CLASSICALLY CONDITIONED HEART RATE IN RATS BASED ON THE INTEROCEPTIVE EFFECTS OF ETHANOL AND LITHIUM CHLORIDE

by

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INTRODUCTION

Drugs are capable of exerting several types of effects on behavior. An effect that has received considerable attention in recent years has been that drugs can serve as stimuli (Thompson & Pickens, 1971). The control of behavior by stimuli associated with internal states of an organism is not a new idea. Bykov (1957) demonstrated the existence of behaviorally significant interoception as early as 1928, by showing that manipulation of an internal organ or a physiological state could serve as either a conditioned stimulus (CS) or as an unconditioned stimulus (US) for the development of conditioned responses (CRs). Since then, the stimulus properties of drugs have been well demonstrated in a variety of learning situations, and some of the parameters of behavioral control by drug stimuli have been tested (see Thompson & Pickens, 1971, for a recent review).

The intent of the present research was to elucidate further the behavioral control by drug stimuli in the context of a classical conditioning situation. More specifically, the objective of the present study was to determine whether classically conditioned changes in heart rate (HR) could be produced in rats using an intraperitoneal (i.p.) injection of ethanol as the CS and an i.p. injection of lithium chloride (LiCl) as the US. Both ethanol and LiCl have been shown to produce major physiological effects that can persist for considerable periods of time (Webb & Degerli, 1969; Jacobs, 1978). As will be pointed out, both drugs appear to possess stimulus consequences that

allow them to participate in learned modifications of behavior. These demonstrations have usually involved changes in a variety of different kinds of skeletal-motor activity. The intent of the current experiment was to see if comparable learned changes could be produced in the cardiovascular system.

This approach is in contrast to what may be considered a more conventional or traditional pharmacological study of the unconditioned effects of drugs on various behaviors. An example of the pharmcological approach might be to study the effects of ethanol or chlordiazepoxide (both drugs which have been hypothesized to be tension reducing) on behavior in aversive conditioning situations (Cicala & Hartley, 1967).

The use of drugs as stimuli in learning experiments is reviewed in the material that follows. The first section covers studies in which instrumental learning procedures were employed and drugs served either as discriminative stimuli or as reinforcers. The second section, which is more detailed, is concerned with the results of classical conditioning studies in which drugs functioned either as signalling (CS) events or as reinforcing (US) events. The intent of these sections is to illustrate the generality of conditioning with drug stimuli, especially in classical conditioning, and to point out many of the relevant parameters.

Instrumental Conditioning

Discriminative Stimulus Properties of Drugs

Pavlov (1927) initially described the principle of stimulus discrimination in dogs within the context of classical conditioning. He first established conditioned salivation to the sound of a bell

which had been consistently followed by food. He continued to reinforce salivation to the original bell by presenting food, but when a different bell or a buzzer was sounded, no reinforcement was presented. Soon the dog learned to salivate only to the sound of the original bell, and not to the other sounds.

Within instrumental learning situations, stimulus discrimination procedures involve reinforcing the subject for a given behavior (e.g., lever pressing) only in the presence of the discriminative stimulus. Successful discrimination is achieved when the subject makes the response in the presence of the discriminative stimulus and fails to make the response when the discriminative stimulus is absent. Drugs have been shown to function very effectively as discriminative stimuli. The generality of the discriminative stimulus function of drugs is well established and reviews may be found in Overton (1971) and Harris and Balster (1971).

Operant discrimination between drug states has been refined to the extent that it may be used to assess the similarity of drugs as stimuli. Barry (1974) and Overton (1971) have shown that it is possible to classify drugs into groups based on similiar discriminable effects, that it is also possible to discriminate between different doses of the same drug, and that different doses of similiar drugs can be behaviorally equated based on their discriminable effects. Many drugs active in the central nervous system (CNS) have been found to act effectively as discriminative stimuli in the control of learned behavior. Control of operant responding by a drug state is not peculiar to specific tasks, schedules, or reinforcement parameters (Overton, 1971; Harris & Balster, 1971; Schuster & Brady, 1971), and

a near equivalence of drug effectiveness in controlling behavior on various schedules of reinforcement has been shown (Harris & Balster, 1971).

The speed with which drugs acquire the ability to control responding is dependent upon the intensity and salience of the drug-state stimuli. For many drugs, stimulus intensity may be related directly to drug dosage, which has been shown to be directly related to the rate of development of drug discrimination learning (Overton, 1971; Barry, 1974). The type of drug employed is also a critical variable influencing the rate of drug discrimination learning. High doses of anesthetic or depressant drugs acquire strong discriminative control very rapidly, requiring as few as 2 to 3 training sessions to achieve a criterion of 8 out of 10 correct T-maze shock avoidance responses (Overton, 1971). High doses of drugs such as antimuscarinics, narcotics or amphetamines, however, exert only moderate discriminative control and require as many as 6 to 10 training sessions to reach the same criterion (Barry, 1974; Overton, 1971).

Some drugs are barely discriminable from absence of the drug, even though they possess clear CNS effects. The major tranquilizers, phenothiazines, and such drugs as imipramine and diphenylhydantoin (Dilantin) require an unusually large number of training sessions before criterion performance is attained (Barry, 1974), and in some cases it may never be attained (Overton, 1971). This latter result points out the fact that the central action of a drug may not, of itself, determine its stimulus value.

An important consideration in the study of drugs is the extent to which they exert their effects on the CNS, as opposed to the peripheral

nervous system. Barry (1974) and Overton (1966) have both presented evidence that suggests a central locus of the discriminative properties of various atropine compounds. After showing strong transfer of drug discrimination learning between the centrally acting compounds, they found that the atropine derivatives that exerted only peripheral actions were not discriminated from absence of the drug (Barry, 1974; Overton, 1966). For example, an attempt to train a rat to discriminate the peripheral-acting atropine methyl-nitrate (10 mg/kg) from saline was not successful (Overton, 1966). However, there are instances in which atropine methyl-nitrate and some other peripheral-acting drugs have acquired response control. These demonstrations involved the use of very high drug doses and large numbers (30 to 50) of training sessions (Overton, 1971).

Overton (1971) stated two general proposals to account for discriminative control by drug states: (a) drugs primarily influence sensory systems that function normally to control behavior; (b) drugs stimulate brain centers controlling different kinds of behavior and the sensory systems are not involved. The fact that drugs possessing strong peripheral actions on organs and nerves seem to be barely discriminable argues against hypotheses that relate drug stimulus control to the induction of sensory or interoceptive stimulation. However, the fact that some peripherally acting drugs can come to control behavior indicates that drug-state control is not exclusively intrinsic to the brain either. Possibly, peripheral and central acting drugs do not produce the same type of stimulus control. Peripherally acting drugs may exert their stimulus effects through a given sensory modality while centrally acting drugs may have undefined central stimulus effects.

Some support for the idea that peripheral and central acting drugs exert separate modes of stimulus effects was provided by several studies described by Overton (1971). When animals were trained to discriminate substantial brightness differences, the learning curves resembled those obtained using peripheral acting drugs as the stimulus agents. In both cases, acquisition required large numbers of trials to achieve criterion performance. Only the difference between very bright light and total darkness produced a sharply-sloped learning curve resembling one produced by pentobarbital (15 mg/kg), a centrally acting drug. This may suggest that a possible source of the stimulus effects which are produced by peripherally acting drugs may be subtle shifts in the sensory modalities.

The stimulus properties of ethanol have been well documented in a number of studies of instrumental conditioning. These studies have shown that ethanol can serve as a discriminative stimulus for several types of responding. These include running in a T-maze to avoid shock (Overton, 1968, 1977) or to obtain food (Kubena & Barry, 1969), approach-avoidance responding (Kubena & Barry, 1969) and two-lever choice responding (Krimmer and Barry, 1973). Overton (1971) listed ethyl alcohol (ethanol), 1 - 3 g/kg, as a drug which exerts strong stimulus control in comparison with 43 other drugs tested for discriminative control. Drug vs no-drug discrimination tasks have been used to show that ethanol possesses some stimulus properties which transfer to the barbiturate pentobarbital (Krimmer & Barry, 1973; Bueno, Carlini, Finkelfarb & Suzuki, 1976), but that the two drugs do not completely overlap (Barry & Krimmer, 1973). Drug vs drug discrimination is a sensitive technique for assessing the differences

between drugs and the use of this technique has shown ethanol to be readily discriminable from the barbiturates (Krimmer & Barry, 1973; Overton, 1977), with which ethanol is most often classed in terms of stimulus similarities. Krimmer and Barry (1973) found differential responding at all doses of ethanol and pentobarbital which they tested. They felt that this indicated the qualitative nature of the differences of ethanol's stimulus properties.

Overton (1977) established a dose-response curve of drug vs no-drug discriminative control for ethanol using a criterion of 8 out of 10 correct avoidance trails in a T-maze. Weak discriminative control (50 sessions-to-criterion) was found at doses of 0.6 g/kg, moderate control (25 sessions-to-criterion) was found at doses of 0.8 g/kg, and strong control (7 or fewer sessions-to-criterion) was found at doses higher than 1.0 g/kg.

Reinforcement Properties of Drugs

A drug may function effectively as an appetitive or aversive reinforcer in much the same way as any other more traditional reinforcer such as food or electric shock. In instrumental learning situations, it has been demonstrated that animals will lever-press to receive intravenous infusions of many of the common drugs of abuse. The narcotics morphine, codeine and methadone (Woods & Schuster, 1971), the stimulants amphetamine, caffeine, cocaine, methylphenidate and tranylcypromine (Woods & Schuster, 1970, 1971; Pickens & Thompson, 1971), the barbiturate pentobarbital (Woods & Schuster, 1970), and ethanol (Smith & Davis, 1974; Smith, Werner, & Davis, 1976, 1977) have each maintained lever-pressing behavior that resulted in drug infusions. Such findings indicate that these drugs have behavioral properties

similiar to those of more conventional appetitive reinforcers (e.g. food and water).

Some drugs may provide punishing or aversive reinforcing effects as measured by a reduction in the behavior that results in drug delivery. A reduction in lever pressing behavior has been reported, paradoxically, for amphetamine (Whitney & Trost, 1970) and ethanol (Thompson, Bigelow, & Pickens, 1971), two drugs which also have some appetitive reinforcing properties. Some drugs are also known to maintain escape-avoidance responding. These include chlorpromazine, nalorphine, cyclazocine, and LSD (Hoffmeister, 1975; Hoffmeister & Wuttke, 1975). Dosage and previous experience may be important factors determining whether a drug serves as an appetitive or as an aversive reinforcer (Woods & Schuster, 1970; Pickens & Thompson, 1971; Thompson, Bigelow, & Pickens, 1971; Pickens, Meisch, & Thompson, 1978). Thompson et al. (1971) reported that variables such as stress, environmental familiarity, reinforcement contingencies, and concurrent reinforcement schedules may also affect self-administration behavior. At present, it is not clear whether these variables influence the reinforcing properties of drugs, or merely the behavioral expressions involved.

Evidence indicates that the rate of development of responding for drug reinforcement may vary according to the drug dosage, the type of drug that is used, and the species being examined. Furthermore, observed interactions between dose and drug type suggest that there may be intrinsic differences in the type of reinforcement provided by different drugs. Woods and Schuster (1970) reported that for self-administration of narcotics and stimulants, the rate of approach to

asymptotic responding was inversely related to the unit dose, with asymptotic levels being reached more rapidly in animals that had previously been exposed to the drugs. With respect to drug type, asymptotic and regularly spaced responding for 1.0 mg/kg of amphetamine was observed in rats within 2 to 4 days of training (Pickens & Thompson, 1971), while rats responding for 1.0 mg/kg of morphine required 6 to 8 days to exceed saline controls (Woods & Schuster, 1971). Responding for morphine continued to increase as tolerance developed, but responding for amphetamine became less frequent with continued drug exposure.

The reinforcing effects of ethanol and barbiturates seem to differ between species. In monkeys, a pattern of steadily increasing responding for several doses of barbiturates was seen beginning with the first day of exposure (Woods & Schuster, 1970). However, responding for ethanol did not become established for 3 to 4 days, and even then the monkeys displayed periodic episodes of decreased responding.

It is well known that rats do not usually consume appreciable quantities of ethanol or barbiturates unless some special contingency or training procedures is arranged to induce self-administration (Meisch, 1977). Rats maintained on an intermittent food schedule with an ethanol solution constantly available come to drink excessive amounts of ethanol, a phenomenon termed schedule-induced polydipsia (Falk, Samson, & Winger, 1972). Forced ethanol consumption has also been used successfully to induce responding for ethanol (Thompson et al., 1971; Meisch,1977; Deutsch & Eisner, 1977), as has electric foot shock (Anisman & Waller, 1974; Davis & Miller, 1963). In general, however, ethanol consumption usually stopped shortly after these procedures were terminated (Meisch, 1977).

