

**THE INFLUENCE OF PAVLOVIAN CONDITIONING
ON THE
MOTIVATIONAL PROPERTIES OF ETHANOL**

by

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ABSTRACT

The development of animal models of alcoholism has encountered limited success. Unlike humans, rats and most other animals are reticent to self-administer alcohol at levels resulting in pharmacological effects. These experiments were designed to investigate the ability of a positive reinforcer, sucrose, to transfer positive motivational properties to ethanol through Pavlovian conditioning. The influence of sucrose pairing on ethanol reward was measured using three different paradigms: self-administration, taste conditioning, and place conditioning.

In Experiment 1, experimental groups received sucrose paired with either a typically aversive concentration of orally-available ethanol or an ethanol-saccharin solution, while controls received sucrose independently of ethanol. Animals receiving ethanol-saccharin paired with sucrose showed the greatest ethanol consumption, followed by those receiving ethanol with sucrose, and lastly by unpaired controls. The effect of sucrose concentration was measured in Experiment 2. Two-bottle choice testing demonstrated significantly greater preference for ethanol-saccharin in subjects receiving 32% sucrose versus 4% sucrose. These results suggest that the aversive orosensory cues of ethanol could be overridden by appetitive associative cues and that ethanol may develop secondary motivational properties as a result of conditioning.

The results of Experiment 2 suggested that sucrose consumption significantly lowered blood ethanol concentration (BEC). To minimize this effect, ethanol was administered intraperitoneally in Experiment 3, and a delay was introduced between ethanol delivery and sucrose access. Ethanol's motivational value was assessed by measuring the degree of taste aversion conditioned to NaCl. Unexpectedly, those subjects receiving ethanol paired with sucrose demonstrated greater taste aversion than unpaired controls. Although BECs were not measured, it is possible that the subjects receiving ethanol paired with sucrose had lower BECs, thus experienced less

ethanol preexposure than the unpaired controls, resulting in greater taste aversion.

In Experiment 4, the effect of sucrose consumption on BEC at various time points following i.p. injection of 0.5, 1.0, and 1.5 g/kg ethanol was examined. While sucrose, in general, significantly reduced blood ethanol levels, little impact was observed when sucrose was given 30 min after i.p. delivery of 1.0 g/kg ethanol.

In Experiment 5, the taste conditioning design used for Experiment 3 was repeated using the parameters suggested by Experiment 4. In contrast to Experiment 3, no significant differences in NaCl consumption were observed between the experimental and control groups, suggesting that ethanol preexposure completely prevented the conditioning of taste aversion. In addition, since consumption was unaffected by ethanol-sucrose pairing, it appears that sucrose was unable to alter the motivational properties of ethanol as measured by this paradigm.

The use of place conditioning to assess the valence of ethanol also failed to reveal a distinction between experimental groups as a result of sucrose pairing (Experiment 6). However, in contrast to the taste conditioning study, a reduction in ethanol preexposure trials did allow aversion to ethanol-paired cues to be observed.

In summary, these studies demonstrate that pairing orally-delivered ethanol with sucrose resulted in increased preference for ethanol solution in self-administration studies. In contrast, associating sucrose with intraperitoneally-delivered ethanol did not alter ethanol's motivational effects as measured by taste conditioning and place conditioning. The ability of sucrose to lower BECs may have accounted for the increased ethanol preference in the ethanol-sucrose groups in Experiments 1 and 2, suggesting that ethanol's pharmacological effects are aversive. The quantitative differences in preference observed following varying concentrations of sucrose would suggest, however, that associative factors also may play a role, and that the motivational properties of ethanol can be conditioned.

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Introduction

Theories of drug abuse often suggest that drugs are generally abused because of their reinforcing pharmacological effects (Stewart, de Wit, & Eikelboom, 1984; Tabakoff & Hoffman, 1988). However, behavior consistent with drug abuse also may arise not from the intrinsic reinforcing properties of abused substances, but through secondary, or learned, motivational effects (McCusker & Bell, 1988; van der Kooy, O'Shaughnessy, Mucha, & Kalant, 1983; Vogel-Sprott, 1972). Principles of learning observed with nonpharmacological stimuli may be applied to our understanding of the reinforcing properties of drugs. A greater appreciation of the role of learning in ethanol reinforcement and the development of methods to investigate and manipulate the reinforcing properties of ethanol may enhance our understanding of the etiology, prevention, and treatment of alcohol abuse and alcoholism (Sherman, Jorenby, & Baker, 1988).

Events or stimuli are defined as reinforcers or punishers in terms of their influences on behavior (Mello, 1983). A stimulus is classified as a reinforcer if it increases the probability of a response, while punishers suppress behavior (Skinner, 1953). The same stimulus event may actually serve as either a reinforcer or as a punisher, depending upon the specific conditions under which it is presented (Morse, McKearney, & Kelleher, 1977). Although some stimuli, such as food and electric shock, tend to maintain consistent roles as reinforcers and punishers through a wide variety of situations (Schwartz, 1984), reinforcing value is not an immutable property. In the extreme, animals can be conditioned to avoid food (Fowler & Miller, 1963; Masserman, 1943) and to approach shock (Brown, Martin, & Morrow, 1964; Melvin & Stenmark, 1968), emphasizing the power of learning in dictating behavior.

There are two general categories of associative learning procedures which differ in terms of the operations established with the reinforcer during training. In classical (Pavlovian) conditioning, the experimenter arranges a contingency between a stimulus and the reinforcer (Rescorla, 1967), with the reinforcing or punishing stimulus presented independently of the subject's behavior (Dickinson, 1980). In contrast, instrumental conditioning involves a response-reinforcer contingency in that the subject must perform a particular response to receive reinforcement or to avoid or escape punishment (Mackintosh, 1983; Sherman et al., 1988).

Thorndike (1911) was one of the first to recognize the importance of motivational influences in learning. He evaluated the ability of certain stimuli to "motivate" behavioral responses by virtue of the consequences of the behavior. He employed instrumental paradigms to investigate the role of response outcomes in controlling behavior. Positive motivational properties would be expected to induce or increase the associated behavior, while negative properties would reduce or inhibit responding.

Thorndike proposed the Law of Effect, hypothesizing that the association between a stimulus and a response was strengthened or weakened as a result of the consequences of the response. He theorized that when an animal is exposed to certain "stimulus situations," it performs a wide variety of behaviors. Through trial and error, the subject may produce a response which results in a satisfying consequence. Thorndike believed that the consequence of a response influenced the learning of an association between environmental stimuli and the response. When a particular response produced a positive event, it was likely to be repeated when those stimuli were experienced again. He hypothesized that behaviors followed by satisfying consequences became more strongly associated with the cues of the situation. When responses were followed by discomfort, the cue-

response association was "stamped out," such that the animal avoided making the same response under those cues. Thus, the presence or absence of reinforcing consequences was viewed as shaping the most adaptive response to a given situation.

Classical conditioning was first systematically studied by Pavlov (translated in 1927). In this paradigm, a neutral stimulus, the conditional stimulus (CS), is presented in temporal contiguity with the delivery of a motivationally significant event, the unconditional stimulus (US). The US unconditionally (i.e. prior to any training) elicits a response, the unconditioned response (UR). With repeated pairings, the CS comes to evoke responses (CR) in anticipation of US delivery. These responses, elicited under the conditions of the training procedure, are evidence that an association between the CS and US has been learned (Stewart & Eikelboom, 1987). In his classic study, Pavlov paired the sound of a bell (the CS) with the presentation of food (the US) in dogs. Food spontaneously triggered salivation (the UR). With repeating pairings of the bell and food, the bell also came to elicit salivation (the CR) in the absence of food delivery.

Pavlov (1927) often referred to the US as the "reinforcer" in recognition of its role in conditioning a behavioral change to the CS; however, many operant theorists reserve use of this term for instrumental paradigms in which performance of a contingent response is increased by presentation of a reinforcer (Winger, Young, & Woods, 1983). Skinner (1948) recognized that reinforcing stimuli did not necessarily have to be the consequences of a response for them to result in an increase in behavior. His writings supported the importance of reinforcement in modifying behavior in both instrumental and classical conditioning paradigms (Skinner, 1937, 1948).

Both classical and instrumental paradigms have been used to study drug-related responding. Pavlov (1927) recognized that the cues surrounding drug

administration could be viewed as stimuli. Repeatedly presenting the drug in the presence of a common set of environmental cues allows those stimuli to become associated with the effects of the drug. In Pavlovian terms, the cues that signal drug delivery function as the CS, while the stimuli elicited by the drug serve as the US. With repeated pairings of the drug and the drug-signaling cues, subjects learn the contingent relationship between these stimuli and perform anticipatory responses upon exposure to the CS (Stewart & Eikelboom, 1987). These responses may be the same as those induced by the drug (Eikelboom & Stewart, 1979; Sherman, 1979), or may actually oppose, or compensate for, the drug response (Schull, 1979; Siegel, 1975, 1976, 1978, 1983, 1987; Solomon & Corbit, 1974) and provide a mechanism for conditioned tolerance.

Instrumental paradigms were first employed in pharmacology to examine the behavioral effects of drugs on performance. The effects of opiates and other drugs on behavior were investigated using maze tasks (Macht, Greenberg, & Isaacs, 1920; Macht & Mora, 1921). Macht (1943) also trained rats to climb a rope for food reinforcement. In later studies, this behavioral task was used as a screening test to establish the side effects of new pharmacological compounds through their influence on task performance (Winter & Flataker, 1949, 1951).

Instrumental and classical conditioning also have been used to evaluate the reinforcing properties of drugs. Instrumental tasks have been advocated as models for human drug-seeking behavior (Samson, Pfeffer, & Tolliver, 1988). Under these paradigms, animals are required to perform a response in order to receive drug delivery. Many of the methods used to assess the reinforcing value of ethanol fall under this rubric, including drinking studies and operant designs. Classical conditioning paradigms typically investigate the influence of drug-related cues on preference or aversion to associated stimuli, and include taste conditioning and place conditioning studies.

Although ethanol administration has been studied in several animal species, especially rodents, review of the literature will focus primarily, though not exclusively, on studies using rat subjects, since this species was selected for the series of experiments to follow.

INSTRUMENTAL CONDITIONING

Drinking Studies

Rats have been shown to consume ethanol and to prefer it to water at concentrations less than 6 to 7% in the absence of food or fluid restriction (Meisch, 1977; Myers, 1961, 1966). The aversive taste and olfactory characteristics of ethanol often have been cited as the reason animals will not readily orally self-administer the drug at higher concentrations (Amit & Stern, 1969; Holman & Myers, 1968; Kahn & Stellar, 1960; Lester & Greenberg, 1952; Myers, 1961; Myers & Carey, 1961; Richter & Campbell, 1940; Samson, 1987). However, even when preferred concentrations of ethanol are self-administered by rats, the subsequent doses are typically too low to result in intoxicating effects (Lester, 1966; Meisch, 1977; Wallgren & Barry, 1970). Some investigators have suggested that the delay of reinforcement inherent in oral self-administration at these lower doses does not allow the animals to absorb enough drug to experience a pharmacological effect, or to associate drug effects with the oral consumption of ethanol. However, Cunningham and Bischof (1987) demonstrated that when rats drank enough ethanol to experience its pharmacological effects (as indicated by drug-induced hypothermia), the animals subsequently decreased consumption to levels that did not induce hypothermia, suggesting that at least some of the pharmacological effects of ethanol are aversive (Cunningham, Hawks, & Niehus, 1988; Stewart, Perlanski, & Grupp, 1988).

Several investigators have induced increased oral self-administration of ethanol by manipulating deprivation state. Ethanol's value as a reinforcer has been

increased by food-restricting subjects (Meisch & Thompson, 1971, 1972; Roehrs & Samson, 1981), presumably increasing the reinforcement provided by ethanol's caloric value. However, food deprivation has been shown to increase the intake of non-nutritive pharmacological agents as well (Carroll, France, & Meisch, 1979; Carroll & Meisch, 1981). It also has been argued that ethanol's food-like properties may be reinforcing even to non-calorically deprived animals (Stewart et al., 1988). Similarly, ethanol consumption has been increased by restricting water access and providing access to ethanol, or by making ethanol the only available liquid (Grant & Samson, 1986; Rick & Wilson, 1966; Veale & Myers, 1969), thereby increasing the reinforcing value of ethanol's fluid properties.

Although manipulations of both food and water availability have been successful in initiating ethanol self-administration, animals typically fail to maintain consumption of ethanol when the deprivation state is removed (Meisch & Thompson, 1973; Roehrs & Samson, 1982), suggesting that, while the caloric and fluid properties of ethanol are reinforcing, the resulting pharmacological effects are either not reinforcing (Stewart et al., 1988), or are not reinforcing to a degree that overcomes its taste or other potentially aversive properties. Alternatively, based on the results of Carroll et al. (1979), perhaps ethanol's pharmacologically rewarding properties are state-dependent, with animals needing to be food- or fluid-restricted to find these effects reinforcing. However, other investigators have demonstrated ethanol self-administration in non-deprived subjects (Grant & Samson, 1985b).

Adulteration of the flavor of ethanol using sucrose or saccharin also has been shown to increase its consumption. This has been demonstrated in both food-restricted (Gilbert, 1974, 1976, 1978) and satiated subjects (Cullen, Croes, & Gillis, 1973; Eriksson, 1969; Geiger & Barker, 1976). Primates also have exhibited increased ethanol consumption when the drug has been presented in

orange, grape, or grapefruit juice (Fitz-Gerald, Barfield, & Warrington, 1968). However, these manipulations did not result in increased ethanol intake when the drug was subsequently presented without the adulterating flavor (Cullen et al., 1973), suggesting that subjects were not consuming the solution solely to experience the pharmacological effects of ethanol. Alternatively, if the doses of ethanol consumed were indeed pharmacologically reinforcing, then the reinforcement provided by ethanol was presumably not great enough to overcome avoidance of its aversive taste (Myers & Carey, 1961).

In drinking studies, in addition to measuring overall consumption, choice testing often is used to assess the relative reinforcing value of ethanol (Myers & Veale, 1972). In one of the first studies examining ethanol preference, Richter and Campbell (1940) allowed animals to drink from two bottles attached to their cages containing either ethanol or water. Ethanol concentration gradually was increased and the relative amounts of ethanol and water consumed were compared to determine those concentrations of ethanol which were preferred to water and those which were the most aversive (Myers & Holman, 1966). Other investigators have used a similar design but have successively decreased the concentration of ethanol over trials and compared consumption to that of water (Myers & Carey, 1961). Fuller (1964) evaluated ethanol preference in mice by allowing simultaneous access to six different concentrations of ethanol. The results from choice tests typically are expressed as preference ratios, which are calculated by dividing consumption of the fluid of interest by total consumption (McClearn & Rodgers, 1959). Relative consumptions from single-bottle tests, where the solutions of interest are presented on alternate trials, also are used to establish preference. However, these tests are not true tests of preference because subjects are not allowed a choice between fluids on any given trial and may be "forced" to

consume a less-preferred fluid by virtue of it being the only fluid available (Dragoin, McCleary, & McCleary, 1971; Grote & Brown, 1971).

Operant Tasks

That ethanol can function as a reinforcer has been shown using intravenous (Deneau, Yanagita, & Severs, 1969; Grupp, 1981; Karoly, Winger, Ikomi, & Woods, 1978; Lyness & Smith, 1992; Numan, 1986; Oei & Singer, 1979; Sinden & LeMagen, 1982; Smith & Davis, 1974; Winger & Woods, 1973) and intragastric (Altshuler, Weaver, & Phillips, 1975; Amit & Stern, 1971; Davis, Smith, & Werner, 1978; Davis, Werner, & Smith, 1979; Smith, Werner, & Davis, 1975, 1976; Yanagita & Takahashi, 1973) routes of administration. Intragastric (i.g.) administration originally was developed to allow ethanol and morphine to be self-administered in such a manner as to bypass the oropharyngeal sensory receptors, and thus avoid the mechanisms thought to be responsible for taste aversion. One additional benefit of this technique is that the physiological pathways for absorption are the same as in oral self-administration (Smith et al., 1975).

Although ethanol is self-administered intravenously and intragastrically, it is only weakly reinforcing compared to such drugs as morphine or cocaine. Numan (1981) induced intravenous (i.v.) self-administration after giving extensive ethanol pre-exposure, while Oei and Singer (1979) employed food-deprivation as a mechanism to stimulate self-administration. In other studies of ethanol self-administration, the operant response has been initiated using other drugs, such as cocaine or methohexital, with ethanol being substituted as the reinforcer once responding has been stabilized (Winger & Woods, 1973). Other investigators have been unsuccessful in demonstrating i.v. ethanol self-administration (Collins, Weeks, Cooper, Good, & Russell, 1984; deNoble, Mele, & Porter, 1985).

In operant paradigms involving oral self-administration, animals are typically required to approach a tube or perform a barpress response for dipper delivery of ethanol (Roehrs & Samson, 1982). Studies employing scheduled delivery of food pellets to induce high ethanol consumptions typically make ethanol access contingent upon a barpress response (Meisch, 1976; Meisch & Beardsley, 1975; Meisch & Thompson, 1971, 1972, 1973, 1974a). Falk (1961) showed that food-restricted rats will consume three times their normal mean 24 h fluid intake during the time they are given intermittent access to food. In this study, rats were placed on a variable interval (VI) schedule in which pressing a bar resulted in delivery of a 45 mg food pellet. Pellets were available on an average of one per minute (VI-1'), and water was freely available from a drinking spout in the operant chamber. Animals consumed a mean of 92.5 ml of water during their 3-h food-restricted sessions versus only 27.0 mls during the 24-h non-restricted sessions. Falk (1966) also demonstrated that, in addition to barpressing for food, animals would barpress for water under this paradigm. He referred to the phenomenon of excessive fluid drinking under intermittent food delivery as "schedule-induced polydipsia."

Lester (1961) substituted ethanol for water under a VI-55 sec schedule of food reinforcement and showed that rats would drink a mean of 9.9 ml of 5.6% ethanol in 1 h drinking sessions. Everett and King (1970) induced even greater ethanol consumptions using this paradigm by flavoring the ethanol solution with Kool-aid. In addition, since food pellets were delivered automatically on a fixed-interval schedule, this study demonstrates that operant responses for food delivery were not necessary for the establishment of schedule-induced polydipsia.

Numerous studies have shown schedule induction to be useful in initiating ethanol consumption. In fact, Falk, Samson, and Winger (1972) were able to induce physical dependence on ethanol by this method. Meisch and Thompson

(1971, 1974a) demonstrated that, following schedule induction, food delivery could be discontinued and animals would continue to barpress for ethanol at concentrations up to 32%. Although schedule-induced responding for ethanol could be maintained in animals that were returned to ad lib feeding conditions, a marked reduction in responding has been observed (Meisch & Thompson, 1973). This supports the belief that the caloric properties of ethanol may have been responsible, at least in part, for its reinforcing efficacy.

Because eating induces fluid consumption ("prandial drinking"), oral consumption of ethanol has been initiated simply by making ethanol available during daily feeding sessions. Meisch and his colleagues used a prandial drinking paradigm to induce ethanol consumption by making ethanol the only fluid available during meals, and showed that rats would continue to drink low concentrations of ethanol even when food was no longer available (Henningfield & Meisch, 1975; Meisch, 1976; Meisch & Beardsley, 1975). Stewart and Grupp (1984) also have demonstrated prandial drinking techniques to be effective in increasing ethanol consumption in the absence of operant conditioning by making food pellets and drinking tubes of ethanol available simultaneously (Macdonall & Marcucella, 1979).

The caloric value of ethanol has been implicated in its role as a reinforcer in both schedule-induced polydipsia and prandial drinking paradigms (Freed, Carpenter, & Hymovitz, 1970; Freed & Lester, 1970; Holman & Myers, 1968). However, in addition to the primary reinforcing value of its caloric properties, ethanol also may provide secondary reinforcement in these paradigms by virtue of its association with food during the induction procedure. The reduction in ethanol consumption observed with removal of the deprivation state is consistent with this dual reinforcement. Just as the reinforcing value of food is reduced in satiated animals, both the primary and conditioned reinforcement provided by ethanol's

properties as a food also might be expected to decline with recovery from deprivation. Although it is not clear how deprivation state acts to alter reinforcement provided by the non-caloric pharmacological effects of ethanol, the results of Carroll et al. (1979) demonstrating that deprivation increased self-administration of non-nutritive pharmacological agents suggests that this possibility cannot be ruled out.

Other operant designs explicitly have employed secondary conditioning procedures to increase the oral self-administration of ethanol. In several studies, the delivery of food pellets was made contingent upon consumption of ethanol (Black & Martin, 1972; Coulson, Koffer, & Coulson, 1971; Keehn, 1969; Persensky, Senter, & Jones, 1968; Senter & Persensky, 1968). Although ethanol-contingent food reinforcement served to increase consumption of ethanol, even to the point of intoxication in some studies, ethanol was not established as a reinforcer and responding for ethanol typically extinguished following removal of the contingency.

Mello and Mendelson (1965) reinforced consumption of 10% ethanol solution with sweetened milk in non-deprived rats in an attempt to induce sufficient ethanol consumption to cause dependency. Animals were trained initially with sucrose-contingent responding. However, when ethanol was substituted for sucrose, responding dropped in three of the seven rats. When ethanol later was substituted for the sweetened milk such that the animals were consuming ethanol for dipper presentations of ethanol, all animals continued to respond, although at greatly decreased levels. No information was presented on consumption so it is not clear if the animals were actually administering the drug.

Senter, Smith, and Lewin (1967) examined the relative influence of positive and negative reinforcement on ethanol consumption. They required groups of rats to ingest a 7% ethanol solution in order to either receive food or avoid shock in an

effort to examine the tension-reducing effects of the drug. Although this design was effective in inducing drug intakes which resulted in overt symptoms of intoxication, associating ethanol delivery with either positive or negative reinforcement did not result in preference for the drug over water, as demonstrated in 24-h choice testing. These secondary conditioning procedures typically failed to establish ethanol as a reinforcer to a sufficient extent that responding for ethanol would be maintained upon removal of the contingency.

Samson and his colleagues have established oral ethanol self-administration in animals that are neither food nor fluid-deprived in a series of operant tasks using sucrose. The success of this technique derives from the fact that food- and fluid-sated rats will barpress for sucrose reinforcement (Samson, 1986). Initial studies employed secondary conditioning procedures with sucrose to establish an operant response for ethanol. Free-feeding Long-Evans rats drank from a lickometer containing 5% ethanol to receive contingent dipper presentations of 20% sucrose. After several such trials, the lickometer was removed and ethanol substituted for sucrose in the dipper. Following this induction procedure, animals barpressed for successively increasing concentrations of ethanol with no further sucrose access, demonstrating that oral ethanol reinforcement could be established in nondeprived rats (Grant & Samson, 1985b).

Grant and Samson (1986) compared ethanol consumption using both response-contingent ethanol reinforcement and sucrose reinforcement. Consumption of 5% ethanol from a lickometer resulted in dipper access to either 5% ethanol for one group or 20% sucrose for the other. Following training, lickometers were removed and both groups were shaped to lever press for 5% ethanol. Both reinforcers were comparably effective in maintaining ethanol responding. As in a prior study (Grant & Samson, 1985a), subjects were water-deprived during the initial training trials, and, thus, were also experiencing some

degree of food deprivation (Fitzsimons & LeMagnen, 1969) during these initial sessions. Ethanol may have been serving as a reinforcer during the induction phase not for its pharmacological effects, but because of its caloric properties, as suggested by the gain in body weight demonstrated by the ethanol-ethanol subjects relative to the ethanol-sucrose group.

A second series of sucrose-induction studies was based on taste adulteration. In most designs examining the influence of adulterating the taste of an ethanol solution on oral self-administration, the adulterating substance is removed in a single trial and its influence on ethanol consumption or preference is reflected in its absence. Samson (1986) proposed that ethanol consumption was not maintained in these studies due to the rapid stimulus change induced by removal of the adulterating flavor. He suggested that the gradual removal of the adulterating flavor may be more effective in maintaining ethanol consumption. In these stimulus-substitution, or "sucrose-fading," procedures, non-deprived animals were trained initially to lever-press for a 20% sucrose solution. Following a series of trials at this concentration, the sucrose concentration gradually was reduced. When responding was stable, low-concentration ethanol was added and animals were given the combined solution as reinforcement. Sucrose concentration and ethanol concentration were manipulated further over trials until the animals eventually were responding for ethanol alone. Once responding for ethanol was established in these studies, ethanol was preferred to water under a concurrent schedule. Equal responding was demonstrated for 1% sucrose and 10% ethanol. However, responding was much greater for 5% sucrose than 10% ethanol under the same schedule, suggesting that, although ethanol was shown to be reinforcing, sucrose, at sufficient concentrations, was still a preferred reinforcer.

Samson et al. (1988) also conditioned ethanol-maintained responding using a "sucrose-switch" procedure. Animals initially were shaped and trained to lever

press for a 20% sucrose reinforcement after which 5% ethanol was immediately substituted for the sucrose. No fading out of sucrose and fading in of ethanol was initiated. Responding was maintained despite the rapid stimulus change. When ethanol concentration was progressively increased to 40% over a series of trials, a decrease in responding was seen with increasing concentration, and overall intakes were comparable to those using the sucrose-fading procedure. However, this initiation procedure was not consistently successful since, in an earlier study (Pfeffer & Samson, 1987), less than half of the animals had been successfully initiated.

Typically, subject populations are small in Samson's operant studies and not all subjects in any given experiment are successfully initiated, possibly due to the use of a heterogeneous population. The Long-Evans stock used in these experiments show a wide range in initial ethanol preferences (Samson, 1986; Tolliver, Sadeghi, & Samson, 1988). However, a study using sucrose-fading in the homozygous alcohol-nonpreferring (NP) line of rats bred at Indiana University also demonstrated differential initiation (Samson, Tolliver, Lumeng, & Li, 1989), suggesting that rats, not unlike humans, may show individual differences in sensitivity to ethanol, irrespective of genetic makeup.

Although various manipulations of sucrose-pairing operant designs have been successful in initiating and maintaining oral ethanol self-administration, the factors which contribute to maintained responding have not been identified. Association of ethanol with sucrose may establish ethanol as a secondary reinforcer. Samson (1987) has argued against this interpretation and suggests that these manipulations simply initiate ethanol consumption, thereby allowing the subjects to experience the reinforcing pharmacological properties of the drug. The pharmacological reinforcement provided by ethanol presumably maintains subsequent responding, since any reinforcement conditioned to ethanol as a result

of its association with sucrose would be expected to extinguish over trials once sucrose was no longer available. Samson's findings that animals continue to respond for ethanol after sucrose has been removed argues against conditioning as an explanation of ethanol reinforcement. However, it is possible that some non-pharmacological properties of ethanol, such as its caloric value, may share similar stimulus characteristics as sucrose. This may allow ethanol to be substituted for sucrose and, thus, delay extinction, even in the absence of food deprivation (Mackintosh, 1973). Use of a non-caloric stimulus, such as saccharin, could help clarify this relationship.

Preference may be demonstrated in operant tasks by the relative response rates for different stimuli presented on alternate days (similar to single-bottle testing) or by relative responding for concurrently available stimuli (Meisch & Beardsley, 1975). Roehrs and Samson (1981) used prandial induction on a concurrent schedule of ethanol and water availability and demonstrated a higher response rate for ethanol relative to water in food-restricted rats, presumably due to ethanol's caloric advantage (Roehrs & Samson, 1982).

As this series of studies demonstrates, it has been difficult to establish pharmacologically significant ethanol self-administration in rat subjects. Although a wide variety of experimental manipulations have been undertaken to stimulate intake, often taking advantage of ethanol's value as a food or as a fluid, it is rare that self-administration has been maintained when the value of these non-pharmacological properties has been reduced by removal of the deprivation state. In studies that have been successful in initiating self-administration, it is unclear if ethanol is being administered for its inherent motivational effects or for appetitive properties that have been conditioned during the induction process. Although the extent of alcohol abuse in the human population would suggest that ethanol's

pharmacological effects are appetitive, this has been difficult to demonstrate in rat subjects.

CLASSICAL CONDITIONING

Two paradigms commonly used to evaluate the reinforcing properties of ethanol, taste conditioning and place conditioning, employ elements of both classical and instrumental conditioning. Although a stimulus-reinforcer contingency is established, an instrumental contingency is also in force, since each subject is required to approach and consume the stimulus fluid in taste aversion conditioning, and to either approach or avoid the drug-paired environment in order to experience the reinforcing (or punishing) cues in place conditioning.

Taste Conditioning

The phenomenon of taste aversion learning was first recognized in the mid-1950's (Garcia, Kimeldorf, & Koelling, 1955; Richter, 1953; Rzoska, 1953). Since its discovery, numerous studies have been performed using this paradigm in both animals and humans to investigate determinants of food selection and to assess motivational effects of drugs and other stimuli, as well as to analyze the unique characteristics of learning associated with this phenomenon (Hunt & Amit, 1987; Riley & Tuck, 1985).

In studies of taste conditioning, a distinctive flavor is presented to fluid-restricted animals followed by exposure to a stimulus whose reinforcing value is of interest. This may be viewed as a classical conditioning paradigm with the novel flavor serving as the CS and the paired stimulus as the US, since presentation of the US is independent of the subject's response to the CS (Rozin, 1969).

Consumption of the flavored solution is monitored over conditioning trials and serves as the CR. A decrease in consumption of the flavor CS over subsequent trials is cited as evidence that the unconditional stimulus and/or its administration have aversive properties. Preference testing also may be performed following

conditioning trials to assess the influence of US pairing on CS consumption. An advantage to the use of a taste conditioning design with pharmacological agents is that, in contrast to self-administration studies, reinforcing value can be evaluated in the absence of the drug of interest such that its pharmacological effects do not interfere with performance during testing.

Much of the interest in taste aversion conditioning is derived from its unique characteristics as a learning paradigm. In contrast to many learning procedures, where several trials are required to establish an association, taste aversion can be established with the single pairing of a taste with an aversive stimulus. In addition, the flavor CS may precede the aversive US by as many as several hours, in some cases, and the association is still evident (Garcia, Ervin, & Koelling, 1966; Revusky & Garcia, 1970; Smith & Roll, 1967).

This paradigm, like some of the operant tasks previously discussed, produces an approach/avoidance conflict. The state of fluid deprivation and the availability of the CS fluid provide stimuli which drive the subjects to approach and consume the distinctive flavored solution. However, the smell and taste of the CS elicit avoidance behavior as a result of their association with the aversive US. Because of the drive to consume the aversive CS in response to fluid deprivation inherent in single-solution tests, Dragoin et al. (1971) and Grote and Brown (1971) have proposed two-choice preference testing as a more sensitive technique for determining the impact of taste aversion conditioning.

Studies on taste conditioning with ethanol as the US show that the drug almost universally produces taste aversion. One of the first studies examining ethanol-induced taste aversion was that of Lester, Nachman, and LeMagen (1970), who gave single doses of ethanol to fluid-restricted rats by intraperitoneal (i.p.), i.g., and intracardiac (i.c.) routes following oral self-administration of 0.1% saccharin solution. Drug infusion via the i.c. route was achieved by direct cardiac

puncture. All routes of administration resulted in decreased saccharin consumption, with the greatest decrease seen in animals receiving ethanol by the i.p. and i.c. routes.

Eckardt, Skurdal, and Brown (1974) demonstrated taste aversion at lower doses of ethanol than those used by Lester et al. (1970). Grape and orange Kool-aid flavors were used as conditioned stimuli and only i.p. injections were given. Subjects were exposed to several flavor-drug pairings, in contrast to the single pairing used in the previous study. The results indicated that taste aversion could be conditioned by low doses of ethanol if several conditioning trials were given, further suggesting that, even at very low doses, ethanol has aversive properties. Eckardt (1975a, 1975b, 1976) also showed that aversion could be conditioned to flavors orally self-administered with ethanol.

Many other studies have supported the finding of conditioned taste aversion to ethanol using different doses and routes of administration (Cunningham, 1979; Cunningham et al., 1988; Froelich, Harts, Lumeng, & Li, 1988; Linakis & Cunningham, 1979; Miceli, Marfaing-Jallat, & LeMagen, 1980; Sinclair, 1984). However, preference for an ethanol-paired flavor has been demonstrated in rats that are food-restricted. A study by Sherman, Hickis, Rice, Rusiniak, and Garcia (1983), in which location and flavor effects of ethanol were conditioned simultaneously, demonstrated place aversion to locations paired with 0.5, 1.0, and 2.0 g/kg ethanol in food-deprived rats. However, animals showed preference for a flavor paired with 0.5 g/kg ethanol and aversion to a flavor paired with 2.0 g/kg, with no significant effect of 1.0 g/kg. Intubation of a glucose solution isocaloric to 0.5 g/kg ethanol in the place of water during conditioning resulted in animals showing preference for the glucose-paired flavor over the ethanol-paired flavor. This suggests that ethanol was only a weak reinforcer and may have been

reinforcing solely for its caloric benefit and not necessarily for any psychopharmacological effects.

Fedorchak and Bolles (1987) gave food-restricted rats access to one flavor in an ethanol solution and a second flavor in saccharin on alternate days as the only available fluid. Following four 23-h exposures to each solution, subjects were given two-bottle choice tests with the flavors in water. Animals showed preference for the flavor paired with ethanol over that paired with saccharin. However, this preference disappeared when the rats were returned to ad lib feeding, possibly due to the loss of ethanol's value as a calorie-source. Preference for the ethanol-paired flavor may have also been a state-dependent phenomenon. Free feeding may have resulted in a change in the animal's physiological state such that the learned flavor-ethanol association did not transfer and was lost. Although it is possible, based on studies of self-administration (Meisch, 1977), that animals find the pharmacological properties of ethanol reinforcing, it appears that the caloric properties of ethanol may mediate conditioned taste preference for the drug (Kulkosky, 1985; Mehiel & Bolles, 1984).

Place Conditioning

Place conditioning is a valuable tool for studying reinforcing value, especially of pharmacological agents. It is relatively simple and conditioning occurs rapidly, with animals learning the drug-environment association with few conditioning trials. The test is also bi-directional, allowing both rewarding and aversive characteristics to be demonstrated. Place conditioning is especially valuable with drug evaluation because, as in taste conditioning, animals can be tested in a drug-free state, thereby eliminating possible interference of drug effects on task performance (Katz & Gormezano, 1979; Mucha, van der Kooy, O'Shaughnessy, & Bucenicks, 1982; van der Kooy, 1987).

In place conditioning paradigms, animals are given the drug of interest prior to placement in a distinctive environment. The drug's pharmacological effects subsequently are experienced in the presence of cues specific to that environment and the motivational properties of the drug are associated with those cues. This paradigm also follows classical conditioning theory, with cues from the drug-paired environment serving as the CS+ and stimuli from the non-drug, or vehicle-paired, environment as the CS-. The pharmacological effects of the drug function as unconditioned stimuli. Following conditioning, subjects are allowed simultaneous access to both environments and the amount of time spent in each environment during the choice period is measured. It is assumed that if a drug has rewarding properties, the cues of the distinctive paired environment will be associated with those properties and will elicit, or reinforce, approach behavior. In contrast, animals will avoid the CS+ environment if the drug had predominantly aversive properties (Mucha et al., 1982).

One difficulty with interpretation of the place conditioning paradigm is the possible influence of drug-induced activity changes on familiarity to the drug-paired environment. This relationship was studied by Swerdlow and Koob (1984), who exposed rats to amphetamine in a place-conditioning paradigm in which animals in one group were restrained in a plastic tube in both the drug-paired and saline-paired environments, while the other group was allowed to explore their surroundings freely. Conditioned hyperactivity was observed in the drug-paired environment in both groups; however, only the freely-moving animals showed preference for the amphetamine-paired environmental cues. The restrained subjects showed neither preference nor aversion, suggesting that locomotor behavior was necessary to produce preference for the drug-paired cues. These differences demonstrate that, while both freely-moving and restrained subjects were able to associate environmental cues with the activity-influencing effects of

the drug, restraint prohibited learning the association between these cues and the motivational properties of amphetamine.

If a drug induces hyperactivity, animals may explore the drug-associated environment to a greater extent than they explore the non-drug or vehicle-associated chamber. This greater familiarity may result in an increased preference for, or a reduced aversion to, the drug-paired chamber during choice testing. This change in preference would not be due necessarily to the reinforcing properties of the drug, but may, instead, be secondary to the drug's influence on activity and, consequently, familiarity with the environmental cues. This hypothesis was investigated by Carr, Phillips, and Fibiger (1988), who revealed an inverse U-shaped function in terms of preference and novelty. They demonstrated that animals showed decreased preference for a completely novel environment as well as one in which they had received several exposures. Rat subjects showed the greatest preference for moderately familiar environmental cues. Their results suggested that restraint in the Swerdlow and Koob (1984) study preserved the novelty of both the drug-paired and saline environments. They also showed that exposure to the drug-paired environment prior to restrained place conditioning resulted in place preference, demonstrating that the stimulant effects of drugs on activity are not required for preference conditioning.

The psychomotor stimulant theory of addiction proposed by Wise and Bozarth (1987) supports a relation between the activating effects of drugs and demonstration of conditioned place preference. However, the basis for this connection is independent of the influence of activity on environmental familiarity. These investigators suggest that the psychomotor stimulant effects of drugs may share common biological mechanisms with the drug's reinforcing properties.

With most substances that are readily abused, such as opiates, amphetamine, and cocaine, there is a high correlation between their ability to

function as reinforcers, as shown by self-administration, and their ability to condition place preference. This correlation is not as clear for ethanol, and animals that have self-administered ethanol have subsequently demonstrated conditioned place aversion to the ethanol-paired environment (Schechter, 1992; Stewart & Grupp, 1986).

