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The Role of Acetaldehyde in the Mediation of Some of the Psychopharmacological Effects of Ethanol

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ABSTRACT

THE ROLE OF ACETALDEHYDE IN THE MEDIATION OF SOME OF THE PSYCHOPHARMACOLOGICAL EFFECTS OF ETHANOL

Karen Spivak

The roles of central acetaldehyde and brain aldehyde dehydrogenase (aldh) were examined with regard to their involvement in the psychopharmacological effects of ethanol. In Experiment 1, it was observed that centrally-administered acetaldehyde produced a time and dose dependent effect on locomotion. Results indicated that acetaldehyde was pharmacologically active in brain. Further evidence for central involvement of acetaldehyde in locomotion induced by ethanol was provided by Experiment 2. It was observed that animals pretreated with cyanamide (an aldh inhibitor or 4-methylpyrazole (an alcohol dehydrogenase inhibitor) + cyanamide, demonstrated suppression of increased locomotor activity induced by ethanol. These results suggested that changes in central levels of acetaldehyde due to brain aldh inhibition, may play a role in mediating this effect. In Experiment 3, the role of central acetaldehyde in mediating the conditioned taste aversion (CTA) induced by ethanol was examined, using the same enzyme manipulations as in Experiment 2. In comparison to a group conditioned with a subthreshold dose of

ethanol (.4 gm/kg), animals pretreated with both cyanamide and 4-methylpyrazole + cyanamide, exhibited a reduction in saccharin intake. Results indicated that centrally-acting and not peripherally-acting acetaldehyde may play a role in the aversive properties of ethanol. The results of these experiments indicate an important role for brain aldh in mediating the central effects of acetaldehyde. A role for central acetaldehyde in mediating the positive reinforcing properties of ethanol has previously been demonstrated. The present studies additionally implicate a role for centrally-acting acetaldehyde in the mediation of the locomotor and CTA inducing effects of ethanol.

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Table of Contents

Table of Contents (Cont'd)

A variety of social, environmental and biological factors have been found to contribute to the individual variations in alcohol consumption. While approximately 90% of adult North Americans and Europeans drink alcohol at some time during their lives, only about 5-15% suffer from persistent and pervasive alcohol related problems that could be considered as constituting alcoholism (Hagnel and Tunvig, 1972; Weissman, Myers and Harding, 1980). Although various psychosocial factors may contribute to the development of alcohol abuse (see Goluke, Landeen and Meadows, 1983), recent research suggests an equally important role for biological factors (for review see von Wartburg and Buhler, 1984).

Some of the well-known acute psychopharmacological effects of alcohol consumption in humans are reflected in reports of euphoria (Freed, 1978), loss of motor control (see Mello, 1983) and lability of mood (Mello, 1983). In some instances, chronic exposure to ethanol can lead to psychological and physical dependence (Goluke et al, 1983). Animal models of human alcohol use have been developed by researchers in an effort to elucidate the precise biochemical mechanisms by which ethanol induces these effects.

Alcohol self-administration has been established in several laboratory species including monkeys (Winger and Wood, 1973), rats (Smith, Werner and Davis, 1976) and dogs (Jones, Essig and Creager, 1970). These studies

demonstrated that under various schedules of reinforcement, animals would acquire an operant response maintained by oral consumption (Meisch and Beardsley, 1975; Sinclair, Walker and Jordan, 1973), intravenous (Karoly, Winger, Ikomi and Woods, 1978; Winger and Woods, 1973) or intragastric (Amit and Stern, 1969; Davis, Werner and Smith, 1979; Smith et al, 1976) infusions of ethanol. Furthermore, laboratory rats have also been shown to voluntarily consume large amounts of ethanol despite its apparent aversive taste (e.g. Richter and Campbell, 1940; Wilson, 1972).

It should be noted that in most of the ethanol self-administration studies, blood alcohol levels were never high enough or continuous enough to produce the blood alcohol levels required for physical dependence (see Amit, Sutherland and White, 1975). These results therefore, suggest that ethanol has positive reinforcing properties which may account for the initiation and maintenance of ethanol self-administration.

In recent years, a great deal of evidence has emerged suggesting an important role for acetaldehyde, the primary metabolite of ethanol, in some of the behavioral, pharmacological and positive reinforcing properties of ethanol (for review, see Lindros, 1978; Amir, Brown and Amit, 1980). Numerous studies have demonstrated that acetaldehyde, when present in the peripheral circulation at high concentrations following ethanol administration, can evoke

various aversive and physiological symptoms in both humans and experimental animals (see Lindros, 1978).

In addition to these well-established aversive effects, it has also been demonstrated that naive laboratory rats will learn to perform an operant maintained by response-contingent intracerebroventricular (Amit, Brown and Rockman, 1977; Brown, Amit and Rockman, 1978) or intravenous (Myers, Ng and Singer, 1985) infusions of acetaldehyde. These results suggest that acetaldehyde may possess positive reinforcing properties (Amir et al, 1980; Brown et al, 1978).

It would appear therefore, that acetaldehyde may act either as an aversive or positive reinforcing agent and may mediate some of ethanol's actions. Given that acetaldehyde is the primary metabolite derived from ethanol, it has been postulated that the enzymes responsible for the formation and degradation of acetaldehyde may play a role in a number of the psychopharmacological effects of ethanol, including voluntary ethanol consumption (Amir, 1977; Aragon and Amit, in press; Lindros, Koivula and Eriksson, 1975), locomotor activity (Aragon, Spivak and Amit, 1985) and toxicosis (Mizoi et al, 1983). Accordingly, the contribution of these enzyme systems in modulating the pharmacological and behavioral effects of ethanol, may provide invaluable information concerning acetaldehyde's role in ethanol's actions. More importantly, it has been suggested that genetic or predispositional factors towards alcoholism may

be potentially related to differences in the metabolism of ethanol. Consequently, a better understanding of the interaction between the enzyme systems and the metabolic fate of acetaldehyde may well provide critical insights into the motivational properties of ethanol and alcohol abuse.

The following sections will review the peripheral mechanisms that mediate the formation and metabolism of acetaldehyde and the evidence for the occurrence of acetaldehyde both in the periphery and the brain. This will be followed by a review of the psychopharmacological effects of acetaldehyde and the evidence suggesting a role for centrally-acting acetaldehyde in some of the pharmacological effects of ethanol.

Metabolism of Ethanol: Acetaldehyde Formation

Ethanol is highly soluble in water and after oral administration in both humans and animals, is readily absorbed from the gastrointestinal tract where it diffuses rapidly and uniformally throughout the body (Erickson, 1979). The elimination of absorbed ethanol is extremely efficient with over 90% being metabolized in the body (Erickson, 1979).

There is general agreement that the principal enzyme responsible for the initial metabolism of ethanol to acetaldehyde is NAD+ - dependent alcohol dehydrogenase (see Hawkins and Kalant, 1972). Alcohol dehydrogenase is

located primarily in the cytosolic region of the cell (Havre, Margolis, Abrams and Landau, 1976) and is abundant in the liver where over 90% of ethanol ingested is oxidized to acetaldehyde (Hawkins and Kalant, 1972). Smaller amounts of alcohol dehydrogenase have been detected in extrahepatic tissue including the kidneys, gastric and intestinal mucosa (Lundquist, 1971; Raskin and Sokoloff, 1972) as well as the brain (Buhler, Pestalozzi, Hess and von Wartburg, 1983; Raskin and Sololoff, 1968, 1970).

Although alcohol dehydrogenase is the principal ethanol-oxidizing enzyme in the liver, ethanol may also be metabolized by the NADPH- and O_2- dependent microsomal ethanol-oxidizing system (MEOS: Lieber and DeCarli, 1968; Lieber, 1977) and by the peroxidatic H_2O_2-dependent catalase system (Keilin and Hartree, 1945; Lieber, 1977). The participation of these non-alcohol dehydrogenase pathways in ethanol metabolism is minimal under normal conditions. However, some investigators argue that at high blood ethanol concentrations (> 20 mM) and after chronic ethanol ingestion, these alcohol metabolizing systems may have a more active role in ethanol metabolism and subsequently enhance acetaldehyde formation (e.g. Hawkins and Kalant, 1972; Lieber, 1977). Nonetheless, other investigators have found very little evidence to support any relevant contribution for synthesis of acetaldehyde from non-alcohol dehydrogenase pathways in vivo at high blood ethanol levels or after chronic administration

(Khanna, Lindros, Israel and Orrego, 1977; Lindros, Salaspuro and Pikkarainen, 1977).

The functional roles of the MEOS and catalase systems in acetaldehyde synthesis is still controversial. For example, the H_2O_2-mediated ethanol peroxidation by catalase is controlled by the rate of peroxide production rather than the amount of catalase (Boveris, Oshino and Chance, 1972). The efficiency of the catalse system in ethanol metabolism may thus be dependent on the presence of sufficient peroxide. The role of MEOS is even less clearly understood. Although there is evidence suggesting a significant contribution of MEOS to ethanol metabolism in vitro (see Lieber, 1983), activity of the MEOS system in intact liver cells has not been clearly demonstrated (Khanna and Kalant, 1977).

Metabolism of Ethanol: Elimination of Acetaldehyde

Acetaldehyde that is formed by the oxidation of ethanol is rapidly metabolized to acetate by the NAD-dependent enzyme aldehyde dehydrogenase (Hawkins and Kalant, 1972; Lundquist, 1971; von Wartburg, 1980). Because aldehyde dehydrogenase is the primary enzyme responsible for oxidizing biogenic aldehydes to acids, it is widely distributed in mammalian tissues (Deitrich, 1966) and can be found in the mitochondrial, cytosolic and endoplasmic reticular regions of the cell (Pettersson and Tottmar, 1982; Tottmar, Pettersson and Kiessling, 1973).

Although a large quantity of aldehyde dehydrogenase is found in the liver, considerable levels are found in the kidney, small intestine and brain as well (Deitrich, 1966; Koivula, Turner, Huttunen and Koisvusalo, 1981). In addition, there are variations of aldehyde dehydrogenase (isozymes) having different subcellular organization, specificity and kinetic constants (Deitrich, 1966; Harada, Agarwal and Goedde, 1978). With improved methods for cellular fractionation and enzyme assays, hepatic mitochondrial aldehyde dehydrogenase, which has a high affinity to acetaldehyde, has been implicated as the principal enzyme responsible for acetaldehyde oxidation (Lindros Vihma and Forsander, 1972; Socaransky, Aragon, Amit and Blander, 1984; Tottmar and Marchner, 1976).

As with ethanol metabolism, the liver is the primary site of acetaldehyde elimination and is capable of metabolizing 90-95% of acetaldehyde produced during ethanol oxidation (see Lindros, 1978). Acetaldehyde metabolism can occur in extrahepatic tissue including the kidney, muscle (Deitrich, 1966) and the brain (Deitrich, 1966; Mukherji, Kashiki, Ohyanagi and Sloviter, 1975). Hepatic aldehyde dehydrogenase however, is so efficient in eliminating acetaldehyde, that only part of the extrahepatic capacity is normally used (Lindros, 1978).

Occurrence of Acetaldehyde in the Periphery

Most of the acetaldehyde formed following the consumption of moderate quantities of ethanol is rapidly oxidized and only minute amounts of acetaldehyde can be detected in the peripheral circulation (Eriksson, 1977; Eriksson and Sippel, 1977). When larger doses of ethanol (> 2 gm/kg) are administered, the rate of ethanol oxidation may exceed the rate of the hepatic acetaldehyde elimination capacity, resulting in peripheral acetaldehyde accumulation (Lindros et al, 1972; Raskin and Sokoloff, 1972). However, contrary to reports of significant blood acetaldehyde levels, a recent study by Eriksson, Atkinson, Petersen and Deitrich (1984) using mice demonstrated that no acetaldehyde could be detected in blood even after a challenge with a high dose of ethanol (3 gm/kg), when proper control samples were included in the assay procedure.

Chronic ingestion of ethanol may also lead to elevated blood acetaldehyde levels (Lindros, Stowell, Pikkarainen and Salaspuro, 1980). It is known that prolonged ethanol exposure may disrupt liver function (e.g. Hasumura, Teschke and Lieber, 1975). One such consequence of this disruptive influence may be a decrease in hepatic mitochondrial aldehyde dehydrogenase activity, thereby reducing the capacity of the liver to oxidize acetaldehyde and subsequently increasing circulating acetaldehyde levels (Hasumura et al, 1975; Jenkins and Peter, 1980). It should be noted however, that demonstrations of increased

(Amir, 1978a) or unchanged (Redmond and Cohen, 1971) aldehyde dehydrogenase activity after prolonged ethanol exposure have also been reported. Due to this discrepancy across studies, only tentative conclusions can be drawn at this time.

