CORTICOSTERONE EFFECTS ON THE ACQUISITION AND EXPRESSION OF ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE IN MICE

by

Julia Ann Chester

A THESIS

Presented to the Department of Behavioral Neuroscience and the Graduate Council of Oregon Health Sciences University School of Medicine

In partial fulfillment of the requirement for the degree of

Master of Science

June, 1996

APPROVED

(Professor in Charge of Thesis)

(Chairperson, Graduate Council)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

First, I would like to thank my mentor, Chris Cunningham, for being the best advisor in the whole world (maybe I am a little biased) and allowing me to pursue my interests. Many thanks go to my committee members, Deb Finn, Ed Keenan, Don Keith, Tamara Phillips, and Fred Risinger for their advice and encouragement.

Cunningham lab members have been very supportive. Thank you Julie, Nancy, Dobrina, Carly, and Kate for all your help and for understanding when times were especially tough. Shelly, thank you for your moral support, and teaching me how stuff works in graduate school and the Cunningham lab. Nick, thanks for being a good friend and for giving me someone to "argue" with without worrying about them taking it the wrong way. Heather and Erika, thanks for supporting me and being understanding roommates. Gwen, thank you for encouraging me and for listening to my concerns. Val, you have inspired me and taught me a lot about the crazy world of science. Thank you for all your support and advice. Paul, thank you for listening to me complain and feeding my sweet tooth (I guess). Ann, thanks for offering me food and being supportive. Sarah, you have helped me a lot. Thanks for all your encouragement. Alex, thank you so much for understanding, supporting, and encouraging me through this whole process. And, of course, nothing could have been accomplished without Ginger!

ABSTRACT

Stress has long been thought to contribute to the development of drug-seeking and addictive behavior. Several recent studies have implicated the stress hormone, corticosterone in modulating the rewarding properties of abused drugs, including amphetamine and ethanol. The experiments in this thesis examined a role for corticosterone in modulating the rewarding effects of ethanol in the place conditioning paradigm. All experiments were conducted with male DBA/2J mice, which are an inbred line of mice that show a strong conditioned place preference with ethanol.

Experiments 1 and 2 tested the hypothesis that high corticosterone levels enhanced the rewarding effects of ethanol during the acquisition of ethanol-induced place preference. For Experiment 1, various doses of corticosterone (0, 1, 5, or 10 mg/kg) was administered prior to the conditioning session with ethanol (2 g/kg). Corticosterone administration was expected to increase the magnitude of place preference in a dose-dependent manner. The results of Experiment 1 did not support this prediction. Experiment 2A examined the possibility that the normal rise in corticosterone in response to handling and ethanol injections is important in modulating ethanol's rewarding effects. The steroid synthesis inhibitor, aminoglutethimide (AMG) was administered prior to conditioning trials to examine the effect of inhibition of corticosterone release on the acquisition of

ethanol place preference. Experiment 2B was conducted to determine plasma corticosterone levels immediately following the 5 min conditioning session and show that AMG was effective in suppressing corticosterone release. The results of Experiment 2A demonstrated that AMG administration did not alter the magnitude of place preference. During conditioning trials, ethanol-stimulated locomotor activity in the AMG-treated group was significantly higher relative to the vehicle-treated group, suggesting that corticosterone may normally inhibit ethanol-stimulated activity. Experiment 2B showed that corticosterone levels in the AMG-treated group were significantly lower than the vehicle-treated group, confirming AMG's suppressive effect on corticosterone synthesis and release.

Experiments 3 and 4 tested the hypothesis that stressor-induced corticosterone release during a preference test facilitates the expression of ethanol-induced conditioned place preference. For Experiment 3, one of two doses of AMG (10 or 50 mg/kg) was administered prior to the preference test. It was predicted that inhibition of corticosterone release would attenuate the expression of place preference. Opposite to the predicted outcome, AMG dose-dependently enhanced the magnitude of preference, suggesting that corticosterone normally inhibits the expression of ethanol place preference. This effect was statistically marginal, therefore, the purpose of Experiment 4 was to replicate the observed effect of AMG on ethanol-induced preference. In order to maximize the chances of observing an increase in the magnitude

of preference, the experiment included another group conditioned with a lower dose of ethanol (1.5 g/kg). In addition, plasma corticosterone levels were determined for all groups immediately following the 60 min test session. Experiment 4 did not replicate the enhancement of preference found in Experiment 3. Consistent with the previous hypothesis, vehicle-treated groups showed a stressor-induced level of corticosterone during the preference test. Corticosterone levels in the AMG-treated groups were significantly reduced relative to vehicle-treated groups. However, inhibition of corticosterone release with AMG did not alter the magnitude of ethanol place preference.

In general, the present studies are not consistent with previous studies that suggested a facilitatory effect of corticosterone on the reinforcing and rewarding properties of abused drugs, such as ethanol. Overall, the experiments in this thesis suggest that corticosterone is not involved in modulating the acquisition or expression of ethanol-induced conditioned place preference in DBA/2J mice.

INTRODUCTION

Stress is thought to be a significant factor that contributes to substance abuse and addictive behavior (see reviews by Grunberg & Baum, 1985; Newcomb & Harlow, 1986; Marlatt & Baer, 1988; Pohorecky, 1990, 1991). In addition, individual variability in the response to stressors may result in a predisposition to abuse drugs (Piazza, Deminière, Maccari, Le Moal, Mormède, & Simon, 1991) . Since both stressors and abused drugs cause a rise in the endogenous hormone corticosterone, this may be an important factor in the development of addictive behavior. This thesis will focus on examining the potential role of corticosterone in mediating the positive motivational properties of one of the most commonly abused drugs, ethanol.

Stressors and the HPA Axis

The term "stress" is often used to describe both physical or psychological stimuli that confront an organism, and the physiological response of the organism to these stimuli. In order to clarify the meaning of stress, this thesis will use the term "stressor" to indicate the stimulus that disturbs the homeostasis of the organism, resulting in a stress response. The primary physiological stress response of the organism is activation of the hypothalamic-pituitary-adrenal (HPA) axis.

The HPA axis plays an important role in maintaining homeostatic balance within the body (see review by Munck & Náray-Fejes-Toth, 1995). Activation of the axis occurs when hypothalamic neurons are stimulated by physical or psychological stressors and synthesize corticotropin releasing factor (CRF) and vasopressin (VP). CRF is secreted and binds to cell membranes on the pituitary gland to stimulate synthesis and release of adrenal-corticotropin-releasing hormone (ACTH). VP also stimulates ACTH secretion, and CRF activity is strongly potentiated by VP (Bilezikjian & Vale, 1987). ACTH is released into the blood by the anterior pituitary to stimulate synthesis of corticosteroids from the adrenal cortex. In humans and monkeys, the principal adrenal corticosteroid is cortisol and in rodents it is corticosterone.

Corticosteroids exert negative feedback control on the hypothalamus and pituitary gland to suppress activation of the axis and further hormone release. This negative feedback loop is a protective mechanism against an overreaction of internal systems, such as the immune system, which could lead to injury if left unchecked. Corticosteroids are essential for homeostatic defense mechanisms and survival of the organism. The functions of corticosteroids have been described in terms of permissive and suppressive effects (Munck, Guyre, & Holbrook, 1984). The permissive actions serve to "prime" homeostatic mechanisms, such as gluconeogenesis, so they are ready to respond when challenged. The suppressive actions prevent the activated

defense mechanisms from overshooting and damaging the organism.

Most cells in the body are influenced by corticosteroids and almost all nucleated cells contain corticosteroid receptors (Munck & Náray-Fejes-Tóth, 1995). The widespread actions of corticosteroids throughout the body, both centrally and peripherally, are mediated via two types of intracellular receptor systems, mineralocorticoid (MR) and glucocorticoid (GR). In the rat brain, these receptors have been distinguished by their localization, binding affinities, and capacity for corticosterone (Reul & de Kloet, 1985).

MR are found to have a high affinity for corticosterone (Kd~0.5nM), six to ten-fold higher than GR, and are located primarily in the lateral septum and hippocampus. MR are thought to primarily control basal activity of the HPA axis throughout the circadian cycle. Approximately 80% of MR are already bound with basal circulating corticosterone levels, rising to >90% following exposure to a stressor or during the circadian peak. GR have a low affinity for corticosterone (Kd ~ 2.5-5 nM) and are widely distributed throughout the brain. The highest density of GR are found in the lateral septum, dentate gyrus, nucleus tractus solitarii, and central amygdala. Substantial amounts of GR are found in the paraventricular nucleus and locus coeruleus. Approximately 10% of GR are occupied with basal levels of CORT, rising to approximately 67-74% with stressor-induced plasma levels (~ 25 μg/100ml). Activation of GR suppress further stimulation of the axis, via negative feedback on CRF and ACTH synthesis (Reul & de Kloet, 1985). MR

have also been found to play a significant role in the feedback control of corticosteroid release (Ratka, Sutanto, Bloemers, & de Kloet, 1989). In addition, it is proposed that GR have inhibitory feedback influences on stressor-activated brain mechanisms (de Kloet & Reul, 1987).

Corticosteroid Action in the Brain

The classic mechanism of steroid action is via the activation of intracellular receptors (e.g., MR and GR) that modulate the transcription and expression of genes and synthesis of new proteins (for a review see Orchinik & McEwen, 1994). The time-course of steroid effects via this traditional genomic mechanism is relatively slow, ranging from hours to days. Recently, however, there have been an increasing number of studies describing rapid responses to steroids, suggesting that steroids also work through non-genomic mechanisms (Schumacher, 1990). These rapid effects of steroids have been shown to occur within minutes to alter neuronal processes and behavior. The mechanisms that mediate rapid steroid effects are still unclear. Because these effects are not altered by protein synthesis inhibitors, it is thought that rapid steroid effects are due to a direct interaction with cell membranes. For example, a cell surface receptor for corticosterone has been discovered that mediates inhibition of reproductive behavior in an amphibian (*Taricha granulosa*) within 5 min (Orchinik, Murray, & Moore, 1991). Steroids have also been shown to have rapid modulatory effects on ion channels, such as Cl- and Ca^{2+}, neurotransmitter receptor functioning, and

neurosecretion (see review by McEwen, 1991; Schumacher, 1990). In addition, it has been shown that rapid actions of certain steroids are due to an interaction between both genomic and non-genomic mechanisms. For example, the rapid facilitatory action of progesterone on female mating behavior in the rat is shown to be dependent on previous activation of the estrogen receptor (Kubli-Garfias & Whalen, 1977).

Many of the studies reviewed in this thesis suggest a rapid and possibly non-genomically mediated effect of corticosterone on the rewarding properties of several abused drugs. The rationale for the experiments conducted in this thesis was based on the idea that corticosterone may exert a rapid effect on the neural substrates mediating ethanol reward.

Corticosterone and Abused Drugs

Much interest has been generated regarding the contribution of the HPA axis and specifically, corticosterone in mediating the behavioral responses and hedonic properties of abused substances. Many abused drugs, such as amphetamine and ethanol, produce a rise in endogenous corticosterone levels in rodents (Swerdlow, Koob, Cador, Lorang, & Hauger, 1993; Tabakoff, Jaffe, & Ritzmann, 1978; Thiagarajan, Mefford, & Eskay, 1989), and several recent studies have demonstrated that corticosterone plays a role in modulating the effects of these drugs.

Corticosterone, Dopamine, and Drug-Stimulated Locomotor Activity

Many drugs of abuse share the property of producing locomotor stimulation. Drug-stimulated locomotor activity, as well as the rewarding properties of abused drugs, is thought to be primarily mediated via the mesolimbic dopaminergic pathway (for a review see Bozarth, 1991). Accordingly, locomotor behavior is frequently studied in order to investigate the neurobiological mechanisms mediating the rewarding and addictive properties of abused drugs.

Cador, Dulluc, and Mormède (1993) investigated the effect of modifications in circulating levels of corticosterone on the locomotor response to peripheral administration of *d*-amphetamine. Adrenalectomy was found to reduce the locomotor response, whereas pellets releasing physiological amounts of corticosterone restored a normal response to *d*-amphetamine. In contrast, pellets releasing high amounts of corticosterone, designed to mimic a chronic stress situation, potentiated the locomotor response to *d*-amphetamine.

The locomotor activating effect of *d*-amphetamine has been shown to depend on activation of the dopaminergic projection to the nucleus accumbens (Kelly, Seviour, & Iversen, 1975). Cador et al. (1993) also demonstrated that locomotor activation induced by central injection of *d*-amphetamine directly into the nucleus accumbens was attenuated by adrenalectomy and restored with pellets releasing constant amounts of

corticosterone. These effects may be mediated, in part, by the intracellular GR found in dopaminergic cell bodies of the ventral tegmental area which projects to the nucleus accumbens (Härfstrand, Fuxe, Cintra, Agnati, Zini, Wikström, Okret, Yu, Goldstein, Steinbusch, Verhofstad, & Gustafsson, 1986). Cools (1991) also found a significant reduction in the locomotor response to *d*-amphetamine with adrenalectomy, and administration of the selective GR agonist dexamethasone potentiated the response.

In addition, a rise in corticosterone levels and GR activation has been shown to be critical for behavioral sensitization to amphetamine (Rivet, Stinus, Le Moal, & Mormède, 1989; Cole, Cador, Stinus, Rivier, Rivier, Vale, Le Moal, & Koob, 1990). Repeated exogenous corticosterone administration has also been shown to potentiate the locomotor response to amphetamine (Deroche, Piazza, Maccari, Le Moal, & Simon, 1992). Recently, GR activation has been shown to play a critical role in locomotor sensitization to the stimulant effects of ethanol (Roberts, Lessov, & Phillips, 1995). Thus, these studies indicate that high corticosterone levels may selectively interact with the dopaminergic system via GR activation. However, the intracellular mechanisms affected by GR activation and ultimately altering dopaminergic reactivity are not known.

Several studies have demonstrated corticosterone-induced increases in dopamine turnover (e.g., Iuvone, Morasco, & Dunn, 1977; Wolkowitz, Sutton, Koulu, Labarca, Wilkinson, Doran, Hauger, Pickar, & Crawley, 1986)

and release. For example, Imperato, Puglisi-Allegra, Casolini, Zocchi, and Angelucci (1989) used brain dialysis techniques to measure dopamine release following restraint-stress in rats. Increases in dopamine release were found in the nucleus accumbens and prefrontal cortex areas of the dopaminergic system. Adrenalectomized rats did not show the same magnitude of dopamine release from these areas, and corticosterone administration was able to reverse the suppressive effects of adrenalectomy on dopamine release. Other studies, however, have shown stressor-induced changes in the dopaminergic system are independent of corticosterone release (Dunn, 1988; Imperato, Puglisi-Allegra, Casolini, & Angelucci, 1991).

Overall, these data suggest that the presence of corticosterone is necessary for the locomotor activation produced by *d*-amphetamine and that corticosterone may be mediating this effect by activation of the dopaminergic system. Furthermore, high levels of corticosterone appear to potentiate the effects of *d*-amphetamine and ethanol, possibly via a selective interaction of corticosterone with GR present in dopamine neurons or a non-genomic effect on dopamine release. Most of the studies to date have only examined a role for corticosterone in modulating the locomotor-stimulant effects of *d*-amphetamine. However, a similar relationship between corticosterone and ethanol-related behaviors would be predicted.

Corticosterone, Dopamine, and Reward

In addition to the locomotor-stimulant effects of drugs, the mesocorticolimbic dopamine system is suspected to play a primary role in mediating the rewarding effects of many psychoactive drugs, as well as other appetitive behaviors such as feeding and drinking, and is often termed the "reward pathway" (Bozarth, 1991). Numerous studies support a role for the dopaminergic system in the rewarding effects of many abused drugs (e.g., Wise & Bozarth, 1982). Di Chiara and Imperato (1988) used brain dialysis in freely moving rats and found that drugs of abuse (e.g., ethanol, amphetamine) increased extracellular dopamine levels in the nucleus accumbens. The place conditioning procedure has also supported the role of the dopamine system in the rewarding properties of many drugs. For example, amphetamine is found to produce a place preference that is blocked by systemic administration of haloperidol and reduced by 6-OHDA lesions of the nucleus accumbens (Spyraki, Fibiger, & Phillips, 1982). Since stressors and abused drugs have been found to activate the dopaminergic system, this system could be the common mechanism responsible for an increased sensitivity to the reinforcing effects of drugs following exposure to stressors. Furthermore, the rise in corticosterone levels produced by stressors and abused drugs may be an important factor in the development of addictive behavior (see review by Piazza, Deminière et al., 1991).

Stressors, Corticosterone, and Drug-Seeking Behavior

A number of studies have shown that previous exposure to stressors (including drugs of abuse) may predispose an organism to acquire drug-seeking behavior. For example, Piazza, Deminière, Le Moal, and Simon (1990) found that rats exposed to repeated tail-pinch or repeated amphetamine had a greater intake of amphetamine during self-administration relative to controls. Repeated amphetamine pre-exposure has also been shown to facilitate acquisition of cocaine self-administration (Horger, Giles, & Schenk, 1992; Valadez & Schenk, 1994). In addition, pre-exposure to other stimulants such as nicotine (Horger et al.), caffeine (Horger, Wellman, Morien, Davies, & Schenk, 1991), and cocaine (Horger, Shelton, & Schenk, 1990) reduced the latency to subsequent cocaine self-administration. Since all of the drugs used in these studies have been shown to stimulate the release of corticosterone (e.g., nicotine: Cam & Bassett, 1983; caffeine: Nicholson, 1989; cocaine: Moldow & Fischman, 1987), it may be that repeated exposure to corticosterone is one common mechanism by which pre-exposure to these drugs facilitates self-administration of amphetamine and cocaine. This interpretation is consistent with the findings that prior exposure to stressors (Antelman, Eichler, Black, & Kocan, 1980) and repeated corticosterone administration (Deroche et al., 1992) sensitize the locomotor response to amphetamine.

Chronic social stress conditions have also been shown to increase amphetamine self-administration in rats. Lemaire, Deminière, and

Mormède (1994) examined the effects of chronic stressors, such as social instability and cohabitation with females, on amphetamine self-administration in male rats. Interestingly, these conditions differentially affected amphetamine self-administration. Cohabitation with females significantly increased amphetamine intake relative to rats exposed to social instability (daily rotation of social group members). In addition, cohabitation with females has been shown to chronically increase HPA axis activity, whereas social instability does not (Mormède, Lemaire, Castanon, Dulluc, Laval, & Le Moal, 1990). Furthermore, Maccari, Piazza, Deminière, Lemaire, Mormède, Simon, Angelucci, and Le Moal (1991) report an association with decreased hippocampal MR number and increased duration of corticosterone secretion in rats with enhanced vulnerability to self-administer amphetamine. The central MR system plays an important role in feedback control of corticosterone release (Ratka, Sutanto, Bloemers, & de Kloet, 1989). Thus, the authors suggest that decreased MR resulting in prolonged corticosterone secretion may be the biological mechanism responsible for an increased susceptibility to self-administer amphetamine. Overall, these studies suggest that high circulating corticosterone levels due to chronic stress and/or a disruption in corticosteroid feedback mechanisms may result in greater self-administration of amphetamine.

Other studies have indicated that individual differences in HPA axis functioning and the adrenocortical response to novelty and environmental

stressors may be a significant factor in the susceptibility to develop drug-seeking behavior. Piazza, Maccari et al. (1991) found rats with a longer duration of corticosterone secretion in response to a novel environment showed an enhanced acquisition and maintenance of amphetamine self-administration. In addition, corticosterone administered to rats that did not show a prolonged corticosterone response facilitated acquisition of amphetamine self-administration in this group. Furthermore, Maccari, Piazza, Deminière, Angelucci, Simon, & Le Moal (1991) found the rats that showed a longer duration of corticosterone secretion in response to novelty and greater amphetamine self-administration (Piazza, Maccari et al.) also had lower hippocampal MR and GR affinities for corticosterone. It may be that a disruption in HPA feedback via MR or GR mechanisms results in higher circulating corticosterone levels and a greater propensity to abuse drugs.

A positive relationship between cocaine self-administration and stress-induced corticosterone levels has been shown. Goeders and Guerin (1994) trained rats to stably respond for food on a fixed-ratio 10 schedule and concurrently receive response-contingent electric footshock on a random-ratio 15 schedule. Another group of rats responded for food on the same schedule of reinforcement and received shock presentation that was yoked to lever responding by rats in the first group. A third group of rats responded on the same schedule for food but never received shock. Following this phase of the experiment, the authors demonstrated that non-contingent electric

Environmental stressors have also been shown to influence the psychomotor effects and self-administration of an opiate drug, morphine. Deroche, Piazza, Le Moal, and Simon (1994) demonstrated that social isolation enhanced morphine-stimulated locomotor activity in rats with an intact HPA axis compared to adrenalectomized rats implanted with pellets releasing a constant amount of corticosterone. Immobilization stress has been shown to significantly increase oral consumption and preference for a morphine solution in rats relative to a no stress group (Shaham, Alvares, Nespor, & Grunberg, 1992). These studies suggest that an increase in corticosterone levels induced by social isolation and immobilization stress may be responsible for an increased sensitivity to the locomotor and reinforcing effects of morphine.

In summary, a high level of corticosterone appears to facilitate self-administration of several abused drugs. Individual differences in the corticosterone response to stressors, such as a prolonged secretion of corticosterone, may predispose an animal to acquire drug-seeking behavior. Exposure to high corticosterone due to environmental stressors, including abused drugs, significantly increases self-administration of amphetamine, cocaine, and morphine. In addition, the control an animal has over a stressor has been shown to influence the magnitude of corticosterone release (Hennessy, King, McClure, & Levine, 1977; Goeders & Guerin, 1994), and appears to be important in the acquisition of cocaine self-administration.

Interestingly, a similar relationship has been demonstrated with unpredictable stressors and ethanol consumption (this will be discussed in the next section).

Stressors, Individual Differences, and Ethanol Reward

Human Alcohol Consumption

Stress, using the general term, has long been suspected to influence human alcohol consumption (Horton, 1943). Research on this issue has provided contradictory evidence regarding the effects of stressors on alcohol consumption, and how alcohol modulates the stress response (for a review see Pohorecky, 1991). Many studies have tried to identify the factors that may contribute to a predisposition to abuse alcohol, such as previous stressful experiences or an altered physiological response to stressors. Several hypotheses have been generated regarding the idea that alcohol may have "stress-relieving" properties, such as the Tension Reduction Hypothesis (TRH). This hypothesis states that alcohol is rewarding because it decreases an aversive internal state, and subsequently alcohol consumption becomes a learned response (Conger, 1956). Although conflicting evidence regarding the TRH has been provided, Cappell and Greely (1987) recently concluded that alcohol does reduce tension.

