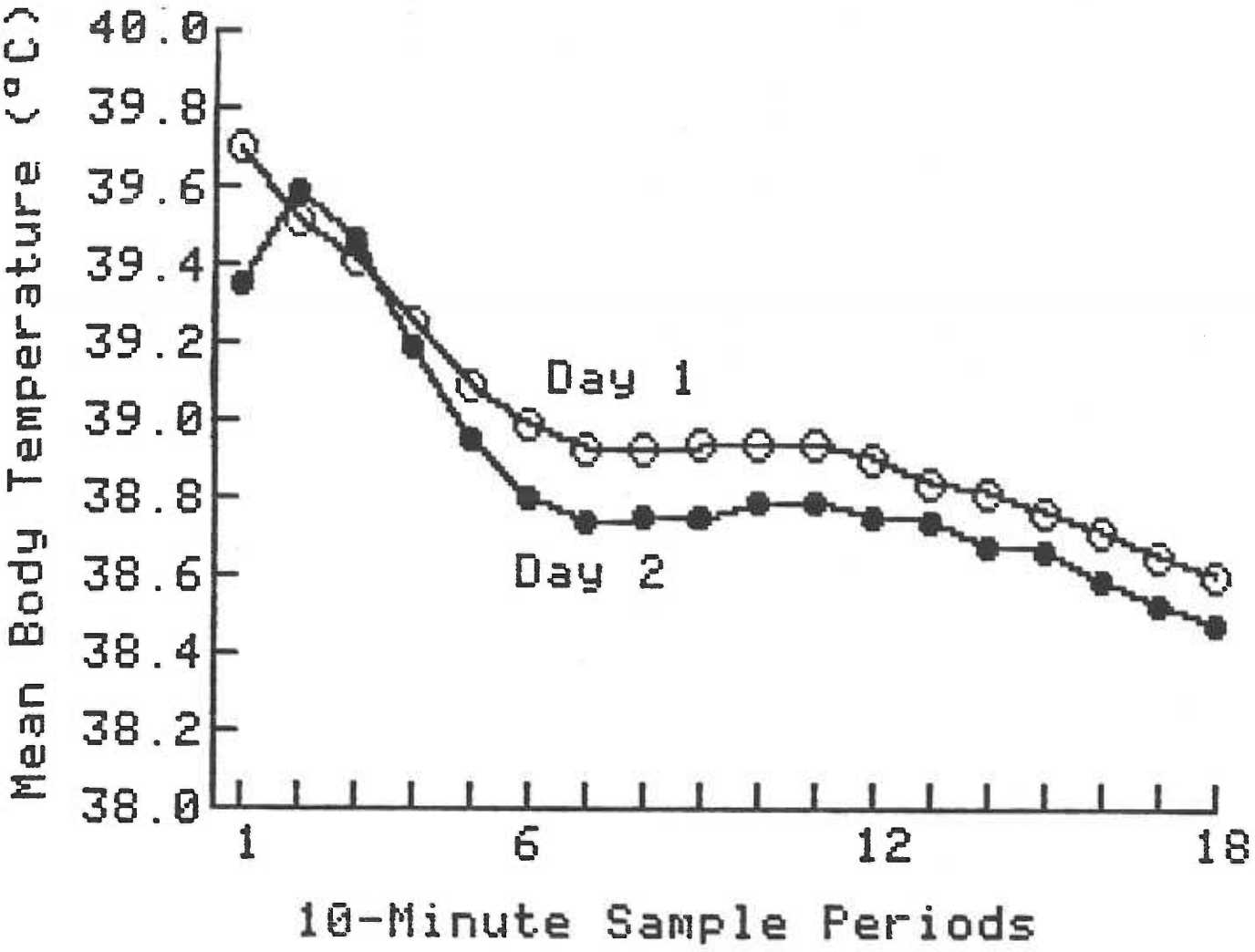

Mean Heart Rate (BPM)
450
435
420
405
390
375
360
345
330
315
300
Day 1
Day 2
1
6
12
18
10-Minute Sample Periods

Figure 2. Mean body temperature during the two habituation sessions. Data are collapsed across groups.


Mean Body Temperature (°C)
40.0
39.8
39.6
39.4
39.2
39.0
38.8
38.6
38.4
38.2
38.0
Day 1
Day 2
1
6
12
18
10-Minute Sample Periods

average body temperature did not.

Baseline Scores

Heart Rate

Heart rate during the first hour (the baseline period), collapsed over the nine acquisition days and both test days, is graphed in Figure 3 for each group over sample periods. Generally, heart rate was elevated in all groups immediately after placement in the chamber and then decreased about 80 bpm over the course of the hour. A three-way ANOVA found a significant Dose x Days x Sample Periods interaction ($F\{100,950\} = 1.58$), a significant Dose x Days interaction ($F\{20,190\} = 1.71$), a significant Dose x Sample Periods interaction ($F\{10,95\} = 2.00$) and a significant Days x Sample Periods interaction ($F\{50,950\} = 5.18$). There were also significant main effects of Days ($F\{10,190\} = 2.56$) and Sample Periods ($F\{5,95\} = 169.1$).

Figure 4 shows baseline heart rate on Day 1 (Panel A) and on Day 10 (Panel B) for all groups. It can be seen that, over days, heart rate decreased about 40 bpm in Group H. The data from Day 1 and Day 10 (the Tolerance Test) were chosen for followup analysis since it appeared that baseline levels on these days were involved in the interaction. A significant Sample Periods effect was present on Day 1 ($F\{5,95\} = 94$) and significant Dose and Sample Periods effects on Day 10 ($F\{2,19\} = 5.95$, $F\{5,95\} = 35.65$, respectively) supporting the observations. When the differences on Day 10 were followed up using a Newman-Keuls comparison, Group H was significantly different from Groups S and L ($Cn\text{-}k\{2,19\} = 39.8$ for S vs H and $Cn\text{-}k\{3,19\} = 42.2$ for L vs H). Groups S and L did not differ.

Figure 3. Mean heart rate of the three groups during baseline periods. The data are collapsed over the nine acquisition and two test sessions.

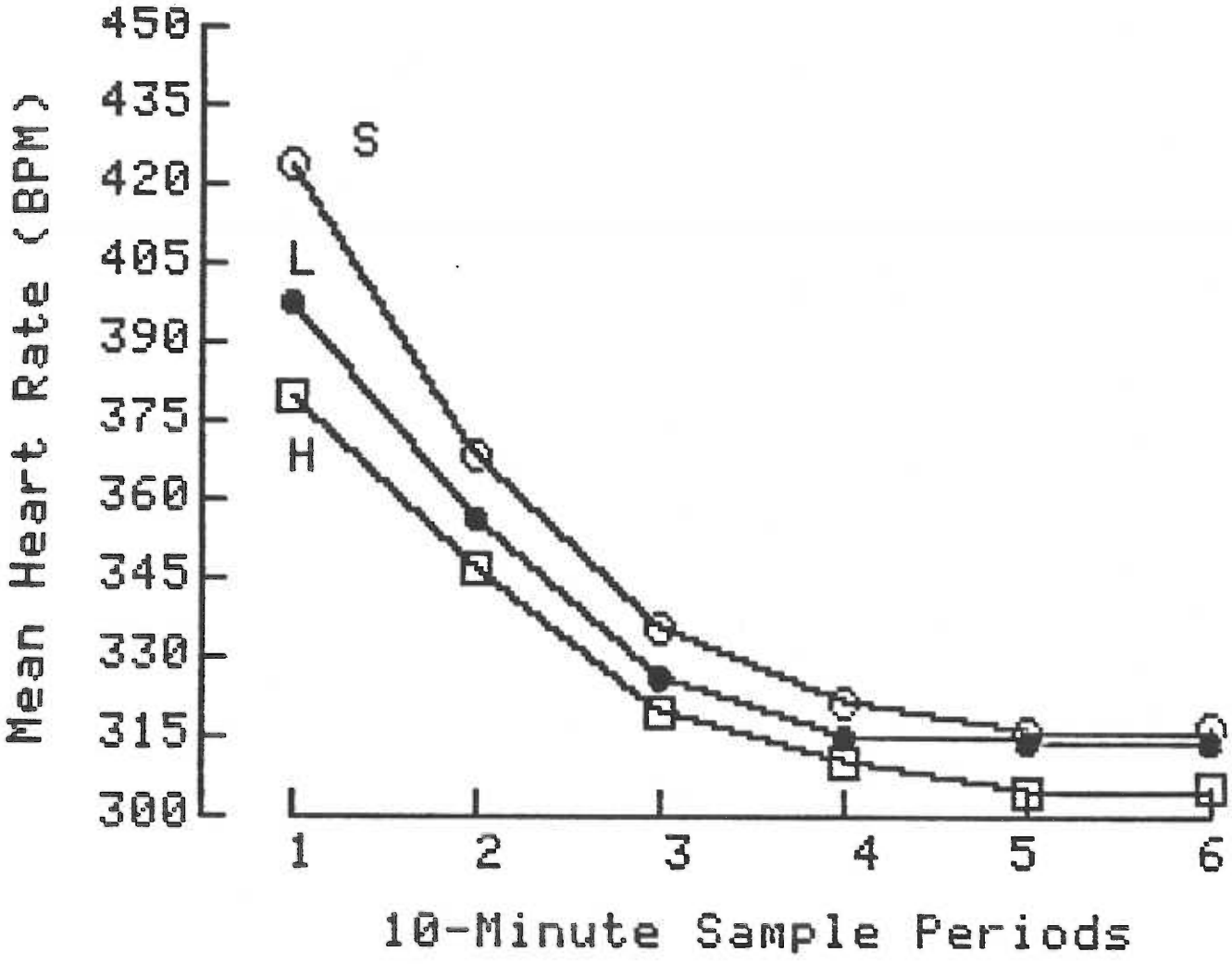
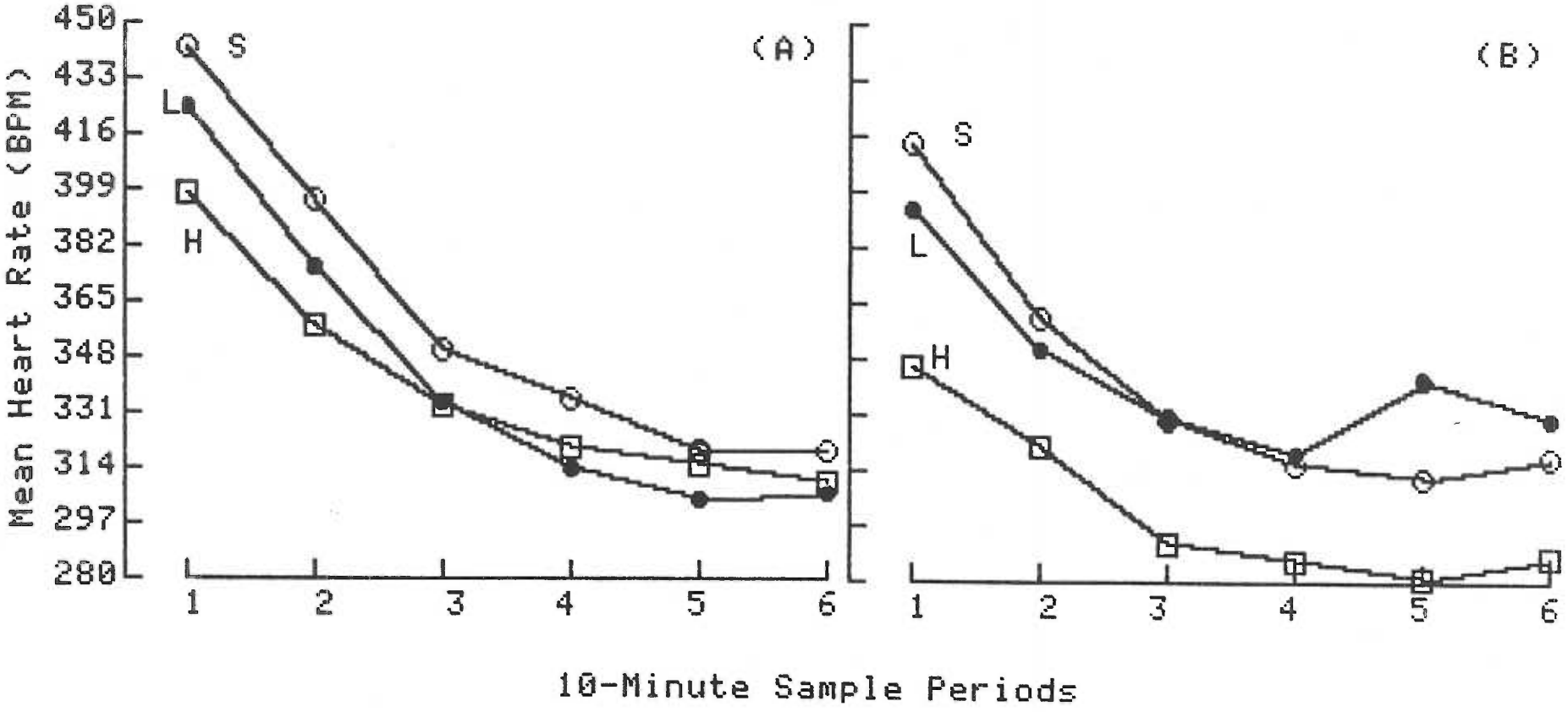

Mean Heart Rate (BPM)
450
435
420
405
390
375
360
345
330
315
300
S
L
H
1
2
3
4
5
6
10-Minute Sample Periods

Figure 4. Mean baseline heart rate of the three groups on Day 1 (Panel A) and Day 10 (Panel B).


Mean Heart Rate (BPM)
450
433
416
399
382
365
348
331
314
297
280
S
L
H
(A)
(B)
1
2
3
4
5
6
10-Minute Sample Periods

Temperature

Baseline temperatures from the first hour of acquisition and test days are graphed in Figure 5 collapsed over days. Temperature increased slightly during the first hour in all groups. This increase disappeared after 50-60 min. Significant interactions of Days x Sample Periods ($F\{50,700\} = 7.21$) and Dose x Days ($F\{20,150\} = 2.84$) were found. Followup within-group analyses revealed a significant effect of Days in Group S ($F\{10,50\} = 10.96$) and Group H ($F\{10,60\} = 3.66$) but not in Group L. This interaction is graphed in Figure 6 showing that whereas temperature in Groups S and H decreased over days, that in Group L did not. This may be due to a combination of Mini-Mitter drift in Group L and habituation in Groups S and H.

Figure 7 illustrates the decrease in baseline body temperature from Day 1 to Day 11 (the placebo test). Over days, there was a greater change in baseline during the early sample periods, accounting for the Days x Sample Periods interaction.

Summary: Baseline

Handling-induced elevation of heart rate was present during the baseline period throughout tolerance acquisition and test phases. The magnitude of this response decreased over days. Although baseline heart rate levels were equal in the three groups at the start of acquisition due to matching, the initial acceleration and final level of basal heart rate were about 30-60 bpm lower in Group H towards the end of the experiment. This change appeared gradually over the course of the experiment in a manner similar to the development of a compensatory CR. This result should be considered when one attempts to

Figure 5. Mean body temperature of the three groups during the baseline periods. Data are collapsed over the nine acquisition and two test sessions.

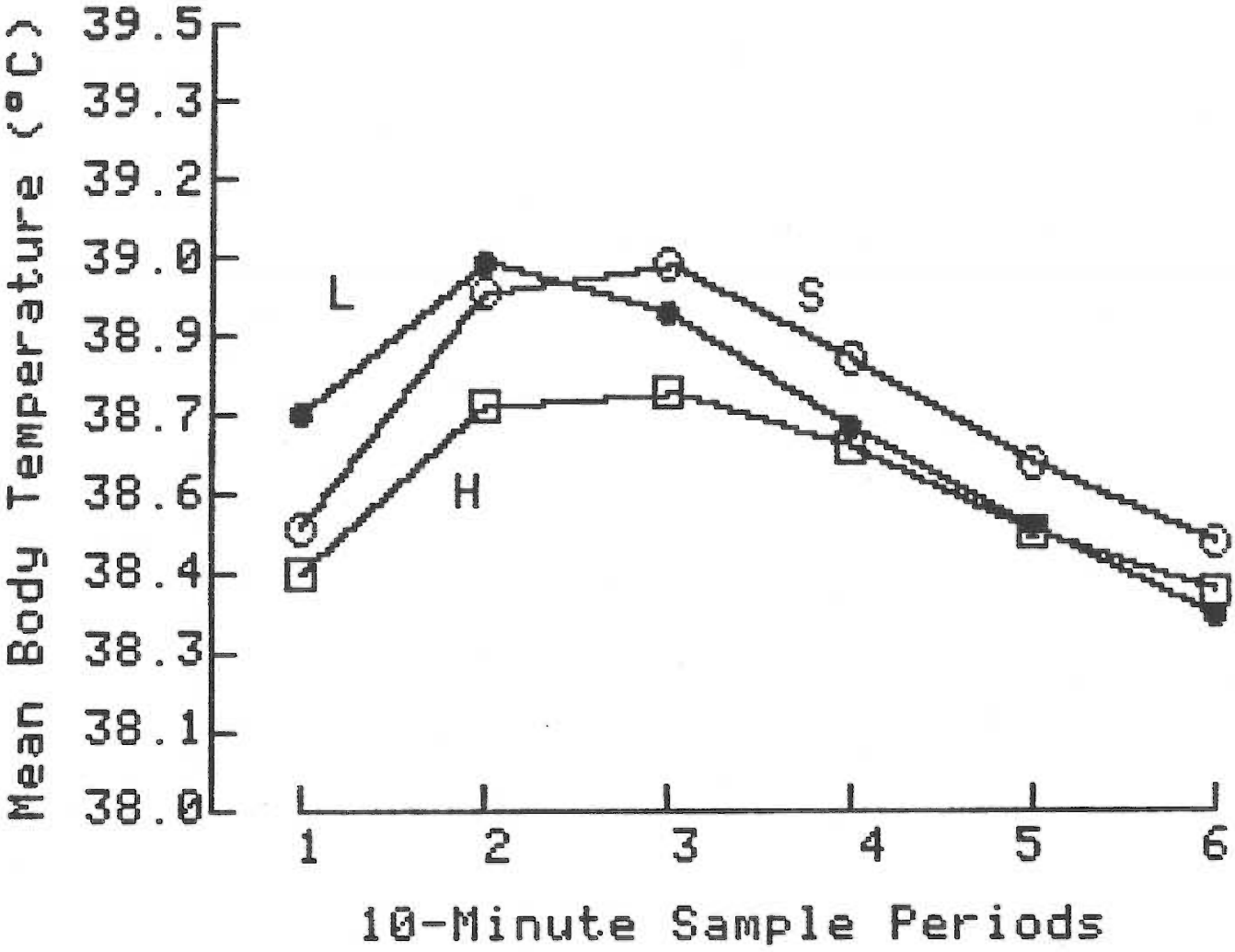

Mean Body Temperature (°C)
39.5
39.3
39.2
39.0
38.9
38.7
38.6
38.4
38.3
38.1
38.0
L
S
H
1
2
3
4
5
6
10-Minute Sample Periods

Figure 6. Mean baseline temperature of the three groups over acquisition and test days. Data are collapsed over 10-minute sample periods.

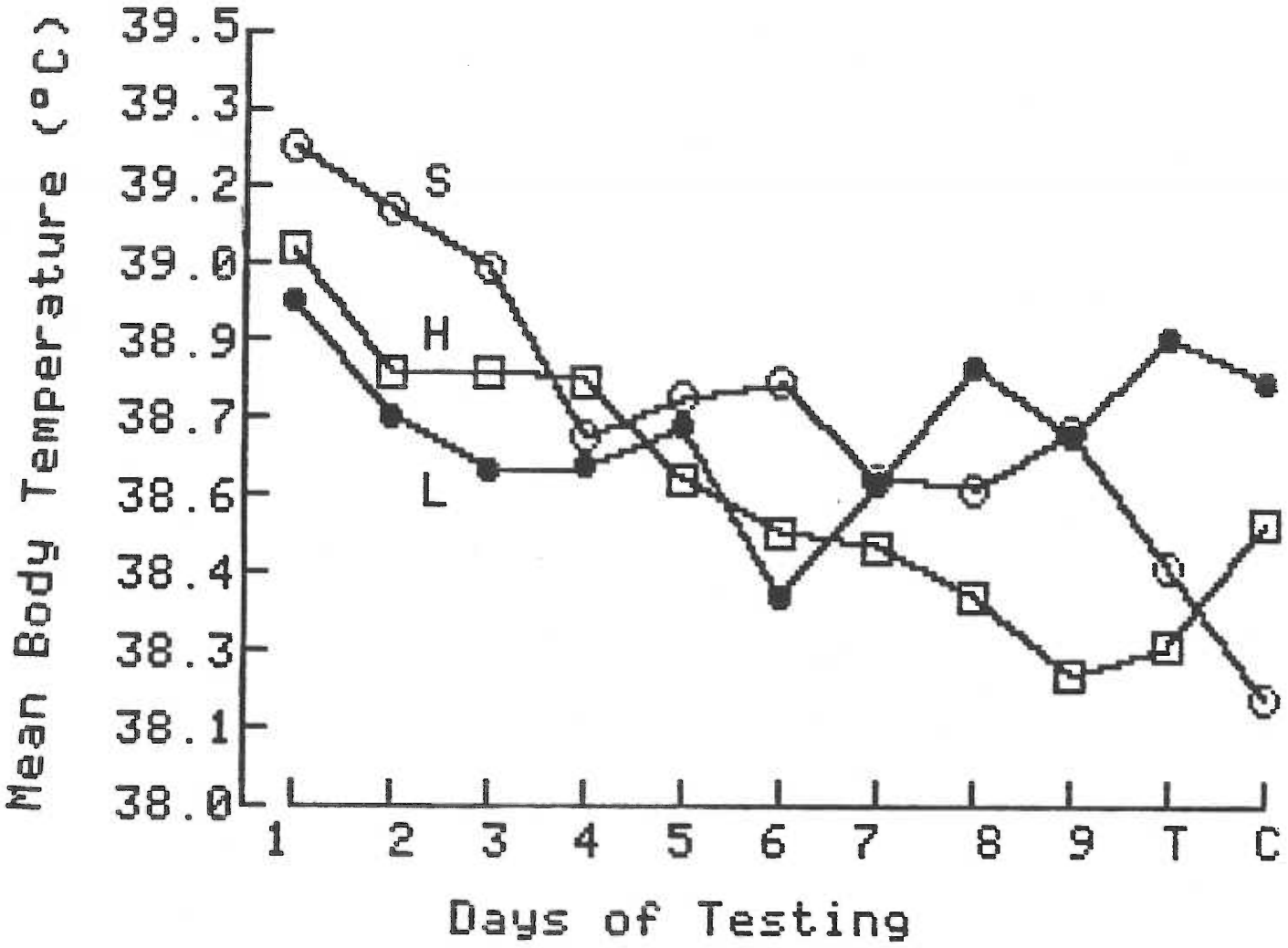
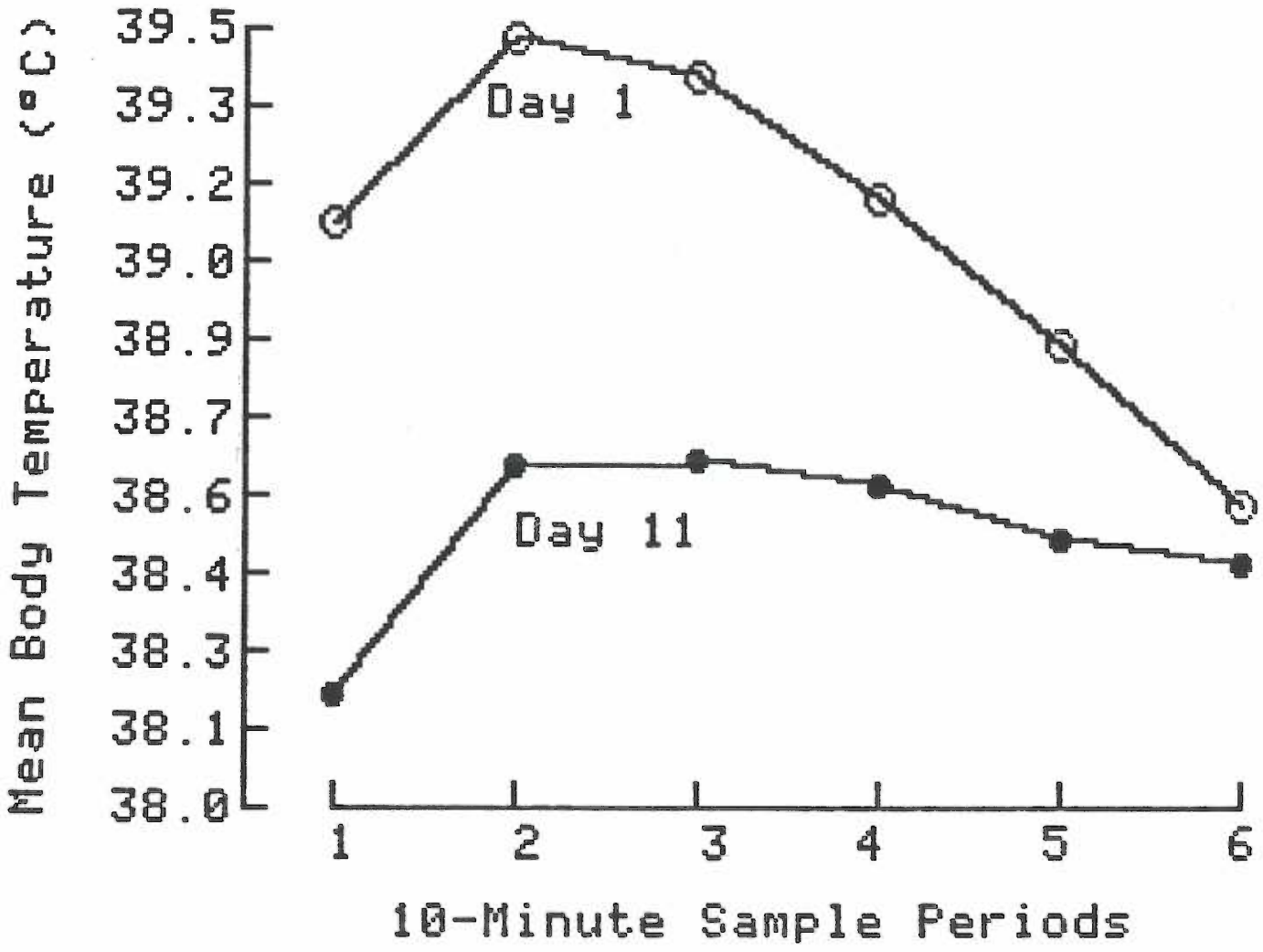

Mean Body Temperature (°C)
39.5
39.3
39.2
39.0
38.9
38.7
38.6
38.4
38.3
38.1
38.0
S
H
L
1
2
3
4
5
6
7
8
9
T
C
Days of Testing

Figure 7. Mean baseline temperature on Day 1 of acquisition and Day 11 (the placebo test). Data are collapsed across groups.


Mean Body Temperature (°C)
39.5
39.3
39.2
39.0
38.9
38.7
38.6
38.4
38.3
38.1
38.0
Day 1
Day 11
1
2
3
4
5
6
10-Minute Sample Periods

account for differences in change scores or post-injection scores between groups during later recording sessions.

Temperature baselines were also affected by repeated handling during tolerance acquisition and test phases, such that the magnitude of the handling-induced hyperthermia decreased over days. In addition, mean baseline temperature decreased over days in Groups S and H but not in Group L. This change may have been due to habituation to the handling procedure or to Mini-Mitter drift or both. Recalibration of Mini-Mitters after the experiment indicated that the linear-regression curve of some of the Mini-Mitters had drifted to the left while some had drifted to the right. There were no specific trends for either direction drift in any group.

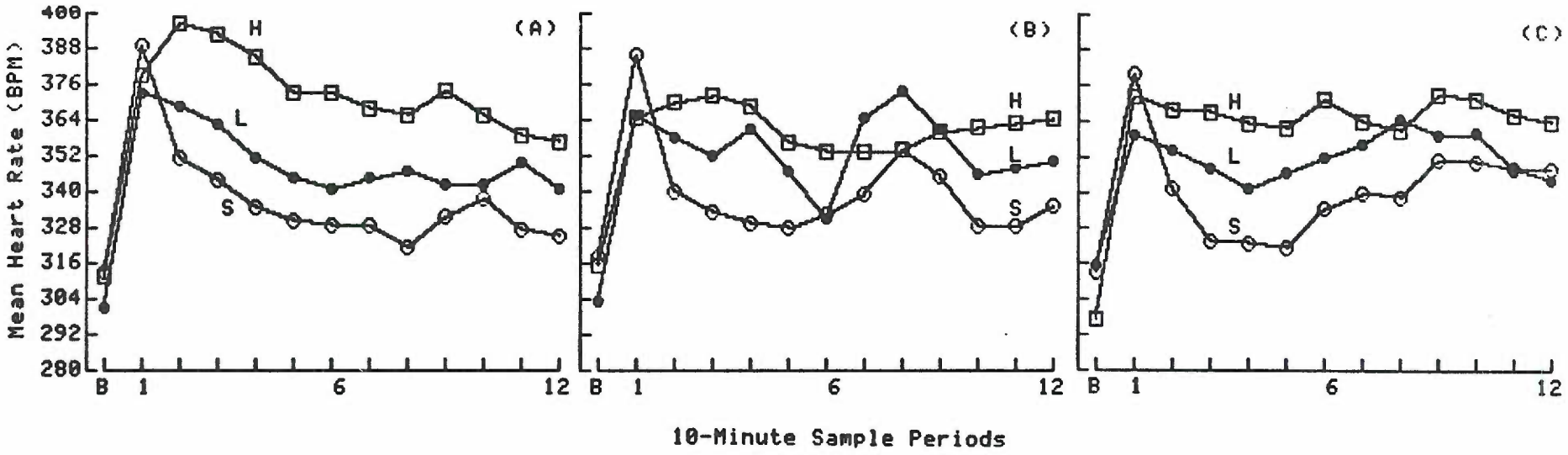
Acquisition

Heart Rate

Post Injection Scores. The mean heart rates of the three groups are graphed in the three panels of Figure 8 over sample periods and collapsed over consecutive 3-day blocks. Heart rate increased (relative to heart rate during the baseline period) in all three groups on all days immediately after injection and then tended to decrease over the 2-hr recording period. This decrease was greatest in Group S, with heart rate remaining fairly high in Group L and quite high in Group H. These differences did not appear to change over days.

A three-way ANOVA performed on these data revealed significant Dose x Sample Periods ($F\{24,228\} = 3.73$) and Days x Sample Periods ($F\{96,192\} = 2.01$) interactions and a significant Sample Periods effect ($F\{12,228\} = 26.97$) supporting these observations.

Figure 8. Mean heart rate of the three groups (S = Group S, L = Group L, H = Group H) during the 10-minute period before (B) and the two-hours after injection. Data are collapsed across Acquisition sessions 1, 2, & 3 (Panel A), sessions 4, 5, & 6 (Panel B) and sessions 7, 8, & 9 (Panel C).


Mean Heart Rate (BPM)
400
388
376
364
352
340
328
316
304
292
280
H
L
S
(A)
(B)
(C)
B
1
6
12
10-Minute Sample Periods

Within-group followup analyses found significant main effects of Sample Periods for all three groups ($F\{12,72\} = 11.58$, $F\{12,84\} = 10.04$, $F\{12,72\} = 12.77$) for Groups S, L, and H, respectively). A significant Dose effect ($F\{2,19\} = 7.37$) after 30 min but not after 10 or 120 min explains the Dose x Sample Period interaction. Newman-Keuls comparisons among the three groups found significant differences in mean heart rate between Group H and Group S at 30 min ($Cn\text{-}k\{3,19\} = 43.8$) and not in other pairwise comparisons.

Change Scores. The heart rate change scores of the three groups during tolerance acquisition are graphed over sample periods in Figure 9 (collapsed over days). Generally, there was an increase in heart rate immediately after injection in all groups. Heart rate remained elevated in Group H, diminished slightly in Group L and returned nearly to baseline levels in Group S. A significant Dose x Sample Periods interaction ($F\{22,209\} = 3.42$) and significant main effects for Dose ($F\{2,19\} = 4.86$) and Sample Periods ($F\{11,209\} = 9.58$) supported these observations. Followups revealed a significant Dose effect ($F\{2,19\} = 7.37$) after 30 min but not after 10 or 120 min. Newman-Keuls comparisons among the three groups indicated that the mean heart rate change at 30 min was greater in Group H relative to Group S ($Cn\text{-}k\{3,19\} = 50.7$) and in Group L relative to Group S ($Cn\text{-}k\{2,19\} = 29.1$). There were no differences during this time period between Groups L and H.

The overall analysis also yielded a significant Days x Sample Periods interaction ($F\{88,176\} = 2.13$). Figure 10 shows heart rate change scores on Day 1 and Day 9 collapsed across groups. There was

Figure 9. Mean change in heart rate of the three groups after injection. Data are collapsed over the nine acquisition days. The mean baseline heart rate prior to injection (collapsed over 9 days) was 323 in Group S, 319 in Group L and 306 in Group H.