Studies of ethanol's ability to mediate place preference in rats are relatively limited and equivocal. Such factors as species differences (Cunningham & Noble, 1992), drug dosage and route of administration (Stewart & Grupp, 1985), timing of drug exposure and conditioning, US preexposure (Bozarth, 1990; Reid, Hunter, Beaman, & Hubbell, 1985), and the simultaneous availability of other reinforcers (Marghlin, MacKechnie, Mattie, Hui, & Reid, 1988; Stewart & Grupp, 1981, 1985, 1986) have been shown to impact the development and expression of preference or aversion to the drug.

The initial study evaluating the role of ethanol in place conditioning reported place preference to ethanol. Black, Albiniaak, Davis, and Schumpert (1973) administered 1.0 g/kg of 10% ethanol i.p. to Sprague-Dawley rats prior to placement in the less preferred of two environments. Saline injections were given on intervening days prior to placement in the alternate environment. Animals were confined to these environments for 15-min trials. On test days, subjects spent more time in the ethanol-paired environment following both ethanol and saline injections, leading the investigators to conclude that the central effects of ethanol were reinforcing.

Cunningham (1979) found ethanol to produce both taste aversion and place aversion. He later attempted to replicate the findings of Black et al. (1973) in Holtzman albino rats using similar experimental parameters, but was unable to condition preference for ethanol-paired environmental cues (Cunningham, 1981). Other investigators, using several dosages and routes of administration of ethanol,

also have been unable to demonstrate place preference in rats. For example, van der Kooy et al. (1983) administered ethanol in different doses and at several concentrations, both i.v. and i.g., but were able to show only conditioned place aversion. They concluded that ethanol, in the doses used, provided only neutral or punishing motivational influences.

Asin, Wirtshafter, and Tabakoff (1985) also failed to observe place preference in rats receiving ethanol i.v., despite demonstrating amphetamine-induced place preference with the same paradigm. They were able to show an increase in time spent in the non-preferred environment following association with ethanol, but this increase was not significantly different from that of saline controls. They hypothesized that if ethanol does have primary reinforcing properties, these rewarding cues are not easily associated with environmental stimuli. Alternatively, ethanol may function as a secondary reinforcer, and is rewarding only through its association with primary reinforcers. They also suggested that rats may find low doses of ethanol reinforcing, but only if they are allowed to control the drug's administration. However, this self-administration hypothesis was not supported. Stewart and Grupp (1986) provided food-restricted animals access to ethanol in place conditioning chambers and demonstrated that, although rats would approach and orally self-administer ethanol, implying that they found the drug reinforcing, they showed conditioned place aversion to the ethanol-paired environment during choice testing.

In addition to the initial report of Black et al. (1973), six other studies have demonstrated conditioned place preference in rats. However, in each case, unique manipulations were employed which may have influenced the demonstration of preference. Stewart and Grupp (1981) gave ethanol and food in one location and food alone in a second location. Using this technique, the animals preferred the location paired with ethanol and food. Alternatively, when food was not present

during conditioning, ethanol did not condition a preference. These same investigators (Stewart & Grupp, 1985) later demonstrated place preference to a location paired with i.p. injections of ethanol in rats given food in both drug-paired and saline-paired environments. Animals having food available in both environments showed increased preference for the environment paired with 0.5 g/kg ethanol and decreased aversion to the environment paired with 1.0 and 1.4 g/kg doses relative to controls. Subjects receiving ethanol without access to food showed either no preference or aversion at lower doses of ethanol (less than 0.7 g/kg) and only aversion at 1.0 and 1.4 g/kg. These findings suggest that environmental conditions, such as the presence of food, may interact with the effects of ethanol to influence the rewarding effects of the drug, although this is not a universal effect. Sherman et al. (1983) provided animals with access to flavored sucrose solutions in distinctive environments following intubation with both ethanol and water, but demonstrated only aversion to the ethanol-paired location, despite the presentation of a food source in both environments. Perhaps the low concentration of sucrose used (5%) was not a strong enough reinforcer to interact with ethanol in an equivalent manner to result in place preference.

Exposure to morphine during trials of place conditioning with ethanol have also been shown to induce conditioned place preference to an ethanol-paired environment. Marglin et al. (1988) exposed rats to doses of morphine (2.0 mg/kg) and ethanol (0.5 g/kg) which do not consistently result in place preference. They exposed subjects to the combination of morphine and ethanol on one side of the place conditioning apparatus, and either morphine, ethanol, or saline on the other side, and demonstrated conditioned place preference to the side paired with the morphine-ethanol combination. Animals receiving either morphine or ethanol alone on one side and saline on the other showed no place preference. These findings suggest that morphine and ethanol may act synergistically to increase

preference and support other studies that demonstrate an impact of opioid activity on ethanol reinforcement (Reid & Hunter, 1984; Hubbell & Reid, 1990).

A fourth study demonstrating ethanol conditioned place preference in rats gave extensive preexposure to the drug prior to place conditioning (Reid et al., 1985). Fluid-restricted rats were given 1 h access to 6% ethanol for 26 d. Following this preexposure phase, animals were exposed to place cues for 4-min sessions 4 min following ethanol injection. These temporal parameters follow the purported period of positive affect to ethanol (Shippenberg & Altshuler, 1985; Shippenberg, Knappenberger, & Altshuler, 1983). Subjects having prior experience with ethanol showed preference for the ethanol-paired environment on two of three tests for preference. Bozarth (1990) was also able to condition preference to ethanol-paired cues in animals that received a large number of conditioning trials prior to preference testing. These studies suggest that extensive exposure to ethanol, either through exposure prior to place conditioning or through the use of multiple conditioning trials, may result in tolerance developing to the aversive aspects of the drug, such that the appetitive aspects are enhanced, resulting in the demonstration of place preference during testing.

The influence of tolerance development on place conditioning was examined by Holloway, King, Bedingfield, and Gauvin (1992). They established tolerance to the behaviorally-disruptive effects of ethanol in animals given daily ethanol or saline injections prior to operant responding for food reinforcement. Ethanol was consistently given in one environmental context, while saline was given in another. The environments differed in olfactory, visual, and auditory characteristics. Ethanol-tolerant and ethanol-naive animals from this study were then trained in a place conditioning paradigm, with compartments that differed in wall design and floor texture. Initial preference scores were assessed during habituation trials, after which animals were given i.p. ethanol prior to placement in

the nonpreferred side of the apparatus in the presence of the context cues paired with ethanol during the tolerance phase of the experiment; saline was given prior to placement on the preferred side with the addition of the saline-paired contextual cues. Subjects received five cycles consisting of a 4-d sequence of conditioning followed by two consecutive days of testing. Testing was performed by allowing animals access to the entire chamber in the presence of the ethanol-paired context cues on one day of each cycle and the saline-paired context cues on the other test day. Following the first cycle, an additional test session was given in the presence of a blue light, a stimulus which had not been previously used.

It was shown that the animals receiving no exposure to ethanol prior to place conditioning spent less time in the ethanol-paired environment in all contexts after both the first and fifth weeks of conditioned place preference training. The subjects made tolerant to ethanol in the first phase of the experiment did not show aversion to the ethanol-paired environment, even when tested in the neutral and saline-paired contexts. Over continued training and testing cycles, the ethanol-tolerant animals developed a conditioned place preference for the ethanol-paired compartment when tested in the presence of the ethanol context cues.

Gauvin and Holloway (1991) studied the influence of prior ethanol experience on place conditioning using both drug discrimination (Overton, 1987) and sucrose-fading procedures (Samson et al., 1988). One group of food-restricted subjects was trained to discriminate between i.p. injections of 1.5 g/kg ethanol and saline to determine the correct lever to earn food reinforcement. Training was carried out over a seven-month period. A second group of animals was trained to self-administer ethanol in combination with sucrose over trials lasting several weeks, during which ethanol concentration was progressively increased to 20% while sucrose was gradually removed from the solution. Following this ethanol preexposure phase, these rats and a group of ethanol-naive subjects were trained in

a place conditioning task. The drug discrimination and ethanol-naïve animals were gavaged with either ethanol or water prior to placement in the appropriate compartment of the place conditioning apparatus, while the self-administration group was given access to water in one compartment and ethanol in the other. One subgroup was sequestered in the respective side compartments during fluid exposure, while another was given access to the entire chamber with a tube containing ethanol mounted on one end and a tube containing water mounted on the other. Following training, animals were tested for place preference in the absence of drug. The ethanol-naïve animals showed conditioned place aversion to the ethanol-paired compartment, while subjects trained in the drug discrimination task showed neither preference nor aversion. Animals receiving self-administration training showed an increase in preference scores for the ethanol-paired environment. These results suggested that extensive experience with ethanol alone was not sufficient to condition place preference and that allowing subjects to control their consumptions may play a critical role in establishing ethanol's hedonic value.

In summary, as with operant studies, it has been difficult to demonstrate motivational properties for ethanol in rat subjects using Pavlovian procedures. Rats consistently demonstrate conditioned taste aversion to ethanol-paired cues at significant doses of ethanol. They also show conditioned place aversion to ethanol in the absence of experimental manipulations that may alter demonstration of the inherent characteristics of the drug. Extensive preexposure and multiple conditioning trials may allow differential tolerance development to the aversive and appetitive effects of ethanol, with more rapid acquisition of tolerance to ethanol's aversive effects. Combining ethanol exposure with access to such appetitive stimuli as food or other drugs may result in the conditioning of

motivational properties to ethanol, which may be mistakenly interpreted as inherent appetitive characteristics.

VARIATION IN THE MOTIVATIONAL PROPERTIES OF ETHANOL

Compared to most abused substances, ethanol does not produce a strong degree of reinforcement following initial exposure. While such drugs as morphine, cocaine, and amphetamine consistently induce a high level of self-administration and conditioned preference under several different paradigms, ethanol administration typically acts as a punisher on first exposure in rats (van der Kooy et al., 1983). Many of the techniques used successfully to initiate ethanol self-administration or to otherwise provide evidence of ethanol's appetitive properties appear to involve manipulations that might be viewed as altering the initial motivational value of ethanol through both non-associative and associative mechanisms.

Non-associative Mechanisms

With repeated exposure to a drug, tolerance or sensitization may develop to its pharmacological effects (Lett, 1989; Tiffany & Baker, 1986). Drug tolerance is defined as a decrease in the effect of a drug resulting from repeated administrations, such that more of the drug is required to achieve the same effect. In contrast, sensitization is seen when multiple drug exposures cause an increased pharmacological effect. Repeated exposures to a given drug may induce tolerance to some pharmacological effects and sensitization to others. In addition, the time course of development of either tolerance or sensitization may differ for varying pharmacological events, creating a very complicated picture when attempting to interpret the influence of drug exposure on drug reinforcement.

Repeatedly exposing an organism to ethanol, under certain conditions, has been shown to increase preference for the drug. Deutsch and Koopmans (1973) administered 6 days of i.g. infusion of ethanol to rats and demonstrated

significantly greater consumption of 10% ethanol over water in subsequent choice testing in these animals compared to water-infused controls. However, it is not clear if the subjects were consuming ethanol for some inherent reinforcing property of the drug or to alleviate withdrawal symptoms.

Chronic ethanol exposure does not always result in an increase in preference. Rats that are restricted to ethanol as their sole fluid source typically fail to demonstrate a preference for alcohol in subsequent choice tests (Myers & Carey, 1961). Veale and Myers (1969) demonstrated that ethanol exposure could either reduce or enhance subsequent preference for ethanol, depending upon the conditions of the exposure. Rats offered 12% ethanol as their sole fluid source for 10 days demonstrated a preference ratio of less than 0.1 in subsequent choice testing between water and progressively increasing concentrations of ethanol. The preference ratio of the ethanol-naïve controls remained above 0.6 until ethanol concentration reached 12%. A second group given simultaneous access to water and progressively increasing concentrations of ethanol from 3% to 30%, demonstrated an increase in their preference for ethanol from levels established prior to exposure. It is possible that presenting animals with aversive concentrations of ethanol as their sole fluid source leads to a conditioned taste aversion to ethanol that cannot be overcome, despite the use of typically preferred concentrations of ethanol in subsequent choice testing. Use of i.g. techniques to forcibly administer ethanol, even though the taste of the drug is presumably experienced to some degree (Linakis & Cunningham, 1979), may not condition a taste aversion as readily as forced oral consumption of nonpreferred concentrations. By progressively increasing the concentration of ethanol in the preference tests, acclimation to the aversive taste of ethanol may occur. The simultaneous presence of water also may enable animals to dilute the ethanol

administered or rinse away its taste such that the experience is less aversive to these subjects.

If tolerance develops more rapidly to aversive properties of ethanol than to appetitive effects, reinforcing qualities may predominate during continued drug exposure. Support for tolerance developing to aversive effects of ethanol comes from studies of the influence of ethanol preexposure on the development of conditioned taste aversion to ethanol. Berman and Cannon (1974) demonstrated weaker ethanol-induced saccharin aversion in rats receiving experience with an ethanol solution as their sole fluid source for several days prior to taste conditioning compared to naive rats. Although this attenuation in aversion may be due to a decremental effect of US exposure on the ability to form an association between ethanol and taste cues (Randich & LoLordo, 1979), it is also possible that preexposure to ethanol altered its reinforcing value such that the drug was less aversive, thus decreasing its potential for conditioning taste aversion.

The study by Reid et al. (1985) demonstrating place preference in rats provided prolonged experience with ethanol prior to place conditioning. This extensive preexposure may have resulted in the development of tolerance to the aversive effects of ethanol or sensitization to its appetitive properties, and may explain the dichotomous findings of this study relative to most studies in which aversion is conditioned to environmental cues paired with ethanol.

The sucrose induction procedures employed by Samson also provided subjects with extensive exposure to ethanol prior to sucrose extinction and testing of ethanol's reinforcing effects. Masking of ethanol's taste with sucrose and slowly altering the relative concentrations of the two fluids may have allowed the subjects to acclimate to the aversive taste of ethanol. In these studies, subjects were allowed to control their ethanol intakes. This may have enabled the animals to

regulate their exposure to ethanol's aversive effects and develop tolerance to these effects prior to testing.

A lack of tolerance to ethanol's aversive effects may account for the difficulty in initiating self-administration that is typically observed. Providing animals with extensive exposure to ethanol prior to testing its reinforcing value may enable its reinforcing characteristics to be revealed.

Associative Mechanisms

The aversive taste of ethanol is generally blamed for the difficulty in inducing its oral self-administration (Kahn & Stellar, 1960). Samson (1987) has suggested that procedures which allow the aversive taste of ethanol solutions to be overcome will result in subjects consuming enough of the drug to appreciate its inherently reinforcing pharmacological effects. These reinforcing properties, once experienced, will maintain ethanol administration in the absence of the initiating procedure. Samson argues that:

"The procedures used to develop oral self-administration should not be taken to suggest that any additional properties are added or changed in ethanol. What is established is oral ethanol self-administration, and not a change in ethanol which turns it into a reinforcer. Such procedures do not establish ethanol as a reinforcer, but rather establish behavior via the reinforcing properties of ethanol. Perhaps a more descriptive term of these procedures is initiation rather than establishment, for the major concern in these procedures is to overcome the aversive taste properties of ethanol solutions."

(Samson, 1987, p. 222)

In other words, Samson suggests that these procedures simply serve to "initiate" ethanol-maintained behavior by increasing oral consumption such that the organism can experience its reinforcing properties. Ethanol's reinforcing pharmacological effects are presumed to maintain consumption.

Support for the view that the pharmacological effects of ethanol are reinforcing comes from studies on i.v. and i.g. administration, where ethanol is self-administered independently of other reinforcers (Grupp, 1981; Sinden & LeMagnen, 1982; Smith & Davis, 1974; Smith et al., 1976). These drug delivery techniques were employed to bypass the oral route such that the aversive taste of ethanol would not interfere with self-administration of the drug. However, the aversive taste characteristics of ethanol may not be completely avoided through these non-oral routes, since Cunningham and Linakis (1980) have demonstrated that i.p. ethanol delivery results in animals experiencing the taste of the drug. This finding suggests that the decreased difficulty in initiating self-administration via non-oral routes is not attributed entirely to taste avoidance. Indeed, i.g. and i.v. delivery often require additional manipulations, such as preliminary training with other drugs like cocaine, to induce consistent levels of responding for ethanol (Woods, Ikomi, & Winger, 1971). Because the concentration of ethanol in the blood is so much lower than the concentration of ethanol solution administered, the taste cues experienced with i.v. or i.g. delivery would be much weaker and less aversive than would be experienced with oral administration, providing an advantage to these non-oral routes.

Alternatively, the effects of ethanol, excluding its caloric properties, may not be reinforcing. Instead, the reinforcing value may be learned, secondary to the association of ethanol with primary reinforcers, such as food, water, or other drugs (van der Kooy et al., 1983). McCusker and Bell (1988) tested this hypothesis in rats using social reinforcement to condition an increase in the reinforcing value of ethanol. One group of subjects was given 30-min access to ethanol in the presence of another rat, while a second group received ethanol while isolated. During the initial conditioning trials, ethanol consumptions were comparable between the two groups. However, the groups diverged with continued conditioning, with the

companion group drinking significantly more ethanol than the isolated group. Choice testing following conditioning demonstrated significantly greater preference for ethanol in the group receiving ethanol paired with social reinforcement. These results suggest that ethanol can become a conditioned reinforcer through its association with other reinforcers.

Support for an influence of conditioning on ethanol reinforcement comes from studies in which oral self-administration of ethanol has been consistently demonstrated. In both schedule-induced polydipsia and prandial drinking, ethanol consumption is paired with access to a food reinforcer. Even when food no longer is delivered during the experimental sessions, ethanol still may be serving as a cue to food availability since the food ration generally is made available in the home cage immediately following the daily operant session (Meisch & Thompson, 1974b). This association, though delayed, may serve to maintain the ethanol-reinforcer contingency and, thus, ethanol self-administration.

An associative interpretation also might be applied to the findings from Samson's sucrose-induction studies, despite the absence of food deprivation in these procedures. It is possible that pairing ethanol with sucrose, whether as separate solutions (Grant & Samson, 1985a, 1985b) or within the same solution (Samson, 1986; Tolliver et al., 1988), transfers reinforcing value to ethanol. Support for this conditioned reinforcement effect comes from the Grant and Samson (1985b) study in which response-contingent sucrose access increased water consumption in control animals. Although water reinforcement did not maintain responding in the absence of sucrose while ethanol did, it is possible that the animals were not responding for ethanol for its pharmacological effect, but, instead, were responding for the drug based on its prior association with sucrose. An increase in reinforcing value may have been conditioned only to ethanol and not water because the stimulus properties of ethanol may have provided a more

salient cue for association with sucrose than did water. Substitution of a more salient, but pharmacologically inactive fluid, such as sodium chloride, for water, also might have resulted in maintained responding in the absence of any possible pharmacological reinforcement.

Grant and Samson (1985a) argue that, in other studies, secondary conditioning generally was not sufficient to result in maintained ethanol ingestion following removal of either the contingency or the deprivation state (Black & Martin, 1972; Coulson et al., 1971; Keehn, 1969; Martin & Myers, 1972; Ramsay & van Dis, 1967; Senter et al., 1967). However, in the study by Mello and Mendelson (1965), pairing ethanol with sweetened milk did result in maintenance of some ethanol consumption when ethanol was later substituted for milk reinforcement, although the level of responding for ethanol was significantly lower than that for milk in all subjects. Pairing ethanol with some reinforcers, such as sweetened fluids, may be more effective in conditioning reinforcing value to ethanol than is pairing the drug with food pellets, possibly due to differences in the degree of reinforcing value or in the novelty of the reinforcer. Grant and Samson (1986) argue against secondary conditioning as an explanation for their results, suggesting that ethanol responding should have extinguished in the absence of ethanol-sucrose pairing. However, some of the stimulus properties of ethanol, such as those induced by its nutritive effects, may be similar to those of sucrose. This generalization between sucrose and ethanol may serve to retard extinction of responding in the absence of sucrose.

The inability of studies using ethanol-contingent reinforcement to demonstrate maintained responding for ethanol following termination of food- or fluid-deprivation (Meisch & Thompson, 1973; Roehrs & Samson, 1982) or after the removal of an adulterating flavor (Cullen et al., 1973) also fail to support Samson's interpretation of the induction procedure. In several of these

experiments, animals consume sufficient ethanol to experience the drug's pharmacological effects. If these effects are reinforcing and are not state-dependent, responding for ethanol should be maintained despite removal of these initial motivating influences in these procedures, just as it presumably does in sucrose-induction.

CONDITIONING OF POSITIVE MOTIVATIONAL PROPERTIES TO ETHANOL

Ethanol has numerous pharmacological effects. The extensive use and abuse of ethanol by humans suggests that these pharmacological effects are appetitive. However, the difficulty in inducing ethanol consumption in animals and the evidence for aversive effects of ethanol provided by taste conditioning and place conditioning paradigms suggests that ethanol reinforcement may not be that simple, especially considering the strong correspondence between animals and humans with regard to self-administration of other drugs of abuse. Most designs in which ethanol has been shown to function as a reinforcer either take advantage of the caloric value of the drug or induce ethanol consumption by pairing it with known reinforcers. In fact, most humans rarely consume unflavored ethanol in the absence of other primary reinforcers. Although Samson argues that pairing procedures serve only to induce subjects to experience ethanol's pharmacological effects and do not condition any reinforcement to ethanol, his studies have not controlled for nor tested this possibility.

The studies which follow attempt explicitly to condition a change in ethanol's motivational value through association of ethanol with sucrose, a highly reinforcing stimulus. That ethanol has appetitive pharmacological properties cannot be ruled out by these studies. Rather, the experiments proposed here will serve to determine whether the motivational value of ethanol can be increased using techniques that have been shown to enhance the value of other

nonpharmacological stimuli. Increased knowledge of the mechanisms of ethanol reinforcement and how this reinforcement is established and changed with experience may contribute to understanding the etiology, prevention, and treatment of alcohol abuse.

Experiment 1: Influence of Sucrose Pairing on Ethanol Self-administration

Rationale

Samson and his colleagues have shown sucrose to be an effective stimulus in operant paradigms for initiating oral ethanol self-administration (Grant & Samson, 1985a, 1985b). In these studies, free-feeding rats drank from a lickometer containing ethanol to receive dipper presentations of 20% sucrose. After several such trials, the lickometer was removed and animals barpressed for successively increasing concentrations of ethanol with no further sucrose access. Samson argued that pairing ethanol with sucrose induces animals to consume greater quantities of ethanol than they might in the absence of sucrose by causing the approach response to ethanol for sucrose reinforcement to override the avoidance response to ethanol's aversive taste. According to Samson, once the animals consume enough ethanol to experience its reinforcing pharmacological effects, these effects maintain responding in the absence of sucrose.

Pairing a relatively neutral stimulus, such as a flavor, with saccharin or sucrose has been shown to increase the reinforcing value of that flavor (Fanselow & Birk, 1982; Holman, 1975, 1980). Although Samson argues that, in sucrose-induction procedures, pairing ethanol with sucrose serves only to induce animals to consume ethanol and does not alter the drug's reinforcing value, his studies have not addressed this possibility.

The purpose of Experiment 1 was to determine if pairing ethanol with a known positive reinforcer could enhance its motivational effect. In contrast to the studies by Grant and Samson (1985a, 1985b), subjects in Experiment 1 were food-restricted to further enhance conditioning by increasing the incentive value of the sucrose reinforcer (Revusky, 1967; Revusky & Garcia, 1970). Food-deprivation

also might be expected to increase preference for ethanol in the absence of conditioning because of the drug's caloric properties (Fedorchak & Bolles, 1987; Freed & Lester, 1970; Kulkosky, 1985; Meisch & Thompson, 1973; Samson & Falk, 1974; Sherman et al., 1983). In some cases, saccharin was added to the ethanol solution to determine if improved palatability would increase consumption of the ethanol solution, thereby, increasing exposure to the CS during conditioning (Geiger & Barker, 1976; Gilbert, 1974, 1978; Samson & Falk, 1974). Addition of saccharin also might be expected to increase the strength of the association between ethanol and sucrose through a within-compound association of the sweet taste of saccharin with ethanol (Rescorla & Cunningham, 1978).

Change in the motivational value of ethanol as a result of sucrose pairing was determined by differences in ethanol consumption between animals receiving ethanol paired with sucrose and those receiving ethanol and sucrose explicitly unpaired. It was hypothesized that pairing access to an ethanol solution with sucrose availability would increase oral self-administration of ethanol in food-deprived rats. It was predicted that the greatest ethanol consumption would be observed in the group receiving ethanol both combined with saccharin and paired with sucrose.

Material and Methods

Subjects

Subjects were 30 adult male Holtzman albino rats weighing between 377 and 431 g prior to food restriction [$X (\pm SEM) = 398 (2) \text{ g}$]. They were individually housed in hanging stainless steel cages (24 cm x 18 cm x 18 cm) with mesh fronts and floors in a temperature-controlled ($22 \pm 1^\circ\text{C}$) colony room maintained on a 12:12 h light/dark cycle (6 am, light on). All procedures took place in the animals' home cages during the light part of the cycle. Water was available ad lib except during treatment and testing periods.

Procedure

Ethanol solutions were prepared by diluting the appropriate volume of 95% ethanol (v/v) with tap water. Commercial table sugar (Spreckels) and sodium saccharin (EM Science) solutions were prepared w/v. Sucrose concentration was 32% and saccharin concentration was 0.15%. All solutions were presented at room temperature in Nalgene tubes (100 ml) with stainless steel drinking spouts.

The experimental procedure included three phases: food restriction (10 d), sucrose pre-exposure (four sessions), and ethanol conditioning (63 sessions). The experimental design is outlined in Table 1.

Food Restriction. After 7 d acclimation to the colony, the food restriction phase was initiated. The goal of this phase was to attain 80% of their free-feeding weight for all subjects. On day 1 of food restriction, subjects were weighed and the food was removed from the front of each cage. This weight was used as the free-feeding weight. Throughout the remainder of the experiment, subjects were weighed daily and given rations of chow (typically two to four pieces weighing approximately 4 g each) appropriate to maintain body weight at 80% of the free-feeding weight.

TABLE 1 -- Design for Experiment 1: Ethanol Self-administration

Phase 1: FOOD RESTRICTION (10 days)

All subjects given restricted feedings to maintain body weight at 80%

Phase 2: SUCROSE PRE-EXPOSURE (4 sessions)

All subjects given access to 32% sucrose solution

15 min access for 2 sessions

5 min access for 2 sessions

Phase 3: ETHANOL CONDITIONING (63 two-d sessions with + and - Trials alternating daily)

<u>Group</u>	<u>+ Trial</u>	<u>- Trial</u>
ES+	Ethanol-Saccharin x 5 min followed by Sucrose x 5 min	Water x 5 min
ES-	Ethanol-Saccharin x 5 min followed by Water x 5 min	Sucrose x 5 min
E+	Ethanol x 5 min followed by Sucrose x 5 min	Water x 5 min
E-	Ethanol x 5 min followed by Water x 5 min	Sucrose x 5 min

Ethanol concentration was initially 3% and increased over trials to 30% with 5-7 trials at each concentration. Sucrose (32%) and saccharin (0.15%) concentrations were held constant.

Sucrose Pre-exposure. After 10 d of restricted feeding, subjects were given brief access to 32% sucrose on each of four successive days. Fifteen min access was permitted on the first 2 d, followed by 5 min access for 2 d. One subject was removed for failure to consume adequate amounts of the fluid (daily mean intake < 5 ml). Remaining subjects were assigned to one of four groups matched for mean sucrose consumption over the 5-min trials [$X (\pm SEM) = 10.3 (0.3)$ ml].

Ethanol Conditioning. The ethanol conditioning phase consisted of 63 2-d trial blocks. Each trial block contained a plus (+) trial and a minus (-) trial. On each trial, water bottles were removed and animals were weighed. Following a 5-min delay, tubes containing ethanol-saccharin (ES) or ethanol (E) alone were placed on the cages at 10-sec intervals. After 5 min access to the fluid, tubes were removed. Subjects immediately were allowed 5 min access to either 32% sucrose or water. After each trial, water bottles were replaced and subjects were fed following a 2-h delay.

On + Trials, two groups of subjects received ES paired either with sucrose (Group ES+, $n = 8$) or with water (Group ES-, $n = 7$), while two other groups received E alone paired with sucrose (Group E+, $n = 7$) or with water (Group E-, $n = 7$). On - Trials, ES+ and E+ subjects received water while ES- and E- animals were given access to sucrose for 5 min. Thus, all groups received equivalent exposure to ethanol, sucrose, and water, but differed in their exposure to saccharin and/or in the temporal relation between ethanol and sucrose.

Ethanol concentration was initially 3% v/v and was slowly increased over the 63 conditioning trials to 30% as follows: 3%, 5%, 8%, 10%, 13%, 16%, 18% (5 trials each); 20% (7 trials); 22%, 24%, 27% (5 trials each); and 30% (6 trials). Saccharin and sucrose concentrations were unchanged.

Results

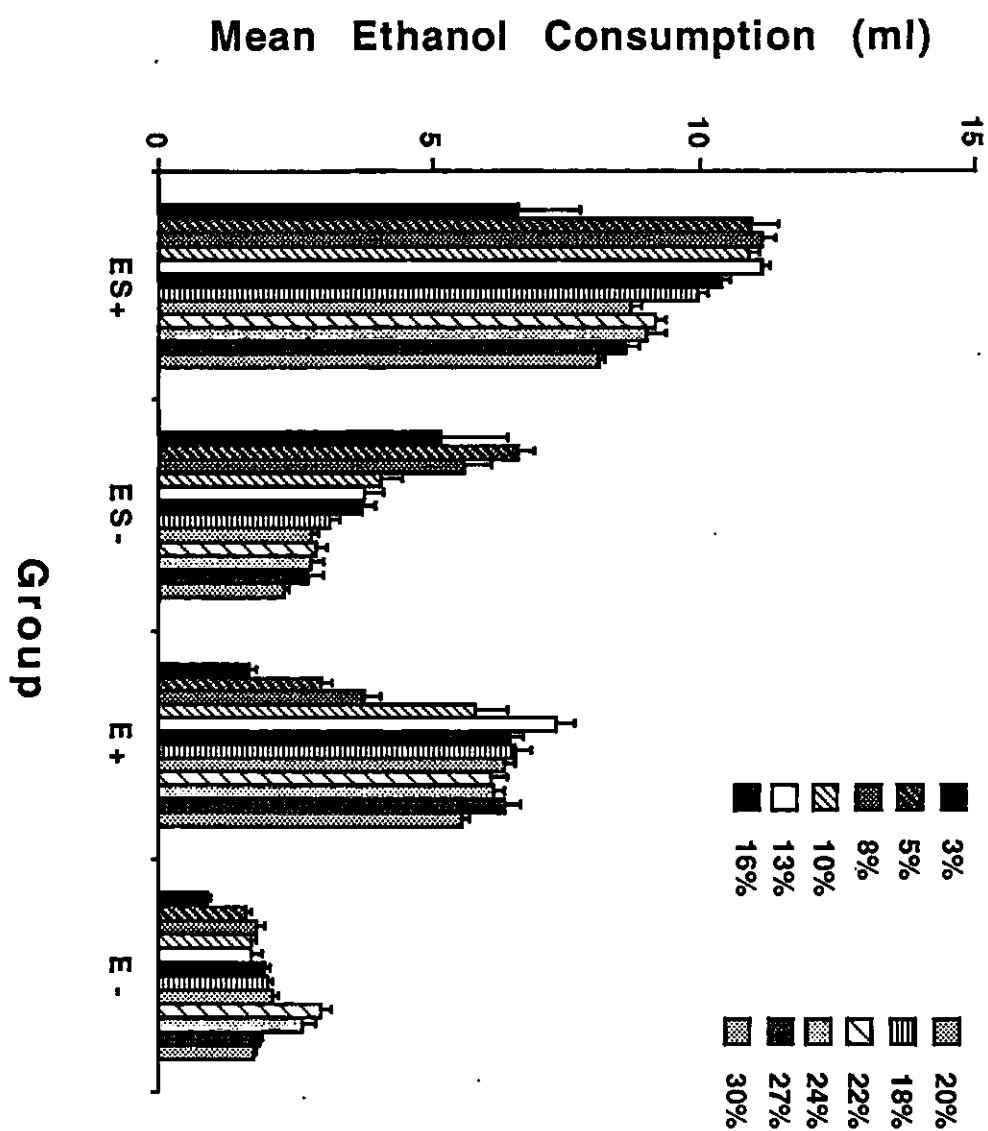
Two subjects were removed following severe weight loss and inability to maintain their weights at 80% of baseline: one subject injured his front teeth and was unable to eat (weight fell to 73% baseline), while a second animal developed a respiratory infection (weight dropped to 75%). This attrition left six subjects each in the ES- and E- groups, while Groups ES+ and E+ contained eight and seven subjects, respectively.

Body Weights. Two-way repeated measures analysis of variance (ANOVA), with group as the between-groups factor and trial as the repeated measures factor, of weights across the 63 conditioning trials showed a significant trials effect [$F(62,1426)=7.4, p<0.01$], with no significant differences between groups and no group $\times$ trial interaction. Mean weights across all groups ranged between 313 and 331 g over the 63 trials.

Ethanol Consumption. Mean ($\pm$ SEM) volumes of ES and E consumed at each concentration during ethanol conditioning are shown in Figure 1. During the early conditioning trials with relatively low ethanol concentrations, presence of saccharin in the ethanol solution appeared to have a marked influence, with Groups ES+ and ES- showing higher intakes of ethanol relative to groups receiving unsweetened ethanol solutions. However, as ethanol concentration increased over trials, presence of saccharin was insufficient to maintain consumption. Association of ethanol with sucrose access appeared more influential on ethanol intake since volume consumed by Group ES- declined and that of Group E+ increased. Ethanol intake by Group ES+, which received ethanol in saccharin paired with sucrose, remained high throughout conditioning. In contrast, consumption by Group E- was consistently low.

These observations were supported by statistical analysis. Three-way repeated measures ANOVA, with saccharin and reinforcement condition as the

Figure 1: Mean consumptions (ml $\pm$ SEM) of ethanol solutions for each group calculated across trials at each concentration of ethanol during conditioning. Group E+ was given access to ethanol while Group ES+ received ethanol-saccharin for 5 min followed by 5 min access to 32% sucrose. The groups were given 5 min access to water on non-ethanol days. Groups ES- and E- received ethanol-saccharin and saccharin, respectively, followed by water and were given sucrose alone on alternate days. Ethanol concentration was initially 3% and was progressively increased over the 63 conditioning trials to 30% with 5-7 trials at each concentration.



between-groups factors and ethanol concentration as the repeated measures factor, of ethanol consumption across the conditioning phase revealed significant main effects of saccharin [$F(1,23) = 22.8, p < 0.01$], reinforcement condition [$F(1,23) = 54.1, p < 0.01$], and ethanol concentration [$F(11,253) = 8.6, p < 0.01$].

There also were significant saccharin x ethanol concentration [$F(11,253) = 15.7, p < 0.01$] and reinforcement condition x ethanol concentration [$F(11,253) = 10.8, p < 0.01$] interactions. The saccharin x ethanol concentration interaction was probably due to the enhancing effect of saccharin on ethanol consumption at low ethanol concentrations and diminution of this influence as ethanol concentration increased. The reinforcement condition x ethanol concentration interaction may reflect the positive influence of sucrose pairing on ethanol intake across trials.

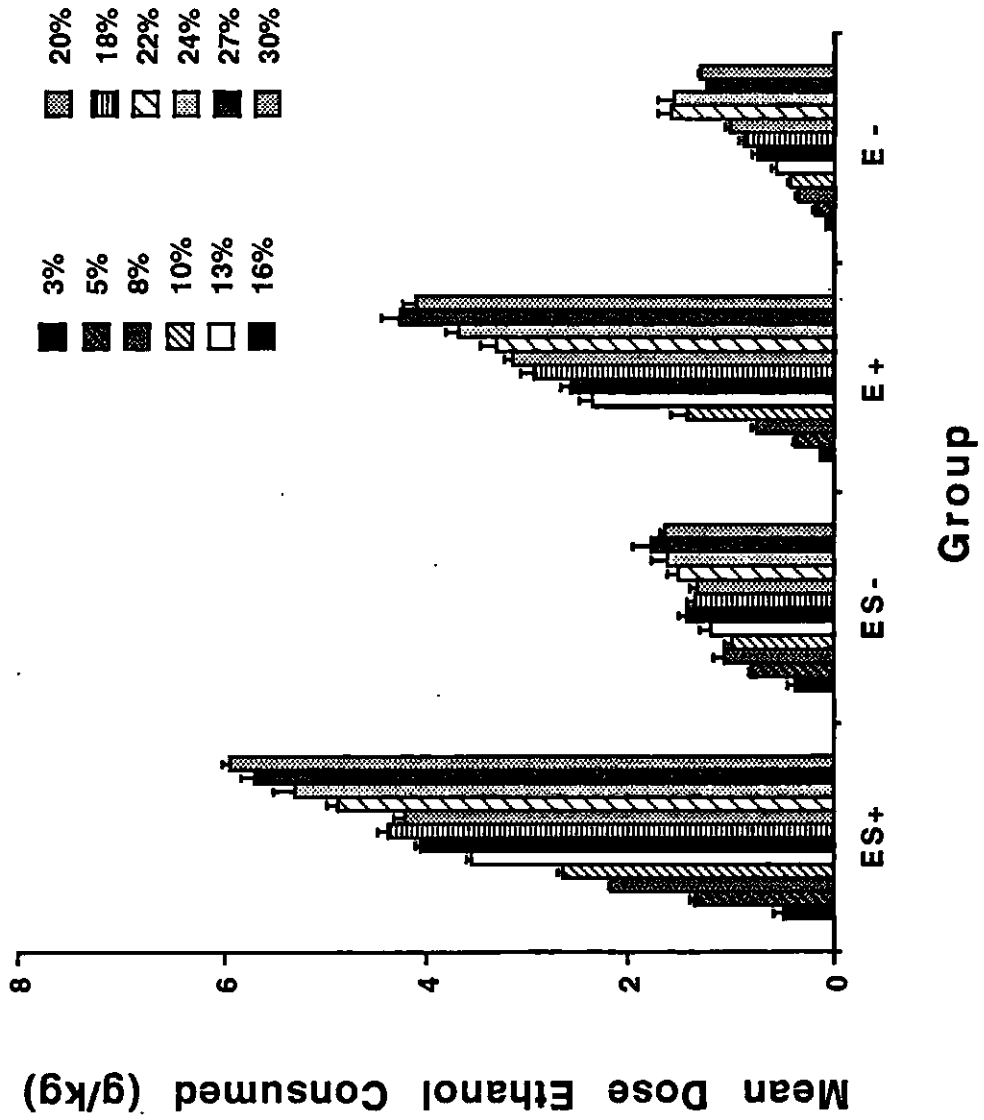
Three-way ANOVAs, with saccharin and reinforcement condition as between-groups factors and trials as the within groups factor, at each ethanol concentration in conditioning showed significant effects of saccharin on consumption of the ethanol solution at concentrations of 3%, 5%, 8%, 10%, 13%, 16%, and 18% [all $F's(1,23) > 9.0, p < 0.01$]. No significant impact of saccharin was seen at 20% and 22% ethanol, although presence of saccharin again had a significant impact at 24%, 27%, and 30% ethanol concentration [all $F's(1,23) > 5.0$], though at a alpha value of $p < 0.05$ instead of 0.01 as seen at the previous concentrations. Mean ethanol consumptions by Groups ES- and E-, which had differed by 2 to 5 ml at ethanol concentrations up to and including 20%, differed by less than 0.2 ml at ethanol concentrations of 22% and 24%, further demonstrating that presence of saccharin was not able to maintain ethanol consumption at high concentrations. Pairing ethanol solution with sucrose had no significant impact on consumption of 3% ethanol solution, although it did significantly enhance consumption at the remaining ethanol concentrations [all $F's(1,23) > 8.5, p < 0.01$].