Alcohol itself may also be considered a stressor because it consistently produces an adrenocortical response in various species, including humans and rodents (Ellis, 1966; Thiagarajan et al, 1989). Individual differences in the physiological responses to stressors and alcohol have frequently been shown to influence human alcohol consumption (e.g., Schuckit, 1984; Schuckit, Gold, & Risch, 1987; Wand & Dobs, 1991). Most studies report a positive correlation between stressors and alcohol use. However, numerous factors such as age, gender, and individual response variability may interact to modify the consumption of alcohol (Pohorecky, 1991). Although factors contributing to human alcohol consumption are complex, animal models provide a means to examine the interactions of alcohol, specific environmental stressors, and individual differences.

Alcohol Consumption in Experimental Animals

Various interactions of ethanol and stressors have been examined in experimental animals (for a review see Pohorecky, 1990). In general, these studies have focused on the effects of ethanol in stressed subjects and the effect of stressors on ethanol intake. In addition, individual differences in the interaction of ethanol and stressors further act to modify the consumption of ethanol. For example, a recent study by Adams (1995) examined the effects of tail pinch on ethanol drinking in female and male Maudsley Reactive (RA) and Nonreactive (NRA) inbred rats. MRA rats have been selectively bred for

"stress" reactivity. Specifically, selection was based on defecation scores in an open field which is often considered a measure of anxiety or "emotionality". These rats have also been found to differ on many behavioral measures such as avoidance conditioning and response to shock (Broadhurst, 1975). Adams found a greater propensity in the RA rats to develop a preference for ethanol compared to NRA rats. However, this ethanol preference has been shown to be variable, suggesting the influence of environmental factors on ethanol consumption. In Adams' study, female and male RA rats consumed more ethanol than NRA rats, and females within each strain showed significantly higher ethanol preference relative to males. Tail pinch moderately increased ethanol preference in MR females and males, but did not affect ethanol preference in NRA rats. Thus, this study provides one example of the complex interactions between genetic and environmental factors in the individual response to ethanol and possibly the susceptibility to abuse ethanol. In addition, the results of this study suggest that a high reactivity to "stress" or anxiety, and possibly their physiological correlates, may be related to an increased sensitivity to the reinforcing effects of ethanol.

Previous exposure to stressors has been shown to influence ethanol consumption in rats. For example, Nash and Maickel (1985) showed a significant increase in ethanol consumption in rats following exposure to unpredictable isolation or immobilization stress relative to rats receiving no stress. Since unpredictable stressors have been shown to result in a higher

level of circulating corticosterone relative to a predictable stressor (Hennessy et al., 1977; Quirce, Odio, & Solano, 1981), exposure to high endogenous corticosterone levels may facilitate subsequent ethanol consumption. However, this increase in ethanol consumption did not occur until after the stressful stimuli were terminated. The authors suggest that the ethanol consumed post-stress may be a compensatory response to the reduction of circulating corticosterone levels because ethanol stimulates corticosterone release. Volpicelli, Ulm, & Hopson (1990) have also shown that rats increase their preference for ethanol following experience with inescapable footshock relative to unshocked rats. It has been suggested (see review by Volpicelli, 1987) that the post-stress increase in ethanol consumption is due to a compensatory opponent process, which involves an increase in endorphin release with uncontrollable shock followed by a deficiency in endorphins post-shock. According to this theory, ethanol consumption is more reinforcing following stress because it stimulates endorphin release and compensates for the deficiency.

A positive relationship between prior exposure to stressors and subsequent ethanol consumption has also been demonstrated in rhesus monkeys (Higley, Hasert, Suomi, & Linnoila, 1991). Monkeys were reared for the first 6 months of life by either their mother or a peer social group and later subjected to social separation. The monkeys reared by their peers showed an increase in fear-related behaviors, higher levels of circulating

cortisol, and consumed significantly more ethanol than mother-reared subjects. Furthermore, ethanol consumption increased in mother-reared subjects following exposure to social separation. Overall ethanol consumption during the separation was positively correlated with distress behaviors and peak plasma cortisol levels in both groups, suggesting a relationship between individual differences in reactivity to stressors and ethanol consumption.

In general, these findings are consistent with studies showing prior exposure to stressors facilitates self-administration of certain drugs of abuse (e.g., Piazza et al., 1990), and suggest that corticosterone may be a common physiological mechanism by which stressors modulate the reinforcing effects of ethanol.

Corticosterone, Dopamine, and Ethanol Reward

The mechanism by which prior exposure to stressors acts to facilitate ethanol drinking in rats and monkeys is not clear. It has been reported that various stressors can activate central dopaminergic systems in rodents (Thierry, Tassin, Blanc, & Glowinski, 1976; Wolkowitz et al., 1986), and certain regions of the system seem to be preferentially activated in response to specific stressors (Roth, Tam, Ida, Yang, & Deutch, 1988). Systemic ethanol administration has been shown to increase the firing rate of dopaminergic neurons of the VTA and substantia nigra (Gessa, Muntoni, Collu, Vargiu, &

Mereu, 1985; Koob & Bloom, 1988) and increase dopamine release in the nucleus accumbens (Di Chiara & Imperato, 1988). As previously suggested with the stimulant drugs, it is possible that corticosterone is sensitizing the same neural substrates that mediate the reinforcing effects of ethanol. For example, corticosterone may increase the reinforcing effects of ethanol by facilitating dopamine transmission (Imperato et al., 1989) via cytosolic GR found in dopamine cell bodies (Markey, Towle, & Sze, 1982). Several studies provide evidence for a direct relationship between GR activation and increased dopamine levels (e.g., Hall & McGinley, 1982). For example, the selective GR agonist, dexamethasone, increases dopamine levels in rat hypothalamus and nucleus accumbens (Rothschild, Schatzberg, Langlais, Cole, & Bird, 1983). In addition, GR are abundantly found in limbic areas of the rat brain, such as the catecholamine-producing locus coeruleus (Reul & de Kloet, 1985). There is evidence that the locus coeruleus can influence the activity of dopamine neurons in the ventral tegmental area and substantia nigra (Grenhoff, Nisell, Ferre, Aston-Jones, & Svensson, 1993), which suggests the locus coeruleus may be a substrate important in mediating drug reward.

The removal of endogenous corticosterone has been found to reduce ethanol consumption in certain rats. Fahlke, Engel, Eriksson, Hård, and Söderpalm (1994a) found adrenalectomy significantly reduced consumption of a 6% ethanol solution in Wistar rats with high preference for ethanol. However, ethanol intake in rats with low ethanol preference was not affected

by adrenalectomy. Corticosterone administered in both fluid bottles restored endogenous corticosterone to a similar level in both high and low ethanol preference groups. Treatment with corticosterone reestablished ethanol intake to the preoperative level in the high-preference rats, but not in the low-preference rats, indicating that the variation in ethanol intake in this strain of rats may not be entirely related to variations in endogenous corticosterone levels. The effect on ethanol intake in the high-preference rats appears to be a specific action of corticosterone because treatment with aldosterone had no effect on ethanol consumption. Furthermore, these authors report the same pattern of results in the high- and low-preference rats treated with the 11β-hydroxylase inhibitor, metyrapone, which blocks the synthesis of corticosterone (Fahlke, Hård, Thomasson, Engel, & Hansen, 1994b).

The finding that adrenalectomy does not affect ethanol consumption in low-preference rats is consistent with an early study by Zarrow, Aduss, and Denison (1960). They report that adrenalectomy did not affect ethanol consumption in rats that did not previously prefer ethanol to water in a choice situation. In the same experiment, adrenally intact rats exposed to chronic severe cold stress showed significantly greater preference for ethanol, suggesting a positive influence of stressor-induced corticosterone levels on ethanol consumption. Other studies have shown a decrease in ethanol consumption following adrenalectomy in male (Mardones, 1960) and female

rats (Morin & Forger, 1982). In the latter study, daily corticosterone injections increased ethanol preference relative to vehicle-injected sham controls.

In general, these studies indicate that corticosterone modulates ethanol consumption in certain rats and stress-induced corticosterone levels potentiate ethanol consumption. The reason for the individual differences in ethanol consumption in high and low-preference rats is unknown. Fahlke et al. (1994a) found no correlation between endogenous corticosterone levels and ethanol intake in sham treated animals. However, it has been shown that the high-preference rats release more dopamine in the nucleus accumbens following ethanol relative to low-preference rats (Engel, Enerbäck, Fahlke, Hulthe, Hård, Johannessen, Svensson, & Söderpalm, 1992). A recent study by Fahlke, Hård, Eriksson, Engel, & Hansen (1995a) found that the high-preference rats show significantly greater amphetamine-stimulated locomotor activity relative to low-preference rats. In addition, corticosterone levels following amphetamine were higher in the high-preference rats compared to the low-preference rats. These studies implicate the mesolimbic dopamine system as the common neural substrate mediating the reinforcing properties of ethanol and the locomotor response to amphetamine. The differential reactivity of the mesolimbic dopamine system may also be influenced by the corticosterone response to these drugs.

Genetics may mediate individual differences in circulating corticosterone levels and the responsiveness of the HPA axis to stressors and

ethanol. For example, Pohorecky (1984) found significant differences between nine different strains of rats in plasma corticosterone levels following various doses of ethanol. In rats, individual differences in levels of circulating corticosterone levels have also been shown to influence ethanol consumption. Prasad and Prasad (1995) demonstrated voluntary ethanol consumption to be correlated with endogenous corticosterone levels and the corticosterone response to stress. Rats with high basal corticosterone levels and an attenuated stressor-induced rise in corticosterone were found to have a greater preference for ethanol relative to rats with low basal corticosterone levels and high stress-induced corticosterone levels. In recombinant inbred mice, a genetic association between the corticosterone response to ethanol and ethanol-related behaviors has been demonstrated (Roberts, Phillips, Belknap, Finn, & Keith, 1995). For example, similar to the finding of Prasad and Prasad (1995), mice that displayed a large corticosterone response to ethanol also showed less preference for ethanol. This relationship appears to be inconsistent with the previous findings that suggest a positive relationship between corticosterone levels and ethanol consumption.

In summary, these studies suggest that corticosterone plays a significant role in modulating the reinforcing properties of ethanol consumption. However, unlike psychostimulants, the relationship between endogenous corticosterone levels and ethanol self-administration is less clear. In general, previous exposure to environmental stressors and high levels of

corticosterone appear to facilitate subsequent ethanol drinking. In addition, individual differences in the adrenocortical response to stressors, including ethanol, may differentially affect neural substrates mediating ethanol reward.

Disadvantages of Ethanol Consumption as a Measure of Ethanol Reward

A significant amount of evidence has implicated corticosterone in mediating the rewarding properties of psychostimulants. Overall, these studies suggest that the rewarding aspects of these drugs, assessed mostly by self-administration paradigms, are enhanced with a high level of circulating corticosterone. In the case of ethanol, however, relatively few studies have examined specific manipulations of endogenous corticosterone levels on ethanol reward-related behaviors. Most of the studies to date have examined the effects of stressors or corticosterone manipulation on ethanol drinking. Similar to psychostimulants, it appears that high corticosterone levels increase ethanol's rewarding properties, as determined by an increase in ethanol self-administration. However, this conclusion is limited to ethanol drinking behavior, and corticosterone may be acting on a mechanism that is specifically affecting the consumption of ethanol. For example, corticosterone may alter taste reactivity to ethanol. Indeed, adrenal corticosteroids have been shown to alter sensory processes such as taste reactivity (Henkin, 1975). Corticosteroids have been shown to decrease conditioned taste aversion produced by drugs (Hennessy, Smotherman, & Levine, 1976; Revusky &

Martin, 1988). Adrenalectomy has also been shown to reduce the consumption of sweet-tasting fluids like sucrose (Seidenstadt & Eaton, 1978) and saccharin (Silva, 1977). These studies illustrate a potential problem in interpreting the effects of corticosterone on ethanol's rewarding properties in that corticosterone may be affecting mechanisms involved in consummatory behavior rather that affecting a mechanism mediating ethanol reward.

Advantages of Conditioned Place Preference as a Measure of Drug Reward

The experiments discussed in this thesis use the place conditioning paradigm to examine the rewarding effects of ethanol. This paradigm is a Pavlovian conditioning procedure that is frequently used to assess the rewarding properties of many drugs (Bozarth, 1987). The place conditioning procedure involves pairing a distinctive environmental stimulus, termed the conditioned stimulus or CS, with the administration of a drug, called the unconditioned stimulus or US. With repeated pairings, a learned association is made between the stimulus properties of the CS and physiological and/or behavioral effects produced by the US. In the absence of the drug, presentation of the CS elicits an conditioned response or CR. In place conditioning, the CR of interest is approach or avoidance behavior to the drug-paired environment. The ability of the drug-paired CS to elicit approach or withdrawal behavior provides information about the drug's affective

properties. A drug produces a place preference and is determined to have rewarding motivational properties if an animal spends more time in the environment previously paired with it. Alternatively, if an animal spends more time in the vehicle-paired environment the drug is said to be aversive and a place aversion has developed.

There are several advantages of this procedure compared to other paradigms, such as self-administration paradigms, that are used to determine the rewarding effects of a drug. For example, testing can be conducted under drug-free conditions. This is important if a drug's pharmacological effects may interfere with the measurement of its rewarding properties (e.g., motor effects that impair responding). The dose of a drug is also controlled by the experimenter rather than the subject, which allows for a more precise assessment of a drug's positive motivational effects and avoids between and within-group dose variability. An advantage of the place conditioning paradigm over the oral self-administration paradigm is that it does not involve ingestive behavior. Thus, it avoids interpretive problems regarding the possible non-specific effect of an agonist or antagonist on consummatory behavior, rather than a selective effect on drug reinforcement or reward. Another advantage of the paradigm is that it can be used to measure both rewarding and aversive drug effects. In this regard, it is also useful for assessing the effect of drugs that may increase or decrease the magnitude of

place conditioning, and these drugs can be assessed independently for their own affective properties as a measure of control.

Ethanol and Conditioned Place Preference

Conditioned place preferences have been observed with many abused drugs such as amphetamine, heroin, and cocaine (Carr, Fibiger, & Phillips, 1989). Place conditioning with ethanol, however, has provided varied results. For example, in rats, ethanol generally produces a place aversion (Cunningham, 1981; van der Kooy, O'Shaughnessy, Mucha, & Kalant, 1983; Stewart & Grupp, 1986) although there are a few reports of place preference (e.g. Bozarth, 1990). In contrast, several inbred and selectively bred lines of mice have shown a reliable and robust place preference for the environment paired with ethanol (e.g., Cunningham, Hallett, Niehus, Hunter, Nouth, & Risinger, 1991; Cunningham, Niehus, Malott, & Prather, 1992). Thus, in mice, the place conditioning procedure appears to be a useful tool to study the rewarding properties of ethanol.

Overall Hypothesis

The following experiments were designed to test the hypothesis that high endogenous corticosterone levels are involved in modulating ethanol's rewarding properties in mice. These experiments examined the effects of various levels of endogenous corticosterone in modulating ethanol-induced

conditioned place preference. The subjects used in the current experiments are an inbred strain of mice (DBA/2J). These mice were chosen because they consistently display a robust conditioned place preference with ethanol (e.g., Cunningham, Niehus, & Noble, 1993; Risinger, Dickinson, & Cunningham, 1992a; Risinger, Malott, Riley, & Cunningham, 1992b). In addition, inbred mice are all genetically identical. Thus, in controlled environmental conditions, behavioral differences between groups within an experiment and between experiments can be attributed mostly to the independent variable (e.g., administration of a drug) and not to genotype. However, a disadvantage in using an inbred strain is that generalization of the obtained results to the mouse species as a whole may be limited. For Experiment 1, various doses of corticosterone were administered during the acquisition of ethanol-induced place preference. Experiment 2 examined the effect of reducing endogenous corticosterone, via the steroid synthesis inhibitor aminoglutethimide (AMG), on the acquisition of place preference. Experiments 3 and 4 assessed the effect of AMG administered prior to the expression of ethanol place preference. Specific predictions for each experiment will be discussed below.

Experiment 1

Effects of Corticosterone on Acquisition of Conditioned Place Preference

Several studies have shown acute effects of corticosterone on drug-related behaviors. For example, Piazza, Maccari et al. (1991) found that infusion of corticosterone 10 min prior to a self-administration session with amphetamine facilitated self-administration in rats that previously did not acquire or maintain amphetamine self-administration. In a different experiment, corticosterone administered simultaneously with amphetamine increased self-administration in low-response rats to a level comparable to high responders.

Fahlke et al. (1994b) demonstrated a significant decrease in ethanol consumption within 6 hrs of administration of the corticosterone synthesis inhibitor, metyrapone. This decrease in consumption was partially counteracted when corticosterone was administered 2 hrs before metyrapone. Furthermore, when metyrapone was omitted from the preinjection treatment, ethanol preference and consumption immediately returned to baseline levels. More recently, Fahlke, Hård, Eriksson, Engel, and Hansen (1995b) investigated the effects of chronic treatment (3 weeks) with corticosterone or the GR agonist dexamethasone on ethanol consumption in adrenalectomized and adrenally intact rats. Subcutaneous corticosterone pellets increased ethanol consumption relative to baseline levels in both

adrenalectomized and adrenally intact rats. It was also found that chronic corticosterone treatment potentiated ethanol drinking in adrenally intact rats with low ethanol preference. In addition, Fahlke et al. (1995b) tested the effects of MR (RU 28318) and GR (RU 38486) antagonists on ethanol drinking in these rats. RU 28318 and RU 38486 administered alone or in combination did not alter ethanol consumption, suggesting that corticosterone's facilitatory actions may be mediated via a membrane mechanism rather than intracellular binding to MR or GR. Thus, these data suggest a relatively rapid, and possibly non-genomically mediated effect of corticosterone on the reinforcing effects of amphetamine and ethanol.

The purpose of Experiment 1 was to examine the effect of corticosterone administration on the acquisition of ethanol-induced conditioned place preference, by administering corticosterone prior to conditioning sessions with ethanol. Various doses of corticosterone were chosen in an attempt to show a dose-response relationship for this effect. It was hypothesized that high corticosterone levels prior to the conditioning session would potentiate the positive effects of ethanol during conditioning and facilitate acquisition of place preference. This hypothesis was based on the above studies that show corticosterone administration facilitates self-administration of amphetamine and ethanol, presumably by increasing their positive motivational properties. Specifically, Piazza, Maccari et al. (1991) demonstrated the rapid facilitatory influence of exogenous corticosterone on

amphetamine self-administration in rats "resistant" to developing self-administration behavior. The prediction for Experiment 1 was that mice receiving the highest dose of corticosterone should subsequently display the largest magnitude of place preference. This prediction was based on the idea that corticosterone is exerting a rapid effect during conditioning on the neural substrate mediating ethanol reward.

Method

Subjects

Subjects were adult male inbred mice (DBA/2J) obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were housed in polycarbonate cages (27.9 X 9.5 X 12.7 cm) in groups of four with corn cob bedding in a Thoren Rack. Animals were allowed free access to food and water and allowed to acclimate to the colony room for 12-14 days before training. Mice were between 54-60 days old on the first day of training (habituation). Ambient temperature was maintained at 21± 1° C. Experimental procedures were conducted during the light phase of a 12:12 light/dark cycle (lights on at 0700).

Apparatus

Twelve identical acrylic and aluminum boxes (30 X 15 X 15 cm) were separately enclosed in ventilated, light and sound-attenuating chambers

(Coulbourn Model E10-20). Six sets of infrared light sources and photodetectors were mounted opposite each other at 5-cm intervals along the length of each box, 2.2 cm above the floor. Occlusion of the infrared light beams was used as a measure of general activity and location of the animal (left or right) within the box. Total activity counts were recorded every minute by an Apple II microcomputer (10 msec resolution). The floor of each box consisted of interchangeable halves of one of two distinct textures. "Grid" floors consisted of 3.18 mm rods mounted 6.4 mm apart in acrylic rails. "Hole" floors consisted of perforated 16 gauge stainless steel with 6.4 mm round holes on 9.5 mm staggered centers. This combination of floor textures was selected on the basis of previous studies showing that drug-naive mice spend approximately equal time on each floor type during drug-free preference tests (Cunningham, 1995; Cunningham et al., 1992; Cunningham & Noble, 1992). The floors and the inside of the boxes were wiped with a damp sponge and the litter paper beneath the floors was changed between animals.

Ethanol Place Conditioning

Ethanol (20% v/v) was prepared from a 95% stock solution using saline as the vehicle. All subjects were randomly assigned to one of two conditioning groups and exposed to a Pavlovian differential conditioning procedure. During the conditioning trials, all mice had access to both sides of

the apparatus on a homogeneous floor type. On alternating days, mice in the G+ group received an IP injection of ethanol (2 g/kg) immediately prior to a 5 min session on the grid floor (CS+ sessions). These mice received saline on intervening days paired with the hole floor (CS- sessions). Conversely, mice in the G- group received ethanol paired with the hole floor and saline paired with the grid floor. Conditioning groups were matched for overall exposure to CS type and drug treatment, and the order of exposure to ethanol and saline was counterbalanced within groups. The 5 min session duration was chosen based on previous studies showing that it produced a stronger conditioned place preference with ethanol than did other session durations (Cunningham & Prather, 1992).

Procedure

Experiment 1 involved one habituation session, eight conditioning sessions, and one test session. A 2-day break separated the first four and the last four conditioning sessions. For the habituation session, mice received an injection of saline (12.5 ml/kg) immediately before being placed in the conditioning box for 5 min on a smooth paper floor.

For conditioning, mice were randomly assigned to one of four corticosterone (CORT) dose groups: 0 mg/kg (n = 20), 1 mg/kg (n = 24), 5 mg/kg (n = 24), and 10 mg/kg (n = 22). Within each of the experimental groups, mice were randomly assigned to one of the two ethanol conditioning

subgroups (G+ or G-) and subjected to standard ethanol conditioning procedures, as described previously. CORT (Sigma) was dissolved in a 20% w/v solution of β-cyclodextrin and saline. All subjects received an IP injection of vehicle (0 group) or CORT (10 ml/kg) 45 min before the CS+ conditioning session. This pretreatment interval was chosen in order to mimic a stressor-induced physiological level of CORT prior to the conditioning session (Kakihana, Noble, & Butte, 1968; Natelson, Tapp, Adamus, Miller, & Levin, 1981; Piazza, Maccari et al., 1991).

For the test session, all mice received an injection of β-cyclodextrin vehicle 45 min before the 60 min preference test. A saline injection was given immediately prior to placement in the apparatus to match the cues during conditioning days. The floor of each box was half grid and half hole with left/right position counterbalanced within groups.

Statistical Analyses

For all experiments, data were analyzed by analysis of variance (ANOVA) with the alpha level set at 0.05.