Peripheral blood concentrations of acetaldehyde can also be increased by pharmacological agents that are capable of inhibiting hepatic aldehyde dehydrogenase. Two such agents widely used in the treatment of alcoholism are disulfiram (Antabuse) and the cyanamide derivative, calcium carbamide (Temposil) (Ritchie, 1970). In the presence of ethanol, these agents induce a reaction identified as the Disulfiram-alcohol-reaction (DAR: Truitt and Walsh, 1971; Kitson, 1977). Manifestations of this reaction include vasodilation, tachycardia, decrease in blood pressure, dizziness , nausea and vomitting (Kitson, 1977). In more severe cases, respiratory depression, cardiovascular collapse and death may occur (Jacobsen, 1952).

The effects of these compounds on both man and animal have been attributed to their ability to markedly inhibit aldehyde dehydrogenase activity, thereby causing an accumulation of acetaldehyde in the blood and tissue after ethahol administration (Kitsqn, 1977; Marchner and Tottmar, 1978). It has been demonstrated both in humans as well as laboratory animals, that the administration of disulfiram or cyanamide reduces voluntary consumption of ethanol (see Lindros, 1978). However, there have been other reports

indicating no change in voluntary ethanol intake in laboratory rodents following treatment with cyanamide (e.g. Amit, Levitan and Lindros, 1976). The discrepancy in studies may be due to procedural differences in cyanamide treatment as well as the use of different drinking schedules.

The widely accepted explanation for the demonstrated reductions in voluntary ethanol consumption is that acetaldehyde, at high levels in the blood (as a consequence of aldehyde dehydrogenase inhibition), is toxic and produces aversive effects (Lindros et al, 1975; Schlesinger, Kakihana and Bennet, 1966; Sellers, Naranjo and Peachey, 1981). This view has recently been challenged by Sinclair and Lindros (1981), who demonstrated that prevention of the accumulation of blood acetaldehyde by concurrent treatment with cyanamide and the alcohol dehydrogenase inhibitor 4-methylpyrazole, still resulted in the suppression of alcohol drinking in rats. These authors concluded that acetaldehyde accumulation in the periphery was not responsible for the suppression of alcohol drinking following cyanamide pretreatment.

However, the contention that high circulating levels of acetaldehyde may limit subsequent ethanol drinking has been strongly supported by investigations of the innate ethanol-sensitivity observed in some Orientals (Goedde, Harada and Agarwal, 1979; Mizoi et al, 1983; Wolff, 1972). These studies revealed that at least 50% of Japanese lack

the hepatic mitochondrial low km enzyme aldehyde dehydrogenase (Harada, Misawa, Agarwal and Goedde, 1980; Mizoi et al, 1983). Ingestion of low to moderate doses of alcohol in these individuals results in much higher blood acetaldehyde levels than that found in Caucasians after ingestion of similar amounts of ethanol. It would appear then, that elevated blood acetaldehyde levels may be a major factor contributing to the lower alcohol consumption observed in some Oriental societies (see Lindros, 1983).

It should be noted that in most cases, elevated blood acetaldehyde levels are detectable only when acetaldehyde levels are abnormally or artificially increased. However, under conditions in which animals voluntarily consume ethanol (i.e. free choice paradigm) (Lindros, 1978) and after moderate consumption in Caucasians, acetaldehyde cannot readily be detected in the blood (for review, see Eriksson, 1980; Lindros, 1983). Consequently, it has not been possible to determine the threshold levels of acetaldehyde in the blood that are necessary to exert some pharmacological effect (see Lindros, 1983).

Presence of Acetaldehyde in the Brain

It has been frequently reported that ethanol diffuses through body tissue and can be readily detected in brain tissue (e.g. Ritchie, 1970). Acetaldehyde, because of its high lipid affinity, can also easily diffuse through various organs including the brain (Akabane, 1971;

Lindros, 1978). However, attempts to measure or detect the presence of acetaldehyde in the brain following exposure to ethanol has yielded ambiguous results. Earlier studies reported the presence of acetaldehyde in the brain of ethanol-treated animals at levels equal to or greater than acetaldehyde levels measured in cerebral blood (Duritz and Truitt, 1966; Kiessling, 1962; Majchrowitz, 1973). The relatively high levels of acetaldehyde observed in these earlier studies have been disputed because of technical and methodological difficulties (Eriksson et al, 1984; Sippel, 1972). The problem with all these procedures was the spontaneous non-enzymatic formation of acetaldehyde by ethanol oxidation during sample preparation (Sippel, 1972; Truitt, 1970). Sippel (1972) demonstrated that the administration of thiourea to the deproteinized brain homogenate prevented the non-enzymatic release of acetaldehyde. When thiourea was used in the assay procedure of subsequent studies, acetaldehyde levels were extremely low or undetectable in brain tissue of rats after an injection of 3 gm/kg of ethanol (Eriksson and Sippel, 1977; Sippel, 1974). Acetaldehyde was detected in the brain only if cerebral blood levels exceeded 200 nM following pretreatment with cyanamide (Eriksson and Sippel, 1977). In contrast, Tabakoff, Anderson and Ritzman (1976) injected mice with 3 gm/kg of ethanol and detected very low brain acetaldehyde levels (about 6 nM) when the concentration of acetaldehyde in the blood was approximately 70 nM. In

addition, cerebrospinal fluid of rats has been shown to contain acetaldehyde at concentrations lower than that found in the blood but at levels appreciably higher than those found in the brain following ethanol administration (3 gm/kg) (Kiianmaa and Virtanen, 1978). Pettersson and Kiessling (1977) also demonstrated the presence of acetaldehyde in cerebrospinal fluid of rats. However, they reported a direct relationship between concentrations of acetaldehyde in the blood and cerebrospinal fluid. The discrepency may be due to the large number of methodological differences between these two studies.

The discrepency in the levels of acetaldehyde found in the brain and cerebrospinal fluid in the studies stated above, may be attributed to different experimental conditions (Eriksson and Sippel, 1977). However, what is apparent from these studies is that blood acetaldehyde levels after acute ethanol administration did not reflect acetaldehyde concentrations found in the brain.

Various theories have been proposed to account for the lack of a direct relationship between levels of acetaldehyde in the blood or cerebrospinal fluid and those found in the brain. It has been suggested that the cerebral capillary walls may act as a special enzymatic blood brain barrier, limiting the entry of circulating acetaldehyde to the brain (Eriksson and Sippel, 1977; Kiianmaa and Virtanen, 1978; Sippel, 1974; Tabakoff et al, 1976). However, the presence of acetaldehyde in cerebrospinal

fluid indicates that acetaldehyde can cross the blood brain barrier. It has been proposed that the presence of high affinity brain aldehyde dehydrogenase may result in lower levels of acetaldehyde in the brain than in the cerebrospinal fluid (Petterson and Kiessling, 1976). This notion is supported by recent work by Westcott, Weiner, Shultz and Myers (1980). Using a push-pull perfusion technique, these authors detected acetaldehyde in the interstitial fluid of rat brain (5-20 nM) after intragastric administration of ethanol (4.5 gm/kg). At the termination of this procedure, the rats were sacrificed and the brains were extracted for acetaldehyde determination using the gas chromatographic technique. Similar to the results of previous studies (Eriksson and Sippel, 1977; Kiianmaa and Virtanen, 1978), Westcott et al (1980) did not detect acetaldehyde in whole brain unless the blood levels of acetaldehyde were artificially elevated using the aldehyde dehydrogenase inhibitor, disulfiram. Westcott et al (1980) concluded that the presence of acetaldehyde in the extracellular fluid of the brain indicated that acetaldehyde could cross the blood brain barrier. Furthermore, the absence of acetaldehyde in whole brain tissue may have resulted from the rapid oxidation of acetaldehyde by brain aldehyde dehydrogenase. In addition, these authors suggested that acetaldehyde present in the interstitial fluid of the brain could impinge upon the environment of the neuron, thereby exerting some pharmacological effect.

Additional studies examining brain enzyme activity have suggested the possible presence of acetaldehyde in cerebral tissue. The brain does possess the necessary oxidative machinery for ethanol metabolism. Ethanol can be metabolized via brain alcohol dehydrogenase. Alcohol dehydrogenase has been detected in the brain (Buhler et al, 1983; Raskin and Sokoloff, 1970; 1972) and its activity has been reported to increase following chronic ethanol administration in mice (Raskin and Sokoloff, 1974). This finding suggested that central acetaldehyde formation may be enhanced during chronic ingestion of ethanol (Amir et al, 1980). Although alcohol dehydrogenase is present in the brain, its capacity to metabolize ethanol is extremely low and very little acetaldehyde is actually formed through this route (see Lindros, 1978).

Ethanol metabolism may also occur via the formation of reactive hydroxyl radicals during the autoxidation of ascorbate (Cohen, 1977). Another potential oxidative pathway may be via cytochrome P-450, whose presence in the brain has been established (Paul, Axelrod and Diliberts, 1977). There is, however, no direct evidence to support the role of these two systems in ethanol metabolism in the brain.

Cohen, Sinet and Heikkila (1980) presented biochemical evidence of ethanol oxidation in rat brain in vivo via the peroxidatic activity of brain catalase. In this study, treatment with ethanol prior to the administration of the

catalase inhibitor 3-amino-1,2,4-tryazole (AT), prevented the inhibition of catalase in rat brain suggesting that ethanol competed successfully with the inhibitor. These authors concluded that their results constituted indirect evidence for ethanol metabolism in the brain.

The brain also possesses the necessary oxidative machinery for the removal of acetaldehyde. Mukherji et al (1975) measured the incorporation of radioactivity in amino acids of the isolated rat brain after perfusion with [14]-c acetaldehyde. These results indicated that the rat brain was capable of metabolizing acetaldehyde. It has been suggested that aldehydes of the brain are the most likely candidates responsible for the elimination of acetaldehyde, since they have a high affinity towards this substrate (Duncan and Tipton, 1971; Erwin and Deitrich, 1966).

In summary, the presence of acetaldehyde in the brain may arise from circulating blood acetaldehyde produced by the hepatic oxidation of ethanol. However, acetaldehyde appears to be detected in the brain only following a very high dose of ethanol or when acetaldehyde metabolism has been blocked with aldehyde dehydrogenase inhibitors (e.g. Eriksson and Sippel, 1977; Tabakoff et al, 1976). Alternatively, the brain does possess the oxidative machinery for the production and degradation of acetaldehyde, suggesting that acetaldehyde metabolism can occur directly in the brain, possibly without producing any measurable levels of acetaldehyde (Eriksson and Sippel, 1977).

Although the presence of appreciable amounts of acetaldehyde in the brain during ethanol administration has not been conclusively demonstrated; the absence of such a demonstration does not preclude the possibility of some quantities occurring.

Psychopharmacological Effects of Acetaldehyde

Aside from its well-known aversive effects (Eriksson, 1980), acetaldehyde has also been demonstrated to possess positive reinforcing properties, implicating this compound in voluntary ethanol consumption (see Amir et al, 1980; Amit, Brown, Rockman, Smith and Amir, 1980b; Lindros, 1978).

In earlier studies, Myers and his coworkers demonstrated that intraventricular infusions of acetaldehyde as well as a variety of alcohols, aldehydes and alkaloids increased ethanol drinking in rats and Rhesus monkeys (Myers and Veale, 1969; Myers, Veale and Yaksh, 1972; Myers and Melchoir, 1977). However, using similar paradigms other investigators have been unable to replicate some of those findings (Friedman and Lester, 1975; Amit, Smith, Brown and Williams, 1982). More recently, it was shown that naive rats would self-administer acetaldehyde into the cerebral ventricles but would not perform the operant task when ethanol infusions were used as the reinforcer (Brown et al, 1979). These same authors also reported that the propensity to self-administer acetaldehdye intraventricu-

larly was positively correlated with ethanol preference (Brown, Amit and Smith, 1980). It was suggested that the central reinforcing effects of acetaldehyde may mediate at least in part, voluntary ethanol consumption in rats (Brown et al, 1980). In support of these findings, Myers et al (1982) reported that animals would self-inject acetaldehyde intravenously.

Further evidence for the central reinforcing effects of acetaldehyde was obtained from a study examining alcohol effects in human subjects (Brown, Amit, Smith, Sutherland and Selvaggi, 1983). After consumption of low doses of alcohol, enhanced euphoria and stimulation were reported in subjects pretreated with the aldehyde dehydrogenase inhibitors disulfiram or calcium carbamide. No such effects were reportd in placebo-treated subjects consuming the same amounts of alcohol. The authors concluded that the potentiation of the euphoric effects of low doses of alcohol with the aldehyde dehydrogenase inhibitors appeared to be attributed to the increased availability of acetaldehyde to the brain.

The implication of these studies is that acetaldehyde may be pharmacologically active at low levels which appear to support intravenous and intraventricular self-administration of acetaldehyde and ethanol consumption (Amit, Brown, Amir, Smith and Sutherland, 1980a; Amit et al, 1980b). It would appear then, that high circulating levels of acetaldehyde may not be necessary to produce

psychopharmacological effects.