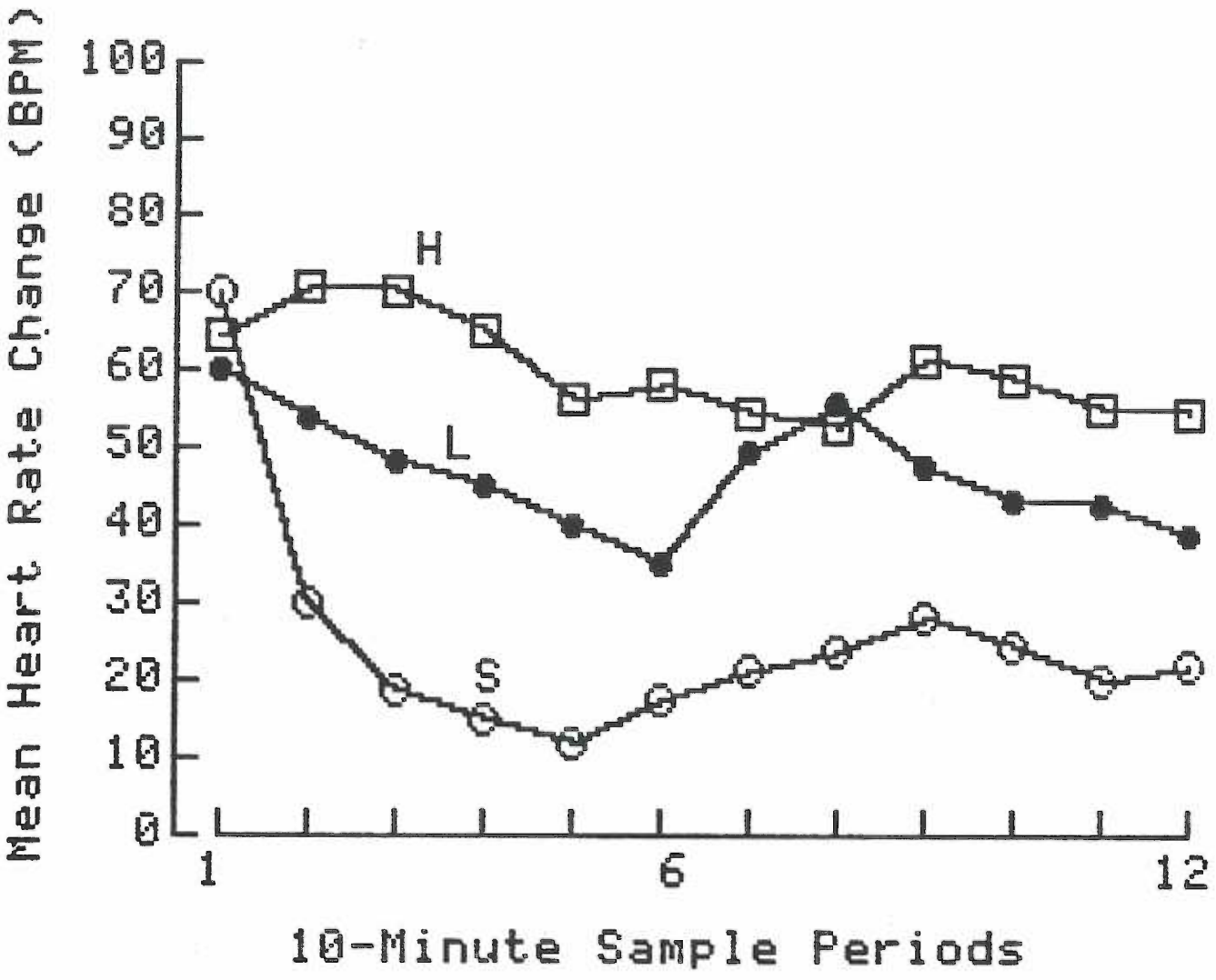
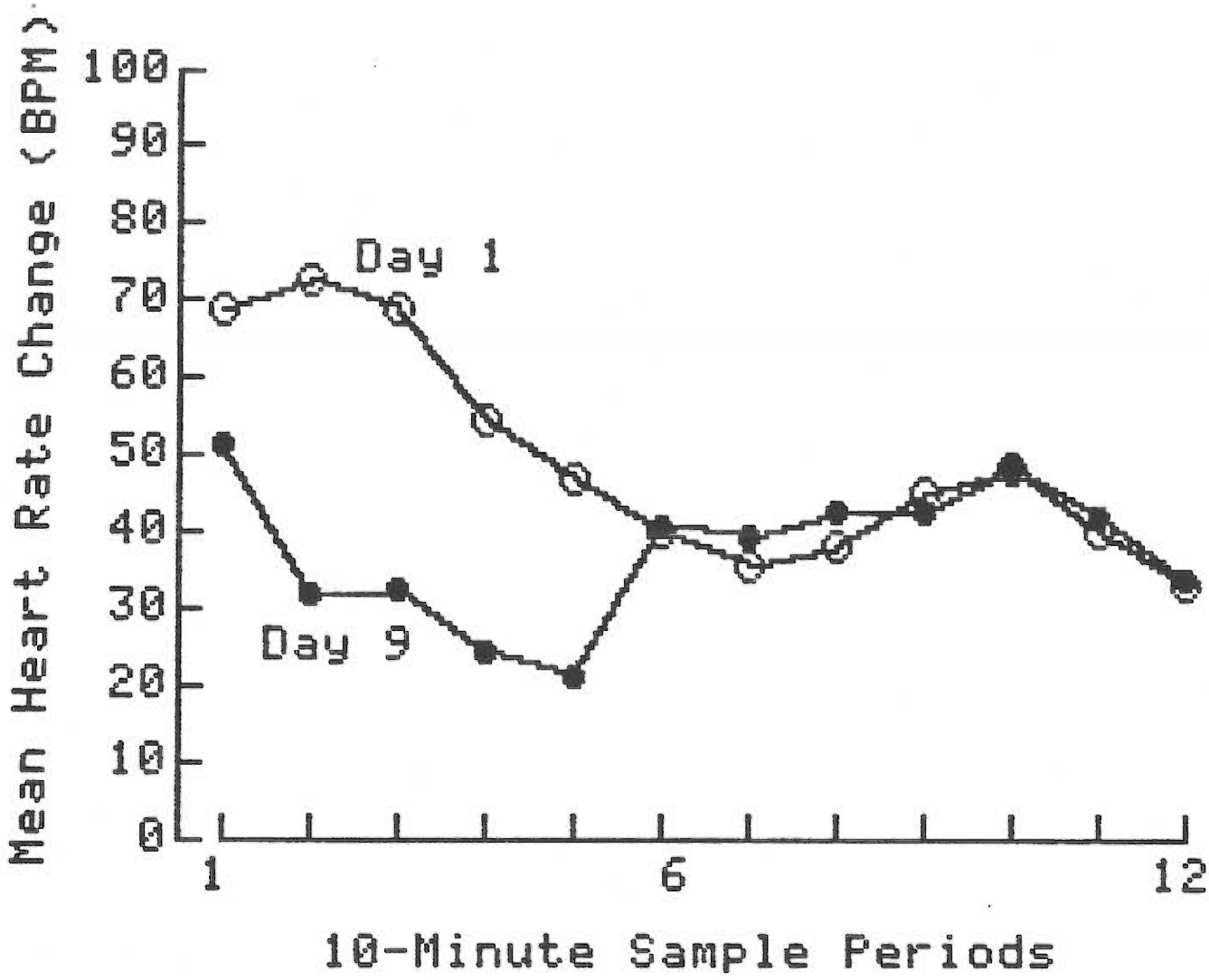

Mean Heart Rate Change (BPM)
100
90
80
70
60
50
40
30
20
10
0
H
L
S
1
6
12
10-Minute Sample Periods

Figure 10. Mean change in heart rate after injection on Day 1 and Day 9. Data are collapsed across groups. Baseline heart rate just before injection on Day 1 was 340 and on Day 9 was 350.


Mean Heart Rate Change (BPM)
100
90
80
70
60
50
40
30
20
10
0
Day 1
Day 9
1
6
12
10-Minute Sample Periods

less of an increase in heart rate during the first hour of Day 9 relative to that during the first hour of Day 1. This effect was present in all groups.

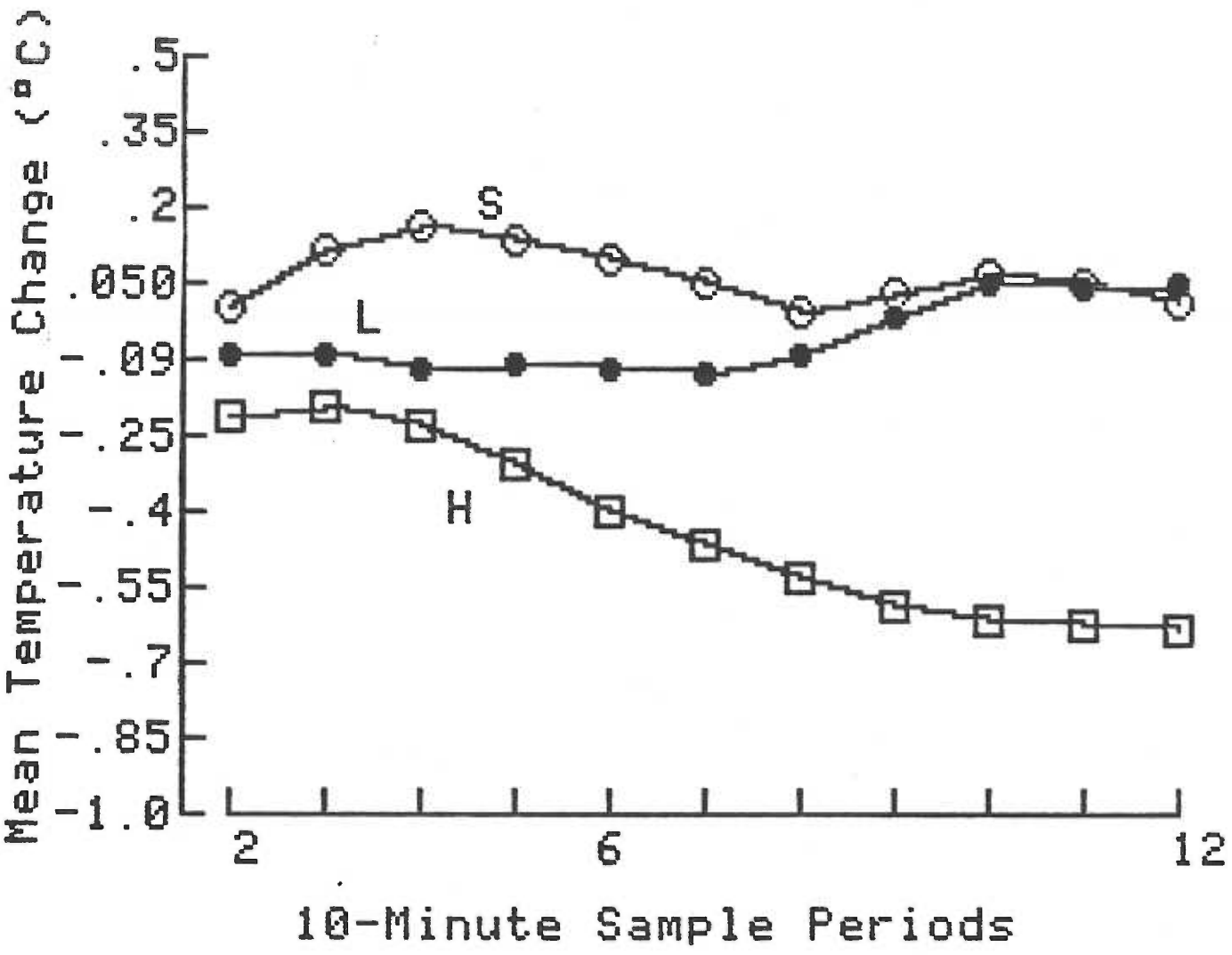
Temperature

Change Scores. The temperature change scores after injection are graphed in Figure 11 over sample periods and collapsed over days. Temperature was lower in both Groups L and H relative to Group S with the greatest difference appearing between Groups S and H. The differences appear to be due to both hypothermia in the ethanol groups and a slight hyperthermia in Group S. The hypothermia was greatest towards the end of the 2-hr recording session while the hyperthermia was greatest during the first hour after injection.

These observations were supported by a significant Dose x Sample Periods interaction ($F\{20,150\} = 4.07$) and main effects of Dose ($F\{2,15\} = 9.57$) and Sample Periods ($F\{10,150\} = 2.41$). Followup within-group comparisons revealed a significant Sample Periods effect in Group H only ($F\{10,60\} = 14.0$). When data from only Sample Period 2 or Sample Period 12 were analyzed, there was a significant Dose effect ($F\{2,15\} = 6.11$) during Sample Period 12 and not Sample Period 2 accounting for the Dose x Sample Period interaction. Newman-Keuls analyses on the data from Sample Period 12 found significant differences between Group H and each of the other groups ($Cn\text{-}k\{2,15\} = 0.6$ for H vs L and $Cn\text{-}k\{3,15\} = 0.64$ for H vs S) but not between Groups S and L.

The overall analysis also showed a Days x Sample Periods interaction ($F\{80,1200\} = 5.59$) indicating a tendency for the

Figure 11. Mean change in body temperature of the three groups after injection. Data are collapsed over the nine acquisition sessions. Baseline temperature was 38.5 in Group S, 38.4 in Group L and 38.45 in Group H.


Mean Temperature Change (°C)
.5
.35
.2
.050
-.09
-.25
-.4
-.55
-.7
-.85
-1.0
S
L
H
2
6
12
10-Minute Sample Periods

magnitude of hypothermia occurring during the last hour to decrease in all groups over days. This is supported by a significant Sample Periods effect on Day 1 ($F\{10,150\} = 20.29$) but not Day 9.

Although the interaction of Dose x Days x Sample Periods was statistically unreliable ($F\{160,1200\} = 1.2$, $p=.055$), an examination of the interaction was pursued for a number of reasons. First, prior data suggest that tolerance to the hypothermic effects of ethanol should have occured over this time course of administration. Second, there is the possibility that Mini-Mitter drift increased between-group variability thus decreasing the probability that such a Dose x Days interaction would be significant. Third, the probability of statistical significance was quite close.

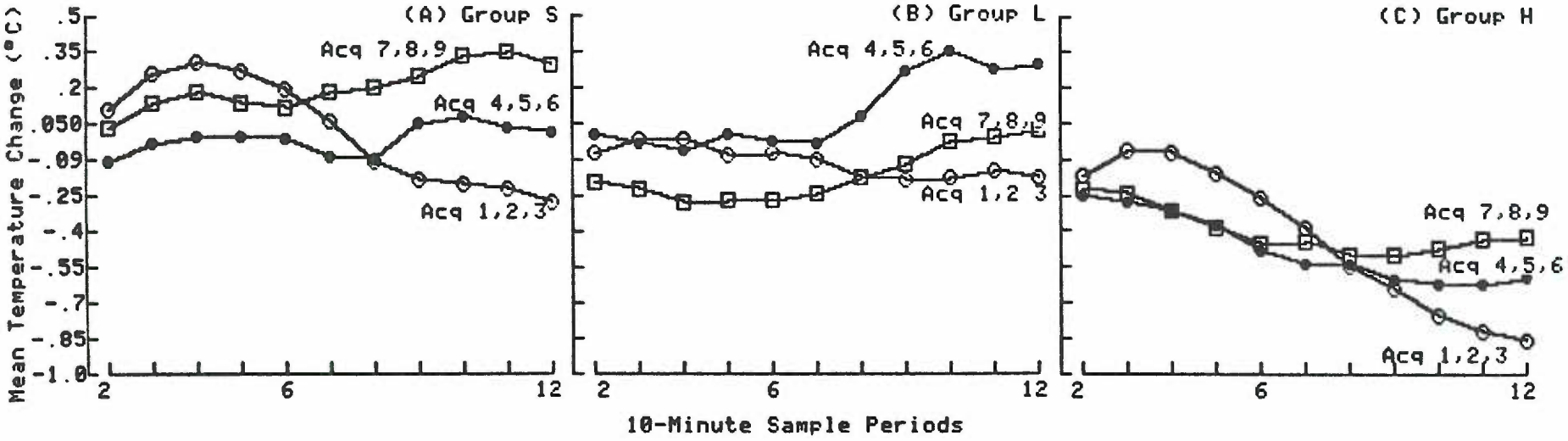
Mean temperature change scores of each of the three groups are graphed in Figure 12 collapsed over consecutive 3-day blocks. A decrease in the magnitude of hypothermia exhibited in Group H during the last 60 min of the 7th, 8th and 9th acquisition sessions may be indicative of tolerance. However, body temperature during the last hour also increased over days in Group S, implying an effect independent of ethanol exposure. In addition, unsystematic changes over days in Group L also occurred.

Summary: Acquisiton

There was a dose-related tachycardia associated with ethanol administration that disappeared within 120 min after injection. The magnitude of this response did not diminish after nine ethanol injections of either dose indicating that tolerance did not develop.

In contrast to the studies cited in the introduction, a 2.0 g/kg

Figure 12. Mean change in body temperature after injection during consecutive 3-day blocks of acquisition sessions. Scores from Group S are graphed in Panel A, Group L in Panel B and Group H in Panel C. Baseline temperature in Group S was 38.65 for Days 1-3, 38.5 for Days 4-6 and 38.45 for Days 7-9. Baseline temperature over the three blocks of days was 38.2, 38.05 and 38.46 for Group L and 38.67, 38.35 and 38.3 for Group H.


(A) Group S
(B) Group L
(C) Group H
Mean Temperature Change (°C)
.5
.35
.2
.050
-.09
-.25
-.4
-.55
-.7
-.85
-1.0
Acq 7,8,9
Acq 4,5,6
Acq 1,2,3
2
6
12
10-Minute Sample Periods

dose of ethanol did not elict hypothermia until 120 min after injection. A 1.0 g/kg dose led to a slight hypothermia but this was not significantly different from temperature changes after a saline injection. Group S exhibited a slight hyperthermia after injection on the first day, reminiscent of the handling-induced hyperthermia that occurred during habituation and baseline periods. However, this effect did not occur consistently over days and was not significant. Based on comparisons of groups during the acquisition phase, it cannot be confidently concluded that tolerance to the hypothermic effect of ethanol developed in this study although the possibility exists.

Tolerance Test

Heart Rate

Post-Injection Scores. Mean heart rate of the three groups after the test injection of 2.0 g/kg of ethanol was elevated (363 bpm relative to levels of 310 measured at the end of the baseline period) immediately after injection. Heart rate generally remained at this level throughout the 2-hr session for Group H, at times increasing to 380 bpm. Heart rate appeared to decrease slightly (to about 350 bpm) over the 2-hr session in Groups S and L.

A two-way ANOVA revealed a Dose x Sample Periods interaction ($F\{24,228\} = 1.86$) and separate within-group followups revealed a significant Sample Periods effect in Groups H and L ($F\{11,66\} = 2.79$ and $F\{12,84\} = 1.94$) but not Groups S. Data from Sample Period 1 and Sample Period 12 were analyzed in order to assess the nature of the Dose x Sample Periods interaction. There was a significant Dose effect during Sample Period 12 ($F\{2,18\} = 3.58$) but not Sample Period 1.

Newman-Keuls comparisons found that the mean heart rate was significantly greater in Group H than in either Group S or L. Mean heart rate of these two groups did not differ. It should be noted that the relatively greater heart rate in Group H occurred despite the fact that its baseline was significantly below those of the other two groups.

Change Scores. Heart rate change scores after a 2.0 g/kg ethanol injection are graphed in Figure 13. Heart rate was increased in all groups after injection and remained elevated in Group H, increasing slightly over the recording session. Heart rate of Groups S and L appeared to decrease slightly over the 2-hr period. A significant Dose x Sample Periods interaction ($F\{22,209\} = 1.71$) supported these observations. Within-group followup comparisons on the three groups found a significant Sample Periods effect in Group H only ($F\{11,66\} = 1.79$). Followup between-group analyses found a significant effect during Sample Period 12 ($F\{2,18\} = 4.07$) but not Sample Period 1 explaining the Dose x Sample Period interaction. Newman-Keuls comparisons found that heart rate of Group H was significantly higher than that of Group S ($Cn\text{-}k\{2,18\} = 65.7$) which is not indicative of tolerance. There were no other group differences.

Temperature

Change Scores. The temperature change scores after a 2.0 g/kg injection are graphed in Figure 14 for all groups. Temperature decreased about 1.0°C in both Groups S and L over the course of the 2-hr recording period. There was only a slight hypothermia (about 0.2 °C) in Group H which disappeared within 2 hr. A two-way ANOVA revealed

Figuer 13. Mean change in heart rate after a 2.0 g/kg ethanol injection in all three groups. Mean baseline heart rate was 318 in Group S, 330 in Group L and 285 in Group H.

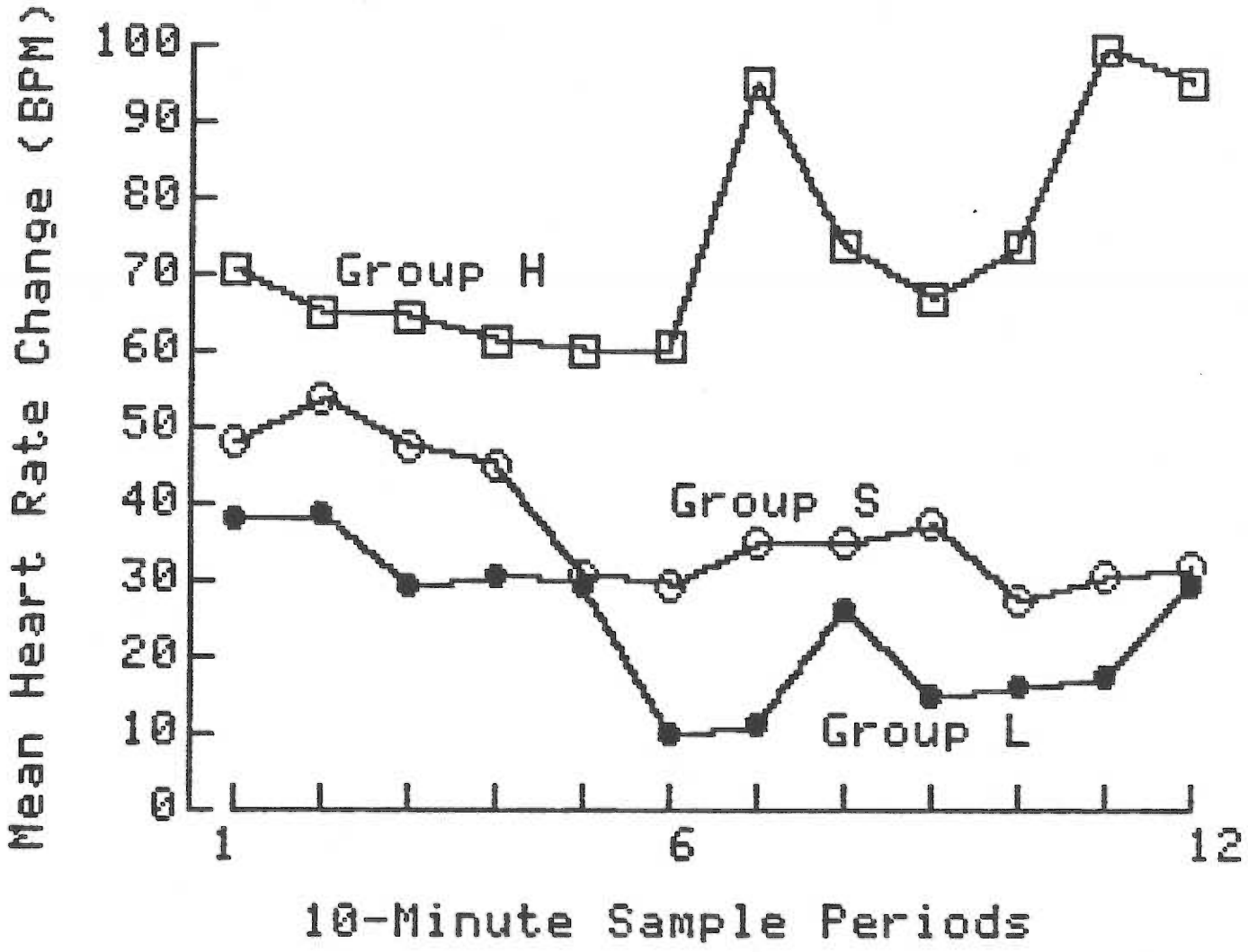
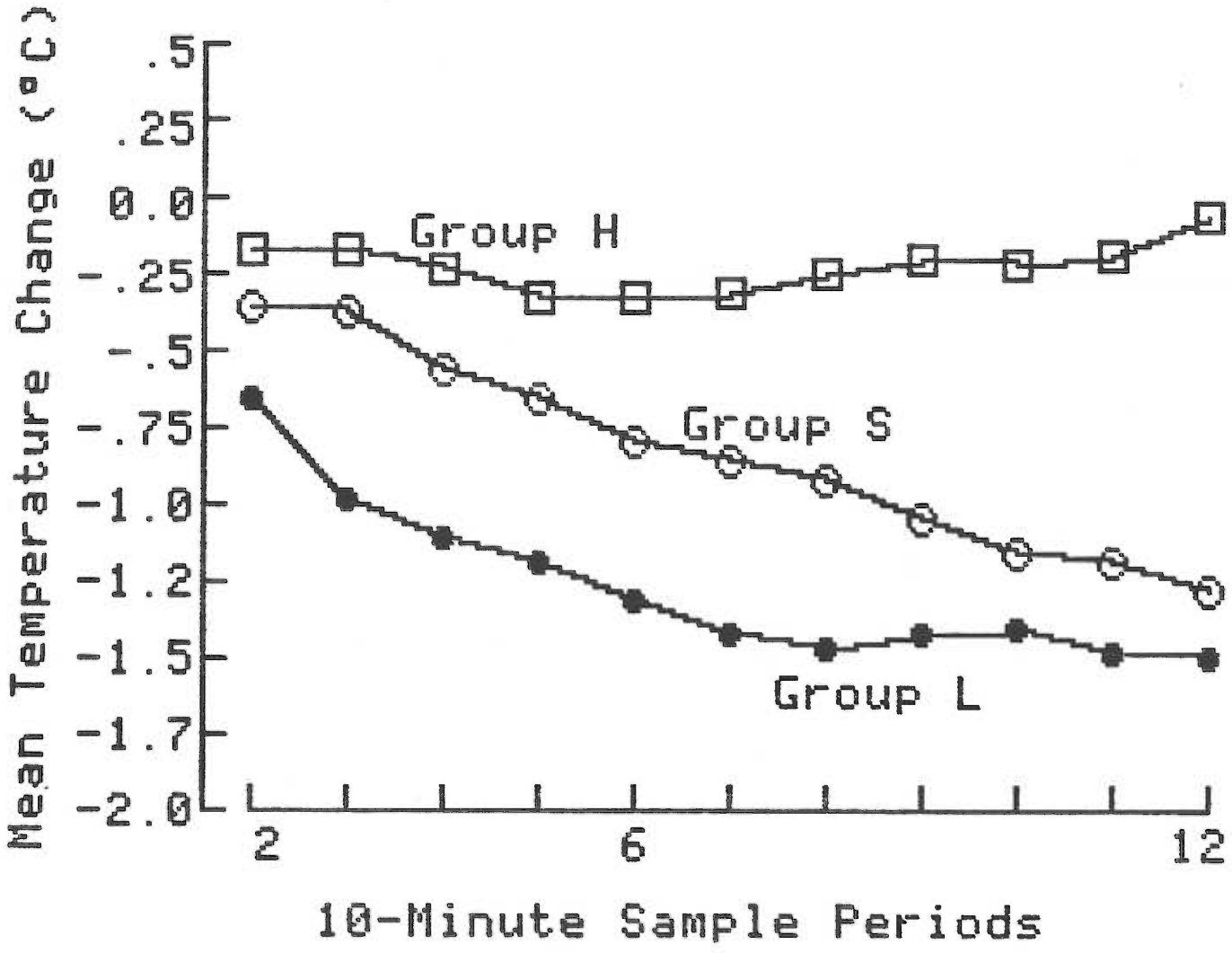

Mean Heart Rate Change (BPM)
100
90
80
70
60
50
40
30
20
10
0
Group H
Group S
Group L
1
6
12
10-Minute Sample Periods

Figure 14. Mean change in body temperature after a 2.0 g/kg ethanol injection in all three groups. Mean baseline temperature was 38.45 in Group S, 39.03 in Group L and 38.32 in Group H.


Mean Temperature Change (°C)
.5
.25
0.0
-.25
-.5
-.75
-1.0
-1.2
-1.5
-1.7
-2.0
Group H
Group S
Group L
2
6
12
10-Minute Sample Periods

a significant Dose x Sample Periods interaction ($F\{20,140\} = 5.97$) and significant main effects of Dose and Sample Periods ($F\{2,14\} = 10.24$ and $F\{10,140\} = 18.1$, respectively). Within-group analyses found a significant main effect of Sample Periods in Groups S and L ($F\{10,40\} = 13.04$ and $F\{10,40\} = 9.53$, respectively) but not in Group H.

Between-group comparisons of Group S versus each of the ethanol groups revealed a significant Dose x Sample Periods interaction for the comparison with Group H ($F\{10,110\} = 4.72$) and not for the comparison with Group L. These results explain the Dose x Sample Periods interaction.

Summary: Tolerance Test

The magnitude of ethanol-induced cardioacceleration was increased by previous ethanol administration in Group H but not Group L. This effect appeared both in the analysis of absolute heart rate and in the analysis of change scores. Post-hoc comparisons were made between heart rate change scores of each group during the tolerance test with heart rate change scores of Group H during the first acquisition session. Heart rate in Group H had increased from the first to the last ethanol exposure but only during later sample periods. Heart rate was lower in Group L during all sample periods after its first high dose of ethanol than that of Group H on Day 1 of acquisition. There was no difference between the initial responses of Groups S and H to the high dose. Therefore, repeated exposure to the high dose led to greater heart rate after the test dose while repeated exposure to the low dose led to lower heart rate. This finding in Group L may be

interpreted as tolerance but this is not supported by the absence of a significant difference between responding of Group L and Group S during the tolerance test.

A 2.0 g/kg dose of ethanol elicited hypothermia in Groups S and L but not Group H. This is indicative of tolerance development to the administration of 2.0 g/kg ethanol after nine injections. There was no difference between the responses in Groups S and L, signifying that nine previous administrations of 1.0 g/kg ethanol spaced 48 hr apart, were not sufficient to induce thermic tolerance to a 2.0 g/kg challenge dose.

Placebo Test

Heart Rate

Post-injection Scores. Mean heart rate after a saline injection in the recording environment was not affected by dose of previous ethanol injections. Heart rate immediately after injection was 385 bpm and decreased to 330 bpm within 20 min. Heart rate remained around 335 bpm for the remainder of the session in all groups. A two-way ANOVA revealed only a significant effect of Sample Periods ($F\{12,216\} = 6.23$).

Change Scores. Heart rate change scores after the saline injection were elevated in all three groups after injection but decreased within the first 30 min. This is supported by a significant main effect of Sample Periods ($F\{11,198\} = 5.56$). There were no significant main effects or interactions involving the dose of previous injections.

Temperature

Change Scores. Temperature change scores from the saline test were not significantly affected by dose of previous ethanol administrations. There was also no difference in temperature change scores across Sample Periods following saline injection.

Summary: Placebo Test

There was no evidence of conditioned responses (either compensatory or not) in either heart rate or temperature after a saline injection. The handling-induced cardioacceleration seen during tolerance acquisition and tolerance test days was still present in all groups after the saline injection. There was no effect of handling and injection procedures on body temperature during the placebo test unlike that seen in Group S during acquisition.

Saline Volume Effects

Baseline, post-injection and change scores for both temperature and heart rate were analyzed from the Subgroups S-lo and S-hi for all phases of the experiment in order to assess the effects of injection volume. There were no effects or interactions due to volume when habituation, baseline or test day data were analyzed but there was a significant volume x Sample Periods interaction ($F\{10,40\} = 3.02$) involving the temperature change scores during tolerance acquisition. This interaction was due to significant temperature increases ($F\{10,20\} = 3.75$) of small magnitude (about 0.3 C) in the high volume subgroup during the last hour of the recording period. Despite this difference, all subjects in these subgroups were analyzed as Group S.

Discussion

Tolerance did not develop to the dose related cardioacceleratory effect of ethanol after nine ethanol injections. There was no trend for decreasing cardioaccelerations over days which might indicate that tolerance would have occurred if additional tolerance acquisition sessions had been given. Although heart rate in Group H decreased just prior to injection (in a fashion similar to a compensatory cardiodeceleratory CR), it did not summate with post-injection cardioacceleration to produce a diminished effect of ethanol on heart rate. Instead, ethanol caused a greater effect after the tolerance acquisition phase. The depressed baseline in Group H could be the result of the development of an anticipatory conditioned response due to the presentation of cues associated with ethanol administration. The effect of this type of CR on subsequent heart rate responses to ethanol and its implication for drug tolerance is not clear. The data from the tolerance and placebo tests support the conclusion that tolerance did not occur when either of the two dosing regimens were employed.

The ethanol-induced cardioacceleration reported in this study replicates those found by Crow (1968) using a 1.8 g/kg dose and by Fitzgerald and Stainbrook (1978) using a 0.8 g/kg dose. The biphasic response reported by Fitzgerald and Stainbrook after a 2.4 g/kg injection of ethanol or by Wilkin et al. (1982) using 0.8 g/kg dose was not replicated in the present study. This difference may have been due to the high degree of restraint that was employed in the studies by

Fitzgerald and colleagues relative to the freely-moving preparation employed in the present study. The development of sensitization to the cardioacceleratory effects of ethanol found in the present study, rather than diminution of this effect (as would be seen if tolerance developed) partially supports the findings of Wilkin, et al. (1982).

In contrast to previous experiments that used similar doses (Crowell, Hinson & Siegel, 1981; Mansfield & Cunningham, 1980), ethanol induced a relatively small magnitude hypothermia which did not peak until almost 120 min after injection. The previous experiments reported a hypothermia of 1.5°C magnitude occurring within 30-60 min after injection. Ambient temperature of the experimental chambers, which has been found to affect ethanol-induced hypothermia (Pohorecky & Rizek, 1981), was comparable to that of other studies and was therefore not suspected for decreasing the initial effect of ethanol or tolerance to that effect.

It can be concluded that tolerance developed to the hypothermic effect of ethanol in Group H only, even though the initial magnitude of this response was less than that seen in other studies. The development of tolerance is supported mostly by the results of the tolerance test, in which the hypothermic response to ethanol in Group H was greatly reduced compared to that seen in Groups S and L. The magnitude of the hypothermia induced by the high dose of ethanol during training also appeared to decrease over tolerance acquisition days, but because of concurrent changes in Group S and Group L, conclusions concerning the development of tolerance could not be supported from the tolerance acquisition phase alone.

One possible explanation for the small magnitude hypothermic response is the relatively small amount of handling in this study. One mechanism of ethanol-induced hypothermia hypothesizes a decreased set point in the temperature regulation system, therefore, a handling-induced elevation of baseline temperature before ethanol injection may appear to cause a more pronounced effect of the drug. For example, if ethanol changes the setpoint from 38°C to 37°C and handling increases temperature to 39°C, then decreases in body temperature after an ethanol injection will appear greater in handled animals relative to those left undisturbed. In the present experiment, ethanol was not given until 60 min after initial handling occurred. Thus baseline had returned almost to normal levels before ethanol administration resulting in an apparent decrease in the magnitude of the ethanol response.

Another possible explanation of the discrepancy between this and previous findings is that the absence of continuous handling (because of the use of the Mini-Mitters for temperature measurement) decreased the magnitude of the unconditioned response to ethanol. A decrease in the magnitude of the UR would be expected to decrease the magnitude of any conditioned compensatory CR (see Mackintosh, 1974) that may develop concurrent with tolerance. This possibility was addressed in subsequent experiments.

EXPERIMENT 2

Experiments 2 and 3 were designed to investigate more closely the effects of handling and ethanol administration on body temperature and

heart rate. Experiment 2 focused on the acute effects of ethanol and handling on body temperature, while Experiment 3 studied both the acute effects and the development of tolerance to chronic exposure to ethanol.

The hypothesis that handling affects body temperature has been addressed by a few studies, some of which also measured the effects of both handling and ethanol on body temperature. Ethanol-induced hypothermia is well-documented, while handling has been shown to induce hyperthermia (Cunningham & Peris, 1983; York & Regan, 1982). There are a number of ways in which handling and intoxication might combine to affect body temperature, one of which is simple algebraic summation. Handling-induced hyperthermia and ethanol-induced hypothermia could summate to result in either a diminished hypothermia or hyperthermia (depending on which component is of the greater magnitude).

York and Regan (1982) found that placement of a caged rat on a benchtop led to hyperthermia that did not decrease in magnitude with repeated treatment. The hypothermic response to 2.0 g/kg of ethanol was lessened by placement of the cage on the benchtop. A 1.0 g/kg dose, that did not significantly decrease body temperature when given alone, decreased the magnitude of the hyperthermia that developed when cages were placed on the benchtop.

If handling is characterized as a stressful procedure (which is not unreasonable since handling raised both heart rate and body temperature in Experiment 1) then the literature reviewing the effects of stressors on the degree of ethanol intoxication must also be considered. There is a general view that stressors decrease the

effects of intoxicating substances (Pohorecky, 1981). According to this view, one would expect that handling would decrease the magnitude of the ethanol-induced hypothermia regardless of the direct effect of handling on temperature. Most of these studies (Frankenhauser, Dunne, Bjustrom & Lundberg, 1974; Leikola, 1961; Wallgren & Tirri, 1963) used behavioral rather than physiological indices of the effects of stress on the degree of ethanol intoxication (e.g., operant responding, motor coordination, analgesia).

On the other hand, the combination of stressful handling procedures and ethanol intoxication may interact to cause a potentiation of changes in body temperature (e.g., handling might increase the magnitude of hypothermia seen after ethanol injection). Myrsten, Lamble, Frankenhauser and Lundberg (1979) found that reward (which was hypothesized to increase achievement stress) counteracted the depressive effects of ethanol on mood and performance but additively combined with the arousing effects of ethanol on physiological variables (e.g., heart rate and catecholamine and cortisol secretion). Blood alcohol levels of the human subjects were not different under stressful and non-stressful conditions.

Myrsten et al. (1979) concluded that stress potentiated ethanol intoxication implying that the combined effects of ethanol and stress were greater than the sum of the effects of these two variables applied separately. In actuality, their data indicated that both physiological and behavioral responses to stress and ethanol were simply additive. When the effects of ethanol and stress on a particular measure were in the same direction, stress appeared to increase the effect of ethanol.