Conditioning Data. Three-way ANOVAs were conducted independently for CS+ and CS- session data, with CORT Dose as a between group factor (CORT Dose groups were collapsed across G+ and G- subgroups) and Trials and Minutes as within group factors. The purpose of this was to separately investigate corticosterone effects on ethanol-stimulated locomotor

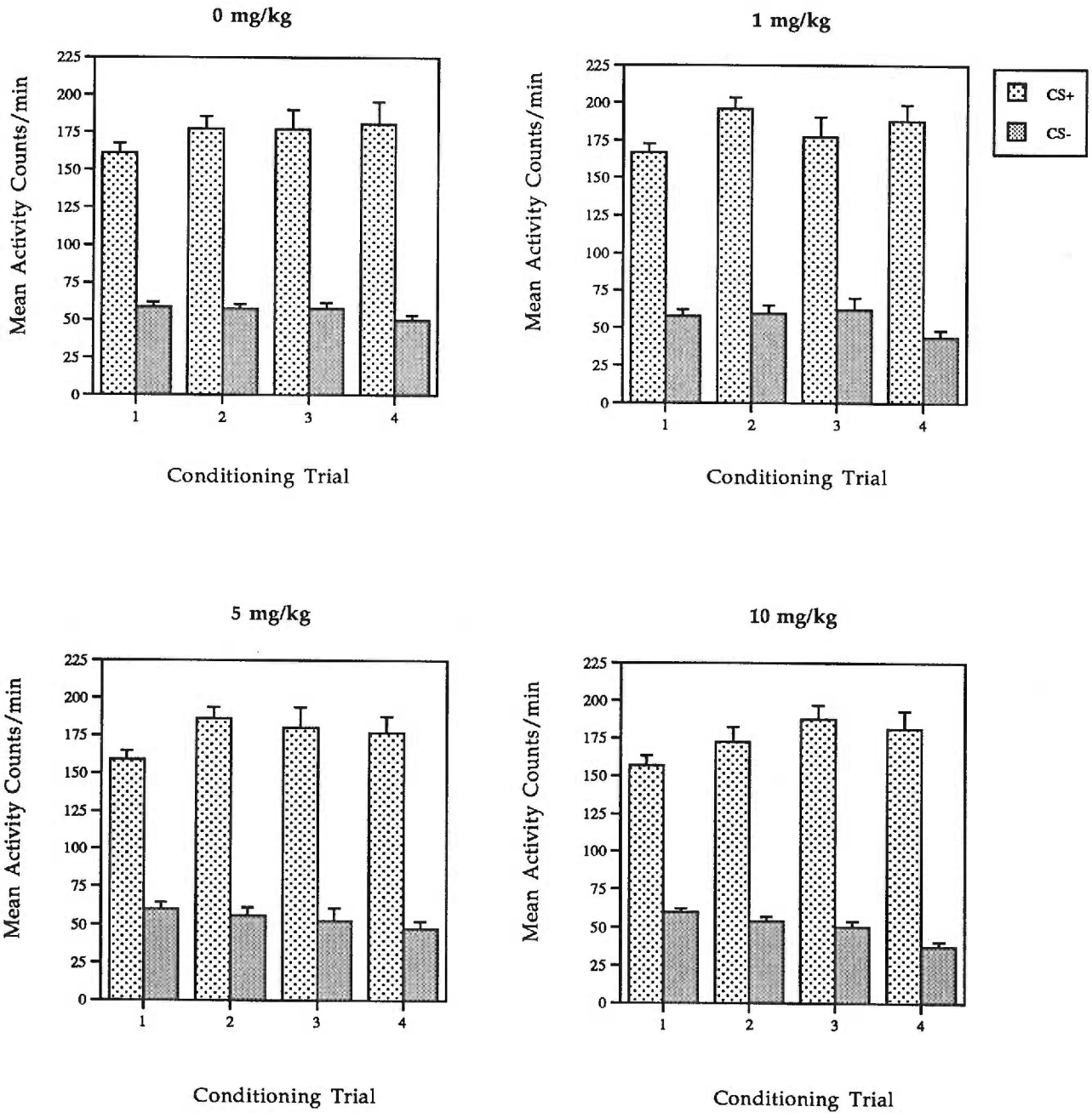
activity during the 5 min conditioning sessions, potential sensitization across trials, and possible effects of corticosterone on saline activity or habituation across trials.

Preference Test Data. Two-way ANOVA was conducted with CORT Dose and Conditioning Group (G+ and G-) as between group factors. The data were collapsed across minutes since the amount of time spent on the grid floor was nearly constant throughout the 60 min test session. One-way ANOVA was conducted for activity data during the test session with CORT Dose as the between group factor.

Results

Conditioning. Figure 1 shows the mean (±sem) activity counts per min collapsed across conditioning subgroup (G+ and G-) for all four CORT dose groups during conditioning trials 1-4 (trial = CS+ session and CS- session). Ethanol produced significant locomotor activation during the CS+ sessions relative to the CS- sessions with saline. As previously observed with DBA/2J mice (e.g., Cunningham & Noble, 1992; Cunningham & Prather, 1992), higher activity counts were observed on the last CS+ session compared to the first CS+ session, indicating that sensitization to the locomotor-activating effects of ethanol occurred across the four trials. Corticosterone did not affect ethanol-stimulated locomotor activity at any dose. Mean (±sem) activity counts per min averaged for all four CORT dose groups were 160.9±3.6 on the

Figure 1. Activity data for all four CORT dose groups during conditioning trials 1-4. Values are mean (±sem) activity counts per min during CS+ and CS- sessions.


0 mg/kg
1 mg/kg
5 mg/kg
10 mg/kg
Mean Activity Counts/min
Conditioning Trial
CS+
CS-
0
25
50
75
100
125
150
175
200
225
1
2
3
4

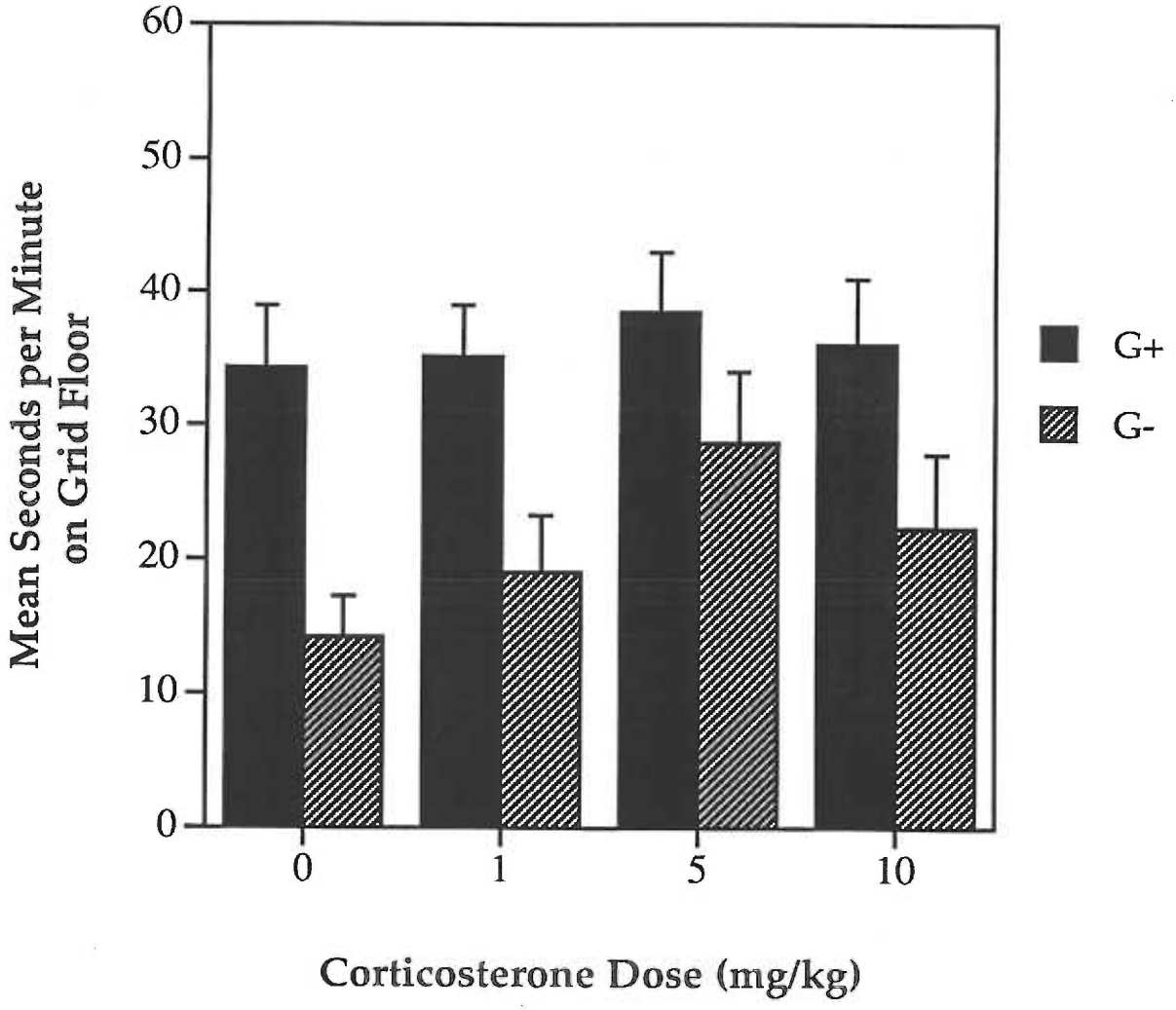
first CS+ session and 183.4±5.7 on the fourth CS+ session. Mean activity counts were 59.5±1.7 and 44.6±2.1 during the first and fourth CS- session, respectively, indicating that mice habituated to experimental procedures.

Three-way ANOVAs (CORT Dose X Trials X Minutes) were separately conducted for CS+ and CS- session data. The CS+ ANOVA yielded significant main effects of Trials [$F(3, 249) = 9.8$, $p < 0.0001$] and Minutes [$F(4,332) = 435.5$, $p < 0.0001$] and a Trials X Minutes interaction [$F(12,996) = 8.3$, $p < 0.0001$]. The CS- ANOVA also showed significant effects of Trials [$F(3,249) = 13.9$, $p < 0.0001$], Minutes [$F(4,332) = 86.7$, $p < 0.0001$], and a Trials X Minutes interaction [$F(12,996) = 3.0$, $p < 0.001$].

Preference Testing. Figure 2 shows the mean (±sem) sec per min spent on the grid floor by both conditioning subgroups of each CORT dose group during the preference test. The amount of time spent on the grid floor by both the G+ and G- subgroups was nearly constant throughout the test session, therefore, the data shown in Figure 2 are collapsed across the 60 min session. Mice in the G+ conditioning subgroup of each CORT dose group spent more time on the ethanol-paired grid floor than the G- subgroup, indicating a conditioned place preference for the grid floor. The magnitude of place preference (demonstrated by G+ vs G- differences) did not differ, suggesting that CORT did not affect the development of place preference at any dose. Overall analysis of the data collapsed across the 60 min test session (two-way ANOVA: CORT Dose X Conditioning Group) yielded a significant

Figure 2. Mean (±sem) sec per min spent on the grid floor by both conditioning subgroups of the four CORT dose groups during the preference test. During conditioning, G+ subjects received an injection of β-cyclodextrin vehicle or CORT (1, 5, or 10 mg/kg) 45 min before an injection of ethanol paired with the grid floor and vehicle preinjection and saline paired with the hole floor. Conversely, G- subjects received vehicle or CORT preinjection and ethanol paired with the hole floor and vehicle preinjection and saline paired with the grid floor. Data shown are collapsed across the 60 min session.

Experiment 1: Preference Test



effect of Conditioning Group [$F(1, 82) = 20.9$, $p < 0.0001$]. No effect of CORT Dose or CORT Dose X Conditioning Group interaction was found, confirming that CORT treatment did not alter the magnitude of place preference expressed.

Mean (±sem) activity counts per min during the 60 min test were 24.6±1.9, 22.3±1.5, 23.1±1.6, and 18.8±1.5 for the 0, 1, 5, and 10 mg/kg CORT groups, respectively. One-way ANOVA showed no effect of previous CORT Dose on activity levels during the test [$F(3,86) = 2.3$, $p > 0.07$].

Discussion

Corticosterone administration prior to conditioning trials did not enhance the magnitude of place preference at any dose. These data indicate that administration of corticosterone does not facilitate the acquisition of ethanol-induced place preference. This does not support the hypothesis that high corticosterone levels increase the rewarding properties of ethanol and produce a greater magnitude of place preference.

Prior to Experiment 1, Chester et al. (unpublished results) examined the possible rewarding properties of corticosterone in the place conditioning paradigm. The same doses of corticosterone (1, 5, and 10 mg/kg) were administered i. p. 45 min before a 15 min conditioning session. Corticosterone did not produce place conditioning with the 1 or 10 mg/kg dose, however, significant place preference was observed with the 5 mg/kg

dose. However, relative to 2 g/kg ethanol, corticosterone produced a smaller magnitude of place preference. Thus, the results of this study suggest that corticosterone may have rewarding properties in the place conditioning paradigm at the 5 mg/kg dose. In the present study, however, the 5 mg/kg of corticosterone did not alter the magnitude of ethanol-induced place preference. It may be that the rewarding effects of ethanol are stronger than the rewarding properties of corticosterone. This could result in ethanol's stimulus properties overshadowing the stimulus properties of corticosterone, so that ethanol is the only salient unconditioned stimulus during acquisition trials.

One limitation of the present study is that corticosterone levels following the conditioning session with ethanol were not determined. It may be difficult to interpret the effect of corticosterone treatment when the basal level of corticosterone in the vehicle pretreated group (0 mg/kg CORT) is unknown. The purpose of the present experiment was to compare the normal magnitude of ethanol-induced place preference in mice that presumably have low or "basal" corticosterone levels (0 mg/kg group) prior to conditioning sessions with the magnitude of ethanol-induced preference in mice that have various "stress-induced" levels of corticosterone (1, 5, and 10 mg/kg) prior to conditioning sessions. It is possible that corticosterone in the 0 mg/kg group was elevated due to experimental procedures, so that corticosterone pretreatment did not appear to alter the acquisition of place

preference because a stress-induced level of corticosterone was achieved in the 0 mg/kg group. There may be a certain concentration of corticosterone that facilitates ethanol conditioning and once this concentration is reached, excess corticosterone may not further potentiate the effects of ethanol.

Although plasma corticosterone was not measured, the doses of corticosterone that were administered were intended to produce various physiological levels of corticosterone within a range that is produced by stressors. Several studies have shown that similar doses of corticosterone produce plasma levels within a stress-induced range. For example, Deroche et al. (1992) demonstrated a stress-induced level of corticosterone (~18 μg/dl) in rats 30 min following an i.p. injection of 1.5 mg/kg corticosterone. This level of corticosterone was comparable to plasma levels 30 min following exposure to novelty stress. A dose of 5 mg/kg corticosterone administered s. c. has been shown to produce approximately twice the level of corticosterone (39.1 μg/dl) induced by novelty stress (Mitchell & Meaney, 1991), and is comparable to the level of corticosterone produced by 30 min restraint stress (~40 μg/dl) (e.g., Piazza, Deroche, Deminière, Maccari, Le Moal, & Simon, 1993). A longer period of restraint stress (e.g., 1-2 hr) produces higher levels of corticosterone in rats, within a range of 45-75 μg/dl (e.g., Kvetnansky, Weise, Thoa, & Kopin, 1979; Haleem, Kennett, & Curzon, 1988). However, there may be a species difference in the corticosterone response to restraint stress, since

Roberts et al. (1995) found much lower corticosterone levels (~ 15 µg/dl) in DBA/2J female mice following a 2 hr restraint period. In rats, a s. c. injection of 10 mg/kg corticosterone has been shown to produce a plasma level of approximately 45 µg/dl corticosterone measured 1 hr following the injection (e.g., Ratka, Sutanto, & de Kloet, 1988).

In addition to producing elevated plasma concentrations of corticosterone, the doses of corticosterone used in the present study have also been shown to rapidly influence various types of behavior. For example, a significant increase in locomotor behaviors (e.g., horizontal activity) in a novel environment has been demonstrated in rats that were administered a dose of 5 mg/kg corticosterone 15 min before testing relative to saline pretreated rats (Sandi & Guaza, 1994). In the present study, there were no effects of corticosterone pretreatment on activity levels. However, since ethanol produces high levels of stimulated activity in DBA/2J mice, any locomotor-stimulant effects of corticosterone may have been masked by the significant activation produced by ethanol.

A dose of 10 mg/kg corticosterone has been shown to produce a rapid effect on behavior in mice. For example, Sze (1993) demonstrated that an acute dose of corticosterone (10 mg/kg) administered 15 min before an injection of ethanol (3 g/kg) antagonized ethanol's sedative effect as measured by a significant decrease in sleep time. Overall, the doses of corticosterone used in the present experiment (1, 5, and 10 mg/kg) are within

a range shown to rapidly influence various behaviors. However, corticosterone at these doses did not significantly enhance the rewarding effects of ethanol by altering the acquisition of place preference.

Experiment 2

Effects of AMG on Acquisition of Conditioned Place Preference

The results of Experiment 1 suggest that stressor-induced levels of corticosterone prior to conditioning sessions do not enhance the rewarding effects of ethanol. The present experiment examined the possibility that the immediate rise in corticosterone produced by acute ethanol administration (Kakihana, et al. 1968; Thiagarajan et al., 1989) is an important factor modulating the acquisition of place preference. Similar to Experiment 1, it was hypothesized that a stressor-induced level of corticosterone may facilitate the acquisition of ethanol-induced place preference. However, Experiment 2 specifically focused on the acute corticosterone-elevating effects of ethanol, as well as handling and injection procedures, as an important factor modulating ethanol's rewarding effects during conditioning. This acute rise in corticosterone may be a critical event that augments ethanol's effects. This rapid facilitatory effect of corticosterone might also explain why administration of exogenous corticosterone in Experiment 1 did not further facilitate ethanol place conditioning.

The purpose of Experiment 2A was to use a different strategy to manipulate corticosterone and investigate its role in the acquisition of ethanol-induced place preference. Specifically, Experiment 2A examined the effects of inhibition of corticosterone release during ethanol conditioning

trials on the acquisition of ethanol place preference. Aminoglutethimide (AMG), a steroid synthesis inhibitor, was used to provide a stable, low level of corticosterone in order to examine the effects of corticosterone release during conditioning. Because AMG prevents the synthesis and release of corticosterone (Dexter, Fishman, Ney, & Liddle, 1967; Roberts, Gallaher, & Keith, 1993), this provides more control over corticosterone levels during conditioning compared to exogenous corticosterone administration in Experiment 1. It was hypothesized that the normal rise in corticosterone levels in response to ethanol injections is important in modulating ethanol's rewarding effects and facilitates the acquisition of conditioned place preference.

There are several ways in which corticosterone could rapidly influence the rewarding properties of ethanol during place conditioning. For example, corticosterone has been shown to alter the functioning of many neurotransmitter systems (see review by Hall, 1982; McEwen, 1991), and may enhance the rewarding properties of ethanol by facilitating the release of neurotransmitters such as dopamine (e.g., Imperato et al, 1989) that may be involved in ethanol reward. Gilad, Rabey, and Gilad (1987) demonstrated *in vitro* that high glucocorticoid concentrations may increase synaptic dopamine concentrations by directly acting on presynaptic dopamine terminals to reduce dopamine re-uptake. Rapid actions of corticosterone may also be mediated via binding to corticosterone membrane receptors. Furthermore, the

receptors that have been localized to the surface of neuronal cells in the amphibian, *Taricha granulosa* are known to be G-protein coupled (see review by Moore & Orchinik, 1994). There is also evidence that corticosterone may exert actions on the neural membrane conductance of various ions via a non-receptor-mediated action (Hall, 1982; McEwen, 1991).

In addition, corticosterone may be important for the acquisition of place preference via activation of a genomically-mediated mechanism. For example, the acquisition of place preference over trials may require the synthesis of new proteins that might be produced by repeated GR activation across conditioning sessions. Indeed, it has been shown that acquisition of ethanol-induced place preference requires at least 3 CS-US pairings (Cunningham et al., unpublished observations). Futhermore, it has been shown that rapid actions of certain steroids are dependent on previous steroid activation of a genomic mechanism (Schumacher, 1990). Thus, it is possible that a rapid facilitatory effect of corticosterone on ethanol's rewarding properties occurs on later conditioning trials due to GR activation on earlier conditioning trials.

Overall, there is a significant amount of evidence that corticosterone may exert a rapid effect on brain functions, including reward-related processes, and behavior. Based on the hypothesis that stressor or ethanol-induced corticosterone release normally facilitates ethanol place conditioning, AMG administration prior to conditioning trials was expected to reduce the

magnitude of place preference, as revealed in the preference without AMG.

AMG has also been shown to effectively inhibit restraint stressor-induced release of corticosterone in mice (Roberts et al., 1993). However, the effects of AMG blockade on the corticosterone response to ethanol have not been studied. Thus, a control experiment (2B) was conducted to determine plasma corticosterone levels and confirm that the AMG dose used in Experiment 2A was effective in suppressing corticosterone synthesis and release in the presence of ethanol.

Subjects

The subjects used in Experiment 2A and 2B were male DBA/2J mice obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were between 54-60 days old on the first day of training (habituation). Housing and environmental conditions were exactly as described in Experiment 1.

Apparatus

The apparatus was exactly as described in Experiment 1.

Procedure

Experiment 2A

Experiment 2A involved one habituation session, eight conditioning sessions, and one test session. A 2-day break separated the first four and the last four conditioning sessions. For the habituation session, mice received an injection of saline (12.5 ml/kg) immediately before being placed in the conditioning box for 5 min on a smooth paper floor.

For conditioning, mice were randomly assigned to one of three groups: AMG (n = 31), ETOH (n = 32), and AMG/ETOH (n = 32). The AMG group served as a control for the possible rewarding or aversive effects of AMG alone. Within each of the experimental groups, mice were randomly assigned to one of the two ethanol conditioning subgroups (G+ or G-) and subjected to standard ethanol conditioning procedures. AMG was dissolved in a 20% w/v solution of β-cyclodextrin and saline. Subjects in the AMG and AMG/ETOH group received an IP injection of AMG (50 mg/kg; 10 ml/kg) 2 hrs before the conditioning session, and the ETOH group received an injection of the vehicle β-cyclodextrin. The pretreatment time and dose of AMG were chosen because they are within an effective range shown to maximally inhibit restraint stressor-induced release of corticosterone in mice (Roberts et al., 1993). Saline (AMG group) or ethanol was given immediately before placement in the apparatus.

For the test session, mice received an injection of vehicle 2 hrs before the 60 min preference test. A second saline injection was given immediately prior to placement in the apparatus to match the cues during conditioning days. The floor of each box was half grid and half hole with left/right position counterbalanced within groups.

