INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

Positive Reinforcement and Conditioned Taste Aversion Induced by Self-Administered Drugs: Are they Related?

Daniel Kunin

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfilment of the Requirements
Of the Degree of Doctor of Philosophy at
Concordia University
Montreal, Quebec, Canada

August 2001

ABSTRACT

Positive Reinforcement and Conditioned Taste Aversion Induced by Self-Administered Drugs: Are they Related?

Daniel Kunin. Ph.D
Concordia University. 2001

The prevalence of alcohol. nicotine and caffeine place these substances among the most widely consumed in the world. Over the years studies have documented a strong positive relationship in the use of these drugs. Increasing attention within both the human and animal literature has been devoted to the study of the potential interactive effects between these recreational substances. The goal of this thesis was to examine the relationship between the positive reinforcing and conditioned taste aversion (CTA)-inducing properties of alcohol. caffeine and nicotine. and in so doing. to further elucidate the nature of the relationship between the phenomena of positive reinforcement and conditioned taste aversion.

Experiments 1a-c and 2a-c showed that acetaldehyde. the putative reinforcing metabolite of ethanol may share common stimulus properties with nicotine and may also mediate the previously observed interaction between nicotine and ethanol in the preexposure CTA paradigm. Experiment 3 demonstrated that caffeine and nicotine shared common stimulus properties in the preexposure CTA procedure. Experiment 4 showed that mecamylamine. the nicotinic acetylcholine receptor antagonist. reversed the nicotine-induced attenuation of caffeine CTA and also blocked the formation of a caffeine-induced

CTA. Experiments 5a and 5b demonstrated that caffeine facilitated the acquisition and maintenance of ethanol drinking in free feeding ethanol naïve animals. Experiments 5c and 5d revealed that the caffeine-induced elevation in ethanol drinking while unrelated to alteration in blood ethanol, might have been related to an increase in corticosterone. Experiments 6a-c showed that caffeine's facilitation of ethanol drinking might have resulted in an increase in the reinforcing efficacy of ethanol as reflected in CTA. Experiments 7a-c indicated that while locomotion responses to an inescapable novel environment appeared to be differentially related to the development of CTAs to amphetamine and morphine in rats it was unrelated to the expression of a LiCl induced CTA. Finally, the results of experiment 8 demonstrated a positive relationship between the amount of saccharin consumed orally and the expression of *c-Fos*-like immunoreactivity (FLI) in the Nucleus of the Solitary Tract suggesting that FLI may be more reactive to positive rather than aversive conditioning effects. Taken together, the studies reported here further shed light on the nature of the relationship between commonly co-used recreational substances and also support the hypothesis that the positively reinforcing and CTA inducing properties of self-administered drugs are indeed related and possibly governed by a shared neurobiological substrate.

Acknowledgements

I have been fortunate to have had the opportunity to work with a number of exceptional individuals who have helped to make this journey most enjoyable and stimulating. I thank Dr Zalman Amit for his advice, constant display of support and encouragement. I would also like to thank Dr Brian Smith for his insights and spirited participation in the many discussions that took place throughout the various stages of this dissertation. A special thanks to Franc, Luc, Maria, Mark, Rick, Stephane, Van and Yuri whose contributions have made this thesis possible.

Table of Contents

Table of Contents (Continued)

Table of Contents (Continued)

List of Figures

List of Figures (Continued)

List of Figures (Continued)

List of Tables

List of Tables (Continued)

Introduction

Multiple drug use of a variety of substances among humans is a frequently observed phenomenon. For example, the use of alcohol, nicotine in the form of tobacco products, and caffeine in the form of coffee consumption, are positively correlated (Kozlowski, 1993). Over the years, increasing attention within both the human and animal literature has been devoted to the study of the potential interactive effects between these recreational substances.

Investigation of the relationship between recreational substances is of much importance to both researchers and clinicians for a number of reasons. First, it has been suggested that the use of one drug may increase the risk in use of a second drug (Madden, Heath, Starmer, Whitfield & Martin, 1995). Second, chronic use of recreational drugs such as smoking and excessive alcohol use is associated with increased risk for some forms of cancer (Brownson & Chang, 1987; Kreek, 1987; Zacny, 1990) and cardiovascular disease (Zacny, 1990), and with increased health and economic costs to society (Kreek, 1987). Third, the use of one drug may make treatment for the dependence of a second drug more difficult and with poorer treatment outcome (Zacny, 1990). In view of the common co-use of alcohol, caffeine and nicotine it would seem important to shed light on possible mechanisms, both behavioral and biochemical, which may help to explain the combined use of these widely available drugs.

Alcohol and Nicotine: Clinical Findings

The co-use of alcohol and nicotine in the form of tobacco products is widespread (Zacny, 1990; Batel et al, 1995). Besides anecdotal reports of the co-use of these drugs, a

large number of clinical and epidemiological studies have confirmed this relationship. For example, it has been reported that roughly 90% of alcoholics smoke (Dresher & Fraser, 1967; DiFranza & Geurerra, 1990; Patten, Martin & Owen, 1996). Smokers also tend to be more likely to use alcohol as compared to non-smokers (Freidman, Siegelaub & Seltzer, 1974). In fact, rates of drinking among non-alcoholic smokers are twice those of non-smokers (Carmody, Brischetto, Matarazzo, O'Donnell, Connor, 1985).

While the precise nature of the relationship between alcohol and nicotine use remains to be determined, the co-relation in the use of these substances is well established (e.g., DiFranza & Geurerra, 1995). One suggestion is that consumption of alcohol may directly influence the effects of nicotine, thereby increasing or decreasing cigarette smoking (Griffiths, Bigelow & Liebson, 1976). In fact, a number of researchers have found that exposure to alcohol increases the frequency of smoking behaviour (Griffiths et al, 1976; Mitchell, deWit & Zacny, 1995; Nil, Buzzi & Battig, 1984). However, it is also possible that the use of nicotine may alter the psychopharmacological effects of alcohol and consequently it's ingestion.

In view of the apparent co-occurrence in the use of these two drugs in humans, it may be of great importance to elucidate the nature of the interaction between these drugs of abuse. A number of studies have attempted to examine the nature of the interaction between these drugs using a variety of behavioral paradigms. For example, the potentiating effects of these drugs have been reported in studies assessing locomotor activity (Blomqvist, Soderpalm & Engel, 1992; Shaefer & Michael, 1992). In addition, nicotine has been shown to produce differential effects on locomotion in rats selectively bred for ethanol preference with depression of activity seen only in non-alcohol

preferring (NP) rats as opposed to alcohol preferring (P) rats that showed no locomotion depression (Gordon, Meehan, Schechter, 1993).

Drug discrimination procedures in animals are a commonly used vehicle to identify the receptor systems implicated in mediating the subjective effects of a variety of drugs. With this procedure, animals can be trained to press one of two levers to obtain food after receiving injections of a drug, and to press the other lever to obtain food after injections of the vehicle (Redila, Smith & Amit, 2000). After the discrimination has been learned, the animal starts pressing the appropriate lever according to whether it has received the training drug or vehicle (Redila et al., 2000). Once stimulus control has been achieved, a substitution (administration of other drugs) test can be performed (Redila et al., 2000). Such studies have demonstrated that rats trained to discriminate the presence or absence of ethanol showed increased ethanol appropriate responses when pretreated with nicotine (Signs & Schechter, 1986). Nicotine has also been shown to partially substitute for the ethanol stimulus in ethanol-preferring (P) rats but not in non-preferring (NP) rats (Gordon, Meehan, Schechter, 1993). Using a preexposure conditioned taste aversion (CTA) procedure, Kunin, Smith and Amit (1999b) reported that preexposure to alcohol or nicotine produced symmetrical attenuation effects on the development of CTA to the opposing agent. Taken together, these studies suggested that nicotine and ethanol shared overlapping psychopharmacological properties.

Recently, Kunin, Smith and Amit (1999b) assessed whether nicotine's ability to augment ethanol drinking occurred because of a nicotine-induced attenuation of ethanol's aversive effects. The authors reported an asymmetrical interaction between ethanol and nicotine in the conditioned taste aversion (CTA) paradigm where nicotine completely

blocked an ethanol-induced CTA while ethanol merely attenuated a nicotine-induced CTA. It was suggested that nicotine's ability to antagonize ethanol's "aversive" effects reflected self-medication whereby one agent ameliorates or neutralizes the effects of a second agent (Zacny, 1990). Interestingly, there have been several studies with humans that have demonstrated that the psychomotor impairing effects of ethanol are reduced by smoking tobacco cigarettes (Leigh & Tong, 1976; Leigh, 1982; Tong, Knott, McGraw, Leigh, 1974).

Another possible explanation for the frequent co-use of alcohol and nicotine in the form of tobacco smoking may reflect the fact that the use of one drug (e.g., nicotine) may increase the positive reinforcing efficacy of a second drug (e.g., alcohol). Although a number of human studies have examined the effects of alcohol intake on cigarette smoking (see Zacny, 1990 for review), the question of whether using alcohol and nicotine in combination increases the rewarding effects of these drugs in humans has rarely been examined. In one study (Glautier, Clements, White, Taylor & Stolerman, 1996), subjects reported greater subjective enjoyment of cigarettes during alcohol consumption but this was not reflected in greater cigarette preference.

While there is a lack of experimental studies with humans demonstrating that alcohol and nicotine in combination increase the rewarding effects of these drugs, there are several studies with rats demonstrating that nicotine potentiates alcohol self-administration. Potthoff, Ellison and Nelson (1983), assessed the direct effect of nicotine treatment on alcohol intake in rats and demonstrated that chronic administration of nicotine through osmotic minipumps, enhanced ethanol consumption in experienced ethanol drinkers. One limitation of this study was that ethanol drinking was not assessed

after the cessation of nicotine treatment. Moreover, these authors failed to include an appropriate non-drug treated control group. However, these findings were recently confirmed in a study by Smith, Horan, Gaskin and Amit (1999) demonstrating that nicotine enhanced ethanol drinking when ethanol was presented to rats in a free choice and ascending concentrations (2-10%) in a 1-hr limited access procedure.

Only recently have attempts been made by researchers to elucidate a possible neuronal mechanism by which these two drugs may interact. One mechanism that has been proposed to account for the interaction between ethanol and nicotine involves activity at the nicotinic-acetylcholine receptor (nAChR). For example, central nicotine administration has been reported to attenuate ethanol-induced motor in coordination in mice and was antagonized by nicotinic receptor antagonists (Dar, Li & Bowman, 1993). It was also demonstrated that the central nAChR antagonist mecamylamine antagonized the effects of nicotine on ethanol induced stimulatory effects in mice (Blomqvist et al., 1992). Finally, ethanol induced activation of the mesolimbic dopamine system was attenuated by local infusions with mecamylamine (Blomqvist, Ericson, Engel & Soderpalm, 1997).

Blomqvist, Ericson, Johnson, Engel and Soderpalm (1996) reported that subcutaneous injections of nicotine in rats increased their intake of a 6% ethanol solution. In the same study it was reported that blockade of central nAChR with mecamylamine resulted in both a decrease in ethanol consumption and also attenuated the observed nicotine induced increase in ethanol drinking. This finding was confirmed by Smith et al., (1999) who reported that pretreatment with mecamylamine attenuated a nicotine-induced enhancement in ethanol drinking. Recently, Ericson, Engel and Soderpalm, (2000)

reported that daily injections with different nicotinic drugs, both central and peripherally acting (e.g., nicotine, mecamylamine, hexamethonium) for a period of 15 days increased 6% ethanol drinking in rats. These results were somewhat surprising because neither antagonist prevented the nicotine effect on ethanol drinking previously observed, and, both antagonists themselves increased ethanol drinking, an effect that was similar to that seen with nicotine.