In a few studies, rats have been trained to self-ingest ethanol without the use of special training arrangements. For example, Smith and Davis (1974) and Smith, Werner and Davis (1976, 1977) reported successful acquisition of lever pressing in rats for intravenous or intragastric infusions of ethanol as reinforcement. Conceivably, these positive outcomes were due to the fact that these administration routes eliminated the unpleasant taste associated with the oral consumption of ethanol (cf. Meisch, 1977).

It has also been demonstrated that drugs may acquire secondary reinforcement properties when they are paired with a primary appetitive reinforcer. Harris, Claghorn and Schoolar (1968) made drug ingestion an operant response for food reinforcement in hungry rats. More specifically, they required rats to lick a spout containing a drug solution in order to receive food. The experimental animals developed a greater preference for meprobamate and chlordiazepoxide, two minor tranquilizers, than did control animals that were force fed the drug without food reinforcement.

Classical Conditioning

In the following section, attention will be focused on the actions of various drugs in three kinds of classical conditioning situations. These situations represent the main types of paradigms that have been used to study classical conditioning processes and are known as taste aversion conditioning, conditioned emotional response (CER) conditioning, and traditional classical conditioning. Although these paradigms have several features in common, it is possible that the way in which drugs enter into the associative processes occurring in each of these paradigms may be different.

A potential problem in the area of drug conditioning is the possibility that the drugs may produce unlearned side effects which can mistakenly be treated as conditioned responses. This is analogous to the problems of sensitization and pseudoconditioning that arise with conventional exteroceptive CSs and USs in classical conditioning. Many investigators have failed to include unpaired control groups which receive equivalent drug exposure to that given the paired experimental group. Potentially, drug exposure may have long term effects on the baseline behaviors that are measured in the conditioning situation. Without a control group that has been equivalently exposed to the drug, it cannot be safely assumed that any difference in responding observed in an experimental group was due to conditioning, rather than to a nonspecific drug effect. For purposes of the present discussion, reference will be made to the nonspecific effects of drugs when the investigator failed to use an appropriate drug control group to rule out these effects.

Taste Aversion Studies

Taste-aversion conditioning, as described by Garcia and Koelling (1966) involves the pairing of a particular taste or flavor (the CS) with an agent (the US) that produces unconditioned illness or discomfort. The presence of conditioning is indicated by a decrease in the amount of flavor solution consumed after the CS-US pairings compared to that consumed before the pairings or to the amount consumed by an appropriate control group. Illness inducing USs that have commonly been used are X-irrdiation and the administration of various drugs and toxins such as apomorphine, emetine, cyclophosphamide and LiCl (Garcia & Koelling, 1966; Garcia, McGowan & Green, 1972; Garcia, Ervin

& Koelling, 1966; Garcia et al., 1972; Elkins, 1974; Revusky & Gorry, 1973; Rozin & Kalat, 1971). The latter drug is one of the most potent and widely used. The unconditioned responses that are produced by these USs include nausea, vomiting and in the case of LiCl, convulsive-like activity. Presumably, it is the aversive nature of these drug-responses that leads to decreased consumption of the flavored CSs with which they are paired.

Paradoxically, some drugs which function as appetitive reinforcers in that they support drug-seeking operant responses can also serve as effective USs in taste aversion learning. Morphine, a powerful appetitive reinforcer for lever pressing, also has some emetic actions in humans and has been used as a US for aversion formation (LeBlanc & Cappell, 1974). Amphetamine is another potential appetitive reinforcer that has been used for taste aversion (LeBlanc & Cappell, 1974; Cappell & LeBlanc, 1975). Ethanol is an additional drug that is often self-ingested in humans, yet is capable of functioning as a US in taste aversion (Eckardt, Skurdal, & Brown, 1974; Lester, Nachman, & LeMagnen, 1970; Davison & House, 1975). It is not known whether depressant drugs such as the barbiturates also possess these bivalent properties. Some preliminary findings of Ionescu and Buresova (1977), in which taste aversion was conditioned with LiCl while rats were in a state of pentobarbital-anesthesia, seemed to indicate that barbiturates may not function as taste aversion USs.

Substantial taste aversions have been observed with intervals as long as 12 h between the delivery of the CS and the presentation of the US (Rozin & Kalat, 1971). Intervals of this length typically do not lead to conditioning of other response systems (Mackintosh, 1974).

A view that has received some support is that the optimum CS-US interval for classical conditioning may vary according to the response system being studied, with the gustatory system having an extended CS-US optimum. (Garcia, et al., 1972; Spiker, 1977; Thompson, 1976).

In taste-aversion learning, the URs elicited by the US are rarely measured. Instead, attention is usually focused on CS-related changes in behavior. This emphasis is unfortunate because an adequate explanation of conditioned taste aversion may depend upon an understanding of the relationship of conditioned and unconditioned behaviors. Studies have shown that one of the URs to an illness US is heightened secretion of adrenocorticotropic hormone (ACTH), a pituitary hormone (Braveman, 1977; Jacobs, 1978; Hennessy, Smotherman, & Levine, 1976). This increased hormonal response has been shown to be component of the conditioned response to forced exposure to a taste-aversion CS (Smotherman, Hennessy, & Levine, 1976a, 1976b).

Some studies suggest that ACTH secretion may be directly involved in the mediation of taste-aversion conditioning. Smotherman et al. (1976) found that the strength of LiCl-induced taste aversion was highly correlated with the level of ACTH secretion produced by the US. When the level of ACTH was raised or lowered there was a corresponding change in the strength of taste-aversion performance. Smotherman et al. (1976) and Hennessy et al. (1976) used dexamethaxone, an inhibitor of ACTH release, to block ACTH secretion while a single taste-aversion conditioning trial was given. After this blocking manipulation, both conditioned taste aversion and conditioned ACTH secretion were attenuated on test trials. Additional evidence of the involvement of ACTH in taste aversion conditioning was provided by Kendler, Hennessy,

Smotherman, & Levin, (1976), who found that administration of ACTH during testing retarded taste aversion extinction.

In a novel demonstration of taste aversion conditioning Cunningham (unpublished manuscript) gave rats an intraperitoneal(i.p.) injection of 0.6 g/kg of ethanol followed 2.5 min later by an i.p. injection of 3 mEq/kg of LiCl. After five such trials, the animals showed a reduced preference for drinking an ethanol solution, indicating successful conditioning presumably based on the interoceptive effects of ethanol and LiCl. Cunningham also found that the effectiveness of ethanol as a taste-aversion US was reduced following these ethanol-LiCl pairings.

CER Studies

In the conditioned emotional response (CER) paradigm, animals are trained to press a lever for food or water reinforcement. Later, a CS which had been paired with a painful US like electric shock is presented while the animal is engaged in lever pressing. The strength of conditioning is measured as a suppression of lever pressing in the presence of the CS (Estes & Skinner, 1941). This suppression is typically attributed to the effects of covert, emotionally-based CRs presumed to be elicited by the CS.

Turner and Altshuler (1976) used amphetamine as a CS in the CER paradigm. They gave rats an i.p. injection of 0.8 mg/kg of amphetamine followed by a series of 200 inescapable shocks that began 15 min after the drug injection. The animals showed strong suppression of lever pressing when tested with amphetamine or cocaine, while a control group given random CS-US presentations showed no suppression of responding to either drug. This demonstrated the effectiveness of a drug (amphetamine)

as a CS for the development of conditioned suppression and the generalization of that effect to a different drug (cocaine) having similar stimulus properties.

Several examples exist of the use of drugs as USs in CER procedures. Conditioned suppression of lever pressing has been observed when rats were presented with tone or light CSs associated with the hallucinogens psilocybin and LSD (Cameron & Appel, 1972a, 1972b, 1976), with the tranquilizer chlorpromazine (Cameron & Appel, 1972a) and with the stimulant amphetamine (Whitney & Trost, 1970). Nalorphine is a drug that induces withdrawal-type reactions in morphine-dependent animals. When tones and lights were paired with nalorphine, conditioned suppression developed in both morphine-dependent rats (Goldberg, 1970, 1971; Goldberg & Schuster, 1967, 1970) and in morphine withdrawn post-dependent rats (Goldberg, 1971; Goldberg & Schuster, 1970). However, nalorphine failed to support conditioning in rats that had never been exposed to morphine (Goldberg & Schuster, 1967). When lever pressing resulted in i.v. infusions of morphine, a light CS signalling the injection of nalorphine produced an increased rate of responding, suggesting that a motivational state had been conditioned by the nalorphine (Goldberg, 1971,1976).

The development of conditioned suppression in CER situations has been reported in as few as three to five trials with amphetamine (Whiteney & Trost, 1970) and nalorphine (Goldberg & Schuster, 1967, 1970). Suppresion developed in 6 to 8 days with hallucinogens and chlorpromazine (Cameron & Appel, 1972, 1976). As with self-administration behaviors, dose may be an important factor in the rate of CER development. A monkey receiving 2 mg/kg of nalorphine as a US

totally suppressed responding after three pairings with a light CS, while two monkeys receiving 1 mg/kg of nalorphine did not completely suppress responding until 6 or 7 pairings (Goldberg & Schuster, 1967).

Traditional Classical Conditioning Studies

In traditional classical conditioning experiments, a variety of different drugs have served as USs. Only one study could be located in which a drug functioned as the CS. The responses that have been examined include skeletal-motor activities, basic physiological changes and reactions of the autonomic nervous system. With some notable exceptions, the range of activities that have been conditioned using drugs as USs in Pavlov's (1927) classical conditioning technique seems only to be limited by the ability of drugs to produce an unconditioned response.

Motor Activity CRs. In the only study of its type that could be located, Cook, Davidson, Davis, and Kellehrer (1960) showed that injections of epinephrine, norepinephrine, or acetylcholine could function as CSs for a conditioned leg-flexion response in dogs. An electric-shock US was delivered to a hind limb 30 sec after the start of an i.v. infusion of one of the drugs. It was found that 10 mg/kg of l-epinephrine, 10 mg/kg of norepinephrine, and 20 mg/kg of acetylcholine came to serve as CSs for a leg-flexion CR. Pre-conditioning tests with the drugs were used to rule out nonassociative explanations of the leg-flexion responses.

Unconditioned increases in motor activity induced by a variety of drugs have been shown to be conditionable. In one of the first studies of this kind, conditioning of increased wheel-turning induced by 2 mg/kg of methamphetamine was reported by Irwin and Armstrong

(1961). Rats were given a single injection of the drug in a test cage (the CS) and their activity levels during a subsequent exposure to the test cage were compared to those of controls that had received saline in the test cage. The control group was not exposed to the drug at any time, however, so that a nonspecific drug effect cannot be ruled out.

In a properly controlled study, Pickens and Crowder (1967) obtained conditioning of running in rats based on an amphetamine US. This result occurred after six pairings of the drug and an alleyway apparatus (CS) for the experimental group. A control group received an equal number of drug exposures 3 to 4 h after being removed from the alleyway.

Schreiber, Wood, and Carlson (1975) used methylphenidate to demonstrate that drug-induced increases in wheel-turning in guinea pigs could be conditioned even when the unconditioned activity was prevented by locking the wheel after the drug was administered. The results showed that conditioning under these circumstances was comparable to that obtained when the wheel was not locked. However, the control groups in this study did not received equivalent drug exposures to methylphenidate, leaving open the possibility that wheel-turning was contaminated by nonspecific drug effects.

A 2-min light was used as the CS and a 0.5 mg/kg i.v. infusion of methamphetamine as the US in an investigation of conditioned activity in rats reported by Pickens and Daugherty (1971). Increased activity during the CS was observed after 10 light-drug pairings. A pseudo-conditioning control group receiving the same number of CS and US presentations in an unpaired fashion was used to rule out nonassociative interpretations of the activity changes in the experimental group.

Changes in skeletal-motor responding other than increased general activity have been studied using drug USs. Irwin and Armstrong (1961) claimed conditioned decreases in wheel-turning produced by perphenazine and chlorpromazine to injection and environmental CSs. However, an appropriate control group was not used to rule nonassociative factors that may have affected motor activity. Using Siamese fighting fish, Braud and Weibel (1969) conditioned reductions in aggressive display behavior induced by phenergan, a tranquilizer, and increases in display behavior induced by morphine by pairing differently colored lights with these drugs. Light-drug pairings and light and drug exposures were counterbalanced such that all animals received equivalent exposures.

General Physiological CRs. By far, the most frequently studied reactions using drug USs have been autonomic and physiological changes. Insulin-induced hypoglycemia has been conditioned to a strong odor CS by Woods (1976). Four rats received 6 pairings of an olfactory CS and an i.p. injection of either insulin, insulin-plus-glucose, glucose, or saline as the USs. The groups given the insulin or the insulin-plus-glucose USs demonstrated conditioned hypoglycemia on a saline-injection test trial, indicating that a hypoglycemic UR was not necessary for the development of a hypoglycemic CR. The control groups did not show a hypoglycemic response to the CS.