Experiment 2B

Mice were subjected to similar experimental procedures described above for the ETOH (n = 6) and AMG/ETOH (n = 6) group. All mice received an acute injection of AMG or vehicle 2 hrs before an injection of ethanol (2 g/kg), and were immediately placed in the apparatus for 5 min. Following the 5 min session, each mouse was removed from the box and approximately 20 µl of tail blood was taken for corticosterone assay.

Corticosterone Radioimmunoassay

Tips of tails were nicked (2 mm) and approximately 20 µl of blood was collected into heparinized capillary tubes. The tubes were centrifuged at 2000 rpm for 5 min to separate the plasma from other blood elements. Five µl of plasma was removed, diluted in 100 µl sterile water and stored at 4°C until assayed for corticosterone. Corticosterone radioimmunoassay was executed following a previously reported method (Keith et al., 1978) described briefly below.

The plasma samples were immersed in boiling water to denature corticosterone binding globulin (Murphy, Engelberg, & Pattee, 1963), which would compete with the antibody for binding with corticosterone. Duplicate standard solutions were made containing 0, 10, 20, 50, 100, 200, 500, 1,000, 2,000, and 10,000 pg corticosterone in 100 µl sodium azide buffer (0.1%). Tubes were also prepared to estimate the total binding capacity and non-specific binding of the assay. One-hundred µl (equal to 9,000 counts per min) of [^{125}I]-corticosterone (ICN Biomedicals) and 100 µl of corticosterone antibody (Ventrex), titrated to bind approximately 40% of the total [^{125}I]-corticosterone, were added to the samples and standards for a total volume of 0.3 ml. The tubes were vortexed and incubated at 4°C overnight. Separation of bound from free corticosterone was achieved by the addition of 1000 µl of dextran-coated charcoal (4°C), 15 min incubation period, and 15 min centrifugation at 2000 rpm. The supernatant, containing bound corticosterone, was decanted into new test tubes and counted (Micromedic Automatic Gamma Counter).

Counts per min were normalized and fit to a least-squares regression equation produced by log-logit transformation of the standards. The minimum concentration of corticosterone detectable within the 95% confidence interval was 0.2 µg/dl. The maximum detectable corticosterone concentration was 200 µg/dl. Intra-assay variability was less than 10%. Assay specificity was very high, with only 4% cross-reactivity to deoxycorticosterone,

1% cross-reactivity to 5β-pregnanedione, and less than 0.6% cross-reactivity to other adrenal steroids.

Statistical Analyses

Because the performance of the ETOH compared to the AMG/ETOH group was of primary interest in Experiment 2, data from these two groups were included in one set of analyses. A separate set of analyses was conducted for the AMG control group in order to determine any effects of AMG alone on locomotor activity or acquisition of place conditioning.

Conditioning Data. For ETOH and AMG/ETOH groups, three-way ANOVAs were conducted independently for CS+ and CS- session data with Drug Treatment as a between groups factor (Drug Treatment groups were collapsed across G+ and G- subgroups) and Trials and Minutes as within groups factors. Significant interactions with Drug Treatment as a factor were further analyzed using two-way and one-way ANOVAs to determine drug treatment effects across the 5 min sessions and across trials.

For the AMG group, three-way repeated measures ANOVA was conducted with Trials, CS Type (CS+ and CS-), and Minutes as the within group factors.

Preference Test Data. Two-way ANOVA was conducted with Drug Treatment (ETOH and AMG/ETOH) and Conditioning Group as between group factors. For the AMG group, one-way ANOVA was conducted with

Conditioning Group as the between group factor. For both sets of analyses, the data were collapsed across minutes since the amount of time spent on the grid floor was nearly constant throughout the 60 min test session. One-way ANOVA was conducted for activity data during the test session with Drug Treatment as the between group factor.

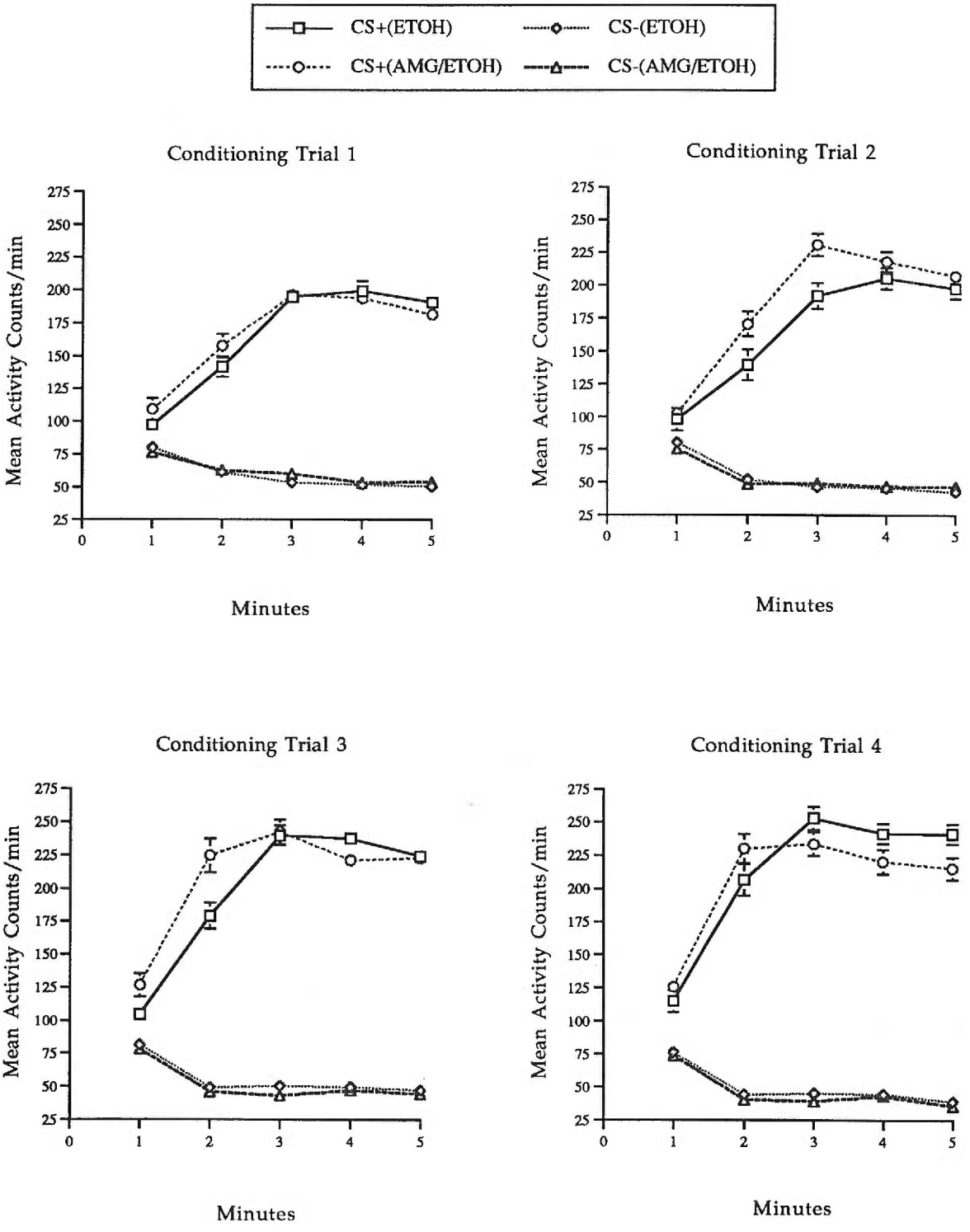
Results

Experiment 2A

Conditioning. Figure 3 shows the mean (±sem) activity counts per min for the ETOH and AMG/ETOH groups during conditioning trials 1-4. Ethanol produced significant locomotor activation during the CS+ sessions relative to the CS- sessions with saline. As previously observed with DBA/2J mice (e.g., Cunningham & Noble, 1992), activity counts were higher on the last CS+ session compared to the first CS+ session in both groups, indicating sensitization to the locomotor-activating effects of ethanol occurred across the four trials. In general, the AMG/ETOH group showed a more rapid development of locomotor activation during the first half of each session, and sensitization to ethanol's stimulant effects appeared to occur more rapidly in the AMG/ETOH group.

Initial analysis of CS+ session data (Three-way ANOVA: Drug Treatment X Trials X Minutes) yielded significant main effects of Trials [$F(3,186) = 40.20$, $p < 0.001$] and Minutes [$F(4,248) = 332.9$, $p < 0.001$], significant

Figure 3. Activity data for the ETOH and AMG/ETOH groups during conditioning trials 1-4. Values are mean (±sem) activity counts per min during CS+ and CS- sessions.


CS+(ETOH)
CS-(ETOH)
CS+(AMG/ETOH)
CS-(AMG/ETOH)
Conditioning Trial 1
Conditioning Trial 2
Conditioning Trial 3
Conditioning Trial 4
Mean Activity Counts/min
Minutes
275
250
225
200
175
150
125
100
75
50
25
0
1
2
3
4
5

two-way interactions of Drug Treatment X Trials [$F(3,186) = 3.0$, $p < 0.05$] and Drug Treatment X Minutes [$F(4,248) = 8.6$, $p < 0.001$], and a significant three-way interaction of Drug Treatment X Trials X Minutes [$F(12,744) = 3.0$, $p < 0.001$]. Separate repeated measures ANOVAs (Trials X Minutes) conducted for each Drug Treatment group yielded a significant main effect of Trials for the ETOH [$F(3,93) = 30.9$, $p < 0.001$] and AMG/ETOH group [$F(3,93) = 14.8$, $p < 0.001$], confirming the development of sensitization to ethanol's stimulant effects in both ethanol-treated groups. To further examine the nature of the three-way interaction, two-way ANOVAs (Drug Treatment X Minutes) were conducted separately for each trial. These analyses yielded a significant main effect of Drug Treatment on Trial 2 only [$F(1,62) = 4.0$, $p < 0.05$]. Significant Drug Treatment X Minutes interactions were found on Trials 2-4 [Trial 2: $F(4,248) = 3.3$, $p < 0.05$; Trial 3: $F(4,248) = 8.2$, $p < 0.001$; Trial 4: $F(2,248) = 6.1$, $p < 0.01$]. No main effect of Drug Treatment or interaction was found on Trial 1.

In order to characterize these Drug Treatment X Minutes interactions, one-way ANOVAs were conducted separately for each minute of each trial. The purpose of these analyses was to investigate the nature of the interaction and determine the point at which ETOH and AMG/ETOH groups differ in the magnitude of ethanol-stimulated locomotor activity during the 5 min conditioning session. These analyses revealed significant Drug Treatment effects at Minutes 2 and 3 on Trial 2 [Minute 2: $F(1,62) = 4.3$, $p < 0.05$;

Minute 3: $F(1,62) = 9.0$, $p < 0.01$], Minutes 1 and 2 on Trial 3 [Minute 1: $F(1,62) = 4.3$, $p < 0.05$; Minute 2: $F(1,62) = 8.0$, $p < 0.01$], and Minute 5 on Trial 4 [$F(1,62) = 5.1$, $p < 0.05$]. For Trials 2 and 3, these effects were due to significantly higher activity counts for the AMG/ETOH group relative to the ETOH group. In contrast, the effect on Trial 4 was due to significantly lower activity counts in the AMG/ETOH group compared to the ETOH group.

In summary, ethanol-treated groups showed sensitization to ethanol's locomotor-activating effects, as evidenced by an increase in activity levels across trials. The effect of Drug Treatment on Trial 2 suggests that sensitization to ethanol occurred more rapidly in AMG-treated animals. The significant enhancement during Trials 2 and 3 indicates that this effect developed over trials. However, activity levels in both groups reached a maximal level by Trial 4.

Three-way ANOVA (Drug Treatment X Trials X Minutes) of CS-session data indicated significant effects of Trials [$F(3,186) = 14.1$, $p < 0.001$] and Minutes [$F(4,248) = 133.8$, $p < 0.001$] and Trials X Minutes interaction [$F(12,744) = 3.1$, $p < 0.001$]. Activity during each session was initially high and decreased over the 5 min period. The interaction appeared to be due to a more rapid decrease in activity over the 5 min session on later trials relative to earlier trials. Mean (±sem) activity counts per min collapsed across Drug Treatment groups were 60.2±1.1 on Trial 1 and 48.1±1.9 on Trial 4, indicating habituation to experimental procedures. Overall, these analyses show that drug treatment

on CS+ days did not affect group activity levels during CS- sessions.

Table 1 shows mean (±sem) activity counts per min during CS+ and CS- sessions for the AMG control group on Trials 1-4. Activity levels during CS+ sessions with AMG were higher relative to CS- sessions. During both CS+ and CS- sessions, activity counts were initially high and decreased over the 5 min session. A three-way repeated measures ANOVA (Trials X CS Type X Minutes) conducted for the AMG group showed significant effects of Trials [$F(3,93) = 34.7$, $p < 0.001$], CS Type [$F(1,31) = 8.1$, $p < 0.01$], and Minutes [$F(4,124) = 92.4$, $p < 0.001$], but no interactions were found. In summary, this analysis indicates a slight activating effect of AMG on activity. Moreover, overall activity counts during CS+ and CS- sessions decreased across trials, showing that mice habituated to experimental procedures. Despite the overall decrease in activity across trials, activity counts during CS+ sessions remained significantly higher relative to CS- sessions. This suggests that mice habituated to experimental procedures across trials but did not become tolerant to the activating effect of AMG.

Preference Testing. Figure 4 shows the mean (±sem) sec per min spent on the grid floor by all conditioning subgroups during the preference test. The data presented in Figure 4 were collapsed across the 60 min test session. Mice in the G+ conditioning subgroup in ethanol-treated groups spent more time on the ethanol-paired grid floor than the G- subgroup, indicating a conditioned place preference for the grid floor. The magnitude of place

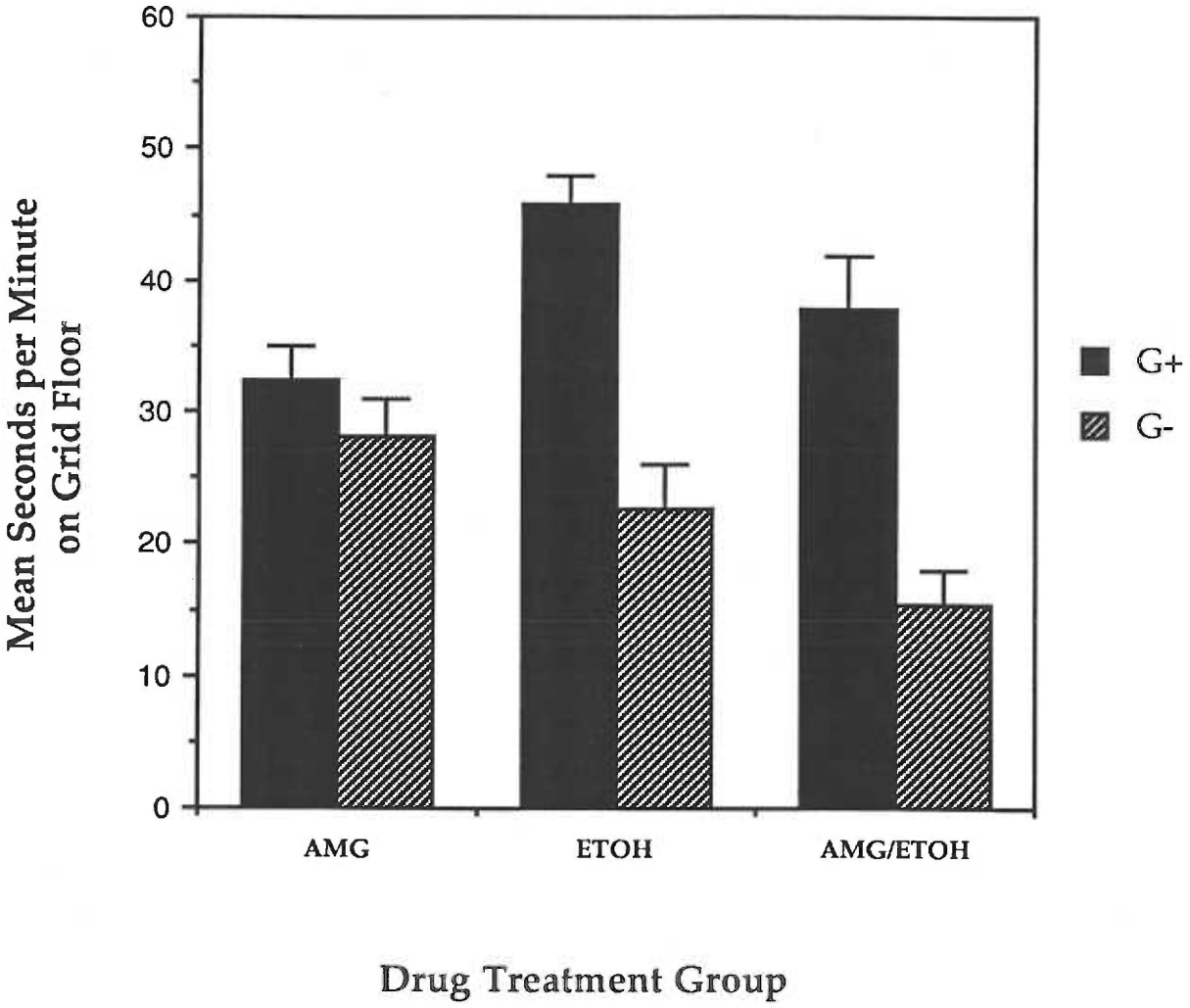
Table 1

Mean (±sem) Activity Counts per min During CS+ and CS- Sessions for the AMG Group on Trials 1-4

CS Type	Trial 1	Trial 2	Trial 3	Trial 4
CS+	59.4±2.7	45.6±2.1	44.0±2.1	40.8±2.3
CS-	56.0±2.2	42.3±1.9	40.3±2.1	35.8±1.9

Figure 4. Mean (±sem) sec per min spent on the grid floor by subjects in both conditioning subgroups of the AMG, ETOH, and AMG/ETOH groups during the preference test. G+ animals received ethanol paired with the grid floor during conditioning and saline paired with the hole floor and G- animals received ethanol paired with the hole floor and saline paired with the grid floor. Data shown are collapsed across the 60 min session.

Experiment 2: Preference Test



preference (demonstrated by G+ vs G- differences) in the ethanol-treated groups did not differ, suggesting that AMG did not affect the development of place preference. AMG conditioning subgroups spent approximately half of the session on each floor type, indicating the G+ subgroup did not acquire a preference or aversion for the AMG-paired grid floor.

The preliminary analysis of ethanol-treated groups (two-way ANOVA: Drug Treatment X Conditioning Group) yielded significant main effects of Drug Treatment [$F(1,60) = 6.5$, $p = 0.013$] and Conditioning Group [$F(1,60) = 56.1$, $p < 0.001$], but no interaction was found [$F < 1$], confirming no group differences in the magnitude of place preference. Figure 4 shows the drug treatment effect was due to significantly less time spent on the grid floor in both conditioning subgroups in the AMG/ETOH group. This effect is possibly the result of a sampling error (i.e., a greater number of mice randomly assigned to the AMG/ETOH subgroups happened to show an unconditioned preference for the hole floor). Alternatively, AMG may alter tactile sensitivity and cause a shift (increase) in the amount of time spent on the hole floor in both conditioning subgroups. The separate one-way ANOVA (Conditioning Group) conducted for the AMG group showed no significant preference for the AMG-paired floor [$F(1,29) = 1.2$, $p > 0.2$]. Thus, these data show that AMG administered prior to conditioning trials does not alter the acquisition of ethanol-induced conditioned place preference. In addition,

AMG administered alone does not cause a conditioned preference for either floor type (grid or hole).

Mean (±sem) activity counts per min during the 60 min test session were 27.1±1.0, 26.1±1.8, and 24.1±1.6 for AMG, ETOH, and AMG/ETOH groups, respectively. One-way ANOVA showed no effect of Drug Treatment (during conditioning) on activity levels during the preference test [$F(2,92) = 1.0$, $p > 0.3$].

Experiment 2B

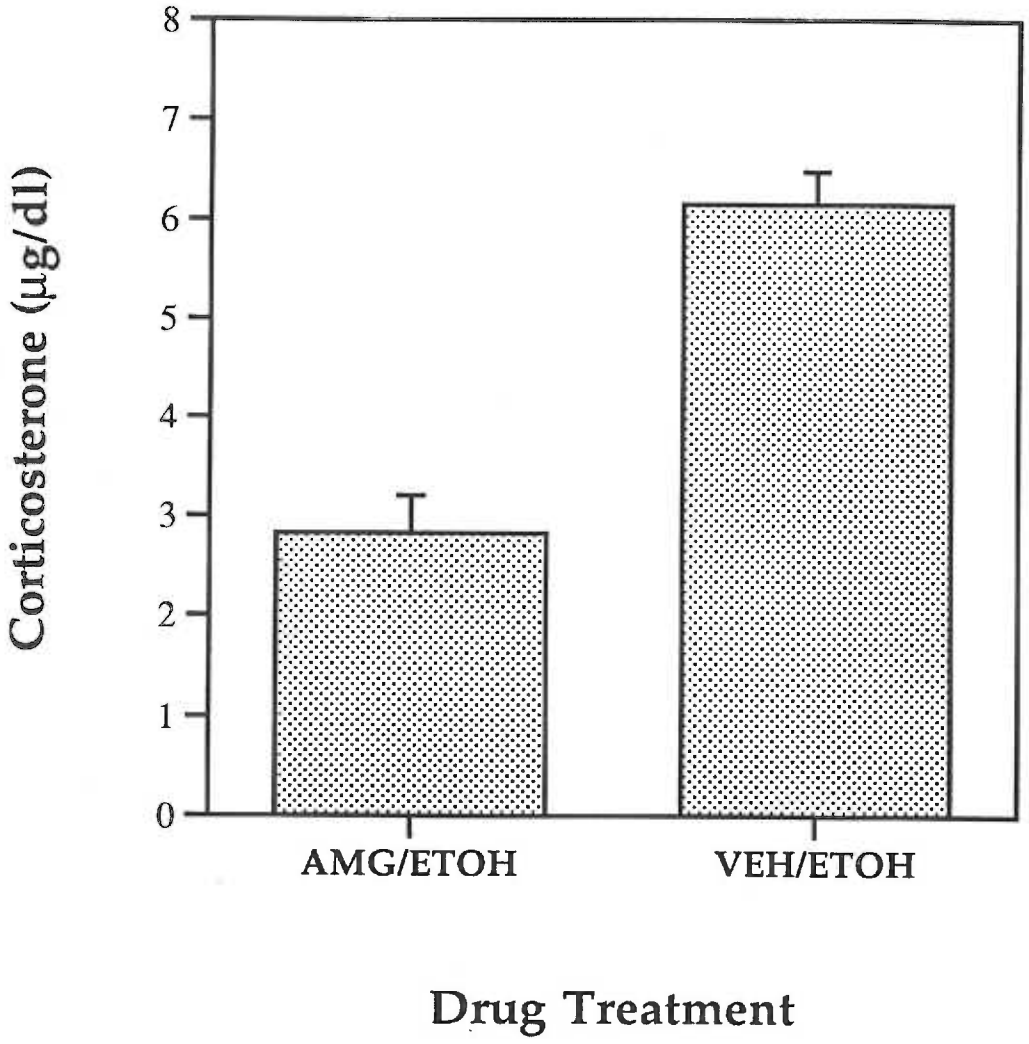
Corticosterone Assay

Figure 5 shows mean (±sem) plasma corticosterone levels in the vehicle and AMG-treated groups immediately following the 5 min session. AMG-treated animals showed significantly lower corticosterone levels following ethanol relative to vehicle-treated animals. One-way ANOVA showed a significant effect of drug treatment on corticosterone levels [$F(1,10) = 44.4$, $p < 0.001$].