The Relationship Between Caffeine and Nicotine

The prevalence of nicotine in the form of tobacco smoking and caffeine in the form of coffee drinking place these drugs among the most widely consumed licit drugs in the world (Garrett & Griffiths, 2001). Over the past several years, a number of studies have documented a strong positive relationship in the concurrent use of these drugs (Budney & Higgins, Hughes, Bickel, 1993; Istvan & Matarazzo, 1984; Swanson, Lee & Hopp, 1994). Moreover, there is evidence that caffeine can interact with nicotine's pharmacological effects and modify the frequency of cigarette smoking (Chait & Griffiths, 1983; Emurian, Nellis, Brady & Ray, 1982).

There are a number of studies, employing laboratory animals, that have examined interactions between caffeine and nicotine and their effects (e.g. Cohen, Welzel & Battig, 1991; White, 1988) on a number of behaviors including food-maintained behavior and locomotor activity. White (1988) found that the co-administration of graded doses of nicotine with a 3-mg/kg dose of caffeine produced increases in response rate on a fixed-interval (FI) schedule of food reinforcement. Higher doses of caffeine, which by themselves failed to alter FI response rates eliminated the rate increasing effects of

nicotine on FI responding. Cohen et al.. (1991) reported that acute administration of caffeine to naive rats abolished the depressant effect of a co-administered dose of nicotine. This was in contrast to nicotine-tolerant rats co-administered nicotine and caffeine that showed increases in locomotion beyond control levels. It is noteworthy that nicotine initially produces locomotor depression in nicotine naive animals but stimulates locomotor activity with repeated exposure to the drug (Clarke & Kumar. 1983).

Studies involving drug discrimination procedures have reported that caffeine and nicotine do not have common drug discriminative properties. For example. it has been demonstrated that caffeine fails to generalize to the nicotine cue in rats trained to discriminate nicotine from saline (Gasior. Shoaib.Yasar. Jaszyna & Goldberg. 1999). Similarly. it has been reported that nicotine fails to generalize to the caffeine cue in rats trained to discriminate caffeine from saline (Modrow. Holloway & Carney. 1981). On the basis of these studies it can be concluded that caffeine and nicotine do not share drug discriminative properties in. at least. classic drug discrimination procedures in rats.

While anecdotal and empirical studies suggest a strong positive correlation in the use of caffeine and nicotine (Budney et al.. 1993: Emurian et al.. 1982: Istvan & Matarazzo. 1984: Kozlowski et al.. 1993: Marshall. Green. Epstein. Rogers & McCoy. 1980: Parsons & Neims. 1978) there is no convincing support for the notion that the effects of these drugs are causally related. However. Emurian et al.. (1982) reported that cigarette smoking occurred more frequently during a 20-min period following coffee consumption than during a 20 min prior to its consumption. Likewise. Marshall et al.. (1980) reported that the consumption of coffee increased the number of cigarettes smoked.

Recently, Shoaib, Swanner, Yasar, and Goldberg (1999) reported that caffeine increased nicotine self-administration in rats. In their study, rats consuming caffeine (3 mg/ml) for 7 days in their drinking water prior to the start of nicotine self-administration acquired intravenous nicotine self-administration (0.03 mg/kg per infusion) more quickly than controls with access to tap water. In a follow-up cross-over design, caffeine was removed from drinking water and rates of nicotine self-administration returned to baseline whereas adding caffeine to the drinking water of animals that had previously received water served to increase their responding for nicotine. Based on these findings, the authors concluded that caffeine enhanced the putative reinforcing effects of nicotine thereby accelerating the acquisition of nicotine self-administration. It is noteworthy that the animals that had ingested caffeine throughout the course of the experiment also showed substantial weight loss compared to control rats. Given that caffeine has anorectic effects (Shoaib et al., 1999), it is possible that caffeine may indirectly promote responding for nicotine by promoting weight loss. Such a notion is consistent with reports that food deprivation may promote drug self-administration (Carroll & Meisch, 1984). An alternative interpretation of this data is that chronic caffeine ingestion may have produced a heightened state of hyperactivity thereby resulting in greater lever pressing for nicotine infusions.

While the mechanism mediating caffeine's facilitory action of nicotine self-administration remains to be elucidated, there are a number of putative explanations for this effect, which may involve alterations in the pharmacokinetics of nicotine or caffeine (Emurian et al., 1982), changes in nicotinic receptor binding by chronic caffeine administration (Shi, Nikodijevic, Jacobson & Daly, 1993) anorectic effects of caffeine

indirectly enhancing responding for nicotine as has been observed for other self-administered drugs (Carroll & Meisch, 1984) and or simply enhancing the hyperactivity of the animals and thus making them more likely to perform more responses.

Caffeine and Alcohol Interaction

Much of the interest in studying the interactive effects of caffeine and alcohol is based on anecdotal reports that individuals who are heavy alcohol consumers are also heavy coffee drinkers. In general it appears that while alcohol consumption is correlated with caffeine intake in humans, the correlation is only robust if either drug is used heavily (see Istvan & Matarazzo, 1984 for review). For example, Klatsky, Friedman, Siegelaub and Gerard (1977) reported that across a variety of ethnic groups, heavy alcohol drinkers ($\geq$ 6 drinks per day) were twice as likely as non-drinkers (26% vs. 13%) to be heavy coffee drinkers ($\geq$ 6 drinks per day).

One popular belief is that heavy alcohol users consume more caffeine only to counteract the behavioral depressant effects associated with heavy alcohol use. This idea that caffeine and alcohol could produce counteracting effects on the central nervous system has been examined in a number of human studies (e.g., Azcona, Barbanoj, Torrent & Jane 1995; Fudin & Nicastro, 1988). In general, the studies examining this issue have yielded contradictory findings and have demonstrated antagonistic effects between these agents in few cases (Fudin & Nicastro, 1988). The reasons for the discrepancy in results are likely due to the diversity in doses and methods employed and also the order and time intervals of the two treatments. While most studies report that caffeine is frequently given following alcohol, the interval between their administration ranged from drugs taken

simultaneously to about 50 minutes (Fudin & Nicastro, 1988). Osborne and Rogers (1983) found that 150 mg of caffeine and 0.8 g/kg of ethanol produced impairments in reaction time when administered together. In contrast, Franks, Hagedorn, Hensley and Starmer (1975) showed that caffeine (300 mg) counteracted impairment in cognitive tasks and motor behavior resulting from alcohol treatment (0.75 g/kg). More recently, Azcona, Barbanoj, Torrent and Jane (1995) showed that the administration of alcohol 0.8 g/kg and caffeine (400 mg) in combination with each other resulted in antagonistic effects on a number of cognitive and psychomotor performance tasks.

Studies assessing the effects caffeine and ethanol combinations on various behaviors in laboratory mice have also yielded mixed results. For example, some studies have reported that caffeine increases the ataxic effects produced by ethanol (Dar, Jones, Close, Mustafa & Wooles, 1987) whereas other studies have reported that caffeine attenuated the loss of motor coordination produced by ethanol (Dar, 1988).

While the use of alcohol appears to be correlated with coffee intake among those who are already heavy users of either alcohol or coffee (Istvan & Matarazzo, 1984) relatively few studies have attempted to determine whether this relationship is causal in nature. There are studies using laboratory rats that have attempted to examine the effects of caffeine on voluntary ethanol ingestion but they have been largely unreliable. For example, while caffeine has been shown to facilitate ethanol drinking in food deprived animals (Gilbert, 1976; Gilbert, 1979) interpretation of these findings is problematic as they are confounded by the fact that food deprivation may itself enhance the self-administration of drugs (Carroll & Meisch, 1984). In addition, caffeine has been shown to decrease ethanol intake but within dose ranges (e.g., 50 mg/kg) producing toxic effects

(Dietze & Kulkosky, 1991; Hederra, Aldunate, Segovia-Riquelme & Maradones, 1975). Thus, these studies not withstanding, it still remains to be determined whether caffeine can directly influence ethanol drinking within dose ranges that are behaviorally relevant in humans. Moreover, a putative mechanism by which such an interaction may occur is yet to be elucidated.

Conditioned Taste Aversion

One of the unique features of self-administered drugs including caffeine, nicotine and alcohol is their ability to support conditioned taste aversion (CTA). In fact, the study of the stimulus properties of self-administered drugs within the context of the CTA paradigm evolved from earlier work in which CTA was employed primarily as a method of studying unique associative learning processes in animals. Garcia, Kimmeldorf and Koelling (1955) are credited with conducting the first experimental analysis of behavior involving the CTA procedure. In their pioneering study, laboratory rats were presented with a novel tasting saccharin fluid (conditioned stimulus) followed by exposure to x-irradiation (unconditioned stimulus). On a later date the same rats were offered the saccharin-flavored fluid and displayed a complete avoidance of it after merely a single pairing with x-irradiation. This was in contrast to control rats that were not exposed to the pairing of x-irradiation with the flavored solution and displayed no avoidance of the saccharin solution. Traditionally it was believed that this reduced preference of the once novel tasting fluid reflected a conditioned taste aversion (Garcia et al., 1955). Furthermore, it was believed that this reduction in fluid preference reflected an

association between the novel tasting fluid and the aversive quality of the conditioning drug (Goudie, 1979).

In contrast to the rapid acquisition of CTA, it was found to be more difficult to train rodents to learn a spatial avoidance response to a distinctive environment paired with x-irradiation (Garcia, Kimmeldorf & Hunt, 1957). In fact, it was believed that rodents were innately "prepared" to associate gustatory cues rather than non-gustatory cues with sickness (Seligman, 1970). Taken together, the interest shown in CTA was based on the relatively rapid rate of acquisition and selective associability of gustatory cues with toxic consequences. Furthermore, unlike other traditional forms of learning, CTA could occur after a single trial and with a long delay between two stimulus events (Etscorn & Stephens, 1973; Revusky & Garcia, 1970). It is worth noting that the robustness of CTA is underscored by the finding that rats will acquire taste aversion even when they are anaesthetized during presentation of the US as well as after US presentation (Buresova & Bures, 1977; Roll & Smith, 1972). In general, these data helped to establish CTA as a relatively unique form of learning.

The nature of the conditioned response in CTA learning

Garcia, Hankins and Rusiniak (1974) proposed that CTA reflected a shift in the hedonic value of the conditioned gustatory stimulus (e.g., saccharin) reflecting a conditioned nausea response. Garcia's conditioned nausea explanation was based on his observations of the behaviour of rats that had encountered taste cues previously associated with poisoning. In general, these animals demonstrated behaviours that could be characterized as "disgust" reactions. Grill and Nogren (1978) subsequently developed

the taste reactivity (TR) test. which measures a rat's orofacial and somatic response to flavours that are infused directly into its mouth. In general. rats that receive a palatable fluid will respond with tongue protrusions. mouth movements and paw licking. Conversely. rats infused with unpalatable solutions (e.g. quinine) respond by chin rubbing. gaping. paw treading and fluid rejection. Berridge. Grill and Nogren (1981). examined the pattern of ingestive behaviours for lithium paired sucrose-flavoured solutions compared to unpaired control animals and reported that while the orofacial response patterns of lithium-paired animals receiving sucrose resembled those of animals receiving quinine. control animals demonstrated no apparent aversive reactions to the sucrose solution. These findings provided support for Garcia's proposal that CTA reflected a shift in the hedonic response to the taste stimulus.

Does CTA really reflect an index of toxicity or conditioned nausea?