Modification of brain metabolism of dopamine, a putative neurotransmitter, has been conditioned to the sound of a buzzer CS by Perez-Cruet (1976). Following 10 trials on which the buzzer CS was paired with an injection of morphine, methadone, or bulbocapnine, dopamine metabolites were measured to the buzzer CS on test trials.

The main finding was that the levels of metabolites in the experimental group were higher than those shown by three separate control groups. Although the three control groups were exposed either to the drugs, to the CS or to the test situation alone, a more appropriate control group given both the CS and the US in an unpaired fashion was not used.

Pavlov (1927) reported conditioning of salivation and emesis following several pairings of a morphine injection (the US) with the test environment. Crisler (1930) also reported conditioning of salivation on the basis of a morphine US after seven to nine pairings with a CS, a further reported the development of the CR when the UR had been suppressed by atropine. When Crisler attempted to maintain the CR by substituting pilocarpine for morphine, the CR extinguished. In contrast to morphine, pilocarpine produces salivation by acting peripherally on the salivary glands themselves. Failure of pilocarpine to support the salivary CR provided some of the earliest evidence that in order for a drug to function effectively as a US, it must exert its effects centrally through the CNS.

Conditioning of cortical evoked potentials has been reported by Stein, Lynch, and Rushkin (1977), who paired a 2-min train of clicks (CS) with the administration of morphine (US) and recorded electrocorticograms and evoked potentials (EPs) from the sensory-motor cortex of rats. Changes in EP magnitudes to the CS were reported in the group of animals that had received pairings of the CS and the US. Comparable changes were not obtained in a group receiving the same procedures except that saline was substituted for morphine.

Reduction of withdrawal symptoms associated with morphine administration in morphine-addicted rats has been conditioned to

auditory and olfactory CSs by Lal, Miksic, Drawbaugh, Numan, and Smith (1976). After pairing the different CSs with the administration of morphine, the CSs were presented during nalorphine-induced withdrawal. The presence of the CSs during withdrawal caused reduction of withdrawal signs as measured by wet-dog shakes, hypothermia, and aggression. Rats that had received unpaired presentations of the CS and the US were used for comparison purposes.

Conditioning of morphine tolerance has been reported by Seigel (1975, 1976, 1979). Following four or eight pairings of a special environment (the CS) with morphine in rats, morphine-induced alteration of pain sensitivity or body temperature was assessed in the same or in a different environment. Analgesic and hyperthermic tolerance were displayed when rats were tested in the environment in which they previously received morphine, but not when they were in the alternative environment.

Using human subjects, O'Brien, Testa, O'Brien, and Greenstein (1975) conditioned elements of the morphine withdrawal syndrome by pairing a tone-odor-injection combination CS with the administration of naloxone at a 5 to 10 min CS-US interval. Naloxone is a drug that induces morphine-withdrawal reactions by displacing morphine in the brain. Withdrawal symptoms during the CS following conditioning were compared to withdrawal symptoms during the CS prior to conditioning. Withdrawal intensity (rhinorrhea, nausea, yawning, lacrimation, goose-flesh, stomach cramps, and sweating) showed a significant increase during the CS test session after conditioning.

Under normal conditions, the spleen contracts in response to endogenous levels of epinephrine. Bykov (1957) reported for a single

dog that pairing of a 6-min sound of a metronome CS with an injection of 0.05 mg/kg of epinephrine which began 3 min after the start of the metronome resulted in conditioned contractions of the spleen after six CS-US pairings. The CR was actually measured as a decrease in the volume of the spleen. No conditioning or drug exposure controls were reported in this study, however.

Cardiac CRs. Heart rate conditioning based on a variety of drug USs has been examined in several experiments. In addition to providing information on the possibility of obtaining classical conditioning on the basis of the interoceptive effects of drugs, these experiments also bear on the question of possible reinforcement mechanisms in the development of conditioned HR responses. In essence, this question concerns the issue of whether a conditioned HR response can be obtained by pairing a CS with a US that simply produces an unconditioned cardiac response or whether the US must also be painful or motivating (Fitzgerald, Martin, & O'Brien, 1973; Fitzgerald & Hoffman, 1976; Gantt, 1970). A related issue concerning the type of US that will serve effectively in HR conditioning deals with the physiological locus of US effects. It has been suggested (Perez-Cruet, Jude, & Gantt, 1966; Gantt, 1970) that USs generating direct CNS activity can produce HR conditioning, but that USs acting on peripheral neural structures will not. Presently, the available evidence favors the view that to obtain HR conditioning, the US must have at least some motivating capabilities (Fitzgerald, Martin, & Hoffman, 1975; Gantt, 1970), and/or be able to trigger CNS processes (Perez-Cruet, 1971).

In the present context, injections of both centrally and peripherally acting drugs have been employed as USs in HR conditioning

experiments. In general, the central acting drugs have supported HR conditioning, whereas those possessing only peripheral actions have not. Claims that conditioning was obtained using peripheral acting drugs have been made (Bykov, 1957) but unfortunately crucial control procedures were not employed to rule out nonassociative explanations of the findings.

Morphine, a drug having major central effects including nausea and vomiting, was used by Bykov (1957) to condition HR deceleration in dogs. Learned reactions in the same direction as the URs were observed after 20 to 30 pairings of the injection environment (the CS) with morphine. Test trials in which saline was substituted for morphine reinforcement were used to measure the conditioned HR response. An unpaired control procedure was not used for comparison purposes.

Bykov (1957) also reported conditiong of HR increases induced by epinephrine, a drug which has both central and peripheral effects. In the first part of the study, the experimental environment served as the CS and conditioned effects were noted following 3 or 4 injections of the drug. In the second part of the study, a trumpet sound was used as a discriminative CS and HR CRs were observed following 30 to 40 CS-US pairings. Again, no controls were included in the study.

Tachycardia and neural conduction changes in the EKG induced by injecting bulbocapnine directly into the heart have been conditioned to tone and light CSs (Perez-Cruet & Gantt, 1964). Bulbocapnine is known to produce HR and EKG changes when injected intracerebrally in the region of the subthalamic nucleus (Perez-Cruet, 1971), suggesting a central locus of action of this drug. After 20 or more trials in

which the tone CS was followed 15 sec later by an intracardiac injection of 5 to 10 mg/kg of bulbocapnine, presentations of the tone without the drug injection elicited tachycardia and EKG changes which were highly similiar to those originally elicited by the drug (Perez-Cruet & Gantt, 1964). In a subsequent investigation, Perez-Cruet (1971) reported conditioning of HR based on a bulbocapnine US after two reinforcements, whereas EKG conduction changes required as many as 20 to 25 trials.

Intracranial injections of acetylcholine and norepinephrine elicit HR increases and decreases, respectively. Schneiderman (1974) has reported differential HR conditioning in rabbits based on these intracranial drug injections. In his study, electrical stimulation of one lateral geniculate thalamic nucleus functioned as the CS+ and was accompanied by an injection of one of the drugs, whereas stimulation of the contralateral geniculate nucleus functioned as the CS- and was accompanied by a saline injection. Differential conditioning occurred using either acetylcholine or norepinephrine as the US. The CR consisted of decreased HR for the norepinephrine US and increased HR for the acetylcholine US.

As previously noted, mixed reports have been published on the ability of peripherally acting drugs to function as effective USs in HR conditioning. Teitelbaum, Gantt & Stone (1956) were unable to obtain a cardiac CR in dogs that had received 200 pairings of an auditory CS with an i.v. injection of acetylcholine despite the presence of consistent and large magnitude cardiodecelerations to the acetylcholine. Similiarly, McKenzie and Gantt (1950) published a brief report indicating that injections of atropine, a peripherally

acting anticholinergic drug, did not serve as an effective US for the development of a HR CR in dogs. Here, the drug produced substantial accelerative HR URs. As both of these experiments involved a relatively large number of conditioning trials, it would be difficult to argue that the absence of conditioned HR was due to insufficient training.

In apparent contrast to these negative outcomes are reports of HR conditioning based on peripherally acting drugs by Bykov (1957). Bykov reported that a dog given 55 pairings of the sound of a trumpet CS with an i.v. injection of acetylcholine showed conditioned cardiodeceleration to the CS. Although acetylcholine is thought to promote neural activity when injected intracranially, it does not cross the blood-brain barrier and its actions are classified as peripheral in nature (Goodman & Gilman, 1975). In the Bykov (1957) study, the HR change to acetylcholine was accelerative in contrast to the deceleration that would have been expected given the sympathomimetic properties of the drug. This raises the possibility that the conditioning effect was based on the pain-produced HR acceleration associated with the injection, rather than on the acetylcholine itself.

Bykov (1957) also reported conditioned EKG changes and HR decreases in a single dog using nitroglycerin as the US. Nitroglycerin is a peripherally acting drug that relaxes smooth muscle and that does not cross the blood-brain barrier (Goodman & Gilman, 1975). Bykov noted that after 30 paired presentations of the sound of a trumpet CS with an injection of nitroglycerin, the decelerative CR began to emerge and became stable after 100 pairings. The possibility that pain from the injection mediated the development of the CR was reduced by the fact that several saline injections were given to habituate the needle-prick reaction.

Strophantin is a cardiac glycoside that acts directly on the myocardium, but is also believed to have some central effects in the brainstem (Somberg & Smith, 1979). Bykov (1957) reported that this drug functioned as a US for the development of a decelerative HR CR in a single dog. In this experiment, the subject was given pairings of the trumpet CS with the drug and conditioning started to appear after "repeated trials." Unfortunately, in neither this study nor in any of the other drug studies that he carried out, did Bykov use unpaired control animals to assess the presence of nonassociative factors that could have contributed to the HR changes that he observed. This failure, along with examining such a small number of subjects, makes it difficult to compare Bykov's findings with those of other classical conditioning experiments.

In most of the traditional classical conditioning studies outlined above, the US was a drug and the CS was either the test environment, an auditory or visual signal, or an odor. In no case did a drug serve as both the CS and the US, and there was only one example of a drug functioning as the CS (Cook, Davison, Davis & Kellehrer, 1960). In the latter study, the CS was an i.v. injection of either epinephrine, norepinephrine or acetylcholine and the CR consisted or HR changes and leg flexion.

The purpose of the present study was to explore the possibility of obtaining classically conditioned changes in HR by pairing the interoceptive effects of a drug CS with the interoceptive effects of a drug US. The drugs selected for the CS and US were ethanol and LiCl, respectively. Ethanol was selected as the CS because of its salient stimulus properties. It has been used as a discriminative

stimulus for many operant tasks (Kubena and Barry, 1969; Overton, 1968, 1977; Krimmer & Barry, 1973) and exerts strong stimulus control over various behaviors (Overton, 1971). Also, ethanol is absorbed and circulated by the blood relatively rapidly (Stainbrook, 1975; Linakis & Cunningham, 1979), making it possible to pair its physiological effects with those of the US using a relatively short CS-US interval.

Lithium chloride was selcted as the US for several reasons. First, the aversive nature of LiCl is well documented by its use in the conditioned taste aversion paradigm (Garcia & Koelling, 1966; Garcia et al., 1972; Revusky & Gorry, 1973). It will be recalled that there is good reason to believe that the occurrence of HR conditioning may depend upon the use of an aversive US. An additional reason for employing LiCl as the US was that it is known to have profound unconditioned effects on the cardiovascular system. McKusick (1950) reported a marked bradycardia, resembling hyperkalemia, in a variety of animals, including intact dogs, rabbits, guinea pigs and a single cat following lithium administration. Electrocardiogram changes included amplified T-waves, auricular standstill, widening of the QRS complex and the appearance of bizarre biphasic QRS-T complexes at a low rate. Preliminary studies with rats in this laboratory have also shown HR decreases of 80 to 100 beats per min following in i.p. injection of 3 mEq/kg of LiCl, the dose that was subsequently used as the US.

The design of the study called for single pairings of ethanol and LiCl on each of several days with HR being measured prior to and following each injection. Subsequent to the HR conditioning phase, the subjects were examined in a taste aversion paradigm similiar

to the one employed by Cunningham in an unpublished investigation. It will be recalled that he reported the presence of a taste aversion CR based on pairings of ethanol and LiCl injections. The inclusion of a test for taste aversion in the present experiment allowed a direct comparison to be made between a consummatory CR and a cardiac CR within the same subjects.

METHOD

Subjects

The subjects were 20 naive, female albino rats purchased from Simonsen Laboratories and maintained on a 12-h light-dark cycle by the Department of Animal Care of the University of Oregon Health Sciences Center. The rats were 90 to 120 days old and ranged in weight from 245 to 285 g. They were given food and water freely during the first two phases of the experiment. During the third phase, the rats were allowed to eat food freely, but access to fluid was limited to a test period ranging from 10 to 20 min each day.

