BEHAVIORAL INFLUENCES ON TOLERANCE TO ETHANOL

JAMES GUY MANSFIELD

A DISSERTATION

Presented to the Department of Medical Psychology

and the Graduate Council of the

University of Oregon Health Sciences Center

in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

July, 1979

Approved:

(Professor in Charge of Thesis)

(Chairman, Graduate Council)

ACKNOWLEDGMENTS

Judson Brown and Chris Cunningham provided the opportunities, guidance, and patient training that formed the foundation for my graduate career. The outstanding examples they set as scholars and scientists will serve me for years to come.

Drs. R. D. Fitzgerald, K. E. Fox, and D. S. Phillips contributed to this project at several stages. Their consideration is appreciated greatly. Drs. M. Meikle and J. H. O'Brien were kind enough to provide some of the equipment necessary for the study.

Throughout this study, I received constant support from my friends and family. Mom, Dad, Grandma, I owe you much more than this thank you. To my wife, June, I will always owe the time we did not have together because of my academic pursuits. I am grateful for her love, patience, and understanding.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Figures (continued)

INTRODUCTION

When organisms experience recurrent presentations of the same stimuli, a prevalent response is adaptation. Tolerance is a term used to describe an organism's adaptation to repeated experiences with a drug, and is customarily defined in two ways. An organism is said to be tolerant (a) when regularly occurring administration of a fixed drug dose results in a diminished effect or (b) following repeated drug administration, a larger drug dose is required to elicit the original response. Tolerance may be observed as a shift to the right of the dose-response curve, a measure providing information not only on the magnitude of adaptation, but also in some cases, on the mechanism involved (Kalant, LeBlanc, & Gibbins, 1971).

Both empirical and theoretical distinctions have been used to classify physiological mechanisms underlying drug tolerance. For example, the terms drug-disposition tolerance or metabolic tolerance refer to adaptation mediated by changes in drug absorption or metabolism that reduce the concentration of the drug at the site of action. Cellular tolerance (also termed pharmacodynamic, functional, or tissue tolerance), on the other hand, refers to reduced sensitivity to drugs, even when the concentration at the site of action is presumed to be unchanged. It is possible, therefore, to distinguish between cellular and metabolic tolerance by relating a

a drug's effect to its concentration in blood or tissue.

Goldstein, Aronow, and Kalman (1974) have suggested that the mechanisms involved in cellular tolerance may be theoretically classified into two types. One type of theory assumes that the drug-receptor interaction remains essentially unchanged and that adaptation is due to homeostatic adjustments in more distal biochemical or neuronal pathways. Martin (1968), for example, has proposed that tolerance may involve the development and functioning of alternate neuronal pathways that substitute for drug-impaired systems. A related suggestion (Kalant, 1977) is that tolerance to ethanol, barbiturates, and opiates reflects a general adaptation to the central nervous system depression caused by these agents rather than specific adaptation to the chemical agents themselves. Alternatively, the other type of theory proposes a change in the configuration or number of available drug receptors, thereby reducing the extent of effective drug-receptor interaction. Along these lines, antagonist-affinity studies (Takemori, 1975) support the suggestion that chronic exposure to morphine results in a change in the structure of the receptor molecule, thereby weakening drug-receptor binding.

Hug (1972), making a theoretical dichotomy similar to that of Goldstein et al., described changes in the cells upon which the drug acted directly as a cellular mechanism, while tolerance due to adjustment in other cells was described as due to homeostatic

mechanisms. The proposal that ethanol's interaction with the cell membrane is countered by an adaptive change in membrane conformation (Hill & Bangham, 1975; Wallgren, Nikander, & Virtanen, 1975) would exemplify the former type of mechanism. At present, many details of the physiological and neurochemical processes mediating drug tolerance remain unknown.

A comprehensive account of the nature of tolerance must involve consideration of a number of issues: (1) variables influencing the time course of the development and loss of tolerance, (2) differential development of tolerance to various actions of the same drug, (3) cross-tolerance (or lack of it) between drugs, and (4) the possible relationship between tolerance and dependence. Traditionally, the factors considered to be importantly related to tolerance have included the extent of contact between the drug and its site of action, the chemical structure of the drug, and the nature of the responses measured. For example, it is maintained that "tolerance development is favored by the continuous presence of drug in adequate concentration" (Goldstein et al. 1974, p. 596); that drugs with similar chemical structures more often show cross-tolerance (Hug, 1972); and that tolerance develops more readily to the disruption of simple versus complex responses (Ferraro, 1978).

Behavioral Tolerance

Recently, attention has been directed to the role of nonpharmacological variables in the development and manifestation of drug tolerance. Researchers working with a variety of drugs have reported that behavioral manipulations, apparently unrelated to the extent of contact between the organism and the drug, can dramatically influence the amount or rate of development of tolerance. The term behavioral tolerance is used here to distinguish instances of adaptation to a drug that are particularly dependent upon behavioral manipulations. It has been suggested that such behavioral manipulations reduce sensitivity to drugs via mechanisms that differ from those thought to be responsible for cellular or metabolic tolerance. These mechanisms have been characterized as involving instrumental learning, adaptation to functional impairment induced by drugs, state-dependent learning, and classical conditioning. Current research and theorizing related to these proposed mechanisms of behavioral tolerance will be outlined below.

Instrumental Learning

Observing that tolerance does not develop uniformly to all behavioral effects of a drug, Schuster (1978) and his co-workers proposed that tolerance will develop only to the extent that a drug's primary actions result in a decrease in the amount of reinforcement received. These investigators reported that while no tolerance was evidenced to amphetamine-induced increases in general

activity or shock-avoidance responding, tolerance did develop to the disruptive effect of amphetamines on DRL (differential reinforcement for low rates of responding) bar-pressing behavior in the same animals (Schuster & Zimmerman, 1961; Schuster, Dockens, & Woods, 1966). Schuster et al. (1966) suggested that this differential development of tolerance could be accounted for by examining the drug's effect on frequency of reinforcement. The amphetamine-induced increases in shock-avoidance responding and general activity yielded no decrement in frequency of reinforcement; however, administration of amphetamine did result in a decrease in food reinforcement received in the DRL task. In such a situation, adaptation to the drug may be viewed as a response which is instrumental in producing an increase in (or normalization of) the amount of reinforcement (LeBlanc & Cappell, 1977).

The role of loss of reinforcement in the acquisition of drug tolerance fits within a broader theoretical framework in which instrumental learning is hypothesized to mediate adaptation to drug effects (Carder, 1978; Ferraro, 1976). Within this framework, when a drug's initial effects result in a decrement in reinforcement, or in other adverse conditions, the stage is set for instrumental strengthening of homeostatic adjustments or adaptive responses that ameliorate the drug-produced deficit. The nature of such responses may range from changes in cellular functioning to gross behavioral adjustments. For example, the influence of several behavioral

manipulations on the development of tolerance to marihuana (or its active agent 1 -Δ^9- tetrahydrocannabinol) may be accounted for by viewing tolerance as an instrumentally reinforced adaptive response, learned in the presence of appropriate contingencies, and manifested in the presence of appropriate stimulus cues (Ferraro, 1978).

Functional Impairment

A second interpretation of the effects of behavioral variables on drug tolerance stresses adaptation to the functional impairment induced by drugs. Kalant et al. (1971) have proposed that three factors contribute to the rate or extent of development of tolerance during drug exposure. Adaptation to drugs is said to be a function of drug concentration in blood or tissue, prior experience with the drug, and the amount of functional impairment induced by the drug. Functional impairment may be described as the lack of ability, at a neural or behavioral level, to respond suitably to demands placed on the organism. Therefore, amount of functional impairment is said to depend on both the magnitude of the primary cellular disturbances caused by the drug, and the amount of functional demand placed on the organism during the period of drug exposure (Kalant, 1977).

A variety of experimental work supports the suggestion that the nature of ongoing activity during drug exposure can importantly influence the development of tolerance. Investigations of tolerance to ethanol (Chen, 1968; Crow & Higbee, 1977; LeBlanc, Gibbins,

& Kalant, 1973; 1975), amphetamines (Carlton & Wolgin, 1973), barbiturates (Tang & Falk, 1978) and morphine (Adams, Yeh, Woods, & Mitchell, 1969; Siegel, 1975, 1976, 1977, 1978a) have consistently shown that when drug administration is paired with, or regularly precedes behavioral testing, the development of tolerance appears to be enhanced. In what was perhaps the first study of its type, Chen (1968) exposed three groups of rats to equal amounts of ethanol on four occasions. On the first three exposures, one group (Before group) received an injection of ethanol (1.2 g/kg) just prior to a training session in a circular maze, while the other group (After group) received an injection of the drug just after the maze session. On the fourth exposure to ethanol, all animals received the drug just before the maze test. As all animals had had equal experience with ethanol (and the test environment) prior to the fourth ethanol challenge, it might be expected that both groups would have displayed equal tolerance to the drug. This was not the case. While both the Before and After groups did show equivalent tolerance to the motor depressant effects of ethanol, only the Before group evidenced tolerance to the maze-performance disruption (a decrement in the number of correct trials) caused by the drug. Chen concluded that physiological theories of tolerance could not account for this outcome and postulated that some unspecified behavioral mechanism might be involved.

More recently, LeBlanc, Gibbins, and Kalant (1973) have confirmed Chen's general finding; moreover, these investigators used a comprehensive experimental procedure that yielded additional information and allowed an interpretation different from that offered originally by Chen. In their study, LeBlanc et al. exposed three groups of rats equally to ethanol and a test situation according to the design outlined below.

GROUP	PRE-TEST INJECTION	TEST	POST-TEST INJECTION
Before	ethanol	maze	saline
After	saline	maze	ethanol
Control	saline	maze	saline

All animals received an injection before and after the daily test session and the development of tolerance was monitored every fourth day over a period of about 10 weeks by testing all groups in the maze after an injection of ethanol. Like Chen, LeBlanc et al. observed that initially only the animals previously exposed to the drug while in the maze evidenced tolerance; however, with continued drug exposure, both the Before and After groups eventually developed tolerance to the same extent. LeBlanc et al. concluded that tolerance shown by the group exposed to ethanol during testing differed from tolerance evidenced by the After group only in rate of development and that postulation of different underlying mechanisms was unnecessary. Further support for this interpretation

was provided in an additional study by LeBlanc, Gibbins, and Kalant (1975) who reported that tolerance developed in rats performing a moving-belt, shock-avoidance task generalized to performance in a circular maze. Such generalization would not be expected if tolerance involved a behavioral mechanism such as a task-specific, learned compensation for drug impairment. Of special interest was the finding that under these conditions, development of drug dependence also appeared to be enhanced.

According to Kalant's model, organisms receiving equal exposure to a drug, but experiencing the drug at different levels of activity (as in the Before and After group designs) may be expected to develop tolerance differentially. The term "behaviorally augmented tolerance" has been coined by LeBlanc and co-workers to refer to the enhanced rates of development of tolerance under these conditions. It is important to note that Kalant maintains that the influence of this behavioral manipulation merely involves enhanced rate of development of physiological tolerance, and not mechanisms such as habituation or learning.

State Dependent Learning

It has been observed that learning which takes place while an organism is under the influence of a drug may fail to transfer to the non-drug state. Conversely, learning in a normal state may fail to be evidenced when the organism is subsequently drugged. (For reviews, see Bliss, 1974; Overton, 1972, 1978). Such lack of

transfer of learning from one state of the organism to another has been termed state-dependent learning, and Kalant et al. (1971) have suggested an alternate interpretation of behavioral tolerance based on this phenomenon. In the Before and After designs described earlier (e.g. Chen, 1968; LeBlanc et al. 1973) animals receiving the drug just prior to training can be thought of as learning the task while in the drug state. The After groups, however, have no such opportunity during training. When both groups are subsequently tested in the drug state, training transfers for the Before animals and they are able to perform well behaviorally (i.e., they appear tolerant to the drug's effects). For the After animals however, training in the non-drug state fails to transfer to the drug state and performance is poor.

Classical Conditioning

A fourth mechanism of behavioral tolerance has been proposed in which classical conditioning is invoked to account for acquired resistance to drug effects (Siegel, 1975, 1976, 1977, 1978b). According to this theory, environmental cues which regularly precede drug administration serve as a conditioned stimulus (CS), while the pharmacological effects of the drug serve as an unconditioned simulus (US) (cf. Pavlov, 1927). After repeated CS-US pairings (repeated drug administration in the same environment), these cues come to elicit a conditioned response. Siegel suggests that this conditioned response involves homeostatic adjustments which

allow the organism to compensate partially for the primary actions of the drug. As the compensatory conditioned response increases in magnitude with repeated pairings, the pharmacological effect of the drug will be counteracted to a greater extent. Consequently, repeated administration of the same drug dose should elicit steadily diminishing effects: One operational definition of tolerance.

Initial Demonstrations. A variety of research lends support to Siegel's model of behavioral tolerance. In an early study, conceptually similar to that of Chen (1968), Adams, Yeh, Woods, and Mitchell (1969) examined the effects of environmental manipulations on the development of tolerance to morphine. In two experiments, these investigators found that experience with the drug in the test environment (a hot-plate apparatus) enhanced the development of tolerance to the analgesic effects of morphine. As in the studies of Chen (1968) and LeBlanc et al. (1973) among animals equally exposed to a drug, only those previously exposed to the drug while in the test situation showed enhanced development of tolerance. This drug-test interaction was evidenced in an additional experiment in which animals experienced morphine effects in the presence of a non-functional analgesiometric device, indicating that the tolerance observed was not due merely to practice of the response while under the influence of the drug.

Siegel (1975) further examined this drug-test interaction effect in an experiment designed specifically to test his

conditioning theory of tolerance. In an initial study, three groups of rats received three injections of morphine sulfate (5 mg/kg), at 48-h intervals, the environment paired with the drug being different for each group. One group (M-HP) was exposed to a hot-plate analgesia test following morphine administration, a second group (M-CP) was injected with morphine and placed briefly on a non-functional hot-plate apparatus, while the third group (M-CAGE) was merely injected with morphine and returned to its home cage. A fourth group (S-HP) received saline injections followed by exposure to the hot plate. When all animals were subsequently tested on a functional hot plate, only the groups (M-HP, M-CP) for which the test environment had been paired with morphine effects demonstrated tolerance. That the M-HP and M-CP groups were equally tolerant indicated that practice in making the paw-lick response (the measure of analgesia used) did not contribute greatly to the tolerance observed. Animals (M-CAGE) receiving morphine in equal amounts, but not paired with the test environment, evidenced a level of analgesia equal to that of animals (S-HP) receiving morphine for the first time in the final tolerance test.

In addition to confirming the general findings of Adams et al. (1969), Siegel included a manipulation designed to test a specific prediction generated from his conditioning theory of tolerance. If a classically conditioned compensatory response was indeed responsible for the observed tolerance, then presentation of the

environmental cues alone, without drug administration, should evoke a hyperalgesic response. In fact, Siegel reported that when the M-HP group was subsequently exposed to the hot plate test following an injection of saline, they showed just such a hyperalgesic response.

Environmental Specificity. Siegel's model of behavioral tolerance incorporates mechanisms which are quite different from those traditionally thought to be associated with drug tolerance. Specifically, it is asserted that one process which mediates tolerance is activated only in the presence of certain environmental cues. Siegel (1976) provided further support for the environmental specificity of morphine tolerance in a study in which two groups of rats experienced morphine injections and analgesia assessment in dissimilar situations. One group received morphine in the home colony room and was tested in paw-pressure device, while the other group was transferred to a distinctive room, injected with morphine, and tested on a hot-plate apparatus. Both groups received eight, 5 mg/kg doses of morphine (followed by analgesia assessment), spaced at 48-h intervals and were then tested (in a counterbalanced order) in both situations for tolerance to the drug. Siegel reported that only animals tested in the environment in which they originally experienced the drug effects evidenced tolerance.

In a more recent study, Siegel (1978a, Exp. 1A) reported that tolerance to the hyperthermic effects of morphine also showed

environmental specificity. In this experiment, two groups of rats were exposed to alternating injections of morphine (5 mg/kg) and saline, one per day for a period of 20 days. On morphine days, one group was transferred to a distinctive room, injected with morphine and subjected to rectal temperature assessment at 10-min intervals for a 2-h period. Rats in the other group were returned to their home cages following morphine injections and were otherwise not treated. On saline days, the environments and procedures were reversed for each group. A third group received daily saline injections in both environments during the tolerance acquisition period. Following this period, all animals were tested in the distinctive room, the saline control group receiving saline, and the other two groups an injection of morphine. Consistent with Siegel's theory, although both groups had previously been exposed to morphine, the two environments, and the temperature assessment procedures, only the group for which the distinctive room cues had been previously paired with morphine effects showed tolerance to the hyperthermic effects of morphine.

There is at least one alternative interpretation of such findings. While the groups in Siegel's (1978a) study received equal amounts of morphine, the group receiving morphine in the distinctive room also experienced more handling and stimulation (e.g., rectal temperature measurement at 10-min intervals) than did animals merely injected and returned to the home cage. Recall that

Kalant et al. have suggested that increased levels of functional demand during drug exposure may enhance the development of tolerance. It may be argued that the animals that evidenced tolerance did so not because of a classical conditioning process, but because increased levels of functional demand (or stress, cf. Carder, 1978) during drug exposure enhanced the rate of development of physiological tolerance. A stronger demonstration of environmental specificity in Siegel's study would have involved further showing that animals tolerant in the distinctive environment were not tolerant in the home cage environment, a prediction which would not follow from Kalant's model.

Siegel's evidence for a conditioning model of tolerance has been criticized on other grounds. Specifically, Hayes and Mayer (1978) have argued that in Siegel's early studies (1975, 1976) experimental and control groups received unequal exposure to the analgesia testing environment or apparatus before the final test for tolerance. Thus, competing responses due to fear, exploratory behavior, etc., may have adulterated the measure of analgesia for these animals, and contributed to between-group differences.

Some of the above criticisms were countered in an experiment designed so that experimental and control animals experienced equal exposure to the test environment and apparatus during conditioning (Siegel, Hinson, & Krank, 1978). Throughout this experiment, rats were individually housed in dark, sound-attenuating

chambers and constantly exposed to white noise. The CS in this study consisted of exposure to room lighting and relative quiet for a 45-min period, and the US was an injection of morphine sulfate (5 mg/kg). All animals in this study received equal exposure to both the CS and the US; however, for one group, the complex CS was specifically paired with the morphine injection, while for the other group, the CS and US were explicitly unpaired. Two other groups received saline injections, paired or unpaired with the CS. Following the tolerance acquisition phase (consisting of a maximum of nine presentations of the CS and US), all animals were tested for tolerance to the analgesic effects of morphine. In the presence of the CS, the rats were tested on a hot-plate apparatus following an injection of morphine. Siegel et al. reported that tolerance (as evidenced by low levels of analgesia) was shown only by the animals for which the CS had been repeatedly paired with morphine injections. Animals in the unpaired group displayed levels of analgesia similar to those of the saline control groups.

Compensatory Conditioned Responses. According to the hypothesis proposed by Siegel, tolerance to a drug is due (at least in part) to a classically conditioned response which counteracts the primary effects of the drug. Thus, it is predicted that following repeated pairings of environmental stimuli with drug administration, presentation of those cues not followed by drug administration (i.e., a CS-alone trial) should elicit a measurable compensatory

conditioned response. Siegel (1978b) has noted that when drugs are used as unconditioned stimuli in classical conditioning experiments, the conditioned response is often in a direction opposite that of the primary pharmacological effect of the drug. For example, the unconditioned response to epinephrine includes tachycardia, hyperglycemia, and decreased gastric secretion. Conditioned responses reported to stimuli paired with epinephrine injections include bradycardia, hypoglycemia, and increased gastric secretion. One critical test of a conditioning theory of tolerance is the demonstration of such a conditioned response.

As previously noted, Siegel (1975, Exp. 2A) has reported a conditioned hyperalgesic response to stimuli associated with injections of morphine. Siegel additionally reported that rats show a hypothermic response when presented with cues previously associated with morphine administration. Recall that in this experiment (Siegel, 1978a) one group of rats had experienced repeated pairings of the pharmacological effects of morphine with the environmental cues of a distinctive room. Over the course of 10 drug administrations in this environment, tolerance to the morphine-induced hyperthermia was observed. Another group of rats received saline injections in the distinctive room and experienced the drug effects in their home cages, and showed no tolerance when subsequently tested with morphine in the distinctive room. Siegel then administered a placebo (saline) injection in the distinctive room to both these

groups (and to a third group which had always received saline). As predicted by a conditioning model of tolerance, the animals which had received morphine injections paired with the distinctive room cues showed a hypothermic response to the presentation of those cues alone. The other groups manifested only the slight rise in body temperature that characteristically followed a subcutaneous injection of saline. Importantly, such compensatory conditioned responses are not predicted by Kalant et al.'s model of behaviorally augmented tolerance and thus form an empirical basis for discriminating between possible mechanisms involved in behavioral tolerance.

Additional Pavlovian Manipulations. Siegel has further suggested that manipulations known to affect Pavlovian conditioning should influence the manifestation of tolerance in a predictable fashion. Following Pavlovian acquisition of a conditioned response, repeated presentation of the CS alone (i.e., not followed by the US) can greatly weaken or extinguish that response. Siegel reasoned that if morphine tolerance were, in fact, due to a classically conditioned compensatory response, repeated presentations of the CS alone would result in extinction of the conditioned response and loss of tolerance. This hypothesis was tested in two similar experiments (Siegel, 1977, Exps. 1 and 2) in which rats acquired tolerance to morphine following repeated pairings of specific environmental cues with the pharmacological actions of

the drug. After tolerance (assessed with a paw-pressure analgesiometer) developed, half of the animals were repeatedly exposed to the drug administration cues alone, while the other half were left undisturbed in their home cages. Siegel reported that in both studies, the groups that received extinction trials showed less tolerance than did the control groups, as would be predicted by a conditioning theory of tolerance. In a similarly designed study (Siegel, 1978a), it was shown that CS-alone presentations weakened the tolerance acquired to the hyperthermic effects of morphine. Siegel (1977, Exps. 3 and 4) has also reported that manipulations involving partial reinforcement and latent inhibition affected acquisition of tolerance to morphine in a manner consistent with a model of tolerance based on classical conditioning.

Although the effects of these Pavlovian manipulations are consistent with predictions offered by the laws of classical conditioning, a problem common to the studies just cited is that experimental and control groups were unequal with respect to handling, number of injections, and experience with analgesiometric procedures used, factors which might also influence the measures of tolerance employed. For example, in Siegel's 1977 study, the stimulus complex uniquely associated with the effects of morphine during acquisition included not only the distinctive environmental cues, but also the stimulation of handling, injection, and analgesia measurement procedures. During the extinction phase, the experimental

group repeatedly experienced this full array of stimulation (unaccompanied by drug effects), while the control group remained undisturbed in their home cages. However, such events as handling, injections, and analgesia assessment serve not just as components of the CS, but may have additional behavioral effects as well; effects which may have contributed to the differences observed between extinction groups and control animals.

Tolerance and Learning

A variety of other evidence suggests that the development of tolerance shares several characteristics with the acquisition of learned responses. This evidence has been reviewed recently (LeBlanc & Cappell, 1977; LeBlanc, Poulos, & Cappell, 1978; Siegel, 1978b) and will be outlined briefly below. Noting that tolerance to opiates sometimes persists for long periods of time, Cochin (1972) suggested that the processes involved may be similar to those of long-term memory. Along these lines, it has been reported that manipulations which appear to prevent consolidation of learning also interrupt the development of tolerance. Cortical ablation, protein synthesis inhibition, serotonin depletion, and electroconvulsive shock are treatments which appear to inhibit learning or memory consolidation and also to retard the development of tolerance to various drugs. Though these findings are suggestive, they do not unambiguously implicate learning in the development of drug tolerance. The above findings may merely indicate that a

physiologically and neurochemically intact nervous system is necessary for either the occurrence of learning or the development of tolerance.

