Alcohol and Opiates: Interaction in the Conventional and Pre-exposure Conditioned Taste Aversion Paradigms

Max J. Ng Cheong Ton

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada

November 1984

ABSTRACT

Alcohol and Opiates:
Interaction in the Conventional and Pre-exposure
Conditioned Taste Aversion Paradigms

J. Max Ng Cheong Ton, Ph.D.
Concordia University, 1984.

The interaction between alcohol and opiates and the role of the opiate receptor in this interaction were examined in the conditioned taste aversion (CTA) and its variant pre-exposure paradigms. Experiment 1 was an attempt to observe the effects of ethanol pre-exposure on morphine-induced CTA and the effects of morphine pre-exposure on ethanol-induced CTA. The results showed that ethanol pre-exposure blocked development of morphine-induced CTA and that morphine pre-exposure also blocked development of ethanol-induced CTA. Experiments 2a and 2b were carried out to examine the effects of naloxazone, a long-acting opiate antagonist, on morphine- and ethanol-induced CTAs respectively. Naloxazone was found to block development of both morphine- and ethanol-induced CTAs suggesting a role for the opiate receptor in the development of CTAs to both drugs. An attempt was then made to find out whether naloxone or naloxazone would block the ability of ethanol pre-exposure to impair the development of morphine

CTA, and the ability of morphine pre-exposure to impair the development of ethanol CTA. The results obtained in Experiments 3a and 3b showed that while naloxone did not affect the pre-exposure interaction between the two drugs, naloxazone reversed these interactions. The latter finding was taken as support for an involvement of the opiate receptor in the pre-exposure interaction between alcohol and opiates. To further substantiate this notion, an attempt was made to find out whether the interaction is mediated by a stereospecific receptor. Experiment 4a was a preliminary observation of the capacities of levorphanol, the active isomer at the opiate receptor, and its inactive counterpart, dextrorphan to induce CTAs at different doses. Levorphanol but not dextrorphan was found to produce CTAs at all doses tested suggesting that opiate-induced CTA is mediated by a stereospecific opiate receptor. In Experiment 4b, pre-exposure to 5 mg/kg of levorphanol was found to block development of both morphine- and ethanol-induced CTAs. Preexposure to the same dose of dextrorphan did not have any effect. Finally, Experiment 5 was a preliminary attempt to examine the role of acetaldehyde as a possible mediator in the ethanol-morphine pre-exposure interaction. The results showed that acetaldehyde, like ethanol, blocked development of morphine-induced CTA. These observations suggest that the opiate receptor may be involved in CTAs produced by both morphine and ethanol and in the pre-exposure interaction between the two drugs. In

addition, acetaldehyde may be a mediator of the actions of ethanol in these paradigms. In conclusion, an interaction between alcohol and opiates has been confirmed and the opiate receptor has been identified as a common element between these two classes of drugs. Possible mechanisms of this ethanol-opiate interaction are discussed.

ACKNOWLEDGEMENTS

I thank my advisor, Dr. Zalman Amit, very deeply. Over the last seven years of my studies, he has provided me with constant material and moral support. I am especially grateful for his expert guidance in research endeavors. I also like to thank all the people in the Zalman's laboratory who in one way or another have contributed to make this thesis possible. My special thanks go to F. Rogan for his technical help, and to Linda Gardner, Way Chew Ho, and Renee Segal for their assistance in my research endeavors. My gratitude also goes to Drs. P. Shizgal and R. Wise for their well-thought advices.

I owe an enormous debt to all members of my family for having supported me in many ways so that I can pursue my studies. To all brothers and sisters, I would like to pay tribute to their help, understanding, and cooperation. Special thanks go to Guy, Madeleine, and Sylvianne and their respective families who have been my most fervent supporters. I hope that they will not be deceived for their labor of love, brotherhood and kindness.

Lastly, I want to pay homage to a most respectful and respected person, my mentor and friend, Reverend Michel de Labauve d'Arifat.

I dedicate this work to my dear mother,

and to the memory of my late father.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF APPENDICES

INTRODUCTION

Drug interaction between various psychoactive drugs is a growing medical concern. According to Green and Jaffe (1977), the concern for an interaction between alcohol and opiates dates back to the last half of the 19th century. Based on numerous observations, medical professionals of that era had been entertaining the idea that alcoholism is an etiological factor in the development of opiate dependence. An epidemiological study of the past few decades reported that 20% of opiate addicts had a well-defined history of periodic alcohol drinking (Kolb, 1962). An additional 19% had suffered marked alcohol-related problems prior to narcotic use. The same study found that one-third of more than a thousand opiate addicts have been alcoholics prior to opiate addiction or returned to alcohol drinking during periods of abstinence from opiate use. Kolb (1962) also noted that, in some cases, alcoholism seemed to have developed following opiate dependence. Most of the above mentioned observations have since been confirmed by other researchers and clinicians (Bihari, 1974; Maddux & Elliott, 1975; Mitcheson, Davidson, Hawks, Hitchins & Malone, 1971; Stimmel, Cohen, & Hanbury, 1978; Weppner & Agar, 1971).

Research on experimental animals has likewise produced evidence for an interaction between opiates and alcohol. For instance, the works by Sinclair and coworkers (Sinclair, 1974; Sinclair, Adkins, & Walker, 1973) have demonstrated that administration of opiates causes a reduction in the self-administration of alcohol in rats. Some

researchers believe that the study of ethanol-opiate interaction in animals will explain the clinical observations of the interactions between these drugs (e.g. Blum, 1980). Another reason for the interest in the study of ethanol-opiate interaction is the present alarming rate of polydrug abuse and its lethal consequences (e.g. Garriott & Sturner, 1973). Clinicians hope that a better understanding of the interaction between those drugs will yield insights into the clinical problems and that new prevention and treatment programmes may be developed from these insights.

Furthermore, the study of the interaction between alcohol and opiates is another way for neuroscientists to verify certain hypotheses about the actions of these drugs individually. Understanding how alcohol and opiates interact may increase our understanding of the mechanisms and of the neural substrates involved in the actions of either drug. For neuropsychopharmacologists, this belief rests on the suspicion that those two classes of drugs share one or more common mechanism of action (Amit & Levitan, 1975; Wise, 1980). Thus, by studying the ethanol-opiate interaction, it is hoped that more information will be gathered on the mechanisms, localizations, and neural substrates of reinforcement or other behavioral parameters in which those drugs may interact.

Over the last decade or so, significant progress has been made in the fields of both opiates and alcohol. From this work, new and

powerful concepts have emerged. These concepts are now generating new hypotheses which attempt to explain the interaction between opiates and alcohol. Indeed, these new concepts have vigorously stimulated research interests in the study of the interaction between alcohol and opiates. These developments will now be reviewed to illustrate the concepts that have been used to explain the interaction between alcohol and opiates.

A landmark in the development of opiate research was the simultaneous demonstration by three independent laboratories (Pert & Snyder, 1973; Simon, Hiller & Edelman, 1973; Terenius, 1973) of a stereospecific opiate receptor in the rat brain. This discovery soon led to a major increase in research on the actions of opiates. The distribution of the opiate receptor has been mapped out in various species of animals (Atweh & Kuhar, 1977a, b, c; Hiller, Pearson & Simon, 1973; Kuhar, Pert & Snyder, 1973; Simantov & Snyder, 1977). These maps of opiate binding sites correlate well with what is known about the brain areas where opiates actions presumably occur (see Simon & Hiller, 1978). From an evolutionary perspective, it was reasoned that since the natural receptors would not exist primarily for the hypothetical case that the organism might consume an exogenous drug, the opiate receptor must serve some normal endogenous physiological functions. Based on this belief, researchers began to search for endogenous ligands that would act on the opiate receptor.

Within two years of the discovery of the opiate receptors, two endogenous pentapeptides were demonstrated to shift the binding curve of radioactive-labeled opiate agonists or antagonists and also to act like morphine in bioassays such as the guinea pig ileum (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975). In the next five years following this discovery, about two dozen endogenous opioids or morphine-like compounds have been identified (see Olson, Olson, Kastin & Coy, 1979), including opiate-like non-peptide compounds (Gintzler, Levy & Spector, 1976). Today, it is known that there are several varieties of opiate receptor, three of which have firmly been established: the mu, delta and kappa receptors (Wood, 1982). Different opiate agonists are believed to have different affinities for the different configurations of the opiate receptors (Hollt & Seizinger, 1983; Magnan, Paterson, Tavani, & Kosterlitz, 1982). In addition, most researchers would tend to agree that the endogenous opioids form three families. Each of these families constitutes a parent long-chain peptide that may degrade to produce a multitude of active opioids (see Akil, Watson, Young, & Lewis, 1984). In summary, the concepts of an opiate receptor, some endogenous opiate-like systems, multiplicity of opiate receptors and endogenous ligands have been firmly established in the opiate research literature.

Important progress has also been made in understanding the

psychopharmcological actions of ethanol. It is now established that several neurotransmitter systems are affected in a number of ways by acute or chronic ethanol administration. For example, acute ethanol injection usually alters turnover of norepinephrine (Carlsson & Lindquist, 1973; Pohorecky, 1974), dopamine (Carlsson et al., 1973; Hunt & Majchrowicz, 1974), and serotonin (Pohorecky, 1974) which are the most extensively studied biogenic amines. Another significant development in alcohol research is the new importance given to the metabolism of alcohol and its byproducts in the mediation of alcohol effects (Amir, Brown, & Amit, 1980). Acetaldehyde, the primary metabolite of ethanol, has long been considered to mediate only the aversive effects of alcohol because of its high degree of toxicity in the periphery (Hald & Jacobsen, 1948; Jacobsen, 1952). Indeed, therapeutic treatments for alcoholism have been developed on the principle that high levels of acetaldehyde will be so highly aversive as to make the alcoholic avoid alcohol consumption (Hald & Jacobsen, 1948). This high level of acetaldehyde is usually ensured by the administration of drugs like disulfiram (see Eneanya, Bianchine, Duran & Andersen, 1981) or calcium carbamide (see Mottin, 1973) that inhibits the actions of aldehyde dehydrogenase, the enzyme that is responsible for the metabolism of acetaldehyde.

Interestingly, acetaldehyde has recently been suggested also as a possible agent in the mediation of the positive reinforcing effects of

ethanol (Myers & Veale, 1969; Smith, 1982). Several lines of evidence support this notion. First, acetaldehyde itself has been demonstrated to be self-administered by naive rats (Brown, Amit, & Rockman, 1979). Second, there is a positive correlation between acetaldehyde self-administration and subsequent voluntary consumption of ethanol in rats (Brown, Amit, & Smith, 1980a). Third, inhibition of acetaldehyde formation leads to reduction in voluntary alcohol consumption (Carr, Brown, Rockman, & Amit, 1980). Fourth, inhibition of acetaldehyde elimination in humans enhances the subjective euphoric effects of low doses of ethanol (Brown, Amit, Smith, Sutherland, & Selvaggi, 1983). In addition, there is evidence that enzymes controlling the conversion of ethanol to acetaldehyde and the degradation of acetaldehyde to acetate can account for individual variation in the reinforcing properties of ethanol (Amir, 1977; 1978a, b; Amir & Stern, 1978). Positive correlations have been reported between the levels of brain catalase, an enzyme that also metabolizes ethanol to acetaldehyde, and voluntary ethanol consumption in rats (Aragon & Amit, in press; Aragon, Sternklar, & Amit, in press). There is also a positive correlation between brain aldehyde dehydrogenase level and ethanol preference in several strains of rats (Socaransky, 1982). Indeed, Amit and coworkers (Smith et al., in press) have proposed that these enzymes may serve as genetic markers for alcoholism in man.

Progress has also been made in the study of the effects of

ethanol on membrane fluidity (Chin & Goldstein, 1977; Harris & Schroeder, 1981), effects that have been proposed as the basis for many of the pharmacological actions of ethanol (e.g. Litteton, 1980). By fluidizing the neuronal membrane, ethanol can disrupt normal neuronal activity which may lead to many different effects depending on the type of cells affected. The changes induced by ethanol in neuronal membrane properties are postulated to affect the activity of membrane proteins which include enzymes, carriers, ion channels, and membrane receptors (Harris, 1984).

It is evident from the foregoing that the research developments in the individual fields of alcohol and opiates have generated some powerful concepts. In turn, these concepts have been used to generate hypotheses to account for possible interactions or commonalities between alcohol and opiates.

Theories of alcohol-opiates interaction

Several theories have been proposed to account for the pharmacological interactions between alcohol and opiates. In the following section, the more recent biobehavioral theories will be briefly outlined. Evidence for each of these theories will subsequently be presented and discussed.

The first hypothesis was presented by Davis and Walsh in their seminal 1970 report. These authors provided evidence for the

formation of tetrahydroisoquinoline (TIQ) compounds from biogenic amines in brain homogenates. Since some of those TIQ compounds had previously been shown to be intermediate precursors of morphine in the poppy plant (Kirby, 1967; Shamma, 1972), Davis and Walsh (1970) suggested that the formation of TIQ compounds in vivo might explain the addictive properties of, or the physical dependence to, ethanol or both either by acting like opiates or by serving as precursors for endogenous opiate-like compounds. A second hypothesis advanced recently by other researchers (e.g. Hoffman, Urwyler, & Tabakoff, 1982; Hiller, Angel & Simon, 1983) was that ethanol modulates the endogenous opiate system by directly affecting the opiate receptors. These researchers proposed that the ability of ethanol to mimic opiate antagonist properties was a consequence of its fluidizing action on neural membranes that contain opiate receptors. By disrupting the membrane structure, the opiate receptors are destroyed, and hence the opiates, endogenous or exogenous, are deprived of their active recognition and activating sites. Alternatively, the interaction between alcohol and opiates may be the result of the effects of ethanol on the release of endogenous opiate-like peptides which would then act on the opiate receptor to produce the opiate-like effects (Gianoulakis et al., 1981; Seizinger, Bovermann, Maysinger, Hollt & Herz, 1983).

These hypotheses are not mutually exclusive. Therefore, in presenting the data, an attempt will be made to assess the usefulness of each of the three hypotheses presented above.

Experimental Evidence for alcohol-opiate interaction

Biochemical studies

TIQs and actions of ethanol. One of the earliest biochemical reports that suggested a possible interaction between alcohol and opiates was the paper by Davis and Walsh (1970). These investigators demonstrated the formation of tetrahydropapaveroline (THP) from the biogenic amine dopamine in rat brain stem homogenates, and showed that this conversion was slightly enhanced by ethanol and by acetaldehyde, the primary metabolite of ethanol. Davis and Walsh (1970) suggested that the enhanced formation of the THP compound is the result of a persistent inhibition of the metabolism of dihydroxyphenyacetaldehyde, the metabolite of dopamine, by acetaldehyde. Such inhibition then leads this metabolite to condense with the parent amine compound, dopamine, to form THP. Since THP has been shown to be an intermediate compound in the synthesis of opiates in the poppy plant (Kirby, 1967; Shamma, 1972), the authors suggested that this compound may lead to morphine-like compounds in the human alcoholic. According to these authors, the formation of these morphine-like compounds would explain the similarities between opiates and alcohol with respect to their addictive properties as well as to the withdrawal symptoms they produced in dependent animals. Another report by Cohen and Collins (1970) revealed the formation of other TIQ compounds in cow adrenals as condensation products of acetaldehyde and the biogenic amines,

norepinephrine and epinephrine. These latter investigators also suggested that these TIQ compounds could play a role in the development of alcohol dependence and withdrawal symptoms. Although no direct evidence was presented in favor of TIQ compounds in the alcohol-opiate interaction, there was a clear statement in Davis and Walsh's (1970) report that the TIQ compounds or their metabolites could have opiate-like properties that would account for the physical dependence or addictive properties of alcohol or both. This hypothesis has been severely critized by other workers (Goldstein & Judson, 1971; Halushka & Hoffman, 1970; Seevers, 1970).

Some studies have examined the biochemical properties of TIQ compounds and their roles in alcohol actions. It is now established that TIQ compounds can act as false transmitters (for a review, see Deitrich & Erwin, 1980). For example, it was observed that they are taken up and stored by catecholamine neurons (Cohen, Mytilineou & Barrett, 1972; Locke, Cohen & Dembiec, 1973). They have been shown to be stored in catecholamine synaptic vesicles (Tennyson, Cohen, Mytilineou & Heikkila, 1973), to inhibit, in a competitive fashion, the enzymatic breakdown of catecholamines by monoamine oxidase and catechol-O-methyl transferase (Collins, Cashaw & Davis, 1973; Giovine, Renis & Bertolino, 1976; Cohen & Katz, 1975). More importantly, the stored TIQs can be released into the synapse by electrical or chemical stimulation (Greenberg & Cohen, 1973; Rahwan, O'Neill & Milner, 1974),

and also can activate catecholamine receptors (Mytilineou, Cohen & Barrett, 1974).

Despite the progress made so far, the issue concerning the in vivo physiological formation of TIQ compounds in an organism still confronts the workers in the field. While some studies have reported the detection of the TIQ compound, salsolinol, under certain nonphysiological conditions (Collins & Bigdeli, 1976; Sandler, Carter, Hunter & Stern, 1973; Turner, Baker, Algeri, Frigenio & Garattini, 1974), others workers have published negative findings (O'Neill & Rahwan, 1977). Recently, Shier, Koda, and Bloom (1983) used tritiated dopamine to assess the formation of THP and salsolinol in the rat brain. They observed that with or without pretreatment with alcohol, no conversion of tritiated dopamine to tritiated THP or tritiated salsolinol was detectable even though the sensitivity level of the technique was in the order of 0.016 pmolar for THP and 0.16 pmolar for salsolinol. Furthermore, the observation that TIQ alkaloids do not readily cross the blood-brain barrier (Rahwan, 1975) makes it unlikely that peripherally-formed TIQ compounds would contribute to the central effects of ethanol. Therefore, whether TIQ compounds are produced physiologically following normal ingestion of alcohol and whether they mediate the effects of ethanol still remain open questions.

Ethanol and endogenous opiate systems. There is now some evidence for an action of ethanol on the release of endogenous opioid

peptides. Acute systemic injections of ethanol have been shown to increase the tissue levels of met-enkephalin and beta-endorphin in distinct areas of the rat brain (Schulz, Wuster, Duka, & Herz, 1980; Seizinger et al., 1983). Similarly, in humans, a 4-fold increase in levels of opioid activity has been reported in the plasma of normal volunteers following an acute ethanol administration (Naber, Soble, & Pickar, 1981).

Chronic effects of ethanol administration on the endogenous opiate system have also been examined. In contrast to the acute effects, chronic ingestion of ethanol diet resulted in decreased levels of beta-endorphin and met-enkephalin in several areas of the brain (Blum, Briggs, Elston, DeLallo, & Sheridan, 1982; Gianoulakis, Chan, Kalant, & Chretien, 1983; Schulz et al., 1980; Seizinger et al., 1983;). Further examination of this action of ethanol indicates that the chronic ingestion of ethanol can cause increases in the biosynthesis and release of beta-endorphin, and its precursors, beta-lipotropin, and pro-opiomelanocortin (Gianoulakis et al., 1981; Gianoulakis et al., 1983; Seizinger et al., 1983). Chronic systemic injections of morphine also reduce the levels of beta-endorphin and met-enkephalin (Hollt, Haarmann, & Herz, 1981; Przewlocki et al., 1979). However, in the case of morphine, a decrease in the biosynthesis of beta-endorphin was also observed in contrast to ethanol (Hollt et al., 1981). Failure to observe an effect of ethanol

on the endogenous opiate systems has also been published. For instance, Ryder, Straus, Lieber, and Yalow (1981) have noted that acute or chronic ethanol administration failed to alter the levels of enkephalin in all areas of brain and gut investigated.

Previous studies have shown that ethanol has a modulatory influence on the dopamine functions (Lai, Carino, & Horita, 1980). On further examination, it was observed that the changes in dopamine activity following chronic alcohol consumption parallel changes in the actions of opiates on the same system. In mice which had been withdrawn for 24 hours from a liquid alcohol diet, the dose-response curve for morphine-induced stimulation of striatal dopamine metabolism was shifted to the right (Hoffman et al., 1982). This action was correlated with a decrease in the high affinity of the opiate receptor of the caudate in ethanol-treated animals. Presumably, the decrease in the opiate receptor affinity was caused by the continuous release of endogenous opiates as a result of the chronic ethanol diet. In agreement with this notion, it was found that acute intragastric administration of ethanol increases striatal dopamine turnover (Lai, Makous, Horita, & Leung, 1979; Reggiani, Barbaccia, Spano, & Trabucchi, 1980). This effect of ethanol can be reversed by pretreatment with naloxone (Barbaccia, Reggiani, Spano, & Trabucchi, 1980). Furthermore, DBA 2J mice, which lack enkephalinergic modulation of dopaminergic activity in the striatum, do not show the

normal change of dopamine metabolism after acute intragastric administration of ethanol which can be observed in the C57 Bl/6J and Swiss Albino strains of mice (Barbaccia et al., 1980). These studies suggest that a possible locus of the ethanol-opiate interaction may be the endogenous opiatergic modulation of dopamine turnover in the striatum. However, while these findings are consistent with the action of ethanol on the release of endogenous opioids in the striatum, they are also congruent with the idea of a modulatory action of ethanol directly on the opiate receptor. Hence, whether these effects of ethanol are caused by an action on the release of endogenous opioids or by a direct action of ethanol on the opiate receptor has yet to be resolved.

Pharmacological Studies

Most of the work that will be reviewed in this section is not exclusively pharmacological in nature. However, all the material relates to the receptor concept that has been proposed to account for the first site of drug actions.

TIQs. Fertel, Greenwald, Schwarz, Wong, and Bianchine (1980) reported that both salsolinol and THP bind to opiate receptors in rat brain with affinities of 62 and 19.5 uM respectively. In the same assay, these workers reported that morphine had a binding affinity of 0.0105 uM. In addition, the abilities of the TIQ compounds to

displace tritiated naloxone was found to decrease by about 4 times by 100 mM of sodium ions, an observation that has characteristically defined an opiate agonist. Thus, it seems that those TIQ compounds have opiate agonistic properties. Earlier studies have observed that the contractions of the guinea pig ileum elicited by electrical stimulation can be reduced, albeit partially, by the TIQ compound, salsolinol (Blum, Hamilton, Hirst, & Wallace, 1978; Hamilton, Hirst, & Blum, 1979). This effect of salsolinol could be reversed by naloxone only if the opiate antagonist was added to the medium first (Hamilton et al., 1979). Furthermore, it was also noted in the same study that salsolinol can reduce the inhibitory activity of morphine in the same bioassay. These results would suggest that the TIQ compounds bind to opiate receptors in an opiate-agonistic fashion, but only act like partial opiate agonists because of their low affinity binding. Since these effects of the TIQ compounds in these bioassays are not naloxone-reversible (North, Collins, Milner, Karras & Koziol, 1981), one can reject the action of TIQs on the release of endogenous opioids as a possible mechanism. By the same argument, it is unlikely that TIQs bind to the opiate receptor in the same manner as common opiate agonists. Based on his work on the actions of ethanol using the same bioassay, Clement (1980) has concluded that the inhibitory effect of ethanol on the electrically-elicited contraction of the guinea-pig does not involve the opiate receptor. Clement (1980) also provided

evidence to suggest that while the main site of morphine's actions in this system is presynaptic, that of ethanol's actions seems to be postsynaptic.

Ethanol actions on opiate receptors. Recent studies have demonstrated a direct action of ethanol on opiate receptor binding. For example, Hiller, Angel, and Simon (1981) have shown that ethanol or other aliphatic alcohols inhibit binding of tritiated D-Ala-Leu-enkephalin, but not the bindings of tritiated dihydromorphine or tritiated naloxone at low concentrations in vitro. The potency of alcohols to inhibit the binding was found to increase with the chain length of the particular alcohol. Elsewhere it has been demonstrated that enkephalins have a significant binding preference for the delta receptor (Smith & Simon, 1980) while dihydromorphine is known to bind selectively to the mu receptor (Kosterlitz, Paterson, & Robson, 1981). Following this line of reasoning, it was suggested that the delta receptors are more sensitive to the action of the alcohols that are mu receptors (Hiller et al., 1981). A more recent study by the same group of investigators (Hiller et al., 1983) has confirmed these results. In addition, it was found that the kappa opiate receptor subtype was similarly insensitive to inhibition by aliphatic alcohols as was the mu receptor subtype (Hiller et al., 1983). Furthermore, the inhibitory effect of alcohol on delta receptor binding was observed to be a result of a decrease in receptor affinity for the

agonists (Hiller et al., 1984). These results have in part been confirmed by other researchers (Tabakoff & Hoffman, 1983). However, in the latter report, ethanol decreased binding of dihydromorphine, the mu receptor agonist, in the mouse caudate membranes and as well as that of D-Ala-D-Leu enkephalin, the delta receptor agonist. It would seem then that there is conclusive evidence that acute ethanol administration inhibits opiate binding to the delta and the mu receptors.

In another study it was reported that etorphine binding to opiate receptors from rat and mouse brains was unchanged by acute ethanol administration in the dose of 0.05 to 3% (Jorgensen & Hole, 1981). This finding need not be seen as discrepant since it is also known that etorphine has preferential binding to the mu receptor (Wuster, Schulz, & Herz, 1979). Hence, the failure of ethanol to affect the binding of etorphine is consistent with the notion that ethanol selectively inhibits binding to the delta receptor.

Chronic alcohol administration also affects opiate receptor binding. Pfieffer, Seizinger, and Herz (1981) found that rats given ethanol in their drinking water for a period of three weeks show a significant increase in binding to the delta receptor but not binding to the mu receptor. This selective increase in delta binding is believed (Gianoulakis, 1983) to be a supersensitive response to the selective inhibitory action of ethanol ingestion on the delta

receptor. This phenomenon of receptor supersensitivity is called receptor up-regulation and has been reported for a number of different receptor systems (see Creese & Sibley, 1981). This chronic effect of ethanol on the delta opiate receptors has been confirmed by other workers (Gianoulakis, 1983; Hynes, Lochner, Bemis, & Hymson, 1983; Lucchi, Rius, Uzumaki, Govoni, & Trabucchi, 1984).

Opposite findings have been reported concerning the effects of ethanol on selective mu agonists. Chronic consumption of ethanol has been found to cause a decrease in the affinity of dihydromorphine for a high affinity receptor in the mouse caudate (Hoffman et al., 1982) and in rat striatum (Lucchi, Bosio, Spano, & Trabucchi, 1981; Lucchi et al., 1984). These results suggest that long-term ethanol treatment affects the mu receptor as well. Presumably, the decrease in mu receptor binding reflects receptor down-regulation following an action of ethanol in enhancing binding to the receptor. Indeed, there is evidence that an acute treatment with low concentrations ethanol increases rather than decreases binding to the mu receptor (Hoffman & Tabakoff, 1983; Levine, Hess & Morley, 1983).

Finally, in an attempt to examine the possible mechanisms for this action of ethanol, Charness and Diamond (1984) have demonstrated that, unlike incubation with opiate antagonists, long-term incubation with ethanol does not disturb agonist-induced down-regulation of opiate receptors in brain homogenates. In conclusion, the evidence

suggests that chronic ethanol administration causes an up-regulation of the delta opiate receptor subtype presumably as a result of an acute inhibitory action of ethanol on binding to these receptors. This inhibitory action of ethanol on receptor binding is likely to be different from the action of an opiate antagonist. Alternatively, Lucchi et al. (1984) have proposed that the supersensitivity of the delta receptor binding following chronic alcohol consumption could be the consequence of the diminished enkephalin release that they detected in the rat striatum. In addition, ethanol also seems to cause an increase in the mu receptor binding which leads to a decrease in mu receptor binding following chronic ethanol treatment.

Physiological Studies

To date only a few studies have been carried out to investigate the physiological interactions between ethanol and opiates. Khanna, Le, Kalant, and LeBlanc (1979) have demonstrated that chronic ethanol diet resulted in tolerance to the hypothermic effects of both ethanol and morphine. However, no tolerance was found to the hyperthermic effect of morphine. According to the authors, these findings suggest that the interaction is response-specific. However, one should note that only one dose of morphine (5 mg/kg) was used to assess cross-tolerance to the hyperthermic effect of morphine. Therefore, a more

systematic study is warranted before any general conclusion can be drawn. In the same study, it was found that chronic morphine also led to tolerance to the hypothermic effect of morphine and ethanol. This symmetrical cross-tolerance between these two classes of drugs is an interesting feature. However, no other report has since been published concerning the mechanism of this symmetrical interaction. Other studies have found that intracisternal administration of beta-endorphin potentiated the hypothermic effects of ethanol in mice while not affecting the blood and brain ethanol concentrations (Luttinger, Frye, Nemeroff & Prange, 1983; Luttinger, Nemeroff, Mason & Frye, 1981). These findings are in agreement with the notions that ethanol affects directly the opiate receptors or that ethanol causes the release of endogenous opioids or both.