As discussed earlier, the presence of centrally-acting acetaldehyde in the brain may arise from circulating blood acetaldehyde levels produced by hepatic oxidation of ethanol. This route however, has been questioned since the levels of acetaldehyde resulting from voluntary ethanol consumption in animals, are below the level of detection in peripheral blood and consequently in the brain (Eriksson and Sippel, 1977; Tabakoff et al, 1976). A second possible route contributing to the central pharmacological actions of acetaldehyde may be direct ethanol oxidation in the brain via the enzyme catalase. Ethanol readily passes into brain tissue (Ritchie, 1970) and catalase has been demonstrated to play a role in the oxidation of ethanol in rat brain (Cohen et al, 1980).

It is possible then, that centrally-produced and centrally-acting acetaldehyde may mediate alcohol-motivated behaviors under conditions of normal voluntary ethanol intake (Amir et al, 1980; Amit et al, 1980a; 1980b). Recently, Aragon, Sternklar and Amit (in press) demonstrated a direct relationship between brain catalase activity and voluntary ethanol consumption in rats. These authors suggested that the central formation of acetaldehyde via brain catalase may play a regulatory role in ethanol intake. In addition, it has been reported that a direct relationship exists between brain aldehyde dehydrogenase activity and ethanol consumption under a variety of

manipulations and conditions (Amir, 1977; 1978b; Amir and Stern, 1978; Sinclair and Lindros, 1979; Sinclair and Lindros, 1981; Socaransky et al, 1984). It has been postulated that the modulation of ethanol intake may be dependent, in part on the capacity to form and eliminate acetaldehyde centrally (Amir et al, 1980; Aragon and Amit, in press; Socaransky et al, 1984).

Two recent studies suggest a role for centrally-formed acetaldehyde in mediating some of the psychopharmacological effects of ethanol. Numerous studies have demonstrated that rats injected with ethanol (intraperitoneally) immediately following the presentation of a novel flavored solution, reduce their intake of that flavored solution upon subsequent presentations (e.g. Cappell, LeBlanc and Endrenyi, 1973; Lester, Nachman and LeMagnen, 1970). This reduction, commonly referred to as "conditioned taste aversion" (CTA), is characteristic of most self-administered drugs (see Goudie, 1979). Work by Aragon et al (1985) has implicated centrally-formed acetaldehyde in mediating CTAs induced by ethanol. Rats pretreated with the catalase inhibitor AT, did not demonstrate a CTA normally induced by ethanol. More directly, this effect seemed specific to ethanol, as pretreatment with AT failed to attenuate or block the CTAs induced by morphine or lithium chloride (an emetic agent). The authors concluded that not only was brain catalase important for ethanol metabolism in the brain, but that centrally-formed

acetaldehyde may mediate the CTAs induced by ethanol.

These same authors investigated the role of central acetaldehyde in the depressant effects of ethanol using the open field paradigm (Aragon et al, 1985). The results indicated that rats pretreated with AT did not demonstrate depression of locomotor activity following ethanol administration (2 gm/kg). These authors suggested that acetaldehyde formed centrally via the peroxidatic activity of brain catalase was also important in mediating the pharmacological effects of ethanol on open field activity.

However, the elimination of acetaldehyde from the brain may also be an important factor in the central effects produced by ethanol. Sinclair and Lindros (1981) concluded from their studies that the suppression of alcohol consumption by cyanamide was due to its direct inhibition of brain aldehyde dehydrogenase and not to elevated blood acetaldehyde levels. They suggested a role for brain aldehyde dehydrogenase in the central regulating mechanisms of alcohol drinking. This notion is consirstant with other reports suggesting a systematic relationship between alcohol intake and brain aldehyde dehydrogenase activity (Amir, 1977; 1978b; Socaransky et al, 1984).

Taken together, these studies implicate a role for centrally-acting acetaldehyde in the mediation of ethanol consumption, and ethanol's effects on locomotor activity and CTA. Furthermore, the enzymes which regulate the production and degradation of acetaldehyde in the brain,

may be a critical link in mediating the psychopharmacological effects of acetaldehyde. The work by Aragon and colleagues (1985) represents a minority of studies that have examined the involvement of centrally-acting acetaldehyde in behaviors other than alcohol consumption.

The present series of experiments was designed to: a) further investigate the potential behavioral effects of centrally-administered acetaldehyde on locomotor activity; b) to demonstrate that ethanol-induced effects on locomotor activity and CTA can be altered by manipulating the enzymes responsible for the oxidation of ethanol and acetaldehyde; c) to attempt to differentiate the relative contribution of central and or peripheral actions of acetaldehyde in mediating some of ethanol's behavioral effects.

The central focus of the present investigation is the role of centrally-acting acetaldehyde and brain aldehyde dehydrogenase in the mediation of ethanol's effects on locomotor activity and CTA. By achieving a better understanding of these phenomena, it is hoped that new light will be shed on the involvement of acetaldehyde in mediating some of the psychopharmacological effects of ethanol.

EXPERIMENT 1

Acetaldehyde, the proximate metabolite of ethanol has been implicated in some of the pharmacological properties of alcohol (Amir et al, 1980; Amit et al, 1980b). This notion has encountered immediate difficulties since numerous attempts to detect or measure the presence of acetaldehyde in the brain following exposure to ethanol have been inconclusive (Eriksson, 1980; Eriksson and Sippel, 1977). However, it has been demonstrated that animals will self-administer acetaldehyde intracerebroventricularly, suggesting that acetaldehyde may have reinforcing properties (Brown et al, 1979) and may play some role in the mediation of voluntary ethanol consumption (Amir et al, 1980; Brown et al, 1980).

Despite these positive findings, only a few studies have examined the central properties of acetaldehyde in different paradigms. Using a conventional CTA paradigm, Brown, Amit, Smith and Rockman (1978) demonstrated that intracerebroventricular infusions of acetaldehyde did not induce a CTA. Smith, Amit and Splawinsky (1984) investigated the effects of multiple infusions of acetaldehyde on conditioned place preference. Earlier studies examining the phenomenon of place conditioning used aversive agents such as irradiation as the unconditioned stimulus (Garcia, Kimeldorf and Hunt, 1957). In a study by Garcia et al (1957), rats showed a clear aversion to environmental cues that had been paired with irradiation. More recently,

conditioned place preference has been shown to occur following the administration of self-administered drugs such as morphine (Blander, Hunt, Blair and Amit, 1984), heroin (Schenk, Hunt, Colle and Amit, 1983) and cocaine (Spyraki, Fibiger and Phillips, 1982). Consistent with these findings, Smith et al (1985) demonstrated that multiple intracerebroventricular infusions of acetaldehyde induced conditioned place preference in rats.

In an attempt to elucidate the possible role of central acetaldehyde in the various actions of ethanol, the present experiment examined the effects of centrally-administered acetaldehyde on locomotor activity.

Experiment 1 consists of two sections (a and b). Because experiments were conducted on two separate occasions, each section represents a different dose of acetaldehyde tested.

Experiment 1a

Method

Subjects

Subjects were 15 male Long Evans rats (Charles River Breeding Farms) weighing 250-275 grams. The animals were individually housed in stainless steel cages with free access to food and water throughout the experiment. The animal colony room was illuminated on a 12 hr day/night schedule.

Surgery

Following an adaptation period to the animal room of at least 3 days, the animals were anaesthesized with sodium pentobarbital (60 mg/kg, i.p.) and simultaneously received a subcutaneous injection of atropine sulphate (.6mg/ml) (Glaxo Laboratories). Under anaesthesia, a 22-guage stainless steel cannula guide (Plastic Products Inc.) stereotaxically aimed at the left lateral cerebral ventricle was implanted in each animal. The stereotaxic co-ordinates were as follows: the incisor bar was set at 0.0, and the stereotaxic co-ordinates were 1.0 mm posterior to Bregma, 1.5 mm lateral to the mid-saggital line and 3.6 mm ventral to the dura. The cannula guide was secured in position by cranioplast cement anchored to the skull by five stainless steel screws. A stainless steel stilette was inserted into the guide to keep it free from obstruction.

Apparatus

The experimental chamber consisted of a clear plexiglass box with a wire mesh floor (30 cm x 30 cm x 38 cm). Thin colored cord divided the floor into four equal squares. A single activity count was recorded by the observer each time the rat crossed the corded line in any direction. This was determined only when both of the rat's hindlegs crossed the line.

Procedure

After a 7 day recovery period, animals were randomly divided into two treatment groups. Animals were infused with acetaldehyde (Ach) or saline (Sal) using a micrometer syringe connected with polyethelyne tubing to a 28-guage cannula which was inserted into the chronically implanted guide. Acetaldehyde was administered intraventricularly at a dose of 640 µg (40 µl of 2% acetaldehyde). The amount of acetaldehdye infused was based on observations reported by Brown et al (1978). They reported that animals treated with a dose of 800 µg of acetaldehyde were sedated within a minute of the infusion, while no observable effects were demonstrated by rats infused with 64 and 320 µg doses. The control animals (Sal) were infused with 40 µl of the vehicle (saline) which was buffered with hydrochloric acid to a pH of 4, equivalent to that of the acetaldehyde solution. All infusions were delivered over a 20 sec interval. One minute after completion of the infusion, the inner cannula was removed and the animal was randomly placed in one of the four squares in the open field chamber. The frequency of crosses was recorded by the observer each minute for a 10 min testing period. At the end of the 10 min session, the rat was removed from the open field box and returned to the home cage.

It should be noted that although only one observer carried out all the open field testing, inter-rater reliability was verified by independent observations. On 3 separate occassions, the regular observer and an

independent observer both recorded the open field activity of a single naive rat subject over a 10 minute testing period. Correlation of the activity counts recorded each minute by the observers indicated inter-rater reliability of $r = .95$ ($t(8) = 5.04$, $p < .001$).

At the termination of the experiment, all animals were sacrificed by decapitation. The brains were extracted rapidly, stored in formalin and then sliced into 40 μ coronal sections for determinations of cannula placement.

Results

Mean activity counts over the 10 min test period for acetaldehyde- and saline-treated animals is presented in Figure 1. Analysis of variance with repeated measures (2 x 10) yielded both a significant Time effect ($F(1,9) = 3.81$; $p < .0005$) and Group x Time interaction ($F(1,9) = 5.32$; $p < .00001$). Group comparisons across days using Tukey tests revealed that the activity level of group Ach was significantly depressed relative to group Sal from Minutes 1 to 4 ($q(2,110) = 3.04$; $p < .05$). However, at Minutes 9 and 10 the activity level of group Ach was significantly higher than group Sal ($p < .05$).

Histological examination of the brains confirmed that all cannula guides were properly implanted in the left lateral ventricle of each rat. Furthermore, no damage to brain tissue was evident in any of the sections.