Ethanol Dose. Mean ($\pm$ SEM) dose ethanol consumed is shown in Figure 2.

Both groups receiving ethanol paired with sucrose access appeared to consume greater amounts of ethanol across conditioning trials, with mean intakes by Group ES+ approaching 6 g/kg during later trials. Individual doses reached a maximum of 9 g/kg in one subject during the 5-min conditioning trial. These consumptions were within the range of lethality for rats not having developed tolerance to ethanol. Tsibulsky and Amit (1993) established an LD₅₀ of 6.1 g/kg (95% confidence interval of 5.7-6.5 g/kg) in male Wistar rats. This increased to 7.9 g/kg in animals given multiple ethanol exposures prior to lethality testing. Saccharin tended to augment ethanol intake throughout conditioning, although this effect appeared to be more consistent in the sucrose-reinforced group than in the non-reinforced group.

Three-way repeated measures ANOVA, with saccharin and reinforcement condition as the between-groups factors and ethanol concentration as the repeated measures factor, demonstrated significant effects of saccharin [$F(1,23) = 11.8$, $p < 0.01$], reinforcement condition [$F(1,23) = 65.8$, $p < 0.01$], and ethanol concentration [$F(11,253) = 108.2$, $p < 0.01$]. There was also a significant reinforcement condition x ethanol concentration interaction [$F(11,253) = 35.3$, $p < 0.01$]. This interaction was probably due to groups receiving ethanol paired with sucrose access consuming larger amounts of ethanol in the later conditioning trials relative to those receiving unpaired exposures. Three-way ANOVAs, with saccharin and reinforcement condition as between-groups factors and trials as the within groups factor, at each ethanol concentration showed, as in the consumption data, that presence of sucrose had no impact on dose of 3% ethanol consumed, but had a significant impact at ethanol consumption at concentrations greater than 3%. Although there was no saccharin by ethanol concentration interaction, presence of

Figure 2: Mean doses (g/kg $\pm$ SEM) of ethanol consumed for each group calculated across trials at each ethanol concentration during conditioning. Group designations are as listed in the legend for Figure 1.



saccharin did not significantly impact consumption of 20% and 22% ethanol solution.

Sucrose Consumption. Overall group differences in sucrose consumption were minimal. All groups demonstrated slightly increased sucrose intake over the first few trials, consistent with acclimation to the conditioning procedure or habituation of neophobia to the taste of sucrose. Mean ($\pm$ SEM) overall sucrose intakes were 13.4 (0.6), 13.6 (0.6), 13.6 (0.5), and 14.1 (0.4) ml for Groups ES+, ES-, E+, and E-, respectively. Three-way repeated measures ANOVA, with saccharin and reinforcement condition as the between-groups factors and trial as the repeated measures factor, showed a significant effect only of trial [$F(62,1426) = 6.4, p < 0.01$] due to the gradual increase in sucrose consumption in all groups over the 63 ethanol conditioning trials [$X (\pm SEM) = 12.4 (0.3)$ ml at 3% and 13.6 (0.4) ml at 30%].

Water Consumption. Water intake remained low for all groups with the mean amount consumed never exceeding 2.5 ml on any trial. Mean consumptions, however, were slightly higher for the paired sucrose groups [$X (\pm SEM) = 1.10 (0.12)$ ml] than for the unpaired sucrose groups [$X (\pm SEM) = 0.27 (0.04)$ ml]. Three-way repeated measures ANOVA, with saccharin and reinforcement condition as the between-groups factors and trial as the repeated measures factor, revealed a significant effect of reinforcement condition [$F(1,23) = 36.7, p < 0.01$]. There was also a significant effect of trial [$F(62,1426) = 2.4, p < 0.01$], reflecting a slight increase in consumption over trials [$X (\pm SEM) = 0.61 (0.10)$ ml at 3% and 0.69 (0.12) ml at 30%].

Discussion

Experiment 1 demonstrated that pairing an ethanol solution with access to sucrose was effective in significantly increasing consumption of ethanol in food-restricted rats in a Pavlovian paradigm. Total amount of ethanol consumed in the 5-min access period approached 6 g/kg/trial in the group receiving ethanol combined with saccharin and paired with sucrose, and 4 g/kg in subjects receiving unsweetened ethanol paired with sucrose.

Although adulterating the taste of ethanol with saccharin was important in stimulating ethanol consumption during the early conditioning trials, consumption by Group ES- did not differ from that of Group E- at higher ethanol concentrations. It may be that saccharin was effective as a taste-enhancer with low concentrations when the taste of ethanol was less aversive, but made a negligible improvement in palatability at higher concentrations.

The combined influence of delivering ethanol in a saccharin solution and pairing it with sucrose provided the optimal condition for ethanol consumption, possibly by increasing the degree of association between the CS and US through similarity of taste. This may have provided stronger and more rapid conditioning, as witnessed by the steeper initial increase in consumption shown by Group ES+ relative to Group E+.

This study suggests that sucrose-pairing plays a larger role than simply eliciting approach to ethanol so as to allow subjects to experience the drug's inherently reinforcing effects, as Samson advocates. Early in conditioning, Group ES- consumed greater quantities of ethanol than did Group E+. If ethanol's effects were indeed reinforcing, one would expect consumption by Group ES- to be maintained. However, with further conditioning trials at increased ethanol concentrations, consumption by Group E+ eclipsed that of Group ES-, suggesting that sucrose-pairing played a more pivotal role than simply seducing animals to

consume ethanol. If ethanol consumption at any given concentration was determined solely on the basis of taste, one would expect that consumption of ES- would consistently surpass that of E+ at all ethanol concentrations. These findings suggest that the consequences of ethanol ingestion play a more influential role than do taste factors in determining ethanol consumption.

The difference in consumptions between Groups ES+ and E+ also suggests that the development of an association between ethanol, functioning as a CS, and sucrose, functioning as a US, increases the reinforcing value of ethanol. Although saccharin may have functioned simply to increase the palatability of ethanol, the similarity in taste between saccharin and sucrose also may have served to enhance the strength of the association between ethanol and sucrose based on a within-compound association between ethanol and saccharin (Rescorla & Cunningham, 1978).

Although Samson argues that ethanol's effects are reinforcing, the results from this study suggest that the drug's pharmacological effects are only mildly appetitive at best and may actually be aversive at higher doses, as suggested by the "ceiling" in dose consumptions by the ES- and E- groups. This is consistent with findings from studies of conditioned taste aversion which demonstrate that, as ethanol concentration increases, consumption of ethanol or of an ethanol-paired stimulus declines. This was seen with both Groups ES+ and ES-, where volume of ethanol solution consumed progressively declined with increasing ethanol concentration. In contrast, consumption by Groups E+ and E- plateaued and remained stable despite increasing concentrations of ethanol. This difference in consumption patterns may be the consequence of ethanol's aversive effects becoming associated with the taste of saccharin in the ES groups and resulting in conditioned taste aversion to this flavor. The significantly greater consumptions

by Groups ES+ and E+ support the hypothesis that sucrose pairing can overpower ethanol's aversive properties.

Experiment 2: Quantitative Influence on Sucrose Pairing on Ethanol Self-administration

Rationale

Experiment 1 demonstrated that pairing ethanol with 32% sucrose was extremely effective in inducing the oral consumption of high concentrations of ethanol in food-restricted rats. To further refine the nature of this induction, in Experiment 2, the influence of sucrose on ethanol reinforcement was assessed quantitatively using a range of sucrose concentrations.

Sucrose previously has been shown to have a concentration-dependent influence on conditioned behavior. Guttman (1953) demonstrated that the time required to condition a barpress response for sucrose reinforcement was inversely related to sucrose concentration. Resistance to extinction and rate of responding were also concentration-dependent. His findings supported previous studies in which manipulation of amount of food reward was effective in altering operant response characteristics (Crespi, 1942, 1944; Grindley, 1929; Wolfe & Kaplon, 1941; Zeaman, 1949). Kraeling (1961) varied the concentration of sucrose, time of exposure, and amount of caloric intake and assessed the effect of these manipulations on running time and number of licks from a drinking tube. She demonstrated that sucrose concentration was the major determinant of running speed and strength of the consummatory response. Neither caloric reinforcement nor time of exposure influenced running speed, although increasing exposure had a positive effect on strength of the consummatory response. Her results suggest that intensity of taste stimulation was most responsible for the relation between amount of reward and instrumental response strength seen with sucrose reinforcement.

Holman (1975) replicated these findings on the importance of sweetness in conditioning preference using saccharin instead of sucrose. He gave food-

restricted rats a flavored saccharin solution for 5 min and a second flavored saccharin solution for 60 min. A choice test between the flavors revealed no difference in preference. However, when distinctive flavors were associated with different concentrations of saccharin solution, using either within-compound or trace conditioning, preference was shown for the flavor paired with 0.32% saccharin over that associated with 0.06% saccharin. His results also demonstrated that flavor preferences could be conditioned without need reduction. However, when he provided a 30-min delay between the flavor and either a saccharin or a dextrose US, preference was demonstrated only with dextrose. These findings suggest that need reduction through the calories provided by the dextrose solution may be a necessary consequence for delayed reinforcement of flavor preferences, while sweetness is an effective reinforcer if presented immediately following the CS.

If pairing ethanol with sucrose serves only to stimulate ethanol consumption and continued ethanol intake is due solely to its pharmacological effects, then no differences in ethanol consumption or preference would be expected between groups receiving varied sucrose concentrations paired with ethanol. However, if ethanol develops secondary reinforcing properties from such pairings, as suggested from the above studies on the relationship between response and degree of reinforcement, it is expected that ethanol consumption and ethanol preference would vary with sucrose concentration.

In addition to comparing ethanol consumptions during conditioning, differences in preference were assessed directly using 2-bottle choice testing. Blood ethanol concentrations were determined at two points during conditioning to gain information on the potential pharmacological significance of the doses consumed.

Material and Methods

Subjects

Subjects were 50 adult male Holtzman albino rats weighing between 400 and 452 g prior to food-restriction [$X (\pm SEM) = 421 (2) \text{ g}$]. They were individually housed as described in Experiment 1. All procedures took place in the animals' home cages during the light part of the cycle. Water was available ad lib except during treatment and testing periods and as described below.

Procedure

Ethanol, saccharin, and sucrose solutions were prepared as described in Experiment 1 and presented at room temperature in Nalgene tubes (100 ml) with stainless steel drinking spouts. The experimental procedure included four phases: food restriction (10 d), sucrose pre-exposure (six sessions), ethanol conditioning (25 sessions), and choice testing (two sessions). The experimental design is outlined in Table 2.

Food Restriction. After 3 d acclimation to the colony, subjects were restricted to 80% of their free-feeding weights by once-daily feedings of Purina rat chow as described in Experiment 1.

Sucrose Pre-exposure. Following 10 d of restricted feeding, subjects were exposed to 4% sucrose solution on each of 6 successive days, with 15 min access for 3 d and 5 min access for 3 d. Subjects were fluid-deprived for 24 h and 21 h, respectively, prior to the first and second exposures and were then returned to ad lib water. Two subjects were removed for failure to consume adequate amounts of sucrose (mean daily intake $< 2 \text{ ml}$). Remaining subjects were assigned to one of five groups matched for mean sucrose consumption over the 5-min trials [$X (\pm SEM) = 9.3 (0.4) \text{ ml}$].

Ethanol Conditioning. The ethanol conditioning phase consisted of 25 2-d trial blocks. Each trial block contained a plus (+) and a minus (-) trial. On each

TABLE 2 -- Design for Experiment 2: Influence of Sucrose Concentration

Phase 1: FOOD RESTRICTION (10 days)

All subjects given restricted feedings to maintain body weight at 80%

Phase 2: SUCROSE PRE-EXPOSURE (6 sessions)

All subjects given access to 4% sucrose solution

15 min access for 3 sessions

5 min access for 3 sessions

Phase 3: ETHANOL CONDITIONING (25 two-d sessions with + and - trials alternating +--+ or -++-)

<u>Group</u>	<u>+Trial</u>	<u>- Trial</u>
E-0	Ethanol-Saccharin x 5 min followed by Water x 5 min	Saccharin
E-4	Ethanol-Saccharin x 5 min followed by 4% Sucrose x 5 min	Saccharin
E-16	Ethanol-Saccharin x 5 min followed by 16% Sucrose x 5 min	Saccharin
E-32	Ethanol-Saccharin x 5 min followed by 32% Sucrose x 5 min	Saccharin
C	Saccharin x 5 min followed by 32% Sucrose x 5 min	Ethanol-Saccharin

Ethanol concentration was initially 3% and increased over trials to 13% with 3-7 trials at each concentration. Sucrose concentration was maintained at the respective concentration for each group throughout conditioning. Saccharin concentration (0.15%) was held constant.

Blood ethanol concentrations were measured after Trials 8 (5% ethanol) and 23 (13% ethanol).

Phase 4: CHOICE TESTING (2 sessions)

All subjects given simultaneous access to ethanol-saccharin (13% ethanol in 0.15% saccharin) and saccharin (0.15%) for 5 min during each session

trial, water bottles were removed and animals were weighed. Following a 5-min delay, tubes containing ethanol-saccharin (ES) solution (ethanol and 0.15% saccharin) or saccharin (S) alone (0.15% saccharin in tap water) were placed on the cages at 10-sec intervals. After 5 min access to the fluid, tubes were removed. All ES Groups received ES followed by 5 min access to sucrose on + Trials; however, the groups differed in the concentration of sucrose paired with ES. Group E-0 ($n = 9$) received ES followed by 5 min access to 0% sucrose (water); Group E-4 ($n = 10$) received 4% sucrose; Group E-16 ($n = 10$) received 16% sucrose; and Group E-32 ($n = 10$) received 32% sucrose. On - Trials, they were given saccharin alone. Control subjects ($n = 9$) received S followed by 32% sucrose on + Trials and ES alone on - Trials. Thus, all groups were given equal exposure to ethanol and saccharin, but differed in their exposure to sucrose and in which fluids were paired with sucrose delivery.

After each trial, water bottles were replaced and subjects were fed following a 2-h delay. Fluid exposures were counterbalanced within each group and alternated after every two exposures over successive trials. Ethanol concentration was initially 3% and was increased over the 25 trials to 13% as follows: 3% (3 trials); 5%, 8%, 10% (5 trials each); and 13% (7 trials). Saccharin and sucrose concentrations were unchanged.

Choice Testing. On 2 consecutive days following the conditioning phase, subjects were given a two-bottle choice test between ES and S. On each day, subjects were allowed 5 min access to the two fluids. Ethanol concentration was 13% (v/v) and saccharin concentration was 0.15% (w/v). Fluid positions were counterbalanced within each group and reversed for each subject from the first to second test trial.

Blood Ethanol Determinations. During Trials 8 (5% ethanol) and 23 (13% ethanol) of ethanol conditioning, blood samples were collected 1 h following

ethanol access by nicking the tip of the tail with a sharp scalpel. The samples were analyzed using a method described by Roach & Creaven (1968) as modified by Crabbe, Kosobud, Tam, Young, and Deutsch (1987). The 20 μ l tail blood samples were placed in a 1.5 ml polyethylene centrifuge tube containing 50 μ l of 5% zinc sulfate, to which was added 50 μ l of 0.3 N barium hydroxide and 300 μ l of cold distilled water. The solution was vortexed and centrifuged at 8-10,000 g for 5 min. The supernatant was removed and placed in an airtight glass vial for analysis by gas chromatography. Due to the necessary delay between collection and analysis, all samples were frozen (-20°C) and thawed prior to testing.

Analysis was performed with a Hewlett-Packard Model 5890 gas chromatograph. Flame ionization detector temperature was 230°C, injection port temperature was 175°C, and oven temperature was 154°C. A Porapak Q column was conditioned by heating overnight at 200°C with nitrogen carrier gas flow. Peak areas were determined with an H-P Model 3393A Integrator. A standard curve for ethanol (range 0.2-2.0 mg/ml) was determined before measuring. The coefficient of variation among five injections of a sample is less than 1% in this assay.¹

¹Blood samples and standards were collected and prepared by the investigator. The samples were run on the gas chromatograph (GC) by Erik Terdal, a research assistant in the laboratory of Dr. John Crabbe, Portland Veterans Affairs Medical Center. The investigator calculated the blood alcohol concentration from the area-under-the-curve produced by the GC for each sample relative to the mean concentration equivalent for the standards.

Results

Body Weights. Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, of mean body weights collapsed across the 2 d of each trial showed a significant trials effect [$F(24,1032) = 3.7, p < 0.01$] across the 25 conditioning trials. Mean weights across all groups ranged between 328 and 344 g over the 25 trials.

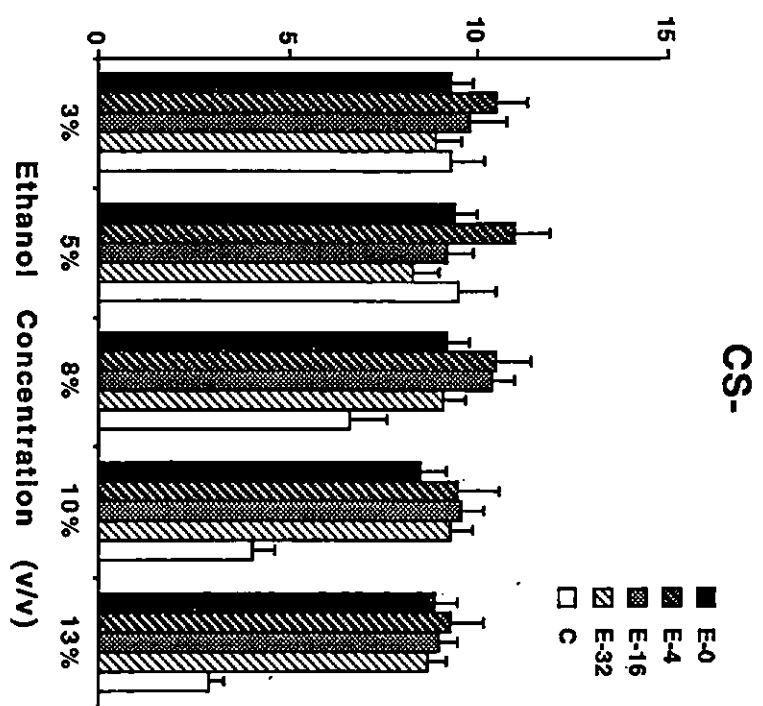
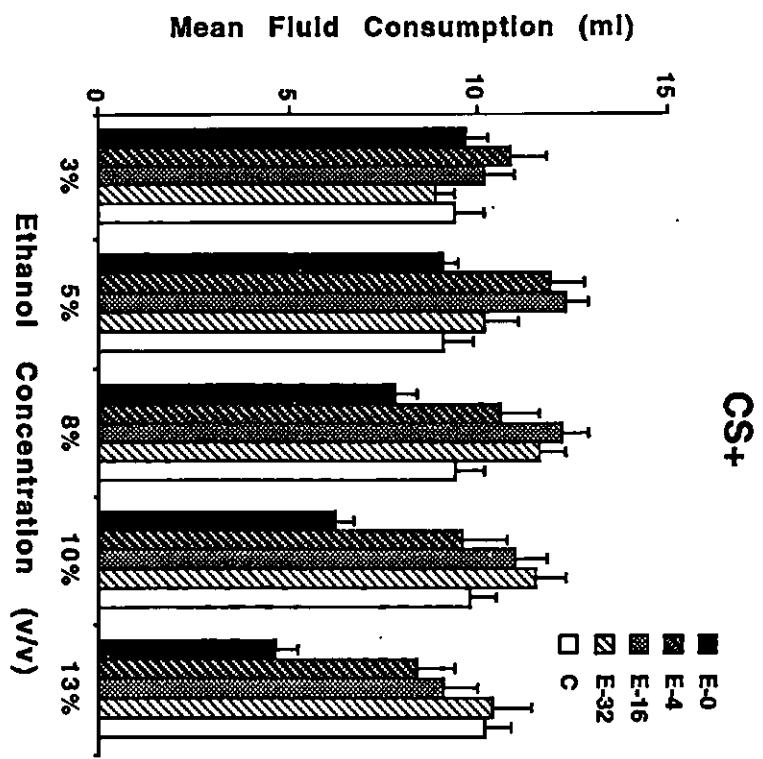
Ethanol Conditioning

Because Groups C and E-0 were similar in that both groups received ethanol-saccharin in the absence of sucrose reinforcement, they were seen conceptually as the unpaired control groups. Similarly, Groups E-4, E-16, E-32, despite their differences in concentration of the sucrose reinforcer paired with ethanol-saccharin, were treated as a single experimental group.

CS Consumption. Mean ($\pm$ SEM) fluid intakes during the ethanol conditioning trials are shown in Figure 3. Consumptions were high during both the + and - Trials for all groups early in conditioning; however, as ethanol concentration increased across trials, consumption of ES decreased in those groups not receiving the fluid followed by sucrose access (Groups E-0 on CS+ days and C on CS- days). Consumption of S was unaltered throughout conditioning.

A three-way repeated measures ANOVA, with group as the between-groups factor and trial type and ethanol concentration as repeated measures factors, of ethanol consumption across conditioning yielded significant effects of group [$F(4,43) = 2.9, p < 0.05$], trial type [$F(1,43) = 15.5, p < 0.01$], and ethanol concentration [$F(4,172) = 28.6, p < 0.01$], and significant interactions of group x trial type [$F(4,43) = 11.0, p < 0.01$], group x ethanol concentration [$F(16,172) = 6.0, p < 0.01$], trial type x ethanol concentration [$F(4,172) = 4.6, p < 0.01$], and group x trial type x ethanol concentration [$F(16,172) = 18.8, p < 0.01$]. Post-hoc two-way ANOVAs with group as the between-groups factor and trial type as the repeated

Figure 3: Mean consumptions (ml $\pm$ SEM) of CS+ and CS- fluids for each group for each concentration of ethanol used during the 25 conditioning sessions. On CS+ trials (left panel), Groups E-0, E-4, E-16, and E-32 received 5 min access to ethanol-saccharin followed by 0%, 4%, 16%, and 32% sucrose, respectively; for 5-min, while Group C received saccharin followed by 5 min access to 32% sucrose. On CS- trials (right panel), Group C received ethanol-saccharin, whereas the experimental subjects were given access to saccharin. Ethanol concentration was initially 3% and progressively increased to 13% with 3-7 trials at each concentration.



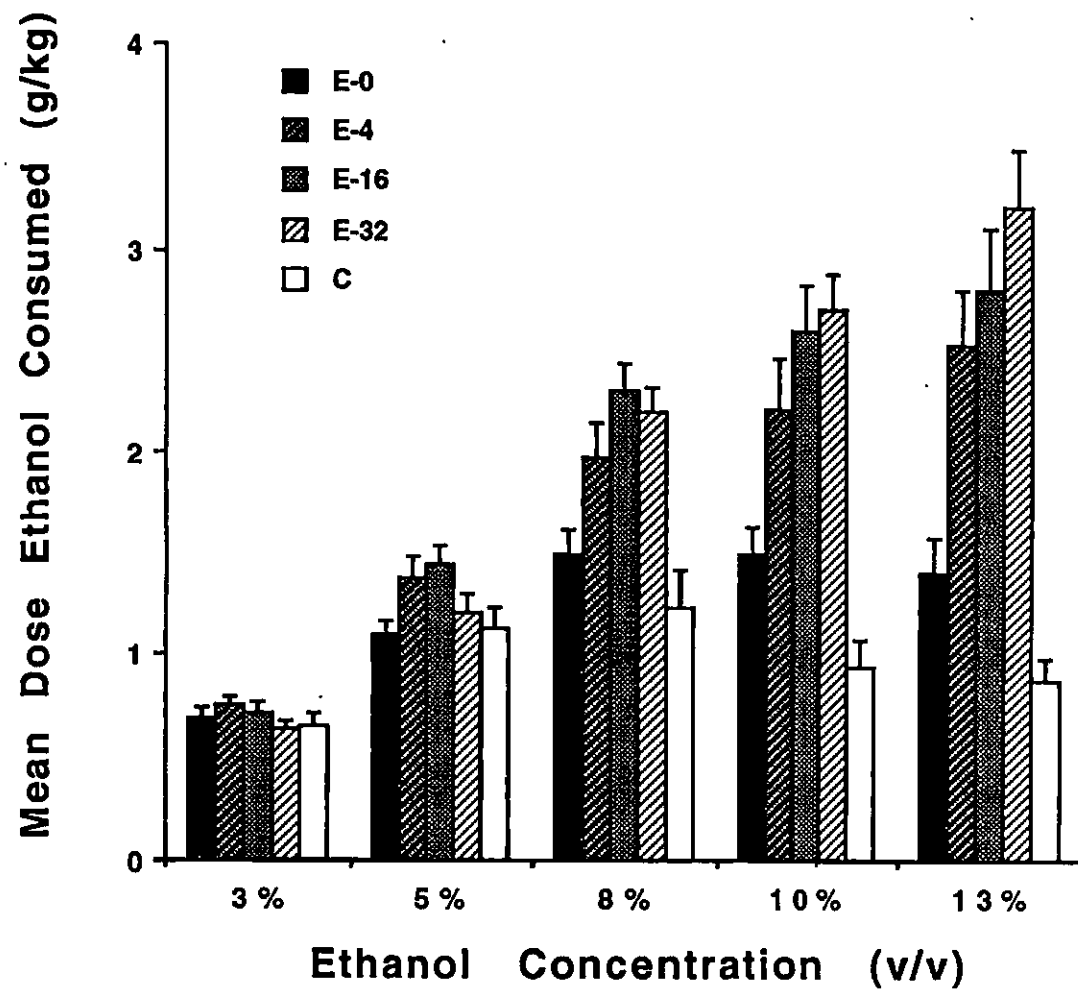
measures factor, at each ethanol concentration showed the interactions to be due to the absence of any significant group or trial type effects in the 3% ethanol trials, with the development of a significant difference in consumption of CS+ and CS- fluids with 13% ethanol in Groups E-0 [$F(1,8) = 38.1, p < 0.01$], E-32 [$F(1,9) = 5.07, p < 0.05$], and C [$F(1,8) = 92.3, p < 0.01$]. More S than ES was consumed by both Group E-0 (CS- vs. CS+) and Group C (CS+ vs. CS-), resulting in a significant group x trial type interaction [$F(4,43) = 29.3, p < 0.01$] with 13% ethanol.

Analysis of ES consumption by those groups receiving ES paired with sucrose (Groups E-4, E-16, and E-32) showed no group differences during conditioning despite the differences in concentrations of sucrose used as a reinforcer. Contrast analyses demonstrated that mean ES consumption by these groups differed from that of the control groups (Groups E-0 and C) at concentrations of 5% or more [all $F_s(1,43) > 8.5, p < 0.01$].

Ethanol Dose. Mean ($\pm$ SEM) dose ethanol consumed across trials at each concentration is shown in Figure 4. While groups consumed similar amounts of 3% ethanol, the ES-sucrose groups consumed considerably more ethanol than the control groups at ethanol concentrations of 8% and above.

Two-way repeated measures ANOVA, with group as the between-groups factor and ethanol concentration as the repeated measures factor, for ethanol dosage showed significant effects of group [$F(4,43) = 12.2, p < 0.01$], ethanol concentration [$F(4,172) = 162.2, p < 0.01$], and group x ethanol concentration [$F(16,172) = 15.2, p < 0.01$]. Contrast analyses of the interaction revealed no difference between ES-sucrose groups and control groups at 3% ethanol, and a significant effect of sucrose pairing at concentrations of 5% ethanol and higher [all $F_s(1,43) > 7.7, p < 0.01$]. There was no significant difference between the three experimental groups in the dose ethanol consumed.

Figure 4: Mean doses (g/kg $\pm$ SEM) of ethanol consumed at each concentration during conditioning for all groups. Group designations are as listed in the legend for Figure 3. Ethanol-saccharin solution was made available to Groups E-0, E-4, E-16, and E-32 during the CS+ trials, while Group C received ethanol-saccharin during the CS- trials.



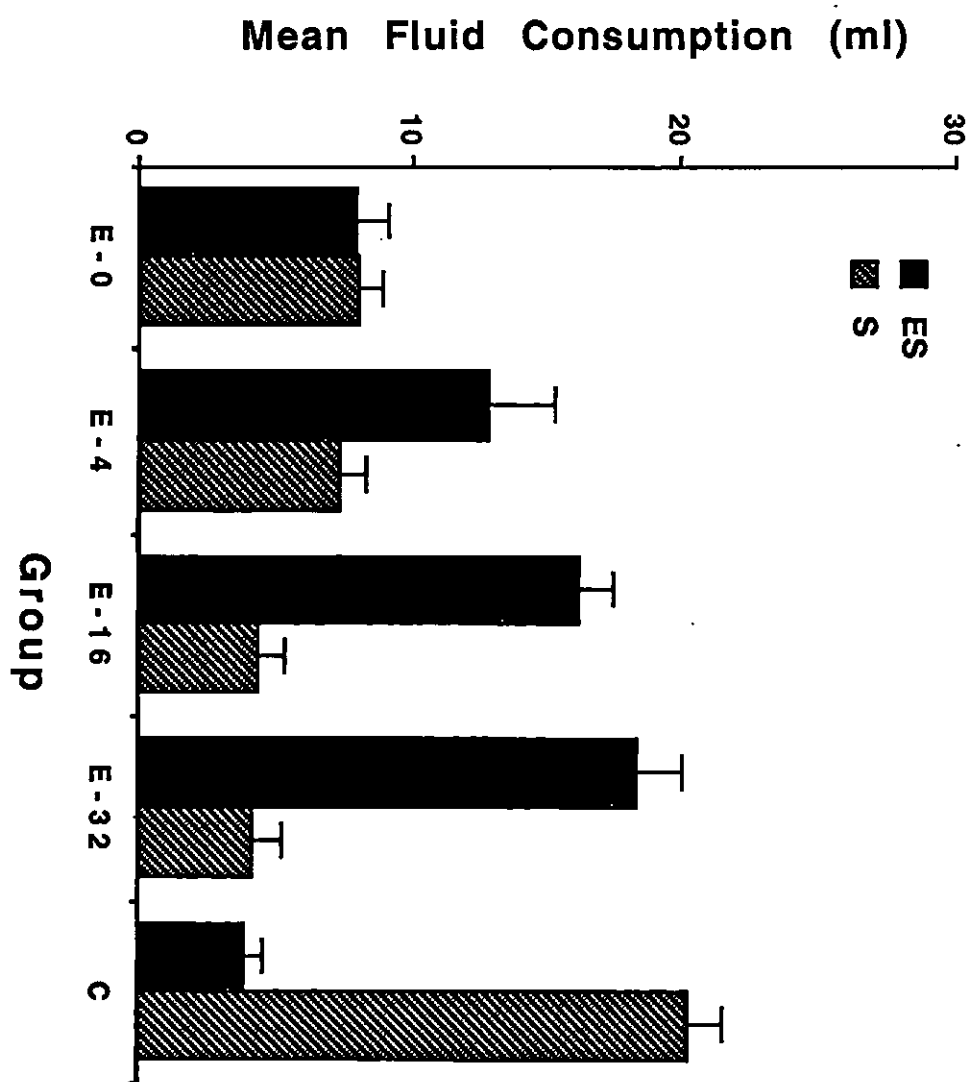
Sucrose Consumption. Mean sucrose consumption ($\pm$ SEM) across the 25 trials was 11.2 (0.7), 14.5 (0.6), 14.2 (0.5), and 14.5 (0.7) ml for Groups E-4, E-16, E-32, and C, respectively. Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, showed a significant effect of group [$F(3,35) = 6.8, p < 0.01$] and trial [$F(24,840) = 13.9, p < 0.01$]. Contrast analyses of the group effect showed sucrose consumption by Group E-4 to be significantly lower than that by the groups receiving 16% and 32% sucrose [$F(1,35) = 20.5, p < 0.01$], which did not differ from one another. The trial effect was due to the slight increase in sucrose consumption observed across the 25 trials [X increase ($\pm$ SEM) = 3.9 (0.5) ml between Trials 1 and 25]. Water consumption by Group E-0 remained low throughout conditioning, with a mean ($\pm$ SEM) consumption of 0.4 (0.03) ml.

Choice Testing

Mean ($\pm$ SEM) total consumptions for the two-bottle choice test are shown in Figure 5. Groups receiving sucrose consumed more of the sucrose-paired fluid than the unpaired fluid. Moreover, among the ES-sucrose groups, ES intake increased as a function of sucrose concentration. Group E-0, which received ES paired with water, consumed similar amounts of ES and S.

Two-way repeated measures ANOVA, with group as the between-groups factor and reinforcement condition as the repeated-measures factor, comparing consumptions of + and - fluids summed across the 2-d choice trial showed significant effects of group [$F(4,43) = 4.5, p < 0.01$], reinforcement condition [$F(1,43) = 87.0, p < 0.01$], and group $\times$ reinforcement condition [$F(4,43) = 8.8, p < 0.01$]. Followup analyses showed significant differences in fluid consumption based on reinforcement condition only in Groups E-16 [$F(1,9) = 39.5, p < 0.01$], E-32 [$F(1,9) = 34.0, p < 0.01$], and C [$F(1,8) = 79.7, p < 0.01$].

Figure 5: Mean total consumptions ($\pm$ SEM) of ethanol/saccharin (ES) and saccharin (S) for the 2 d of 5-min two-bottle choice testing for all groups. Group designations are as listed in the legend for Figure 3. Ethanol-saccharin was the CS+ fluid for Groups E-0, E-4, E-16, and E-32, while saccharin was the CS+ fluid for Group C. Ethanol concentration was 13%.



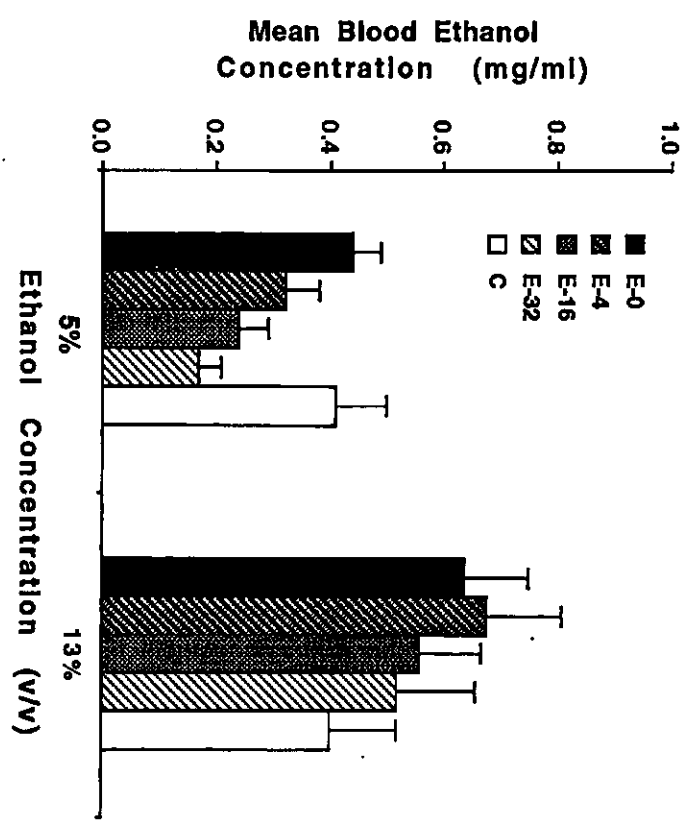
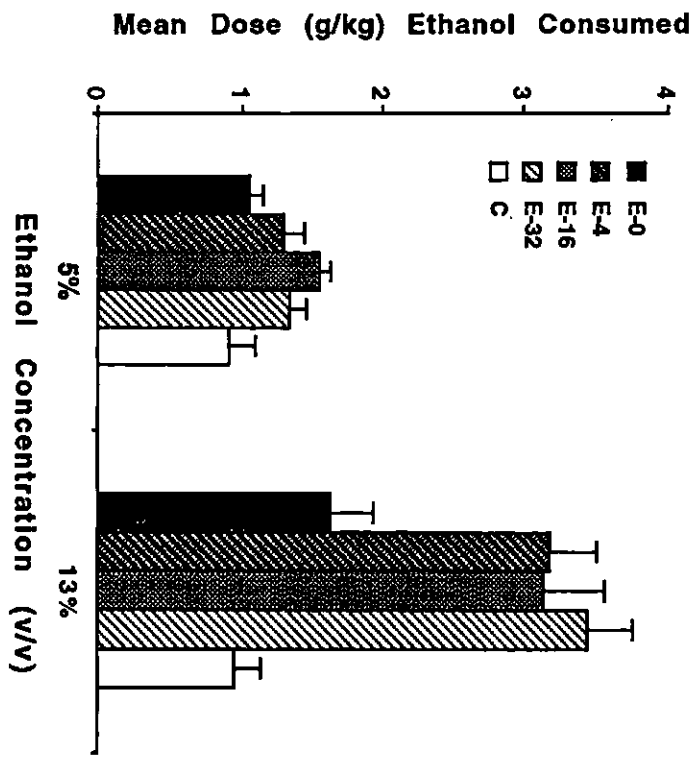
Contrast analyses revealed significant differences in ES consumption between groups receiving ES paired with sucrose and the control groups (Groups E-0 and C) [$F(1,43) = 43.9, p < 0.01$]. Group 32 also consumed significantly more ethanol than Group E-4 [$F(1,43) = 6.2, p < 0.05$]. There were no significant differences in ES consumption between Groups E-4 and E-16; between Groups E-16 and E-32; or between Groups E-0 and C. Contrast analyses of saccharin consumption showed Group C to consume significantly more than any other group [all F 's(1,43) $> 6.6, p < 0.01$]. Groups E-0 and E-4 each drank significantly more saccharin than Groups E-16 and E-32 [all F 's $> 4.4, p < 0.05$].