Discussion

AMG did not alter the acquisition of conditioned place preference with ethanol. Thus, this outcome does not support the hypothesis that a rise in corticosterone levels facilitates the conditioning of ethanol place preference by enhancing ethanol's rewarding effects. In addition, this finding suggests that

Figure 5. Mean (±sem) plasma corticosterone levels immediately following a 5 min session. Mice were pretreated with either AMG or vehicle 2 hrs before an injection of ethanol (2 g/kg).


Corticosterone (µg/dl)
8
7
6
5
4
3
2
1
0
AMG/ETOH
VEH/ETOH
Drug Treatment

the acquisition of ethanol-induced place preference is independent of ethanol's corticosterone-elevating effects. The AMG control group did not develop a preference or aversion for the drug-paired floor, showing that AMG does not possess any rewarding or aversive properties of its own in the place conditioning paradigm.

The AMG dose (50 mg/kg) has been found to maximally inhibit restraint stress-induced release of corticosterone (Roberts et al., 1993). The results of Experiment 2B show that this dose of AMG effectively suppresses ethanol-induced corticosterone release (Figure 4). These data are in agreement with Lessov and Phillips (unpublished observations) who found AMG at a dose of 30 mg/kg completely blocked the rise in corticosterone produced by ethanol (1.5 mg/kg) over 9 consecutive days in mice. In addition, these data indicated that tolerance to AMG's effects on corticosterone inhibition does not develop over a 9 day period. Lessov and Phillips also found a potentiated corticosterone response to both saline and ethanol (1.5 mg/kg) injections following chronic treatment with AMG, possibly due to an accumulation of steroid precursors.

The preference test data suggest that a rise in corticosterone levels and presumed GR activation during conditioning trials does not normally influence the acquisition of ethanol place preference. The corticosterone data appear to support this since the AMG pretreated animals showed significantly lower levels of circulating hormone. GR is normally minimally occupied

(~10%) with basal corticosterone levels (1-3 μg/100ml during the morning) (Reul & de Kloet, 1985). Approximately 70% of GR is bound at the circadian peak or stressor-induced levels of corticosterone (25-35 μg/100ml). Thus, the AMG pretreated group had a level of corticosterone comparable to a non-stressed state. However, even though the vehicle pretreated group had a significantly higher level of corticosterone than the AMG pretreated group, this level of corticosterone is still within a non-stressed range (e.g., Sapolsky, Krey, & McEwen, 1984). This low level of corticosterone following ethanol is probably due to the fact that blood was sampled 5 min following the ethanol injection, but the peak in corticosterone following ethanol normally occurs after 30 min (e.g., Kakihana et al., 1968). It may be that the difference in corticosterone levels between the two groups during the 5 min conditioning sessions was not large enough to affect the magnitude of place preference. However, there were significant differences in ethanol-stimulated locomotor activity between AMG and vehicle-treated groups.

Similar to the finding of Lessov and Phillips (unpublished observations), it is possible that AMG treatment during conditioning trials resulted in an enhanced release of corticosterone during the preference test. This could possibly explain why an alteration in the expression of preference was not found in the AMG/ETOH group. However, a second preference test was also conducted in order to examine the expression of preference following a 2 hr pretreatment with AMG. The magnitude of preference in

each drug treatment group was almost identical to the first preference test (data not shown). Thus, these data suggest that inhibition of corticosterone release (during a second preference test) with AMG does not affect the expression of ethanol-induced place preference.

AMG administration prior to conditioning trials increased ethanol-stimulated activity during the first half of the 5 min conditioning session. A possible explanation for this effect is that ethanol-stimulated activity is normally slightly suppressed via higher corticosterone levels and GR activation. In addition to the function of GR in inhibitory feedback control of glucocorticoid release (see reviews by Munck, Guyre, Holbrook, 1984; de Kloet, Rosenfeld, Van Eekelen, Sutanto, & Levine, 1988), GR has been proposed to have inhibitory feedback influences on stress-activated brain mechanisms (de Kloet & Reul, 1987). AMG may result in a critically lower amount of occupied GR relative to the vehicle-treated group, which would possibly remove an inhibitory influence of this receptor on brain mechanisms that mediate ethanol-stimulated activity. This effect appeared to develop across trials, suggesting that GR activation may normally delay the development of sensitization.

These data are in contrast to the findings of Roberts et al. (1995) who significantly attenuated ethanol-induced locomotor sensitization with administration of the GR antagonist RU 38486. These contradictory findings suggest that AMG and RU 38486 may be working via different mechanisms to

modulate ethanol-induced sensitization. However, differences in experimental procedures could also account for these discrepancies across laboratories. For example, Roberts et al. administered RU 38486 prior to ethanol on a daily basis, whereas in the present study, AMG and ethanol were given at 48 hr intervals. The differences in time interval between ethanol exposures may activate different mechanisms that mediate locomotor sensitization produced by ethanol. For example, longer intervals between ethanol exposure may primarily involve associative mechanisms of locomotor sensitization, whereas daily exposure to ethanol may activate a non-associative mechanism that mediates sensitization to ethanol. Furthermore, associative mechanisms of ethanol sensitization may not involve GR activation, which would explain why AMG did not attenuate ethanol-induced locomotor sensitization. In contrast, non-associative mechanisms of ethanol sensitization, produced by daily ethanol exposure, may critically depend on GR activation and protein synthesis. This idea is supported by the finding that RU 38486 significantly decreased the development of ethanol-induced locomotor sensitization.

In summary, AMG did not affect the acquisition of ethanol-induced conditioned place preference. However, the results of this study suggest that AMG administration may facilitate the development of locomotor sensitization with repeated ethanol exposure. Thus, consistent with other recent studies using this paradigm (Cunningham, 1995; Risinger et al., 1992a;

Risinger, Malott, Prather, Niehus, & Cunningham, 1994), there is no relationship between the acute stimulant response to ethanol and the magnitude of conditioned place preference.

Experiment 3

Effects of AMG on Expression of Conditioned Place Preference

The results of Experiments 1 and 2 suggest that modulation of corticosterone does not alter the rewarding ethanol effects responsible for the acquisition of conditioned place preference. However, high corticosterone levels may still be an important factor modulating the expression and maintenance of conditioned ethanol reward. Handling and injection procedures prior to a preference test may result in a substantial release of corticosterone, or they may become conditioned stimuli (after repeated pairings with ethanol) that trigger a conditioned release of corticosterone. In addition, exposure to the floor CS may cause a conditioned corticosterone release, or there may be non-specific arousal effects resulting in elevated corticosterone. Such increases in corticosterone may be important in activating mechanisms responsible for the expression of conditioned reward. These mechanisms could be the same as those activated during conditioning trials (e.g., dopamine release), or they could be independent. For example, because activation of GR has been found to promote memory recall (de Kloet, de Kock, Schild, & Veldhuis, 1988), GR binding during the preference test could facilitate retrieval of the learned association made between ethanol and tactile stimuli.

Previous studies have shown that exogenous corticosterone administration (1, 10, or 20 mg/kg) prior to a preference test does not produce consistent changes in the magnitude of ethanol-induced place preference (Cunningham et al., unpublished results). One limitation of exogenous corticosterone administration is that the desired level of corticosterone can only be approximated due to fluctuations in endogenous corticosterone in the experimental groups. Even though this factor is controlled for with appropriate experimental design, the amount of circulating corticosterone in the control group may be higher than what is presumed to be a "basal" level. The level of corticosterone in the control group may also fluctuate across experiments, which could make it difficult to obtain a consistent effect of exogenous corticosterone administration on the expression of place preference. Furthermore, if corticosterone levels are normally elevated during a preference test, then administration of additional corticosterone may not have any further effect on behavior. The purpose of Experiment 3 was to examine the effect of AMG on the expression of conditioned ethanol place preference. Because elevated corticosterone levels during a preference test might normally facilitate the expression of place preference, it was hypothesized that inhibition of corticosterone release with AMG would attenuate the expression of place preference.

Subjects

The subjects used in Experiment 3 were male DBA/2J mice obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were between 54-60 days old on the first day of training (habituation). Housing and environmental conditions were exactly as described in Experiment 1.

Apparatus

The apparatus was exactly as described in Experiment 1.

Procedure

Experiment 3 consisted of three phases: one habituation session, eight conditioning sessions, and one test session, with sessions conducted on consecutive days. For the habituation session, mice received an injection of saline (12.5 ml/kg) immediately before being placed in the conditioning box on a smooth paper floor. During the conditioning phase, all subjects were randomly assigned to one of three AMG treatment groups: 0 (n = 31), 10 (n=31) and 50 (n = 32). Within each of the experimental groups, mice were randomly assigned to one of the two ethanol conditioning subgroups (G+ or G-) and subjected to standard ethanol place conditioning procedures, as described previously. AMG was not administered during this phase of the study.

For the 60 min test session, mice received one of three doses of AMG (0, 10, or 50 mg/kg). AMG was dissolved in a 20% w/v solution of β-cyclodextrin and saline and injections were administered IP (10 ml/kg) 2 hrs before the preference test. A second saline injection was given immediately prior to placement in the apparatus to match the cues during conditioning days. During the test, the floor was half grid and half hole with left/right position counterbalanced within groups.

Statistical Analyses

Conditioning Data. Two-way repeated measures ANOVAs were conducted separately for CS+ and CS- session data (collapsed across AMG Dose group), with Trials and Minutes as within group factors.

Preference Test Data. Two-way ANOVA was conducted with AMG Dose and Conditioning Group as between group factors. The data were collapsed across minutes since the amount of time spent on the grid floor was nearly constant throughout the 60 min test session. It was hypothesized that AMG would attenuate the magnitude of place preference in a dose-dependent manner. However, it is often difficult to observe smooth dose-response curves with conditioned place preference, thus, planned pairwise comparisons were conducted between AMG dose groups. One-way ANOVA was conducted for activity data during the test session with AMG Dose as the between group factor.

Results

Conditioning. Figure 6 shows mean (±sem) activity counts per min during conditioning trials 1-4 averaged across all groups. Ethanol produced significant locomotor activation during the CS+ session relative to the CS- session with saline, and sensitization to the locomotor-activating effects of ethanol occurred with repeated exposure. Mean (±sem) activity counts per min on the first CS+ session was 158.6±3.6 and on the fourth CS+ session it was 200.6±3.9. Activity levels during CS- session decreased across trials, indicating that mice habituated to procedures. Activity counts were 60.6±1.2 and 48.6±1.6 for the first and fourth CS- session, respectively. Two-way repeated measures ANOVAs (Trials X Minutes) were separately conducted for CS+ and CS- session activity data. The CS+ ANOVA indicated a significant activity increase across Trials [$F(3,273) = 34.7$, $p < 0.001$] and Minutes [$F(4, 364) = 248.9$, $p < 0.001$], and a significant interaction [$F(12,1092) = 12.4$, $p < 0.001$]. The CS- ANOVA showed a significant decrease in activity across Trials [$F(3,273) = 28.5$, $p < 0.001$] and Minutes [$4,364) = 250.4$, $p < 0.001$], and a significant interaction [$F(12,1092) = 11.3$, $p < 0.001$].

Preference Testing. Figure 7 shows the mean (+sem) sec per min spent on the grid floor by both conditioning subgroups of the 0, 10, and 50 mg/kg groups during the preference test. The amount of time spent on the grid floor by both G+ and G- subgroups was fairly constant throughout the test, therefore, the data shown in Figure 7 are collapsed across the 60 min session.

Figure 6. Mean (±sem) activity counts per min during conditioning trials 1-4 averaged across all groups. On CS+ days, animals received ethanol paired with one floor type (grid or hole). Saline was paired with the alternate floor type on CS- days.

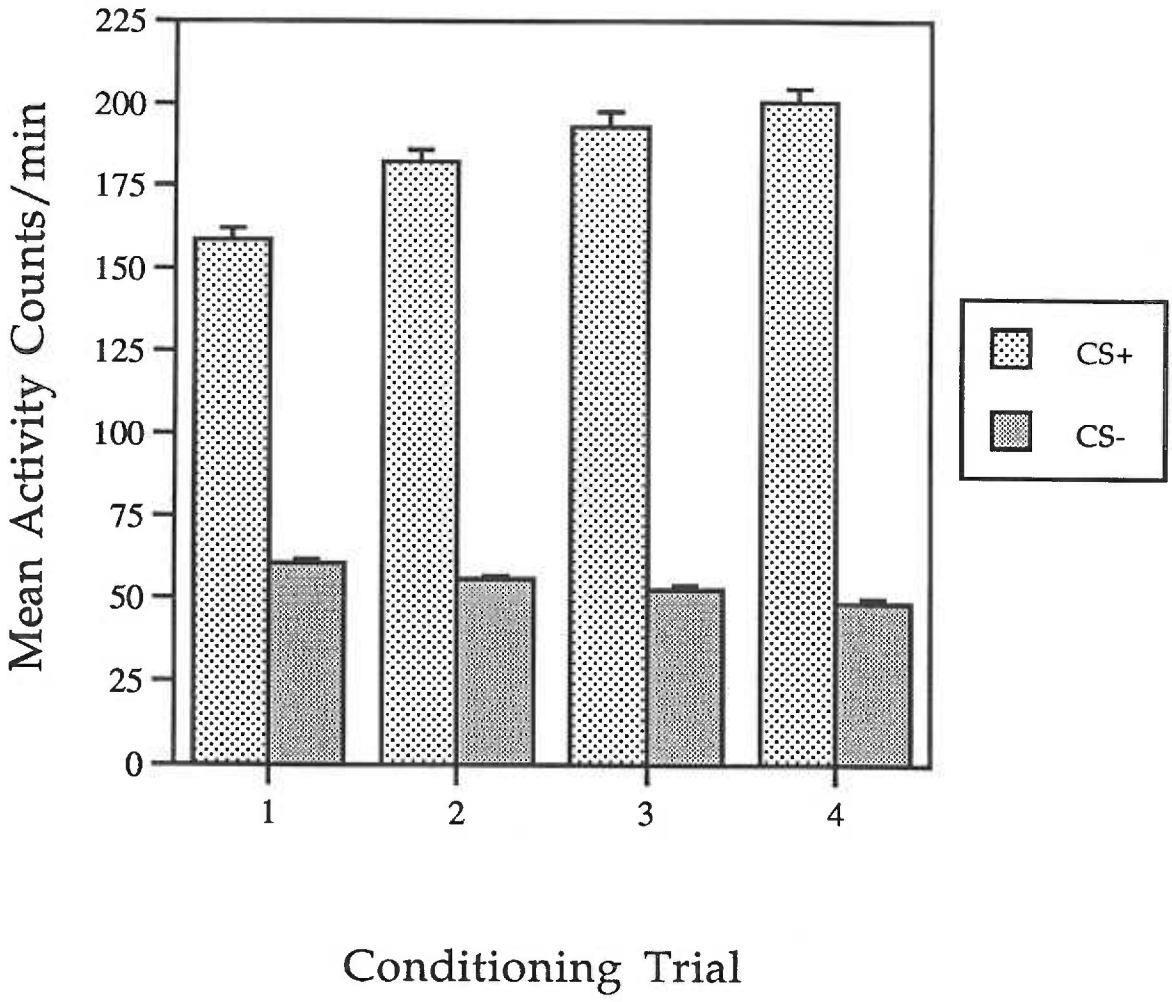
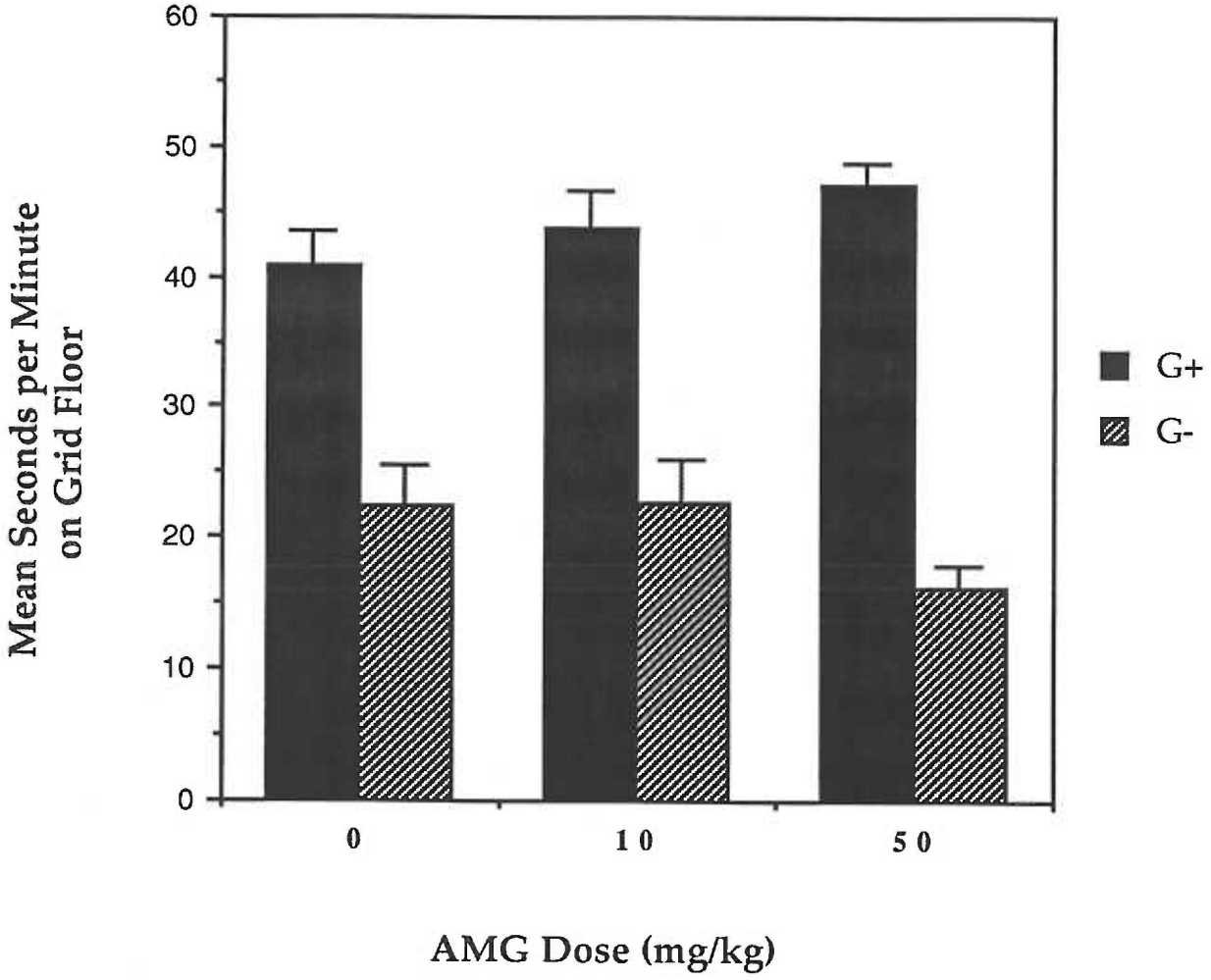

Mean Activity Counts/min
225
200
175
150
125
100
75
50
25
0
1
2
3
4
CS+
CS-
Conditioning Trial

Figure 7. Mean (±sem) sec per min spent on the grid floor by subjects in both conditioning subgroups of the 0, 10, and 50 mg/kg groups during the preference test. During conditioning, G+ animals received ethanol paired with the grid floor and saline paired with the hole floor and G- animals received ethanol paired with the hole floor and saline paired with the grid floor. Data shown are collapsed across the 60 min session.

Experiment 3: Preference Test



The G+ subgroups in all three AMG treatment groups spent more time on the grid floor relative to G- subgroups, showing the development of preference for the grid floor.

Initial analysis of the data collapsed across the 60 min test session (two-way ANOVA: AMG Dose X Conditioning Group) yielded a significant effect of Conditioning Group [$F(1,88) = 117.3$, $p < 0.001$] and a marginally significant AMG Dose X Conditioning Group interaction [$F(2,88) = 3.0$, $p = 0.053$]. Although the AMG Dose X Conditioning Group interaction approached but did not reach significance, this interaction was further investigated. Separate one-way ANOVAs (Conditioning Group) were conducted for each AMG Dose group (0, 10, and 50). These analyses indicated that G+ subgroups in all three AMG Dose groups showed significant preference for the grid floor compared to mice in the G- subgroups [0 group: $F(1,29) = 18.8$, $p < 0.001$; 10 group: $F(1,29) = 23.4$, $p < 0.001$; 50 group: $F(1,30) = 170.4$, $p < 0.001$]. Planned pairwise comparisons of the three AMG dose groups were conducted (two-way ANOVA: AMG Dose X Conditioning Group). These analyses revealed a significant AMG Dose X Conditioning Group interaction, indicating significantly greater preference in the 50 group relative to the 0 group [$F(1,59) = 6.6$, $p = 0.012$]. The planned comparison between the 50 group and the 10 group yielded an interaction that approached significance [$F(1,59) = 3.8$, $p = 0.052$]. However, the magnitude of preference in the 10 group did not significantly differ from the 0 group [$F < 1$].

One-way ANOVA indicated no effect of AMG Dose on activity levels during the 60 min preference test [$F(2,91) = 1.5$, $p = 0.22$]. Mean activity counts (±sem) per minute during the test were 28.5±1.0, 26.6±1.6, and 25.3±1.3 for the 0, 10, and 50 groups, respectively.

Discussion

AMG administration prior to the preference test did not attenuate the expression of place preference. This finding suggests that the assumed rise in corticosterone levels and possible GR activation during preference testing does not normally facilitate the expression of conditioned preference. Contrary to the predicted outcome, these data suggest that AMG enhanced the expression of place preference in a dose dependent manner. The highest dose of AMG used (50 mg/kg) is within the range shown to maximally inhibit restraint stressor-induced release of corticosterone, whereas intermediate stressor-induced corticosterone levels were found with the 10 mg/kg dose (Roberts et al., 1993). Thus, is is possible that different levels of endogenous corticosterone in the three groups may account for the observed differences in magnitude of preference.