Genetic models of alcoholism and certain actions of alcohol have been developed by selective inbreeding of mice that have differential responses to alcohol on several behavioral or biochemical measures (Deitrich & Collins, 1977). One such model is the short-sleep and long-sleep mice. Short-sleep mice are very resistant to the narcotic effect of ethanol while the long-sleep mice generally show extreme sensitivity to the same effect of ethanol (McClearn & Kakihana, 1973). Different sensitivities to the hypothermic effect of ethanol in these two genetic lines of mice have also been reported (Moore & Kakihara, 1978). Brick and Horowitz (1982) found that morphine, in contrast to

ethanol, induced a more potent hypothermic effect in short-sleep mice than in long-sleep mice. The same study reports that naloxone reversed the morphine effect in both genotypes and the alcohol effect only in the short-sleep mice.

Kalant and his collegues (Mayer, Khanna, Kalant, & Spero, 1980) have demonstrated similar cross-tolerance between alcohol and morphine in another physiological system. They found that chronic administration of morphine for three days caused tolerance to the inhibitory effect of morphine in vitro on the longitudinal muscle-myenteric plexus preparation as well as to the inhibitory effect of ethanol in the same preparation. Conversely, chronic administration of ethanol for two weeks resulted in tolerance to the inhibitory effects of ethanol and morphine in the same preparation. The effects of opiate antagonists in this symmetrical interaction were not reported, however.

The mechanism of this cross-tolerance remains to be investigated. Nevertheless, like opiate agonists, ethanol, at least in this preparation, inhibits the electrically-elicited contractions of the longitudinal muscle. It seems unlikely that the cross-tolerance is mediated by a direct action of ethanol on the delta opiate receptor. From the studies reviewed earlier, it was evident that the action of ethanol was to inhibit binding to the delta receptor in an analogous manner to an opiate antagonist (e.g. Hiller et al., 1981; Hiller et

al., 1983). On the other hand, ethanol was found to increase mu receptor binding (e.g. Hoffman & Tabakoff, 1983). Therefore, the mechanism for the effect of ethanol on the guinea-pig ileum may be related to its action on the mu receptor.

The cross-tolerance between ethanol and morphine in the guinea-pig ileum may be mediated by the formation of TIQ compounds that act like opiate agonists in the preparation. In support of this notion, Hamilton et al. (1979) have demonstrated opiate agonistic properties of salsolinol in the guinea pig ileum preparation. Another possibility is that both drugs produce their effects through their action on the same neurotransmitter system. One candidate transmitter for a such possibility is acetylcholine, which has been implicated in the action of morphine in this preparation. Studies have demonstrated that morphine presynaptically inhibits the release of acetylcholine and that this action of morphine underlies its inhibition of the electrically-elicited contractions of the guinea pig ileum (Paton, 1975). On the other hand, ethanol, unlike morphine, inhibits acetylcholine-induced contractions in the guinea pig thus suggesting a postsynaptic site of action (Clement, 1980). It is probable that the action of ethanol occurs at some steps subsequent to receptor activation since it did not affect binding of radiolabeled acetylcholine agonists to the receptor (Clement, 1980). It would be interesting to examine whether this action of ethanol on the receptor-

effector coupling process is universal in terms of different receptor systems and tissues.

Evidence for an interaction between alcohol and opiates has also come from endocrine studies. Simultaneous injections of naloxone and ethanol produced a rise in cortisol, corticotrophin and prolactin in humans while ethanol administration alone did not affect the levels of the hormones (Jeffcoate, Platts, Ridout, Hastings, MacDonald, & Selby, 1981). The administration of naloxone alone produced inconsistent effects. Knych and Prohaska (1981) similarly reported that co-administration of naloxone and ethanol prevented the development of tolerance to the immediate stimulatory action of ethanol on corticosterone levels in rats. In another report, ethanol suppressed the increase in serum luteinizing hormone levels evoked by naloxone at extremely low blood ethanol concentrations (Cicero, Newman, Gerrity, Schmoeker, & Bell, 1982). These authors suggested that ethanol may enhance the synthesis or release of endogenous opioids which in turn override the action of naloxone on luteinizing hormone-releasing hormone (Cicero et al., 1982).

Behavioral Studies

Self-administration. A recent report has shown that rats that readily self-administered morphine also readily self-administered ethanol (Smith, Werner, & Davis, 1981). In addition, the same study

reported that rats that failed to self-administer morphine also failed to self-administer ethanol. This finding is consistent with an early observation that two strains of rats that have been selectively bred for a difference in their susceptibility to morphine addiction, also differed in their susceptibility to alcohol addiction (Nichols & Hsiao, 1967). Some common mechanisms for the actions of ethanol and morphine were hypothesized.

There is also experimental evidence to show that intraperitoneal injection of 60 mg/kg of morphine decreased the voluntary consumption of ethanol (Sinclair et al., 1973; Sinclair, 1974). This effect has successfully been replicated in hamsters (Ross, Hartmann, & Geller, 1976) and in mice (Ho, Chen, & Morrison, 1977). A more recent confirmation of this finding with injections of the opiate agonist-antagonist, buprenorphine on intravenous self-injection of ethanol has also been obtained (Martin, Pilotto, Singer, & Oei, 1983).

In an attempt to examine whether this ethanol-morphine interaction in the self-administration paradigm is symmetrical, Gelfand and Amit (1976) investigated the effects of ethanol intraperitoneal injections on morphine self-administration. They failed to observe any change in morphine preference following the administration of ethanol. Nevertheless, the finding that ethanol treatment also induced a significant suppression of voluntary ethanol drinking (Miceli, Marfaing-Jallat, & LeMagnen, 1980; Sinclair, Walker,

& Jordan, 1973) suggests that both ethanol and morphine act in similar fashions on the voluntary consumption of ethanol. Ho & Rossi (1982) found that met-enkephalin injected into the rat lateral ventricle significantly suppressed the volitional consumption of ethanol, thus implicating the endogenous opioid peptides in this phenomenon. In contrast to the study of Miceli et al. (1980) where naloxone enhanced the suppression of ethanol on voluntary ethanol-drinking, Ho and Rossi (1982) observed that naltrexone, a longer acting opiate antagonist than naloxone, partially reversed the effect of met-enkephalin on ethanol drinking. These data would seem to suggest that the opiate-induced suppression of voluntary intake of ethanol is mediated by the opiate receptor while that produced by ethanol is not. However, even in the case of opiates, the large doses of agonists used to suppress the drinking of ethanol cast some doubt on the role of the opiate receptor in this phenomenon.

Another confusing finding is the report that naltrexone blocked the intravenous self-administration of ethanol in rhesus monkeys (Altshuler, Phillips & Feinhandler, 1980). This effect has also been reported with the short acting opiate antagonist, naloxone (Sinclair, Altshuler, & Rusi, 1981; Doyle & Samson, 1984). Thus, it seems that opiate agonists and antagonists act in a similar way on ethanol self-administration. These findings are inconsistent with the concept of a receptor-mediated action where the agonist produces a certain effect

and an antagonist blocks it or produces the opposite action. Doyle and Samson (1984) also rejected the idea of an opiate receptor-mediated effect because of the high dose of naloxone that was necessary to suppress alcohol drinking in the research paradigm they used. This dose of naloxone was also found to decrease sucrose self-administration as well. Although most of these studies use a preference measure to control for disruption of operant performance due to the drugs' effects, the possibility that the reduction in self-administration of ethanol or morphine may have been due to taste factors was not examined (Miceli et al., 1980).

The idea that TIQ compounds can serve as links in the ethanol-opiate interaction also has some difficulties (cf. Blum, 1980). As discussed earlier, these TIQ compounds have been detected in vivo after administration or ingestion of ethanol only when the biosynthesis of acetaldehyde or the degradation of monoamines with enzyme inhibitors or both was arrested (e.g. Collins & Bigdeli, 1975). However, one other prerequisite for a role for these TIQ compounds in the mediation of the addictive properties of ethanol is that they would increase ethanol self-administration. Conflicting data are available on this issue (see Smith, 1982). According to Smith (1982), it seems that TIQ compounds do not affect voluntary ethanol intake. It is noted also that naloxone (Myers & Critcher, 1982), naltrexone, and morphine blocked (Critcher, Lin, Patel, & Myers, 1983) the

apparent effects of TIQ compounds in increasing alcohol drinking. These findings are inconsistent with the notion that the reinforcing properties of ethanol are mediated by the actions of TIQ compounds at the opiate receptors since an agonist and two antagonists acted the same way. Furthermore, reservation should be exercised in interpreting this finding, since to date no other laboratory apart from that of Myers has been able to fully replicate the finding that TIQ compounds increase alcohol drinking (Brown, Amit, & Smith, 1980b; Dunçan & Deitrich, 1980; Sinclair & Myers, 1982).

In support for some common actions of ethanol and morphine on neurotransmitters, Amit and Levitan (1975) have provided evidence that showed that while morphine self-administration is severely disrupted by dopamine depletion, a selective norepinephrine depletion caused a slight decrease in morphine drinking. In the same study, it was found that ethanol self-administration was more sensitive to norpinephrine depletion than to dopamine depletion. Nevertheless, other studies have implicated norepinephrine as a subtrate in self-administration of both ethanol and morphine. FLA-57 (4-methyl-1-homo-piperazinedithiocarboxylic acid), a dopamine-beta-hydroxylase inhibitor, has been found to suppress both voluntary intake of morphine (Brown, Amit, Sinyor, Rockman, & Ogren, 1978) and ethanol (Amit, Brown, Levitan, & Ogren, 1977; Brown, Amit, Levitan, Ogren, & Sutherland, 1977). The neurotransmitter serotonin also seems to be implicated in the

mediation of both ethanol and morphine self-administration. Zimelidine, a putative serotonin uptake inhibitor which had previously been shown to block ethanol consumption (Rockman, Amit, Bourque, Brown, & Ogren, 1979), also blocked morphine consumption in naive (Rockman, Amit, Bourque, Brown, & Ogren, 1980) and morphine-addicted rats (Ronnback, Zeuchner, Rosengren, Wronski, & Ogren, 1984). The fact that zimelidine does not have any direct action on opiate receptors (Hall & Ogren, 1981) in the rat brain is another argument that common action on the serotonin system may be responsible for this suppression of ethanol and morphine drinking.

Locomotor activity. A report by Middaugh, Read and Boggan (1977) showed that naloxone at a dose of 3 mg/kg lowered the increase in locomotor activity induced by ethanol in the C57BL/6 mice. This finding has recently been replicated by Kiianmaa, Hoffman, and Tabakoff (1983) who demonstrated that a 1 mg/kg of naltrexone reversed an ethanol-induced increase in locomotor activity in two strains of mice. The results from these two studies seem to support the notion that the opiate receptor is involved in the effects of ethanol on motor activity.

Analgesia. Mehar, Parker, and Tubas (1974) have shown that oral administration of ethanol increased the effects of morphine on the threshold current for avoiding footshock, particularly at the high doses of morphine. This finding could be interpreted as an

enhancement of the analgesic action of morphine by ethanol. An attempt to examine the role of the opiate receptor in the ethanol-opiate interaction in this paradigm resulted in a failure of naloxone to reverse ethanol-induced analgesia (Bass, Friedman, & Lester, 1978). Ethanol administration was found to reverse the increase in startle response and vocalization due to naloxone. Nonetheless, the authors argued that this action of ethanol cannot be attributed to its analgesic or motoric effects (Bass et al., 1978). Failure of naloxone to reverse the analgesic effects of ethanol has also been reported in a study by Jorgensen and Hole (1981). In that study, naloxone in a range of 0.5 to 10 mg/kg did not affect the analgesic effect of ethanol in the tail-flick paradigm. It does not seem then that ethanol and opiates interact in terms of their analgesic properties. Another explanation for these conflicting findings may relate to the methodological difficulties of these studies. The behavioral correlates of nociception usually show high variability, particularly when rats are stressed with multiple drug injections as the case would be in such drug interaction studies.

The analgesic properties of TIQ compounds have also been investigated. In the study by Fertel and coworkers (1980), it was reported that salsolinol and THP had analgesic potencies similar to those of enkephalins. In addition, these investigators showed that analgesia produced by those two TIQ compounds was reversible by

naloxone. This finding is consistent with earlier reports that demonstrated blockade of the analgesic effects of 3-carboxy salsolinol and salsolinol with naloxone (Marshall, Hirst, & Blum, 1977). These findings provide additional support for the notion that the action of TIQ compounds at the opiate receptors may affect a variety of behavioral measures. Nevertheless, the data do not give direct support for their roles as "links" in the interaction between ethanol and opiates (cf. Blum, 1980).

Intracranial Self-Stimulation. A comprehensive discussion of intracranial self-stimulation (ICSS) would be beyond the scope of this dissertation (for a review see Gallistel, 1983). In addition, for a complete treatment of the effects of drugs on ICSS, the reader is referred to reviews by Esposito and Kornetsky (1978), and Wauquier (1976). Instead, the following paragraphs will focus on studies that are related directly or indirectly to the interaction between alcohol and opiates.

Low doses of several drugs with abuse potential, including amphetamine (Carey, Goodall, & Lorens, 1975; Holtzman, 1976), ethanol (Carlson & Lydic, 1976; Vrtunski, Murray, & Wolin, 1973), and morphine (Glick & Rapaport, 1974; Lorens, 1966) have been reported to decrease the threshold for electrical brain stimulation. To account for these findings, it is proposed that drugs with rewarding properties will lower the threshold whereas drugs with punishing properties will raise

the threshold for ICSS. Using this paradigm, Lorens and Sainati (1978) studied the effects of ethanol on ICSS. They found that ethanol increased the percent change in bar-presses from predrug baseline for lateral hypothalamic ICSS; a 5 mg/kg of naloxone prevented this effect of ethanol. The dose of naloxone used did not affect lateral hypothalamic ICSS by itself and also did not alter the blood ethanol level. Based on these findings, these researchers proposed that ethanol may release endogenous opioids whose action at opiate receptors results in an excitatory behavioral (euphorigenic) effect which is naloxone-reversible. This interpretation should be assessed with caution since the increased responding in ICSS following ethanol administration may have been due to hyperactivity rather than potentiation of reward. A recent study by Lewis (1984) reported that a decrease in brain stimulation threshold in the lateral hypothalamus produced by 0.25 g/kg of ethanol was unaffected by 1.6 mg/kg of naloxone. An increase in response rate by 1.5 g/kg of ethanol was also not altered by the same dose of naloxone. The use of a threshold measure helps avoid the confounding factor of motoric capacity on brain stimulation thus making the data concerning the effects of ethanol in Lewis' study less ambiguous. However, Lewis' (1984) conclusion that ethanol and opiates do not share some common mechanism or mechanisms is unwarranted because of the single dose of naloxone used.

Motoric Effects. A number of measures have been used to evaluate the motoric effects of ethanol. Using the roto-rod as a measure of motor performance, Hymson and Hynes (1982) failed to find any evidence for a role of an opioid mechanism in the mediation of the debilitating effects of ethanol on motor performance. These workers found that naloxone at 10, 30, and 100 mg/kg dose failed to antagonize motor impairment induced by ethanol in mice, and that there was no cross-tolerance between ethanol and morphine in this model. Jorgensen and Hole (1981) also failed to find any change produced by naloxone pretreatment on ethanol-induced impairment of the righting reflex. Another failure of naloxone to alter the motoric effects of ethanol in several psychomotor tasks was reported by Bird, Chescher, Perl, and Starmer (1982). The evidence seems to favor a lack of interaction between alcohol and opiates on this measure. Nonetheless, two studies did report positive findings. The first report showed that naloxone antagonized ethanol-induced impairment of the aerial righting reflex in rats (Vogel, Frye, Koepke, Mailman, Mueller, & Breese, 1981). However, the reversal of the motor impairment produced by ethanol occurred only at high doses (20-60 mg/kg) of naloxone, possibly indicating a nonspecific effect of the opiate antagonist. The second report is a study by Kiianmaa et al. (1983) in which the investigators demonstrated that a high dose of naltrexone differentially reduced the duration of loss of righting reflex due to ethanol in three strains of

mice. Again, the high dose of naltrexone required to antagonize the effects of ethanol warrants caution in the interpretation of the results. Overall, it seems unlikely that the opiate receptor is involved in the effects of ethanol on this behavioral measure.

Drug Discriminating Properties. In this paradigm, all the published studies to date have failed to observe an interaction between ethanol and opiates or produce any supporting evidence for such interaction. For instance, Chipkin, Stewart, and Channabasavaiah (1980) have shown that 5 mg/kg naloxone did not alter ethanol-appropriate responding. In a substitution test for ethanol, D-Ala(2)-Met(5)-enkephalin-ol, D-Ala(2)-Met(O)(5)-enkephalin-ol, two synthetic opioids did not generalize to the ethanol cue. York and Bush (1982) showed that in monkeys pentobarbital and barbital given intragastrically mimicked ethanol cues, but intraperitoneal injections of morphine sulphate over a wide dose range failed to elicit the response associated with ethanol. In addition, naloxone and naltrexone at 1 and 10 mg/kg doses failed to change the discriminating properties of ethanol in monkeys (Altshuler, Applebaum, & Shippenberg, 1981). Even the TIQ compounds, such as salsolinol, 3-carboxy-salsolinol, and THP, do not show generalization to fentanyl, another synthetic opioid (Shearman & Herz, 1983). From these studies, it is very likely that the pharmacological cues of those two classes of drugs differ markedly.

Toxicity. Clement (1978) has demonstrated that pretreatment with ethanol, pentobarbital or morphine can protect mice from the lethal effects of hemicholinium, a choline uptake inhibitor (Clement, Lockwood, Rylett, & Colhoun, 1979). Since the lethal effects of curare were not prevented by any of these drugs, the authors argued that the protective mechanism of these pretreatment drugs was central rather than peripheral. Thus, it would seem that ethanol and morphine may act on the same protective mechanism to prevent the lethal effects of hemicholinium. Another study reported that 10 mg/kg of naloxone increased the lethal dose of ethanol in mice from 9.2 g/kg to 10.8 g/kg (Ho & Ho, 1979) suggesting a role of the opiate receptor in the lethal effects of ethanol. Supporting this notion, it was found that alpha-l-acetylmethadol (LAAM), a synthetic opioid, increased ethanol lethality even though it also increased the disappearance of blood and brain ethanol levels (Ho, Chen, & Ho, 1978). Nevertheless, the fact that in the same study ethanol was found to decrease LAAM lethality confuses the issues. The authors made no attempt to speculate on the mechanisms underlying these findings. Since little is known about the mechanism of drug lethality, it is difficult to reconcile these findings with theories of ethanol-opiate interaction.

Other Behavioral Studies. Other studies have addressed the issue of ethanol-opiate interaction using different behavioral indices. For instance, using the conditioned taste aversion paradigm, Miceli,

Marfaing-Jallat and LeMagnen (1979) found that naloxone, while not affecting lithium-induced conditioned taste aversion (CTA), enhanced the CTA produced by ethanol. Since the agonistic properties of opiates are believed to produce CTAs, it was expected that naloxone would antagonize the ethanol CTA. The enhancement of ethanol CTA with naloxone was therefore suggested by the investigators to be the result of a nonspecific effect of naloxone (Miceli et al., 1979). Sklar and Amit (1977) also found that both alpha-methyl-para-tyrosine, a tyrosine hydroxylase inhibitor, and pimozide, a dopamine receptor blocker, prevented the induction of CTAs by morphine and ethanol thus implicating dopamine as a common neurotransmitter in the actions of morphine and ethanol. Other investigators have studied the ethanol-opiates interaction by examining the narcosis produced by ethanol. A study by Luttinger et al. (1983) showed that beta-endorphin induced sleep when followed by administration of a subthreshold dose of ethanol, presumably by potentiating the effects of ethanol. Jorgensen and Hole (1981) failed to observe a change in the narcotic effects of ethanol following naloxone administration. Moreover, only very high doses of naloxone in the range of 300 to 400 mg/kg seemed to produce significant reductions in the narcotic effects of ethanol (Khanna, Mayer, Kalant, & Shah, 1982). Since naloxone alone had marked convulsant effects at these doses, the antagonism of ethanol-induced sleep was suggested to be due to the analeptic actions of naloxone

(Khanna et al., 1982). Another report (Ho, Chen & Kreek, 1979) produced evidence that ethanol suppressed morphine withdrawal signs, like diarrhea, and intestinal motility, in a dose-dependent fashion.

In summary, from the behavioral studies reviewed, it would seem that there is some evidence for an interaction between alcohol and opiates. One notes that there are more inconsistent findings from the behavioral studies than from the biochemical or pharmacological studies. Nevertheless, even in studies that demonstrated an alcohol-opiate interaction, the data do not clearly indicate the mechanisms of the interaction.

Clinical Studies

Some clinical observations for an interaction or some commonalities between alcohol and the opiates have already been mentioned at the beginning of this paper. These observations have drawn many researchers to study the interaction between these two classes of drugs more systematically.

Brown, Kozel, Meyers, and Dupont (1973) have studied a group of narcotic addicts and found that addicts' use of alcohol prior to their use of heroin was significantly higher than that of a normal sample group. The study revealed that the addicts' use of alcohol decreased as they became more involved with heroin but did not increase during treatment to terminate heroin addiction. Similar patterns have been

observed elsewhere (Bess, Janus, & Rifkin, 1972; Perkins & Bloch, 1970; Schut, File, & Wohlmuth, 1973). In addition, others have reported excessive alcohol abuse following treatment for opiate addiction (Kreek, 1976; O'Donnell, 1964). These observations were thought to be expressions of an underlying common interaction between alcohol and opiates. In a review of the literature pertaining to alcohol use among opiate addicts by Green and Jaffe (1977), the authors also came to the conclusion that there may be a biological disorder that underlies both alcohol and narcotic use. It was speculated that the endogenous opiates may play a role in the development of both drug dependence (opiates or alcohol or both) and deviant behavior (unipolar depression or sociopathy or both) (Green & Jaffe, 1977).

Based on animal research, Ho and Rossi (1982) have proposed that endogenous opioids may act as modulators on the voluntary consumption of ethanol. Blum and coworkers (Blum, Briggs, DeLallo, Elston, & Ochoa, 1982; Blum, Briggs, Elston, DeLallo, & Sheridan, 1982; Blum, Elston, DeLallo, Briggs, & Wallace, 1983) have further extended this notion by providing some support for the notion that "alcohol-seeking" behavior is a function of endogenous levels of opioids. Two clinical studies from the same laboratory have received support for this hypothesis. In these studies, it was observed that chronic alcoholics had one third of the normal level of beta-endorphin in the

cerebrospinal fluid (Genazzani et al., 1982; Savoldi et al., 1983). These findings are in agreement with the hypothesis that ethanol causes the release of endogenous opioids. Presumably, by releasing those endogenous opioids, ethanol restores the levels of these peptides at optimal levels and thereby reinforces alcohol drinking. However, in another study examining the biochemical correlates of alcoholism, a negative correlation was obtained between level of endorphin and level of blood alcohol in healthy alcoholics (Borg, Kvande, Rydberg, Terenius, & Wahlstrom, 1982).

In some clinical reports, naloxone was shown to reverse the intoxicating effects of ethanol (Barros & Rodriquez, 1981; Jefferys, Flanagan, & Volans, 1980; Mackenzie, 1979; Schenk et al., 1978; Sorenson & Mattisson, 1978). However, in a systematic study of this interaction between naloxone and ethanol in which choice retention, tracking, flicker fusion, body sway on an electronic table, lateral gaze nystagmus, and the balance of extraoculor muscles were assessed, Mattila, Nuotto, and Seppala (1981) failed to observe a change in the effects of ethanol following naloxone administration. In that same study, naloxone also failed to influence the subjective assessment of inebriation produced by ethanol. Bird and coworkers (1982) similarly failed to find any effect of naloxone, given before or after ethanol ingestion, on the blood level of ethanol or on ethanol-induced psychomotor impairment. In a review of the literature, a panel of

investigators made the observation that the literature does not adequately reflect the negative experience of emergency ward physicians who have used naloxone to treat patients intoxicated with alcohol (Dole, Fishman, Goldfrank, Khanna, & McGivern, 1982).

The findings of these clinical studies are as contradictory as those from the behavioral studies. Again, this contrasts with the more coherent picture presented by biochemical and pharmacological studies. It is also becoming evident that the criterion of naloxone-reversibility is a weak one for inferring an involvement of the opiate receptor. That is, the demonstration that an effect of alcohol can be reversed by naloxone is not sufficient evidence for a role of the opiate receptor in this effect. The main reason for this is the accumulated evidence that naloxone has nonspecific effects independent of its action at the opiate receptor (see Sawynok, Pinsky, & LaBella, 1979). For example, naloxone was shown to lower the blood level of ethanol by changing the redox states of the hepatic nicotinamide-adenine dinucleotide phosphate system (Badawy & Evans, 1981). This action of naloxone may explain some of the clinical findings reported in the literature. The difficulties in demonstrating a clear interaction between alcohol and opiates seem to relate to the choice of appropriate behavioral paradigms and to the sensitivity of the behavioral measures.

The Present Investigation

To summarize the review of the studies that have been presented, it seems that the evidence for ethanol-opiate interaction is stronger in terms of the biochemical and pharmacological data than it is in terms of the physiological data. More importantly, it is also evident that the behavioral studies have produced the most conflicting data. Two possibilities can be entertained. One way to reconcile such discrepancies in the behavioral findings is to disregard all instances of positive results and to argue that the biochemical, pharmacological and physiological data are artifacts or may be irrelevant for behavior and for the study of alcohol and opiates abuse in man. The other possibility is that the degree of conflict in the data is related to the discipline of investigation. That is, as the level of the scientific examination becomes more molar there is a larger number of factors that can affect a given behavior. As such, assuming that the reliability of a phenomenon is dependent on the controllability of the factors other than the ones being manipulated, it may be suggested that more conflicting data would be expected the more molar the unit of scientific investigation. Furthermore, certain behavioral paradigms may not be suitable for the observation of drug interaction because the measure used is very often close to one end of its range of values. Such a situation makes it difficult to uncover a bidirectional change in the measure of interest as a function of the

drug interaction. For example, in the runway paradigm, if the rats under certain conditions are already running at their maximum speed down the runway to eat food, it would then be very difficult to show an additive effect of two drugs that are supposed to increase running speed.

Given the conflicting nature of the behavioral data, the present investigation attempts to examine the interaction between alcohol and opiates in the conditioned taste aversion paradigm (CTA) and in the pre-exposure CTA paradigm; the role of the opiate receptor in the interaction will also be studied. It will be argued in the next section that the CTA paradigm, and the pre-exposure CTA paradigm in particular, are very sensitive paradigms and may constitute suitable behavioral assays for the study of drug interactions.

Experiment 1 was conducted to examine the effect of morphine pre-exposure on ethanol-induced CTA, and also the effect of ethanol pre-exposure on morphine-induced CTA. In the second experiment, the effect of a long-acting opiate antagonist, naloxazone, on morphine, and ethanol CTAs was studied. The first part of the third experiment examined the effect of the opiate antagonist, naloxone on the preeposure interaction between ethanol and morphine. In the second part of the third experiment, naloxazone was investigated for its effect on ethanol-morphine pre-exposure interaction in the CTA paradigm. The fourth experiment assessed whether two opiate isomers,

one active and the other inactive at the opiate receptor, would differentially influence morphine and ethanol CTAs as pre-exposure agents. In the first part of the fourth experiment, dextrorphan, the inactive opiate isomer, and levorphanol, its active counterpart, were studied for their abilities to produce a CTA at three different doses. The most effective dose of levorphanol to produce a CTA was noted. Using this dose, the effect of levorphanol pre-exposure on morphine and ethanol CTAs was assessed in the second part of the fourth experiment. Dextrorphan was used as the control drug and was administered at the same dose as levorphanol. Finally, in the fifth experiment, the pre-exposure interaction between acetaldehyde, the primary metabolite of ethanol, and morphine was examined. It was hypothesized that, like pre-exposure to ethanol, acetaldehyde pre-exposure would block a morphine CTA. It was also hypothesized that pre-exposure to morphine would block an acetaldehyde-induced CTA. The latter hypotheses are based on finding that acetaldehyde is responsible for some of the psychopharmacological actions of ethanol (e.g. Amir et al., 1980).