Mean total dose ($\pm$ SEM) of ethanol consumed during the two days of choice testing was 2.4 (0.4), 3.8 (0.7), 4.9 (0.4), 5.6 (0.5), and 1.2 (0.2) g/kg for Groups E-0, E-4, E-16, E-30, and C, respectively. One-way ANOVA revealed a significant group effect [$F(4,43) = 14.5, p < 0.01$]. Contrast analyses showed that, although the ethanol doses consumed by Groups E-0 and C did not differ significantly, both consumed a significantly lower dose than did the other groups [all F 's(1,43) > 4.1 , all p 's < 0.05]. In addition, dose consumption by Group E-4 was significantly less than that of Group-32 [$F(1,43) = 7.4, p < 0.01$]. Group E-16 did not differ from either Group E-4 or Group E-32.

Ethanol Self-administration and Blood Ethanol Concentrations

Blood ethanol concentrations (BECs) and corresponding ethanol consumptions following Trials 8 (5% ethanol) and 23 (13% ethanol) are shown in Figure 6. Data were discarded for four subjects from Trial 8 and one subject from Trial 23 due to inability to obtain blood samples. On Trial 8, the mean ethanol dose consumed was slightly higher in the ES-sucrose groups than in the control groups. However, BEC was lower in the ES-sucrose groups than in the control groups and was inversely related to sucrose concentration. On Trial 23, the ES-

Figure 6: Mean doses (g/kg $\pm$ SEM) of ethanol self-administered (left panel) and resultant mean blood ethanol concentrations (mg/ml $\pm$ SEM - right panel) for each group for Trials 8 (5% ethanol) and 23 (13% ethanol) of ethanol conditioning. Group designations are as described in the legend for Figure 3.



sucrose groups consumed higher amounts of ethanol than the control groups.

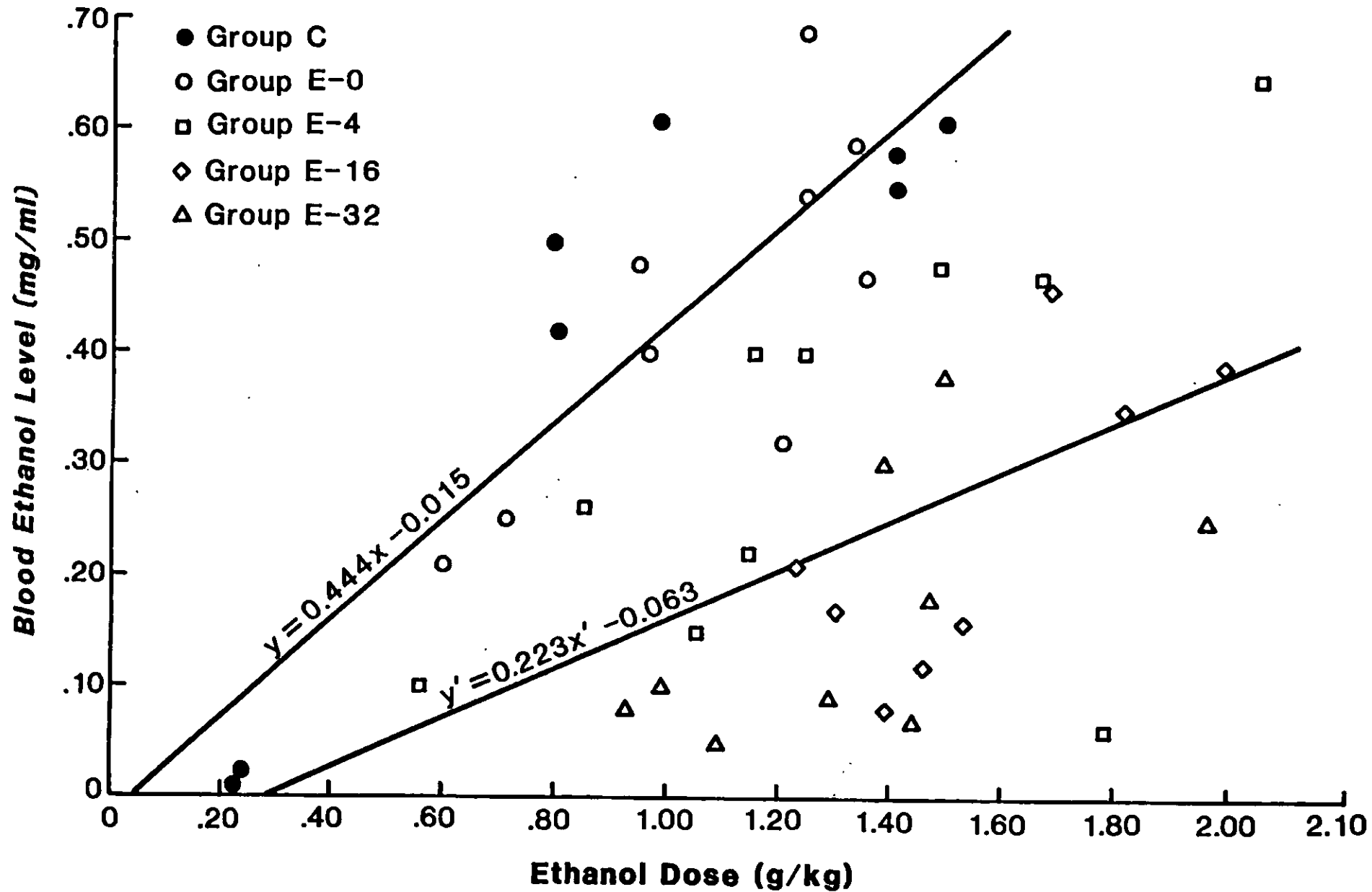
BECs, however, were generally similar across groups.

One-way ANOVA of BEC showed a significant group effect for Trial 8 [$F(4,39) = 11.4, p < 0.01$], but not for Trial 23. Followup contrast analyses showed blood ethanol levels to be significantly higher on Trial 8 in control subjects [$F(1,39) = 11.4, p < 0.01$] relative to ES-sucrose groups, despite the lower ethanol intake by controls. One-way ANOVA of ethanol dose showed a significant group effect for both Trial 8 [$F(4,39) = 3.8, p < 0.05$] and Trial 23 [$F(4,42) = 11.3, p < 0.01$]. Followup contrast analyses showed higher consumptions of both 5% ethanol [$F(1,39) = 12.2, p < 0.01$] and 13% ethanol [$F(1,42) = 41.0, p < 0.01$] in the pooled ES-sucrose groups than in the control groups.

Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, comparing ethanol dose consumed between sucrose-paired and control groups for Trials 8 (5% ethanol) and 23 (13% ethanol) showed significant effects of group [$F(1,42) = 46.8, p < 0.01$], trial [$F(1,42) = 44.2, p < 0.01$], and group $\times$ trial [$F(1,42) = 21.7, p < 0.01$]. Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, comparing BECs for the pooled ES-sucrose groups to the pooled controls for Trials 8 and 23 showed a significant trial effect [$F(1,42) = 11.7; p < 0.01$], but no significant difference between groups.

A scatterplot of BEC (mg/ml) versus dose ethanol consumed (g/kg) for Trial 8 is shown in Figure 7. The data for the two control groups and for the three ES-sucrose groups were pooled separately for computing the regression equations shown in the figure. As can be seen, the slope for the regression line was steeper for control subjects than for ES-sucrose subjects. Calculation of the t value based on these regressions (Cohen and Cohen, 1983) showed the slopes of these lines to be significantly different ($t(40) = 3.2; p < 0.01$). There was also a higher correlation

Figure 7: Scatterplot of blood ethanol concentration (mg/ml) versus dose of ethanol consumed (g/kg) during Conditioning Trial 8 (5% ethanol). The line corresponding to $y' = 0.223x' - 0.063$ describes the regression for animals receiving ethanol-saccharin solution paired with sucrose (Groups E-4, E-16, and E-32). Line $y = 0.444x - 0.015$ describes the relationship between blood ethanol concentrations and ethanol dose in control subjects (Groups E-0 and C) given ethanol-saccharin solution without subsequent sucrose access. Group designations are more thoroughly described in the legend for Figure 3.



between ethanol dose and blood ethanol levels for control animals than for those receiving ethanol with sucrose. The correlation coefficient for the ES+ groups was .50 ($df=25$, $p<0.01$), while that for the control groups was .86 ($df=15$, $p<0.01$).

The scatterplot of BEC versus dose ethanol consumed for Trial 23 (data not shown) also demonstrated a slightly steeper slope for control subjects relative to the ES-sucrose groups. The regression equations were $y = 0.363x + 0.051$ for the pooled control groups and $y' = 0.24x' - 0.193$ for the pooled ES-sucrose groups. However, analysis showed that the difference in the slopes of these regression lines was not significant. This suggests that sucrose consumption may interfere less in the relationship between ethanol dose and blood ethanol levels at higher ethanol concentrations. Correlation between ethanol dose and blood ethanol level again varied between experimental and control groups at this ethanol concentration, although the difference was smaller than that seen with 5% ethanol. The correlation coefficient was .86 ($df=15$, $p<0.01$) for the pooled controls, which matched that from Trial 8. The correlation increased to .67 ($df=28$, $p<0.01$) for the experimental subjects.

Discussion

The results from Experiment 2 support Experiment 1 in demonstrating that ethanol paired with sucrose access increased the oral consumption of ethanol and increased preference for ethanol over vehicle relative to animals receiving the fluids unpaired. This was a robust phenomenon, with mean ethanol dosages delivered exceeding 3 g/kg in a 5-min period at some ethanol concentrations.

Comparison of Group E-0 with Groups E-4, E-16, and E-32 showed that temporal pairing of ethanol with sucrose was necessary for the maintenance of ethanol consumption at higher ethanol concentrations. Although all groups consumed similar amounts of ethanol when the concentration was 3%, consumption by Groups E-0 and C, which received ethanol paired either with water (E-0) or alone (C), declined rapidly as ethanol concentration exceeded 5%.

Although ES consumption during ethanol conditioning by the three groups receiving ethanol paired with sucrose was ordered with respect to sucrose concentration at 10% and 13% ethanol, the difference between groups was not significant. In fact, 4% sucrose appeared just as effective in maintaining ethanol consumption as 32% sucrose. However, the groups did differ in the amount of sucrose solution consumed during conditioning, with subjects in groups E-16 and E-32 consuming higher mean levels of sucrose solution. Although Groups E-4, E-16, and E-32 did not differ with respect to ES consumption during ethanol conditioning, in two-bottle choice testing between ES and S following conditioning, Group E-32 consumed significantly more ES than Group E-4. Although putative differences in reinforcing value did not impart a difference in ES consumption during conditioning, the more sensitive two-bottle test did reveal differences in preference based on differences in associated reinforcement.

Analysis of BEC during Trial 8 suggests that, in early trials, the ethanol-sucrose experimental groups experienced lower BECs for a given dose of ethanol

consumed than did Groups E-0 and C, which did not receive ethanol with sucrose access. However, during later trials at higher ethanol concentration, pairing ethanol with sucrose allowed the ethanol-sucrose subjects to consume much greater volumes of ethanol and maintain blood ethanol concentrations equivalent to those of control subjects. This contrast between ethanol consumption and resultant BECs may have resulted from the large bolus of sucrose either diluting the orally delivered ethanol or otherwise altering ethanol's absorption or metabolism. Thus, in addition to its reinforcing taste, sucrose may have reduced ethanol's pharmacological impact by altering pharmacokinetics (see Experiment 4, Discussion).

Although some investigators, such as Samson, might argue that ethanol's pharmacological effects are appetitive in rats, the results of this experiment suggest, instead, that those effects are aversive. The mean dose of ethanol consumed by control animals did not exceed 1.5 g/kg, despite increasing ethanol concentrations, suggesting avoidance of higher doses. It is unclear if ethanol's pharmacological effects are bivalent (i.e. appetitive at lower doses and aversive at higher doses), or if its effects are consistently aversive and that its appetitive caloric properties supersede its aversive pharmacological effects at lower dosages. Further support comes from the finding that diluting ethanol with sucrose resulted in a lower blood ethanol concentration for a given dose of drug, thereby reducing its pharmacological and, potentially, aversive effects. This appeared to allow the ethanol-sucrose experimental animals to increase their ethanol consumption to achieve the same pharmacological effect, as indicated by the equivalent BECs measured for all groups consuming 13% ethanol. As a result, animals receiving ethanol and sucrose in combination show increased "preference" for ethanol as measured by increased consumption relative to those not experiencing this dilutional effect.

Although the dilutional effects of sucrose could explain the reduced aversive impact of ethanol consumption, choice testing revealed that, as with non-pharmacological stimuli, the motivational value of ethanol can be directly altered through conditioning. Ethanol consumptions did not differ between groups receiving different sucrose concentrations during conditioning; however, subjects experiencing the drug paired with 32% sucrose showed greater preference for ethanol in two-bottle choice testing than did animals receiving 4% sucrose. The demonstration that blood ethanol concentrations did not vary between the experimental groups, or between experimental and control subjects, argues against any differential experience with ethanol's pharmacological effects as being responsible for this difference in preference. Thus, variations in the appetitive value of the sucrose reinforcer directly influenced the degree of conditioned change in the motivational value of ethanol.

These findings suggest that instances of ethanol self-administration should be examined for possible contributions of conditioned enhancement of ethanol's motivational value before attributing reinforcement solely to the drug's pharmacological effects. Sucrose was shown to be a strong stimulus for conditioning, suggesting that, in studies where ethanol is paired with sucrose or some other highly reinforcing stimulus such as food, appetitive value may be readily acquired by ethanol as a result of conditioning. These results also suggest that the potential for altered ethanol pharmacokinetics should be a consideration in conditioning paradigms in which orally-delivered ethanol is presented with a second orally-acquired solution. Furthermore, animals may regulate ethanol intake so as not to exceed a certain BEC, as revealed by the approximately equal BECs seen for all groups at 13% ethanol (and seen for Groups E-0 and C at 5% ethanol). This regulation is presumably a learned skill and acquired over several conditioning trials (and adjusted to changes in ethanol concentration), which may

explain the absence of consistency in BECs across all groups with 5% ethanol, but not with 13% ethanol. Recurrent exposure to ethanol would be expected to result in an upward shift in "target BEC" as subjects developed tolerance to ethanol's aversive effects.

Experiment 3: Influence of Sucrose Pairing on Taste Conditioning with Ethanol

Rationale

Experiments 1 and 2 established that the motivational value of ethanol can be increased by pairing the drug with a strong positive reinforcer. This was demonstrated in animals receiving ethanol paired with sucrose relative to subjects receiving the fluids unpaired. However, as shown in Experiment 2, sucrose pairing also lowered the blood ethanol levels of the ethanol-sucrose experimental groups compared to the unpaired controls, which may have contributed to the differences in preference observed by decreasing the aversiveness of ethanol in the paired groups. Experiment 3 investigated further the influence of pairing ethanol access with sucrose availability on the motivational value of ethanol. However, the route of ethanol administration and the interval between ethanol delivery and sucrose access were altered from previous experiments, to control for the possible influences of sucrose on ethanol pharmacokinetics.

The results from Experiment 2 suggest that sucrose consumption altered the absorption or metabolism of orally-administered ethanol. In Experiment 3, ethanol was administered i.p. to prevent its dilution in the stomach by the sucrose bolus that the animals subsequently consume. Administering the drug by injection instead of allowing oral consumption also provided more experimental control over the dose of ethanol given to each animal, thus minimizing the variability in drug level experienced by each subject. In addition, the interval between drug delivery and sucrose access was increased. Instead of subjects receiving sucrose immediately following ethanol, a 15-min delay was introduced between the injection and sucrose availability to allow for greater absorption of the drug prior to sucrose consumption.

In contrast to the choice testing employed in Experiment 2, taste conditioning was used in Experiment 3 to assess the influence of sucrose pairing on the motivational value of ethanol. Use of this paradigm allowed the cues of drug administration to be consistent between ethanol conditioning and testing, with the drug being administered i.p. in both phases.

Three groups of subjects were employed. During ethanol conditioning, Group E+ received an ethanol injection followed by sucrose and a saline injection followed by water on alternate days; Group E- received a saline injection followed by sucrose and an ethanol injection followed by water on alternate days; and Group S received either sucrose or water following saline and did not experience ethanol during conditioning. In the taste conditioning phase, all groups received access to a sodium chloride (NaCl) solution followed by an injection of ethanol. NaCl was chosen because of its "primary" taste properties (Pfaffmann, 1960) in an effort to minimize stimulus generalization between the CS used in taste conditioning and the sucrose US from the ethanol conditioning phase. This concentration of NaCl has been shown to be an effective stimulus in taste conditioning studies (Rescorla & Cunningham, 1978).

It was expected that Groups E+ and E-, which received ethanol during the ethanol conditioning phase, would show more protracted acquisition of taste aversion compared to saline controls as a result of US pre-exposure attenuating conditioning (Barker & Johns, 1978; Berman & Cannon, 1974; Braveman, 1975; Cannon, Berman, Baker, & Atkinson, 1975; Randich & LoLordo, 1979). Group E+, by virtue of receiving ethanol paired with sucrose, was expected to show an even greater retardation of taste aversion because ethanol presumably would be an even weaker aversive stimulus to these subjects as a result of the ethanol-sucrose association.

Material and Methods

Subjects

Subjects were 42 adult male Holtzman albino rats weighing between 353 and 414 g [$X (\pm \text{SEM}) = 384 (2) \text{ g}$] prior to food restriction. They were housed as described in Experiment 1. All procedures took place in the animals' home cages during the light part of the cycle. Water was available ad lib except during treatment and testing periods and during taste conditioning, as described below.

Procedure

Ethanol and sucrose solutions were prepared as described in Experiment 1. Ethanol concentration was maintained at 15% (v/v) throughout the experiment, while concentration of sucrose was 32% w/v. Sodium chloride (NaCl - Mallinckrodt) solutions (0.2 M) were prepared w/v. Water, NaCl, and sucrose solutions were presented at room temperature in Nalgene tubes (100 ml) with stainless steel drinking spouts inserted through the front of the home cage.

The experimental procedure included five phases: food restriction (12 d), sucrose pre-exposure (five sessions), ethanol conditioning (eight sessions), fluid restriction (eight sessions), and taste conditioning (seven sessions). The experimental design is outlined in Table 3.

Food Restriction. After 6 d acclimation to the colony, subjects were restricted to 80% of their free-feeding weights over 12 d as described in Experiment 1. They were maintained throughout the remainder of the experiment on once-daily feedings of Purina rat chow given 140 min after the experimental sessions.

Sucrose Pre-exposure. After 12 d of restricted feeding, animals were given brief access to 32% sucrose on each of 5 successive days. Fifteen min access was permitted for 2 d. Access was reduced to 5 min on the next 3 d. Subjects were

TABLE 3 -- Design for Experiment 3: Taste Conditioning

Phase 1: FOOD RESTRICTION (12 days)

Subjects restricted to 80% of their free-feeding weights

Phase 2: SUCROSE PREEXPOSURE (5 sessions)

All animals given access to 32% sucrose

15 min access for 2 sessions

5 min access for 3 sessions

Phase 3: ETHANOL CONDITIONING (8 two-d sessions with + and - trials alternating daily)

<u>Group</u>	<u>+ Trial</u>	<u>- Trial</u>
E+	Ethanol (i.p.) --15-min delay --> Sucrose x 30 min	Saline (i.p.) --15-min delay --> Water x 30 min
E-	Saline (i.p.) --15-min delay--> Sucrose x 30 min	Ethanol (i.p.) --15-min delay--> Water x 30 min
S+	Saline (i.p.) --15-min delay--> Sucrose x 30 min	Saline (i.p.) --15-min delay--> Water x 30 min

Dose ethanol injected was initially 0.75 g/kg and increased in 0.25 g/kg increments each session to 1.5 g/kg where it remained for the remaining sessions (Trials 4-8)

Phase 4: FLUID RESTRICTION (8 sessions)

Subjects limited to 30-min access to water daily

Phase 5: TASTE CONDITIONING (7 two-d sessions)

All animals given 15 min access to NaCl (0.2 M) followed by i.p. injection of ethanol (2.0 g/kg) on alternate days. Given 15 min access to water on intervening drug-free sessions

assigned to one of three groups equated for mean sucrose consumption on day 5 of pre-exposure [$X (\pm \text{SEM}) = 13.0 (0.3) \text{ ml}$].

Ethanol Conditioning. The ethanol conditioning phase consisted of eight 2-d trial blocks, which began on the day following sucrose pre-exposure. On each trial, water bottles were removed and subjects were weighed. Following a 5-min delay, animals were given an i.p. injection of either ethanol or saline. Fifteen min after injection, subjects were allowed 30 min access to either sucrose or water. On reinforced trials, Group E+ received an i.p. injection of ethanol followed by access to sucrose, while Group E- received a saline injection followed by sucrose. On alternate days, Group E+ received a saline injection paired with access to water, while Group E- received an ethanol injection followed by water. Thus, both groups received equal access to all fluids, but differed in the fluid paired with sucrose access. The ethanol dose administered was 0.75 g/kg on Trial 1, 1.0 g/kg on Trial 2, 1.25 g/kg on Trial 3, and 1.5 g/kg on Trials 4 through 8. Ethanol concentration was held constant and volume was varied to alter dose in order to avoid possible concentration-dependent effects (Linakis & Cunningham, 1979). Control subjects (Group S) received saline injections prior to both sucrose access and water access. Order of exposure to trial type was counterbalanced within each group. Tail blood samples were collected for assessment of blood ethanol concentration; however, an error in preparation made the samples unusable.

After an intervening day of no treatment, all subjects were given 10 min access to sucrose with no preceding injection to determine whether conditioning had affected sucrose consumption in the absence of ethanol. Water bottles were not returned following sucrose access to begin the fluid restriction phase.

Fluid Restriction. During fluid restriction, subjects were weighed daily and given 30 min access to water. Their food rations were delivered following a 2-h delay. When stable weights were established (7 d), taste conditioning began.

Taste Conditioning. On alternate days, all subjects were given 15 min access to 0.2 M NaCl followed immediately by i.p. injection of 2.0 g/kg ethanol. Animals were given seven exposures to NaCl, the first six followed by ethanol injection. Consumption of NaCl was monitored over the seven trials. Subjects were allowed 15 min access to water 5-6 h after each session on conditioning days and at the same time as the conditioning procedure on intervening days. Food was returned 2 h after conditioning or intervening water access.

Results

Body Weight. Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, of weights across the seven trials of taste conditioning showed a significant trials effect [$F(6,234) = 14.9, p < 0.01$], with no significant groups difference and no interaction. Mean weight ($\pm$ SEM) was 283 (2) g on the first trial of taste conditioning and 284 (2) g during Trial 7.

Sucrose Consumption. Mean sucrose intakes during ethanol conditioning are shown in Figure 8. Although all groups consumed the same amount during the first conditioning trial, consumption by Group E- decreased with continued trials. Groups E+ and S appeared to consume similar amounts across the 8 conditioning periods.

Two-way repeated measures ANOVA, with group as the between-groups factor and day as the repeated measures factor, yielded significant effects of group [$F(2,39) = 4.7, p < 0.05$] and day [$F(7,273) = 6.4, p < 0.01$] with no interaction. Post-hoc analysis revealed that both Group E+ [$F(1,26) = 9.1, p < 0.01$] and Group S [$F(1,26) = 4.7, p < 0.01$] drank significantly more sucrose than did Group E-.

During the 10-min sucrose exposure following conditioning, Groups E+, E, and S consumed 14.2, 16.8, and 19.1 ml, respectively. One-way ANOVA showed a significant group effect [$F(2,39) = 4.3, p < 0.05$]. Although consumption by the two experimental groups did not differ, Group S consumed significantly more sucrose than did Group E+ [$F(1,26) = 11.1, p < 0.01$], but did not differ from Group E-

NaCl Consumption. Mean ($\pm$ SEM) NaCl intakes during taste conditioning are shown in Figure 9. The groups did not differ in NaCl consumption on first exposure. Taste aversion developed very rapidly in Group S, which had received no previous exposure to ethanol. Groups E+ and E- showed similar consumptions

Figure 8: Mean consumptions ($\text{ml} \pm \text{SEM}$) of 32% sucrose across the eight ethanol conditioning trials for Groups E+, E-, and S. Group E+ received 30 min access to sucrose 15 min after ethanol injection, while sucrose followed saline injection in Groups E- and S. Group E- received ethanol injections on non-sucrose trials followed by 30 min water access. Groups E+ and S were given saline injections followed by water on - Trials. Dose of ethanol (g/kg) injected was gradually increased over trials (Trial 1 = 0.75, Trial 2 = 1.0, Trial 3 = 1.25, and Trials 4-8 = 1.5).

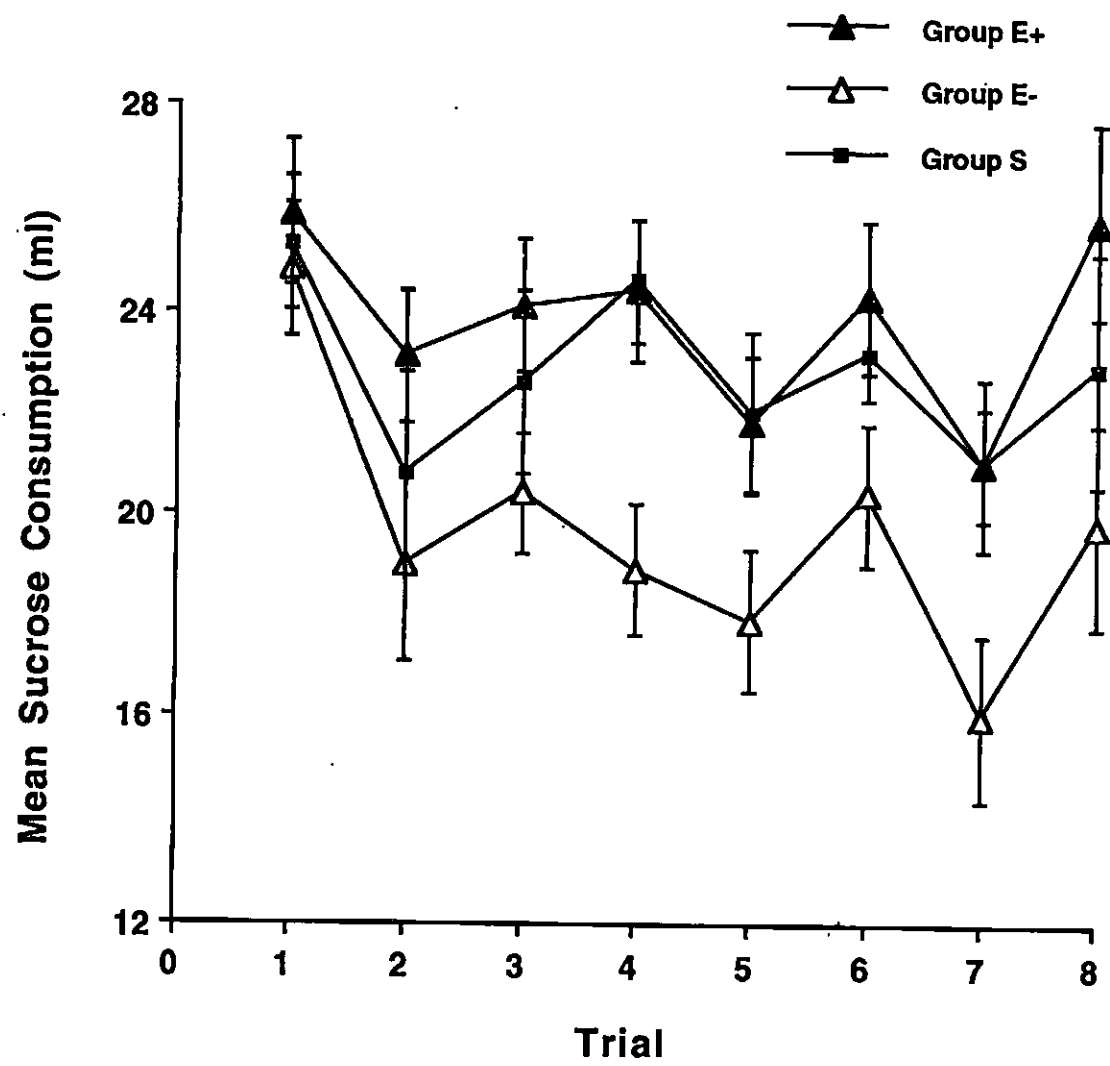
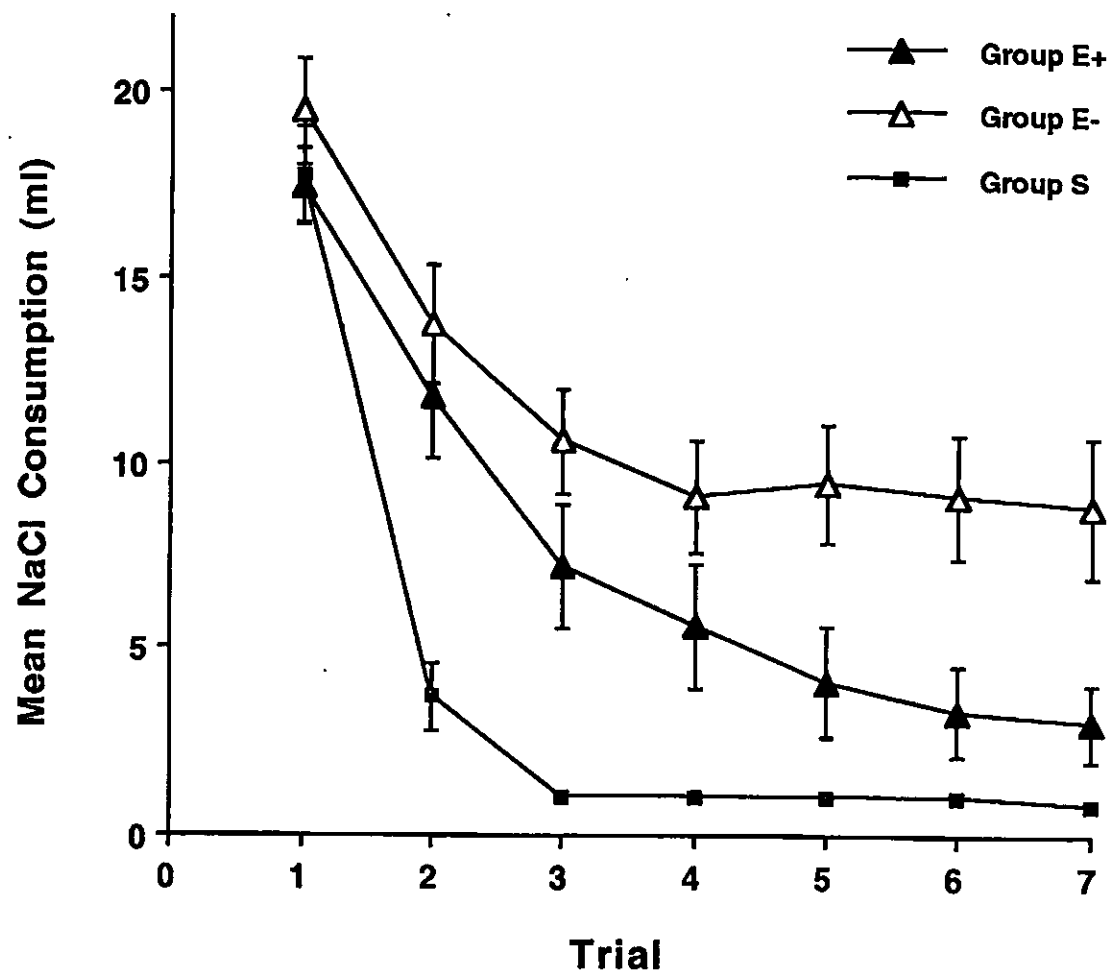


Figure 9: Mean consumptions ($\text{ml} \pm \text{SEM}$) of 0.2M NaCl across the seven taste conditioning trials for Groups E+, E-, and S. Groups were designated on the basis of their differential treatment during the ethanol conditioning phase, as described in the legend for Figure 8. Treatment during the taste conditioning phase was identical with all groups receiving 15 min access to NaCl followed by i.p. injection of 2.0 g/kg ethanol.



during initial taste conditioning trials with both groups demonstrating conditioned taste aversion. However, the groups appeared to diverge during later trials with Group E+ demonstrating stronger aversion to NaCl than Group E-, which is opposite to what was expected on the basis of sucrose pairing.

Two-way repeated measures ANOVA, with group as the between-groups factor and day as the repeated measures factor, examining NaCl consumptions across the seven taste conditioning trials yielded significant effects of group [$F(2,39) = 13.5, p < 0.01$] and day [$F(6,234) = 119.6, p < 0.01$], as well as a significant group x day interaction [$F(12,234) = 4.8, p < 0.01$]. Followup contrast analysis showed no significant difference between groups on their initial exposure to NaCl. However, Groups E+ and E- drank significantly more than Group S on Trial 2 after only a single exposure to ethanol [$F(2,39) = 26.3, p < 0.01$]. Consumption by Group E-, with the exception of the first trial which was prior to ethanol exposure, remained significantly higher than that by Group S throughout the remaining taste conditioning trials [all F 's(1,26) > 18.8]. This group also consumed significantly more than did Group E+ on Trials 5, 6, and 7 [all F 's(1,26) $> 8.7, p < 0.01$]. Consumption by Group E+ did not differ from Group S on these trials. These findings suggest that the significant group x day interaction reflects the rapidly acquired difference between Group S and Groups E+ and E- over the initial trials, with the more protracted emergence of a difference between Groups E+ and E- during the later exposures.

Discussion

In contrast to the preceding two experiments, in which subjects were allowed to self-administer ethanol, animals in Experiment 3 were subjected to progressively increasing dosages of ethanol delivered intraperitoneally. This difference in drug delivery may have accounted for the development of conditioned taste aversion to sucrose observed in this study. During ethanol conditioning, consumption of sucrose declined following the first trial and remained significantly lower in Group E- over several of the ethanol conditioning trials. Although Group E- did not receive sucrose paired with ethanol, the association of procedural cues, including injection, may have generalized across both ethanol and saline trials to allow for the development of conditioned taste aversion to sucrose and a decline in sucrose consumption.

While Group E+ could be expected to develop a similar or even greater conditioned taste aversion than Group E- because of the more explicit pairing of ethanol and sucrose, the pharmacological effect of ethanol delivered immediately prior to sucrose exposure may have somehow interfered with the expression of taste aversion to sucrose on conditioning trials. Further support for this hypothesis is the finding that Group E+ consumed significantly less sucrose than did Group S during the 10-min sucrose exposure trial during which ethanol injection was withheld, a difference which was not seen during the ethanol conditioning trials.

A change in deprivation state between the ethanol conditioning and taste conditioning phases of this experiment may have interfered with demonstration of any associative effects. During ethanol conditioning, subjects were food-deprived. Although food-deprivation results in fluid-deprivation, animals are typically not fluid-restricted to the degree that they were during taste conditioning. If the association between ethanol and sucrose was state-dependent, this association may have been lost with a change in deprivation state. Although this could explain the

lack of a difference between experimental groups despite sucrose pairing, state dependent effects do not explain the more rapid development of taste aversion in Group E+. Similarly, a change in stimulus cues due to altering the ethanol dose between ethanol conditioning and taste conditioning could interfere with the demonstration of appetitive conditioning from sucrose pairing, but, again, would not explain the greater aversion to the taste stimulus in Group E+.

In contrast to findings from other studies pairing orally ingested flavor solutions with access to sweetened fluids (Fanselow and Birk, 1982; Holman, 1975, 1980), pairing ethanol injection with sucrose did not increase the motivational value of the drug as assessed by acquisition of taste aversion. Both experimental groups demonstrated more protracted development of aversion to a NaCl solution in response to subsequent ethanol injection than did Group S, which was naive to ethanol. This finding is consistent with numerous studies demonstrating attenuation of conditioning, including taste conditioning, with US preexposure (Barker and Johns, 1978; Berman and Cannon, 1974; Braveman, 1975; Cannon et al., 1975; Randich and LoLordo, 1979). However, it was expected that pairing ethanol with sucrose would further retard development of taste aversion in Group E+; instead, this group actually demonstrated more rapid acquisition of taste aversion to an ethanol-paired flavor than did Group E-.