When they were in opposite directions, stress decreased ethanol intoxication. They did not analyze the data in a way that would reveal significant interactions of stress and ethanol which would support the potentiation hypothesis nor did it appear from the data that a potentiation effect occurred.

An interaction of stress and drug intoxication was found by McDougal, Marques and Burks (1981) using morphine and restraint stress. In unrestrained rats, morphine caused predominantly hyperthermia while there was a dose-related biphasic effect in restrained rats. Low doses of morphine led to hyperthermia while higher doses caused hypothermia. They did not conclude that stress was the cause of this interaction since plasma corticosteroid levels did not differ between stressed and non-stressed animals 2 hr after placement in the restrainers or non-restraining chambers. Instead, they suggested that the inability of the restrained animals to minimize heat loss through postural adjustment (decreasing skin surface exposure to air) led to a greater decrease in temperature after morphine.

Rationale and Method Summary

The effects of ethanol and handling stress on body temperature and any interaction of these two variables was the focus of Experiment 2. Animals were freely moving; therefore, postural adjustments were not prevented. In order to test how handling-induced hyperthermia and ethanol-induced hypothermia combine to affect body temperature in rats, two designs were employed that compared the effects of two handling procedures on the body temperature of rats when intoxicated or when not. In the design used in Phase 1, body temperature was recorded from

two groups of rats after either a saline or ethanol injection (2.0 g/kg). One group (Group N) was not handled after initial injection and placement into the chamber. The other group (Group P) was handled and probed as if for rectal temperature measurement, prior to, and at 30-min intervals after, injection. The design used in Phase 2 employed a within-group comparison in which the effects of probing and non-probing procedures were measured on two consecutive days when animals were given an ethanol injection.

Method

Subjects

Subjects were 12 adult male albino rats (Holtzman Co, Madison, Wisconsin) which were 160 days old and weighed about 638 g at the start of testing. They had previously participated in an experiment designed to measure the effects of various handling procedures on body temperature. This experiment is more fully described in the appendix (Cunningham & Peris, 1983). The animals were housed and maintained as in Experiment 1.

Apparatus

Experimental chambers and temperature monitoring apparatus were those described in Experiment 1. Mini-Mitters were Model M and were equilibrated so that they no longer drifted before implantation. The rectal probe was a small flexible temperature monitoring type (YSI, Model 402).

Surgical Preparation

Mini-Mitters were surgically implanted in the intraperitoneal cavity as described in Experiment 1. Due to the length of the previous

experiment, Mini-Mitters had been implanted 9 days before the first test day of this experiment.

Procedure

Phase 1. On each day of testing, rats were brought to the experimental room in standard shoebox cages on a cart and were then weighed. The transfer procedure lasted about 5-10 min and the weighing procedure about 2-3 min. After a 30-min waiting period in the shoeboxes, all rats were given either a saline or ethanol injection (2.0 g/kg, 17.8 % v/v with saline, maintained at 25°C). They were then placed in the chambers for 150 min during which body temperature was recorded. This constituted the treatment for Group N (n = 6). Group P (n = 6) was handled for rectal temperature measurement immediately before injection and 30, 60, 90 and 120 min after injection. This probing procedure involved removing the rat from the recording chamber, snugly wrapping it in a towel, and then inserting the lubricated tip of the probe 6 cm into the animal's rectum for 60 sec. Injections were given on consecutive days with half of the rats in each group receiving ethanol first and the other half receiving saline first.

Phase 2. One week after Phase 1 was completed, 10 of the same animals were used in a within-group comparison of the effects of ethanol on body temperature under both handling conditions (2 rats were discarded because their Mini-Mitters stopped transmitting after Phase 1). Transporting and weighing procedures were the same as those used in Phase 1. All animals received an injection of ethanol before placement in the chamber on two consecutive days. Rectal probing took place on one day; on the other day, the rats were left undisturbed.

Four rats received the probing procedure on the first day and six rats received it on the second day. The chamber doors were left open during this phase of the experiment to equate for possible differences in ambient temperature in the chambers.

Results

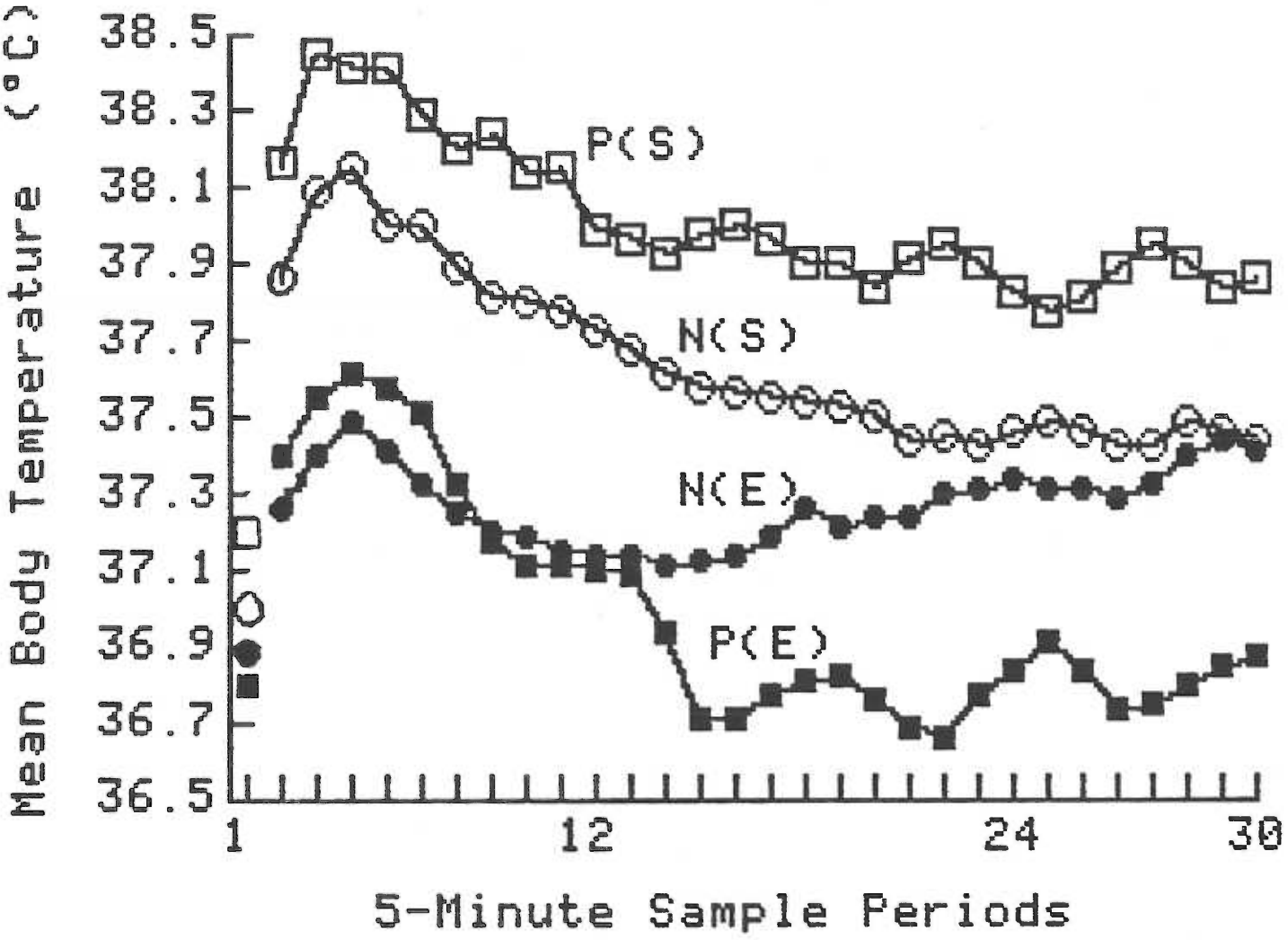
The data from two rats were discarded before Phase 2 due to loss of the Mini-Mitter signal between phases. Therefore, data from only 10 rats were included in the analyses of Phase 2. Mean body temperature was calculated for each 5-min period of the 150-min test sessions. Because baseline (i.e., pre-injection) scores were not obtained from either phase of this experiment, only the post-injection scores were analyzed.

A three-way ANOVA was performed on the data from Phase 1. The between-group variable was handling treatment (Group N vs Group P) and the within-group variables were drug (Saline vs Ethanol) and 5-min sample periods. A two-way ANOVA was performed on data from Phase 2 which included handling treatment (Not Probed vs Probed) and 5-min sample periods as within-group variables. Analyses of order effects and habituation are included in the Appendix.

Phase 1

The mean body temperatures of the two groups after either a saline or an ethanol injection are graphed in Figure 15 over sample periods. Temperatures were lower in both groups after an ethanol injection than those after a saline injection. While temperatures in Group N were lower than those of Group P after a saline injection, the

Figure 15. Mean body temperatures of Group P and Group N after an ethanol injection (E) or after a saline injection (S). The first unconnected point illustrates the artifactual drop in temperature due injection of the cool fluid.


Mean Body Temperature (°C)
38.5
38.3
38.1
37.9
37.7
37.5
37.3
37.1
36.9
36.7
36.5
P(S)
N(S)
N(E)
P(E)
1
12
24
30
5-Minute Sample Periods

reverse was true after an ethanol injection. Temperature was lower in Group P than in Group N when the animals were intoxicated and this difference was greatest during the last 90 min of the session.

These observations were supported by significant Groups x Drug x Sample Periods ($F\{29,290\} = 4.62$), Groups x Drug ($F\{1,10\} = 14.30$), Drug x Sample Periods ($F\{29,290\} = 2.82$), and Groups x Sample Periods ($F\{29,290\} = 1.66$) interactions. There were also significant Drug ($F\{1,10\} = 64.20$) and Sample Periods ($F\{29,290\} = 18.57$) main effects. There were significant Drug x Sample Periods interactions in both Groups N and P ($F\{29,145\} = 5.79$ and 2.43, respectively) but only a main effect of Drug in Group P ($F\{1,5\} = 123.52$).

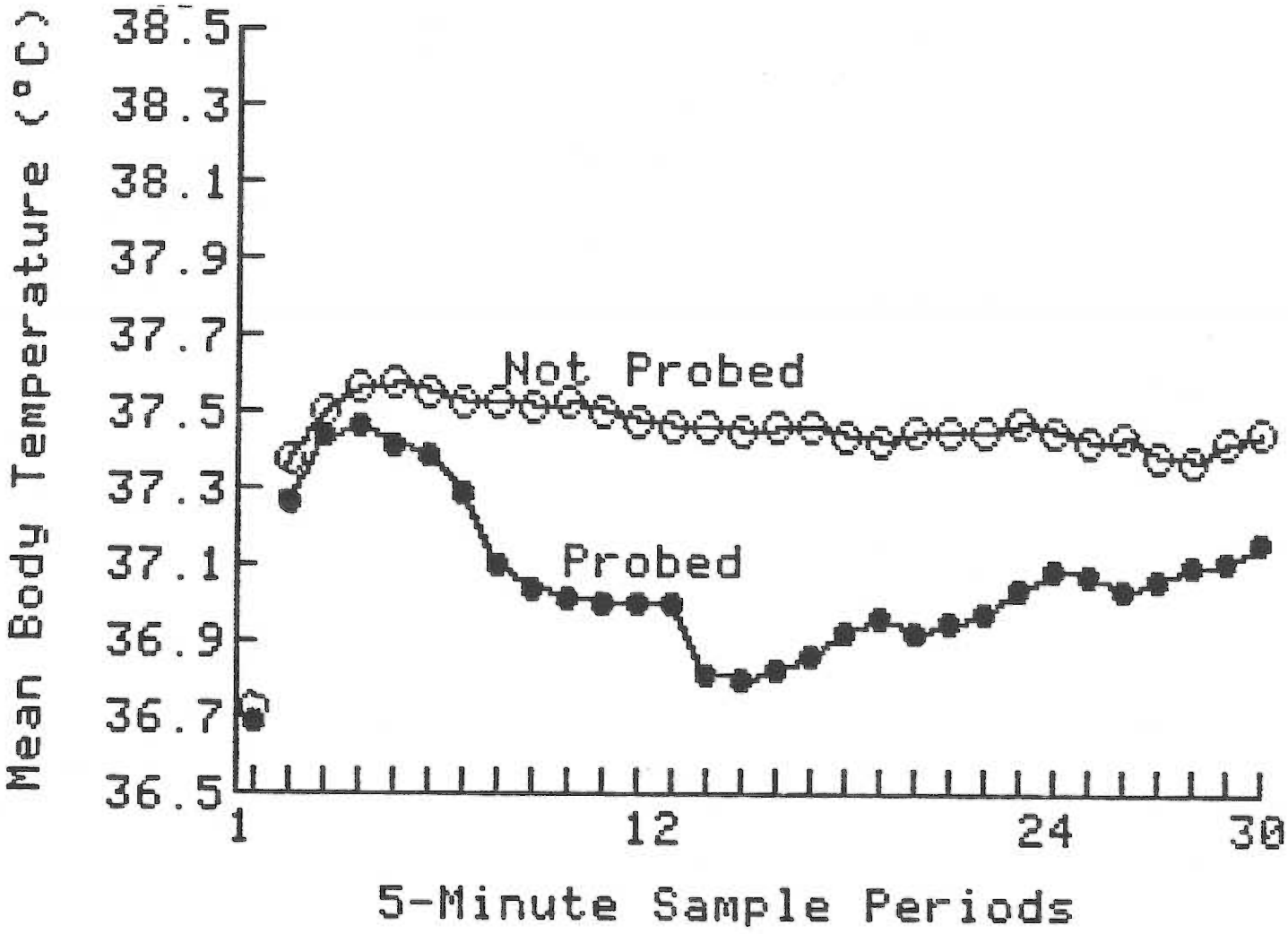
Separate followups on the Saline vs Ethanol conditions revealed Groups x Sample Periods interactions for the Ethanol comparison only ($F\{29,290\} = 5.32$). Therefore, the handling treatment affected temperature significantly only while rats were intoxicated. When rats received a placebo, there was a trend for probing-induced hyperthermia but this was not significant.

Phase 2

Body temperature throughout both handling treatments and after a 2.0 g/kg injection are graphed in Figure 16 over 5-min sample periods. Temperature decreased about 0.7°C during the Probed condition over the course of the 2-hr recording period but only about 0.2°C during the Not Probed condition. Temperature was lower throughout the Probed test condition relative to the Not Probed condition with the greatest difference occurring about 75 min after injection.

A two-way ANOVA revealed a significant Handling Treatment x

Figure 16. Mean body temperature after an ethanol injection when animals are left undisturbed (N) or probed (P) just before and every 30 min after injection. The first unconnected point illustrates the injection artifact.


Mean Body Temperature (°C)
38.5
38.3
38.1
37.9
37.7
37.5
37.3
37.1
36.9
36.7
36.5
Not Probed
Probed
1
12
24
30
5-Minute Sample Periods

Sample Periods interaction ($F\{29,261\} = 4.54$) and significant main effects of Handling Treatment ($F\{1,9\} = 7.24$) and Sample Periods ($F\{29,261\} = 10.04$). These statistics support the observations made from the graph.

Discussion

Findings from both the between-group (Phase 1) and within-group (Phase 2) designs indicated that while probing alone induced a slight hyperthermia and ethanol alone elicited hypothermia, probing of intoxicated rats led to a large magnitude hypothermia. The peak hypothermic response occurred sooner after injection when rats were probed than when they were not probed. This interaction between probing and ethanol intoxication is contrary to the summation hypothesis suggested by York and Regan (1982). Such an interaction has not been considered in studies where rectal temperature measurement procedures have been the sole means of measuring the hypothermic effects of ethanol. Handling not only exacerbated the direct effect of ethanol but also the difference between temperatures after saline and ethanol injections.

EXPERIMENT 3

Experiment 3 studied the effects of rectal probing on the acute and chronic effects of ethanol on heart rate and body temperature. In addition, the effects of handling procedures on conditioned tolerance were also examined in order to determine whether handling, usually considered a nonassociative behavioral mechanism, might actually be involved associatively (i.e., serve as a cue for the evocation of

conditioned compensatory responses).

If handling interacts with ethanol intoxication to potentiate hypothermia, then it is possible that tolerance may also be affected by handling during intoxication. Mansfield and Cunningham (1980) compared ethanol tolerance in rats that received ethanol and saline injections on different days. They found that tolerance was greater in rats handled during ethanol exposure than in rats that were not handled during ethanol exposure. Conditioned hyperthermia was also less in rats not handled during ethanol exposure. One might hypothesize that handling-induced augmentation of ethanol-induced hypothermia enhanced tolerance development in groups that were handled while intoxicated. If handling increases the magnitude of the unconditioned response to ethanol (similar to a dose-related increase in UR magnitude), then a conditioned compensatory response developing during tolerance acquisition would have to be of greater magnitude. Tolerance would then be greater in handled rats relative to non-handled rats.

When handling is characterized as a stressful situation, a number of hypotheses about the interaction of stress with drug tolerance may be applied to the effects of handling on tolerance. Siegel (1983) discusses several ways that stress may be relevant to drug addiction and tolerance: states induced by stressors may serve as cues predicting drug intoxication, as compensatory CRs and/or as direct potentiators of the drug effect. For example, stress present during drug intoxication may become associated with the systemic effects of the drug, and like similarly paired exteroceptive cues, may come to elicit drug-compensatory CRs.

Carder (1978) found that drug exposure contiguous with a stressor or arousing event produced a greater degree of tolerance than drug exposure in the home cage, even if the stressful experience was dissimilar to the tolerance test procedure. Exposure to stressful procedures without previous drug administration also decreased the effect of the drug during the tolerance test in a manner consistent with the development of tolerance. Carder concluded that increased stress (caused by placement in the drug-paired environment) during a period of intoxication facilitates tolerance development and that exposure to Pavlovian conditioning trials or the opportunity to obtain instrumental reinforcement may have minimal effects on the development of drug tolerance.

Other investigators, however, do not feel that stress is important in the development of tolerance. For example, Gebhart, Sherman and Mitchell (1972) studied tolerance to the analgesic effect of morphine in stressed rats (restraint was one method used) for four consecutive days. The rats were not exposed to analgesic testing until the fifth day. The analgesic effect of morphine in the stressed rats was not different from that in nonstressed rats receiving morphine daily (but also not tested on the hot plate until the fifth day). The analgesic response to morphine in rats tested all five days on the hot plate was significantly lower than that observed in the stressed animals. It was concluded that stress _per se_ does not significantly contribute to the results obtained with the hot plate procedure but that opportunity for intoxicated practice of the response was important.

The effect of stress on tolerance developing in studies using classical conditioning designs has not yet been addressed. Kesner and Baker (1981) believe that a major problem with Carder's hypothesis for stress-induced tolerance is that it is unable to account for the elicitation of drug CRs when saline is administered in the presence of cues formerly associated with the drug. His hypothesis is also refuted by those studies like Mansfield and Cunningham (1980) that give drug and saline in environments other than the home cage, thereby equating all groups for the arousal stress that would be predicted by Carder to occur outside of the home cage. Their design limits explanations of tolerance to those including context-specificity of anticipatory drug responses. No studies have yet addressed the possibility that stress and other affective states may interact with ethanol intoxication and that the effects of this interaction may be classically conditioned to environmental and/or handling cues.

Rationale and Method Summary

In Experiment 2, handling associated with the measurement of rectal temperature augmented hypothermia induced by acute exposure to ethanol. An interaction of this type may affect the development of tolerance to ethanol-induced hypothermia. This hypothesis was addressed in Experiment 3. In addition, the effects of handling on the cardioacceleration induced by both acute and chronic administration of ethanol was also studied. Gliner, Horvath and Browe (1978) examined the interaction of a stressor (tailshock) and ethanol on a number of cardiovascular measures and found that although shock increased heart rate, neither ethanol alone nor in combination with the shock stressor

affected heart rate levels differently from the saline controls (with or without shock). However, both stressed and unstressed rats in this study were restrained which may have increased heart rate to near maximum levels.

Stressor levels have been found to affect the cardiovascular response to morphine (Cunningham, Peris & Schwarz, in preparation). The heart rate responses to five doses of morphine (infused through the jugular vein) were measured in two groups of rats: one group was restrained throughout the infusion and recording procedures and the other was freely-moving. Heart rate generally decreased after drug infusion, but to a greater degree in the restrained rats. Unrestrained animals then showed a dose-dependent acceleration that was of a longer duration than the decelerative phase. If directionality of heart rate responses to ethanol is affected by handling procedures in the same way as restraint procedures, then this would increase the generality of the hypothesis that stressful stimuli change the physiological responses to drugs.

Experiment 3 also included procedures which were intended to permit replication of the findings of Experiment 1 concerning changes in heart rate before and after repeated ethanol administrations. That study showed that after repeated exposure to ethanol, baseline heart rate of animals was depressed just before injection and the magnitude of ethanol-induced cardioacceleration was increased. Both of these changes occurred only after repeated administration of the high dose of ethanol (2.0 g/kg).

The procedure of this experiment was very similar to that of

Experiment 1 except that the amount of handling during intoxication as well as type of injection (saline vs ethanol) were used as between-group variables. Only one dose of ethanol (2.0 g/kg) was given, since this dose was most effective in eliciting both cardiovascular and thermic changes in the first study. There were three groups (see Table 1): Group AP+ was handled for rectal temperature measurement while intoxicated in the test environment during tolerance acquisition; Group AN+ received ethanol during the session but was handled and rectally probed only on alternate days while unintoxicated in the home cage; and, Group AP- was rectally probed during the test sessions but received saline injections throughout tolerance acquisition.

As in Experiment 1, all groups underwent tolerance testing during which ethanol was given to all animals and conditioned response testing during which saline was given. Each rat was tested twice for tolerance and twice for conditioned responses, once while undisturbed and once while rectally probed. These tests were designed to indicate whether handling-induced augmentation of tolerance is cue specific and also whether it increased the magnitude of the CR. This would support the hypothesis that the cues provided by the handling procedure (possibly including the presence of an affective state such as stress) could support the development of conditioned ethanol tolerance.

Method

Subjects

The subjects were 24 adult male albino rats (Holtzman Co., Madison, Wisconsin), 70 days old at the start of testing and weighing

Table 1. A summary of group treatments during Experiments 3 & 4. For the tolerance and conditioned response tests, half of each acquisition group was tested first under the same handling condition that was paired with the drug, and half was tested first under a different handling condition. All rats were tested under both handling conditions. A = Recording environment; B = Home environment; (+) = ethanol injection; (-) = saline injection; P = rectally probed; and N = not probed. Experiment 3 included Groups AP+, AN+ and AP- with 8 subjects/group at the start of the experiment. Experiment 4 included all 4 groups with 6 subjects/group at the start of the experiment.

Group	Habituation Phase	Acquisition Phase	Tolerance Tests		CR Tests	
			1	2	1	2
AP+	AN	AP+ / BN	AP+	AN+	AP-	AN-
			AN+	AP+	AN-	AP-
AN+	AN	AN+ / BP	AP+	AN+	AP-	AN-
			AN+	AP+	AN-	AP-
AP-	AN	AP- / BN	AP+	AN+	AP-	AN-
			AN+	AP+	AN-	AP-
AN-	AN	AN- / BP	AP+	AN+	AP-	AN-
			AN+	AP+	AN-	AP-

an average of 350 g. The animals were housed and maintained as in Experiment 1.

Surgical Preparation

Heart rate electrodes and Mini-Mitters were surgically implanted as in Experiments 1.

Apparatus

Experimental chambers and heart rate and temperature monitoring apparatus were those used in Experiment 1. All Mini-Mitters were Model M and were equilibrated for drift before implantation.

Procedure

A detailed outline of the experimental design for the three groups is given in Table 1. The procedure used during this experiment was similar to that used in Experiment 1. Following surgery, rats were distributed to individual cages where they remained undisturbed for 48 hrs. This was followed a series of habituation sessions, tolerance acquisition training sessions and two phases of test sessions. As in Experiment 1, all sessions were 48 hr apart. The habituation phase (Column 2, Table 1) was identical to that given in Experiment 1 except that it lasted 3 days. After that phase, rats were distributed to three groups based on basal heart rate and body temperature during the first 60 min of the habituation sessions and on body weight (measured before surgery).

During the acquisition phase of the experiment (Column 3, Table 1), rats were transferred to the testing area and weighed as in Experiment 1. Animals were placed in the test chamber (Environment A in Table 1) and then 60 min later were briefly removed and injected.

All solutions were maintained and injected at 35°C to minimize the artifactual drop in intraperitoneal temperature following injection of a cool fluid seen in Experiments 1 and 2. Rats in Groups AP+ and AN+ received 2.0 g/kg ethanol, while those in Group AP- received an equivalent volume of saline (15 ml/kg). During the tolerance acquisition phase, animals were injected once every 48 hr.

Rats in Groups AP+ and AP- were rectally probed immediately before, and at 30, 60, and 90 min following injection. This procedure was that described in Experiment 2. Rats in Group AN+ were not probed before the injection and were left undisturbed during the remainder of the session. Body temperature and heart rate were automatically monitored each minute of the 3-hr session.

After testing, the animals were transferred back to their home cages (Environment B in Table 1) in the colony room. About 24 hr after the start of recording sessions, animals in Group AN+ were removed from their home cages and rectally probed 4 times at 30-min intervals. Groups AP+ and AP- were left undisturbed at these times.

After 14 tolerance acquisition training sessions (28 days total), two tolerance tests (Column 4, Table 1) and two conditioned response tests (Column 5, Table 1) were administered. For the tolerance tests, all groups received 2.0 g/kg ethanol 60 min after placement in the chamber. During one tolerance test, rats were probed every 30 min and during the other, they were left undisturbed. Therefore, during one tolerance test, the animals were tested under the same handling condition that occurred while previously intoxicated and during the other test, under the opposite handling condition.

Following this phase of the experiment, two conditioned response tests were administered during which all the animals received an injection of saline, once while probed every 30 minutes and once while undisturbed. Thus, during one test, the animals received the injection under a handling condition similar to that occurring while previously intoxicated and during the other test, under a different handling condition. The order of test treatments was counterbalanced for both test phases, such that half of each group received the Probed condition first and half received the Not Probed condition first.

Data Analysis

Heart rate and temperature data were analyzed as in Experiment 1. The three treatment groups were the between-group variable and rectal probing was included as a within groups variable in the tolerance test and conditioned response test analyses.

Results

The data from two rats were discarded due to death: one died after surgery and one during the tolerance test phase. Both subjects were from Group AN+. Body temperature data were discarded from two rats in Group AP- due to loss of the Mini-Mitter signal during tolerance acquisition. Body temperature data were discarded from one rat in Group AP+ during the Conditioned Response test phase due to loss of signal just prior to this phase. This left 7 rats in Group AP+, 6 rats in Group AN+ and 6 rats in Group AP- by the end of the Conditioned Response Phase.

Mean heart rate and temperature were calculated for each 10-min

period of the 3-hr habituation, tolerance acquisition and test sessions. Baseline scores, post-injection scores, and change scores were analyzed separately for tolerance acquisition and test phases. To simplify presentation of the results, only the analyses of baselines and change scores are presented here; analyses of the post-injection scores are included in the Appendix. In all three-way ANOVAs performed on the data from the tolerance acquisition phase, the between-group variable was tolerance treatment group (AP+ vs AN+ vs AP-) and the within-group variables were days and 10-min sample periods. The three-way ANOVAs performed on data from tolerance test and conditioned response test phases included tolerance treatment groups as the between-group variable and test treatment (Probed vs Not Probed) and 10-min sample periods as the within-group variables.

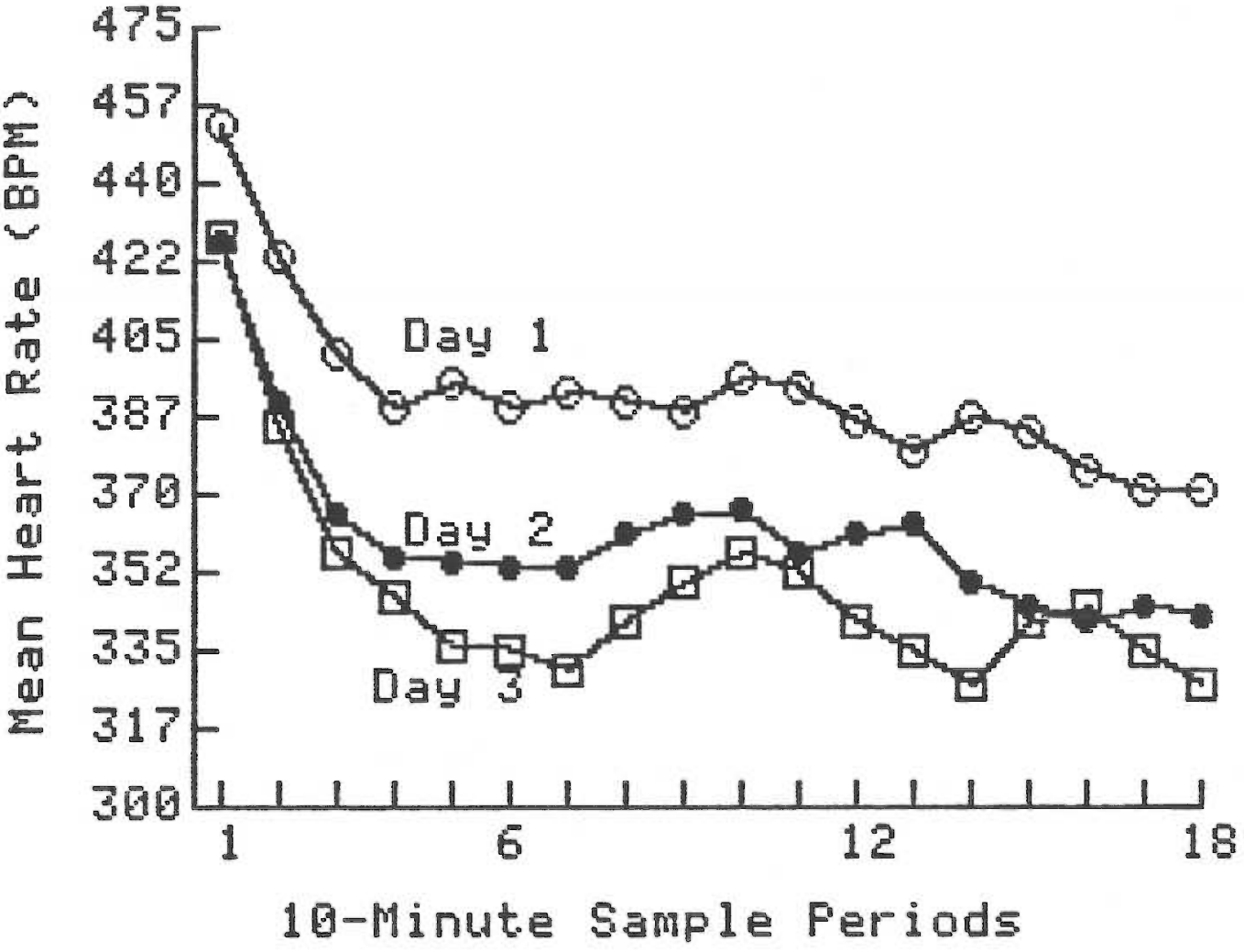
Habituation

Heart Rate

Heart rate during the three habituation sessions is graphed in Figure 17 over days and sample periods (collapsed over groups). Heart rate was initially elevated after placement in the chambers on all three days, decreasing about 70-90 bpm within the first 40 min, after which heart rate generally remained at a constant level. Both the initial level and the final level of heart rate decreased from the first to the second day. Heart rate was also slightly lower on Day 3 than on Day 2.

A three-way ANOVA on these data revealed a significant Days x Sample Periods interaction ($F\{34,646\} = 2.37$) and significant main effects of Days ($F\{2,38\} = 41.65$) and Sample Periods ($F\{17,323\} =$

Figure 17. Mean heart rate during the three habituation days. Data are collapsed over groups.


Mean Heart Rate (BPM)
475
457
440
422
405
387
370
352
335
317
300
Day 1
Day 2
Day 3
1
6
12
18
10-Minute Sample Periods

54.87). There were no significant main effects or interactions involving Groups (which was a dummy variable during habituation). Followup analyses support the observation that heart rate decreased over days with the least change occurring during the earlier sample periods.

Temperature

Mean body temperature during the three habituation sessions is shown in Figure 18 over days and sample periods (collapsed over groups). Temperature was elevated during the earlier portions of each session but tended to decrease after the first 30 min. Temperature was generally greatest on Day 2. A three-way ANOVA revealed a significant Days x Sample Periods interaction ($F\{34,646\} = 4.85$) and a significant main effect of Sample Periods ($F\{17,323\} = 28.55$). Followup analyses support these observations.

Summary: Habituation

As in Experiment 1, both temperature and heart rate were temporarily elevated due to placement in the recording chamber. Heart rate generally decreased over days, especially during later sample periods, while temperature increased from Day 1 to Day 2 and then decreased from Day 2 to Day 3. This is similar to the findings from the first experiment in which heart rate consistently decreased over habituation sessions and body temperature did not.

Baseline Scores

Heart Rate

Baseline heart rate of the three groups is graphed in Figure 19 over sample periods (data are collapsed over the 14 acquisition days

Figure 18. Mean body temperature during the three habituation days. Data are collapsed across groups.

