EFFECTS OF ETHANOL ON THE DEVELOPMENT OF CLASSICALLY CONDITIONED HEART RATE IN THE RAT

by

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INTRODUCTION

Although the debilitating effects of ethanol on general behavior are well known, very little information is available concerning the possible influence of ethanol on processes involved in learning and memory. In many experiments primary interest has been on the magnitude of ethanol induced changes in baseline skeletal activity and in physiological reactions such as respiration, heart rate, blood pressure, and the galvanic skin response. Moreover, a considerable number of studies have been concerned with the influence of ethanol on motor reaction times and on visual, auditory, and pain thresholds. In general, these investigations have revealed that the changes in physiological and behavioral responses produced by ethanol are dose dependent. For example, at low doses, ethanol may function as a general stimulant and increase the level of both autonomic and gross-motor activity. At high doses, ethanol functions as an anesthetic and significantly depresses physiological and behavioral measures.

Currently, ethanol concentrations are commonly specified in grams or cubic centimeters of ethanol per kilogram of subject weight (gm/kg). Circulating levels of ethanol in the blood are expressed in terms of the number of milligrams of absolute ethanol per 100 milliliters of plasma (mg/100 ml).

The range of ethanol doses which has been used in behavioral experiments has varied from approximately .25 gm/kg to about 10 gm/kg. Although it is impossible to generalize across species and response

measures within species, the following approximate categories are presented to facilitate comparisons between studies. Doses from .25 gm/kg to .8 gm/kg which produce peak blood ethanol levels of approximately 75 mg/100 ml are considered low, doses from .8 gm/kg to 1.6 gm/kg which produce peak blood ethanol levels of approximately 140 mg/100 ml are considered intermediate, and doses from 1.6 gm/kg to 10.0 gm/kg which produce peak blood ethanol levels of over 140 mg/100 ml are considered high.

Prior to the last decade, the amount of research in behavioral pharmacology was relatively small. During the past 10 years there has been a dramatic increase in the search for experimental designs and response measures which are sensitive and specific indicators of ethanol effects. Classical conditioning and instrumental training procedures have only come into systematic use within the past several years with the number of experiments employing instrumental procedures far outnumbering those that have used classical conditioning paradigms. Furthermore, very few of the classical conditioning studies have been concerned with autonomic nervous system responses. Broadly viewed, the objective of the present experiment was to provide new information regarding the actions of ethanol on classically conditioned heart rate.

The review of the literature which is presented below focuses upon three basic types of experimental paradigms which have been employed to investigate the influence of ethanol on the classical conditioning process. The first type used the traditional Pavlovian technique of pairing a conditioned stimulus such as a tone with an unconditioned stimulus such as food or electric shock. Early

investigators using this paradigm were primarily interested in the effects of ethanol on established classically conditioned responses and on their extinction. In more recent studies of this type emphasis has been placed on the possible effects of ethanol on fear and anxiety as indexed by the level of conditioning observed. The second type included experiments that dealt with the effects of ethanol on the ability of subjects to form and maintain a discrimination between two conditioned stimuli. The third type represents experiments that were concerned with the effects of ethanol on the magnitude and persistence of orienting responses. In many of the experiments within these three classifications an effort was made to evaluate the effects of ethanol in terms of its influence on inhibitory processes. Because of their potential relevance to a more complete understanding of how alcohol affects the process of conditioning, experiments involving the use of depressant drugs such as sodium bromide, sodium amytal, and sodium pentobarbital have also been surveyed here.

Pavlov (1927) was one of the first investigators to stress that inhibitory processes may play an important role in determining the detailed characteristics of classically conditioned responses. According to Pavlov, the progressive increase in the latency of conditioned responses known as the inhibition of delay phenomenon and the gradual reduction in responding to a nonreinforced conditioned stimulus that occurs during discrimination conditioning were due to the build up of a state of cortical inhibition. He also felt that habituation of the orienting response, which consists of skeletal-motor,

cortical, and autonomic components that are elicited by a novel low intensity stimulus, was produced by an active process of inhibition.

Although Pavlov (1927) recognized that the internal physiological condition of an organism might influence conditioned responses and orienting responses, he performed very few studies to investigate the effects of motivational variables or drugs on these responses. However, several experiments designed to study drug effects on conditioned responses were carried out in Pavlov's laboratory. These studies represent the first reported experiments dealing with the effects of ethanol on classical conditioning and they have provided the theoretical framework for many subsequent investigations.

Zavadski (1908), a student of Pavlov, examined the effects of low and high doses of ethanol on delayed conditioned salivary reflexes in dogs. He used .2 gm/kg and 1.6 gm/kg doses of 96 percent ethyl alcohol which were introduced into the stomach. He found that the small dose completely suppressed the conditioned salivary response for a brief period shortly after its administration, but only slightly decreased the unconditioned salivary reaction. Five minutes after introduction of the high dose both conditioned and unconditioned reflexes were inhibited. After 30-60 min. the unconditioned reflexes returned whereas a period of from 2-4 hr. elapsed before the reappearance of the conditioned response. In brief, he concluded that ethanol produced a depression of central nervous system activity and that the degree of depression increased as a function of dose level. He also emphasized that the effects of ethanol varied with the time of testing following the introduction of the drug.

Nikiforovski (1910) studied the effects of ethanol on several positive and negative conditioned responses and unconditioned responses in nine adult dogs. He employed a discrimination paradigm in which two tone conditioned stimuli at different frequencies were used. One of the conditioned stimuli was consistently followed by a food reinforcement (positive stimulus) and the other was never reinforced (negative stimulus). After stable differential responding was developed by the dogs, e.g. salivation to the positive stimulus and no salivation to the negative stimulus, the effects of ethanol were examined on these responses. Two dose levels of ethanol in 10% solution were introduced via the rectum. The low dose was from 2-5 gm of 99% ethanol and the high dose 6 gm or more of 99% ethanol. It was observed that 10-15 min. after the introduction of the low dose, differentiation between the two stimuli was abolished, in that the dogs began to salivate to the negative stimulus. The large dose greatly diminished or abolished all responses. It was noted that the day after the administration of the large dose the magnitude of the conditioned salivary response was greater than its normal pre-ethanol levels. The conclusion which was drawn on the basis of these results was that cortical inhibitory processes were more sensitive to the effects of ethanol than were processes of excitation, and that ethanol had disinhibitory properties. In a second part of the study, Nikiforovsky found that sodium bromide and other depressant type drugs tended to retard the rate of conditioning of new responses and accelerated the rate of extinction of established conditioned responses. On the other hand, certain excitants such as caffeine and benzedrine

were found to facilitate the development of conditioned responses and slow down the process of extinction.

A similar investigation (Lebdinsky, 1927) indicated that in sufficient concentration urethane and chloral hydrate, both narcotics, depressed the orienting response to the conditioned stimulus, the conditioned response, and the unconditioned response to the unconditioned stimulus. It was noted that as the subject progressed toward complete narcosis the conditioned response was affected first, followed by the orienting response. The unconditioned response was the last reaction to be affected. As the subject recovered from the narcosis the order of reappearance of responses was unconditioned response, orienting response, and conditioned response. It was also observed that regardless of drug state, low intensity, short duration stimuli were less effective in eliciting responses than were high intensity, long duration stimuli.

Andreyev (1934) conducted an extended investigation of the effects of various doses of ethanol on a classically conditioned salivary response in one dog. He used a discrimination procedure in which one conditioned stimulus was followed by food and a second stimulus was always presented alone. The doses used ranged from approximately 1.0 gm/kg to 5 gm/kg and were given through a chronic gastric fistula. In control experiments, an equivalent amount of water was introduced into the stomach through the fistula.

It was observed that following the administration of a low dose of ethanol (1.0 gm/kg) the dog showed marked agitation and restlessness lasting for 30-40 min. It was also noted, that during the first

90 min. the low dose produced a marked increase in rate of salivation to the nonreinforced conditioned stimulus but that salivation to the reinforced stimulus was not affected. Immediately following larger doses, such as 2.5 gm/kg or 4.0 gm/kg of ethanol, responding to the nonreinforced stimulus increased. Within 15 min. salivation to both conditioned stimuli was greatly reduced. Andreyev concluded that ethanol could have both excitatory and inhibitory effects on the central nervous system, and that the type of behavioral effect obtained depended upon the dose given and when testing was started. In addition, he recognized that an ethanol related increase in responding to a non-reinforced conditioned stimulus could be due to an increase in excitation or to a decrease in inhibition.

Gantt (1935) investigated the effects of three levels of 20% ethanol (.4 gm/kg, 1.2 gm/kg, and 3.0 gm/kg) on the performance of conditioned motor and salivary responses in dogs. The different concentrations of ethanol were prepared by diluting 95% ethanol with milk and water. The solutions were taken orally by the animals over a period of several months during which time conditioning sessions were conducted. Gantt found that the low concentration caused a slight increase in the latency of motor and salivary conditioned responses. The moderate dose caused slight ataxia, some loss of coordination, an increase of 25% in the latency of both conditioned responses, a 20% reduction in the magnitudes of both conditioned responses, and a 5-10% decrease in the magnitudes of the unconditioned responses. At the high dose the latencies of the motor and salivary conditioned responses were almost three times normal, the magnitudes of the conditioned

responses were reduced by 80% and the unconditioned responses were reduced in magnitude approximately 20%. It was also observed that the orienting response, turning of the dogs' heads toward the conditioned stimulus, was stronger than usual at the high dose. He suggested that this was due to a loss of inhibition which had been developed during prior repeated presentations of the conditioned stimulus.

In explaining his results, Gantt postulated that orienting responses and unconditioned responses reflected primarily subcortical activities and that the formation of conditioned responses was dependent on higher cortical function. Since he found that the conditioned responses were more affected by the ethanol than were the other reactions he concluded that ethanol's major action was cortical.

Dworkin, Bourne, and Raginsky (1937) studied the effects of several drugs including ethanol on a food-reinforced lid-lifting response in dogs. Subjects were trained to lift the lid of a box containing food in the presence of a particular auditory stimulus but not in the presence of a second auditory stimulus. They were also trained not to touch the lid during the intertrial intervals. With low, moderate, and high doses of ethanol administered by stomach tube, several well marked stages of drug action were noted. At 2.0 mg/kg ataxia and asynergia occurred and the dogs began to touch the lid during the intertrial intervals. At 2.5 mg/kg the ability to discriminate between the two conditioned stimuli was lost, and finally at the 4.0 mg/kg dose the dogs would not eat the food

when it was given to them. The conclusion of these authors was that ethanol and other depressant drugs weakened the process of cortical inhibition which was responsible for the suppression of behavior.

Settlage (1936) found that cats which were trained under the influence of moderate doses of sodium amytal failed to demonstrate a classically conditioned leg-flexion response. However in the absence of the drug the conditioned response was easily evoked with no additional training. It thus appeared that the sodium amytal depressed the performance of the conditioned response without significantly interferring with the learning of the response.

Girden and Culler (1937) established classically conditioned leg flexion responses in dogs that were drugged with low doses of raw curare. They observed that the conditioned response was performed better under the drug than under the non-drug condition. This study, which stands as perhaps the first example of state-dependent learning and the experiment by Settlage (1936) which indicated that a peripheral acting drug could prevent conditioned response performance without blocking the learning of the response are important guideposts for research dealing with drugs and the conditioning process. These findings provide clear examples of the difficulty of specifying the exact locus of drug effects and the problem of separating performance from learning deficits.

The experiments outlined above represent the earliest attempts that were made to investigate the effects of ethanol on classical conditioning. A number of factors should be noted in evaluating the results of these studies. First, the number of subjects that was employed was generally

very small, with the same subjects being used in a wide variety of different conditioning situations. Second, the descriptions of the procedures that were employed were generally incomplete making it difficult to know exactly what was done to the subjects. Third, with the exception of those experiments in which a discrimination paradigm was employed adequate controls for sensitization and pseudo-conditioning were not included in the design of these studies. Although these early experiments do not measure up to today's standards, they nevertheless provided hypotheses and techniques that have been useful in subsequent work.

Recent investigators have continued to employ classical conditioning procedures in attempting to characterize the specific effects of ethanol on learning and behavior. Many of these studies have been based on the assumption that fear and anxiety are important emotional factors in aversive classical conditioning situations and that conditioning performance might be influenced if the level of emotionality could be reduced by giving subjects ethanol or other depressant drugs. The basis for this assumption can be traced to earlier investigations reported by Masserman and Yum (1943) and Conger (1951) that seemed to show that ethanol had anxiety or fear-reducing properties.

A series of experiments has been reported by Franks and his colleagues in which the effects of various drugs on the development and performance of a classically conditioned eyeblink response in humans were examined. In the first of these (Franks and Laverty, 1955) two groups of subjects received either 3.0 mg/kg or 6.0 mg/kg of sodium amytal, a drug having sedative-depressant properties. The conditioned stimulus was a 1.1 KHz, 65 dB tone, and the unconditiond stimulus a

.5-sec. air puff delivered at a pressure of approximately 75 mm of mercury from a distance of 2 cm from the eye. Thirty conditioning trials and 18 test trials were given. The results showed that both concentrations of the drug reduced the number of conditioned eyeblink responses during acquisition and extinction. There were no differences between the groups.

Franks and Trouton (1958) administered sodium amytal, a depressant, or dexamphetamine sulfate, a stimulant, to separate groups of human subjects receiving classical eyeblink conditioning training. There were three treatments given 45 min. prior to the start of the experiment. These included 4.5 gm of sodium amytal, 10 mg. of dexamphetamine sulfate or a distilled water placebo. A fourth treatment administered 2 hr. before conditioning consisted of 10 mg of dexamphetamine sulfate. All treatments were given orally. The conditioning procedure was the same as that used in the prior study. The authors reported that the sodium amytal reduced the number of conditioned responses in both acquisition and extinction whereas the dexamphetamine sulfate increased the frequency of responding during acquisition and extinction. The authors stated that the depression of conditioning by sodium amytal was consistent with the results of a prior experiment (Franks & Laverty, 1955). It was concluded that it was impossible to determine if the results were due to the effects of the drugs on peripheral motor mechanisms or to direct effects on central nervous system associative processes.

The influence of methyl-pentyl-alcohol on the development of classically conditioned eyeblink and galvanic skin responses was investigated by Barthalomew, Franks, and Marley (1958). The subjects

were 25 patients hospitalized for treatment of neurotic and psychosomatic disorders. Subjects were given .5 gm of methyl-pental-alcohol four times each day for four days prior to conditioning. The results revealed that the drug depressed eyeblink conditioning and sympathetic nervous system activity as measured by galvanic skin response levels. The authors proposed that the drug might have blocked the formation of central nervous system changes associated with conditioning and at the same time interferred with the peripheral performance of the conditioned response.

Franks (1963) examined the influence of ethyl alcohol on the formation of a classically conditioned eyeblink response in 88 paid male volunteers. The experimental group received 1 ml of 90 proof blended whiskey per kg of body weight mixed with ice and a carbonated beverage. A placebo-control group received 5 ml of the whiskey placed directly on the surface of the ice and beverage mixture just before the subjects began drinking. Solutions were given orally and subjects were allowed to drink at their own pace. After an interval of approximately 90 min. conditioning was begun. For the experimental group the mean pre- and post-conditioning blood ethanol levels were 86 mg/100 ml and 80 mg/100 ml, respectively. Relative to prior findings, the levels of conditioning achieved by the experimental and placebo-control groups were both suppressed and equally so. The authors suggested that the amount of alcohol given the placebo-group was probably too large in comparison to that which the experimental group received and that this may have been the reason a difference between the groups failed to be present.

An experiment examining the effects of ethanol on classically conditioned eyeblink in humans was reported by Hobson (1966). The experimental design employed was a 2 x 3 factorial with a single control group in which one dimension was ethanol dose level (.68 gm/kg or 1.4 gm/kg) and the other dimension the number of unpaired unconditioned stimulus-alone trials given prior to conditioning (0, 35, 70). Each ethanol dose was administered as a mixture of whiskey and iced ginger ale in a 1:2 ratio while the single control group received 4 gm/kg of iced ginger ale. The mean blood-ethanol level shown by the low-ethanol subjects was 49 mg/100 ml and that of the high-ethanol subjects 99 mg/100 ml. The conditioned stimulus was a 500 Hz tone delivered to both ears through headphones, and the unconditioned stimulus a .05 sec., 80 mm of Hg pressure air puff delivered to the cornea. Following drug treatments and adaptation trials, each subject received 100 acquisition trials. The results of the study demonstrated that both ethanol doses and the 35 and 70 adaptation trials reliably depressed the level of conditioned responding. It was suggested that the ethanol may have decreased the noxiousness of the unconditioned stimulus, and that further research should be done on the relationship between ethanol dose and unconditioned stimulus intensity.

The effects of ethanol on the acquisition of a classically conditioned galvanic skin response in adult male human subjects was reported by McGonnell and Beach (1968). There were 32 participants randomly assigned to the four cells of a 2 x 2 factorial design: alcohol-conditioning, placebo-conditioning, alcohol-pseudoconditioning, and placebo-pseudoconditioning. The drug treatment consisted of

.5 gm/kg of ethanol mixed with soda water and grapefruit juice. The placebo contained soda water, grapefruit juice, and 3 ml of ethanol placed on the surface of the liquid immediately before it was given to the subjects. The conditioned stimulus was a 5-sec. 1 KHz tone approximately 30 dB above threshold, and the unconditioned stimulus a .2-sec 50-volt electric shock applied to the wrist. The interval between the conditioned and unconditioned stimuli was 4.8 sec. Training consisted of 25 paired conditioning trials and five test trials. The main findings of this study were that ethanol inhibited the acquisition and maintenance of the conditioned galvanic skin response in the experimental group. Ethanol did not depress the magnitude of the unconditioned response to the unconditioned stimulus. Even though the magnitude of the unconditioned response of the experimental group was not depressed the authors suggested that the ethanol had perhaps reduced fear and that this resulted in inferior conditioning performance.