Conditioned Taste Aversion (CTA)

Numerous studies have demonstrated that if a drug that has aversive properties is administered to rats immediately following the presentation of a novel taste solution, the rats will avoid the novel

flavour on subsequent trials. Intestinal malaise is an example of the aversive properties that appear to act as the unconditioned stimulus in these experiments. The conditioned change in consumption of the novel substance is referred to as the conditioned taste aversion (CTA) and was first demonstrated in animals by Garcia and colleagues (Garcia, Kimeldorf, & Koelling, 1955). In their first study, Garcia et al. (1955) presented rats with saccharin followed by exposure to gamma radiation which presumably made the animals sick. These rats were found to avoid the saccharin solution on subsequent occasions. Two important features characterize the CTA phenomenon. First, because there was a delay of several hours between the sweet taste of the saccharin solution and the toxic effect of radiation, the CTA phenomenon was considered incongruent with the traditional learning theories that emphasized close temporal contiguity between the conditioned stimulus and the unconditioned stimulus (Seligman, 1970). Second, it was reported that some stimuli are more relevant than others in particular situations (Garcia & Koelling, 1966). For example, rats were apparently predisposed to associate tastes with illness. Although there is still controversy about the nature of this phenomenon (see Gamzu, 1977), it is regarded by many researchers as an adaptive mechanism for animals enabling them to quickly learn to avoid poisonous substances (Seligman, 1970). It is important to note that for the purpose of this investigation, aversion will be defined as

some noxious state which is expressed as an avoidance of some stimuli by the animal. More specifically, aversion will be operationally defined as a significant decrease in the consumption of the saccharin solution from the baseline intake at the first presentation of the saccharin solution.

It has also been established that animals previously pre-exposed to a drug will not develop CTA associated with that particular drug. This pre-exposure effect has been reported in the cases of lithium (Cannon, Berman, Baker, & Atkinson, 1975; Holman, 1976; Batson & Best, 1979), morphine (Cappell, LeBlanc & Herling, 1975; Stewart & Eikelboom, 1978; Brown, Amit, Smith & Rockman, 1979), ethanol (Berman & Cannon, 1974; Cannon et al., 1975; Barker & Johns, 1978). Cross-drug pre-exposure effects have also been reported. For example, when rats had prior exposure to lithium, attenuation of an ethanol CTA was observed; similarly, prior exposure to ethanol decreased the aversion associated with lithium (Cannon, Baker, & Berman, 1977). Switzman, Fishman, and Amit (1981) have reported an asymmetrical cross-drug pre-exposure effect between morphine, diazepam, and delta-9-tetra-hydro-cannabinol.

The cross-drug pre-exposure effect in this paradigm allows a further examination of drug interactions. There are several advantages in this paradigm. First, the drug administrations are given separately at long time intervals. Such a procedure avoids, or

at least minimizes, the possible effect of one drug in altering the pharmacokinetics of the other drug. Naloxone was observed to lower blood-ethanol concentration by reversing the disturbances in the redox states of the hepatic nicotiamide-adenine dinucleotide caused by the metabolism of ethanol in rats (Badawy & Evans, 1981). This finding suggests that some effects of ethanol that have been shown to be naloxone-reversible may be explained by this mechanism and do not necessarily involve the opiate receptor. At the same time, the cross-drug pre-exposure paradigm minimizes the stressful reactions that are likely to be associated with multiple injections of drugs. Second, since this is a conditioning paradigm, the animals are tested in a drug-free state. Thus, unlike a number of other paradigms that measure the direct positive reinforcing properties of drugs, the CTA paradigm and the pre-exposure CTA paradigm are not confounded by motor-debilitating effects of the drugs being investigated. Third, because CTA is such a robust, stable and reliable phenomenon, it provides a ready and easy behavioral tool to examine the ethanol-morphine interaction.

Psychoactive drugs that are known to be self-administered, and, by definition, possess positive reinforcing properties, are also capable of producing a CTA. LeMagnen (1969) was first to demonstrate a CTA caused by a low dose of the psychoactive drug, amphetamine. Since then the psychoactive drugs that have been shown to produce CTAs

are opiates (Cappell, LeBlanc & Endrenyi, 1973; Jacquet, 1973), amphetamine (Berger, Wise & Stein, 1973; Cappell et al., 1975), cocaine (Booth, Pilcher, D'Mello & Stolerman, 1977; Goudie, Dickins & Thornton, 1978), ethanol (Cappell et al., 1973; Eckardt, 1975), barbiturates (Vogel & Nathan, 1975), benzodiazapines (Cappell et al., 1973; Vogel & Nathan, 1975), cannabinoids (Corcoran, Bolotow, Amit & McCaughran, 1974; Elsmore & Fletcher, 1972; Kay, 1975), fenfluramine (Booth et al., 1977; Goudie, Taylor & Atherton, 1975), methylscopolamine (Berger et al., 1973; Braveman, 1975), and nitrous oxide (Goudie & Dickens, 1978). Some researchers believe that there is a close relationship between the positive reinforcing effects of drugs and their aversive properties, as measured by their abilities to produce a CTA (Switzman, 1980; White, Sklar, & Amit, 1977; Wise, Yokel, & DeWit, 1976). The above discussion is only peripherally relevant to the present dissertation. Therefore, an elaboration of this thesis is not appropriate at this stage. However, one should note that there are major differences between CTAs produced by self-administered drugs and those induced by non-self-administered drugs (e.g. Dacanay & Riley, 1982; Sklar & Amit, 1977). Nevertheless, the present dissertation will attempt to address certain issues concerning mechanisms of CTAs induced by self-administered drugs like ethanol or morphine.

One can argue that the study of CTAs induced by psychoactive

drugs can potentially have important ramifications in psychopharmacology. First, an examination of the CTA induced by a particular drug could add important information as to its aversive qualities, its mode of action, and the mechanisms that mediates aversion in general. Second, the study of drug-induced CTAs may also give some clues as to the mechanisms of the reinforcing effects of that drug. Third, drug-induced CTAs can be used as a behavioral tool to investigate the relationship between reinforcement and aversion in the central nervous system.

From the above discussion, it is likely that CTA and the pre-exposure effect are very sensitive and reliable phenomena that may provide suitable paradigms for the study of alcohol-opiate interaction. Hopefully, the use of these paradigms will provide a stronger test for the existence of a behavioral interaction between alcohol and opiates, and thus offer a basis for further exploration of the underlying common mechanism or mechanisms of their actions.

EXPERIMENT 1

The studies that have been reviewed so far suggest that alcohol and opiates interact on several behavioral dimensions (e.g. Ho & Rossi, 1982; Kiianmaa et al., 1983; Lorens & Sainati, 1978; Middaugh et al., 1977; Sinclair et al., 1973). However, a large number of studies have produced contradictory findings (e.g. Doyle & Samson, 1984; Hymson & Hynes, 1982; Jorgensen & Hole, 1981; Lewis, 1984; York & Bush, 1982). We have argued that the pre-exposure CTA paradigm is a very sensitive behavioral paradigm for observing drug interaction. To capitalize on the advantage of this paradigm, it was used as an attempt to examine the relationship between alcohol and morphine.

Early studies have shown that pre-exposure to a drug will prevent the development of a CTA to that same drug (Cappell et al., 1975). This phenommenon called the pre-exposure effect in the CTA literature has been reported, among others, for drugs like lithium (Batson & Best, 1979; Riley, Jacobs, & LoLordo, 1976), amphetamine (Cappell & LeBlanc, 1975; Goudie & Thornton, 1975), and morphine (Cappell et al., 1975; Jacobs, Zellner, LoLordo, & Riley, 1981). The pre-exposure effect between different drugs has also been demonstrated. For instance, prior exposure to lithium has been shown to prevent the development of CTA produced by ethanol (Cannon et al., 1977).

Experiment 1 was carried out to examine the relationship between alcohol and opiates in the pre-exposure conditioned taste aversion

paradigm (CTA). More specifically, the effect of ethanol pre-exposure on morphine-induced CTA was investigated. In addition, the effect of morphine pre-exposure on ethanol-induced CTA was examined. It was hypothesized that prior experience with ethanol will block the formation of a CTA associated with morphine administration. Similarly, prior experience with morphine was hypothesized to block the development of a CTA associated with ethanol injection.

Method

Subjects

Subjects were 67 male Sprague Dawley rats (Charles River Breeding Laboratories Ltd.) weighing between 250-275g at the beginning of the experiment. The animals were housed individually in stainless-steel cages with free access to water and food (Purina Rat Chow). The animal colony was illuminated on a 12 hour day-night schedule. After one week of adaptation to the animal colony environment, the rats were placed on a water-deprivation schedule with free access to food. Every day at the same time, the rats were presented with water for 30 minutes, and water intake was measured.

Drugs and Injections

Morphine hydrochloride (Merck, Sharp and Dohme Ltd.) was dissolved in injectable Ringer's solution to a concentration of 12 mg/ml. A 20% (w/v) ethanol solution was prepared by mixing a 95% ethanol solution with distilled water. The doses of morphine and ethanol used in this experiment and those to be described have been selected based on data from preliminary studies. These doses of each drug respectively have been found to reliably produce CTAs of moderate magnitude.

All injections were given intraperitoneally (i.p.). Morphine injection was given in a volume of 1 ml/kg and ethanol was administered in a volume of 7.5 ml/kg.

Procedure

After two weeks of adaptation to the water-deprivation schedule, animals were divided into 2 batches. Each batch was further subdivided into two groups. An injection of 1.2 g/kg of 20% ethanol (E) or distilled water (W) was administered i.p. to rats in batch 1 on Days 1, 3, and 5. Similarly, 12 mg/kg of morphine (M) or Ringer's solution (S) were given i.p. to animals in batch 2. On Day 7, animals in both batches were exposed to the novel taste of a 0.1% saccharin solution for 30 minutes. Immediately following the saccharin presentation, animals in batch 1 (pre-exposed to either E or W) were

injected with either 1.2 mg/kg of M or S (groups E-M, n=9; E-S, n=8; W-M, n=8, W-S, n=8) while animals in batch 2 (pre-exposed with either M or S) were injected with either 1.2 g/kg of E or W (groups M-E, n=9; M-W, n=8; S-E, n=9; S-W, n=8). The latter trial constituted the first conditioning trial. The pre-exposure treatment were repeated on Days 8, 10, and 12. On Day 14, a second conditioning trial was carried out in manner identical to the first conditioning trial. Saccharin solution (0.1%) was presented to the animals on three more occasions without drug injections at 7-day intervals (Extinction trials, Days 21, 28, 35). The consumption of saccharin solution was measured to the nearest ml at each presentation. A summary of the procedure is provided through the schematic diagram in Figure 1. Except for Experiment 1 where the fourth pre-exposure injection was given one day following the first conditioning trial (Day 8) and where the time interval between trials was 7 days, all the other pre-exposure experiments to be reported followed closely the procedure detailed by the schematic diagram (see Figure 1).

Statistical Analysis

Saccharin intake on the first conditioning trial was used as baseline. The baseline values for the different groups were first compared using Tukey HSD test (Kirk, 1968). If no significant difference was found between the baseline values, then the scores for

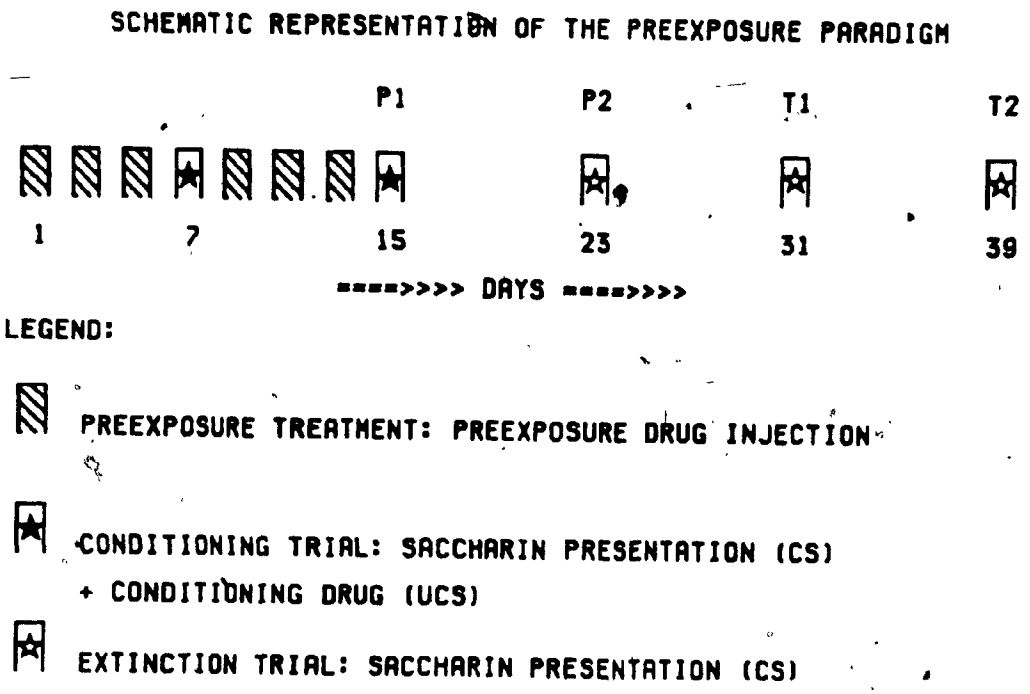

FIGURE 1. Schematic diagram representing the preexposure conditioned taste aversion paradigm. Saccharin consumption on first conditioning trial was used as baseline. P1, P2 represent the % change scores for conditioning trials and T1 and T2 represent the % change scores for extinction trials. Note that for Experiment 1, the fourth preexposure treatment took place on the day following the first conditioning trial and that the intertrial interval was seven days long.

saccharin intake on all other trials were computed as percent change scores from the baseline values for each animal. Statistical analyses were performed on these transformed percent scores. A one-way Dunnett's test (Kirk, 1968) was used to evaluate the deviation of the group means of the percent change scores from baseline for each trial. A significant decrease in saccharin consumption would indicate an aversion while an increase would indicate a preference for the saccharin solution. In addition, an a priori t-test (Kirk, 1968) was used to make between-groups comparisons.

Results

Means of raw scores and of percent change scores of saccharin consumption for all trials are shown in Appendix A. The means and standard errors of baseline saccharin intake of all the groups are presented in Table 1. Using Tukey HSD test, no significant difference was observed between the groups for the baseline scores ($p > 0.05$). P1 and P2 represent the mean percent change scores in saccharin intake for the first and second conditioning trials respectively (see Figure 1). T1 and T2 represent the mean percent change scores in saccharin intake for the first and second extinction trials respectively (see Figure 1). Using Dunnett's test, it was found that animals pre-exposed with distilled water and conditioned with Ringer's solution (W-S) showed high increases in saccharin intake for all trials ($p < 0.05$).

Table 1

Means and standard errors of baseline saccharin intake of animals preexposed with morphine (M-), saline (S-), ethanol (E-) or distilled water (W-) and conditioned with either one of the drugs or control solutions.

Group	Mean	Standard Error
W-S (n=8)	17.13	0.91
E-S (n=8)	16.13	0.52
W-M (n=8)	16.25	1.10
E-M (n=9)	14.44	1.21
S-W (n=8)	17.88	0.64
M-W (n=8)	14.88	1.47
S-E (n=9)	17.89	0.98
M-E (n=9)	16.44	0.71

* n between brackets refers to the number of animals in each group.

Table 1

Means and standard errors of baseline saccharin intake of animals preexposed with morphine (M-), saline (S-), ethanol (E-) or distilled water (W-) and conditioned with either one of the drugs or control solutions.

Group	Mean	Standard Error
W-S (n=8)	17.13	0.91
E-S (n=8)	16.13	0.52
W-M (n=8)	16.25	1.10
E-M (n=9)	14.44	1.21
S-W (n=8)	17.88	0.64
M-W (n=8)	14.88	1.47
S-E (n=9)	17.89	0.98
M-E (n=9)	16.44	0.71

* n between brackets refers to the number of animals in each group.

The E-S group also revealed significant increases in saccharin consumption for all trials ($p < 0.05$). Rats in the W-M group displayed significant decreases in saccharin consumption for the two conditioning trials (P1 and P2, $p < 0.05$) and on the first extinction trial (T1, $p < 0.05$). However, rats pre-exposed to E and conditioned to M only exhibited a significant decrease in saccharin intake on second conditioning trial (P2, $p < 0.05$) (see Figure 2a). In addition, the percent change scores for the W-M group were significantly smaller algebraically than those for the E-M group after all trials (a priori one-tailed t-test, $p < 0.05$).

The results for batch 2 revealed a similar pattern to that in the batch 1. Tukey HSD test showed no significant difference between the baseline values. Animals that did not receive E as the conditioning drug showed significant increases in saccharin consumption (S-W and M-W groups, $p < 0.05$). Significant decreases in saccharin intake were observed in the S-E group on the two conditioning trials (P1 and P2, $p < 0.05$). When the rats were pre-exposed to M, they did not show significant decreases in saccharin associated with the ethanol conditioning (M-E group) on both conditioning trials (P1 and P2, $p > 0.05$). Instead, the M-E group exhibited a significant increase in saccharin consumption on the second extinction trial (T2, $p < 0.05$, see Figure 2b). Furthermore, the M-E group also had significantly higher percent change scores than the S-E group for all trials (one-

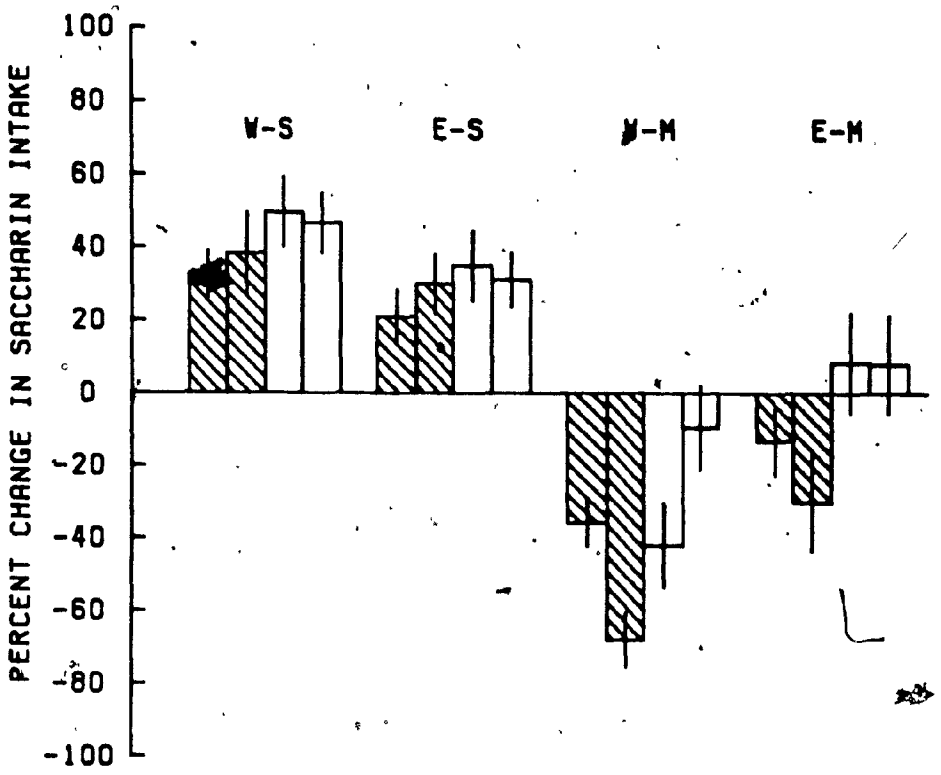

FIGURE 2a. Means and standard errors of percent change scores in saccharin intake of groups pre-exposed with ethanol (E-) or distilled water (W-) and conditioned with morphine (-M) or saline (-S) for conditioning (hatched bars) and extinction (opened bars) trials.

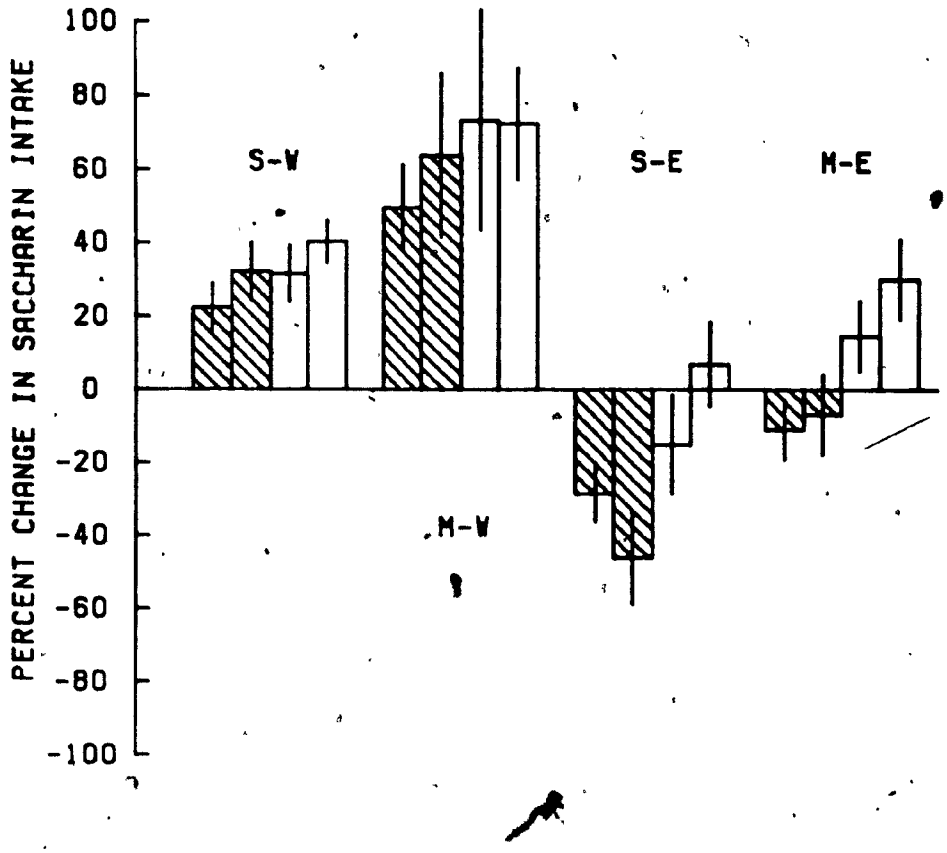

FIGURE 2b. Means and standard errors of percent change scores in saccharin intake of groups pre-exposed with morphine (M-) or saline (S-) and conditioned with ethanol (-E) or distilled water (-W) for conditioning (hatched bars) and extinction (opened bars) trials.

tailed a priori t-test, $p < 0.05$).

Further analyses using two-tailed a priori t-test revealed two unexpected but interesting findings. First, it was found that pre-exposure to E alone (E-S) caused some reductions in saccharin consumption compared to the W-S group which was significant for the second extinction trial (T2, $p < 0.05$). Second, animals that were pre-exposed to M alone (M-W) showed significantly larger increases in saccharin consumption than the S-W group for all trials ($p > 0.05$).

Discussion

The data indicate that prior exposure to morphine blocked the conditioned taste aversion to ethanol. Conversely, it was observed that prior exposure to ethanol blocked the conditioned taste aversion to morphine. The results confirmed other reports that have demonstrated an interaction between alcohol and the opiates (e.g. Hoffman et al., 1982; Lucchi et al., 1984; Mayer et al., 1980; Sinclair et al., 1974).

The symmetrical interaction between alcohol and opiates in this pre-exposure conditioned taste aversion paradigm interestingly stands in contrast to most of the interactions between different classes of drugs that have been observed so far in this paradigm. For example, Switzman et al. (1981) reported that prexposure to delta-9-tetra-hydro-cannabinol (THC) blocked its own CTA, as well as CTAs induced by

diazepam and morphine. However, neither diazepam nor morphine pre-exposure was able to block the CTA induced by THC. Similarly, pre-exposure to diazepam blocked the CTAs associated with diazepam, or morphine but pre-exposure to morphine did not block the CTA induced by diazepam. According to Switzman and coworkers (1981), the likelihood that a CTA induced by one drug will be blocked by pre-exposure to other drugs seems to be negatively correlated with the relative liabilities of the two drugs to be self-administered by animals. For instance, Switzman (1980) showed that morphine, the compound with highest potential for self-administration of the three drugs tested, produced a CTA that was easily disrupted by THC and diazepam. On the other hand, diazepam, which is more easily self-administered by animals than THC, induced a CTA that was blocked by diazepam itself and by THC but not by morphine.

If one extends those arguments to the results of the present experiment, one can speculate that the symmetrical interaction between alcohol and morphine may reflect the fact that the two drugs have about the same potential for self-administration in animals.

The fact that morphine pre-exposure alone led to an increase in saccharin consumption, the possibility of an asymmetrical interaction between morphine and ethanol should be considered. That is, the apparent attenuating effect of morphine pre-exposure on development of CTA induced by ethanol as observed in the M-E group could be the

summation of independent effects of the two drugs on saccharin consumption. Because morphine pre-exposure increased saccharin consumption and the conditioned response associated with ethanol was a decrease in saccharin intake, one would observe no change in saccharin intake from baseline levels. On the other hand, the pre-exposure to ethanol seemed to decrease consumption of saccharin in the same direction as the conditioned response associated with morphine. Yet, in the latter case, an attenuation of morphine CTA was observed. Hence, a case can be made for asymmetry rather than symmetry in the psychopharmacological interaction between ethanol and morphine. Nevertheless, the symmetrical interaction hypothesis seems to offer a more parsimonious explanation for the data from experiments to be described.

EXPERIMENT 2a

The interaction between alcohol and morphine observed in Experiment 1 puts into question the mechanism of ethanol-induced and of morphine-induced CTAs. While there is still some debate about the processes underlying the pre-exposure effect in the CTA paradigm, it can be argued that CTA to drug A should be blocked or attenuated by drug B only if drug B shares some pharmacological or drug-discriminative properties of drug A (Braveman, 1975; Gamzu, 1977). Since ethanol pre-exposure attenuates morphine CTA, and conversely, morphine pre-exposure blocks ethanol CTA, it follows that ethanol and morphine must share some pharmacological or drug-discriminative properties.

This view is inconsistent with available evidence suggesting that ethanol and morphine do not have any common drug-discriminative properties (Miksic, Shearman & Lal, 1978; Shearman & Herz, 1983; Winter, 1975). These studies used ethanol or morphine as the discriminative stimulus and found no evidence that one drug can substitute for the other. Nevertheless, it is still possible that ethanol and morphine share some common actions that are either irrelevant for drug-discrimination or that are masked by others of their discriminating actions. Since ethanol is a simple molecule that does not have the structure specificity of opiates, it would seem more likely that the opiate-like actions of ethanol are mediated by

modulation of an endogenous opiate system rather than by directly activating the opiate receptor. However, it is still important to establish whether the blockade of the opiate receptor will antagonize the opiate-like actions of ethanol.

In an attempt to examine the involvement of the opiate receptor in the CTA produced by ethanol, LeMagnen and coworkers (Miceli et al., 1979) observed that pretreatment with naloxone, an opiate antagonist, enhanced the CTA induced by ethanol. These authors postulated that naloxone nonspecifically caused release of adrenocorticotropic hormone (ACTH) which mediates the enhancement of ethanol CTA. The difficulty with this intrepretation is that naloxone itself has been shown to produce a CTA (LeBlanc & Cappell, 1975; Van der Kooy & Phillips, 1977). In the report by Miceli et al. (1979), naloxone alone did not significantly produce a CTA. Still, it can be argued that a CTA produced by naloxone may have masked its own actions in reversing ethanol-induced CTA.