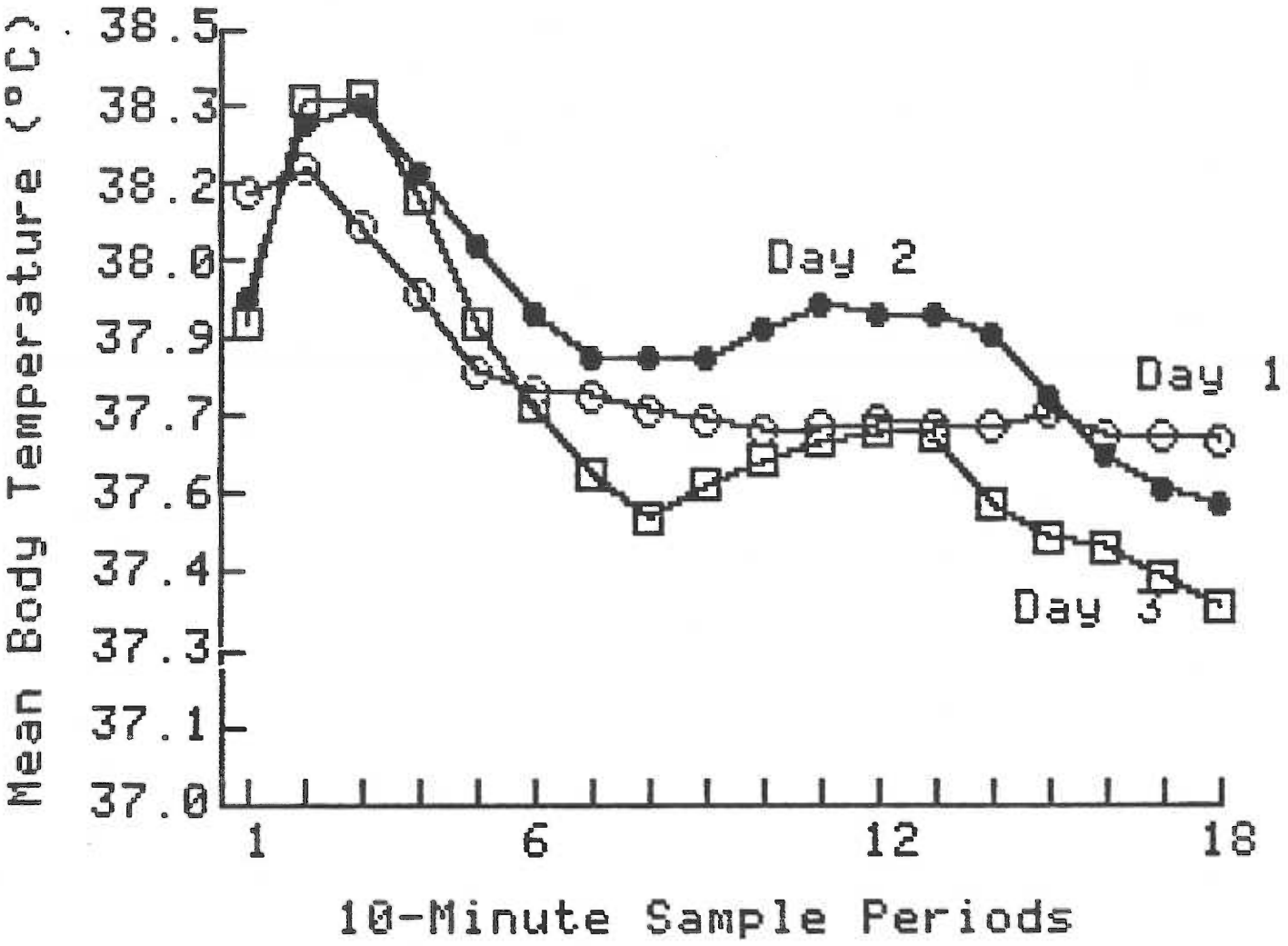
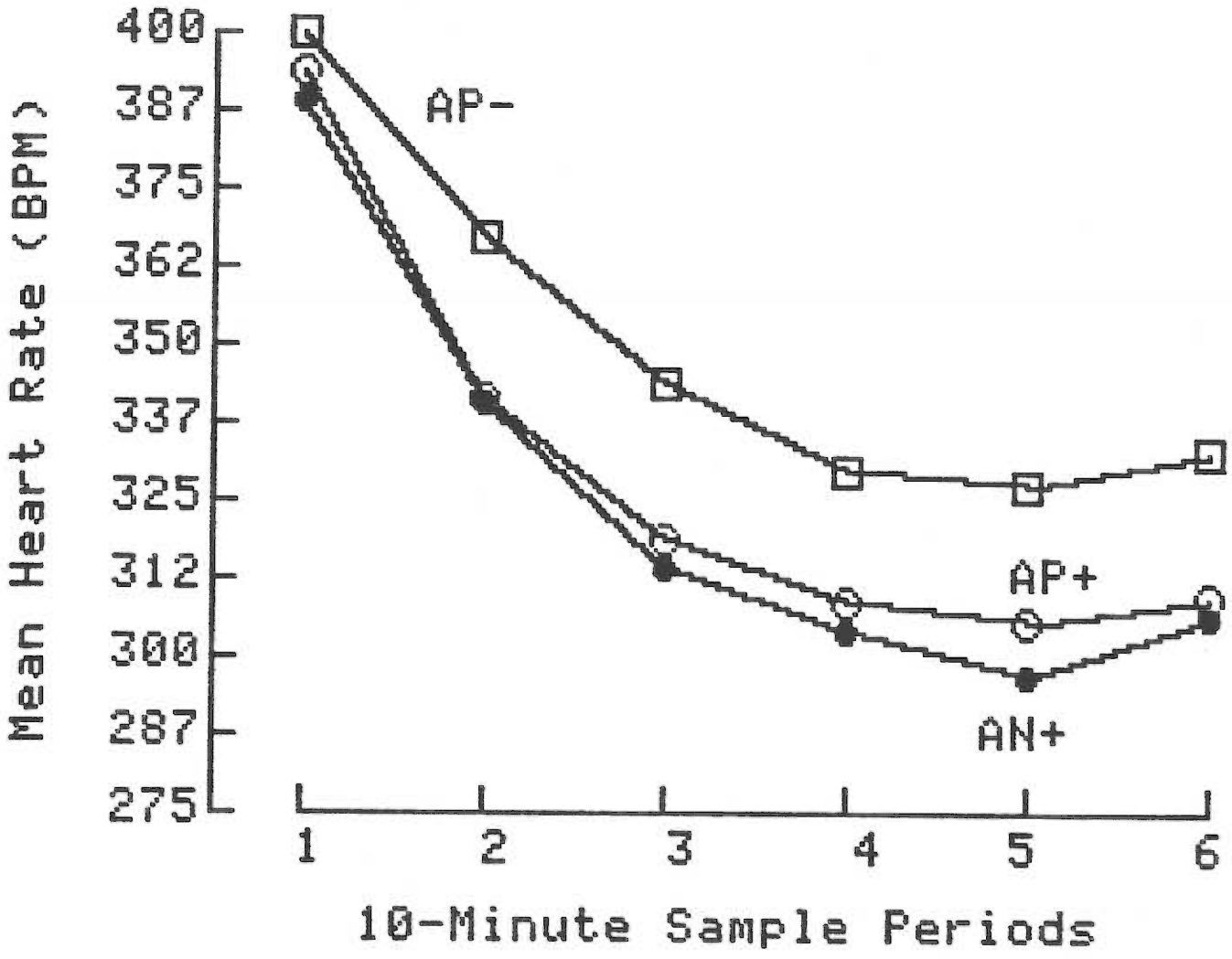

Mean Body Temperature (°C)
38.5
38.3
38.2
38.0
37.9
37.7
37.6
37.4
37.3
37.1
37.0
Day 2
Day 1
Day 3
1
6
12
18
10-Minute Sample Periods

Figure 19. Mean baseline heart rate of the three groups during the 60 min preceding injection. Data are collapsed over Days 1-18 (Tolerance Acquisition Phase plus both test phases).


Mean Heart Rate (BPM)
400
387
375
362
350
337
325
312
300
287
275
AP-
AP+
AN+
1
2
3
4
5
6
10-Minute Sample Periods

and the four test days). Generally, heart rate was elevated in all groups immediately after placement in the chamber. Heart rate then decreased about 80 bpm over the course of the hour. Heart rate was slightly lower at the end of 60 min in the ethanol groups (AP+ and AN+) than in the saline group (AP-). A three-way ANOVA found a significant Days x Sample Periods interaction ($F\{85,1615\} = 2.75$) and significant main effects of Days ($F\{17,323\} = 10.22$) and Sample Periods ($F\{5,95\} = 114.77$). The apparent differences between groups were not significant.

Figure 20 shows baseline heart rate on Day 1 and on Day 17 (collapsed over groups). Day 17 was chosen for followup because it was the last baseline period before treatment changed. It can be seen that, between these two days, heart rate decreased about 40 bpm during earlier sample periods but only 10 bpm during later sample periods. When data from Days 1 and 17 were analysed, a significant Days x Sample Periods interaction was found ($F\{5,95\} = 8.24$) as were main effects of Days ($F\{1,19\} = 23.51$) and Sample Periods ($F\{5,95\} = 87.75$). Significant Sample Periods effects were present on both Days 1 and 17 ($F\{5,95\} = 113.43$ and 29.5, respectively) due to the decrease in heart rate from early sample periods to later ones.

Temperature

Baseline temperatures from the first hour of acquisition and test days are graphed in Figure 21 (collapsed over days). Temperature increased slightly during the 30 min in all groups, returning to lower levels after 50-60 min. A significant Days x Sample Periods interaction was found ($F\{85,1445\} = 5.05$) as were significant main

Figure 20. Mean baseline heart rate on Days 1 and 17. Data are collapsed over groups.

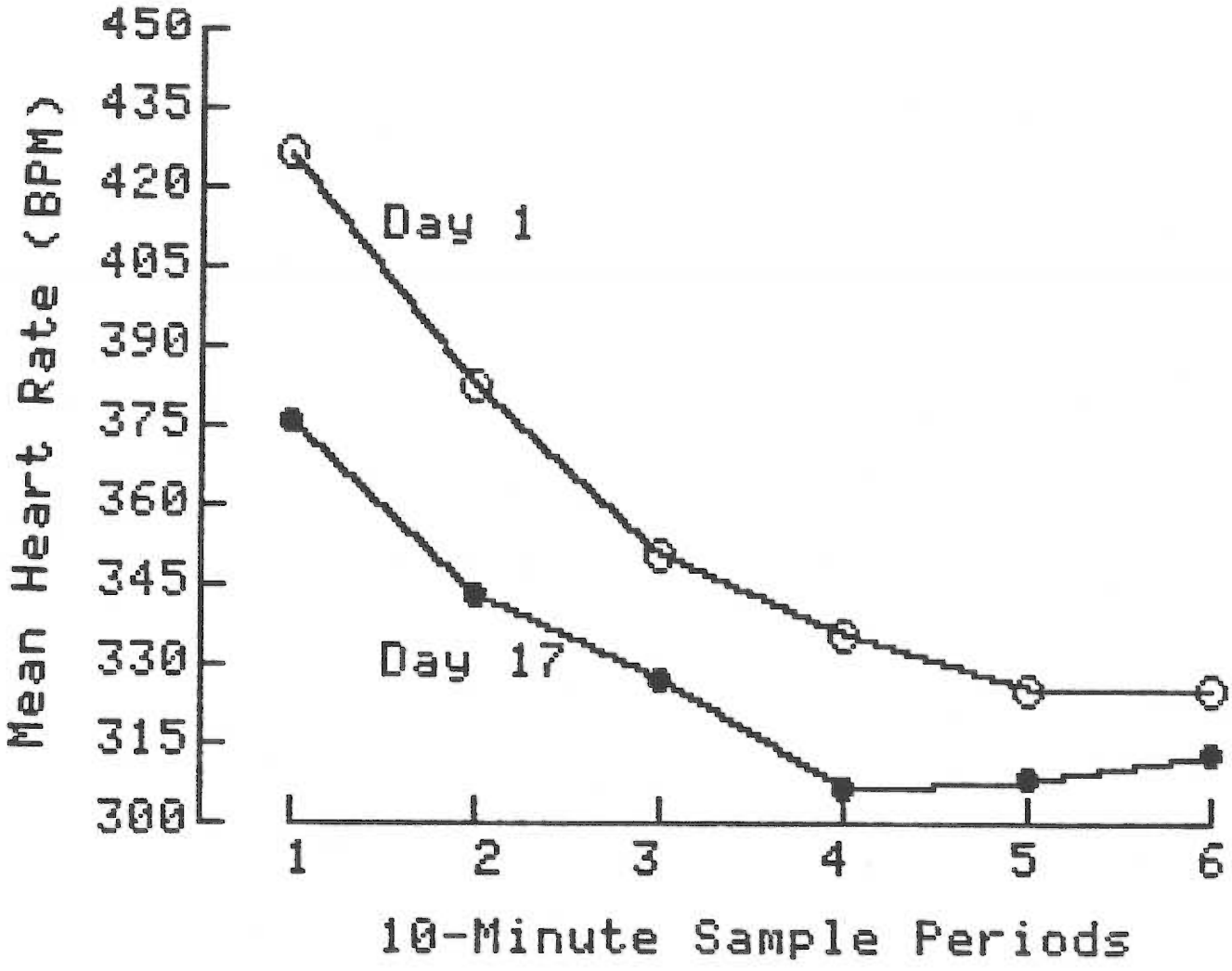
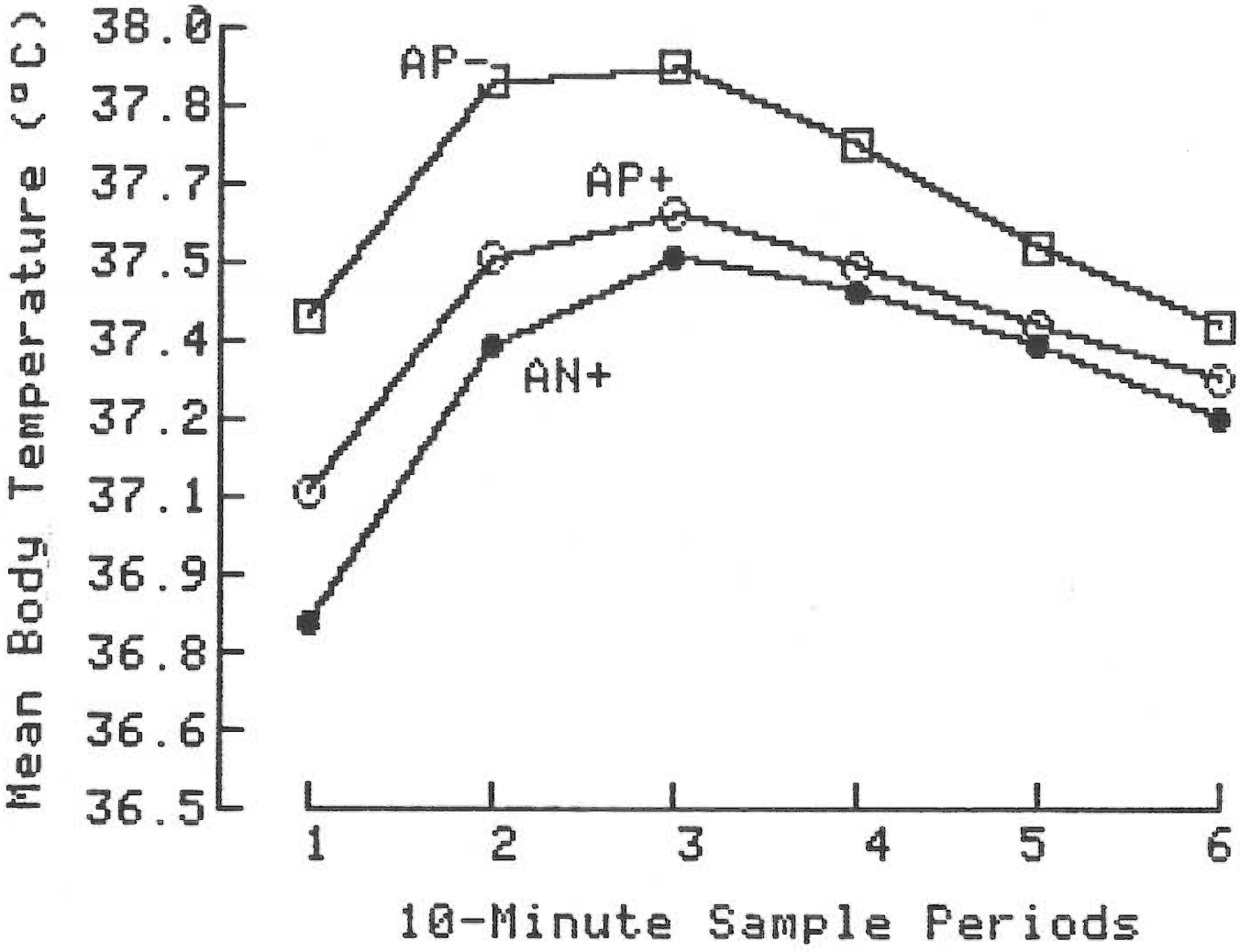

Mean Heart Rate (BPM)
450
435
420
405
390
375
360
345
330
315
300
Day 1
Day 17
1
2
3
4
5
6
10-Minute Sample Periods

Figure 21. Mean baseline body temperature of the three groups during the 60 min preceding injection. Data are collapsed over acquisition and test days.


Mean Body Temperature (°C)
38.0
37.8
37.7
37.5
37.4
37.2
37.1
36.9
36.8
36.6
36.5
AP-
AP+
AN+
1
2
3
4
5
6
10-Minute Sample Periods

effects of Days ($F\{17,289\} = 2.66$) and Sample Periods ($F\{5,85\} = 27.19$). There were no reliable differences among groups.

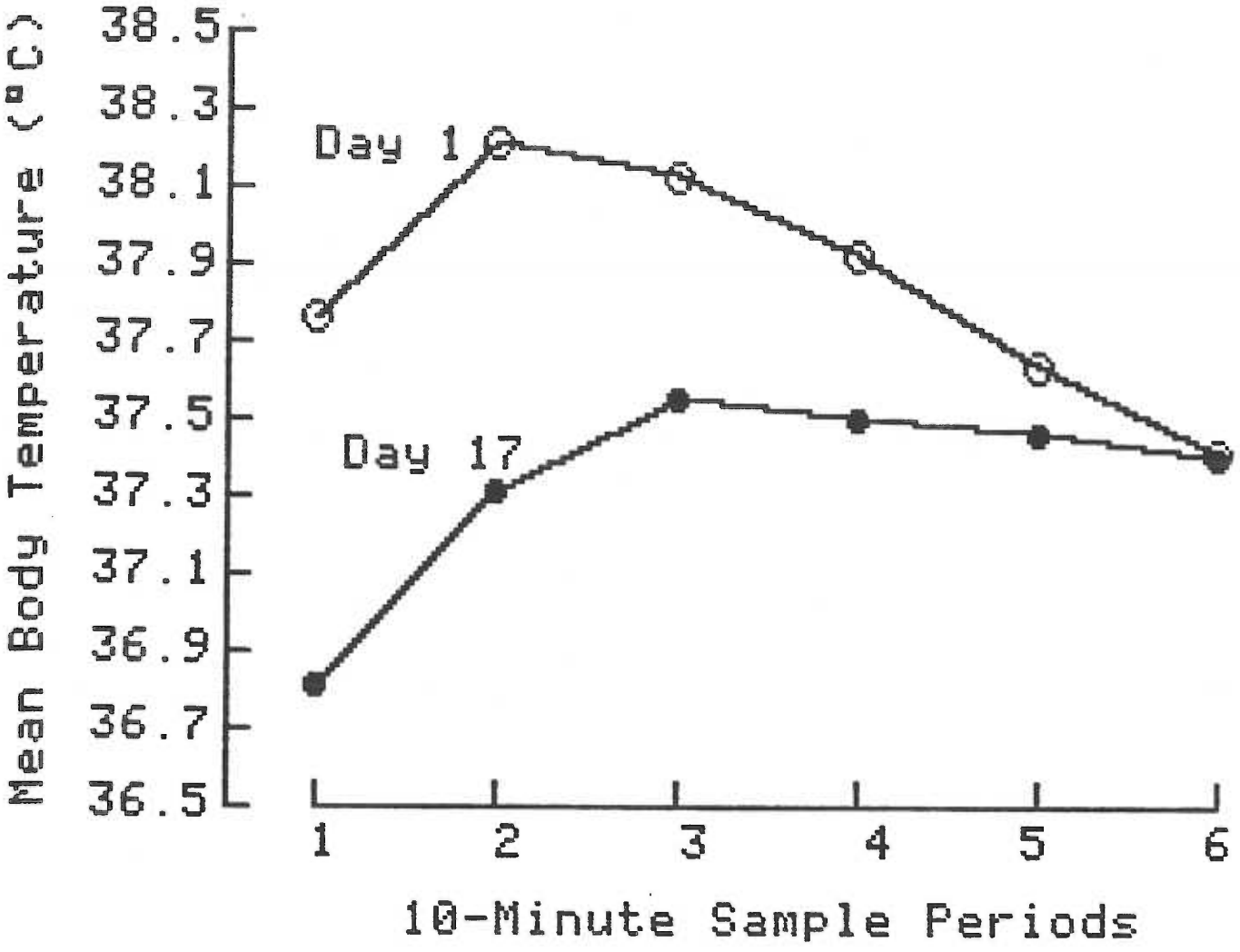
Figure 22 illustrates the decrease in baseline body temperature from Day 1 to Day 17 (the first conditioned response test). There was a greater decrease in baseline during the early sample periods (0.95°C) relative to later sample periods (0.0°C), explaining the Days x Sample Periods interaction. Analysis of data from Days 1 and 17 revealed a significant Days x Sample Periods interaction ($F\{5,85\} = 17.89$) plus main effects of Days ($F\{1,17\} = 20.99$) and Sample Periods ($F\{5,85\} = 17.32$). Within-group analyses revealed significant Sample Periods effects on both days due to the higher body temperature during earlier sample periods.

Summary: Baseline

Heart rate was initially elevated during the baseline period throughout tolerance acquisition and test phases, presumably due to the handling associated with placing the animal in the experimental chamber. The magnitude of this response decreased over days, but a significant acceleration was still present on the last day. Temperature baselines changed in a similar manner over days, such that the magnitude of the initial hyperthermia decreased over days, but was still present on the last day.

There were no group differences in baseline scores at any time during Experiment 2. This does not replicate the finding from Experment 1 of a greater decrease in baseline heart rate (that developed over days) in the group receiving a high dose of ethanol. There was a similar trend for heart rate of Groups AP+ and AN+ in this

Figure 22. Mean baseline body temperature on Days 1 and 17. Data are collapsed over groups.


Mean Body Temperature (°C)
38.5
38.3
38.1
37.9
37.7
37.5
37.3
37.1
36.9
36.7
36.5
Day 1
Day 17
1
2
3
4
5
6
10-Minute Sample Periods

study, but this was not significant.

Acquisition

Heart Rate Change Scores

The mean heart rate change scores of the three groups are graphed over sample periods in the seven panels of Figure 23 (data are collapsed into consecutive 2-day blocks). Heart rate change scores of the three groups increased in the positive direction and remained elevated during the 2 hr following injection. Level of heart rate was greatest in Group AP+ and fluctuated the most within days in Group AP-. There was a general decrease in the magnitude of cardioacceleration of all groups over days but no systematic changes in ethanol groups relative to the saline group. Significant Groups x Sample Periods ($F\{22,209\} = 2.81$) and Days x Sample Periods ($F\{143,2717\} = 3.08$) interactions help support these observations. There were also significant main effects of Days ($F\{13,247\} = 2.27$) and Sample Periods ($F\{11,209\} = 9.58$).

The data graphed in Figure 24 (collapsed over days) illustrate the Groups x Sample Periods interaction. Heart rate change scores of Group AP- are considerably lower than those of the other two groups except every 30 min during probing. Followup comparisons between groups revealed a significant Groups x Sample Periods interaction for the AN+ vs AP- ($F\{11,132\} = 3.92$) and AP+ vs AP- ($F\{11,154\} = 2.62$) comparisons but not for the AP+ vs AN+ comparison. Followup analyses of the data during Sample Periods 1, 3 and 12 revealed that Group AP- was significantly lower than Group AP+ during Sample Periods 3 and 12 ($F\{1,14\} = 9.13$ and 5.58, respectively) but not Sample Period 1.

Figure 23. Mean heart rate change scores of the three groups during acquisition for each consecutive 2-day block (Panels A-G). Baseline heart rate for consecutive 2-day blocks was 319, 321, 313, 324, 294, 295 and 295 for Group AP+, 307, 304, 314, 306, 298, 308 and 295 for Group AN+ and 328, 323, 334, 323, 333, 321 and 336 for Group H.

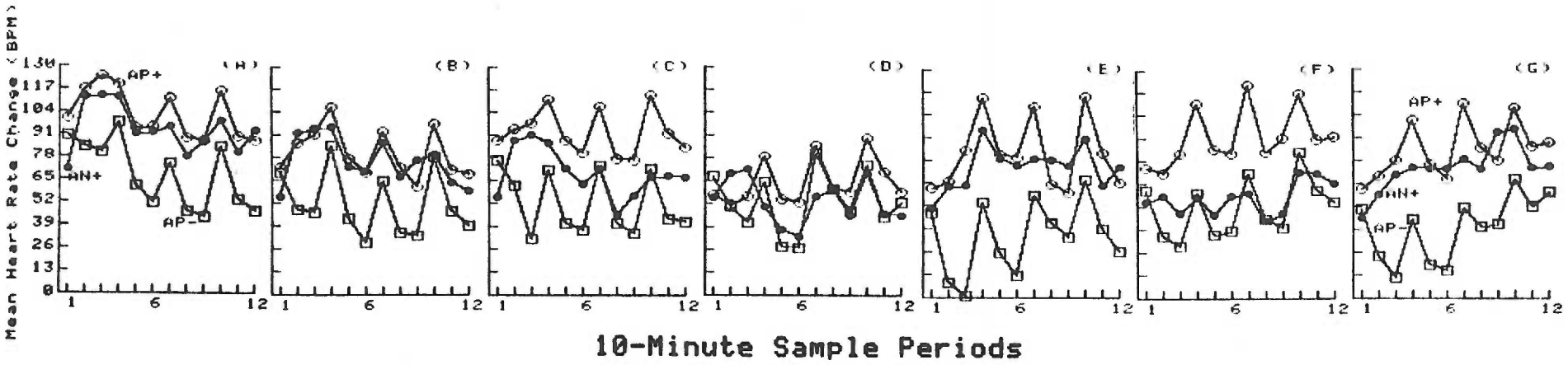
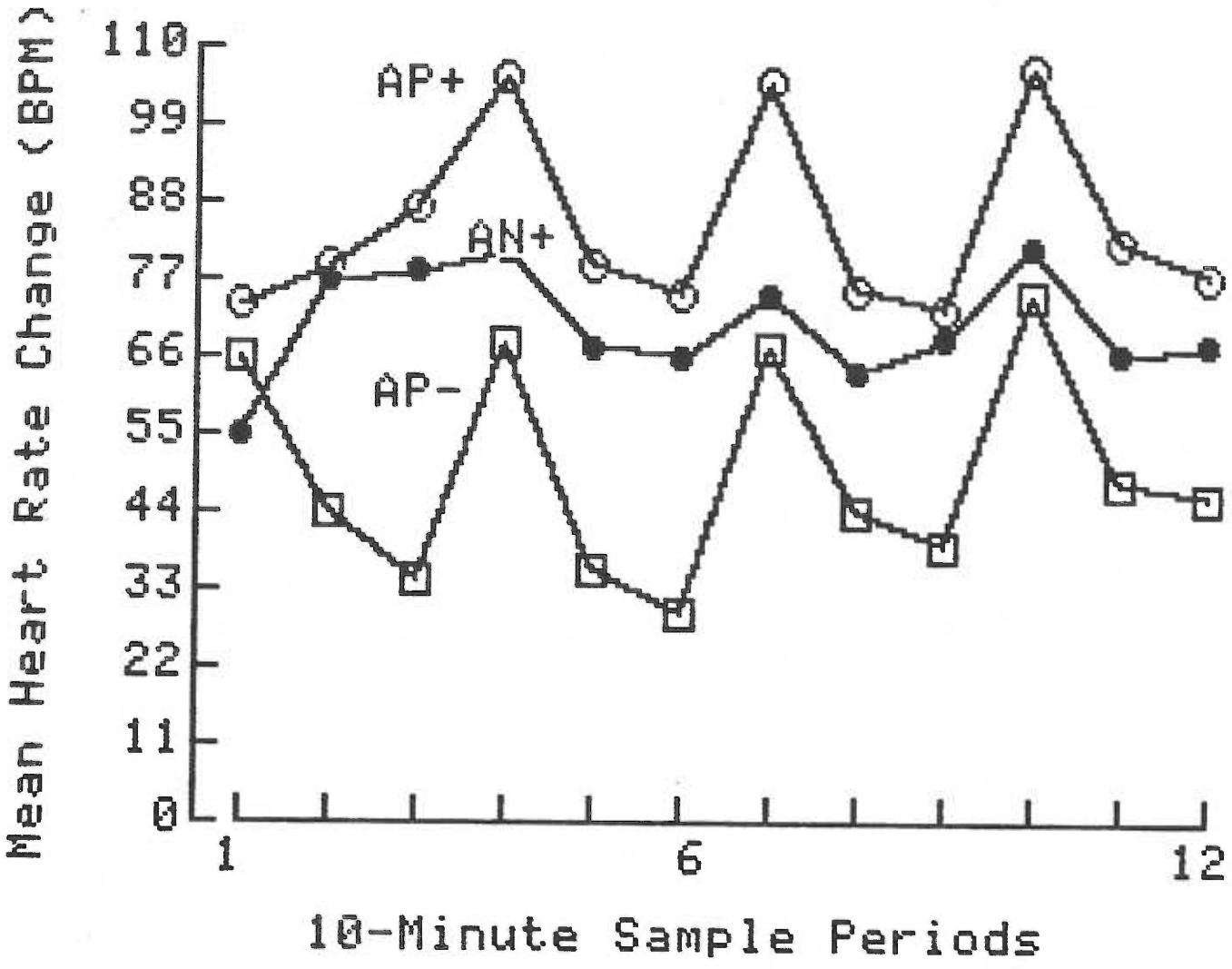

Mean Heart Rate Change (BPM)
130
117
104
91
78
65
52
39
26
13
0
AP+
AN+
AP-
(A)
(B)
(C)
(D)
(E)
(F)
(G)
1
6
12
10-Minute Sample Periods

Figure 24. Mean heart rate change scores of the three groups during the Tolerance Acquisition Phase. Data are collapsed over days. Mean baseline heart rate was 306 in Group AP+, 304 in Group AN+ and 331 in Group AP-.


Mean Heart Rate Change (BPM)
110
99
88
77
66
55
44
33
22
11
0
AP+
AN+
AP-
1
6
12
10-Minute Sample Periods

Group AP- was significantly lower than Group AN+ during Sample Period 3 ($F\{1,12\} = 4.95$).

The Days x Sample Periods interaction was analysed by comparing data during Sample Periods 1 and 12. This revealed a significant effect of Days during Sample Period 1 ($F\{13,272\} = 2.72$) but not Sample Period 12. The data are graphed in Figure 25 (collapsed over Groups and into 30-min Sample Periods). It can be seen that a greater decrease over days occurred during the first hour relative to the second hour after injection.

Temperature Change Scores.

The temperature change scores after injection are graphed in Figure 26 over sample periods and 2-day blocks. Temperature was lower in both Group AP+ and AN+ relative to Group AP-. The differences appear to be due to both hypothermia in the ethanol groups and a slight hyperthermia in the saline group. The hypothermia was greatest towards the end of the 2-hr recording session on all days and the magnitude of this change decreased over days, especially in Group AN+. The hyperthermia in Group AP- was greatest during the first hour after injection on earlier sessions but gradually became greatest at the end of the second hour. During later sessions, Group AP+ was more hypothermic than Group AN+.

These observations were supported by significant Groups x Days x Sample Periods ($F\{143,2717\} = 1.16$) and Groups x Sample Periods ($F\{22,209\} = 16.34$) interactions and by significant Groups ($F\{2,19\} = 33.17$) and Sample Periods ($F\{11,209\} = 8.4$) main effects. Pairwise group comparisons revealed Groups x Days x Sample Periods interactions

Figure 25. Mean heart rate change scores after injection on Days 1 and 2 and Days 13 and 14. Data are collapsed over groups and into 30-minute periods. Mean baseline heart rate on Days 1 and 2 was 330 and on Days 13 and 14 was 321.

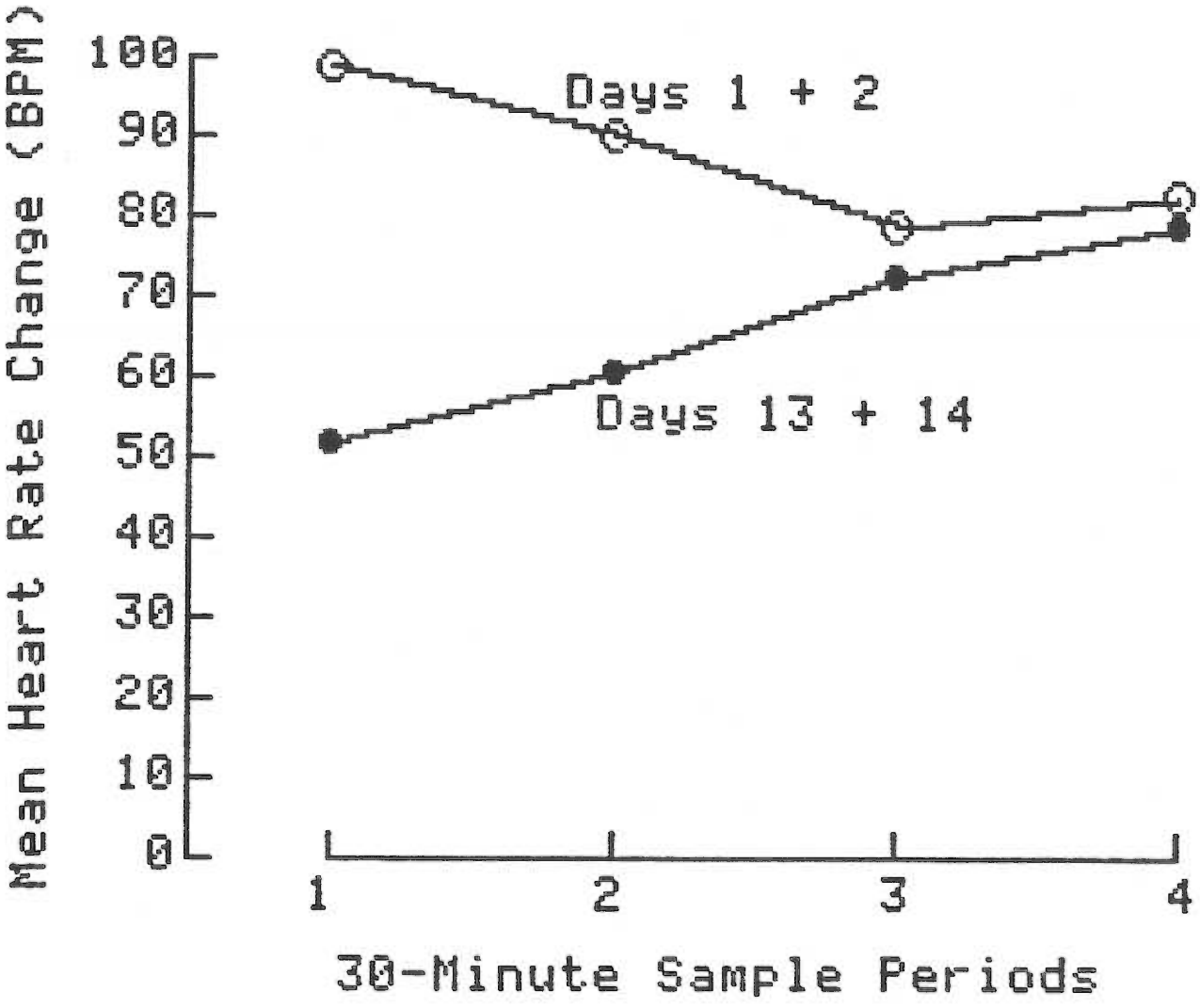
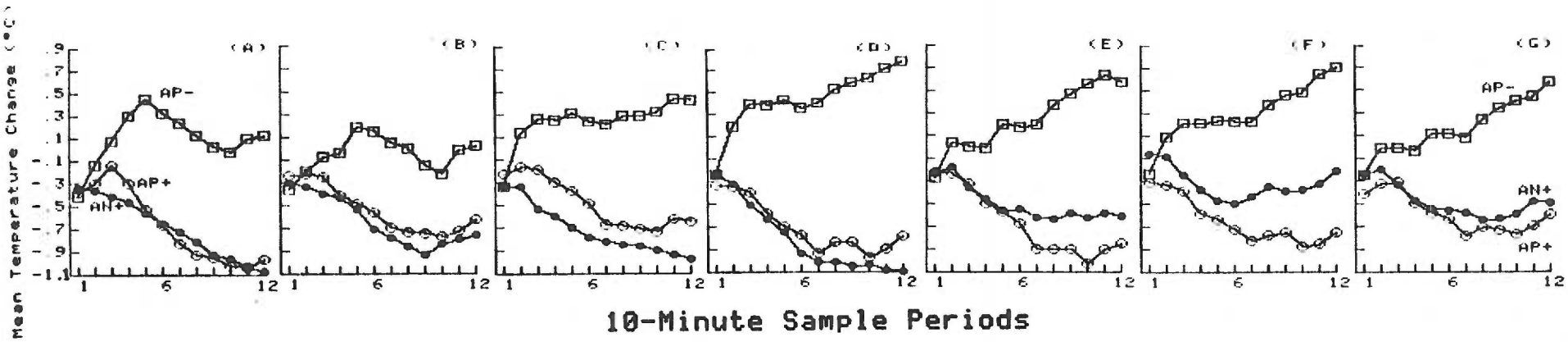

Mean Heart Rate Change (BPM)
100
90
80
70
60
50
40
30
20
10
0
Days 1 + 2
Days 13 + 14
1
2
3
4
30-Minute Sample Periods

Figure 26. Mean body temperature change scores of the three groups during acquisition for each consecutive 2-day block (Panel A-G). Mean baseine body temperature for each consecutive 2-day block was 37.5, 37.3, 37.1, 37.3, 37.2, 37.3 and 37.4 for Group AP+, 37.3, 37.2, 37.1, 37.3, 37.2, 36.9 and 37.2 for Group AN+ and 37.3, 37.2, 37.4, 37.3, 37.5, 37.4 and 37.6 for Group AP-.