Only one experiment could be located in which the effects of alcohol on conditioned heart rate was examined. Hildago, Tarleton, Dileo, and Thompson (1969) used 4 adult mongrel dogs that were restrained in a Pavlovian-type stock. The dogs were given classical conditioning discrimination trials in which CS+ was consistently followed by a 1-sec. shock to the forepaw and CS- was always presented alone. After four to twenty sessions, the dogs showed an increase in heart rate to CS+ and very little change in heart rate to CS-. Subsequently a large number of drugs including ethanol were administered to the dogs and their effects on conditioning performance examined.

The dose of ethanol used was .5 gm/kg administered orally. This was a relatively low dose and it produced a slight ataxia in the four dogs which were tested. It also blocked the performance of the conditioned heart-rate response in two of the dogs. The authors concluded that moderate doses of ethanol, meprobamate, chlordiazepozide, and diazepam were more effective in reducing fear as indexed by level of heart-rate conditioning than were comparable doses of a number of other potential fear or anxiety reducing drugs.

In spite of the fact that orienting responses may play an important role in the conditioning process, relatively little is known about the effects of ethanol on the magnitude and persistence of such reactions. Pavlov (1927) viewed the orienting response as an alerting or "what is it" type of reaction that was elicited by unfamiliar stimuli. Sokolov (1960, 1963) provided an elaboration of Pavlov's views and noted that the total orienting response consisted of skeletal motor, cortical and autonomic components. Although orienting or alerting reactions have usually been associated with awake, behaving subjects, recent evidence has indicated that similar reactions can be obtained during sleep. In addition, Gantt (1936) reported that decorticate dogs were capable of showing what he labelled orienting responses.

Several Russian studies, cited by Sokolov (1960), demonstrated that an orienting response which had been habituated during the awake state could be elicited during sleep. Hord, Lubin and Johnson (1966) examined the effect of sleep stages on the magnitude of the evoked heart-rate response in humans. The subjects were five adult males who spent three consecutive full nights of sleep in a sound-attenuated,

air-conditioned room. Stages of sleep were determined from electroencephalogram recordings and rapid eye movement recordings. The stimulus was a 3-sec. 1 KHz tone presented randomly at 30- and 45-sec. intervals for the entire night. The intensity of the tones was approximately 30 dB above threshold. The experiment demonstrated that the evoked heart-rate response was maximal during the rapid-eye movement stage of sleep. The form of the heart-rate response was biphasic with a small initial acceleration followed by a larger magnitude and longer duration deceleration. Contrary to expectation, the heart-rate response did not habituate with repeated stimulus presentations. The authors concluded that the maximum heart-rate response during the rapid eye movement sleep stage distinguished it from electroencephalographic electrodermal, and plethysmographic components of the orienting response Habituation of a number of other evoked autonomic, somatic, and central nervous system responses was also retarded, but since their maxima occurred during different stages and their rates of habituation were variable it was suggested that the underlying response systems did not necessarily respond as a unit.

In a follow-up study, Johnson and Lubin (1967) investigated the effects of sleep stages in humans on several components of the orienting response. The subjects were 17 adult males who participated in both daytime-awake and all night sleep sessions. The stimulus was a 3-sec. 1-KHz tone presented randomly at 30- and 45-sec. intervals during the daytime and sleep sessions. Heart rate, electroencephalographic, electrodermal, and finger plethysmographic components of the orienting response were measured. Results of the experiment indicated that all responses

habituated to some extent during the awake sessions. With the onset of sleep the responses were again elicited and thereafter showed little habituation. It was noted that although most orienting response measures were smallest during the first rapid eye movement sleep stage, the heart-rate orienting response was maximal during this stage. The authors emphasized the apparent dissociation between the cardiovascular components of the orienting response and those of other response systems.

Taken together these experiments indicate that rather sizeable cardiovascular reactions can be obtained during sleep states and that such reactions show very little reduction in magnitude with repeated stimulus presentations. Decreased rates of habituation of orienting responses have also been reported in subjects that received moderate doses of sodium pentobarbital, or ethanol.

Teitlebaum, Newton, and Gantt (1970) examined the effects of 15-18 mg/kg of sodium pentobarbital on the heart-rate orienting responses of dogs. The dogs were placed in hammocks located in a sound-proof chamber and a variety of non-noxious stimuli were introduced. The results showed that consistent heart-rate orienting responses were made by all dogs under moderate anesthesia to a variety of stimuli e.g., the opening of the test chamber door, three different auditory stimuli, and one visual stimulus. Similar to the findings of the sleep studies described above, the heart-rate orienting responses did not habituate under moderate anesthesia. In addition, all of the auditory stimuli were more effective in producing decelerative heart-rate changes in the drug state than in the non-drug state. Of all the stimuli which were tested the most effective was opening the door, which produced heart-rate increases in

awake dogs and heart-rate decreases in moderately anesthetized dogs. It was further noted that the magnitudes of the orienting responses depended on the depth of anesthesia. During the deepest stage there was no orienting response, whereas with moderate anesthesia the response was again demonstrated. Magnitude of heart-rate changes did not depend on baseline heart rate during anesthesia, as the baseline remained quite stable regardless of the depth of anesthesia.

In one of the few other studies of this type, Powell, Goodwin, Janes, and Hoine (1971) investigated the effects of ethanol on the galvanic-skin response, finger-pulse volume, and heart-rate components of the orienting response in human subjects. One ethanol group and one control group were tested for orienting responses to twenty 15-sec. 600-Hz tone stimuli presented at an average intertrial interval of 30 sec.. Prior to testing, the alcohol subjects consumed between 8-10 oz. of 80-proof vodka diluted in a soft drink, over a 1 hr. period. Control subjects consumed equivalent amounts of soft drink. For the alcohol subjects blood-alcohol levels determined 30 min. after termination of drinking varied from 80-140 mg/100 ml, and most subjects appeared moderately intoxicated. The authors found a nonsignificant overall depression of all response measures in the ethanol group.

Ingle (1973) studied the effects of ethanol on the habituation of prey-catching activity in adult leopard frogs. The activity was elicited by a rotating dummy prey object. Prior to testing the experimental group (N = 9) sat in water containing 400 mg/100 ml of ethanol whereas the control group (N = 9) sat in a container of pure water. The exposure time for both groups was 2-2.5 hrs. Subjects

were tested 1 min. after removal from the solutions. The frogs receiving ethanol emitted approximately twice the number of prey-catching responses during the first two minutes of the test period than did the control frogs. Another finding was that the ethanol group reacted more frequently to the test object when it was placed in a familiar position than when it was placed in an unfamiliar location. The author concluded that the increased frequence of prey-catching activity was probably due to the disinhibitory effects of ethanol on the visual-motor system in the frog.

The experiments reviewed above indicate that ethanol may have pronounced effects on the various responses which may be measured in a classical conditioning situation. In some cases, moderate doses of ethanol were shown to reduce the frequency of conditioned eyeblink responses, to decrease the magnitude of conditioned salivary, motor, and galvanic skin responses, and to increase the latency of learned salivary and motor reactions. High doses of ethanol were uniformly shown to depress both autonomic and skeletal conditioned responses. Discrimination responding was impaired at low ethanol concentrations and completely eliminated at high concentrations. Orienting responses to novel stimulation and unconditioned responses to intense stimulation, both thought to be unlearned reactions, were generally reduced in magnitude by high concentrations of ethanol. However, there was some indication that low to moderate doses prolonged the habituation of orienting responses. It is important to note that systematic evaluations

of more than one concentration of ethanol were made in very few of the experiments and that most of the studies were concerned with skeletal-motor responses. Furthermore, in almost all of the studies ethanol was administered to the subjects after the conditioned response was fully established. Thus, there is little information regarding the effects on ethanol of the acquisition process of conditioned responses.

The purpose of the present experiment was to provide a detailed analysis of the effects of ethanol on classically conditioned heart rate in rats. Two concentrations of ethanol and two intensities of shock were employed in an effort to determine whether the possible debilitating effects of ethanol could be diminished by the use of an intense reinforcing stimulus. An examination of the effects of ethanol on the heart-rate orienting response was also made.

A series of prior experiments (Fitzgerald & Teyler, 1970; Fitzgerald & Martin, 1971) revealed that the direction of the conditioned heart-rate response exhibited by restrained rats was decelerative. It was further observed that the direction of the heart-rate orienting response was also decelerative. The orienting response and conditioned response were differentiated on the basis of their topographies. Response topographies were determined by examining the magnitude of the heart-rate change in successive time intervals during the conditioned stimulus. Initially, the orienting heart-rate decrease was greatest immediately following the onset of the conditioned stimulus. After several presentations of the stimulus, the overall magnitude of the orienting response was generally reduced even though the form of the response remained essentially the same.

On early conditioning trials the largest heart-rate decelerations occurred at the beginning or middle periods of the conditioned stimulus. By the end of the conditioning session maximum heart-rate decreases were found during the last portion of the conditioned stimulus. Thus, the final form of the conditioned heart rate response appeared to demonstrate the presence of inhibition of delay. Although there is not complete agreement on the nature of the mechanisms underlying inhibition of delay, many investigators have argued that an active process of inhibition may be involved. It was anticipated that the ethanol employed in the present study might interfere with the development of this process.

METHOD

Subjects

The subjects were 129 naive female Long-Evans hooded rats, 90-120 days of age, ranging in weight from 150-225 gm. They were purchased from the University of Oregon Health Sciences Center Department of Animal Care and housed two per cage under conditions of continuous illumination with free access to food and water.

Apparatus

During the conditioning session, the subjects were restrained in an adjustable animal holder manufactured by E & M Instrument Company. The restrainer consisted of a U-shaped plastic housing contoured to fit sungly around the rat's body. The housing was mounted on a plastic base which had a removable plastic plate on the rear-half. Guillotine-type plastic inserts were positioned in slots at both ends of the housing to hold the subjects and to keep movement at a minimum. To prevent extraneous auditory signals from reaching subjects, the restrainer was placed in a small Industrial Acoustic sound-isolation chamber. The chamber was equipped with a ventilating fan, a 60-w house light recessed in the ceiling, and a 10 cm speaker mounted in the ceiling. To further mask unwanted auditory signals, white noise measuring approximately 75 dB, sound pressure level (re. .0002 dyne/cm^2) was presented continuously through the 10 cm speaker.

The electrocardiogram was recorded on a Grass polygraph from 20-gauge hypodermic needles inserted through a fold of skin on either side of the rat's thoracic cavity. Heart rate was measured by means of an automated on-line system that provided a punched paper tape

tabulation of the heart-beat totals occurring in successive time periods on each trial. The details of this system have been described at length by Fitzgerald, Vardaris, and Teyler (1968) and will only be briefly described at this time.

The system consisted of a sensitive low-force-lever type Microswitch positioned on the writer unit of the Grass polygraph in such a way that the Microswitch was actuated when the electrocardiogram polygraph pen was deflected by the R wave of the QRS complex. The Microswitch closure triggered a transistorized pulse shaper whose output was fed to a transistorized BCD counter. At the end of each counting period the contents of the counter were punched out on an eight bit Tally high speed paper-tape punch. To provide a visual check on the reliability and accuracy of the system, the output of the pulse shaper was used to operate a relay whose contacts switched a small voltage onto one pen of the polygraph. The resulting spikes were compared to the electrocardiogram polygraph records and to the punched heart-beat totals. The accuracy of the counting circuit was regularly checked by substituting a 10-Hz signal for the incoming electrocardiogram signal.

The conditioned stimulus was a 6.5-sec., 2.9 KHz tone presented at 85 dB sound pressure level (re. .0002 dyne/cm^2) through a 2.5 cm Mallory Sonalert mounted directly in front of the subject. The unconditioned stimulus was a .5-sec., 60 Hz a.c. electric shock produced by a Grason Stadler E 6070B stimulator, and delivered through electrodes spaced 2 cm apart on the base of the rat's tail. The electrodes were constructed of two 1.5-cm lengths of .75-cm diameter elastic-rubber tubing. Electrical contact with the tail was made

through the heads of two no. 6 machine screws fastened to the rubber tubing with a lock washer and nut. The rat's tail was scrubbed with a gauze pad soaked in 95% lab-alcohol and Harvard electrode paste was vigorously rubbed into the tail before the shock electrodes were positioned. After electrode placement a resistance reading was taken to insure proper contact of the electrode. If the resistance measured more than 20-K ohms, additional electrode paste was applied and the electrodes were slightly repositioned.

Two subjects were conditioned concurrently, through the use of two identically equipped chambers with trials alternating between subjects. Trials were automatically initiated by a film tape programmer with events occurring within a trial programmed and timed by Massey Dickinson transistorized logic modules. The repeat accuracy of the timing modules was approximately .05%. Periodically during the course of the experiment the intensity of the white noise and conditioned stimulus was measured on the C-scale of a Scott sound pressure meter. The shock levels were also monitored at regular intervals with an oscilloscope by measuring the voltage drop across a 100 ohm resistor in series with the subject.

Procedure

There were six experimental groups of 15 subjects each and three pseudo-conditioning control groups of five subjects each. The six experimental groups comprised the cells of a 3 x 2 factorial design, with one dimension consisting of the drug treatment (saline, 10% ethanol, and 30% ethanol) and the other dimension consisting of unconditioned stimulus intensity (.6 ma or 1.2 ma). The three control groups received either saline, 10% ethanol, or 30% ethanol.

Each subject was placed in the conditioning chamber, and given 15 min. to adapt to the restrainer. Following adaptation, the door of the chamber was opened and the subject was injected. A small detachable plastic plate on the floor of the restrainer was removed, a fold of skin lateral to the ventral mid-line and slightly anterior to the hind legs on the right side was held with the left forefinger while the 1.5 cm syringe needle was advanced through the skin and the solution injected i.p. with the right hand. Following an additional 15-min. post-injection period, all subjects were given 10 presentations of the conditioned stimulus at intertrial intervals of 70, 90, or 110 sec. (M = 90 sec.). Finally, 20 paired presentations of the conditioned and unconditioned stimuli were given to all of the experimental subjects at randomly spaced intertrial intervals of 160, 180, or 200 sec. (M = 180 sec.). A delayed conditioning paradigm was used in which the .5-sec. unconditioned stimulus overlapped the final .5 sec. of the 6.5-sec. conditioned stimulus.

The control subjects were given treatment identical to that of the experimental animals with the exception that they received unpaired presentations of the conditioned and unconditioned stimuli with the unconditioned stimulus following the conditioned stimulus by an interval of 70, 90, or 110 sec. (M = 90 sec.). In order to provide maximum control for sensitization and pseudoconditioning, all of the control subjects received the 1.2-ma. unconditioned stimulus.

A blind injection procedure was employed. The solutions of 10% and 30% ethanol were made from a base of 95% laboratory ethanol diluted in normal saline. After the solutions were prepared and placed in

5 ml vials, another experimenter placed code numbers on the vials. All doses were given on the basis of 1 cc/100 gm of body weight. Therefore, subjects given a 10% ethanol injection received approximately .8 gm of ethanol per kilogram of body weight and subjects given the 30% ethanol injection received approximately 2.4 gm of ethanol per kilogram of body weight.

Baseline heart rate in the pre- and post- 15-min. adaptation periods was tabulated in five 6-sec. time intervals spaced 3-min. apart. Heart beats were counted in eight consecutive time periods on each trial of the pretest and conditioning phases of the experiment. The duration of the first period was 6 sec. and that of the remaining seven periods 2 sec. The 6-sec. period occurred immediately prior to onset of the conditioned stimulus (pre-conditioned stimulus period) and provided a measure of baseline heart rate. The first three 2-sec. periods represented the 6-sec. interval between the conditioned and unconditioned stimuli and the final four 2-sec. periods were located after the termination of the unconditioned stimulus. For purposes of data analysis, the beat per minute rates during the interval prior to the conditioned stimulus weresubtracted from the beat per minute heart rate during each of the 2-sec. intervals when the CS was present.

Blood ethanol analyses

Prior to the beginning of the main experiment, blood ethanol concentrations were established for 24 female Long-Evans hooded rats of the same age and weight range as those used in the main experiment. This was done to obtain an estimate of circulating levels of ethanol in the subjects employed in the main experiment. The subjects

were randomly assigned to the cells of a 2 x 2 factorial design with one of the dimensions being alcohol concentration (10% and 30%) and the other dimension being US intensity (.6 ma and 1.2 ma). All subjects received classical conditioning training using exactly the same procedure as that employed in the main experiment. However, because of the frequent interruptions that were necessary to obtain the blood samples, the heart-rate data for these subjects were not analyzed.

Prior to the injection of ethanol, the subjects were lightly anesthetized with ether, and a mid-ventral incision approximately 2.5 cm in length was made at a point about 2.5 cm from the base of the tail. The integument was reflected and the connective tissue and caudal vein were exposed. Procaine (10 mg procaine hydrochloride/ml) was applied topically to the area as a local anesthetic, with repeated applications every .5 hr. Two hr. after the completion of the surgery the subjects were placed in the plastic restrainers, and after a 15-min. adaptation period in the sound isolation chamber they were given ethanol injections.

Approximately 10 min. after the injection, the door of the chamber was re-opened, the exposed tail vein severed, and a blood sample was collected with a heparinized microcapillary tube, with a .050 capacity. Additional samples were taken at .5-hr, 1-hr, and 2-hr intervals after the injection. Each of these samples was collected during the intervals between classical conditioning trials. Immediately after the blood sample was taken, one end of the capillary tube was sealed with a clay-sealant and the tube was labelled. All blood samples were stored in a refrigerator (4° C) until analyzed, with all analyses being conducted within 48 hr after the samples had been collected.