The secondary effects of drug administration may include not only tolerance (and dependence) but an enduring change in the organism which may alter further interaction with the drug. For example, it has been reported that rats that had previously developed and lost tolerance to ethanol acquired tolerance at a faster rate during subsequent cycles of exposure to the drug (Kalant, LeBlanc, & Gibbins, 1971; Kalant, LeBlanc, Gibbins, & Wilson, 1978). This similarity between the redevelopment of tolerance and the reacquisition of learned responses provides further, albeit indirect, support for a mediating role of conditioning processes. LeBlanc and Cappell (1975) have observed that when tolerance initially develops following chronic exposure to a consistent level of drug (as produced by pellet implantation or gas inhalation techniques) reacquisition of tolerance appears not to be enhanced. By contrast, in studies where improved reacquisition is reported, drugs have been administered intermittently (e.g., via a series of injections) during each tolerance development period. LeBlanc and Cappell speculated that the basis for this discrepancy is that "the frequent intermittent stimulation of the adaptive process caused it to consolidate more effectively." Equally likely, however, is that the repeated drug administrations involved in

these studies represent repeated conditioning trials which facilitate subsequent relearning.

In summary, adaptation to drugs is generally considered to be mediated by non-associative processes. That is, the metabolic or cellular adjustments that render an organism less sensitive to a drug's actions are thought to be dependent primarily on the extent of contact between the drug and the physiological systems involved (cf. Cicero, 1978; Goldstein et al. 1974; Hug, 1972). There is a growing body of evidence, however, that environmental or behavioral manipulations can significantly influence the development or manifestation of drug tolerance. Although the mechanisms by which such variables influence tolerance have yet to be determined, the studies reviewed above indicate a possible role of an associative process such as intrumental or classical conditioning. Additionally, it appears that factors such as level of activity during drug exposure, or past history of tolerance can influence the rate of development of tolerance.

Present Study

The purpose of the present investigation was to examine the influence of behavioral variables on the development of tolerance to ethanol. Administration of ethanol and presentation of environmental stimuli were scheduled such that the role of physiological or metabolic tolerance was minimized, and so that specific tests of a Pavlovian conditioning model of tolerance (Siegel, 1978b)

could be conducted. These tests included determination of the environmental specificity of the observed tolerance, a test for compensatory conditioned responses that might mediate tolerance, and an assessment of the influence of extinction procedures on tolerance. Additionally, level of activity and stimulation were varied between groups, in order to assess the possible contribution of behaviorally augmented tolerance (Kalant et al. 1971; Kalant, 1977).

Throughout the study, the drug's effect (and resistance to it) was assessed by monitoring body temperature and motor capabilities. Ethanol produces a dose-related fall in body temperature, reaching a maximum some 60 to 90 min following administration (Freund, 1973; Linakis & Cunningham, in press; Ritzmann & Tabakoff, 1976). This hypothermia results from a combination of peripheral vasodilation and disturbance of hypothalamic temperature regulating mechanisms. depending upon the dose administered (Ritchie, 1974). Such thermal responses have been used successfully to monitor development of drug tolerance (Crabbe, Rigter, Vijter, & Strijbos, 1979; Ritzmann & Tabakoff, 1976; Siegel, 1978a) and may be less sensitive than some behavioral measures to motivational or practice effects. A relatively low dose of ethanol was used throughout the study for several reasons. When higher doses (2 to 3 g/kg) were chronically administered in pilot studies, rats too frequently became ill, or died. A lower dose (1.4 g/kg) was chosen for the present study to avoid this problem, and to minimize development of metabolic or

cellular tolerance due simply to exposure to the drug.

A rod-hanging test was used to assess the effect of ethanol on motor capabilities. It was expected that the ataxia initially produced by ethanol would be reflected in an inability to balance on, or hang from a metal rod. Pre-training on this task was included to minimize the contribution of practice effects during the tolerance acquisition phase (cf. Moskowitz & Wapner, 1964).

The experiment consisted of four phases: (1) a tolerance acquisition phase, (2) a series of tolerance and conditioned response tests, (3) an extinction phase, and (4) post-extinction tolerance and conditioned response tests. During tolerance acquisition, injections of ethanol were consistently paired with one set of distinctive environmental cues, while control injections of saline were consistently paired with a different set of environmental cues. Administration of drug and vehicle alternated, as did the injection environment, and groups of rats were counterbalanced such that the drug environment for one group was the saline environment for the other. Of the three groups receiving ethanol during the tolerance acquistion phase, one experienced temperature and motor testing following both ethanol and saline injections, a second group experienced tests only after injections of ethanol, and the third was tested only after injections of saline. Thus, although the groups were equated for total exposure to ethanol and the conditioning environments, they differed with respect to the level of

activity and stimulation during drug exposure periods. A fourth group experienced saline injections and testing in both environments during the acquisition phase.

Following the tolerance acquisition phase, all animals were tested on two occasions for tolerance to ethanol, once in the environment which had previously been paired with drug effects, and once in the environment previously paired with saline injections. A difference in the level of tolerance between animals tested in the presence of drug cues and animals tested in the presence of saline cues should reflect the contribution of a conditioning mechanism that is dependent upon specific environmental cues. Such a difference would not be predicted by traditional theories of tolerance, or by a model involving behavioral augmentation of tolerance. The influence of varied levels of activity during drug exposure should be illuminated by comparison of the levels of tolerance evidenced by the three ethanol acquisition groups. Moreover, the effects of repeated saline injections should be reflected in the response of the group receiving saline throughout the acquisition phase.

According to a conditioning analysis of tolerance, the cues regularly paired with drug effects should elicit a conditioned response which acts to mitigate the drug's primary pharmacological actions. By exposing animals to these drug cues, not followed by drug administration, the compensatory conditioned response should

be observable in isolation. To test this prediction, all animals received an injection of saline, half of each acquisition group in the environment previously paired with ethanol, half in the environment paired with saline. A conditioning theory of tolerance would be supported if the animals given a placebo injection in the drug environment were more hyperthermic than animals tested in the saline environment.

The conditioned response tests constituted the first day of an extinction phase during which animals received only injections of saline. Half of the animals experienced these injections in the environment previously paired with drug effects, while the other half received the injections in the environment previously paired with saline. According to the conditioning hypothesis, animals experiencing placebo injections in the drug environment should undergo extinction of the compensatory conditioned response, and hence lose tolerance. During the extinction phase, both extinguished and non-extinguished animals (in Group I) experienced an equal amount of handling and injections so that the non-associative effect of these manipulations should have been minimized. Following the extinction trials, all animals received a post-extinction tolerance test. For this test, animals were returned to the original drug environment, in which temperature and motor responses to an injection of ethanol were assessed. A subsequent conditioned

response test was performed by again returning all animals to the drug environment and administering a placebo injection of saline.

METHOD

Subjects

The subjects were 56 male albino rats from King Animal Laboratories, Inc., Oregon, Wisconsin, 60 days old at the beginning of the experiment and weighing between 240 and 280 g. The animals were housed in a temperature-controlled colony room with a 12-h light/dark cycle. Water was always available to the rats in the home cages; however, they were maintained on a mild food-deprivation schedule, with each rat receiving 20-25 g of food (University of Oregon Rat Chow) immediately following the habituation or conditioning period each day. This procedure was adopted to reduce the chance of injection injury to the gastrointestinal system (cf. Lewis, Kunz, & Bell, 1966).

Apparatus

An electronic thermometer (Yellow Springs Instrument Co., Model 46) with a small flexible probe (YSI Model 402) was used to assess rectal temperature. The rod-test apparatus consisted of a 47-cm high, 18-cm wide, 20-cm deep wooden box. This box had a hardware cloth floor, a clear Plexiglas top, and was open in front. A 1.2-cm diameter steel rod was mounted horizontally across the inside of the box, 36 cm above the floor and 10 cm out from the back wall.

Two distinctive environments were used during the drug conditioning trials and the subsequent tolerance tests. Environment A

was a relatively spacious room (7.6 m long, 3.4 m wide, 5.9 m high) well illuminated by overhead fluorescent lights. Background noise (40 dB, re 20 uN/m^2) was provided by the room's ventilation system. Each rat was placed in a stainless steel cage (24 cm long, 17 cm wide, and 18 cm high) with mesh front and floor panels and solid back and sides. These cages were identical to the home cages, and were arranged in rows on a metal rack.

Environment B was the inside of a sound-deadened refrigerator shell (.8 m long, .5 m wide, and 1.0 m high) which was dark except for a 200-msec flash of a 7.5-W incandescent bulb at 4-sec intervals. Background noise (65 dB, re 20 uN/m^2) was provided by two ventilation fans, and the click of a microswitch at 4-sec intervals. A distinctive odor was added to Environment B by placing 7 g of ground orange peel (Schilling) in an open dish on the floor of the chamber prior to each trial. Inside the chamber the rats were housed individually in clear plastic boxes (30 cm long, 18 cm wide, and 13 cm high) arranged in rows on two shelves. Each box was covered with a ventilated metal top and had a layer of clean wood chips on the floor. Rats in Environment B were briefly exposed, during temperature measurement and rod tests, to the dimly-lit room in which the sound-attenuating chamber was housed.

Procedure

A schedule of the procedures used during the experiment is

outlined in Table 1. Following a 5-day quarantine period in the Animal Care Department, the rats were distributed to individual cages, and were handled (2 min/rat) and weighed daily for the next 5 days. Food deprivation began on the fourth handling day. The sixth through tenth days following the quarantine period were habituation days, on which each rat experienced one temperature measurement, two rod-hanging trials, and was weighed. In order to habituate the animals to injection procedures, each rat also received a .5-ml injection of saline (i.p.), approximately 20 min after the temperature measurement.

For the acquisition phase of the experiment, the rats were randomly distributed to the groups diagrammed in Table 2. Rats in Groups I, II, and III (N = 16/group) consistently experienced the effects of an injection of ethanol in one environment, and the effects of an injection of saline in the alternate environment. During the tolerance acquisition phase, the animals were injected at 48-h intervals, with injections alternating between saline and ethanol. On days between injection days, the rats were left undisturbed (with the exception of feeding) in their home cages. The animals were run in four squads of 14. Conditioning days for Squads 1 and 2 were rest days for Squads 3 and 4, and vice versa. Squads 1 and 3 received conditioning and test trials in the mornings, whereas Squads 2 and 4 received these trials in the afternoons.

Table 1. An outline of the experimental schedule.

DAYS	PROCEDURES
1-5	Handling
6-10	Habituation to measurements and procedures
11-66	Tolerance Acquistion Phase: Alternating saline and ethanol conditioning trials at 48-h intervals (14 sets)
67-68	First tolerance test
69-72	One set of saline and ethanol conditioning trials
73-74	Second tolerance test
75-78	One set of saline and ethanol conditioning trials
79-80	Conditioned response test
81-104	Extinction Phase: 12 saline trials at 48-h intervals
105-106	Post-extinction tolerance test
107-108	Post-extinction conditioned response test

Table 2. A summary of group treatments during the experiment. For the tolerance test, half of each acquisition group were tested in the environment previously paired with the drug, and half were tested in the environment previously paired with saline. A = Environment A; B = Environment B; (+) = ethanol injection; (-) = saline injection; and underlined letter (e.g. A) indicates that following injections in this environment, the animals experienced temperature and motor tests.

Group (n)	Acquisition Phase	Tolerance Tests		CR Test	Extinction Phase	Post-extinction Tests	
		1	2			Tolerance	CR
Ia (8)	A+ / B-	A+	B+	A-	A-	A+	A-
		B+	A+	B-	B-	A+	A-
Ib (8)	B+ / A-	A+	B+	A-	A-	B+	B-
		B+	A+	B-	B-	B+	B-
IIa (8)	A+ / B-	A+	B+	A-	A-	A+	A-
		B+	A+	B-	B-	A+	A-
IIb (8)	B+ / A-	A+	B+	A-	A-	B+	B-
		B+	A+	B-	B-	B+	B-
IIIa (8)	A+ / B-	A+	B+	A-	A-	A+	A-
		B+	A+	B-	B-	A+	A-
IIIb (8)	B+ / A-	A+	B+	A-	A-	B+	B-
		B+	A+	B-	B-	B+	B-
IVa (4)	A- / B-	A+	B+	A-	A-	A+	A-
		B+	A+	B-	B-	A+	A-
IVb (4)	B- / A-	A+	B+	A-	A-	B+	B-
		B+	A+	B-	B	B+	B-

Groups I, II, and III differed with respect to the amount of handling and stimulation experienced during the drug and saline conditioning periods. Rats in Groups Ia and Ib experienced repeated handling and stimulation (temperature measurement and rod-hanging tests) during the 135-min period following injections in both environments. Groups IIa and IIb experienced temperature measurement and rod tests only following injections in the drug environments, while Groups IIIa and IIIb received this additional handling only following injections in the saline environments. Within each acquisition group, treatments were counterbalanced such that one half of the rats consistently experienced injections of ethanol in Environment A and injections of saline in Environment B, while the other half experienced the drug and saline injections in the opposite environments. An additional group of rats (Group IV, N = 8) experienced saline injections, followed by temperature assessment and rod tests, in both environments (see Table 2).

On conditioning days, body temperatures were measured in the colony room 60 min prior to injections. Additionally, each rat's weight was recorded at this time. Approximately 15 min before injections, rats scheduled for Environment B were placed into small individual carrying boxes, and rats scheduled for Environment A were placed into metal cages on the transfer rack. The animals were then transferred to their scheduled environments. Injections were administered at 1.5-min intervals to animals in Environment A,

then to animals in Environment B. Throughout the experiment, all ethanol injections were 1.4 g/kg (as a 20% v/v solution), administered intraperitoneally. Saline injection volumes corresponded to those for a 1.4 g/kg ethanol injection (8.86 ml/kg). All solutions were maintained and injected at room temperature. Following the 135-min conditioning period, the animals were transferred back to their home cages in the colony room, where they received their daily food ration.

Rats in the groups not handled following injections were left undisturbed during the conditioning period. In other groups, body temperature was measured immediately before, and at 30, 60, 90, and 120 min following the injection. Additionally, following temperature measurement at the 60 and 120 min intervals, these animals experienced two rod-hanging tests.

Body temperatures were measured by inserting the lubricated tip of a small thermistor probe 4 cm into the animal's rectum. The animal was held gently against the experimenter's coat and a reading (to the nearest 10th of a degree, Celsius) was taken 30 sec following insertion of the probe.

Rod-hanging tests were conducted in the following manner. To begin each trial, the rat was placed in the rod-test apparatus with its front legs draped over the horizontal rod, and its rear legs hanging below. The animal was then released and the time spent on the bar (up to a maximum of 15 sec) was measured with a stopwatch

to the nearest second. The second rod test began 5 sec after the end of the first.

After 14 pairs of saline and ethanol acquisition trials, tests for the environmental specificity of tolerance were administered (Table 2, column 3). For these tests, each of the six acquisition groups was further divided into two groups, matched for their thermic response to the drug on the last three ethanol acquisition trials. One subgroup was transferred to the environment previously associated with ethanol (i.e., one-half of Groups Ia, IIa, and IIIa was transferred to Environment A), while the other subgroup was transferred to the saline environment (i.e., one-half of Groups Ia, IIa, and IIIa was transferred to Environment B). Animals in Groups IVa and IVb were similarly distributed. All animals were then injected with ethanol (1.4 g/kg) and their body temperatures and rod-hanging capabilities were assessed. The first tolerance test was administered 48 h after the last acquisition trial. Following this first tolerance test, an additional set of saline and ethanol conditioning trials was administered. A second tolerance test was then administered, identical to the first, except that the groups receiving drug in the drug environment in the first test now received ethanol in the saline environment, and vice versa.

Following an additional set of saline and ethanol conditioning trials (see Table 1), a conditioned response test was administered. For this test, each acquisition group was redivided into two groups

(see Table 2, column 4) equated for order of exposure to drug and saline environment on the previous tolerance tests. For the conditioned response test, all the animals received an injection of saline, one group in the saline environment, one group in the drug environment. During all tolerance and conditioned-response tests, temperatures were measured at 0, 30, 60, 90, and 120 min, and rod-hanging tests were administered at 60 and 120 min following injections, for all animals.

In the extinction phase of the study (Table 2, column 5) no ethanol was administered, and all animals received injections of saline, at 48-h intervals. In this phase the "extinction" groups experienced 12 saline injections in the environment previously associated with ethanol effects, while the "control" groups received an equal number of saline injections in the saline environment. During the extinction phase of the experiment, groups experienced the temperature and motor tests only if in the environment in which these tests were given during acquisition (see Table 2). For example, in Group II, only "extinction" animals underwent temperature and motor tests during the post-injection periods.

A post-extinction tolerance test was administered 48-h after the last extinction trial. During this 135-min test, all animals received an ethanol injection in the environment originally associated with drug effects. A post-extinction conditioned response test was administered 48-h after the tolerance test. All animals were again

returned to their original drug environment, and injected with a placebo (saline). During all tolerance tests and conditioned response tests, the animals remained in the test environment for 135 min following the injections, and all rats experienced temperature measurements and rod tests.

RESULTS

Six rats were discarded due to death or illness during the course of the experiment. Two rats from Group IIIb and one from Group IIIa were discarded in the last week of the acquisition phase. One rat from Group IIb died just prior to the extinction phase; one rat from Group Ia and one from Group Ib were discarded during the extinction phase. In the following statistical analyses, group means were substituted for any animal's missing scores and the degrees of freedom associated with each error term were reduced appropriately for purposes of determining critical values.

The results will be outlined in five major sections, ordered to correspond to the chronology of treatments imposed during the experiment. The first section details the patterns of responding observed in the acquisition phase, and is followed secondly by the tolerance test results and thirdly by the findings of the conditioned response test. The fourth major section outlines the extinction phase results and is followed by a description of responding in the post-extinction tolerance and conditioned response tests.

Four measures were used to assess the effects of repeated injections of ethanol and saline during all phases of the experiment: (1) Pre-trial body temperatures were measured in the colony room 60-min prior to injections (colony room temperatures), and in the conditioning environments immediately before injections (pre-injection

temperatures); (2) Post-injection temperatures were assessed at 30, 60, 90, and 120 min following injections; (3) Temperature change (pre-injection temperature minus post-injection temperature) at each time interval was also calculated. (4) In addition to these physiological measures, rod-hanging tests were administered at 60 and 120 min following injections. Pre-trial temperatures, temperature change scores, and rod-test scores will be presented in detail in this section, while post-injection temperatures will be detailed in Appendix B.

Body weights increased gradually during the experiment, for all groups, and are presented in Appendix A. There were no significant weight differences between groups during the acquisition phase, the extinction phase, or on any of the test days. Ambient temperatures in the conditioning environments were monitored throughout the study, and are also detailed in Appendix A. Environment B was generally about 2-3° C warmer than Environment A.

Acquisition Phase

During the acquisition phase of the study Groups I, II, and III experienced alternating injections of saline and ethanol; the effects of each substance being consistently paired with one of the two conditioning environments. Group IV received saline injections in both environments and was not treated with ethanol during the acquisition phase. According to the experimental design (see Table 2), behavioral and physiological measures were obtained for Groups

I and II on ethanol days, for Groups I and III on saline conditioning days, and on all trials for Group IV. Colony room body temperatures were measured for all animals on all conditioning days.

Four-way analyses of variance involving the factors of room assignment (drug paired with Environment A versus drug paired with Environment B), group (I vs II, or I vs III), time after injection, and trial blocks (seven blocks of two trials), were used to evaluate the acquisition phase data. Separate analyses were used for ethanol and saline trials. The physiological and behavioral responses of Group IV, which received saline in both conditioning environments, were analyzed separately.

Pre-trial Temperatures. Pre-trial temperatures during ethanol and saline acquisition trials are detailed in Table 3. Several similarities between pre-trial temperatures on ethanol and on saline conditioning trials should be noted. During the interval between temperature measurement in the colony room (60 min prior to injections) and measurement in the conditioning environment (immediately before injections), body temperatures rose on the average between 0.5 and 1.0° C, on both ethanol conditioning days, and on saline conditioning days, Fs (1, 28) = 367.06, 635.89, $ps < .001$. Although body temperatures in the colony room were similar for all groups, the increase in temperature during the hour preceding injections was always greater for groups in Environment B, regardless of whether this environment was associated with injections of saline, or ethanol,

Table 3. Pre-trial temperatures during the tolerance acquisition phase. The time intervals -60 and 0, refer respectively to temperature measurement in the colony room 60-min prior to injections, and temperature measurement in the conditioning environment, immediately before injections. All values are expressed in degrees Celsius. Subgroup designations (a or b) refer to the environment paired with ethanol for that group.

	ENVIRONMENT A		ENVIRONMENT B	
ETHANOL TRIALS	(Groups Ia, IIa)		(Groups Ib, IIb)	
Trial Block	-60	0	-60	0
1	37.0	37.7	36.9	37.9
2	37.0	37.3	36.8	37.6
3	36.8	37.2	36.8	37.6
4	36.7	37.3	36.8	37.7
5	36.7	37.2	36.6	37.5
6	36.7	37.2	36.6	37.5
7	36.5	37.0	36.3	37.4

SALINE TRIALS	(Groups Ib, IIIb)		(Groups Ia, IIIa)	
Trial Block	-60	0	-60	0
1	37.1	37.9	37.0	38.0
2	36.9	37.5	36.9	37.8
3	36.9	37.4	36.7	37.7
4	36.8	37.3	36.8	37.7
5	36.8	37.4	36.7	37.7
6	36.7	37.3	36.8	37.8
7	36.6	37.0	36.7	37.6

Fs (1, 28) = 30.31, 30.85, *ps* < .001. This difference was consistent with the observed disparity in ambient temperatures (see Appendix A).

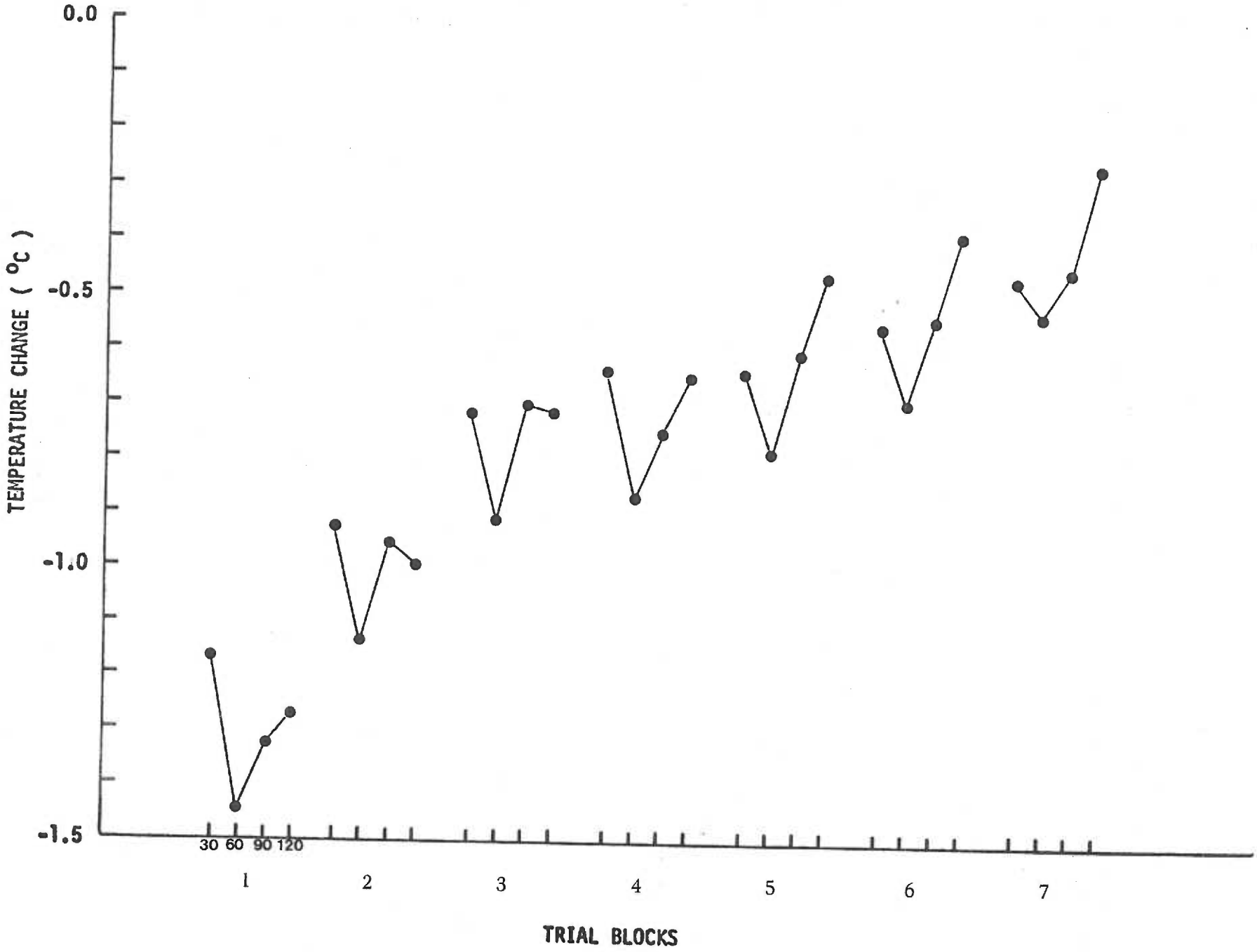
During the acquisition phase, colony room body temperatures and pre-injection temperatures decreased significantly over trials, on both ethanol and saline conditioning days, *Fs* (6, 168) = 26.61, 17.77, *ps* < .001, an effect which may be seen by looking down the columns of Table 3. This decrease in pre-trial body temperatures was also seen in Group IV, *F* (6, 36) = 5.87, *p* < .05, and may reflect habituation to the handling, transferring, and temperature measurement procedures.