Although ethanol was given i.p. so as to better control the dose administered and to minimize potential influences of sucrose on blood ethanol levels, it is possible that subsequent access to sucrose still altered ethanol pharmacokinetics (Jones, 1983; Experiment 2). Animals receiving sucrose following ethanol injection during ethanol conditioning may have experienced consistently lower peak BECs or had elevated blood ethanol levels for a shorter duration of time than did subjects in Group E-. This differential experience with ethanol may have resulted in weaker development of tolerance to the aversive

effects of ethanol in Group E+ relative to Group E-, which would contribute to Group E+ acquiring taste aversion more rapidly during subsequent ethanol exposures.

Another possible explanation for these results is that tolerance to ethanol in Group E+ was conditioned through an association between sucrose and ethanol. If sucrose were a component of the broad environmental context to which ethanol tolerance was conditioned, the absence of the sucrose stimulus during taste conditioning could have resulted in diminished tolerance for Group E+ relative to Group E-. Absence of sucrose during testing may have further increased aversion to the ethanol-paired flavor in Group E+ by inducing a state of frustration in these animals (Amsel, 1958), since they had come to expect delivery of sucrose following ethanol injection as a result of the ethanol conditioning trials.

Experiment 4: Assessment of Blood Ethanol Concentration Following Sucrose Pairing

Rationale

Several investigators have shown blood ethanol concentrations to be influenced by various sugars, even when administered by different routes (Clark, Hughes, & Letley, 1973; Jones, 1983; Jones, Goldberg, & Neri, 1979; Rawat, 1977). Effects on both absorption (Clark et al., 1973; Jones et al., 1979) and metabolism (Tygstrup, Winkler, & Lundquist, 1965) have been implicated, with Jones (1983) demonstrating that sugar was capable of delaying ethanol absorption and accelerating metabolism such that peak BECs were decreased. Blood ethanol concentrations measured in Experiment 2 and the unexpected results seen in Experiment 3 suggest that the simple associative effects expected from presenting sucrose in combination with ethanol may be confounded by the influence of this sugar on ethanol metabolism.

Experiment 4 examined the influence of sucrose on blood ethanol concentration at various time points following i.p. administration of ethanol using a design similar to that used during ethanol conditioning in Experiment 3. The time interval between ethanol injection and sucrose access was varied across groups during the ethanol conditioning phase to determine if the sucrose-ethanol interaction was temporally dependent. In contrast to Experiment 3, however, sucrose availability was limited to 10 ml so as to minimize any differences in sucrose consumption between subjects.

Material and Methods

Subjects

Subjects were 73 adult male Holtzman albino rats weighing between 359 and 479 g [$X (\pm SEM) = 418 (3) \text{ g}$] prior to fluid restriction. They were housed as described in Experiment 1. All procedures took place in the animals' home cages during the light part of the cycle. Food was available ad lib except during treatment and testing periods, as described below.

Procedure

Solutions were prepared as described in Experiment 1. Water and sucrose (32% w/v) solutions were presented at room temperature in Nalgene tubes (100 ml) with stainless steel drinking spouts inserted through the front of the home cage.

The experimental procedure included two phases: fluid restriction (6 d) and ethanol conditioning/blood ethanol assessment (three sessions). The experimental design is outlined in Table 4.

Fluid Restriction. After 14 d acclimation to the colony, a fluid deprivation schedule was initiated. Animals were weighed daily; their food was removed; and they were given 30 min access to water. Food was returned approximately 6.5 h after drinking. Temporary food restriction was begun to familiarize the animals with this procedure prior to the blood ethanol assessment phase.

Ethanol Conditioning/Blood Ethanol Assessment. During this phase, mean blood ethanol levels were assessed at intervals following i.p. injection of ethanol and access to 32% sucrose. The temporal interval between injection and sucrose access varied depending upon group assignment.

Only a quarter of the subjects could be tested each day due to the time constraints imposed by fluid exposure and blood collection. To accommodate this, subjects were divided into four sets of 18. Rats from each set were matched for

TABLE 4 -- Design for Experiment 4: Blood Ethanol Concentration Assessment

Phase 1: FLUID RESTRICTION (6 days)

Subjects given 30-min access to water daily

Phase 2: ETHANOL CONDITIONING/BLOOD ETHANOL ASSESSMENT (3 sessions spaced 7 d apart)

Conditioning: Ethanol (i.p.) -----*delay*-----> Sucrose x 15 min

<u>Group</u>	<u>Delay</u>
0	0 min
15	15 min
30	30 min
60	60 min
90	90 min
360	360 min

Ethanol dose was 0.5 g/kg on Session 1; 1.0 g/kg on Session 2; and 1.5 g/kg on Session 3
Sucrose concentration was 32% and subjects were limited to 10 ml

Blood ethanol assessment: Tail blood sampling performed 15, 30, 60, 90, 180, 240, and 360 min after ethanol injection. Groups 0 and 360 had samples taken at each time point. With the exception of Group 360, no samples were collected prior to sucrose exposure.

mean body weight and mean water consumption on day 6 of fluid restriction and assigned to one of the six ethanol-sucrose experimental groups such that each group was represented by three animals on each day of testing. Each rat was tested three times with increasing doses of ethanol (0.5, 1.0, and 1.5 g/kg) with 7 d between each ethanol exposure. Concentration of ethanol was maintained at 15% v/v and injection volume was altered to produce a change in dose to avoid the potential complication of concentration-dependent ethanol effects (Linakis & Cunningham, 1979).

Each day, all animals were weighed and their food was removed. Following a 10-min delay, test subjects were given an i.p. injection of ethanol and returned to their cages. Animals from Group 0 were given 15 min access to 10 ml of 32% sucrose immediately after injection; Group 15 experienced a 15-min delay between injection and sucrose access, while Groups 30, 60, 90, and 360 experienced delays of 30 min, 60 min, 90 min, and 360 min, respectively. Thus, all groups received equal access to ethanol and sucrose, but differed in the temporal relationship between the two stimuli.

At various intervals following injection and sucrose access, test subjects were removed from their cages and the tips of their tails cut with a sharp scalpel to collect 20 μ l of blood for analysis of ethanol levels. Blood samples were collected at 15, 30, 60, 90, 180, 240, and 360 min after ethanol injection. With the exception of Group 360, subjects were not tested at any time points prior to their sucrose exposure. Group 360 served as the non-sucrose control and was sampled at every time point. Two samples were drawn from each subject 180 min after injection to determine reliability of the sampling procedure. Samples were processed and analyzed as described in Experiment 2.

When animals were not being tested, they received 30 min access to water with no ethanol injection. Food was returned to all animals immediately after

Group 360 received sucrose access on test days and approximately 6.5 h after water access on intervening days.

Results

Body Weight. One subject was removed from the experiment because of inability to maintain body weight with fluid deprivation (weighed 283 g), leaving 72 subjects. Mean body weight ($\pm$ SEM) on the first day of conditioning across all groups was 355 (3) g, while that at the completion of conditioning (17 d later) was 368 (6) g. Two-way repeated measures ANOVA, with group as the between-groups factor and trials as the repeated measures factor, across the 18 d of ethanol conditioning showed a significant effect of trials on body weight [$F(17,816) = 12.8, p < 0.01$]. There were no significant group differences.

Blood Ethanol Concentration. Mean ($\pm$ SEM) blood ethanol concentrations following injection of 0.5 g/kg ethanol are shown in Figure 10. Subjects in Group 360 showed slightly higher blood concentrations at the various post-injection intervals than the other groups. Concentrations rose between 15 and 30 min then gradually declined. There was a failure to detect blood ethanol at all time points assessed beyond 90 min.

One-way ANOVA performed at each sampling interval showed no significant group effect at any post-injection period. A two-way repeated measures ANOVA, with group as the between-groups factor and sampling interval as the repeated measures factor, comparing blood ethanol concentrations for Groups 0 and 360 at 15, 30, 60, and 90 min showed no significant group effect, although there was a significant effect of sample interval [$F(3,66) = 36.4, p < 0.01$] with no interaction.

Mean ($\pm$ SEM) blood ethanol concentrations following injection of 1.0 g/kg are shown in Figure 11. Group 360 showed a higher level than Group 0 only at 15 min post-injection. All groups appeared to have comparable concentrations at the other intervals tested. Ethanol was still detectable in blood at 180 min post-injection, but not 240 min.

Figure 10: Mean blood ethanol concentrations (mg/ml $\pm$ SEM) for each group at designated sampling intervals following i.p. injection of 0.5 g/kg ethanol. Groups differed in terms of the delay between ethanol injection and 15 min access to 32% sucrose. Group 0 received sucrose immediately after injection, while Groups 15, 30, 60, 90, and 360 experienced delays of 30 min, 60 min, 90 min, and 360 min, respectively, between injection of ethanol and sucrose access.

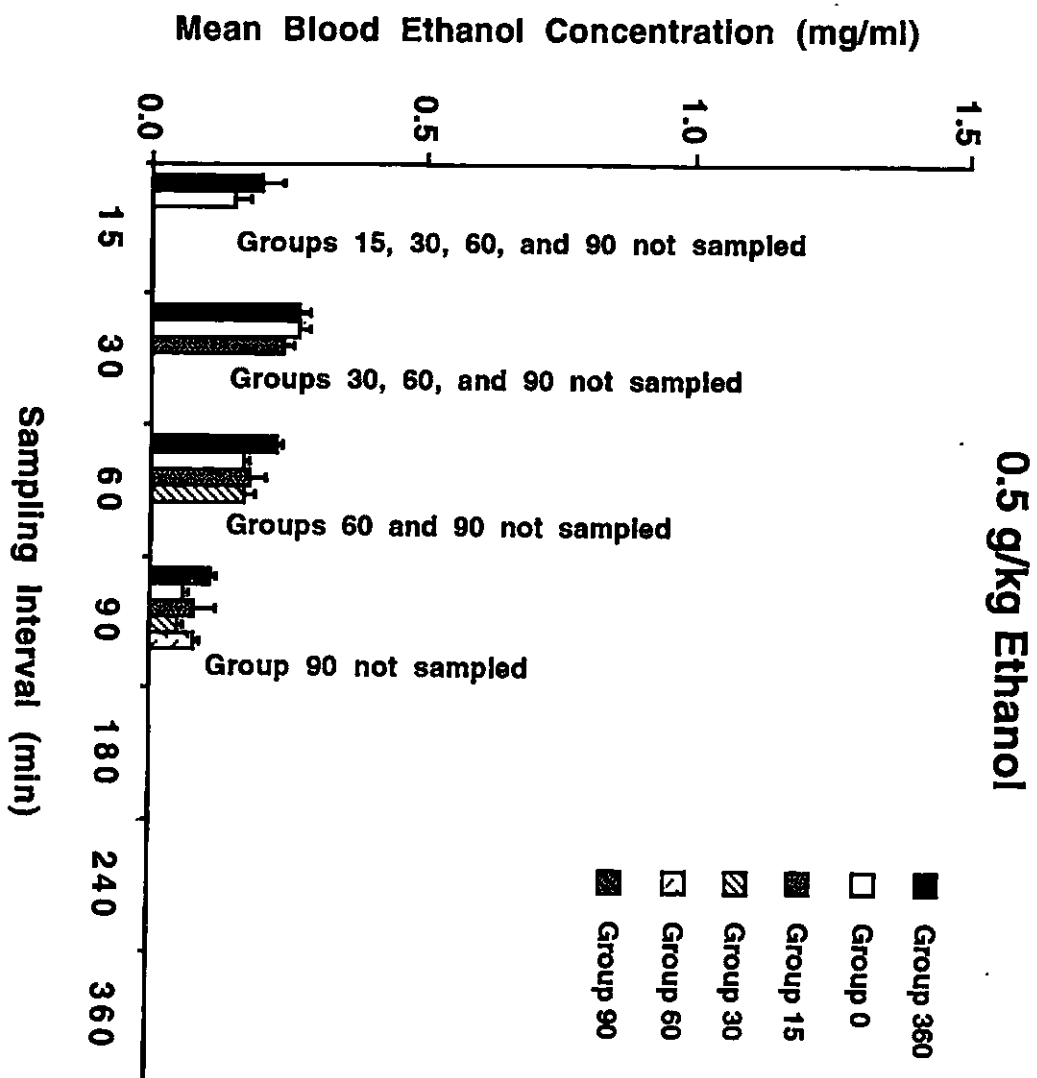
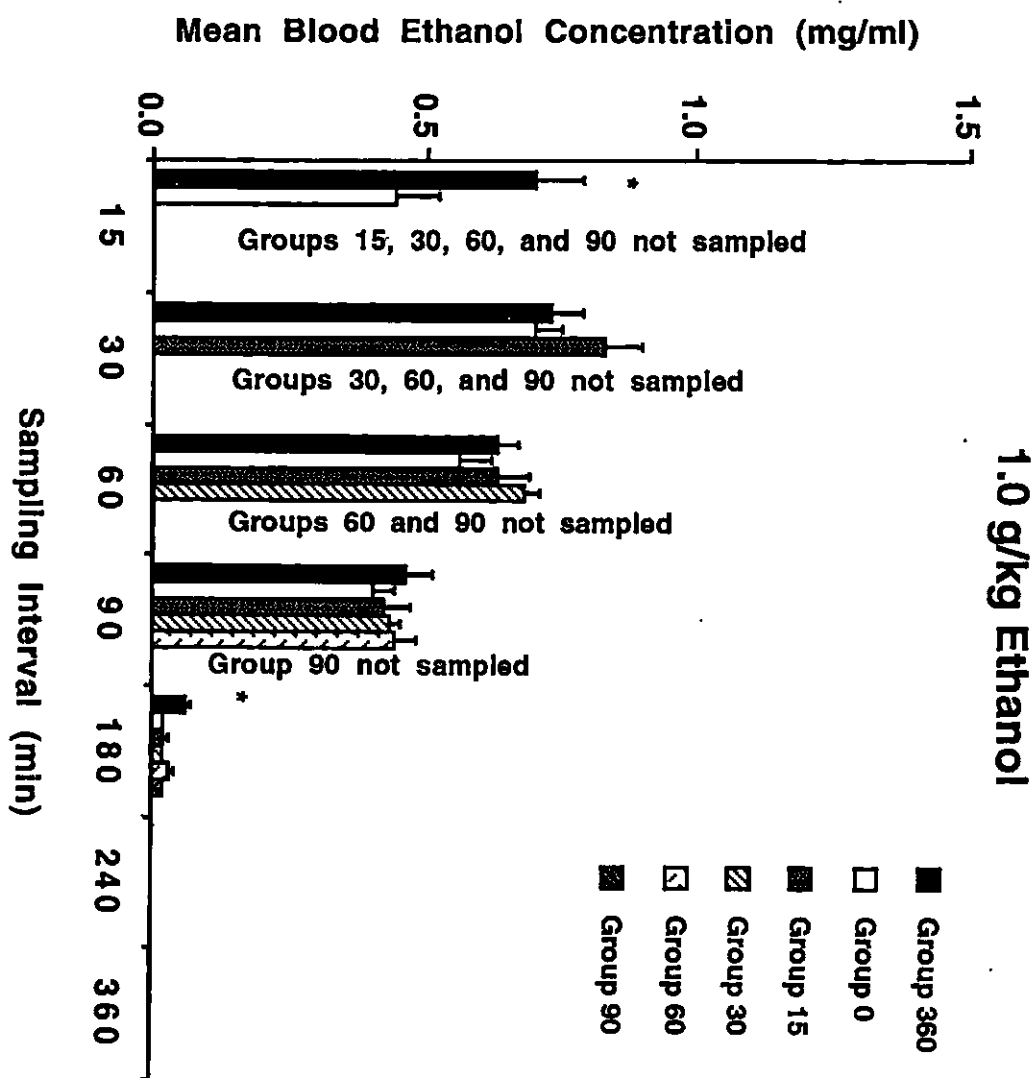


Figure 11: Mean blood ethanol concentrations (mg/ml $\pm$ SEM) for each group at designated sampling intervals following i.p. injection of 1.0 g/kg ethanol. The groups and sampling procedures are as described in the legend for Figure 10. The asterisk (*) indicates a significant difference from Group 0 at the same sampling interval.

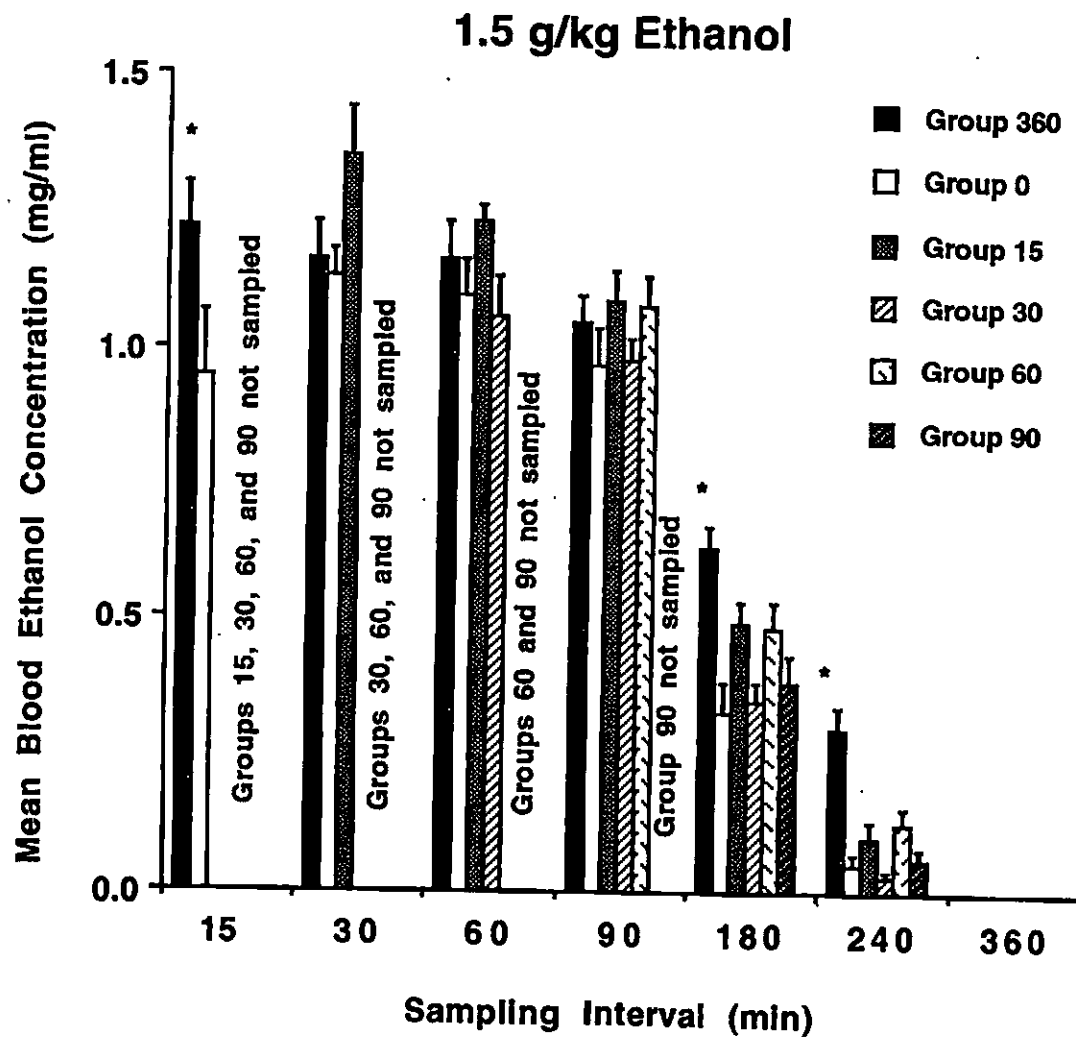


One-way ANOVA comparing groups at different sampling intervals confirmed that Group 360 had a significantly higher blood ethanol concentration at 15 min post-injection than did Group 0 [$F(1,22) = 4.9, p < 0.05$]. There was also a significant group effect at 180 min [$F(5,66) = 4.0, p < 0.01$], due to Group 360 having slightly, but significantly, higher concentrations than any other group, as supported by contrast followup [$F(1,66) = 9.9, p < 0.01$]. Blood ethanol concentrations were not significantly different at any other interval tested. A two-way repeated measures ANOVA, with group as the between-groups factor and sampling interval as the repeated measures factor, comparing blood ethanol concentrations between Groups 0 and 360 across the six sampling intervals tested showed no significant differences between the groups. In addition, there was no significant difference in blood ethanol concentrations between the two samples drawn from each subject at 180 min, indicating good reliability. This was further supported by the finding of a significant correlation between the two samples collected ($r = .62, df=70, p < 0.01$).

Determination of drug clearance based on the area under the curve of blood ethanol concentration plotted against time following injection of 1.0 g/kg ethanol gave a clearance of 15.7 ml/min/kg for the pooled experimental groups and 13.0 ml/min/kg for the controls, providing further evidence of more rapid ethanol metabolism in the sucrose-paired subjects.

Mean ($\pm$ SEM) blood ethanol levels following injection of 1.5 g/kg ethanol are shown in Figure 12. Group 360 had higher levels than Group 0 at 15 min, and higher levels than all groups at 180 and 240 min post-injection. Blood ethanol concentrations remained fairly constant through the first 90 min following injection, then declined such that blood ethanol was detectable in only 6 of 72 animals at 360 min post-injection.

Figure 12: Mean blood ethanol concentrations (mg/ml $\pm$ SEM) for each group at designated sampling intervals following i.p. injection of 1.5 g/kg ethanol. The groups and sampling procedures are as described in the legend for Figure 10. The asterisk (*) indicates a significant difference from Group 0 at the same sampling interval.



One-way ANOVA performed at each sampling interval showed significant group effects at 180 [$F(5,66) = 6.8, p < 0.01$] and 240 min [$F(5,66) = 14.2, p < 0.01$]. Followup contrast analysis confirmed that Group 360 had significantly higher blood concentrations than the other groups at these testing intervals [$F(1,66) = 10.8$ at 180 min, $p < 0.01$; $F(1,66) = 62.6$ at 240 min, $p < 0.01$]. A two-way ANOVA, with group as the between-groups factor and sampling interval as the repeated measures factor, comparing Groups 0 and 360 across the six sampling periods showed significant main effects of group [$F(1,22) = 7.8, p < 0.05$] and sampling interval [$F(5,110) = 116.1, p < 0.01$], as well as a group x sampling interval interaction [$F(5,110) = 2.6, p < 0.05$]. Followup analyses confirmed this interaction to be due to the development of a group effect at 180 min [$F(1,22) = 21.6, p < 0.01$] and 240 min [$F(1,22) = 39.3, p < 0.01$]. Again, a significant correlation was observed between the two samples collected at 180 min post-injection ($r = .85, df = 83, p < 0.01$).

Evaluation of drug clearance rates following a dose of 1.5 g/kg revealed a rate of 9.5 ml/min/kg for Group 0 and 7.2 ml/min/kg for Group 360, further suggesting that sucrose may have acted to increase the rate of ethanol metabolism in the sucrose-paired subjects..

There was no difference in sucrose consumption either between groups receiving sucrose access at different times post-injection or following different doses of ethanol. Mean fluid consumptions ranged between 8.7 and 9.1 ml [$X (\pm SEM) = 8.9 (0.04)$ ml].

Discussion

Experiment 4 demonstrated that oral self-administration of sucrose altered blood ethanol levels in rats when ethanol was administered intraperitoneally following a prolonged delay between ethanol and sucrose exposure. These results are consistent with other studies demonstrating the influence of sugars on ethanol pharmacokinetics (Clark et al., 1973; Jones, 1983; Jones et al., 1979). In particular, significantly lower BECs were observed in the experimental groups relative to Group 360 at 180 and 240 min following injection of 1.5 g/kg ethanol.

At the lower doses of ethanol, there was very little difference between groups. Following injection of 0.5 g/kg ethanol, there were no group effects at any of the intervals examined. Following 1.0 g/kg ethanol, there was a significant difference between Group 0 and Group 360 only at 15 min post-injection. All groups then showed comparable blood levels at all other intervals tested until 180 min post-injection. Group 360 had a significantly higher mean BEC ($X = 0.05$ mg/ml) than did the experimental groups, whose means ranged from 0.02 to 0.03 mg/ml. Although this difference was statistically significant, it was probably not physiologically important.

Of greater interest were the differences in BEC following injection of 1.5 g/kg, the same dose as was used in Experiment 3. Group 360 had higher blood levels at this dose than any of the experimental groups at both 180 min ($X = 0.63$ vs. 0.41 mg/ml) and 240 min ($X = 0.30$ vs. 0.07 mg/ml) post-injection. The treatment given to Group 360 was similar to that received by Group E- in Experiment 3 (i.e. unpaired exposures of ethanol and sucrose). Blood ethanol levels from this experiment suggest that Group E- may have experienced elevated BECs over a longer period than did Group E+, which received sucrose 15 min after ethanol injection. The impact of sucrose may have been even greater in the previous experiment, because consumption was not limited and subjects consumed

more than twice the volume of sucrose as was allowed in Experiment 4.

One of the few substances demonstrated to alter the typically constant rate of ethanol metabolism by the liver is fructose. Initial work on the influence of sugars on ethanol metabolism was published in 1937 by Carpenter and Lee, who demonstrated that the rate of metabolism in humans, as revealed by carbon dioxide production from ^{14}C -ethanol, was increased in the presence of fructose. Since then, many investigators have confirmed these findings with fructose delivered either orally or intravenously (Tygstrup et al., 1965; Merry & Marks, 1967; Pawan, 1968; Lowenstein, Simone, Boulter, & Nathan, 1970; Brown, Forrest, & Roscoe, 1972; Clark et al., 1973; Soterakis & Iber, 1975). In addition to fructose, sucrose also has been shown to increase ethanol metabolism, presumably because of its composition as a fructose-containing disaccharide (Pawan, 1968; Soterakis & Iber, 1975). However, the equivalent dose of sucrose is twice that of fructose since its other breakdown product, glucose, has been shown to have no effect on ethanol metabolism (Pawan, 1968; Patel, Paton, Rowan, Lawson, & Linton, 1969; Crownover, LaDine, Bradford, Glassman, Forman, Schneider, & Thurman, 1986).

Several possible mechanisms for the effect of fructose on ethanol metabolism have been proposed. Although some investigators have suggested that fructose simply delays the absorption of ethanol (Crownover et al., 1986), most suggest that fructose has a more direct influence on ethanol metabolism. Typically, dehydrogenation of ethanol to acetaldehyde is limited by the dissociation of the complex alcohol dehydrogenase and reduced nicotinamide adenine dinucleotide (ADH-NADH). In the presence of ethanol metabolism, glyceraldehyde, formed from fructose and typically dehydrogenated to glycerate, is, instead, reduced to glycerol by this alcohol dehydrogenase complex, which accelerates the dissociation process and speeds ethanol metabolism (Tygstrup et al., 1965; Lowenstein et al., 1970). Alternatively, Scholz and Nohl (1976)

proposed that conversion of fructose into fructose-1-phosphate via fructokinase converts ATP to ADP, which is transported to the mitochondria. Once there, ADP stimulates oxygen uptake and reoxidation of NADH, which produces additional NAD^+ for alcohol dehydrogenase-dependent oxidation of ethanol to acetaldehyde.

A study by Crownover et al. (1986) revealed the importance of the relative timing of ethanol and fructose deliveries. When human or rat subjects were given fructose (1.0 g/kg) and ethanol (0.85 g/kg) orally and simultaneously, there was a significant reduction in peak blood ethanol levels, although the slope of the ethanol elimination curve was unaffected. However, when a 1-h delay was introduced between ethanol and fructose delivery, peak blood ethanol concentration was unchanged, although the slope of the ethanol elimination curve was significantly increased. Portal vein and vena caval blood samples from rats receiving the fluids simultaneously revealed a transhepatic decline in ethanol concentration in animals receiving fructose that was 3 times that of control rats, suggesting that fructose stimulates a "first-pass" elimination of ethanol.

These studies support the findings from this experiment that sucrose administration reduces blood alcohol levels. Because of the varying time intervals between ethanol and sucrose delivery between groups, it is possible that several of these mechanisms may have altered ethanol pharmacokinetics. For example, since Group 0 received sucrose immediately following ethanol delivery, influences on blood flow and absorption, as well as fructose effects on first-pass elimination of ethanol, may have been active in reducing blood ethanol levels. The time course of this first pass effect of fructose has not been fully established, so it is unclear if additional groups (i.e. Groups 15 and 30) may have been influenced by this mechanism. Based on Crownover et al.'s results, it is presumed that Groups 60 and 90 experienced similar peak blood alcohol levels as Group 360 (although peak BECs were not determined); however, ethanol elimination curves would be

expected to have an increased slope due to the secondary influences of fructose metabolism on NAD⁺/NADH pathways involved in ethanol metabolism.

Although deprivation state is less of a factor in Experiment 4, where subjects are fluid restricted, than in the preceding experiments, where animals were food deprived, Pawan (1972) investigated the influence of starvation on ethanol metabolism in obese humans and demonstrated that a 7-d period of starvation reduced ethanol metabolism by a rate of 22%. He hypothesized that nutritional restrictions could affect metabolism through several pathways, including depletion of the necessary enzymes and cofactors involved in the formation of NAD; an inhibition of ADH and aldehyde dehydrogenase by free fatty acids, which are increased in a state of starvation; and enhancement of the reduced redox state of liver cells. It is difficult to determine if these factors might be present in the relative state of food restriction employed in the present studies. However, this suggests another process by which access to a food source, sucrose, in combination with ethanol may have altered the rate of metabolism and, subsequently, overall experience with ethanol's pharmacological effects between the paired and unpaired groups in the preceding experiments.

Experiment 5: Influence of Sucrose Pairing on Taste Conditioning
with 1.0 g/kg Ethanol

Rationale

Experiment 5 was designed to determine whether pairing ethanol delivery with access to sucrose alters ethanol's reinforcing value using the taste conditioning procedure. However, experimental conditions were altered from those used in Experiment 3 to minimize differential tolerance development (as suggested by differences in BEC revealed in Experiment 4) and to provide greater consistency of drug-associated cues between ethanol conditioning and testing (Fedorchak & Bolles, 1987).

In Experiment 3, the dose of ethanol administered during ethanol conditioning was gradually increased from 0.75 g/kg to 1.5 g/kg over the eight conditioning trials, and a dose of 2.0 g/kg was used in the taste conditioning phase. In Experiment 5, ethanol dose was maintained at 1.0 g/kg across the conditioning trials, since in Experiment 4, this resulted in very little difference in blood ethanol concentration between groups beyond the 15-min sampling interval. Although this dose resulted in statistically significant differences in BEC between the sucrose-paired groups and Group 360 at 180 min post-injection, the differences were probably too small to be physiologically important. The delay between ethanol injection and access to sucrose was increased to 30 min, since results from Experiment 4 also demonstrated that a 30-min delay provided more comparable BECs between groups than did the 15-min delay used in Experiment 3. In Experiment 4, there was a significant difference in BEC between Group 360 and Group 0 at 15 min following injection of 1.0 g/kg ethanol, but not between Groups 360 and 30. Imposing a 30-min delay between ethanol and sucrose would be expected to decrease the impact of sucrose on blood ethanol concentration. An

increase in the CS-US interval from 15 to 30 min would not be expected to adversely affect conditioning, since Holman (1975) has shown a 30-min delay to be effective in preference conditioning with dextrose. Sucrose access again was restricted in this experiment to 10 ml to limit its influence on blood ethanol levels.

In addition to holding fluid restriction constant throughout the experiment, taste conditioning procedures were altered to provide a more consistent drug cue between ethanol conditioning and testing. In contrast to Experiment 3, where the ethanol dose was increased from 1.5 g/kg during ethanol conditioning to 2.0 g/kg during taste conditioning, the ethanol dose was maintained at 1.0 g/kg during both phases of this experiment. This strategy was intended to maximize generalization of ethanol-sucrose pairing from conditioning to the testing phase. This dose of ethanol has been shown to be adequate in conditioning a taste aversion in ethanol-naive rats (Eckardt et al., 1974).

In addition to measuring flavor intakes on successive conditioning trials, taste conditioning was assessed using a two-stimulus test. Choice tests have been shown to be more sensitive than single-stimulus tests in revealing conditioned taste aversions (Dragoin et al. 1971; Grote and Brown, 1971). In single-stimulus tests, animals are motivated to drink the aversive fluid by virtue of their fluid-deprived state. By providing an alternative, neutral fluid, two-stimulus tests may more readily allow for conditioned differences to be seen.

Experiment 5 also differed from the previous study in that a second control group was added. In Experiment 3, Group S received saline injections both with and without sucrose access during ethanol conditioning. Group S then was given ethanol following NaCl access during taste conditioning trials. This same group (Group S-E) was used as a control in the present experiment; however, a second control group (Group S-S) was added to provide a no-drug baseline for taste conditioning assessment. Group S-S was treated like Group S-E during ethanol

conditioning, but continued to receive only saline injections during taste conditioning. This provided a baseline for determining the degree of aversion developing in the groups receiving ethanol.

It was hypothesized that temporally pairing ethanol injections with access to sucrose would condition an increase in the motivational valence of the drug. This change in appetitive value was assessed using taste conditioning. It was expected that animals receiving ethanol paired with sucrose would show more attenuated acquisition of taste aversion to an ethanol US than would subjects receiving unpaired exposures of ethanol and sucrose. Group S-S was expected to show no aversion to the novel flavor, and to demonstrate an increase in consumption after the initial trial as these animals overcame neophobia to the novel CS (Domjan, 1976). Finally, Group S-E was expected to show taste aversion most rapidly since subjects in this group had not experienced previous exposure to ethanol.

Material and Methods

Subjects

Sixty-seven adult male Holtzman albino rats were housed as described in Experiment 1. All procedures took place in the animal's home cages during the light part of the cycle. Food was available ad lib except during treatment and testing periods. Water access was restricted as described below. Mean weight ($\pm$ SEM) prior to fluid restriction was 389 (3) g.

Procedure

Solutions were prepared and presented as described in Experiments 1 and 3. The experimental procedure included four phases: fluid restriction (16 d), ethanol conditioning (eight sessions), taste conditioning (three sessions), and choice testing (two sessions). The experimental procedure is outlined in Table 5.

Fluid Restriction. Following a 6-d period of acclimation to the colony, a fluid deprivation schedule was initiated. On each of the 16 d of this phase, subjects were weighed; their food was removed; and they were given access to water (Session 1). Food was returned 4 h later, and a second session of water access was provided (Session 2). During the first 7 d of this phase, 30 min access to an unlimited volume of water was given during both sessions; however, subjects were unable to maintain weight on this schedule. On each of the remaining 9 d, subjects were given 30 min access to 10 ml of water during Session 1 and 60 min access to 40 ml during Session 2. Water volume was limited during Session 1 to the volume of sucrose available during the ethanol conditioning phase. Seven subjects were removed from the study during this phase as a result of weight loss (mean loss of 41 g or 12% of original weight: from $X = 351$ g to $X = 310$ g).

Ethanol Conditioning. Subjects were divided into four groups ($n = 15$ /group) matched for body weight and mean water consumption for both sessions during the final day of fluid restriction.

TABLE 5 -- Design for Experiment 5: Taste Conditioning with 1.0 g/kg Ethanol

Phase 1: FLUID RESTRICTION (16 days)

All subjects given limited period of access to water daily

Phase 2: ETHANOL CONDITIONING (8 two-d sessions with + and - trials alternated daily)

<u>Group</u>	<u>+ Trial</u>	<u>- Trial</u>
E+	Ethanol (i.p.) --30-min delay--> Sucrose x 15 min	Saline (i.p.)
E-	Saline (i.p.) --30-min delay--> Sucrose x 15 min	Ethanol (i.p.)
SE	Saline (i.p.) --30-min delay--> Sucrose x 15 min	Saline (i.p.)
SS	Saline (i.p.) --30-min delay--> Sucrose x 15 min	Saline (i.p.)

Ethanol concentration was held at 1.0 g/kg; saline volume was 8.4 ml/kg; and sucrose concentration was 32% with access limited to 10 ml.

Phase 3: TASTE CONDITIONING (3 two-d sessions)

Groups E+, E-, SE	NaCl (p.o.) x 15 min followed by Ethanol (i.p.)
Group SS	NaCl (p.o.) x 15 min followed by Saline (i.p.)

Ethanol concentration was 1.0 g/kg and NaCl concentration was 0.2 M. Injections were given every other day with animals receiving 15 min access to water on the intervening day

Phase 4: CHOICE TESTING (2 sessions run 48 h apart with 30 min access to water on the intervening day)

All subjects given simultaneous access to NaCl and Water x 30 min

The ethanol conditioning phase consisted of eight 2-d trial blocks. On each day, subjects were weighed and their food was removed. Following a 5-min delay, animals were given an i.p. injection of either 1.0 g/kg ethanol (15% v/v) or an equivalent volume of normal saline (8.4 ml/kg). Thirty min after injection on reinforced trials, subjects were given 15 min access to 10 ml of 32% sucrose. Group E+ was given sucrose access following ethanol injection, while Groups E-, SE, and SS received sucrose after saline injection. On the intervening non-reinforced trials, Groups E+, SE, and SS were given saline injections while subjects in Group E- received ethanol. Thus, both experimental groups were given equal access to all fluids, but differed in fluid paired with ethanol access. The control groups received saline both with and without sucrose. In contrast to Experiment 3, in which subjects were food-restricted but not fluid-restricted during the ethanol conditioning phase, no fluid was made available on non-reinforced trials, since water could serve as a reinforcer to the fluid-restricted animals used in this study. Ethanol dose was held constant at 1.0 g/kg across the conditioning trials. Order of exposure to trial type was counterbalanced within each group. Food was returned and animals were given 60 min access to water 5 h after their daily injections.