The microcapillaries were centrifuged in a micro-hematocrit centrifuge for 5 min. at approximately 11,500 RPM. This yielded about .03 ml plasma and .02 ml packed cells, as the mean rat hematocrit is approximately .37 (37% red blood cells). After centrifugation, a .02 ml aliquot of plasma was removed from the capillary tube by means of a 0.10 ml syringe, and added to 1 ml of 0.9 N NaCl. Exactly .1 ml of the plasma-NaCl mixture was then added to previously prepared reagent solution which contained 1 ml nicotinamide-adenine dinucleotide (NAD), 1.5 ml phosphate buffer (pH = 8.7), and .4 ml of alcohol dehydrogenase (ADH) prepared from a yeast extract. The phosphate buffer was composed of the following: 1 gm sodium pyrophosphate, .25 gm semicarbazide-HCL, 1.5 gm glycine. This mixture was dissolved in 25 ml of distilled water and to it was added 2 ml of 1 N NaOH. If the pH of this solution was above 8.7 it was titrated with 1 N HCL. The NAD was prepared by the addition of 14.2 mg NAD to 10 ml of the phosphate buffer. Since the ADH has a shelf life of only about 24 hr., it was prepared daily by the addition of 1.5 mg ADH/10 ml phosphate buffer.

The final reaction mixture of plasma-NaCl, NAD, ADH, and phosphate buffer was kept at room temperature, approximately 25 C, for 1 hr. in order to insure completion of the following reaction:

$$\underset{\text{(plasma)}}{\text{ethyl alcohol}} \xrightarrow[\text{NAD}]{\text{ADH}} \text{acetaldehyde}$$

NAD: colorless at 340 nm

NADH: colored at 340 nm

The semicarbazide-HCl contained in the phosphate buffer prevented the acetaldehyde end product formed in this reaction from undergoing a reverse reaction to form ethanol.

The amount of ethanol in each plasma sample was determined photometrically. A standard Beckman spectrophotometer was set at 340 nanometers and the indicator was set at zero absorbance by using a plasma blank taken from randomly selected animals prior to their injection with ethanol. On each test day prior to the examination of ethanol samples the spectrophotometer was zeroed first with a water blank and next with a plasma blank, which was treated in a manner identical to the ethanol containing samples. Therefore, on each test day the experimental samples were evaluated for absorbance relative to plasma blanks obtained from randomly selected animals before their injection. Spectrophotometer readings were taken at 340 nm because the values obtained at this setting are specific for NADH, relative to the other chemicals present in this reaction. Prior to the initiation of this reaction there is no NADH present in the mixture, but as alcohol is converted to acetaldehyde, the coupled reaction of NAD--NADH also occurs. Therefore, the final amount of NADH present in a sample after completion of the previously diagrammed reaction is a specific and quantitative measure of the amount of ethanol contained in the plasma sample.

The formula used to relate the absorbance value of the experimental sample (A) to mg of alcohol per 100 ml sample is:

$$\frac{A}{2.07} \times \frac{50}{.1} \times \frac{46.07}{10^6} \times 100 = \text{g alcohol/100 ml plasma}$$

where A is the absorbance of the sample relative to the plasma blank. The 2.07 value in the above equation was determined as follows: the absorbance of one micromole of NADH/1 ml solution in a one cm light path at 340 nm is 6.22. Thus, the conversion of one micromole of NADH in the 3.0 ml of reaction mixture of alcohol corresponds to an absorbance change of $\frac{6.22}{3.0} = 2.07$. Because the .1 ml sample was composed of 1 part plasma to 50 parts NaCl, the total number of micromoles in a 1 ml plasma sample is $\frac{A}{2.07} \times \frac{50}{.1}$ micromoles. In order to convert micromoles of alcohol to (g); the last formula is multiplied by the molecular weight of ethyl alcohol (46.07) and divided by 10^6 (µg to g). Multiplying the result by 100 converts this last value into grams of ethanol/100 ml plasma.

RESULTS

Blood ethanol determinations from preliminary study

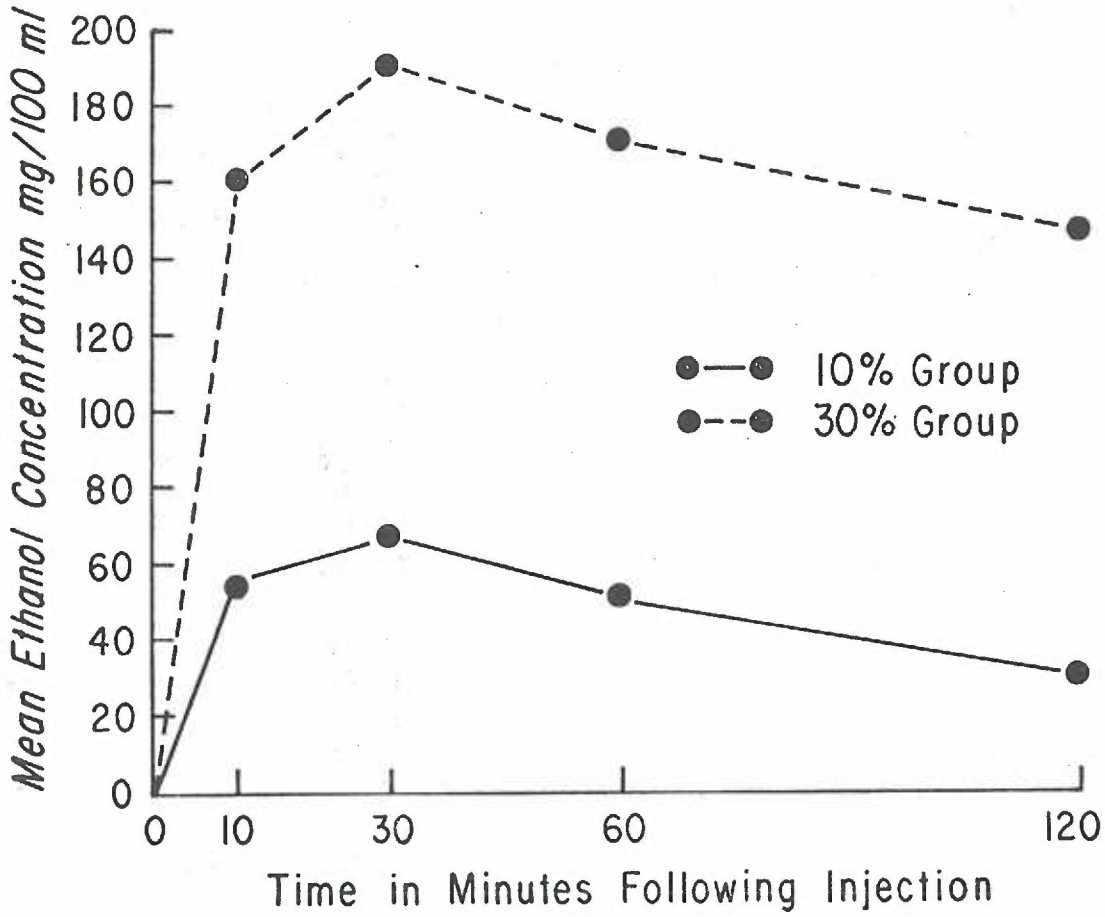
Figure 1 shows mean blood-ethanol levels of the 10% and 30% ethanol groups given conditioning training prior to the beginning of the main experiment as a function of time after injection. In each case, a single group was formed after separate analyses of variance demonstrated that intensity of the unconditioned stimulus had no effect on blood-ethanol levels. Observation of the figure clearly indicates that blood-ethanol concentrations of the 30% group were considerably higher at each sampling point than those of the 10% group. Both groups showed substantial blood-ethanol levels 10 min. after being injected, with peak concentrations being reached within approximately 30 min. Elimination of the ethanol was gradual with approximately 50% of the peak concentration remaining after 2 hr. for the 10% group and approximately 80% remaining for the 30% group.

An analysis of variance comparing the blood ethanol levels of the two groups provided a significant effect of drug treatment (F = 217.84, df = 1/22, $p < .001$) and a significant effect of sampling points (F = 4.44, df = 3/66, $p < .01$) indicating that blood-ethanol concentration changed reliably over the 2-hr. period. The Drug treatment x Sampling periods interaction was not significant, demonstrating that the changes in ethanol levels over sampling periods of the two groups were not reliably different.

Adaptation.

Since the unconditioned stimuli were not presented during adaptation or during the pretest trials with the conditioned stimulus,

Figure 1. Mean blood-ethanol concentrations of the 10% and 30% groups as a function of time in minutes following injection.


Mean Ethanol Concentration mg/100 ml
200
180
160
140
120
100
80
60
40
20
0
0
10
30
60
120
Time in Minutes Following Injection
10% Group
30% Group

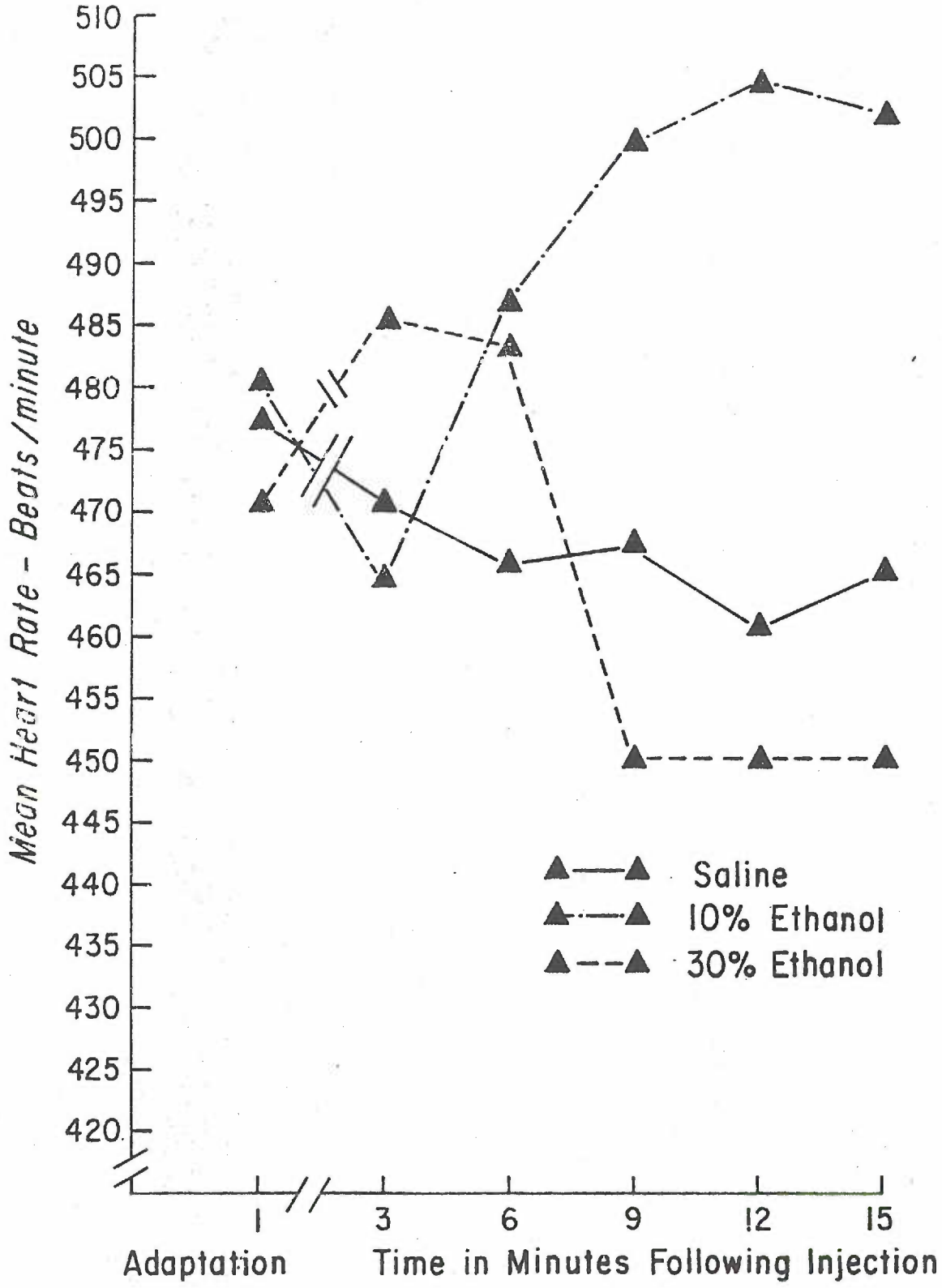
the data for the separate groups within each drug treatment were combined during these phases of the experiment.

None of the experimental groups or the control groups exhibited a significant change in baseline heart rate during the 15-min. pre-ethanol adaptation period. The heart-rate range for all groups during this period was between 471 and 481 beats per minute (bpm). The mean heart rates of the combined saline, 10%, and 30% ethanol experimental groups during the adaptation period are displayed in the left side of Figure 2. An analysis of variance on these points indicated that there were no significant differences among the groups.

Figure 2 also presents the heart-rate baselines of the three experimental groups during the 15-min. period following the injection. It is clear from this part of the figure that the two concentrations of ethanol had opposite effects on heart rate. Relative to the pre-injection level, the 10% groups showed a decrease in heart rate 3 min. after injection followed by a rapid increase that reached an asymptotic level of approximately 500 bpm. In contrast, heart rate of the 30% group showed a small initial increase followed by a sharp decline to approximately 450 bpm. The saline group showed a slight decrease in heart rate across the 15-min. post-injection period.

Although different subjects were involved, it was possible to compare the heart-rate changes shown in Figure 2 with the blood ethanol concentrations shown in Figure 1. This comparison revealed that the post-injection heart-rate increase in the 10% group occurred at approximately the same time that the blood alcohol concentration of the 10% group in the preliminary study showed the greatest increase.

Figure 2. Mean baselevel heart rate in beats per minute of the saline, 10%, and 30% groups during the pre- and post-injection adaptation periods.


Mean Heart Rate - Beats/minute
510
505
500
495
490
485
480
475
470
465
460
455
450
445
440
435
430
425
420
Saline
10% Ethanol
30% Ethanol
1
3
6
9
12
15
Adaptation
Time in Minutes Following Injection

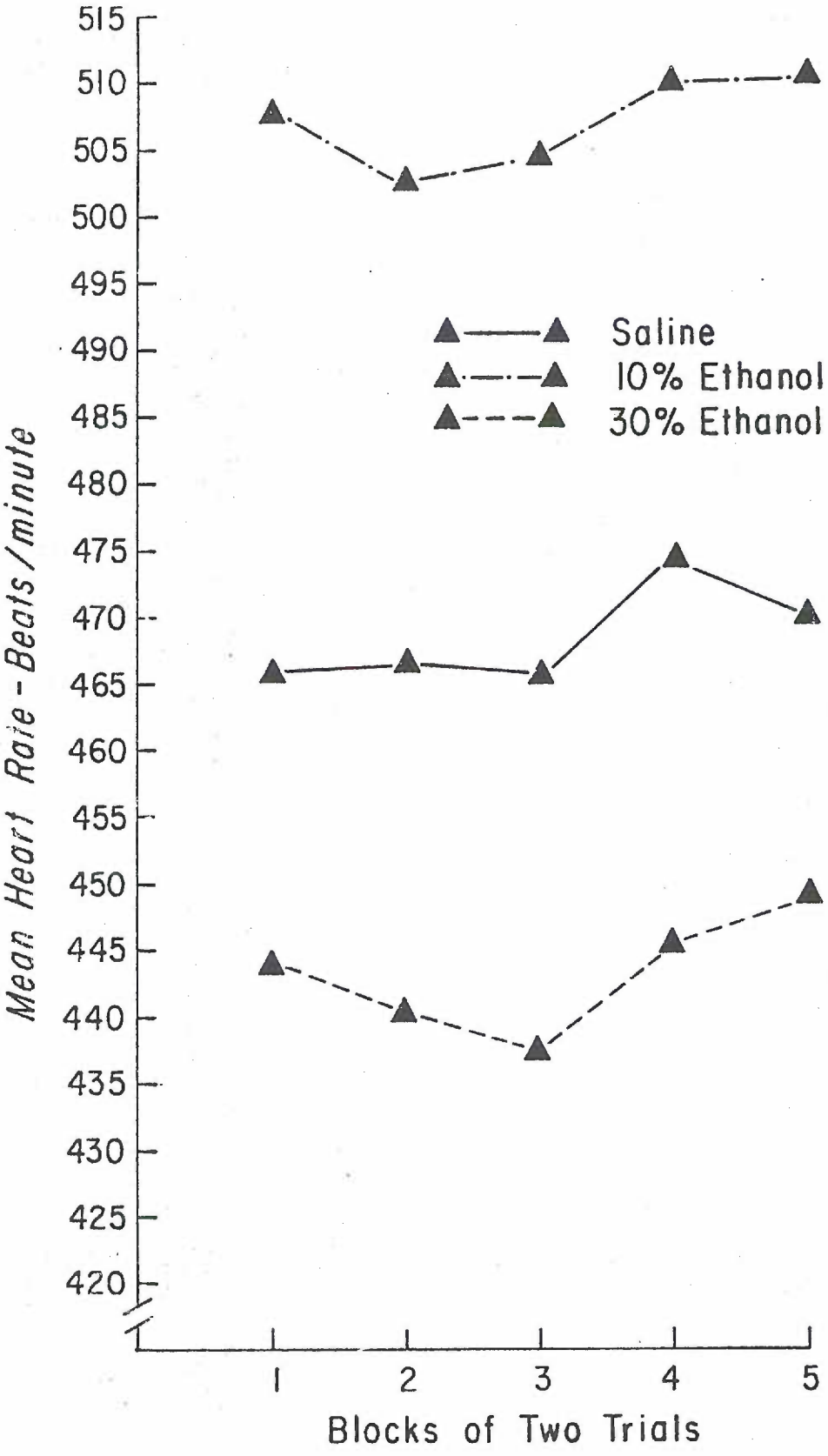
Similarly, the heart-rate decrease in the 30% group was also temporally related to the rapid rise in blood-ethanol concentration of the 30% group in the preliminary study.