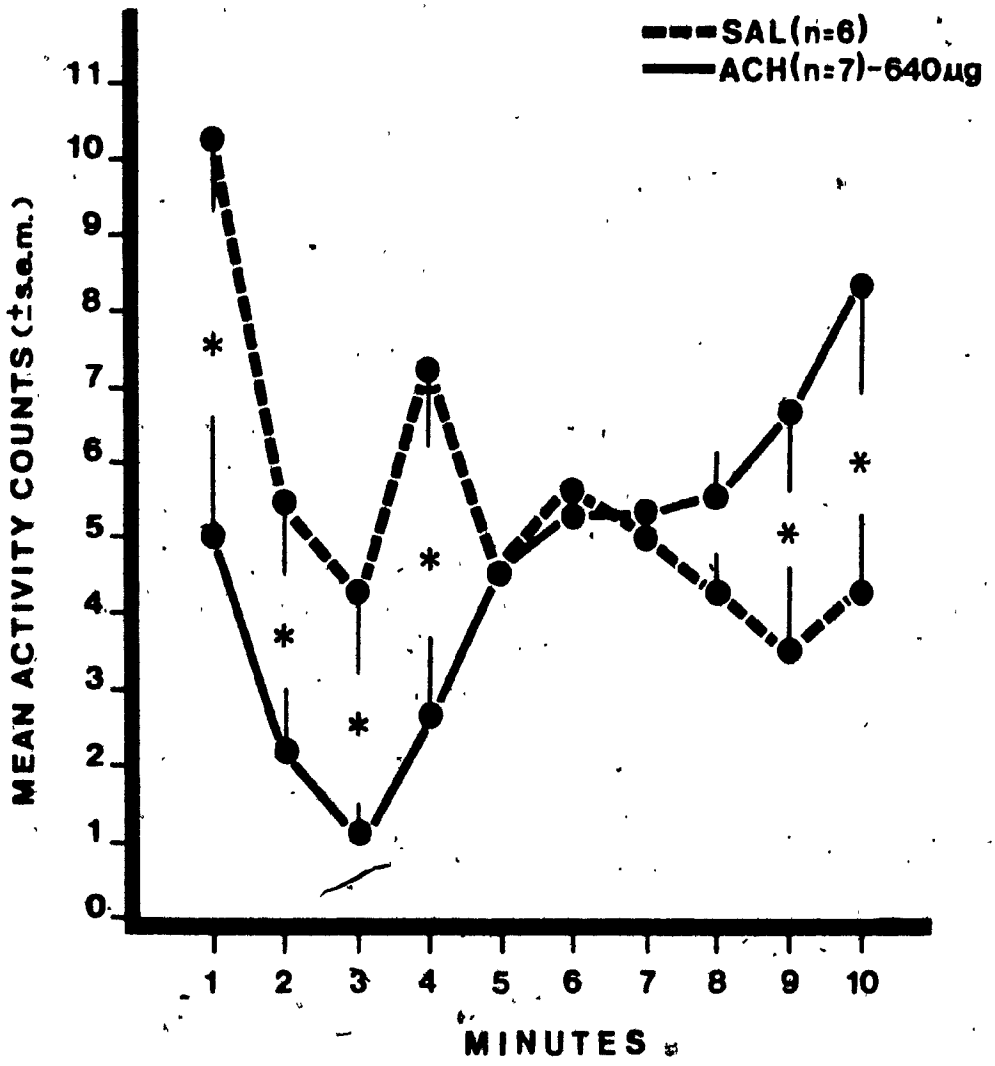

Figure 1. Mean activity counts for acetaldehyde and saline treated groups over a 10 min testing period.
* - $p < .05$

Discussion

The results of the present study demonstrated that intracerebroventricular administration of acetaldehyde at a dose of 640 µg produced both depressant and stimulatory effects on locomotor activity. Both phases of this biphasic effect on motor activity were of short duration as both sedation and excitation occurred within a 10 min testing period.

Ethanol is characterized as a depressant of central nervous system function (see Pohorecky, 1977; Wallgren and Barry, 1970) but its spectrum of action has been shown to include an excitatory component (e.g. Carlsson, Engel and Svensson, 1972; Sanders, 1976). There is evidence to indicate that moderate to large doses of ethanol (1.5 - 6.0 g/kg) have time-dependent biphasic effects on both experimental animals (e.g. Crabbe, Johnson, Gray, Kosobud and Young, 1982) and man (Ekman, Frankenhaeuser, Goldberg, Hagdahl and Myrsten, 1964). However, the time course for this ethanol-induced biphasic effect has been reported to occur over several hours (for review, see Pohorecky, 1977).

Results of the present study indicated that central acetaldehyde administration induced both depressant and stimulatory effects on locomotor activity similar to the time-dependent biphasic effect induced by ethanol administration. However, the rate of onset and duration of the biphasic effect occurred more quickly following acetaldehyde treatment.

The time courses for ethanol's effects and acetaldehyde's actions in the present experiment are so different that it may be difficult to argue in support of a role for acetaldehyde in the locomotor effects produced by ethanol. However, in the present experiment acetaldehyde was administered directly into the brain whereas ethanol in the studies stated above was administered orally or intraperitoneally (i.p.). The process of absorption and metabolism of ethanol would be much longer in these studies when compared to central acetaldehyde administration.

The results of the present study are consistent with other findings that the psychopharmacological consequences of exposure to acetaldehyde are generally more rapid than those observed with ethanol. Holtzman and Schneider (1974) reported that the depression of motor activity in mice following intravenous administration of acetaldehyde was relatively brief in comparison to ethanol-treated mice. Oritz, Griffith and Littleton (1974) demonstrated that acute acetaldehyde inhalation induced a faster peak of excitation and depression on locomotor activity than ethanol inhalation in mice. In addition, these authors reported that the onset and duration of withdrawal after chronic acetaldehyde inhalation treatment was much faster compared to ethanol-treated mice.

To examine whether the results observed in the present study were due to some non-specific effects of the high dose of acetaldehyde used (640 µg), Experiment 1b was

conducted using a lower dose of acetaldehyde.

Experiment 1b

Method

Subjects

Subjects were 14 male Long Evans rats (Charles River Breeding Farms) weighin 250-275 grams. The housing conditions were the same as Experiment 1a. The surgical procedure was identical to that performed in Experiment 1a as well.

Apparatus

Open field boxes as described in Experiment 1a were used.

Procedure

The identical procedure to that used in Experiment 1a was conducted. However, acetaldehyde was administered intracerebroventricularly at a dose of .64 µg (4 µl of 2% acetaldehyde). The saline control animals were infused with 4 µl of saline vehicle which was buffered with hydrochloric acid to a pH of 4, equivalent to that of the acetaldehyde solution. All the infusions were delivered over a 20 sec interval. One addition to the procedure was to extend the open field testing period to 20 min instead of 10 min used in Experiment 1a. At the termination of the experiment, animals were sacrificed by decapitation and the brains were extracted for determinations of cannula placement.

Results

Mean activity counts in 2 minute blocks for acetaldehyde and saline treated animals is presented in Figure 2. A two-way analysis of variance (ANOVA) with repeated measures yielded a significant Group effect ($F(1,12)= 6.21$, $p<.03$) and a significant Time effect ($F(9,108)= 11.28$, $p<.00001$). Although both groups demonstrated a progressive decrease in activity over time, the activity counts of group Ach were lower than saline-treated animals (see Figure 2).

To compare these results with that of Experiment 1a, a two-way ANOVA was conducted on the first 10 min of open field testing. The analysis yielded only a significant Time effect ($F(4,48)= 14.10$, $p<.00001$). No significant differences in activity counts were found between the groups in the first 10 min of testing. A two-way ANOVA conducted on the last 10 min of open field testing yielded only a significant Group effect ($F(1,12)= 6.10$, $p<.03$). The onset of locomotor depression for group Ach appeared to occur 10 min after the drug infusion.

Histological examination of the brains confirmed that all cannula guides were properly implanted in the left lateral ventricle of each rat.

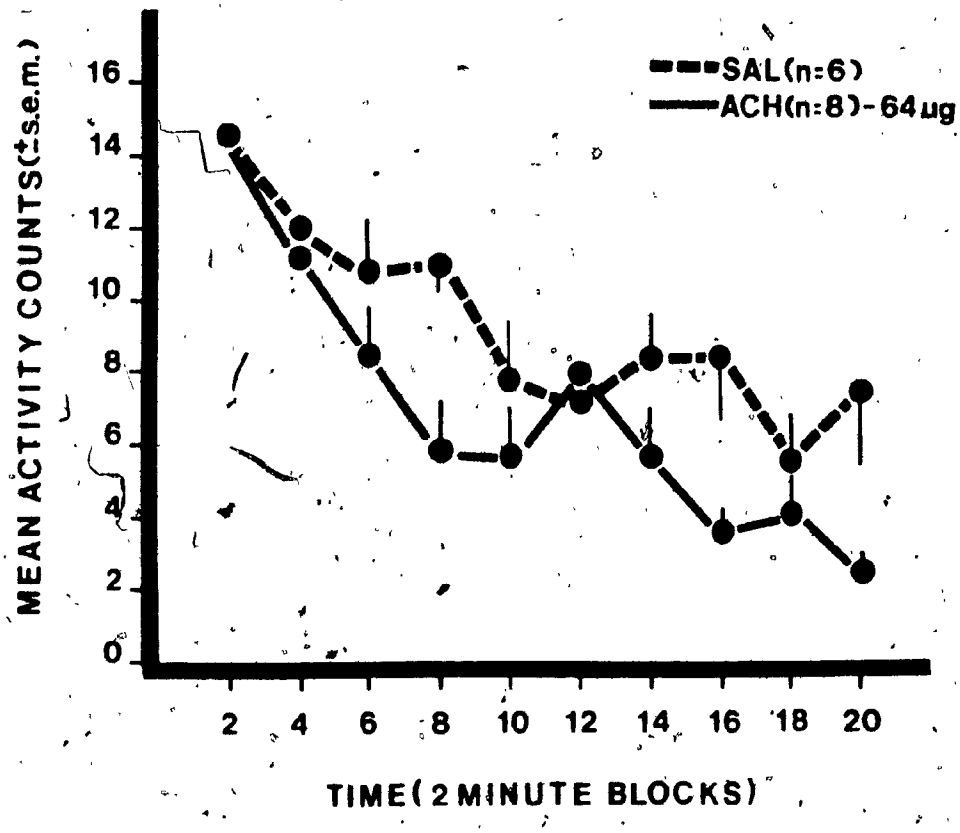

Figure 2. Mean activity counts in 2 min blocks for acetaldehyde and saline treated groups

Discussion

The results of Experiment 1b demonstrated that intracerebroventricular administration of 64 µg of acetaldehyde did not induce changes in locomotor activity similar to that observed with a dose of 640 µg. Although both acetaldehyde and saline treated animals demonstrated a decrease in activity over the 20 min test period, potentiation of this decrease was observed with group Ach during the last 10 min of open field testing.

These data indicated that a lower dose of acetaldehdye (64µg) infused directly into the brain, may produce some locomotor depression. However, unlike animals infused with 640 µg of acetaldehyde, the rate of onset of locomotor depression was much slower.

The results of Experiments 1a and 1b suggest that centrally-administered acetaldehyde may have dose-dependent effects on locomotor activity. These findings are consistent with other reports of dose-related locomotor effects. Morphine administered at high doses (i.p.) to rats, produced a time-dependent biphasic effect characterized by locomotor depression followed by locomotor stimulation (Babbin and Davis, 1972). At low doses, morphine induced only locomotor excitation (Babbins and Davis, 1972). The same effect for high and low doses of ethanol (i.p.) have been reported in mice (Carlsson et al, 1972; Matchett and Eriksson, 1977). However, locomotor excitation induced by ethanol is more variable and less

evident in rats (see Frye and Breese, 1981). Consequently, even at low doses of ethanol (.5 or 1.0 gm/kg), rats have been shown to demonstrate locomotor depression when compared to saline control animals (e.g. Mason, Corcoran and Fibiger, 1979). In the present experiment then, animals treated with 64 µg of acetaldehyde may be displaying the "normal" depressant component of a low dose of acetaldehyde.

The results of Experiments 1a and 1b demonstrated that acetaldehyde when administered intracerebroventricularly can be pharmacologically active in the brain. The next step was to investigate whether endogenously-produced acetaldehyde could also affect locomotor behavior.

EXPERIMENT 2

In the preceding experiment, it was shown that a single intracerebroventricular infusion of acetaldehyde produced changes in locomotor activity of rats. These results suggested that acetaldehyde can be pharmacologically active in the brain and may play some mediational role in ethanol's actions.

Administration of high doses of ethanol or acetaldehyde cause depression of the central nervous system (Truitt and Walsh, 1971) and consequently locomotor activity (Holtzman and Schneider, 1974). It would be of interest then to establish whether some of the actions traditionally ascribed to ethanol are in part produced by acetaldehyde or whether these two compounds produce their effects, though similar in nature, independently of each other.

As mentioned in the introduction, Aragon et al (1985) have recently demonstrated that pretreatment with the catalase inhibitor 3-amino-1,2,4-tryazole (AT) blocked ethanol-induced locomotor depression in rats. These authors concluded that the central formation of acetaldehyde via brain catalase may be involved in ethanol's effects on locomotor activity.

The present experiment was designed to investigate the role of centrally-acting acetaldehyde in mediating the locomotor effects produced by ethanol. It was hypothesized that acetaldehyde levels both in the brain and in the periphery could be manipulated in the rat by the

administration of agents which inhibit oxidative enzymes responsible for ethanol metabolism.

It has been reported that the administration of 4-methylpyrazole, an alcohol dehydrogenase inhibitor (Magnusson, Nyberg, Bodin and Hansson, 1972) decreases the rate of elimination of ethanol in the liver and consequently limits the production of acetaldehyde in the periphery (Lindros, Stowell, Pikkarainen and Salaspuro, 1981; Sinclair and Lindros, 1981). This treatment is not expected to interfere with the central formation of acetaldehyde by the peroxidatic activity of catalase (Cohen et al, 1980; Aragon et al, 1985). Therefore, in the brain, ethanol can be metabolized by catalse to produce acetaldehyde. Treatment with cyanamide has been shown to interfere with the metabolism of acetaldehyde in the liver by inhibiting aldehyde dehydrogenase, thereby causing elevated levels of acetaldehyde in the periphery (Marchner and Tottmar, 1978). Cyanamide treatment has also been reported to inhibit brain aldehyde dehydrogenase activity (Sinclair and Lindros, 1981).