Mean Temperature Change (°C)
.9
.7
.5
.3
.1
-.1
-.3
-.5
-.7
-.9
-1.1
(A)
(B)
(C)
(D)
(E)
(F)
(G)
AP-
AP+
AN+
1
6
12
10-Minute Sample Periods

for AP+ vs AP- ($F\{143,1859\} = 1.26$) and AN+ vs AP- ($F\{143,1716\} = 1.24$). However, there were no significant effects or interactions for the comparison between Groups AN+ and AP+. There were significant Days x Sample Periods interactions in Group AN+ and AP- ($F\{143,858\} = 1.8$ and 2.07, respectively) supporting the observations that temperature increased during later sample periods. There was not an effect or interaction of Days in Group AP+ which is not consistent with the hypothesis that hypothermia decreased over days in this group.

Summary: Acquisiton

Tachycardia was associated with both ethanol administration and rectal probing and occurred throughout the 120-min post-injection period. The magnitude of this response was not initially different in Groups AP+ and AN+ relative to Group AP- nor did it diminish at a different rate after 14 injections. This implies that neither tolerance nor sensitization to the cardioacceleratory effects of ethanol developed. There was a general decrease in heart rate in all three groups which may indicate that habituation developed to the general handling procedures. This decrease was more pronounced during earlier sample periods right after handling and injection than during later ones.

As in Experiment 1, a 2.0 g/kg dose of ethanol elicted hypothermia that reached a peak magnitude 120 min after injection. Rectal probing produced a short term hyperthermia in the saline group (relative to baseline levels) but did not affect the temperature of intoxicated animals. This is not consistent with the findings from Experiment 2 but still supports an interaction hypothesis. The effect

of probing on body temperature was different in sober rats relative to intoxicated rats.

A decrease in the magnitude of hypothermia exhibited in the ethanol groups during the last 30 min of later acquisition sessions may be indicative of tolerance. However, as in the first experiment, body temperature during the later sample periods also increased over days in Group AP-, implying a possible effect independent of ethanol exposure.

Tolerance Test

Heart Rate Change Scores

Heart rate change scores after a 2.0 g/kg ethanol injection are graphed in Figure 27 (collapsed over groups). Heart rate increased after injection (independent of groups) and remained elevated throughout the 2-hr period. Heart rate of probed animals fluctuated between accelerations of 50-97 bpm, while accelerations of unprobed animals remained between 50-60 bpm. A significant Test Treatment x Sample Periods interaction ($F\{11,209\} = 5.09$) and Test Treatment ($F\{1,19\} = 9.4$) and Sample Periods ($F\{11,209\} = 5.03$) main effects were found. Followup within-group analyses found a significant effect of Sample Periods for Probed animals only ($F\{11,66\} = 1.79$). These findings support the observation that the heart rate of probed animals fluctuated within a session more than did the heart rate of non-probed animals.

Temperature Change Scores

The temperature change scores after a 2.0 g/kg injection are graphed in Figure 28 for all groups and test treatments. Temperature decreased about 2.0°C in Group AP- over the course of the 2-hr

Figure 27. Mean heart rate change scores during Probed (P) and Not Probed (N) conditions during the Tolerance Test phase. Data are collapsed over groups. Mean baseline heart rate was 320 before the Probed condition and 321 before the Not Probed condition.

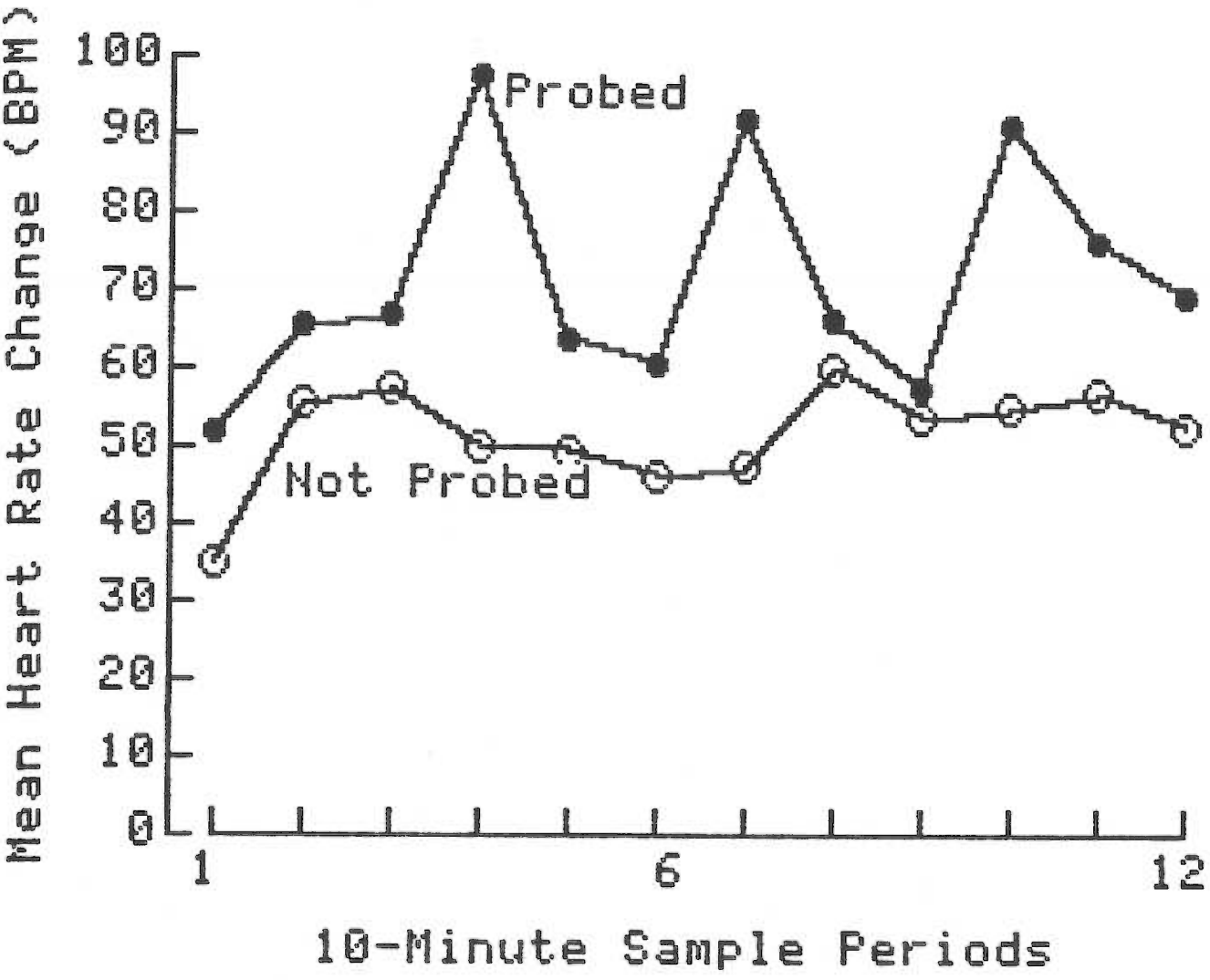
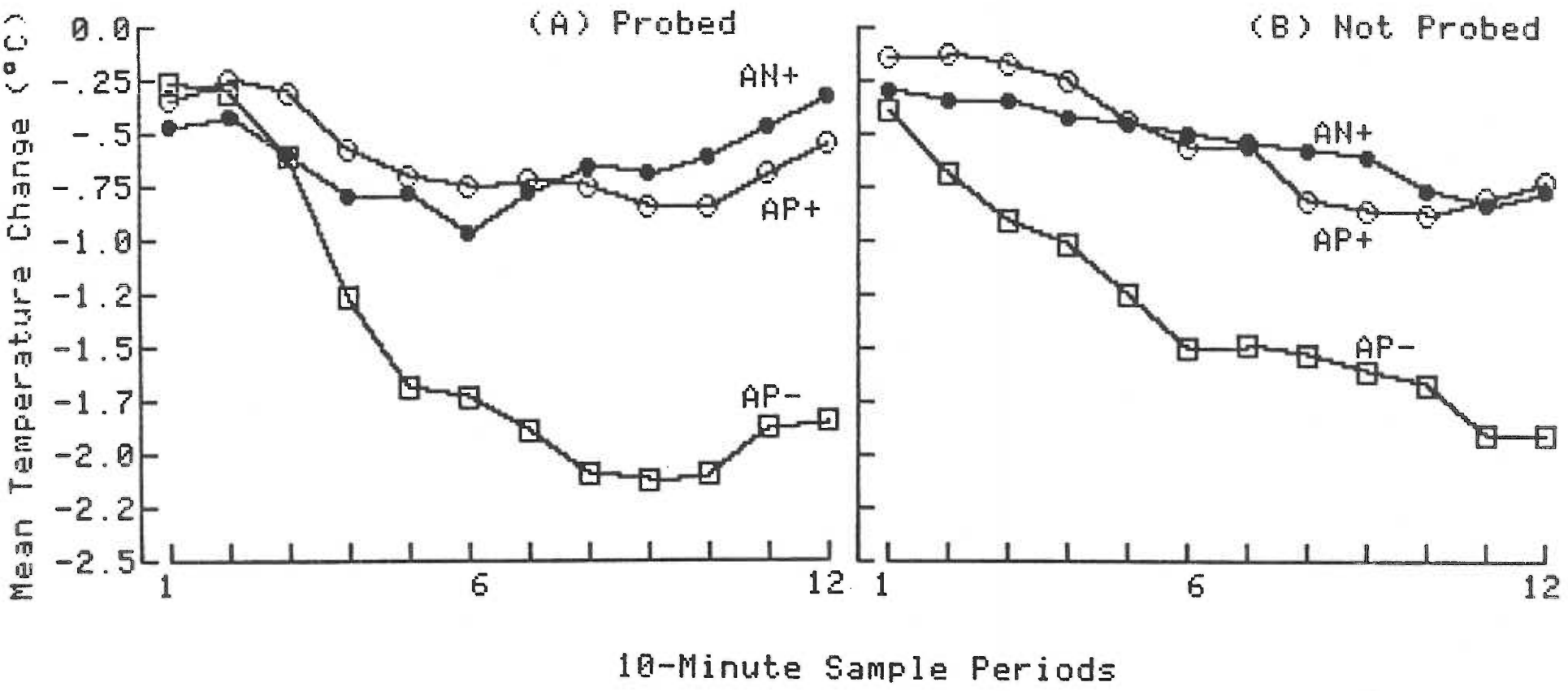

Mean Heart Rate Change (BPM)
100
90
80
70
60
50
40
30
20
10
0
Probed
Not Probed
1
6
12
10-Minute Sample Periods

Figure 28. Mean body temperature change scores of the three groups during Probed (Panel A) and Not Probed (Panel B) conditions of the Tolerance Test phase. Mean baseline temperature before the Probed condition was 37.4 in Group AP+, 37.45 in Group AN+ and 37.54 in Group AP-. Temperature before the Not Probed condition was 37.35 for Group AP+, 37.18 for Group AN+ and 37.49 for Group AP-.

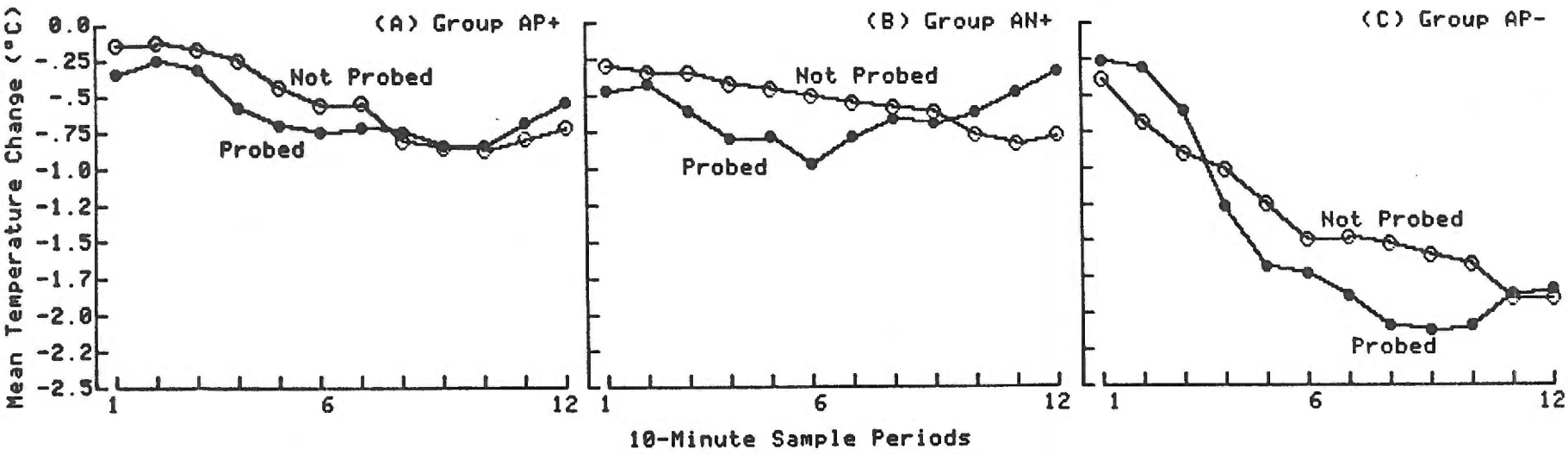

(A) Probed
(B) Not Probed
Mean Temperature Change (°C)
0.0
-.25
-.5
-.75
-1.0
-1.2
-1.5
-1.7
-2.0
-2.2
-2.5
AN+
AP+
AP-
1
6
12
10-Minute Sample Periods

recording period. A smaller degree of hypothermia also occurred in Groups AP+ and AN+ which reached peak magnitude (-0.8°C) before the end of the 2-hr session. Although not apparent in this figure, temperature was lower during the Probed test condition in Groups AN+ and AP-. When the data are graphed as in Figure 29, the effect of probing is more obvious.

A three-way ANOVA revealed significant interactions of Groups x Test Treatment x Sample Periods ($F\{22,187\} = 1.96$), Groups x Sample Periods ($F\{22,187 = 5.12$) and Test Treatment x Sample Periods ($F\{11,187\} = 4.02$). There were also significant main effects of Groups ($F\{2,17\} = 8.24$) and Sample Periods ($F\{11,187\} = 22.23$). Within-group analyses found significant Test Treatment x Sample Periods interactions in Groups AN+ and AP- ($F\{11,55\} = 2.35$ and $F\{11,55\} = 5.18$, respectively) but not in Group AP+. These findings support the observation that probing during the test increased the magnitude of hypothermia but only in Groups AN+ and AP-.

Between-group comparisons revealed a significant Groups x Test Treatment x Sample Periods interaction for the comparison between Groups AP+ vs AP- ($F\{11,132\} = 2.81$). This was due to a greater hypothermia in Group AP- during middle sample periods of the Probed condition relative to the Not Probed condition. Group AP+ was less hypothermic than Group AP- during both conditions but there was no difference between conditions. There was a significant Groups x Test Treatment x Sample Periods interaction in the comparison between Groups AN+ vs AP- ($F\{11,110\} = 2.84$). This was due to a larger hypothermia during later sample periods of the Probed treatment by Group AP- than

Figure 29. Mean body temperature change scores of the three groups during the Probed and Not Probed conditions of the Tolerance Test phase. Data from Group AP+ are graphed in Panel A, Group AN+ in Panel B and Group AP- in Panel C. The baseline scores were the same as those reported for Figure 29.


Mean Temperature Change (°C)
0.0
-.25
-.5
-.75
-1.0
-1.2
-1.5
-1.7
-2.0
-2.2
-2.5
(A) Group AP+
Not Probed
Probed
(B) Group AN+
Not Probed
Probed
(C) Group AP-
Not Probed
Probed
1
6
12
10-Minute Sample Periods

during that by Group AN+. There were no groups effects or interactions for a similar comparison between Groups AP+ vs AN+.

Summary: Tolerance Test

There was no effect of previous tolerance treatment (groups) on heart rate changes after a test injection of ethanol. Thus, it can be concluded that neither tolerance nor sensitization occurred within the 28-day course of ethanol administration. The cardioacceleratory effects of ethanol injection and rectal probing were still present during the tolerance test.

Previous ethanol treatment decreased the magnitude of hypothermia induced by the test injection, which was indicative of ethanol tolerance in Groups AP+ and AN+. There were no differences between tolerance in Group AP+ and AN+ during either of the test treatment conditions. Thus, probing did not act as a cue for conditioned tolerance. If it had, tolerance would have been greater during the Probed condition in Group AP+ and during the Not Probed condition in Group AN+.

The presence of the Probed condition during testing tended to decrease temperature, but only in Groups AN+ and AP-. This supports the interaction effect found in Experiment 2 as does the trend for lower temperature in Group AP+ relative to Group AN+ seen during the last half of tolerance acquisition. Together, these findings support the hypothesis that probing interacts with ethanol's hypothermic effect, but for some reason this was not a pronounced effect in Experiment 3.

Conditioned Response Test

Heart Rate Change Scores

Heart rate change scores after the saline injection were elevated by 70 bpm in all three groups after injection. Heart rate then decreased under both handling conditions and remained at near baseline levels in the Not-Probed condition but increased 60 bpm every 30 min in the Probed condition.

These observations are supported by a significant Test Treatment x Sample Periods interaction ($F\{11,209\} = 6.27$) and a main effect of Sample Periods ($F\{11,209\} = 12.83$). There was also a main effect of Groups ($F\{2,19\} = 4.74$) which is graphed in Figure 30. It can be seen that heart rate was greater in Group AN+ relative to the other groups. A Neuman-Keuls analysis revealed a significant difference between Groups AN+ and AP- ($Cn\text{-}k\{3,19\} = 36.16$) but not between any other group comparisons.

Temperature Change Scores

Temperature change scores of the three groups are plotted in Figure 31 over test treatment conditions and sample periods. The degree of hyperthermia was greater in the ethanol groups especially Group AN+. Temperature was particularly elevated in rats of this group during the Probed test condition. A three-way ANOVA found significant Groups x Test Treatment x Sample Periods ($F\{22,176\} = 2.6$) and Test Treatment x Sample Periods ($F\{11,176\} = 3.38$) interactions and a significant main effect of Sample Periods ($F\{11,176\} = 27.39$).

Within-group followups revealed a significant Test Treatment x Sample Periods interaction for Group AN+ only ($F\{11,55\} = 5.41$) supporting the observation that temperature was higher during probing

Figure 30. Mean heart rate change scores of the three groups during the Conditioned Response test. Data are collapsed over the test treatment variable. Mean baseline heart rate was 311 in Group AP+, 307 in Group AN+ and 343 in Group AP-.

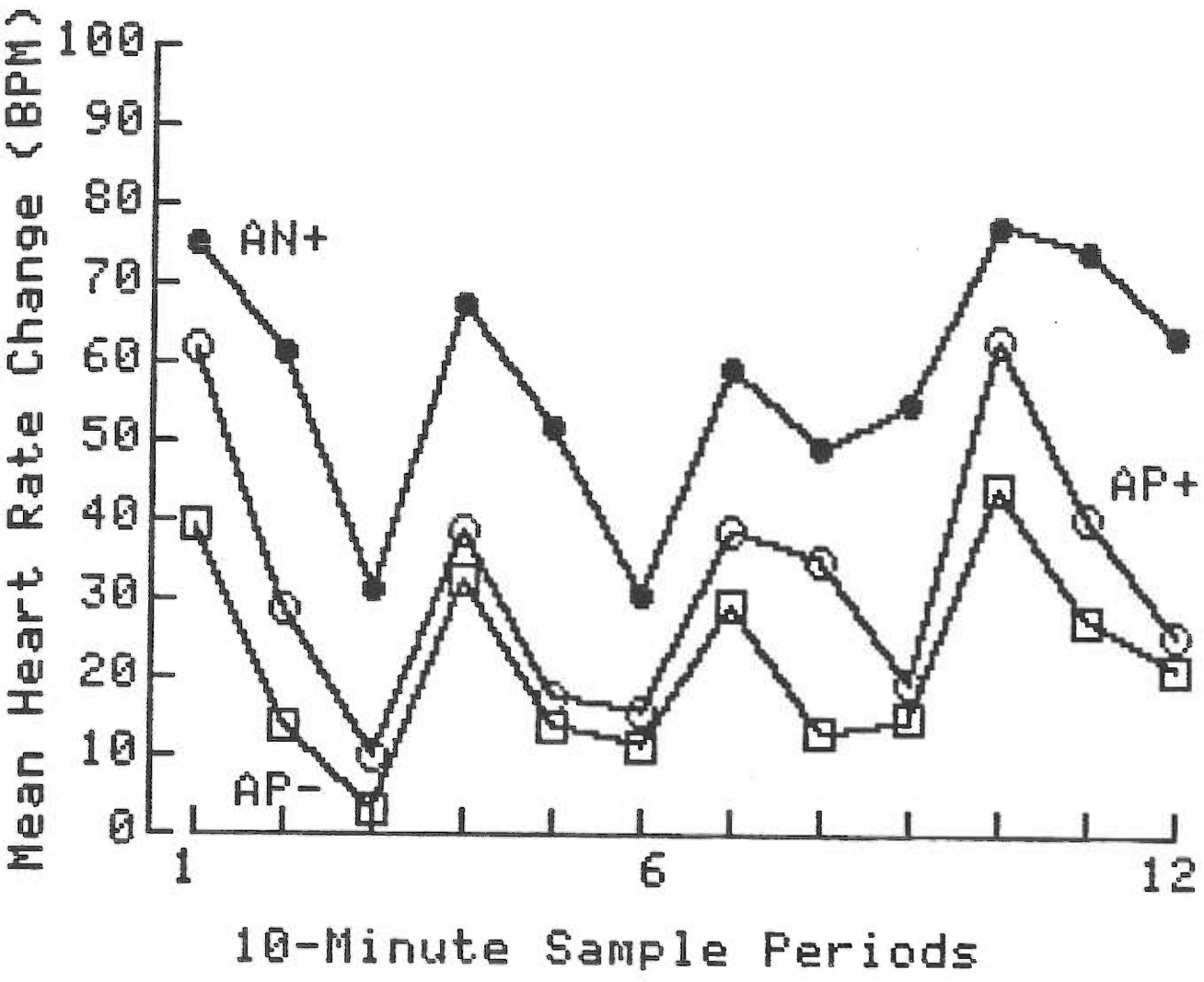
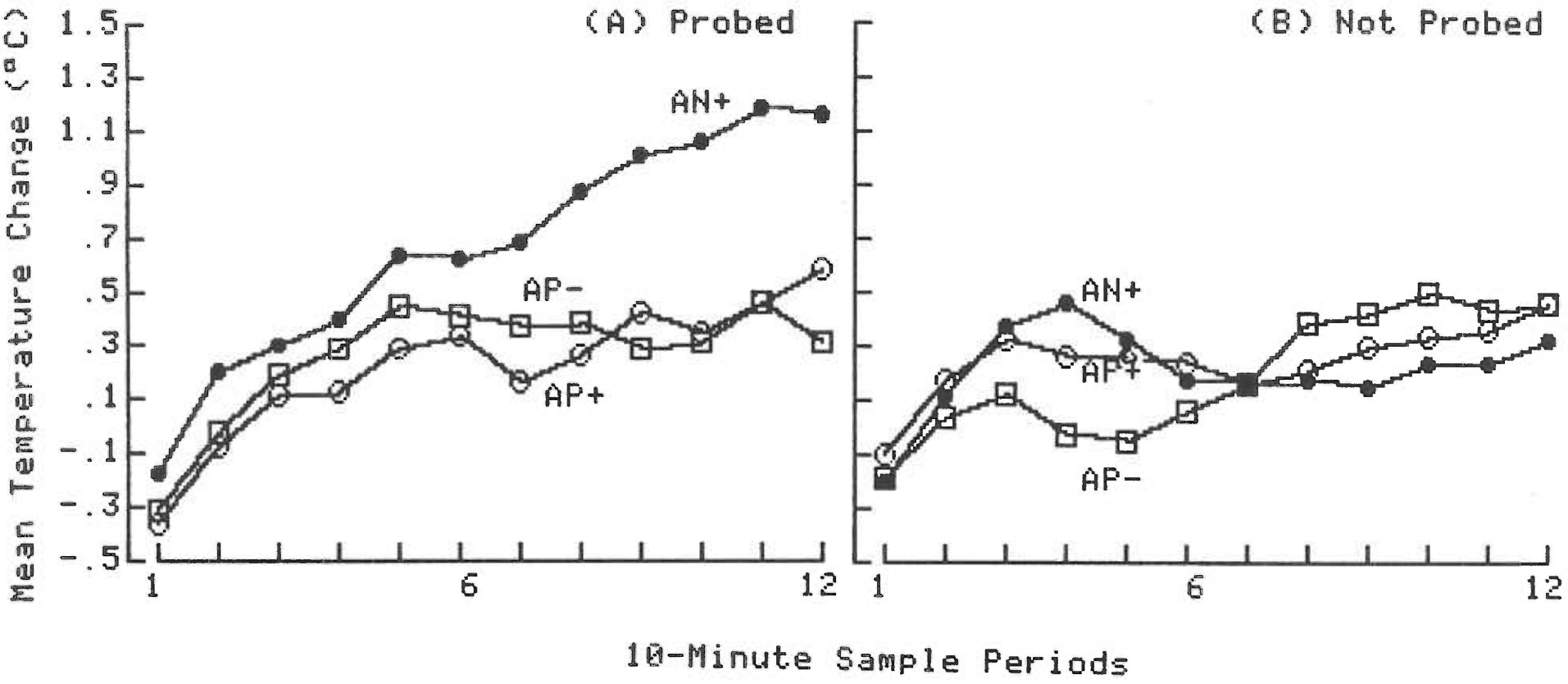

Mean Heart Rate Change (BPM)
100
90
80
70
60
50
40
30
20
10
0
AN+
AP+
AP-
1
6
12
10-Minute Sample Periods

Figure 31. Mean body temperature change scores of the three groups during the Probed (Panel A) and Not Probed (Panel B) conditions of the Conditioned Response Test phase. Mean baseline temperature before the Probed condition was 37.3 for Group AP+, 37.4 for Group AN+ and 37.3 for Group AP-. Temperature before the Not Probed condition was 37.3 for Group AP+, 37.4 for Group AN+ and 37.8 for Group AP-.


(A) Probed
(B) Not Probed
AN+
AP-
AP+
Mean Temperature Change (°C)
1.5
1.3
1.1
.9
.7
.5
.3
.1
-.1
-.3
-.5
1
6
12
10-Minute Sample Periods

but only in this group. Pairwise group comparisons revealed a significant Groups x Test Treatment x Sample Periods interaction for the comparison between Groups AN+ vs AP- only ($F\{11,110\} = 4.17$) supporting the observation that hyperthermia was greater in Group AN+ during later sample periods of the Probing condition but only when compared to Group AP-. The pairwise comparison of Groups AP+ and AN+ revealed only a Test Treatment x Sample Periods interaction ($F\{11,121\} = 6.18$). Thus, temperature was increased relatively more during probing but did not differ between Groups AP+ and AN+ even though the difference between these groups under the Probed condition looks as large as that between Groups AN+ and AP-. There were no Group or Treatment effects or interactions for the Group AP+ vs AP- comparison.

Followup analyses at Sample Periods 1, 3 and 12 of the AN+ vs AP- comparison revealed a significant Group x Test Treatment interaction for Sample Period 12 ($F\{1,10\} = 4.85$) but not for Sample Periods 1 or 3. This supports the observation that the greatest increase in temperature occurred during later sample periods of the Probed condition of Group AN+.

Summary: Conditioned Response Test.

Heart rate during the conditioned response test was not affected by previous tolerance acquisition treatments. It increased in a cyclical manner as a result of repeated probing during the test. When heart rate change scores were compared, there was a difference between groups, with Group AN+ having a higher level of heart rate than either Group AP+ or AP-. This could be due to the novelty of being probed in

the test environment as opposed to the home cage even though the increase was not specific to the Probed condition. It is unlikely that this increase is due to conditioning since it was not specific to either of the handling cues nor was evidence of conditioning present during the Tolerance Acquisition or Test Phases.

Temperature was increased by probing during the test and this difference was greatest in Group AN+. If this increase was a conditioned compensatory response, it should have been present only during the Not Probed condition since these were the cues to which tolerance should have been conditioned. If hyperthermia was conditioned to cues other than handling (which would explain the hyperthermia present during both handling conditions) then hyperthermia should also have been present in Group AP+. There was no difference between mean temperatures of Groups AP+ and AP- during the Conditioned Response Test; thus conditioned compensatory responses did not develop in Group AP+. It is therefore unlikely that the general increase in both heart rate and body temperature of Group AN+ are conditioned compensatory responses. They may be interpreted as reflections of an increase in overall stress in these animals. After 6 weeks, some of the electrode preparations had become slightly infected which may have increased stress. Unfortunately, it is not known whether the problem was greater in Group AN+ than in the other groups.

Discussion

As seen previously, long-duration increases in both heart rate and body temperature occurred repeatedly in response to the various

handling procedures used in Experiment 3. Handling-induced elevation of heart rate was present during the early portion of habituation sessions and during baseline periods of the tolerance acquisition and test phases. Temperature was also increased during these same periods. The magnitude of these responses decreased over days, but increases were still present after 18 sessions. Handling-induced cardioacceleration and hyperthermia were also seen in Group AP- in response to rectal probing throughout acquisition. However, only the cardioacceleratory effect of probing was seen in Group AP+. There was no difference in temperature between Groups AP+ and AN+.

Unlike Experiment 1, there were no group differences in baseline heart rate at any time during Experiment 3. Thus, the evidence that a compensatory decrease in heart rate develops just prior to a cardioacceleratory dose of ethanol is weak. There was a trend towards depression in baseline heart rate just prior to injection in Groups AP+ and AN+ in Experiment 3, but this effect was not significant. The difference between the findings of the two studies may be due to changes induced by probing in the saline control group included in Experiment 3. Comparison of responses of Groups AP+ and AN+ with a not probed control group (as in Experiment 1) may have revealed an apparently greater depression in baseline heart rate. For example, there may have been a compensatory deceleration in baseline scores that developed in Group AP- due to the subsequent probing-induced acceleration. This would decrease apparent differences between groups whereas a comparison with a Group AN- would not.

Sustained tachycardia was associated with both ethanol

administration and rectal probing but the pattern of heart rate changes varied with the treatment. Ethanol induced a long-term tachycardia that remained fairly constant throughout the 120-min post-injection period. Probing led to a sharp peak in heart rate that returned to pre-probed levels within 10 min after probing. The repeated probing procedure resulted in a cyclic variation in heart rate (1 cycle per 30 min). Complete summation of these two responses did not occur (i.e., Group AP+ was not significantly different from Group AN+). It is possible that a ceiling may be imposed on the magnitude of heart rate accelerations (e.g., limit on the length of the refractory period of pacemaker cells) such that two acceleratory responses cannot completely summate.

The magnitude of the cardioacceleratory responses did not change differently in ethanol groups relative to the saline group over a course of 14 injections nor did it differ between groups during a test for tolerance. Thus, the data from both experiments lead to the conclusion that a sustained regimen of ethanol administration does not result in tolerance to the cardioacceleratory effects of ethanol. It is possible that the response may become sensitized as was seen in Experiment 1, but this finding was not replicated in Experiment 3. Again, changes in Group AP- due to expectation of the probing procedures could have obscured comparisons with Groups AP+ and AN+. Although Group AN+ did exhibit increased heart rate accelerations during the conditioned response test, this response was not related to either of the handling procedures. It is therefore not clear whether it is a conditioned response (it may be conditioned to cues other than

handling previously paired with ethanol injection).

As in Experiment 1, 2.0 g/kg of ethanol elicted a long-lasting hypothermia that diminished by about one third with repeated intoxication. The combination of rectal probing and ethanol intoxication did not result in a statistically different degree of temperature change during the Tolerance Acquisition Phase. This is not consistent with a summation hypothesis (as supported by York & Regan, 1982) and is not strong evidence for an interaction hypothesis (as suggested by Experiment 2). It should be noted that if a group was included that was not handled and that received saline injections, a Groups x Probing interaction might have been significant on the assumption that handling alone produces hyperthermia (see Cunningham & Peris, 1983). During the Tolerance Test there was a significant decrease in the temperature of rats while rectally probed relative to when unprobed. The effect was most notable in Group AP- which had never been probed while intoxicated. These latter findings replicate those from Experiment 2. However, the delayed occurrence of this effect in Group AN+, its absence in Group AP+ and and its weakness during tolerance acquisition implies that the design of Experiment 3 was not optimal for revealing the interaction of ethanol and stress.

Groups AP+ and AN+ exhibited two responses indicative of ethanol tolerance. There was a decrease in the magnitude of hypothermia that developed during the last 30 min of later acquisition sessions and there was a decreased magnitude of hypothermia in these groups relative to the saline group during the Tolerance Test.

In summary, it has been established that tolerance to the

hypothermic effects of ethanol can develop over a period of time, during which no change is seen in the cardioacceleratory effects of ethanol. This documents the dissociation of tolerance to two robust effects of ethanol under two relatively moderate dosing schedules of ethanol. The absence of learned tolerance or physiological compensation to the cardioacceleratory effect of ethanol may be due to the strong reflexive control exerted on heart rate. If the cardioacceleratory mechanism of ethanol is primarily reflexive in nature (e.g., increased heart rate due to decreased blood pressure due to increased vasodilation), then the amount of higher control might be expected to be minimal. It is unlikely that smooth muscle in the arterioles would exhibit a large degree of cellular tolerance (e.g., increased resistance to vasodilation). Vasodilation is an important homeostatic mechanism for the fine-tuning of cardiac output and absence of this response would seriously decrement cardiovascular balance.

Chan and Sutter (1983) found that repeated oral alcohol consumption (up to 5.8 g/day after 12 weeks of exposure) led to increased systolic blood pressure in rats. They identified the mechanism of this increase as a 24% expansion of plasma volume whch they suggested was caused by ethanol-induced elevation of plasma arginine-vasopressin and renin activity. In vitro smooth muscle responsivity to norepinephrine was examined and there was no difference between ethanol-treated and control animals. This supports the previous hypothesis that vascular responsiveness is not changed by ethanol exposure even when a higher dose and a more frequent dosing schedule (relative to those in the present studies) were used.

Over the same time course of ethanol administration, tolerance to the hypothermic effects of ethanol developed, although not completely. The magnitude of this response was not affected by probing stress which produces hyperthermia in sober animals. The weak interactive effect of stress did not appear to affect either the rate or magnitude of tolerance differentially. This could be because stress caused by probing does not affect tolerance mechanisms or because the weakness of the initial effect prevented the development of an obvious effect. If the initial effect of stress on ethanol-induced hypothermia could be enhanced, then perhaps an effect on tolerance development would also be more obvious. This hypothesis was addressed in Experiment 4.

EXPERIMENT 4

It seemed possible that the failure to replicate the interactive effect of handling on ethanol-induced hypothermia in Experiment 3 might have been due to differing dose-response relations obtained in between- vs within-subject designs (e.g., a lower effective dose in a within-subject design). If the interactive effect shown in Experiment 2 is a true one, presumably there is some point at which the dose-response functions for stressed and non-stressed subjects cross. At this dose, stressed and non-stressed animals would show equivalent changes in temperature after an ethanol injection. From the findings of Experiment 3 (a between-group design), one would conclude that this dose was about 2.0 g/kg, while the findings of Experiment 2 (a within-group design) suggest the dose is less than 2.0 g/kg.