Following ethanol conditioning, two non-conditioning days were given prior to the start of the next phase. Animals were weighed and given 60 min access to water at the usual times, though no drug injections were given.

Taste Conditioning. In this phase, subjects were given three taste conditioning trials at 48-h intervals. On alternate days, following weighing and food removal, subjects in Groups E+, E-, and SE were given 15 min access to 0.2 M NaCl followed immediately by an i.p. injection of 1.0 g/kg ethanol. Group SS was given access to the NaCl solution followed by an i.p. injection of saline. NaCl consumption was monitored over trials. On intervening days, animals received 15

min access to water. Food was returned 5 h after the daily conditioning trials and 60 min access to water was given.

Choice Testing. Following taste conditioning, two 2-bottle choice tests were given. Following weighing and food removal, subjects were allowed simultaneous access to tubes containing NaCl (0.2 M) and water for 30 min. Bottle positions were counterbalanced within groups and across days to minimize the effects of position preference. The two-bottle tests were run 48-h apart with subjects receiving 30 min access to water alone on the intervening day. On all days, food was returned 5 h after the daily drinking session and 60 min access to water was given.

Results

Body Weight. One subject died during the ethanol conditioning phase, leaving 14 subjects in Group E+ and 15 subjects each in Groups E-, SE, and SS. Two-way repeated measures ANOVA, with group as the between-groups factor and day as the repeated measures factor, of daily weights through conditioning and testing trials showed that animals gained a significant amount of weight from the first day of conditioning [$X (\pm SEM) = 378 (4) \text{ g}$] to the final day of testing [$X (\pm SEM) = 447 (4) \text{ g}$] [$F(27,1485) = 344.9, p < 0.01$]. Weights did not differ between groups and there was no significant group x day interaction.

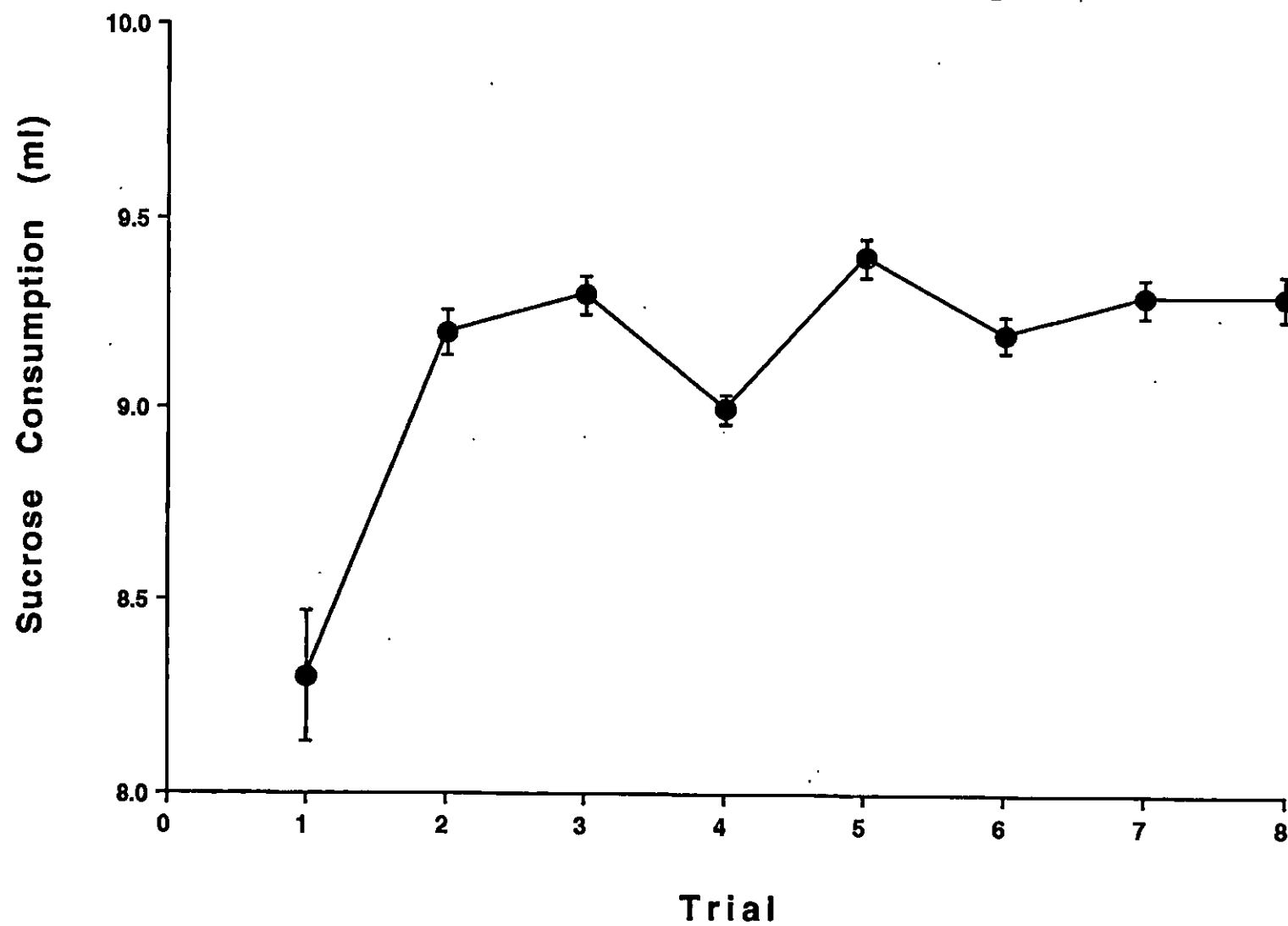
Ethanol Conditioning

Figure 13 shows mean ($\pm SEM$) sucrose consumptions collapsed across groups for the eight conditioning trials. Intake appeared to increase following the first trial, but remain stable during the seven remaining trials. Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, yielded a significant effect of trial [$F(7,385) = 21.5, p < 0.01$], with all groups consuming less sucrose on Trial 1 than on subsequent trials, presumably due to neophobia to the taste of sucrose experienced on initial exposure. There was no significant group effect or group x trial interaction.

Mean daily water consumptions differed between sucrose and non-sucrose conditioning trials with subjects consuming more water on days they had not received sucrose [$X (\pm SEM) = 13.9 (0.3) \text{ ml}$ vs. $18.7 (0.3) \text{ ml}$]. Three-way ANOVA, with groups as the between-groups factor and sucrose and trial as repeated measures factors, revealed a significant difference between sucrose and non-sucrose trials [$F(1,55) = 409.1, p < 0.01$]. There was no overall difference between conditioning groups although there was a significant trial effect, with subjects drinking less water on the final conditioning trial than they had initially [$X (\pm SEM) = 15.9 (0.4) \text{ ml}$ vs. $16.5 (0.4) \text{ ml}$]. There was also a significant group

Figure 13: Mean ($\pm$ SEM) sucrose consumption for the eight ethanol conditioning trials preceding taste conditioning. Because consumptions between groups did not differ significantly, the data were collapsed across groups. Subjects received 15 min access to 10 ml of 32% sucrose 30 min following i.p. injection of either ethanol or saline.

Ethanol Conditioning



x sucrose interaction [$F(3,55)=4.1$, $p<0.05$], presumably due to some groups showing a greater difference in consumption than others (see Table 6).

Taste Conditioning

Figure 14 demonstrates the mean ($\pm$ SEM) consumptions of NaCl by each group during the three trials of taste conditioning. All groups showed similar consumptions on the first trial, preceding any exposure to ethanol. Over trials, however, consumption of NaCl by Group SE, which had received no prior exposure to ethanol, declined following the first NaCl-ethanol pairing. Consumptions by Groups E+ and E- did not appear to differ from that of Group SS, which did not receive saline-ethanol pairings, suggesting that the ethanol preexposure received by Groups E+ and E- during the ethanol conditioning phase reduced the aversiveness of the drug in these animals.

Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, of NaCl consumptions during ethanol conditioning yielded significant effects of group [$F(3,55)=3.9$, $p<0.05$], trial [$F(2,110)=14.8$, $p<0.01$], and group x trial [$F(6,110)=6.6$, $p<0.01$]. Followup analyses of consumptions on individual trials revealed group effects for Trial 2 [$F(3,55)=3.2$, $p<0.05$] and Trial 3 [$F(3,55)=8.1$, $p<0.01$]. Contrast analyses showed these effects to be due to the decreased NaCl consumption in Group SE relative to the other groups.

Choice Testing

Mean ($\pm$ SEM) NaCl (left panel) and water consumptions (right panel) during two-bottle choice testing are shown in Figure 15. Group SE appeared to consume slightly less NaCl than did other groups during the first test, while the remaining groups, including the drug-naïve control, Group SS, consumed similar amounts, supporting the finding suggested during the conditioning phase that ethanol preexposure attenuated conditioned taste aversion. Since Groups E+ and

TABLE 6 -- Mean water consumptions (ml $\pm$ SEM) for sucrose and non-sucrose conditioning trials:

<u>Group</u>	<u>Sucrose Trial</u>	<u>Non-sucrose Trial</u>
E+	14.1 (0.5)	17.5 (0.6)
E-	13.5 (0.8)	19.1 (0.8)
SE	13.9 (0.6)	19.0 (0.5)
SS	14.1 (0.6)	19.1 (0.7)

Figure 14: Mean NaCl consumptions (ml $\pm$ SEM) during the three taste conditioning trials for Groups E+, E-, and SE, who received 15 min NaCl access followed by an i.p. injection of 1.0 g/kg ethanol, and Group SS, who was given NaCl followed by saline. Both Groups E+ and E- had received prior exposure to ethanol during the ethanol conditioning phase, with Group E+ experiencing i.p. ethanol paired with 32% sucrose access and Group E- having been given saline paired with sucrose and ethanol alone. Groups SE and SS had received saline followed by sucrose during ethanol conditioning. Group SS remained ethanol-naive throughout both phases of the experiment, while Groups SE was first exposed to ethanol during taste conditioning.

Taste Conditioning

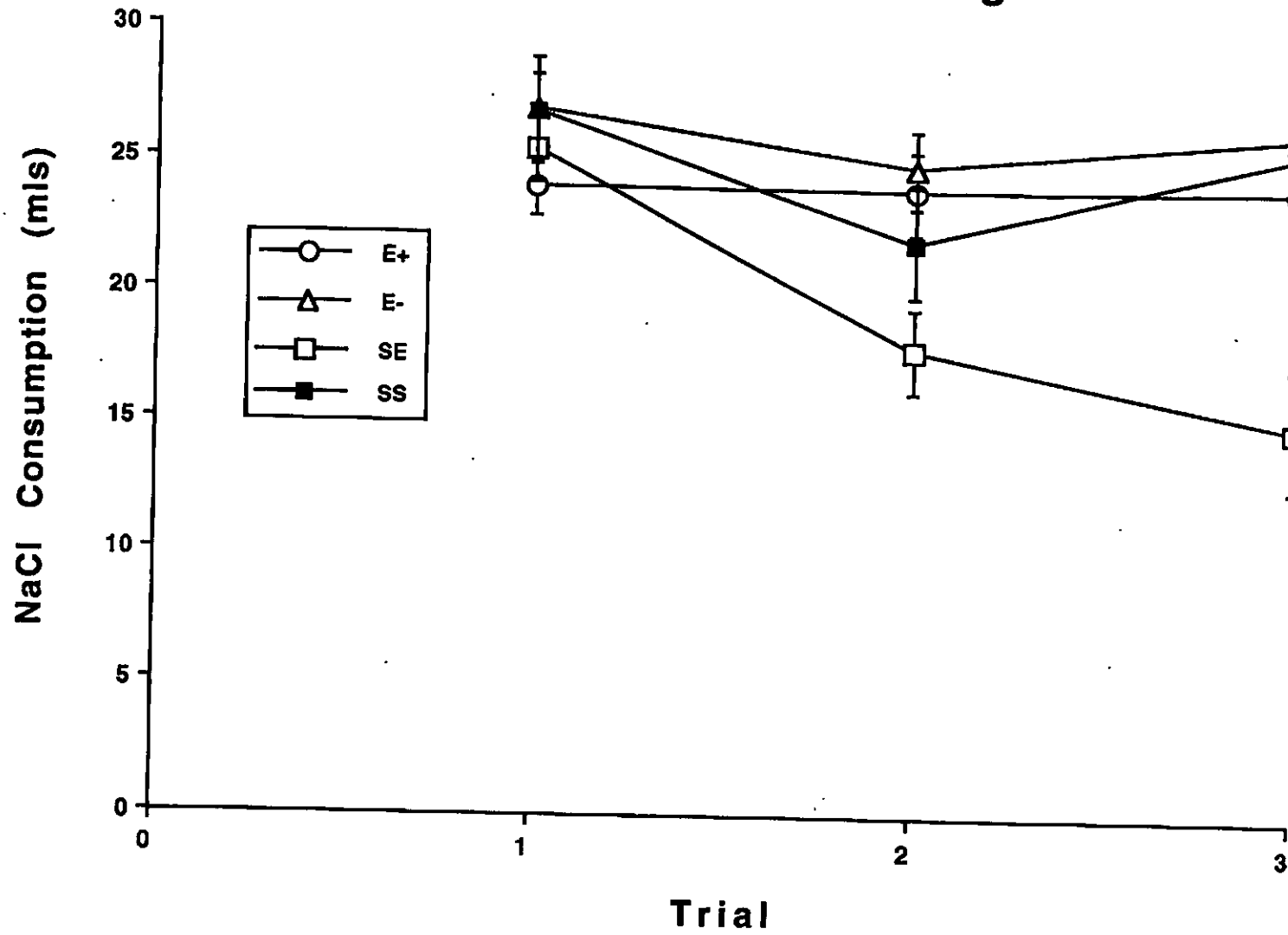
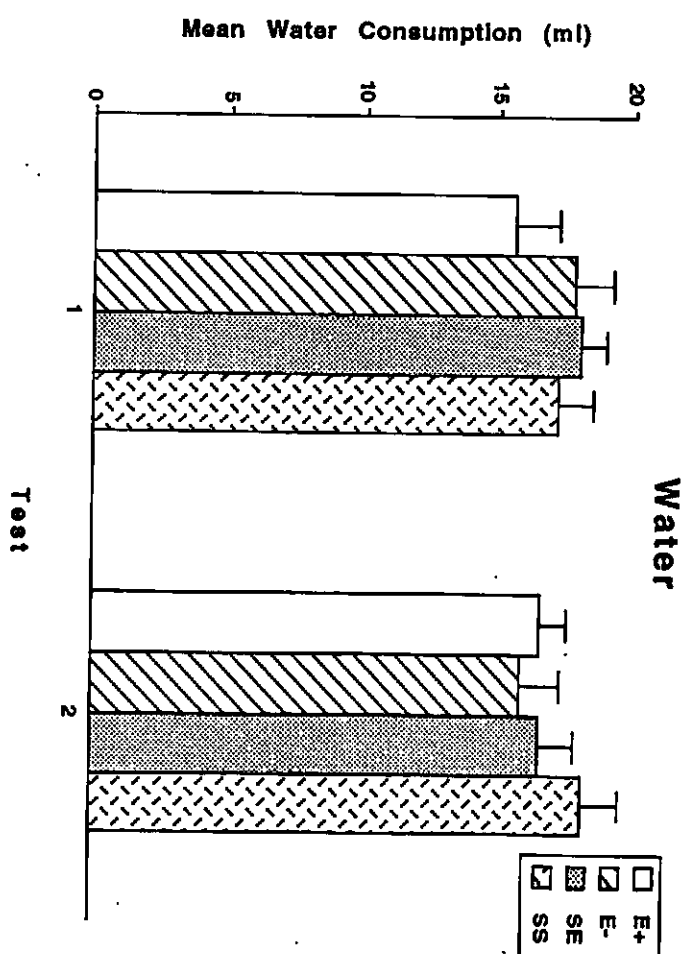
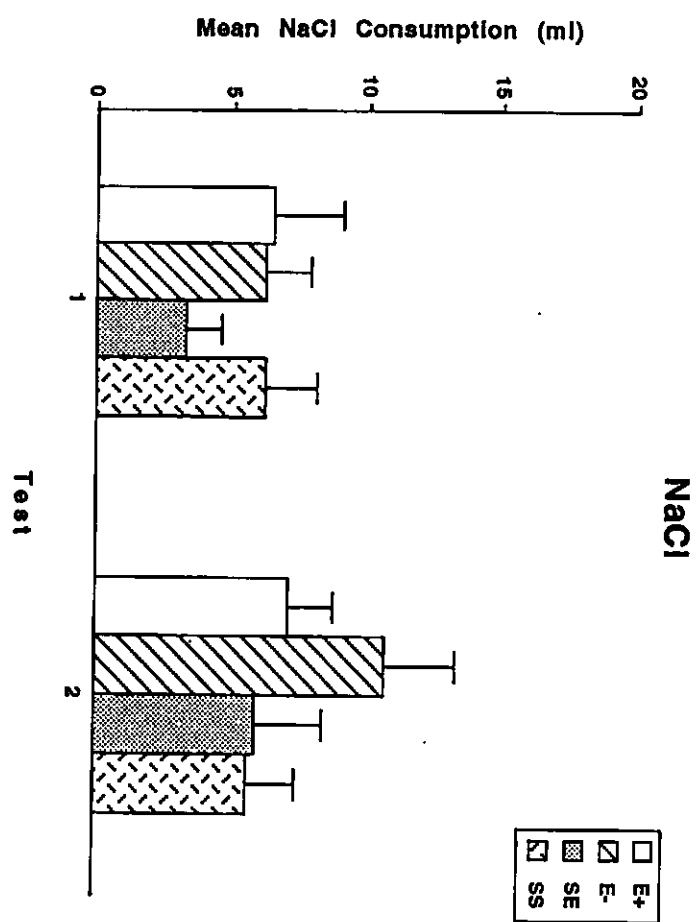


Figure 15: Mean NaCl (left panel) and water (right panel) consumptions (ml $\pm$ SEM) for all groups during Trials 1 and 2 of two-bottle choice testing given subsequent to the taste conditioning phase. Group designations are as outlined in the legend for Figure 14. All subjects received simultaneous access to NaCl (0.2 M) and Water for 30 min. Test trials were run 48 h apart.



E- did not differ in NaCl consumption, it appears that pairing ethanol with sugar during the ethanol conditioning phase does not produce a measurable change in the drug's aversive value as determined by choice testing. NaCl consumption by all three conditioning groups appeared to increase on the second test, probably as a result of extinction of taste aversion through exposure to the conditioned stimulus in the absence of ethanol during Test 1. Water consumptions appeared similar for all groups and in both tests. However, two-way repeated measures ANOVAs, with group as the main factor and test as the repeated measures factor, of both NaCl and water consumption showed no significant differences between groups nor between Tests 1 and 2 with either fluid.

Mean preference ratios [NaCl consumption/NaCl + water consumption ($\pm$ SEM)] collapsed across the two days of two-bottle preference testing were 0.26 (0.05), 0.30 (0.05), 0.17 (0.05), and 0.22 (0.04) for Groups E+, E-, SE, and SS, respectively. Two-way repeated measures ANOVA, with group as the between-groups variable and test as the repeated measures variable, showed no difference between groups or between test days.

Discussion

Blood ethanol concentration results from Experiment 4 suggested that the dose of ethanol and the timing of the CS and US exposures used in the previous taste conditioning study, Experiment 3, may have resulted in the experimental groups being exposed to varying concentrations of the drug. In Experiment 5, ethanol dose and the time between ethanol and sucrose exposures were adjusted to provide more equivalent exposure to ethanol in the experimental and control groups, thereby leaving sucrose pairing as the only independent variable examined. It was expected that the subjects receiving ethanol paired with sucrose would show weaker aversion for the ethanol-paired flavor than would animals receiving unpaired exposures to ethanol and sucrose. All groups consumed similar amounts of sucrose during the ethanol conditioning phase, reassuring that pairing intraperitoneal ethanol with sucrose did not reduce the motivational value of sucrose.

During the taste conditioning phase, NaCl consumption diminished in Group SE, although it was much less precipitous than the decline seen in Experiment 3, where a dose of 1.5 g/kg ethanol was used. Consumption of NaCl did not differ between Groups E+ and E-, suggesting that pairing ethanol with sucrose during ethanol conditioning did not alter the motivational value of a second ethanol-paired stimulus. However, NaCl consumption by these two groups did not differ from that of the unpaired control. Because of the absence of any difference between these groups, only three taste conditioning trials were employed to minimize extinction of the ethanol-sucrose association in Group E+ prior to two-bottle choice testing.

The lack of a difference in NaCl consumption between Groups E+, E-, and SS suggests that previous exposure to ethanol resulted in the development of tolerance to the aversive effects of this dose in these subjects, such that a ceiling

effect may have occluded any observable differences between experimental groups. It is also possible that ethanol preexposure prevented conditioning of the ethanol US to NaCl during taste conditioning. However, Berman and Cannon (1974) demonstrated reduced taste aversion to saccharin in animals receiving ethanol preexposure and found decreased behavioral measures of intoxication in these subjects. They also showed the reduction in aversion to be greater with higher doses of ethanol used in preexposure. These findings suggest that the development of tolerance to the aversive effects of ethanol during preexposure was at least partially responsible for the decrease in taste aversion conditioning.

With the use of a lower dose of ethanol in this experiment than in Experiment 3, it may have been necessary to give the experimental groups fewer exposures to the drug during the ethanol conditioning phase. This may have attenuated the development of tolerance to ethanol's aversive effects during this phase, such that some degree of aversion could have been conditioned to the taste stimulus during taste conditioning. This might have allowed a difference to be seen between Groups E+ and E- as a result of sucrose pairing. As it was, the groups that had received ethanol preexposure showed no difference from the non-ethanol control group in terms of NaCl consumption. Use of a slightly higher dose of ethanol during taste conditioning in this experiment also may have helped overcome the effects of tolerance development to reveal some degree of aversion between the two groups and separated their NaCl consumptions from that of Group SS, and possibly from each other. However, it could be argued that using a higher dose of ethanol introduces a new stimulus, such that any effects of having paired the previous dose with sucrose during ethanol conditioning would not transfer to this new stimulus and, thus, would not be seen during taste conditioning. Although a difference was seen between Groups E+ and E- in Experiment 3 despite the introduction of a novel dose of ethanol during the taste

conditioning phase, this was probably due to differential tolerance to ethanol between the groups resulting from sucrose-pairing reducing blood ethanol concentrations in Group E+, as previously discussed.

Two-bottle choice testing between NaCl and water revealed greater preference for water than for NaCl in all groups. Despite the strong aversion to NaCl seen in Group SE during taste conditioning, no significant differences were seen between groups during either test, nor within groups between Test 1 and Test 2. The lack of evidence of significant taste aversion in Group SE may have been the result of having had only three conditioning trials and having used such a low dose of ethanol that only mild taste aversion developed.

Results from this experiment show that the differences in taste aversion between Groups E+ and E- seen in Experiment 3 were lost when experimental conditions were changed to minimize differences in blood ethanol concentration between the groups. It is difficult to know if the absence of any group differences reflects an inability of sucrose pairing to alter ethanol's motivational effects, or if ethanol preexposure inherent in this conditioning process results in the development of tolerance to the aversive effects of ethanol such that such alterations are masked. Alternatively, taste conditioning, by virtue of its unique characteristics in terms of learning, may not be an appropriate model for assessing conditioned changes in ethanol's appetitive value.

Experiment 6: Influence to Sucrose Pairing on Place Conditioning with Ethanol

Rationale

Experiment 6 employed a different behavioral task to evaluate if pairing ethanol with sucrose alters the motivational value of ethanol. In this experiment, a place conditioning paradigm was used, with the expectation that temporally pairing ethanol with access to sucrose would reduce aversion to, or increase preference for, an ethanol-paired environmental stimulus.

Tactile cues were used as the distinctive environmental stimuli paired with ethanol administration. Vezina and Stewart (1987a) assessed the valence of morphine in rats using differing tactile cues as the discriminative stimulus and demonstrated a robust place preference, with some subjects spending greater than 80% of the test trial on the morphine-paired surface. Cunningham (Crabbe, Phillips, Cunningham, & Belknap, 1992; Cunningham, Hallett, Niehus, Hunter, Nouth, & Risinger, 1991; Cunningham, Niehus, Malott, & Prather, 1992; Cunningham, Niehus, & Noble, 1993, Cunningham & Noble, 1992; Cunningham & Prather, 1992; Risinger, Dickinson, & Cunningham, 1992; Risinger, Malott, Riley, & Cunningham, 1992) has also been very successful in demonstrating place preference to ethanol-paired tactile cues in mice.

In many studies of place conditioning using visual or olfactory cues, for example, environmental preference is significant, but weak, with animals spending less than 60% of the testing period in the preferred environment (Bozarth, 1987; Cunningham, 1981; Vezina and Stewart, 1987a, b). One possible explanation for this minimal demonstration of preference may be related to the types of cues used as discriminative stimuli in place conditioning. For example, if visual or olfactory manipulations are used, animals can approach and experience these drug-

associated stimuli while positioning themselves on the non-drug side of the apparatus. Thus, animals may express preference for the drug-associated environmental cues, but their behavior will not be measured as such. Use of a tactile cue as the distinguishing stimulus forces subjects to position themselves on the drug-paired side of the apparatus to experience the drug-associated stimulus, allowing this demonstrated preference to be appropriately measured.

In contrast to many studies of place conditioning in which drug is paired with the less-preferred environment (with the expectation of drug conditioning a place preference) or without respect to inherent differences in preference for environmentally-paired stimuli (Bozarth, 1987; Lett, 1989), a balanced design was employed in this experiment such that tactile cues were chosen which were equally preferred by drug-naive subjects in the absence of conditioning. A pilot study (Experiment 7; see Appendix) was performed in which tactile cues were manipulated and tested to establish two floor textures with equal unconditioned preferences. Minimizing inherent preferences between the distinctive environmental cues used avoids problems in interpreting how changes in time spent on the less-preferred side reflects preference (van der Kooy, 1987); and also avoids ceiling and floor limitations in evaluating preference and aversion, respectively. The establishment of different but equally preferred discriminative stimuli also allows for the use of an unbiased procedure (Shippenberg, Bals-Kubik, Spanagel, & Herz, 1989). Comparably-preferred environments enable counterbalancing within groups such that each floor type can serve as the CS+ for half the subjects in each group.

The trial length was chosen to be 30 min, based on the results of a pilot study (Experiment 8; see Appendix), in which relative aversion to ethanol-paired environmental cues were compared for trial lengths of 5 min and 30 min. This experiment demonstrated greater ethanol-conditioned place aversion to

environmental cues to which the animals were exposed for 30 min following ethanol injection than when the trial length was only 5 min. It was felt that factors potentially reducing ethanol aversion could be more readily observed under conditions where greater aversion at baseline had been established.

Dosage and parameters for ethanol conditioning followed those used in Experiment 5, based on the blood ethanol concentration findings of Experiment 4. A dose of 1.0 g/kg ethanol has been shown to be adequate for establishing differential place conditioning in rats (Stewart and Grupp, 1985). The ethanol conditioning phase was conducted in the home cages to prevent association of either ethanol or sucrose with cues of the place conditioning apparatus during this phase. It was hoped that the use of tactile cues would allow a more definitive and sensitive demonstration of place conditioning such that the potentially mild influence of sucrose-pairing on preference in Group E+ relative to Group E- could be appreciated.

Material and Methods

Subjects

Subjects were 64 adult male Holtzman albino rats weighing between 375 and 470 g prior to fluid restriction [$X(\pm\text{SEM}) = 418(2)$ g]. They were individually housed as described in Experiment 1. Food and water were available ad lib only during initial acclimation to the colony, after which subjects were placed on a fluid restriction schedule, as described below.

Apparatus

Place conditioning and testing occurred in four identical acrylic and aluminum boxes (47.5 cm x 16.5 cm x 17.5 cm) housed on separate shelves within a large sound- and light-attenuating enclosure (0.9 m x 1 m x 2 m). The chambers had aluminum sides (15.5 cm x 17.5 cm), plexiglas front and rear walls (50.5 cm x 17.5 cm), and a plexiglas lid (50.5 cm x 16.5 cm). Five pairs of infrared light sources and photodetectors were mounted along the front and rear walls at 7-cm intervals and 5 cm above the floor. One detector was located at the midpoint, with the others spaced equally on either side. Occlusion of infrared light beams was used to measure general activity and to monitor the subjects' location (left vs. right side) within the box. An animal was considered to have changed sides when both of the outer photobeams on one side were released and at least one of the outer beams on the opposite side was occluded (the midpoint detector was used only to record activity). Total activity counts and amount of time spent on each side of the chamber were recorded every min by an Apple II microcomputer (10 msec resolution).

Floor Stimuli. The floor of the apparatus was composed of two interchangeable halves made up of one of three textures, each measuring 26.7 cm x 20.3 cm. The "mesh" (M) floor consisted of galvanized-wire hardware cloth (6.5 mm squares) mounted over an acrylic frame; the "grid" (G) floor of 2-mm stainless

steel rods mounted 12.5-mm apart in acrylic rails; and the "hole" (H) floor of perforated stainless steel (16 GA) with 13-mm round holes on 19-mm staggered centers mounted on acrylic. A previous study on texture preference (see Experiment 7; Appendix) revealed that drug-naïve rats demonstrated an inherent preference for the M surface and little bias between the G and H textures. Thus, the M floor was used only for the habituation phase; and the G and H textures were used as discriminative stimuli for place conditioning and testing.

An ethanol solution was prepared for injection by diluting the appropriate volume of 95% ethanol with normal saline to yield a concentration of 15% ethanol (v/v). Dosage was maintained at 1.0 g/kg (8.4 ml/kg of 15% ethanol) throughout the experiment.

Procedure

The experimental procedure consisted of five phases: fluid restriction (6 d), ethanol conditioning (four sessions), habituation (one session), place conditioning (four sessions), and place testing (two sessions). The experimental procedure is outlined in Table 7.

Fluid Restriction. Following 6 d acclimation to the colony room, during which the animals were weighed daily, a 6-d fluid restriction phase was begun. Subjects were weighed and their racks were transported from the colony room to a holding room near the place conditioning testing room. Following a 3-h delay, subjects were given 15 min access to water. Approximately 2 h after water access, the animals were returned to the colony room and, after a 2-h delay, given 60 min access to water. Food was available ad lib during this phase.

Subjects were randomly assigned to one of four groups matched for mean body weight on the final day of the fluid restriction phase.

Ethanol Conditioning. Four 2-d trial blocks of ethanol conditioning were given. Subjects were weighed, their food was removed, and they were transported

TABLE 7 -- Design for Experiment 6: Place Conditioning

Phase 1: FLUID RESTRICTION (6 days)

All subjects given limited period of access to Water daily

Phase 2: ETHANOL CONDITIONING (4 sessions)

<u>Group</u>	<u>Sucrose Trial</u>	<u>No Sucrose Trial</u>
E+	Ethanol (i.p.) --30-min delay--> Sucrose	Saline (i.p.)
E-	Saline (i.p.) --30-min delay--> Sucrose	Ethanol (i.p.)
SE	Saline (i.p.) --30-min delay--> Sucrose	Saline (i.p.)
SS	Saline (i.p.) --30-min delay--> Sucrose	Saline (i.p.)

Ethanol dose was 1.0 g/kg and Saline volume was 8.4 ml/kg

Phase 3: HABITUATION (one 30-min session)

All subjects injected with Saline (8.4 ml/kg) and exposed to Mesh floor

Phase 4: PLACE CONDITIONING (four 2-d trials of 30-min duration)

<u>Group</u>	<u>Subgroup</u>	<u>+ Trial</u>	<u>- Trial</u>
E+	Grid+	Ethanol--->Grid	Saline--->Hole
	Grid-	Ethanol--->Hole	Saline--->Grid
E-	Grid+	Ethanol--->Grid	Saline--->Hole
	Grid-	Ethanol--->Hole	Saline--->Grid
SE	Grid+	Ethanol--->Grid	Saline--->Hole
	Grid-	Ethanol--->Hole	Saline--->Grid
SS	Saline--->Grid alternating with Saline--->Hole		

Both Ethanol (1.0 g/kg) and Saline (8.4 ml/kg) were given by i.p. injection

Phase 5: PLACE TESTING (two 30-min sessions)

All subjects injected with Saline and given simultaneous access to the Grid and Hole textures

from the colony room to the holding room. After a 2-h delay, each animal was given an i.p. injection of either ethanol or saline (8.4 ml/kg) and returned to its cage. Subjects in Groups E+ and E- received ethanol and saline injections on alternate days, while animals in Groups SE and SS received daily saline injections. Fifteen min access to 10 ml of 32% sucrose was given to Group E+ 30 min following ethanol injection, and to Group E- 30 min following saline. Groups SE and SS received sucrose on alternate days (Sucrose). No fluid exposure was given on the non-sucrose trials (No Sucrose). Ethanol injections were counterbalanced such that half the subjects in Groups E+ and E- received ethanol and half received saline each day. Sucrose exposures also were counterbalanced within groups.

Animals were returned to the colony room 2 h after fluid exposure. Their food was returned after a 2-h delay and they were given 60 min access to water.

Habituation. Following ethanol conditioning, exposure to the place conditioning apparatus was initiated. A single habituation trial was given to reduce the stress and novelty of handling, injection, and exposure to the place conditioning apparatus.

Subjects were weighed, their food removed, and they were transported to the holding room. After a 60-min delay, the first group of four subjects was taken to the testing room, injected with saline, and given 30 min access to the chamber. During this phase, all boxes contained M floors. After the 30-min exposure, subjects were returned to the animal carrier for 10 min while the chambers were cleaned and prepared for the subsequent squad. Following this delay, the animals were transported back to the holding room and the next squad of subjects obtained. Sixty min after the final squad was returned to the home rack, the rack was returned to the colony room. Food and 60 min access to water were given following a 3-h delay.

Place Conditioning. Place conditioning consisted of four 2-d trial blocks.

The procedure differed from habituation in that subjects in Groups E+, E-, and SE were given i.p. injections of ethanol alternating over days with i.p. saline prior to placement in the conditioning chambers for 30-min periods. During this phase, the floors on each side of the chamber were homogeneous. However, in contrast to the habituation phase, in which all animals were exposed to the M floor, half the subjects in each group received ethanol prior to exposure to the G surface (Grid+ subgroups) and half received ethanol prior to experience with the H texture (Grid- subgroups) (see Table 7). Group SS served as the no-drug control, and received saline prior to exposure to both floor types.

Drug exposures were counterbalanced such that, on any given day, half the subjects received ethanol (CS+ trial) and half were given saline (CS- trial). Floor textures also were counterbalanced such that half the boxes during any given session contained G floors and half contained H floors.

As in the habituation phase, each rack of animals was returned to the colony room 60 min after its final squad had completed conditioning. Following a 3-h delay, food and 60 min access to water were provided.

Place Testing. The place testing phase consisted of a single 2-d trial block. The procedure differed from the conditioning phase in that all subjects were injected with saline and placed into the conditioning chambers for 30 min with both the G and H floor textures present simultaneously. The amount of time spent on each surface was measured to determine relative preference for the two textures. Floor positions were counterbalanced and were reversed for each subject from the first to the second test trial. The primary dependent variable was the amount of time spent on the grid (G) floor during the 30-min test session.

Results

Body Weight. One subject died during the place conditioning phase, leaving 15 subjects in Group E- and 16 subjects each in Groups E+, SE, and SS. Two-way repeated measures ANOVA, with group as the main factor and day as the repeated measures factor, of daily weights showed that animals gained a significant amount of weight from the first day of conditioning [$X(\pm\text{SEM}) = 413(2) \text{ g}$] to the final day of testing [$X(\pm\text{SEM}) = 440(3) \text{ g}$] [$F(18,1062)=122.0$, $p<0.01$]. Weights did not differ between groups, although there was a significant group x day interaction [$F(54,1062)=1.7$, $p<0.05$], presumably due to the slightly greater increase in weight in Group SE (36 g) than in the other groups (19 to 28 g).