A 3 x 5 analysis of variance (Drug treatment x Trials) with repeated measures on trials performed on the post-injection baseline heart-rate data shown in Figure 2 yielded a significant interaction of Drug treatment x Trials (F = 9.31, df = 8/336, $p < .001$). This interaction reflects the reliability of the differential change in heart rate of the saline, 10%, and 30% groups. A second analysis comparing just the 10% and 30% groups also showed a significant Drug treatment x Trials interaction (F = 14.22, df = 4/232, $p < .001$).

Pretest trials with the conditioned stimulus

Baseline heart rate. Figure 3 depicts the mean heart rates of the three experimental groups during the 6-sec. pre-conditioned stimulus period in blocks of two trials. It is obvious from an inspection of this figure that the between-group differences in baseline heart rate that occurred during habituation (see Figure 2) continued to be present during the pretest phase of the study. The overall means of the saline, 10%, and 30% groups during this phase were 466, 507, and 443 bpm, respectively. Figure 3 also reveals that the heart-rate levels of the three groups increased slightly from the beginning to the end of the pretest trials. In order to test the reliability of the differences between the three experimental groups a 3 x 5 (Drug treatment x Trials) analysis of variance with repeated measures on trials was performed on these data. The results of this analysis provided a significant effect of drug treatment (F = 10.59, df = 2/87, $p < .001$) and a

Figure 3. Mean pre-conditioned stimulus heart rate in beats per minute of the saline, 10%, and 30% experimental groups as a function of successive two-trial blocks during the pretest phase of the experiment.

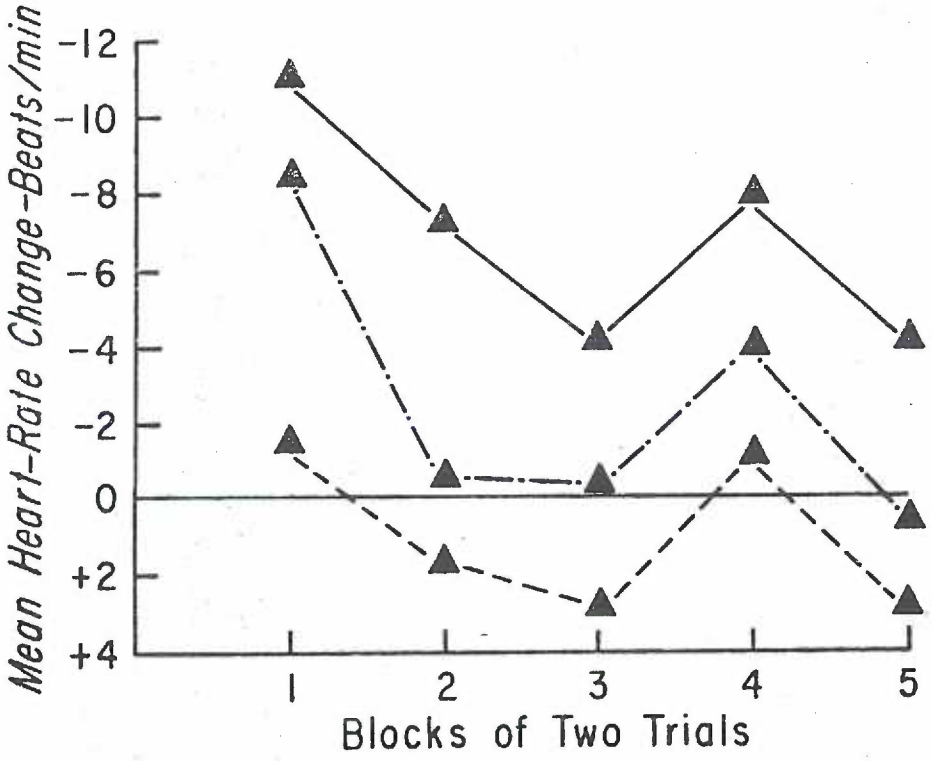

515
510
505
500
495
490
485
480
475
470
465
460
455
450
445
440
435
430
425
420
Mean Heart Rate - Beats/minute
Saline
10% Ethanol
30% Ethanol
1
2
3
4
5
Blocks of Two Trials

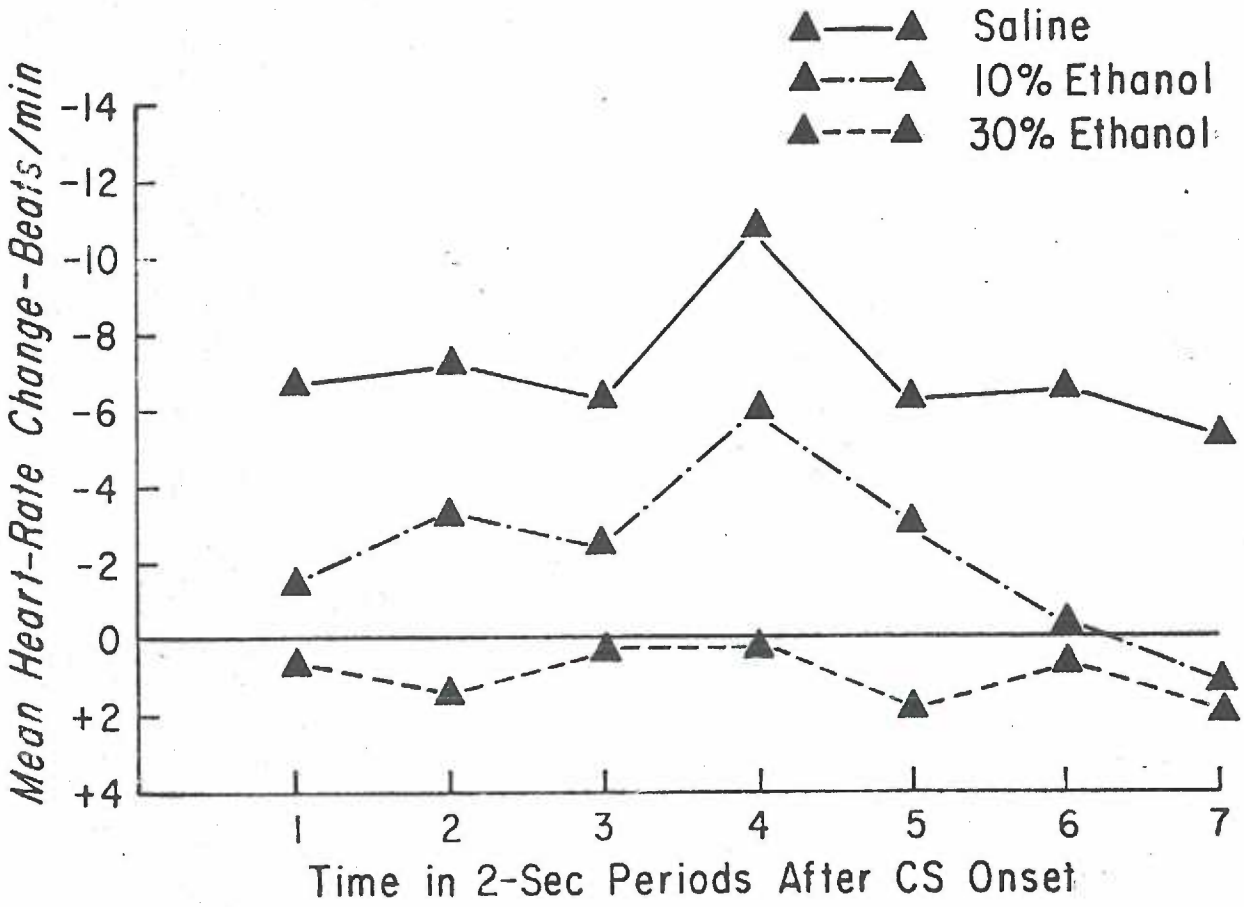
significant effect of trials (F = 3.50, df = 4/348, $p < .001$). A Newman-Keuls test indicated that the overall heart-rate means of the saline, 10%, and 30% groups were significantly different from each other ($p < .01$).

Orienting response. The orienting responses of the experimental groups in blocks of two trials are presented in the top half of Figure 4. It is apparent from this figure that the 30% groups showed no evidence of an orienting response to the tone. The direction of the heart-rate reactions of this group fluctuated between deceleration and acceleration with the magnitudes of each type of response being very small. Both the saline and 10% groups showed decelerative orienting responses which decreased in magnitude across trials. The magnitude of the response in the 10% group was consistently lower than that of the saline group and appeared to habituate at a faster rate. A 3 x 3 (Drug treatment x Trials) analysis of variance with repeated measures on trials performed on these data demonstrated a significant effect of drug treatment (F = 8.51, df = 2/360, $p < .001$), and a significant effect of trials (F = 7.08, df = 4/348, $p < .001$). A Newman-Keuls test indicated that the overall mean magnitude of the orienting responses of the three groups were significantly different from each other ($p < .01$).

The bottom half of Figure 4 illustrates the form of the heart-rate orienting responses of the experimental groups in successive 2-sec. counting periods averaged over the 10 tone-alone trials. Counting periods 1, 2, and 3 represent the 6 sec. of the conditioned stimulus, and periods 4, 5, 6, and 7 represent the 8 sec. following the offset of the

Figure 4, top. Mean beat per minute heart-rate change of the saline, 10%, and 30% groups to the conditioned stimulus on the pre-test trials. Figure 4, bottom. Mean beat per minute heart-rate change of the saline, 10%, and 30% groups in successive 2-second periods after the onset of the conditioned stimulus averaged over the 10 pre-conditioning trials.


Mean Heart-Rate Change-Beats/min
-12
-10
-8
-6
-4
-2
0
+2
+4
1
2
3
4
5
Blocks of Two Trials


Saline
10% Ethanol
30% Ethanol
Mean Heart-Rate Change-Beats/min
-14
-12
-10
-8
-6
-4
-2
0
+2
+4
1
2
3
4
5
6
7
Time in 2-Sec Periods After CS Onset

conditioned stimulus. The level of heart-rate deceleration in the saline and 10% groups was fairly constant across the three 2-sec. periods of the conditioned stimulus. With the offset of the conditioned stimulus (counting period 4), the magnitude of heart-rate deceleration in both groups increased substantially. Immediately following this enhanced decelerative change heart rate returned toward baseline. The magnitude of heart-rate deceleration of the 10% group was below, by a relatively constant amount, that of the saline group in each of the seven counting periods. The 30% group showed very little heart-rate change in any of the counting periods. A 3 x 7 (Drug treatment x Counting periods) analysis of variance with repeated measures on counting periods was carried out on these data and indicated a significant effect of drug treatment (F = 11.87, df = 2/540, $p < .001$) and a significant effect of counting periods (F = 5.33, df = 6/522, $p < .001$).

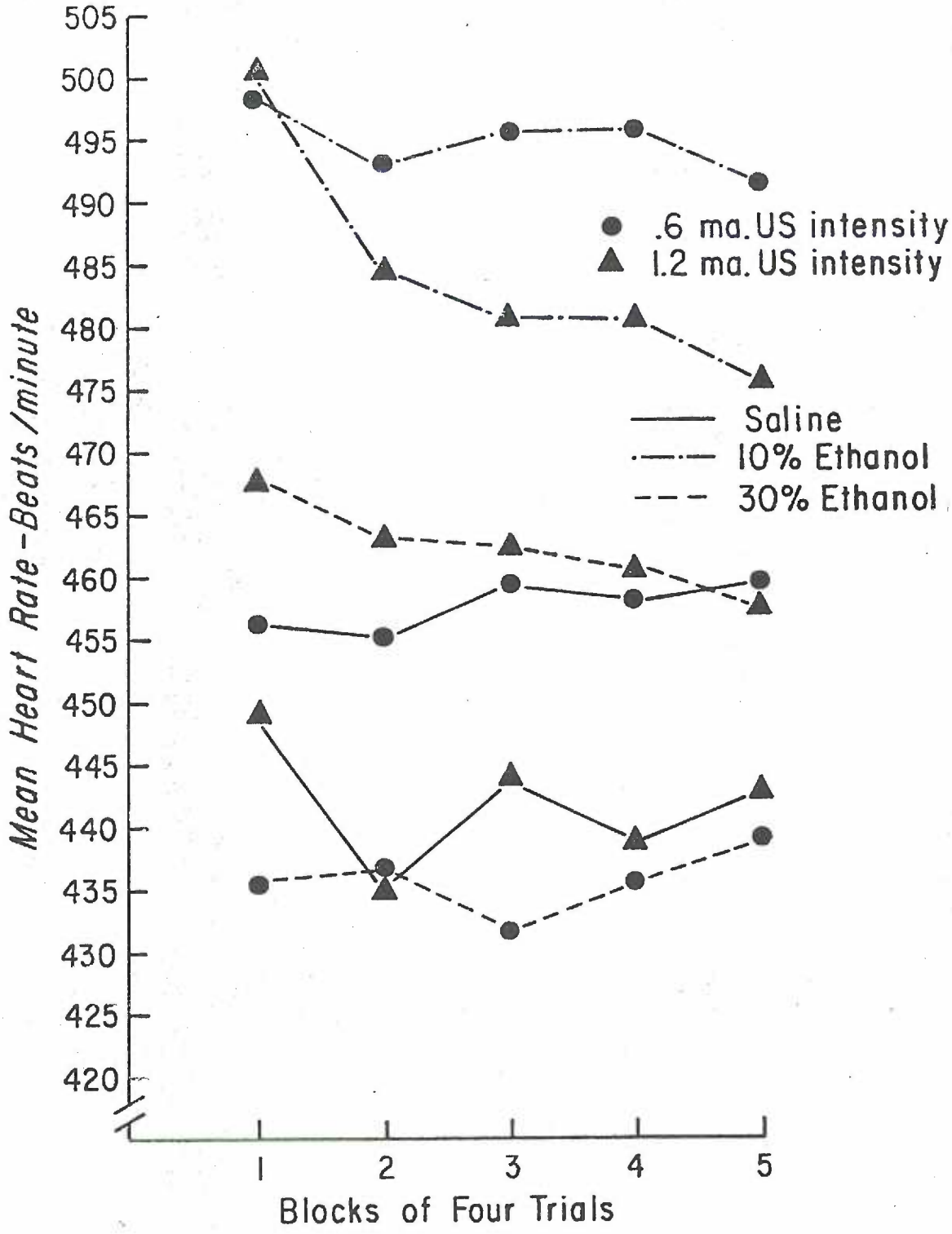
In order to determine the reliability of the heart-rate reactions of the saline and 10% groups to the offset of the tone, separate t tests were carried out comparing the mean heart-rate responses during the fourth counting period with the responses during the second counting period. The second counting period was selected because both groups showed the next largest heart-rate change during this time. These tests were significant for both groups: saline group (t = 2.20, df = 29, $p < .025$); 10% group (t = 1.92, df = 29, $p < .05$). Further inspection revealed that the magnitudes of the responses to the offset of the tone did not decrease over the pretest trials.

Acquisition

Baseline heart rate. Figure 5 presents mean pre-conditioned-stimulus (baseline) heart rate for the six experimental groups in blocks of four acquisition trials. It is apparent from this figure that, as was the case during the habituation and pretest phases of the study, overall baseline heart rate in the 10% ethanol treatment condition was above that in the saline and 30% conditions. It is also obvious that baseline heart rate of the 10%-1.2 ma group decreased during acquisition and was lower than that of the 10%-.6 ma group. Similarly, baseline of the saline-1.2 ma group was lower than that of the saline-.6-ma group. Thus, contrary to what might be expected, the high intensity unconditioned stimulus led to a lower baseline level of heart rate with saline and 10% than did the low intensity unconditioned stimulus. In contrast, the 30% groups showed the opposite relationship, with baseline heart rate of the 1.2-ma group being consistently higher than that of the .6-ma group. The overall mean baseline heart rates of the experimental groups were as follows: saline-1.2 ma = 442, saline-.6 ma = 457 bpm, 10%-1.2 ma = 484, 10%-.6 ma = 495, 30%-1.2 ma = 462, and 30%-.6 ma = 437.

The reliability of the data presented in Figure 5 was tested in a 3 x 2 x 5 (Drug treatment x Shock intensity x Trials) analysis of variance with repeated measures on trials. This analysis provided a significant effect of drug treatment ($F = 7.20$, $df = 2/84$, $p < .005$), a significant effect of trials ($F = 2.913$, $df = 4/336$, $p < .05$) and a significant Shock intensity x Trials interaction ($F = 2.37$, $df = 4/336$, $p < .05$). A Newman-Keuls test showed that the mean baseline heart-rate

Figure 5. Mean pre-CS heart rate in beats per minute of the six experimental groups as a function of four-trial blocks during conditioning.