Experiments 2a and 2b were attempts to examine the direct effect of an opiate antagonist on CTAs induced by morphine and alcohol. The opiate antagonist used in these experiments was naloxazone, a very long-acting opiate antagonist. Naloxone, the most commonly used opiate antagonist, was not appropriate for this experiment because of its short half-life. A study using high-performance liquid chromatography with electrochemical detection has confirmed an earlier

report (Berkowitz, Ngai, Hempstead, & Spector, 1975) that the serum half-life of naloxone in the rat is about 40 minutes (Tepperman, Hirst, & Smith, 1983). If naloxone were used, it would have been important that the pretreatment be given close to the conditioning drug injection on conditioning days. Presumably, even if the naloxone injection is given prior the saccharin presentation, the change in the internal state of the animal due to the drug's actions would occur after the saccharin cue. Given these favourable temporal parameters for forward conditioning, it would have been difficult to avoid the association between the conditioned stimulus (usually a novel tasting substance, e.g. saccharin) and the action of naloxone; and hence, the induction of a CTA due to the naloxone alone. In their report, Pasternak and Hahn (1980) observed that mice pretreated with naloxazone did not show evidence for analgesia when morphine was given 24 hours later. By using such a long-acting opiate antagonist as naloxazone, it was possible to administer the drug a few hours prior to the injection of the conditioning drug. Any change in the internal state of the animals due to the naloxazone injection would occur before the presentation of the conditioned cue. This procedure makes the induction of a CTA to the pretreatment of the opiate antagonist less likely; hence the interaction between the pretreatment drug and the conditioning will be less confounded.

Method

Subjects

Twenty male Sprague-Dawley rats (Canadian Breeding Laboratories Ltd.) were housed under similar laboratory conditions to those in Experiment 1 for one week.

Drugs and Injections

Naloxazone hydrochloride was prepared from naloxone (Endo Laboratories Ltd.) as described by Pasternak & Hahn (1980). Naloxone was slowly added to anhydrous hydrazine dissolved in absolute ethanol. The solution was stirred at room temperature for 90 minutes, after which time it was added carefully to a 5% sodium borate solution. The aqueous solution was extracted three times with chloroform and the combined organic extracts were then back-washed with 5% sodium borate solution, dried over anhydrous sodium sulphate, and concentrated by evaporation in vacuo. Petroleum ether was added to the residue and the solution was allowed to stand while naloxazone crystallized. The naloxazone was dissolved in saline solution to yield a concentration of 10 mg/ml and injections were administered i.p. in a volume of 1 ml/kg. Ethanol and morphine HCl preparations and administrations were similar to those in Experiment 1.

Procedure

The rats were placed on water-deprivation schedule during which access to water was restricted to 30 minutes per day. Since no data were available concerning the lowest active dose of naloxazone in this paradigm, the dose used in this experiment was determined by assessing its unconditioned effect on saccharin intake. It was found that naloxazone, at a dose of 10 mg/kg, decreased saccharin consumption.

When water intake had stabilized, animals were randomly divided in three groups. On Day 1, animals in groups N-S (n=6) and N-M (n=7) were injected i.p. with naloxazone (10 mg/kg, N) while animals in group S-M (n=7) received saline (S). Four hours later, the rats were presented with a novel tasting 0.1% saccharin solution for 30 minutes. Immediately following the saccharin presentation, the rats were injected with either 12 mg/kg morphine, M (groups N-M and S-M) or saline, S (group N-S) (conditioning trial 1). The second and third conditioning trial took place at intervals of six days (Days 7 and 13). Thereafter, the rats were presented with a saccharin solution without drug injection three more times at 6-day intervals (Days 19, 25, and 31).

Statistical Analysis

As in Experiment 1, saccharin intake on this first presentation was used as a baseline. However, because of significant differences between the baseline values, the data were transformed into change scores from baseline. Dunnett's test was used to test the change scores for significant departure from baseline.

Results

Means of the raw scores and of the change scores are tabulated in Appendix B. Naloxazone significantly decreased saccharin drinking on the first presentation (Tukey HSD test, $p < 0.05$; see Table 2a). Figure 3a illustrates the means of change scores for all trials. In the group of animals that received saline as pretreatment and morphine on conditioning trials (S-M), there were substantial and significant decreases in saccharin intake from baseline level for the second and third conditioning trials (P2 and P3, $p < 0.05$). Gradual recovery was evident after the two extinction trials (T1 and T2). Both the N-S and the N-M groups showed no decrease on the conditioning trials. These two groups showed significant increases in saccharin consumption for conditioning and extinction trials ($p < 0.05$). Although naloxazone as a pretreatment produced a decrease in baseline saccharin intake (see Table 2a), it did not cause any decrease in saccharin intake as percent change from the baseline value for the conditioning and extinction trials as

Table 2a

Means and standard errors of baseline saccharin intake of groups that received naloxazone (N-) or saline (S-) as pretreatment and morphine (-M) or saline (-S) as conditioning drug.

Group	Mean	Standard Error
N-S (n=6)	14.17	0.95
S-M (n=7)	19.00	1.99
N-M (n=7)	12.29	1.11

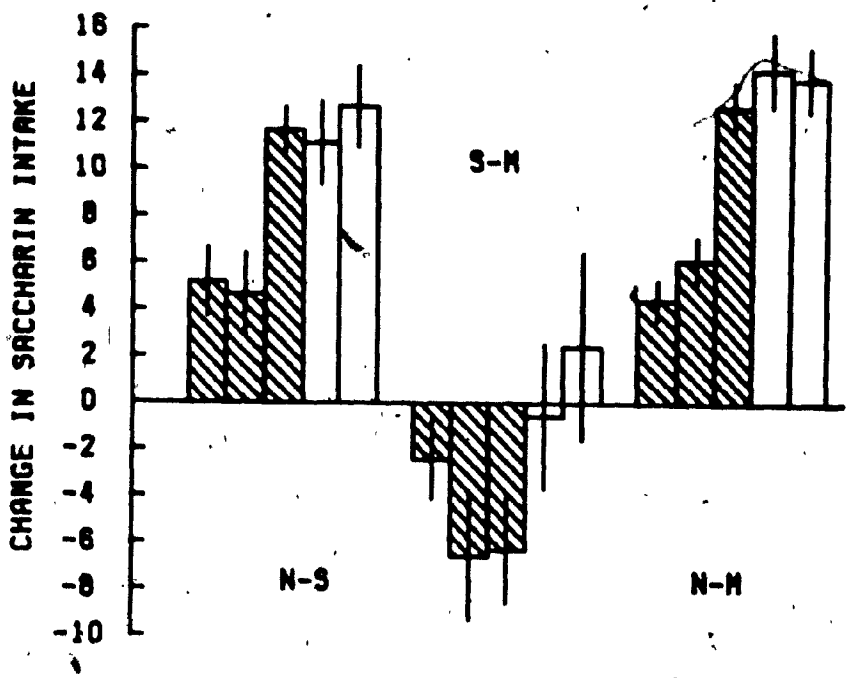

FIGURE 3a. Means and standard errors of change scores for saccharin intake of groups that received naloxazone (N-) or saline (S-) as pretreatment and saline (-S) or morphine (-M) as the conditioning drug for conditioning (hatched bars) and extinction (opened bars) trials.

observed in the N-S group. Instead, the N-S group showed significant increases in saccharin consumption for all trials ($p < 0.05$). These large increases in saccharin intake may be partly due to a rebound effect following the suppressing actions of naloxazone on fluid consumption during the first three pairings, a common effect that has been observed with a number of psychoactive drugs (Switzman et al., 1981).

Discussion

Previous studies have shown that naloxone can also block the CTA induced by morphine (LeBlanc & Cappell, 1975; Van der Kooy & Phillips, 1977). However, the fact that naloxone itself produced a CTA in these same reports has confused the interpretation of the results. Since an opiate agonist and an opiate antagonist can both produce the same behavior, one may conclude that the receptor does not mediate the pharmacological effects of these drugs related to this behavior. One could argue that a nonspecific effect of naloxone would enhance morphine CTA rather than antagonize it. The fact naloxone has been shown to reverse the development of opiate-induced CTAs would instead give more support for the importance of the opiate receptor in opiate-induced CTA. Experiment 2a confirmed the involvement of the opiate receptor in the CTA produced by morphine and possibly by other opiates. This can be seen by the complete reversal of the decrease in

saccharin intake produced by the morphine administration as a result of pretreatment with the long-acting opiate antagonist, naloxazone.

In Experiment 2a, naloxazone alone did not produce a CTA. Because of its long course of action, it was possible to administer the naloxazone a long time before the injection of the conditioning drug. Thus, in so doing, one is assured that conditioning will not develop to the pretreatment drug because the change in internal state (the unconditioned stimulus) due to the naloxazone would occur long before the conditioned stimulus. Because backward conditioning is very weak compared to forward conditioning, such procedure minimizes CTA produced by the pretreatment alone. The absence of a CTA associated with naloxazone alone support the above hypothesis. The complete reversal of the morphine CTA by the naloxazone pretreatment in the present experiment can thus be taken as stronger evidence for the involvement of the opiate receptor in the CTA produced by morphine.

Some early work by Martin and collegues (Martin, Eades, Thompson, Huppler, & Gilbert, 1976) have led those investigators to propose several subtypes of opiate receptors in the mediation of opiates' effects. Other studies have confirmed the existence of three subtypes (see Wood, 1982). Furthermore, naloxazone was reported to bind to the high affinity mu opiate receptor (Pasternak, Childers, & Snyder, 1980). One can speculate then the complete reversal of morphine-

induced CTA may mean that the mu high affinity receptor is principally responsible for the opiate-induced CTA.

EXPERIMENT 2b

In the first part of the second experiment, it was shown that naloxazone pretreatment blocked the CTA produced by morphine. This result was interpreted as a support for the notion that the opiate receptor may be involved in the mediation of the morphine-induced CTA. In the second part of this experiment, the effect of naloxazone pretreatment on an ethanol-induced CTA was examined. If the ethanol-induced CTA has an opiate-like mediational component, one would expect that pretreatment with naloxazone will block or attenuate ethanol-induced CTA.

Method

Subjects

Twenty-one male Sprague-Dawley rats (Canadian Breeding Laboratories Ltd) were randomly divided into two groups. Laboratory conditions were the same as in Experiment 1.

Drugs and injections

The drugs and their preparations were the same as in the previous experiments.

Procedure

The procedure was the same as in Experiment 2a except that only two conditioning trials were carried out, and ethanol was used as the unconditioned stimulus. Group N-E (n=11) received 10 mg/kg of naloxazone as pretreatment and 1.2 g/kg of ethanol as the conditioning drug while the animals in group S-E (n=10) were injected with saline as pretreatment and with 1.2 g/kg of ethanol as the conditioning drug.

Statistical analysis

Dunnett's test was once again used to test for significant difference between change scores from baseline within each group.

Results

Means of the raw data and of the change scores for saccharin consumption are given in Appendix B. It was observed that naloxazone produced a decrease in baseline saccharin intake (Tukey HSD test, $p < 0.05$; see Table 2b). The results, illustrated in Figure 3b, show that both the S-E and N-E groups demonstrated significant decreases of equal magnitude in saccharin consumption for the first conditioning trial (P1, $p < 0.05$). However, for the second conditioning trial (P2), while the N-E group showed the same decrease in saccharin intake, the S-E group showed a greater decrease in saccharin drinking. Using an a priori t-test, this difference in change scores between

Table 2b

Means and standard errors of baseline saccharin intake of groups that received naloxazone (N-) or saline (S-) as pretreatment and ethanol (-E) as the conditioning drug.

Group	Mean	Standard Error
S-E (n=10)	12.73	0.95
N-E (n=11)	19.38	0.88

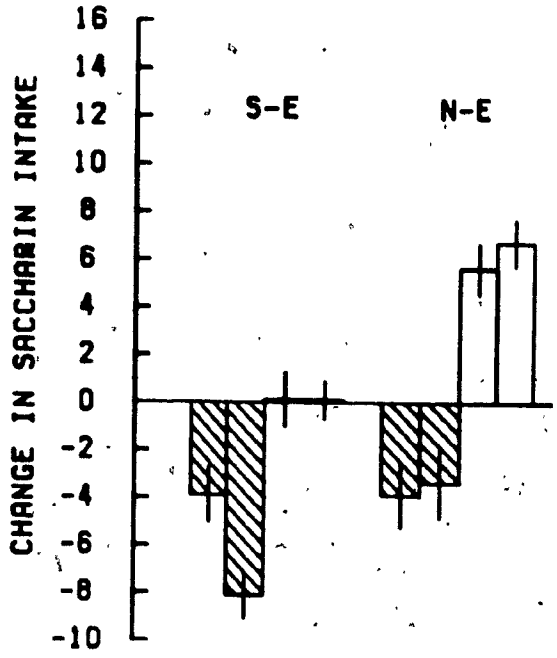

FIGURE 3b. Means and standard errors of change scores for saccharin intake of groups that received naloxazone (N-) or saline (S-) as pretreatment and ethanol as the conditioning drug for conditioning (hatched bars) and extinction (opened bars) trials.

these two groups was found to be significant ($p < 0.05$). In addition, the differences between the two groups in terms of percent change in saccharin intake for the extinction trials (T1 and T2) were also significant ($p < 0.05$).

Discussion

The data obtained in Experiment 2b suggest a common central basis for the actions of alcohol and morphine in the induction of CTA. While in the case of morphine the CTA was completely reversed by the antagonist nalaxazone, the latter drug only partially reversed the ethanol-induced CTA. It would seem that this interaction involved the high-affinity, low-capacity mu opiate receptor. Unpublished work in our laboratory that was designed to investigate the role of opiate receptors in the mediation of both morphine- and ethanol-induced CTAs using the opiate antagonist naloxone, yielded mixed results. One plausible explanation for this inconsistency is that naloxone itself when administered contiguously with saccharin intake induces a CTA (LeBlanc & Cappell, 1975; Van der Kooy & Phillips, 1977). Therefore, in order to eliminate or at least weaken the CTA induced by the opiate antagonist, it was necessary to increase the time interval between injections of the antagonist and the conditioning drugs. Unfortunately, this requirement precluded the use of naloxone as the opiate antagonist because of its short duration of action. Naloxone

has been found to have a serum half-life of about 40 minutes (Berkowitz et al., 1976; Tepperman et al., 1983). On the other hand, naloxazone can completely block opiate receptors for at least 24 hours (Pasternak & Hahn, 1980). At the dose selected, the drug produced some antidipsogenic effect, but it did not induce CTA by itself thus making the interpretation of the present data less ambiguous than in the case of naloxone studies.

In summary, the findings obtained in this experiment suggest that the high affinity binding opiate receptor might be a common element mediating both morphine and ethanol CTAs. The mechanism of the pre-exposure effect has not so far been elucidated. One hypothesis is the opiate receptor may be a common element in morphine- and ethanol-induced CTAs and in the ethanol-morphine pre-exposure interaction. The next experiments were conducted to examine this possibility.

EXPERIMENT 3a

In the previous experiment, naloxazone, a long-acting opiate antagonist, was found to block and attenuate morphine- and ethanol-induced CTAs respectively, thus implicating the opiate receptor in the CTAs associated with these drugs. It would seem to follow that the pre-exposure interaction between morphine and ethanol in the CTA paradigm may also be mediated through the opiate receptor. The following experiments were carried out to test this hypothesis using two opiate receptor antagonists, naloxone and naloxazone.

Method

Subjects

Sixty-six male Sprague-Dawley rats (Charles River Ltd.) weighing between 250g and 310g at the beginning of the experiment were randomly divided into 8 groups. Laboratory conditions were identical to those that prevailed in Experiment 1.

Drugs and injections

Naloxone hydrochloride (Endo Laboratories) was dissolved in saline solution to yield a concentration of 5 mg/ml and injected i.p. in a volume of 1 ml/kg. Ethanol and morphine HCl injections were prepared and administered as described in Experiment 1.

Procedure

The animals were handled and were allowed to habituate to the animal colony prior to experimentation. They were then placed on a water deprivation schedule which restricted them to 30 minutes of water access daily. The procedure was similar to that described in Experiment 1. Therefore, unless specified otherwise, the same procedure was used as in Experiment 1. When water intake had stabilized, the rats were injected with naloxone (N) or saline (S) and immediately after with either ethanol (E), distilled water (W), morphine (M) or saline (S) on Days 1, 3, and 5. On Day 7, the rats were presented with a novel tasting 0.1% saccharin solution for 30 minutes immediately followed by the appropriate conditioning drug injection (first conditioning trial). In denoting the groups, note that the first two letters in the abbreviations stand for the pre-exposure treatment and the last letter represents the conditioning drug. Four groups of animals that received ethanol or distilled water with naloxone (N-E-M, n=8; N-W-M, n=8) or without naloxone (S-E-M, n=9; S-W-M, n=8) on pre-exposure days, were injected with 12 mg/kg of morphine HCl. Similarly, the other four groups (N-M-E, n=9; N-S-E, n=7; S-M-E, n=9; S-S-E, n=8) were injected with 1.2 g/kg of ethanol. The pre-exposure treatment resumed on Days 9, 11, and 13. A second conditioning trial was performed 8 days later. Three more saccharin presentations were made without drug injections at intervals of 8 days

(extinction trials).

Statistical Analysis

Scores are expressed in terms of percent change from baseline intake values. The statistical analyses performed were similar to those described in Experiment 1.

Results

Appendix C represents the means of the raw scores and of the percent change scores of saccharin intake for the different groups. The baseline values are shown in Table 3a. The data for groups that received morphine as the conditioning drug and the data for those that received ethanol as the conditioning drug were analyzed separately. For those groups that were administered morphine on conditioning trials there were no significant differences between group baseline values (Tukey HSD test, $p > 0.05$). Figure 4a illustrates the percent change in saccharin consumed for the conditioning and extinction trials of all groups that received morphine on conditioning trials. No significant decrease in saccharin intake was observed for the first conditioning trial in all groups (P1, $p > 0.05$), but significant decreases in intake were found in all groups on the second conditioning trial (P2, $p < 0.05$). As shown in Figure 4a, the group that received saline and ethanol as pre-exposure drugs (S-E-M) had a

Table 3a

Means and standard errors of baseline saccharin intake of groups that received a two-injection combination of saline (S), water (W), naloxone (N), ethanol (E), or morphine (M) and conditioned with either morphine (-M) or ethanol (-E).

Group	Mean	Standard Error
S-W-M (n=8)	16.25	2.71
N-W-M (n=8)	16.62	4.37
S-E-M (n=9)	18.44	2.92
N-E-M (n=8)	16.50	1.69
S-S-E (n=8)	18.88	1.25
N-S-E (n=7)	22.14	2.34
S-M-E (n=9)	15.56	2.51
N-M-E (n=9)	15.78	2.99

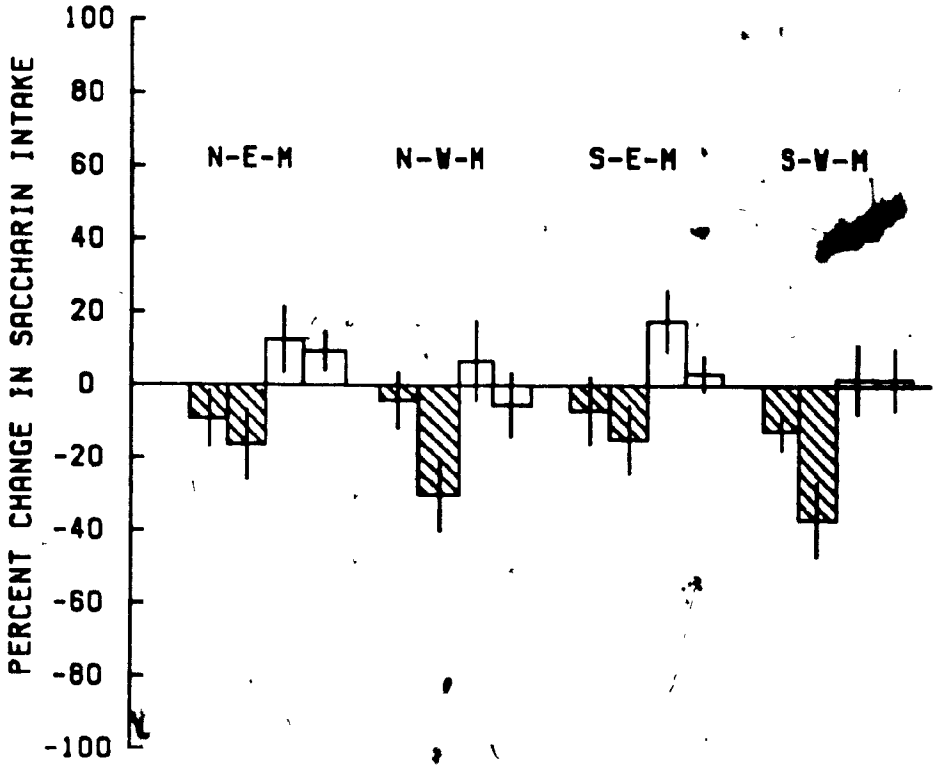

FIGURE 4a. Means and standard errors of percent change scores in saccharin intake of groups pre-exposed with a combination of either saline-water (S-W-), naloxone-water (N-W-), saline-ethanol (S-E-), or naloxone-ethanol (N-E-) and conditioned with morphine (-M) for conditioning (hatched bars) and extinction (opened bars) trials.

significantly smaller decrease in saccharin intake on conditioning trial 2 than the control group that received saline and distilled water (S-W-M) ($p < 0.05$). However, while naloxone-pre-exposed animals (N-W-M) did not differ from the saline controls (S-W-M), the naloxone-ethanol animals (N-E-M) also showed no difference from their respective controls (S-E-M) on the second conditioning trial.

Figure 4b illustrates the mean percent change scores of the groups that received ethanol on conditioning trials. Naloxone pre-exposure caused a significant increase in baseline saccharin consumption (Tukey HSD test, $p < 0.05$). In addition, rats that received a combination of either saline-morphine or naloxone-morphine showed significant reductions in baseline saccharin intake (Tukey HSD test, $p < 0.05$). Analysis of the data in terms of change scores from baseline value showed no significant difference from the results obtained in terms of percent change scores as presented below.

Rats that were pre-exposed to a combination of saline and saline and conditioned with ethanol (S-S-E) significantly decreased their saccharin intake after the two conditioning trials and after the first extinction trial (P1, P2 and T1, $p < 0.05$). Similarly, the group that received a combination of naloxone and saline as a pre-exposure treatment and ethanol as the conditioning drug (N-S-E) also showed significant decreases for the two conditioning trials and the first extinction trial (P1, P2 and T1, $p < 0.05$). This finding suggested

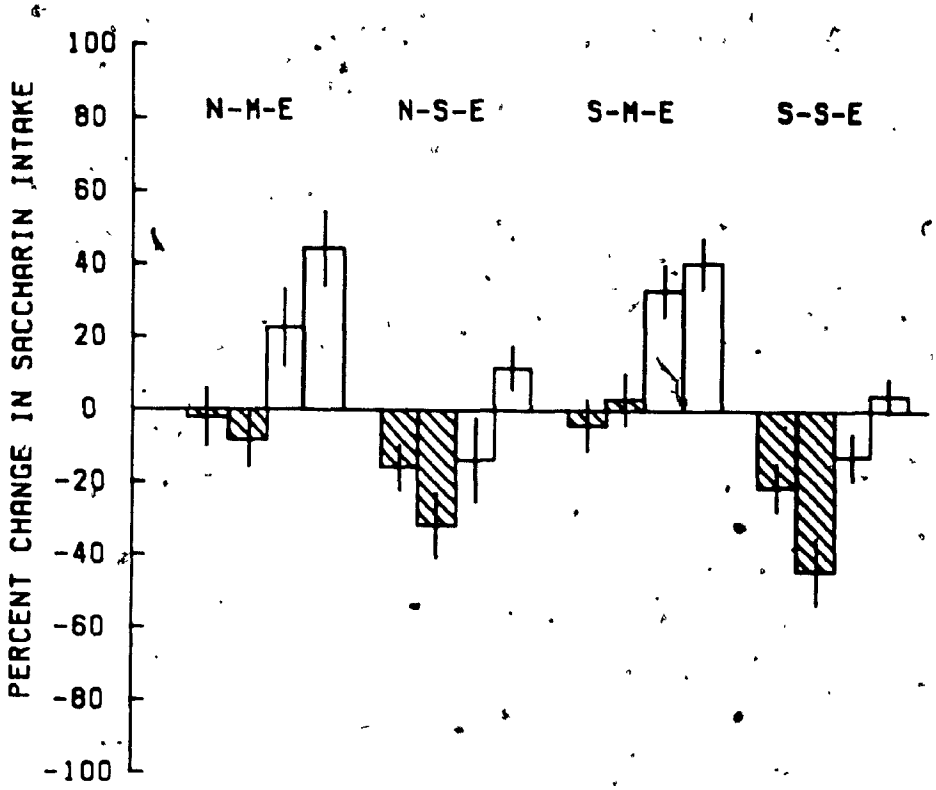

FIGURE 4b. Means and standard errors of percent change scores in saccharin intake of groups pre-exposed with a combination of either saline-saline (S-S-), naloxone-saline (N-S-), saline-morphine (S-M-), or naloxone-morphine (N-M-) and conditioned with ethanol (-E) for conditioning (hatched bars) and extinction (opened bars) trials.

that naloxone alone did not have any effect on ethanol-induced CTA. As reported in Experiment 1, morphine pre-exposure attenuated the CTA associated with ethanol as evidenced by the absence of significant decrease in saccharin intake in the S-M-E group for all trials ($p > 0.05$). Naloxone pretreatment injections during the pre-exposure treatment failed to affect morphine's attenuating pre-exposure effect since no significant decreases were observed in the N-M-E group ($p > 0.05$).

Discussion

Naloxone alone during the pre-exposure session did not affect either morphine- or ethanol-induced CTAs. It also failed to reverse the attenuating effect of morphine pre-exposure on ethanol-induced CTA.

There are two possible reasons for the failure of naloxone to affect the symmetrical interaction between alcohol and morphine. One reason may be the dose of naloxone used in Experiment 3a. Other studies have established that a dose of 5 mg/kg is more than sufficient to block morphine's analgesic effect when given intraperitoneally (e.g. Szekely, 1983). However, it seems that the dose of this opiate antagonist sufficient to block the effects of morphine may also be dependent on the behavior being measured. It is possible then that in the present paradigm a higher dose was required

to antagonize the symmetrical attenuating pre-exposure effect of alcohol and morphine.

Another reason for this failure of naloxone to affect the alcohol-morphine interaction may be related to the different subtypes of opiate receptors. It is possible that some receptor subtypes are more important than others in the induction of a morphine CTA and the effect of pre-exposure to morphine in the CTA paradigm. Since naloxone preferentially binds to the mu receptor (Magnan et al., 1982), it would be interesting to study the selective contribution of each receptor subtype to the morphine-induced CTA and to the pre-exposure effect. The results obtained in Experiments 2a and 2b suggested that the high affinity mu opiate receptor plays an important role in the induction of CTAs by morphine and ethanol. The next experiment was therefore conducted in an attempt to examine the involvement of the high affinity mu receptor in the ethanol-morphine pre-exposure interaction.

EXPERIMENT 3b

The data from the previous experiment suggested that the opiate antagonist, naloxone, was ineffective in blocking the pre-exposure effect of alcohol on a morphine-induced CTA or in blocking the pre-exposure effect of morphine on an alcohol-induced CTA. Experiment 3b was therefore carried out to examine whether the long-lasting opiate antagonist, naloxazone, would be able to reverse this symmetrical interaction between alcohol and morphine in the pre-exposure CTA paradigm. A previous study by Pasternak et al. (1980) has shown that naloxazone binds to the high-affinity mu receptor site, and has a duration of action of at least 24 hours.

Method

Subjects

Thirty-three male Sprague-Dawley rats (Charles River Ltd.), weighing between 250g and 300g, were individually housed in stainless steel cages in the animal colony. The rats were handled several times on different days before the start of the experiment and were divided randomly into 4 experimental groups.

Drugs and injections

Naloxazone hydrochloride was prepared as described in Experiment 3a. Ethanol and morphine HCl injections were prepared and administered as described in Experiment 1.

Procedure

The animals were first adapted to a water-deprivation schedule consisting of a daily access to water for a period of 30 minutes. Unless specified, the procedure is identical to that described for Experiment 3a. When water intake had stabilized, the animals were pretreated with either 10 mg/kg of naloxazone or saline 4.5 hours prior to the presentation of water. Following water presentation, the rats were pre-exposed to either 12 mg/kg of morphine or 1.2 g/kg of ethanol i.p.. This combination of pretreatment and pre-exposure was carried out for a total of 6 times on alternate days as in Experiment 1. On the second day following the third pretreatment, the rats were presented with a novel tasting 0.1% saccharin solution for 30 minutes and immediately thereafter injected i.p. with either 1.2 g/kg of ethanol or 12 mg/kg of morphine HCl or saline. A second conditioning trial and three extinction trials, consisting only of saccharin presentations, were carried out at intervals of 8 days as in Experiment 1. The four experimental groups were S-M-E (n=9), N-M-E (n=8), S-E-M (n=8), and N-E-M (n=8).