The adequacy of the model of CTA as an index of conditioned illness has been challenged by a number of studies including that of Berger (1972). who first demonstrated that behavioral measures of drug induced toxicity correlated poorly with the strength of conditioned taste aversions. That is. a variety of psychoactive drugs including amphetamine. scopolamine and chlordiazepoxide induced strong taste aversions at doses that produced no observable signs of illness. It was subsequently demonstrated that several highly toxic substances (e.g.. strychnine and cyanide) induced weak or no aversions to a saccharin flavored solution paired with these agents (Ionescu & Buresova. 1977: Nachman & Hartley. 1975). More recently it was reported that treatment with the anti-nausea agent ondansetron failed to attenuate a LiCl-induced CTA in a

sensitive two-bottle test (Limebeer & Parker. 2000). Finally, a large number of studies have demonstrated CTAs to a wide variety of positively reinforcing drugs such as amphetamine, cocaine, morphine, alcohol, barbituates, cannabis and more within dose ranges normally self-administered by laboratory rats (e.g., Cappell & LeBlanc, 1973; Cappell, LeBlanc & Endrenyi, 1973; Goudie, Dickens & Thornton, 1978; Sklar & Amit, 1977; Vogel & Nathan, 1975). Together, these findings suggested that an explanation of CTA based upon illness, toxicity or nausea was neither necessary nor sufficient for the establishment of CTA.

The Paradox of Self-Administered Drugs

The observation that drugs, which are self-administered by laboratory rats, and therefore presumed to possess positively reinforcing properties, are also endowed with the capacity to induce CTA has been termed "paradoxical" (Goudie, 1979; Hunt & Amit, 1987). These observations were considered paradoxical because they challenged the conventional wisdom of hedonic drug properties. As was suggested elsewhere (e.g., Goudie, 1979; Hunt & Amit, 1987), the paradoxical nature of CTAs to positively reinforcing drugs can be conceptualized as contradicting the prediction that flavours associated with positively reinforcing drugs should become preferred and not avoided.

This apparent paradox was underscored by a set of landmark experiments demonstrating that injections of self-administered drugs could simultaneously produce reinforcement and aversion. Wise, Yokel and DeWit (1976) trained rats to lever press for injections of apomorphine after having ingested a novel-tasting saccharin solution. Subsequently, when tested, it was shown that the rats avoided the saccharin solution but

also maintained bar pressing for apomorphine injections. In a related study, White, Sklar and Amit (1977) demonstrated that injections of morphine given to rats after they had run down an alleyway for food reward increased their running speed down the alleyway on subsequent trials but also induced a CTA to the flavoured food consumed in the goal box prior to the morphine injections. Switzman, Amit, White and Fishman (1978) subsequently demonstrated that the two effects were positively correlated. That is, rats that showed the greatest increase in running speed also showed the most profound CTA.

This paradox has led to a number of studies that have attempted to explain the seemingly contradictory observation that the same presentation of a positively reinforcing drug such as morphine and or amphetamine can induce CTAs and act simultaneously to produce positive reinforcement in the same animal (e.g., White et al., 1977). Attempts to explain the nature of CTA to positively reinforcing drugs have not been entirely successful. In general, the capacity of positive reinforcing drugs to produce CTA has been found to be unrelated to other behavioral effects of these same drugs (Hunt & Amit, 1987). For example, amphetamine CTA has been found to be unrelated to amphetamine's anorectic effects (Stolerman & D'Mello, 1978). In addition, CTA to hypnotics (e.g. phenobarbital) has been found to be unrelated to their disabling motoric effects (Vogel & Nathan, 1976). Moreover, the capacity of opioids to elicit CTA has been found to be unrelated to their analgesic effects (Stewart & Eikelbloom, 1978; Switzman, Hunt & Amit, 1981). Finally, the CTA inducing capacities of psychoactive drugs do not appear to be correlated with their duration of action (D'Mello, Goldberg, Goldberg & Stolerman, 1981).

On the other hand, there is some evidence to suggest that the rate of onset of a drug's action may be related to its capacity to elicit CTA. For example, relative to heroin, morphine, which has a slower rate of onset, appears to yield a stronger CTA (Switzman, Hunt & Amit, 1981), this despite the fact that these two drugs are pharmacologically identical once they reach the brain. Taken together, while there does not appear to be a correlation between the CTA inducing properties of positively reinforcing drugs and their other known behavioral effects, there is some suggestion that the rate of onset of a drug's action may be related to its potential as a CTA inducing agent.

One earlier proposition suggested that control (i.e., experimenter administration vs. self-administration) over the drug administration could be critical in determining why drugs have opposing actions. For example, Vogel and Nathan (1975) suggested that the "aversive" effects of reinforcing drugs reflected the fact that animals could not control their drug experience. However, this hypothesis was challenged on the basis of a study demonstrating that CTA can occur while animals self-administer the same drug (Wise et al., 1976). Moreover, stimuli associated with experimenter-administered injections of positively reinforcing drugs can support conditioned positive reinforcement (Bardo & Bevins, 2000).

Procedural differences between CTA and self-administration paradigms involving route of drug administration have also been thought to play a role in distinguishing between the different effects of positively reinforcing drugs. However, the same route of drug administration has been shown to simultaneously induce CTA and self-administration in the same animal (Wise et al., 1976).

Evidence for the involvement of positive reinforcement in the mediation of CTA to positively reinforcing drugs

To date, no theory has been able to fully account for the finding that a given self-administered drug can elicit both positive reinforcement and CTA. However, the observation that positively reinforcing drugs can also support CTAs has led some researchers to propose that CTAs to positively reinforcing drugs may actually reflect their rewarding nature (e.g., Hunt & Amit, 1987). A large body of data has and continues to point in this direction (e.g., Grigson, 1997).

Overlap in the neurochemical systems mediating the expression of CTA and positive reinforcement

The putative involvement of similar neurochemical systems in the mediation of positive reinforcement and CTA has added support for the notion that these apparently discrete drug effects are functionally related. In fact, a large body of data (e.g., Goudie, Thornton & Wheatley, 1975; Roberts & Fibiger, 1975; Sklar & Amit, 1977) supports the view that central catecholamine systems, implicated in the mediation of drug self-administration to a variety of drugs including morphine, amphetamine and ethanol, are also involved in the induction of CTA to these same drugs. Earlier demonstrations of a relationship between catecholamine systems and CTA, showed that catecholamine depletion produced by injections of alpha-methyl-para-tyrosine (an inhibitor of tyrosine hydroxylase) blocked the formation of CTA induced by amphetamine, morphine and ethanol but not lithium chloride (Goudie, Thornton & Wheatley, 1975; Sklar & Amit, 1977). Catecholamine depletions resulting from ICV administrations of the neurotoxin 6-

hydroxydopamine (6-OHDA) have also been shown to disrupt CTA to amphetamine but not to LiCl (Roberts & Fibiger. 1975). Wagner. Foltin. Seiden and Schuster (1981) demonstrated that selective depletion of dopamine resulting from ICV 6-OHDA administration in combination with desmethylimipramine. a noradrenergic uptake blocker. attenuated a CTA induced by methylamphetamine but not LiCl. Sklar and Amit (1977) demonstrated that depletion of brain norepinephrine by administartion of FLA-57. a dopamine beta-hydroxylase inhibitor. attenuated a CTA induced by ethanol and morphine but not LiCL. Pimozide. a dopamine receptor antagonist. has also been found to block CTA to a variety of self-administered drugs. Sklar and Amit (1977) reported that pretreatment with pimozide selectively attenuated CTA to ethanol and morphine but not to LiCl. Pimozide has also been shown to attenuate CTA to amphetamine as well as cocaine (Grupp. 1977: Hunt. Switzman & Amit. 1985). Taken together. the latter evidence supports the view that central catecholamine systems appear to be involved in mediation of CTA to a variety of self-administered drugs. Likewise. these same catecholamine systems have also been implicated in self-administion of the very same drugs (Wise. 1980: Phillips. 1984).

Central cholinergic systems have also been implicated in the mediation of CTA and self-administration of a variety of positively reinforcing drugs. Pretreatment with atropine. a centrally acting cholinergic receptor antagonist has been shown to attenuate a morphine induced CTA. enhance an amphetamine CTA and produce no effect on a LiCl induced CTA (Hunt. Segal & Amit. 1987). In a direct parallel to these findings. Davis and Smith (1975) demonstrated that atropine decreased morphine self-administration but enhanced amphetamine self-administration.

Qualitative differences in the nature of CTAs induced by positively reinforcing drugs and by agents with no established properties of positive reinforcement

There are a number of behavioral findings reported within the CTA literature that support a distinction between the CTA's induced by positively reinforcing drugs and CTAs induced by drugs with no reported properties of positive reinforcement (Hunt & Amit, 1987). In contrast to CTA's induced by non-self-administered drugs (e.g., emetic agents) which typically exhibit a linear dose-response function, CTA's induced by positively reinforcing drugs tend to yield a curvilinear dose-response relationship (Farber et al. 1976; Nachman & Ashe, 1973b). It has also been shown that the relative magnitude of CTA is greater for emetic substances than for positively reinforcing drugs (Farber et al. 1976; Nachman & Ashe, 1973b). Similarly, CTA's induced by positive reinforcing drugs appear to show greater variability in strength of CTA produced within a group of subjects being tested (Riley, Jacobs & Lolordo, 1978). This is in contrast to reports that CTA's induced by emetic agents such as LiCl result in greater within group consistency. Finally, CTA's to positively reinforcing drugs compared to emetic agents are more likely to be attenuated by prior exposure to the same drug (Farber et al. 1976; Hunt & Amit, 1987; Switzman, Fishman & Amit, 1981).

Another distinctive feature characterizing CTAs induced by self-administered drugs when compared to emetic agents is derived from a recent study examining the effects of stress on CTA. Bowers, Gingras and Amit (1996) assessed the effects of footshock on amphetamine CTA at different time parameters. In their study, footshock was administered either 30 minutes prior to conditioning with amphetamine or several days prior to saccharin-amphetamine pairing. The authors reported that while shock delivered

30 minutes prior to conditioning had no effect in attenuating an amphetamine CTA. more distal shock pre-exposure (2 and 4 days pre-exposure) increased the magnitude of amphetamine CTA and retarded its extinction. These results were in contrast to findings demonstrating that pretreatment with shock attenuated a LiCl induced CTA under conditions that do not influence amphetamine CTA (Revusky & Reilly, 1989). It is notable that shock pretreatment has also been demonstrated to have no effect on a morphine CTA under the same conditions that attenuate a LiCl CTA (Revusky & Reilly, 1989). Together, these results support the notion that CTAs resulting from emetic agents such as LiCl are differentially influenced by the application of stress as compared to their effects on CTA's induced by self-administered drugs like amphetamine and morphine.

It is well-documented that food deprivation will increase the likelihood of self-administration to a variety of drugs (Carroll & Meisch, 1984; Carroll, France & Meisch, 1979; Meisch & Kliner, 1979). It has been suggested that rather than self-administer drugs in order to compensate for decreased calories, food deprived rats self-administer drugs at a higher rate because of an increased reinforcing efficacy of the drug during the food deprived state (Carroll, France & Meisch, 1979). The results of a recently conducted study reveal that food deprivation will also modulate the acquisition of a CTA induced by amphetamine and chlordiazepoxide but not LiCl (Bell, Thiele, Seeley, Bernstein & Woods, 1998). In this study, rats were either food deprived (maintained at 80% free-feeding body weight) or had access to food ad lib and then conditioned with the above mentioned drugs. It was shown that rats that had been food deprived at the time of conditioning exhibited an attenuated CTA relative to those rats that had not been food

deprived. It is notable that differences were only observed when a rewarding drug (e.g., amphetamine) was used as the conditioning agent.