Apparatus

The animals were restrained in a plastic, inverted U-shaped holder purchased from Narco Bio-Systems, Inc. Guillotine-type plastic inserts in the holder were adjusted to fit snugly directly in front of and behind the animals. The holder was placed inside an Industrial Acoustics Corp., sound-isolation chamber (41 cm x 58 cm x 34 cm) equipped with a 7.5-cm ventilation fan. Extraneous sounds were masked by white noise measuring about 75 dB (re .0002 dyne/cm^2, A-scale, Scott Instruments Type 3 sound level meter) and presented through a 8.3-cm speaker mounted on one wall of the chamber.

The electrocardiogram (EKG) was recorded on a Grass Model-5 Polygraph from 20-gauge hypodermic needles inserted subcutaneously on either side of the thoracic cavity of the rats. An automated

recording system, described in more detail by Fitzgerald, Vardaris and Teyler (1968), provided a record of the heart beats occurring within each trial. The system contained a low-force lever-type Microswitch that was mounted directly above the EKG polygraph pen so that the R wave of the QRS complex of each cardiac cycle activated the switch. Each triggering of the Microswitch was recorded by a solid state counter. At the end of selected time periods, the accumulated heart-beat totals were punched on paper tape by a Tally tape perforator. The accuracy of the recording system was checked periodically by substituting a 10-Hz signal for the incoming EKG signal.

Two rats were conditioned concurrently in two identically equipped chambers. Time intervals during which HR was measured were initiated by a film-tape programmer and controlled with Massey Dickinson logic modules having a repeat accuracy of .05 per cent.

Procedure

The experiment was divided into three phases. The first phase, the anticipatory conditioning phase, involved repeated pairings of the ethanol CS with the lithium chloride (LiCl) US. The primary purpose of this phase was to determine whether an anticipatory HR CR could be developed to the ethanol CS. The second phase involved the use of special test trials in which the nature of the US event was unexpectedly changed. These trials were given to see if evidence of HR conditioning could be found during the period when the UR was normally present. The third phase comprised testing for the presence of conditioned aversion to the oral ingestion of the ethanol CS. Also examined during this phase was the effectiveness of ethanol as a US for the development of

conditioned taste aversion. This higher order taste aversion conditioning test was included to assess any differences in the potency of ethanol as a taste aversion US resulting from previous ethanol-LiCl pairings.

The ethanol CS consisted of an i.p. injection of 0.8 g/kg of ethanol (10 ml/kg, 10.17%, v/v, in normal saline), a dose that has been shown to be discriminable by rats lever pressing for food reinforcement (Overton, 1977). The LiCl US consisted of an i.p. injection of 3 mEq/kg of LiCl (10 ml/kg of 0.3 M LiCl in distilled water). This dose is similar to what has been used to obtain taste aversion in rats. Equivalent volumes of normal saline (10 ml/kg) were injected in place of CS and US events at various times during the conditioning and HR test phase.

The experiment was comprised of one experimental group and one control group. The two groups were formed by pairwise matching of the animals for body weight and then randomly assigning one member of each pair to one group or the other. A coin toss was used to assign one group to the experimental procedure and the other group to the control procedure.

Phase 1-HR Conditioning. During the anticipatory conditioning phase, the experimental group received 10 ethanol-LiCl (E-L) conditioning trials and 10 saline-saline (S-S) trials. The control group received 10 ethanol-saline (E-S) trials and 10 saline-LiCl (S-L) trials. For both groups, one trial was given every other day over 40 successive days, with the two trial types within each group being alternated. With this design, the two groups were equated in terms of handling, number of injections, and exposure to ethanol and LiCl. The principal

difference between the groups was that ethanol was always followed by LiCl in the experimental group and by saline in the control group.

At the start of each session, the animal was placed in the restrainer, the EKG electrodes were inserted and the animal was then positioned inside the isolation chamber. After a 20-min adaptation period, the rat was given the CS injection. The door of the conditioning chamber was opened and the rat restrainer was tilted 45° to 90° from horizontal. The injection was administered via a 25-ga x 5/8" hypodermic needle inserted through a 10-mm round hole in the bottom of the restrainer. The rat was then repositioned inside the chamber and the door was closed. The US injection was given in a similar manner 10 min later. Twenty min after the US injection, the animal was removed from the chamber and returned to its home cage. Heart rate was recorded during fourty 10-sec periods spaced 1-min apart beginning 10 min before the CS injection and lasting 20 min after the US injection. The first HR sample period after each injection occurred approximately 30 sec following the injection. The number of heart beats in each 10-sec period was converted to beats per minute. Table 1 provides a summary of the events that occurred during the conditioning phase.

Phase 2-HR Test Trials. At the end of the conditioning phase, both groups received four test trials, one every other day over 8 successive days. Two of these trials within each group were exactly the same as those given during the previous conditioning phase; E-L and S-S for the experimental group, and E-S and S-L for the control group. The other two trials differed from those given during the conditioning phase in that the US event was changed. Thus,

Table 1. Summary of procedures and events within each type of trial during the anticipatory conditioning phase.

Group	Type of Trial	Placed in chamber	1st injection	2d injection	Removed from chamber
		20 min	10 min	20 min	
Experimental	E-L S-S	adaptation adaptation	ethanol saline	LiCl saline	
Control	E-S S-L	adaptation adaptation	ethanol saline	saline LiCl	

for the experimental group, saline was substituted for LiCl on the E-L trial, making it an E-S trial, and LiCl was substituted for saline on the S-S trial, making it an S-L trial. For the control group, LiCl was substituted for saline on the E-S trial, making it an E-L trial, and saline was substituted for LiCl on the S-L trial, making it an S-S trial. On these test trials attention was focused on the HR changes occurring in the 20-min post-US period to see if the HR URs of the two groups were the same. Differences between the groups at this point would presumably reflect prior differences in the conditioning procedure that each group received.

Both groups were divided in half during the HR test phase. One half of each group received the test trials in the order E-L, E-S, S-L, S-S, while the other half of each group received the test trials in the order E-S, E-L, S-S, S-L. This was done in an attempt to balance any effects that may have accrued as a result of either a conditioning or an extinction-type of test trial preceding the other. In keeping with the anticipatory conditioning phase, HR was recorded in 10-sec periods at 1-min intervals beginning 10 min before the CS injection and lasting 20 min after the US injection.

Phase 3-Oral Aversion. As a further measure of conditioning to the ethanol CS, the experimental and control groups were tested using an oral aversion procedure. There were two phases to this procedure. In the first phase, and without additional training, the amount of ethanol consumed by both groups was measured in an ethanol drinking test. In the second phase, injected ethanol was used as the US and paired with oral saccharin that served as the CS. Saccharin consumption was then measured.

Prior to these tests, all of the animals in both groups received restricted fluid access for 7 days. On these days, the animals were fluid-deprived and allowed 20 min to drink water at approximately the same time each day. On the eighth day, both groups received a solution of 4.75% ethanol in tap water. The solution was presented in a Nalgene tube holding 20 ml of fluid. A steel spout fitted to the end of the tube projected through the front of the home cage in the position normally occupied by the spout of the regular water bottle. The first test of conditioned aversion to ethanol was composed of two 5-min drinking periods separated by a 2-min interval of no drinking. A fresh bottle of solution was placed on the cage before each test period. Following this phase, both groups were placed back on their regular drinking schedule of 20-min access to tap water for 2 days.

The second taste aversion phase was begun after this 2-day rest period. The procedure for this phase consisted of allowing the animals 10 min to drink a 0.1% (w/v) saccharin solution in tap water. At the end of the 10 min, the animals were picked up and given an i.p. injection of ethanol (2 g/kg, 25 ml/kg of 10.17%, v/v ethanol in sterile saline). Although this was the concentration of ethanol that was used in the ethanol-LiCl (i.e., E-L) conditioning trials, the dose was 2.5 times larger. The reason for increasing the dose was to enhance the overall aversiveness of ethanol. Both groups received a total of 6 taste aversion conditioning trials. The trials were separated by 2 days on each of which the animals were permitted to drink tap water for 20 min. The amount of fluid consumed on each of the 6 conditioning trials was used as the index of conditioned aversion.

Subject Attrition. Two rats in the experimental group died during the course of the experiment. One animal appeared to die of asphyxiation in the restrainer during the ethanol-LiCl conditioning phase, while the other died of kidney failure secondary to infection which developed following the initiation of water deprivation during the oral aversion phase. To achieve an equal number of rats in each group for statistical analyses, two animals were randomly eliminated from the control group.

RESULTS

Phase 1--HR Conditioning

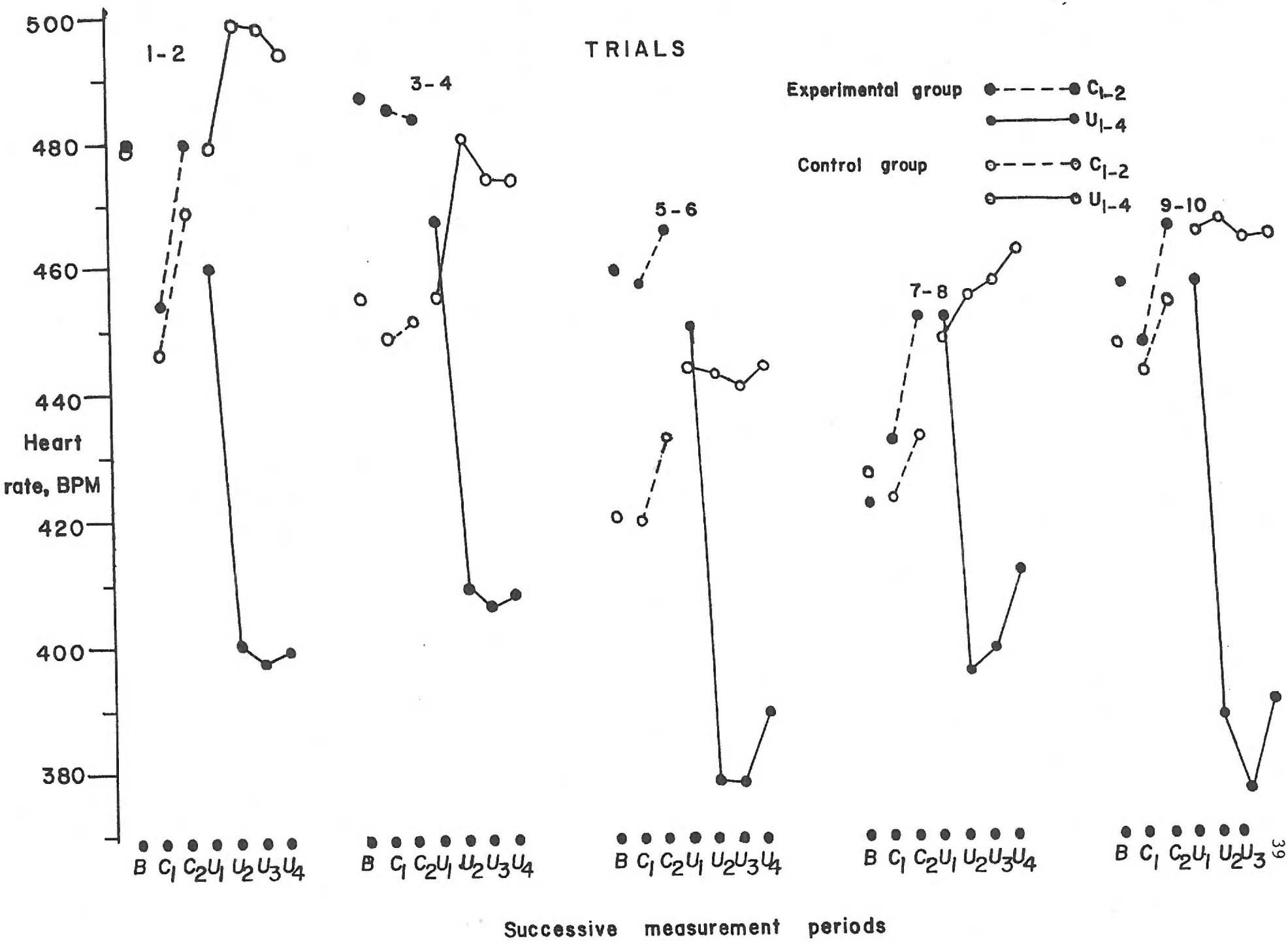
Ethanol-CS Trials. Figure 1 depicts the mean HR responses of the experimental and control groups on the conditioning trials in which ethanol was given as the CS. The data were averaged over five blocks of two trials each. The first point on the left in each panel, labelled B, represents the baseline HR as measured during the 10-min period prior to the ethanol injection. The next two points, labelled C_1 and C_2, represent the HR responses in successive 5-min periods following the ethanol injection. The remaining four points, designated U_1, U_2, U_3, and U_4, refer to the HR responses in successive 5-min periods following the injection of LiCl for the experimental group and of saline for the control group. In general, this figure shows that the HR responses (with respect to baseline) of the two groups to the ethanol CS were similar to each other on most of the trial blocks. During the first period of Trial Block 1, the ethanol produced major HR decreases from baseline in both groups. Separate t tests on the HR decreases from baseline for the experimental and control groups established that both decreases were significantly different from zero: Experimental group, $t(7) = 3.38$, p .01; Control group, $t(7) =$ 2.36, p .05. After Trial Block 1 the HR decreases in the first 5-min period became substantially smaller in both groups. The HRs of the two groups in the second 5-min period after the ethanol injection were near baseline on Trials Blocks 1 and 2. On Trial

Blocks 3, 4, and 5, both groups showed HR accelerations in the second period. However, separate t tests for each group established that only the HR increase of the experimental group on the fourth trial block was significantly different from zero during this period, $t(7) = 3.30$, $p < .02$.