This difference could be due to the high variability that exists

in the hypothermic response to ethanol between animals. A within-group design might be more likely to find a significant interaction effect because this variability would be more homogeneous in data from stressed and non-stressed conditions. A between-group study (which could not equate stressed and nonstressed animals for variability in ethanol reponsivity) would be more likely to lead to nonsignificant results especially at lower doses where the interaction was not as pronounced. Another possibility is that rats in Experiment 2 were not harnessed for heart rate measurement as were those in Experiment 3. If this slightly restraining procedure increases stress in all animals then it may have helped to decrease apparent differences between stressed and non-stressed groups.

Results from a pilot study using unharnessed animals in a design similar to Phase 2 of Experiment 2 suggested that a higher dose of ethanol (plus perhaps the lower degree of restraint) would increase the interactive effect of stress and ethanol intoxication. Therefore in Experiment 4, a dose of 3.0 g/kg ethanol was used to study the interaction of stress on the acute and chronic hypothermic effects of ethanol.

Method

Subjects

The subjects were 24 adult male albino rats (Holtzman Co., Madison, Wisconsin), 80 days old at the start of testing and weighing an average of 400 g. Mini-Mitters were surgically implanted and the animals were housed and maintained as in the previous experiments.

Apparatus

Experimental chambers and temperature monitoring apparatus were those used in the previous experiments. All Mini-Mitters were Model M and were equilibrated for drift before implantation.

Procedure

The experimental design included all four groups shown in Table 1. Rats in Groups AP+ and AN+ received 3.0 g/kg ethanol (20% v/v in saline, 20 ml/kg volume) while those in Groups AP- and AN- received saline injections. Rats in Groups AP+ and AP- were rectally probed immediately before, and at 30, 60, and 90 min following injection. Rats in Groups AN+ and AN- were not probed before the injection and were left undisturbed during the remainder of the session.

About 24 hr after the start of each recording session, animals in Groups AN+ and AN- were removed from their home cages and rectally probed four times at 30-min intervals. Groups AP+ and AP- were left undisturbed at these times.

After 14 tolerance acquisition training sessions (28 days), two tolerance tests and two conditioned response tests were administered. The procedure for these tests was exactly those in Experiment 3 except the dosage of ethanol was 3.0 g/kg.

Data Analysis

Temperature data were analyzed similarly to those in the previous experiments, except rectal probing was included as a between-groups variable throughout the experiment and also as a within-groups variable in the tolerance test and conditioned response test analyses.

Results

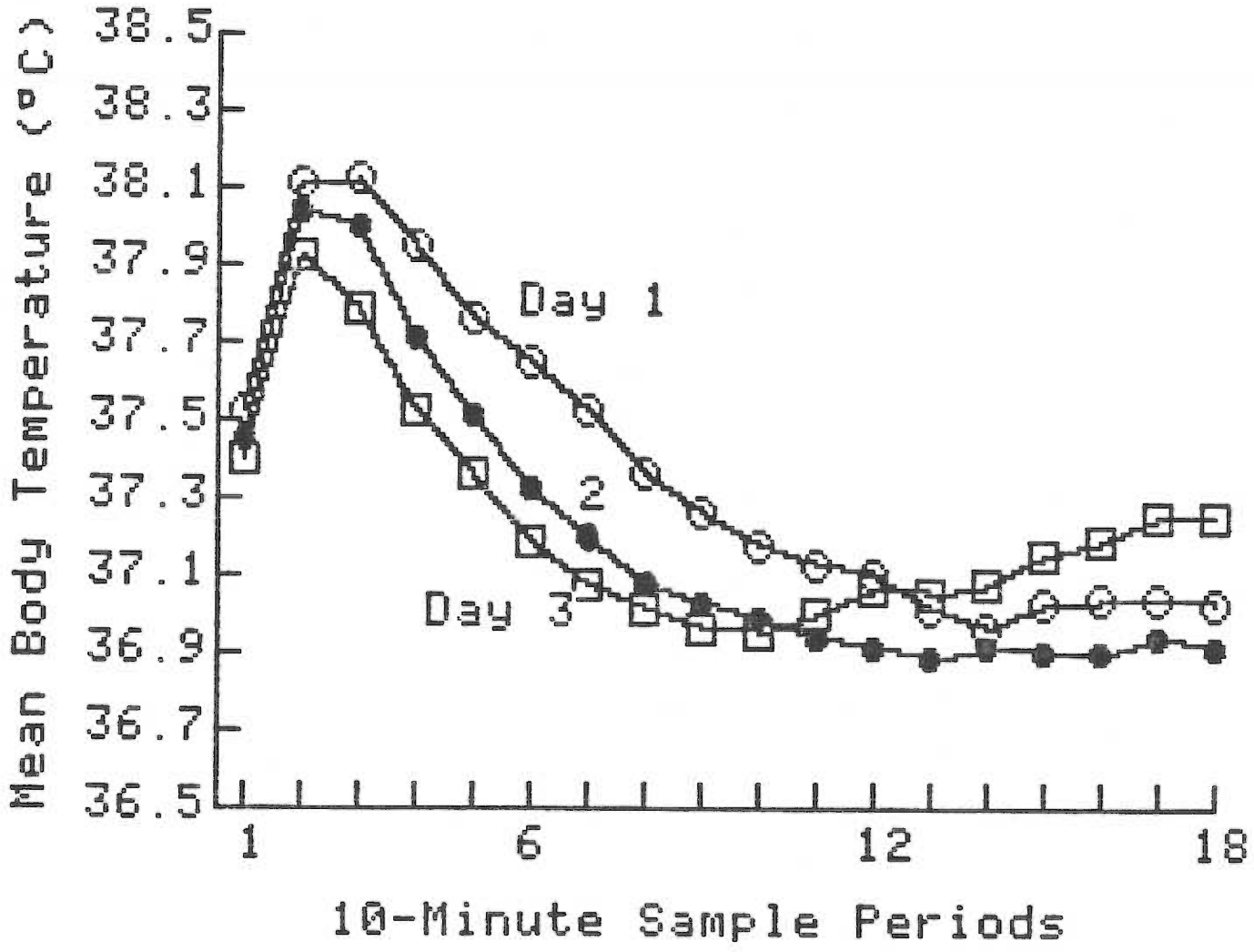
Two rats died during the tolerance acquisition phase and their data were discarded from all phases. One rat was from Group AN+ and one from Group AP-. The data from one rat in Group AP+ were also discarded after the tolerance acquisition phase due to loss of the Mini-Mitter signal. Therefore, data from 22 rats were included in analyses of habituation and tolerance acquisition phases (n = 6, 5, 5, 6) and data from 21 rats were included in test phases (n = 5, 5, 5, 6). Mean body temperature was calculated for each 10-min period of the 180-min test sessions. Baseline scores, change scores and post-injection scores were analysed.

Four-way ANOVAs were performed on the data from habituation and tolerance acquisition phases of Experiment 4. The between-group variables were drug treatment (Ethanol vs Saline) and tolerance handling treatment (Probed vs Not Probed) and the within-group variables were days and 10-min sample periods. Four-way ANOVAs were performed on data from the two test phases. These included the same between-group variables as in the earlier phases, but used test handling treatment (Probed vs Not Probed) and 10-min sample periods as within-group variables.

Habituation

Mean body temperatures of rats on the three days of the habituation phase are graphed in Figure 32 over sample periods. Temperature increased at the start of each session and decreased within 60 - 70 min. The magnitude of the initial increase was smaller on Days

Figure 32. Mean body temperature of rats on the three days of habituation, Data are collapsed across groups.


Mean Body Temperature (°C)
38.5
38.3
38.1
37.9
37.7
37.5
37.3
37.1
36.9
36.7
36.5
Day 1
2
Day 3
1
6
12
18
10-Minute Sample Periods

2 and 3 than on Day 1.

These observations were supported by a significant Days x Sample Periods ($F\{34,612\} = 7.88$) interaction and a significant Sample Periods ($F\{17,306\} = 70.02$) main effect. There were no effects or interactions of Drug or Tolerance Handling groups which were dummy variables at this time. Followups on the Days x Sample Periods interaction supported the observations from the figure.

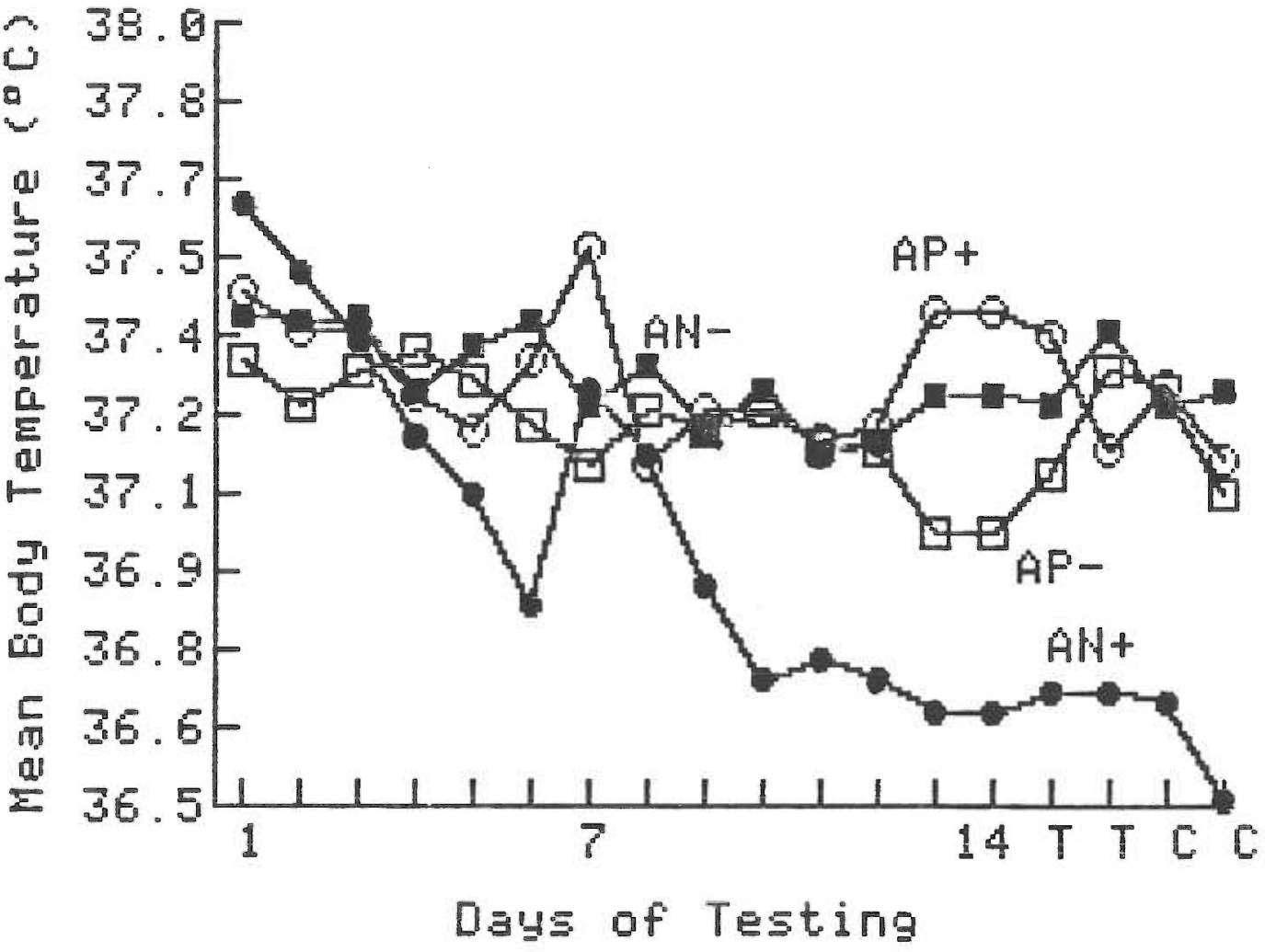
Baseline Scores

Mean body temperatures of the four groups during the 60-min baseline period are graphed in Figure 33 (collapsed over sample periods). Baseline temperature decreased considerably over days in group AN+ and increased slightly over days in Group AP+ relative to the two saline groups. At the start of tolerance acquisition, all groups had relatively equal baseline scores.

Statistical analysis revealed a significant Drug x Tolerance Handling x Days interaction ($F\{17,306\} = 1.83$) which supports these observations. There were also significant Drug x Days ($F\{17,306\} = 1.82$) and Days x Sample Periods ($F\{85,1530\} = 5.55$) interactions and Days ($F\{17,306\} = 3.93$) and Sample Periods ($F\{5,90\} = 25.58$) main effects.

Followup within-group analyses of the Drug x Tolerance Handling x Days interaction revealed a main effect of Days in Group AN+ ($F\{17,68\} = 4.56$) but not in the other three groups. This was due to the overall decrease in baseline of this group during all sample periods, whereas the other three groups changed over days only during some sample periods. All groups had significant Days x Sample Periods

Figure 33. Mean baseline body temperatures of the four groups over aqcuisition (1-14) and test phases (T and C). Data are collapsed over 10-minute sample periods.


Mean Body Temperature (°C)
38.0
37.8
37.7
37.5
37.4
37.2
37.1
36.9
36.8
36.6
36.5
AP+
AN-
AP-
AN+
1
7
14 T T C C
Days of Testing

interactions and Sample Periods effects which support this conclusion.

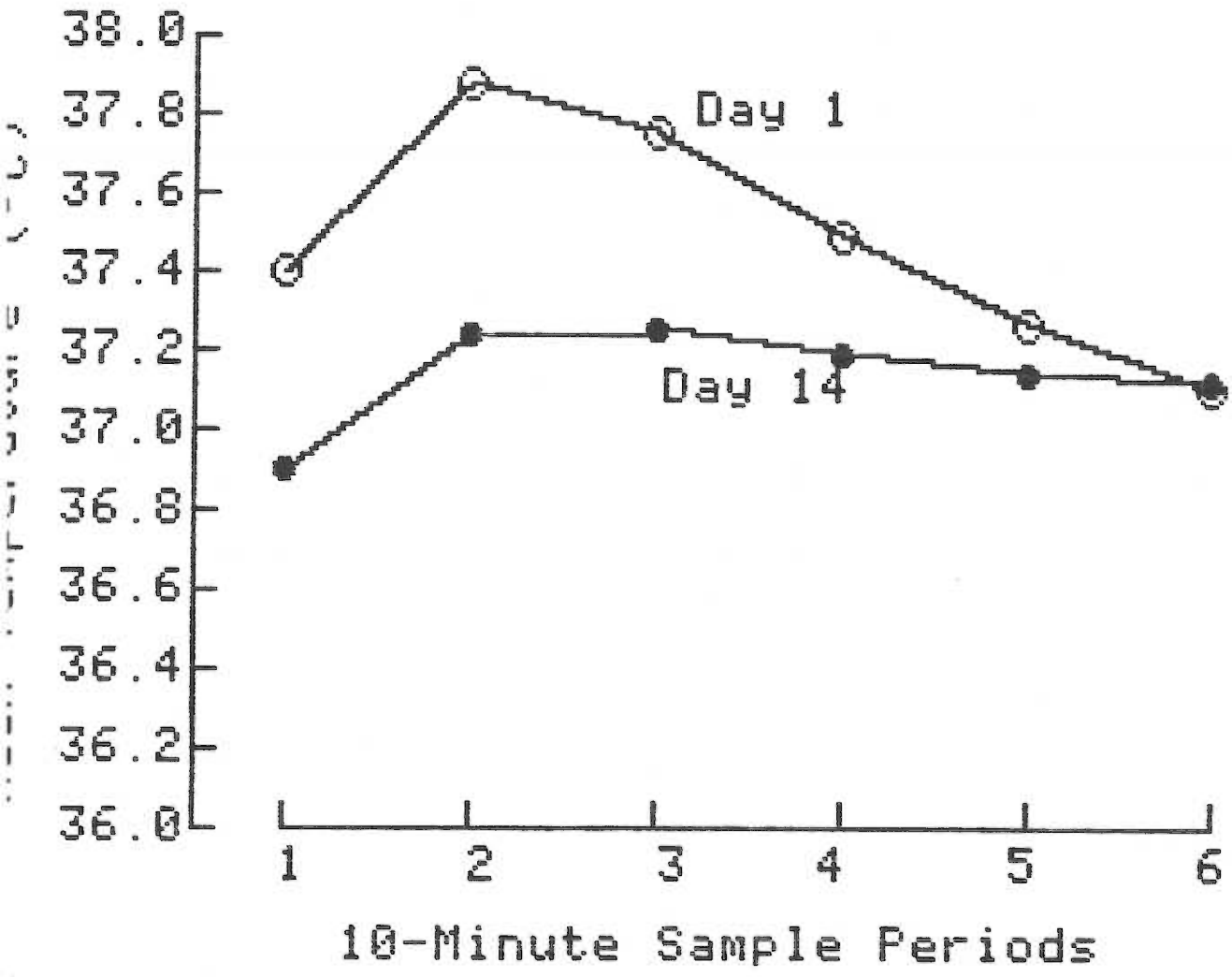
Followup analyses were performed on the data from Days 1 thru 4 and Days 13 thru 16. These blocks of days were chosen because there appeared to be no systematic differences between groups early in the experiment but large differences between groups during these later days. There was a significant Drug x Tolerance Handling interaction ($F\{1,18\} = 4.44$) during the later block of days and no significant group differences during the first block. This supports the observation that baseline temperatures of the four groups were not different during earlier acquisition sessions, but by later sessions, Group AN+ had much lower baselines than the saline groups and Group AP+ had slightly higher baseline levels. Neuman-Keuls analyses on data from the later four days support the difference between Groups AP+ and AN+ but not between these groups and any other groups.

The nature of the Days x Sample Periods interaction is apparent from analyses of Days 1 and 14 (the first and last days of tolerance acquisition). Mean baseline temperatures (collapsed across groups) on Day 1 vs Day 14 of tolerance acquisition are graphed in Figure 34 over sample periods. Temperature was initially 37.4°C on Day 1 and increased about 0.5°C during the first 20 min of the baseline period after which baseline decreased to about 37°C. On Day 14, initial baseline was about 36.9°C and the magnitude of the increase was only about 0.3°C. Final levels were equal on both days. There were significant Sample Periods effects on both days.

Tolerance Acquisition

Post-injection scores. Mean body temperatures of the four

Figure 34. Mean baseline body temperatures on Days 1 and 14. Data are collapsed across groups.

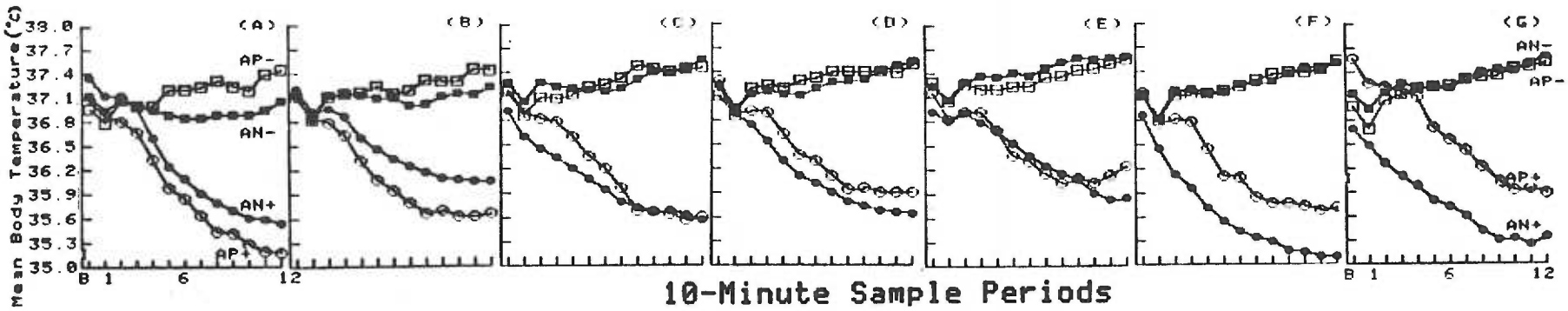

38.0
37.8
37.6
37.4
37.2
37.0
36.8
36.6
36.4
36.2
36.0
Day 1
Day 14
1
2
3
4
5
6
10-Minute Sample Periods

groups during tolerance acquisition are graphed in the seven panels of Figure 35 (collapsed into 2-day blocks). Temperature was lower in the ethanol groups relative to the saline groups. During early sessions, hypothermia was greater in Group AP+ relative to Group AN+ even though probing increased temperature in Group AP-. Mean temperature generally increased over days in Group AP+ especially during later sample periods, whereas, it appeared as if temperature of Group AN+ decreased over days during all sample periods. Hyperthermia in Group AP- remained fairly constant over days while a small magnitude hyperthermia developed in Group AN-. Thus, at the end of this phase there was no difference in temperature between the saline groups and the relative temperatures of the ethanol groups had reversed.

Statistical analyses revealed significant interactions of Drug x Tolerance Handling x Days x Sample Periods ($F\{156,2808\} = 1.47$), Tolerance Handling x Days x Sample Periods ($F\{156,2808\} = 0.74$), Drug x Days x Sample Periods ($F\{156,2808\} = 1.56$), Days x Sample Periods ($F\{156,2808\} = 1.99$), Drug x Sample Periods ($F\{12,216\} = 110.48$), Drug x Tolerance Handling x Days ($F\{13,234\}$ 3.35) and Tolerance Handling x Days ($F\{13,234\} = 1.95$). There were significant main effects of Drug ($F\{1,18\} = 43.68$), Days ($F\{13,234\} = 2.52$) and Sample Periods ($F\{12,216\} = 41.61$).

Followup within-group analyses revealed significant main effects of Days in Groups AN+ and AN- ($F\{13,52\} = 3.45$ and $F\{13,65\} = 3.15$) but not in the probed groups which support the observation that temperature in Group AN+ decreased and that in Group AN- increased in all sample periods over days. There were significant Days x Sample

Figure 35. Mean body temperatures of the four groups during the Tolerance Acquisition Phase. Data are graphed over 10-minute sample periods and 2-day blocks.


Mean Body Temperature (°C)
38.0
37.7
37.4
37.1
36.8
36.5
36.2
35.9
35.6
35.3
35.0
(A)
(B)
(C)
(D)
(E)
(F)
(G)
AP-
AN-
AN+
AP+
B 1 6 12
10-Minute Sample Periods

Periods interactions and Sample Periods effects in all groups indicating that the changes were not equal in all sample periods over days. Followup analyses on selected pairs of days (e.g., early, middle and late) revealed a significant Drug x Tolerance Handling x Sample Periods interaction on Days 1 and 2 ($F\{12,216\} = 2.63$) but not on Days 7 and 8 or Days 13 and 14. There were significant Drug effects in these last two analyses but no significant effect or interactions due to Tolerance Handling. This supports the observation that probing affected temperature differently after a saline or an ethanol injection during earlier acquisition sessions. It does not support the observation that there was a difference between temperature in Groups AP+ and AN+ during later sessions although from the graph, this seems like quite an obvious effect.

When the data from the ethanol groups on Days 1 and 2 were analysed, there was a significant Tolerance Handling x Sample Periods interaction ($F\{12,108\} = 1.84$). Data from the saline groups on these days revealed a significant Tolerance Handling x Sample Periods interaction ($F\{12,108\} = 2.01$). As stated before, there were significant Sample Periods effects in all groups. When the Probed groups and Not Probed groups were compared separately, there were significant Drug x Sample Periods interactions and main effects of Drug and Sample Periods on all days for both comparisons.

The interactions are explained by bigger decreases in temperature over days in Group AN+ during middle and later sample periods while Group AN- had increased body temperature over days only during later sample periods. This is supported by significant effects of Days in

Group AN+ in the analyses of Sample Periods 3 and 13 while in Group AN- there was only a significant effect of Days in the analysis of Sample Period 13. These comparisons support the observations that Groups AN+ and AN- changed over Days in all sample periods while Group AP+ only changed during some sample periods and Group AP- did not change over days at all.

The development of tolerance in Group AP+ but not AN+ is clearly seen in Figure 36 (data are shown for Sample Period 13 only). On the first few days, temperature was lower in Group AP+ relative to AN+. Over days, temperature in Group AP+ increased while that of AN+ decreased. Temperature of Group AN- increased until it was equal to Group AP+ which remained constant over days. The Days x Sample Periods interaction in the overall analysis is due to a general increase in temperature during the later sample periods that developed over days.

Change Scores. Mean body temperature change scores of the four groups are graphed in Figure 37 over 2-day blocks and 10-min sample periods. Hypothermia was greater in the ethanol groups than in saline groups. Initially, probing caused greater hyperthermia in Group AP- than in Group AN- but slightly increased hypothermia in Group AP+ relative to Group AN+. During later sessions, there was no difference between Groups AP- and AN- and both Groups AP+ and AN+ had become slightly less hypothermic. There were no consistent differences between these two groups during later sessions. Generally, hypothermic responses decreased and hyperthermic responses increased in all groups over days especially during the last sample periods.

The analysis revealed significant interactions of Drug x Days x

Figure 36. Mean body temperatures of the four groups over days of tolerance acquisition training. Data are collapsed over 10-minute sample periods.

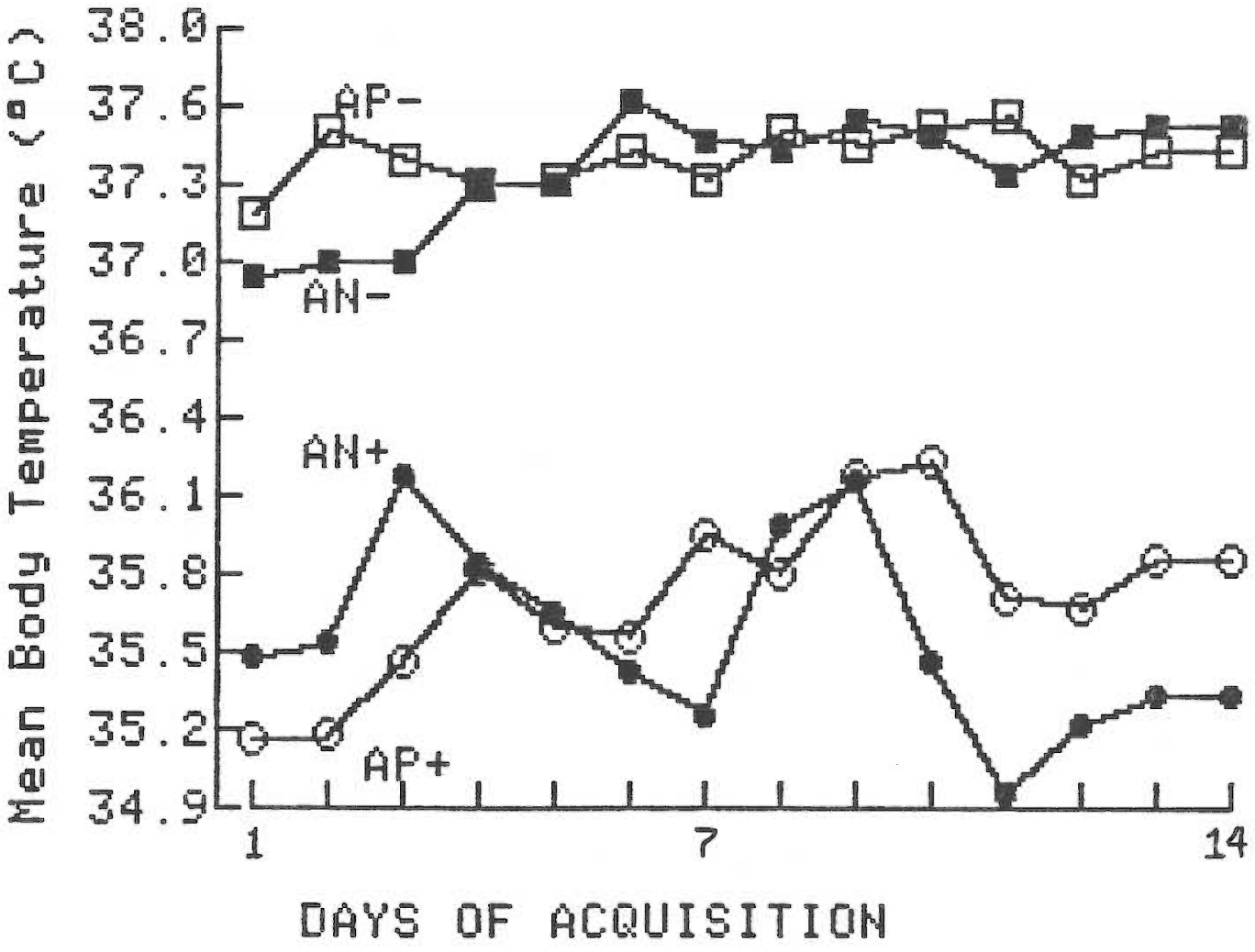
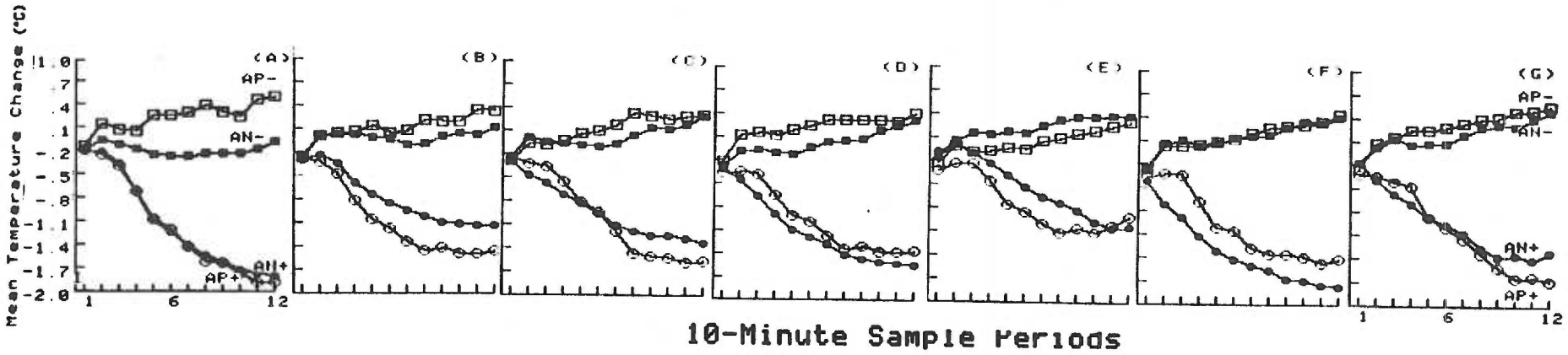

Mean Body Temperature (°C)
38.0
37.6
37.3
37.0
36.7
36.4
36.1
35.8
35.5
35.2
34.9
AP-
AN-
AN+
AP+
1
7
14
DAYS OF ACQUISITION

Figure 37. Mean temperature change scores of the four groups during the Tolerance Acquisition Phase. Data are graphed over 10-minute sample periods and 2-day blocks. Mean baseline temperatures on each consecutive 2-day block were 37.05, 37.1, 37.1, 37.3, 37.1, 37.2 and 37.5 for Group AP+, 37.4, 37.2, 36.9, 37.2, 36.9, 36.8 and 36.7 for Group AN+, 37.0, 37.1, 37.1, 37.1, 37.3, 37.1 and 36.9, for Group AP-, and 37.1, 37.1, 37.3, 37.2, 37.2, 37.1 and 37.1 for Group AN-.


Mean Temperature Change (°C)
+1.0
.7
.4
.1
-.2
-.5
-.8
-1.1
-1.4
-1.7
-2.0
1
6
12
(A)
(B)
(C)
(D)
(E)
(F)
(G)
AP-
AN-
AN+
AP+
10-Minute Sample Periods

Sample Periods ($F\{143,2574\} = 1.56$), Drug x Sample Periods ($F\{11,198\} = 109.6$), Drug x Days ($F\{13,234\} = 2.11$) and Days x Sample Periods ($F\{143, 2574\} = 1.97$). There were main effects of Drug ($F\{1,18\} = 133.42$), Days ($F\{13,234\} = 2.0$) and Sample Periods ($F\{11,198\} = 33.16$). The Drug x Handling x Days x Sample Periods interaction approached significance ($F\{143,2574\} = 1.18$, $p = .07$) but in contrast to the analyses based on post-injection scores, there were no effects or interactions of Tolerance Handling Treatment.

In general, ethanol groups became less hypothermic over days during middle and later sample periods and saline groups more hyperthermic over days during later sample periods. The changes over days were more gradual in the saline groups while those in the ethanol groups decreased in a variable manner with the largest decrease occurring around Days 9 and 10.

Followup within-group analyses revealed a significant Days effect in Group AN+ ($F\{13,52\} = 2.18$) but not in the other three groups. There were significant Days x Sample Periods interactions in Groups AP+, AN+ and AN- only and significant effects of Sample Periods in all groups. The absence of any Days effect or interaction in Group AP- supports the observation that the magnitude of hyperthemia did not change in this group over tolerance acquisition. Temperature change scores in Group AN- were initially equal to zero throughout all sample periods but hyperthermia progressively appeared during later sample periods which is supported by the Days x Sample Periods interaction in this group.

Summary: Tolerance Acquisition Phase

The acute hypothermic effect of ethanol on body temperature was increased if rats were probed while intoxicated. However, rats probed after a saline injection were more hyperthermic relative to rats that were not probed after a saline injection. From the post-injection scores, one may conclude that tolerance developed only in Group AP+ because temperature after an ethanol injection increased over days in this group while temperature of Group AN+ actually decreased over days. However, when change scores were analysed, both groups showed diminishing hypothermia over days, although these changes were smaller in Group AN+.

When change score results are considered, the general decrease in hypothermia in ethanol groups is consistent with the development of tolerance in both groups. However, there was also an increase in temperature and decrease in hypothermic changes in Group AN- but not in Group AP- over days. Therefore, the changes that occurred over days in the Not Probed groups were not totally drug specific while those in the Probed groups occurred only after an ethanol injection. It can be concluded from the post-injection scores that tolerance developed in Group AP+ but not in Group AN+. The change scores also support the conclusion that tolerance developed only in Group AP+ and not in Group AN+.

The difference between post-injection and change scores was due mostly to the large decrease in baseline body temperature of Group AN+. Such a change in baselines could have decreased the apparent drop in temperature after an ethanol injection. The trend for higher baselines in Group AP+ may have masked the decrease in hypothermia over days.

This effect on baselines did not occur in Experiment 3 when a lower dose of ethanol was used (although a slight trend in the same direction was present).