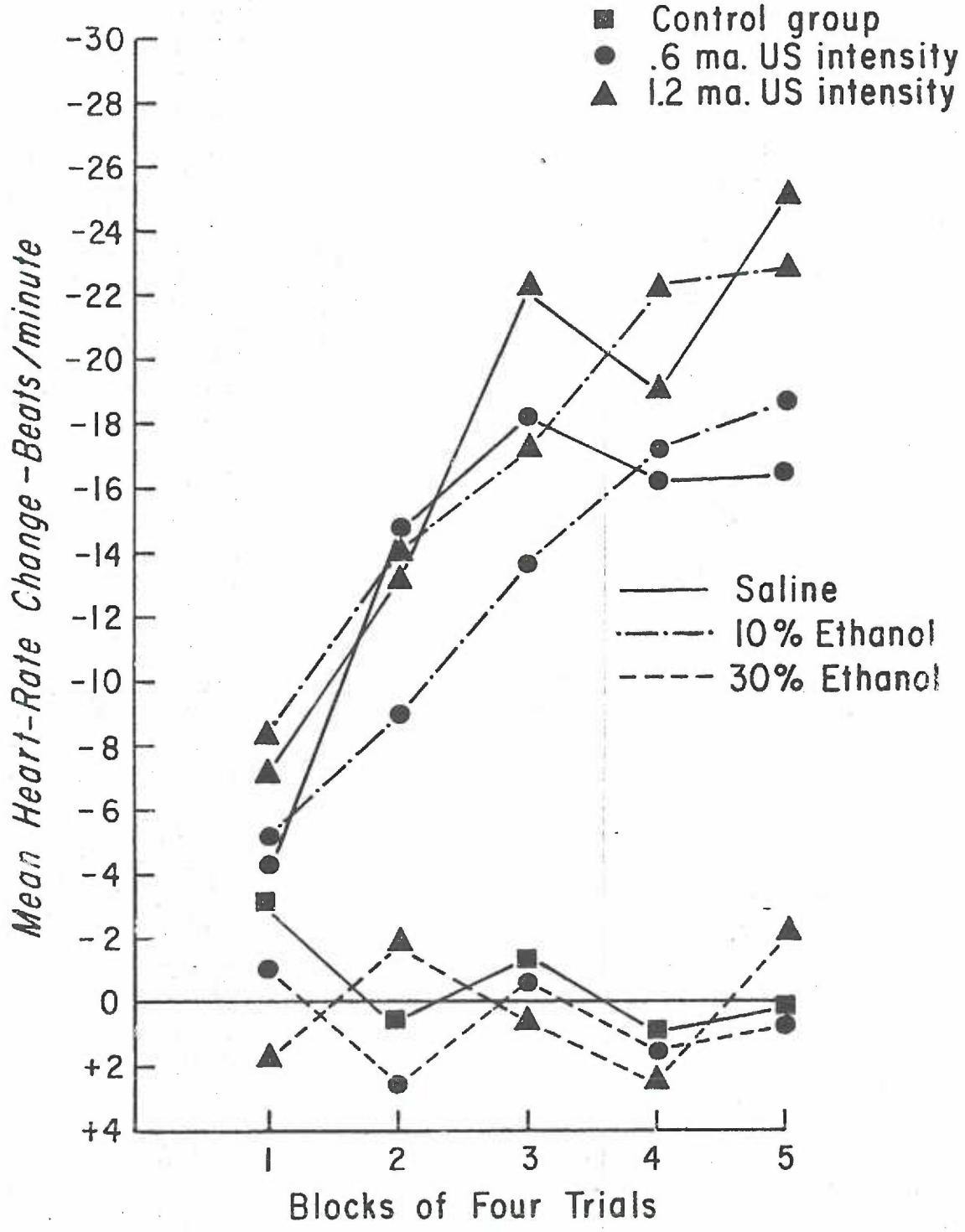

505
500
495
490
485
480
475
470
465
460
455
450
445
440
435
430
425
420
Mean Heart Rate-Beats/minute
.6 ma. US intensity
1.2 ma. US intensity
Saline
10% Ethanol
30% Ethanol
1
2
3
4
5
Blocks of Four Trials

levels of the saline, 10%, and 30% groups collapsed across shock intensity were all significantly different from each other ($p < .05$).

Conditioned responding. Figure 6 presents mean conditioned-stimulus minus pre-conditioned-stimulus heart-rate changes of the six experimental groups and the combined-control groups plotted as a function of four-trial blocks during acquisition. A single control group was formed after it was determined by an analysis of variance that the three control groups did not differ significantly from each other. Inspection of this figure indicates that both the saline and 10% groups demonstrated decelerative heart-rate conditioned responses which increased in magnitude across conditioning trials. There was no evidence that the magnitudes of the conditioned responses of the 10% group were depressed relative to those of the saline group. However, neither of the 30% groups showed conditioning, with their heart-rate reactions fluctuating around zero and being comparable to those of the control group. Observation of this figure also shows that the intensity of the unconditioned stimulus had very little if any systematic effect on the magnitudes of the conditioned responses.

Initially, a 3 x 2 Drug treatment x Shock intensity analysis of variance with a single control group (Winer, 1971, p. 201) was carried out on the overall mean heart-rate changes of the groups reflected in Figure 6. The outcomes of this analysis demonstrated that the control group versus all other groups' effect was significant ($F = 15.56$, $df = 1/98$, $p < .001$). Subsequently, Dunnett's "t" statistic was used to compare the mean heart-rate changes of each experimental group with the control groups combined. This analysis provided the

Figure 6. Mean beat per minute heart-rate change of the six experimental groups and combined control groups as a function of four-trial blocks during conditioning.

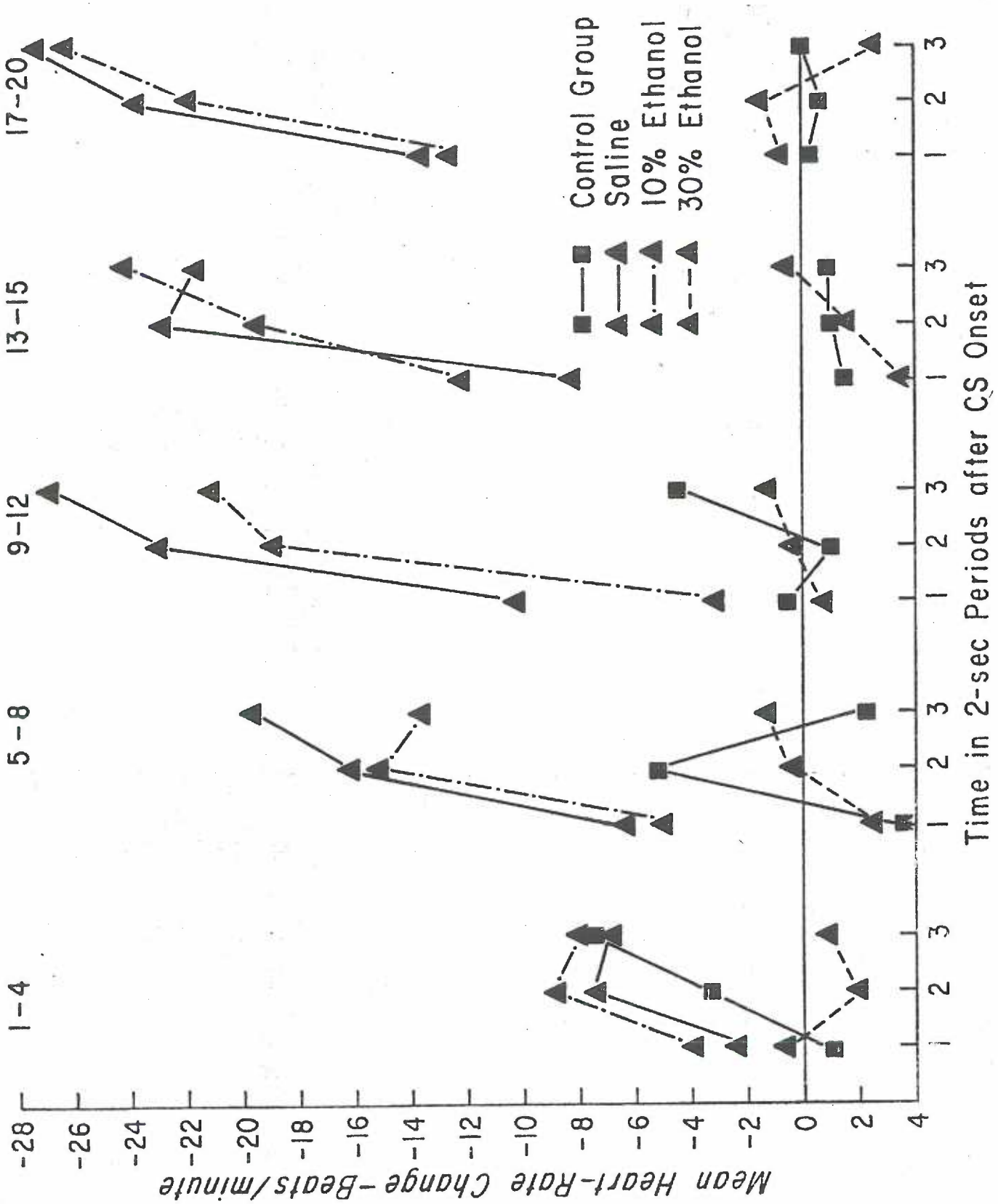

Control group
.6 ma. US intensity
1.2 ma. US intensity
Saline
10% Ethanol
30% Ethanol
Mean Heart-Rate Change - Beats/minute
-30
-28
-26
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0
+2
+4
1
2
3
4
5
Blocks of Four Trials

following results: the two saline and the two 10% groups were significantly different from the control group ($p < .01$); neither of the 30% groups was significantly different from the control group. On the basis of this analysis, it was concluded that the saline and 10% experimental groups demonstrated reliable heart-rate conditioning whereas the 30% groups did not.

Having established that reliable conditioning occurred, a 3 x 2 x 5 (Drug treatment x Shock intensity x Trials) analysis of variance with repeated measures on trials was performed on the data of just the experimental groups. The results of this analysis demonstrated a significant effect of drug treatment ($F = 27.58$, $df = 2/84$, $p < .001$), a significant effect of trials ($F = 25.03$, $df = 4/336$, $p < .001$) and a significant Drug treatment x Trials interaction ($F = 6.74$, $df = 8/336$, $p < .001$). There was no significant effect of shock intensity. A Newman-Keuls test on mean conditioned response levels revealed that all saline and 10% groups were significantly different from the two 30% groups ($p < .01$). There were no reliable differences between the saline and 10% groups.

An additional attempt was made to differentiate the performance of the experimental groups by means of a detailed analysis of the heart-rate changes that occurred in the presence of the conditioned stimulus. Figure 7 presents mean heart-rate changes in the three 2-sec. counting periods of the tone averaged over successive blocks of four acquisition trials. The different shock-intensity groups within each drug treatment condition were combined after three separate 2 x 3 x 5 (Shock intensity x Counting periods x Trial blocks) analyses of variance determined that shock intensity did not influence the form of the heart-rate changes.

Figure 7. Mean beat per minute heart-rate change of the three combined experimental and control groups in 2-second counting periods after onset of the conditioned stimulus during acquisition.


Trials
1-4
5-8
9-12
13-15
17-20
Control Group
Saline
10% Ethanol
30% Ethanol
Mean Heart-Rate Change -Beats/minute
-28
-26
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
1 2 3
Time in 2-sec Periods after CS Onset

It is readily apparent from Figure 7 that the topographies of the conditioned responses of the saline and 10% groups were highly similar with both groups showing evidence at the end of conditioning of inhibition of delay. It is also clear that the heart-rate responses of these groups were markedly different from those of the 30% groups and control group. During the first four-trial block, conditioned response magnitudes of both the saline and 10% groups were greatest during the second 2-sec. counting period. On later trials maximum conditioned response magnitude of both of these groups shifted to the third 2-sec. counting period, which immediately preceeded delivery of the shock. The heart-rate reactions of the 30% experimental and control groups failed to show any systematic changes. It may be noted, however, that the control groups showed a response similar to the conditioned responses of the saline and 10% groups during the first four-trial block.

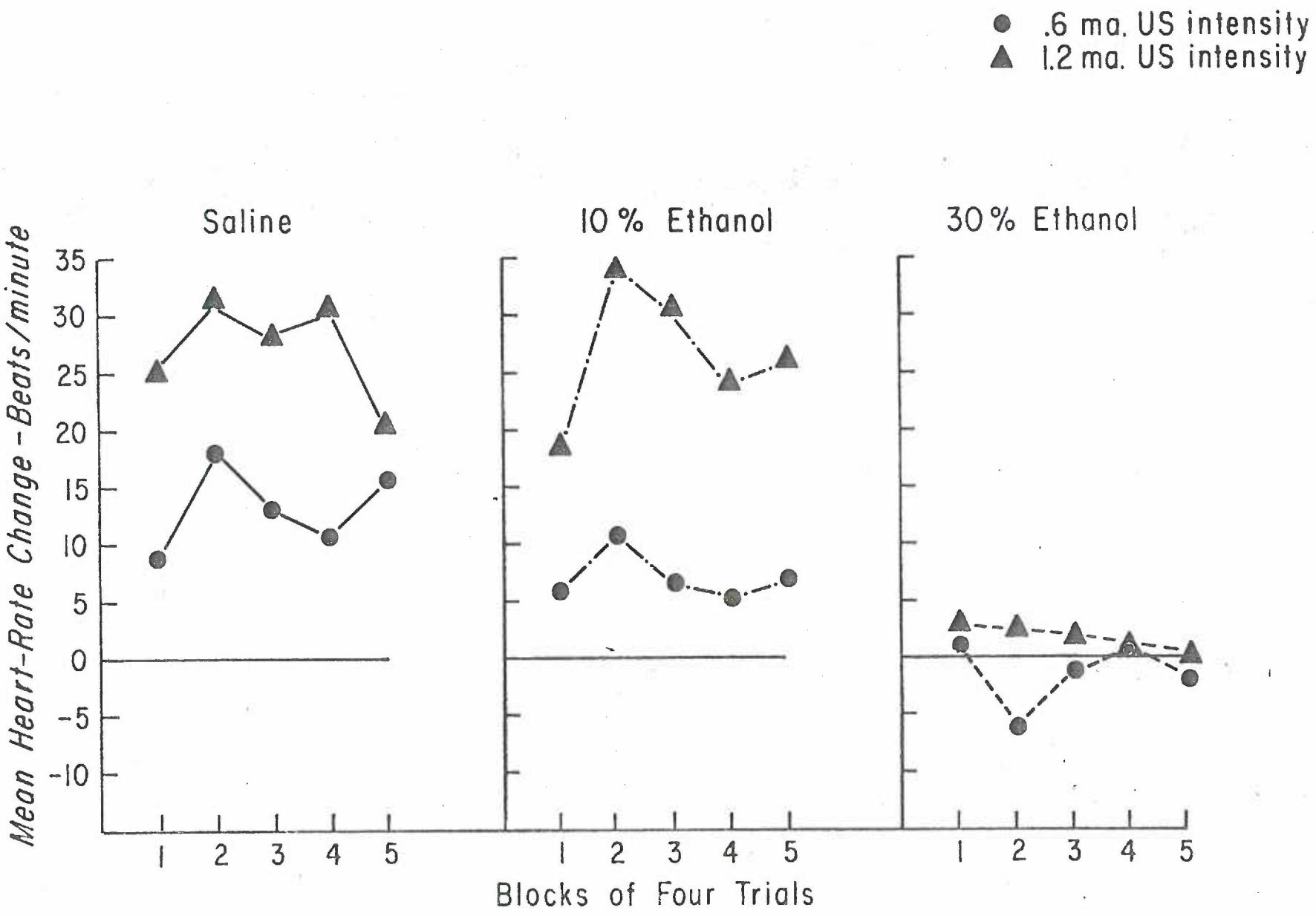
To test the reliability of the group differences shown in Figure 7 a 3 x 5 x 3 (Drug treatment x Trial blocks x Counting periods) analysis of variance with repeated measures on trial blocks and counting periods was carried out on the data of the three experimental groups. The significant outcomes of this analysis relating to the forms of the conditioned responses were: counting periods (F = 57.54, df = 2/174, $p < .001$), Drug treatment x Counting periods interaction (F = 10.14, df = 4/174, $p < .001$), Trials x Counting periods interaction (F = 3.86, df = 8/696, $p < .01$) and Drug treatment x Trial blocks x counting periods interaction (F = 1.67, df = 16/696, $p < .05$). These outcomes indicate that the experimental groups showed a reliable change in heart rate across the three counting periods; that there were significant differences

among the experimental groups in the way in which heart-rate changed across counting periods; and that the change in form of the conditioned heart-rate responses of the experimental groups was reliably different across trials.

An additional analysis performed on the data of the saline and 10% groups only demonstrated that the topographies of their conditioned responses were not reliably different. However, the analysis did indicate a significant effect of counting periods ($F = 55.52$, $df = 2/116$, $p < .001$) and a significant Trials x Counting periods interaction ($F = 4.87$, $df = 8.464$, $p < .001$). This demonstrated that the forms of the conditioned responses of these groups changed reliably across conditioning trials.

Unconditioned response. The unconditioned heart rate to shock was measured in beats per minute on each conditioning trial during an 8-sec. period beginning .7 sec. after the termination of the shock. Figure 8 presents the mean unconditioned heart-rate responses minus the baseline (pre-conditioned-stimulus) heart-rate levels of the six experimental groups plotted as a function of four-trial blocks. It is evident that the accelerative unconditioned responses of the 10% groups were comparable to those of the saline groups. In both cases, the 1.2-ma shock produced a larger increase in heart rate than did the .6-ma shock. Additionally, it should be noted that the magnitudes of the unconditioned responses of the 10% and saline groups increased from the first trial block to the second trial block and then generally declined. There was no evidence that the shocks elicited reliable changes in heart rate in either of the 30% groups.