A growing body of data has demonstrated that the orofacial reactions of rats that have ingested flavors paired with positively reinforcing drugs also differ qualitatively from their reactions when conditioned with agents possessing no established properties of positive reinforcement (Parker, 1995). Parker (1982) compared the orofacial reactions of rats who had ingested flavors that were paired with the LiCl or amphetamine and showed that while flavors paired with amphetamine failed to elicit conditioned rejection responses, flavors paired with LiCl did. The different reactions observed in animals conditioned with these substances was not a result of the putative differences in the strength of flavor-drug associations because both drugs produced CTAs that were of equal magnitude.

Subsequent investigations using the taste reactivity procedure have lent support to the notion that orofacial responses resulting from the ingestion of flavors paired with a variety of positively reinforcing drugs when compared with non-reinforcing drugs differ quantitatively. For example, nicotine (Parker, 1991; Parker & Carvell, 1986) morphine (Parker, 1988; Parker, 1991), cocaine (Parker, 1993), and methamphetamine (Parker, 1993) fail to elicit aversive orofacial responding within dose ranges that serve to produce positive reinforcement. However, high doses of nicotine will yield aversive orofacial responses but at doses producing place aversion (Jorenby, Steinpreis, Sherman & Baker, 1990). Taken together, these latter observations support the notion that taste aversions induced by positively reinforcing drugs are qualitatively distinct from CTA's induced by

emetic substances such as LiCl and do not simply reflect the aversive or illness inducing properties of these drugs.

The Nature of CTA to positively reinforcing drugs

One explanation for the nature of CTA to positively reinforcing drugs may be related to the novelty of the drug state (Amit & Baum, 1970; Gamzu, 1977; Hunt & Amit, 1987) which is to be distinguished from viewing CTA to positively reinforcing drugs as reflecting aversion per se. The novelty hypothesis posits that CTA to positively reinforcing drugs may be indicative of fear induced by a novel drug state, which, after a number of experiences, habituates (Amit & Baum, 1970). This interpretation suggests that CTA's induced by drugs possessing properties of positive reinforcement are a function of the internal stimulus complex provided by the drug, which may also include those same stimulus properties that can be positively reinforcing (Gamzu, 1977). Indeed, there are a large number of studies already cited in this thesis that would support the view that the rewarding and CTA inducing properties of drugs are inextricably tied (e.g., Hunt & Amit, 1987).

Hunt and Amit (1987), suggested that the reduction in the consumption of the CS following pairing with a positive reinforcing US could be more accurately described as taste shyness. According to the taste shyness hypothesis, taste specific responses to a drug stimulus, result in a conservative consummatory response, signalling possible danger to the rat and subsequent avoidance of the fluid, which is to be distinguished from viewing CTA as reflecting aversion. The taste shyness hypothesis serves to provide a possible explanation for the paradoxical nature of CTA's to positively reinforcing drugs.

An alternative explanation of the nature of CTA's to positively reinforcing drugs has been suggested to reflect reward comparison (Grigson, 1997). According to this view, the suppression of a CS (e.g., saccharin) following repeated pairings with a positively reinforcing drug (e.g., morphine) occurs because the value of the gustatory CS is outweighed by the availability of a highly preferred psychoactive drug. It is notable that the "Reward Comparison Hypothesis" (Grigson, 1997) cannot account for all instances of CTA induced by positive reinforcing drugs. For example, the "Reward Comparison Hypothesis" cannot account for the observation that US drug preexposure will block CTA to the same drug. In fact, there is evidence that the US preexposure effect with some positively reinforcing drugs may reflect drug sensitization where multiple preexposure injections with the US increase the reinforcing efficacy of the US drug thereby attenuating the drug's aversive effects (Gaiardi, Bartoletti, Bacchi, Gubellini, Costa, & Babbini, 1991). According to the Reward Comparison Hypothesis, drug preexposure should augment CTA to the US because of the enhanced rewarding efficacy of the US drug. Also, while Grigson (1997) infers that "the hedonic value of saccharin pales in comparison to a positively reinforcing drug" she never directly compares their hedonic values and therefore the inference is not really warranted.

Physiology of CTA: The role of emetic mechanisms

Early interpretations of CTA supported the notion that CTA's induced by stimuli such as x-irradiation and LiCl reflected the emetic properties of these agents (Garcia & Koelling, 1966). CTA's were thought to result from a selective association between taste and sensations of visceral, particularly gastrointestinal sickness that appeared to make

taste stimuli less palatable. According to Garcia and Ervin (1968), agents induced taste aversion to the extent that they act upon a particular internal neural system that mediated visceral, gastrointestinal distress. This was in contrast to external stimuli (e.g., light, shock) that provided input to an alternative neural system that regulates interactions with the external environment.

In formulating their emetic-UCS hypothesis, which holds that CTA occurs via activation of pathways that mediate emesis (vomiting), Garcia and Colleagues (1977) relied on a model of emesis developed by Borison and Wang (1953) who proposed two major inputs to the emetic center, an area that is situated in the lateral reticular formation adjacent to the Nucleus of the Solitary Tract (NTS). The first route is by gastric irritation where information is conveyed through the vagus nerve and is then transmitted via vagal afferents to the NTS and from there to the emetic center, triggering emesis (Grant, 1987). A second route in which a stimulus can elicit emesis is blood borne. Here, toxins may enter the blood, but are then detected by the Area Postrema (AP), the site of chemoreceptors for detection of circulating toxins, otherwise known as the trigger zone (Grant, 1987). The AP, which is located on the floor of the fourth ventricle, is suited for detecting blood-borne toxins because it is not shielded by the blood-brain barrier (Grant, 1987). Furthermore, because of its proximity to the fourth ventricle, cerebrospinal fluid (CSF) is continuously being monitored and any toxins emanating from the CSF will be detected by the AP and thereby trigger emesis (Grant, 1987).

Support for the emetic-UCS hypothesis in rats has been mixed. Coil, Rogers, Garcia and Novin (1978) showed that severing the vagus nerve, thus denervating the GI tract, abolished emesis normally induced by intubated copper sulphate but not by copper

sulphate administered intravenously. In contrast. Rabin. Hunt and Lee (1985). demonstrated that copper sulphate administered intragastrically (IG) produced no CTA in rats with an intact vagus nerve but a pronounced CTA in vagotomized rats. Despite these contradictory reports on whether vagal routes mediate CTA's induced by copper sulphate. there is evidence that lesions of the AP attenuate CTA induced by copper sulphate in rats (Coil & Nogren. 1981; Rabin et al.. 1985).

Another chemical frequently used in CTA is LiCl. a drug known for producing robust taste aversions. It has been reported that while lesions of the AP attenuated a LiCl-induced CTA (McGlone. Ritter & Kelly. 1980; Rabin. Hunt & Lee. 1983). vagotomy failed to attenuate a LiCl-induced CTA (Martin. Cheng & Novin. 1978). Together. it may appear that LiCl-induced CTA's operate via a blood-borne route. inducing emesis at the trigger zone.

As was mentioned earlier. amphetamine. a psychostimulant. characteristically known for its positive reinforcing properties can also elicit CTA. According to the UCS-emetic hypothesis (Coil & Garcia. 1977). amphetamine CTA should thus be mediated by emetic mechanisms. However. amphetamine. which crosses the blood-brain barrier. does not appear to elicit CTA via activation of the trigger zone. Support for this notion is derived from studies demonstrating that lesions to the AP fail to attenuate an amphetamine induced CTA (Berger. Wise & Stein. 1973; Ritter. McGlone & Kelly. 1980). However. Carr and White (1986) reported that microinjections of amphetamine into a medullary region near the AP produced CTA. Taken together. it would appear that although amphetamine acts centrally. it has yet to be determined whether the mechanisms mediating amphetamine CTA are nevertheless emetic and peripheral in nature.

Like amphetamine, morphine induces CTA (Cappell, LeBlanc & Endrenyi, 1973) and is also self-administered (Weeks & Collins, 1964). Consistent with the results reported for amphetamine, morphine CTA does not appear to be mediated by the trigger zone. That is, rats with lesions to the AP do not show an attenuated morphine CTA (van der Kooy, 1984). While it is known that morphine elicits CTA centrally, as shown by studies demonstrating that opiate antagonists that cross the blood-brain barrier reduce a morphine-induced CTA (Ng Cheong Ton & Amit, 1984), as of yet, there is no evidence that morphine induces CTA at the trigger zone. In addition, no studies have implicated the vagus route in the mediation of a morphine induced CTA. Together, it would seem that while some agents such as LiCl and copper sulfate, appear to produce CTA's via recognized emetic structures, it can be argued that CTA's resulting from positively reinforcing drugs such as amphetamine and morphine do not appear to depend upon the integrity of these same structures.

As was previously stated, Garcia's emetic-US theory of CTA (Coil et al., 1978) holds that CTA occurs via activation of pathways that mediate emesis. This, despite the fact that rats are incapable of vomiting. Still, it is notable that receptor sites and afferent pathways similar to those mediating emesis in other species (e.g. cats, dogs) are found in rats and other mammals that do not vomit (Grant, 1987). Moreover, surgical procedures that attenuate emesis in non-rat species are also capable of attenuating CTA in these same species, which may suggest overlapping mechanisms between the structures mediating emesis and CTA. In fact, Rabin, Hunt, Chedester and Lee (1986) showed that AP lesioned cats showed both attenuated radiation induced emesis and CTA. This finding

lends support to the view that emesis and CTA appear to be mediated by the trigger zone, and are parallel phenomenon.

An important role for other central mechanisms in the mediation of CTA has also been demonstrated. For example, the NTS, a site in the brainstem where both taste and visceral information converge has been postulated to play a critical role in the mediation of CTA (Bernstein, 1999). The functioanl significance of the NTS in the mediation of CTA has been difficult to assess, particularly with surgical ablation techniques, because the NTS appears to mediate cardiovascular and other vital functions. Grigson, Shimura and Nogren (1997) reported that lesions of the rostral portion of the NTS, the gustatory zone, failed to impair CTA learning in rats. These results are somewhat surprising in view of the fact that this region appears to be essential for the reception of gustatory input, which appears critical for CTA learning. One explanation for their findings may be related to the fact that their lesions were not sufficiently extensive and thus did not completely eliminate incoming gustatory signals.

The pontine parabrachial nucleus (PBN) is another site that appears to be critical for taste aversion learning. Reilly, Grigson and Nogren (1993) reported that rats with lesions to the PBN failed to acquire a CTA. In a subsequent study it was demonstrated that if conditioning preceded PBN lesioning, there was no disruption in CTA expression (Grigson et al., 1997) These results suggested that while the PBN appears critical for CTA acquisition, it may not be critical for the expression of CTA.

Another brain structure that has been of much interest to researchers studying CTA is the amygdala (AMYG). Studies examining the effect of lesions of the AMYG on CTA learning have been inconsistent. For example, while some studies report that lesions of

the AMYG impair CTA learning (Gallo, Roldan & Bures, 1992; Lasiter & Glanzman, 1985; Nachman & Ashe, 1973a; Schafe & Bernstein, 1996; Simbayi, Boakes & Burton, 1986) others report little or no effect (Bermudez-Rattoni & McGaugh, 1991; Dunn & Everitt, 1988; Galaverna, Seeley, Berridge, Grill, Epstein & Schulkin, 1993). One possible explanation for the discrepant research findings with the AMYG may have to do with the locus of the lesion. It has been suggested that when AMYG lesions impair CTA learning, the effect is principally a result of damage to fibers passing from the insular cortex (IC) through the AMYG (Dunn & Everitt, 1988). A second explanation for the discrepant results with AMYG lesions may be related to the conditioning protocol itself. Schafe and Bernstein (1998) demonstrated that the effects of AMYG lesions on taste aversion learning depend on whether a standard bottle procedure is employed or whether the intraoral method (I/O) is used. In the I/O method, a procedure that conforms most closely to a classical conditioning procedure, the taste CS is presented through an intraoral cannula without any response requirement. This is in contrast to the bottle method which requires the rat to approach and drink the solution from a bottle, an instrumental type learning. It appears that the AMYG may be less critical in the latter conditioning procedure.