Over saline days, the decrease in pre-trial temperatures was greater for groups in Environment A than for groups receiving injections in Environment B. Additionally, pre-injection temperatures decreased more over trials than did colony room body temperatures. These observations were supported statistically by reliable room assignment x block, *F* (6, 168) = 2.73, *p* < .05, and time x block, *F* (6, 168) = 3.94, *p* < .01, interactions. For ethanol trials, the decrease in pre-trial temperatures during the acquisition phase was similar for both colony room and pre-injection temperatures. A significant time x block interaction, *F* (6, 168) = 5.30, *p* < .001, reflected the fact that on Blocks 2 and 3, temperature elevation during the hour preceding injections was slightly less than on Trial Blocks 1, and 4-7.

For animals in Group IV pre-injection temperatures were also about 1.0° C higher than colony room body temperatures, F (1, 6) = 230.08, $p < .001$. Analysis of significant room assignment x environment, and room assignment x environment x time interactions, Fs (1, 6) = 89.75, 58.13, ps < .001 indicated that while colony room temperatures did not differ, pre-injection temperatures were generally higher in Environment B than in Environment A.

Temperature Change. Temperature change scores (combined for Groups I and II), during ethanol acquisition trials are shown in Figure 1. As indicated by the time-effect curves in this figure, ethanol (1.4 g/kg) initially produced a fall in body temperature, with a maximum of approximately 1.5° C of hypothermia occurring 60 min following injections on Trial Block 1. The magnitude of this ethanol-produced hypothermia decreased in a regular manner over trial blocks, F (6, 168) = 60.00, $p < .001$, indicating the development of tolerance. Post-injection temperatures, which increased reliably over trial blocks (see Appendix B), also reflected the development of tolerance to the hypothermic effects of the drug. Temperature change also varied significantly as a function of time after injection, F (3, 84) = 31.25, $p < .001$, and it is apparent from Figure 1 that the form of the time effect curve changed over trials, with a more pronounced recovery from hypothermia occurring in later trial blocks. This observation was supported statistically by a significant interaction between time and trial block factors, F (18, 504) = 3.11, $p < .01$.

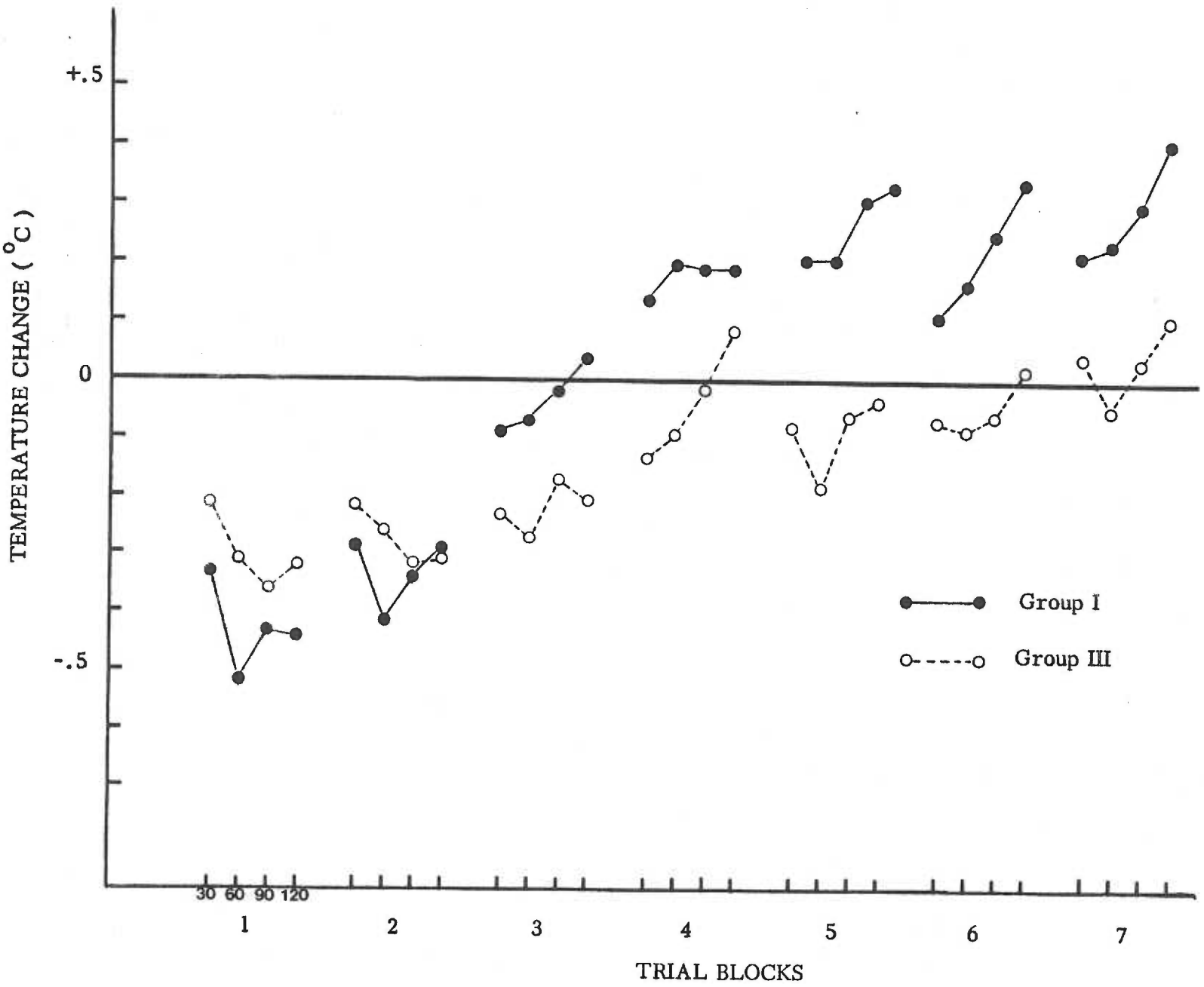
Figure 1. Temperature change (pre-injection minus post-injection temperature) during ethanol acquisition trials. Successive points within each trial block represent temperature change at 30, 60, 90, and 120 minutes after injection. Each point represents a mean score for 32 animals (Groups I and II).


0.0
-0.5
-1.0
-1.5
TEMPERATURE CHANGE (°C)
30 60 90 120
1
2
3
4
5
6
7
TRIAL BLOCKS

The change in the shape of the time-effect curves over trial blocks varied as a function of room assignment. Initially, animals receiving the drug in Environment B (Groups Ib, IIb) showed less recovery from hypothermia than did animals receiving the drug in Environment A (Groups Ia, IIa). However, the increase across blocks, in recovery from hypothermia was more dramatic for Groups Ib and IIb so that for Trial Blocks 3 to 7, recovery was greater for these groups than for Groups Ia and IIa. These relationships were revealed by graphic analysis of significant room assignment x time, F (3, 84) = 3.50, $p < .05$, and room assignment x time x trial block, F (18, 504) = 3.42, $p < .01$, interactions. These variations in recovery from hypothermia may be partially responsible for the finding that while the overall hypothermia was greater during early trials for groups in Environment B, by the sixth or seventh trial block, these groups showed less hypothermia than animals in Environment A, an observation supported statistically by a significant room assignment x trial block interaction, F (6, 168) = 3.03, $p < .01$.

Temperature changes during saline acquisition trials are shown in Figure 2, plotted separately for Groups I and III. In this figure it can be seen that injections of saline initially produced slight hypothermia, which gradually decreased during the course of the acquisition phase, F (6, 168) = 18.09, $p < .001$. Temperature change following saline injections varied significantly as a function of time after injection, F (3, 84) = 4.41, $p < .01$. Initially,

Figure 2. Temperature change during saline acquisition trials. Successive points within each trial block represent temperature changes at 30, 60, 90, and 120 minutes after injection. Each point represents a mean score for 16 animals.


+.5
0
-.5
TEMPERATURE CHANGE (°C)
30 60 90 120
1
2
3
4
5
6
7
TRIAL BLOCKS
Group I
Group III

body temperatures tended to fall during the 2-h period following injections. During the later acquisition trials, however, this trend reversed and body temperatures tended to increase during the post-injection period. A significant time x block interaction, F (18, 504) = 1.99, $p < .05$, confirmed this observation statistically.

It is apparent from Figure 2 that body temperature changes following saline injections differed between Groups I and III, F (1, 28) = 7.12, $p < .05$. While initial hypothermia was slightly greater in rats handled on both saline and ethanol trials (Group I), than for those handled only after saline injections (Group III), the change from a hypothermic to hyperthermic response was more dramatic for Group I as evidenced by a significant group x block interaction, F (6, 168) = 3.22, $p < .005$.

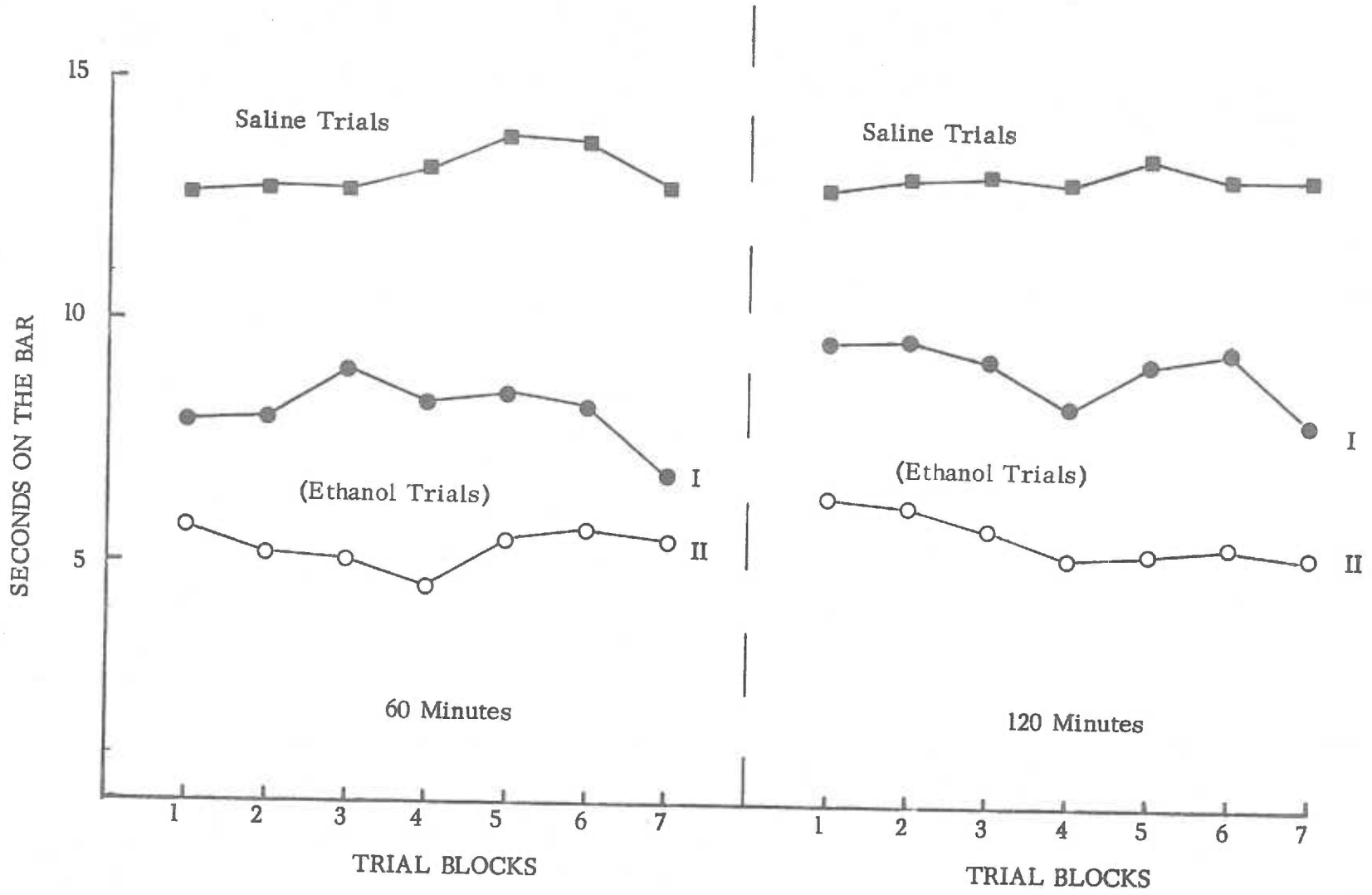
The magnitude and direction of temperature changes displayed by Group IV (saline only) during the acquisition phase were similar to those shown in Figure 2. Temperature change scores also varied significantly over trial blocks, F (6, 36) = 11.38, $p < .001$, from hypothermia in early trial blocks to a slight hyperthermia at the end of the acquisition phase. In early trials a fairly regular fall in body temperature was observed during the 2-h conditioning period. In Trial Blocks 4 to 7 however, temperatures tended to increase as a function of time, giving rise to a significant time x trial block interaction, F (18, 108) = 2.13, $p < .01$. Graphic analysis of a

room assignment (IVa vs IVb) x environment (A vs B), x trial block interaction, F (6, 36) = 2.87, $p < .05$, indicated that while hypothermia was greater in Environment B in early trial blocks, just the opposite was true in Blocks 5-7. This change over trials was more dramatic for animals in Group IVb.

Rod-test. Rod-test scores during acquistion trials are shown in Figure 3. Rod-test scores (on ethanol conditioning days) did not vary significantly over trial blocks, though rats generally stayed on the bar longer at 120 than at 60 min after injections, F (1, 28) = 12.53, $p < .005$. Thus there was no evidence for the development of tolerance to the motor effects of the drug. Rats in Groups I and II were treated identically, with the exception of the additional handling on saline trials experienced by Group I. From Figure 3, it is evident that Group I animals stayed on the bar longer than Group II animals, F (1, 28) = 4.43, $p < .05$, a disparity apparently due to this handling difference. Though not shown in Figure 3, rats in Environment A stayed on the bar longer than rats in Environment B, (8.8 vs 5.2 sec), F (1, 28) = 5.69, $p < .05$.

For comparison purposes, the rod-test performance on saline acquisition days (collapsed across groups) is included in Figure 3. Performance on the rod-test following an injection of saline remained constant across trial blocks, and did not vary significantly as a function of group, room assignment, or time. For groups IVa and IVb, (not shown in Figure 3), rod-hanging times were always slightly

Figure 3. Rod-test scores during the tolerance acquisition phase. The lower two sets of points (open and solid circles) in each panel represent rod-test scores on ethanol acquisition trials for Groups I and II (n = 16). The upper set of points (solid squares) in each panel represent rod-test scores for Groups I and III on saline trials.


15
10
5
SECONDS ON THE BAR
Saline Trials
(Ethanol Trials)
I
II
60 Minutes
120 Minutes
1 2 3 4 5 6 7
TRIAL BLOCKS

higher in Environment A (13.9 sec) than in Environment B (13.6 sec), F (1, 6) = 6.21, $p < .05$, but did not change significantly over trials.

Acquisition Summary. In brief, it was observed that temperature measurement and handling before acquisition trials elicited an increase in body temperature of about 1° C. This pre-trial hyperthermia habituated somewhat during the acquisition phase, but was observed throughout the remainder of the experiment, and was consistently greater for groups in Environment B. Decreases in the magnitude of hypothermia, increases in post-injection temperatures, and greater recovery from hypothermia, all indicated the development of tolerance to ethanol during the acquisition phase. A change in responding over trial blocks, from hypothermia to hyperthermia was observed on saline trials and for Group IV (saline only) animals.

During drug trials, animals in Group I had higher rod-test scores than those in Group II, a difference that may be attributable to more frequent exposure to the rod-test for Group I. No changes in rod-test scores were observed during the acquisition phase.

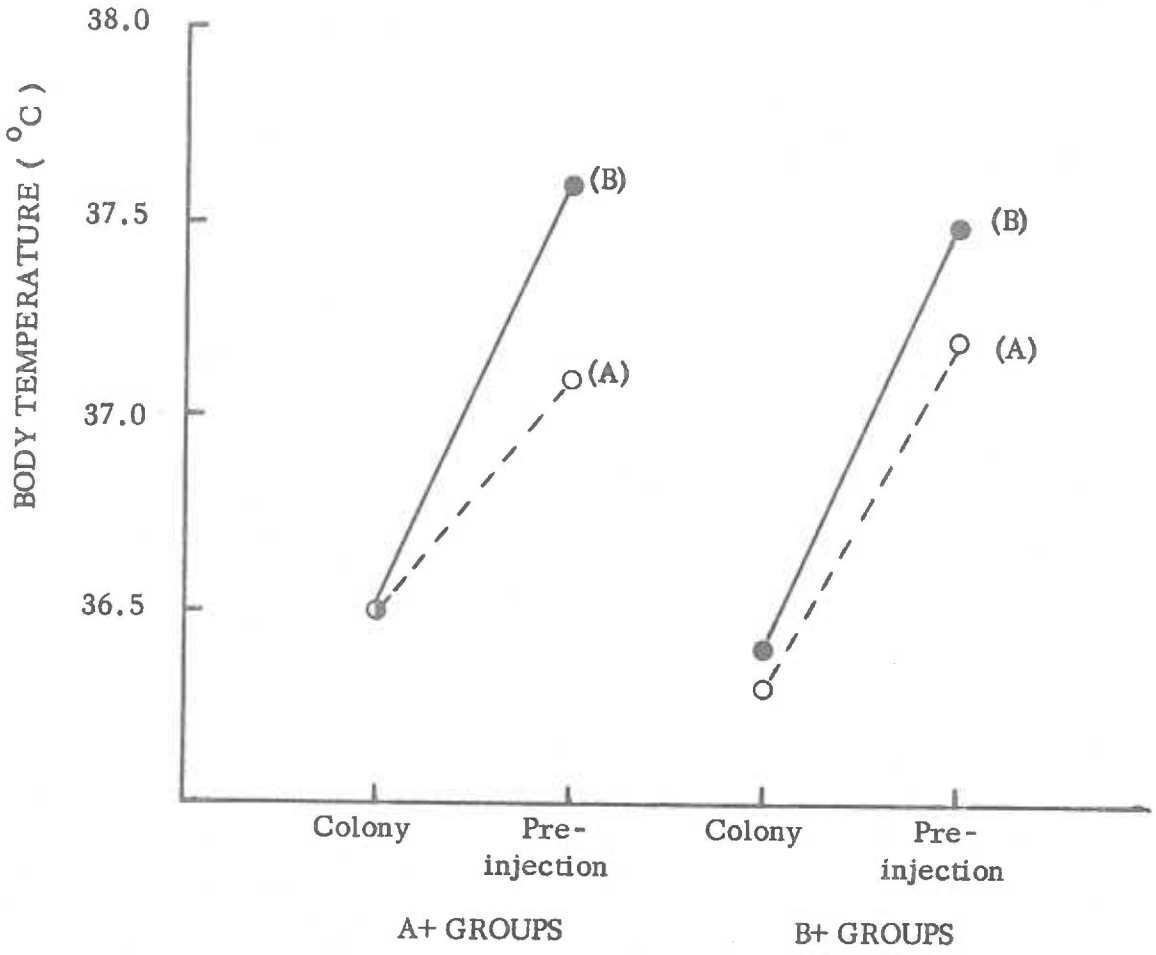
Tolerance Tests

Following the acquisition phase, two tolerance tests were administered. In these tests, one half of each acquisition group was returned to the drug environment and one half returned to the saline environment; all animals were injected with 1.4 g/kg of ethanol.

Animals tested in the presence of ethanol cues in the first test were tested in the presence of saline cues in the second test, and vice versa. Data from both post-acquisition tolerance tests were subjected to a four-way analysis of variance with two between-subjects factors (room assignment : A + drug, or B + drug; and acquisition group : I, II, or III), and two within-subjects factors, (test cue : drug vs saline; and time after injection). In this manner, each animal's response to ethanol in the presence of drug cues was compared to its response in the presence of saline cues.

Pre-test Temperatures. Body temperatures in the colony room, 60 min prior to injections, and in the conditioning environments, immediately before injections are shown in Figure 4, collapsed across Groups I, II, and III, but plotted separately, for animals in each room assignment condition. It is apparent that pre-injection temperatures were, on the average, about 1° C higher than colony room body temperatures. This elevation in body temperature during the hour preceding injections was highly significant, F (1, 39) = 1105.82, $p < .001$. It is also evident from Figure 4 that in general, pre-injection temperatures were higher in Environment B, regardless of its association with drug or saline, and consequently body temperature elevation during the pre-test hour was greater for groups scheduled for Environment B. These findings are consistent with the measured differences in ambient temperature (see Appendix A), and were supported statistically by significant

Figure 4. Colony room body-temperatures and pre-injection temperatures for the post-acquisition tolerance tests. Each point represents a mean for the 24 animals of Groups I, II, and III in each room assignment condition.