Statistical Analysis

The statistical analyses performed were identical to those described in Experiment 1

Results

Means of the raw scores and of the percent change scores are given in Appendix D. No significant difference was found between the baseline values of all groups (Tukey HSD test, $p > 0.05$; see Table 3b). Percent change scores are illustrated in Figure 4c. Significant decreases in saccharin intake were observed for the second conditioning trial only in groups that were treated with naloxazone prior treatment with the pre-exposure drug (N-M-E and N-E-M) ($p < 0.05$) indicating that naloxazone partially reversed both the effects of morphine and ethanol as pre-exposure agents in blocking the CTA induced by the other drug. In support of this finding, the S-M-E group displayed a significantly smaller decrease in saccharin intake than the N-M-E group for the second conditioning trial (P2, $p < 0.05$). In the S-E-M and N-E-M groups, significant differences were observed for the extinction trials (T1 and T2, $p < 0.05$) where the naloxazone-pretreated animals showed lesser increases in saccharin consumption, hence confirming the effect of naloxazone in attenuating the pre-exposure effect of ethanol on the morphine-induced CTA.

Table 3b

Means and standard errors of baseline saccharin intake of groups that received a two-injection combination of saline (S), naloxazone (N), morphine (M) and ethanol (E), and conditioned with either morphine (-M) or ethanol (-E).

Group	Mean	Standard Error
S-M-E (n=9)	21.89	1.90
N-M-E (n=8)	18.88	0.69
S-E-M (n=8)	16.63	1.43
N-E-M (n=8)	20.63	1.13

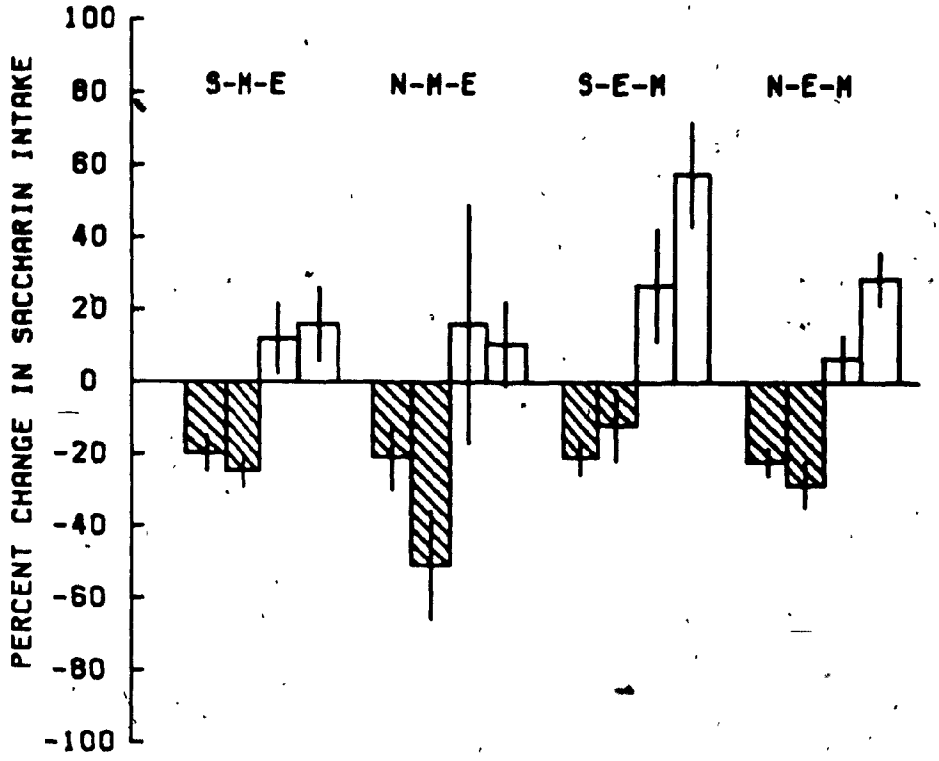

FIGURE 4c. Means and standard errors of percent change scores in saccharin intake of groups pre-exposed with a combination of either saline-morphine (S-M-), naloxazone-morphine (N-M-), saline-ethanol (S-E-), or naloxazone-ethanol (N-E-) and conditioned with ethanol (-E) or morphine (-M) for conditioning (hatched bars) and extinction (opened bars) trials.

Discussion

The data from Experiment 3b show that naloxazone pretreatment partially reversed the attenuating effect of morphine pre-exposure in ethanol-induced CTA, and also blocked the attenuating effect of ethanol pre-exposure on morphine-induced CTA. In contrast, naloxone, a short-acting opiate antagonist, failed to produce either of these effects in Experiment 3a. As mentioned earlier, it is possible that the dose of naloxone used was too low to be effective in this particular paradigm. However, it is also possible that the naloxone was less effective in blocking the receptor subtype that is most critical in ethanol-morphine interaction responsible for the pre-exposure effects. Although naloxazone has been shown to bind preferentially to the high affinity mu receptor, the present data suggest that the high-affinity mu receptor type may be principally responsible for the ethanol-morphine interation. Further experiments should be carried out to resolve this issue.

Some studies have began to question the use of naloxone as a probe for the role of the opiate receptor in the actions of opiates because of its nonspecific effects (see Sawynok et al., 1979). Because naloxazone is a derivative of naloxone, it is likely that naloxazone also shares the nonspecific properties of naloxone. Such a possibility would weaken the conclusion that the opiate receptor is involved in the ethanol-morphine pre-exposure interaction. Another

criterion for assessing the contribution of the opiate receptor in the actions of opiates is the stereospecificity of the opiates' actions. Using this criterion, the next experiments were carried out to further examine the role of the opiate receptor in the pre-exposure effect of opiates on ethanol-induced CTA.

EXPERIMENT 4a

Using an opiate antagonist, like naloxazone, is one way to examine the role of the opiate receptor in the interaction between morphine and alcohol in the CTA paradigm. However, the interpretation of data generated by such studies is not straightforward. The reason is that naloxone has been shown to have nonspecific effects (see Sawynok et al., 1979). Since most of the opiate antagonists like naltrexone and naloxazone are derived from naloxone, it is probable that they too could have nonspecific effects unrelated to their actions at the opiate receptor.

Another way to examine the role of the opiate receptor in behavior is to compare the effects of an active and a nonactive opiate stereoisomers. Stereoisomers, that is isomers that are mirror-images of each other, can be classified into two categories according to the way they affect a beam of polarized light. Isomers that rotate the plane of polarized light to the left are called levo-isomers while isomers that rotate the plane of polarized light to the right are called dextro-isomers. It is well-established that only the levo-isomers of opiates bind with high affinity to the opiate receptor. This property of stereoisomers has been used as an additional criterion in the identification of various opiate receptors in binding studies (Pert & Snyder, 1973; Simon et al., 1973; Terenius, 1973).

To date only a few behavioral studies have used this criterion

for establishing the role of the opiate receptor in the mediation of an opiate-induced effect. One study by Shannon and Holtzman (1976) reported that rats trained to discriminate morphine from saline generalized their responses to levorphanol, an opiate levo-isomer, and not to dextrorphan, its equivalent dextro-isomer. This suggests that discriminating properties of morphine were mediated by a stereospecific receptor.

To further examine the role of the opiate receptor in opiate-ethanol interaction, the effects of two opiates enantiomers, the active isomer levorphanol and the nonactive isomer dextrorphan, as pre-exposure agents on both ethanol- and morphine-induced CTAs were compared. If ethanol- and morphine-induced CTAs are mediated at least in part by a common that involves the opiate receptor, then the active isomer levorphanol should block the aversion to either morphine or ethanol. On the other hand, the nonactive isomer dextrorphan should not affect morphine- or ethanol-induced CTAs.

Experiment 4a was first conducted to establish dose-response curves for the CTAs induced by levorphanol and dextrorphan. It has previously been observed that the dose response curve for a morphine-induced CTA is similar in shape to a U-curve (Farber, Gorman, & Reid, 1976). Following these studies, it was expected that a U-curve relationship would be observed between doses of levorphanol and aversion to the conditioned stimulus and little or no aversion to the

doses of dextrorphan.

Method

Subjects

Forty-four male Sprague-Dawley rats (Charles River Ltd.) weighing between 272g and 330g at the beginning of the experiment were randomly divided into 7 groups. Housing conditions were similar to those in Experiment 1.

Drugs and injections

Both levorphanol tartrate and dextrorphan tartrate (Hoffman-LaRoche Ltd) were dissolved in saline solution to a concentration of 1 mg/ml, 5 mg/ml, or 10 mg/ml and injected in a volume of 1 ml/kg. The salts of the drugs were used for the next two experiments.

Procedure

The animals were handled and given a few days to habituate to the animal colony prior to experimentation. They were then placed on a water deprivation schedule that restricted access to only 30-minute per day. Unless specified otherwise, the procedure was identical to that described in Experiment 2. The rats were presented with a novel tasting 0.1% saccharin solution for 30 minutes immediately followed by

a drug injection (first conditioning trial), Three groups of animals received either 1 (n=6), 5 (n=6), or 10 mg/kg (n=7) of levorphanol tartrate; another three groups (n=6 for each group) were injected with either 1, 5, or 10 mg/kg of dextrorphan tartrate; saline was administered to the last group of rats (n=7). A second conditioning trial was performed 6 days later. Three saccharin presentations were made without drug injection at intervals of 6 days (extinction trials).

Statistical analysis

Scores were expressed in terms of percent change from baseline intake. Statistical analyses were performed as described in Experiment 1.

Results

There were no significant differences between baseline saccharin intake among the groups (Tukey HSD test, $p > 0.05$; see Table 4a). Means of the raw scores and of the percent change scores are presented in Appendix D. Percent change scores are illustrated in Figure 5a. The saline group showed significant increases in saccharin intake for all conditioning and extinction trials, $p < 0.05$. There were no significant decreases in saccharin intake for all the groups that received dextrorphan as conditioning drug for all trials ($p < 0.05$).

Levorphanol produced significant decreases in saccharin intake at all three doses tested after the second conditioning trial (P2, $p < 0.05$). The largest decrease in saccharin consumption was observed with the 5 mg/kg dose. When compared to the saline group however, dextrorphan at all doses did cause a decrease in saccharin consumption, particularly at the highest dose (a priori t-test, $p < 0.05$).

Table 4a

Means and standard errors of baseline saccharin intake of groups that received saline, or one of the various doses of dextrorphan (D), or levorphanol (L).

Group	Mean	Standard Error
Saline (n=7)	19.00	1.88
D (1 mg/kg) (n=6)	22.33	1.69
D (5 mg/kg) (n=6)	20.17	1.56
D (10 mg/kg) (n=6)	23.17	0.95
L (1 mg/kg) (n=6)	20.17	0.98
L (5 mg/kg) (n=6)	21.83	0.79
L (10 mg/kg) (n=7)	22.29	1.04

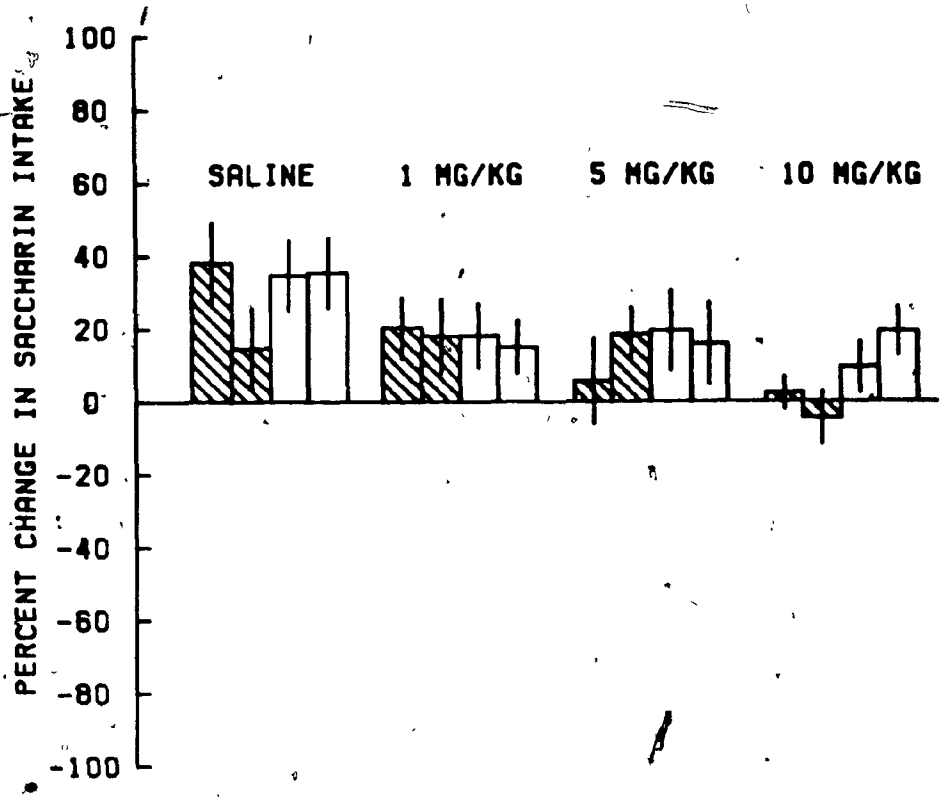

FIGURE 5a. Means and standard errors of percent change scores in saccharin intake of groups that were conditioned with either saline, 1, 5, or 10 mg/kg of dextrorphan for conditioning (hatched bars) and extinction (opened bars) trials.

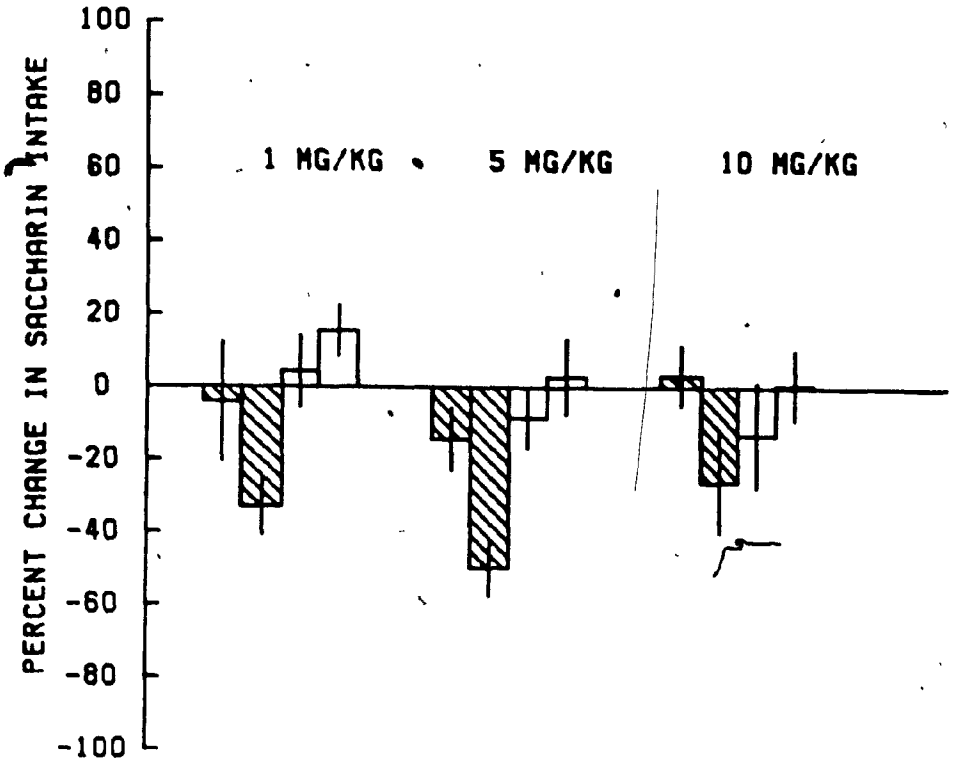

FIGURE 5b. Means and standard errors of percent change scores in saccharin intake of groups that were conditioned with either 1, 5, or 10 mg/kg of levorphanol for conditioning (hatched bars) and extinction (opened bars) trials.

Discussion

The data obtained in Experiment 4a showed that dextrorphan at the range of doses tested did not produce a CTA as defined by a decrease in saccharin intake from baseline value. However, all doses of dextrorphan used did reduce saccharin intake when compared to saline controls. This would suggest that dextrorphan may have produced a decreased preference for saccharin. Since this effect is not germane to the objectives of the experiments and does not interfere with the interpretation of the other findings, it was not examined further.

A comparison of the dextrorphan and levorphanol groups clearly showed that the two drugs have different potencies in producing an aversion to saccharin. All doses of levorphanol induced a CTA on the second conditioning trial. The greatest aversion to saccharin occurred at the 5 mg/kg dose. As predicted, and as has been demonstrated for morphine, the magnitude of the aversion to the saccharin was related to th dose of levorphanol by a U-shaped function.

This difference in potencies between levorphanol and dextrorphan in inducing a CTA is believed to be related to their differential abilities to bind to and activate the opiate receptor. Although no data are available concerning the pharmacokinetics of the two drugs, it is unlikely that the differences observed in the CTA paradigm are due to differences in absorption, distribution, metabolism or

excretion of the drugs. There is evidence that the dextro-isomer and levo-isomer of pentazocine, another opiate agonist, are very similar in terms of their pharmacokinetic properties (Berkowitz & Way, 1971). Furthermore, it is well known that CTA is a robust phenomenon that does not required the close temporal contiguity between the conditioned stimulus and the unconditioned stimulus that is characteristic of other classical conditioning paradigms.

Thus, the results from Experiment 4a support the role of the opiate receptor in the CTA produced by opiates, and in addition, provided evidence to satisfy the criterion of receptor stereospecificity for an alcohol-opiate interaction.

EXPERIMENT 4b

The results of Experiment 4a indicate that levorphanol can produce a conditioned taste aversion in the dose range of 1-10 mg/kg. The levorphanol data described a U-curve dose-related function which was also shown with morphine (Farber et al., 1976). Experiment 4b was designed as an attempt to further examine the role of the opiate receptor in the pre-exposure interaction between morphine and ethanol. The approach used was to compare levorphanol and dextrorphan in terms of their effectiveness in blocking morphine- and ethanol-induced CTAs. The dose of levorphanol and dextrorphan selected was 5 mg/kg, the dose at which levorphanol produce the strongest CTA. Dextrophan at that dose did not produce a CTA although it decreased saccharin intake as compared to the control saline group.

Method

Subjects

Forty-nine male Sprague-Dawley rats (Charles River Ltd.), weighing between 285g and 326g, were individually housed in stainless steel cages in the animal colony. Housing conditions were similar to those in Experiment 1.

Drugs and injections

Levorphanol tartrate and dextrophan tartrate (Hoffman-LaRoche Ltd) were dissolved in saline solution to yield a concentration of 5 mg/ml. The volume of injection was 1 ml/kg for both drugs. Morphine hydrochloride (BDH Chemicals Canada Ltd) was dissolved in saline to yield a concentration of 12 mg/ml and was injected in a volume of 1 ml/kg. A 95% solution of ethanol was diluted in distilled water to yield a concentration of 20% (w/v) and was injected in a volume of 7.5 ml/kg.

Procedure

The rats were handled four times on different days before the start of the experiment. The animals were first adapted to a water-deprivation schedule consisting of daily access to water for a period of 30 minutes. Unless specified otherwise, the procedure was similar to that described in Experiment 1. The animals were pre-exposed to 5 mg/kg of levorphanol or dextrorphan i.p. (the dose was the most effective dose of levorphanol shown to induce a CTA in Experiment 4a) once a day on three alternate days following the 30-minute access to water. On the second day following the last pre-exposure treatment, the rats were presented with a novel tasting 0.1% saccharin solution for 30 minutes and were injected i.p. immediately thereafter with either 1.2 g/kg of ethanol or 12 mg/kg of morphine HCl or saline

(first conditioning trial). Pre-exposure treatment with levorphanol and dextrorphan resumed for another three sessions two days after the first conditioning trial. A second conditioning trial similar to the first one and three extinction trials, consisting only of saccharin presentations, were carried out at intervals of 8 days. The groups were D-S (n=8), L-S (n=8), D-M (n=9), L-M (n=8), D-E (n=7), and L-E (n=9).

Statistical analysis

The data were analyzed as described in Experiment 1

Results

There were no significant differences in baseline values among the groups (Tukey HSD test, $p > 0.05$; see Table 4b). The means of the raw values and of the percent change scores are tabulated in Appendix E. Figure 5c illustrates the means of percent change scores in saccharin intake for the two conditioning trials and the two extinction trials. As can be seen from Figure 5c, the two groups that received either dextrorphan or levorphanol as the pre-exposure drug and saline on conditioning trials (D-S and L-S) showed significant increases in saccharin consumption for all conditioning and extinction trials ($p < 0.01$). The group of animals that were injected with dextrorphan as the pre-exposure drug and with morphine as the

Table 4b

Means and standard errors of baseline saccharin intake of groups that were preexposed with dextrorphan (D-), or levorphanol (L-), and conditioned with saline (-S), morphine (-M), or ethanol (-E).

Group	Mean	Standard Error
D-S (n=8)	19.00	1.89
L-S (n=8)	18.38	1.31
D-M (n=9)	20.89	1.07
L-M (n=8)	18.00	1.24
D-E (n=7)	20.29	1.58
L-E (n=9)	18.00	1.21

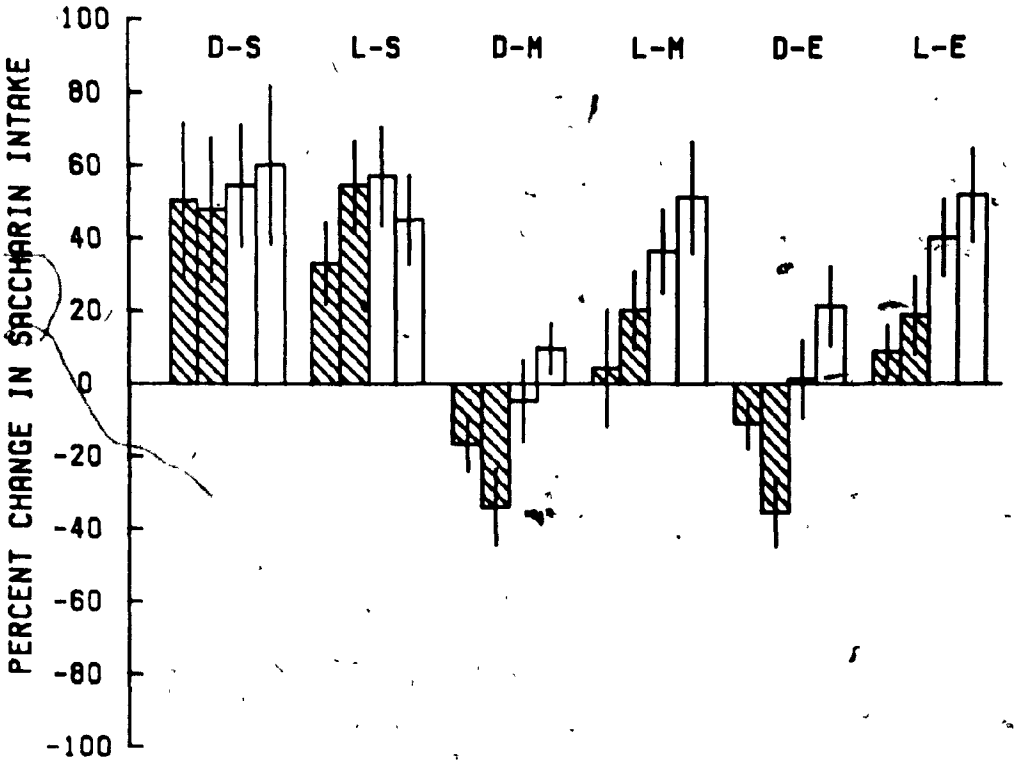

FIGURE 5c. Means and standard errors of percent change scores in saccharin intake of groups that were pre-exposed with either dextrorphan (D-) or levorphanol (L-) and conditioned with either saline (-S), morphine (-M), or ethanol (-E) for conditioning (hatched bars) or extinction (opened bars) trial.

conditioning drug (D-M) displayed substantial and significant decreases in saccharin intake following the two conditioning trials ($p < 0.05$). Rats that were pre-exposed with levorphanol, the active isomer and conditioned with morphine (L-M) showed increases in saccharin intake for all trials. Significant increases in saccharin consumption were observed in this group after the two extinction trials T1 and T2 ($p < 0.05$). Similarly, the dextrorphan-pre-exposed group (D-E) that received ethanol on conditioning trials demonstrated decreases in saccharin intake that were significant after the second conditioning trial, P2 ($p < 0.05$). The levorphanol-pre-exposed animals that were conditioned with ethanol (L-E) showed significant increases in saccharin intake for both conditioning trials (P1 and P2, $p < 0.05$).

Discussion

The results of Experiment 4b showed that a stereospecific opiate receptor was involved in the pre-exposure effect of opiates on morphine-induced CTA and on ethanol-induced CTA. In summary, it was found that prexposure to the active opiate isomer levorphanol, blocked completely the CTA induced by morphine and that produced by ethanol as well. Dextrorphan, the inactive opiate isomer, did not block the morphine or ethanol CTAs. Although it is possible that dextrorphan attenuated the CTAs to those two drugs, the fact that there were no

saline pre-exposure controls in this experiment made it impossible to assess the effect of dextrorphan or levorphanol in absolute terms. Nevertheless, the relative effectiveness of levorphanol when compared to dextrorphan in blocking both morphine- and ethanol-induced CTAs strongly supports the notion that a stereospecific opiate receptor is involved in the ethanol-morphine interaction in the pre-exposure CTA paradigm.

An alternative explanation for these findings may be that the two enantiomers have different pharmacokinetic properties. One would not expect dextrorphan to reverse either morphine- or ethanol-induced CTAs if the drug does not reach the relevant brain sites in sufficient time and dose whatever the mechanism of the blockade. According to Berkowitz and Way (1971), optical enantiomers possess highly similar physicochemical properties but differ in their pharmacological activities. Presumably, differences in pharmacological activities would be attributed to their differential affinities for specific receptors which mediate the actions of the drugs. In their study (Berkowitz & Way, 1971), the distribution of levo-pentazocine and dextro-pentazocine did not differ significantly in different areas of the brain over the time course of drug action. Since no data are available about the pharmacokinetics of dextrorphan and levorphanol, the findings of Berkowitz and Way (1971) may be tentatively extended to other opiates, and in this case, to dextrorphan and levorphanol.

So far the investigation has examined the role of the opiate receptor in the interaction between opiates and alcohol. Whether ethanol directly or indirectly acts on the opiate receptor to produce CTA or the pre-exposure effect is yet to be resolved. Several lines of evidence now support the notion that acetaldehyde, the primary metabolite of ethanol, mediates some actions of ethanol (Amir et al., 1980). Experiment 5 was therefore conducted as a preliminary attempt to examine whether acetaldehyde also mediates the ethanol pre-exposure effect.

EXPERIMENT 5

Acetaldehyde is the primary metabolite of ethanol (Hald & Jacobsen, 1948). Some studies have focused on the role of this metabolite as a possible mediator of the actions of ethanol (Amir et al., 1980). While it was thought for a long time that acetaldehyde was mainly contributing to the aversive effects of ethanol (Hald & Jacobsen, 1948; Jacobsen, 1952), some more recent studies have provided evidence that acetaldehyde may play an important role in the positive reinforcing properties of ethanol as well (e.g. Smith, 1982).

Most researchers would consider CTA as a paradigm most suitable for examining the aversive properties of drugs. The classic example is the induction of a CTA by lithium chloride, an emetic drug which is known to produce intestinal malaise (Nachman, 1970). To explain this phenomenon, Seligman (1970) proposed the "preparedness" hypothesis which says that organisms are predisposed to associate flavors with delayed illness. However, the fact that self-administered drugs can also produce CTAs (e.g. Berger et al., 1973; Cappell et al., 1973) has led some investigators to propose that the stimulus properties of those self-administered drugs that promote CTA may be related to the properties which underlie their positive reinforcing effects (Switzman, 1980). According to Switzman (1980), it would seem that any drug that is self-administered will also produce a CTA. It should be noted that the converse does not hold in all cases. Hence, drugs

like lithium produce strong CTAs but are not self-administered.