The insular cortex (IC) is also thought to play a role in the expression of CTA. The IC has strong reciprocal connections with the NTS and PBN (Bernstein, 1999) and studies employing lesioning techniques have consistently shown that ablation of the IC attenuated taste aversion learning (Bermudez-Rattoni & McGaugh, 1991; Dunn & Everitt, 1988; Lasiter & Glanzman, 1982; Naor & Dudai, 1996). Most recently, Cubero, Thiele and Bernstein (1999) reported that electrolytic lesions of the IC blocked the

behavioral expression of a CTA both when lesions occurred prior to conditioning but also when they were made after conditioning but before testing. Together, these results suggest that the IC appears necessary for the behavioral expression of CTA.

The US drug Preexposure effect

One feature of CTA of particular significance to learning theorists involves the disruptive effects of drug preexposure on subsequent taste aversion. As predicted from the US preexposure effect, CTA should be attenuated by prior exposure with the US that will subsequently serve as the conditioning agent. The US preexposure effect has been shown with a wide variety of agents including amphetamine (Goudie, Taylor & Atherton, 1975), diazepam (Switzman, Fishman & Amit, 1981), ethanol (Berman & Cannon, 1974), fenfluramine (Goudie, Taylor, Atherton, 1974), lithium chloride (Riley, Jacobs & LoLordo, 1976), morphine (Cappell, LeBlanc & Herling, 1975) methamphetamine (Goudie, Thornton & Wheeler, 1976) and nicotine (Iwamoto & Williamson, 1984).

In addition, while drug preexposure attenuates CTA to the same drug, cross drug preexposure effects have also been reported in the literature (Cannon, Baker & Berman, 1977; Cappell, LeBlanc & Herling, 1975; Goudie & Thornton & Wheatley, 1975; Rabin, 1996; Rabin, Hunt & Lee, 1988; Switzman, Fishman & Amit, 1981; Vogel & Nathan, 1976). For example, Cannon, Baker and Berman (1977) demonstrated that prior exposure to ethanol attenuated a LiCl-induced CTA. Switzman et al., (1981) demonstrated asymmetrical cross drug preexposure effects between diazepam, delta-9-tetra-hydrocannabinol (Δ^9- THC) and morphine. That is, morphine preexposure blocked aversions normally induced by morphine by not Δ^9- THC or diazepam. In contrast,

diazepam preexposure attenuated both the morphine and diazepam induced CTAs to a greater degree than the taste aversion induced by delta-9-tetra-hydrocannabinol. Finally, Δ^9- THC preexposure attenuated aversions to diazepam, Δ^9- THC and morphine. In the case of such asymmetrical findings involving self-administered drugs, Switzman et al., (1981) proposed that unlike poorly self-administered drugs such as diazepam, morphine, which is more readily self-administered, is less effective as a disruptive drug preexposure agent and more susceptible to drug preexposure in the preexposure CTA paradigm.

The preexposure CTA technique has also been proven to be useful in evaluating the relationship between the stimulus properties of reward and those of CTA. Hunt, Spivak and Amit (1985) demonstrated that preexposure to a low dose of morphine (still within a range self-administered by laboratory rats) was just as effective as a preexposure to a higher dose of morphine in serving to disrupt morphine CTA's. The authors suggested that a commonality exists between the stimulus properties of morphine as a CTA inducing agent and an agent with established properties of positive reinforcement.

A number of explanations have been offered to account for the CTA preexposure phenomenon and those have variously emphasized the importance of habituation to novelty and or drug state (Gamzu, 1977), drug tolerance (Dacanay & Riley, 1982) and associative blocking (Braveman, 1977). Although there is little consensus concerning the mechanism mediating this effect, the US preexposure paradigm has proven to be a useful tool for psychopharmacologists interested in evaluating the similarity between drug effects. It is possible that the extent to which preexposure to one drug disrupts CTA to a second drug may be determined by the similarity in the psychopharmacological effects of the two drugs including their stimulus properties. If this hypothesis is correct, it follows

that pharmacologically similar drugs should yield greater CTA disruption than pharmacologically dissimilar drugs (see Aragon, Abitbol & Amit, 1986, Aragon, Abitbol & Amit, 1991; Bienkowski, Piasecki, Koros, Stefanski, & Kostowski, 1998; Kunin, Smith & Amit, 1999a; Kunin, Smith & Amit, 1999b; Ng Cheong Ton & Amit, 1984; Ng Cheong Ton & Amit, 1985). Finally, the preexposure paradigm has been used in the evaluation of the similarity of the effects of new drugs to those drugs with known properties (DeBeun, Lohmann, Schneider & De Vry, 1996; DeBeun, Rijk & Broekkamp, 1993; Kirby, Rowan, Smith & Lucki, 1994).

There are a number of advantages to the use of the preexposure CTA paradigm in the examination of drug interactions. First, in this procedure, the preexposure and conditioning drugs are administered separately over a sufficiently long interval such that there is no opportunity for one drug to alter the pharmacokinetic properties of the second drug. Second, unlike other procedures that measure the direct effects of positively reinforcing drugs (i.e., self-administration procedures, drug discrimination (DD) procedures), that may be confounded by the motor impairing effects of these drugs, in the CTA preexposure paradigm, animals are tested in a drug free state. That is, the final preexposure drug administration is typically given at least 24-hr prior to the onset of the first conditioning day, and on conditioning and test days, the conditioning drug is given following a time limited exposure to the novel tasting fluid. In this protocol, the animal is never drugged while being tested. Third, the use of the drug preexposure paradigm minimizes stress to the animal that is typically associated with multiple drug injections, a feature that is more typical of the drug discrimination procedure. At the same time, the CTA preexposure paradigm is a relatively rapid method, requiring fewer experimental

sessions, unlike the DD procedure, which typically requires a large number of experimental sessions. Finally, the CTA preexposure paradigm is a robust and reliable procedure, which can be used to further elucidate the nature of the interaction between the CTA inducing and positive reinforcing stimulus properties of drugs, including an elucidation of their putative underlying common mechanisms of action.

The Present Investigation

As was alluded to earlier in this thesis, the use of alcohol, nicotine in the form of tobacco products, and caffeine in the form of coffee consumption, are positively correlated. Over the years, increasing attention has been devoted to the study of their potential interactive effects. While a number of mechanisms have been proposed to explain the co-occurrence of the use of these drugs, to date, none appears sufficient. The first goal of this thesis was to elucidate the nature of the relationship between alcohol, caffeine and nicotine in an attempt to shed light on possible common mechanisms, both behavioral and biochemical, that may help explain the combined use of these drugs. In order to achieve this, a variety of behavioral procedures were employed including the CTA paradigm. In view of the already previously observed functional relationship between the positively reinforcing and CTA inducing properties of self-administered drugs (e.g., Hunt & Amit, 1987), it is argued that the CTA procedure may permit an indirect measure of the positive reinforcing properties of drugs and thereby provide clues as to the mechanisms, both behavioral and biochemical, involved in mediating the potential interactive effects between commonly used pairs of drugs.

A second goal of this thesis was to investigate the nature of the relationship between the phenomenon of positive reinforcement and conditioned taste aversion induced by self-administered drugs. It has been approximately 30 years since the initial demonstrations of the paradoxical nature of many self-administered drugs. Since then, a number of studies, including that of White et al., (1977) have typified this paradox by demonstrating a positive relationship between the magnitude of drug reinforced responding and drug induced CTA. In addition, behavioral and neurochemical data, reviewed in this thesis, have ultimately lent support for the notion that the positive reinforcing and CTA inducing stimulus properties of a variety of self-administered drugs may be interrelated. While the evidence appears to support a functional relationship between the properties of CTA and those of positive reinforcement, a question of primary relevance is, what is the nature of this relationship? In other words, how are these apparently dichotomous stimulus properties interrelated and how do they interact with each other? Such questions may ultimately help to elucidate the nature of CTA to self-administered drugs.

Experiment 1a-c and 2a-2c were conducted in order to determine whether acetaldehyde, the putative reinforcing metabolite of ethanol (Smith, Aragon and Amit, 1997) would interact with nicotine in the preexposure CTA procedure. It was argued that if the reinforcing metabolite of ethanol mediates many of ethanol's effects (Smith et al., 1997), then acetaldehyde should interact with nicotine in a similar fashion to ethanol. Experiment 3 was designed to expand on the understanding of the stimulus properties of nicotine, specifically with regard to its interaction with caffeine. It was argued that if the perceived psychopharmacological effects of nicotine share unique stimulus properties

with caffeine, then the nature of their interaction should be qualitatively different from that of nicotine and acetaldehyde. Experiment 4 examined whether nicotine's interaction with a caffeine-induced CTA involved nicotine's action at the nicotinic acetylcholine receptor (nAChR). It was hypothesized that if nicotine's effect on a caffeine-induced CTA is mediated through the nAChR, previously shown to mediate the putative positive reinforcing effects of nicotine (Shoaib et al., 1997), then, pretreatment with mecamylamine, a non-competitive nAChR antagonist would reverse the nicotine-induced effect on caffeine CTA.

Experiments 5a and 5b examined the effect of caffeine exposure, at low to moderate doses, on the aquisition and maintenance of ethanol drinking behavior in free-feeding ethanol naive rats. Experiments 5c and 5d examined a possible biological process that could account for caffeine's action on ethanol intake. Experiments 6a-c assessed whether caffeine's facilitation of ethanol drinking observed in Experiments 5a and 5b reflected an attenuation of ethanol's "aversive effects", or whether it was related to an increase in the reinforcing efficacy of ethanol as reflected in CTA.

Experiments 7a-c examined whether differences in locomotion response to an inescapable novel environment, previously shown to predict sensitivity to drug self-administration would also predict sensitivity to amphetamine and morphine induced CTAs but not to LiCl. It was hypothesized that if the CTA inducing and positive reinforcing properties of self-administered drugs are functionally related and mediated by a shared substrate then novelty induced locomotion should predict sensitivity of CTA's to amphetamine and morphine, drugs possessing positive reinforcing effects, but not to LiCl a drug without positive reinforcing properties.

The final experiment of this thesis examined whether c-Fos immunoreactivity (FLI) is expressed differentially in both the nucleus of the solitary tract (NTS) and the area postrema (AP) following CTA's to amphetamine and LiCl. It was hypothesized that LiCl-induced CTA, whose effects are mediated through the AP, would elicit greater elevations in FLI in this region compared with amphetamine CTA, which does not depend on the integrity of this structure. It was also predicted that while amphetamine and LiCl would elicit CTA's of equal magnitude, there would be no differences in the expression of FLI in the NTS, as this structure appears critical for CTA conditioning with both these drugs (Swank, Schafe, & Bernstein, 1995).

Experiment 1a-1c : Preexposure effects of nicotine on an acetaldehyde-induced conditioned taste aversion

An accumulated body of data has pointed to an interaction at some level between nicotine and ethanol (e.g., Zacny, 1990). During the past decade, several studies have documented both behavioural as well as biochemical interactions between these two substances (Burch et al., 1988; Collins et al.,1988; Dar et al.,1993; deFiebre & Collins, 1993; Blomqvist et al., 1996 ; Kunin et al., 1999b; Smith, Horan, Gaskin & Amit, 1999). Recently, Kunin et al., (1999b) reported that nicotine and ethanol blocked the conditioned taste aversions (CTA) of one another. This symmetrical interaction suggested that nicotine and ethanol share common stimulus properties.