38.0
37.5
37.0
36.5
BODY TEMPERATURE (°C)
(B)
(A)
(B)
(A)
Colony
Pre-injection
Colony
Pre-injection
A+ GROUPS
B+ GROUPS

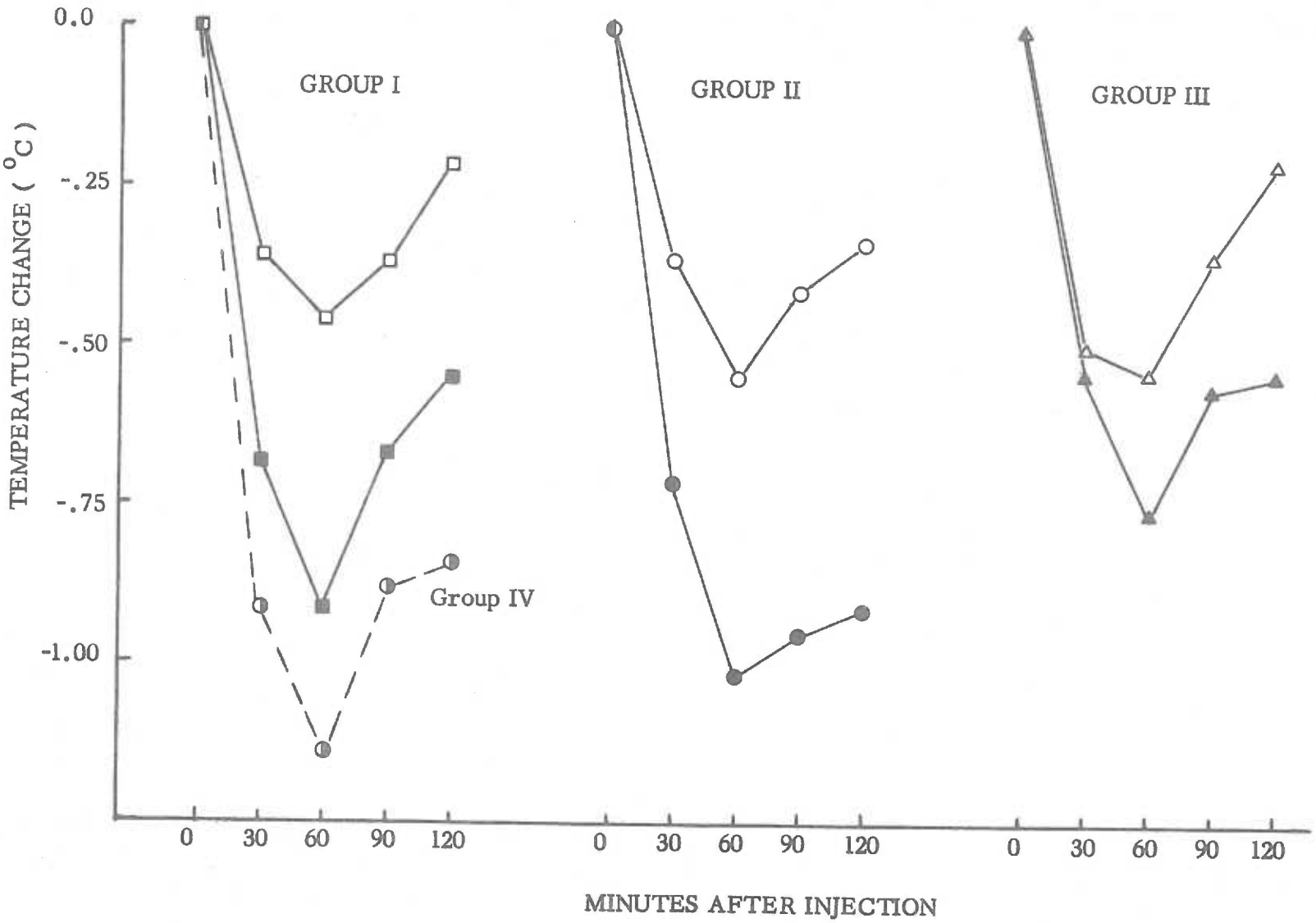
cue x time, room assignment x cue, and room assignment x cue x time interactions, Fs (1, 39) = 6.71, 20.07, 55.52, ps < .05, .001, .001.

Though not immediately apparent from Figure 4, the mean temperature increase (for both saline and ethanol cue tests) was greater for B+ groups (1.00° C) than for A+ groups (0.85° C), as indicated by a significant room assignment x time interaction, F (1, 39) = 6.54, p < .05. Graphic analysis of a significant room assignment x group x cue interaction, F (2, 39) = 3.26, p < .05, revealed one exception to the finding that pre-injection temperatures were higher in Environment B than in Environment A. For Group IIIb, pre-test temperatures were slightly lower for drug-cue (B) tests, than for saline-cue (A) tests.

Temperature Change. As indicated by the time-effect curves in Figure 5, rats tested in the presence of cues previously associated with ethanol (open symbols) were less sensitive to the hypothermic effects of the drug than those tested in the presence of saline cues, (solid symbols), F (1, 39) = 22.29, p < .001. While Groups I, II, and III were relatively tolerant to the thermic effect of ethanol when in the presence of drug cues, the response of these animals to ethanol administered in the presence of saline cues did not differ significantly from the response of rats receiving the drug for the first time (Group IV).

The response to ethanol administered during the tolerance tests was characterized by a maximum hypothermia occurring 60 min after

Figure 5. Temperature change during the post-acquisition tolerance tests. While open symbols represent the response to ethanol in the presence of drug cues, solid symbols represent responding in the presence of saline cues. Each point represents a mean score for eight animals, with all groups collapsed across the room assignment condition.

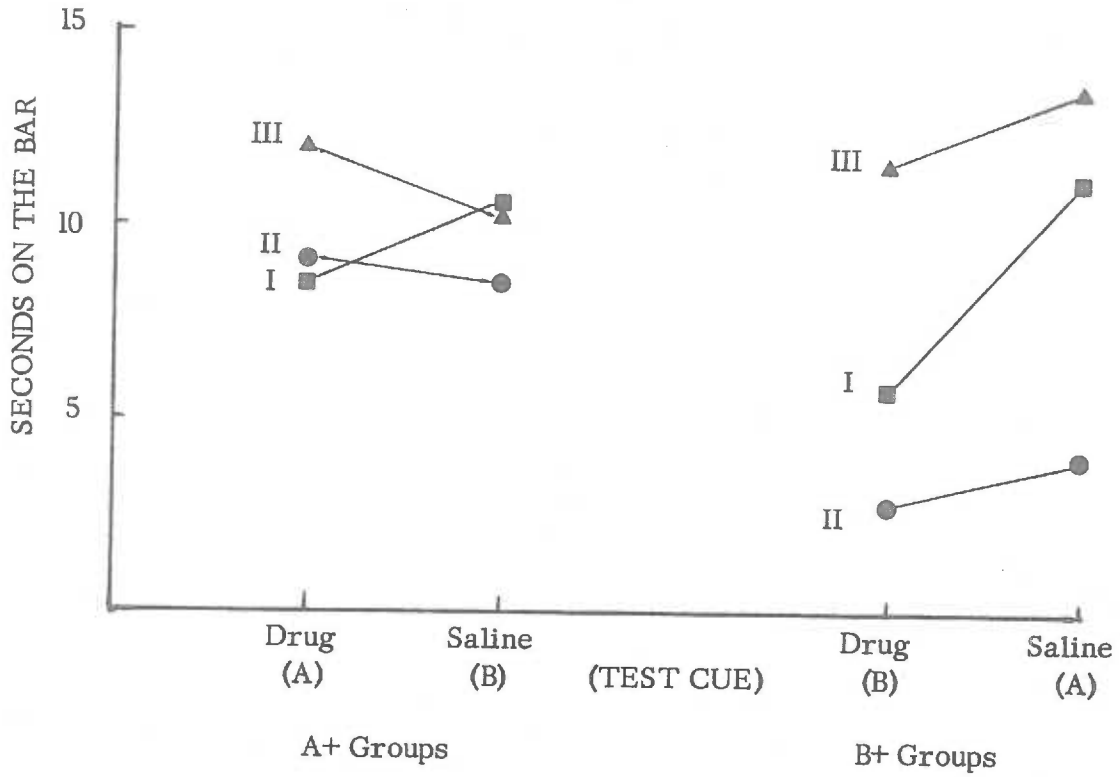

0.0
-.25
-.50
-.75
-1.00
TEMPERATURE CHANGE (°C)
GROUP I
GROUP II
GROUP III
Group IV
0
30
60
90
120
MINUTES AFTER INJECTION

injection of the drug, followed by some degree of recovery. Though not shown in Figure 5, this recovery was relatively dramatic for B+ animals in the presence of drug cues, resulting in a greater disparity between drug and saline cue responding later during the test period. By contrast, drug cue--saline cue differences remained rather constant over time for A+ groups. A statistically significant main effect of time, F (3, 117) = 15.56, $p < .001$, and a significant interaction involving the factors of room assignment, test cue, and time, F (3, 117) = 4.20, $p < .05$, were associated with this pattern of results.

Differences among the three differently handled acquisition groups were not apparent in the combined tolerance test analysis. Separate statistical analyses for the first and second tolerance tests (in which test cue was a between-subjects factor), yielded outcomes similar to the combined analysis, with one exception. In the second tolerance test, animals that experienced a low level of activity during acquisition drug trials (i.e., Group III, which was not handled) were relatively tolerant in the presence of both drug and saline cues. This finding was supported statistically by a significant test cue x group x time interaction, F (6, 96) = 3.30, $p < .01$.

Rod-Test. Tolerance test rod scores are portrayed graphically in Figure 6, where it may be seen that, with the exception of Groups IIa and IIIa, rats stayed on the bar longer in the presence of cues

Figure 6. Rod-test performance during the tolerance tests. In each room assignment condition (A+ groups vs B+ groups), each point represents a mean score for eight animals.


15
10
5
SECONDS ON THE BAR
III
II
I
III
I
II
Drug
(A)
Saline
(B)
(TEST CUE)
Drug
(B)
Saline
(A)
A+ Groups
B+ Groups

previously associated with saline than when in the presence of drug cues. Note however, that for five out of the six groups, rod-hanging times were higher in Environment A, regardless of its association with drug or saline, a finding consistent with differences observed during the acquisition phase. A significant effect of test cue, F (1, 39) = 5.82, $p < .05$, and significant cue x room assignment, F (1, 39) = 6.48, $p < .05$, and cue x group, F (2, 39) = 3.93, $p < .05$, interactions were associated with this observation.

Closer examination of Figure 6 reveals that Group III generally had higher rod scores than Group I, which in turn had higher scores than Group II, F (2, 39) = 8.89, $p < .001$. Though not shown in the figure, these group differences were more pronounced at 120 min after injections than at 60 min, an observation supported by a significant group x time interaction, F (2, 39) = 4.12, $p < .05$. With the exception of Group IIb (which displayed aberrantly low rod-hanging times), Groups I, II, and III did not differ from Group IV in the rod tests. Though not shown in Figure 6, rod-test scores at 120 min following injections were generally higher than those at 60 min, F (1, 39) = 6.67, $p < .05$.

Tolerance Test Summary. During the hour preceding tolerance test injections, body temperatures rose in a manner similar to that observed during the acquisition phase. This temperature elevation was generally more pronounced in Environment B, regardless of whether this environment had been previously paired with drug or saline

administration. Both within-subject and between-subject comparisons indicated that rats were more tolerant (i.e., showed less hypothermia), when tested in the presence of drug cues than when in the presence of saline cues. The one exception to this finding was Group III, which in the second tolerance test appeared relatively tolerant, regardless of the cues present. Rod-hanging times were generally higher in Environment A than in Environment B. Animals not handled previously during drug trials (Group III), had the highest rod-test scores, followed by rats handled on both saline and ethanol acquisition trials (Group I), and rats handled only on ethanol trials (Group II) in that order.

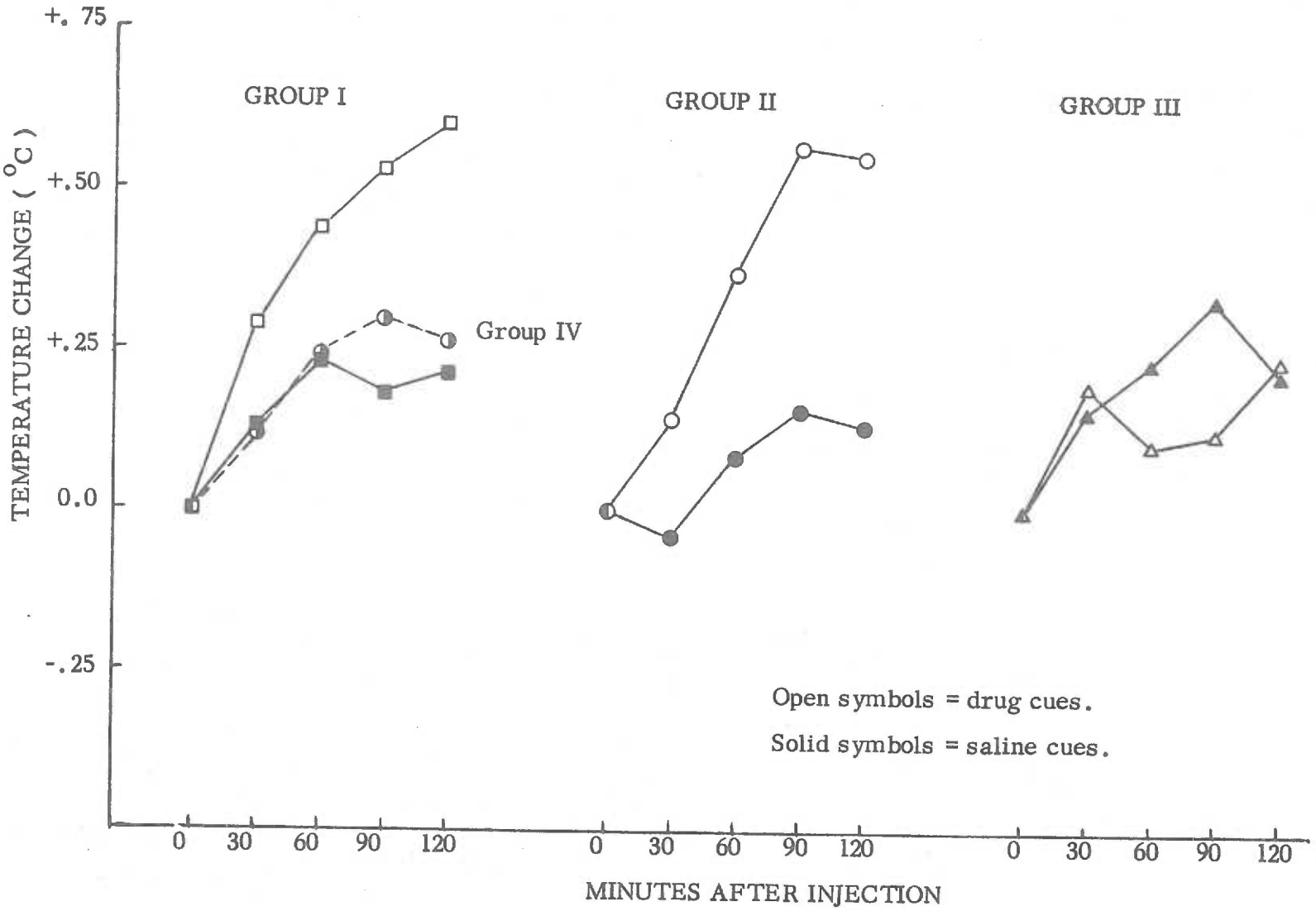
Conditioned Response Test

In the conditioned response test, responding to placebo (saline) injections in the presence of drug and saline cues was compared. Half of each acquisition group received these injections in the environment originally paired with ethanol, half in the environment paired with saline. To analyze conditioned response test data, a four-way analysis of variance, with three between-subjects factors, (room assignment: A+ vs B+; test cue: drug vs saline; and group: I, II, and III), and one within-subjects factor (time after injection), was utilized. Significant interactions were analyzed graphically (Linton & Gallo, 1975; Winer, 1971) or with follow-up analyses of variance and *t* tests.

Pre-test Temperatures. Body temperatures in the colony room 60 min prior to injections were similar for all groups. In the hour preceding injections, body temperatures rose an average of about 1° C, and this increase was always greater for rats in Environment B, regardless of the cue condition. These findings were supported by a significant main effect of time, F (1, 32) = 426.09. $p < .001$, and significant cue x time, F (1, 32) = 8.13, $p < .01$, and room assignment x cue x time, F (1, 32) = 30.30, $p < .001$ interactions. Analysis of a cue x group x time interaction F (2, 32) = 3.89, $p < .05$, revealed that for animals in Group I the pre-test elevation in body temperature was greater for rats in the saline environment than for those in the drug environment (1.2° C vs 0.6° C). Temperatures for Groups II and III were similar both in the colony room and immediately preceding injections, regardless of cue condition.

Temperature Change. Thermal responding in Groups I, II, and III to an injection of saline in the presence of drug or saline cues is shown in Figure 7. It may be seen in this figure that during the conditioned response test period, body temperatures generally increased over time, and that for Groups I and II, animals were more hyperthermic when in the presence of cues previously paired with ethanol injections. For purposes of clarity the data in Figure 7 have been graphed collapsed over room assignment conditions. However, significant room assignment x time, and room

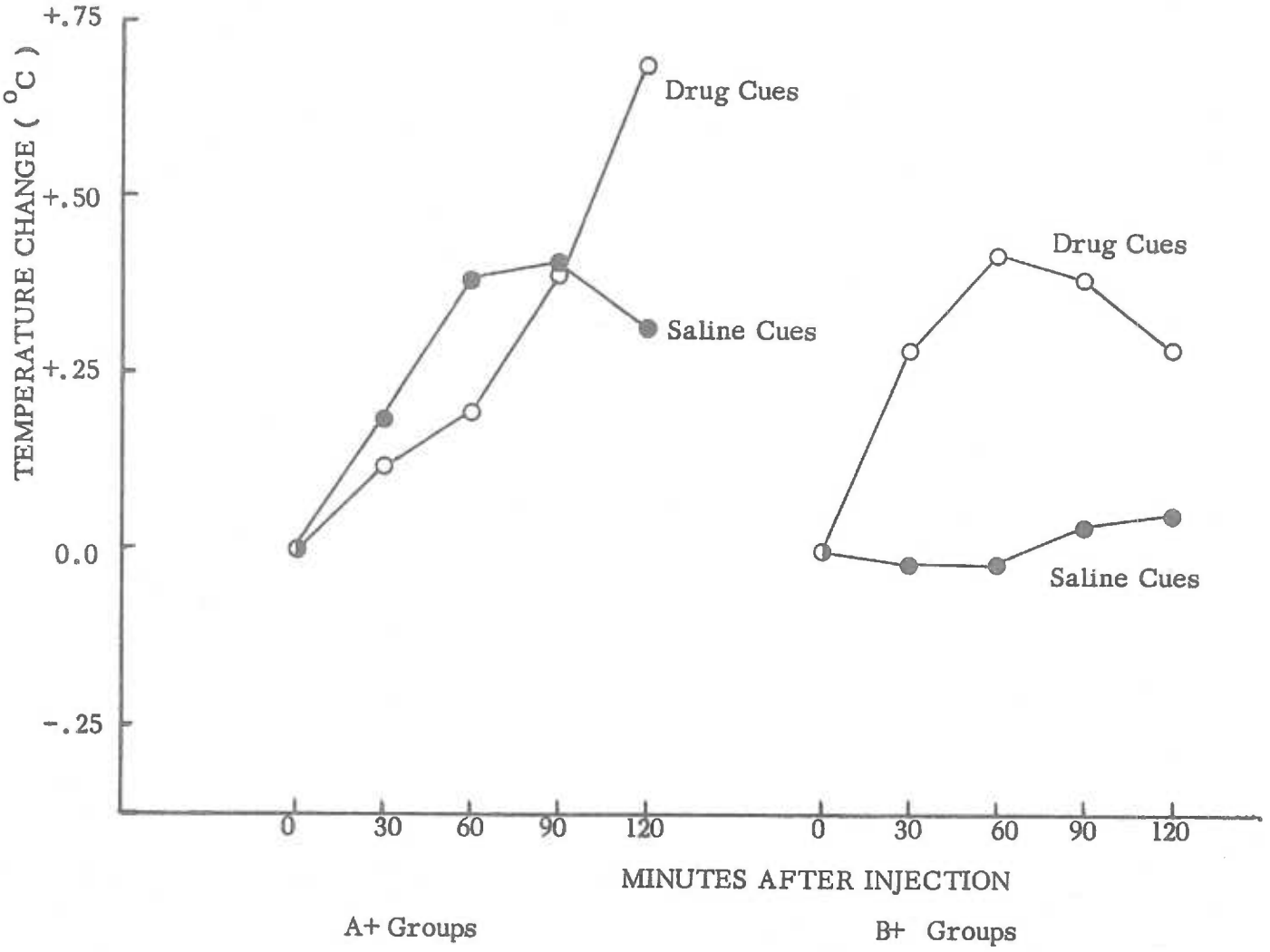
Figure 7. Temperature change during the conditioned response test. Open symbols represent the response to saline injections in the presence of drug cues while solid symbols represent responding in the presence of saline cues. The data are graphed collapsed across room assignment conditions, and each point represents a mean for eight animals.


+. 75
+.50
+.25
0.0
-.25
TEMPERATURE CHANGE (°C)
GROUP I
GROUP II
GROUP III
Group IV
Open symbols = drug cues.
Solid symbols = saline cues.
0
30
60
90
120
MINUTES AFTER INJECTION

assignment x test cue x time interactions, *Fs* (3, 96) = 7.72, 10.66, *ps* < .001, indicated that the influence of test cues differed as a function of both room assignment and time after injection. These relationships may be seen in Figure 8, where responding to drug and saline cues is shown separately for A+ and B+ groups. To further analyze these interactions, separate three-way analyses of variance, involving the factors, test cue, group, and time, were computed for each room assignment condition. Significant test cue x time interactions in each separate analysis, *Fs* (3, 45) = 7.96, 3.20, *ps* < .05, were further examined by comparing saline cue vs drug cue differences at each time interval following injections. These comparisons indicated that B+ animals were more hyperthermic in the presence of drug cues at 30, 60, and 90 min following injections, *ts* (22) > 2.45, *ps* < .05. For A+ groups, animals tested in the presence of drug or saline cues did not differ significantly until 120 min following injections, at which time hyperthermia was more pronounced in the presence of drug cues, *t* (22) = 2.77, *p* < .05.

One observation that may partially account for this pattern of results is that responding to drug cues in A+ groups was an almost linear increase in hyperthermia during the test period, while in B+ groups a maximum hyperthermia was reached 60 min after injections, followed by a slight return toward pre-injection levels.

Figure 8. Temperature change during the conditioned response test, plotted separately for animals in each room assignment condition. Open symbols represent the response to saline injections in the presence of drug cues while solid symbols represent responding in the presence of saline cues. The data are graphed collapsed across the three ethanol acquisition groups, and each point represents a mean for 12 animals.

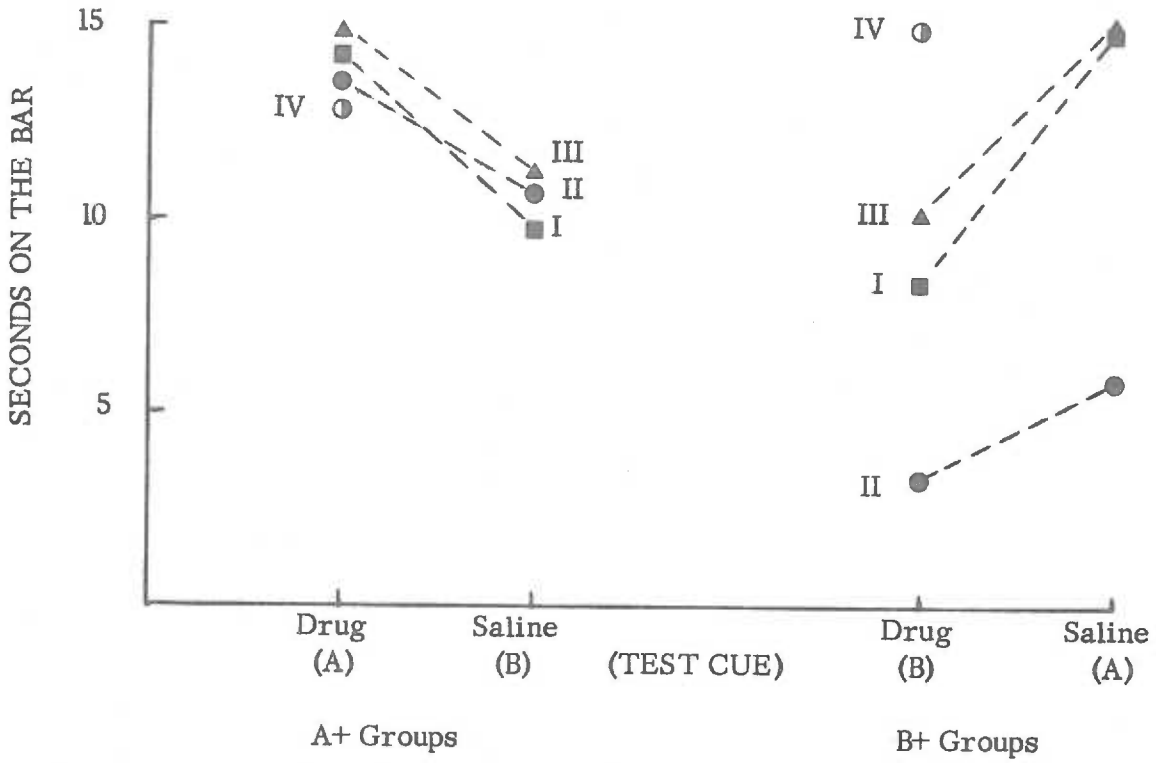

+.75
+.50
+.25
0.0
-.25
TEMPERATURE CHANGE (°C)
Drug Cues
Saline Cues
Drug Cues
Saline Cues
0
30
60
90
120
0
30
60
90
120
MINUTES AFTER INJECTION
A+ Groups
B+ Groups

There were group differences in the time-effect curves generated by saline injections, and a significant room assignment x group x time interaction, F (6, 96) = 2.79, $p < .05$, indicated that these group differences varied with room assignment condition. Graphic analysis revealed that while Groups Ia, IIa, IIIa, Ib, and IIb showed a relatively uniform increase in temperature over time, Group IIIb showed very little change.