A number of studies have implicated acetaldehyde as a possible mediator of the reinforcing properties of ethanol. This was demonstrated in a study where acetaldehyde was shown to be self-administered by rats (Brown et al., 1979; Brown et al., 1980). Like other psychoactive drugs which are self-administered, acetaldehyde was also shown to produce a CTA (Brown, Amit, Smith, & Rockman, 1978).

If one assumes that a functional relationship exists between the reinforcing properties of a drug and its ability to produce a CTA, or as an extension, the liability of its CTA to be blocked by prior experience with another self-administered drug, one would then expect acetaldehyde to behave in the same way as ethanol in the pre-exposure paradigm. Experiment 5 is an attempt to examine whether acetaldehyde will display the same pre-exposure CTA interaction with morphine as ethanol did in Experiment 1. Thus, according to the hypothesis that acetaldehyde mediates the positive effects of ethanol, and following the assumption that the CTA pre-exposure paradigm is a model capable of measuring drug reinforting effects, acetaldehyde pre-exposure should block a morphine-induced CTA and morphine pre-exposure should block an acetaldehyde-induced CTA.

Method

Subjects

Thirty-two male Sprague-Dawley rats (Charles River Ltd.), weighing between 215g and 295g, were individually housed in stainless steel cages in the animal colony room. Housing conditions were similar to those in Experiment 1.

Drugs and injections

Morphine hydrochloride (BDH Chemicals Canada Ltd) was dissolved in saline in a concentration of 12 mg/ml and injected in a volume of 1 ml/kg. Acetaldehyde was prepared by diluting a stock solution to a concentration of 5% (w/v) with distilled water. The volume of injection was 5 ml/kg.

Procedure

The rats were handled four times on different days before the start of the experiment. The animals were first adapted to a water-deprivation schedule consisting of daily access to water for a period of 30 minutes. Unless specified otherwise, the procedure was similar to that described in Experiment 1. The animals were pre-exposed to 0.2 g/kg of acetaldehyde or 12 mg/kg of morphine i.p. once a day on three alternate days following the 30-minute access to water. On the

second day following the last pre-exposure treatment, the rats were presented with a novel tasting 0.1% saccharin solution for 30 minutes and were injected i.p. immediately after with either 0.2 g/kg acetaldehyde (S-A, n=8 and M-A, n=8) or 12 mg/kg of morphine HCl (W-M, n=8 and A-M, n=8) (first conditioning trial). Pre-exposure treatment with acetaldehyde and morphine resumed for another three sessions two days after the first conditioning trial. A second conditioning trial similar to the first one and three extinction trials, consisting only of saccharin presentations, were carried out at intervals of 8 days.

Statistical analysis

The data were analyzed as described in Experiment 1

Results

There were no significant differences between the baseline values (Tukey HSD test, $p > 0.05$; see Table 5). The means of the raw values and of the percent change scores are tabulated in Appendix F. Figure 6 illustrates the means of the percent change scores in saccharin consumption for the two conditioning trials and the two extinction trials. The reduction in saccharin intake observed in the S-A group on the second conditioning trial, P2, was not statistically significant ($p > 0.05$). However, a comparison of the M-A group and the S-A group showed that the M-A group obtained a percent change

Table 5

Means and standard errors of baseline saccharin intake of groups that were preexposed with saline (S-), morphine (M-), water (W-), or acetaldehyde (A-) and conditioned with morphine (-M) or acetaldehyde (-A).

Group	Mean	Standard Error
S-A (n=8)	17.88	1.14
M-A (n=8)	17.25	1.76
W-M (n=8)	18.13	1.42
A-M (n=8)	16.00	1.86

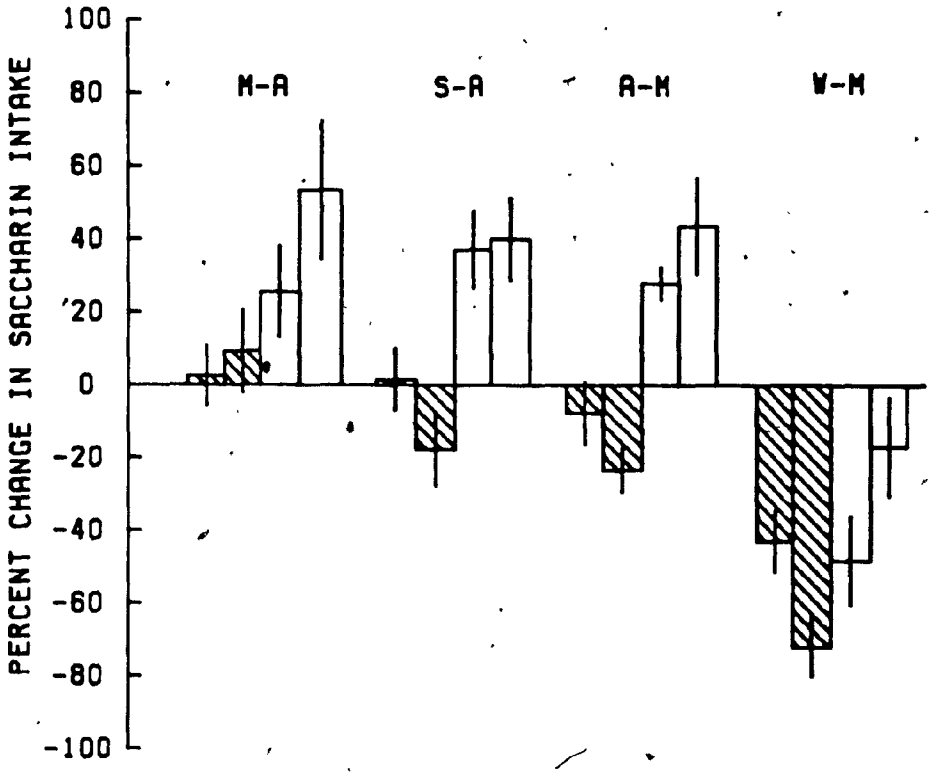

FIGURE 6. Means and standard errors of percent change scores in saccharin intake of groups that were pre-exposed with either saline (S-), morphine (M-), water (W-), or acetaldehyde (A-) and conditioned with either acetaldehyde (-A) or morphine (-M) for conditioning (hatched bars) and extinction (opened bars) trials.

score significantly higher than that of the S-A group ($p < 0.05$). This suggested that the morphine pre-exposure attenuated the acetaldehyde effect despite the fact that the dose of acetaldehyde used was not sufficient to induce a strong aversion to the saccharin solution.

On the other hand, large significant reductions in saccharin consumption were observed in the W-M group after the two conditioning trials and the first extinction trial (P1, P2 and T1, $p < 0.05$). Pre-exposure to acetaldehyde attenuated this effect as can be seen in the smaller reductions in saccharin intake in the A-M group. The decrease in saccharin intake in the A-M was only significant after the second conditioning trial (P2, $p < 0.05$). In addition, significant increases were observed in the A-M group but not in the W-M group after the extinction trials (T1 and T2, $p < 0.05$). Between-group comparisons showed that the reduction in saccharin intake was greater in the W-M group than the A-M group after the second conditioning trial (P2, $p < 0.05$). These results then suggest that the pre-exposure to acetaldehyde attenuated the conditioned taste aversion to morphine, and possibly vice versa.

Discussion

The data obtained in Experiment 5 show that acetaldehyde pre-exposure attenuated the development of a morphine-induced CTA, and that morphine pre-exposure attenuated the development of an apparent acetaldehyde-induced CTA. The failure to observe a CTA produced by acetaldehyde is believed to be because of the low dose of acetaldehyde used in this experiment. Other studies have demonstrated that higher doses of acetaldehyde produced strong CTAs (Aragon, Abitbol, & Amit, 1984; Brown et al., 1978). Since the morphine-pre-exposed group had a significantly greater percent change in saccharin intake than the saline-pre-exposed groups, it is very likely that an acetaldehyde CTA would have been reduced by the morphine pre-exposure.

A number of studies have now implicated acetaldehyde in the positive reinforcing properties of ethanol (Brown et al., 1979; Brown et al., 1980). Thus, the observed interaction between acetaldehyde and morphine in the CTA pre-exposure paradigm may be indicating that this metabolite of ethanol underlies the ethanol-morphine interaction responsible for the pre-exposure effect. If the hypothesis that acetaldehyde is an important factor in the reinforcing properties of ethanol holds true, one may speculate that the interaction between alcohol and opiates in the pre-exposure effect may also reflect an an overlap in the reinforcing properties of those two classes of drugs.

GENERAL DISCUSSION

The present investigation sought to examine the interaction between alcohol and opiates. The main objectives of the experiments were to examine the interaction between opiates and alcohol in the conventional conditioned taste aversion (CTA) paradigm and the pre-exposure CTA paradigm, and the role of the opiate receptor in alcohol-opiate interaction in these two paradigms.

The results of Experiment 1 suggest that pre-exposure to morphine blocks the development of CTA to ethanol, and that conversely, prior exposure to ethanol attenuates the development of CTA to morphine. This finding is consistent with the results of previous studies that have demonstrated an interaction between ethanol and morphine using various biochemical (e.g. Hoffman et al., 1982), pharmacological (e.g. Khanna et al., 1979), physiological (e.g. Cicero et al., 1982) and behavioral measures (e.g. Blum et al., 1983). In Experiment 2, it was found that naloxazone, a long-acting high-affinity mu agonist, antagonized both morphine- and ethanol-induced CTAs. Thus, it was suggested that the high-affinity mu opiate receptor was involved in the mechanisms underlying both morphine and ethanol CTAs (cf. Miceli et al., 1979). The data obtained in Experiment 3b showed that naloxazone decreased the effects of morphine as a pre-exposure agent in attenuating an ethanol-induced CTA. Similarly, naloxazone also decreased the attenuating effect of ethanol on a morphine-induced CTA.

It seems then that the high-affinity binding mu opiate receptor plays an important role in conventional CTAs to ethanol and morphine as well as in the pre-exposure CTA effects of these two drugs. This hypothesis received further support from the results of Experiment 4a in which levorphanol, an active opiate-receptor isomer, induced a CTA, but its inactive isomer, dextrorphan, did not. This is the first demonstration that the CTA induced by opiates is mediated by a stereospecific opiate receptor. The role of the opiate receptor in a given opiate-induced behavior has usually been examined with the use of an opiate antagonist like naloxone. There is an increasing body of data on nonspecific actions of naloxone, the classical opiate antagonist (Sawynok et al., 1979). It is likely that other opiate antagonists may also possess nonspecific actions because most of them are derived from naloxone. Thus, it is becoming more important to demonstrate stereospecificity effect in receptor research. Experiment 4b demonstrated that levorphanol but not dextrorphan blocked the development of CTAs induced by both morphine and ethanol. Therefore, it was concluded that the pre-exposure effect of opiates on the ethanol-induced CTA is mediated, at least in part, by a stereospecific opiate receptor. Finally, in Experiment 5, it was observed that acetaldehyde, the primary metabolite of ethanol, also showed the same symmetrical interaction with morphine in the pre-exposure CTA paradigm. It is possible, therefore, that the interaction between

ethanol and morphine in the pre-exposure CTA paradigm is mediated by the action of acetaldehyde as has been argued for other psychopharmacological effects of ethanol (e.g. Amir et al., 1980; Carr et al., 1980).

In the tradition of psychopharmacology, it would be interesting and important to examine the dose-response curve for the interaction between opiates and alcohol. However, the design imposed by the paradigm is such that the addition of one more dose for each drug would expand the study to an unmanageable degree in terms of cost, time, and information processing. It is argued that the fact that a symmetrical interaction was observed between alcohol and opiates rather than an asymmetrical, or a lack, of interaction makes this shortcoming less problematic.

Recent studies have supported the notion that ethanol may act on the opiate receptor both following acute and chronic treatment. The data suggest that acute alcohol treatment inhibits binding of opioids to the delta receptors (Hiller et al. 1981, 1984; Gianoulakis, 1983) and that chronic alcohol treatment results in receptor supersensitivity which is expressed in an increased number of opiate receptors (Hoffman et al., 1982; Gianoulakis, 1983). Some workers proposed that the disordering action of ethanol on the lipid bilayer that makes up biological membranes may underlie its actions on the opiate receptors (Hiller et al., 1984; Hoffman et al., 1982). By

disturbing the membrane containing the opiate receptor, one expects that ethanol would render the receptor nonfunctional and thus acts in a similar fashion to an opiate antagonist. This hypothesis would be consistent with the observation that chronic treatment with ethanol produces supersensitivity of the opiate receptors analogous to the actions of opiate antagonists (Schulz et al., 1979). However, the findings of the present investigation are not consistent with this hypothesis. In the conventional CTA paradigm, ethanol, like morphine, produced a CTA at low doses and therefore behaved like an opiate agonist. This argument for an opiate agonistic nature of ethanol may be weak since naloxone, the classical opiate antagonist, can also induce CTA (LeBlanc & Capell, 1975; Van der Kooy & Phillips, 1977). Nevertheless, the fact that ethanol pre-exposure attenuates morphine CTA in a manner similar to the blockade of morphine CTA by morphine pre-exposure (Experiment 1; Brown et al., 1979; LeBlanc & Cappell, 1974; Parker et al., 1973; Switzman et al., 1981) argues against the hypothesis that the membrane-fluidizing action of ethanol that mediates its effects on delta binding plays a role in either ethanol-induced CTA or its pre-exposure effect on morphine-induced CTA. It was noted that pre-exposure to the opiate antagonist, naloxone, did not affect CTAs induced by either morphine or ethanol. Again, this contrasts with the effects of ethanol, and argues against the hypothesis that ethanol is acting like an opiate antagonist in the CTA

paradigm.

On the other hand, ethanol seems to act on the mu opiate receptors in a manner similar to an opiate agonist. Acute ethanol treatment caused an increase in mu receptor binding (e.g. Levine et al., 1983). As it has been shown for chronic treatment with opiate agonist (e.g. Davis, Akera, & Brody, 1979), chronic ethanol diet produced subsensitivity of these mu receptors (e.g. Tabakoff et al., 1981). These findings are consistent with the present results. As argued above, ethanol behaves like an opiate agonist in producing CTA, and in attenuating the development of both ethanol- or morphine-induced CTAs. One may then hypothesize that the mu receptor mediates the effects of ethanol in the conventional and pre-exposure CTA paradigms. This hypothesis is further supported by the results of the experiments with naloxazone. Previous studies have demonstrated that naloxazone is a selective opiate antagonist for the high affinity mu opiate receptor (e.g. Pasternak et al., 1980). The results obtained from the present investigation showed that naloxazone blocks the development of both morphine- and ethanol-induced CTAs, and prevents the pre-exposure interaction between morphine and ethanol.

More research is necessary to find out whether the mu receptor exclusively mediates the pre-exposure interaction between ethanol and morphine. There is now substantial evidence for, at least, three types of opiate receptors (Wood, 1982). Speculation abounds

concerning the functions of these different types of opiate receptors (Gintzler & Pasternak, 1982; Herz, 1983; Ling, Spiegel, Nishimura, & Pasternak, 1983; Pert, 1981; Schmauss & Yaksh, 1984). The understanding of their differential contributions to CTAs produced by self-administered drugs, and to the pre-exposure effects between self-administered drugs may possibly increase our knowledge about those drugs and reinforcement. The other concern that should be addressed is the brain area or areas responsible for the interaction observed in the pre-exposure CTA paradigm. Indeed, studies have shown that the different types of receptors have different distribution patterns in the brain (Duka, Schubert, Wuster, Stoiber, & Herz, 1981; Goodman, Snyder, & Kuhar, 1980; Quirion, Weiss, & Pert, 1983; Quirion, Zajac, Morgat, & Roques, 1983). There are possible candidate locations in the brain that would be responsible for the interaction. However, systematic studies would be necessary to confirm the importance of these brain regions.

Other studies provided support for the idea that endorphins and enkephalins are released under acute pharmacological actions of ethanol (Schulz et al. 1980; Seizinger et al. 1983; Naber et al. 1981; Blum et al., 1982). For instance, Seizinger et al. (1983) reported that acute ethanol administration resulted in a significant increase in met-enkephalin in the hypothalamus, striatum, and midbrain. On the other hand, chronic ethanol resulted in a dramatic decrease in tissue

levels of several endogenous peptides in different brain areas. The latter action of ethanol is similar to the action of chronic morphine administration on endorphin levels (Przewlocki et al. 1979). This proposed biochemical action of ethanol is also in agreement with the behavioral findings of the present investigation. According to this view, ethanol may indirectly activate the opiate receptors by releasing those endogenous opioids and thus act as an opiate agonist in blocking development of morphine-induced CTA. Similarly, the ethanol-induced CTA could be mediated through the same action of ethanol on the endogenous opiates, and hence, could be blocked by prior exposure to morphine, other opiate agonists, or by acute pretreatment with an opiate antagonist. Furthermore, the actions of endogenous opioids released by the actions of ethanol can be blocked by opiate antagonists. This hypothesis would explain the attenuation of the pre-exposure effects of ethanol on morphine-induced CTA by naloxazone.

There seems to be a close relationship between the reinforcing properties of self-administered drugs and their abilities to produce CTAs (Switzman, 1980; White, Sklar, & Amit, 1977; Wise et al., 1976). Both opiates and ethanol are readily self-administered by animals and humans (see Schuster & Thompson, 1968). Like other self-administered drugs, these drugs also produce CTAs (Cappell et al., 1973). As suggested by Switzman (1981), pre-exposure to a self-administered drug

can block the formation of a CTA to another self-administered drug. This relationship apparently does not hold for non-self-administered drugs. That is, prior experience with a self-administered drug will not block the CTA to a non-self-administered drug.

The present investigation provides further evidence for the relationship between self-administration liability and effectiveness in the CTA paradigm either as the unconditioned stimulus or as a pre-exposure agent.

Several studies have supported this notion. Wise et al. (1976) demonstrated that CTA developed to saccharin when a saccharin solution was ingested before access to amphetamine self-administration. Reicher and Holman (1977) observed that injections of amphetamine on one side of a shuttlebox induced both a preference for the location of the injection and a CTA in the same animals. When rats were injected with morphine after running down a runway for flavored food in a goal-box, it was found that morphine increased running speed to the goal box on subsequent trials. At the same time, a reduction in food intake was observed (White et al., 1977). In a further investigation of White et al.'s finding, Switzman (1980) has shown that rats that displayed the highest degree of positive reinforcement, as measured by fastest running speed in the runway also displayed the highest degree of aversion, as measured by the smallest amount of the food consumed in the goal-box. The interpretation of the present findings may

therefore be extended to implicate mechanisms underlying the common reinforcing properties of ethanol and the opiates.

In the light of this relationship between positive reinforcement and aversion, as observed in the conventional and the pre-exposure CTA paradigms, the data from the present investigations would also provide strong support for the involvement of a stereospecific opiate receptor in drug reinforcement, or possibly in reinforcement in general. The conventional and pre-exposure CTA paradigms may become powerful tools to study the central mechanism or mechanisms underlying drug reinforcement.

It is evident that the interaction between alcohol and opiates is not manifested in every behavioral measure. The available evidence suggests that the opiate receptor mediates the effects of ethanol on locomotor activity (e.g. Middaugh et al., 1977), and that ethanol may act on the endogenous opioids in effecting changes in brain stimulation parameters (e.g. Lorens & Sainati, 1978). To a lesser extent but yet significant is the asymmetrical interaction between alcohol and opiates in the drug self-administration paradigm (e.g. Sinclair, 1974; Gelfand & Amit, 1976). On the other hand, failures to observe an alcohol-opiate interaction are generally reported for such behaviors as analgesia (e.g. Bass et al., 1978), motor performance (e.g. Hymson & Hynes, 1982), and drug discrimination (e.g. Chipkin et al., 1980). In light of the present findings supporting an

interaction between alcohol and opiates, one may reexamine the hypotheses for which contradictory findings have been reported in the literature with particular attention to the measures used in terms of their sensitivity, reliability and validity. Another issue that will have to be addressed is the importance of the symmetrical interaction observed in the pre-exposure CTA paradigm as opposed to the asymmetrical interaction in the drug self-administration paradigm (e.g. Gelfand & Amit, 1976). Whether this symmetry or lack of it will help to increase our understanding of the actions of these drugs will have to be investigated.

The findings of the present investigation are in agreement with previous clinical reports supporting the idea of an interaction between alcohol and opiates. Naloxone has been observed in several reports to reverse the intoxicating effects of ethanol (e.g. Jefferys et al., 1980). It was also found that narcotic addicts have usually a high incidence of alcohol-related problems before, during or after opiate addiction (e.g. Brown et al., 1973). Based on work with animals, Blum and coworkers (1977) have proposed that a lack of central beta-endorphin plays a role in the alcohol-seeking behavior typical of chronic alcoholism. There is now clinical evidence in support of this hypothesis. Two studies have reported reduced beta-endorphin levels in cerebrospinal fluid of chronic alcoholics (Genazzani et al., 1982; Savoldi et al., 1983). Based on the results

obtained in the present experiments, one may speculate that the reduced levels of beta-endorphin in alcoholics is a result of continual release of these endogenous opioids by alcohol. One may even predict an increased number of opiate receptors in chronic alcoholics as has been observed in animals maintained chronically on an ethanol diet (Gianoulakis, 1983).

In summary, the present investigation confirms the notion of an interaction between alcohol and opiates in a behavioral paradigm. It also suggests a common role for a stereospecific opiate receptor in the mechanisms of actions of ethanol and opiates. Furthermore, there is some preliminary support for the suggestion that the high affinity mu receptor may play a selective role in alcohol-opiate interaction. Finally, based on the results obtained, one may speculate that the mechanism of the interaction of ethanol with the opiate system or systems involves the release of endogenous opioids that subsequently act on the opiate receptors. Thus, some of the actions of ethanol could be mimicking the actions of opiates at the opiate receptor. In conclusion, the findings obtained in the present investigation warrant continued future research into the ramifications of the observed interactions between ethanol and opiates in both basic and applied areas of research.

REFERENCES

Akil, H., Watson, S.J., Young, E., Lewis, M.E., Khatchaturian, & Walker, J.M. (1984). Endogenous Opioids: Biology and Function. Annual Review of Neuroscience, 7, 223-255.

Altshuler, H.L., Applebaum, E., & Shippenberg, T.S. (1981). The effects of opiate antagonists on the discriminative stimulus properties of ethanol. Pharmacology Biochemistry & Behavior, 14, 97-100.

Altshuler, H.L., Phillips, P.E., & Feinhandler, D.A. (1980). Alteration of ethanol self-administration by naltrexone. Life Sciences, 26, 679-688.

Amir, S. (1977). Brain and liver aldehyde dehydrogenase: Relation to ethanol intake in the Wistar rat. Neuropharmacology, 16, 781-784.

Amir, S. (1978a). Brain and liver aldehyde dehydrogenase activity and voluntary ethanol consumption by rats: Relations to strain, sex and age. Psychopharmacology, 57, 97-102.

Amir, S. (1978b). Brain and liver aldehyde dehydrogenase: Adaptive increase following prolonged ethanol administration in rats. Neuropharmacology, 17, 463-467.

Amir, S., Brown, Z., & Amit, Z. (1980). The role of acetaldehyde in the psychopharmacological effects of ethanol. In H. Rigter and J.C. Crabbe, Jr. (Eds.), Alcohol Tolerance and Dependence (pp.

137-337). Amsterdam: Elsevier/North-Holland

Amir, S., & Stern, M.H. (1978). Electrical stimulation and lesions of the medial forebrain bundle of the rat: Changes in voluntary ethanol consumption and brain aldehyde dehydrogenase activity. Psychopharmacology, 57, 167-174.

Amit, Z., Brown, Z.W., Levitan, D.E., & Ogren, S.O. (1977). Noradrenergic mediation of the positive reinforcing properties of ethanol: Suppression of ethanol consumption in laboratory rats following dopamine-beta-hydroxylase inhibition. Archives Internationales de Pharmacodynamie et de Therapie, 230, 65-75.

Amit, Z., & Levitan, D.E. (1975). Ethanol and morphine self-administration. A possible relationship based on differential involvement of catecholamines. In J.D. Sinclair and K. Kiianmaa (Eds.), The Effects of Centrally Active Drugs on Voluntary Alcohol Consumption (pp.85-100). Helsinki: Rutgers Center for Alcohol.

Aragon, C.M.G., Abitbol, M., & Amit, Z. (1984). Acetaldehyde may mediate both the reinforcing and aversive properties of ethanol: An examination using the preexposure conditioning taste aversion paradigm. Manuscript submitted for publication.

Aragon, C.M.G., & Amit, Z. (in press). A two dimensional model of alcohol consumption: Possible interaction of brain catalase and aldehyde dehydrogenase. Alcohol.

Aragon, C.M.G., Sternklar, G., & Amit, Z. (in press). A correlation between voluntary ethanol consumption and brain catalase activity in the rat. Alcohol.

Atweh, S.F., & Kuhar, M.J. (1977a). Autoradiographic localization of opiate receptors in rat brain. I. Spinal cord and lower medulla. Brain Research, 124, 53.

Atweh, S.F., & Kuhar, M.J. (1977b). Autoradiographic localization of opiate receptors in rat brain. II. The brain stem. Brain Research, 129, 1-12.

Atweh, S.F., & Kuhar, M.J. (1977c). Autoradiographic localization of opiate receptors in rat brain. III. The teleencephalon. Brain Research, 134, 393-405.

Badawy, A.A.-B., & Evans, M. (1981). The mechanism of the antagonism by naloxone of acute alcohol intoxication. British Journal of Pharmacology, 74, 514-516.

Barbaccia, M.L., Reggiani, A., Spano, P.F., & Trabucchi, M. (1981). Ethanol-induced changes of dopaminergic function in three strains of mice characterized by a different population of opiate receptors. Psychopharmacology, 74, 260-262.

Barbaccia, M.L., Reggiani, A., Spano, P.F., & Trabucchi, M. (1980). Ethanol effects on dopaminergic function: Modulation by the endogenous opioid system. Pharmacology Biochemistry & Behavior, 13, 303-306.

Barker, L.M., & Johns, T. (1978). Effect of ethanol preexposure on ethanol-induced conditioned taste aversion. Journal of Studies on Alcohol, 39, 39-46.

Barros, S., & Rodriquez, G. (1981). Naloxone as an antagonist in alcohol-intoxication. Anesthesiology, 54, 174.

Bass, M.B., Friedman, H.J., & Lester, D. (1978). Antagonism of naloxone hyperalgesia by ethanol. Life Sciences, 22, 1939-1946.

Batson, J., & Best, P. (1979). Drug-preexposure effects in flavor-aversion learning: Associative interference by conditioned environmental stimuli. Journal of Experimental Psychology: Animal Behavior Processes, 3, 273-283.

Berger, B.D., Wise, C.D., & Stein, L. (1973). Area postrema damage and bait shyness. Journal of Comparative and Physiological Psychology, 82, 475-479.

Berkowitz, B.A., Ngai, S.H., Hempstead, J., & Spector, S. (1975). Disposition of naloxone: Use of a new radioimmunoassay. Journal of Pharmacology and Experimental Therapeutics, 195, 499-504.

Berkowitz, B.A., & Way, E.L. (1971). Analgesic activity and central nervous system distribution of the optical isomers of pentazocine in the rat. Journal of Pharmacology and Experimental Therapeutics, 177, 500-508.

Berman, R.F., & Cannon, D.S. (1974). The effect of prior ethanol experience on ethanol-induced saccharin aversions. Physiology

and Behavior, 12, 1041-1044.

Bess, B., Janus, S., & Rifkin, A. (1972). Factors in successful narcotics renunciation. American Journal of Psychiatry, 128, 861.

Bihari, B. (1974). Alcoholism and methadone maintenance. American Journal of Drug and Alcohol Abuse, 1, 79-87.

Bird, K.D., Chesher, G.B., Perl, J., & Starmer, G.A. (1982). Naloxone has no effect on ethanol-induced impairment of psychomotor performance in man. Psychopharmacology, 76, 193-197.

Blum, K. (1980). Alcohol and opiates: A review of common mechanisms. In L. Manzo (Ed.), Advances in Neurotoxicology (pp. 71-90). London: Pergamon Press.

Blum, K., Briggs, A.H., Delallo, L., Elston, S.F.A., & Ochoa, R. (1983). Whole brain methionine enkephalin of ethanol-avoiding and ethanol-preferring C-57BL mice. Experientia, 38, 1469-1470.