It has been previously demonstrated that the simultaneous administration of the alcohol dehydrogenase and aldehyde dehydrogenase inhibitors 4-methylpyrazole and cyanamide respectively, prevents the accumualtion of acetaldehyde in the periphery (Sinclair and Lindros, 1981; Sinclair, Lindros and Tehro, 1980). Moreover, brain aldehyde dehydrogenase activity is inhibited (Sinclair and

Lindros, 1981; Sinclair et al, 1980) and this manipulation may serve to increase central acetaldehyde levels formed via brain catalase (Aragon et al, 1985). It was therefore hypothesized that this possible accumulation of brain acetaldehyde would potentiate the locomotor effects of a moderate dose of ethanol (.8 gm/kg) in animals pretreated with 4-methylpyrazole + cyanamide or cyanamide alone. If however, peripherally-produced acetaldehyde (levels which can be potentiated by cyanamide pretreatment) is a major contributive factor in mediating the locomotor effects induced by ethanol, then differential activity levels between the various treatment groups should be observed.

The focus of the present experiment then, was to investigate the potential roles of centrally- and or peripherally-acting acetaldehyde in the locomotor effects induced by ethanol.

Methods

Subjects

Subjects were 51 male Long Evan rats weighing 275-300 grams. The housing conditions were identical to those used in Experiment 1a.

Drugs

4-methylpyrazole (Sigma Chemicals, St. Louis) was dissolved in saline and the pH was adjusted to 7.0 with 1N sodium hydroxide to yield a final concentration of 10 mg/ml. Sodium cyanamide (Sigma Chemicals, St. Louis) was also dissolved in saline and the pH was adjusted to 7.0

with 1N sodium hydroxide to yield a final concentration of 25 mg/ml. Saline when required as a pretreatment vehicle, was injected at a volume of 1 ml/kg.

Apparatus

The open field boxes were the same as those used in Experiment 1a.

Procedure

After 7 days adaptation to the laboratory housing conditions, the experiment began. 2 hr prior to testing in the open field, animals were randomly assigned to 6 groups. Animals then received intraperitoneal (i.p.) injections of ethanol (.8 gm/kg; 30% v/v ethanol solution) or saline (5 ml/kg) and were placed in the open field one minute after ethanol or saline administration. A summary of the treatment groups is presented in Table 1. The frequency of crosses was recorded by the observer each minute for a 10 min testing period.

At the end of the 10 minute testing session, animals were sacrificed by decapitation. Trunk blood was collected for gas chromatographic analysis of blood ethanol and acetaldehyde levels using the procedures outlined below. The brains were rapidly extracted, rinsed in ice cold saline and blotted lightly on dry filter paper.. Brain samples were stored at -70°c, then assayed for aldehyde dehydrogenase activity levels.

Preparation of Brain Tissue: Frozen brain samples were weighed and then placed into the homogenation medium.

Table 1. Summary of Treatment Groups.

GROUPS	PRETREATMENT	TREATMENT
S+S--E (n=8)	saline	ethanol
4MP+S--E (n=8)	4-methylpyrazole	ethanol
C+S--E (n=8)	sodium cyanamide	ethanol
4MP+C--E (n=8)	4-methylpyrazole + cyanamide	ethanol
S+S--S (n=8)	saline	saline
4MP+C--S (n=9)	4-methylpyrazole + cyanamide	saline

Whole brains were homogenized (Teflon on glass) in sufficient .25M sucrose containing 1% Triton-X100 to make 10% brain homogenates. Homogenates were centrifuged for 60 min at 100,000 x g, at 0° c and then the clear supernatant was used as the enzyme source. All samples were frozen at -70° c until assayed.

Assay of Aldehyde dehydrogenase: Aldehyde dehydrogenase activity was assayed spectrophotometrically by measurement of the rate of the enzyme-catalyzed NAD+-dependent production of NADH (modified from Deitrich, Troxell, Worth and Erwin, 1976). A reaction mixture consisting of 0.03 M pyrophosphate buffer (pH 9.6), 1.0 mM NAD and 0.2 ml of the enzyme substrate was incubated for 10 minutes at 23° C. The reaction was initiated by the addition of 3.3 mM acetaldehyde, bringing the total volume to 2.0 ml.

Protein content was measured following the method of Lowry et al (1951) and bovine serum albumin was used as the standard. All assays were carried out in duplicate for both the enzyme activity and protein determinations.

Determination of blood ethanol and acetaldehyde: The procedure used was based on that of Stowell (1979) with modifications by Iversen and Damagaard (1983). These modifications were used to ensure accurate ethanol and acetaldehyde levels by protecting acetaldehyde and ethanol degradation during sample preparations. A brief outline of the procedure is discussed. Animal trunk blood was collected into test tubes which contained 150 I.U. of

sodium heparin. Blood (1 ml) was mixed with 4 ml of ice-cold semicarbizide reagent, containing 25 mM of thiourea. This mixture was spun in a refrigerated centrifuge at 400 g to separate blood cells from serum. Serum (2 ml) was added to 0.5 ml of 3.0 M perchloric acid and then spun at 11,000 g to obtain a clear protein-free supernatant. At this time, 0.5 ml of the supernatant was pipeted into an 8 ml vial, stoppered and stored at -70° c until assayed for ethanol and acetaldehdye by head-space gas chromatography.

Results

An inherent component of the open field paradigm is the interaction between the drug effect and individual responses to a novel environment (Denenberg, 1969). Compounding this interaction in the present experiment is the variability in the rates of absorption of ethanol following administration. Consequently, it was decided apriori to eliminate animals 1) whose total activity counts were 2 standard deviations above or below the group mean (based on cumulative counts over the 10 minute testing period), or 2) whose blood ethanol levels were 2 standard deviations above or below the group mean. Using this criterion, one animal from group 4MP+C--E and one animal from group S+S--E were removed from the experiment. A total of 49 out of 51 animals were included in the statistical analyses.

Mean activity counts in 2 minute blocks for all groups

is presented in Figure 3. A 2-way analysis of variance with repeated measures yielded a significant Group effect ($F(5,43)=5.24$, $p<.001$), a significant Time effect ($F(4,172)=82.03$, $p<.00001$) and a signficant Group x Time interaction ($F(20,172)=1.94$, $p<.01$). The interaction indicated that the activity counts of the various groups were different at some blocks of time and over time. Pairwise comparisons using Tukey tests revealed that in Block 1, group 4MP+S--E displayed significantly higher levels of activity than all the other groups except group S+S--E ($q(6, 215)= 5.74$, $p<.05$). In addition, activity of group 4MP+C--E was significantly depressed compared to group S+S--E ($p<.05$). Furthermore, Scheffé tests applied to the data in Block 1 revealed that the activity counts of groups C+S--E and 4MP+C--E were significantly depressed compared to that of groups 4MP+S--E and S+S--E ($F(5, 215)= 11.05$, $p<.05$).

Although the activity levels were more depressed for ethanol-treated animals in the second 2 minute block (Block 2), the pattern of locomotor activity was similar to that observed in Block 1. (see Figure 2). Tukey tests revealed that activity of group 4MP+C--E was depressed compared to groups 4MP+S--E ($p= .05$) and S+S--E ($p<. 05$). Additional Tukey tests indicated that all groups treated with ethanol demonstrated locomotor depression within 4 minutes (Block 2) in comparison to their activity levels in Block 1 ($q(5,172)=4.94$, $p<.05$). This effect was observed

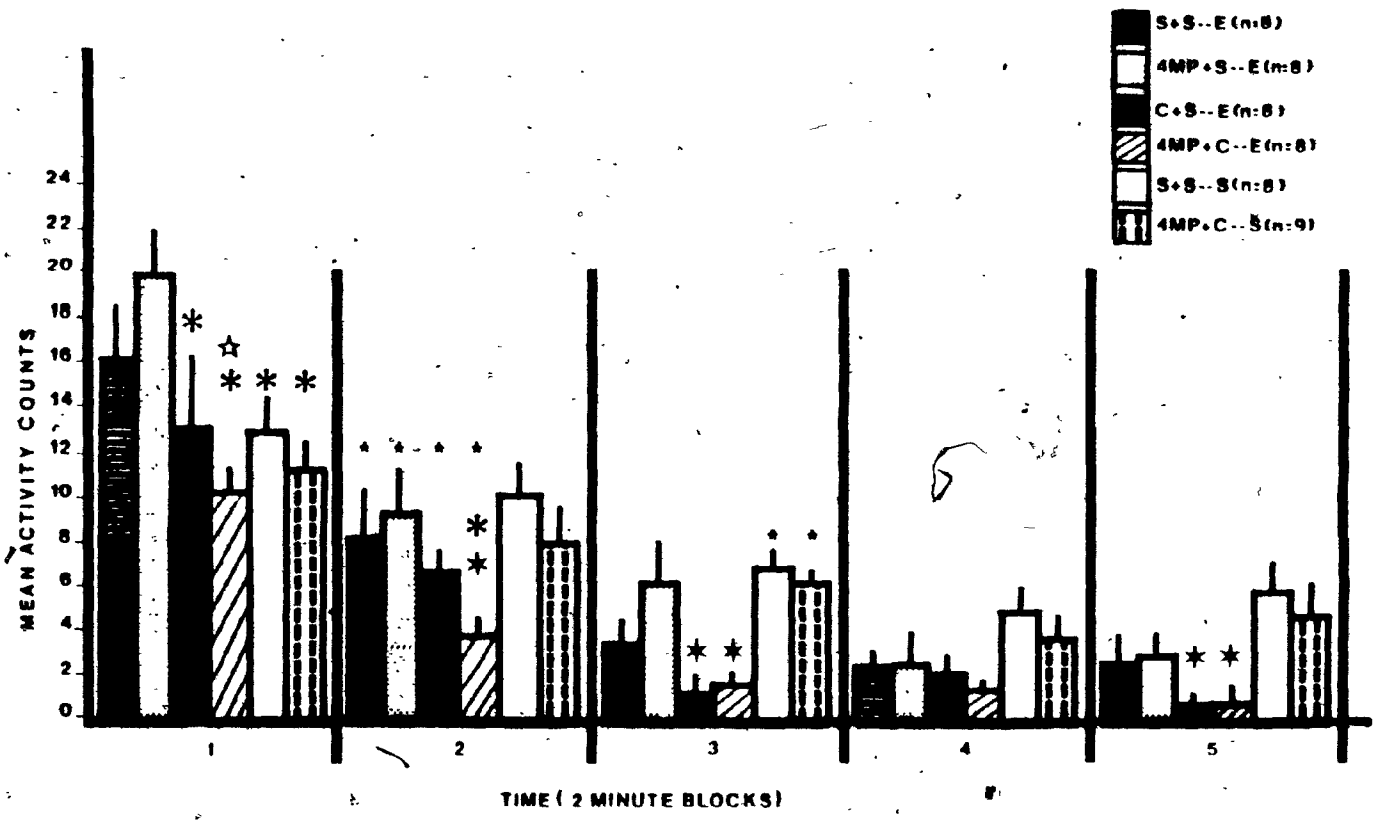

Figure 3. Mean activity counts in 2 min blocks for all treatment groups. S= saline, 4MP= 4-methylpyrazole, C= cyanamide, E= ethanol.
* - significantly different compared to group 4MP+S--E.
☆ - significantly depressed compared to group S+S--E.
✱ - significantly depressed compared to group S+S--S.
★ - activity is significantly depressed compared to Block 1.
significance level- $p < .05$.

in the saline-treated animals only at Block 3. In terms of overall activity within the 10 minute testing session, Scheffe tests indicated that the activity levels of groups 4-MP+C--E and C+S--E were significantly lower compared to groups S+S--E and 4-MP+S--E and compared to the saline control groups ($F(5,43)= 12.25$, $p<.05$).

Blood ethanol and acetaldehyde levels and brain aldehyde dehydrogenase activity for all groups is presented in Table 2. Blood acetaldehyde levels were undetectable for all groups except group C+S--E. Blood acetaldehyde levels for these animals were significantly elevated in comparison to the other groups. No significant differences were found in blood ethanol levels between groups.

Brain aldehdye dehydrogenase activity was analyzed using the apriori t-test for orthogonal comparisons among means. It was revealed that aldehyde dehydrogenase activity was significantly inhibited (20-30%) in all groups pretreated with cyanamide compared to the saline control (S+S--S) and ethanol control (S+S--E) groups ($t(40)= 2.74$, $p<.005$, one tailed test).

Discussion

The results of the present experiment suggest that centrally-acting acetaldehyde may play some role in mediating the locomotor effects induced by ethanol.

In the first two minutes (Block 1), animals pretreated with 4-methylpyrazole (4MP+S--E) displayed more activity

TABLE 2. Blood ethanol and acetaldehyde levels and brain aldehyde dehydrogenase activity for all groups.