It can be seen in Figure 7 that Group IV (which received saline only during the acquisition phase), responded much the same as the other groups did in the presence of saline cues. Responding in Groups I, II, and III to an injection of saline in the presence of drug cues (solid symbols in Figure 7) was compared to Group IV responding in the same environment. Comparisons at 120 min following injections, where differences were most dramatic, indicated that Groups I and II were significantly more hyperthermic than Group IV, ($ps < .05$), however, Group III was not.

Rod-Test. While Groups Ia, IIa, Ib, and IIIb had similar rod-hanging times (mean scores = 12.3 ± .7 sec), Group IIb times were considerably lower (4.5 sec), a pattern of results yielding significant main effects of group, F (2, 32) = 6.55, $p < .01$, and room assignment, F (1, 32) = 7.60, $p < .01$, and a significant room assignment x group interaction, F (2, 32) = 5.57, $p < .01$. In Figure 9, it may be seen that the poor rod-test performance of Group IIb was similar to that observed in the previous tolerance test.

Figure 9. Rod-test performance during the conditioned response test. In each room assignment condition (A+ groups vs B+ groups, each points represents a mean score for four animals.


SECONDS ON THE BAR
15
10
5
IV
III
II
I
IV
III
I
II
Drug
(A)
Saline
(B)
(TEST CUE)
Drug
(B)
Saline
(A)
A+ Groups
B+ Groups

Closer examination of Figure 9 reveals that regardless of cue condition, rod-hanging scores were generally lower in Environment B, an observation consistent with tolerance test findings, and supported by a significant room assignment x cue interaction, $F(1, 32) = 16.13$, $p < .001$. This was not the case for Group IV, however. As shown by the isolated symbols in Figure 9, rod-hanging times for this group were actually slightly higher in Environment B than in Environment A.

Conditioned Response Test Summary. An elevation in body temperature was again observed prior to the conditioned response test. This increase in temperature was always greater in the warmer environment (B), regardless of cue conditions. Animals experiencing placebo (saline) injections in the presence of drug cues showed a greater hyperthermic response than those in the presence of saline cues. This hyperthermic response to drug-paired cues appeared to reach a maximum during the first hour after injection in Environment B, but was still increasing in Environment A at the end of two hours. In the drug environment Groups I and II showed a greater hyperthermia than Group IV (saline only during acquisition); Group III did not.

Extinction Phase

During the extinction phase all animals received a total of 12 injections of saline, spaced at 48-h intervals. Extinction groups experienced these injections in the environment previously paired

with the drug while control groups experienced these injections in the saline environment. Temperatures and rod-hanging capabilities were monitored for Group I in both extinction and control conditions. Responding in Groups II and III was measured only under extinction or control conditions, respectively, as outlined in Table 2. Separate statistical analyses were performed to compare the responses of the following groups: a) Group I, extinction vs control, b) Group I vs Group II (extinction), c) Group I vs Group III (control). For these analyses extinction data were grouped into six blocks of two trials.

Pre-trial Temperatures. During the extinction phase, pre-injection temperatures were higher than colony room temperatures. This pre-trial elevation in body temperatures was significant for Group I $F(1, 10) = 204.76$, $p < .001$, Group II (extinction), $F(1, 9) = 168.05$, $p < .001$, and Group III (control), $F(1, 10) = 901.79$, $p < .001$. In the Group I comparison (extinction vs control), graphic analysis of significant interactions involving factors of extinction condition, time, and trial block $F(5, 48) = 2.86$, $p < .01$, and extinction condition, room assignment, and time $F(1, 10) = 6.66$, $p < .05$, indicated (a) that pre-trial temperature elevation was slightly greater for control animals, but only in extinction blocks 1-3, and (b) that extinction groups in Environment B had higher pre-injection temperatures than their counterparts in Environment A.

For the Group I vs II (extinction) comparison, and the Group I vs Group III (control) comparison, colony room temperatures did not differ, but pre-injection temperatures were always higher in Environment B, giving rise to significant room assignment x time interactions, F (1, 9) = 5.16, $p < .05$, F (1, 10) = 22.75, $p < .001$.

Temperature Change. During the extinction phase, the response to an injection of saline was a regular increase in hyperthermia during the 2-h post-injection period, similar to that observed in the conditioned response test, but of slightly reduced magnitude. This temperature elevation was significant for Group I (extinction vs control), F (3, 30) = 17.23, $p < .001$; for Group II (extinction) F (3, 27) = 40.91, $p < .001$; and for Group III in control conditions, F (3, 30) = 12.02, $p < .001$. In the groups for which thermal responses were measured during the extinction phase, temperature change following injections did not change over trial blocks, or vary as a function of group or extinction condition. Because the conditioned response test itself may have served as an extinction trial, a further analysis was performed, in which hyperthermic responding during the conditioned response test was compared to responding on the last extinction trial, for Groups I and II. A significant room assignment x trial (conditioned response test vs extinction trial 12) x time interaction, F (3, 30) = 5.64, $p < .01$, was further analyzed with separate t comparisons at each time interval, for A+ and B+ groups. During the last hour in B+ groups,

and at 60 min after injections in A+ groups, the hyperthermia shown in the last extinction trial was less than that in the conditioned response test, *ts* (14) > 2.29, *ps* < .05.

The difference in ambient temperature between Environments A and B again influenced temperature change scores. For all groups, hyperthermia was greater in Environment B than in Environment A, *Fs* (1, 10) = 6.68, 8.68, *ps* < .05. During the extinction phase, Group IV's response to saline injections was a slight hyperthermia which did not vary over trial blocks.

Rod-Test. During the extinction phase, rod-hanging times did not differ between groups, or vary as a function of time after injection. In all three comparisons, (Group I, extinction vs control; Group I vs II, extinction; Group I vs III, control), animals stayed on the bar longer in Environment A than in Environment B, *Fs* (1, 10) = 7.84, 44.64, 6.86, *ps* < .05, respectively. For the Group I vs III comparison, analysis of a significant trial block effect, *F* (5, 50) = 3.03, *p* < .05, and a significant trial block x room assignment interaction, *F* (5, 50) = 2.76, *p* < .05, indicated that rod-hanging times in Environment A (but not B) changed over trial blocks. This variation was seen as a decrease in hanging times from Blocks 1-4, followed by an increase in Blocks 4-6. Group IV rod-hanging times did not change during the extinction phase.

Extinction Phase Summary. In general, pre-trial temperatures, temperature change scores, and rod-test performance did not change

during the extinction phase. However, in Groups I and II, hyperthermic responding in the last extinction trial was somewhat diminished relative to responding in the conditioned response test. Body temperatures again increased during the hour preceding injections, and the hyperthermia following injections did not differ between extinction and control groups.

Post-extinction Tests

Following the extinction phase, all animals were returned on two occasions to the environment originally paired with the drug. In the post-extinction tolerance test, responding in extinction and control groups was compared, in order to assess the effects of extinction treatment on the retention of tolerance. Evidence for conditioned responding following placebo injections in the drug environment was examined in the subsequent conditioned response test. Data from both post-extinction tests were examined with four-way analyses of variance, involving three between-subjects factors, (room assignment: A+ vs B+; extinction condition: extinction vs control; and group: I vs II vs III), and one within-subjects factor, (time after injection).

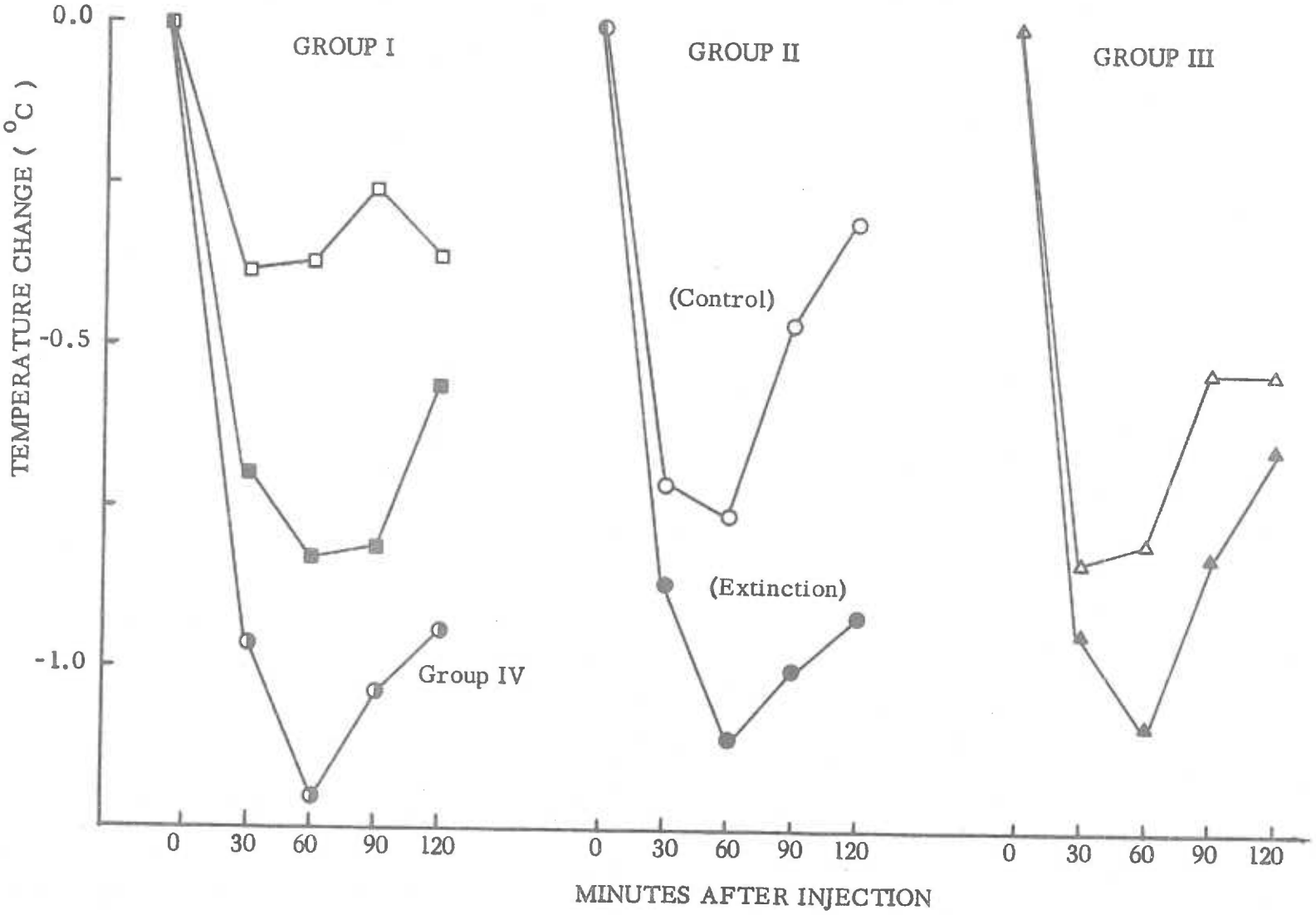
Post-extinction Tolerance Test

Pre-test Temperatures. Groups in Environment B showed a greater pre-test elevation in temperature (+1.3° C) than groups in Environment A (+0.9° C). Both the main effect of time, F (1, 30) = 519.72,

$p < .001$, and the room assignment x time interaction, F (1, 30) = 23.23, $p < .001$, were significant. Analysis of a group x time interaction, F (1, 30) = 12.37, $p < .005$, revealed that although colony room body temperatures were identical for Groups I, II, and III (36.4° C), there was a small but regular difference in pre-injection temperatures, ordered from Group I (37.4° C), to Group II (37.5° C), to Group III (37.6° C).

Temperature Change. Temperature change scores during the post-extinction tolerance test are shown in Figure 10, where it may be seen that in general, rats that had experienced repeated injections of saline in the presence of drug cues (solid symbols) were less resistant to ethanol-produced hypothermia than were control animals (open symbols), F (1, 31) = 10.19, $p < .005$. This hypothermia generally reached a maximum at 60 min following injections, and was followed by some degree of recovery, a reliable variation over time, F (3, 90) = 9.34, $p < .001$. A significant room assignment x extinction condition x group interaction was examined by computing separate analyses of variance (involving the factors room assignment, extinction condition, and time) for each acquisition group. In these analyses the pooled between-subjects error term from the overall analysis was used. Significant main effects of extinction treatment for Groups I and II, Fs (1, 30) = 4.70, 5.16, ps $< .05$, but not for Group III were obtained, indicating that the extinction--control difference observed for Group III,

Figure 10. Temperature change during the post-extinction tolerance test. Open and closed symbols represent responding in control and extinction groups, respectively, to injections of ethanol in the drug environment. The data are plotted collapsed across room assignment conditions, and each point represents a mean score for eight animals.


0.0
-0.5
-1.0
TEMPERATURE CHANGE (°C)
GROUP I
GROUP II
GROUP III
(Control)
(Extinction)
Group IV
0
30
60
90
120
MINUTES AFTER INJECTION

although in the same direction as for other groups, was not statistically reliable. For Group II, analysis of a significant room assignment x extinction condition interaction, F (1, 30) = 12.73, $p < .01$, revealed that while extinction--control differences were dramatic for Group IIa, (-1.3° C vs -0.3° C), this effect was slightly reversed in Group IIb.

Separate two-way analyses of variance were used to compare responding in Group IV to that of extinction and control animals in Groups I, II, and III. Animals in Groups I, II, and III that had experienced the extinction treatment did not differ from Group IV in the amount of hypothermia induced by ethanol ($Fs < 1$). By contrast, control animals in Groups I and II displayed significantly less hypothermia than Group IV, Fs (1, 14) = 8.50, 5.58, $ps < .05$; Group III controls did not.

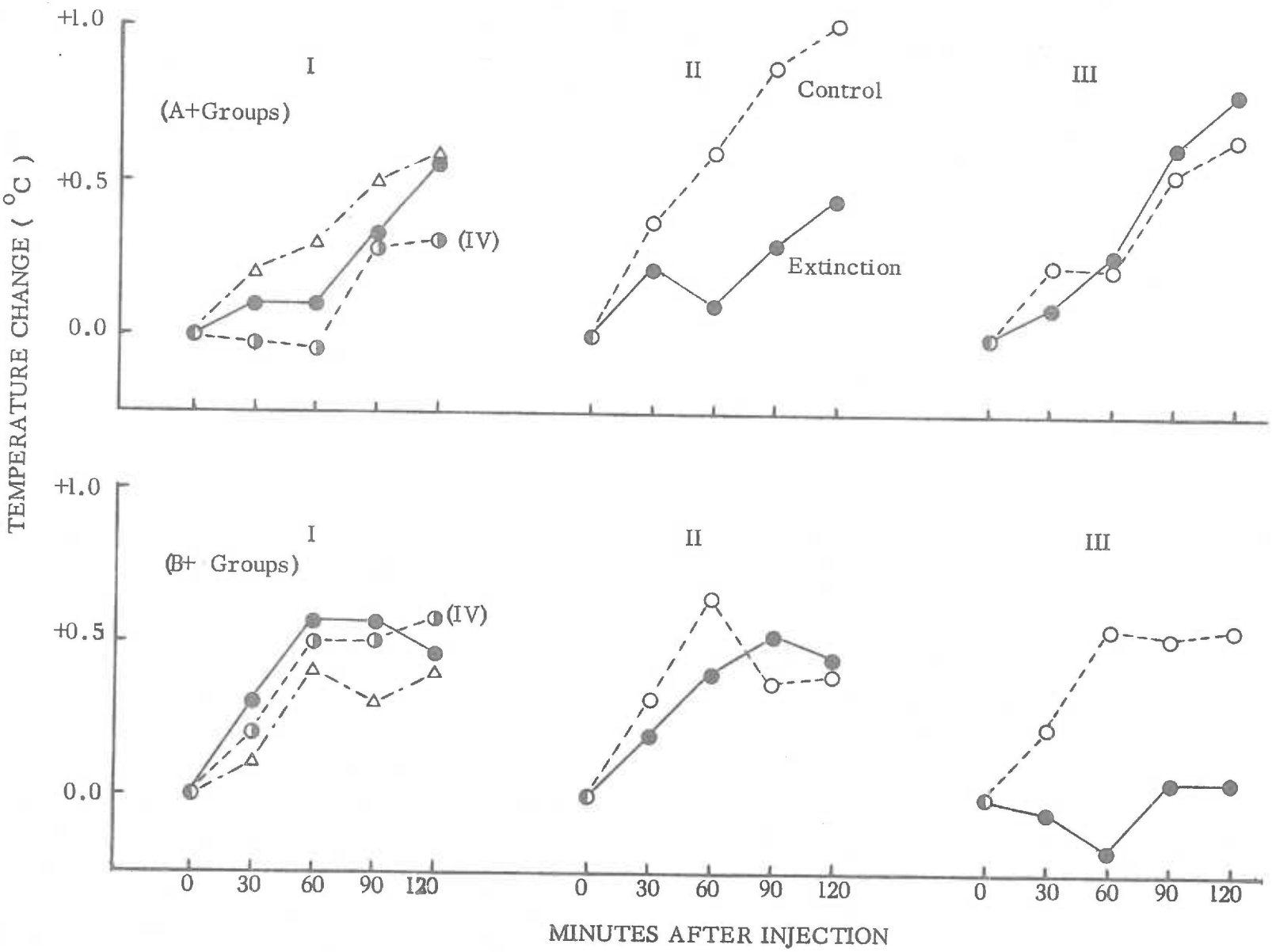
Rod-test. Rod-test performance was not affected by treatment during the extinction period. No significant main effects of extinction treatment were obtained, nor did this factor significantly interact with any of the other factors. Graphic analysis of a significant main effect of room assignment, and a significant room assignment x group x time interaction, Fs (1, 30) = 4.45, 5.18, $ps < .05$, revealed that rod-hanging times were higher in Environment A than in Environment B, for Groups II and III, but not for Group I. Moreover, for Group III, this difference was more pronounced at 60 min following injections than at 120 min.

Post-extinction Conditioned Response Test

Pre-test Temperatures. Prior to the conditioned response test, body temperatures again rose about 1° C, a change which was highly significant, F (1, 30) = 258.45, $p < .001$, and more pronounced for rats in Environment B, as indicated by a significant main effect of room assignment, F (1, 30) = 4.40, $p < .05$, and a significant room assignment x time interaction, F (1, 30) = 26.94, $p < .001$.

Temperature Change. Temperature changes following saline injections in the post-extinction conditioned response test are shown in Figure 11 plotted separately for each acquisition group in each room assignment condition. It is apparent from this figure that in general, the response to these injections was mild hyperthermia which gradually increased over time, F (3, 90) = 46.27, $p < .001$, and that with the possible exceptions of Groups IIa and IIIb, there were no differences among extinction and control groups. A significant main effect of group, F (1, 30) = 32.72, $p < .001$, a significant room assignment x time interaction, F (3, 90) = 14.35, $p < .001$, as well as a significant four-way interaction involving the factors room assignment, extinction condition, group, and time, F (6, 90) = 3.57, $p < .01$, were obtained. The four-way interaction was analyzed by first computing three-way analyses of variance (with room assignment, extinction condition, and time as factors), for each group, with follow-up extinction condition x time analyses attending any significant interaction involving room assignment,

Figure 11. Temperature change during the post-extinction conditioned response test. Responding is plotted separately for each acquisition group, in each room assignment condition. Each point represents a mean score for four animals (except where attrition led to reduced group size). Open and solid symbols represent responding in control and extinction groups, respectively.


+1.0
+0.5
0.0
TEMPERATURE CHANGE (°C)
I
II
III
(A+Groups)
Control
Extinction
(IV)
(B+ Groups)
0
30
60
90
120
MINUTES AFTER INJECTION

extinction condition, and time.

In these subsequent analyses, significant extinction condition x room assignment x time interactions, Fs (3, 36) = 4.48, 3.27, ps < .05, were obtained for Groups II and III. These interactions may be seen by comparing Group II and III extinction vs control responding in A+ and B+ groups in Figure 11. The subsequent two-way analyses revealed that only for Group IIa was there a significant extinction condition x time interaction, F (3, 18) = 4.35, p< .05, indicating that extinction--control differences increased over time. While Group IIIb differences appear to be of the same magnitude in Figure 11, they were not statistically reliable, probably due to a reduced group size (n = 3). Responding in Group IV was similar to that in the other groups.

Rod-test. The room assignment condition interacted in a complex fashion with the rod-hanging performance of extinction and control animals. While control group performance for both A+ and B+ groups was similar, (9.9 vs 10.3 sec), extinction group rod-hanging times were elevated for A+ groups (14.3 sec), and depressed for B+ groups (7.4 sec). This pattern of results gave rise to a significant main effect of room assignment, F (1, 30) = 9.19, p < .01, and a significant room assignment x extinction condition interaction, F (1, 30) = 11.68, p < .005.

Groups Ia, IIa, IIIa, Ib, and IIIb had similar rod-test scores, however, the scores for Group IIb were again aberrantly low. A

significant main effect of group and a significant room assignment x group interaction reflected this outcome, Fs (2, 30) = 7.51, 4.45, ps < .05.

Post-extinction Test Summary. In the post-extinction tests, pre-test elevation in body temperature was similar to that observed during the preceding phases of the experiment. In control animals in Groups I and II, tolerance to the hypothermic effects of ethanol was retained over a period of three weeks during which no drug was administered. By contrast, animals that experienced repeated extinction trials had lost tolerance. Extinction and control animals in Group III did not differ significantly. Differential responding in the conditioned response test was observed only in Group IIa, in which control rats showed greater hyperthermia than those which had experienced the extinction treatment. As in the preceding phases, rod-test scores were generally higher in Environment A than Environment B, but were not influenced by the extinction treatment.

DISCUSSION

The treatments employed in this study were designed to assess the possible contributions of behavioral augmentation of tolerance, and of Pavlovian conditioning, to tolerance developed to the physiological and behavioral effects of ethanol. To this end, the important behavioral manipulations in the tolerance acquisition phase were varying the level of activity (between groups) during drug exposure, and consistently pairing one set of distinctive cues with the sequelae of ethanol administration. Kalant et al.'s (1971) behavioral augmentation theory predicts that animals experiencing higher levels of stimulation and activity while under the influence of the drug (e.g., Groups I and II, which were handled during ethanol acquisition trials), should develop tolerance at a faster rate than animals equally exposed to the drug, but at lower levels of activity (e.g., Group III, handled only during saline acquisition trials). Siegel's classical conditioning model of tolerance predicts that animals should be more tolerant in the presence of cues previously paired with the drug; moreover, this tolerance is said to be mediated by a conditioned response which offsets the drug's primary effects. Throughout the experiment, efforts were made to reduce the contribution of cellular and metabolic tolerance by (1) minimizing the intensity and duration of exposure to the drug (administering relatively low doses at long inter-dose intervals)

and (2) equating the ethanol acquisition groups (I, II, and III) for total experience with the drug (and conditioning environments).