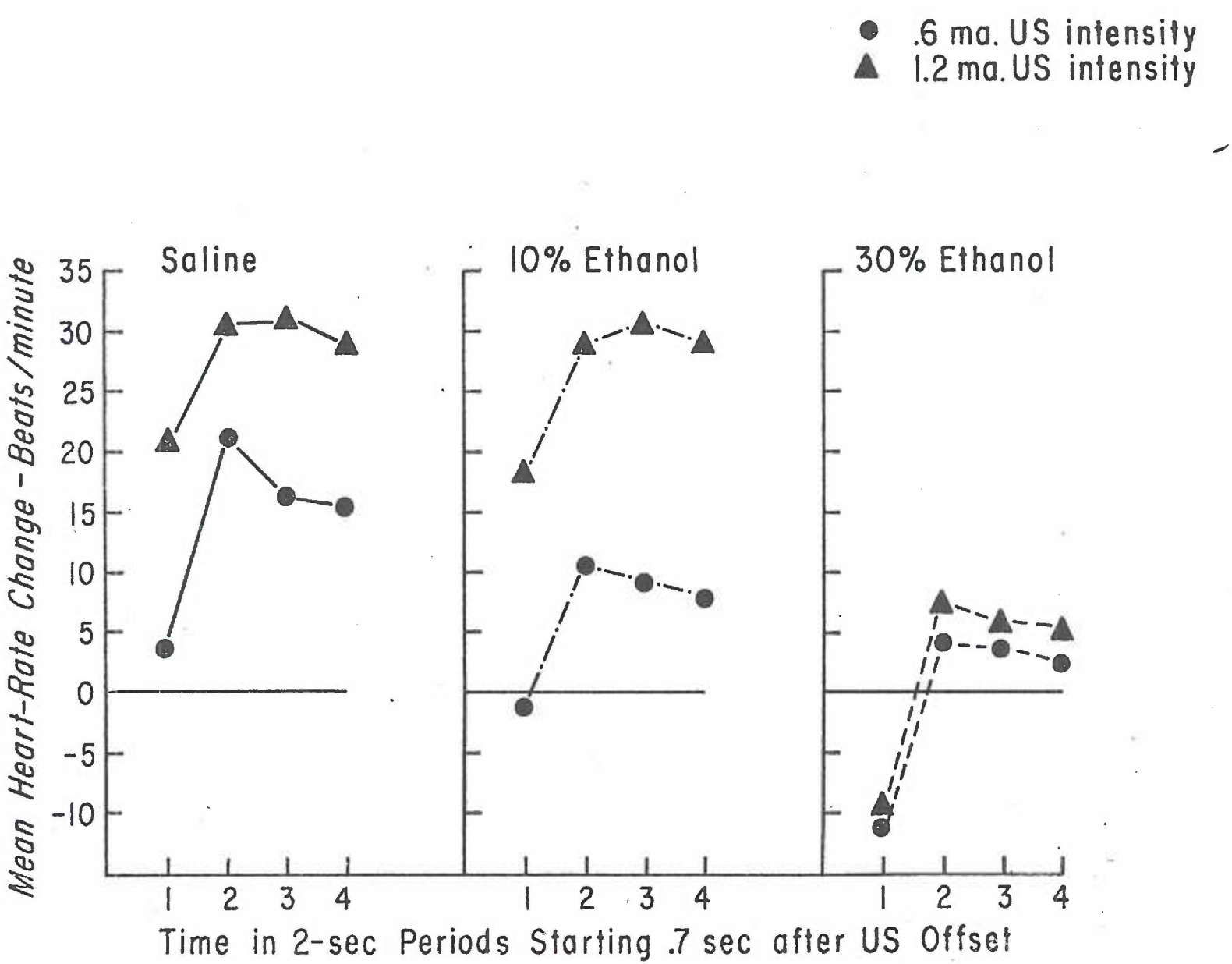
Figure 8. Mean beat per minute heart-rate reaction of the six experimental groups to the unconditioned stimulus plotted as a function of four-trial blocks during conditioning.


.6 ma. US intensity
1.2 ma. US intensity
Saline
10 % Ethanol
30 % Ethanol
Mean Heart-Rate Change - Beats/minute
35
30
25
20
15
10
5
0
-5
-10
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
Blocks of Four Trials

A 3 x 2 x 5 (Drug treatment x Shock intensity x Trial blocks) analysis of variance, indicated that there was a significant effect of drug treatment (F = 12.31, df = 2/84, $p < .001$), a significant effect of shock intensity (F = 11.47, df = 1/84, $p < .001$) and a significant effect of trial blocks (F = 3.04, df = 4/336, $p < .05$). A Newman-Keuls test demonstrated that the unconditioned responses elicited by the 1.2-ma shocks in the 10% and saline groups were reliably greater than those elicited by the .6-ma shocks ($p < .05$). The unconditioned responses of all the saline and 10% groups were reliably greater than those of the two 30% groups ($p < .05$). There was no significant difference between the two 30% groups.

Figure 9 shows the mean unconditioned heart-rate responses of the six experimental groups in four successive 2-sec. post-shock counting periods averaged over the 20 acquisition trials.
This figure reveals that the reactions of the saline and 10% groups were similar, with the smallest changes in heart rate occurring in the first 2-sec. counting period. Heart-rate accelerations were maximum in the second or third periods, and declined slightly in the final period. In sharp contrast, both of the 30% groups showed a biphasic reaction to the shocks with heart rate decelerating in the first counting period and then accelerating in the second and succeeding counting periods. An examination of individual records of the 30% subjects revealed that all 15 subjects in the .6-ma group and 11 subjects in the 1.2-ma group showed heart-rate decelerations during the first post-shock counting period.

Figure 9. Mean beat per minute heart-rate change of the six experimental groups as a function of time in 2-second counting periods after termination of the unconditioned stimulus.


.6 ma. US intensity
1.2 ma. US intensity
Saline
10% Ethanol
30% Ethanol
Mean Heart-Rate Change - Beats/minute
35
30
25
20
15
10
5
0
-5
-10
1 2 3 4
1 2 3 4
1 2 3 4
Time in 2-sec Periods Starting .7 sec after US Offset

A 3 x 2 x 4 analysis of variance (Drug treatment x Shock intensity x Counting periods) carried out on the data shown in Figure 9 produced a significant drug effect (F = 11.93, df = 2/84, $p < .001$), a significant shock intensity effect (F = 11.64, df = 1/84, $p < .001$), and a significant counting-periods effect (F = 73.24, df = 3/252, $p < .001$). Separate t tests comparing the heart-rate decreases in the first 2-sec. period against zero were significant for both 30% groups: .6-ma group (t = 8.5, df = 14, $p < .005$), 1.2-ma group (t = 3.9, df = 14, $p < .005$). Individual t tests performed on the mean heart-rate accelerations of these groups during the last three 2-sec. counting periods were also significant: .6-ma group (t = 3.23, df = 14, $p < .005$) and 1.2-ma group (t = 6.75, df = 14, $p < .005$). Taken together, these tests indicate that the biphasic unconditioned heart-rate responses of the two 30% groups were highly reliable.

DISCUSSION

The principal findings of the present experiment were: (a) Peak blood ethanol levels of the 10% (.8 gm/kg) and 30% (2.4 gm/kg) groups tested prior to the main experiment were 68 mg/100 ml and 190 mg/100 ml, respectively and were reached within 30 min. post injection. Ethanol elimination rates of the groups did not differ with the ethanol levels at 2 hr. post injection being 33 mg/100 ml for the 10% group and 148 mg/100 ml for the 30% group. (b) In the main experiment 10% and 30% ethanol had opposite effects on baseline heart rate with the 10% concentration elevating heart rate and the 30% concentration depressing heart rate. These differences existed from 10 min. post injection to the end of conditioning. (c) The heart-rate orienting response was depressed in the 10% group and absent in the 30% group. The orienting responses of the 10% and saline groups were decelerative and similar in form. Both of these groups showed a reduction in the magnitude of the orienting responses across the pre-conditioning trials. The largest component of the orienting response which occurred to the offset of the conditioned stimulus did not habituate in either group. (d) No evidence of a conditioned heart rate response was found in the 30% ethanol groups. The conditioned responses demonstrated by the 10% and saline were highly similar in magnitude and form. The 10% ethanol groups showed evidence of inhibition of delay which was comparable to that obtained in the saline group. (e) Both shock intensities elicited heart-rate deceleration in the 30% groups as measured shortly after the offset of the shock. Heart-rate deceleration was then followed by a small acceleration. In contrast,

the unconditioned heart-rate responses of the 10% and saline groups at both shock intensities were similar and generally accelerative. The magnitudes of the accelerations elicited by the high intensity were greater than those produced by the low intensity shock.

Blood Ethanol Determinations

Peak blood-ethanol levels and rates of elimination of ethanol shown by the 10% and 30% groups in the preliminary study were similar to established standards for adult rats (Wallgren, 1970). The fact that the blood-ethanol values were comparable to these standards suggests that the tight restraint and the repeated shocks delivered over a period of one hour did not alter the normal metabolism of alcohol. Prior studies (Wallgren, 1970) have also shown that ethanol metabolism was not influenced by the activity level of subjects.

Systematic determinations of blood-ethanol levels throughout a classical conditioning procedure have not been previously reported. In those few studies in which blood-ethanol levels were specified they were measured prior to and following conditioning rather than during the experimental session as was done in the present experiment. This information allowed detailed comparisons to be made between changes in heart-rate that occurred during various phases of the experiment and blood-ethanol levels.

Knowledge of the time course of the absorption and elimination of ethanol was used to plan the procedure of the main experiment. Studies with humans (Goldberg, 1943; Jones & Vega, 1972; and Jones, 1973) have shown that greater behavioral deficits may occur on the absorption-ascending limb of the ethanol curve than on the elimination-descending limb of the curve. It was not possible to complete conditioning

in the present experiment during the time that ethanol was being absorbed. Therefore, rather than having to combine heart-rate responses from the absorption and elimination phases of the blood-ethanol curve, all conditioning trials were carried out after the blood-ethanol levels had reached peak values and while subjects were on the more gradual descending limb of the curve. For similar reasons the pre-test trials with the conditioned stimulus alone were presented only during the absorption phase of the blood-ethanol curve. It was hoped that this procedure would reduce intersubject variability and provide a more uniform assessment of the effects of ethanol on heart rate during classical conditioning.

In attempting to compare the effects of ethanol in different studies it is important to consider the species and the age of the subjects employed, since both of these factors have been shown to influence the rate of metabolism of ethanol. For example, the rate of elimination of ethanol in rats has been estimated at 300 mg/kg/hr which is approximately three times as rapid as that in humans (Wallgren, 1970). Such differences require that actual blood-ethanol levels be considered and not simply the time after injection.

Baseline Heart Rate

Finding that 10% (.8gm/kg) ethanol increased heart-rate baseline is in agreement with the results of several studies in which low doses of ethanol produced mild tachycardia. Hebbelink (1962) found that a dose of .6 gm/kg which produced a peak blood-ethanol level of 30 mg/100ml increased resting heart rate in humans. Administration of 1.5 gm/kg to

humans was found to produce linear increases in heart rate with blood ethanol levels ranging from 20 mg/100 ml to 100 mg/100 ml (Doctor, Naitoh, & Smith, 1966). Clarification of the mechanisms underlying these increases in heart rate may be provided by considering studies in which a variety of measures of cardiovascular activity have been obtained following the administration of ethanol.

A number of investigations have been conducted to examine the effects of low and moderate doses of ethanol on peripheral resistance, blood pressure, cardiac output, stroke volume, and heart rate. Webb and Degerli (1965) found that .5 gm/kg of ethanol administered to anesthetized dogs produced a moderate decrease in peripheral resistance, a significant increase in cardiac output, and small increases in stroke volume and heart rate. A dose of 1.5 gm/kg was associated with a significant decrease in peripheral resistance, and significant increases in cardiac output, stroke volume, and mean arterial blood pressure. No further increases in heart rate were produced by this larger dose of ethanol.

Riff, Jain, and Doyle (1969) examined the effects of .4 gm/kg of bourbon on cardiovascular function in humans. Peak blood-ethanol levels were reached within 30 minutes and ranged from 85 mg/100 ml to 136 mg/100 ml. Ethanol produced a significant decrease in peripheral resistance and a significant increase in cardiac output which was accounted for mainly by an increase in heart rate since stroke volume did not change. Juchems and Klobe (1969) studied the effects of ethanol doses in the range from .75 gm/kg to 1.5 gm/kg on peripheral resistance, cardiac output, heart rate and stroke volume in humans. Blood ethanol levels up to 85 mg/100 ml produced a decrease in peripheral resistance and an increase in cardiac

output and heart rate. It was observed that peripheral resistance decreased, and that cardiac output increased with increasing doses of ethanol. Since no increases in stroke volume were observed at these levels, increased cardiac output was related directly to the rise in heart rate. Higher blood ethanol levels (120mg/100ml) were accompanied by further decreases in peripheral resistance and increases in cardiac output but heart rate did not increase. However, there was a relatively large increase in stroke volume.

In general, these studies showed that moderate doses of ethanol consistently decreased peripheral resistance , increased cardiac output and heart rate. High doses of ethanol decreased peripheral resistance and increased cardiac output even further but failed to elevate heart rate. In such cases stroke volume was observed to increase (Juchems & Klobe, 1969). On the basis of these results it has been suggested that low and moderate doses of ethanol depress vasomotor activity which in turn leads to decreased peripheral resistance and to reflexive tachycardia (Goodman & Gilman, 1970; Juchems & Klobe,1969, Murphree, 1973). Similar adjustments in the cardiovascular system could account for the heart-rate increases seen in the 10% groups in the present study.

As noted above, high doses of ethanol may increase stroke volume rather than heart rate (Juchems & Klobe, 1969). An increase in stroke volume could account for the fact that the baseline heart rate of the 30% group in the present study was not elevated above that of the 10% group. Studies have shown that in sufficient concentrations (5-10gm/kg) ethanol depresses respiration, produces severe bradycardia, and finally causes death(Haggard, Greenberg, Cohen, & Rakieten, 1941). However, it seems unlikely that the 2.4gm/kg dose administered to the 30% subjects

produced these effects as it was considerably below the potentially lethal dose. Furthermore, it should be noted that baseline heart rate of the 30% group was only depressed relative to that of the saline and 10% groups and was well within the normal range for adult rats (Hoskins et al., 1927). It is possible that the depression of baseline heart rate in the 30% group was due to a reduction in the level of emotionality following the administration of the ethanol.

It has also been noted that both ethanol and acetaldehyde, a metabolic product of ethanol, are catecholamine releasers, and that the catecholamines tend to cancel the hypotensive effects of ethanol and have an additive effect with ethanol on heart rate (Murphree, 1973). Some support exists for the notion that acetaldehyde may be more important than ethanol in producing heart-rate increases. James & Bear (1967) have shown increases in heart rate after infusion of acetaldehyde while comparable amounts of ethanol produced no effect. Asmussen, Hald, & Larsen (1948) have also demonstrated that infusion of acetaldehyde in humans is followed by increased heart rate. It has been proposed that these effects are produced by catecholamines released by the myocardium (Eade, 1959).

Orienting Response

Magnitude, direction, and rate of habituation of the orienting responses of the saline group were similar to those observed in previous investigations in which an auditory conditioned stimulus of moderate intensity was employed (Stern & Word, 1961; Fitzgerald & Martin, 1971). Finding that the largest deceleration in heart rate occurred at the offset of the conditioned stimulus has also been reported in other experiments (Graham & Clifton, 1966; Fitzgerald & Teyler, 1970). In the Fitzgerald and Teyler study the offset of a 1-sec. tone produced a decelerative heart-rate change that was almost twice as large as that

which occurred during the tone. Furthermore, as in the current study the offset reaction appeared to be more resistant to habituation than did the response occurring to the presence of the stimulus. These results suggest that the heart-rate orienting response in rats may consist of two components having different strengths.

Although both components of the orienting reactions of the 10% group were depressed relative to those of the saline group, there was no difference between the groups in rate of habituation of the onset reactions. Both groups showed a gradual reduction in magnitude of these responses across the pretest trials. In general, these outcomes agree with the results of a previous study (Powell, Goodwin, & James, 1971) in which low doses of ethanol in humans reduced the magnitudes of heart-rate and galvanic-skin responses to a nonreinforced conditioned stimulus but did not affect the rate of habituation of the two responses.

As pointed out earlier, the orienting responses of the 10% and 30% groups were measured during the period of time that the ethanol was being absorbed. The rapidly changing levels of circulating ethanol during the absorption phase might be expected to produce a fluctuating internal stimulus condition (Overton, 1972) that could possibly slow down the habituation of orienting responses. That this did not occur could indicate that the internal cues produced by ethanol were not a salient part of the stimulus complex eliciting the responses or that they were masked by the depressant effects of ethanol on the central nervous system.

Baseline heart rate of the 10% group (510 beats per minute) was considerabl higher than that of the saline group (470 beats per minute) during the pretest trials. On the basis of the Law of Initial Values (Wilder, 1967) an elevated heart-rate level might be expected to facilitate decelerative responses to a stimulus normally eliciting deceleration.

That this did not occur in the 10% group is consistent with the results of experiments showing that the magnitude of orienting responses and conditioned responses were not systematically related to the level of baseline heart rate (Fitzgerald & Teyler;1970: Fitzgerald & Martin, 1971). However, the possibility remains that those cardiovascular adjustments underlying changes in baseline heart rate of the 10% groups may also have interferred wtih vagal circuits known to be involved (Fitzgerald, Martin, & O'brien, 1973) in the control of decelerative orienting responses in rats.

In a recent comprehensive review of the literature Graham & Clifton (1966) cited evidence indicating that the magnitude of orienting behaviors increased with moderate increases in the intensity of the eliciting stimulus. Other experiments have shown that ethanol can interfere with central nervous system responses to auditory stimuli. For example, Grenell (1959) found that high doses of ethanol and a variety of other alcohols depressed auditory cortical responses to repetitive click stimuli in cats. In addition, DiPierri, Dravid, Schweigert, & Himwich (1968) observed that a 1gm/kg dose of ethanol depressed auditory responses to click stimuli in both the inferior colliculus and primary auditory cortex of cats. Gross, Begleiter, Tobin, & Kissin (1966) found maximum reduction of auditory evoked responses in humans 15-30 minutes after ingestion of a moderate amount of ethanol. In light of these results, it is conceivable that the effective intensity of the conditioned stimulus may have been reduced by the ethanol given the 10% group and that this in turn decreased the magnitudes of their orienting responses.

Although the above effects would of course be magnified substantially by the higher dose of ethanol administered to the 30% group, it should

not be assumed that the subjects in this group failed to show orienting responses because they could not hear the auditory conditioned stimulus. In establishing the 30% dose, preliminary observations were made of the behavior of rats in an open field situation and these indicated that while the overall level of general activity was sharply reduced in a 30 min. period following the injection of ethanol the subjects nevertheless appeared reactive to auditory and tactile stimulation.