Over the past two decades, it has been documented that acetaldehyde, the primary reinforcing metabolite of ethanol, may mediate many of ethanol's behavioural effects (Smith et al., 1997), including an ethanol induced CTA (Aragon, Abitbol & Amit, 1986). If one assumes a functional relationship between the reinforcing properties of a drug and its ability to support CTA (Hunt & Amit, 1987), then it follows logically that acetaldehyde, the putative reinforcing metabolite of ethanol, would interact with nicotine in the preexposure CTA paradigm in a manner resembling ethanol. In a series of 3 separate studies, we examined the effect of nicotine preexposure (0.8, 1.2 or 2 mg/kg), on conditioned taste aversion induced by acetaldehyde (0.2 or 0.3 mg/kg). Because, nicotine was previously shown to block an ethanol-induced CTA, it was hypothesized that nicotine should also block an acetaldehyde-induced CTA.

Method

Subjects

Subjects were 96 male Wistar rats (Charles River, Quebec). N =32 per experiment, weighing between 225-250 g at the start of the experiment. The animals were individually housed in stainless steel cages and had free access to lab chow (PMI Nutrition International, Oakville, ON) and water for a 7-day acclimatization period. The animals were maintained in a room regulated for constant temperature and humidity on a 12-hour light-dark cycle. All subjects used in the present set of experiments were treated in accordance with the guidelines of the Canadian Council for Animal Care. In addition, the care and use of, as well as all procedures involving animals in the present study, were approved by the Concordia University Animal Care Committee.

Drugs

Nicotine ditartrate salt (Sigma Chemical Co.) was dissolved in 1ml/kg of 0.9% saline solution and was injected in doses of 0.8 mg/kg, 1.2 mg/kg and 2 mg/kg. All doses of nicotine were calculated as the nicotine salt and all nicotine injections were administered subcutaneously (s.c). Acetaldehyde 5% (v/v) was diluted with saline from a 99% stock solution (Aldrich Chemical Company). Acetaldehyde was injected at doses of 0.2g/kg and 0.3 g/kg. All acetaldehyde injections were administered intraperitoneally (i.p).

Procedure

Following 7 days of acclimatization to the colony room conditions, rats were placed on a 23 hour and 40 minute water deprivation schedule. Tap water was presented to the animals in two stoppered plastic tubes fitted with stainless steel ball bearing spouts for 20 minutes beginning at noon each day. The spouts were inserted through the fronts of the home cages and were presented in this manner at the same time daily. Fluid was measured to the nearest millilitre. A two bottle free choice was used throughout the experiment since such a procedure is thought to be more sensitive to the detection of less robust CTA's (Dragoin, McCleary, & McCleary, 1971; Grote & Dickins, 1971).

After three days of adaptation to the water deprivation schedule, rats were randomly assigned to one of the treatment conditions. The preexposure injections, which began on day 4, were repeated on days 5 and 6 following the 20-minute water session (Kunin et al., 1999b). Eight animals were assigned to each group. Animals assigned to groups nicotine-acetaldehyde (N-A) and nicotine-vehicle (N-V) were preexposed to nicotine while, animals assigned to group vehicle-acetaldehyde (V-A) and vehicle-vehicle (V-V) were preexposed to saline. On day 7, 24 hours after the final preexposure injection, rats were presented with a novel tasting 0.1% saccharin solution for 20 minutes at noon. Within one minute after completion of the 20- minute saccharin drinking session, animals in groups N-A and V-A were injected with acetaldehyde while, animals in groups V-V and N-V were injected with an equivalent volume of saline. A second, third, and fourth pairing of the saccharin solution and the drug or vehicle injections were repeated on days 10, 13 and 16 of this experiment. Days 19, 22, 25 and 28 comprised drug free test days. On these days, animals were presented with a choice of water or saccharin solution. The

position of the tubes was rotated on every test day in order to control for the development of a side preference (Etscorn, Moore, Scott, Hagen, Caton, Sanders, & Devine, 1987). On intervening days, between conditioning and test days, animals were presented with water for 20 minutes beginning at noon.

Experiment 1a examined the effects of nicotine (0.8 mg/kg) preexposure on a 0.2 g/kg acetaldehyde-induced CTA. Experiment 1b examined the effects of nicotine (1.2 mg/kg) preexposure on a 0.2 g/kg acetaldehyde-induced CTA. Experiment 1c examined the effects of nicotine (2 mg/kg) preexposure on a 0.3 g/kg acetaldehyde-induced CTA. Both doses of acetaldehyde (0.2g/kg and 0.3 g/kg) have previously been shown to produce CTA (Aragon et al., 1986).

Data Analysis

A saccharin preference ratio (total saccharin consumed/total fluid) was calculated for each group. Consistent with a two-bottle test (Etscorn et al., 1987), a CTA was defined as a significant decrease in saccharin preference relative to group V-V. Preference scores were obtained by collapsing the average of two successive test days (i.e., test days 1 and 2 and test days 3 and 4). The two sets of test days were collapsed because the position of the drinking tubes was rotated in order to control for the development of a side preference (Etscorn et al., 1987). By collapsing the test days as opposed to reporting on individual test days (which may underestimate or overestimate actual saccharin preference due to position bias) a more accurate assessment of saccharin preference is obtained (Etscorn et al., 1987). In addition, saccharin intake data obtained

over 4 CS-US (saccharin-drug) pairings, for each experiment, was also subject to separate analysis.

Three separate two-way analyses of variance (ANOVAs) with repeated measures were conducted on saccharin intake and saccharin preference data. Newman-Keuls Post Hoc analysis was performed where appropriate for pairwise comparisons and significant interaction effects. An alpha level of .05 was used for all statistical tests.

Results

Experiment 1a: Nicotine (0.8 mg/kg) – Acetaldehyde (0.2g/kg)

A two-way (4 x 4) analysis of variance (ANOVA) with repeated measures across 4 CS-US pairings was conducted on saccharin intake data. The analysis revealed a significant effect of group, $F(3, 27) = 7.991$, $p < .05$, no significant effect of day, $F(3, 81) = 1.828$, $p > .05$, and a significant group x day interaction, $F(9, 81) = 5.059$, $p < .05$. Table 1 shows that groups V-A and N-A suppressed their saccharin intake across the four CS-US pairings, thus suggesting that nicotine (0.8 mg/kg) preexposure failed to attenuate saccharin intake resulting from conditioning with acetaldehyde.

Figure 1a shows that nicotine (0.8 mg/kg) preexposure failed to alter the development of an acetaldehyde-induced CTA. This analysis revealed a significant effect of group, $F(1, 30) = 7.535$, $p < .05$, no significant effect of day, $F(1, 30) = 2.378$, $p > .05$, and no significant group x day interaction, $F(1, 30) = 2.034$, $p > .05$. Post hoc analysis using a Newman-Keuls test revealed that acetaldehyde (0.2 g/kg) promoted a CTA as revealed by a significant decrease in saccharin preference for group V-A compared to group V-V ($p < .05$). However, saccharin preference did not differ

significantly between groups V-A and N-A ($p > .05$) thus suggesting that nicotine preexposure failed to block the development of an acetaldehyde-induced CTA.

Table 1

Effect of Nicotine (0.8 mg/kg) Preexposure on an Acetaldehyde-Induced CTA (0.2 g/kg) As Reflected in Mean Saccharin Consumption (Millilitres) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-V	19.63 ± 0.84	21.88 ± 0.77	21.88 ± 0.83	22 ± 0.76
V-A	18.38 ± 1.13	16.75 ± 1.19	14.88 ± 1.81	15.38 ± 1.74
N-A	17.29 ± 1.34	18.43 ± 1.04	18.29 ± 1.17	14.14 ± 1.28
N-V	18.63 ± 1.12	20.75 ± 1.24	22.5 ± 0.63	22.38 ± 0.96

Note. A= acetaldehyde; N= nicotine; V= vehicle

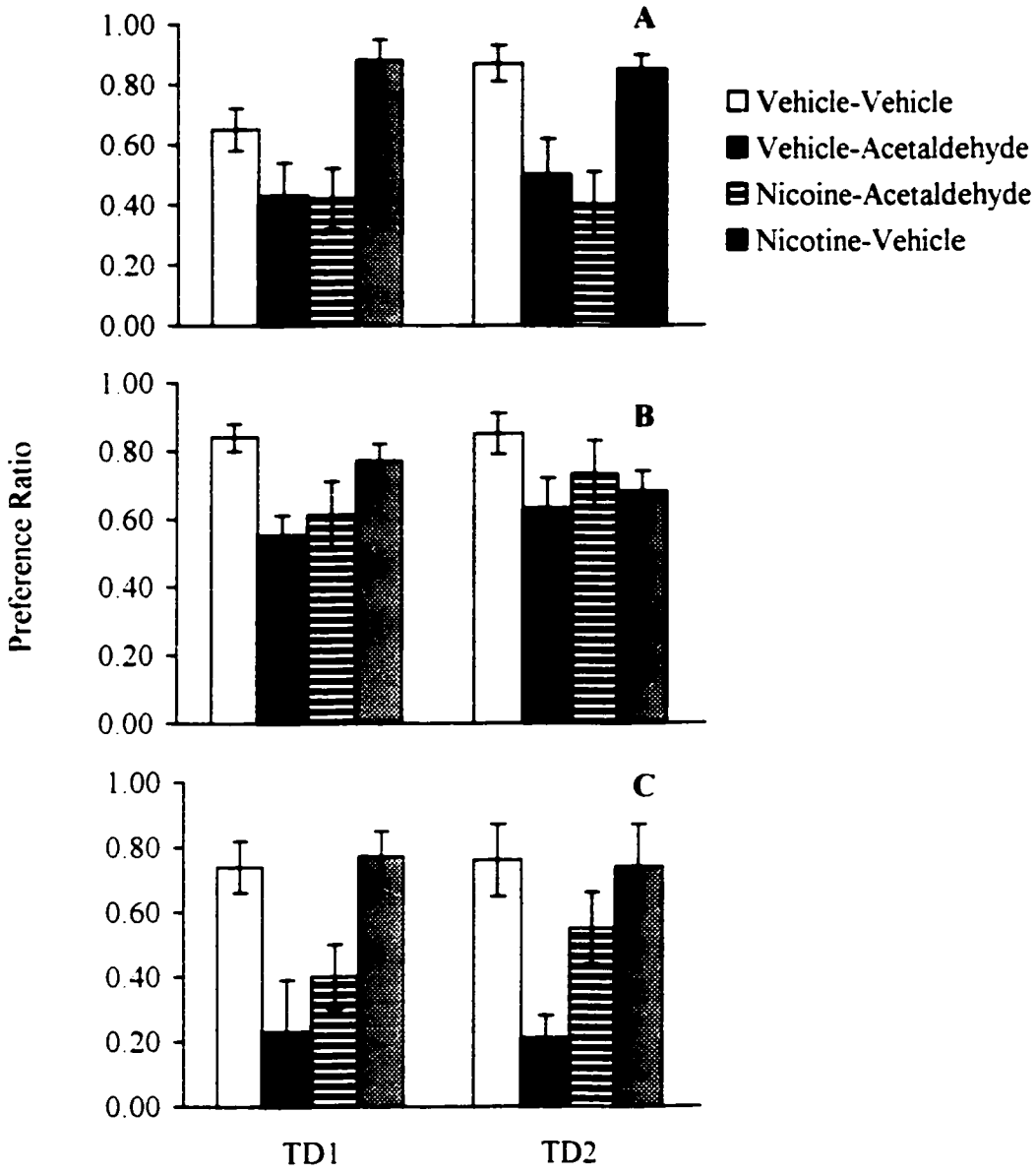


Figure 1. Effect of preexposure with one of three doses of nicotine [0.8 mg/kg (A), 1.2 mg/kg (B), or 2 mg/kg (C)] on a conditioned taste aversion induced by acetaldehyde [0.2 (A,B) or 0.3 mg/kg (C)] as reflected in mean preference ratio. TD1 is collapsed across test days 1 and 2 and TD2 is collapsed across test days 1and 2 and TD2 is collapsed across test days 3 and 4. Vertical lines represent the SEM.