The enhancement of preference with AMG administration suggests corticosterone normally has an inhibitory influence on the expression of conditioned place preference. This could be due to the absence of GR activation, which may mediate inhibitory feedback mechanisms on stressor-

activated brain processes and behavioral responses (de Kloet & Reul, 1987). Activation of GR have been found to suppress various behaviors (e.g., Porsolt, Le Pichon, & Jalfre, 1977), which is thought to reflect a behavioral adaptation in response to stressors (McEwen, Brinton, & Sapolsky, 1988). Corticosterone has also been shown to rapidly (within 1 hr) alter the performance of many aspects of learned behaviors, such as facilitation of extinction of passive and active avoidance responses (see review by McEwen, de Kloet, & Rostene, 1986).

In summary, these data suggest that inhibition of corticosterone release via AMG during the preference test enhances the magnitude of ethanol-induced place preference. This effect may be due to a reduction in corticosterone levels, which may normally act to suppress the expression of place preference. Alternatively, the enhancement could be due to a non-specific inhibitory effect of AMG on adrenal steroid synthesis. For example, since the synthesis of all other adrenally secreted steroids is inhibited with AMG administration (Dexter et al., 1967), this effect could also be due to a deficiency in circulating levels of another steroid, and not to a reduction in corticosterone levels. Because corticosterone levels were not determined in this experiment, interpretation of these data is limited. Experiment 4 will address this issue.

Experiment 4

Effects of AMG on Expression of Conditioned Place Preference

The results of Experiment 3 indicated that AMG enhanced the expression of ethanol-induced conditioned place preference in a dose-dependent manner. However, this effect was relatively small and statistically marginal. Therefore, the purpose of Experiment 4 was to replicate the effect of AMG on ethanol preference. One potential difficulty in demonstrating an enhancement of preference with AMG is that the standard dose of ethanol (2 g/kg) often produces a near-maximal effect. Thus, in order to optimize the possibility of observing an increase in the magnitude of place preference, a lower ethanol dose group (1.5 g/kg) was included in this experiment. The lower dose of ethanol was expected to produce a smaller magnitude of preference, leaving more room for preference to be increased by AMG pretreatment.

The results of Experiment 3 led to the hypothesis that stress-induced corticosterone release during the preference test normally attenuates expression of place preference. Thus, it was predicted that AMG would enhance the magnitude of preference in both ethanol dose groups. To obtain a measure of plasma corticosterone in each experimental group and confirm AMG's suppressive effect on corticosterone release, blood was taken from subjects in each group following the preference test for corticosterone

radioimmunoassay. It was expected that AMG-treated groups would have significantly lower corticosterone levels relative to vehicle-treated animals.

Subjects

The subjects used in Experiment 3 were male DBA/2J mice obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Mice were between 54-60 days old on the first day of training (habituation). Housing and environmental conditions were exactly as described in Experiment 1.

Apparatus

The apparatus was exactly as described in Experiment 1.

Procedure

The experiment consisted of three phases: one habituation session, eight conditioning sessions, and one test session, with sessions conducted on consecutive days. For the habituation session, mice received an injection of saline (12.5 ml/kg) immediately before placement in the conditioning box for 5 min on a smooth paper floor.

For the conditioning phase, all subjects were randomly assigned to one of two ethanol dose groups: 1.5 g/kg and 2 g/kg. Within each of the experimental groups, mice were randomly assigned to G+ and G-

conditioning subgroups (n = 27-30) and subjected to standard ethanol place conditioning procedures, as previously described.

For the 60 min test session, mice from each ethanol dose group were assigned to one of two AMG dose groups (0 or 50 mg/kg). AMG was dissolved in a 20% w/v vehicle solution of β-cyclodextrin and saline, and injections of either vehicle or AMG were administered IP (10 ml/kg) 2 hrs before the preference test. A second saline injection was given immediately before the test session to match the cues during conditioning days. During the test, the floor was half grid and half hole with left/right position counterbalanced within groups. Immediately following the test session, approximately 20 µl of tail blood was taken from each mouse for corticosterone assay. The method for corticosterone radioimmunoassay was exactly as described in Experiment 2.

Statistical Analyses

Conditioning Data. Two-way ANOVAs were conducted separately for CS+ and CS- session data, with ETOH Dose as between group factors (collapsed across G+ and G- subgroups) and Trials as a within group factor.

Preference Test Data. Three-way ANOVA was conducted with AMG Group, ETOH Dose, and Conditioning Group as between group factors. The data were collapsed across minutes since the amount of time spent on the grid floor was nearly constant throughout the 60 min test session. Two-way

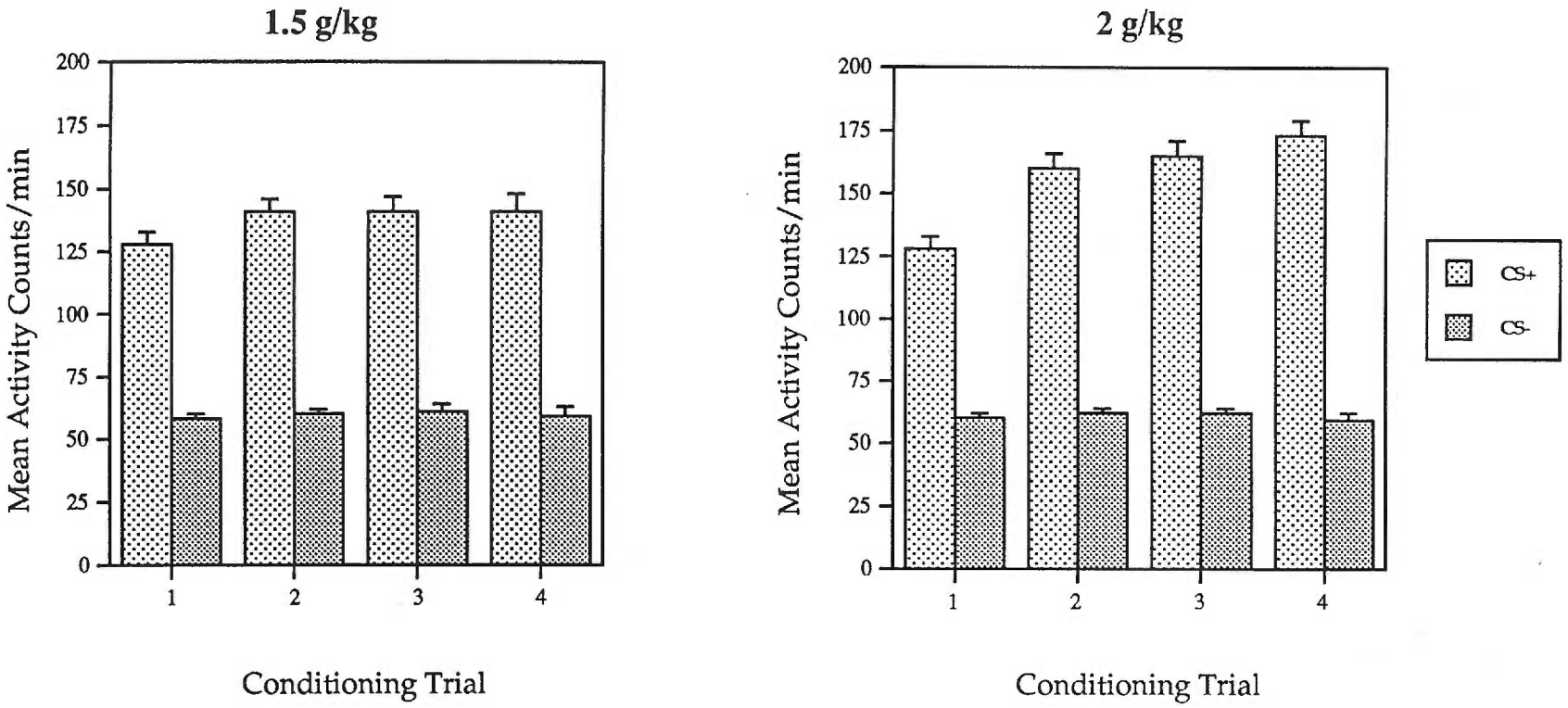
ANOVA was conducted for activity data during the test session with AMG Group and ETOH Dose as between group factors.

Results

Conditioning. Figure 8 shows mean activity counts per min during conditioning trials 1-4 averaged across each ethanol dose group. Ethanol produced significant locomotor activation during CS+ sessions relative to CS- sessions in both the 1.5 and 2 g/kg dose groups. In the 2 g/kg ethanol dose group, locomotor activity on the fourth CS+ session was greater relative to activity levels observed on the first CS+ session.

Two-way ANOVAs (ETOH Dose X Trials) were separately conducted for CS+ and CS- session data. The CS+ ANOVA revealed a significant effect of ETOH Dose [$F(1,113) = 9.8$, $p < 0.01$] and Trials [$F(3,339) = 21.9$, $p < 0.0001$] and a significant interaction [$F(3,339) = 5.9$, $p < 0.001$]. To further investigate the ETOH Dose X Trials interaction, one-way repeated measures ANOVAs were conducted for each ETOH Dose group (1.5 and 2 g/kg). A significant increase in activity across trials was found in the 2 g/kg dose group [$F(3,168) = 24.1$, $p < 0.0001$], confirming the development of sensitization with repeated ethanol exposure. Significant locomotor sensitization did not occur across trials in the 1.5 mg/kg group [$F(3,171) = 2.8$, NS]. The CS- ANOVA showed no significant main effects and no interactions [$Fs < 1$].

Figure 8. Activity data averaged separately for 1.5 and 2 g/kg ethanol dose groups during conditioning trials 1-4. Values are mean (±sem) activity counts per min during CS+ and CS- sessions.

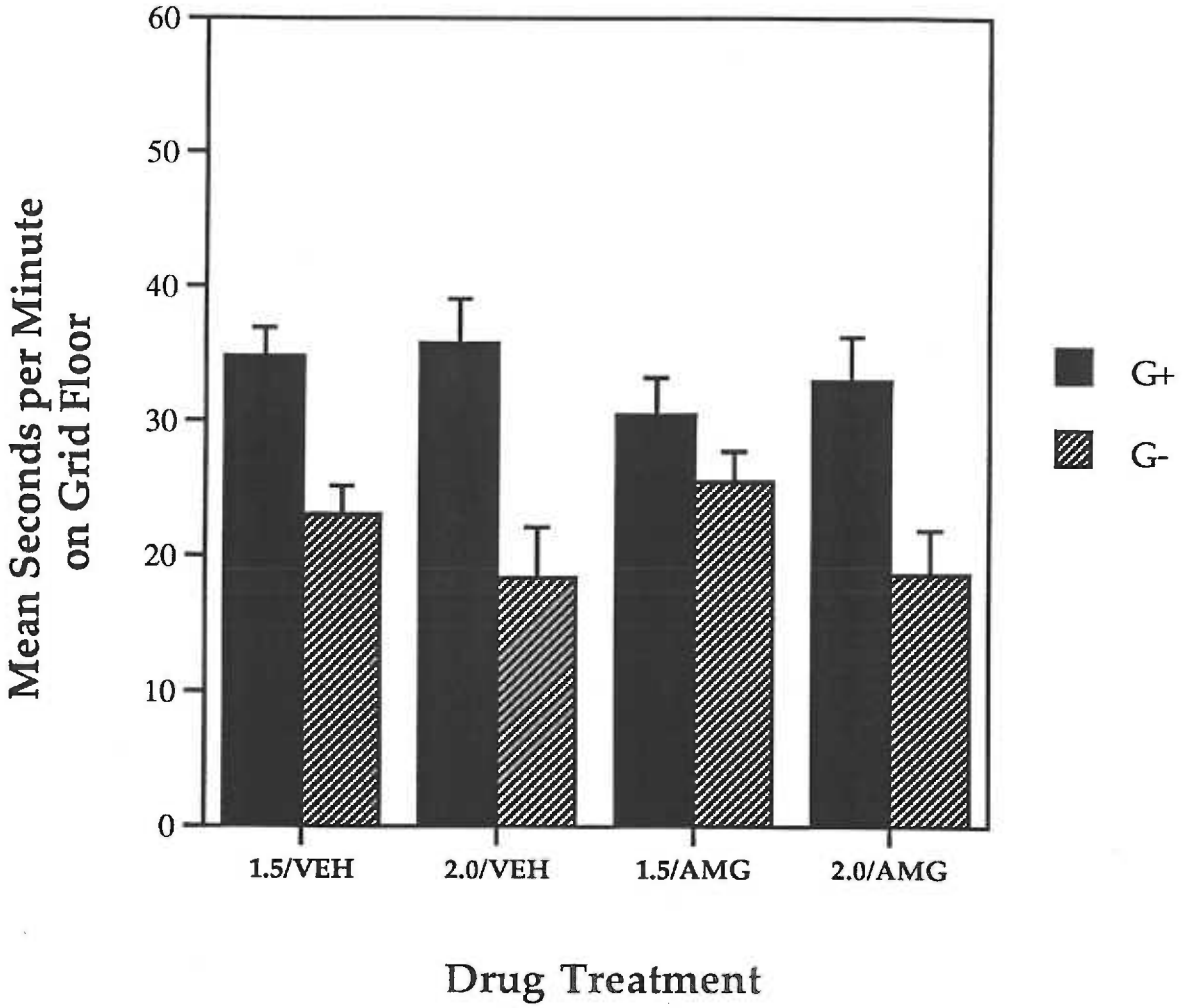

1.5 g/kg
Mean Activity Counts/min
200
175
150
125
100
75
50
25
0
1
2
3
4
Conditioning Trial
2 g/kg
Mean Activity Counts/min
200
175
150
125
100
75
50
25
0
1
2
3
4
Conditioning Trial
CS+
CS-

Preference Testing. Figure 9 shows the mean (+sem) sec per min spent on the grid floor by both conditioning subgroups in the four drug treatment groups during the preference test. The data shown are collapsed across the 60 min session. G+ subgroups in each drug treatment group spent significantly more time on the grid floor relative to G- subgroups, indicating the development of ethanol-induced preference for the grid floor. The magnitude of place preference (demonstrated by G+ vs G- differences) within the 2.0 g/kg dose groups did not differ, suggesting that AMG did not enhance preference. Within the 1.5 g/kg dose groups, the AMG- treated group showed slightly attenuated preference relative to the vehicle-treated group, suggesting that AMG may have actually decreased the magnitude of place preference in this group.

Overall analysis of the data collapsed across the 60 min test session (three-way ANOVA: AMG Group X ETOH Dose X Conditioning Group) yielded a significant effect of Conditioning Group [$F(1,107) = 34.9$, $p < 0.0001$]. No significant effects of AMG Group or ETOH Dose were found. Although not statistically justified due to the absence of a significant interaction, the 1.5 and 2.0 g/kg ETOH Dose groups were analyzed separately to investigate a possible effect of AMG within one of the ETOH Dose groups. These two-way ANOVAs (AMG Group X Conditioning Group) yielded a significant effect of Conditioning Group for the 1.5 g/kg [$F(1,54) = 12.91$, $p = 0.001$] and 2.0 g/kg

Figure 9. Mean (±sem) sec per min spent on the grid floor by subjects in both conditioning subgroups of the four drug treatment groups during the preference test. During conditioning, G+ subjects received ethanol (1.5 or 2.0 g/kg) paired with the grid floor and saline paired with the hole floor and G- subjects received ethanol paired with the hole floor and saline paired with the grid floor. Two hours before the preference test, subjects received an injection of AMG or vehicle. Data shown are collapsed across the 60 min session.

Experiment 4: Preference Test



ETOH Dose group [$F(1,53) = 21.84$, $p < 0.001$]. However, no significant effect of AMG Group or interactions were found.

Activity levels during the preference test were higher in the 1.5 g/kg ETOH Dose group relative to the 2.0 g/kg group. Activity levels were also higher in AMG-treated groups relative to vehicle-treated groups. Mean (±sem) activity counts per min during the 60 min test were 34.3±1.3, 29.4±1.5, 39.0±1.6, and 34.1±1.6 for the 1.5/vehicle, 2.0/vehicle, 1.5/AMG, and 2.0/AMG groups, respectively. Two-way ANOVA (AMG Group X ETOH Dose) revealed a significant effect of AMG Group [$F(1,111) = 10.0$, $p < 0.01$] and ETOH Dose [$F(1,111) = 11.1$, $p < 0.01$] on activity levels during the test.

Corticosterone Assay. Figure 10 shows mean (+sem) plasma corticosterone levels in each drug treatment group immediately following the 60 min preference test. AMG-treated groups showed reduced corticosterone levels compared to vehicle-treated groups. Three-way ANOVA (AMG Group X ETOH Dose X Conditioning Group) showed a significant effect of AMG on corticosterone levels [$F(1,104) = 146.95$, $p < 0.0001$]. No significant effect of ETOH Dose or Conditioning Group on corticosterone levels was observed.

Figure 11 shows the relationship between corticosterone level and mean percent time spent on the drug-paired floor for each subject in vehicle ($n = 57$) and AMG ($n = 55$)-treated groups. Corticosterone levels in vehicle-treated animals were significantly higher and more variable than in AMG-

Figure 10. Mean (±sem) plasma corticosterone levels immediately following the 60 min preference test. Mice received an injection of AMG or vehicle 2 hrs before and a saline injection immediately before the preference test.

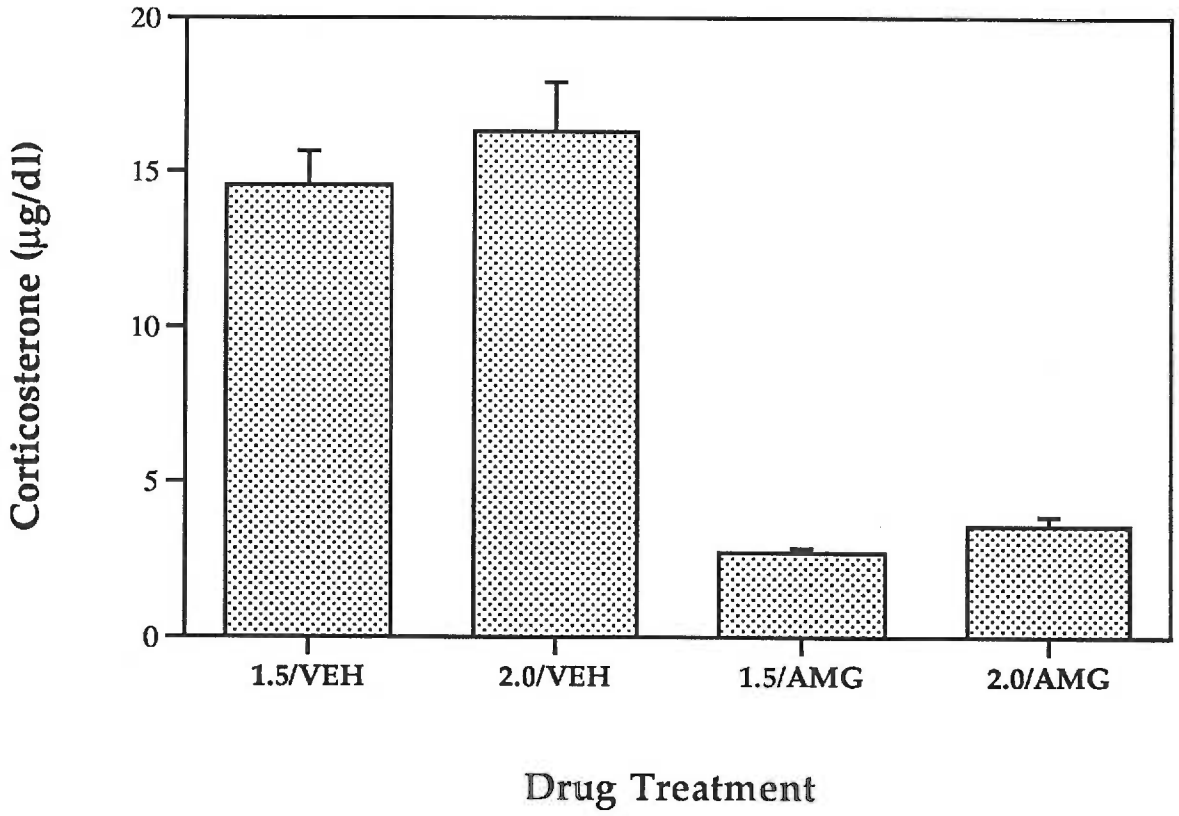
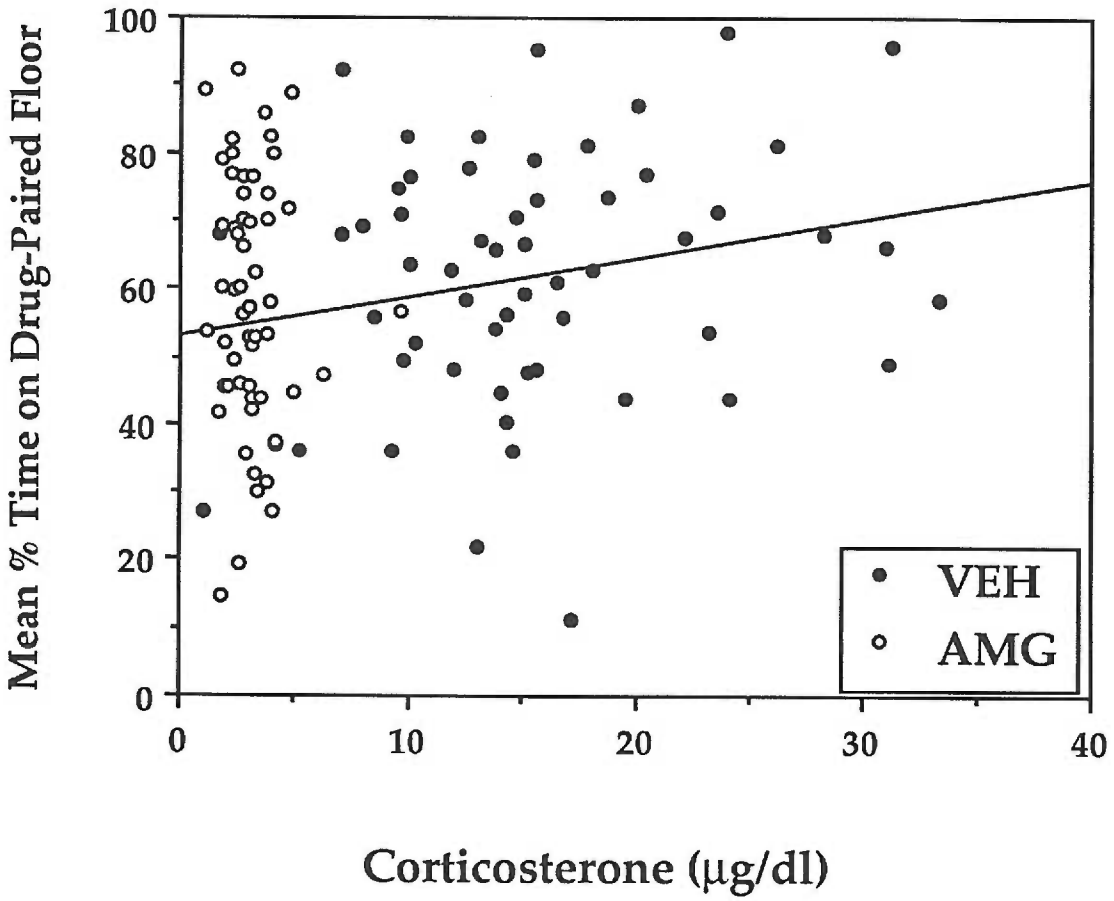

20
15
10
5
0
Corticosterone (µg/dl)
1.5/VEH
2.0/VEH
1.5/AMG
2.0/AMG
Drug Treatment

Figure 11. Scatter plot showing the relationship between corticosterone level following the 60 min test session and mean percent time spent on the drug-paired floor in vehicle and AMG-treated animals. The line that best fits the data for vehicle-treated animals only is shown (r = +0.22, p = 0.098).