A 2 by 5 by 2 (experimental vs control by trials by counting periods) analysis of variance was carried out on the C_1 and C_2 results shown in Figure 1 using CS minus baseline difference scores as the data. This test contained a significant trials effect, $F(4, 56) = 5.85$, $p < .01$, and a significant counting periods effect, $F(1, 14) = 14.48$, $p < .01$ indicating that the HR responses of the two groups changed reliably over trials and over counting periods. The analysis also contained a significant trials by counting periods interaction, $F(4, 56) = 5.99$, $p < .001$, indicating that there was a reliable shift in the directions of the HR responses over trials. None of the outcomes involving groups as a factor were significant.

The URs to the LiCl and saline USs following the ethanol CS appear as the last four points in each panel in Figure 1. An inspection of these points reveals that LiCl produced pronounced HR decreases in the experimental group. These cardiodecelerations began within 5 min of the LiCl injection, attained a maximum approximately 10 min after the injection, and showed little recovery by the end of the 20-min recording period. Although the overall magnitude of the HR decreases varied from trial to trial, there did not appear to be a consistent pattern of habituation or facilitation with repeated exposures to the LiCl. Figure 1 shows that the saline US given the control group was followed by cardioacceleration. It should be recalled

Figure 1. Mean heart rate in beats per min of the experimental and control groups as a function of recording periods and of two-trial blocks during ethanol-CS conditioning trials.

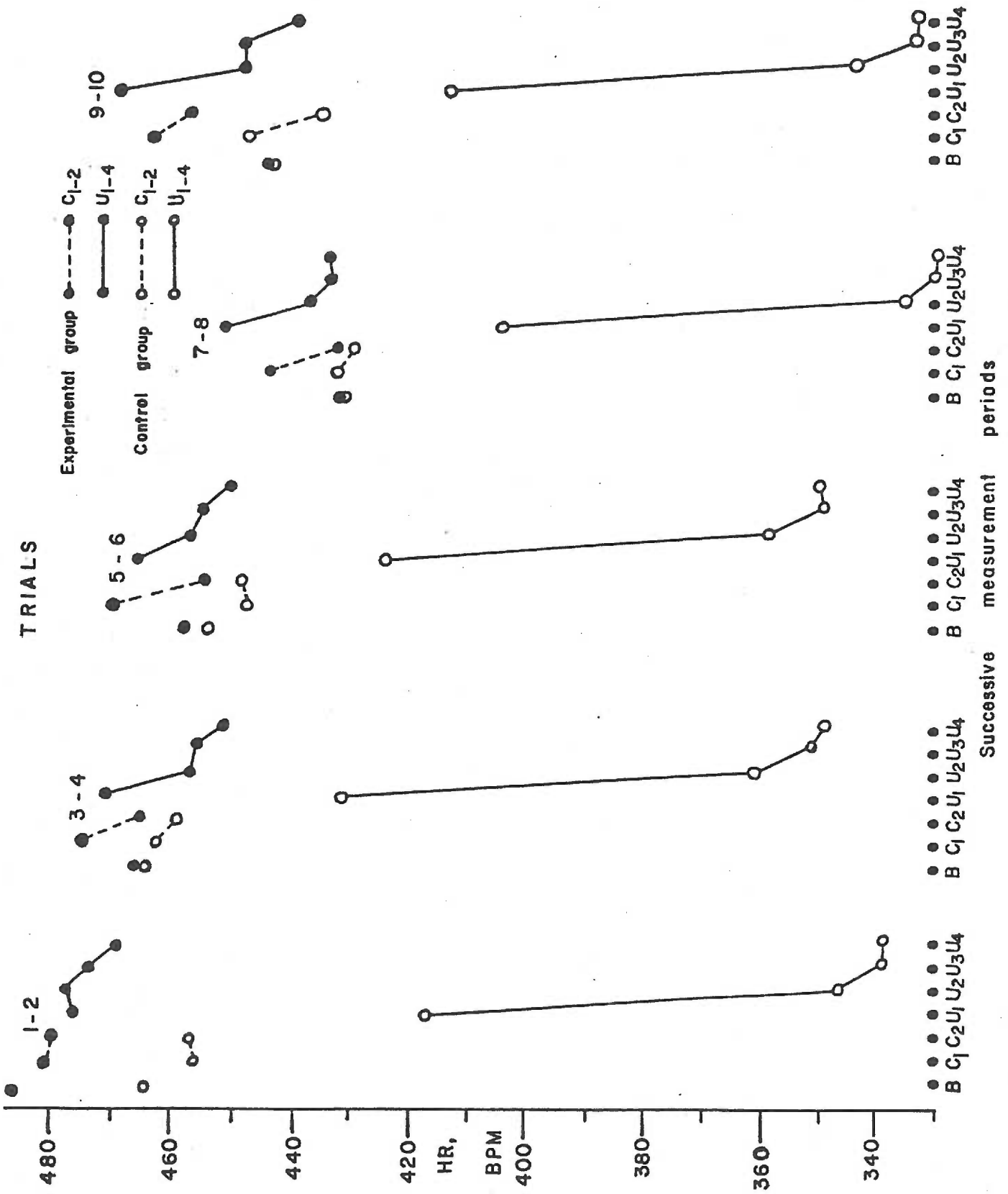

TRIALS
1-2
3-4
5-6
7-8
9-10
Experimental group
C_{1-2}
U_{1-4}
Control group
C_{1-2}
U_{1-4}
500
480
460
440
420
400
380
Heart rate, BPM
B C_1 C_2 U_1 U_2 U_3 U_4
Successive measurement periods

that each of these saline injections for this group was preceded 10 min earlier by an injection of ethanol and that HR was already accelerative or moving in that direction by the time saline was given. A *t*-test comparing the mean HR response of the control group in the four post-saline periods averaged over the five blocks showed that the HR increase (mean = 18.4) was significant, $t(7) = 3.05$, $p < .02$. Further statistical analyses of the URs depicted in Figure 1 will be given after the URs of the two groups on the saline-CS trials are presented in the next section.

Baseline HR during the ethanol-CS trials appears at the far left in each panel of Figure 1. With the exception of Trial Blocks 2 and 3, baseline HRs of the two groups were similar to each other, with both groups showing first a decrease and then an increase in HR. On Trial Block 3, baseline HR of the control group was 60 bpm below the level of the first trial block. A comparable decrease was shown later by the experimental group on Trial Block 4. Despite the substantial changes in baseline HR, the magnitudes of the responses, expressed as differences from baseline, remained fairly constant for both groups. A 2 by 5 (experimental vs control by trials) analysis of variance yielded a significant effect of trials, $F(4, 56) = 18.46$, $p < .001$, and a significant experimental vs control by trials interaction, $F(4, 56) = 3.38$, $p < .05$, supporting the reliability of the differential changes in the baseline HRs of the two groups across trials.

Saline-CS Trials. Shown in Figure 2 are the HR responses of the experimental and control groups on the conditioning trials in which saline was given as the CS. The points in each panel are labelled as in Figure 1 and the data were also averaged over five

Figure 2. Mean heart rate in beats per min of the experimental and control groups as a function of recording periods and of two-trial blocks during saline-CS trials of conditioning.


TRIALS
1-2
3-4
5-6
7-8
9-10
Experimental group
Control group
C1-2
U1-4
C1-2
U1-4
480
460
440
420
400
380
360
340
HR, BPM
B C1 C2U1 U2U3U4
B C1 C2U1 U2U3U4
B C1 C2U1 U2U3U4
B C1 C2U1 U2U3U4
B C1 C2U1 U2U3U4
Successive measurement periods

blocks of two trials each. On the first trial block, both groups displayed equivalent HR decreases from baseline in the two 5-min periods following the saline CS injections. Beginning with the second trial block and continuing for the remaining blocks, the responses of the two groups appeared to diverge during C_1. This divergence was mainly due to the fact that the experimental group showed HR accelerations in the first 5-min period following saline injections, whereas the control group continued to show mainly small HR decelerations or no change in HR.

A 2 by 5 by 2 (experimental vs control by trials by counting periods) analysis of variance of the CS minus baseline HR responses to saline yielded a significant effect of counting periods, $F(1, 14) = 4.62$, $p < .05$, indicating that the change in HR across the CS periods was reliable. The apparent trend for the responses of the two groups to become different across trials was not supported by a significant experimental vs control by trials by counting periods interaction.

The URs to the LiCl and saline USs on the saline CS trials appear as the last four points in each panel of Figure 2. As in the experimental group shown in Figure 1, the LiCl injections produced large magnitude HR decelerations in the control group which were visible in the first 5-min period following the injection. Maximum deceleration occurred 15 to 20 min after the injection. The saline-US injection in the experimental group produced HR acceleration in the first post-injection period with HR then returning to baseline or to a point slightly below baseline. Figure 2 also reveals that the HR baseline of the groups were similar to each other on the saline-CS trials with both groups showing a gradual decrease in HR over trials. A 2 by 5

(experimental vs control by trials) analysis of variance on baseline HR provided a significant effect of trials, $F(4, 56) = 7.31$, $p < .001$, indicating that this decrease was reliable.

A comparison of Figures 1 and 2 reveals that the HR decelerations that were produced by LiCl in the control group were larger than those occurring in the experimental group. The mean HR decrease of the control group averaged over the five trial blocks and over the four post-LiCl injection periods was 90 bpm, whereas for the experimental group the decrease was 50 bpm. In evaluating this difference it should be recalled that an injection of ethanol preceded the LiCl in the experimental group but not in the control group, and that ethanol by itself triggered HR increases (see E-S trials of control group in Figure 1) that could have diminished the HR decreases of the experimental group to LiCl.

A 2 by 5 by 4 (groups by trials by counting periods) analysis of variance comparing the LiCl US minus baseline HR responses shown in Figures 1 and 2 contained a significant experimental vs control effect, $F(1, 14) = 18.66$, $p < .001$, indicating that the group difference in the magnitude of the HR deceleration to LiCl was reliable. The test also provided a significant effect of trials, $F(4, 56) = 6.81$, $p < .01$, and a significant effect of counting periods, $F(3, 42) = 17.81$, $p < .001$. These effects indicate that there was a reliable change in the magnitude of the URs over trials and over counting periods. Further significant outcomes included the experimental vs control by trials interaction, $F(4, 56) = 3.05$, $p < .05$, and the experimental vs control by counting periods interaction, $F(3, 42) = 2.92$, $p < .05$. These findings indicate that the magnitude

of the HR differences between the groups varied reliably over trials and over counting periods.