GROUPS	Blood levels (ug/100ml) Ach	Etoh	Brain Aldh activity (nM NADH/min/mg)		%inhibition to control group S+S--E
S+S--E	---	100 ± 5	1.07 ± .07		
4MP+S--E	---	97 ± 6	.99 ± .06		8
C+S--E	1214	94 ± 9	.75 ± .05	*	30
4MP+C--E	---	102 ± 5	.84 ± .08	*	21
S+S--S	---	----	1.05 ± .05		2
4MP+C--S	---	----	.86 ± .05	*	21

S= saline, 4MP= 4-methylypyrazole, C= cyanamide, E= ethanol.
Ach= acetaldehyde; Etoh= ethanol, Aldh= aldehyde dehydrogenase.
--- = undetectable.
* - Aldh activity significantly inhibited compared to S+S--E, $p < .05$

than all other groups except the ethanol control group (S+S--E). This effect is consistent with a previous finding that mice pretreated with 4-methylpyrazole demonstrated increased locomotor excitation following ethanol administration (Svensson and Waldeck, 1973). These authors suggested that this effect may be due to increased circulation of blood ethanol levels as a consequence of alcohol dehydrogenase inhibition. However, in the present experiment, no detectable increases in blood ethanol levels were evident for animals pretreated with 4-methylpyrazole. It is difficult then to speculate why group 4MP+S--E displayed more activity than the other groups.

In the present experiment, pretreatment with cyanamide reduced the increases in spontaneous locomotor activity produced by ethanol. This reduction in locomotor activity can not be attributed to peripheral acetaldehyde accumulation since locomotor activity of group C+S--E, who showed elevated blood acetaldehyde levels, was not different from group 4MP+C--E.

The dose of ethanol administered (.8 gm/kg) was also an important factor contributing to the pharmacological effects observed in the present experiment. There is considerable controversy in the literature concerning ethanol-induced increases in spontaneous locomotor activity in rodents (for review see Pohorecky, 1977; Frye and Breese, 1981). As mentioned earlier, locomotor excitation is more variable and less reproducible in rats than in mice

(see Pohorecky, 1977). A methodological problem in most of these studies may be the lack of a sensitive measure to examine locomotor activity. In particular, many of the studies which examined locomotor excitation in rats were based on recordings of cumulative activity counts ranging from 5 minutes (e.g. Mason et al, 1979) to one hour (e.g. Frye and Breese, 1981). Using this measure in the present experiment, the overall activity (cumulative counts at 10 minutes) of the ethanol control and 4MP+S--E groups was not significantly different from saline control animals. However, by presenting activity levels at 2 minute intervals, qualitative differences between ethanol-treated and saline-treated groups could be observed. In particular, these data indicated that activity in minutes 3 and 4 (Block 2) for all ethanol-treated groups was significantly lower than activity in the first two minutes. No significant change in activity levels was observed for saline-treated groups from Block 1 to Block 2. These data therefore demonstrated that some pharmacological effects of ethanol on locomotor activity at a dose of .8 gm/kg can occur.

More importantly, the significant effects observed in the first 4 minutes of open field testing for ethanol-treated animals conforms to the diffusion kinetics of ethanol. It has been reported that immediately following ethanol administration alcohol is rapidly absorbed by the brain from arterial blood (Sunhara, Kalant, Shofield and

Grupp, 1978). Equilibrium between arterial blood ethanol and brain ethanol concentrations has been demonstrated to occur within 5-10 minutes after ethanol administration (Erickson, 1976; Sunhara et al, 1978). Animals in the present study were tested one minute following ethanol administration. Consequently, the increased locomotor activity observed with groups 4MP+S--E and S+S--E in Block 1, may reflect this influx of ethanol available to the brain. Animals pretreated with C+S--E and 4MP+C--E should also experience this initial influx of ethanol to the brain. However, changes in central acetaldehyde metabolism due to aldehyde dehydrogenase inhibition, may have contributed to the suppression of the increased locomotor activity induced by ethanol.

It would appear then, that the locomotor activity displayed in the first 4 minutes of open field testing may reflect sensitive changes induced by a moderate dose of ethanol. Once equilibrium between brain ethanol and blood ethanol concentrations are reached, these effects may become less dramatic. These subtle effects would have clearly been masked had only cumulative activity counts over an extended period of time been recorded.

Aragon et al (1985) demonstrated that animals pretreated with the catalase inhibitor AT, failed to demonstrate locomotor depression produced by ethanol. In the present experiment, animals pretreated with cyanamide or 4-methylpyrazole + cyanamide demonstrated less activity

than the other groups. Together, these results support a role for centrally-acting acetaldehyde and brain aldehyde dehydrogenase in mediating the locomotor effects induced by ethanol. If central acetaldehyde formation is an important variable mediating ethanol-induced locomotor effects (Aragon et al, 1985), perhaps the degradation of acetaldehyde via brain aldehyde dehydrogenase plays some regulatory role in maintaining this behavior as well (Amir et al, 1980).

To examine whether this hypothesis can be generalized to other behaviors influenced by ethanol, Experiment 3 investigated the effects of centrally-acting acetaldehyde and brain aldehyde dehydrogenase in the conditioned taste aversion produced by ethanol.

EXPERIMENT 3

Results of the previous experiments provided some evidence for acetaldehyde involvement in the locomotor effects induced by ethanol. These results further support the notion that acetaldehyde may be pharmacologically active in the brain and may be directly involved in some of the pharmacological consequences of ethanol administration (Amir et al, 1980; Aragon et al, 1985).

As mentioned earlier, acetaldehyde has been shown to possess both aversive and reinforcing properties (Eriksson, 1980). A common property shared by a variety of self-administered drugs including ethanol and acetaldehyde, is the ability to induce a conditioned taste aversion (CTA) (for review, see Goudie, 1979). Earlier studies demonstrated that rodents exposed to a novel flavored solution and then injected with an aversive or emetic agent (i.e. lithium chloride), reduced their intake of that flavored solution on subsequent presentations (e.g. Nachman, 1963; Nachman and Ashe, 1973). This reduction in intake of the test substance was assumed to be due to an association between the taste of the substance (gustatory cue) and some aversive action of the drug.

While the CTA phenomenon has been demonstrated with a variety of self-administered drugs (see Riley and Clarke, 1978), the nature of the conditioned response involved in CTA learning has been shown to be different in self-

administered and non self-administered or emetic agents (see Goudie, 1979). For example, different neurochemical systems have been implicated in subserving CTAs induced by self-administered drugs such as amphetamine, morphine and ethanol and those induced by lithium chloride (Goudie, Thornton and Wheatley, 1975; Sklar and Amit, 1977). Moreover, it has been demonstrated that neurochemical interventions which disrupted positive reinforcement produced by self-administered drugs also disrupted CTAs induced by the same drug (Goudie et al, 1975; Sklar and Amit, 1977). These data in conjunction with the finding that positive reinforcement and CTA can be demonstrated in the same animal at the same time (White, Sklar and Amit, 1977), has led some investigators to suggest that a functional relationship exists between the positive reinforcing and aversive effects of self-administered drugs (Switzman, Amit, White and Fishman, 1978; White et al, 1977).

There are numerous studies demonstrating CTAs induced by ethanol (e.g. Cappell et al, 1973; Cunningham, 1979). The role of acetaldehyde if any, in mediating this effect is at present unclear. Brown et al (1978) reported that i.p. administration of acetaldehyde (.2 and .3 gm/kg) produced CTAs in rats, whereas intracerebroventricular infusions of acetaldehyde did not induce a CTA. These authors concluded that the aversive effects of acetaldehyde were mediated by peripheral toxicity rather than by its

pharmacological actions in the brain. More recently, Aragon, Abitbol and Amit (in press) reported that the involvement of peripheral toxicity in the CTAs induced by acetaldehyde was dependent on the dose of acetaldehyde administered. These authors demonstrated that the nature of the CTA induced by .2 gm/kg acetaldehyde (i.p.) was more similar to that produced by ethanol (1.2 gm/kg) than was the CTA induced by .3 gm/kg acetaldehyde.

Because of its toxic properties, it is conceivable that circulating blood acetaldehyde levels produced by ethanol metabolism may play some role in ethanol-induced CTAs. However, acetaldehyde is self-administered by rats indicating that this metabolite may mediate the positive reinforcing effects of ethanol (Brown et al, 1978). It is possible then that both ethanol self-administration and ethanol-induced CTA may be mediated by acetaldehyde and are functionally related to the positive reinforcing and aversive properties of acetaldehyde. Support for this notion has recently been provided by Aragon et al (1985). As mentioned in the introduction, these authors provided indirect evidence for central acetaldehyde involvement in CTAs induced by ethanol. Animals pretreated with the catalase inhibitor AT did not demonstrate an ethanol-induced CTA, suggesting that centrally-formed acetaldehyde via activity of catalase may mediate CTAs produced by ethanol.

In an attempt to elucidate the nature of CTAs induced

by ethanol, the present experiment investigated acetaldehyde's involvement in ethanol-induced CTAs. Blood acetaldehyde levels were manipulated with the alcohol dehydrogenase and aldehyde dehydrogenase inhibitors 4-methylpyrazole and sodium cyanamide respectively, in an attempt to assess the putative roles of peripherally- and centrally-acting acetaldehyde in CTAs induced by ethanol.

Experiment 3 consists of two sections. Because experiments were conducted on two separate occasions, each section represents a different dose of ethanol tested. Each section consists of two phases. Phase 1 investigated the behavioral effects of ethanol on taste aversion. In phase 2, naive animals were used to determine blood acetaldehyde and ethanol levels.

Experiment 3a- Phase 1

Methods

Subjects

Subjects were 56 male Long Evans rats (Charles River Breeding Farms) weighing 250-275 grams. The animals were individually housed in stainless steel cages with free access to food and water prior to the onset of the experiment. The animal colony room was illuminated on a 12 hr day/night schedule

Drugs

4-methylpyrazole and sodium cyanamide were prepared

and administered in the same manner as that used in Experiment 2.

Procedure

After 7 days adaptation to laboratory housing conditions, the animals were placed on a 23 hr 40 min water deprivation schedule. For the following 7 days, tap water was available to the rats for a 20 min drinking period in the home cage. On day 8 (Pairing day 1), a 0.1% (w/v) sodium saccharin solution was substituted for water during the 20 min drinking period. 4 hr prior to saccharin presentation, animals were randomly assigned to 4 groups (n= 14 per group) and received various drug treatments. The pretreatment conditions were as follows: Group S+S received two successive injections of saline (one injection immediately following the other), group 4MP+S received two successive injections of 4-methylpyrazole (10 mg/kg) and saline, group C+S received two successive injections of sodium cyanamide (25 mg/kg) and saline and group 4MP+C received two successive injections of 4-methylpyrazole and cyanamide. All injections were administered intraperitoneally (i.p.). Within a minute after termination of the 20 min drinking period, animals in each pretreatment group received i.p. injections of either ethanol (.8 gm/kg; 30% solution v/v, n= 8 per group), or saline vehicle (5 ml/kg; n= 6 per group). The saline conditioning groups were included to investigate potential confounding effects of the various enzyme manipulations on saccharin consumption.

For 5 days following the first pairing day, tap water continued to be available for 20 min drinking periods. On day 14 (Pairing day 2), and identical procedure to that used on Pairing day 1 was conducted. The cycle of pairing day followed by 5 intervening water days was repeated until 3 pairings had been completed. Saccharin solution was presented on 2 more occasions followed by 5 intervening water days (Test days 1 and 2), however, no pretreatment or conditioning agents were administered.

Phase 2

Subjects

Subjects were 24 male Long Evans rats (Charles River Breeding Farms) weighing 300-350 grams. The housing conditions were the same as those in Phase 1.

Drugs

4-methylpyrazole and cyanamide were prepared and administered in the same manner as that used in Phase 1.

Procedure

After 7 days adaptation to the laboratory housing conditions, animals were randomly assigned to 4 groups (n= 6 per group) and received various drug treatments as described in Phase 1. The pretreatment groups were: S+S, 4MP+S, C+S and 4MP+C. 4 hours after the pretreatment regimen, all groups were administered .8 gm/kg ethanol (30% solution v/v). Twenty minutes after the ethanol injection, subjects were sacrificed by decapitation. Trunk blood of each animal was collected for blood acetaldehyde and

ethanol determinations. These determinatations were assayed by the same procedure used in Experiment 2.