Experiment 1b: Nicotine (1.2 mg/kg) – Acetaldehyde (0.2 g/kg)

A two-way (4 x 4) analysis of variance (ANOVA) with repeated measures across 4 CS-US pairings conducted on saccharin intake data revealed a significant group, F (3, 28) = 3.593, $p < .05$, day, F (3, 84) = 24.477, $p < .05$, and group x day interaction, F (9, 84) = 2.840, $p < .05$. Table 2 shows that with the exception of group V-A, all groups significantly increased their saccharin intake across the four conditioning days.

Figure 1b shows nicotine (1.2 mg/kg) preexposure failed to alter an actealdehyde (0.2 g/kg) CTA. The analysis revealed a significant effect of group F (3, 27) = 3.136, $p < .05$, no significant effect of day, F (1, 27) = .584, $p > .05$, and no significant group x day interaction, F (3, 27) = 1.429, $p > .05$. Newman-Keuls Post hoc analysis revealed that consistent with the results of experiment 1a, acetaldehyde (0.2 g/kg) suppressed saccharin preference as revealed by a significant decrease in saccharin preference for group V-A compared to group V-V across the test days ($p < .05$). However, the observation that group N-A failed to differ significantly from group V-A ($p > .05$) suggested that nicotine (1.2 mg/kg) preexposure failed to alter an acetaldehyde (0.2 g/kg) CTA.

Table 2

Effect of Nicotine (1.2 mg/kg) Preexposure on an Acetaldehyde-Induced CTA (0.2 g/kg)As Reflected in Mean Saccharin Consumption (Millilitres) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-V	19.25 ± 0.96	25.38 ± 2.02	28.50 ± 1.24	26.88 ± 0.95
V-A	22.25 ± 1.32	21.75 ± 0.82	24.88 ± 1.23	25.00 ± 2.25
N-A	18.50 ± 1.24	19.75 ± 1.32	20.38 ± 2.2	25.13 ± 1.43
N-V	19.13 ± 0.9	21.38 ± 0.89	28.0 ± 0.71	26.63 ± 0.91

Experiment 1c: Nicotine (2mg/kg) – Acetaldehyde (0.3 g/kg)

A two-way (4 x 4) analysis of variance (ANOVA) with repeated measures across 4 CS-US pairings conducted on saccharin intake data revealed a significant group, F (3, 29) = 9.337, $p < .05$, a non-significant day, F (3, 87) = .008, $p > .05$, and a significant group x day interaction effect, F (9, 87) = 3.055, $p < .05$. Table 3 shows that acetaldehyde (0.3 g/kg) suppressed saccharin intake across the pairing days. In contrast nicotine (2 mg/kg) preexposure failed to block the saccharin suppression resulting from conditioning with this higher dose of acetaldehyde.

Figure 1c shows that nicotine (2 mg/kg) preexposure blocked the formation of an acetaldehyde (0.3 g/kg) CTA. The analysis revealed a significant effect of group F (3, 26) = 11.217, $p < .01$, no significant effect of day, F (1, 26) = .169, $p > .05$, and no significant group x day interaction effect, F (3, 26) = .325, $p > .05$. Newman-Keuls Post hoc analysis revealed that while acetaldehyde (0.3 g/kg) promoted robust CTA as revealed by a significant decrease in saccharin preference for group V-A compared to group V-V ($p < .05$), nicotine (2 mg/kg) preexposure blocked the acetaldehyde-induced CTA as shown by a significant difference in saccharin preference for group V-A compared to group N-A across the two test days ($p < .05$).

Table 3

Effect of Nicotine (2 mg/kg) Preexposure on an Acetaldehyde-Induced CTA (0.3 g/kg) As Reflected in Mean Saccharin Consumption (Millilitres) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-V	20.5 ± 1.40	23.50 ± 1.0	24.25 ± 0.94	24.88 ± 1.44
V-A	20.25 ± 0.94	15.38 ± 2.56	14.50 ± 2.51	13.88 ± 2.40
N-A	20.00 ± 0.55	18.78 ± 1.53	17.56 ± 1.52	16.89 ± 2.01
N-V	18.75 ± 0.98	21.84 ± 1.32	23.75 ± 1.18	24.13 ± 2.79

Discussion

Experiments 1a-c assessed the effect of preexposure with nicotine (0.8, 1.2 or 2 mg/kg) on an acetaldehyde-induced CTA (0.2 or 0.3 g/kg). The results of these experiments revealed a dose dependent interaction, with the lower nicotine doses (0.8, 1.2 mg/kg) failing to interact with the lower acetaldehyde dose (0.2 g/kg), but the higher nicotine dose (2 mg/kg) attenuating a rather robust acetaldehyde-induced CTA (0.3 g/kg). Although the present results may suggest overlapping stimulus properties of acetaldehyde and nicotine at the highest doses tested, the failure of nicotine (1.2 mg/kg) to interact with the lower dose of acetaldehyde (0.2 g/kg) is somewhat at odds with the findings of Kunin et al., (1999b) who reported that a dose of 1.0 mg/kg of nicotine blocked the formation of an ethanol-induced CTA (1.2 g/kg). In view of studies (e.g., Aragon et al., 1986) demonstrating overlapping stimulus properties of acetaldehyde (0.2 g/kg) and ethanol (1.2 g/kg), it was predicted that nicotine (1.2 mg/kg) should interact optimally with a dose of 0.2 g/kg acetaldehyde. One explanation for the lack of generalization effects across studies may have to do with differences in methodology. Specifically, Kunin et al., (1999b) used a single bottle procedure whereas in the present study, a two-bottle procedure was used which may be more sensitive to the detection of CTA's.

An alternative explanation for the discrepant findings may reflect the fact that the mode of drug administration differed across studies with nicotine being administered i.p in the Kunin et al., (1999b) study and nicotine administered s.c in the present experiment. Indeed, the s.c route generally produces a slower rate of onset of drug action with a longer duration when compared to i.p with its fairly rapid rate of absorbtion (Etscorn et

al., 1987). Previous studies (e.g., Goudie 1979; Hunt & Amit, 1987) have suggested that the rate of onset of a drug's action may play a role in the development of CTA.

Experiments 2a-c: Preexposure effects of acetaldehyde on a nicotine-induced conditioned taste aversion

Experiments 2a-2c examined the effect of preexposure with acetaldehyde (0.2 g/kg or 0.3 g/kg) on the development of a CTA induced by nicotine (0.8 mg/kg. 1.2 mg/kg. or 2.0 mg/kg).

Method

Subjects

A total of 96 male (N =32 per experiment) Wistar rats weighing between 225-250 g were used in the following three experiments and were housed in conditions described in Experiments 1a-c.

Procedure

The experimental procedures used in this experiment were identical to those described in Experiment 1a-c with the following exceptions. The preexposure injections consisted of injections of acetaldehyde for groups A-N and A-V and saline for groups V-N and V-V. On pairing days. animals assigned to group A-N and V-N were conditioned with nicotine and animals assigned to group A-V and V-V were conditioned with saline.

Results

Experiment 2a: Acetaldehyde (0.2 mg/kg) – Nicotine (0.8 mg/kg)

A two-way (4 x 4) analysis of variance (ANOVA) with repeated measures across 4 CS-US pairings conducted on saccharin intake data revealed no significant effect of

group. F (3, 28) = 1.883, $p > .05$, a significant effect of day, F (3, 84) = 37.098, $p < .05$, and a significant group x day interaction, F (9, 84) = 2.672, $p < .05$. Table 4 shows that with the exception of group V-N, all groups significantly increased ($p < .05$) their saccharin intake across the 4 CS-US pairing days.

Figure 2a displays the saccharin preference data for the groups across the two test days. The results of this data revealed no significant effect of group, F (1, 30) = 1.462, $p > .05$, a significant effect of day, F (1, 30) = 7.043, $p < .05$, and no significant group x day interaction, F (1, 30) = 1.944, $p > .05$. The failure to observe a nicotine (0.8 mg/kg) CTA precluded any meaningful statements about the preexposure effects of acetaldehyde on nicotine-induced saccharin suppression.

Table 4

Effect of Acetaldehyde (0.2 g/kg) Preexposure on a Nicotine-Induced CTA (0.6 mg/kg) As Reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-V	15.25 ± 1.52	21.63 ± 1.19	21.13 ± 1.27	22 ± 0.25
V-N	16.44 ± 1.04	18.78 ± 1.08	18.67 ± 0.96	16.67 ± 1.03
A-N	15.13 ± 1.29	21.13 ± 0.9	22.25 ± 1.08	21.25 ± 0.86
A-V	15.57 ± 1.63	21.57 ± 1.99	23.43 ± 1.90	22.14 ± 0.77

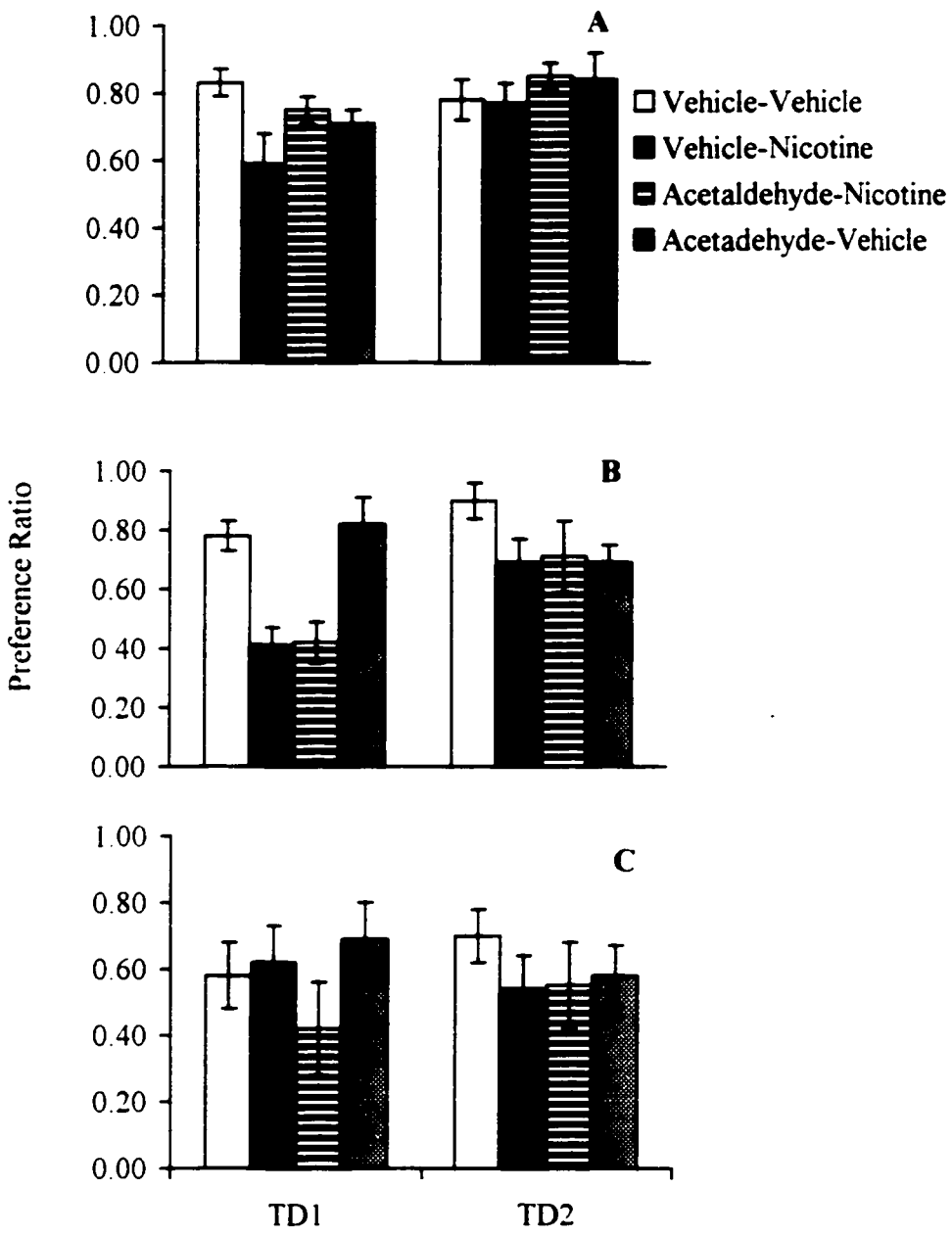


Figure 2. Effect of preexposure with one of two doses of acetaldehyde [0.2 g/kg (A, B) or 0.3 g/kg (C)] on conditioned taste aversion induced by nicotine [0.8 mg/kg (A) 1.2 mg/kg (B) or 2 mg/kg (C)] as reflected in mean preference ratio. TD1 is collapsed across test days 1 and 2 and TD2 is collapsed across test days 3 and 4. Vertical lines represent the SEM.