In contrast to what was found in the present experiment, Gantt (1936) reported that 2-4gm/kg of ethanol increased the vigor of a partially habituated head-turning response in dogs. He suggested that ethanol produced a loss of cortical inhibition which had developed during the original presentations of the conditioned stimulus. Furthermore, Ingle (1973) demonstrated that ethanol delayed the habituation of a prey-catching response in frogs. He attributed this outcome to ethanol induced disinhibition within the viso-motor system of frogs. The only other drug study providing evidence bearing on this issue was that of Teitlebaum, et al., (1970). They found that approximately 7-15 min. after the administration of 15-18mg/kg of phenobarbital sodium, the decelerative heart-rate component of the orienting response in dogs was of larger magnitude than it had been prior to drug introduction. It was proposed that the drug blocked the normal inhibitory activity of the reticular formation and that this augmented and prevented habituation of the heart-rate response.

The general suggestion offered in these studies was that ethanol as well as phenobarbital magnified unlearned responses through a disinhibiting action on inhibitory processes. In the Gantt & Ingle experiments the test stimuli were presented immediately after ethanol was administered and although blood ethanol levels were not reported it can be assumed that the orienting responses were measured while the ethanol

was still being absorbed. In the present study, the pretest trials were started approximately 15 min. after the ethanol injection and suppression rather than augmentation of the orienting response occurred. Therefore, one factor which may help explain this apparent divergence is the difference in time after ethanol administration that the pretest stimuli were presented.

Several studies have shown that general motor activity increases shortly after the administration of ethanol. Eriksson & Wallgren (1967) found that ethanol increased open field activity in rats. As noted above, Gantt (1936) observed that skeletal motor activity as measured by head turning toward an auditory stimulus was augmented by a large dose of ethanol. These results raise the possibility that in the present experiment 10% ethanol produced increased motor activity to the conditioned stimulus during the pretest trials. Such an increase would have worked against the normal decelerative heart-rate orienting response. However, a comparison of the orienting responses of the 10% and saline groups indicated that the degree of depression of response magnitude produced by 10% ethanol was highly consistent over the seven counting periods of the pretest trials. Such consistent differences cannot be easily explained by reference to differences in skeletal motor activity. Furthermore, a check of the polygraph records showed no obvious differences in movement artifacts between the 10% and saline groups during the pretest trials.

In addition to the previously discussed considerations it is also possible that the decrement in orienting response magnitude produced by 10% ethanol was due to a general reduction in motivation level. If this were the case it seems possible that random presentation of shocks during the pretest trials might have attenuated the response depression shown by the 10% subjects.

Conditioned Responding

In contrast to its debilitating effects on orienting responses, 10% ethanol had no visible influence on conditioning. In each of the 10% groups, the magnitudes of the conditioned responses, the rates at which they developed and their basic form were all similar to those found in the saline groups. This was true for both intensities of shock.

It is possible to mention several factors that may have contributed to the failure of 10% ethanol to influence conditioning while depressing the magnitude of the orienting response. Based on the results provided by the preliminary study it was possible to estimate that the blood-ethanol levels of the 10% group averaged approximately 62mg/100ml during the pretest trials with the conditioned stimulus alone and approximately 50mg/100ml during conditioning. Although the overall concentration of ethanol in the blood was, therefore, slightly lower during conditioning than during pretest it seem unlikely that this could account for the failure of 10% ethanol to affect conditioning performance. Further evidence against this possibility was provided by the fact that the conditioned responses of the 10% and saline groups were similar during the first part of acquisition even though blood ethanol levels of the 10% group at this point were comparable to those present during the pretest trials.

A related consideration is that the pretest trials were presented during the ascending-absorption phase of the blood-ethanol curve while conditioning was carried out during the descending elimination phase of the curve. This distinction may be important since studies (Jones & Vega, 1972; Jones, 1973) have shown that at identical blood-ethanol levels memory tasks in humans were more severely impaired when ethanol was being absorbed than when it was being eliminated. One interpretation

offered for their findings was that organisms may develop a tolerance to the presence of ethanol which might reduce its effects on behavior. It is conceivable that in the present study such adjustments to ethanol occurred during the 30 minute interval between the administration of ethanol and the onset of the acquisition trials.

An additional factor that may have contributed to the differential effects of 10% ethanol was baseline heart rate. The mean heart rate of the 10% groups to the conditioned stimulus during pretest trials was approximately 510 beats per minute whereas during conditioning it was 480 beats per minute. As noted previously, the 510 beat per minutes heart rate was well above levels reported in previous studies and may have interferred with the performance of the decelerative orienting response. On the other hand, the 480 beat per minute rate was comparable to levels reported in prior investigations in which robust conditioning was obtained (Fitzgerald, Vardaris, & Brown, 1966).

Failure to find that 10% ethanol influenced classically conditioned heart rate is in contrast with the results of prior studies in which comparable doses of ethanol seemed to suppress conditioning. In attempting to account for this apparent discrepancy, it should be noted that none of the earlier studies employed adequate control procedures to rule out such factors as sensitization and placebo effects. Without these controls it is not possible to provide an accurate assessment of the influence of ethanol on conditioning.

Franks (1963) reported that the conditioned eyeblink responses of humans receiving 1.0 ml of 90 proof whiskey (.4 gm/kg of ethanol) were not different from those of a placebo group receiving a total of 5 ml of whiskey (approximately .05 gm/kg of ethanol). However, Franks mentioned

that the level of conditioning achieved by both of these groups was somewhat depressed relative to that obtained in a prior experiment (Franks, 1960) employing non-treated humans. This suggests the possibility that while the .4 gm/kg dose of ethanol may have interferred with conditioning a similar effect may have been produced by the placebo treatment.

In a later study Hobson (1966) demonstrated that two doses of ethanol: .68 gm/kg and 1.4 gm/kg, administered in a carbonated beverage, depressed the development of a conditioned eyeblink response in humans. In contrast to Franks (1963), the placebo group was given a carbonated beverage with no ethanol. In view of the possible placebo effect shown by Franks it is difficult to conclude that the depression in conditioning observed in the .68 gm/kg group was in fact due specifically to the effects of ethanol.

In discussing his results, Hobson noted that the air-puff unconditioned stimulus that he used which measured 80 mm of Hg was less than one-half as intense as the 170 mm of Hg air puff employed by Franks. Hobson suggested that the high intensity puff may have prevented the ethanol from suppressing the conditioned eyeblink response in the Franks study. In this context, it is conceivable that the .6 ma shock in the present experiment exerted an overriding influence on the possible suppressive effects of ethanol on heart-rate conditioning.

McGonnell and Beach (1968) found that .6 gm/kg of ethanol mixed in a carbonated beverage produced a decrement in magnitude of the conditioned galvanic skin response in humans. This effect was based on a comparison of the ethanol group with a placebo group receiving 3 ml of ethanol floated on the surface of the carbonated beverage. As a control for

sensitization, a non-drug group receiving unpaired presentations of the conditioned and unconditioned stimuli was included but statistical comparisons between this group and the ethanol and placebo groups were not made. Visual inspection of the published data indicated that when compared to the response level of the non-drug sensitization group the ethanol group actually appeared to show no conditioning. Although it seems unlikely that a low dose of .6 mg/kg of ethanol would have completely blocked conditioning of the galvanic skin response, these results demonstrate the crucial importance of employing appropriate control conditions when studying the effects of drugs on conditioning.

Most prior studies concerned with the actions of ethanol on classical conditioning have focused on the magnitude or frequency of conditioned responses. In one of the few studies examining other aspects of conditioning performance, Gantt (1936) found that doses of ethanol higher than the low dose employed in the present study increased the latencies of both motor and salivary responses. Latency of the maximum decelerative component of the conditioned responses of the 10% group in the present experiment was like that shown in the saline group. On early acquisition trials maximum heart-rate deceleration in both the saline and 10% groups occurred during the second 2-second counting period of the 6-second conditioned stimulus interval. Thus, even when blood-ethanol levels of the 10% group were maximal conditioning performance was not different from the saline group. By the end of acquisition the topographies of the conditioned responses changed with the locus of maximum heart-rate decelerations of both groups shifting to the third or final 2-second period. Previous studies have shown that the detailed

detailed topographies of conditioned heart-rate reactions in rats were influenced by the type of conditioning procedure, e.g. trace or delay, and by intensity of the unconditioned stimulus. Progressive changes in the topography of the conditioned responses have also been used to help distinguish conditioning from sensitization (Fitzgerald & Teyler, 1970; Fitzgerald & Martin, 1971). These results suggest that topography of the conditioned heart-rate response may reflect a basic process of conditioning. The present findings indicate that this process was not disturbed by a .6 gm/kg dose of ethanol.

The final point to be made with respect to the conditioning results has to do with the fact that the 30% groups showed no evidence of conditioned changes in heart rate. As indicated earlier, there is considerable evidence that the 2.4 gm/kg dose of ethanol given the 30% group would have marked effects on the cardiovascular system (Juchems & Klobe, 1969). Conceivably, conditioning in the 30% group may have occurred but the performance of the response was not demonstrated because of the state of the cardiovascular system. On the other hand, the ethanol may have disrupted central nervous system activity to such an extent that conditioned associations were not established. To answer this question it would have been necessary to test for conditioning after the effects of ethanol had worn off.

Doses of ethanol comparable to that given the 30% subjects are also known to interfere with the neural processing of auditory stimulation (Begleiter, Tobin, & Kissin, 1966) and to raise pain thresholds (Wickler, et al., 1945). Since there was a complete absence of responding to the conditioned stimulus by the 30% subjects both during habituation and

conditioning it might be that these subjects were not able to detect the tone stimulus against the white noise background. Obviously, under these circumstances conditioning could not occur.

Unconditioned Responding

The results of the present investigation provided no evidence that 10% ethanol influenced the unconditioned responses elicited by the .6-ma and 1.2-ma unconditioned stimuli. The magnitudes and detailed characteristics of these responses in the 10% groups were very similar to those shown by the saline groups. In contrast, 30% ethanol produced a marked reduction in the magnitude and altered the form of the unconditioned responses of both the .6-ma and 1.2-ma groups.

An ethanol produced increase in the pain thresholds of the 30% subjects would have reduced the painfulness or activating consequences of the reinforcing shocks. Evidence that this may have occurred was suggested by the fact that magnitude of unconditioned responses of the 30% group were greatly depressed. Such a mechanism could account for the absence of conditioning since results of several recent investigations indicate that it may be necessary to use an unconditioned stimulus that has motivational or painful components to establish conditioned heart rate responses (Teitlebaum, Gantt, & Stone, 1956; Fitzgerald, Martin, & Hoffman, 1975). The fact that the unconditioned stimulus may elicit an unconditioned response does not insure that conditioning will occur.

The accelerative responses to shock exhibited by the 10% and saline subjects were consistent with the results of several previous investigations. Stern and Word (1961), Holdstock and Schwartzbaum (1965), and

Fitzgerald and Martin (1971) all reported accelerative heart-rate responses to moderate and high intensity shocks in restrained rats. It has also been observed that the magnitude of the unconditioned heart-rate response increased with the intensity of shock (Holdstock & Schwartzbaum, 1965; Fitzgerald & Martin, 1970), and that the level of conditioning increased up to a point with the intensity of shock (Fitzgerald & Teyler, 1970). Finding that 10% ethanol did not reduce the magnitude of the heart-rate response elicited by the .6-ma shock perhaps indicates that this intensity was set too high in relation to the low dose of ethanol.

Further support for the potency of the .6-ma shock was provided by the fact that it led to the same level of conditioning as that obtained with the 1.2-ma shock. On the basis of these results it appears that under some circumstances, there may be an optimal level of unconditioned stimulus intensity beyond which conditioning is no longer improved.

Although the mean unconditioned responses to shock averaged across all counting periods and conditioning trials is usually accelerative, it should be noted that exclusive emphasis on this measure may obscure finer characteristics of the response. In both the Stern and Word (1961) and Fitzgerald and Teyler (1970) studies, detailed analysis of the unconditioned responses showed that initial responses to shock presentations were decelerative. The results of the Fitzgerald and Teyler (1970) study indicated that the mean unconditioned response to six different US intensities was heart-rate deceleration to the first shock presentation. Additionally, the magnitude of the deceleration was greater in those groups which received the more intense shocks. By the

third shock presentation the responses of the four highest intensity shock groups had changed to acceleration while the initial decelerative responses of the two lowest groups habituated and did not change to accelerations. Although mean-group decelerative unconditioned responses have not been observed in subsequent studies (Fitzgerald & Martin, 1971), varying numbers of individual rats have shown the effect. An adequate explanation of heart-rate deceleration to painful shock remains to be formulated.

In the present study, marked decelerative responses in the first 2-sec. interval after shock appeared only in the 30% groups, although the responses of the 10%-.6-ma. group during the same interval were slightly decelerative. Inspection of the individual records of subjects in the saline and 10% groups showed that approximately one-half the subjects displayed decelerative responses to the presentation of the first unconditioned stimulus as measured shortly after the stimulus. The reason for the variability among subjects in the direction of the response is not known. On the other hand, there was very little variability among the 30% subjects in the direction of the response to shock during the first 2-sec. period after unconditioned stimulus presentation. All 15 subjects in the 30%-.6-ma group and 11 of 15 subjects in the 30%-1.2-ma group showed consistent decelerations across conditioning trials. The decelerative component of the response was limited to the first 2-sec. counting period as in subsequent postshock periods these subjects displayed a small increase in heart rate.

Several factors may have been important in producing the consistent decelerative responding demonstrated by the 30% subjects. First, it is

highly likely that the 2.4 gm/kg dose of ethanol significantly reduced the aversiveness of the tail shock. Considerable support exists for the analgesic and anesthetic properties of ethanol (Wickler, Goodell, & Wolff, 1945; Dundee, 1970; Goodman & Gilman, 1970). The presentation of a painful shock is usually associated with a burst of skeletal motor activity and physiological stress responses. These reactions often overlap temporally with post shock heart-rate accelerations and may in fact contribute to these increases. Buckalew and Cartwright (1968) demonstrated that doses of ethanol lower than 2.4 gm/kg greatly reduced general skeletal motor activity in freely-moving rats. Personal observations during the preliminary phase of the present study also indicated that 30% ethanol greatly reduced shock-produced skeletal-motor activity. Miller and DiCara (1968) reported that curare in a dose that completely paralyzed skeletal responses reduced the accelerative heart-rate conditioned response of rats to an intense 3.0 ma tail shock. This suggests that pain produced skeletal motor activity may be an important factor contributing to heart-rate acceleration. Conceivably, the absence of motor activity in the 30% groups contributed to the heart-rate decelerations shown by this group.

Pappas, DiCara and Miller (1972) provided evidence that increased sympathetic output may be an important factor in the control of accelerative heart-rate responses of rats to shock. They found that in curarized, chemically sympathectomized rats the magnitude of the unconditioned response was greatly attenuated in comparison to that of normals. The unconditioned stimulus employed was a .5-ma, .3-sec. shock to both hind paws. The authors suggested that the reductions in response

magnitude were due primarily to reduced sympathetic output. It is quite possible that 30% ethanol in the current experiment interferred with the normal integrative action of the sympathetic and parasympathetic divisions of the autonomic nervous system and that this contributed to the decelerative component of the unconditioned response and to the reduction in magnitude of the accelerative component.

Another factor that may have helped determine the characteristics of the responses of the 30% subjects was that ethanol may have increased the latency of the responses to shock. Evidence has been provided that ethanol in the range of the 30% dose increased the latency of both unconditioned and conditioned motor and salivary responses in dogs (Gantt, 1936). In the present study, the persistence of the initial decelerative component of the biphasic response to shock may have been due to a considerable increase in the latency of this component. It is possible that the normal instantaneous response of restrained rats to shock is heart-rate deceleration but that measurement techniques which lock out electrocardiogram recording during shock presentations prevent it from being observed. Individual differences in latency of responding to the shock may account for the fact that some subjects show initial decelerations while others show accelerations.

Although a clear distinction between orienting responses and unconditioned responses does not exist, several operational criteria do allow some general distinctions to be made. Firstly, orienting responses normally habituate to repeated presentations of the eliciting stimulus while unconditioned responses are much more resistant to habituation. In heart-rate conditioning studies where restrained rats were used, low

intensity stimuli normally produced decelerative responses which habituated as a function of repeated presentations of the stimuli (Graham & Clifton, 1966; Fitzgerald & Teyler, 1970; Fitzgerald & Hoffman, 1975). Secondly, unconditioned responses are capable of supporting the development of conditioned responses while orienting responses are not assumed to have this property. It appears that whether a response occurring to a given stimulus is an orienting response or an unconditioned response depends to a large extent on the intensity and/or motivational significance of the eliciting stimulus. On the basis of these criteria the decelerative heart-rate response to shock exhibited by the 30% subjects would seem to be more appropriately labelled an orienting response rather than an unconditioned response. A similar suggestion has been offered to account for the temporary decelerations to shock observed in prior studies (Fitzgerald & Teyler, 1970).

In this context, it is also possible that the conditioning procedure employed in the current study may have contributed to the initial decelerative component of the unconditioned responses shown by some of the subjects in the 10% and saline groups. In this paradigm the conditioned stimulus overlapped the unconditioned stimulus with both stimuli terminating together. Since the relatively large decelerative orienting responses to the offset of the conditioned stimulus on the pre-test trials did not habituate, it is possible that this response persisted during acquisition and contributed to the decelerative responses observed during the first 2-sec. post-shock counting period.

REFERENCES

Andreyev, L.A. The effect of single and repeated doses of alcohol on conditioned reflexes in the dog. Archives Internationales de Pharmacodynamie et de Therapie, 1934, 48, 117-128.

Amussen, E., Hald, J., & Larsen, V. The pharmacological action of acetaldehyde on the human organism. Acta Pharmacologica et Toxicologica (Scandanavia), 1948, 4, 311-320.

Barthalomew, A.A., Franks, C.M., & Marley, E. Succeptibility to methylpentynol: Eyelid conditioning and P.G.R. response. Journal of Mental Science, 1958, 104, 1167-1173.