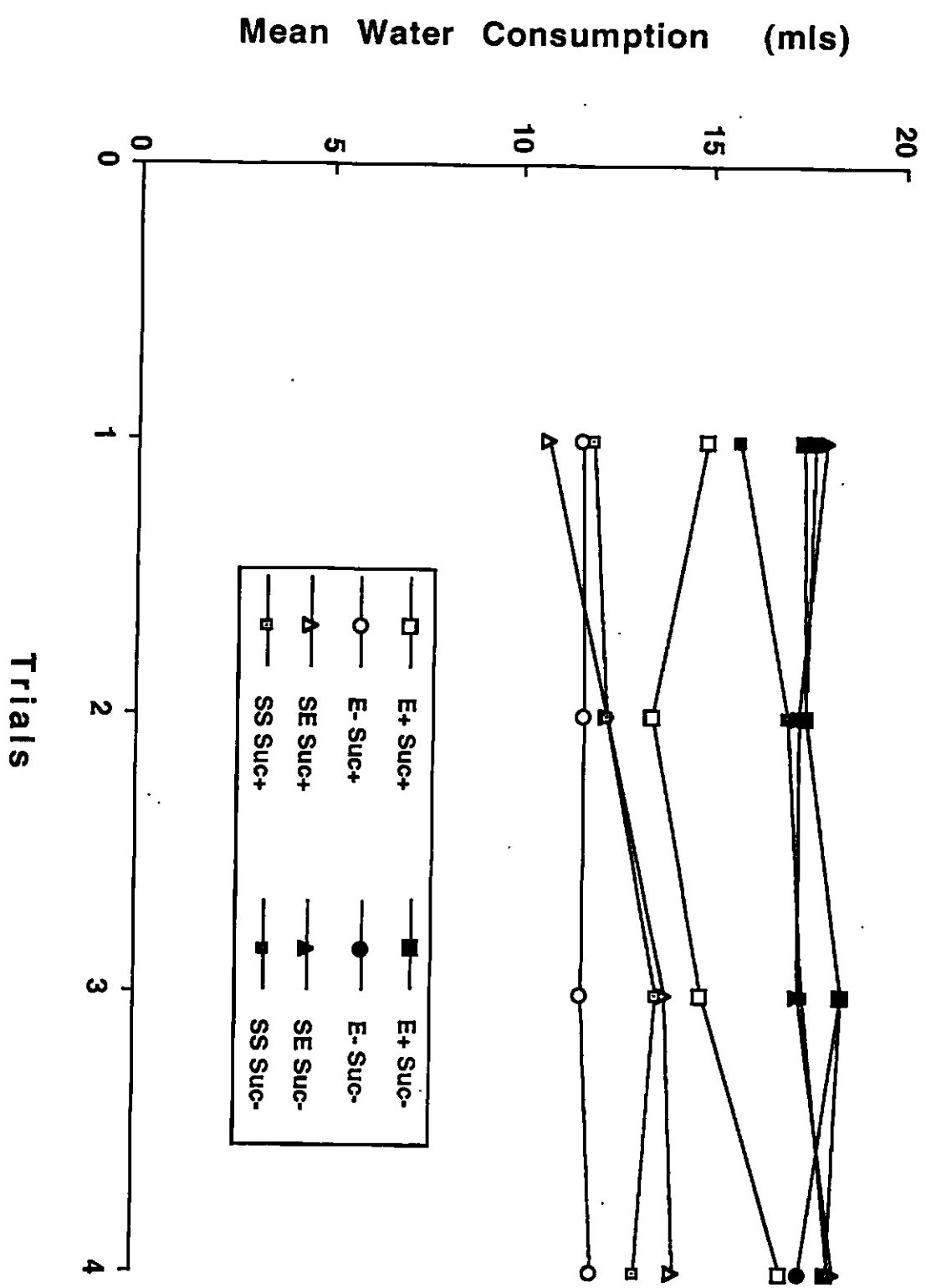
Ethanol Conditioning

Mean ($\pm\text{SEM}$) sucrose consumption increased from 8.1 (0.2) ml on the first ethanol conditioning trial to 8.9 (0.1) ml on the final trial, presumably due to subjects overcoming neophobia to the taste of sucrose over trials. Two-way repeated measures ANOVA, with group as the between-groups factor and trial as the repeated measures factor, showed this increase over trials to be significant [$F(3,177)=16.2$, $p<0.01$]. Group consumptions did not differ significantly and there was no significant group x trial interaction.

Mean ($\pm\text{SEM}$) daily water consumptions during post-conditioning access periods are shown in Figure 16. Consumptions differed between sucrose and non-sucrose conditioning trials, with subjects consuming more water on days they had not received sucrose. Two-way repeated measures ANOVA, with group as the between-groups factor and sucrose as the repeated measures factor, indicated no group effect, but revealed a significant effect of sucrose [$F(1,59)=295.6$, $p<0.01$], as well as a group x sucrose interaction [$F(3,59)=6.4$, $p<0.01$], due to greater differences in water consumption between groups after sucrose than after non-

Figure 16: Mean water consumptions (ml $\pm$ SEM) during the 60-min post-conditioning access period comparing consumptions following sucrose with those when no sucrose was given during the ethanol conditioning trials. Group E+ received ethanol followed by sucrose, while Groups E+, SE, and SS received saline prior to sucrose access. Group E- was given ethanol during the No Sucrose trials. Groups SE and SS remained ethanol naive during this phase of the study.

Sucrose vs. No Sucrose



sucrose trials. Exposure to ethanol during conditioning did not significantly influence post-conditioning water consumptions.

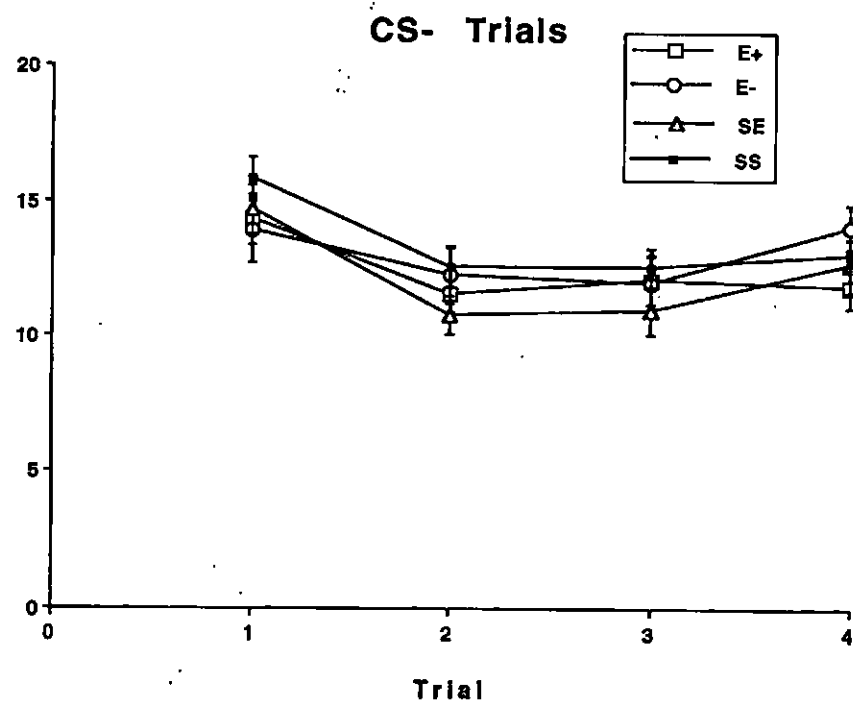
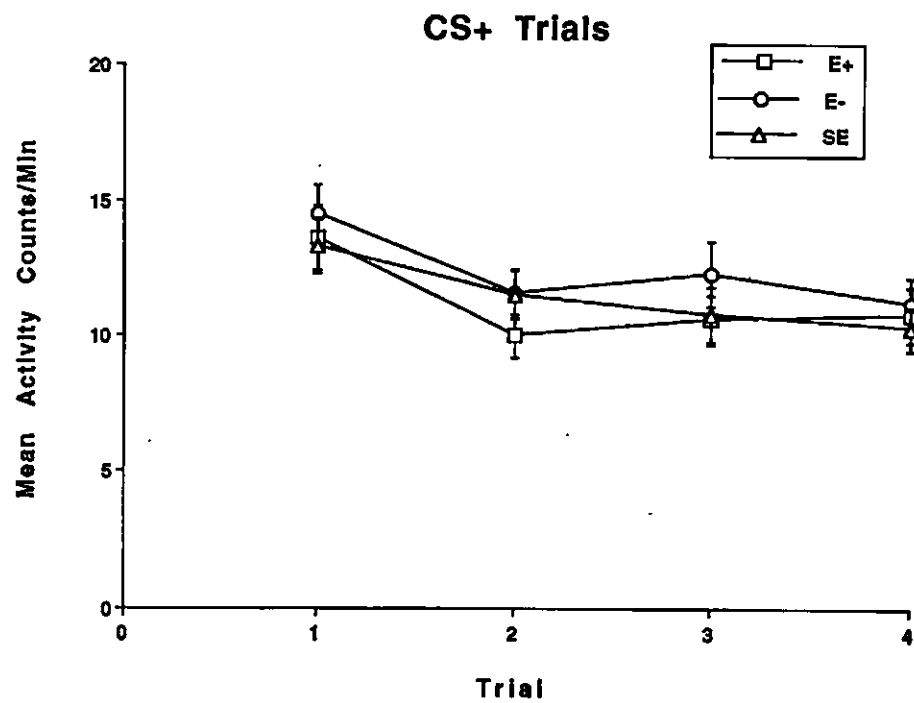
Place Conditioning

Mean activity counts/min ($\pm$ SEM) during place conditioning are shown in Figure 17 for the CS+ trials (left panel) and CS- trials (right panel) for Groups E+, E-, and SE. Mean activity counts collapsed across the two saline exposures in each trial for Group SS, the ethanol-naive control, are included with the CS- trials for the other groups. Across both CS+ and CS- trials, activity declined slightly in all groups following the first trial. There was no significant difference between groups with either CS type, although there was generally a lower level of activity following ethanol exposure than during CS- trials, especially during Trial 4. Three-way repeated measures ANOVA, with group as the between-groups factor and CS type and trial as repeated measures factors, for Groups E+, E-, and SE (Group SS was excluded because subjects received only saline) revealed no significant group effect, but indicated a significant effect of CS type [$F(1,44)=4.3$, $p<0.05$]. Fewer activity counts were recorded during CS+ than CS- trials in Groups E+, E-, and SE, indicating ethanol suppressed activity. There was also a significant trial effect [$F(3,132)=22.0$, $p<0.01$], with activity counts decreasing from the first to subsequent trials.

Place Testing - Activity

Mean ($\pm$ SEM) activity counts/min collapsed across samples were 11.8 (0.7), 12.9 (0.9), 11.3 (1.2), and 12.7 (0.8) during Test 1, and 11.6 (1.1), 11.9 (1.0), 11.9 (1.1), and 12.1 (1.0) during Test 2 for Groups E+, E-, SE, and SS, respectively. Two-way repeated measures ANOVA, with group as the between-groups factor and test trial as the repeated measures factor, indicated no group differences and no difference in activity between test trials.

Figure 17: Mean ($\pm$ SEM) activity counts per minute during place conditioning for the four CS+ (left panel) trials for Groups E+, E-, and SE; and the four CS- (right panel) trials for Groups E+, E-, SE, and SS. Group designations are as described in the legend for Figure 16. Groups E+, E-, and SE received ethanol on CS+ trials and saline on CS- trials during place conditioning, while Group SS was a drug-naive control and received only saline throughout the experiment.

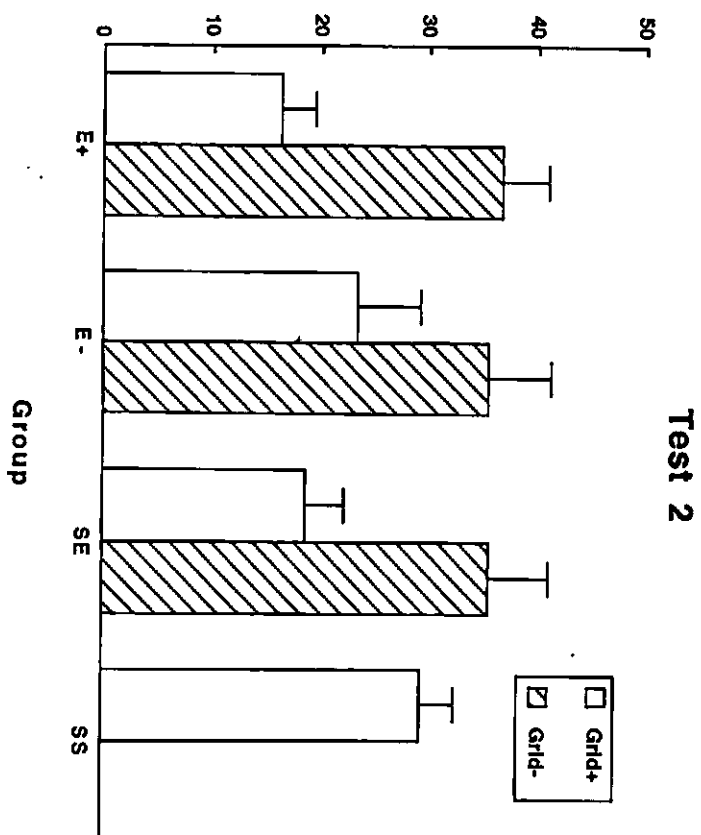
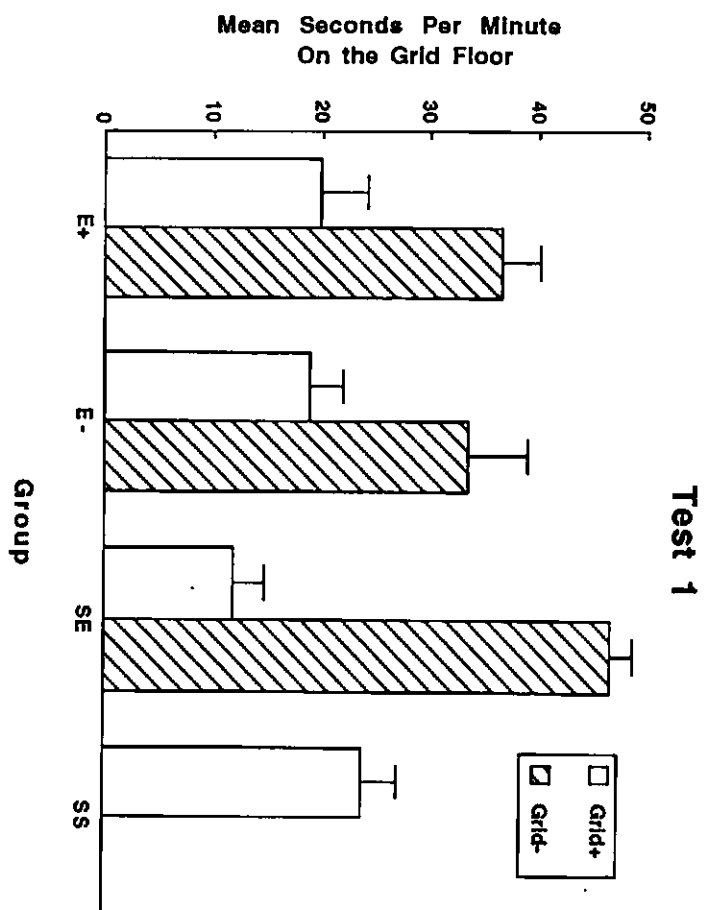


Place Testing - Choice

The mean ($\pm$ SEM) seconds per minute spent on the G floor during the 30 sample periods from Test 1 (left panel) and Test 2 (right panel) are shown in Figure 18 for all groups. Mean scores greater than 30 s/min indicate that the G floor was preferred over the H floor, while mean scores less than 30 s/min indicate a preference for the H floor. The difference between time spent on the grid floor between the Grid+ and Grid- groups indicates the amount of conditioned preference or aversion to the ethanol-paired environment. Subjects in the Grid-conditioning subgroups spent more time on the G floor than did animals in the Grid+ subgroups, demonstrating that ethanol produced a conditioned place aversion. A greater difference between Grid+ and Grid- conditioning subgroups was observed in Group SE than in Groups E+ and E-, suggesting that ethanol preexposure during the ethanol conditioning phase in these groups attenuated the degree of conditioned aversion to the ethanol-paired texture during the place conditioning phase. Time spent on the grid floor by conditioning subgroups in Groups E+ and E- did not differ, suggesting that pairing ethanol with sugar during preexposure did not further reduce aversion. Conditioned place aversion was the same or slightly decreased on Test 2 as compared with Test 1, as a result of extinction arising from exposure to the floor cues in the absence of ethanol during the first choice test.

Two-way ANOVA, with group and conditioning subgroup as between-groups factors, of mean seconds per minute spent on the grid floor for Groups E+, E-, and SE during Test 1 indicated that groups did not differ, although there were significant effects of conditioning subgroup [$F(1,41)=52.9$, $p<0.01$] and group x conditioning subgroup [$F(2,41)=4.5$, $p<0.05$]. Followup two-way ANOVA, with group and conditioning subgroup as between-groups factors, examining mean time spent on the grid floor for Groups E+ and E- showed a significant effect of

Figure 18: Mean ($\pm$ SEM) seconds per minute spent on the grid floor during the first (left panel) and second (right panel) choice tests collapsed across the 30-min testing periods for the Grid+ and Grid- conditioning groups for Groups E+, E-, and SE, and for Group SS. All subjects received saline injections followed by simultaneous access to the Grid and Hole textures. Group designations are as described in the legend for Figure 16.



conditioning subgroup [$F(1,27)=13.8$, $p<0.01$], but no overall group effect and no interaction, suggesting that the interaction seen with all groups reflects the greater aversion observed in the conditioning subgroups of Group SE relative to those of Groups E+ and E-.

Two-way ANOVA, with group and conditioning subgroup as between-groups factors, of mean seconds per minute spent on the grid floor for Groups E+, E-, and SE during Test 2 revealed a significant effect of conditioning subgroup [$F(1,41)=17.8$, $p<0.01$], but no significant interaction. The lack of a significant group $\times$ conditioning subgroup interaction in Test 2 versus Test 1 reflects the partial extinction of aversion to the ethanol-associated floor cues resulting from a single extinction trial (Test 1). Although all groups experienced exposure to floor cues in the absence of ethanol, the greatest extinction was seen in Group SE, which also showed the most profound aversion to the ethanol-paired cues.

Discussion

The results of Experiment 6 mirrored those of Experiment 5: exposure to ethanol during the ethanol conditioning phase in Groups E+ and E- decreased aversion to the drug, as shown by the significantly greater aversion to the ethanol-paired stimuli seen in Group SE relative to Groups E+ and E-. However, loss of aversive conditioning was not as complete with only four conditioning trials as it had been with the eight trials used in Experiment 5, as suggested by the difference in place preference seen between Groups E+ and E- and the ethanol-naive controls, Group SS. In Experiment 5, there was no difference in NaCl consumption between the groups receiving NaCl paired with ethanol and the control group. Although blood ethanol concentrations were not measured, in retrospect, these might have been helpful in determining if the decrease in aversiveness resulting from ethanol conditioning was due to tolerance to ethanol's aversive effects or if ethanol preexposure had decreased the ability to condition environmental cues to the ethanol stimulus in Groups E+ and E-.

As discussed in Experiment 5, Berman and Cannon (1974) demonstrated behavioral tolerance in taste aversion studies with ethanol preexposure. Additionally, place preference has been conditioned to ethanol following extensive ethanol preexposure (Reid et al., 1985), which would suggest that prior exposure did not interfere with the ability of the ethanol US to be conditioned to place cues. These findings suggest that the reduced place aversion in Groups E+ and E- was due to tolerance developing to the aversive properties of ethanol during preexposure.

Pairing ethanol injections with sucrose did not result in a lesser degree of aversion to ethanol-associated cues as measured by place conditioning, as had been predicted. That is, Groups E+ and E- did not differ in their aversion to the ethanol-

paired environmental cues, despite Group E+ having received prior exposures of i.p. ethanol followed by orally self-administered sucrose.

Use of 1.0 g/kg ethanol resulted in only mild place aversion, as demonstrated by the decrease in aversion in Group SE from Test 1 to Test 2. The difference between Groups E+ and E- and Group SE was lost after only a single extinction trial (Test 1) during which the animals were exposed to the environmental cues in the absence of ethanol.

Greater activity was seen during the first trials of place conditioning with both the CS+ and CS- than on any of the subsequent trials, as might be expected due to increased exploration of the experimental apparatus on first exposure to each of the floor types. Exposure to 1.0 g/kg ethanol was found to decrease activity during place conditioning. This response to ethanol has been demonstrated in previous place conditioning studies in rats (Frye & Breese, 1981), and is opposite to that seen in mice, where ethanol has been shown to increase both activity and preference for drug-paired cues (Cunningham et al., 1992). While Groups E+, E-, and SE revealed no significant differences in activity during place conditioning, significant differences in floor preference were observed, which suggests that activity and preference are not directly associated in rats. The lack of activity differences between the experimental and control groups during place conditioning also suggests that previous ethanol exposure did not induce tolerance to ethanol-induced locomotor changes even when tolerance to the drug's aversive effects may have been evident.

Summary and Conclusions

Pairing ethanol with access to sucrose in Pavlovian paradigms was effective in inducing oral consumption of ethanol and conditioning preference for ethanol in two-bottle choice testing (Experiments 1 and 2). However, ethanol-sucrose pairing was apparently ineffective in increasing the appetitive value of ethanol in taste conditioning (Experiments 3 and 5) and place conditioning (Experiment 6) paradigms.

Several factors may be contributed to the differential effect of ethanol-sucrose pairing observed with the various conditioning paradigms. Lower blood ethanol concentrations after sucrose consumption were demonstrated following both oral and intraperitoneal administration of ethanol. In Experiment 2, blood ethanol concentrations were significantly lower in subjects receiving sucrose following consumption of 5% ethanol than in animals receiving ethanol paired with water, despite the paired groups having consumed a significantly greater dose of ethanol at this concentration than the unpaired animals (Figure 6). The slope of the regression line for blood ethanol concentration versus dose of 5% ethanol consumed (Figure 7) also was significantly steeper for control subjects (Groups 0 and C) than for experimental groups (Groups 4, 16, and 32), demonstrating the reduction in blood ethanol concentrations observed following sucrose consumption. Although ES-sucrose groups consumed a dose of 13% ethanol that was 2.5 times that of the control animals, their mean blood ethanol level at this concentration was remarkably similar to that of the control subjects (0.56 mg/ml for ES-sucrose groups and 0.53 mg/ml for controls; see Figure 6). This finding suggests that animals control their ethanol intake to maintain blood ethanol concentrations below a certain level, presumably to limit the pharmacological impact of the drug.

The correlation between ethanol consumption and blood ethanol concentration was greater for control subjects than for the experimental groups with both 5% and 13% ethanol. With 5% ethanol, the correlation for the control groups was .86 and only .50 for the experimental subjects. The correlation between ethanol dose and blood ethanol concentration remained .86 for the controls with 13% ethanol, and rose to .67 for experimental groups.

The mechanism by which sucrose reduced blood ethanol concentration may have involved the dilution of ethanol with the subsequent sucrose bolus, thus slowing absorption. Also, as outlined in the discussion following Experiment 4, sucrose and other sugars have been shown to have a significant impact on ethanol metabolism through a variety of possible mechanisms. As a result, the increased preference for ethanol seen following sucrose-pairing may not have been due solely to an increase in ethanol's appetitive value following the association of sucrose with ethanol, but may have been secondary to a lowering of blood ethanol concentration. A lower blood ethanol concentration may have allowed sucrose-paired subjects to consume significantly greater amounts of ethanol without experiencing the pharmacological effects to the same degree as they would in the absence of sucrose. These findings would suggest further that ethanol's pharmacological effects are aversive, since greater preference for the ethanol solution was observed in two-bottle choice testing in the presence of lower blood ethanol concentrations and presumably decreased pharmacological effects.

Although lowering of blood ethanol concentrations by sucrose may have accounted for some of the increase in preference for ethanol in the sucrose-paired groups, the increase in ethanol preference was shown to be quantitatively determined by sucrose concentration, suggesting that associative factors also were involved. In Experiment 2, animals receiving a 32% sucrose solution showed increased preference for ethanol in two-bottle choice testing relative to subjects

experiencing ethanol paired with 4% sucrose (Figure 5). This demonstration of a quantitative difference in ethanol preference based on sucrose concentration supports the hypothesis that the rewarding value of ethanol can be positively manipulated through associating the drug with a known positive reinforcer.

As outlined in the Introduction, the reluctance of animals to self-administer ethanol at concentrations exceeding 6 to 7% has been attributed to the fluid's aversive olfactory and taste characteristics. However, results from Experiments 1 and 2 suggest that associative factors may be more important than taste characteristics in determining ethanol consumption. In Experiment 1, rats orally self-administered ethanol at concentrations of up to 30%. If taste characteristics were the sole limiting variable, one would have expected higher consumptions by those animals receiving ethanol-saccharin solution without regard to other associative factors. The observation that animals receiving unadulterated ethanol paired with sucrose consumed more than did subjects receiving sweetened ethanol solution suggests that other factors are important.

In a strategy to reduce the impact of sucrose on blood ethanol concentrations, ethanol was delivered intraperitoneally in Experiment 3 with a 15-min delay introduced between ethanol injection and sucrose access. The appetitive influence of sucrose pairing was measured using taste conditioning instead of consumption and choice testing. Although it was expected that association with sucrose would decrease the aversiveness of ethanol, and thus reduce the degree of taste aversion conditioned to a NaCl solution paired with ethanol, animals receiving ethanol paired with sucrose showed greater taste aversion than did subjects receiving the fluids unpaired (Figure 9).

Although efforts had been made to reduce the influence of sucrose on ethanol pharmacokinetics, the intermediate degree of aversion seen in Group E+ in Experiment 3 most likely was due to sucrose lowering blood ethanol

concentrations during ethanol conditioning such that they experienced intermediate pharmacological effects of ethanol relative to Group E- and the ethanol-naive subjects, Group S.

A second factor which may have impacted on the demonstration of taste aversion was the experience with ethanol during the ethanol conditioning phase. Preexposure to both drug and non-drug US's has been shown to result in decreased taste aversion during subsequent taste conditioning (Berman & Cannon, 1974; Cain & Baenninger, 1977; Barker & Johns, 1978). In general, animals have greater difficulty associating a familiar US with a CS than if the US is novel (Domjan & Burkhard, 1986). There are several theories that attempt to explain the mechanisms responsible for this US preexposure effect. One proposed mechanism suggests that animals learn to associate the US with the situational cues present during preexposure, such as the environment in which the US is delivered. This association then interferes with the learning of an association between the CS and US (Wagner, 1976). Another theory suggests that repeated exposure to the US in the absence of other stimuli trains animals that presentation of the US is irrelevant. This learned irrelevance subsequently disrupts the learning of an association between the CS and US (Baker & Mackintosh, 1977). A third mechanism proposed for drug stimuli suggests that preexposure results in the development of tolerance to pharmacological effects of the drug, such that the stimulus presented during conditioning may actually have different characteristics from that to which the animals were preexposed by virtue of tolerance development (Berman & Cannon, 1974; Barker & Johns, 1978).

If animals in Group E+ experienced lower blood ethanol concentrations, it could be argued that they had less preexposure to some of ethanol's effects than did subjects in Group E-. As a result, it may have been easier for them to associate the ethanol US with the NaCl CS because of reduced associative interference

relative to Group E-. Additionally, because ethanol was presented in conjunction with a significant stimulus, sucrose, in Group E+, while Group E- received ethanol by itself, a greater amount of learned irrelevance may have interfered with taste conditioning in Group E+. It also can be argued that preexposure to ethanol induced tolerance development, especially tolerance to ethanol's aversive effects. Group E- by virtue of these animals experiencing greater blood ethanol concentrations, may have developed greater tolerance to ethanol such that ethanol was less aversive in these subjects relative to Group E+, thereby resulting in decreased aversion to the CS during taste conditioning.

Blood ethanol concentrations were not been measured directly in Experiment 3. However, when the impact of various delays between ethanol injection and sucrose access on blood ethanol concentrations was examined in Experiment 4, the results suggested that blood ethanol concentrations had been lower in Group E+. In this experiment, oral access to sucrose 15 min after i.p. administration of either 1.0 or 1.5 g/kg doses of ethanol significantly lowered blood ethanol levels compared to subjects not receiving sucrose access. Sucrose delivery had no impact on a 0.5 g/kg dose of ethanol at any time interval measured (Figure 10). However, sucrose consumption had a significant influence on blood ethanol concentration at 15 min and 180 min following a 1.0 g/kg dose (Figure 11), and at 15, 180, and 240 min following a 1.5 g/kg dose of ethanol (Figure 12). These results suggest that the delivery of sucrose 30 minutes following a 1.0 g/kg ethanol dose does not have a significant effect on blood ethanol concentration. Thus, this dose and access schedule were used in the subsequent taste conditioning and place conditioning studies.

No differences in degree of taste aversion were seen with sucrose pairing in Experiment 5. In fact, conditioned taste aversion did not develop to the ethanol-paired stimulus in either Group E+ or E-. During taste conditioning, NaCl

consumption by these groups was no different from that of the no-drug control, Group SS (Figure 14), suggesting that US preexposure interfered completely with the association of ethanol with the CS during taste conditioning, or that absolute tolerance to the aversive effects of 1.0 g/kg ethanol developed during the eight ethanol conditioning trials. Because the effect was so complete, any potential influence of sucrose pairing in reducing ethanol aversion may have been masked. One might predict that sucrose pairing would result in an increased preference for the ethanol-paired stimuli resulting in an increase in NaCl consumption in Group E+ relative to Groups E- and SS. This effect, if present, may have been precluded by a ceiling effect, with all subjects consuming maximal amounts of NaCl in the time allotted. However, even if a ceiling effect precluded demonstration of increased valence during taste conditioning, a conditioned increase in preference, if present, could have been seen during two-bottle choice testing, but was not. In fact, even the difference in taste aversion between the experimental groups and the drug-naïve control, Group SE, observed during the taste conditioning trials, was absent during choice testing (Figure 15).

The results from place conditioning were similar to those from taste conditioning: no influence of sucrose pairing was evident (Figure 18) with both experimental groups spending similar amounts of the test trial in contact with the ethanol-paired environmental cues during testing. However, unlike Experiment 5, some degree of aversion was seen to the ethanol-paired environment. This difference may have been due to the use of only four ethanol conditioning trials in Experiment 6 and eight in Experiment 5. The US preexposure effect was relatively weak, since only a single extinction trial was necessary to overcome the difference between Groups E+ and E- and Group SE, which had received no ethanol preexposure.

Although the impact of sucrose on blood ethanol concentration may have accounted for the increased preference or reduced aversion seen in the ethanol-sucrose groups in the self-administration studies, other differences in experimental design may have accounted for the lack of an effect of sucrose pairing in the taste conditioning and place conditioning paradigms. In Experiments 1 and 2, ethanol was delivered orally, followed immediately by the oral delivery of sucrose. In contrast, ethanol was delivered intraperitoneally in the subsequent experiments, with sucrose available orally after a 15- or 30-min delay. This difference in route of drug administration may have had a significant impact beyond the pharmacological implications. The cues surrounding ethanol administration were identical to those signaling sucrose access in Experiments 1 and 2. In both instances, bottles containing the two fluids were placed on the front of the cages and subjects approached and consumed the fluids in a like manner. In Experiments 5 and 6, ethanol was delivered by i.p. injection, while sucrose was presented orally. The similarity of cues between presentation of the CS and US may have increased the strength of the association between the ethanol and sucrose solutions in the self-administration studies (Rescorla & Furrow, 1977), and was lacking in the later experiments.

The presence or absence of control over ethanol administration may, in itself, determine the rewarding properties of the drug. In Experiments 1 and 2, subjects orally self-administered the ethanol solution and were able to control the dose. In the remaining experiments, during which ethanol was given by i.p. injection, drug dosage was controlled by the investigator and not by the animal. Moolten and Kornetsky (1990) demonstrated increased sensitivity to brain-stimulation reward in animals that orally self-administered an ethanol-sucrose solution, but not in yoked-control subjects matched for consumption and receiving the solution through an implanted gastric cannula (although a significant effect

was not seen until after six weeks of ethanol exposure). Control over consumption also may have contributed to ethanol self-administration and conditioned place preference seen with the sucrose-fading technique developed by Samson (Samson et al., 1988; Gauvin & Holloway, 1991), in that animals were able to limit the amount of ethanol administered and minimize its pharmacological effects.

In addition to changes in route and control of drug administration, the temporal contiguity of the CS and US was altered between studies. In Experiments 1 and 2, exposure to the CS was followed immediately by exposure to the US. In the taste and place conditioning experiments, a 15- or 30-min delay was introduced between ethanol injection and sucrose access. It is possible that this delay decreased the strength of the association between the CS and US by changing the cues that were associated. With no delay between ethanol and sucrose delivery, the taste of the ethanol solution was the most salient CS cue and, presumably, became associated with the taste cues of the sucrose solution. This provided a strong degree of similarity between CS and US cues, but also may have increased the contingency between CS and US, since the taste of ethanol consistently and reliably predicted access to sucrose. With i.p. delivery of ethanol and a 30-min delay before sucrose access, subjects were experiencing the aversive physiological effects of ethanol paired with sucrose, rather than the immediate orosensory cues. The stimulus complex resulting from ethanol injection may have been less salient than a simple taste cue and could have resulted in decreased associative strength between ethanol and sucrose during the taste conditioning and place conditioning experiments.

A change in the experimental design from first-order conditioning in the self-administration experiments to second-order conditioning in the place and taste conditioning studies also may have contributed to the different results observed (Rescorla, 1980). In Experiments 1 and 2, an orally-delivered ethanol solution

(CS) was paired directly with an orally-delivered sucrose solution (US), with the impact of this association determined by consumption of and preference for the CS. In the other experimental paradigms, ethanol was paired with sucrose, but measurement of the impact of this association was based on pairing ethanol with another stimulus, which necessitated the transfer of the appetitive association from sucrose to ethanol (CS₁) and then from ethanol to another stimulus (CS₂). In the case of taste conditioning, the change in the appetitive value of ethanol had to impact on the degree of taste aversion conditioned to NaCl by ethanol pairing. In the place conditioning paradigm, the conditioned change in ethanol reward resulting from its association with sucrose during ethanol conditioning had to transfer to the cues of the ethanol-paired environment during place conditioning. The association between ethanol and sucrose in Experiments 5 and 6 may have been already fairly weak due to the factors discussed above, and may have been rendered undetectable with second-order conditioning, especially considering the minimal degree of aversion that was conditioned with a dose of 1.0 g/kg of ethanol.

The experiments also differed in the extent of experience with ethanol during the ethanol conditioning phase. Experiments 1 and 2 provided multiple exposures to ethanol with 63 conditioning trials in Experiment 1 and 25 conditioning trials in Experiment 2. In contrast, Experiments 3 and 5 provided eight ethanol conditioning trials, and Experiment 6 provided only four.

The practice of giving subjects multiple exposures to ethanol has been an effective manipulation for conditioning place preference to an ethanol-paired environment (Bozarth, 1990; Reid et al., 1985), whereas most studies have demonstrated place aversion to ethanol in the absence of other manipulations (see Introduction). The multiple exposures used in the initial two experiments may have been effective in conditioning preference for ethanol because of the

development of tolerance to ethanol's aversive effects. Multiple drug exposures reduce the pharmacological impact of ethanol through both metabolic and neurobiological mechanisms. Following the development of tolerance, more ethanol must be consumed for the same pharmacological effect. In self-administration studies, this may be interpreted as an increase in preference for ethanol when, in fact, the rewarding value of ethanol has not changed. The pharmacological of the drug may remain aversive, but animals can take in more drug before reaching the threshold where the aversiveness overrides any preferential caloric or taste properties and self-administration ceases. Some investigators have suggested, however, that tolerance develops differentially to ethanol's motivational effects, with more rapid development of tolerance to the drug's aversive properties.

Providing multiple trials during ethanol conditioning also may have allowed a stronger association to form between ethanol and sucrose. The few conditioning trials used in the taste and place conditioning studies may not have allowed the association to be learned as extensively, although the eight trials used in taste conditioning appeared effective in producing either associative interference or the development of tolerance to ethanol's aversive effects, since neither experimental group developed conditioned taste aversion to the ethanol-paired stimulus (Figure 14).

Despite the fact that these experiments did not demonstrate conditioned ethanol reward in all instances, the demonstration of quantitative differences in preference for ethanol based on the concentration of sucrose with which it was paired, showed that the motivational properties of ethanol could be altered as a result of learning. This finding also suggests that associative factors that might influence its rewarding properties must be considered in experiments where ethanol self-administration is manipulated using other reinforcers. In addition,

when other reinforcers are presented simultaneously with ethanol access, the possible impact of these substances on blood ethanol concentration must be evaluated. Manipulations which alter blood ethanol levels consequently influence ethanol's pharmacological effects and, as a result, may color interpretations of ethanol's valence.

The variability in demonstration of ethanol's rewarding and aversive effects depending upon the experimental design is highlighted by a series of studies using the ethanol preferring (P) and non-preferring (NP) rat lines developed by T. -K. Li and colleagues at Indiana University. These lines were bred based on their consumption of 10% ethanol in choice tests between ethanol and water, with P rats showing preference for the ethanol solution. Although the lines are selectively bred based on differences in oral consumption of ethanol, with rats of the P line drinking more and barpressing more for a 10% ethanol solution than rats of the NP line, both lines show conditioned taste aversion to moderate and high doses of ethanol, and neither preference nor aversion to ethanol doses less than 1.0 g/kg (Froelich et al., 1988). Similarly, both lines demonstrate conditioned place aversion to an environment paired with i.p. injection of 1.0 g/kg ethanol (Schechter, 1992). This series of studies highlights the common finding that self-administration and preference for ethanol in drinking tasks does not necessarily predict the response seen in taste conditioning or place conditioning paradigms. Although a wide range of pharmacological agents that are self-administered by animals and abused by humans cause conditioned taste aversion (Hunt & Amit, 1987), they typically condition place preference (Vezina & Stewart, 1987a,b). This paradox is not as commonly seen with other drugs of abuse and highlights the difficulties in understanding the motivational properties of ethanol.

In summary, several factors which differed between the experiments may have resulted in the demonstration of appetitive conditioning in the initial

experiments and loss of sucrose impact in the later experiments. Independent of any effects of sucrose on ethanol metabolism, these include an alteration in cues signaling exposure to the CS and US, a variation in control over ethanol dose, a difference in stimulus characteristics (taste of the CS with the oral route, pharmacological effects with the i.p. route), a change in the CS-US interval, a decrease in the number of CS-US pairings, and a shift from first-order to second-order conditioning,

The results from the experiments presented here suggest that the pharmacological effects of ethanol are aversive in rats. Unlike most substances that humans abuse, it is very difficult to induce rats to self-administer ethanol or to show preference for ethanol-paired stimuli. It may be that measures of preference based on taste conditioning may be inappropriate for evaluating motivational effects of drugs. This is supported by the demonstration of conditioned taste aversion with multiple drugs of abuse, such as morphine, cocaine, and amphetamines, in addition to ethanol (Cappell & LeBlanc, 1973; Cappell, LeBlanc, & Endrenyi, 1973; Hunt & Amit, 1987). Most drugs are thought to have mixed euphoric and dysphoric effects, such that they may be seen as being appetitive or aversive, depending upon the characteristics measured. It may be that conditioned taste aversion, because of its adaptive necessity, is more sensitive to the aversive, dysphoric, effects irrespective of any appetitive properties of a given substance.