Tolerance Acquisition Phase

During the acquisition phase of the study, rats were exposed at 4-day intervals to a distinctive set of environmental cues, paired with a relatively low dose of ethanol. Interspersed between these drug trials were exposures to an alternate set of cues, paired with injections of saline. A reduced sensitivity (i.e. tolerance) to the hypothermic effects of ethanol developed during the acquisition phase, as evidenced by reliable changes in post-injection temperatures, temperature change scores, and alterations in the form of the time-effect curves following an injection of ethanol.

One measure of ethanol-induced hypothermia was the change from pre-injection to post-injection temperatures. These temperature change scores indicated that the magnitude of response to a fixed dose of ethanol decreased by 70 to 80% during the acquisition phase. Such adaptation is consistent with tolerance mediated by behavioral or physiological mechanisms, although the dosage regimen was unfavorable toward the latter types. One possibility is that the decrease observed in these difference scores was simply an artifact of the fall in pre-injection temperatures during the acquisition phase. This criticism may be tempered by a number of considerations.

First, post-injection temperatures by themselves showed a significant increase across acquisition trial blocks. Therefore the observed decreases in temperature change scores during the acquisition phase reflected both a decrease in pre-injection temperatures and an increase in post-injection temperatures.

Second, it is not clear that the change in pre-injection temperatures served only to exaggerate the magnitude of the tolerance observed; it is also possible that these baseline changes served to mask tolerance. It is possible for example, that the pre-trial temperature elevation produced by handling, transportation to conditioning environments, etc., may have initially competed with the hypothermia induced by the drug. During the acquisition phase this arousal-produced hyperthermia habituated somewhat, as evidenced by the decrease in pre-trial temperatures across trial blocks. Thus, arousal-induced hyperthermia may have partially obscured the development of tolerance, by reducing the initial hypothermia produced by the drug and allowing the drug to produce a greater effect during later trials.

A particularly interesting finding was the change in form of the time-effect curve during the course of the acquisition phase. While maximum hypothermia always occurred at 60 min following injections, not only did the fall in temperature from 0 to 60 min decrease across trials, but in addition, recovery from hypothermia in the last hour appeared to increase. Under other circumstances,

this finding might be interpreted as evidence that metabolic tolerance had developed (cf. Kalant et al. 1971). If the rate of metabolism of ethanol increased during the acquisition phase, one would expect a corresponding decrease in blood-ethanol levels during the post-injection period. Because blood-drug levels were not monitored, it is impossible to rule out this interpretation entirely. However, the lack of evidence for metabolic tolerance in the post-acquisition tolerance tests, and the low exposure level (i.e., a low drug dose administered at long intervals), argue against this interpretation.

One finding which complicates interpretation of acquisition phase results is the pattern of responding to saline injections. Saline injections also initially produced a mild hypothermia. (Previous pilot work has suggested that hypothermia following i.p. injections of room-temperature saline is to some extent volume dependent). During the course of the acquisition phase, this hypothermic response changed to one of hyperthermia, and the magnitude of response change over trial blocks was similar to that observed for ethanol trials. (Post-injection temperatures on saline acquisition trials also increased from trial blocks 2 to 5.) Group IV, receiving only saline throughout the acquisition phase, showed a similar change in responding.

A unitary account of the changes in the magnitude and form of responding observed on ethanol and saline trials (and in Group IV),

involves a classically conditioned response to cues which reliably predict hypothermia induced by either ethanol or saline injections. While this suggestion is consistent with Siegel's Pavlovian model of tolerance, an added assumption is that saline also serves as an effective, albeit weak, unconditioned stimulus, and the cues regularly preceding its effects come to elicit a compensatory hyperthermia. An additional possibility is that responding to the environment paired with ethanol generalized to the saline environment (although such a mechanism would not account for the changes observed in Group IV).

One observation that is consistent with this generalization hypothesis is that Group III showed less change from hypothermic to hyperthermic responding during saline acquisition trials than did Group I. For Group I, the cues attending handling and temperature measurement were common to both drug and saline environments; Group III however, was handled only in the saline environment. Group III therefore would be expected to show less generalization between ethanol-paired and saline-paired cues. Differences in total amount of handling, however, may also have contributed to this disparity.

Although ethanol initially reduced rod-hanging times (relative to saline responding), no tolerance to this effect was observed during the acquisition phase. There may have been several reasons why tolerance was observed to the hypothermic effects of ethanol but not to the motor effects of the drug. It may be that tolerance to the

thermic effects of the drug develops at a relatively faster rate. Along these lines, it has been suggested recently that measurement of changes in the thermic response to ethanol may be a more sensitive indicant of tolerance (Crabbe et al. 1979; Ritzmann & Tabakoff, 1976). LeBlanc et al. (1976) have reported that doses of 1.4 or 2.5 g/kg of ethanol administered at 4-day intervals resulted in no tolerance on a moving belt task, a finding consistent with the present data.

Another possibility is that motivational influences obscured changes in sensitivity to ethanol's effects (cf. Moskowitz & Wapner, 1964). According to this view, even though animals may have developed tolerance to the motor effects of the drug, the motivation for responding (staying on the bar) waned over trials as the fear of falling habituated. During later acquisition trials, some rats were observed to quickly pull up onto the bar, and jump off the other side--behavior which resulted in low rod-test scores, and might otherwise have been interpreted as reflecting motor debilitation.

Tolerance Tests

The experiment was designed so that several important comparisons could be made during the post-acquisition tolerance tests. Animals were challenged with ethanol on two occasions during the tolerance test series, once in the presence of cues previously

associated with the drug, and once in the presence of cues previously paired with saline injections. This manipulation was included as a specific test of a Pavlovian conditioning model of tolerance (Siegel, 1978b). A critical assumption inherent in the current tests was that tolerance due to metabolic or cellular mechanisms would be equivalent regardless of the cues present. (Because blood-drug levels were not measured, the possibility that this assumption was incorrect cannot be ruled out entirely.) In addition to this within-subjects comparison, tolerance displayed by the three differently handled acquisition groups was examined to determine if the level of activity during the drug exposure periods influenced the amount, or rate of development of tolerance (cf. Kalant et al. 1971). Lastly, the response of animals receiving drug throughout the acquisition phase (Groups I, II, and III) was compared to responding in Group IV, receiving ethanol for the first time. This comparison provided another between-subjects measure of the amount of tolerance displayed, and also gave some indication of the effects of repeated saline injections on the initial sensitivity to ethanol.

During the tolerance tests, rats evidenced a reduced sensitivity to the hypothermic effects of ethanol when the drug was administered in the presence of environmental cues which had been paired with the drug during the acquisition phase. When tested in the presence of saline cues, responding did not differ from that

of rats receiving the drug for the first time (Group IV; see figure 5). These findings are consistent with the environmental specificity predicted by the conditioning model of tolerance advanced by Siegel. According to this model, cues regularly preceding drug administration come to elicit a compensatory conditioned response. This conditioned response acquires strength with repeated cue-drug pairings and serves to render the organism tolerant when in the presence of the drug cues.

During the tolerance tests, the influence of a disparity in the ambient temperature in Environments A and B was seen in consistently higher body temperatures in the latter test environment. Had warmer body temperatures in the presence of drug cues been obtained only in B+ groups, the most parsimonious explanation would involve only reference to the ambient temperature differences. This was not the case, however. Regardless of the environment paired with the drug during the acquisition phase, animals showed greater resistance to the thermic effects of ethanol when in the presence of drug cues.

Although rod-test performance was different in the presence of drug and saline cues, these differences appeared largely due to an unexpected interaction between the environments used and rod-hanging performance. Throughout the study, rod-hanging times were consistently higher in Environment A than in Environment B. As the same apparatus was used in both environments, other factors such as

lighting, ambient temperature, or perhaps subtle handling differences presumably were responsible for this discrepancy.

An effect of varied levels of activity during the acquisition drug exposure periods was seen in the second tolerance test. While Groups I and II responded differentially to drug and saline cues, animals in Group III appeared relatively tolerant, regardless of the cues present. One factor that may have contributed to the relative tolerance shown by Group III rats in the presence of saline cues was their experience in the first tolerance test. In this test, Group III animals experienced handling cues paired with drug effects for the first time. As a result of this association, the handling cues present during the second tolerance test may have elicited some conditioned responding (i.e., hyperthermia) in the saline environment, thus rendering the animals tolerant.

There were also group differences in rod-hanging performances during the tolerance tests. Group III, which experienced rod-hanging tests only during saline acquisition trials had higher rod-scores than Group II, which had previously experienced these tests only under the influence of ethanol. Group I (tested after ethanol and saline injections), had intermediate scores. These results are not consistent with models based on state-dependent learning, or behavioral augmentation of tolerance, which would predict enhanced performance for animals which had the opportunity to practice responding or were more active during periods of drug

exposure. Recall, however, that rod-hanging scores did not improve during the acquisition phase. Because no tolerance to the drug's effect on motor capabilities was evidenced during this phase, it is doubtful that the group differences observed during the later tolerance tests were directly related to differential tolerance. During the tolerance tests, Group III experienced rod-hanging trials, under the influence of ethanol and in the drug environment for the first time. This combination of new stimulation and novel circumstances may have elevated rod-test scores by increasing emotionality, or by otherwise arousing the animals.

Conditioned Response Test

A key assumption of the Pavlovian conditioning model of tolerance is that reduced sensitivity to a drug's actions is mediated by the development of a conditioned response which counteracts the drug's effects. This conditioned response is theoretically observable when the organism is presented with all the features regularly paired with drug administration, except for the drug itself. In Pavlovian terms, this would be called a "CS-alone" trial. Applied to the current situation, the conditioning model of tolerance predicts that in the presence of ethanol cues, a hyperthermic response should be observed after an injection of saline, relative to responding in the presence of saline cues.

Some evidence for such a compensatory response was obtained during the conditioned response test (see Figure 7). Animals

receiving placebo (saline) injections in the presence of drug cues showed greater hyperthermia than those in the presence of saline cues. Although this difference was relatively small (0.5°C), its magnitude was sufficient to account for the disparity in tolerance observed in the presence of drug and saline cues in the earlier tolerance tests. Ambient temperature differences also influenced responding during this test. The pattern of results obtained is consistent with the notion that environmental temperature differences enhanced drug cue--saline cue differences in B+ groups, and diminished these differences in A+ groups. Increased hyperthermia in the presence of drug cues was observed early during the test trial in B+ groups, and only at the end of the two-hour test period in A+ groups, suggesting that the ambient temperature differences altered the time course of the hyperthermic response.

Responding in Group III however, did not support a conditioning interpretation. Recall that in the second tolerance test, this group appeared to be tolerant, regardless of the cues present. If this tolerance were mediated by a hyperthermic conditioned response, one would expect to see that response evidenced in the presence of both ethanol and saline cues. In the conditioned response test, levels of hyperthermia in Group III were relatively low, more like responding in Groups I and II to saline cues. Thus the relative tolerance displayed by Group III in the second tolerance test was not accompanied by evidence for conditioned hyperthermia in the

subsequent conditioned response test, and may well have been mediated by other mechanisms.

Badia and Defran (1970) have pointed out a potential methodological difficulty with the CS-alone technique for measuring isolated conditioned responses. They suggest that in addition to a conditioned response elicited by the CS, responding in such a situation may also reflect the contribution of an orienting (or "what is it?") response, elicited by omission of the otherwise regularly scheduled unconditioned stimulus.

While such an interpretation of the current findings cannot be ruled out entirely, a number of features of the present experiment argue against it. First, the temporal parameters of the current study are grossly different from those considered by Badia and Defran. The empirical basis for their model was the GSR (galvanic skin response) conditioning paradigm, employing discrete stimulus presentations, and relatively short (0.5 - 5.0 sec) CS - US intervals. In the present experiment, however, portions of the CS complex were presented some 5 to 10 minutes before injections, while the drug effects themselves did not begin for another several minutes. These effects would not have had an abrupt onset, but probably developed gradually with rising blood-drug levels. An additional consideration is that in early saline acquisition trials, saline injections appeared to cause hypothermia by themselves, and may have acted as weak unconditioned stimuli. If this were the

case, then the US was not omitted during the conditioned response tests, but merely presented in a weaker form. In summary, the conditions in the present experiment, and the conditions upon which the US-omission model are based, although superficially related, are considerably different. As a final consideration, this model does not provide a unified account of the responding observed during the acquisition phase, the tolerance tests, and the post-extinction tests, and in this respect is less parsimonious than the conditioning model of tolerance.

No clear patterns emerged from the rod-hanging trials during conditioned response tests, other than elevated scores in Environment A (relative to Environment B), and depressed responding in Group IIb. These differences are not well understood, but do not appear to be directly related to tolerance, or the mechanisms thereof.

Extinction Phase

During the extinction phase of the experiment the rats received one of two treatments. Half of each group experienced repeated saline injections in the presence of drug cues (extinction condition) and the other half received these saline injections in the presence of saline cues (control condition). For the extinction groups, this treatment represented nonreinforced presentations of the CS, conditions that should have served to diminish the magnitude of the conditioned response.

Throughout the extinction phase, the response to saline injections was hyperthermic, but the magnitude of this hyperthermia did not change significantly over trials. Evidence for an extinction effect was seen when responding in the conditioned response test was compared to responding in the last extinction trial. Because conditions in the conditioned response test (i.e., non-reinforced presentation of the CS) were similar to those in extinction trials, some diminution of responding may have resulted. In this comparison a significant reduction in the magnitude of the hyperthermic response was observed in extinction groups, but not for control animals or in Group IV.

One possible reason for the lack of a more pronounced effect of treatment during the extinction phase is that the effects of the saline injections served somehow to perseverate responding. Because these injections may have functioned as weak unconditioned stimuli, the extinction treatment may have constituted continued conditioning trials, with a weak US replacing a strong one. In a more conventional conditioning paradigm, replacement of a strong US with a weak one has been reported to maintain responding at relatively high levels (Baker, 1978).

There were no group differences in rod-test performance during the extinction phase, nor did rod scores change regularly over trials. In A+ groups, rod-hanging times fell off slightly during the middle extinction trial blocks, but then returned to their

original levels. No ready explanation for this deviation is at hand.

Post-extinction Tests

The post-extinction tests were included to assess the effects of extinction procedures on the retention of the tolerance developed during the acquisition phase. If tolerance were in fact due to a conditioned compensatory response, animals experiencing a treatment designed to diminish this response would be expected to show a corresponding loss of tolerance. For both post-extinction tests, all animals were returned to the environment originally paired with the drug, where they would be expected to be maximally tolerant. (Note that this manipulation differs from the earlier tolerance tests in which responding in the presence of drug vs saline cues was compared.) In the post-extinction tolerance test, rats were challenged with ethanol, and responding in groups which had received extinction or control treatments was compared. In the subsequent conditioned response test, the responses of these groups to injections of saline were compared in an effort to assess the relationship between tolerance and conditioned hyperthermic responding.

Extinction treatment did influence the level of tolerance displayed during the post-extinction tolerance test. While control groups showed little loss of tolerance following the extinction

phase, extinction groups showed increased sensitivity to ethanol, as evidenced by greater levels of hypothermia. Not all groups showed these extinction-control differences however. Group III differences, although in a similar direction, were not statistically reliable, and extinction-control differences in Group IIb were slightly, but not significantly reversed. An influence of extinction treatment on responding in the conditioned response test was seen only in Group IIa, where control animals showed a greater hyperthermic response than extinction groups.

In the conditioned response test, evidence of a greater hyperthermic response in control relative to experimental animals was seen in Group IIa, but not in Groups Ia and Ib, which had also showed differential responding during the tolerance test. Thus, the retention of tolerance seen following the extinction phase was not uniformly associated with higher levels of hyperthermia during the conditioned response test, an association which would have been predicted by the Pavlovian conditioning model. One interpretation of this discrepancy is that the relative tolerance observed in the post-extinction test was not mediated by conditioning mechanisms, and that the extinction treatment weakened tolerance, not by mitigating conditioned responding, but by some other mechanism.

It is also possible that learning influenced the sensitivity to ethanol, but that other factors combined to obscure these influences during the extinction phase and the subsequent tests.

One event which may have altered responding in the post-extinction conditioned response test was the tolerance test immediately preceding. Because this tolerance test was in effect, a conditioning trial, it is possible that conditioned responding to drug cues was rapidly reestablished, and thus evidenced by both extinction and control groups during the subsequent conditioned response test. Consistent with this suggestion is the observation that responding in most groups in the conditioned response test was hyperthermia of a similar magnitude.

The low dose of ethanol used in this study may have served as a relatively weak unconditioned stimulus, leading only to poor conditioning. In this regard, a study employing several dose levels (several US intensities) might provide further information. Higher dose levels would also provide for a greater discrepancy between the effects of the unconditioned stimulus and the effects of the "neutral" saline injections. Recall that there was some evidence that saline injections alone supported the development of hyperthermic responding. In an ideal Pavlovian discrimination paradigm, the CS- would not be paired with events having properties similar to the unconditioned stimulus.

General Conclusions

Many of the results obtained in this study can be subsumed by a model of drug tolerance involving conditioned responding to cues

regularly preceding drug effects. While such a model has been developed to account for morphine tolerance (Siegel, 1978a, 1978b), its general principles should be applicable to a variety of pharmacological agents. Despite the use of an administration regimen usually considered unfavorable for the development of physiologic or metabolic tolerance, reduced sensitivity to ethanol was observed to develop in the present experiment. This tolerance was shown to be environmentally specific, that is, animals were tolerant to the hypothermic effects of ethanol only when in the presence of cues previously paired with the drug. Moreover, this specificity appeared to be related to hyperthermia, evidenced when placebo injections were administered in the presence of drug cues. Subsequently, an extinction treatment designed specifically to weaken tolerance mediated by conditioning was found to be effective, but not for all groups in the study.

The persistence of tolerance developed during the acquisition phase is another feature supportive of a conditioning interpretation. By comparing the responding in control animals during the post-extinction tolerance test (Figure 9, open symbols) to the responding of rats in the drug environment in the first tolerance tests (Figure 5, open symbols) it may be seen that little loss of tolerance occurred during the 23-day extinction period. In Group I, in fact, responding appears unchanged. By contrast, LeBlanc et al. (1969, 1976) have reported complete loss of tolerance to ethanol

(assessed with a moving-belt task) in 12 to 17 days. While the retention of tolerance observed in the present study is consistent with a conditioning theory of tolerance, differential hyperthermic responding associated with this tolerance was obtained only for one group.

Several features of the present study, though experimentally disappointing, were still informative. The rod-hanging test turned out to be relatively insensitive, yielding little information about the development of tolerance. A number of features probably contributed to this insensitivity. As a first consideration, the task was probably too easy--any of a variety of behaviors sufficed to maintain the animal on the bar. In addition, motivational influences may have masked changes in responding due to altered drug sensitivity, and it was apparent that environmental conditions or subtle handling differences somehow influenced rod-test responding.

Measurement of body temperatures during the 60 min prior to injections was included for two reasons: It provided an indication of the stability of body temperatures prior to the conditioning periods, and theoretically might have provided evidence for anticipatory conditioned responding. The consistently observed pre-trial elevation in body temperatures revealed that this measure was quite sensitive to handling influences. Although this response habituated somewhat during the acquisition phase, it was still observable at the end of the experiment. Evidence for anticipatory

conditioned responding would have been obtained if pre-injection temperatures in the presence of drug cues were higher than in the presence of saline cues. No such evidence was obtained. It is possible, however, that the 10-min period between entering the conditioning environments and receiving the injections was too short to observe such an effect.

Post-injection temperatures (detailed in Appendix B), generally paralleled the patterns of responding seen in the temperature change scores, but were more sensitive to the ambient temperature disparity between Environments A and B. During the acquisition phase, post-injection temperatures generally increased over trials on both ethanol and saline days. In the tolerance tests and conditioned response test that followed the acquisition phase, differential responding to drug and saline cues was largely overshadowed by the influence of warmer ambient temperatures in Environment B, (see Figures A3 and A4). In the post-extinction tests, where extinction-control comparisons were not confounded with environmental temperature differences, post-injection temperatures showed patterns of responding quite similar to those revealed by the temperature change scores.

While body temperature change served as a more sensitive index of tolerance than rod-test performance, this measure too was influenced by handling and other factors. Merely handling the rats (e.g., transferring them to the conditioning environments), induced a temperature change which, although opposite in direction, was

similar in magnitude to the change induced by ethanol (at this dose level). This arousal-induced hyperthermia no doubt interacted with responding in ways not fully understood. That handling or amount of activity also interacted with thermic responding in the drug-paired environments was also apparent. Differential handling during the acquisition phase appears to have had an influence on the later display of tolerance. Animals that experienced low levels of stimulation and activity (i.e., Group III, which was not handled) during ethanol acquisition trials, generally failed to provide support for the conditioning model of tolerance, nor did their responding appear consistent with behavioral augmentation of tolerance. The exact nature of the interaction between activity and tolerance in the current situation remains unclear.

In the present study a group was included which experienced an equal amount of handling and activity in all situations. This design feature was included specifically to control for the type of interaction just discussed. Groups II and III (which were included in an attempt to assess the influence of differential stimulation on tolerance), are representative of many of the earlier behavioral tolerance studies. In some of these studies, (e.g., LeBlanc et al. 1973, 1976) this activity differential has been treated as an independent variable, and as such was carefully monitored. Unfortunately, in other studies handling differences have often been ignored while other comparisons were made. For example, in Siegel's (1978a)

demonstration of the environmental specificity of tolerance to the thermic effects of morphine, the critical comparison was between groups analogous to Groups II and III in the present study. Moreover, Siegel's manipulation may be likened to comparing Group II's responding in the drug environment with Group III's responding in the saline environment. In light of the current findings, such a comparison seems too susceptible to extraneous factors. It seems clear that regardless of the mechanisms thought to be involved in tolerance, in any attempt to measure this phenomenon, careful attention must be paid to experimental control of these influences.

Although evidence for a contribution of associative processes to drug tolerance is gradually increasing, the relationship between tolerance mediated by conditioning and tolerance mediated by cellular or metabolic changes, remains unclear. It is possible that "conditioned" tolerance is manifested only when low drug doses are administered, or perhaps under similar limiting circumstances, and thus is not importantly related to the way in which an organism adapts to recurrent drug exposure. At the opposite extreme is the possibility that such a conditioning mechanism contributes importantly to long term retention and enhanced redevelopment of drug tolerance. Tolerance mediated by learning should be a relatively enduring phenomenon, unless actively weakened with a specific extinction treatment. Some evidence for relatively persistent tolerance was obtained in the present study. In addition,

accelerated development of tolerance in organisms which have previously acquired and lost tolerance (cf. Kalant et al. 1971, 1978), suggests the presence of long-term residual effects of chronic drug exposure. These residual effects may well be related to the high incidence of relapse in previously addicted patients. Enhanced development of tolerance bears some resemblance to reacquisition of learned responses. If a learning mechanism is indeed involved in this phenomenon, further study may lead to improved treatment strategies designed specifically to counteract the conditioning that has been acquired.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to assess the possible contributions of behavioral augmentation of tolerance, and of Pavlovian conditioning, to tolerance developed to the physiological and behavioral effects of ethanol. During the tolerance acquisition phase of this study, rats were exposed at 4-day intervals to a distinctive set of environmental cues, paired with injections of ethanol (1.4 g/kg, i.p.). Interspersed between these drug trials were exposures to an alternate set of cues, paired with injections of saline. According to a recently advanced conditioning model of tolerance (Siegel, 1978b), such pairings should lead to the development of compensatory conditioned responses that render animals tolerant when in the presence of cues previously associated with the drug. As an additional manipulation, three groups experienced different levels of handling and stimulation during the tolerance acquisition phase, to determine if increased levels of activity during drug exposure periods would enhance the rate of development of tolerance (cf. Kalant et al. 1971). An additional group of rats received only saline injections during the acquisition phase.