Mean % Time on Drug-Paired Floor
100
80
60
40
20
0
0
10
20
30
40
VEH
AMG
Corticosterone (μg/dl)

treated animals following the 60 min test session. For this reason, a Pearson *r* correlation coefficient was computed separately for AMG and vehicle-treated animals relating corticosterone level and mean percent time spent on the drug-paired floor. No significant correlation between corticosterone level and mean percent time on the drug-paired floor was found for vehicle-treated animals (r = +0.22, p = 0.098, df = 55) or AMG-treated animals (r = -0.06, p = 0.674, df = 53).

Discussion

AMG administration did not enhance the magnitude of ethanol place preference in the 1.5 or 2.0 g/kg ethanol dose groups. Thus, this finding does not replicate the effect of AMG in Experiment 3 and suggests the statistically marginal enhancement of preference in Experiment 3 may have been due to sampling error, rather than to a true effect of AMG on ethanol place preference. These inconsistent results do not support the hypothesis that stress-induced corticosterone levels inhibit the expression of ethanol place preference.

AMG-treated groups showed significantly higher activity levels during the preference test relative to vehicle-treated groups within the 1.5 and 2.0 g/kg ETOH Dose groups. This finding is consistent with the conditioning trial activity data from Experiment 2 that showed a locomotor-activating effect of AMG administered alone. However, the present data are not

consistent with the results of Experiment 3 that found no significant effect of AMG on activity levels during the preference test and a trend towards lower activity levels in AMG-treated groups. Within the AMG and vehicle-treated groups, activity levels during the preference test were also higher in the 1.5 g/kg ETOH Dose group relative to the 2.0 g/kg group. This is possibly due to a conditioned suppression of activity levels in the 2.0 g/kg group relative to the 1.5 g/kg group. Alternatively, this effect could be due to sampling error.

Corticosterone levels in AMG-treated groups were significantly lower relative to vehicle-treated groups. Vehicle-treated groups showed a stress-induced level of corticosterone. Plasma corticosterone in these groups was comparable to those observed 30 minutes following novelty stress or 1.5 mg/kg corticosterone (~13-15 μg/dl) (Deroche et al., 1992). The present data support the hypothesis that plasma corticosterone is elevated during a preference test. However, reducing the level of corticosterone with AMG did not alter the expression of place preference. Overall, these data suggest that corticosterone does not modulate the expression of ethanol-induced conditioned place preference.

GENERAL DISCUSSION

The goal of this research was to examine a role for corticosterone in modulating the rewarding effects of ethanol in mice. Experiment 1 demonstrated that corticosterone administration prior to conditioning sessions with ethanol did not increase the magnitude of ethanol-induced place preference. Similarly, Experiment 2 found no effect of AMG, a steroid synthesis inhibitor, on the acquisition of ethanol place conditioning. Experiments 3 and 4 examined the effect of AMG on the expression of ethanol-induced place preference. The results of Experiment 3 suggested a marginal enhancement of preference with inhibition of corticosterone. However, this effect was not replicated in Experiment 4, which tested the effects of AMG on the expression of preference using two doses of ethanol. Overall, these findings suggest that corticosterone is not involved in either the acquisition or expression of ethanol-induced conditioned place preference in DBA/2J mice.

Experiment 1 was designed to test the effects of corticosterone administration prior to conditioning sessions with ethanol. It was hypothesized that high levels of circulating corticosterone prior to the conditioning session would potentiate the rewarding effects of ethanol during conditioning and facilitate the acquisition of ethanol place preference. The results of Experiment 1 did not support this hypothesis. Corticosterone (1, 5,

and 10 mg/kg) did not enhance the magnitude of ethanol-induced place preference. In fact, there was a non-significant trend in the opposite direction. The higher doses of corticosterone (5 and 10 mg/kg) appeared to decrease the magnitude of place preference (Figure 2). Although the trend is not statistically significant, an inhibitory effect of corticosterone on place preference would be consistent with de Kloet, Joëls, & Sutanto (1991) who discuss suppressive effects of high corticosterone levels on brain functioning. For example, high concentrations of corticosterone increase the amplitude of the afterhyperpolarization in CA1 pyramidal cells following a depolarizing current. This effect appears to be mediated by corticosterone binding to GR (de Kloet et al., 1988) and results in a suppression of stimulated neuronal activity. In general, the results of Experiment 1 are not consistent with the findings of Piazza, Maccari et al. (1991) who found that exogenous administration of corticosterone rapidly increases the rewarding properties of amphetamine based on its facilitation of amphetamine self-administration behavior.

The mechanism for corticosterone's rapid effect on amphetamine or ethanol self-administration in the previous studies is unknown. Piazza, Maccari et al (1991) suggest that corticosterone may be facilitating amphetamine self-administration via an interaction with the dopaminergic system. For example, corticosterone administration immediately before a self-administration session may prime or "sensitize" dopamine neurons and

result in a greater release of dopamine in response to amphetamine. Similarly, corticosterone administered simultaneously with amphetamine may facilitate amphetamine-stimulated dopamine release. Fahlke et al. (1994a) also suggest that alterations in the dopaminergic system (e.g., decreased neurotransmission) in high ethanol preference rats following adrenalectomy could account for the reduction in ethanol intake. Thus, corticosterone may have a specific role in mediating the rewarding effects of ethanol in high preference rats due to inherent differences in dopaminergic reactivity in high preference rats.

It is not known whether the various corticosterone manipulations influenced dopaminergic functioning in Experiment 1. However, based on several lines of evidence, it is safe to speculate that corticosterone increased synaptic dopamine levels (e.g., Gilad, et al., 1987; Rothschild et al., 1985; Versteeg, Van Zoest, & de Kloet, 1984). Thus, the results of Experiment 1 suggest that dopamine/corticosterone interactions are not important in mediating ethanol's unconditioned rewarding properties in the place preference paradigm. This is consistent with studies that investigated the effects of the dopamine antagonist haloperidol on ethanol-induced conditioned place preference. Haloperidol did not alter the acquisition (Risinger et al., 1992a) or expression (Cunningham, Malott, Dickinson, & Risinger, 1992) of ethanol-induced conditioned place preference in DBA/2J mice. However, haloperidol did decrease ethanol-stimulated locomotor

activity during conditioning trials, suggesting a dissociation between ethanol's rewarding and locomotor-activating effects. Taken together, these studies suggest that dopamine is not important for the development or expression of ethanol place preference in this strain of mice.

Experiment 2A further examined the role of corticosterone in modulating the rewarding properties of ethanol. Instead of exogenous administration of corticosterone, the normal stressor-induced rise in corticosterone produced by ethanol and handling procedures was prevented via the steroid synthesis inhibitor, AMG. It was hypothesized that the acute rise in corticosterone during conditioning may augment the rewarding effects of ethanol and increase the magnitude of place preference. Thus, AMG was expected to attenuate ethanol's rewarding effects and decrease the magnitude of preference. The results of Experiment 2A did not support this hypothesis and confirm the conclusion from Experiment 1 that elevated plasma corticosterone is not important in the acquisition of place preference.

Experiment 2B showed that AMG administered 2 hr before ethanol conditioning sessions completely inhibited the acute ethanol-induced rise in corticosterone. The level of endogenous corticosterone in the vehicle-treated group was significantly higher relative to the AMG-treated group (Figure 5). However, the level of corticosterone in the vehicle-treated group, measured following the 5 min conditioning session, was still below a stress-induced range. For example, in DBA/2J mice, Kakihana et al. (1968) demonstrated that

a dose of 1.8 g/kg ethanol produces a plasma level of ~ 30 μg/dl corticosterone 60 min following i. p. injection. In addition, Thiagarajan et al. (1989) showed that corticosterone in response to 3.2 g/kg ethanol reached a level of ~ 20 μg/dl 10 min following intragastric administration. These findings suggest that plasma corticosterone probably did not reach a peak ethanol-induced level until after the conditioning session was over and explain the relatively low level of corticosterone in the vehicle-treated group.

AMG administration did not alter the acquisition of place preference, however, ethanol-stimulated activity during conditioning sessions was significantly higher in the AMG-treated group relative to the vehicle treated group. There are several ways to interpret AMG's effect on ethanol-stimulated locomotor activity. For example, these data suggest that corticosterone may normally inhibit ethanol-stimulated locomotor activity. If so, the concentration of corticosterone necessary for an inhibitory effect is not very high since plasma corticosterone in the vehicle-treated group (~ 6 μg/dl) was only slightly elevated relative to the AMG-treated group (~ 3μg/dl). Alternatively, the increase in ethanol-stimulated activity in AMG-treated animals could be due to an effect of AMG not related to its effect on corticosterone release. AMG blocks the synthesis and release of corticosterone by inhibiting the conversion of cholesterol to pregnenolone, the first step in the adrenal steroid synthesis pathway (Dexter et al., 1967). Since

pregnenolone is the precursor for every adrenally derived steroid (White, Pescovitz, & Cutler, 1995), AMG also inhibits the synthesis of many other steroids, such as mineralocorticoids, androgens, and estrogens, which could be important in mediating locomotor activity. These present data, however, are not consistent with Wallis, Anton, & Randall (1984) who found that adrenalectomy significantly decreased stimulated locomotor activity in response to 1.5 g/kg ethanol in female mice. The discrepancy between these studies may be due to different physiological effects of adrenalectomy vs aminoglutethimide. For example, the biochemical deficits produced by adrenalectomy are permanent and probably more severe than the effects of acute administration of AMG. In addition, because AMG does not completely eliminate adrenally derived steroids, including corticosterone, it may produce a more normal physiological state (Roberts et al., 1993).

Finally, AMG's effects on ethanol-stimulated activity do not support the suggestion that corticosterone may rapidly increase synaptic dopamine levels. Consistent with this hypothesis, the vehicle-treated group might be expected to show a higher activity response following ethanol due to corticosterone's facilitatory effect on dopamine levels, whereas the AMG-treated group would show lower stimulated activity due to the absence of corticosterone's dopamine-elevating effect. In fact, these activity data might suggest that corticosterone normally has a tonic inhibitory influence on dopaminergic functioning, and the reduction in circulating corticosterone

with AMG administration results in potentiated dopamine levels following ethanol.

In summary, the results of Experiment 2A show that the acute rise in corticosterone following ethanol does not modulate ethanol's unconditioned rewarding effects in the place conditioning paradigm. Consistent with previous studies (e.g., Cunningham, 1995; Risinger et al., 1992a), these data also suggest a dissociation between ethanol's rewarding and locomotor effects. However, the mechanism by which AMG increases ethanol-stimulated locomotor activity is unclear.

Experiments 3 and 4 tested the hypothesis that high plasma corticosterone during a preference test is an important factor that facilitates the expression of conditioned ethanol reward. Prior to the preference test in Experiment 3, AMG (10 or 50 mg/kg) was administered in order to examine the effects of inhibition of corticosterone release on the expression of preference. It was predicted that AMG administration would dose-dependently attenuate the expression of place preference relative to the "control" group (0 mg/kg) that presumedly had significantly higher plasma corticosterone levels induced by handling, injection, and exposure to the test apparatus. The results of Experiment 3 were not consistent with the predicted outcome and suggested that corticosterone normally inhibits the expression of place preference. AMG dose-dependently enhanced the magnitude of ethanol-induced place preference, however, the effect was marginally

significant. Concern over the reliability of this finding provided the rationale for Experiment 4.

The purpose of Experiment 4 was to replicate the observed effect of AMG on ethanol-induced place preference. In order to optimize the chances of observing an increase in the magnitude of preference with AMG, the experiment included another group of subjects conditioned with a lower dose of ethanol (1.5 g/kg). In addition, plasma corticosterone was determined in all groups following the preference test. Based on the outcome of Experiment 3, it was hypothesized that stressor-induced corticosterone release during the preference test normally attenuates the expression of preference. The results of Experiment 4 did not support this hypothesis. Indeed, the vehicle-treated groups showed a stressor-induced level of corticosterone during the preference test (Figure 10), which supports the hypothesis that corticosterone was elevated during a preference test. However, the AMG treatment did not enhance the expression of place preference. Taken together, the inconsistent results of Experiment 3 and Experiment 4 suggest that corticosterone is not involved in the expression of ethanol-induced conditioned place preference. In addition, it is possible that other adrenal steroids might have rapid neural effects that alter behavior. Since AMG inhibits all other steroids derived from the adrenal cortex, these findings suggest that the presence of other circulating steroids (e.g., aldosterone) may not be important for the expression (or acquisition) of ethanol place preference. Many of these steroids have a short

half-life (approximately 20 min) and would be expected to be virtually absent 2 hrs following AMG administration (White et al, 1995).

Overall, the experiments in this thesis do not support the hypothesis that corticosterone is important in modulating ethanol's unconditioned and conditioned rewarding properties by exerting a rapid effect on the neural substrate mediating the acquisition or expression of ethanol-induced conditioned place preference. In general, these data are inconsistent with previous studies that demonstrated a facilitatory effect of corticosterone on the rewarding properties of several abused drugs, most notably amphetamine and ethanol. There are several possible reasons for the discrepancies between the previous and present studies. For example, the effect of corticosterone in the previous studies may be unique to rats that show a predisposition for self-administration, possibly due to individual differences in dopaminergic reactivity to these drugs. These individual differences may be due to genetic variability in the rats strains that were utilized. In the present studies, the inbred DBA/2J mouse strain was used because these mice are highly sensitive to ethanol's rewarding properties in the place conditioning paradigm. In addition, since these mice are genetically identical, they exhibit a stable phenotype to examine the neuropharmacological basis of ethanol reward. However, the findings in the present studies may not be generalizable to other strains of mice or other species, such as rats. Consequently, corticosterone may indeed facilitate the rewarding effects of abused drugs in

other species, as suggested by the findings of Piazza, Maccari et al. (1991) and Fahlke et al. (1994a).

Another possibility is that the facilitatory effect of corticosterone on amphetamine and ethanol self-administration is specific to the animal models used to examine the rewarding properties of these drugs. For example, the rapid effects of corticosterone on amphetamine's rewarding or reinforcing properties in the i. v. self-administration paradigm may be due to the temporal contiguity between corticosterone's rapid delivery to the brain in relationship to the onset of amphetamine's pharmacological effects. The effect of corticosterone on ethanol consumption could be related to a selective interaction with the mechanism involved in mediating ethanol's rewarding effects in the oral self-administration paradigm. Alternatively, corticosterone may affect mechanisms not directly involved in the rewarding aspects of oral ethanol self-administration, such as consummatory behavior. In the present studies, it may be that corticosterone did not enhance ethanol's rewarding properties in the place conditioning paradigm due to a different route of administration (i.p.) of these drugs and possibly different time course of effects relative to the i.v. and oral self-administration paradigm. In addition, the subject's control over exposure to these drugs may be an important factor in determining corticosterone's facilitatory effect. For example, in the Piazza, Maccari et al. (1991) and Fahlke et al. (1994a) studies, rats self-administered amphetamine and ethanol, whereas in the present studies exposure to

ethanol was controlled by the experimenter. Thus, corticosterone may specifically interact with neural pathways that mediate the reinforcing and rewarding properties of i. v. amphetamine and oral ethanol in the self-administration paradigm, and these pathways may be distinct from those mediating the rewarding effects of ethanol in the place conditioning paradigm.

CONCLUSIONS

The experiments in this thesis tested the effects of various manipulations of endogenous corticosterone levels on the acquisition and expression of ethanol-induced conditioned place preference. Initially, it was hypothesized that high levels of endogenous corticosterone would enhance ethanol's positive motivational effects. Experiments 1 and 2 tested this hypothesis. Exogenous corticosterone administration (Experiment 1) and inhibition of corticosterone release (Experiment 2) did not affect the acquisition of ethanol place preference. Experiment 3 tested the hypothesis that corticosterone release during a preference test facilitates the expression and maintenance of conditioned ethanol reward. The results of Experiment 3 suggested an enhancement of the expression of place preference with inhibition of corticosterone release. However, Experiment 4 did not replicate this effect and showed that inhibition of corticosterone release did not alter

the expression of place preference. Overall, the present studies suggest that corticosterone is not involved in modulating the unconditioned and conditioned rewarding properties of ethanol in DBA/2J mice in the place conditioning paradigm.

REFERENCES

Adams, N. (1995). Sex differences and the effects of tail-pinch on ethanol drinking in Maudsley rats. Alcohol, 12(5), 463-468.

Antelman, S. M., Eichler, A. J., Black, C. A., & Kocan, D. (1980). Interchangeability of stress and amphetamine in sensitization. Science, 207, 329-331.

Bilezikjian, L. M., & Vale, W. W. (1987). Regulation of ACTH secretion from corticotrophs: The interaction of vasopressin and CRF. Annals of the New York Academy of Sciences, 512, 85-96.

Bozarth, M. A. (1991). The mesolimbic dopamine system as a model reward system. In P. W. J. Scheel-Kruger & J. Willner (Eds.), The Mesolimbic Dopamine System: From Motivation to Action, (pp. 301-330). Chichester: John Wiley & Sons Ltd.

Bozarth, M. A. (1990). Evidence for the rewarding effects of ethanol using the conditioned place preference method. Pharmacology Biochemistry & Behavior, 35, 485-487.

Bozarth, M. A. (1987). An overview of assessing drug reinforcement. In M. A. Bozarth (Ed.), Methods of Assessing the Reinforcing Properties of Abused Drugs, (pp. 635-658). New York: Springer-Verlag.

Broadhurst, P. (1975). The Maudsley reactive and nonreactive strains of rats. Behavior Genetics, 5, 299-319.

Cador, M., Dulluc, J., & Mormède, P. (1993). Modulation of the locomotor response to amphetamine by corticosterone. Neuroscience, 56(4), 981-988.

Cam, G. R., & Bassett, J. R. (1983). The effect of acute nicotine administration on plasma levels of the thyroid hormones and corticosterone in the rat. Pharmacology Biochemistry & Behavior, 19(3), 559-561.

Cappell, H., & Greeley, J. (1987). Alcohol and tension reduction: An update on research and theory. In H. T. Blane & H. E. Leonard (Eds.), Psychological Theories of Drinking and Alcoholism (pp. 15-24). New York: Guilford.

Carr, G. D., Fibiger, H. C., & Phillips, A. G. (1989). Conditioned place preference as a measure of drug reward. In J. M. C. Liebman, S. J. Cooper (Eds.), Neuropharmacological basis of reward, (pp. 264-319). New York: Oxford Press.

Cole, B. J., Cador, M., Stinus, L., Rivier, C., Rivier, J., Vale, W., Le Moal, M., & Koob, G. F. (1990). Critical role of the hypothalamic pituitary adrenal axis in amphetamine-induced sensitization of behavior. Life Sciences, 47, 1715-1720.

Conger, J. J. (1956). Reinforcement theory and the dynamics of alcoholism. Quarterly Journal of Studies on Alcohol, 17, 296-305.

Cools, A. R. (1991). Differential role of mineralocorticoid and glucococorticoid receptors in the genesis of dexamphetamine-induced sensitization of mesolimbic, a-1 adrenergic receptors in the ventral striatum. Neuroscience, 43, 419-428.

Cunningham, C. L. (1981). Spatial aversion conditioning with ethanol. Pharmacology Biochemistry & Behavior, 14, 263-264.

Cunningham, C. L. (1995). Localization of genes influencing ethanol-induced conditioned place preference and locomotor activity in BXD recombinant inbred mice. Psychopharmacology, 120, 28-41.

Cunningham, C. L., Hallett, C. L., Niehus, D. R., Hunter, J. S., Nouth, L., & Risinger, F. O. (1991). Assessment of ethanol's hedonic effects in mice selectively bred for sensitivity to ethanol-induced hypothermia. Psychopharmacology, 105, 84-92.

Cunningham, C. L., Malott, D. H., Dickinson, S. D., & Risinger, F. O. (1992). Haloperidol does not alter expression of ethanol-induced conditioned place preference. Behavioral Brain Research, 50, 1-5.

Cunningham, C. L., Niehus, D. R., Malott, D. H., & Prather, L. K. (1992). Genetic differences in the rewarding and activating effects of morphine and ethanol. Psychopharmacology, 107, 385-393.

Cunningham, C. L., Niehus, J. S., & Noble, D. (1993). Species difference in sensitivity to ethanol's hedonic effects. Alcohol, 10, 97-102.

Cunningham, C. L., & Noble, D. (1992). Conditioned activation induced by ethanol: Role in sensitization and conditioned place preference. Pharmacology Biochemistry & Behavior, 43, 307-313.

Cunningham, C. L., & Prather, L. K. (1992). Conditioning trial duration affects ethanol-induced conditioned place preference in mice. Animal Learning and Behavior, 20, 187-194.

de Kloet, E. R., de Kock, S., Schild, V., & Veldhuis, H. D. (1988). Antiglucocorticoid RU 38486 attenuates retention of a behavior and disinhibits the hypothalamic-pituitary-axis at different sites. Neuroendocrinology, 47, 109-115.

de Kloet, E. R., Joëls, M., & Sutanto, W. (1991). Implication of brain corticosteroid receptor diversity for the adaptation syndrome concept. In G. C. Jasmin, M. (Ed.), Methods and Achievements in Experimental Pathology, (1 ed., Vol. 14, pp. 104-132). Basel: Karger.

de Kloet, E. R., & Reul, J. M. H. M. (1987). Feedback action and tonic influence of corticosteroids on brain function: A concept arising from the heterogeneity of brain receptor systems. Psychoneuroendocrinology, 12(2), 83-105.

de Kloet, E. R., Rosenfeld, P., Van Eekelen, J. A., Sutanto, W., & Levine, S. (1988). Stress, glucocorticoids and development. Progress in Brain Research, 73, 101-120.