Results

A three way analysis of variance (Pretreatment x Conditioning x Days) with repeated measures yielded a significant Pretreatment x Conditioning x Days interaction ($F(9,144)= 2.08$, $p<.035$). Subsequently, individual two-way analyses of variance (ANOVA) were conducted on all pretreatment groups receiving saline or ethanol as the conditioning agent. A two-way ANOVA for the saline conditioned groups yielded a significant days effect ($F(3,60)= 3.58$, $p<.02$). There was no significant Pretreatment effect nor Pretreatment x Days interaction, indicating that the pretreatment manipulations did not affect saccharin intake. Mean saccharin intake (expressed as percent change from baseline) for these control groups is presented in Figure 4. A two-way ANOVA with repeated measures for the ethanol conditioned groups revealed a significant Days effect ($F(3,84)=12.35$, $p < .00001$) and a significant Group x Days interaction ($F(9,84)=2.45$, $p< .045$). Saccharin intake (expressed as percent change from baseline) for all ethanol conditioned groups is presented in Figure 5 panel A. Dunnett's test comparing the control group (S+S) to the various experimental groups revealed that group 4MP+S drank significantly less saccharin than group S+S on days P3, T1 and T2 ($d(4,84)=29.2$, $p<.01$). Group C+S demonstrated a

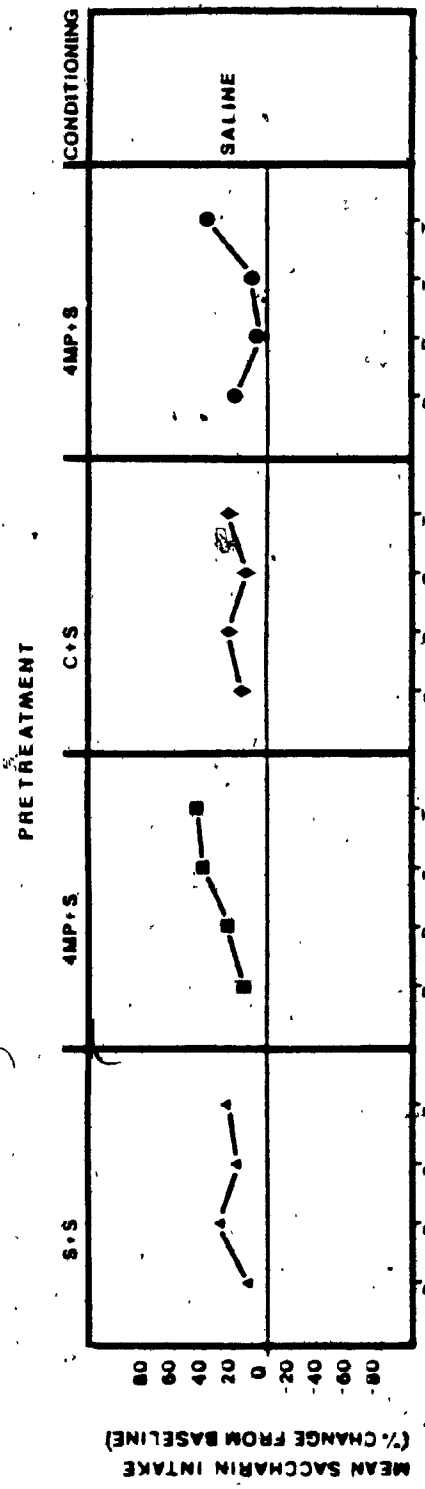

Figure 4. Mean saccharin intake (expressed as percent change from baseline) for all groups conditioned with saline. S= saline, 4MP= 4-methylpyrazole, C= cyanamide. P= pairing days, T= test days.

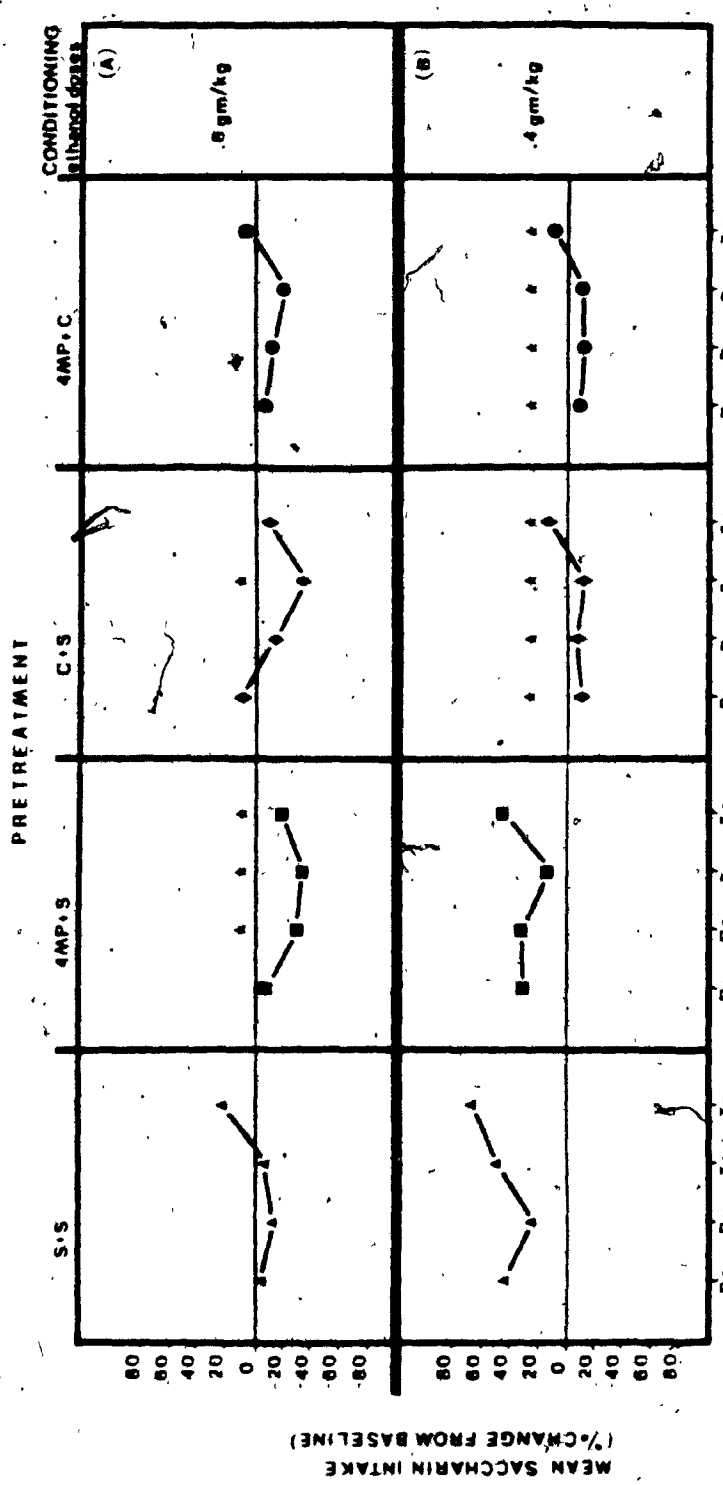

Figure 5. Mean saccharin intake (expressed as percent change from baseline) for all groups conditioned with ethanol (panel A= .8 gm/kg; panel B= .4 gm/kg).
S= saline; 4MP= 4-methylpyrazole, C= cyanamide.
P= pairing days, T= Test days.
★ - significantly different compared to groups S+S
$p < .05$

significant reduction in saccharin intake on Test day 1 when compared to the control group.

Blood acetaldehyde and ethanol levels for all groups is presented in Table 3a. Peripheral acetaldehyde was only detected in animals pretreated with cyanamide (C+S). These levels were considerably elevated in comparison to the blood acetaldehyde levels of the other groups. A one-way ANOVA on blood ethanol levels yielded no significant differences between groups.

Discussion

The results of the present study demonstrated that peripherally-produced acetaldehyde, and hence peripheral toxicity may not be a factor in mediating CTAs produced by ethanol at a dose of .8 gm/kg. Blood acetaldehyde determinations indicated that only animals pretreated with cyanamide (C+S) had significant increases in blood acetaldehyde levels. However, these animals demonstrated a similar pattern and magnitude of saccharin intake as group 4MP+C, whose blood acetaldehyde levels were undetectable. Although group C+S did demonstrate a significant reduction in saccharin intake on T1, the same reduction was observed in group 4MP+S, whose blood acetaldehye levels were undetectable with this pretreatment manipulation. It is possible then, that the reduction on T1 for group C+S may be a spurious finding.

Animals pretreated with 4MP+S demonstrated a

TABLE 3a. Blood ethanol and acetaldehdye levels for all groups treated with .8 gm/kg ethanol.

GROUPS (n=6 per group)	Blood levels (ug/100 ml) Ach	Etoh
S+S	---	120 ± 4
4MP+S	---	123 ± 5
C+S	930 ± 79	127 ± 3
4MP+C	---	113 ± 5

S= saline, 4MP= 4-methylpyrazole, C= cyanamide.
Ach= acetaldehyde, Etoh= ethanol.
--- = undetectable.

Table 3b. Blood ethanol and acetaldehyde levels for all groups treated with .4 gm/kg ethanol.

GROUPS (n=6 per group)	Blood levels (ug/100 ml) Ach	Etoh
S+S	---	43 ± 2
4MP+S	---	50 ± 2
C+S	632 ± 35	46 ± 2
4MP+C	---	48 ± 6

S= saline, 4MP= 4-methylpyrazole, C= cyanamide.
Ach= acetaldehyde, Etoh= ethanol
--- = undetectable.

significant reduction over days, indicating that this pretreatment manipulation potentiated the effect normally induced by ethanol. This effect may be due to an increase in circulating blood ethanol levels as a consequence of alcohol dehydrogenase inhibition (Magnusson et al, 1972). However, blood ethanol determinations indicated that pretreatment with 4-methylpyrazole did not alter blood ethanol levels, suggesting that another mechanism may underlie this effect.

It is unclear why cyanamide pretreatment failed to alter the effects of ethanol conditioning on saccharin intake. However, one must consider that the establishment of a CTA is not based on one particular pharmacological effect of the drug but is related to a constellation of stimulus properties of the drug (Coelpart, 1978). The dose of ethanol used in the present experiment may have been sufficient to produce discriminable pharmacological effects that were insensitive to subtle or discrete changes induced by the enzyme manipulations. To examine this possibility further, Experiment 3b was conducted using a very low dose of ethanol (.4 gm/kg).

Experiment 3b- Phase 1

Method

Subjects

Subjects were 24 male Long Evans rats (Charles River Breeding Farms) weighing 250-275 grams. The housing

conditions were the same as Experiment 3a.

Drugs

4-methylpyrazole and cyanamide were prepared and administered in the same manner as that used in Experiment 3a.

Procedure

The identical procedure to that used in Experiment 3a was conducted. Animals were placed on a water deprivation schedule and water was available to the rats for a 20 min drinking period. On day 8 (Pairing day 1), 4 hr prior to saccharin presentation, animals were randomly assigned to 4 groups (n= 6 per group) and received various drug treatments as described in Experiment 3a. The four pretreatment groups were as follows: S+S, 4MP+S, C+S and 4MP+C. Immediately following saccharin presentation, all animals received i.p. injections of ethanol (.4 gm/kg; 30% solution, v/v). The cycle of pairing day followed by 5 intervening water days was repeated until 3 pairings had been completed. The saccharin solution was presented to the animals on two more occasions without the administration of pretreatment or conditioning agents.

Phase 2

Subjects

Subjects were 24 male Long Evans rats (Charles River Breeding Farms) weighing 300-350 grams. The housing conditions were the same as Experiment 3a.

Drugs

4-methylpyrazole and cyanamide were prepared and administered in the same manner as that used in Experiment 3a.

Procedure

The identical procedure to that used in Experiment 3a was employed. Animals were randomly assigned to 4 groups (n= 6 per group) and received various drug treatments as described in Experiment 3a. The pretreatment groups were as follows: S+S, 4MP+S, C+S and 4MP+C. 4 hr after the pretreatment regimen, all groups received i.p. injections of ethanol (.4 gm/kg). 20 min after ethanol administration, animals were sacrificed by decapitation. Trunk blood of each animal was collected for blood acetaldehyde and ethanol determinations. These determinations were assayed by the same procedure used in Experiment 2.

Results

A two-way ANOVA with repeated measures for the ethanol conditioning dose of .4g/kg yielded a significant Group effect ($F(3,20)=4.98$, $p < .01$), a significant Days effect ($F(3,60)=12.25$, $p < .00001$) and a significant Group x Days interaction ($F(9,60)=2.18$, $p < .04$). Mean saccharin intake (expressed as percent change from baseline) for all groups is presented in Figure 5 panel B. Multiple comparisons using Scheffe tests revealed that saccharin intake of groups C+S and 4MP+C was significantly reduced compared to

groups S+S and 4MP+S on all pairing and test days ($F(3,80)=8.22$, $p<.05$). As shown in Figure 5 panel B, groups C+S and 4MP+C consumed less saccharin than the other two groups across days.

Blood acetaldehyde and ethanol levels for all groups is presented in Table 3b. Peripheral acetaldehyde was only detected in animals pretreated with cyanamide (C+S). A one-way ANOVA on blood ethanol levels yielded no significant differences between groups.