Throughout the study, body temperatures and rod-hanging capabilities were monitored in an effort to assess the animals' sensitivities to ethanol. During the acquisition phase, tolerance to ethanol developed, as evidenced by decreases in the amount of

hypothermia induced by the drug, increases in post-injection body temperatures, and changes in the form of the time-effect curve reflecting more rapid recovery from hypothermia. No tolerance to the motor effects of ethanol was evidenced.

Following 14 pairs of saline and ethanol acquisition trials, all animals were challenged on two occasions with an injection of ethanol (1.4 g/kg), once in the presence of cues previously paired with the drug, and once in the presence of cues previously paired with saline. In these tolerance tests, rats were significantly less hypothermic (i.e., more tolerant) when tested in the presence of the drug cues than when tested in the presence of saline cues. Levels of hypothermia in rats injected with ethanol in the saline environment did not differ from those of animals receiving the drug for the first time. Moreover, in a subsequent conditioned response test, rats showed a greater hyperthermia when administered a placebo (saline) injection in the drug environment, than when tested in the saline environment.

Following the conditioned response test all rats received 12 saline injections, spaced at 2-day intervals. Half of the rats (extinction groups) in each group experienced these injections in the presence of cues previously associated with the drug, while the other half (control groups) received these injections in the presence of the saline cues. After this extinction phase all rats were returned to the environment originally paired with drug injections,

where they were tested first for sensitivity to ethanol, then subsequently for their response to placebo (saline) injections.

In these post-extinction tests, control animals from Groups I and II showed considerable retention of tolerance, despite a 3-week period during which no drug was administered. By contrast, animals which had experienced treatment designed specifically to weaken conditioned responding (i.e., the extinction groups), showed levels of tolerance comparable to those in the saline-only groups. While the conditioning theory predicted greater levels of hyperthermia in animals which were tolerant in the post-extinction test, this was observed for only one of the six subgroups.

Many of the present findings were consistent with the Pavlovian conditioning model of tolerance. During the tolerance acquisition phase, a reduced sensitivity to ethanol was displayed, even though the dosage regimen employed was not favorable toward the development of physiological or metabolic tolerance. Moreover, this tolerance was shown to be specific to the environmental cues previously paired with the drug, and associated with hyperthermic responding, revealed when saline was injected in the presence of these cues. While tolerance developed during the acquisition phase showed marked perseverance in control groups, extinction groups (which underwent treatment designed to weaken conditioned responding), showed increased sensitivity to the drug. In the subsequent conditioned response test, evidence for higher levels of hyperthermia in control groups

was relatively weak, a finding not consistent with the conditioning model. While many of the present findings were supportive of a conditioning model of tolerance, the measures employed were sensitive to handling, and other factors, pointing out the necessity for careful control of such influences.

REFERENCES

Adams, W.J., Yeh, S.Y., Woods, L.A., & Mitchell, C.L. Drug-test interaction as a factor in the development of tolerance to the analgesic effect of morphine. The Journal of Pharmacology and Therapeutics, 1969, 168, 251-257.

Badia, P., & Defran, R.H. Orienting responses and GSR conditioning; a dilemma. Psychological Review, 1970, 77, 171-181.

Baker, J.S. The bimodality and hysteresis properties of response mapping rules based on the cusp catastrophe. Unpublished doctoral dissertation, Northwestern University, 1978. (Cited in P.W. Frey & R.J. Sears, Model of conditioning incorporating the Rescorla-Wagner associative axiom, a dynamic attention process, and a catastrophe rule. Psychological Review, 1978, 85, 321-340.

Bliss, D.K. Theoretical explanations of drug-dissociated behaviors. Federation Proceedings, 1974, 33, 1787-1796.

Carder, B. Environmental influences on marihuana tolerance. In N.A. Krasnegor (Ed.), Behavioral tolerance: Research and implications. NIDA Research Monograph No. 18. Washington, D.C.: U.S. Government Printing Office, 1978.

Carlton, P.L., & Wolgin, D.L. Contingent tolerance to the anorexic effects of amphetamine. Physiology and Behavior, 1971, 7, 221-223.

Chen, C-S. A study of the alcohol effect and an introduction of a new behavioral technique. Psychopharmacologia, 1968, 12, 433-440.

Cicero, T.J. Tolerance to and physical dependence on alcohol: Behavioral and neurobiological mechanisms. In M.A. Lipton, A. DiMascio, & K.F. Killam (Eds.), Psychopharmacology: A generation of progress. New York: Raven Press, 1978.

Cochin, J. Some aspects of tolerance to the narcotic analgesics. In J.M. Singh, L. Miller, & H. Lei (Eds.), Drug addition 1: Experimental pharmacology. New York: Future Press, 1972.

Crabbe, J.C., Rigter, H., Uijlen, J., and Strijbos, C. Rapid development of tolerance to the hypothermic effect of ethanol in mice. The Journal of Pharmacology and Experimental Therapeutics, (In press).

Crow, L.T., & Higbee, M.W. Behavioral augmentation of tolerance to alcohol and the response measure. Bulletin of the Psychonomic Society, 1977, 10, 5-8.

Ferraro, D.P. A behavioral model of marihuana tolerance. In M.C. Braude and S. Szara (Eds.), Pharmacology of marihuana. New York: Raven Press, 1976.

Ferraro, D.P. Behavioral tolerance to marihuana. In N.A. Krasnegor (Ed.), Behavioral tolerance: Research and implications. NIDA Research Monograph No. 18. Washington, D.C.: U.S. Government Printing Office, 1978.

Freund, G. Hypothermia after acute ethanol and benzyl alcohol administration. Life Sciences 1973, 13, 345-359.

Goldstein, A., Aronow, L., & Kalman, S.M. Principles of drug action: The basis of pharmacology (2nd ed.). New York: John Wiley & Sons, 1974.

Hayes, R.L., & Mayer, D.J. Morphine tolerance: Is there evidence for a conditioning model? Science, 1978, 200, 343-344.

Hill, M.W., & Bangham, A.D. General depressant drug dependency: A biophysical hypothesis. In M.M. Gross (Ed.), Alcohol intoxication and withdrawal: Experimental Studies II. New York: Plenum Press, 1975.

Hug, C.C. Characteristics and theories related to acute and chronic tolerance development. In S.J. Mule & H. Brill (Eds.), Chemical and biological aspects of drug dependence. Cleveland: CRC Press, 1972.

Kalant, H. Comparative aspects of tolerance to, and dependence on, alcohol, barbiturates and opiates. In M.M. Gross (Ed.), Alcohol intoxication and withdrawal (Vol. 3). New York: Plenum Press, 1977.

Kalant, H., LeBlanc, A.E., & Gibbins, R.J. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacological Reviews, 1971, 23, 135-191.

Kalant, H., LeBlanc, A.E., Gibbins, R.J., & Wilson, A. Accelerated development of tolerance during repeated cycles of ethanol exposure. Psychopharmacology, 1978, 60, 59-65.

LeBlanc, A.E., & Cappell, H.D. Historical antecedents as determinants of tolerance to and dependence upon psychoactive drugs. In H.D. Cappell and A.E. LeBlanc (Eds.), Biological and behavioral approaches to drug dependence. Toronto: Addiction Research Foundation, 1975.

LeBlanc, A.E., & Cappell, H.D. Tolerance as adaptation: Interactions with behavior and parallels to other adaptive processes. In K. Blum (Ed.), Alcohol and opiates: Neurochemical and behavioral mechanisms. New York: Academic Press, 1977.

LeBlanc, A.E., Gibbins, R.J., & Kalant, H. Behavioral augmentation of tolerance to ethanol in the rat. Psychopharmacologia, 1973, 30, 117-122.

LeBlanc, A.E., Gibbins, R.J., & Kalant, H. Generalization of behaviorally augmented tolerance to ethanol, and its relation to physical dependence. Psychopharmacologia, 1975, 44, 241-246.

LeBlanc, A.E., Kalant, H., & Gibbins, R.J. Acquisition and loss of behaviorally augmented tolerance to ethanol in the rat. Psychopharmacology, 1976, 48, 153-158.

LeBlanc, A.E., Kalant, H., Gibbins, R.J., & Berman, M.D. Acquisition and loss of tolerance to ethanol by the rat. The Journal of Pharmacology and Experimental Therapeutics, 1969, 168, 244-250.

LeBlanc, A.E., Poulos, C.X., & Cappell, H.D. Tolerance as a behavioral phenomenon: Evidence from two experimental paradigms. In N.A. Krasnegor (Ed.), Behavioral tolerance: Research and implications, NIDA Research Monograph No. 18. Washington, D.C.: U.S. Government Printing Office, 1978.

Lewis, R.E., Kunz, A.L., & Bell, R.E. Error of intraperitoneal injections in rats. Laboratory Animal Care, 1966, 16, 505-509.

Linakis, J.G., & Cunningham, C.L. Effects of concentration of ethanol injected intraperitoneally on taste aversion, body temperature, and activity. Psychopharmacology, (In press).

Linton, M., & Gallo, P.S., Jr. The practical statistician: Simplified handbook of statistics. Monterey, California: Wadsworth Publishing Co., 1975.

Martin, W.R. In A. Wikler (Ed.), Addictive states. Baltimore: Williams & Wilkins, 1968.

Moskowitz, H., & Wapner, M. Studies on the acquisition of behavioral tolerance to alcohol. Quarterly Journal of Studies on Alcohol, 1964, 25, 619-624.

Overton, D.A. State dependent learning produced by alcohol and its relevance to alcoholism. In B. Kissin, & H. Begleiter (Eds.), The biology of alcoholism (Vol. 2). New York: Plenum Press, 1972.

Overton, D.A. Major theories of state dependent learning. In B.T. Ho, D.W. Richards, III, & D.L. Chute (Eds.), Drug discrimination and state dependent learning. New York: Academic Press, 1978.

Pavlov, I.P. Conditioned reflexes. (Translated and edited by G.V. Anrep) New York: Dover Publications, 1927.

Ritchie, J.M. The aliphatic alcohols. In L.S. Goodman, & A. Gilman (Eds.), The pharmacological basis of therapeutics (5th Ed.). New York: Macmillan Publishing Co., 1975.

Ritzmann, R.F., & Tabakoff, B. Body temperature in mice: A quantitative measure of alcohol tolerance and dependence. The Journal of Pharmacology and Experimental Therapeutics, 1976, 199, 158-170.

Schuster, C.R. Theoretical basis of behavioral tolerance: Implications of the phenomena for problems of drug abuse. In N.A. Krasnegor (Ed.), Behavioral tolerance: Research and implications. NIDA Research Monograph No. 18. Washington, D.C.: U.S. Government Printing Office, 1978.

Schuster, C.R., Dockens, W.J., & Woods, J.H. Behavioral variables affecting the development of amphetamine tolerance. Psychopharmacologia, 1966, 9, 170-182.

Schuster, C.R., & Zimmerman, J. Timing behavior during prolonged treatment with dl-amphetamine. Journal of Experimental Analysis Behavior, 1961, 4, 327-330.

Siegel, S. Evidence from rats that morphine tolerance is a learned response. Journal of Comparative and Physiological Psychology, 1975, 89, 498-506.

Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. Science, 1976, 193, 323-325.

Siegel, S. Morphine tolerance acquisition as an associative process. Journal of Experimental Psychology: Animal Behavior Processes, 1977, 3, 1-13.

Siegel, S. Tolerance to the hyperthermic effect of morphine in the rat is a learned response. Journal of Comparative and Physiological Psychology, 1978a, 92, 1137-1149.

Siegel, S. A Pavlovian conditioning analysis of morphine tolerance. In W.A. Krasnegor (Ed.), Behavioral tolerance: Research and implications. NIDA Research Monograph No. 18. Washington, D.C.: U.S. Government Printing Office, 1978b.

Siegel, S., Hinson, R.E., & Krank, M.D. The role of predrug signals in morphine analgesic tolerance: Support for a Pavlovian conditioning model of tolerance. Journal of Experimental Psychology: Animal Behavior Processes, 1978, 4, 188-196.

Takemori, A.E. Neurochemical basis for narcotic tolerance and dependence. Biochemical Pharmacology, 1974, 24, 2121-2126.

Tang, M., & Falk, J.L. Behavioral and pharmacological components of phenobarbital tolerance. In N.A. Krasnegor (Ed.), Behavioral tolerance: Research and implications. NIDA Research Monograph No. 18. Washington, D.C.: U.S. Government Printing Office, 1978.

Wallgren, H., Nikander, P., & Virtanen, P. Ethanol-induced changes

in cation-stimulated adenosine triphosphatase activity and lipid-proteolipid labeling of brain microsomes. In M.M. Gross (Ed.), Alcohol intoxication and withdrawal: Experimental studies II. New York: Plenum Press, 1975.

Winer, B.J. Statistical principles in experimental design (2nd ed.). New York: McGraw-Hill, 1971.

APPENDIX A

BODY WEIGHTS AND CONDITIONING ENVIRONMENT TEMPERATURES

Table A1. Environmental temperatures during the experiment. All temperatures are expressed in degrees Celsius, and were measured 1 h following injections. Each number is a mean of measurements on two consecutive days. Temperatures prior to acquisition trial 11 were not recorded. (-) or (+) following a trial number refers respectively to a saline or ethanol acquisition trial.

		AM TRIALS		PM TRIALS	
TRIAL	Environment:	A	B	A	B
11-	(Acquisition Trials)	21.1	24.5	22.5	24.8
11+		21.8	24.8	22.9	24.6
12-		21.2	23.9	22.3	24.6
12+		21.2	23.9	22.3	24.2
13-		20.5	24.1	21.1	24.5
13+		20.6	23.7	22.8	23.8
14-		20.0	24.0	22.4	24.6
14+		20.2	25.0	21.4	24.4
T1	(Tolerance Test 1)	22.0	25.1	22.2	25.5
15-		21.1	24.9	21.6	24.8
15+		21.1	24.2	21.6	24.6
T2	(Tolerance Test 2)	21.7	24.1	21.8	24.4
16-		20.8	23.8	21.5	24.2
16+		20.6	23.4	21.8	23.9
CR	(CR-test)	21.2	24.4	21.2	24.8
EX 1		20.8	23.7	21.6	23.7
EX 2		20.8	23.0	21.2	23.5
EX 3		20.5	23.3	20.7	23.4
EX 4		21.2	24.0	21.5	24.6
EX 5		20.6	24.1	21.5	24.2
EX 6	(Extinction Trials)	20.9	24.1	21.5	24.2
EX 7		20.9	23.8	22.4	24.2
EX 8		21.3	24.2	22.0	24.4
EX 9		21.8	23.8	23.1	24.0
EX 10		20.9	22.7	23.0	23.6
EX 11		21.8	23.7	23.1	24.2
EX12		21.9	23.2	23.1	23.5
P-T (Post-ext. Tolerance Test)		21.8	24.3	22.7	24.7
P-CR (Post-ext. CR-Test		20.8	23.8	21.7	24.3

Table A2. Body weights during the acquisition phase, tolerance tests, and conditioned response test. Each number represents a mean for eight, or four rats, for Groups I, II, and III, and Group IV, respectively. All weights are expressed to the nearest gram.

GROUP	ACQUISITION TRIAL BLOCKS							Tolerance Tests		CR Test
	1	2	3	4	5	6	7			
Ia	257	266	275	282	290	295	301	296	300	315
IIa	252	266	274	280	284	289	295	291	293	294
IIIa	258	279	282	289	295	301	309	304	306	304
IVa	260	275	283	288	294	302	307	305	307	305
Ib	252	263	270	276	282	288	291	289	289	292
IIb	261	274	279	281	289	296	302	298	302	307
IIIb	257	271	278	284	289	295	300	300	302	303
IVb	255	275	281	283	292	302	307	306	308	312

Table A3. Body weights during the extinction phase and post-extinction tests. Each number represents a mean for eight, or four rats, for Groups I, II, III, and Group IV, respectively. All weights are expressed to the nearest gram.

GROUP	EXTINCTION TRIAL BLOCKS						Post-extinction Tests	
	1	2	3	4	5	6	Tol.	CR
I extinction	298	300	300	312	315	320	322	323
I control	300	301	302	306	310	315	318	316
II extinction	298	302	303	306	307	316	317	316
II control	305	310	310	319	320	326	322	322
III extinction	307	310	312	316	319	325	328	331
III control	300	304	305	312	312	318	322	331
IVa	308	310	309	315	316	320	327	325
IVb	314	315	317	323	325	331	335	335

APPENDIX B

POST-INJECTION TEMPERATURES

APPENDIX B

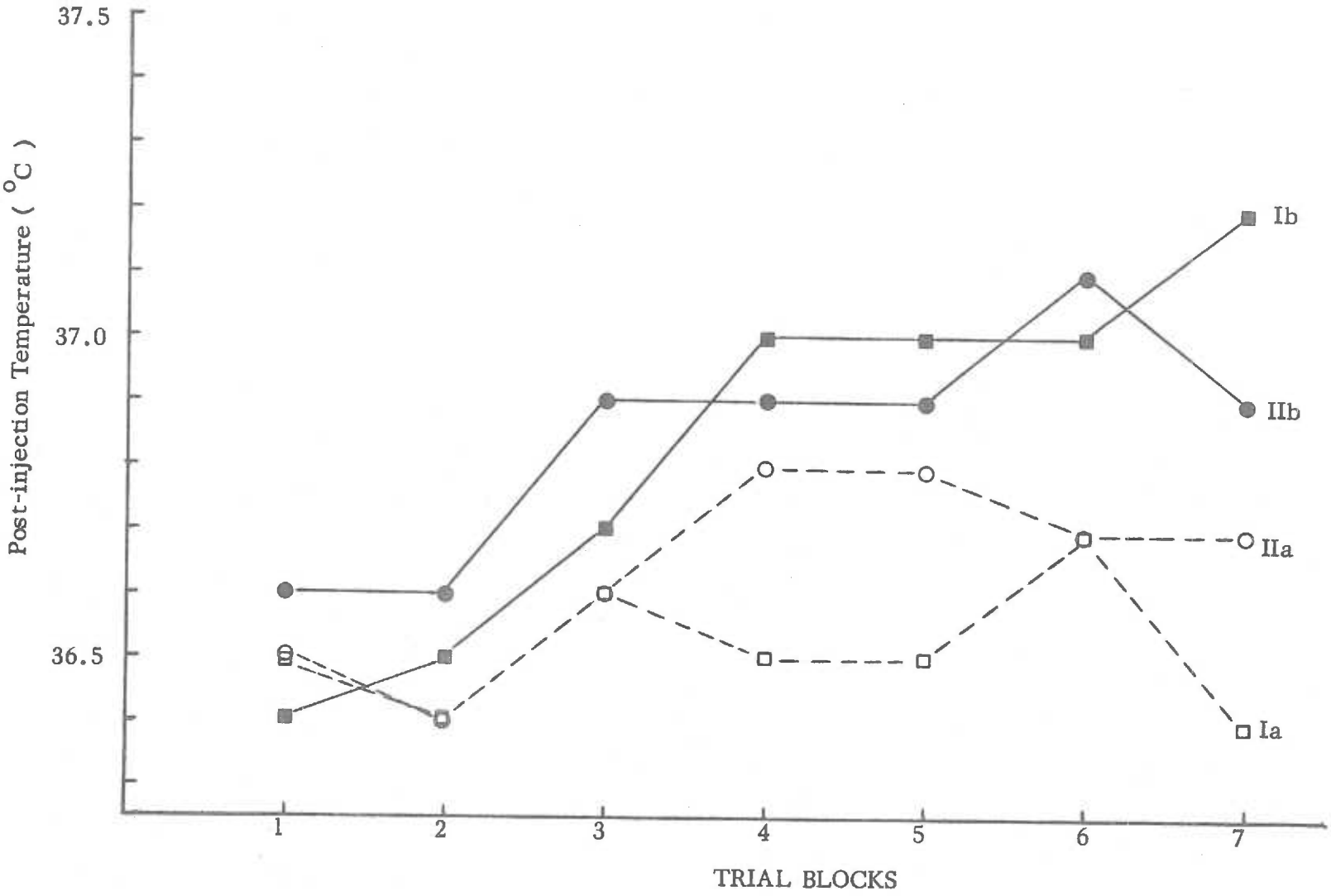
Post-injection Temperatures

Acquisition Phase. Post-injection body temperatures during ethanol acquisition trials are shown in Figure A1, where each point represents the mean body temperature during the 135-min trial period. It may be seen in this figure that post-injection temperatures generally increased across trial blocks, and that the increase was more pronounced for Groups Ib and IIb, which received ethanol injections in Environment B. These observations were supported statistically by a significant trial blocks effect, F (6, 168) = 14.90, $p < .001$, and a significant room assignment x trial blocks interaction, F (6, 168) = 3.82, $p < .05$.

While the increase in temperatures over trial blocks was similar for Groups IIa and IIb, Group Ib showed a much greater increase than Ia, giving rise to a significant room assignment x group x trial block interaction, F (6, 168) = 3.35, $p < .01$.

Post-injection temperatures varied significantly as a function of time, F (3, 84) = 3.10, $p < .001$; with a maximum hypothermia occurring 60 min following injections, and followed by some degree of recovery. This recovery was more pronounced in groups in Environment B, an observation supported by a significant room assignment x time interaction (F (3, 84) p 2.76, $p < .05$. During ethanol acquisition trials, body temperatures were generally slightly

Figure A1. Post-injection body temperatures during ethanol acquisition trials. The data are graphed collapsed across the time after injection factor, and each point represents a mean score for eight animals.


37.5
37.0
36.5
Post-injection Temperature (°C)
Ib
IIb
IIa
Ia
1
2
3
4
5
6
7
TRIAL BLOCKS

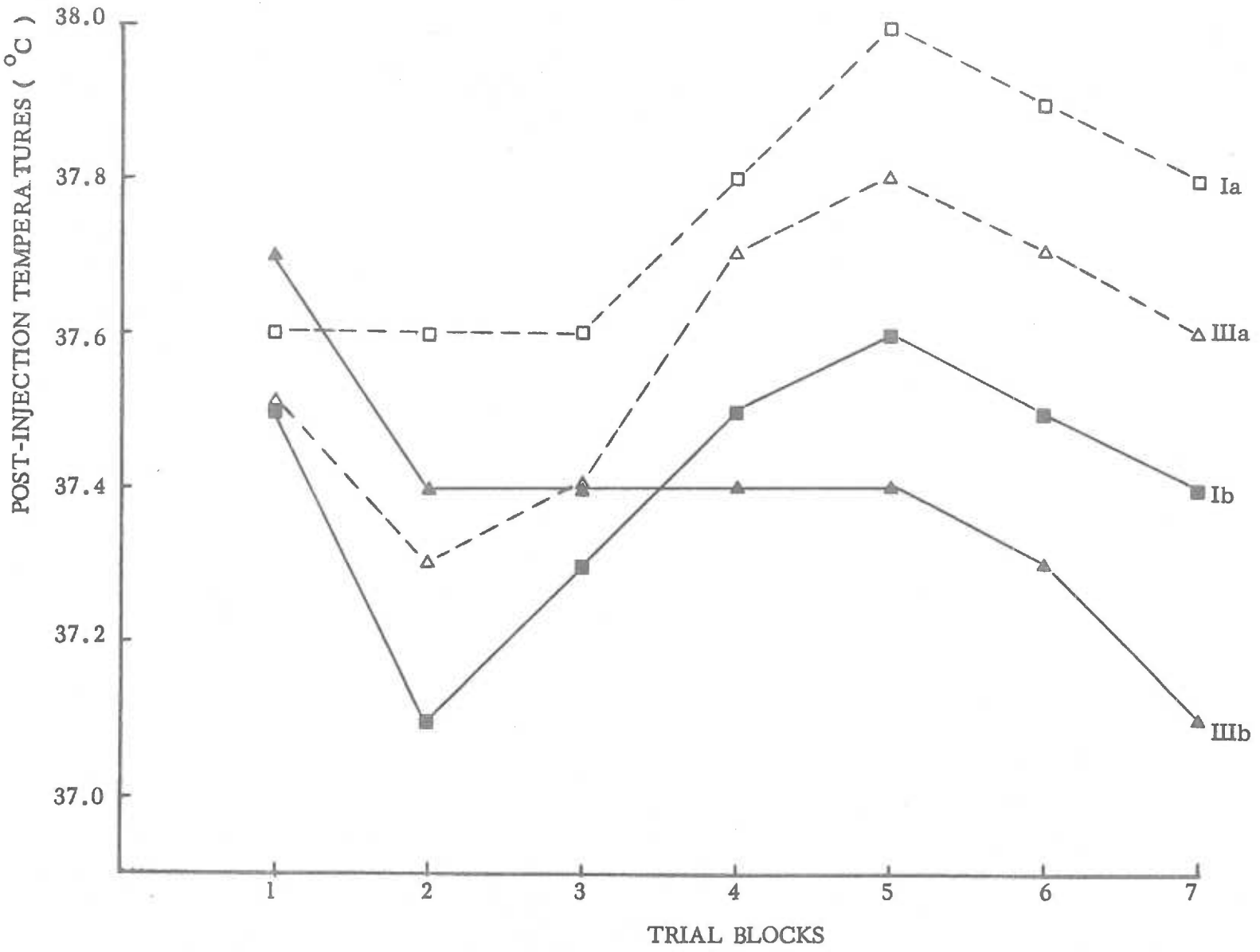
higher in Environment B than A, F (1, 28) = 5.50, $p < .05$.