Deroche, V., Piazza, P. V., Le Moal, M., & Simon, H. (1994). Social isolation-induced enhancement of the psychomotor effects of morphine depends on corticosterone secretion. Brain Research, 640, 136-139.

Deroche, V., Piazza, P. V., Maccari, S., Le Moal, M., & Simon, H. (1992). Repeated corticosterone administration sensitizes the locomotor response to amphetamine. Brain Research, 584, 309-313.

Dexter, R. N., Fishman, L. M., Ney, R. L., & Liddle, G. W. (1967). Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: Studies of the mechanism of action. Journal of Clinical Endocrinology, 27, 473-480.

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Neurobiology, 85, 5274-5278.

Dunn, A. J. (1988). Stress-related changes in cerebral catecholamine and indolamine metabolism: Lack of effect of adrenalectomy and corticosterone. Journal of Neurochemistry, 51(2), 406-412.

Ellis, F. W. (1966). Effect of ethanol on plasma corticosterone levels. Journal of Pharmacology and Experimental Therapeutics, 53, 121-127.

Engel, J. A., Enerback, C., Fahlke, C., Hulthe, P., Hard, E., Johannessen, K., Svensson, L., & Soderpalm, B. (1992). Serotonergic and dopaminergic involvement in ethanol intake. In C. A. Naranjo & E. M. Sellers (Eds.), Novel Pharmacological Interventions for Alcoholism, (pp. 68-82). Berlin: Springer.

Fahlke, C., Engel, J. A., Eriksson, C. J. P., Hård, E., & Söderpalm, B. (1994a). Involvement of corticosterone in the modulation of ethanol consumption in the rat. Alcohol, 11(3), 195-202.

Fahlke, C., Hård, E., Eriksson, C. J. P., Engel, J. A., & Hansen, S. (1995a). Amphetamine-induced hyperactivity: Differences between rats with high or low preference for alcohol. Alcohol, 12(4), 363-367.

Fahlke, C., Hård, E., Eriksson, C. J. P., Engel, J. A., & Hansen, S. (1995b). Consequence of long-term exposure to corticosterone or dexamethasone on ethanol consumption in the adrenalectomized rat, and the effect of type I and type II corticosteroid receptor agonists. Psychopharmacology, 117, 216-224.

Fahlke, C., Hård, E., Thomasson, R., Engel, J. A., & Hansen, S. (1994b). Metyrapone-induced suppression of corticosterone synthesis reduces ethanol consumption in high-preferring rats. Pharmacology Biochemistry & Behavior, 48(4), 977-981.

Gessa, G. L., Muntoni, F., Collu, M., Vargiu, L., & Mereu, G. (1985). Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Research, 348, 201-203.

Gilad, G. M., Rabey, J. M., & Gilad, V. H. (1987). Presynaptic effects of glucocorticoids on dopaminergic and cholinergic synaptosomes. Implications for rapid endocrine-neural interactions in stress. Life Sciences, 40, 2401-2408.

Goeders, N. E., & Geurin, G. F. (1994). Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology, 114, 63-70.

Grenhoff, J., Nisell, M., Ferre, S., Aston-Jones, G., & Svensson, T. H. (1993). Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. Journal of Neural Transmission, 93, 11-25.

Grunberg, N. E., & Baum, A. (1985). Biological commonalities of stress and substance abuse. In S. W. Shiffman, T. A. (Ed.), Coping and Substance Abuse, (pp. 25-62). Orlando: Academic Press, Inc.

Haleem, D. J., Kennett., G., & Curzon, G. (1988). Adaptation of female rats to stress: Shift to male pattern by inhibition of corticosterone synthesis. Brain Research, 458, 339-347.

Hall, E. D. (1982). Glucocorticoid effects on central nervous excitability and synaptic transmission. International Review of Neurobiology, 23, 165-195.

Hall, E. D., & McGinley, P. A. (1982). Effects of a single intravenous glucocorticoid dose on biogenic amine levels in cat lumbar spinal cord. Journal of Neurochemistry, 38, 1787-1790.

Härfstrand, A., Fuxe, K., Cintra, A., Agnati, L. F., Zini, I., Wikström, A., Okret, S., Yu, Z., Goldstein, M., Steinbusch, H., Verhofstad, A., & Gustafsson, J. (1986). Glucocorticoid receptor immunoreactivity in monoaminergic neurons of the rat brain. Proceedings of the National Academy of Science, 83, 9779-9783.

Henkin, R. I. (1975). The role of adrenal corticosteroids in sensory processes. In R. O. G. E. B. Astwood (Ed.), Handbook of Physiology, (Vol. 6, pp. 209-230). Washington D. C.: American Physiological Society.

Hennessy, J. W., King, M. G., McClure, T. A., & Levine, S. (1977). Uncertainty, as defined by the contingency between environmental events, and the adrenocortical response of the rat to electric shock. Journal of Comparative and Physiological Psychology, 91(6), 1447-1460.

Hennessy, J. W., Smotherman, W. P., & Levine, S. (1976). Conditioned taste aversion and the pituitary-adrenal system. Behavioral Biology, 16, 413-424.

Higley, J. D., Hasert, M. F., Suomi, S. J., & Linnoila, M. (1991). Nonhuman primate model of alcohol abuse: Effects of early experience, personality, and stress on alcohol consumption. Proceedings of the National Academy of Science, 88, 7261-7265.

Horger, B. A., Giles, M. K., & Schenk, S. (1992). Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology, 107, 271-276.

Horger, B. A., Shelton, K., & Schenk, S. (1990). Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacology Biochemistry & Behavior, 37, 707-711.

Horger, B. A., Wellman, P. J., Morien, A., Davies, B. T., & Schenk, S. (1991). Caffeine exposure sensitizes rats to the reinforcing effects of cocaine. NeuroReport, 2, 53-56.

Horton, D. J. (1943). The function of alcohol in primitive societies. Quarterly Journal of Studies on Alcohol, 4, 199-320.

Imperato, A., Allegra-Puglisi, S., Casolini, P., & Angelucci, L. (1991). Changes in brain dopamine and acetylcholine release and following stress are independent of the pituitary-adrenocortical axis. Brain Research, 538, 111-117.

Imperato, A., Puglisi-Allegra, S., Casolini, P., Zocchi, A., & Angelucci, L. (1989). Stress-induced enhancement of dopamine and acetylcholine release in limbic structures: Role of corticosterone. European Journal of Pharmacology, 165, 337-338.

Iuvone, P. M., Morasco, J., & Dunn, A. J. (1977). Effect of corticosterone on the synthesis of [3H] catecholamines in the brains of CD-1 mice. Brain Research, 120, 571-576.

Kakihana, R., Noble, E. P., & Butte, J. C. (1968). Corticosterone response to ethanol in inbred strains of mice. Nature, 218, 360-361.

Kalivas, P. W., & Duffy, P. (1989). Similar effects of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. Biological Psychiatry, 25, 913-928.

Keith, L. D., Winslow, J. R., & Reynolds, R. W. (1978). A general procedure for estimation of corticosteroid response in individual rats. Steroids, 31(4), 523-531.

Kelly, P. H., Sevior, P. W., & Iversen, S. D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research, 94, 507-522.

Koob, G. F., & Bloom, F. E. (1988). Cellular and molecular mechanisms of drug dependence. Science, 242, 715-723.

Kubli-Garfias, C., & Whalen, R. E. (1977). Induction of lordosis behavior in female rats by intravenous administration of progestins. Hormones & Behavior, 9(3), 380-386.

Kvetnansky, R., Weise, V. K., Thoa, N. B., & Kopin, I. J. (1979). Effects of chronic guanethidine treatment and adrenal medullectomy on plasma levels of catecholamines and corticosterone in forcibly immobilized rats. Journal of Pharmacology and Experimental Therapeutics, 209(2), 287-291.

Lemaire, V., Deminière, J., & Mormède, P. (1994). Chronic social stress conditions differentially modify vulnerability to amphetamine self-administration. Brain Research, 649, 348-352.

Maccari, S., Piazza, P. V., Deminière, J. M., Angelucci, L., Simon, H., & Le Moal, M. (1991). Hippocampal type I and type II corticosteroid receptor affinities are reduced in rats predisposed to develop amphetamine self-administration. Brain Research, 548, 305-309.

Maccari, S., Piazza, P. V., Deminière, J. M., Lemaire, V., Mormède, P., Simon, H., Angelucci, L., & Le Moal, M. (1991). Life events-induced decrease of corticosteroid type I receptors is associated with reduced corticosterone feedback and enhanced vulnerability to amphetamine self-administration. Brain Research, 547, 7-12.

Mardones, J. (1960). Experimentally induced changes in the free selection of ethanol. International Review of Neurobiology, 2, 41-76.

Markey, K. A., Towle, A. C., & Sze, P. Y. (1982). Glucocorticoid influence on tyrosine hydroxylase activity in mouse locus coeruleus during postnatal development. Endocrinology, 111(1519-1523).

Marlatt, G. A., & Baer, J. S. (1988). Addictive behaviors: Etiology and treatment. Annual Review of Psychology, 39, 223-252.

McEwen, B. S. (1991). Non-genomic and genomic effects of steroids on neural activity. Trends in Pharmacological Sciences, 12(4), 141-147.

McEwen, B. S., de Kloet, E. R., & Rostene, W. (1986). Adrenal steroid receptors and actions in the nervous system. Physiological Reviews, 66(4), 1121-1188.

Mitchell, J. B., & Meaney, M. J. (1991). Effects of corticosterone on response consolidation and retrieval in the forced swim test. Behavioral Neuroscience, 105(6), 798-803.

Moldow, R. L., & Fischman, A. J. (1987). Cocaine-induced secretion of ACTH, beta-endorphin, and corticosterone. Peptides, 8(5), 819-822.

Moore, F. L., & Orchinik, M. (1994). Membrane receptors for corticosterone: A mechanism for rapid behavioral responses in an amphibian. Hormones & Behavior, 28(4), 512-519.

Morin, L. P., & Forger, N. G. (1982). Endocrine control of ethanol intake by rats or hamsters: Relative contributions of the ovaries, adrenals and steroids. Pharmacology Biochemistry & Behavior, 17, 529-537.

Mormède, P., LeMaire, V., Castanon, N., Dulluc, J., Laval, M., & Le Moal, M. (1990). Multiple neuroendocrine responses to chronic social stress: Interaction between individual characteristics and situational factors. Physiology & Behavior, 47, 1099-1105.

Munck, A., Guyre, P. M., & Holbrook, N. J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Reviews, 5(1), 25-44.

Munck, A. & Náray-Fejes-Tóth, A. (1995). Glucocorticoid action. In L. J. DeGroot, M. Besser, H. G. Burger, J. L. Jameson, D. L. Loriaux, J. C. Marshall, W. D. Odell, J. T. Potts, Jr., A. H. Rubenstein, G. F. Cahill, L. Martini, & D. H. Nelson (Eds.), Endocrinology (pp. 1642-1656). Philadelphia: W. B. Saunders Company.

Murphy, B. P., Engelberg, W., & Pattee, C. J. (1963). Simple method for the determination of plasma corticoids. Journal of Clinical Endocrinology, 23, 293-300.

Nash, J. F. J., & Maickel, R. P. (1985). Stress-induced consumption of ethanol by rats. Life Sciences, 37, 757-765.

Natelson, B. H., Tapp, W. N., Adamus, J. E., Mittler, J. C., & Levin, B. E. (1981). Humoral indices of stress in rats. Physiology & Behavior, 26(6), 1049-1054.

Newcomb, M. D., & Harlow, L. L. (1986). Life events and substance abuse among adolescents: Mediating effects of perceived loss of control and meaningless in life. Journal of Personality and Social Psychology, 51, 564-577.

Nicholson, S. A. (1989). Stimulatory effect of caffeine on the hypothalamo-pituitary-adrenocortical axis in the rat. Journal of Endocrinology, 122(2), 535-43.

Orchinik, M. & McEwen, B. S. (1994). Rapid steroid actions in the brain: A critique of genomic and nongenomic mechanisms. In M. Wehling (Ed.) Genomic and Nongenomic Effects of Aldosterone, CRC Series on Pharmacology and Toxicology, (pp. 77-108). Boca Raton, FL.

Orchinik, M., Murray, T. F., & Moore, F. L. (1991). A corticosteroid receptor in neuronal membranes. Science, 252, 1848-1851.

Piazza, P. V., Deminière, J., Maccari, S., Le Moal, M., Mormède, P., & Simon, H. (1991). Individual vulnerability to drug self-administration: Action of corticosterone on dopaminergic systems as a possible pathophysiological mechanism. In P. W. J. Scheel-Kruger & J. Willner, (Eds.), The Mesolimbic Dopamine System: From Motivation to Action, (pp. 473-495). New York: John Wiley & Sons, Ltd.

Piazza, P. V., Deminière, J. M., Le Moal, M., & Simon, H. (1990). Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Research, 514, 22-26.

Piazza, P. V., Deroche, V., Deminière, J., Maccari, S., Le Moal, M., & Simon, H. (1993). Corticosterone in the range of stress-induced levels possesses reinforcing properties: Implications for sensation-seeking behaviors. Proceedings of the National Academy of Science, 90, 11738-11742.

Piazza, P. V., Maccari, S., Deminière, J., Le Moal, M., Mormède, P., & Simon, H. (1991). Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proceedings of the National Academy of Science, 88, 2088-2092.

Piazza, P. V., Marinelli, M., Jodogne, C., Deroche, V., Rougé-Pont, F., Maccari, S., Le Moal, M., & Simon, H. (1994). Inhibition of corticosterone synthesis by metyrapone decreases cocaine-induced locomotion and relapse of cocaine self-administration. Brain Research, 658, 259-264.

Pohorecky, L. A. (1990). Interaction of ethanol and stress: Research with experimental animals-An update. Alcohol & Alcoholism, 25(2/3), 263-276.

Pohorecky, L. A. (1991). Stress and alcohol interaction: An update of human research. Alcoholism: Clinical and Experimental Research, 15(3), 438-459.

Pohorecky, L. A. (1984). Genetic differences in the effect of ethanol on plasma corticosterone and nonesterified fatty acids in rats. Alcoholism: Clinical and Experimental Research, 8(5), 500-504.

Porsolt, R., Le Pichon, M., & Jalfre, M. (1977). Depression: A new animal model sensitive to anti-depressant treatments. Nature Lond., 266, 730-732.

Prasad, C., & Prasad, A. (1995). A relationship between increased voluntary alcohol preference and basal hypercorticosteronemia associated with an attenuated rise in corticosterone output during stress. Alcohol, 12(1), 59-63.

Quirce, C. M., Odio, M., & Solano, J. M. (1981). The effects of predictable and unpredictable schedules of physical restraint upon rats. Life Sciences, 28(17), 1897-1902.

Ratka, A., Sutanto, W., Bloemers, M., & de Kloet, E. R. (1989). On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. Neuroendocrinology, 50, 117-123.

Ratka, A., Sutanto, W., & de Kloet, E. R. (1988). Long-lasting glucocorticoid suppression of opioid-induced antinociception. Neuroendocrinology, 48, 439-444.

Reul, J. M. H. M., & de Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology, 117(6), 25052511.

Revusky, S., & Martin, G. M. (1988). Glucocorticoids attenuate taste aversions produced by toxins in rats. Psychopharmacology, 96, 400-407.

Risinger, F. O., Dickinson, S. D., & Cunningham, C. L. (1992a). Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. Psychopharmacology, 107, 453-456.

Risinger, F. O., Malott, D. M., Prather, L. K., Niehus, D. R., & Cunningham, C. L. (1994). Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. Psychopharmacology, 116, 207-216.

Risinger, F. O., Malott, D. M., Riley, A. L., & Cunningham, C. L. (1992b). Effect of Ro 15-4513 on ethanol-induced conditioned place preference. Pharmacology Biochemistry & Behavior, 43, 97-102.

Rivet, J. M., Stinus, L., Le Moal, M., & Mormède, P. (1989). Behavioral sensitization to amphetamine is dependent on corticosteroid receptor activation. Brain Research, 498, 149-153.

Roberts, A. J., Gallaher, E. J., & Keith, L. D. (1993). Dissociation of the effect of aminoglutethimide on corticosterone biosynthesis from ataxic and hypothermic effects of DBA and C57 mice. Neuroendocrinology, 58(3), 303-309.

Roberts, A. J., Lessov, C. N., & Phillips, T. J. (1995). Critical role for glucocorticoid receptors in stress- and ethanol-induced locomotor sensitization. Journal of Pharmacology and Experimental Therapeutics, 275(2), 790-797.

Roberts, A. J., Phillips, T. J., Belknap, J. K., Finn, D. A., & Keith, L. D. (1995). Genetic analysis of the corticosterone response to ethanol in BXD recombinant inbred mice. Behavioral Neuroscience, 109(6), 1199-1208.

Roth, R. H., Tam, S., Ida, Y., Yang, J., & Deutch, A. Y. (1988). Stress and the mesocorticolimbic dopamine systems. Annals of the New York Academy of Sciences, 15, 138-147.

Rothschild, A. J., Langlais, P. J., Schatzberg, A. F., Miller, M. M., Saloman, M. S., Lerbinger, J. E., Cole, J. O., & Bird, E. D. (1985). The effects of a single acute dose of dexamethasone on monoamine and metabolite levels in rat brain. Life Sciences, 36, 2491-2501.

Rothschild, A. J., Schatzberg, A. F., Langlais, P. J., Cole, J. O., & Bird, E. D. (1983). Dexamethasone elevates dopamine in human plasma and rat brain. Paper presented at the Annu. Meet. Am. Col. Neuropsychopharmacol., San Juan.

Sandi, C., & Guaza, C. (1994). Evidence for nitric oxide-mediated rapid locomotor effects of corticosterone in a novel environment. Annals of the New York Academy of Sciences, 746, 398-399.

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1984). Stress down-regulates corticosterone receptors in a site-specific manner in the brain. Endocrinology, 114(1), 287-292.

Schuckit, M. A. (1984). Differences in plasma cortisol after ingestion of ethanol in relatives of alcoholics and controls: Preliminary results. Journal of Clinical Psychiatry, 45(9), 374-376.

Schuckit, M. A., Gold, E., & Risch, C. (1987). Plasma cortisol levels following ethanol in sons of alcoholics and controls. Archives of General Psychiatry, 44, 942-945.

Schumacher, M. (1990). Rapid membrane effects of steroid hormones: An emerging concept in neuroendocrinology. Trends in Neurosciences, 13, 359-362.

Seidenstadt, R. M., & Eaton, K. E. (1978). Adrenal and ovarian regulation of salt and sucrose consumption. Physiology & Behavior, 21, 313-316.

Shaham, Y., Alvares, K., Nespor, S. M., & Grunberg, N. E. (1992). Effect of stress on oral morphine and fentanyl self-administration in rats. Pharmacology Biochemistry & Behavior, 41, 615-619.

Silva, M. T. A. (1977). Saccharin aversion in the rat following adrenalectomy. Physiology & Behavior, 19, 239-244.

Spyraki, C., Fibiger, H. C., & Phillips, A. G. (1982). Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Research, 253, 185-193.

Stewart, R. B., & Grupp, L. A. (1986). Conditioned place aversion mediated by orally self-administered ethanol in the rat. Pharmacology Biochemistry & Behavior, 24, 1369-1375.

Swerdlow, N. R., Koob, G. F., Cador, M., Lorang, M., & Hauger, R. L. (1993). Pituitary-adrenal axis responses to acute amphetamine in the rat. Pharmacology Biochemistry & Behavior, 45, 629-637.

Sze, P. Y. (1993). Glucocorticoids antagonize the sedative action of ethanol in mice. Pharmacology Biochemistry & Behavior, 45, 991-993.

Tabakoff, B., Jaffe, R. C., & Ritzman, R. F. (1978). Corticosterone concentrations in mice during ethanol drinking and withdrawal. Journal of Pharmacy and Pharmacology, 30, 371-374.

Thiagarajan, A. B., Mefford, I. N., & Eskay, R. L. (1989). Single-dose ethanol administration activates the hypothalamic-pituitary-adrenal axis: Exploration of the mechanism of action. Neuroendocrinology, 50, 427-432.

Thierry, A. M., Tassin, J. P., Blanc, G., & Glowinski, J. (1976). Selective activation of the mesocortical DA system by stress. Nature, 263, 242-244.

Valadez, A., & Schenk, S. (1994). Persistence of the ability of amphetamine preexposure to facilitate acquisition of cocaine self-administration. Pharmacology Biochemistry & Behavior, 47, 203-205.

van der Kooy, D., O'Shaughnessy, M., Mucha, R. F., & Kalant, H. (1983). Motivational properties of ethanol in naive rats as studied by place conditioning. Pharmacology Biochemistry & Behavior, 19, 441-445.

Versteeg, D. H., Van Zoest, I., & de Kloet, E. R. (1984). Acute changes in dopamine metabolism in the medio-basal hypothalamus following adrenalectomy. Experientia, 40(1), 112-114.

Volpicelli, J. R. (1987). Uncontrollable events and alcohol drinking. British Journal of Addiction, 82, 381-392.

Volpicelli, J. R., Ulm, R. R., & Hopson, N. (1990). The bidirectional effects of shock an alcohol preference in rats. Alcoholism: Clinical and Experimental Research, 14(6), 913-916.

Wallis, C. J., Anton, R. F., & Randall, C. L. (1984). Adrenalectomy reduces alcohol-stimulated activity: Blood and brain alcohol content. Pharmacology Biochemistry & Behavior, 20, 883-886.

Wand, G. S., & Dobs, A. S. (1991). Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. Journal of Clinical Endocrinology and Metabolism, 72(6), 1290-1295.

White, P. C., Pescovitz, O. H., & Cutler, G. B. J. (1995). Synthesis and metabolism of corticosteroids. In K. L. Becker, J. P. Bilezikian, W. J. Bremner, W. Hung, C. R. Kahn, D. L. Loriaux, E. S. Nylén, R. W. Rebar, G. L. Robertson, & L. Wartofsky (Eds.), Principles and Practice of Endocrinology and Metabolism, (second ed., pp. 647-662). Philadelphia: J. B. Lippincott Company.

Wise, R. A., & Bozarth, M. A. (1982). Action of drugs of abuse on brain reward systems: An update with specific attention to opiates. Pharmacology Biochemistry & Behavior, 17(2), 239-243.

Wolkowitz, O., Sutton, M., Koulu, M., Labarca, R., Wilkinson, L., Doran, A., Hauger, R., Pickar, D., & Crawley, J. (1986). Chronic corticosterone administration in rats: Behavioral and biochemical evidence of increased central dopaminergic activity. European Journal of Pharmacology, 122, 329-338.

Zarrow, M. X., Aduss, H., & Denison, M. E. (1960). Failure of the endocrine system to influence alcohol choice in rats. Quarterly Journal of Studies on Alcohol, 21, 400-413.