Discussion

The results of Experiments 3b suggest that centrally-acting acetaldehyde and brain aldehyde dehydrogenase may play some role in the CTAs induced by ethanol. Animals pretreated with cyanamide reduced saccharin intake following the administration of a subthreshold dose of ethanol, whereas no reduction from baseline was observed for groups 4MP+S and S+S. This effect cannot be attributed to elevated blood acetaldehyde levels since animals pretreated with 4MP+C and whose blood acetaldehyde levels were undetectable at this dose, demonstrated a reduction in saccharin intake as well.

The results of Experiments 3a and 3b suggest that centrally-acting acetaldehyde and brain aldehyde dehydrogenase may contribute to ethanol's distinct aversive stimulus properties as measured by conditioned taste aversion. Visual inspection of Figure 5 panels A and B

demonstrate that the lower dose of ethanol (.4 gm/kg) was not an effective CTA inducing agent for groups 4MP+S and S+S. However, animals pretreated with cyanamide demonstated a reduction in saccharin intake compared to these groups. These results suggest that changes in central acetaldehyde metabolism (due to brain aldehyde dehydrogenase inhibition), may have potentiated the pharmacological properties of a subthreshold dose of ethanol. This suggestion is supported by the finding that human subjects pretreated with calcium carbamide or disulfiram (aldehyde dehydrogenase inhibitors) reported elevated euphoria following a low dose of ethanol in comparison to placebo-pretreated subjects who reported no euphoric effects (Brown et al, 1983).

At the higher dose tested (.8 gm/kg) in the present study, the ethanol control group displayed a similar reduction in saccharin intake as was observed with the cyanamide pretreated groups at the .4 gm/kg dose. This suggests that central levels of acetaldehyde at a dose of .8 gm/kg may be sufficient to be pharmacologically discriminable to the rats. Because cyanamide pretreatment proved effective at attaining this threshold of discriminability at the lower dose, the same threshold was demonstrated at the .8 gm/kg dose. However, the magnitude of the CTA was potentiated in animals pretreated only with 4-methylpyrazole. The mechanism underlying this effect is at present unclear. This magnitude may reflect more salient

pharmacological effects induced by this enzyme manipulation once the threshold level has been reached.

GENERAL DISCUSSION

The present series of experiments examined the involvement of centrally-acting acetaldehyde and brain aldehyde dehydrogenase in the mediation of some of the psychopharmacological effects of ethanol.

In Experiment 1, it was shown that centrally-administered acetaldehyde resulted in a time and dose dependent biphasic effect on locomotor activity. These results support and extend those of previous reports demonstrating that acetaldehyde can be pharmacologically active in the brain (Amir et al, 1980; Amit et al, 1980b). For example, it has been demonstrated that animals will perform an operant for intracerebroventricular infusions of acetaldehyde (Brown et al, 1978; 1980) and that centrally-administered acetaldehyde induced a conditioned place preference in rats (Smith et al, 1985).

To determine if endogenously-produced acetaldehyde contributes to ethanol's central effects, Experiment 2 investigated the role of centrally-acting acetaldehyde in the mediation of the locomotor effects induced by ethanol. It was observed that pretreatment with cyanamide, an inhibitor of aldehyde dehydrogenase suppressed the increase in spontaneous locomotor activity observed in the saline-treated and 4-methylpyrazole + saline-treated animals. This effect could not be attributed to elevated circulating blood acetaldehyde levels since animals pretreated with

4-methylpyrazole + cyanamide (a condition which prevents the accumulation of peripheral blood acetaldehyde) demonstrated depression of locomotor activity as well.

The results of Experiments 1 and 2 suggest that acetaldehyde may have central pharmacological effects which appear to be involved in mediating some of the locomotor effects produced by ethanol. Furthermore, these studies indicate that in addition to its role in mediating the positive reinforcing properties of ethanol, (Amir et al, 1980), acetaldehyde may also mediate other central actions of ethanol. In this context, it was of interest to examine the role of acetaldehyde in mediating the aversive properties of ethanol. This question gains importance given the suggested relationship between positive reinforcement and CTA (White et al, 1977). Experiment 3 investigated the contribution of acetaldehyde in the CTAs induced by ethanol.

Animals pretreated with cyanamide demonstrated a reduction in saccharin intake following the administration of a subthreshold dose of ethanol. It is possible that this potentiation effect may be due to some alteration in the rate of central acetaldehyde metabolism (i.e. central accumulation of acetaldehyde) due to brain aldehyde dehydrogenase inhibition. However, it is presently unclear why the cyanamide pretreated groups did not show a potentiation of the CTA at the .8 gm/kg dose of ethanol. Recently, it has been reported that catalase may play a role in the conversion of cyanamide to an active meta-

bolite, the latter being the actual inhibitor of aldehyde dehydrogenase (Demaster, Redfern, Shirota and Nagasawa, 1985). Perhaps then, the failure of cyanamide pretreatment to potentiate an ethanol CTA at a dose of .8 gm/kg may be due to some interactive effect between brain catalase and brain aldehyde dehydrogenase inhibition.

However, it is evident that peripherally-produced acetaldehyde at high concentrations did not potentiate the effects of ethanol at either dose tested. These results indicated that acetaldehyde accumulation in the periphery was not a contributing factor in the ethanol-induced reductions in saccharin intake.

The results of the present series of experiments indicate that centrally-acting acetaldehyde may play some role role in ethanol's effects on locomotor activity and CTA. These data suggest that the enzymes responsible for the formation and degradation of central acetaldehyde, perhaps by regulating acetaldehyde levels in the brain, may be an important physiological mechanism in the control of ethanol-related behaviors. This notion is supported by evidence of a systematic relationship between voluntary ethanol consumption and brain catalase activity (Aragon and Amit, in press; Aragon et al, in press) as well as brain aldehyde dehydrogenase activity (Amir, 1977; 1978b; Sinclair and Lindros, 1981; Socaransky et al, 1984). If levels of acetaldehyde in the brain are a physiological parameter that control some of the effects of ethanol, then varia-

tions in brain catalase activity or in brain aldehyde dehydrogenase activity may give rise to differences in behavioral responding to an ethanol challenge. Aragon and colleagues (1985) have demonstrated that inhibition of catalase activity (hence alterations in the levels of acetaldehyde produced) blocked both the locomotor depression and CTA induced by ethanol. Sinclair and Lindros (1981) demonstrated that brain aldehyde dehydrogenase inhibition resulted in the suppression of voluntary ethanol consumption in rats. Furthermore, in the present series of experiments animals pretreated with cyanamide responded differently than did control animals to ethanol's effects on locomotor activity and CTA.

The role of the enzymes mediating acetaldehyde's actions may be a critical link in understanding the psychopharmacological effects of ethanol. These interactions not only describe a possible role for central acetaldehyde in mediating ethanol's effects, but may also account for the individual variations in behavioral responses so often observed following ethanol exposure (for review, see von Wartburg and Buhler, 1984). Thus, the metabolic processes involved in the formation and elimination of central levels of acetaldehyde may play an important regulatory role in the putative psychopharmacological actions of ethanol.

The present series of experiments provide evidence that acetaldehyde is pharmacologically active in the brain and may be involved in some of the pharmacological

consequences of ethanol administration. Furthermore, these studies indicate that in addition to its role in mediating the positive reinforcing properties of ethanol, centrally-acting acetaldehyde may also mediate the locomotor and CTA inducing effects of ethanol. These findings support the recent work by Aragon and colleagues (1985) who demonstrated a role for central acetaldehyde in mediating ethanol's effects on locomotor activity and CTA.

It is not the intention of this present discussion to conclude that acetaldehyde mediates all of ethanol's effects. The emphasis of this dissertation has been that centrally-acting acetaldehyde is a critical factor mediating some of alcohol's central actions. To date, the evidence suggests that centrally-acting acetaldehyde mediates alcohol reinforcement, and alcohol's effects on locomotor activity and CTA. The present data provide only indirect evidence for central acetaldehyde involvement in some of ethanol's central effects. However, the present series of experiments together with evidence of a systematic relationship between voluntary ethanol consumption and brain catalase activity (Aragon et al, in press), as well as brain aldehyde denydrogenase activity (Amir, 1977; Socoransky et al, 1984), and with evidence demonstrating that the inhibition of brain catalase (possibly preventing the central production of acetaldehyde) disrupted ethanol-induced locomotor depression and CTA (Aragon et al, 1985), clearly implicates

an important role for centrally-acting acetaldehyde in mediating some of ethanol's central effects.

The precise nature of the involvement of acetaldehyde in mediating some of ethanol's psychopharmacological effects is at present unclear. Brain aldehyde dehydrogenase has been shown to be a major route of monoamine deamination (Duncan and Sourkes, 1974; Tabakoff and Gelpke, 1975). It has been postulated that acetaldehyde in the brain may competitively inhibit brain aldehyde dehydrogenase, giving rise to increases in steady state levels of its endogenous substrates- biogenic aldehydes (see Deitrich and Erwin, 1975; Thandani and Truitt, 1977). The accumulation of these biogenic aldehydes may subsequently affect neuronal functioning and the behavioral response to ethanol (Deitrich and Erwin, 1975; Amir, 1977; 1978). This hypothesis receives some support from studies demonstrating that acetaldehyde like ethanol, alters the turnover of brain catecholamines (Duritz and Truitt, 1966; Oritz et al, 1974; Thandani and Truitt, 1977; Walsh, 1971). Brain catecholamines have been demonstrated to play a role in ethanol consumption (e.g. Amit, Brown, Levitan and Ogren, 1977; Corcoran, Lewis and Fibiger, 1983; Kiianmaa, 1980), locomotor activity (Carlsson et al, 1972; Mason et al, 1979) and CTA (Sklar and Amit, 1977). Therefore, the most logical extension of this line of research in the future should focus on the possible interaction between central acetaldehyde and the biogenic amine systems in the brain.

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Appendix A

ANOVA Summary Tables for Experiment 1

Two-way ANOVA Summary Tables

Acetaldehyde (640 ug)

Source	df	SS	MS	F Ratio	Sign. Level
Group (G)	1	21.048	21.048	1.29	.2802
Error	11	179.521	16.320		
Minute (M)	9	213.947	23.772	3.81	.0004
M x G	9	299.04	33.224	5.32	.00001
Error	99	618.407	6.247		

Acetaldehyde (64 ug)

Source	df	SS	MS	F Ratio	Sign. Level
Group (G)	1	186.001	186.001	6.21	.0284
Error	12	359.571	29.964		
Minute (M)	9	1145.967	127.323	11.28	.00001
M x G	9	140.339	15.593	1.38	.2053
Error	108	1218.804	11.285		

Appendix B

ANOVA Summary Table for Experiment 2

Two-way ANOVA Summary Table

Source	df	SS	MS	F Ratio	Sign. Level
Group (G)	5	715.486	143.097	5.24	.0008
Error	43	1173.469	27.290		
Minute (M)	4	4385.754	1096.439	82.03	.00001
M x G	20	518.342	25.917	1.94	.0125
Error	172	2298.989	13.366		

Appendix C

ANOVA Summary Tables for Experiment 3

Three-way ANOVA Summary Table

Saline and Ethanol (.8 gm/kg) Conditioning Groups

Source	df	SS	MS	F Ratio	Sign. Level
Pretr (P)	3	1590.078	530.026	.221	
Cond. (C)	1	65002.465	65002.465	27.169	.0009
P x C	3	10651.956	3550.652	1.484	.229
Error	48	114841.065	2392.522		
Days (D)	3	10425.042	3491.682	9.334	.0009
P x D	9	5439.585	604.398	1.615	.115
C x D	3	6063.188	2021.062	5.403	.001
P x C x D	9	6997.231	777.470	2.078	.034
Error	144	53862.984	374.048		

Two-way ANOVA Summary Tables

Saline Conditioning

Source	df	SS	MS	F Ratio	Sign. Level
Group (G)	3	3211.268	1070.423	.380	.7710
Error	20	56889.430	2844.472		
Days (D)	3	3944.642	1314.881	3.58	.0189
G x D	9	5657.619	628.624	1.71	.1060
Error	60	22028.587	367.143		

Ethanol (.8 gm/kg)

Source	df	SS	MS	F Ratio	Sign. Level
Group (G)	3	9999.557	3333.186	1.61	.2092
Error	28	57950.557	2069.663		
Days (D)	3	14036.567	4678.856	12.35	.00001
G x D	9	6965.058	773.895	2.04	.0443
Error	84	31833.562	378.971		

Two-way ANOVA Summary Table

Ethanol (.4 gm/kg)

Source	df	SS	MS	F Ratio	Sign. Level
Group (G)	3	43597.318	14532.439	4.98	.0097
Error	20	58366.312	2918.316		
Days (D)	3	8775.021	2925.007	12.25	.00001
G x D	9	4679.235	519.915	2.18	.0363
Error	60	14328.613	238.810		