Begleiter, H., Brauchey, M.H., & Kissin, B. Effects of ethanol on evoked potentials in the rat. Behavioral Biology, 1972, 7, 137-142.

Buckalew, L.W., & Cartwright, G.M. General and differential effects of five ethanol dosages on the albino rat. Psychological Reports, 1968, 23, 1151-1154.

Conger, J.J. The effects of alcohol on conflict behavior in the albino rat. Quarterly Journal of Studies on Alcohol, 1951, 12, 1-29.

DiPierri, R., Dravid, A., Schweigert, A., & Himwich, H.E. Effects of alcohol on evoked potentials of various parts of the central nervous system of cat. Quarterly Journal of Studies on Alcohol, 1968, 29, 20-37.

Doctor, R.F., Naitoh, P., & Smith, J.C. Electroencephalographic changes and vigilance behavior during experimentally induced intoxication with alcoholic subjects. Psychosomatic Medicine, 1966, 28, 605-615.

Dundee, J.W. Intravenous ethanol anesthesia: a study of dosage and blood levels. Anesthesia and Analgesia (Cleveland), 1970, 49, 467-475.

Dworkin, S., Bourne,W., & Raginsky, B.B. Changes in conditioned responses brought about by anesthetics and sedatives. Canadian Medical Association Journal, 1937, 37, 136-139.

Eade, N.R. Mechanism of sympathomimetic action of aldehydes. Journal of Pharmacology and Experimental Therapy, 1959, 127, 29-34.

Eriksson, K., & Wallgren, H. Behavior of rats under the influence of ethyl alcohol in an open-field situation. Scandanavian Journal of Psychology, 1967, 8, 257-267.

Gantt, W.H., Effect of alcohol on cortical and subcortical activity measured by the conditioned reflex method. Bulletin of the Johns Hopkins Hospital, 1935, 56, 61-83.

Gimeno, A.L., Gimeno, M.F., & Webb, I.L. Effect of ethanol on cellular membrane potential and contractility of isolated rat atrium. American Journal of Physiology, 1962, 203, 194-200.

Girden, E. & E. Culler. Conditioned responses in curarized striate muscle in dogs. Journal of Comparative Psychology, 1937, 23, 261-274.

Goldberg, L. Quantitative studies on ethanol tolerance in man: the influence of ethyl alcohol on sensory, motor and psychological functions referred to blood alcohol in normal and habituated individuals. Acta Physiologica Scandanavia, 1943, 5, Suppl. No. 16, 1-128.

Goodman L.B., & Gilman, A. (Eds.). The Pharmacological Basis of Therapeutics. New York: MacMillan Co., 1970.

Graham, F.K., & Clifton, R.K. Heart-rate change as a component of the orienting response. Psychological Bulletin, 1966, 65, 305-320.

Grenell, R.G. Alcohols and the activity of cerebral neurons. Quarterly Journal of Studies on Alcohol, 1959, 20, 421.

Gross, M.M., Begleiter, H., Tobin, M., & Kissin, B. Changes in auditory evoked response induced by alcohol, Journal of Nervous & Mental Disease, 1966, 143, 152-156.

Fitzgerald. R.D., & Hoffman, J.W. Classically conditioned heart rate in rats following preconditioning exposure to the CS. (In Press).

Fitzgerald, R.D., & Martin, G.K. Heart-rate conditioning in rats as a function of inter-stimulus interval. Psychological Reports, 1971, 29, 1103-1110.

Fitzgerald, R.D., Martin, G.K., & Hoffman, J.W. Classical conditioning of heart rate in rats using direct vagal stimulation as a US. Physiology and Behavior, 1975, 14, 449-456.

Fitzgerald, R.D., Martin, G.K., & O'Brien, J.H. Influence of vagal activity on classically conditioned heart rate in rats. Journal of Comparative and Physiological Psychology, 1973, 83, 485-491.

Fitzgerald, R.D. & Teyler, T.J. Trace and delayed heart-rate conditioning in rats as a function of US intensity. Journal of Comparative and Physiological Psychology, 1970, 70, 242-253.

Fitzgerald, R.D., Vardaris, R.M., & Brown, J.S. Classical conditioning of heart-rate deceleration in the rat with continuous and partial reinforcement. Psychonomic Science, 1966, 6, 437-438.

Fitzgerald, R.D., Vardaris, R.M., & Teyler, T.J. An on-line method for measuring heart rate in conditioning experiments. Psychophysiology, 1968, 4, 352-353.

Franks, C.M. Conditioning and abnormal behavior. In H.J. Eyesenck (Ed.), Handbook of abnormal psychology. London: Pitman Medical Publishers, 1960, 457-487.

Franks, C.M. The apparent failure of ethyl alcohol to inhibit the formation of conditioned eyeblink responses in man. Psychopharmacologica, 1963, 4, 433-440.

Franks, C.M., & Laverty, S.G. Sodium amytal and eyelid conditioning. Journal of Mental Science. 1955, 101, 654-663.

Franks, C.M., & Trouton, D. Effects of amobarbital sodium and dexamphetamine sulfate on the conditioning of the eyeblink response. Journal of Comparative and Physiological Psychology, 1958, 51, 220-222.

Haggard, H.W., Greenberg, L.A., Cohen, L.H., & Rakieten, N. Studies on the absorption, distribution and elimination of alcohol; IX, The concentration of alcohol in the blood causing primary cardiac failure. Journal of Pharmacology and Experimental Therapeutics, 1941, 71, 358-361.

Hebbelink, M., The effects of a small dose of ethyl alcohol on certain basic components of human physical performance: I, the effect on cardiac rate during muscular work. Archives of International Pharmacodynamics, 1962, 140, 61

Hildago, J., Tarelton, W., Dileo, R.J., & Thompson, C.R. Effects of drugs on the cardiac conditioned response of dogs. Behavior Research and Therapy, 1969, 6, 461-471.

Hobson, G.N. Ethanol and conditioning. Quarterly Journal of Studies on Alcohol, 1966, 27, 612-619.

Holdstock, T.L., & Schwartzbaum, J.S. Classical conditioning of heart rate and galvanic skin response in the rat. Psychophysiology, 1965, 2, 25-38.

Hord, D.J., Lubin, A., & Johnson, L.C. The evoked heart-rate response during sleep. Psychophysiology, 1966, 3, 46-54.

Hoskins, R.G. Lee, M.O., & Durrant, E.P., The pulse rate of the normal rat. American Journal of Physiology. 1927, 82, 621-629.

Ingle,D. Reduction of habituation of prey-catching activity by alcohol intoxication in the frog. Behavioral Biology, 1973, 8, 123-129.

James, T.N. & Bear, E.S. Effects of Ethanol and Acetaldehyde on the heart. American Heart Journal, 1967, 74, 243-255.

Johnson, L.C. & Lubin, A. The orienting reflex during waking and sleeping. Conditional Reflex, 1967, 2, 160.

Jones, B.M. Alcohol and memory impairment: a reinterpretation of the dose-response phenomenon. Biological Psychology Bulletin, 1973, 3. 2-8.

Jones, B.M., & Vega, A. Cognitive performance measured on the ascending and descending limbs of the blood alcohol curve. Journal of Abnormal Psychology, 1973, 82, 24-32.

Juchems, R., & Klobe, R. Hemodynamic effects of ethyl alcohol in man. American Heart Journal, 1969, 78, 133-134.

Kalant, H. & Czaja, C. The effect of repeated alcoholic intoxication on adrenal ascorbic acid and cholesterol in the rat. Canadian Journal of Biochemistry and Physiology, 1962, 40, 975-981.

Kimble, G.A.(Ed.) Hilgard and Marquis' Conditioning and Learning (rev. ed.). New York: Appleton-Century, 1961, 238-280.

Lebdinsky, N. (Anesthetic and narcotic effects on conditioned responses) In G.V. Anrep (Ed. and trans.) I.P. Pavlov: Conditioned Reflexes. New York: Dover, 1927.

Masserman, J.H. & Yum, K.S. An analysis of the influence of alcohol on experimental neurosis in cats. Psychosomatic Medicine, 1946, 8, 36-52.

McGonnell, P.C. & Beach, H.D. The effects of ethanol on the acquisition of a conditioned GSR. Quarterly Journal of Studies on Alcohol, 1968, 29, 845-855.

Mendoza, L.C., Hellberg, K., Rickart, A., Tillich, G., & Bing R.J. The effect of intravenous ethyl alcohol on coronary circulation and myocardial contractility of the human and canine heart. Journal of Chemical Pharmacology, 1971, 11, 165-176.

Miller, N.E. & DiCara, L.V. Instrumental learning of heart-rate changes in curarized rats: shaping, and specificity to a discriminitive stimulus. Journal of Comparative and Physiological Psychology, 1967, 63, 12-19.

Murphree, H.B. Electroencephalographic and other evidence for mixed depressant and stimulant actions of alcoholic beverages. Annals of the New York Academy of Sciences, 1973, 215, 325-331.

Nakai, Y. Effects of intravenous infusion of central depressants on the evoked potentials of the auditory cortex in cats. Japanese Journal of Pharmacology, 1964, 14, 235.

Nikiforovsky, P.M.(Ethanol effects on classically conditioned salivary responses) Thesis, St. Petersburg, 1910. (Archives Internationales de Pharmacodynamie et de Therapie, 1934, 48, 118.

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) Physiology and Behavior. New York: Plenum, 1972, 193-217.

Pappas, B.A., DiCara, L.V. & Miller, N.E. Acute sympatechtomy by 6-hydroxydopamine in the adult rat: effects on cardiovascular conditioning and fear retention. Journal of Comparative and Physiological Psychology.1972, 79, 230-236.

Pavlov, I.P. Conditioned Reflexes. (G.V. Anrep, Ed. and trans.) New York: Dover, 1927.

Powell, B.J., Goodwin, D.W., Janes, C.L.,& Hoine, H. State-dependent effects of alcohol on autonomic orienting responses. Psychonomic Science, 1971, 25, 305-306.

Regan, T.J., Levinson, G.E., Oldewurtel, H.A., Frank, M.J., Weisse, A.B., & Moschos, C.B. Ventricular function in noncardiacs with alcoholic fatty liver: role of ethanol in the production of cardiomyopathy. Journal of Clinical Investigation, 1969, 48, 397-407.

Riff, D.R., Jain, A.C., & Doyle, J.T. Acute hemodynamic effects of ethanol on normal human volunteers. American Heart Journal, 1969, 78, 592-597.

Settlage, P.H. The effect of sodium amytal on the formation and elimination of conditioned reflexes. Journal of Comparative Psychology, 1936, 22, 339-343.

Sokolov, E.N. Neuronal models and the orienting reflex. In M.A. Brazier (Ed.), The Central Nervous System and Behavior. New York: Josiah Macy Jr. Foundation, 1960, 187-276.

Sokolov, E.N. Higher nervous functions: the orienting reflex. Annual Review of Physiology, 1963, 25, 545-580.

Stern, J.A. & Word, T.J. Changes in cardiac response of the albino rat as a function of electroconvulsive seizures. Journal of Comparative and Physiological Psychology, 1961, 54, 389-394.

Teitlebaum, H.A., Gantt, W.H., & Stone, S. Cardiac conditional reflexes can be formed to pain but not to acetylcholine. Journal of Nervous and Mental Disease, 1956, 123, 484-490.

Teitlebaum, H.A., Newton, J.E.O., & Gantt, W.H. Effects of pentobarbital sodium anesthesia and neurohumoral agents on the cardiac orienting reflex. Conditional Reflex, 1970, 5, 6-26.

Wallgren, H. Relative intoxicating effects on rats of ethyl, propyl, and butyl alcohols. Acta Pharmacologica and Toxicologica, 1960, 16, 217-222.

Wallgren H., & Barry, H. III, Actions of Alcohol (Vol. 3). New York: Elsevier Pub. Co., 1970, 36-73.

Webb, W.R. & Degleri, I.U. Ethyl alcohol and the cardiovascular system. Journal of the American Medical Association, 1965, 191, 77-80.

Webb, W.R. & Degleri, I.U. Ethyl alcohol and the cardiovascular system; effects on coronary blood flow. Journal of the American Medical Association, 1969, 191, 77-80.

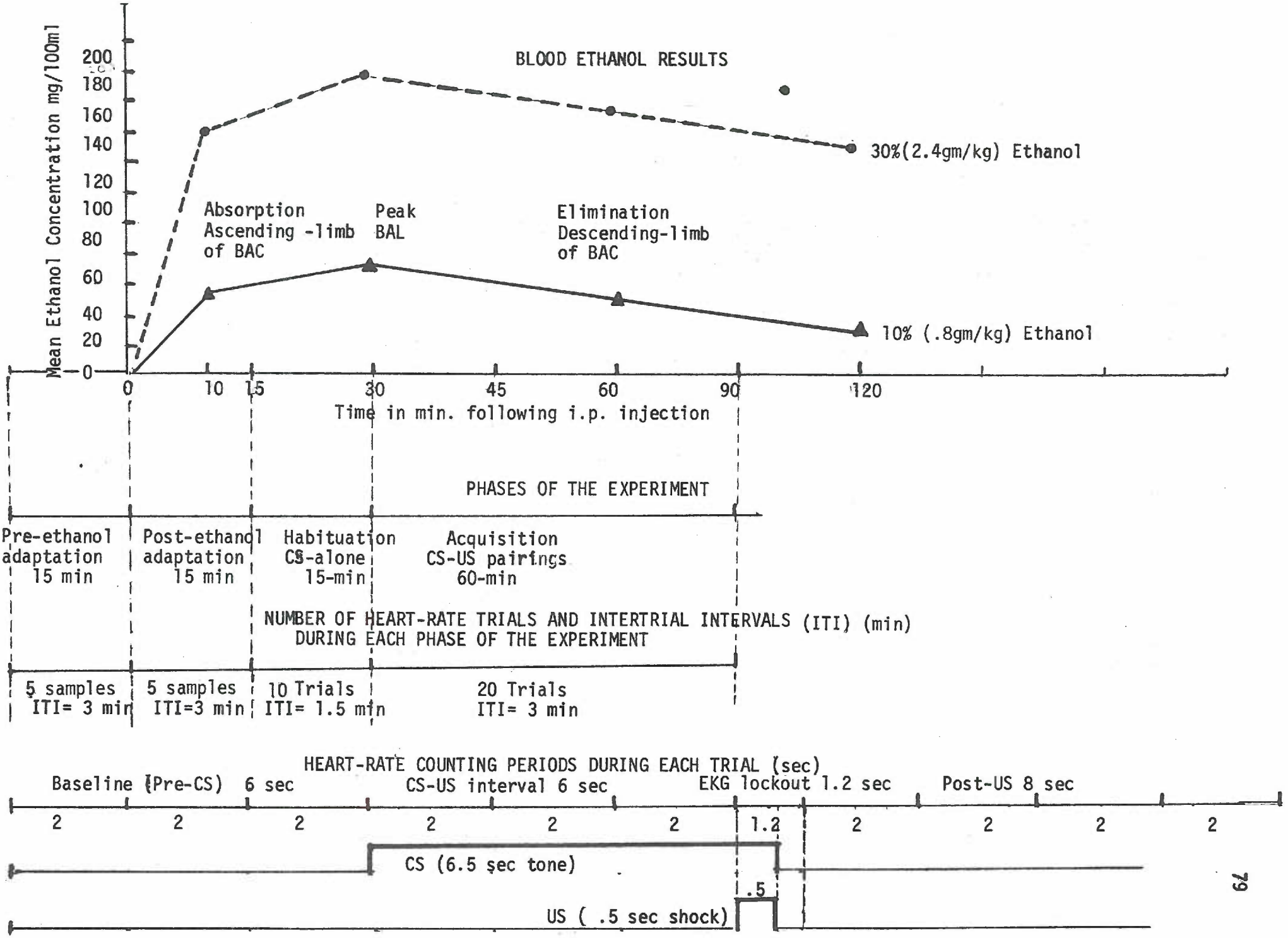
Wilder, J. Stimulus and response: the law of initial value. Bristol: John Wright & Sons, 1967.

Wikler, A., Goodell, H., & Wolff, H.G. Studies on pain: the effects of analgesic agents on sensations other than pain. Journal of Pharmacology and Experimental Therapeutics, 1945, 83, 294-299.

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

Woodworth, R.S. & Schlossberg,H. Experimental Psychology (Rev. ed.). New York: Holt , Reinhart, and Winston, 1954.

Zavadski, I.V. (The effect of ethanol on classical conditioning), Transactions of the Society of Russian Physicians, 1908. (Archives Internationales de Pharmacodynamie et de Therapie, 1934, 48, 117-118.


BLOOD ETHANOL RESULTS
Mean Ethanol Concentration mg/100ml
200
180
160
140
120
100
80
60
40
20
0
30%(2.4gm/kg) Ethanol
Absorption
Ascending -limb
of BAC
Peak
BAL
Elimination
Descending-limb
of BAC
10% (.8gm/kg) Ethanol
0
10
15
30
45
60
90
120
Time in min. following i.p. injection
PHASES OF THE EXPERIMENT
Pre-ethanol
adaptation
15 min
Post-ethanol
adaptation
15 min
Habituation
CS-alone
15-min
Acquisition
CS-US pairings
60-min
NUMBER OF HEART-RATE TRIALS AND INTERTRIAL INTERVALS (ITI) (min)
DURING EACH PHASE OF THE EXPERIMENT
5 samples
ITI= 3 min
5 samples
ITI=3 min
10 Trials
ITI= 1.5 min
20 Trials
ITI= 3 min
HEART-RATE COUNTING PERIODS DURING EACH TRIAL (sec)
Baseline (Pre-CS)
6 sec
CS-US interval 6 sec
EKG lockout 1.2 sec
Post-US 8 sec
2
2
2
2
2
2
1.2
2
2
2
2
CS (6.5 sec tone)
.5
US (.5 sec shock)