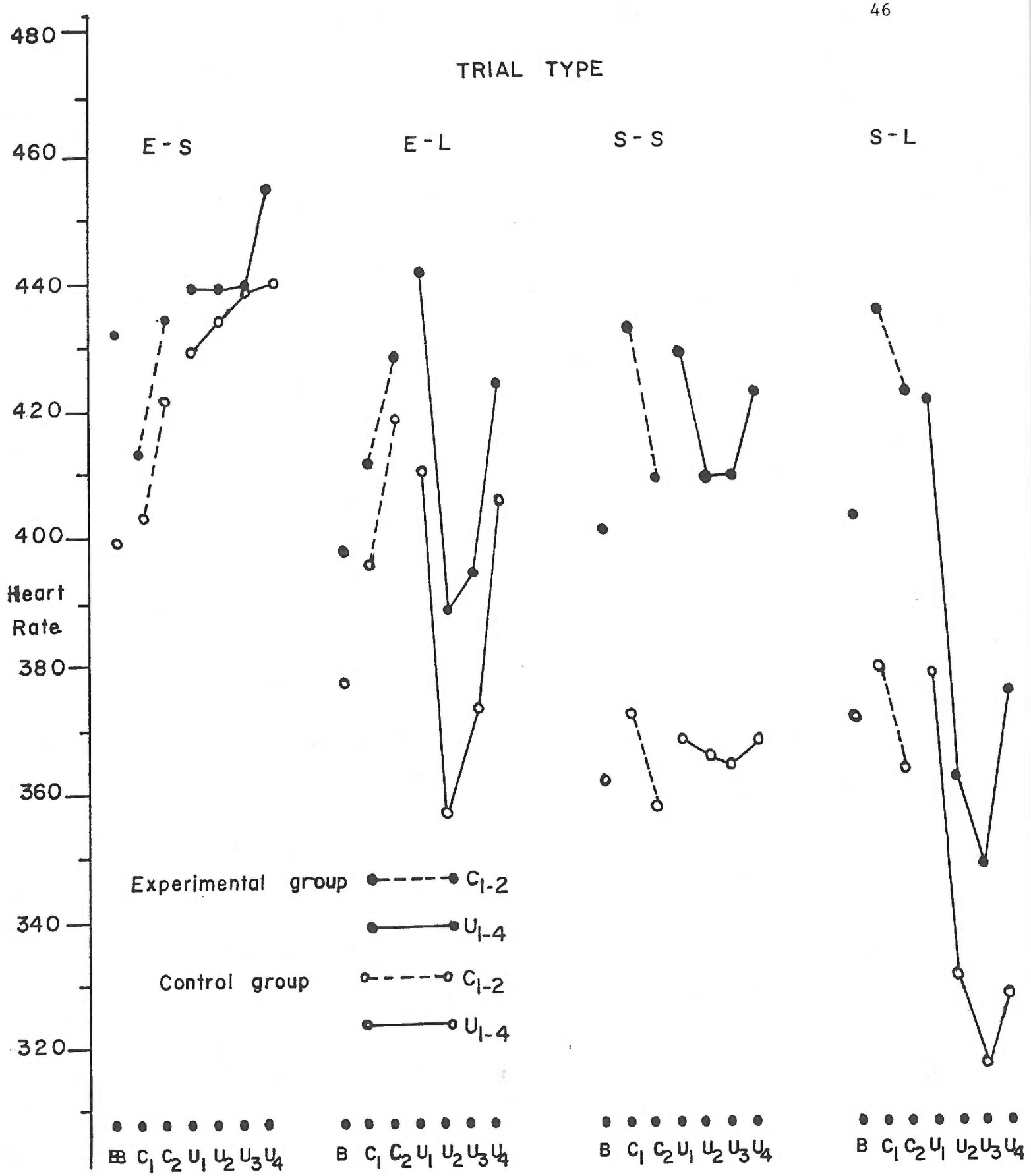
A comparison of the saline URs in Figures 1 and 2 reveals HR increases in both the experimental and control groups. However, the increase in the experimental group was restricted primarily to the first post-saline injection interval, whereas for the control group, it was present in all four intervals. A 2 by 5 by 4 (experimental vs control by trials by counting periods) analysis of variance of the US minus baseline HR responses following saline provided a significant effect of groups, $F(1, 14) = 7.52$, $p < .05$, establishing that the overall magnitudes of URs of the two groups were reliably different. There was also a significant groups by counting periods interaction, $F(3, 42) = 11.39$, $p < .01$, indicating that the pattern of the HR changes were not the same. Finally, there was a significant effect of trials, $F(4, 56) = 3.13$, $p < .05$, which can be attributed to a relatively small increase in the overall magnitudes of the HR increases that occurred over trials.

Phase 2 - HR Test Trials

Figure 3 depicts the mean HR responses of the experimental and control groups on the four test trials that were given following Phase 1. The points in each panel are labelled as in Figures 1 and 2. The first panel on the left of Figure 3 represents the E-S test trials, the second panel the E-L test trials, the third panel the S-S test trials, and the fourth panel the S-L test trials. Separate 2 by 2 by 2 by 2 analyses of variance with two between- and two within-group factors were carried out to test the significance of the anticipatory responses (C_1 and C_2 HR changes) on the ethanol-CS and the saline-CS test trials. The experimental vs control and the order of presentation of the test trials (E-L, E-S vs E-S, E-L) were the between-group factors and trials and counting periods were the within-group factors. Two separate 2 by 2 by 2 by 4 (groups by order of presentation by type of US by counting periods) analyses of variance were carried out to test the significance of the URs (U_1, U_2, U_3, and U_4 HR changes). For all analyses, the data were difference scores computed by subtracting baseline HR from the two CS counting periods or the four post-US counting periods.

Anticipatory HR Changes. Inspection of points C_1 and C_2 on the E-S and E-L test trials reveals that the overall anticipatory HR response of the control group to the ethanol CS was acceleration with respect to baseline on both trials. For the experimental group, the overall response was slight deceleration on the E-S test trial and acceleration on the E-L test trial. The four-way analysis of variance carried out on the C_1 and C_2 difference scores of the ethanol-CS test trials contained a significant effect of counting

Figure 3. Mean heart rate in beats per min of the experimental and control groups as a function of successive measurement periods during the four test trials following conditioning.


TRIAL TYPE
E - S
E - L
S - S
S - L
480
460
440
420
400
380
360
340
320
Heart Rate
Experimental group
Control group
B C1 C2 U1 U2 U3 U4
Successive measurement periods

periods, $F(1, 12) = 13.24$, $p < .01$, indicating that the pattern of responding to the ethanol CS was reliable in both groups. This analysis also contained a significant four-way interaction, $F(1, 12) = 4.90$, $p < .05$, indicating that one of the four subgroups responded in a reliably different manner on one of the test trials. To verify this conclusion, followup analyses were conducted which included separate 2 by 2 by 2 (trials by order of presentation by counting periods) analyses of variance for each group and separate 2 by 2 (order of presentation by counting periods) analyses of variance for each ethanol test trial for the experimental group. The three-way analysis for the control group contained only a significant effect of counting periods, $F(1, 6) = 7.81$, $p < .05$, as in the original analysis, while the three-way analysis for the experimental group contained a significant three-way interaction, $F(1, 6) = 9.85$, $p < .05$, indicating that the HR responses of the experimental subgroups changed in a reliably different manner between trials. In the subsequent two-way analyses of the individual trials for the experimental subgroups, no significant effects were found for the first ethanol test trial, while the analysis of the second test trial contained a significant effect of the order-of-presentation subgroups, $F(1, 6) = 8.71$, $p < .05$, and a significant effect of counting periods, $F(1, 6) = 25.39$, $p < .01$. These results indicate that although there was not a reliable response by either experimental subgroup on the first ethanol test trial, the subgroup which first received the extinction-type test trial showed a reliably larger HR acceleration on the second test trial than the subgroup which first received a consistent test trial.

The anticipatory HR changes on the S-S and S-L test trials indicate that the overall response of the experimental group to the

saline CS was HR acceleration, whereas for the control group it was near zero change. It should be noted that the apparent group differences on the saline test trials were comparable to those that were present on the final saline-CS trials during the previous conditioning phase. For both groups, HR appeared to be higher during C_1 than during C_2 of the saline-CS test trials. The four-way analysis of variance carried out on the C_1 and C_2 difference scores of the saline-CS test trials provided only a significant effect of counting periods, $\underline{F}(1, 12) = 16.51$, $\underline{p} < .01$, supporting the reliability of the HR response pattern to the saline CS but not of the apparent group difference.

UR Changes on the Ethanol Test Trials. The E-S test trial represented the first time that the ethanol CS was followed by saline in the experimental group. For the control group, ethanol was always followed by saline. The substitution of saline for LiCl in the experimental group was meant to provide information on the possible existence of a conditioned response in the experimental group that was being masked by the presence of the LiCl US. Such a conditioned response would appear as a difference between the experimental and control groups during the post-saline periods (i.e., U_1, U_2, U_3, and U_4). As Figure 3 shows, the HR changes in the four post-saline periods were highly similiar for the two groups. Both groups showed HR accelerations with respect to baseline, that were no doubt mainly due to the circulating levels of ethanol that had been injected previously. In general, it can be noted that the HR changes that occurred on the E-S test trials were not unlike those that were present on the E-S trials given the control group during the previous conditioning phase and depicted in Figure 1.

It will be recalled that the E-L test trial was the first occasion in which the control group received LiCl following ethanol. What was being searched for on this trial was some indication that the UR of the control group to LiCl would be different from that of the experimental group. Such a difference could have reflected the presence of a conditioned response to ethanol in the experimental group that was modifying the UR to LiCl. It is clear from Figure 3 that no such evidence was found as the reactions of the two groups to LiCl were highly similiar to each other both in terms of the overall magnitude of the reactions and their time courses over the four post-LiCl injection periods. In each case, cardiodeceleration reached its peak in the second period (i.e., U_2), with HR then going back towards baseline.

The four-way analysis of variance carried out on the U_1, U_2, U_3, and U_4 difference scores of the ethanol-CS test trials did not contain a significant effect of groups, $F(1, 12) = 0.0$, n.s. However, the analysis did contain a significant effect of the type of US, $F(1, 12) = 9.24$, $p < .05$, a significant effect of counting periods, $F(1, 36) = 6.20$, $p < .01$, and a significant US by counting periods interaction, $F(1, 36) = 12.74$, $p < .01$. These results indicate that for both groups, the HR responses to each US on the ethanol test trials were reliably different, that the UR to each US had a reliable pattern, and that these patterns were reliably different from each other.

UR Changes on the Saline Test Trials. In principle, little if any change in HR was expected to occur on the S-S test trials. Neither the experimental nor the control gruop had experienced saline in such a way that a conditioned HR response would have been anticipated.

The results shown for the S-S trial in Figure 3 were consistent with this view, in that neither group showed lasting HR reactions following saline. Both groups exhibited brief HR accelerations in the first 5-min periods after the saline injections, with HR in the remaining periods generally being near baselevel.

For the experimental group, the S-L test trial was the first instance in which LiCl was not preceded by ethanol. However, the control group had received 10 S-L trials prior to the one shown in Figure 3. For the experimental group, the use of saline instead of ethanol on this trial provided an opportunity to measure the HR reaction triggered by LiCl when LiCl was given unexpectedly in the absence of the ethanol CS. Conceivably, a major difference in the LiCl URs of the experimental and control groups on the S-L test trial could reflect some kind of conditioned compensatory response in the experimental group that resulted from previous pairings of the ethanol CS injection with the LiCl US injection on earlier trials, or a compensatory response by the control group. However, as Figure 3 reveals, the LiCl URs of the two groups shared several major features. Basically, they were both decelerative, they followed similiar time courses and their magnitudes were in general about the same. This was true even though the baselevel HR in the control group was substantially below that of the experimental group.

The four-way analysis of variance carried out on the U_1, U_2, U_3, and U_4 difference scores of the saline test trials contained a significant effect of the type of US, $F(1, 12) = 29.29$, $p < .01$, a significant effect of counting periods, $F(3, 36) = 29.07$, $p < .01$, and a significant US by counting periods interaction, $F(3, 36) = 36.23$,

$p < .01$. Thus, as for the URs on the ethanol test trials, these results indicate that for both groups the HR responses to each type of US on the saline test trials were reliably different, that the UR to each US had a reliable pattern and that these patterns were reliably different.

Baseline HR During the Test Trials. Baseline HR during the test trials appears at the far left in each panel of Figure 3. The baseline HRs of the two groups appear to be different; the control group was consistently below the experimental group. Also, the baseline HRs of both groups were lower on the E-L trials than on the E-S trials and were higher on the S-L trials than on the S-S trials. Separate 2 by 2 by 2 (groups by order of presentation by trials) analyses of variance were carried out on the baseline HRs of the ethanol and the saline test trials. This analysis for the ethanol test trials yielded a significant effect of trials, $F(1, 12) = 8.35$, $p < .05$, and a significant order by trials interaction, $F(1, 12) = 6.44$, $p < .05$, indicating that there was a reliable change of baseline between the ethanol test trials and that the order of presentation of these trials affected the magnitude of the change, but that the two groups were not reliably different. The analysis carried out on the baseline HRs of the saline test trials contained only a significant effect of trials, $F(1, 12) = 4.76$, $p < .05$, indicating that the change of baseline HR between the two saline test trials was reliable but that the apparent group difference was not.

Phase 3 - Taste Aversion with Ethanol

Ethanol Aversion. Figure 4 depicts the mean ethanol consumption of both groups during the two 5-min ethanol drinking periods of the ethanol aversion test. During the first 5-min period, the mean consumption of the experimental group was 5.9 ml of ethanol and that of the control group 10.2 ml of ethanol. A comparison of these means using a *t*-test indicated that the difference between groups was significant, $t(14) = 3.35$, $p < .01$. During the second 5-min test period, the experimental group drank 6.0 ml of ethanol, whereas the control group drank 4.3 ml of ethanol. This difference was not significant. The total ethanol consumption of each group, shown in the right of Figure 4, was 11.9 ml for the experimental group and 14.4 ml for the control group. This difference was also not significant. The results of the first period indicate that the experimental group acquired some aversion to ethanol during the ethanol-LiCl conditioning phase.