Blum, K., Briggs, A.H., Elston, L., DeLallo, L., & Sheridan, P.J. (1982). Reduced leucine-enkephalin-like immunoreactive substance in hamster basal ganglia after long-term ethanol exposure. Science, 216, 1425-1427.

Blum, K., Elston, S.F.A., Delallo, L, Briggs, A.H., & Wallace, J.E. (1983). Ethanol acceptance as a function of genotype amounts of brain methionine enkephalin. Proceedings of the National Academy of Sciences of the United States of America, 80, 6510-6512.

Blum, K., Hamilton, M.G., Hirst, M., & Wallace, J.E. (1978). Putative role of isoquinoline alkaloids in alcoholism: A link to opiates. Alcoholism: Clinical and and Experimental Research, 2, 113-120.

Booth, D.A., Pilcher, C.W.T., D'Mello, G.D., & Stolerman, I.P. (1977). Comparative potencies of amphetamine, fenfluramine and related compounds in taste aversion experiments in rats. British Journal of Pharmacology Biochemistry & Behavior, 61, 669-677.

Borg, S., Kvande, H., Rydberg, U., Terenius, L., & Wahlstrom, A. (1982). Endorphin levels in human cerebrospinal fluid during alcohol intoxication and withdrawal. Psychopharmacology, 78, 101-103.

Braveman, N.S. (1975). Formation of taste aversion in rats following prior exposure to sickness. Learning and Motivation, 6, 512-534.

Brick, J., & Horowitz, G.P. (1982). Alcohol and morphine induced hypothermia in mice selected for sensitivity to ethanol. Pharmacology Biochemistry & Behavior, 16, 473-479.

Brown, B.S., Kozel, N.J., Meyers, M.B., & Dupont, R.L. (1973). Use of alcohol by addict and nonaddict populations. American Journal of Psychiatry, 130, 599-601.

Brown, Z.W., Amit, Z., Levitan, D.E., Ogren, S.-O., & Sutherland, E.A. (1977). Noradrenergic mediation of the positive reinforcing properties of ethanol: II. Extinction of ethanol-drinking behavior in laboratory rats by inhibition of dopamine-beta-

hydroxylase. Implications for treatment procedures in human alcoholics. Archives Internationales de Pharmacodynamie et de Therapie, 230, 76-82.

Brown, Z.W., Amit, Z., & Rockman, G.E. (1979). Intraventricular self-administration of acetaldehyde, but not ethanol, in naive laboratory rats. Psychopharmacology, 64, 271-276.

Brown, Z.W., Amit, Z., Sinyor, D., Rockman, G.E., & Ogren, S.-O. (1978). Suppression of voluntary ingestion of morphine by inhibition of dopamine-beta-hydroxylase. Archives Internationales de Pharmacodynamie et de Therapie, 232, 102-110.

Brown, Z.W., Amit, Z., & Smith, B. (1980a). Intraventricular self-administration of acetaldehyde and voluntary consumption of ethanol in rats. Behavioral and Neural Biology, 28, 150-155.

Brown, Z.W., Amit, Z., & Smith, B. (1980b). Examination of the role of tetrahydroisoquinoline alkaloids in the mediation of ethanol consumption in rats. In H. Begleiter (Ed.), Biological Effects of Alcohol (pp. 103-120). New York: Plenum.

Brown, Z.W., Amit, Z., Smith, B., & Rockman, G. (1978). Differential effects on conditioned taste aversion learning with peripherally and centrally administered acetaldehyde. Neuropharmacology, 17, 931-935.

Brown, Z.W., Amit, Z., Smith, B., & Rockman, G. (1979). Disruption of taste aversion learning by pretreatment with diazepam and

morphine. Pharmacology Biochemistry & Behavior, 10, 17-20.

Brown, Z.W., Amit, Z., Smith, B., Sutherland, E.A., & Selvaggi, N. (1983). Alcohol-induced euphoria enhanced by disulfiram and calcium carbamide. Alcoholism: Clinical and Experimental Research, 7, 276-278.

Cannon, D.S., Baker, T.B., & Berman, R.F. (1977). Taste aversion disruption by drug pretreatment: Dissociative and drug-specific effects. Pharmacology Biochemistry & Behavior, 6, 93-100.

Cannon, D.S., Berman, R.F., Baker, T.B., & Atkinson, C.A. (1975). Effect of preconditioning unconditioned stimulus experience on learned taste aversions. Journal of Experimental Psychology: Animal Behavior Processes, 104, 270-284.

Cappell, H.D., & LeBlanc, A.E. (1975). Conditioned aversion by amphetamine: Rates of acquisition and loss of the attenuating effects of prior exposure. Psychopharmacologia, 43, 157-162.

Cappell, H.D., LeBlanc, A.E., & Endrenyi, L. (1973). Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazepoxide. Psychopharmacologia, 29, 239-246.

Cappell, H.D., LeBlanc, A.E., & Herling, S. (1975). Modification of the punishing effects of psychoactive drugs in rats by previous drug experience. Journal of Comparative and Physiological Psychology, 89, 347-356.

Carey, R.J., Goodall, E., & Lorens, S.A. (1975). Differential effects

of amphetamine and food deprivation on self-stimulation of the lateral hypothalamus and medial frontal cortex. Journal of Comparative and Physiological Psychology, 85, 224-230.

Carlson, R.H., & Lydic, R. (1976). The effects of ethanol upon threshold and response rate for self-stimulation. Psychopharmacology, 50, 61-64.

Carlsson, A., & Lindquist, F. (1973). Effect of ethanol on the hydroxylation of tyrosine and tryptophan in rat brain in vivo. Journal of Pharmacy and Pharmacology, 25, 437-440.

Carr, G., Brown, Z.W., Rockman, G.E., & Amit, Z. (1980). Reduction in voluntary ethanol consumption by treatment with alcohol with alcohol dehydrogenase inhibitors. Substance and Alcohol Actions/Misuse, 1, 187-196.

Charness, M.E., Gordon, A.S., & Diamond, I. (1983). Ethanol modulation of opiate receptors in cultured neural cells. Science, 222, 1246-1248.

Chin, J.H., & Goldstein, D.B. (1977). Effects of low concentrations of ethanol on the fluidity of spin-labeled erythrocyte and brain membranes. Molecular Pharmacology, 13, 435-441.

Chipkin, R.E., Stewart, J.M., & Channabasavaiah, K. (1980). The effects of peptides on the stimulus properties of ethanol. Pharmacology Biochemistry & Behavior, 12, 93-98.

Cicero, T.J., Newman, K.S., Gerrity, M., Schmoeker, P.F., & Bell, R.D.

(1982). Ethanol inhibits the naloxone-induced release of luteinizing hormone-releasing hormone from the hypothalamus of the male rat. Life Sciences, 31, 1587-1596.

Clement, J.G. (1980). Investigation into the mechanism of morphine and ethanol inhibition in the guinea pig ileum longitudinal muscle strip. Canadian Journal of Physiology and Pharmacology, 58, 265-270.

Clement, J.G. (1979). Differences in purity of commercial samples of hemicholinium-3: Effects of high affinity choline uptake, neuromuscular transmission, acetylcholinesterase and carbachol and acetylcholine contractions. Life Sciences, 24, 615-620.

Clement, J.G. (1978). Effect of ethanol, pentobarbital and morphine on the toxicity of hemicholinium-3 and d-tubocurarine in mice. Archives Internationales de Pharmacodynamie et de Therapie, 236, 60-65.

Cohen, G., & Collins, M. (1970). Alkaloids from catecholamines in adrenal tissue: Possible role in alcoholism. Science, 167, 1749-1751.

Cohen, G., & Katz, S. (1975). 6,7-dihydroxytetrahydro-isoquinoline: Evidence for in vivo inhibition of intraneuronal monoamine oxidase. Journal of Neurochemistry, 25, 719-722.

Cohen, G., Mytilineou, C., & Barrett, R.E. (1972). 6,7-dihydroxytetrahydroisoquinoliné: Uptake and storage by peripheral

sympathetic nerve in the rat. Science, 175, 1269-1272.

Collins, M.A., & Bigdeli, M.G. (1975). Tetrahydro-isoquinolines in vivo. I. Rat brain formation of salsolinol, a condensation product of dopamine and acetaldehyde, under certain conditions during ethanol intoxication. Life Sciences, 16, 585-602.

Collins, A.C., Cashaw, J.L., & Davis, V.E. (1973). Dopamine-derived tetrahydroisoquinoline alkaloids: Inhibition of neuroamine metabolism. Biochemical Pharmacology, 22, 2337-2348.

Corcoran, M.E., Bolotow, I., Amit, Z., & McCaughran, J.A., Jr. (1974). Conditioned taste aversions produced by active and inactive cannabinoids. Pharmacology Biochemistry & Behavior, 2, 725-728.

Creese, I., & Sibley, D.R. (1981). Receptor adaptations to centrally acting drugs. Annual Review of Pharmacology and Toxicology, 21, 357-391.

Critcher, E.C., Lin, C.I., Patel, J., & Myers, R.D. (1983). Attenuation of alcohol drinking in tetrahydroisoquinoline-treated rats by morphine and naltrexone. Pharmacology Biochemistry & Behavior, 18, 225-229.

Dacanay, R.J., & Riley, A.L. (1982). The UCS preexposure effect in taste aversion learning: Tolerance and blocking are drug specific. Animal Learning & Behavior, 10, 91-96.

Davis, M.E., Akera, T., & Brody, M. (1979). Reduction of opiate binding to brainstem slices associated with development of

tolerance to morphine in rats. Journal of Pharmacology and Experimental Therapeutics, 211, 112-119.

Davis, V.E., & Walsh, M.J. (1970). Alcohol, amines, and alkaloids: A possible biochemical basis for alcohol addiction. Science, 167, 1005-1007.

Deitrich, R.A., & Collins, A.C. (1977). Pharmacogenetics of alcoholism. In K. Blum (Ed.), Alcohol and Opiates: Neurochemical and Behavioral Mechanisms (pp. 109-139). New York: Academic Press.

Deitrich, R.A., & Erwin, V. (1980). Biogenic amine-aldehyde condensation products: Tetrahydroisoquinolines and tryptolines (Beta-carbolines). Annual Review of Pharmacology and Toxicology, 20, 55-80.

Dole, V.P., Fishman, J., Goldfrank, L., Khanna, J., & McGivern, R.F. (1982). Arousal of ethanol intoxicated comatose patients with naloxone. Alcoholism: Clinical and Experimental Research, 6, 275-279.

Doyle, T., & Samson, H. (1984, June). Acute naloxone effects on ethanol self-administration. Paper presented at the Second Congress of the International Society for Biomedical Research on Alcoholism, Santa Fe, NM.

Duka, T.H., Schubert, P., Wuster, M., Stoiber, R., & Herz, A. (1981). A selective distribution pattern of different opiate receptors in

certain areas of rat brain as revealed by in vitro autoradiography. Neuroscience Letters, 21, 119-124.

Duncan, C., & Deitrich, R.A. (1980). A critical evaluation of tetrahydroisoquinoline-induced ethanol preference in rats. Pharmacology Biochemistry & Behavior, 13, 265-282.

Eckardt, M.J. (1975). Conditioned taste aversion produced by the oral ingestion of ethanol in the rat. Physiological Psychology, 3, 317-321.

Elsmore, T.F., & Fletcher, G.V. (1972). Delta-9-tetrahydro-cannabinol: Aversive effects in rat at high doses. Science, 175, 911-912.

Eneanya, D.I., Bianchine, J.R., Duran, D.O., & Andresen, B.D. (1981). The actions and metabolic fate of disulfiram. Annual Review of Pharmacology and Toxicology, 21, 575-596.

Esposito, R.U., & Kornetsky, C. (1978). Opioids and rewarding brain stimulation. Neuroscience & Biobehavioral Reviews, 2, 115-122.

Farber, P.D., Gorman, J.E., & Reid, L.D. (1976). Morphine injections in the taste aversion paradigm. Physiological Psychology, 4, 365-368.

Fertel, R.H., Greenwald, J.E., Schwarz, R., Wong, L., & Bianchine, J. (1980). Opiate receptor binding and analgesic effects of the tetrahydroisoquinolines salsolinol and tetrahydropapaveroline. Research Communications Chemical Pathology and Pharmacology, 27,

3-16.

Gallistel, C.R. (1983). Self-stimulation. In J.A. Deutsch (Ed.), The Physiological Basis of Memory (pp. 269-349). New York: Academic Press.

Gamzu, E. (1977). The multifaceted nature of taste-aversion inducing agents: Is there a single common factor? In L.M. Barker, M.R. Best, & M. Domjan (Eds.), Learning Mechanisms in Food Selection (pp. 477-509). Waco, TX: Baylor University Press.

Garcia, J., Kimeldorf, D.J., & Koelling, R.A. (1955). Conditioned aversion to saccharin resulting from exposure to gamma radiation. Science, 122, 157-158.

Garriott, J.C., & Sturner, W.Q. (1973). Morphine concentrations and survival periods in acute heroin fatalities. New England Journal of Medecine, 289, 1276-1278.

Gelfand, R., & Amit, Z. (1976). Effects of ethanol injections on morphine consumption in morphine-preferring rats. Nature, 259, 415.

Genazzani, A.R., Nappi, G., Facchinetti, F., Mazzella, G.L., Parrini, D., Sinforiani, E., Petraglia, F., & Savoldi, F. (1982). Central deficiency of beta-endorphin in alcohol addicts. Journal Clinical Endocrinology and Metabolism, 55, 583-586.

Gianoulakis, C. (1983). Long term ethanol alters the binding of 3H-opiates to brain membranes. Life Sciences, 33, 725-733.

Gianoulakis, C., Chan, J.S.D., Kalant, H., & Chretien, M. (1983). Chronic ethanol treatment alters the biosynthesis of beta-endorphin by the rat neurointermediate lobe. Canadian Journal of Physiology and Pharmacology, 61, 967-976.

Gianoulakis, C., Woo, N., Drouin, J.N., Seidah, N.G., Kalant, H., & Chretien, M. (1981). Biosynthesis of beta-endorphin by the neurointermediate lobes from rats treated with morphine or alcohol. Life Sciences, 29, 1973-1982.

Gintzler, A.R., Levy, A., & Spector, S. (1976). Antibodies as a means of isolating and characterizing biologically active substances: Presence of a non-peptide, morphine-like compound in the central nervous system. Proceedings of the National Academy of Sciences of the United States of America, 73, 2132.

Gintzler, A.R., & Pasternak, G.W. (1983). Multiple mu receptors: Evidence for mu(2) sites in the guinea pig ileum. Neuroscience Letters, 39, 51-56.

Giovine, A., Renis, M., & Bertolino, A. (1976). In vivo and in vitro studies on the effect of tetrahydro-papaveroline and salsolinol on COMT and MAO activity in rat brain. Pharmacology, 14, 86-94.

Glick, S.D., & Rapaport, G. (1974). Tolerance to the facilitatory effect of morphine on self-stimulation of the medial forebrain bundle in rats. Research Communications in Chemical Pathology and Pharmacology, 9, 647.

Goldstein, A., & Judson, B.A. (1971). Alcohol dependence and opiate dependence: Lack of relationship in mice. Science, 172, 290-292.

Goodman, R.R., Snyder, S.H., Kuhar, M.J., & Young, W.S. (1980). III: Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. Proceedings of the National Academy of Sciences of the United States of America, 77, 6239-6243.

Goudie, A.J., & Dickins, D.W. (1978). Nitrous oxide-induced conditioned taste aversions in rats: The role of duration of drug exposure and its relation to taste aversion-self-administration "paradox". Pharmacology Biochemistry & Behavior, 9, 587-592.

Goudie, A.J., Dickins, D.W., & Thornton, E.W. (1978). Cocaine-induced conditioned taste aversions in rats. Phamacology Biochemistry & Behavior, 8, 757-761.

Goudie, A.J., Taylor, M., Atherton, H. (1975). Effects of prior drug experience on establishment of taste aversions in rats. Pharmacology Biochemistry & Behavior, 3, 947-952.

Goudie, A.J., & Thornton, E.W. (1975). Effects of drug experience on drug induced conditioned taste aversions: Studies with amphetamine and fenfluramine. Psychopharmacologia, 44, 77-82.

Green, J., & Jaffe, J.H. (1977). Alcohol and opiate dependence. Journal of Studies on Alcohol, 38, 1274-1293.

Greenberg, R.S., & Cohen, G. (1973). Tetrahydro-isoquinoline

alkaloids: Stimulated secretion from the adrenal medulla. Journal of Pharmacology and Experimental Therapeutics, 184, 119-128.

Hald, J., & Jacobsen, E. (1948). A drug sensitizing the organism to ethyl alcohol. Lancet, 2, 1001.

Hall, H., & Ogren, S.-O. (1981). Effects of antidepressants drugs on different receptors in the brain. European Journal of Pharmacology, 70, 393-407.

Halushka, P.V., & Hoffman, P.C. (1970). Alcohol addiction and tetrahydro-papaveroline. Science, 169, 1104-1105.

Hamilton, M.G., Hirst, M., & Blum, K. (1979). Opiate-like activity of salsolinol of the electrically stimulated guinea pig ileum. Life Sciences, 25, 2205-2210.

Harris, R.A. (1984). Defining the membrane pathology produced by chronic alcohol consumption. Laboratory Investigation, 50, 113-114.

Harris, R.A., & Schroeder, F. (1981). Ethanol and the physical properties of brain membranes: Fluorescence studies. Molecular Pharmacology, 20, 128-137.

Herz, A. (1983). Multiple opiate receptors and their functional significance. Journal of Neural Transmission, 18, 227-233.

Hiller, J.M., Angel, L.M., & Simon, E.J. (1983). Characterization of the selective inhibition of the delta subclass of opioid binding

sites by alcohols. Molecular Pharmacology, 25, 249-255.

Hiller, J.M., Angel, L.M., & Simon, E.J. (1981). Multiple opiate receptors: Alcohol selectively inhibits binding to delta receptors. Science, 214, 468-469.

Hiller, J.M., Pearson, J., & Simon, E.J. (1973). Distribution of stereospecific binding of the potent narcotic analgesic etorphine in human brain, predominance in the limbic system. Research Communications in Chemical Pathology and Pharmacology, 6, 1052.

Ho, A.K.S., Chen, R.C.A., & Morrison, J.M. (1977). Opiate-ethanol interaction studies. In K. Blum (Ed.), Alcohol and Opiates: Neurochemical and Behavioral Mechanisms (pp. 189-202). New York: Academic Press.

Ho, A.K.S., Chen, R.C.A., & Ho, C.C. (1978). Interaction toxicity between ethanol and narcotics in mice with reference to alpha-l-acetylmethadol (LAAM). Pharmacology Biochemistry & Behavior, 9, 195-200.

Ho, A.K.S., & Chen, R.C.A., & Kreek, M.J. (1979). Morphine withdrawal in the rat assessment by quantitation of diarrhea and modification by ethanol. Pharmacology, 18, 9-17.

Ho, A.K., & Ho, C.C. (1979). Toxic interactions of ethanol with other central depressants: Antagonism by naloxone to narcosis and lethality. Pharmacology Biochemistry & Behavior, 2, 111-114.

Ho, A.K.S., & Rossi, N. (1982). Suppression of ethanol consumption by

methionine-enkephalin in rats. Journal of Pharmacy and Pharmacology, 34, 118-119.

Hoffman, P.L., & Tabakoff, B. (1983). Regulation of opiate receptor function effects of ethanol. Federation Proceedings, 42, 1207.

Hoffman, P.L., Urwyler, S., & Tabakoff, B. (1982). Alterations in opiate receptor function after chronic ethanol exposure. Journal of Pharmacology and Experimental Therapeutics, 222, 182-189.

Hollt, V., Haarmann, I., & Herz, A. (1981). Long-term treatment of rats with morphine reduces the activity of messenger ribonucleic acid coding for the beta-endorphin/ACTH precursor in the intermediate pituitary. Journal of Neurochemistry, 31, 619-626.

Hollt, V., & Seizinger, B.R. (1983). Receptor selectivities of the three opioid peptide families. In G. Koch and D. Richter (Eds.), Biochemical and Clinical Aspects of Neuropeptides: Synthesis, Processing and Gene Structure (pp. 59-72). New York: Academic Press.

Holman, E.W. (1976). The effect of drug habituation before and after taste aversion learning in rats. Animal Learning and Behavior, 4, 329-332.

Holtzman, S.G. (1976). Comparison of the effects of morphine, pentazocine, cyclazocine and amphetamine on intracranial self-stimulation in the rat. Psychopharmacologia, 46, 223-227.

Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan,

B.A., & Morris, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature, 258, 577.

Hunt, W.A., & Majchrowicz, E. (1974). Alterations in the turnover of brain norepinephrine and dopamine in alcohol-dependent rats. Journal of Neurochemistry, 23, 549-552.

Hymson, D.L., & Hynes, M.D. (1982). Evidence that ethanol-induced impairment of roto-rod performance is not mediated by opioid mechanisms. Progress in Neuro-psychopharmacology and Biological Psychiatry, 6, 159-165.

Hynes, M.D., Lochner, M.A., Bemis, K.G., & Hymson, D.L. (1983). Chronic ethanol alters the receptor binding characteristics of the delta opioid receptor ligand, D-Ala(2)-D-Leu(5) enkephalin in mouse brain. Life Sciences, 33, 2331-2337.

Jacobs, W.J., Zellner, D.A., LoLordo, V.M., & Riley, A.L. (1981). The effect of post-conditioning exposure to morphine on the retention of a morphine-induced conditioned taste aversion. Pharmacology Biochemistry & Behavior, 14, 779-785.

Jacobsen, E. (1952). The metabolism of ethyl alcohol. Pharmacological Review, 4, 107-135.

Jacquet, Y.F. (1973). Conditioned aversion during morphine maintenance in mice and rats. Physiology and Behavior, 11, 527-541.

Jeffcoate, W.J., Platts, P., Ridout, M., Hastings, A.G., MacDonald, I., & Selby, C. (1981). Endocrine effects of ethanol infusion in normal subjects: Modification by naloxone. Pharmacology Biochemistry & Behavior, 13, 145-148.

Jefferys, D.B., Flanagan, R.J., & Volans, G.N. (1980). Reversal of ethanol-induced coma with naloxone. Lancet, 1, 308-309.

Jorgensen, H.A., & Hole, K. (1981). Does ethanol stimulate brain opiate receptors? Studies on receptor binding and naloxone inhibition of ethanol-induced effects. European Journal of Pharmacology, 75, 223-229.

Kay, E.J. (1975). Aversive effects of repeated injections of THC in rats. Psychological Reports, 37, 1051-1054.

Khanna, J.M., Le, A.D., Kalant, H., & LeBlanc, A.E. (1979). Cross tolerance between ethanol and morphine with respect to their hypothermic effects. European Journal of Pharmacology, 59, 145-147.

Khanna, J.M., Mayer, J.M., Kalant, H., & Shah, G. (1982). Effect of naloxone on ethanol-induced narcosis and pentobarbital-induced narcosis. Canadian Journal of Physiology and Pharmacology, 60, 1315-1318.

Kiianmaa, K., Hoffman, P.L., & Tabakoff, B. (1983). Antagonism of the behavioral effects of ethanol by naltrexone in BALB/c, C57BL/6, and DBA/2 mice. Psychopharmacology, 79, 291-294.

Kirby, G.W. (1967). Biosynthesis of morphine alkaloids. Science, 55, 170-173.

Kirk, R.E. (1968). Experimental Design: Procedures for the Behavioral Sciences. California: Brooks/Cole.

Kolb, L. (1962). Drug addiction; a medical problem. Illinois: Thomas.

Kosterlitz, H.W., Paterson, S.J., & Robson, L.E. (1981). Characterization of the sigma-subtype of the opiate receptor in the guinea-pig brain. British Journal of Pharmacology, 73, 939-949.

Knych, E.T., & Prohaska, J.R. (1981). Effect of chronic intoxication and naloxone on the ethanol-induced increase in plasma corticosterone. Life Sciences, 28, 1987-1994.

Kreek, M.J. (1981). Metabolic interactions between opiates and alcohol. Annals of the New York Academy of Sciences, 362, 36-49.

Kuhar, M., Pert, C.B., & Snyder, S.H. (1973). Regional distribution of opiate receptor binding in monkey and human brain. Nature, 245, 447.

Lai, H., Makous, W.L., & Horita, A. (1980). Effects of ethanol on central dopamine functions. Life Sciences, 27, 299-304.

Lai, H., Makous, W.L., Horita, A., & Leung, H. (1979). Effects of ethanol on turnover and function of striatal dopamine. Psychopharmacology, 61, 1-9.

LeBlanc, A.E., & Cappell, H. (1975). Antagonism of morphine-induced aversive conditioning by naloxone. Pharmacology Biochemistry & Behavior, 3, 185-188.

LeMagnen, J. (1969). Peripheral and systemic actions of food in caloric regulation of intake. Annual New York Academic of Sciences of the United States of America, 157, 1126-1157.

Levine, A.S., Hess, S., & Morley, J.E. (1983). Alcohol and the opiate receptor. Alcoholism: Clinical and Experimental Research, 7, 83-84.

Lewis, M.J. (1984, June). Naloxone fails to block ethanol effects on BSR. Paper presented at the Second Congress of the International Society for Biomedical Research on Alcoholism, Santa Fe, New Mexico.

Ling, G.S.F., Spiegel, K., Nishimura, S.L., & Pasternak, G.W. (1983). Dissociation of morphine's analgesic and respiratory depressant actions. European Journal of Pharmacology, 86, 487-488.

Littleton, J.M. (1980). The effects of alcohol on the cell membrane: A possible basis for tolerance and dependence. In D. Richter (Ed.), Addiction and Brain Damage (pp. 46-74). Baltimore: University Park Press.

Locke, S., Cohen, G., & Dembiec, D. (1973). Uptake and accumulation of H-6,7-dihydroxytetrahydroisoquinoline by peripheral sympathetic nerves in vivo. Journal of Pharmacology and

Experimental Therapeutics, 187, 56-67.

Lorens, S.A. (1966). Effect of lesions in the central nervous system on lateral hypothalamic self-stimulation in the rat. Journal of Comparative and Physiological Psychology, 62, 256-262.

Lorens, S.A., & Sainati, S.M. (1978). Naloxone blocks the excitatory effect of ethanol and chlordiazepoxide on lateral hypothalamic self-stimullation behavior. Life Sciences, 23, 1359-1364.

Lucchi, L., Bosio, A., Spano, P.F., & Trabucchi, M. (1981). Action of ethanol and salsolinol on opiate receptor function. Brain Research, 232, 506-510.

Lucchi, L., Rius, R.A., Uzumaki, H., Govoni, S., & Trabucchi, M. (1984). Chronic ethanol changes opiate receptor function in rat striatum. Brain Research, 293, 368-371.

Luttinger, D., Nemeroff, C.B., Mason, G.A., Frye, G.D., Breese, G.R., & Prange, A.J., Jr. (1981). Enhancement of ethanol-induced sedation and hypothermia by centrally administered neurotensin, beta-endorphin and bombesin. Neuropharmacology, 20, 305-309.

Mackenzie, A.I. (1979). Naloxone in alcohol intoxication. Lancet, 1, 733.

Magnan, J., Paterson, S.J., Tavani, A., & Kosterlitz, H.W. (1982). The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. Naunyn-Schmiedeberg's Archives of Pharmacology, 319, 197-205.

Marfaing-Jallat, P., & LeMagnen, J. (1982). Decrease of ethanol consumption by naloxone in naive and dependent rats. Alcoholism: Clinical Experimental Research, 6, 304.

Marshall, A., Hirst, M., & Blum, K. (1977). Morphine analgesia augmentation by and direct analgesia with 3-carboxysalsolinol. Experientia, 33, 754-755.

Martin, A., Pilotto, R., Singer, G., & Oei, T.P.S. (1983). The suppression of ethanol self injection by buprenorphine. Pharmacology Biochemistry & Behavior, 19, 985-986.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., & Gilbert, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. Journal of Pharmacology and Experimental Therapeutics, 197, 517-532.

Mattila, M.J., Nuotto, E., & Seppala, T. (1981). Naloxone is not an effective antagonist of ethanol. Lancet, 1, 775-776.