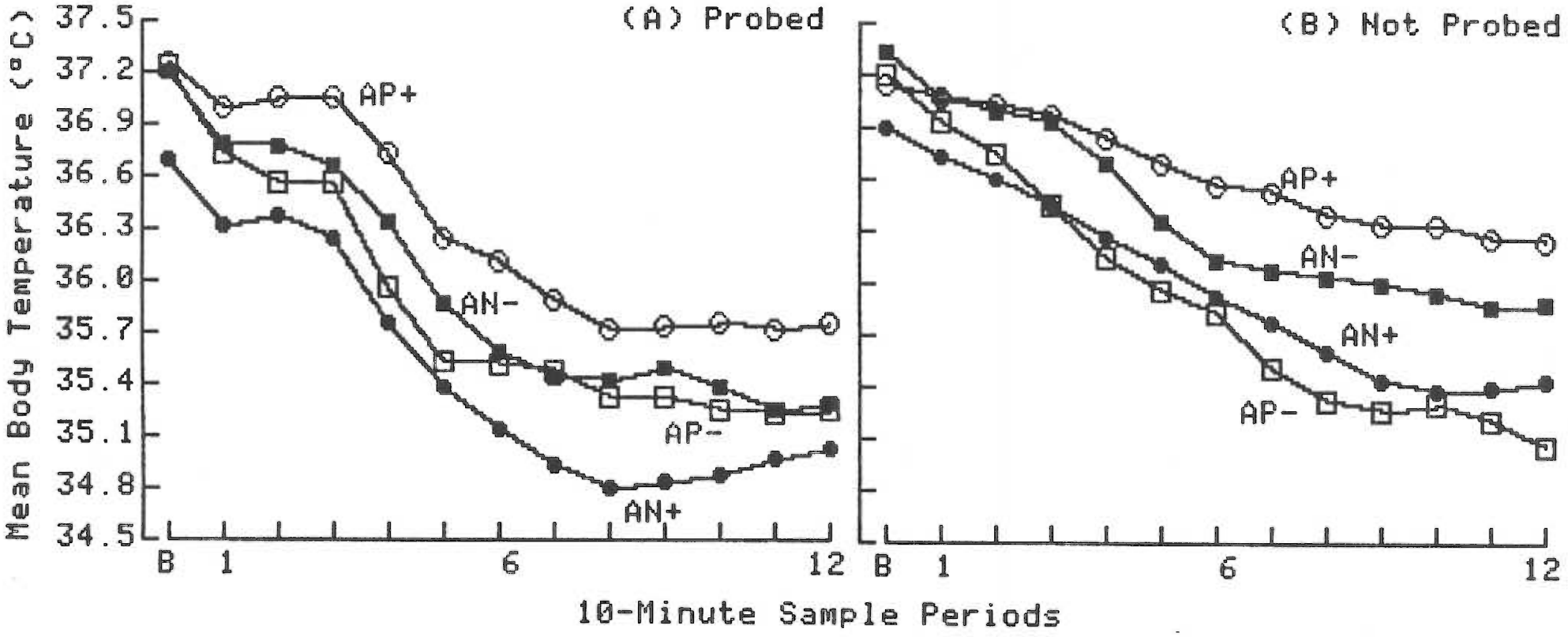
Tolerance Test

Post-injection Scores. Mean body temperature of the four groups during both Probed and Not Probed test handling conditions of the Tolerance Test Phase are graphed over sample periods in the two panels of Figure 38. Generally, the magnitude of hypothermia was greater during the Probed condition relative to the Not Probed condition in all groups. Group AP+ was less hypothermic than the other three groups. Group AN+ was at least as hypothermic as the saline groups and during the Probed condition had lower temperatures than any group.

The statistical analysis revealed significant interactions of Test Handling x Sample Periods ($F\{12,204\} = 3.47$) and Drug x Tolerance Handling ($F\{1,17\} = 4.83$) plus main effects of Test Handling ($F\{1,17\} = 7.86$) and Sample Periods ($F\{12,204\} = 102.46$). These finding support the observations made from the figure. Followup analysis of the Drug x Tolerance Handling interaction revealed that Group AP+ had a higher temperature compared to Group AN+ ($F\{1,8\} = 5.31$) and a higher temperature than Group AP- ($F\{1,8\} = 5.99$). There were no differences in any of the other group comparisons including that between Groups AN+ and AN-.

Followup analysis of the Test Handling x Sample Periods interaction supported the observation that probing lowered temperature relative to that during the Not Probed condition. This effect was

Figure 38. Mean body temperature of the four groups during the Probed (Panel A) and Not Probed (Panel B) conditions of the Tolerance Test Phase. Data are grasphed over 10-minute sample periods.


(A) Probed
(B) Not Probed
Mean Body Temperature (°C)
37.5
37.2
36.9
36.6
36.3
36.0
35.7
35.4
35.1
34.8
34.5
AP+
AN-
AP-
AN+
B
1
6
12
10-Minute Sample Periods

greatest during the later sample periods.

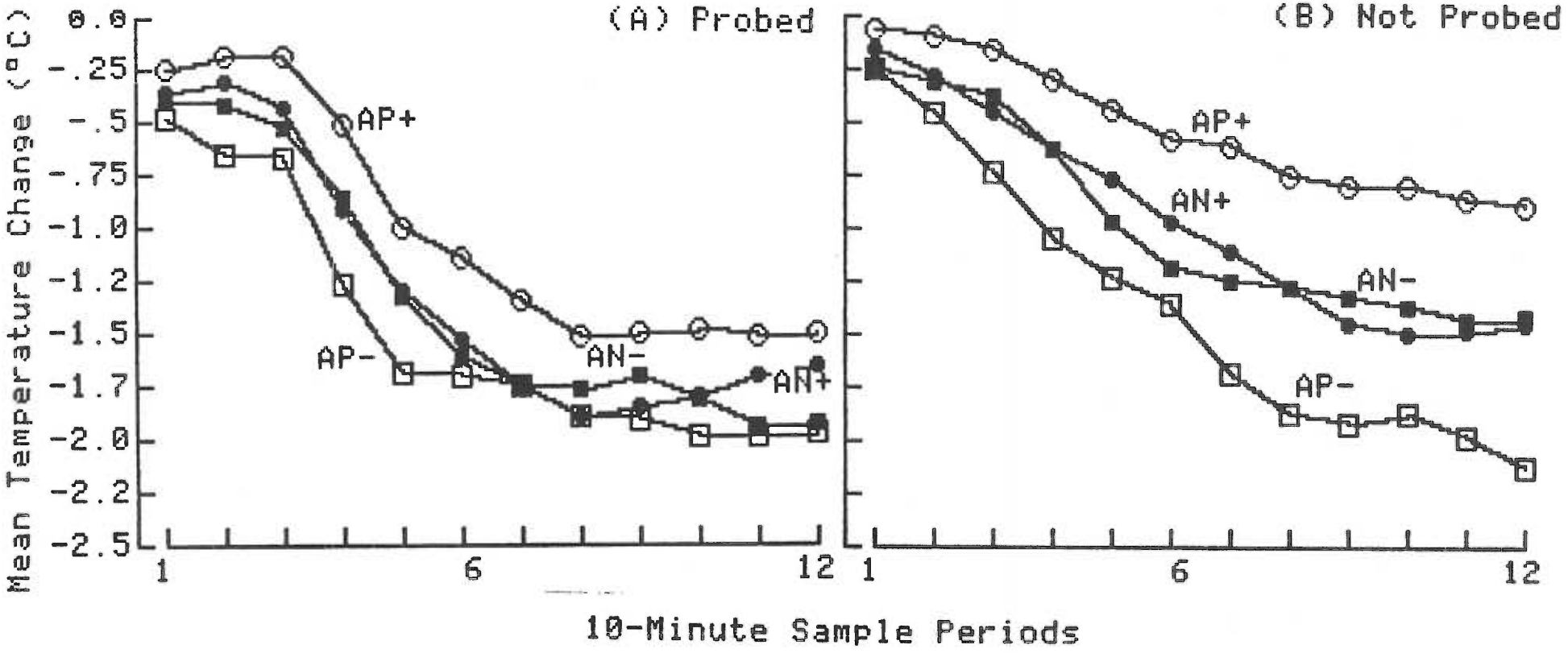
Change Scores. Mean temperature change scores during the Tolerance test phase are graphed in Figure 39. Changes occurred in the Drug and Tolerance Handling groups that were similar to those described for the post-injection scores (i.e., Group AP+ appeared less hypothermic than the other groups). However, analysis of these data revealed only a significant Test Handling Treatment x Sample Periods interaction ($F\{11,187\} = 2.97$) and significant main effects of Test Handling ($F\{1,17\} = 6.46$) and Sample Periods ($F\{11,187\} = 87.44$). There were no effects or interactions of Drug or Tolerance Handling groups.

The nature of the Test Handling x Sample Periods interaction is obvious from the figure. It can be seen that temperature during the Probed condition was much lower relative to the Not Probed condition during later sample periods. Temperature during earlier sample periods was fairly equal during both test conditions.

Summary: Tolerance Test Phase

Group AP+ had a higher mean body temperature after a test injection of ethanol relative to Group AN+ although both groups had equal exposure to ethanol. Hypothermia in Group AN+ was not different after the test injection from that in Group AN- while hypothermia was less in Group AP+ relative to Group AP-. This indicates that tolerance was greater in Group AP+ than in Group AN+. There was no difference in responsivity to the test injection in Groups AN- and AP-. Thus, the decreased responsivity to the test injection of ethanol due to tolerance handling treatment was seen only to animals that had been

Figure 39. Mean temperature change scores of the four groups during the Probed (Panel A) and Not Probed (Panel B) conditions of the Tolerance Test Phase. Mean baseline temperature before the Probed condition was 37.3 for Group AP+, 36.7 for Group AN+, 37.3 for Group AP- and 37.2 for Group AN-. Temperature before the Not Probed condition was 37.1 for Group AP+, 36.9 for Group AN+, 37.2 for Group AP- and 37.3 for Group AN-.


(A) Probed
(B) Not Probed
Mean Temperature Change (°C)
0.0
-.25
-.5
-.75
-1.0
-1.2
-1.5
-1.7
-2.0
-2.2
-2.5
AP+
AN-
AN+
AP-
1
6
12
10-Minute Sample Periods

probed only after receiving ethanol during tolerance acquisition sessions.

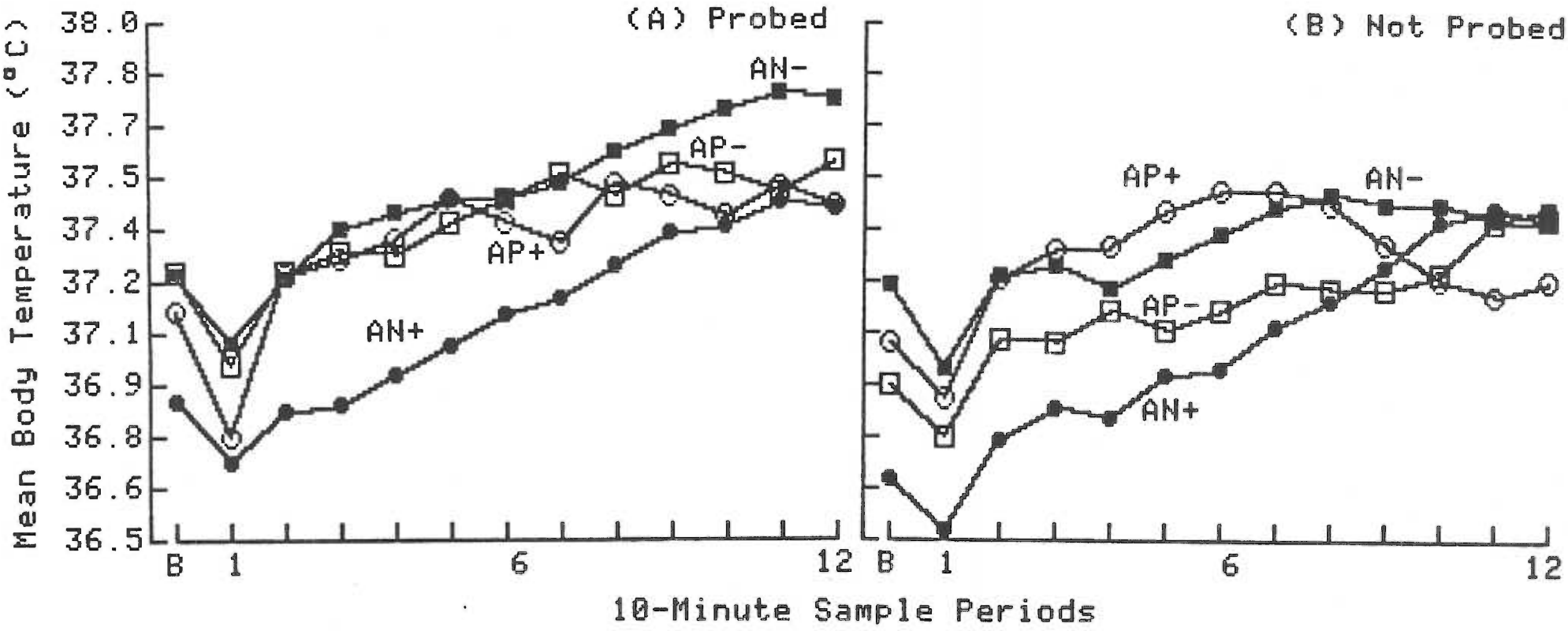
These effects were only significant when post-injection scores were analysed and not when change scores were analysed (although the same trends were present). The changes in baseline temperature diminished the magnitude of change scores similar to the effect of baseline levels on change scores during the Tolerance Acquisition Phase.

As in Experiment 2 and earlier sessions of the Tolerance Acquisition Phase of Experiment 4, probing induced a greater hypothermic effect in intoxicated rats during the Tolerance Test Phase relative to that exhibited during the Not Probed test condition. There was no interaction of Drug Treatment x Tolerance Handling Treatment x Test Handling Treatment. This implies that tolerance was not affected by handling cues that were either different or the same as those occurring after ethanol injections during the acquisition phase. In other words, tolerance was not conditioned to specific handling cues or affective states caused by handling which had been presented contiguously with ethanol injection.

Conditioned Response Test

Post-injection Scores. The mean temperature of the four groups during both Test Handling conditions of the Conditioned Response Test Phase are graphed in Figure 40. Temperature generally increased over sample periods during both test conditions. Temperature was lower in Group AN+ relative to the other groups with the greatest differences occurring during early and middle sample periods. There are no obvious

Figure 40. Mean body temperature of the four groups during the Probed (Panel A) and Not Probed (Panel B) conditions of the Conditioned Response Test Phase.


(A) Probed
(B) Not Probed
Mean Body Temperature (°C)
38.0
37.8
37.7
37.5
37.4
37.2
37.1
36.9
36.8
36.6
36.5
AN-
AP-
AP+
AN+
B
1
6
12
10-Minute Sample Periods

differences between the other three groups.

The statistical analysis revealed significant Drug x Tolerance Handling x Test Handling x Sample Periods ($F\{12,204\} = 1.95$), Drug x Tolerance Handling x Sample Periods ($F\{12,204\} = 1.84$) and Tolerance Handling x Sample Periods ($F\{12,204\} = 1.84$) interactions and a main effect of Sample Periods ($F\{12,204\} = 25.71$). Within-group analyses revealed only significant effects of Sample Periods in all groups and no effect or interaction of Test Treatment.

A separate comparison of Groups AP+ vs AN+ revealed a significant Tolerance Handling x Sample Periods interaction ($F\{12,96\} = 3.14$) and Sample Periods main effect ($F\{12,96\} = 11.92$). There were significant Sample Periods effects in both groups. This supports the observation that Group AN+ had a lower mean temperature than did Group AP+ especially during earlier sample periods. Separate analyses of Groups AP- vs AN-, Group AP+ vs AP-, or Group AN+ vs AN- revealed only Sample Periods main effects in all analyses. Therefore, the four-way interaction was due in part to lower temperature in Group AN+ relative to that in Group AP+ during earlier sample periods. Although the Test Handling factor was not significant in these followup comparisons, it was involved in the four-way interaction. This appears to be due to lower mean temperature in Group AP+ when probed (and therefore less of a difference between Groups AP+ and AN+) during the first few sample periods and higher mean temperature in Group AN+ when not probed (and less of a difference between groups) during the last few sample periods.

The Tolerance Handling x Sample Periods interaction is

illustrated in Figure 41. Temperature was slightly greater during earlier sample periods in groups that had been probed during tolerance acquisition sessions relative to those that had not been probed. During later sample periods, temperature was slightly greater in groups that had not been probed during tolerance acquisition sessions.

Change Scores. The mean temperature change scores of the four groups during both handling treatments of the Conditioned Response Test Phase are graphed in Figure 42. Hyperthermia was greater during later sample periods. There was not an obvious effect of test handling treatments as when post-injection scores were analysed. Group AN+ was considerably more hyperthermic during later sample periods of the Not Probed condition relative to the other groups. There were no other obvious differences between groups.

Statistical analyses revealed significant Drug x Tolerance Handling x Test Handling x Sample Periods ($F\{11,187\} = 2.16$), Drug x Tolerance Handling x Sample Periods ($F\{11,187\} = 2.11$) and Tolerance Handling x Sample Periods ($F\{11,187\} = 2.73$) interactions. There was also a main effect of Sample Periods ($F\{11,187\} = 26.53$). Within-group analyses revealed only significant Sample Periods effects in all groups.

Separate analyses of the ethanol groups revealed a significant Tolerance Handling x Sample Periods effect ($F\{11,88\} = 3.0$) similar to that found when post-injection scores were analysed. Separate analyses of the saline groups, the groups probed during tolerance acquisition and the groups that were not probed during tolerance acquisition sessions revealed only significant Sample Periods main

Figure 41. Mean body temperature of the two tolerance handling treatment groups (Probed = P, Not Probed = N) during the Conditioned Response Phase.

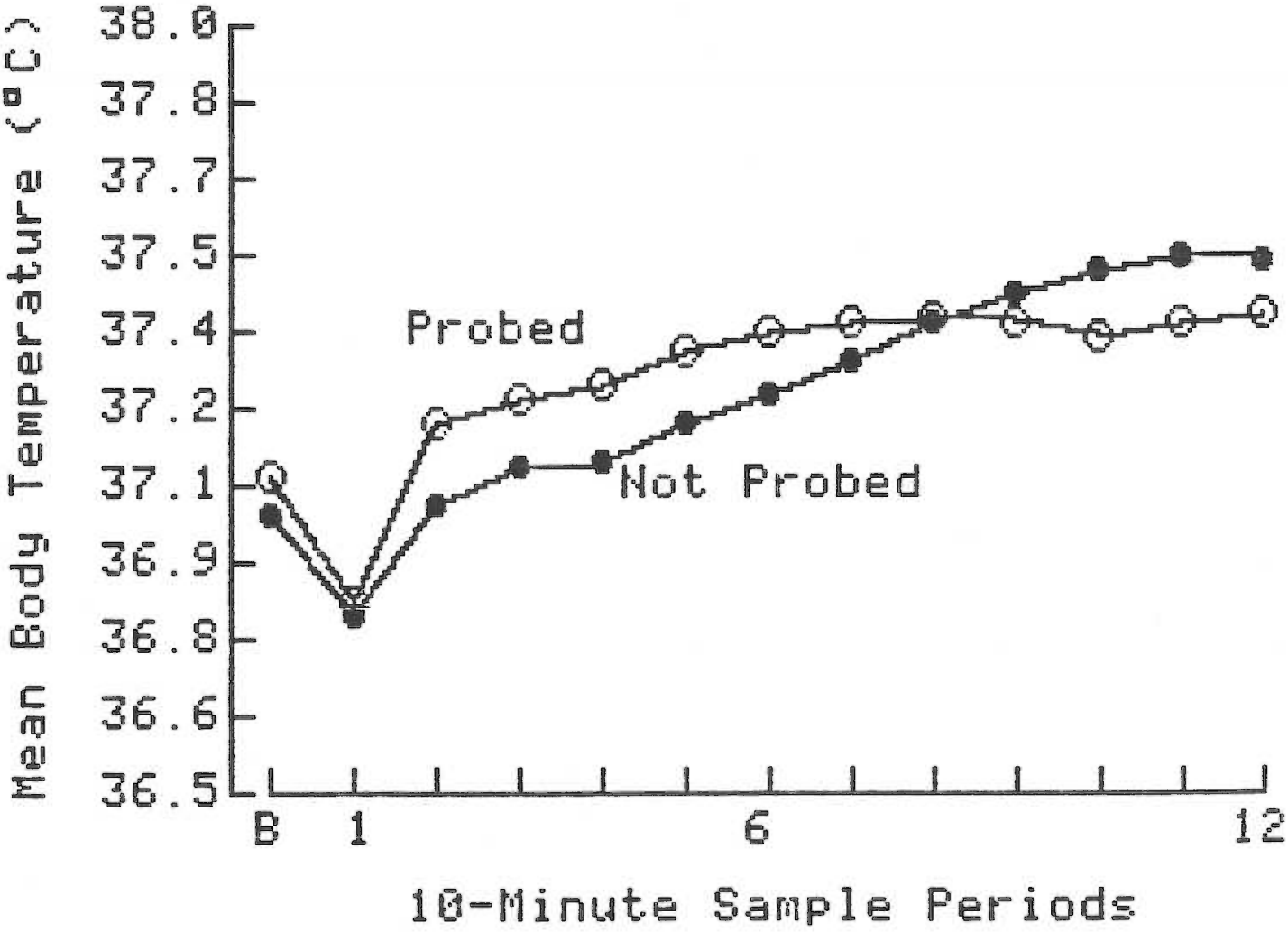
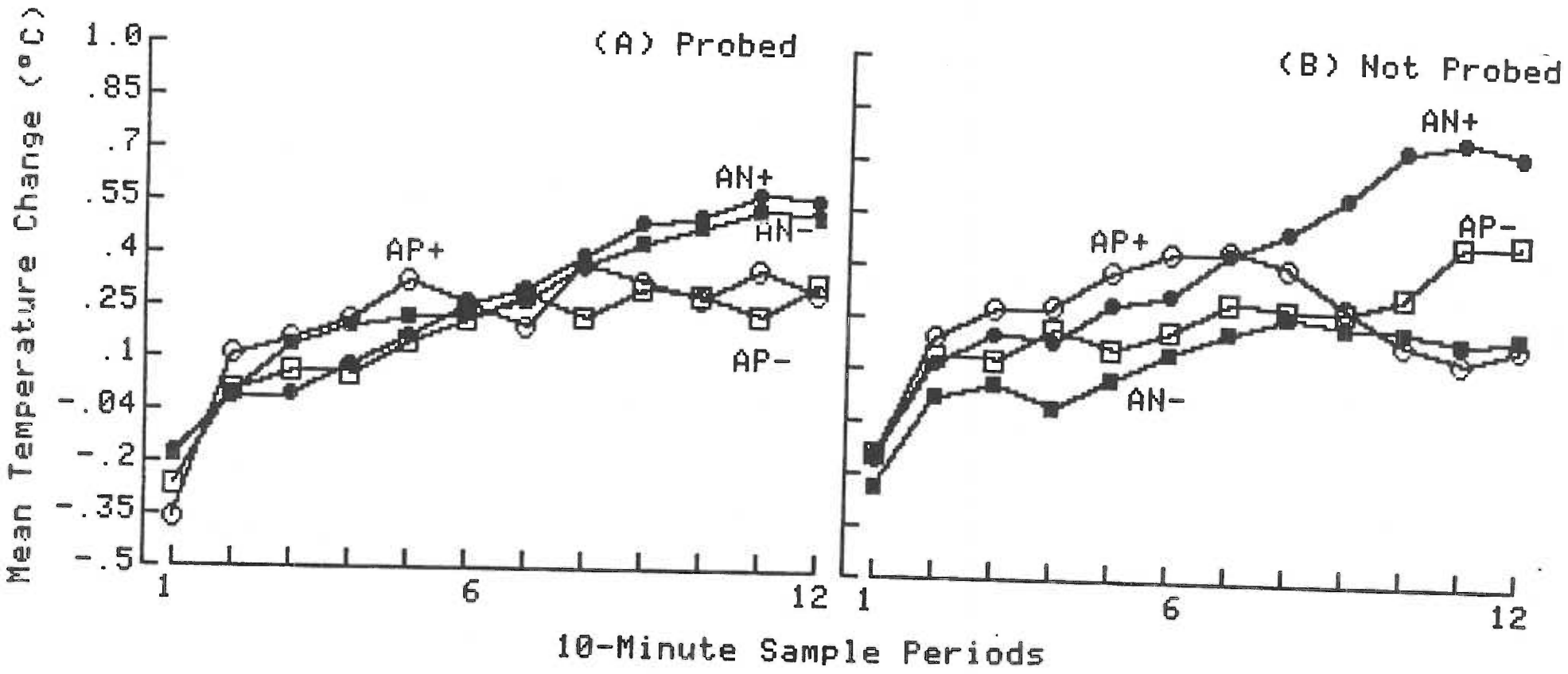

Mean Body Temperature (°C)
38.0
37.8
37.7
37.5
37.4
37.2
37.1
36.9
36.8
36.6
36.5
Probed
Not Probed
B
1
6
12
10-Minute Sample Periods

Figure 42. Mean temperature change scores of the four groups during the Probed (Panel A) and Not Probed (Panel B) conditions of the Conditioned Response Test Phase. Mean baseline temperature before the Probed condition was 37.18 for Group AP+, 36.9 for Group AN+, 37.2 for Group AP- and 37.25 for Group AN-. Temperature before the Not Probed condition was 37.09 for Group AP+, 36.66 for Group AN+, 36.95 for Group AP- and 37.25 for Group AN-.


Mean Temperature Change (°C)
1.0
.85
.7
.55
.4
.25
.1
-.04
-.2
-.35
-.5
(A) Probed
AN+
AN-
AP+
AP-
1
6
12
(B) Not Probed
AN+
AP+
AP-
AN-
1
6
12
10-Minute Sample Periods

effects in all three comparisons.

The Tolerance Handling x Sample Periods interaction in the overall analysis was of the same nature as that found in the analysis of post-injection scores. Hyperthermia was greater during earlier sample periods in groups that were probed during Tolerance acquisition while hyperthermia was greater during later sample periods in groups not probed during tolerance acquisition.

Summary: Conditioned Response Phase.

There was no evidence of a conditioned hyperthermic response in Group AP+ (which exhibited tolerance during earlier phases) while hyperthermia was greatest in Group AN+ (which did not show evidence of tolerance). This hyperthermic response was greatest during later sample periods of the Not Probed condition. When post-injection scores were analysed, temperature was lower in Group AN+ than in other groups. This was mainly due to a lower baseline level that existed in Group AN+ at the time of the saline test injection. It is interesting that the same type of hyperthermic response was present in Group AN+ during the Conditioned Response Test Phase of Experiment 3. In that case, however, the greatest hyperthermia was exhibited during the Probed Test condition rather than the Not Probed condition as seen in Experiment 4. These findings do not support the conclusion that tolerance was greater in Group AP+ relative to Group AN+ due to the development of a conditioned compensatory response in Group AP+ and not in Group AN+.

Discussion

Findings from both tolerance acquisition and tolerance test phases of Experiment 4 indicated that while probing alone induced a

slight hyperthermia and ethanol alone elicited hypothermia, probing of intoxicated rats led to a large magnitude hypothermia. This interaction between handling and ethanol intoxication replicates the findings from Experiments 2 and 3 and supports the interaction hypothesis. These findings also support the hypothesized cross-over type dose-response curves for stressed or non-stressed animals given increasing doses of ethanol.

The magnitude of hypothermia decreased over days in Group AP+ but not in Group AN+. In addition, data from the tolerance test phase revealed that rats in Group AP+ exhibited higher temperatures after an ethanol injection than those in Group AN+ even though both groups had equal experience with ethanol. This is indicative of tolerance in Group AP+ but not in Group AN+. Because these effects were not affected differently by Test Handling conditions, it cannot be concluded that handling was a cue for conditioned tolerance exhibition.

From these data, it seems reasonable to conclude that tolerance developed only in Group AP+. Since initially, ethanol caused a larger magnitude UR in Group AP+ relative to that in Group AN+, it is possible that a greater CR developed in this group. There was slight evidence that an increased hyperthermia had developed prior to ethanol injections (during baseline periods) in this group but this effect was not pronounced relative to the changes that occurred in Group AN+ during all three phases (especially since Group AN+ exhibited a greater degree of hyperthermia than did Group AP+ during the Conditioned Response Test Phase).

The difference in ethanol responsivity seemed to be due mostly to

the development of a lower baseline temperature in Group AN+ and therefore lower temperature during the post-injection period. This effect developed gradually over tolerance acquisition sessions. Informal behavioral observations and body weight monitoring support the conclusion that these animals were more affected by continued exposure to the high dose of ethanol. They lost more weight and slept longer after ethanol injections relative to weight loss and sleep time of Group AP+ near the end of the experiment. The increased weight loss indicates these animals were generally of poorer health status than the other animals. This may possibly have affected baseline temperatures. These informal findings also support the general hypothesis that increased stress or activity during ethanol exposure increases the rate of tolerance development.

GENERAL DISCUSSION

This project was concerned with the development of tolerance to the cardiovascular and thermic effects of ethanol in the rat, both under conditions where drug administration could be anticipated and when it could not. In addition, the interaction of a nonassociative factor (i.e., stress induced by rectal probing) with conditioned anticipatory responses to ethanol and the resulting effect on tolerance development were studied in Experiments 2, 3 and 4.

The first experiment found that two commonly employed doses of ethanol affected heart rate and body temperature in a dose-related fashion for 2 hr after ethanol administration. A schedule of repeated injections in both Experiments 1 and 3 allowed for the assessment of

tolerance to the effects of ethanol on these physiological responses. Tolerance did not develop to the dose-related cardioacceleratory effect of ethanol while the same dosing schedules led to development of tolerance to ethanol's hypothermic effect. Although a response resembling a compensatory cardiodeceleratory CR developed in Group H of Experiment 1, this did not result in a diminished effect of ethanol on heart rate.

These findings correspond with some of the literature reporting the effects of ethanol on heart rate in awake animals. The absence of tolerance can be due to the strong reflexive control exerted on heart rate as seen by Chan and Sutter (1983). Sensitization to the cardioacceleratory effects of the high dose of ethanol in Experiment 1 supports the findings of Wilkin et al. (1982) although these findings were not replicated in Experiment 3.

Tolerance is expected to increase in proportion to the dose given during tolerance acquisition (LeBlanc, Kalant & Gibbins, 1969). This was found to be true in the thermic system since the high dose elicited tolerance while the low dose did not. This was not found to be true in the cardiovascular system since tolerance did not develop to either dose.

Ethanol-induced hypothermia and handling-induced hyperthermia found in these experiments support previous reports in the literature (Cunningham & Peris, 1983; Mansfield & Cunningham, 1980; York & Regan, 1982). In addition, stressful handling procedures interacted with alcohol rather than simply decreasing its effects (Frankenhauser, Dunne, Bjustrom & Lundberg, 1974; Leikola, 1961; Pohorecky, 1981;

Wallgren & Tirri, 1963). In general, these results support the findings of Cunningham, Peris and Schwarz (1984), McDougal, Marques and Burks (1981), and Myrsten, Lamble, Frankenhauser and Lundberg (1979) that stress may interact with the effects of drugs on physiological responses.

In Experiment 1, ethanol induced a relatively small magnitude hypothermic response which seemed to be due to the small amount of handling. Findings from Experiments 2, 3 and 4 indicated that probing of intoxicated rats led to a larger magnitude hypothermia relative to that in not probed animals. When the dose was increased in Experiment 4, a greater effect of probing stress on both acute effects of ethanol and tolerance development was seen. These findings support the hypothesized cross-over of the dose-response curves for stressed and non-stressed animals given ethanol.

The data from the tolerance acquisition and test phases of Experiment 4 led to the conclusion that tolerance developed in Group AP+ but not in Group AN+. The difference in ethanol responsivity seemed to be due mostly to the development of a lower baseline temperature in Group AN+ and therefore lower temperature during the post-injection period. This effect developed gradually over tolerance acquisition sessions and could have decreased the apparent drop in temperature after an ethanol injection. A similar trend was seen in Experiment 3 but this was not a significant finding.

Findings from the placebo or Conditioned Response tests provided an opportunity to determine the animal's reaction to the cues provided by the test environment, by handling, and by injection in the absence

of any direct effects of ethanol. Associative and nonassociative mechanisms of tolerance could not be differentiated from the results of the tests presented in any of the experiments. Thus differences in the magnitude of any compensatory CR could not be compared for increases proportional to the size of the ethanol dose or to the UR elicited under different stressful conditions. It was hypothesized that in either case, an increase would be needed to offset the larger magnitude UR in order for tolerance to develop. It was therefore unknown whether the differences in tolerance development in Groups H and AP+ (of Experiments 1 and 4) were due to the development of a larger compensatory CR under the different circumstances. Since tolerance was present to different degrees in Experiments 1 and 4, there should have been a difference in CRs between the ethanol groups if tolerance were conditioned to the specific handling or environmental cues. There was no evidence that CRs had developed to any degree, however, so this may have been the problem. If CRs could have been elicited to the proper cues then the hypothesis could have been better tested.

The effects of probing on the development of ethanol tolerance support the hypothesis by Carder and others that stress can increase the magnitude of tolerance. It does not support the hypothesis by Carder that stress alone increases tolerance to ethanol (i.e., stress leads to the development of cross-tolerance to ethanol) because there was no difference in hypothermia to a test dose of ethanol in Groups AP- and AN-. In order to support the generalized hypothesis that stress interacts with the effects of drugs, stressors other than rectal probing, physiological responses other than hypothermia, and drugs

other than ethanol should be employed in experiments with designs similar to this one. This type of general hypothesis may help to discern the risks of drug use and the development of tolerance in stressed and non-stressed individuals.

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A microcomputer system for temperature biotelemetry

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A microcomputer (Apple II) system for recording body temperature measured by a commercially made, implantable biotelemetry device (Mini-Mitter) is described. The system includes an inexpensive radio receiver interface circuit and software written in BASIC and 6502 assembly language. The utility of the system is illustrated in a study that shows that various handling procedures (including that normally involved in rectal temperature measurement) elevate body temperature in rats.

Body temperature often serves as the primary dependent variable in studies of thermoregulation, circadian rhythms, and drug action. In recent years, it has become an increasingly popular measure in studies of drug tolerance and the role of behavioral processes (e.g., learning) in the development of tolerance (see review by Cunningham, Crabbe, & Rigter, in press). However, the most common technique for recording core body temperature from rodents over extended periods of time is rather tedious and time-consuming, inasmuch as it requires repeated manual insertion of a rectal probe (see, e.g., Mansfield & Cunningham, 1980; Siegel, 1978). Not only is this technique prone to error from several sources (e.g., variability in depth and duration of probe insertion, incorrect reading of thermometer), but there also is evidence that the handling involved in taking a rectal temperature actually alters body temperature (see, e.g., Briese & de Quijada, 1970).

This paper describes a microcomputer-based system for using a commercially made, implantable biotelemetry device to record body temperature automatically in freely moving rats. The system uses an inexpensive transistor radio that has been modified in a simple way to provide a digital signal input to an Apple II microcomputer. A software package consisting of an Applesoft BASIC program and a clock-interrupt-driven machine language routine permits continuous real-time evaluation of body temperature, both for visual display and permanent storage on floppy disk. In order to illustrate the utility of the system and to show the advantage of using biotelemetry to record body temperature, we also report data on the thermic effects of various handling procedures, including rectal temperature measurement.

This research was supported in part by a research grant from the Medical Research Foundation of Oregon and NHLBI Training Grant HL07332. Requests for additional information should be addressed to the authors at: Department of Medical Psychology L470, Oregon Health Sciences University, Portland, Oregon 97201.

BIOTELEMETRY DEVICE

The biotelemetry device (Mini-Mitter Co., Inc., Sunriver, Oregon, Model M; approximate cost = $38) consists of two thermistors and a battery-operated transmitter contained in a small, nontoxic, waterproof plastic capsule (12 x 19 mm, 2.3 g). The device emits an AM band signal at a rate proportional to the surrounding temperature (approximately 2-4 Hz between 35-40 °C). Each Mini-Mitter unit must be calibrated beforehand in a heated water bath, and the time interval between signal pulses (or the number of pulses per unit time) can be used to index temperature (.1°C resolution). The manufacturer specifies a transmission range of 2-3 ft and a battery life of 2-3 months.

RECEIVER INTERFACE CIRCUIT

The signal transmitted by the Mini-Mitter can be detected as a clicking sound from the speaker of an ordinary AM radio. In order to provide a digital input signal for the Apple II, the speaker from a 9-V transistor radio was replaced by a small board containing an IC circuit. This circuit (shown in Figure 1) detects the Mini-Mitter signal and outputs a short, positive square-wave pulse that can be delivered directly to a TTL ("pushbutton") input on the Apple II game-connector port.