Ethanol as a US. Figure 5 displays the mean saccharin consumption of both groups on each of the 6 taste aversion conditioning trials in which ethanol was paired with saccharin. It can be seen that the consumption of saccharin in the control group was approximately 2.5 to 3.0 ml below that of the experimental group throughout the taste aversion conditioning phase. The saccharin consumption of both groups decreased over the 6 trials. The mean of both groups for the first trial was 12.4 ml of saccharin and for the sixth trial it was 7.2 ml of saccharin, which was a significant decrease, $t(15) = 4.75$, $p < .001$. Individual *t*-tests for each trial revealed that the saccharin consumption was not significantly different between the two groups at any time during the taste-aversion phase.

Figure 4. Mean oral ethanol consumption of the experimental and control groups during the test periods of the ethanol aversion test.

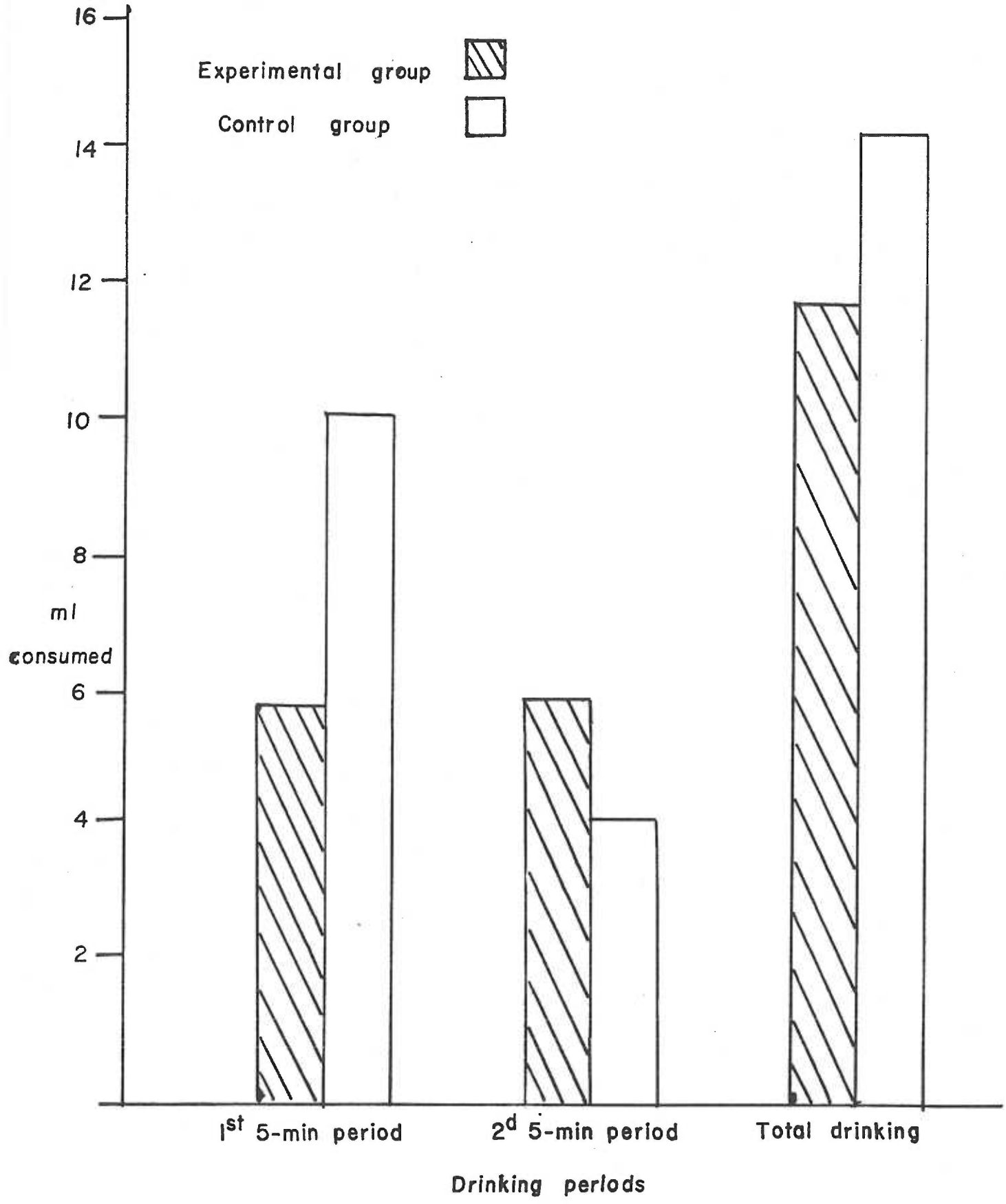
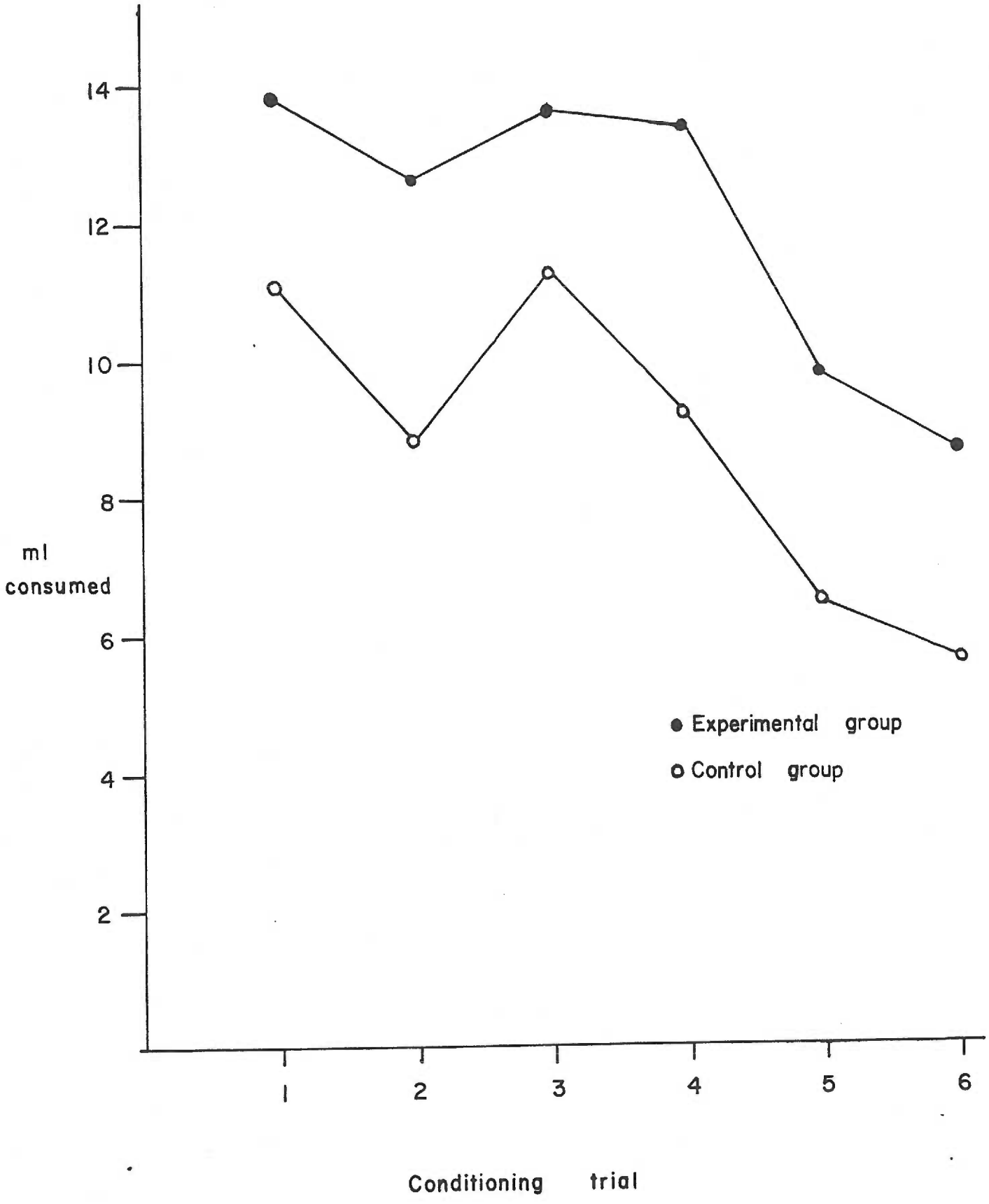

16
14
12
10
8
ml
consumed
6
4
2
Experimental group
Control group
1st 5-min period
2d 5-min period
Total drinking
Drinking periods

Figure 5. Mean saccharin consumption of the experimental and control groups as a function of successive trials during taste-aversion conditioning with ethanol as the US.


14
12
10
8
6
4
2
ml
consumed
Experimental group
Control group
1
2
3
4
5
6
Conditioning trial

DISCUSSION

The principal findings of the present study were that: (a) the HR responses to the ethanol CS on the early presentations of ethanol were decelerative. On later trials, ethanol tended to produce HR accelerations; (b) conditioned changes in HR were not obtained as a result of pairing ethanol with LiCl. There was neither evidence of conditioned HR in anticipation of LiCl nor of conditioned HR during the post-LiCl periods; (c) the experimental subgroup that received an extinction-type test trial first differed reliably on the second test trial from the subgroup that received a consistent test trial first; (d) the unconditioned HR responses to LiCl consisted of profound deceleration lasting for the entire 20-min recording period. The magnitudes of HR decelerations produced by LiCl were attenuated by prior injection of ethanol; (e) oral aversion to ethanol was shown by the experimental group in a special drinking test that was administered after ethanol and LiCl had been paired with each other. No conditioned change in the potency of ethanol as a taste-aversion US was found.

The original, decelerative HR responses to the ethanol CS were pronounced in both groups on the first two trials (not shown individually) that ethanol was administered. Both groups displayed HR decreases of approximately the same magnitude, with some animals in each group attaining decreases of up to 60 bpm during the first 5-min period after ethanol injection. These HR responses were of relatively short

duration, with HR returning to near baseline during the second 5-min period after the injection. After the first two trials, the short-latency decelerative HR responses to ethanol did not occur and only small, unreliable decreases or increases with respect to baseline were seen during the first 5-min post injection period. On later trials, there was a tendency toward ethanol-induced HR acceleration in the second 5-min period after the drug administration, although this tendency was largely unreliable.

It does not seem likely that the decelerative HR response to ethanol that was observed here was due to the novelty of the injection procedure. This is supported by the lack of a comparable deceleration on the first trial in which saline was administered. The ethanol and saline trials were given in an alternating manner and much less HR deceleration was seen on the first and second saline trials than on the first and second ethanol trials. While not statistically significant, the small HR decreases following saline injections suggest that the novelty of the injection procedure may have contributed to the larger HR decreases seen after the ethanol injections, but only partially.

The initial HR decrease following ethanol administration may have been caused by internal physiological changes associated with rising blood-ethanol concentrations. Several authors have reported that the behavioral and physiological effects observed during the absorption of ethanol may differ from those observed during the elimination of ethanol (Eggleton, 1942; Mirsky, Piker, Rosenbaum & Lederer, 1941; Kalant, LeBlanc, & Gibbins, 1971a, 1971b; LeBlanc, Kalant, & Gibbons, 1975; Kulig, 1977). In the present case, the novelty of the internal

state of the rat shortly after the administration of ethanol may have triggered a type of cardiac orienting response. A number of studies (Black, 1964, Cunningham, Fitzgerald, & Francisco, 1977; Teyler, 1971) have shown that rats exhibit decelerative HR orienting response to novel or new stimulus events presented without warning. The disappearance of the HR decrease after the first two ethanol trials could have been due to the fact that tolerance to the internal changes produced by ethanol is known to occur quite rapidly (Kalant, LeBlanc, and Gibbins, 1971b).

Previous reports have indicated that the cardiac responses of rats (Fitzgerald & Stainbrook, 1978, Crow, 1978), of dogs (Webb & Degerli, 1965) and of humans (Riff, Jain, & Doyle, 1969; Juchems & Klobe, 1969) to low doses of ethanol comparable to that employed here consists primarily of HR acceleration. Consistent with the present study, however, Stainbrook (1975) and Fitzgerald and Stainbrook (1978) also observed short latency HR decreases after a single administration of ethanol. In the other experiments, HR was averaged over relatively long time intervals such that a short-latency decelerative component may have been overlooked.

The ethanol-induced HR increases in the second 5-min period that were seen on later trials in the present study are consistent with findings of other investigators who have reported cardioacceleration in response to ethanol. Except for a single trial block, the HR increases shown by the experimental group were not significant. However, 10 min was probably not sufficient for maximum HR increases to occur. Any further increases beyond that point would, of course, be masked by the powerful effects of LiCl on the cardiovascular system.

For the control group, which received saline following ethanol, maximum HR was attained 15 to 20 min after ethanol was injected and these increases were significant.