Cunningham et al. (1991) examined conditioned place preference in two lines of mice selectively bred for their sensitivity (COLD) and insensitivity (HOT) to ethanol-induced hypothermia (Crabbe et al., 1987). Tactile floor cues that have been used successfully in conditioned place preference paradigms (Vezina & Stewart, 1987a, 1987b) were used as discriminative stimuli. Following four pairings of 2.25 g/kg ethanol with one floor type and saline with the other, a choice

test revealed a significant preference for the ethanol-paired floor in the HOT line, while the COLD line showed neither preference nor aversion. This study and another by Cunningham (Cunningham et al. 1992) utilizing genetically distinct strains of mice, as well as the distinct difference in place preference between mouse and rat subjects, suggest that the strength of ethanol's rewarding effect in conditioned place preference paradigms is dependent upon the genetic contributions of an animal.

The paradox between extensive ethanol abuse in humans and the difficulty in initiating self-administration and conditioning place preference to ethanol in rats is not as commonly seen with other drugs of abuse and highlights the complexities in understanding the motivational properties of ethanol. By definition, ethanol is reinforcing because animals will perform an operant response to obtain it. However, ethanol has several properties which make it unique from other drugs of abuse and make interpretation of its motivational effects more complex. Unlike other drugs, ethanol has nutritional value and can serve as a calorie source. Similarly, it also has fluid properties which, in some circumstances, may be rewarding. What is not clear and what is truly of interest, is whether ethanol's pharmacological effects are rewarding. The results from this series of experiments, although not aimed at evaluating this aspect, would suggest that they are not. Animals experiencing lower ethanol blood concentrations preferred ethanol to a greater degree than did subjects experiencing higher blood ethanol concentrations. In these studies and other, rats have consistently avoided taste and environmental cues paired with ethanol, unless other manipulations, such as extensive preexposure or use of other appetitive substances, such as food or sucrose, are also employed. As Experiments 2 and 4 suggested, use of other reinforcers to induce ethanol consumption or preference may actually be altering ethanol's pharmacological effects via their potential impact on blood ethanol

concentrations. Because of their increased complexity, humans, apparently unlike rats, may find some pharmacological properties of ethanol reinforcing based on characteristics not appreciated by rats. The decreased inhibition produced by ethanol, though helpful in social arenas, would not generally benefit animals, and may actually be detrimental to their survival. Ethanol is often used to "treat" emotional and psychological symptoms and to induce sleep in humans, factors presumably unappreciated by rats subjects

As Experiment 2 demonstrated, the motivational properties of ethanol are subject to conditioning through association with a rewarding stimulus. This may not be a surprising finding, considering the associations that are made with alcohol in humans, as television advertising would attest. However, studies in which ethanol is purported to be reinforcing that include manipulations to alter its consumption and preference through use of other rewarding substances should be interpreted carefully with respect to the impact of these manipulations on pharmacological effect and the potential for conditioned motivational influences.

APPENDIX

Establishment of Experimental Parameters for Place Conditioning (Experiments 7 and 8)

Experiment 7: Establishment of Floor Textures for Place Conditioning

Rationale

The purpose of this experiment was to evaluate preferences for different tactile stimuli in drug naive animals in the absence of conditioning. The goal of the study was to establish two floor textures of relatively equal inherent valence to employ as discriminative stimuli in a Pavlovian differential conditioning study of place preference using ethanol as the unconditioned stimulus. The rationale and advantages to using this type of design are discussed in Experiment 6.

Material and Methods

Subjects

Subjects were 12 adult male Holtzman albino rats weighing between 370 and 399 g prior to habituation [$X (\pm SEM) = 383 (3) \text{ g}$]. They were housed individually as described in Experiment 1. Food and water were available ad lib, except during the 30-min experimental sessions as described below.

Apparatus

The apparatus used in this study was that described in Experiment 6.

Procedure

Following 2 d acclimation to the colony room, during which the animals were weighed each morning and then left undisturbed, exposure to the place chambers was initiated. The experimental procedure consisted of two phases: habituation (one session) and preference testing of the previously described grid (G), hole (H), and mesh (M) floors (six sessions). The experimental design is outlined in Table 8.

The general procedure was similar for both phases. Subjects were weighed daily and the home rack was transferred from the colony to a holding area near the place conditioning room. They remained in the holding room undisturbed for 60 min prior to the start of the daily sessions.

Animals were divided into three groups of four subjects each. Each group experienced a single daily session, during which they were transported to the testing room and immediately placed into one of the four individual conditioning chambers. They remained in the boxes undisturbed for 30 min while their activity levels and positions were recorded. Following the 30-min session, subjects were removed and returned to the animal carrier where they remained for 10 min while the boxes were cleaned and prepared for the next group. Following this delay, the animals were returned to the home rack and the subsequent group obtained. After

TABLE 8 -- Design for Experiment 7: Floor Texture Preference

Phase 1: HABITUATION (one 30-min session)

All subjects --> Solid aluminum floor

Phase 2: PREFERENCE TESTING (six 30-min sessions)

All subjects --> GM, GH, HM, HG, MG, and MH in a series
of random exposures

the final daily session, all subjects remained in the home rack for 60 min before the rack was returned to the colony room.

Habituation. The purpose of this single 30-min session was to acclimate the animals to the novelty and stress associated with handling and exposure to the apparatus. The floor of the place conditioning chamber consisted of a solid aluminum sheet. This floor texture was not used during floor preference testing.

Floor Preference. Six d of floor preference testing followed the single habituation trial. During this phase, the floor halves in each conditioning chamber were of different textures. Exposure to the three floor textures (G, H, and M) was such that all subjects were tested with all possible combinations and positions of the textures (GM, GH, HM, HG, MG, and MH). Subjects were exposed to all three textures before any combination was repeated with the floor positions reversed. Each subject received four exposures to each texture. Animals were assigned randomly for exposure to the possible sequences, with each subject receiving a different order of exposures. The amount of time spent on each surface during the 30-min test sessions was measured to determine texture preference.

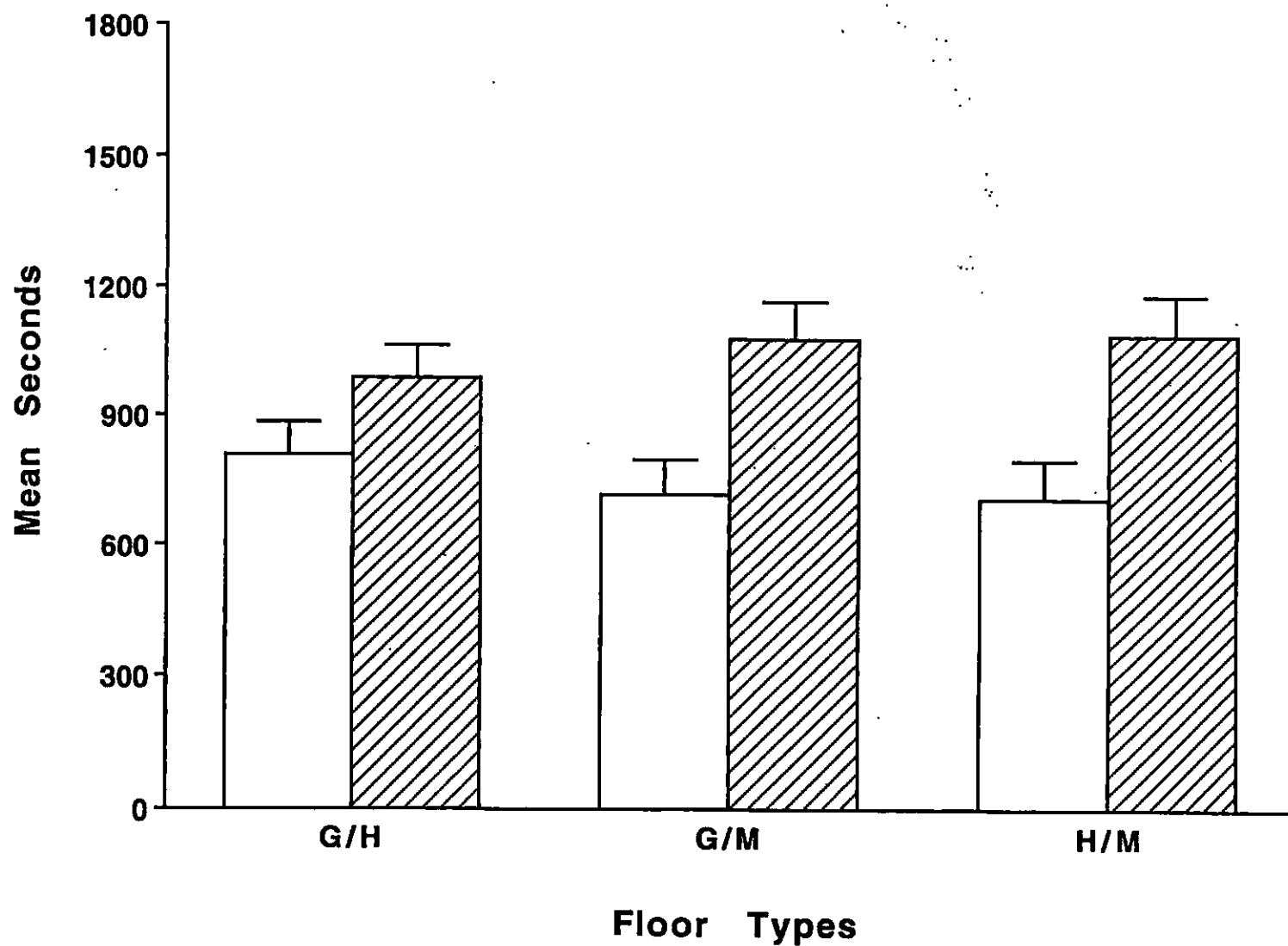
Results

Figure 19 demonstrates the mean time spent on each floor texture presented in various combinations. The maximum number of seconds per session was 1800. Subjects preferred the M surface over both the G and H floors to a similar degree, but showed similar preference for the H and G textures when they were presented together.

Time spent on each floor type in the various combinations was compared by t-test with an expected mean value of 900 sec, which is the value predicted if there are no differences in floor preference. Analysis demonstrated that the period of time in contact with the G floor, when it was presented with the M surface [$t(df=11) = 1.89, p < 0.05$, one-tailed], was significantly less than the mean. Difference in time spent on the H surface relative to the mean when the M texture was the alternative, approached significance [$t(df=11) = 1.77, 0.05 < p < 0.10$]. However, time on the G floor, when presented with the H texture [$t(df=11) = 1.24, p > 0.10$] most closely approximated the value expected on the basis of chance, suggesting that preference for these textures was not significantly different.

Figure 19: Mean ($\pm$ SEM) seconds spent over two 30-min trials on grid (G), hole (H), and mesh (M) textures presented in various combinations. All subjects were exposed to all three textures before any combination was repeated with floor positions reversed.

Relative Floor Preference



Discussion

The purpose of this experiment was to identify tactile cues of equivalent valence to employ as conditioned stimuli in a balanced paradigm of conditioned place preference (van der Kooy, 1987). In a series of 30-min trials in which drug-free subjects were given simultaneous exposure to two textural surfaces, rats spent less than half of the trial on the G and H surfaces when the M floor was the alternative. When G and H floors were presented together, animals spend about half of the trial on each surface. The inherent preference for M could be due to the similarity between this floor type and that of the home cage. The lack of a significant difference in the amount of the trial spent on the G and H floors suggests that these surfaces have equivalent valence prior to place conditioning.

Based on these findings, it was determined that the G and H textures would serve as discriminative stimuli in a place conditioning paradigm, while the M surface would be used during the habituation phase.

Experiment 8: Establishment of Trial Duration for Place Conditioning

Rationale

The purpose of this experiment was to evaluate the impact of duration of exposure to ethanol-paired tactile cues on the degree of aversion to those stimuli.

Mice have been reported to demonstrate greater place preference to ethanol-paired tactile cues when exposed to the cues for 5 min immediately following injection with ethanol versus when the trial duration was 30 min (Cunningham & Prather, 1992). The 5-min exposure group also demonstrated increased sensitization to the activating effects of ethanol over conditioning trials relative to the 30-min group.

Although previous experiments consistently failed to induce place preference to ethanol-paired cues in rats in the absence of such manipulations as extensive ethanol pre-exposure (Reid et al., 1985; Bozarth, 1990) or pairing ethanol with food delivery (Stewart & Grupp, 1981, 1985), as previously discussed, it was hoped that by altering the duration of exposure to the ethanol-associated tactile cues, a difference in the degree of place aversion could be measured. Once a place conditioning paradigm is established, the influence of further manipulations of ethanol valence, such as secondary conditioning, could be appreciated.

Material and Methods

Subjects

Subjects were 32 adult male Holtzman albino rats weighing between 379 and 431 g prior to habituation [$X (\pm \text{SEM}) = 400 (2) \text{ g}$]. They were housed individually as described in Experiment 1. Food and water were available ad lib, except during the experimental sessions as described below.

Apparatus

The apparatus used was that described in Experiment 6. The results of Experiment 7 (see Appendix) revealed that rats showed an inherent preference for the M surface over the G and H floors, but little bias between the G and H textures. Thus, the M floor was used for the habituation phase and the G and H textures were used as discriminative stimuli for place conditioning in this experiment.

An ethanol solution was prepared for injection by diluting the appropriate volume of 95% ethanol with normal saline to yield a concentration of 15% ethanol (v/v). Dosage was maintained at 1.0 g/kg (8.4 ml/kg of 15% ethanol) throughout the experiment.

Procedure

Following 9 d acclimation to the colony room, during which the animals were weighed daily, exposure to the place apparatus was initiated. The experimental procedure consisted of three phases: habituation (one session), place conditioning (four sessions), and place testing (two sessions). The experimental procedure is outlined in Table 9. In all three phases, the animals were weighed daily and the home rack was transferred from the colony to a holding area near the place conditioning testing room. Subjects remained in the holding room undisturbed for 60 min prior to the start of the daily sessions, and for 60 min after the last group had finished before returning to the colony room.

TABLE 9 -- Design for Experiment 8: Trial Duration Effects

Phase 1: HABITUATION (1 session)

All subjects injected with Saline (8.4 ml/kg) and given 30-min access to Mesh floor

Phase 2: PLACE CONDITIONING (8 two-d sessions with + and - trials alternating daily)

<u>Group</u>	<u>Subgroup</u>	<u>+ Trial</u>	<u>- Trial</u>
5	Grid+	Ethanol-->Grid x 5 min	Saline-->Hole x 5 min
5	Grid-	Ethanol-->Hole x 5 min	Saline-->Grid x 5 min
30	Grid+	Ethanol-->Grid x 30 min	Saline-->Hole x 30 min
30	Grid-	Ethanol-->Hole x 30 min	Saline-->Grid x 30 min

Ethanol dose was 1.0 g/kg and Saline volume was 8.4 ml/kg

Phase 3: PLACE TESTING (two 60-min sessions)

All subjects injected with Saline and given simultaneous access to the Grid and Hole textures

Habituation. A single habituation trial was given. The purpose of this phase was to decrease the stress and novelty of handling, injection, and exposure to the place conditioning apparatus. A mesh floor was used in the conditioning apparatus during the habituation trial to avoid latent inhibition to the textured floors used in place conditioning and testing.

During habituation, subjects were transported in groups of four from the holding room to the testing room. Each subject was given an i.p. injection of normal saline (8.4 ml/kg) and placed immediately into the conditioning apparatus for the 30-min session. At the end of the 30-min trial, subjects were removed and returned to the animal carrier for 10 min while the chambers were thoroughly cleaned and prepared for the subsequent group. Following this delay, the animals were returned to the home rack and the next group obtained. This process was repeated for each of the eight squads of animals.

Place Conditioning. The place conditioning phase consisted of four 2-d trial blocks. The procedure was similar to that of the habituation phase; however, subjects were given i.p. injections of ethanol (15% v/v) alternated daily with injections of saline (8.4 ml/kg) prior to placement in the apparatus. Subjects were divided into two groups which differed on length of the conditioning sessions. Animals in Group 5 were allowed 5 min of exposure to the conditioning boxes and floor textures following injection, while Group 30 received 30 min of access.

A single floor texture, either G or H, was used on both sides of the chambers during place conditioning. Half the subjects received ethanol prior to exposure to the G floor (Grid+) and half received ethanol prior to experience with the H surface (Grid-). Saline was paired with the alternate floor type.

Fluid exposures were counterbalanced such that, on any given day, half the subjects received ethanol and half were given saline. Floor textures also were counterbalanced such that half the boxes during any given session contained G and

half contained H floors. The textures were each present in each chamber for four sessions over the eight daily conditioning periods.

Place Testing. Two trials of place testing were given. This phase differed from conditioning in that all subjects were injected with saline (8.4 ml/kg) and placed into the conditioning chambers for 60 min. Both G and H floor textures were present in each chamber simultaneously. The amount of time spent on each surface was measured to determine relative preference. Floor positions were counterbalanced within each squad and for each box over the eight squads, and were reversed for each subject from the first to the second test trial.

Results

Conditioning

Figure 20 shows the mean ($\pm$ SEM) activity counts per minute during the first 5 min after injection for Groups 5 and 30 during CS+ (ethanol - left panel) and CS- (saline - right panel) trials. Data are collapsed across Grid+ and Grid- groups since they did not differ significantly. During the first 5 min, there was no significant difference in activity between CS+ and CS- trials in either group. An overall decrease in activity across trials was seen with both ethanol and saline, suggesting that the changes in activity were due to habituation to the novelty of the preference chamber and to the activating effects of the handling and injection procedures and not to the development of tolerance or sensitization to the effects of ethanol on activity. Three-way repeated measures ANOVA, with trial duration as the between-groups factor and CS trial type and trials as repeated measures factors, of activity counts collapsed across sample periods during the four conditioning trials for Groups 5 and 30 yielded a significant effect of trial [$F(3,90)=15.1$, $p<0.01$] with a decrease in activity across trials for all groups and trial types. There were no significant effects of trial duration or interactions.

Figure 21 shows mean ($\pm$ SEM) activity counts per minute for the entire 30-min exposure during Conditioning Trials 1 (left panel) and 4 (right panel) for Group 30. A consistent decline in activity was observed in both CS+ and CS- conditions over the 30-min trials. However, in contrast to what was seen in Group 5, where there were no activity differences between CS+ and CS- exposures, rats exposed to ethanol in the CS+ condition were, in general, less active than when tested with saline in the CS- condition. Two-way repeated measures ANOVA, with CS trial type and trials as repeated measures factors, yielded significant

Figure 20: Mean ($\pm$ SEM) activity counts per minute during the first 5 min after injection for Groups 5 and 30 for each of the four CS+ (ethanol - left panel) and CS- (saline - right panel) trials. Group 5 received i.p. ethanol followed by access to the conditioning chamber for 5 min after injection, while Group 30 was exposed to the place conditioning apparatus for 30 min following injection. Half the animals in each group received ethanol injections paired with the Grid texture and saline in association with the Hole surface (Grid+). The remaining subjects in each group had this combination reversed (Grid-). Data are collapsed across Grid+ and Grid- groups.

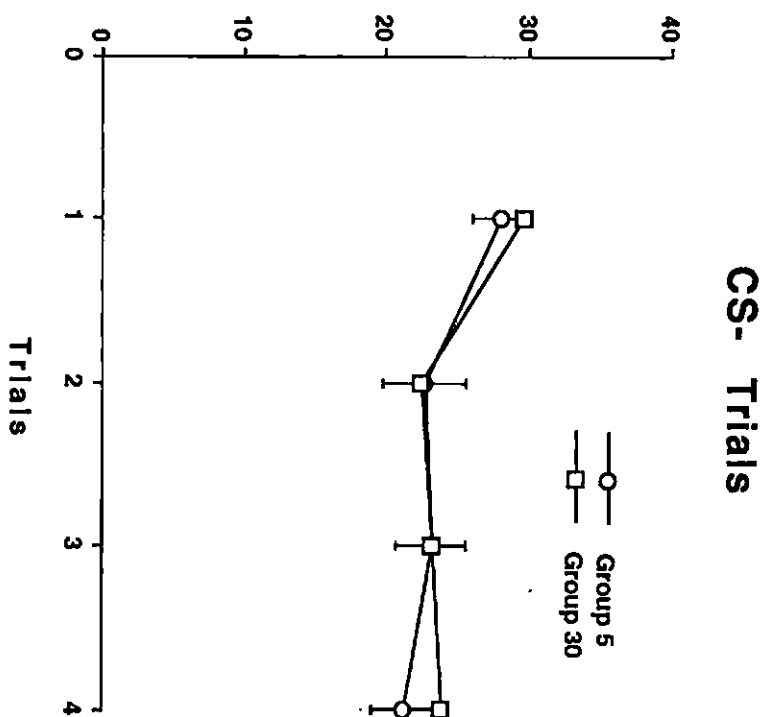
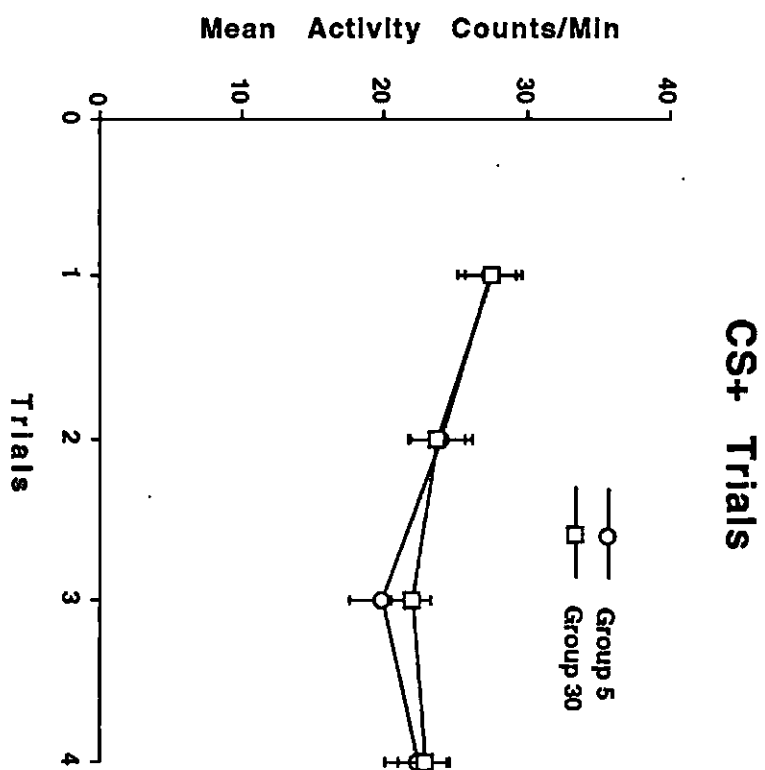
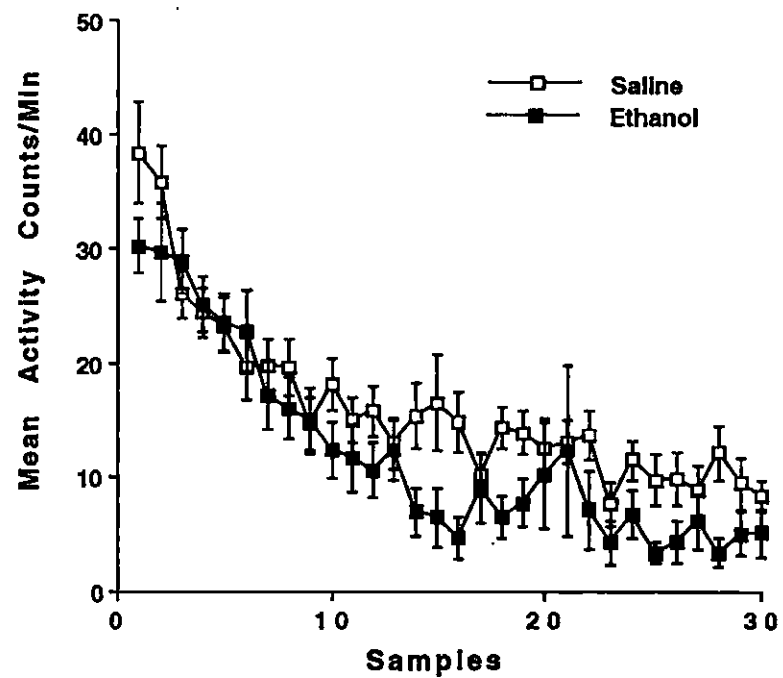
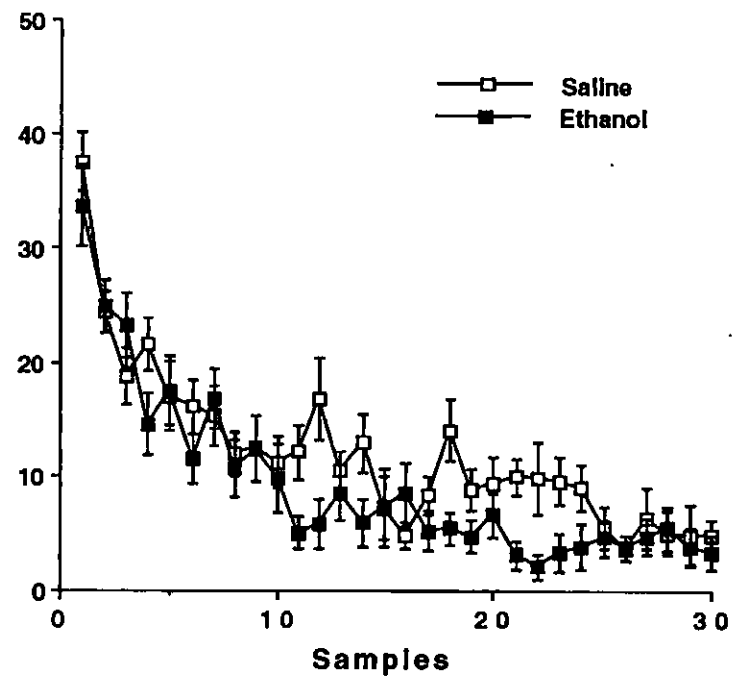


Figure 21: Mean ($\pm$ SEM) activity counts per minute following both ethanol and saline injections during 30-minute place conditioning trials 1 (left panel) and 4 (right panel) for Group 30. Data are collapsed across Grid+ and Grid- subgroups. Subgroup designations are outlined in the legend for Figure 20.

Group 30 - Trial 1



Group 30 - Trial 4



effects of trial type [$F(1,15)=17.7, p<0.01$] and trials [$F(3,45)=15.6, p<0.01$], with animals showing lower activity in response to the CS+ than to the CS-.

Place Testing - Activity

Figure 22 shows the mean ($\pm$ SEM) activity counts for Groups 5 and 30 during Tests 1 and 2. During both tests, Group 5 was more active than Group 30, with both groups showing lower activity on the second test trial than on the first. Two-way repeated measures ANOVA, with trial duration as the between-groups factor and test as the repeated measures factor, revealed significant effects of trial duration [$F(1,30)=10.4, p<0.01$] and test [$F(1,30)=20.1, p<0.01$], with no significant interaction.

Place Testing - Choice

Figure 23 depicts the mean ($\pm$ SEM) seconds per minute spent on the grid floor by each conditioning group during the first (left panel) and second (right panel) 60-min preference tests. Rats receiving ethanol paired with the grid floor (Grid+) during conditioning spent less time on the grid floor than did rats receiving ethanol paired with the alternate floor type (Grid-) during both tests, demonstrating an aversion to the texture paired with ethanol. During Test 1, the difference between Grid+ and Grid- rats in time spent on the grid surface was greater for Group 30 than for Group 5. This suggests that greater aversion to the ethanol-paired texture occurred following a 30-min than a 5-min trial. The degree of aversion shown toward the ethanol-paired surface decreased from the first to the second test as a result of extinction arising from exposure to the CS in the absence of ethanol during Test 1.

Two-way ANOVA with trial duration and conditioning subgroup as between-groups factors, of mean sec per minute spent on the grid floor collapsed across the 60 min of choice testing in Test 1, indicated a significant effect of

Figure 22: Mean ($\pm$ SEM) activity counts per minute for Groups 5 and 30 during the two 60-min place testing trials, during which simultaneous access to the Grid and Hole textures was provided. Group designations are as described in the legend for Figure 20.

Activity - Tests 1 and 2

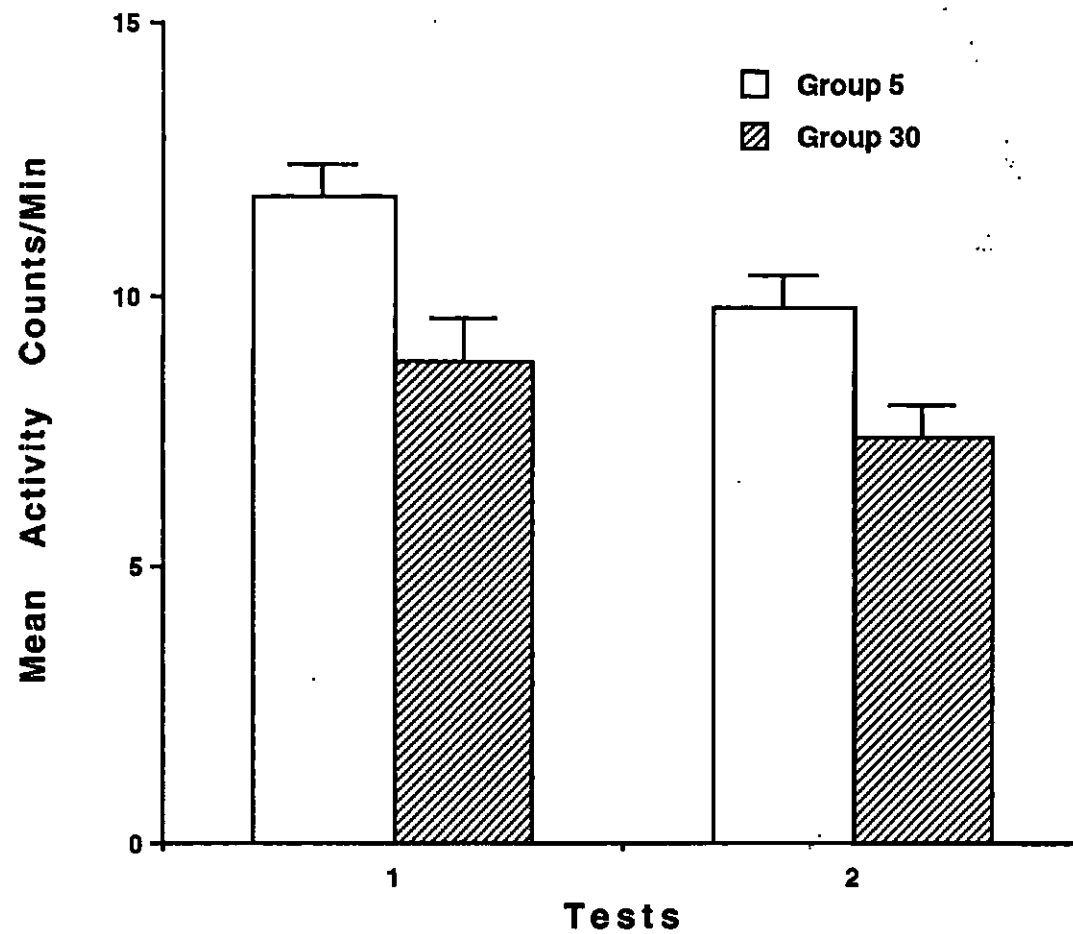
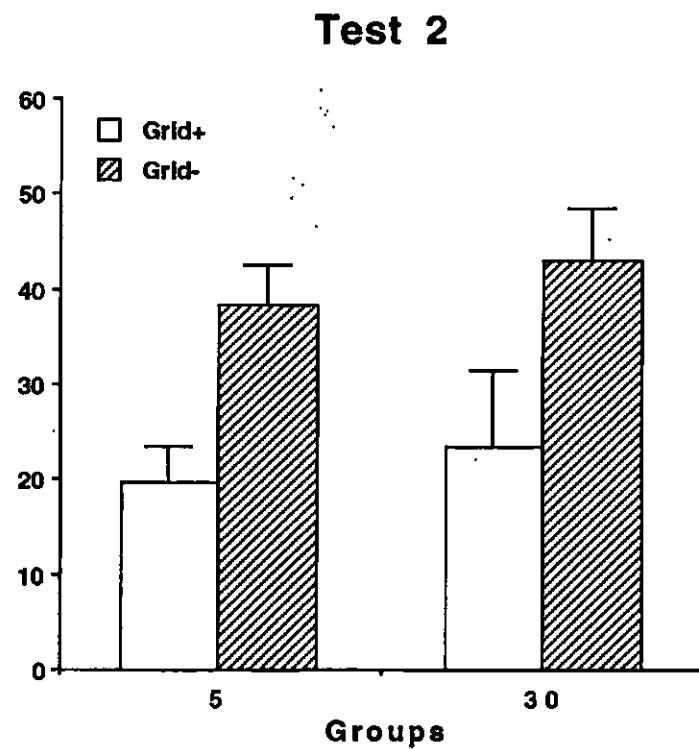
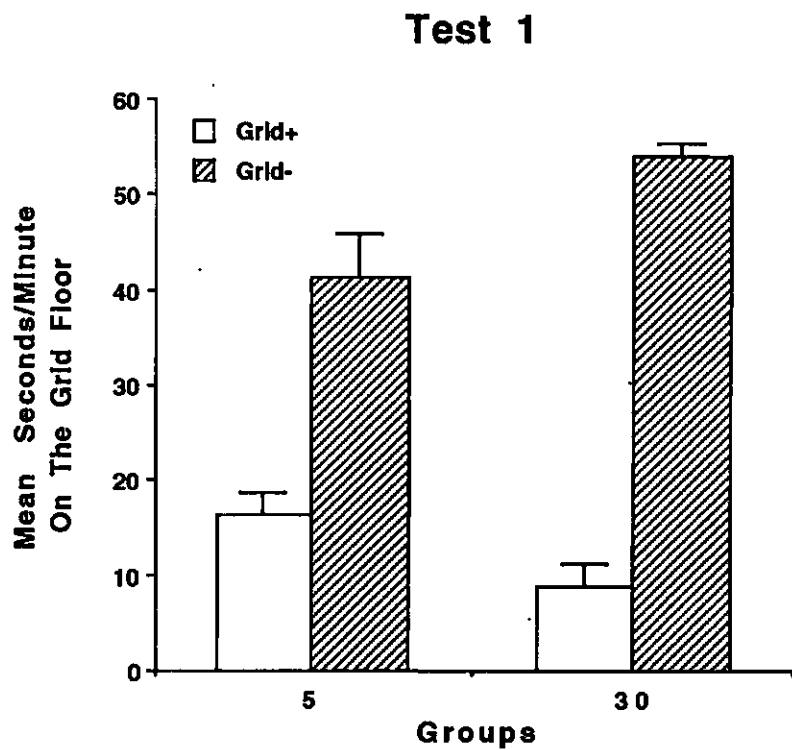


Figure 23: Mean ($\pm$ SEM) seconds per minute spent on the grid floor by each conditioning subgroup during the first (left panel) and second (right panel) 60-min preference tests. Group and subgroup designations are as outlined in the legend for Figure 20.



conditioning subgroup [$F(1,28)=148.2$, $p<0.01$], with the Grid+ subgroups spending less time on the grid floor relative to the Grid- subgroups. There was also a significant interaction of trial duration x conditioning subgroup [$F(1,28)=12.1$, $p<0.01$], due to the greater difference between the Grid+ and Grid- subgroups in Group 30 than in Group 5 in time spent on the grid floor. Direct comparisons between conditioning subgroups revealed that the Grid+ conditioning subgroup spent significantly less time on the grid floor in Group 30 compared with Group 5 [$F(1,14)=4.5$, $p<0.05$], while the Grid- conditioning subgroup in Group 30 spent significantly more time on the grid floor than did Group 5 [$F(1,14)=7.6$, $p<0.05$].

Two-way ANOVA, with trial duration and conditioning subgroup as between-groups factors, of mean sec per minute on the grid floor in Test 2 showed a significant effect of conditioning subgroup [$F(1,28)=11.5$, $p<0.01$] with no significant effects of trials duration or interactions. Grid+ rats spent significantly less time on the grid floor than Grid- rats in Test 2, with this difference being unaffected by trial duration.

Discussion

In this study, rats demonstrated an aversion to a tactile stimulus paired with a peritoneal dose of 1.0 g/kg ethanol. Significantly greater aversion was seen in subjects given 30-min exposures to the ethanol-paired cues following injection than in those given only 5-min exposures. A similar study done in mice revealed neither preference nor aversion to 1.0 g/kg ethanol, and preference at doses between 2.0 and 4.0 g/kg (although identical dosages in mice and rats are not generally equivalent). Mice also demonstrated greater preference for ethanol-paired tactile cues if exposed to those cues for only 5 min than for 15 or 30 min following ethanol injection (Cunningham et al., 1992; Cunningham & Prather, 1992).

In mice, activity levels have been shown to be highest during the first few minutes after injection and to decline over the course of the trial. Sensitization to this ethanol-induced activation also has been observed over successive trials. This association between higher activity and greater preference is consistent with the suggestion that activity and preference may share a common mechanism (Wise & Bozarth, 1987). In contrast, similar doses of ethanol appear to inhibit activity in rats (Frye & Breese, 1981; Cunningham et al., 1992). Although ethanol did not affect activity during the first 5 min following injection in this experiment, significantly lower levels of activity were observed over the 30-min trial relative to rats receiving saline. In addition, rats which had received only 5-min exposure to the ethanol-paired cues during conditioning showed significantly greater activity during the 60-min preference test than did those receiving 30-min access. This may reflect increased exploratory behavior due to their decreased familiarity with the experimental apparatus relative to Group 30.

Because a greater degree of aversion was demonstrated in subjects receiving a 30-min exposure to the tactile cues, this trial length was chosen for

place conditioning in Experiment 6 so that factors potentially attenuating ethanol's aversive effects could be observed more easily.

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