The circuit consists of two main stages. In the first stage, a 20-ohm resistor (R1) has replaced the radio speaker, and the signal is capacitively coupled to the inverting input of a precision op-amp (LM308N) that is wired for single-polarity operation. The noninverting input is tied directly to the positive side of the power supply, which results in a normally high output level in the absence of an input signal. An input signal of sufficient magnitude will drive the output of the op-amp low. This will trigger the second stage of the circuit, a simple one-shot (NE555) that provides a timed output pulse for the computer. The duration of the one-shot

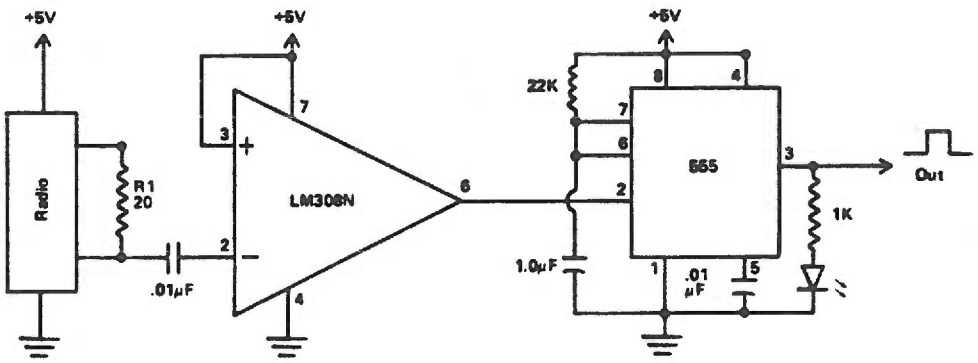

Figure 1. Circuit diagram for the radio receiver interface. The numbers next to the leads from each IC indicate the pin numbers on 8-pin DIPs. The output lead, +5-V, and ground terminals all connect to pins on the Apple II game-connector port.

pulse must be shorter than the shortest interval between signal pulses, but of sufficient duration to be detected by the computer program (see Software section). In the circuit shown in Figure 1, the duration of the output pulse is approximately 30-40 msec and can be varied by changing the values of the resistor and capacitor connected to Pins 6 and 7. The output pulse also activates an LED mounted on the side of the radio. This provides visual feedback that is helpful for adjusting the radio controls.

In order to minimize noise, the radio should be tuned to a relatively "quiet" part of the AM band (the lowest frequency, 550 kHz, works well). Sensitivity (range) can be controlled simply by adjusting the volume knob on the radio. The entire circuit, including the radio, can be powered by the +5-V supply available at the game-connector port of the Apple II. The range of the receiver can be increased somewhat by increasing the supply voltage, up to 9-10 V. However, this requires an external power supply and additional circuitry to convert the one-shot pulse to TTL levels.

The radio used in this application (Dyna-Tone, Model 768) was obtained from a local variety store at a cost under $4.00. The two IC chips are available in 8-pin DIPs and can be mounted end to end in a single 16-pin DIP socket on a small circuit board (e.g., Radio Shack No. 276-159) that fits inside the radio case. The total cost for the radio and parts was less than $9.00.

SOFTWARE

The software for recording body temperature consists of an Applesoft BASIC program (SAMPLE.MITTER) and a clock-interrupt routine written in 6502 assembly language (MINIMITTER.1). Complete commented listings appear in the Appendix. These programs were developed for an Apple II+ microcomputer with 48K RAM memory and at least one disk drive (DOS 3.3). In addition to the receiver interface circuit, the only other required hardware is a clock card capable of providing a 100-Hz interrupt pulse. The system uses a simple homemade clock card plugged into peripheral slot 4, but it also works with a Mountain Hardware or similar commercially made clock card.

The software is designed to run an experimental session in which multiple timed samples (up to 255 samples, each 1-256 sec long) are taken at fixed intervals (0-255 sec) and stored in memory. The raw datum is the average interval between signal pulses during each sample period (.01-sec resolution). Using the slope and intercept of the Mini-Mitter's calibration curve, these values are transformed to temperature (in °C), and both the mean interval and the temperature are displayed on the monitor screen at the end of each sample period. The program also displays a countdown timer (showing the number of seconds remaining in the sample or intersample period), a signal counter, the duration of the most recent intersignal interval (in milliseconds), the sample number, and whether the sample is "on" or "off." The session terminates automatically after the appropriate number of samples has been collected, and the experimenter is given an opportunity to store the data on disk.

The heart of the program is the machine language clock-interrupt routine that is responsible for precise timing of the sample and intersample periods and for timing and evaluating the intervals between Mini-Mitter signals. This routine is loaded into memory (as a binary file) by the Applesoft BASIC program (Line 90). After the BASIC program receives the session parameters from the experimenter and initializes the program variables, the interrupt routine is initialized (Line 220). A screen message then prompts the experimenter to "press any key" to begin the session. The session actually begins 2-3 sec later with the first sample period. At this point, the BASIC program enters a loop (Lines 250-370) that provides a continuous updating of the screen information described earlier.

While Applesoft executes the BASIC program loop, the clock-interrupt pulse "simultaneously" enables the machine language routine to be executed 100 times per second. Each time it is entered, this routine checks the status of the Mini-Mitter signal and updates the session timers. The interval between successive Mini-Mitter signals is timed simply by counting the number of clock pulses that occur between the onsets of one-shot pulses from the receiver interface. Because those pulses will not be synchronized with the clock card, their duration must be at least as long as the interval between clock pulses. Otherwise, input signals may be missed. Since the program looks for signal onsets, longer duration input pulses will cause no problem as long as there is at least one clock period (10 msec) between the offset of one input pulse and the onset of the next.

While monitoring Mini-Mitter signals, the clock-interrupt routine does more than simply count the number of input pulses. It also provides a way of dealing with the two major kinds of signal error. One kind of error comes from sources of radio interference in the laboratory environment. For example, the operation of an electromechanical device (relay, solenoid) in the same room as the receiver is quite capable of triggering an output pulse. The other kind of error occurs when the Mini-Mitter is temporarily out of range (i.e., a missing signal). Changes in the orientation of the transmitter coil relative to the antenna coil can sometimes result in signal loss when the animal moves around the chamber. Reception is best when the loops of both coils are in the same plane. To combat these problems, the program evaluates the time interval between successive input pulses against two kinds of criteria—one based on the absolute value of the intersignal interval and the other based on the relative change in interval duration from one signal to the next. If the interval of time preceding a given input signal does not meet both criteria, it is not included in the computation of average intersignal interval for that sample period. Thus, when errors occur, the sample value is based on less than 100% of the specified sample period.

The criterion values are passed to the machine language routine during the initial portion of the BASIC program (Line 200) and, if necessary, can be changed easily by the experimenter. In our studies, the absolute lower and upper limits for intersignal intervals have been set at 200 and 500 msec, respectively. On average, across Mini-Mitters, this permits temperatures ranging between 33-43 °C. The relative change criterion has been set at 20 msec. In other words, a given interval is accepted only if it is within 20 msec (in either direction) of the most recent "good" interval (and within the absolute limits). If the error condition persists for a long period of time, it is possible for the program to begin to reject legitimate inputs on the basis of the relative-change criterion. To minimize this problem, the program automatically readjusts the criterion signal interval whenever an error condition lasts longer than 2 consecutive seconds (i.e., the most recent interval is used as the value for the "last good interval"). This ordinarily corrects any problem in a matter of seconds if the signal is good. So far, this overall approach for handling signal errors has been quite useful.

At the end of each sample period, the machine language routine automatically passes the sample data (i.e., sample number, signal count, sum of "good" intersignal intervals) to BASIC integer variables (SN%, LC%, and LS%, respectively). The BASIC program then computes the mean intersignal interval, converts that to temperature, and stores the information in a two-dimensional array, D( ) (Lines 310-360). The BASIC program also provides feedback on the extent of any error problems by displaying and storing the percentage of time during each sample period in which "acceptable" signals were recorded.

The session ends when the sample number equals the number of samples originally requested by the experimenter (Line 370). If desired, the contents of the data array can then be transferred to a sequential text file stored on floppy disk (Lines 390-440). The BASIC program shown in the Appendix can be expanded easily to provide summary statistics and a high-resolution graphics display of successive sample values.

EFFECT OF HANDLING ON BODY TEMPERATURE

In order to illustrate the utility of the present system and to show the advantages of biotelemetry, body temperature was recorded automatically from implanted Mini-Mitters in rats after they were picked up, pricked with a hypodermic needle, handled for rectal temperature measurement, or left undisturbed.

Method

The subjects were 12 adult male albino rats weighing an average of 638 g. Each rat was anesthetized with halothane gas, and a Mini-Mitter was implanted surgically. The device was coated with Parafin/Elvax® to protect it from corrosion and had been previously calibrated in a heated water bath (range = 35-41°C). A 1-2-cm ventral midsagital incision was made about 5 cm below the diaphragm through both the skin and peritoneum, and a Mini-Mitter was inserted into the intraperitoneal cavity.

Temperature monitoring took place inside one of four identical clear plastic cages (23 x 20.5 x 21 cm) centered on an acrylic platform over the transistor radio. With this arrangement, an implanted Mini-Mitter always remained less than 21 cm from the radio antenna. Each cage was contained in a darkened, ventilated, sound-attenuating chamber (ambient temperature = 24 °C).

On each of 7 consecutive days, each rat was placed in the experimental chamber for 150 min. On the first 3 days and on the final day, the animals were not disturbed. On the other days, each rat was subjected to one of three handling procedures 60 min after the session began. In one condition (Handled), the rat was picked up, placed in a plastic shoebox cage for 75 sec, and then returned to the test chamber. A second condition (Handled/Injection) involved a similar sequence, but after 60 sec in the shoebox, the rat was picked up and a 25-ga, 5/8-in.

hypodermic needle was inserted into the abdomen for 15 sec to mimic an ip injection (no fluid was actually injected). In the third condition (Handled/Rectal Probe), the rat was picked up and wrapped in a towel for 60 sec while a lubricated, flexible thermistor probe (YSI Model 402) was inserted 6 cm into the rectum. The rats were assigned randomly to groups of four, and each group received these three treatments in a different order.

The Mini-Mitter output was sampled during consecutive 1-min periods (i.e., 0-sec intersample interval), and temperature was averaged over 5-min blocks during each session. These data were collected with a version of our software written for the PDP-8/F, such that four subjects could be monitored simultaneously.

Results

On the handling treatment days, an average of 80.5% (SEM = 2.4) of each sample period was found to be acceptable according to the error-detection criteria. Body temperatures were elevated at the beginning of each session (mean = 38.6°C), presumably due to the handling involved in transporting the rat to the experimental room and placing it in the test chamber. After the animal was placed in the chamber, temperatures declined gradually over time within each session, with the greatest changes occurring during the 1st hour (about 0.8°C).

Figure 2 shows mean body temperatures recorded for 5-min periods just before and for 90 min after each of the handling treatments. The data shown for the Not Handled comparison were obtained by averaging temperatures recorded on the days just before and just after the handling-treatment days. As can be seen, body temperatures continued to decline under the Not Handled condition. However, each of the three handling treatments produced a hyperthermic reaction that persisted long after the animal was returned to the test chamber. Furthermore, the magnitude and duration of the increase in temperature varied as a function of handling treatment. Rectal temperature measurement (Handled/Rectal Probe) produced the largest and most persistent elevation.

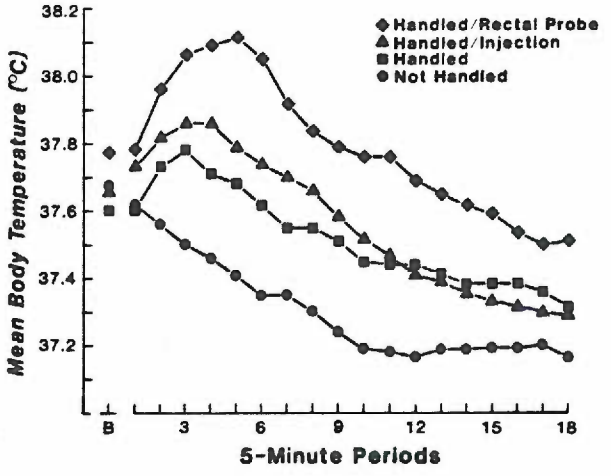

Figure 2. Mean body temperature (in °C) recorded for 5-min periods just before (B) and for 90 min after various handling treatments (n = 12).

The data shown in Figure 2 were subjected to a treatment x time periods analysis of variance that yielded a significant main effect of time periods [$F(18,198) = 25.4$] and a significant interaction [$F(54,592) = 2.3$]. Follow-up analyses that separately compared each handling treatment with the Not Handled condition also revealed statistically significant interactions, indicating that each handling procedure affected body temperature.

CONCLUSION

The microcomputer-controlled biotelemetry system described here offers a relatively inexpensive means of obtaining continuous measures of core body temperature. This approach not only eliminates the need to handle the animal, but it also reduces the experimenter's labor and increases the reliability of the temperature measurements. The data presented here establish the utility of this system and confirm previous findings that handling (including that commonly used to obtain rectal temperature measurements) can have a dramatic impact on core temperature (Blasig, Hollt, Bauerle, & Herz, 1978; Briese, 1965; Briese & de Quijada, 1970; Miles, 1962).

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APPENDIX
Program Listings

```
10  REM  SAMPLE.MITTER
20  REM  C.L. CUNNINGHAM (1983)
30  REM
40 LCX = 0:LSX = 0:SNZ = 0: REM  RESERVE FOR DATA STORAGE
50 VARIABLES = 37376: REM  VARIABLE LIST
60  MEMEM: VA = 1: REM  PROTECT INTERRUPT ROUTINE
70  DEF  FN A(X) = 5 * LEN ( STR$ (X))
80 D$ = CHR$ (4):G$ = CHR$ (7)
90  PRINT D$;"BLOAD MINIMITTER.1.OBJO": REM  LOAD MACHINE CODE
100 SESFLG = VA:SD = VA + 2:IS = VA + 3:SF = VA + 4:HS = VA + 5:TI = VA + 6:ES = VA + 7:C = VA + 8:L = VA + 13
110  TEXT : HOME : INVERSE : PRINT "SAMPLE.MITTER": NORMAL : PRINT
120  REM   X=INTERVAL IN MSEC; Y=TEMP IN DEGREES C
130  PRINT : PRINT "ENTER CALIBRATION PARAMETERS:"
140  PRINT "     SLOPE = ";G$;: INPUT SLOPE: PRINT "     INTERCEPT = ";G$;: INPUT INERCEPT
150  REM  INPUT SESSION PARAMETERS
160  PRINT : PRINT "TOTAL # OF SAMPLES (<256) = ";G$;: INPUT TN
170  PRINT : PRINT "SAMPLE DURATION (SEC)(1-255) = ";G$;: INPUT D: POKE SD,D
180  PRINT : PRINT "SECONDS BETWEEN SAMPLES (<256) = "G$;: INPUT X: POKE IS,X
190  DIM D(TN,2): REM  DATA STORAGE (DEGREES, MEAN INTERVAL, % REJECT)
200  POKE VA + 14,50: POKE VA + 15,20: POKE VA + 16,2: POKE VA + 20,1: REM   SET REJECTION LIMITS & RESET FLAG
210  FOR X = C TO C + 3: POKE X,0: NEXT : POKE HS,1: POKE TI,3: REM    CLEAR DATA STORAGE AND SET TIMERS
220  CALL VA + 21: REM   SETUP INTERRUPT & CLEAR FLAGS
230  PRINT : PRINT "PRESS ANY KEY TO BEGIN SESSION:";G$;: GET A$: POKE SE,1: REM   BEGIN SESSION WITH A SAMPLE
240  HOME : INVERSE : PRINT "SAMPLE.MITTER": NORMAL
250  POKE 36,0: POKE 37,3: PRINT : IF  PEEK (SF) = 0 THEN  PRINT "SAMPLE OFF";: GOTO 270
260  INVERSE : PRINT "SAMPLE ON ";: NORMAL
270 X =  PEEK (TI): PRINT "  (SECONDS REMAINING = "; SPC(  FN A(X));X;")"
280 X =  PEEK (C) +  PEEK (C + 1) * 256: PRINT : PRINT "CURRENT SIGNAL COUNT = "; SPC(  FN A(X));X
290 X =  PEEK (L): PRINT : PRINT "CURRENT SIGNAL INTERVAL (MSEC) = "; SPC(  FN A(X));X * 10
300  IF  PEEK (ES) = 0 THEN 250: REM  LOOP TIL END OF SAMPLE
310  POKE ES,0: PRINT : PRINT : PRINT "LAST SAMPLE DATA (#";SNZ;"):": PRINT
320  IF LCZ = 0 THEN X = 0: GOTO 340
330 X =  INT (LSX / LCZ * 10): REM  COMPUTE MEAN INTERVAL
340  PRINT "MEAN INTERVAL (MSEC) = "; SPC(  FN A(X));X:D(SNZ,1) = X
350 X = SLOPE * X + INERCEPT:X =  INT (X * 10): PRINT "MEAN TEMPERATURE (C) = "; SPC(  FN A(X));X / 10:D(SNZ,0) = X / 10
360 X =  INT (LSX * 10 / (D * 10)): PRINT "SAMPLE PERIOD ACCEPTED = "; SPC(  FN A(X));X;"%":D(SNZ,2) = X
370  IF SNZ <  > TN THEN 250: REM   LOOP TIL END OF SESSION
380  PRINT : PRINT "SESSION OVER": PRINT "STORE DATA TO DISK? (Y/N) ";G$;: INPUT A$: IF A$ <  > "Y" THEN  END
390  PRINT : PRINT "FILENAME = ";G$;: INPUT F$
400  PRINT : PRINT "INSERT STORAGE DISK IN DRIVE AND": PRINT "PRESS ANY KEY TO SAVE DATA: ";G$;: GET A$: PRINT
410  PRINT D$;"OPEN ";F$
420  PRINT D$;"WRITE ";F$
430  FOR X = 1 TO TN: FOR Y = 0 TO 2: PRINT D(X,Y): NEXT : NEXT
440  PRINT D$;"CLOSE ";F$
450  END
```

```
SOURCE FILE: MINIMITTER.1
                    1 ********************************************************
0000:               2 *
0000:               3 * MINIMITTER.1
0000:               4 *   CLOCK-INTERRUPT ROUTINE
0000:               5 *   C.L. CUNNINGHAM (1983)
0000:               6 *
                    7 ********************************************************
C061:               8 PBO     EQU  $C061     ;GAME PORT PB0 INPUT
C400:               9 CLRCLK  EQU  $C400     ;CLOCK IN SLOT 4
----- NEXT OBJECT FILE NAME IS MINIMITTER.1.OBJO
9200:              10         ORG  $9200
9200:00            11 SESFLG  DFB  $0        ;SESSION FLAG
9201:00            12 SMPNUM  DFB  $0        ;SAMPLE # (MAX=255)
9202:00            13 SMPDUR  DFB  $0        ;SAMPLE DURATION (1-256 SEC)
9203:00            14 INTSMP  DFB  $0        ;INTER-SAMPLE INTERVAL (0-255 SEC)
9204:00            15 SMPFLG  DFB  $0        ;SAMPLE FLAG
9205:00            16 HNDSEC  DFB  $0        ;10-MSEC TIMER
9206:00            17 TIMER   DFB  $0        ;SECONDS TIMER
9207:00            18 EOSFLG  DFB  $0        ;END-OF-SAMPLE FLAG
9208:00 00         19 COUNT   DFB  $0,$0     ;SIGNAL COUNTER
920A:00 00         20 SUMISI  DFB  $0,$0     ;SUM SIGNAL INTERVALS
920C:00            21 SIGTIM  DFB  $0        ;SIGNAL TIMER (10-MSEC)
920D:00            22 LASTIM  DFB  $0        ;LAST SIGNAL INTERVAL
920E:00            23 HILIM   DFB  $0        ;HIGH INTERVAL LIMIT
920F:00            24 LOLIM   DFB  $0        ;LOW INTERVAL LIMIT
9210:00            25 RELLIM  DFB  $0        ;RELATIVE CHANGE LIMIT
9211:00            26 INPUT   DFB  $0        ;CURRENT INPUT
9212:00            27 LSTINP  DFB  $0        ;LAST INPUT
9213:00            28 SUMREJ  DFB  $0        ;SUM REJECTED INTERVALS
9214:00            29 RESET   DFB  $0        ;CRITERION RESET FLAG
                   30 ********************************************************
9215:A9 2F         31 SETUP   LDA  #>CLOCK   ;SETUP INTERRUPT POINTERS
9217:8D FE 03      32         STA  $3FE
921A:A9 92         33         LDA  #<CLOCK
921C:8D FF 03      34         STA  $3FF
921F:A9 00         35         LDA  #0        ;CLEAR FLAGS
9221:8D 00 92      36         STA  SESFLG
9224:8D 04 92      37         STA  SMPFLG
9227:8D 07 92      38         STA  EOSFLG
922A:8D 00 C4      39         STA  CLRCLK    ;CLEAR CLOCK INTERRUPT
922D:58            40         CLI            ;ENABLE INTERRUPT
922E:60            41         RTS
                   42 ********************************************************
922F:98            43 CLOCK   TYA            ;SAVE Y REGISTER
9230:48            44         PHA
9231:AD 00 92      45         LDA  SESFLG    ;SESSION ON?
9234:D0 03         46         BNE  RUN
9236:4C 28 93      47         JMP  EXIT      ;NO:EXIT INTERRUPT ROUTINE
9239:EE 0C 92      48 RUN     INC  SIGTIM    ;YES:INCREMENT SIGNAL TIMER
923C:AD 61 C0      49         LDA  PB0       ;INPUT SIGNAL ON?
923F:8D 11 92      50         STA  INPUT
9242:30 03         51         BMI  CHKSIG
9244:4C C6 92      52         JMP  CHKTIM    ;NO:CHECK TIMING
```

```
9247:AD 12 92   53 CHKSIG  LDA  LSTINP    ;YES:OFF-ON TRANSITION?
924A:10 03      54         BPL  NEWSIG
924C:4C C6 92   55         JMP  CHKTIM    ;NO:CHECK TIMING
924F:AD 0C 92   56 NEWSIG  LDA  SIGTIM    ;YES:CHECK SIGNAL LIMITS
9252:CD 0F 92   57         CMP  LOLIM     ;CHECK VS ABSOLUTE LIMITS
9255:90 4E      58         BCC  REJECT
9257:CD 0E 92   59         CMP  HILIM
925A:B0 49      60         BCS  REJECT
925C:CD 0D 92   61         CMP  LASTIM    ;CHECK VS RELATIVE LIMITS
925F:F0 1C      62         BEQ  OKSIG
9261:B0 0B      63         BCS  LONGER
9263:6D 10 92   64 SHRTER  ADC  RELLIM
9266:CD 0D 92   65         CMP  LASTIM
9269:B0 12      66         BCS  OKSIG
926B:4C A5 92   67         JMP  REJECT
926E:18         68 LONGER  CLC
926F:AD 0D 92   69         LDA  LASTIM
9272:6D 10 92   70         ADC  RELLIM
9275:CD 0C 92   71         CMP  SIGTIM
9278:B0 03      72         BCS  OKSIG
927A:4C A5 92   73         JMP  REJECT
927D:EE 08 92   74 OKSIG   INC  COUNT     ;"GOOD" SIGNAL
9280:D0 03      75         BNE  OK        ;INCREMENT COUNTER
9282:EE 09 92   76         INC  COUNT+1   ;(DOUBLE PRECISION)
9285:18         77 OK      CLC
9286:AD 0C 92   78         LDA  SIGTIM    ;RESET CRITERION
9289:8D 0D 92   79         STA  LASTIM
928C:6D 0A 92   80         ADC  SUMISI    ;UPDATE SUM OF SIGNAL INTERVALS
928F:8D 0A 92   81         STA  SUMISI
9292:A9 00      82         LDA  #0
9294:6D 0B 92   83         ADC  SUMISI+1  ;(DOUBLE PRECISION)
9297:8D 0B 92   84         STA  SUMISI+1
929A:A9 00      85         LDA  #0        ;CLEAR RESET VARIABLES
929C:8D 13 92   86         STA  SUMREJ
929F:8D 14 92   87         STA  RESET
92A2:4C C1 92   88         JMP  CLEAR     ;CLEAR SIGNAL TIMER
92A5:AD 14 92   89 REJECT  LDA  RESET     ;RESET FLAG SET?
92A8:D0 11      90         BNE  RSET      ;YES:READJUST CRITERION
92AA:18         91         CLC            ;NO:SUM REJECT INTERVALS
92AB:AD 0C 92   92         LDA  SIGTIM
92AE:6D 13 92   93         ADC  SUMREJ
92B1:8D 13 92   94         STA  SUMREJ
92B4:C9 C8      95         CMP  #200      ;<2 CUMULATIVE SEC ERROR?
92B6:90 09      96         BCC  CLEAR     ;YES:CLEAR SIGNAL TIMER
92B8:EE 14 92   97         INC  RESET     ;NO:SET RESET FLAG
92BB:AD 0C 92   98 RSET    LDA  SIGTIM    ;READJUST CRITERION
92BE:8D 0D 92   99         STA  LASTIM
92C1:A9 00      100 CLEAR  LDA  #0
92C3:8D 0C 92   101        STA  SIGTIM    ;CLEAR SIGNAL TIMER
92C6:CE 05 92   102 CHKTIM DEC  HNDSEC    ;DECREMENT TIMERS
92C9:D0 5D      103        BNE  EXIT      ;EXIT UNLESS INTERVAL IS OVER
92CB:A9 64      104        LDA  #100
92CD:8D 05 92   105        STA  HNDSEC
92D0:CE 06 92   106        DEC  TIMER

92D3:D0 53      107        BNE  EXIT
92D5:AD 04 92   108        LDA  SMPFLG    ;END OF INTERVAL:A SAMPLE PERIOD?
92D8:D0 1D      109        BNE  STOP      ;YES:END SAMPLE
92DA:EE 04 92   110 START  INC  SMPFLG    ;NO:START SAMPLE (SET FLAG)
92DD:EE 01 92   111        INC  SMPNUM    ;INCREMENT SAMPLE COUNTER
92E0:AD 02 92   112        LDA  SMPDUR
92E3:8D 06 92   113        STA  TIMER     ;SET SAMPLE DURATION TIMER
92E6:A9 00      114        LDA  #0        ;CLEAR DATA REGISTERS
92E8:8D 08 92   115        STA  COUNT
92EB:8D 09 92   116        STA  COUNT+1
92EE:8D 0A 92   117        STA  SUMISI
92F1:8D 0B 92   118        STA  SUMISI+1
92F4:4C 28 93   119        JMP  EXIT      ;AND EXIT
92F7:A9 00      120 STOP   LDA  #0        ;END SAMPLE (CLEAR FLAG)
92F9:8D 04 92   121        STA  SMPFLG
92FC:EE 07 92   122        INC  EOSFLG    ;SET END-OF-SAMPLE FLAG
92FF:A0 02      123        LDY  #2        ;TRANSFER DATA TO APPLESOFT
9301:AD 09 92   124        LDA  COUNT+1
9304:91 69      125        STA  ($69),Y
9306:C8         126        INY
9307:AD 08 92   127        LDA  COUNT
930A:91 69      128        STA  ($69),Y
930C:A0 09      129        LDY  #9
930E:AD 0B 92   130        LDA  SUMISI+1
9311:91 69      131        STA  ($69),Y
9313:C8         132        INY
9314:AD 0A 92   133        LDA  SUMISI
9317:91 69      134        STA  ($69),Y
9319:A0 11      135        LDY  #17
931B:AD 01 92   136        LDA  SMPNUM
931E:91 69      137        STA  ($69),Y
9320:AD 03 92   138        LDA  INTSMP    ;IF INTERSAMPLE INTERVAL = 0,
9323:F0 B5      139        BEQ  START     ;THEN START NEXT SAMPLE
9325:8D 06 92   140        STA  TIMER     ;OTHERWISE, SET INTERVAL TIMER
9328:AD 11 92   141 EXIT   LDA  INPUT     ;UPDATE INPUT MEMORY
932B:8D 12 92   142        STA  LSTINP
932E:68         143        PLA            ;RESTORE Y-REGISTER
932F:A8         144        TAY
9330:AD 00 C4   145        LDA  CLRCLK    ;CLEAR CLOCK INTERRUPT
9333:A5 45      146        LDA  $45       ;RESTORE A
9335:40         147        RTI            ;RETURN FROM INTERRUPT

*** SUCCESSFUL ASSEMBLY: NO ERRORS
```

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EXPERIMENT 3 POST-INJECTION SCORES

Acquisition

Heart Rate

The mean heart rates of the three groups increased immediately after injection and remained elevated during the 2-hr period. Level of heart rate was greatest in Group AP+ and fluctuated the most within days in Group AP-. There was a general decrease in the magnitude of cardioacceleration of all groups over days but no systematic changes in ethanol groups relative to the saline group.

A three-way ANOVA performed on these data revealed significant Groups x Sample Periods ($F\{24,228\} = 2.88$) and Days x Sample Periods ($F\{156,2964\} = 2.98$) interactions and significant Days ($F\{13,247\} = 9.57$) and Sample Periods ($F\{12,228\} = 44.09$) main effects. Subsequent comparisons between groups revealed significant Groups x Sample Periods interactions for the comparisons between Groups AN+ vs AP- ($F\{12,144\} = 3.35$) and Groups AP+ vs AP- ($F\{12,168\} = 3.71$) but no group effects for the comparison between Groups AP+ and AN+. Followup analyses of Sample Periods 1, 3 and 13 revealed significant group differences during Sample Period 1 (the baseline period) only ($F\{1,12\} = 4.65$ for Group AN+ vs Group AP- and $F\{1,14\} = 4.95$ for Group AP+ vs Group AP-).

Followups on the Days x Sample Periods interaction revealed a Days effect during Sample Period 13 ($F\{13,273\} = 2.01$) but not during Sample Period 1. This is due to a general decrease in the heart rate reactions of all groups to injection and/or handling over days.

Temperature

Mean body temperature of each group during the 10 min immediately prior to injection and during the two hours after injection were analysed. During earlier tolerance acquisition sessions, temperature slowly decreased in Group AP+ and AN+ reaching a maximum hypothermia by the end of the 2-hr period. Differences in body temperature of between these two groups are minimal. Temperature was elevated in Group AP- during the first 60 min after injection but returned to normal levels within the 2-hr period. After a number of sessions, the magnitude of the hypothermia in the ethanol groups diminished and the peak effect occurred earlier. This effect is less noticeable in Group AP+ relative to the changes seen in Group AN+. The hyperthermia exhibited by Group AP- during the first hour decreased while temperature during the second hour increased.

A threeway ANOVA revealed significant Groups x Days x Sample Periods ($F\{312,2964\} = 1.35$), Groups x Sample Periods ($F\{24,228\} = 18.42$) and Days x Sample Periods ($F\{156,2964\} = 2.54$) interactions. There were also main effects of Groups ($F\{2,19\} = 4.76$) and Sample Periods ($F\{12,228\} = 15.27$). When separate within-group analyses were performed, significant Days x Sample Periods interactions were found in all three groups. Pairwise group comparisons revealed significant Groups x Days x Sample Periods interactions for AP+ vs AP- ($F\{156,2028\} = 1.28$) and AN+ vs AP- ($F\{156,1872\} = 1.29$) but not AP+ vs AP-.

There was a significant Groups x Days interaction ($F\{13,169\} = 2.05$) for the comparison of Groups AP+ vs AP- during Sample Period 13 but not 1 or 3. This was due to a significant increase in temperature during later sample periods that developed over days in Group AP- ($F\{13,78\} =$

2.44) but not in Group AP+. There were no changes over days in either group during earlier sample periods. The followup analysis of the Group AN+ vs Group AP- comparison during these sample periods revealed significant main effects of Groups ($F\{1,12\} = 30.86$) and Days ($F\{13,156\} = 2.95$) but no interaction. An additional analysis was performed at Sample Period 6 and 7 and again no interactions were found.

Tolerance Test

Heart Rate

Mean heart rate of the three groups was elevated immediately after injection (410 bpm when probed and 350 bpm when not probed relative to baseline levels of 310). Heart rate during the Not-Probed condition remained elevated throughout the 2-hr session, while that of probed animals fluctuated between 420 and 370 bpm. There was no effect of previous tolerance treatments.

A three-way ANOVA revealed a Test Treatment x Sample Periods interaction ($F\{12,228\} = 5.42$) and main effects of Test Treatment ($F\{1,19\} = 11.23$) and Sample Periods ($F\{12,228\} = 18.6$). Separate within-group followups revealed a significant Sample Periods treatment for the Probing condition ($F\{12,252\} = 21.75$).

Temperature

Body temperature after a 2.0 g/kg test injection of ethanol was generally decreased in all groups relative to baseline levels but to a greater degree in Group AP- (36.2'C) compared to the ethanol groups (36.86 and 38.77'C). Temperature of rats while probed (relative to that of rats while not probed) was elevated during later periods in Group AN+, depressed in Group AP- and during earlier periods in Group AN+ and not

different in Group AP+.

These observations are supported by Groups x Test Treatment x Sample Periods ($F\{24,204\} = 1.87$), Groups x Sample Periods ($F\{24,204\} = 5.61$) and Test Treatment x Sample Periods ($F\{12,204\} = 3.95$) interactions. Within-group followup analyses revealed Test Treatment x Sample Periods interactions in Groups AN+ and AP- ($F\{12,60\} = 2.19$ and 5.06, respectively) but no effects of Test Treatment in Group AP+.

Pairwise group comparisons revealed significant Groups x Test Treatment x Sample Periods interactions for AP+ vs AP- ($F\{12,144\} = 1.89$) and AN+ vs AP- ($F\{12,120\} = 2.66$) but no effects of Groups or Test Treatment for AP+ vs AN+. Followup analyses of tolerance groups under the Probed condition revealed significant Groups x Sample Periods interactions for Group AP+ vs Group AP- ($F\{12,144\} = 6.97$) and for Group AN+ ($F\{12,120\} = 8.43$). Followup analyses of groups under the Not-Probed condition revealed a significant Groups x Sample Period interaction for the Group AN+ vs AP- comparison ($F\{12,120\} = 2.48$) but not for the Group AP+ vs AP- comparison.

Within-group analyses revealed a main effect of Sample Periods in all groups. Pairwise group comparisons revealed a significant Groups x Sample Periods interactions for AP+ vs AP- ($F\{12,144\} = 1.89$) and AN+ vs AP- ($F\{12,120\} = 2.66$) but no group effects for AP+ vs AN+. Followups at Sample Periods 1, 3, and 13 revealed significant group differences only during the final Sample Period ($F\{1,12\} = 5.35$ for Group AP+ vs AP- and $F\{1,10\} = 5.95$ for Group AN+ vs AP-).

Conditioned Response Test

Heart Rate

Mean heart rate after a saline injection was not affected by previous tolerance treatment. Heart rate in the undisturbed condition was 370 bpm immediately after injection, decreasing to 345 bpm within 20 min. Heart rate during the Not-Probed condition generally stayed at this level for the remainder of the session while it fluctuated between 330 and 400 bpm during the Probed condition. A three-way ANOVA revealed a significant Test Treatment x Sample Periods interaction ($F\{12,228\} = 5.93$) and a main effect of Sample Periods ($F\{12,228\} = 17.84$). There were no effects or interactions due to tolerance treatment groups.

Temperature

The mean body temperature of the three tolerance treatment groups after a saline injection increased slightly after 2 hr in all groups and appears to be greatest in Group AN+. Temperature was elevated during the Probed condition relative to the Not-Probed condition and this difference was greatest in Group AN+.

A three-way ANOVA revealed significant Groups x Test Treatment x Sample Periods ($F\{24,192\} = 2.38$), Groups x Test Treatment ($F\{2,16\} = 2.38$) and Test Treatment x Sample Periods ($F\{12,192\} = 3.09$) interactions. There were also main effects of Test Treatment ($F\{1,16\} = 6.45$) and Sample Periods ($F\{12,192\} = 25.81$). Pairwise group comparisons revealed a significant Groups x Test Treatment x Sample Periods interaction for the AN+ vs AP- comparison ($F\{12,120\} = 3.88$) and a Groups x Test Treatment interaction for the AP+ vs AN+ comparison ($F\{1,11\} = 7.0$). Within-group followup analyses revealed a significant Test Treatment x Sample Periods interaction ($F\{12,60\} = 5.11$) and Test Treatment main effect ($F\{1,5\} = 6.79$) in Group AN+ only.