Post-injection temperatures on saline acquisition trials are shown in Figure A2. To simplify exposition, the data have been plotted collapsed over the time after injection factor. It can be seen in Figure A2 that the variation in temperature over trial blocks was small in magnitude, but fairly regular for all groups. Post-injection temperatures following saline injections fell from Blocks 1-2, rose again, then fell off in Blocks 5-7. Post-injection temperatures were generally higher in Environment B than in Environment A, and this difference was greater in later trial blocks. These observations were supported by a significant main effect of blocks, F (6, 168) = 4.94, $p < .001$, and room assignment, F (1, 28) = 5.32, $p < .05$, and a room assignment x blocks interaction, F (6, 168) = 4.10, $p < .001$.

Body temperatures varied during the two hour post-injection period, F (3, 84) = 5.34, $p < .005$. In early trial blocks, temperatures continued to fall until 60 min after injections, while in later blocks, body temperatures increased from 30 min onward, giving rise to a significant time x block interaction, F (18, 504) = 1.78, $p < .05$.

In the saline only groups (IVa & IVb) post-injection temperatures were always higher in Environment B than A, and this difference was more pronounced in later acquisition trial blocks. Significant room assignment x environment, F (1, 6) = 108.40,

Figure A2. Post-injection body temperatures during saline acquisition trials. The data are graphed across the time after injection factor, and each point represents a mean score for eight animals.

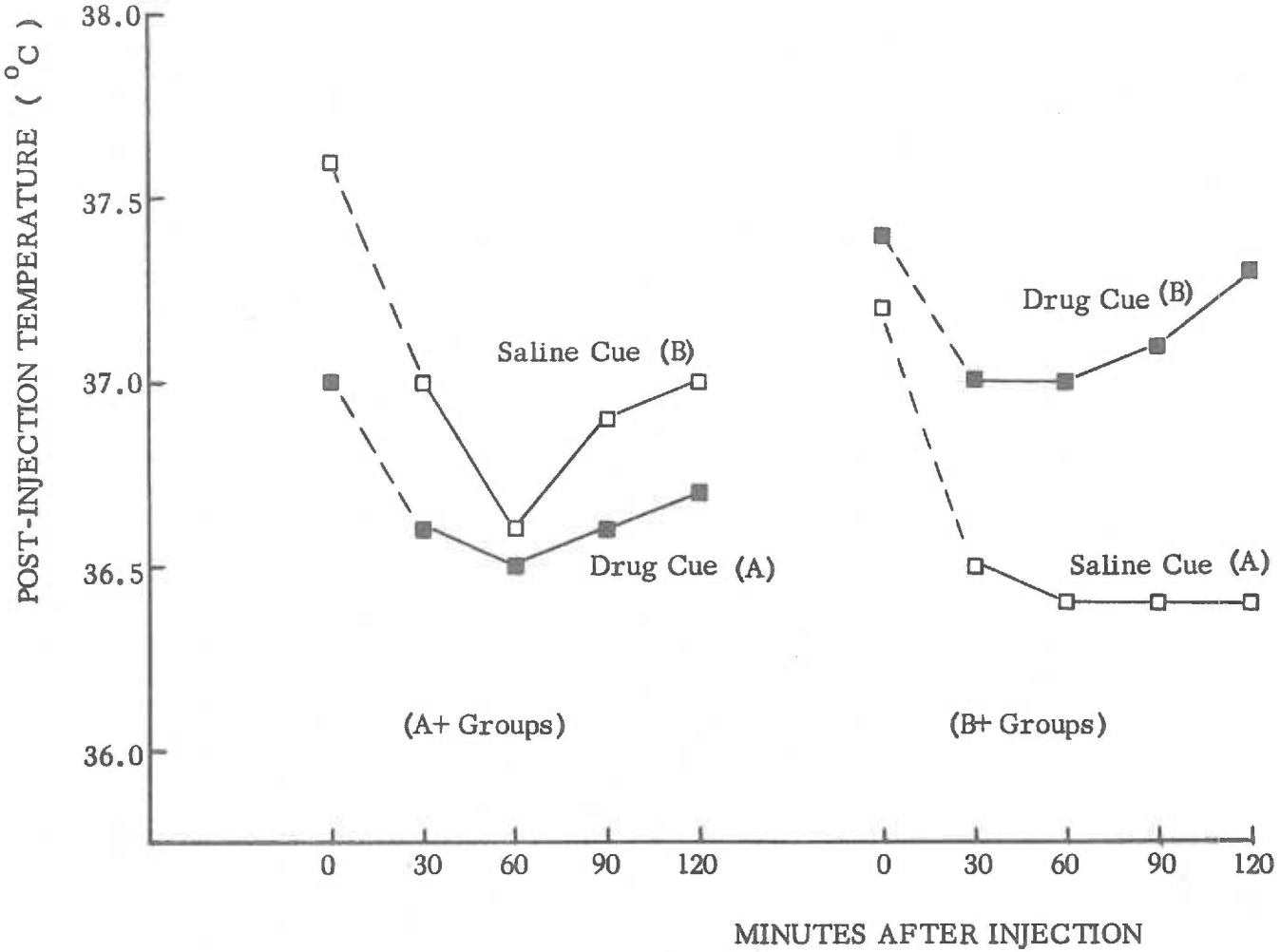

POST-INJECTION TEMPERATURES (°C)
38.0
37.8
37.6
37.4
37.2
37.0
1
2
3
4
5
6
7
TRIAL BLOCKS
Ia
IIIa
Ib
IIIb

$p < .001$, and room assignment x environment x blocks, F (6, 36) = 6.13, $p < .001$, interactions confirmed these observations. Post-injection temperatures in Group IV varied as a function of time after injection. This effect differed over trial blocks in an irregular manner, however. Following injections, temperatures tended to fall on Trial Blocks 2 & 3, remain unchanged on Blocks 1 & 6, and rise on Blocks 4, 5, & 7. These variations yielded a significant time x blocks interaction, F (18, 108) = 2.14, $p < .05$.

Tolerance Test

Post-injection temperatures during the tolerance tests are shown in Figure A3, (collapsed across the three acquisition groups). Pre-injection temperatures (not included in the statistical analysis) are included for comparison purposes, connected with dashed lines. From this figure, it may be discerned that A+ groups in the presence of saline cues (B), and B+ groups in the presence of drug cues (B) had higher post-injection temperatures, regardless of the cue condition this environment represented, yielding a significant main effect of cue, F (1, 39) = 7.69, $p < .01$, and a significant room assignment x cue interaction, F (1, 39) = 47.57, $p < .001$. Note that when pre-injection temperatures are considered, it becomes apparent that, regardless of room assignment, hypothermia produced by an injection of ethanol was always greater in the presence of saline cues than in the presence of drug cues, a finding reflected in the temperature change scores detailed in the

Figure A3. Post-injection body temperatures during the tolerance tests. The response to ethanol injections in the presence of drug cues and saline cues is represented by solid and open symbols, respectively. The data are plotted collapsed across the three acquisition groups, and each point represents a mean score for 24 animals. Pre-injection temperatures are shown connected with dashed lines.


POST-INJECTION TEMPERATURE (°C)
38.0
37.5
37.0
36.5
36.0
Saline Cue (B)
Drug Cue (A)
Drug Cue (B)
Saline Cue (A)
(A+ Groups)
(B+ Groups)
0
30
60
90
120
0
30
60
90
120
MINUTES AFTER INJECTION

results section.

Post-injection temperatures varied significantly over time, F (3, 117) = 15.09, $p < .001$, and an increasing drug cue--saline cue difference in B+ groups was reflected in a significant room assignment x cue x time interaction, F (3, 117) = 4.51, $p < .01$. No differences between the differently handled acquisition groups were obtained.

Conditioned Response Test

Post-injection temperatures during the conditioned response test (collapsed across Groups I, II, & III) are shown in Figure A4. Again, temperatures were always higher in Environment B than A, giving rise to significant room assignment x cue interaction, F (1, 32) = 27.83, $p < .001$. Post-injection temperatures changed significantly during the 2-h test period, F (3, 96) = 12.61, $p < .001$. While responding in Environment B (top lines in Figure A4) was similar for drug cue and saline cue conditions, drug cue groups in Environment A showed a linear increase in temperature, while saline cue groups in this environment changed little over time. This pattern of results yielded significant room assignment x time, F (3, 96) = 7.67, $p < .001$, and room assignment x cue x time, F (3, 96) = 10.75, $p < .001$, interactions. A significant room assignment x group x time interaction, F (6, 96) = 2.86, $p < .05$, was analyzed graphically. Group Ib had higher temperatures than Ia, while IIIa had higher temperatures than IIb, and

Figure A4. Post-injection temperatures during the conditioned response test. The response to saline injections in the presence of drug cues and saline cues is represented by solid and open symbols, respectively. The data are plotted collapsed across the three acquisition groups, and each point represents a mean score for 12 animals. Pre-injection temperatures are shown connected with dashed lines.

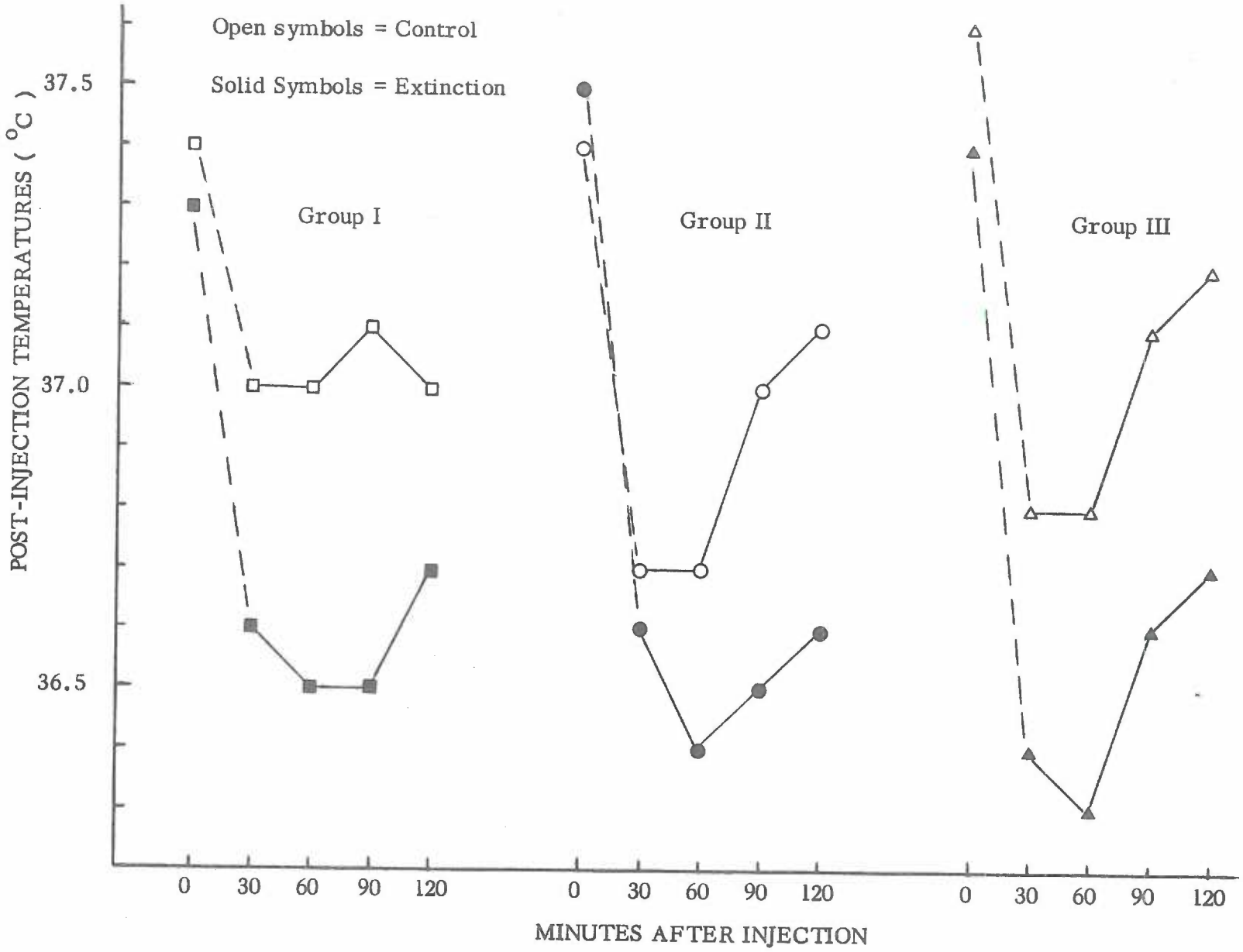

Open symbols = Control
Solid Symbols = Extinction
POST-INJECTION TEMPERATURES (°C)
37.5
37.0
36.5
Group I
Group II
Group III
0
30
60
90
120
MINUTES AFTER INJECTION

Figure A5. Post-injection body temperatures during the post-extinction tolerance test. Open symbols represent body temperatures in control groups following an injection of ethanol, while solid symbols represent responding in extinction groups. The data are plotted collapsed across room assignment condition, with each point representing a mean score for 8 animals (except where subject attrition led to a reduced group size).

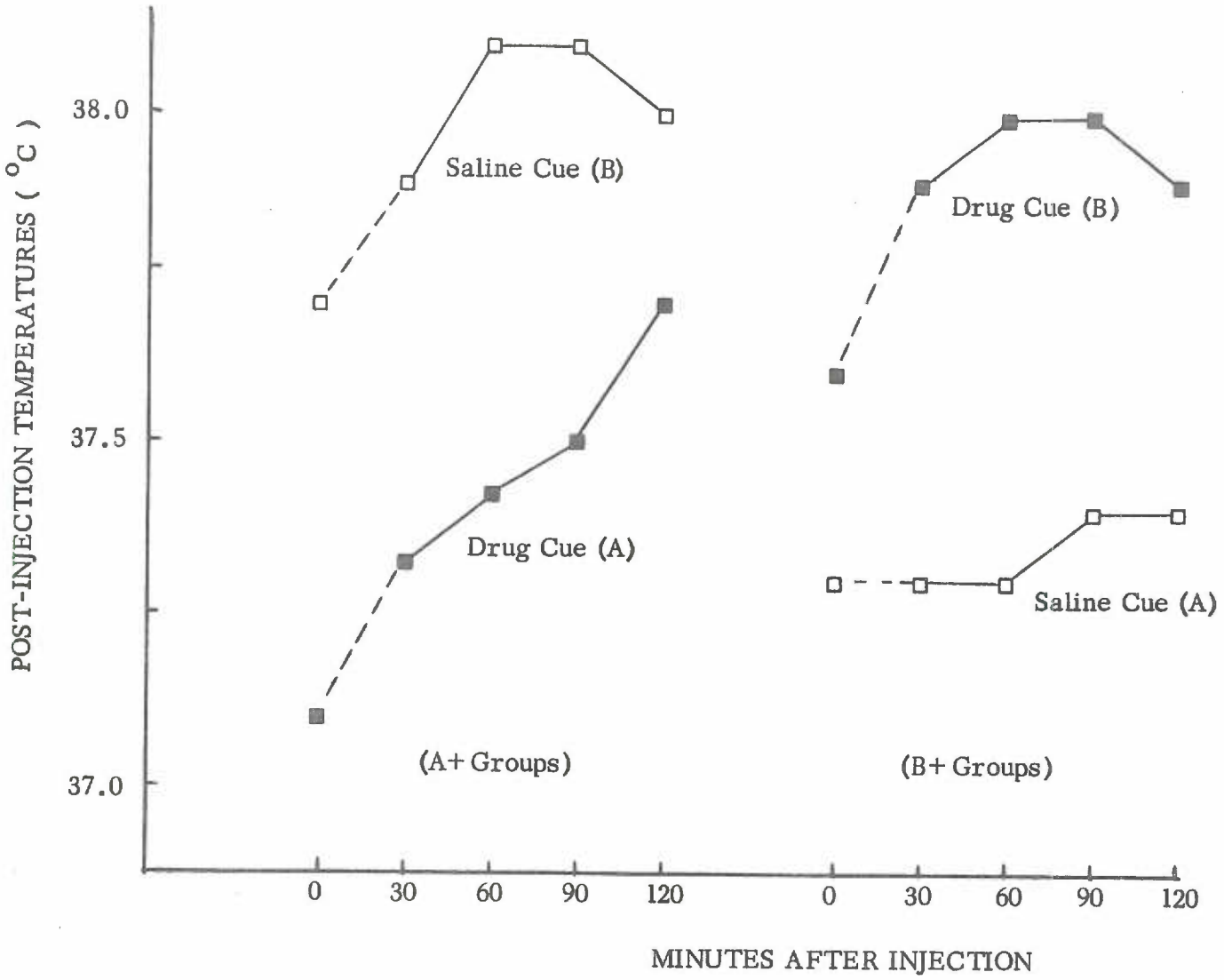
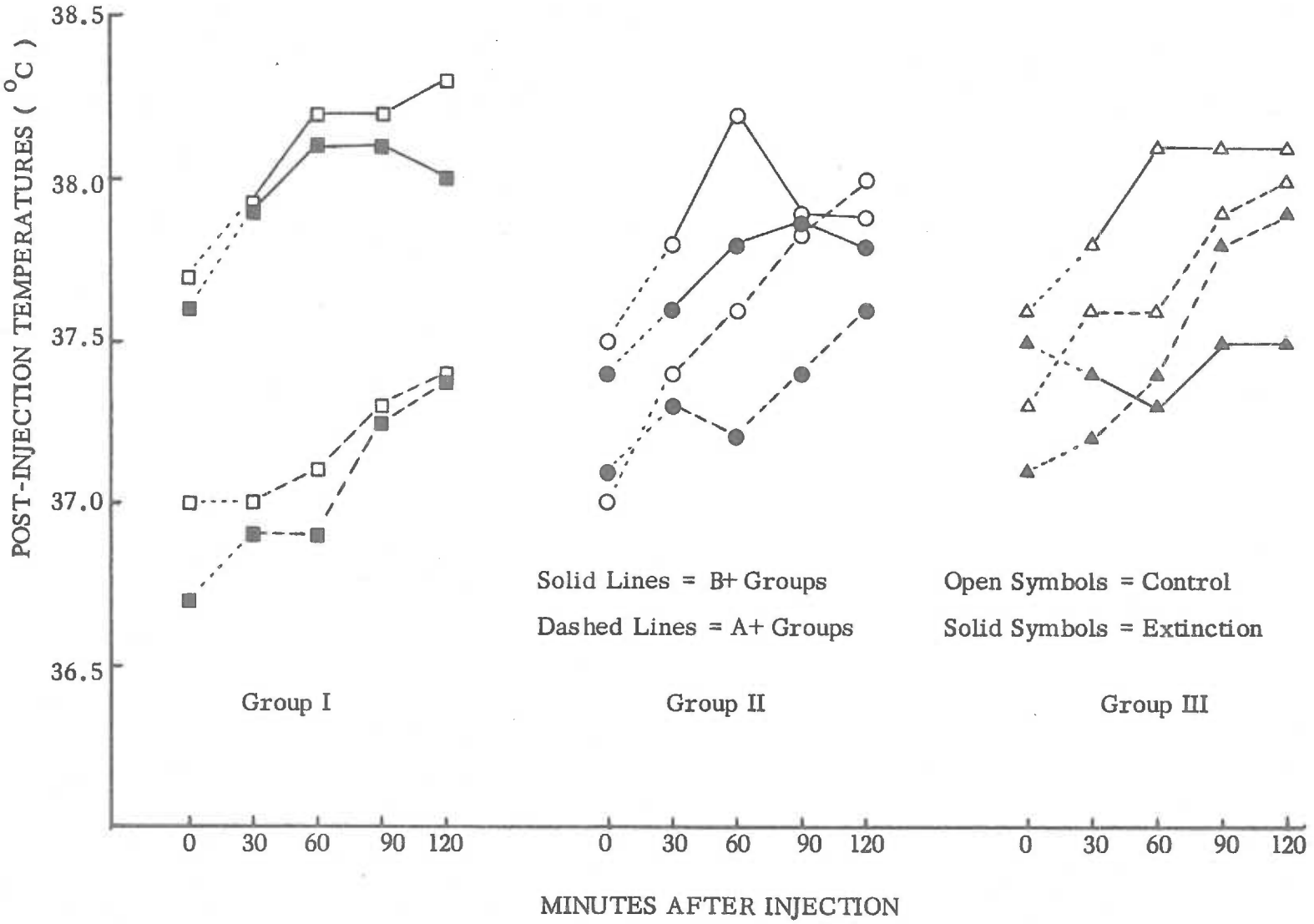

POST-INJECTION TEMPERATURES (°C)
38.0
37.5
37.0
Saline Cue (B)
Drug Cue (A)
(A+ Groups)
Drug Cue (B)
Saline Cue (A)
(B+ Groups)
0
30
60
90
120
0
30
60
90
120
MINUTES AFTER INJECTION

Figure A6. Post-injection body temperatures during the post-extinction conditioned response test. The data are plotted separately for each acquisition group in each room assignment condition, with each point representing a mean score for four animals (except where subject loss led to a reduced group size). Open and solid symbols represent the responses of control and extinction groups to an injection of saline in the drug environment. Points connected with solid lines depict responding in B+ groups, while points connected with dashed lines depict responding in A+ groups. Pre-injection temperatures are shown, connected with dotted lines.


POST-INJECTION TEMPERATURES (°C)
38.5
38.0
37.5
37.0
36.5
Solid Lines = B+ Groups
Dashed Lines = A+ Groups
Open Symbols = Control
Solid Symbols = Extinction
Group I
Group II
Group III
0 30 60 90 120
0 30 60 90 120
0 30 60 90 120
MINUTES AFTER INJECTION

analyzed by computing separate three-way analyses of variance for each room assignment condition. In these analyses the error term from the overall analysis was used to compute between-subjects *F* ratios. For A+ groups, a significant extinction treatment x group x time interaction, with follow-up two-way analyses indicated that only for Group IIa (center panel, dashed lines), at later time intervals, were extinction--control differences reliable. Group IIIb extinction--control differences, although apparently of the same magnitude, were not statistically reliable.