Mayer, J.M., Khanna, J.M., Kalant, H., & Spero, L. (1980). Cross-tolerance between ethanol and morphine in the guinea pig ileum longitudinal-muscle/myenteric-plexus preparation. European Journal of Pharmacology, 63, 223-227.

McClearn, G.E., & Kakihana, R. (1973). Selective breeding for ethanol sensitivity in mice. Behavior Genetics, 3, 409-410.

McDougal, J.N., Marques, P.R., & Burks, T.F. (1982). The thermic

effects of morphine but not ethanol are altered by restraint. Federation Proceedings, 41, 4695.

Mehar, G.S., Parker, J.M., & Tubas, T. (1974). Interaction between alcohol, minor tranquilizers and morphine. International Journal of Clinical Pharmacology, Therapy, and Toxicology, 9, 70-74.

Miceli, D., Marfaing-Jallat, P., & LeMagnen, J. (1979). Non-specific enhancement of ethanol-induced taste aversion by naloxone. Pharmacology Biochemistry & Behavior, 11, 391-394.

Miceli, D., Marfaing-Jallat, P., LeMagnen, J. (1980). Ethanol aversion induced by parenterally administered ethanol acting both as conditioned stimulus and unconditioned stimulus. Physiological Psychology, 8, 433-436.

Middaugh, L.D., Read, E., & Boggan, W.P. (1978). Effects of naloxone on ethanol-induced alterations of locomotor activity in C57Bl/mice. Pharmacology Biochemistry & Behavior, 9, 157-160.

Miksic, S., Shearman, G., & Lal, H. (1978). Generalization study with some narcotic and non-narcotic drugs in rats trained for morphine-saline discrimination. Psychopharmacology, 60, 103-104.

Mitcheson, M., Davidson, J., Hawks, D., Hitchins, L., & Malone, S. (1971). Sedative abuse by heroin addicts. Journal of Psychedelic Drugs, 4, 123-131.

Moore, J.A., & Kakihana, R. (1978). Ethanol-induced hypothermia in mice: Influence of genotype on development of tolerance. Life

Sciences, 23, 2331-2338.

Mottin, J.L. (1973). Drug induced attenuation of alcohol consumption. Quarterly Journal of Studies on Alcohol, 34, 444-472.

Myers, R.D., & Critcher, E.C. (1982). Naloxone alters alcohol drinking induced in the rat by tetrahydropapaveroline (THP) infused ICV. Pharmacology Biochemistry & Behavior, 16, 827-836.

Myers, R.D., & Veale, W.L. (1969). Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. Archives Internationales de Pharmacodynamie et de Therapie, 180, 100-113.

Mytilineou, C., Cohen, G., & Barrett, R. (1974). Tetrahydroisoquinoline alkaloids: Uptake and release by adrenergic nerves in vivo. European Journal of Pharmacology, 25, 390-401.

Naber, D., Soble, M.G., & Pickar, D. (1981). Ethanol increases opioid activity in plasma of normal volunteers. Pharmacopsychiatria, 14, 160-161.

Nachman, M. (1970). Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. Journal of Comparative and Physiological Psychology, 73, 22-30.

Nichols, J.R., & Hsiao, S. (1967). Addiction liability of albino rats: Breeding for quantitative differences in morphine drinking. Science, 157 , 561-563.

North, R.A., Collins, M.A., Milner, J.D., Karras, P.J., & Koziol, D.J.

(1981). Tetrahydrosioquinolines (TIQs) do not act on opiate receptors in the guinea-pig ileum. European Journal of Pharmacology, 71, 489-493.

O'Donnell, J.A. (1964). A follow-up of narcotic addicts; mortality, relapse and abstinence. American Journal of Orthopsychiatry, 34, 948-954.

O'Neill, P.J., & Rahwan, R.G.. (1977). Absence of formation of brain salsolinol in ethanol-dependent mice. Journal of Pharmacology and Experimental Therapeutics, 200, 306-313.

Olson, G.A., Olson, R.D., Kastin, A.J., & Coy, D.H. (1979). Endogenous opiates: 1979. Peptides, 1, 365-379.

Pasternak, G.W., Childers, S.R., & Snyder, S.H. (1980). Opiate analgesia: Evidence for mediation by a subpopulation of opiate receptors. Science, 208, 514-516.

Pasternak, G.W., & Hahn, E.F. (1980). Long-acting opiate agonists and antagonists: 14-hydroxydihydromorphinone hydrazones. Journal of Medical Chemistry, 23, 674-676.

Paton, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea pig ileum. British Journal of Pharmacology, 11, 119-127.

Perkins, M.E., & Bloch, H.L. (1970). Survey of a methadone maintenance treatment program. American Journal of Psychiatry, 126, 1389-1396.

Pert, C.B. (1981). Type 1 and Type 2 opiate receptor distribution in

brain - What does it tell us? In J.B. Martin, S. Reichlin, & K.L. Bick (Eds.), Neurosecretion and Brain Peptides (pp. 117-131). New York: Raven Press.

Pert, C.B., & Snyder, S.H. (1973). Opiate receptor: Demonstration in nervous tissue. Science, 179, 1011-1014.

Pfeiffer, A., Seizinger, B.R., & Herz, A. (1981). Chronic ethanol imbibition interferes with delta-, but not with mu-opiate receptors. Neuropharmacology, 20, 1229-1232.

Pohorecky, L.A. (1974). Effects of ethanol on central and peripheral noradrenergic neurons. Journal of Pharmacology and Experimental Therapeutics, 189, 380-391.

Przewlocki, R., Hollt, V., Duka, Th., Kleber, G., Gramsch, Ch., Haarmann, I., & Herz, A. (1979). Long-term morphine treatment decreases endorphin levels in rat brain and pituitary. Brain Research, 174, 357-361.

Quirion, R., Weiss, A.S., & Pert, C.B. (1983). Comparative pharmacological properties and autoradiographic distribution of 3H-ethylketocyclazocine binding sites in rat and guinea pig brain. Life Sciences, 33, 183-186.

Quirion, R., Zajac, J.M., Morgat, J.L., & Roques, B.P. (1983). Autoradiographic distribution of mu and delta receptors in rat brain using highly selective ligands. Life Sciences, 33, 227-230.

Rahwan, R.G. (1975). Toxic effects of ethanol: Possible role of

acetaldehyde, tetrahydroisoquinolines and tetrahydro-beta-carboliness. Toxicology and Applied Pharmacology, 34, 3-27.

Rahwan, R.G., O'Neill, P.J., & Milner, D.D. (1974). Differential secretion of catecholamines and tetra-hydroisoquinolines from the bovine adrenal medulla. Life Sciences, 14, 1927-1938.

Reggiani, A., Barbaccia, M.L., Spano, P.F.., & Trabucchi, M. (1980). Dopamine metabolism and receptor function after acute and chronic ethanol. Journal of Neurochemistry, 35, 34-37.

Reicher, M.A., & Holman, E.W. (1977). Location preference and flavor aversion reinforced by amphetamine in rats. Animal Learning and Behavior, 5, 343-346.

Riley, A.L., Jacobs, W.J., & LoLordo, V.M. (1976). Drug exposure and the acquisition and retention of a conditioned taste aversion. Journal of Comparative and Physiological Psychology, 90, 799-807.

Rockman, G.E., Amit, Z., Bourque, C., Brown, Z.W., & Ogren, S.O. (1980). Reduction of voluntary morphine consumption following treatment with Zimelidine. Archives Internationales de Pharmacodynamie et de Therapie, 244, 123-129.

Rockman, G.E., Amit, Z., Carr, G., & Ogren, S.-O. (1979). Attenuation of ethanol intake by 5-hydroxytryptamine uptake blockade in laboratory rats. II. Possible interaction with brain norepinephrine. Archives Internationales de Pharmacodynamie et de Therapie, 241, 260-265.

Ronnback, L., Zeuchner, J., Rosengren, L., Wronski, A., & Ogren, S.O. (1984). Decreased morphine intake by opiate addicted rats administered zimelidine, a 5-hydroxytryptamine uptake inhibitor. Psychopharmacology, 82, 30-35.

Ross, D.H., Hartmann, R.J., & Geller, I. (1976). Ethanol preference in the hamster: Effects of morphine sulfate and naltrexone, a long-acting morphine antagonist. Paper presented at the meeting of the Western Pharmacology Society, San Francisco.

Ryder, S., Straus, E., Lieber, C.S., & Yalow, R.S. (1981). Cholecystokinin and enkephalin levels following ethanol administration in rats. Peptides, 2, 223-226.

Salvoldi, F., Mazzella, G.L., Facchinetti, F., Nappi, G., Petraglia, F., Sinforiani, E., Parrini, D., & Genazzani, A.R. (1983). Beta-endorphin, beta-lipotropin and adrenocorticotropic hormone levels in cerebrospinal fluid, and brain damage in chronic alcoholics. European Neurology, 22, 265-271.

Sandler, M., Carter, S.G., Hunter, R., & Stern, G.M. (1973). Tetrahydroisoquinoline alkaloids: In vivo metabolites of L-dopa in man. Nature, 241, 439-443.

Sawynok, J., Pinsky, C., & LaBella, F.S. (1979). Minireview on the specificity of naloxone as an opiate antagonist. Life Sciences, 25, 1621-1632.

Schenk, G.K., Enders, P., Engelmeier, M.P., Ewert, T., Herdemerten,

S., Kohler, K.H., Lodemann, E., Matz, D., & Pach, J. (1978). Application of the morphine antagonist naloxone in psychic disorders. Arzneimittel-Forshung, 28, 1274-1276.

Schmauss, C., & Yaksh, T. (1984). In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. Journal of Pharmacology and Experimental Therapeutics, 228, 1-12.

Schuster, C.R., & Thompson, T. (1968). Self-administration of and behavioral dependence on drugs. Annual Review of Pharmacology, 9, 483-502.

Schulz, R., Wuster, M., Duka, T., & Herz, A. (1980). Acute and chronic ethanol treatment changes endorphin levels in brain and pituitary. Psychopharmacology, 68, 221-227.

Schut, J., File, K., & Wohlmuth, T. (1973). Alcohol use by narcotic addicts in methadone maintenace treatment. Quarterly Journal of Studies on Alcohol, 34, 1356-1359.

Seevers, M.H. (1970). Morphine and ethanol physical dependence: A critique of a hypothesis. Science, 170, 1113-1114.

Seizinger, B.R., Bovermann, K., Maysinger, D., Hollt, V., & Herz, A. (1983). Differential effects of acute and chronic ethanol treatment on particular opioid peptide systems in discrete

regions of rat brain and pituitary. Pharmacology Biochemistry & Behavior, 18, 361-369.

Seligman, M.E.P. (1970). On the generality of the laws of learning. Psychological Review, 77, 406-418.

Shamma, M. (1972). The Isoquinoline Alkaloids: Chemistry and Pharmacology. New York: Academic Press.

Shannon, H.E., & Holtzman, S.G. (1976). Evaluation of the discriminative effects of morphine in the rat. Journal of Pharmacology and Experimental Therapeutics, 198, 54-65.

Shearman, G.T., & Herz, A. (1983). Ethanol and tetrahydroisoquinoline alkaloids do not produce narcotic discriminative stimulus effects. Psychopharmacology, 81, 224-227.

Shier, W.T., Koda, L.Y., & Bloom, F.E. (1983). Metabolism of tritium labeled dopamine following intracerebroventricular injection in rats pretreated with ethanol or chloral hydrate. Neuropharmacology, 22, 279-286.

Simantov, R., & Snyder, S.H. (1977). Opiate receptor binding in the pituitary gland. Brain Research, 124, 178.

Simon, E.J., & Hiller, J.M. (1978). The opiate receptors. Annual Review of Pharmacology and Toxicology, 18, 371-394.

Simon, E.J., Hiller, J.M., & Edelman, I. (1973). Stereospecific binding of the potent narcotic analgesic 3H-etorphine to rat brain homogenate. Proceedings of the National Academy of

Sciences USA, 70, 1947-1949.

Sinclair, J.D. (1974). Morphine suppresses alcohol drinking regardless of prior alcohol access duration. Pharmacology Biochemistry & Behavior, 2, 409-412.

Sinclair, J.D., Adkins, J., & Walker, S. (1973). Morphine-induced suppression of voluntary alcohol drinking in rats. Nature, 246, 425.

Sinclair, J.D., Altshuler, H., & Rusi, M. (1981). Ethanol and opiates: Interactions with consumption but not with intoxication. Paper presented at the meeting of the Second Finnish Symposium on Biological and Medical Effects of Alcohol, Helsinki.

Sinclair, J.D., Walker, S., & Jordan, W. (1973). Alcohol intubation and its effect on voluntary consumption by rats. Quarterly Journal of Studies on Alcohol, 34, 726-743.

Sklar, L.S., & Amit, Z. (1977). Manipulations of catecholamine systems block the conditioned taste aversion induced by self-administered drugs. Neuropharmacology, 16, 649-655.

Smith, B.R. (1982). Acetaldehyde and norepinephrine interactions in the mediation of some of the psycho-pharmacological properties of ethanol. Doctoral Dissertation, Concordia University, 1982. Montreal.

Smith, B.R., Amit, Z., Aragon, C.M.G., & Socaransky, S.M. (in press). Neurobiological correlates of ethanol self-administration: The

role of acetaldehyde. In C.A. Naranjo and E.M. Sellers (Eds.), Research Advances in Psychopharmacological Treatments for Alcoholism. New York: Elsevier Press.

Smith, B.R., Brown, Z.W., & Amit, Z. (1980). Chronic intraventricular administration of tetrahydroisoquinoline alkaloids: lack of effect on voluntary consumption in the rat. Substance and Alcohol Actions/Misuse, 1, 209-221.

Smith, J.R., & Simon, E.J. (1980). Selective protection of stereospecific enkephalin and opiate binding against inactivation by N-ethylaleimide - Evidence for 2 classes of opiate receptors. Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences, 77, 281-284.

Smith, S.G., Werner, T.E., & Davis, W.M. (1981). Intragastric drug self-administration by rats exposed successively to morphine and ethanol. Drug and Alcohol Dependence, 7, 305-310.

Socaransky, S.M. (1983). An investigation of the possible role of aldehyde dehydrogenase as a regulator of voluntary ethanol consumption. Unpublished master's thesis, Concordia University, Montreal.

Sorensen, S.C., & Mattisson, K.W. (1978). Naloxone as an antagonist in severe alcohol intoxication. Lancet, 2, 688-689.

Stewart, J., & Eikelboom, R. (1978). Pre-exposure to morphine and the attenuation of conditioned taste aversion in rats. Pharmacology

Biochemistry & Behavior, 9, 639-645.

Stimmel, B., Cohen, M., & Hanbury, R. (1978). Alcoholism and polydrug abuse in persons on methadone maintenance. Annual New York Academy of Sciences, 311, 99-109.

Switzman, L. (1980). The relationship between positive reinforcement and conditioned taste aversion produced by self-administered drugs. (Doctoral dissertation, Concordia University, 1980). Dissertation Abstracts International, 41, 1486-B.

Switzman, L., Fishman, B., & Amit, Z. (1981). Pre-exposure effects of morphine, diazepam and delta-9-THC on the formation of conditioned taste aversion. Psychopharmacology, 74, 149-157.

Szekely, J.I. (1983). The most characteristics in vivo effects of opiates. In J.I. Szekely & A.Z. Ronai (Eds.), Opioid Peptides: Volume I. Research Methods. Florida: CRC Press.

Tabakoff, B., & Hoffman, P.L. (1983). Alcohol interactions with brain opiate receptors. Life Sciences, 32, 197-204.

Tennyson, R.M., Cohen, G., Mytilineou, C., & Heikkila, R. (1973). 6,7-dihydroxytetrahydroisoquinoline: Electron microscope evidence for uptake into the amine binding vesicles in sympathetic nerves of the rat iris and pineal gland. Brain Research, 51, 161-169.

Tepperman, F.S., Hirst, M., & Smith, P. (1983). Brain and serum levels of naloxone following peripheral administration. Life Sciences, 33, 1091-1096.

Terenius, L. (1973). Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. Acta Pharmacologica et Toxicologica, 32, 317-320.

Turner, A.J., Baker, K.M., Algeri, S., Frigerio, A., & Garattini, S. (1974). Tetrahydropapaveroline: Formation in vivo and in vitro in rat brain. Life Sciences, 14, 2247-2257.

Van der Kooy, D., & Phillips, A.G. (1977). Temporal analysis of naloxone of morphine-induced taste aversion. Pharmacology Biochemistry & Behavior, 6, 637-641.

Vogel, R.A., Frye, G.D., Koepke, M., Mailman, R.B., Breese, G.R., & Mueller, R.A. (1982). Differential effects of TRH amphetamine, naloxone, and fenmetozole on ethanol actions attenuation of the effects of punishment and impairment of aerial righting reflex. Alcoholism: Clinical and Experimental Research, 5, 386-392.

Vogel, J.R., & Nathan, B.A. (1975). Learned taste aversions induced by hypnotic drugs. Pharmacology Biochemistry & Behavior, 3, 189-194.

Vrtunski, P., Murray, R., & Wolin, L.R. (1973). The effect of alcohol on intracranially reinforced response. Quarterly Journal of Studies on Alcohol, 34, 718-725.

Wauquier, A. (1976). The influence of psychoactive drugs on brain self-stimulation in rats: A review. In A. Wauquier and E.T.

Rolls (Eds.), Brain Stimulation Reward (pp. 123-170). New York: Elsevier.

Weppner, .S., & Agar, M.H. (1973). Immediate precursors to heroin addiction. Journal of Health and Social Behavior, 12, 10-18.

White, N., Sklar, L., & Amit, Z. (1977). The reinforcing action of morphine and its paradoxical side effect. Psychopharmacology, 52, 63-66.

Winter, J.C. (1975). The stimulus properties of morphine and ethanol. Psychopharmacologia, 44, 209-214.

Wise, R.A. (1980). Action of drugs of abuse on brain reward systems. Drug and Alcohol Dependence, 6, 82-83.

Wise, R.A., Yokel, R.A., & deWit, H. (1976). Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. Science, 191, 1273-1275.

Wood, P.L. (1982). Multiple opiate receptors: Support for unique mu, delta and kappa sites. Neuropharmacology, 21, 487-497.

Wuster, M., Schulz, R., & Herz, A. (1979). Specificity of opioids towards the mu-, delta- and epsilon-opiate receptors. Neuroscience Letters, 15, 193-198.

York, J.L., & Bush, R. (1982). Studies on the discriminative stimulus properties of ethanol in squirrel monkeys. Psychopharmacology, 77, 212-216.

*** Note that for all appendices (A-E):

C1, C2, (and C3 for Experiment 2a) represent the mean raw saccharin intake on the trial after first, second (and third) conditioning trial respectively.

P1, P2, (and P3 for Experiment 2a) represent the mean percent change in saccharin intake from baseline on the trial after the first, second (and third) conditioning trial respectively.

E1 and E2 represent the mean raw saccharin intake on the trial after the first, and second extinction trial respectively.

T1 and T2 represent the mean percent change saccharin intake on the trial after the first, second extinction trial respectively.

APPENDIX A

Mean and standard error of percent change scores and raw scores

for Experiment 1

Group	Baseline	C1	P1	C2	P2	E1	T1	E2	T2
S-W	17.9	21.6	22.3	23.4	32.1	23.3	31.6	24.9	40.3
	0.6	0.8	6.8	1.1	7.9	0.7	7.7	0.7	5.7
M-W	14.9	21.1	49.6	22.5	63.8	23.4	73.5	24.3	72.6
	1.5	1.0	11.7	1.1	22.4	1.5	29.9	1.1	15.2
S-E	17.9	12.9	-28.6	9.9	-46.1	15.4	-15.1	19.2	6.8
	1.0	1.5	7.6	2.4	12.8	2.5	13.4	2.2	11.5
M-E	16.4	14.7	-11.1	15.4	-6.9	18.9	14.7	21.4	30.3
	0.7	1.5	8.3	2.1	11.0	1.8	9.5	2.0	11.0
W-S	17.1	22.8	32.8	23.3	38.2	25.3	49.6	24.8	46.7
	0.9	1.7	6.4	1.3	11.3	1.3	9.6	1.1	8.3
E-S	16.1	19.4	21.0	21.0	30.1	21.5	35.0	21.1	31.2
	0.5	1.0	7.4	1.2	8.2	1.0	9.6	1.3	7.4
W-M	16.2	10.6	-35.7	5.2	-68.1	9.5	-42.0	14.3	-9.6
	1.1	1.4	6.4	1.4	7.5	2.2	11.6	1.7	11.5
E-M	14.4	12.4	-13.1	9.8	-30.1	15.2	8.4	15.2	8.1
	1.2	1.5	9.4	1.9	13.5	2.0	13.6	1.9	13.4

*** see note on p. 170

APPENDIX B

Mean and standard error of percent change scores and raw scores

for Experiments 2a & 2b

Group	Baseline	C1	P1	C2	P2	C3	P3	E1	T1	E2	T2
N-S	14.2	19.3	5.2	18.8	4.7	25.8	11.7	25.5	11.3	26.8	12.7
	0.9	0.7	1.5	1.5	1.7	1.1	1.0	1.6	1.8	1.5	1.7
S-M	19.0	16.6	-2.4	12.4	-6.6	12.7	-6.3	18.4	-0.6	21.4	2.4
	2.0	2.3	1.7	3.2	2.7	2.9	2.3	2.8	3.1	4.7	4.0
N-M	12.3	12.3	4.4	18.4	6.1	25.0	12.7	26.6	14.3	26.1	13.9
	1.1	1.1	0.8	0.8	1.0	0.5	1.0	1.3	1.6	1.1	1.3
S-E	19.4	15.5	-3.9	11.3	-7.6			19.5	0.1	19.5	0.1
	0.5	0.9	1.1	0.9	0.9			1.1	1.1	0.4	0.8
N-E	12.7	8.8	-3.9	9.4	-3.4			18.4	5.6	19.5	6.7
	0.5	1.0	1.3	1.3	1.5			0.9	1.0	0.4	0.9

*** see note on p. 170

APPENDIX C

Mean and standard error of percent change scores and raw scores

for Experiments 3a

Group	Baseline	C1	P1	C2	P2	E1	T1	E2	T2
S-W-M	16.3	14.1	-12.3	10.0	-36.8	16.4	1.8	16.2	1.7
	1.0	1.0	5.2	1.6	10.1	1.6	9.4	1.2	8.4
N-W-M	16.6	15.6	-4.2	11.5	-30.2	17.5	6.8	15.7	-5.5
	1.5	1.2	9.2	1.4	9.2	1.0	8.2	0.6	4.8
S-E-M	18.4	16.7	-6.9	15.3	-14.9	21.2	17.7	18.8	3.3
	1.0	1.2	9.2	1.4	9.2	1.0	8.2	0.6	4.7
N-E-M	16.5	14.9	-9.1	13.9	-16.3	18.5	12.6	18.1	9.4
	0.6	1.1	7.6	1.7	9.5	1.4	8.8	1.3	5.4
S-S-E	18.9	15.0	-21.1	10.6	-44.1	16.5	-12.5	19.6	4.3
	0.4	1.4	6.4	1.8	9.0	1.2	6.3	0.9	4.7
N-S-E	22.1	18.4	-15.7	15.1	-31.7	18.7	-13.7	24.6	11.5
	0.9	1.0	6.2	2.0	8.9	2.2	11.4	1.2	5.9
S-M-E	15.6	14.8	-4.2	15.8	3.1	20.3	33.0	21.6	40.5
	0.8	1.2	6.8	1.0	7.0	0.8	7.0	1.0	6.9
N-M-E	15.8	15.1	-2.2	14.6	-8.2	18.8	22.6	22.1	44.4
	1.0	1.1	7.9	1.4	7.1	1.1	10.4	0.8	10.2

*** see note on p. 170

APPENDIX C (Continued)

Mean and standard error of percent change scores and raw scores

for Experiments 3b

Group	Baseline	C1	P1	C2	P2	E1	T1	E2	T2
S-M-E	21.9	17.0	-19.7	16.4	-24.6	23.4	12.0	24.2	16.2
	1.9	0.9	4.9	1.6	4.4	1.2	9.5	1.2	9.9
N-M-E	18.9	14.7	-20.9	9.1	-50.8	19.9	16.0	20.7	10.6
	0.4	1.5	9.0	2.8	14.9	5.2	32.9	2.1	11.5
S-E-M	16.6	13.5	-20.9	14.7	-11.9	20.5	26.9	25.4	57.6
	1.4	1.8	4.6	2.1	9.7	2.3	15.4	2.8	14.3
N-E-M	20.6	16.0	-22.0	14.6	-28.5	21.7	6.7	26.2	28.9
	1.1	1.0	3.5	1.2	6.1	1.2	6.2	1.3	7.2

*** see note on p. 170

APPENDIX D

Mean and standard error of percent change scores and raw scores

for Experiments 4a

Group	Baseline	C1	P1	C2	P2	E1	T1	E2	T2
Saline	19.0	24.9	36.0	20.0	14.7	24.9	34.7	25.1	35.4
	1.9	1.4	11.2	1.4	11.1	1.7	9.6	2.1	9.6
D-1	22.3	26.3	20.3	25.7	18.0	25.8	18.1	25.2	15.0
	1.7	1.3	8.3	1.3	10.1	1.4	8.7	1.0	7.2
D-5	20.2	21.8	5.6	23.3	18.4	23.3	19.4	22.7	15.9
	1.6	3.2	11.7	1.0	7.3	1.2	11.1	1.6	11.1
D-10	23.2	23.7	2.3	22.0	-4.6	25.2	9.4	27.5	19.2
	1.0	1.2	4.5	1.6	7.1	1.3	6.8	1.5	6.6
L-1	20.2	19.2	-4.1	13.5	-33.0	20.7	9.7	23.0	15.6
	1.0	3.1	16.3	2.1	10.5	1.4	9.7	1.0	6.9
L-5	21.8	18.5	-14.2	11.0	-49.8	19.8	8.3	22.1	2.8
	1.0	1.4	8.4	1.8	7.6	1.6	8.3	1.8	10.3
L-10	22.3	22.7	3.3	16.3	-26.2	18.9	-13.1	22.1	0.6
	1.0	1.3	8.1	3.0	13.8	2.7	14.3	1.8	9.5

*** see note on p. 170

APPENDIX D (Continued)

Mean and standard error of percent change scores and raw scores

for Experiments 4b

Group	Baseline	C1	P1	C2	P2	E1	T1	E2	T2
D-S	19.0	25.9	50.3	25.9	47.8	27.4	54.5	27.6	60.1
	1.9	0.6	21.4	2.8	19.6	1.3	16.6	0.9	21.8
L-S	18.4	23.6	33.1	27.9	54.6	27.9	57.2	25.9	45.2
	1.3	1.2	11.2	2.0	12.1	1.1	13.5	1.3	12.1
D-M	20.9	17.4	-16.7	13.9	-34.2	20.2	-5.0	23.1	9.7
	1.1	1.7	7.3	2.2	10.4	2.5	11.1	1.9	7.0
L-M	18.0	17.9	4.3	20.9	20.3	23.7	36.5	26.1	51.4
	0.4	2.7	16.0	2.8	10.7	0.9	12.0	1.4	15.3
D-E	20.3	17.9	-11.0	13.3	-35.6	20.4	1.1	23.9	21.4
	1.6	1.5	6.8	2.4	9.3	2.4	10.6	1.2	10.8
L-E	18.0	19.8	8.8	21.2	18.8	24.6	40.4	26.6	52.0
	1.2	2.0	7.2	2.1	10.6	1.5	10.3	1.7	12.6

*** see note on p. 170

APPENDIX E

Mean and standard error of percent change scores and raw scores for Experiment 5

Group	Baseline	C1	P1	C2	P2	E1	T1	E2	T2
S-A	17.9	17.8	1.5	15.8	-17.7	24.0	37.2	24.4	40.2
	1.1	1.3	8.5	2.4	10.0	1.5	10.4	1.1	11.2
M-A	17.3	17.0	2.5	17.8	9.3	20.3	25.6	24.5	53.4
	1.8	1.5	8.3	1.5	11.2	0.8	12.4	1.6	19.0
W-M	18.1	10.3	-43.0	5.1	-72.2	9.1	-48.4	14.1	-17.0
	1.4	1.9	8.4	1.9	8.2	2.5	12.1	2.2	13.6
A-M	16.0	14.1	-7.6	12.0	-23.4	20.1	27.9	21.4	43.6
	1.9	1.3	8.6	1.5	6.2	2.1	4.4	0.9	13.2

*** see note on p. 170