Two possible mechanisms may account for the non significant ethanol-induced HR increases. First, studies in dogs (Webb & Degerli, 1965) and in humans (Riff, Jain, & Doyle, 1969; Juchems & Klobe, 1969) demonstrated that low and moderate doses of ethanol produced decreases in peripheral vascular resistance as well as increased HR. Juchems and Klobe (1969) suggested that the decrease in peripheral resistance may have led to reflexive tachycardia. A second mechanism was suggested by the fact that ethanol administration to cats and rabbits was found to produce significant elevations in plasma and urinary levels of epinephrine and norepinephrine (Walsh & Truitt, 1968). These sympathomimetic agents exert actions directly on the heart and include increased HR and contractile force (Walsh, Hollander, & Truitt, 1969; Walsh, Truitt, & Hollander, 1968).

There was no evidence on the conditioning trials of the development of a conditioned anticipatory HR response as a result of pairing ethanol and LiCl injections. Although there were changes in the HR response to ethanol over trials, both groups showed approximately the same pattern of changes, with very little divergence between the two groups being observed. It is not known whether a conditioned HR response based on a LiCl US would be in the same direction as the UR, i.e., decelerative, or if it would be opposite, i.e., accelerative.

Siegel (1977) has argued that for some kinds of drug conditioning situations, the CR may represent a compensatory physiological adjustment to the forthcoming US and therefore be quite unlike the UR. Predicting

the nature of the CR in the current study was made difficult by the effects of the ethanol CS on HR. These effects could have interfered with the development or performance of a decelerative HR CR. However, it seems likely that the development of an accelerative HR CR would have been aided by the naturally occurring HR increase to the ethanol CS. Had it been present, such an accelerative CR would have been opposite the decelerative UR and supported Siegel's (1977) compensatory response hypothesis.

The test trials provide the only reliable evidence of HR changes which might indicate conditioning. Thus, for the experimental subgroup which received an extinction-type test trial first, the ethanol HR response on the second test trial was elevated significantly above the experimental subgroup which had received a consistent test trial first. An analogous effect was not observed between the control subgroups. This seeming "extinction effect" may be explained, however, by the fact that the baseline HR of the experimental subgroup that received the extinction-type test trial first decreased reliably between test trials, giving the appearance of an exaggerated HR acceleration on the second test trial.

Evidence of the presence of HR conditioning during the UR periods on the test trials was not found. Although the experimental group showed smaller HR decreases to the LiCl US than did the control group on the regular conditioning trials, this difference was not present on the test trials when both groups received the same CS prior to the LiCl injection. On test trials in which LiCl was omitted following administration of the ethanol CS, the two groups also showed similar response patterns. However, inspection of the performance of individual

experimental animals on these trials revealed the presence of profound HR decreases to the absence of LiCl, suggesting that some conditioning may have occurred in the experimental group.

Finding that the direction of the unconditioned HR response to LiCl was a deceleration is consistent with the reported effects of lithium on HR in rabbits (McKusick, 1954), in dogs (Riccuitti, 1971), and in humans (Tilkian, Schroeder, Kao, & Hultgren, 1976). An attenuating effect of ethanol on the effects of LiCl has not been reported previously. Ho and Ho (1978) reported that ethanol potentiated lithium toxicity in mice. In their study, acute administration of ethanol reduced urinary excretion of lithium, resulting in higher tissue levels of lithium. However, this effect did not appear until 3 to 6 h after both drugs were given. In the present study, it seems likely that the catecholamine release induced by ethanol (Walsh & Truitt, 1968) was responsible for the attenuation of LiCl-produced HR decreases.

The principal cardiovascular effects of lithium may be mediated peripherally by alterations of ion transport across myocardial cell membranes. The intracellular-extracellular potassium ratio is critical for the maintenance of normal cardiac activity (Vander, Sherman, & Luciano, 1970). McKusick (1954) described severe hyperkalemia accompanying decreased HR following lithium administration. It has recently been shown that lithium decreases inward potassium transport in rabbit atria (Kunze, 1977) and in sheep cardiac Purkinje fibers (Aronson & Gelles, 1977).

As a part of the present experiment, a preliminary study of the effects of LiCl on the isolated rat heart was carried out. The purpose

of this study was to determine the extent to which lithium is capable of exerting decelerative effects directly on the myocardium, without involvement of central cardiovascular control mechanisms. In this study, rat hearts were removed and maintained with a Langendorf preparation and a modified Krebbs perfusion medium (c.f., Tanz, 1974). After establishing baseline HR, perfusion mediums containing various additional concentrations of LiCl were substituted for the normally-used Krebbs solution. The results of this study were that LiCl slowed the hearts in a dose-dependent manner. Maximum HR decreases of approximately 125 bpm were obtained, usually within 10 to 25 min after exposure to LiCl solutions. These responses of the isolated myocardium to LiCl paralleled those seen in the intact in vivo preparation. Thus, it seems likely that the myocardial actions of LiCl are mainly peripheral and are not mediated via a CNS mechanism.

Successful conditioning was obtained during the oral aversion test. The experimental group consumed significantly less ethanol than did the control group in the first of two 5-min drinking periods following the ethanol-LiCl pairing phase of the study. This finding is consistent with results observed by Cunningham (unpublished manuscript) and with other demonstrations indicating that animals may form oral aversions to flavor stimuli (i.e., saccharin) that have been injected intraperitoneally (e.g., Buresova & Bures, 1977). The formation of taste aversions to injected flavor stimuli has been presumed to be mediated vascularly, i.e., by the secretion of the flavor from the bloodstream into saliva (Cunningham, 1978). It is likely that the same process occurred with ethanol in the present experiment.

It is generally acknowledged that taste aversion represents an association between the taste of a flavored stimulus and illness

(Rozin & Kalat, 1971; Domjan & Wilson, 1972; Mackintosh, 1974; Spiker, 1977). It is possible, however, that aversion to oral ethanol in the present experiment did not simply represent aversion to the taste of ethanol, as this was the first occasion on which ethanol was presentedorally. Injected ethanol possesses many discriminable interoceptive effects other than taste (Overton, 1971, 1977), such as intoxication. Reduced ethanol consumption during the oral test may have been due to the onset of the non-taste interoceptive effects of ethanol, rather than the taste, which had been previously paired with LiCl. The group difference appeared early in the taste test, however, before appreciable onset of ethanol's non-taste interoceptive effects, and was practically abolished at a time in the test when these interoceptive effects were highest. The oral doses consumed by the experimental and control groups averaged 0.86 g/kg and 1.76 g/kg, respectively, at the end of 5 min, and averaged 1.8 g/kg and 2.2 g/kg, respectively,at the end of 10 min. This finding suggests that taste, not intoxication, was the effective stimulus.

The effectiveness of ethanol as a taste aversion US was not found to be reduced following ethanol-LiCl pairings, in contrast to the findings of Cunningham (unpublished manuscript). This difference might be explained on the basis of procedural differences between the present study and those of Cunningham. Further studies by Cunningham (unpublished results) in which the procedures were more closely equated with the present study did not demonstrate a change in the US effectiveness of ethanol following pairings with LiCl.

Consideration should be given to the fact that different outcomes were obtained for the HR and taste aversion measures of conditioning

that were used in the current experiment. This apparent conflict is not unlike outcomes of previous studies using adrenaline (Gantt, Katzenelbogen, & Loucks, 1937) and atropine (Rush, Pearson, & Lang, 1970) as drug USs. In discussing such findings it is necessary to examine the question of centrally versus peripherally mediated drug effects in classical conditioning. Gantt (1970), McKenzie and Gantt (1950) and Lynch, Fertziger, Teitelbaum, Cullen, and Gantt (1973) have claimed that classical conditioning is not obtainable using agents or stimuli which only exert effects peripheral to the CNS. More specifically, Gantt (1970) has claimed that if a drug exerts mixed central and peripheral actions, only the centrally mediated effects of the drug may be conditionable. This view has received some experimental support from studies using drugs having both central and peripheral actions (Gantt, Katzenelbogen, & Loucks, 1937; Rush, Pearson, & Lang, 1970).

The central and peripheral actions of LiCl have not been well delineated in the past. The effectiveness of lithium in the treatment of organically-based affective disorders such as manic-depressive psychosis implies that lithium is capable of exerting effects within the CNS. Although the HR effects of LiCl appear to be peripheral in nature, there is evidence to suggest that lithium affects several neurophysiological functions. Parameters of catecholamine metabolism (Murphy, 1976), ion complexing with proteins (Williams, 1976), and ion transport channels in nerve cell membranes (Hill, 1976) have all been shown to be altered by lithium. Its mechanism of action in the effective treatment of mania is still unknown (Murphy, Costa, & Bunney, 1976).

It appears that LiCl is capable of exerting some actions within the CNS that can be conditioned. One of these central actions is the ability of LiCl to produce increased secretion of ACTH, a pituitary hormone that has been shown to be involved in taste aversion conditioning. To the extent that the presence of ACTH and the phenomenon of taste aversion conditioning are based on emotional or aversive characteristics of LiCl, then HR conditioning using LiCl as a US might also be expected, as other studies have shown that the aversiveness of a US is a major variable controlling the development of HR CRs (Fitzgerald, Martin, & Hoffman, 1976; Gantt, 1970; Bruner, 1969; Sideroff, Elster, & Schneiderman, 1972). The absence of HR conditioning in the present experiment suggests that factors in addition to aversiveness or emotionality may be required for HR conditioning to occur.

In addition to the potency of the US, there were other parameters in the present experiment that should have facilitated the development of a conditioned HR response. For example, the use of an interoceptive drug stimulus as the CS should have enhanced the possibility of conditioning with LiCl, as taste aversion studies have indicated that interoceptive stimuli function more effectively as CSs for LiCl than do exteroceptive stimuli (Garcia & Koelling, 1966; Morrison & Collyer, 1974). Moreover, ethanol is known to have clearly discriminable stimulus effects (Overton, 1971, 1977) that could become associated with a US. A second factor was the CS-US interval that was selected. Drug effects do not have an immediate onset, so it was deemed appropriate to delay the injection of the LiCl US for 10 min following the delivery of the ethanol CS. At this interval, the internal effects of the ethanol should have reached their maximum point, based on

blood-ethanol absorption curves (Stainbrook, 1975; Linakis & Cunningham, 1979). The interval between the ethanol effects and those of LiCl, which probably required less than 1 min to be present, was probably well within the range of CS and US intervals that will support HR conditioning. As a case in point, Goldberg and Schuster (1971) described conditioned HR reactions to nalorphine when a light regularly preceded the drug by as much as 5 min. Conceivably, however, shorter CS-US intervals that overlapped the rising portion of the blood-ethanol curve and the rapid onset of the LiCl effects would increase the chances of obtaining HR conditioning to ethanol and LiCl stimulus events.

The fundamental pharmacological principle that no drug has a single action cannot be ignored. In the analysis of behavior, no stimulus has a single action, and this is closely paralleled by the multiple effects of drug stimuli. Catania (1971) has stated that it is not appropriate to say that a drug produces a stimulus, but that a drug effect is a stimulus complex. Gantt (1970) claims that when this stimulus complex consists of peripherally and centrally mediated effects, only the centrally mediated effects can become conditioned.

In general, the results of the present study may be viewed as being consistent with those previous studies reporting "fragmentary" conditioning of central and peripheral drug effects and with assertions that the central actions of drugs are more readily conditionable than are peripheral actions. Thus, taste aversion with LiCl, which appears to have a centrally mediated ACTH component (Smotherman et al., 1976), was found, whereas LiCl effects on HR, which may be mainly peripheral in nature, did not lead to HR conditioning.

SUMMARY AND CONCLUSIONS

The purpose of the present investigation was to determine whether classically conditioned changes in HR could be produced in rats using an i.p. injection of ethanol as the CS and an i.p. injection of LiCl as the US. Additionally, oral aversion to the ethanol CS was assessed following the conditioning trials to allow a comparison to be made between a consummatory CR and a cardiac CR. Experimental rats received 10 pairings of ethanol and LiCl injections while control rats received equivalent drug exposures in an explicitly unpaired fashion. Subsequently, four test trials were given in which both groups received the CS alone, the CS and US, the US alone, and neither CS nor US. A test of taste aversion to an ethanol solution followed the HR test trials, along with a subsequent test of the strength of ethanol as a US for taste aversion to a saccharin CS.

The main findings were that evidence of taste aversion to oral ethanol was found in the experimental group while conditioned HR responses to the i.p. ethanol CS were not obtained. The experimental and control groups showed initial HR deceleration in response to ethanol which was replaced by a tendency toward HR acceleration on later trials. The HR responses of both groups to LiCl consisted of large-magnitude cardiodecelerations.

The pattern of experimental outcomes concerning the presence of conditioning was discussed in relation to the central and peripheral effects of the LiCl US. Evidence was reviewed which suggests that

the effects of LiCl in taste aversion are centrally mediated while the cardiac effects may be mediated directly on the myocardium. The outcomes of conditioning were consistent with the view that centrally mediated drug effects are conditionable while peripherally mediated drug effects are not.

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