Experiment 2b: Acetaldehyde (0.2 g/kg) – Nicotine (1.2 mg/kg)

A two-way (4 x 4) analysis of variance (ANOVA) with repeated measures across 4 CS-US pairings conducted on saccharin intake data revealed no significant effect of group, F (3, 27) = 2.055, $p > .05$, a significant effect of day, F (3, 81) = 16.199, $p < .05$, and a non-significant group x day interaction, F (9, 81) = .865, $p > .05$. Table 5 shows that saccharin intake remained unaltered across the four conditioning days for all groups.

Figure 2b shows that acetaldehyde (0.2 g/kg) preexposure failed to attenuate a 1.2 mg/kg nicotine-induced CTA. The analysis of this data revealed a significant effect of group, F (1, 30) = 6.235, $p < .05$, day, F (1, 30) = 8.418, $p < .05$, and group x day interaction, F (1, 30) = 4.317, $p < .05$. Test of simple effects revealed that while group V-N and A-N differed significantly from group V-V on TD1 ($p < .05$), group V-N failed to differ significantly from group A-N, suggesting that preexposure with acetaldehyde failed to attenuate a nicotine-induced CTA.

Table 5

Effect of Acetaldehyde (0.2 g/kg) Preexposure on a Nicotine-Induced CTA (1.2 mg/kg) As Reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-V	19.63 ± 1.43	23.63 ± 0.80	25.38 ± 1.16	22.88 ± 1.2
V-N	21.13 ± 2.95	21.50 ± 1.00	22.00 ± 0.76	18.63 ± 2.01
A-N	17.57 ± 1.29	22.57 ± 2.05	25.57 ± 2.13	18.86 ± 1.70
A-V	17.38 ± 0.73	21.88 ± 1.13	24.38 ± 0.84	20.38 ± 1.55

Experiment 2c: Acetaldehyde (0.3 g/kg) – Nicotine (2.0 mg/kg)

A two-way (4 x 4) analysis of variance (ANOVA) with repeated measures across 4 CS-US pairings conducted on saccharin intake data revealed a significant effect of group. F (3, 25) = 4.055, $p < .05$, day, F (3, 75) = 15.118, $p < .05$, and group x day interaction, F (9, 75) = 2.291, $p < .05$. Table 6 shows that with the exception of group V-N, all other groups significantly increased ($p < .05$) their saccharin consumption across the 4 pairing days.

Figure 2c shows the saccharin preference data for the groups across the two test days. The analysis of this data revealed no significant effect of group, F (3, 25) = .837, $p > .05$, day, F (1, 25) = .042, $p > .05$, and group x day interaction, F (3, 25) = .718, $p > .05$. The failure to observe a 2 mg/kg nicotine-induced CTA precluded any meaningful statements about the preexposure effects of acetaldehyde (0.3 g/kg) on nicotine-induced saccharin suppression.

Table 6

Effect of Acetaldehyde (0.3 g/kg) Preexposure on a Nicotine-Induced CTA (2.0 mg/kg) As Reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-V	21.00 ± 1.50	24.57 ± 1.17	28.86 ± 1.08	26.86 ± 1.16
V-N	21.00 ± 1.41	20.00 ± 1.28	21.00 ± 1.61	20.25 ± 1.71
A-N	17.29 ± 1.23	18.86 ± 3.44	23.14 ± 1.12	21.14 ± 1.75
A-V	17.86 ± 2.09	23.29 ± 1.36	26.29 ± 1.63	26.43 ± 1.07

Discussion

Experiments 2a-c were essentially replications of experiments 1a-c but examined the effect of preexposure with one of two doses of acetaldehyde (0.2 or 0.3 g/kg) on a nicotine-induced CTA (0.8, 1.2 or 2.0 mg/kg). The results of these experiments did not allow a meaningful interpretation of the effect of acetaldehyde preexposure on a nicotine-induced CTA (0.8 and 2.0 mg/kg) as nicotine failed to support CTA at these doses. However, the results with the intermediate dose of nicotine (1.2 mg/kg) revealed that while it supported a CTA, it was unaffected by acetaldehyde (0.2 g/kg) preexposure. This finding is at odds with Kunin et al., (1999b) who reported that ethanol (1.2 g/kg) blocked the formation of a nicotine induced CTA (1.0 mg/kg). Because acetaldehyde (0.2 g/kg) was previously shown to mediate an ethanol-induced CTA (1.2 g/kg) (Aragon et al., 1986), then it can be argued that acetaldehyde (0.2 g/kg) should have blocked the formation of a nicotine-induced CTA (1.2 mg/kg). On the basis of these results one may conclude that the putative metabolite of ethanol, acetaldehyde, may not be involved in the previously observed interaction between nicotine and ethanol in the preexposure CTA procedure.

However, it is worth noting that preexposure with nicotine (2.0 mg/kg), which in itself was unable to support CTA (but see Parker, 1991) also proved capable of attenuating a fairly robust acetaldehyde-induced CTA (0.3 g/kg). This finding is at odds with the notion implicit in an associative interference account of CTA where the strength of the disruptive effects of drug preexposure are thought to be directly related to the CTA inducing strength of a given drug (Braveman, 1977; Cannon et al., 1977). Instead, it can be argued that the ability of nicotine (2.0 mg/kg) to attenuate an acetaldehyde-induced

CTA (0.3 g/kg) is likely related to some similarity in the effects involved in the preexposure and conditioning drug treatments but unrelated to their ability to elicit aversion as nicotine (2.0 mg/kg) failed to produce a CTA.

It has previously been shown that doses of morphine that are insufficient to promote CTA are nevertheless capable of attenuating a morphine-induced CTA (Hunt et al., 1985). On the basis of these findings, it was argued that certain properties of morphine preexposure were detected despite the fact that they were incapable of inducing a CTA. Experiments 1a-c and 2a-c demonstrated that the same phenomenon occurred even with seemingly unrelated drugs. The present results regarding the interaction of acetaldehyde (0.3 g/kg) and nicotine (2.0 mg/kg) underscore the notion that the interaction between some drugs in the CTA paradigm may be unrelated to their aversive properties. It follows then that the signals that may give rise to CTAs to drugs possessing positively reinforcing properties need not be aversive, a point argued elsewhere (Hunt & Amit, 1987).

Experiment 3: Preexposure effects of nicotine on a caffeine-induced conditioned taste aversion

As was mentioned earlier in this thesis, the preexposure CTA procedure, may permit the assessment of whether different drugs share common stimulus properties. If preexposure to a drug for several days prior to taste aversion conditioning with a second drug can disrupt the development of CTA to the second drug, it can be said that the two drugs may share some common stimulus properties. The purpose of experiment 3 was to determine whether nicotine's ability to disrupt an acetaldehyde induced-CTA peviously observed in Experiment 1c would extend to CTA's induced by other drugs such as caffeine. In other words, does nicotine share common stimulus properties with other drugs such as caffeine in the preexposure CTA procedure. The importance of this experiment was threefold. First, to determine whether the nature of nicotine's interaction with acetaldehyde, the putative reinforcing metabolite of ethanol (Smith et al., 1997), is unique to these drugs. If the perceived psychopharmacological effects of nicotine share unique stimulus properties with acetaldehyde, the nature of the disruption of an acetaldehyde-induced CTA should be qualitatively different from that of a disruption of a caffeine-induced CTA. A second, goal of this experiment was to expand on the knowledge of the stimulus properties of nicotine, specifically with regard to its interaction with caffeine. Finally, if one assumes that a functional relationship exists between the positive reinforcing properties of a drug and its ability to support CTA (e.g., Sklar & Amit, 1977) then the study of drug-induced CTAs may provide some important clues as to the mechanisms of the reinforcing effects of that drug.

Experiment 3 assessed the effect of preexposure with nicotine (0.6 mg/kg. 1.2 mg/kg. and 2.0 mg/kg) on a caffeine-induced CTA (20 mg/kg and 30 mg/kg). It was hypothesized that if the perceived psychopharmacological effects of nicotine share overlapping stimulus properties with caffeine. then prexposure with nicotine should attenuate the formation of a caffeine-induced CTA.

Method

Subjects

Subjects were 72 male Wistar rats (Charles River. Quebec). n =36 per experiment. weighing between 225-250 g at the start of the experiment. The animals were individually housed in stainless steel cages and had free access to lab chow (PMI Nutrition International. Oakville. ON) and water for a 7-day acclimatization period. The animals were maintained in a room regulated for constant temperature and humidity on a 12-hour light-dark cycle. All subjects used in the present experiment were treated in accordance with the guidelines of the Canadian Council for Animal Care. In addition. the care and use of. as well as all procedures involving animals in the present study. were approved by the Concordia University Animal Care Committee.

Drugs

Nicotine ditartrate salt (Sigma Chemical Co.) was dissolved in 1ml/kg of 0.9% saline solution and was later injected at doses of 0.6 mg/kg. 1.2 mg/kg and 2.0 mg/kg. All doses of nicotine were calculated as the nicotine salt and all nicotine injections were administered s.c. Caffeine (Sigma Chemical Co.) was dissolved in 0.9% saline and

injected in a volume of 1 ml/kg. All caffeine injections were administered i.p and injection volumes were constant across doses (20 and 30 mg/kg).

Procedure

Following 7 days of acclimatization to the colony room conditions, rats were placed on a 23 hour and 40 minute water deprivation schedule. Tap water was presented to the animals in two stoppered plastic tubes fitted with stainless steel ball bearing spouts for 20 minutes beginning at noon each day. The spouts were inserted through the fronts of the home cages and were presented in this manner at the same time daily. A two bottle free choice procedure was used. After three days of adaptation to the water deprivation schedule, animals were randomly assigned to one of the treatment conditions. The preexposure injections, which began on day 4, were repeated on days 5 and 6 following the 20-minute water session (Kunin et al., 1999b). Six animals were assigned to each group. Animals assigned to groups nicotine-caffeine (N-C) were preexposed to nicotine (06, 1.2, or 2.0 mg/kg), animals assigned to groups vehicle-caffeine (V-C) and vehicle-vehicle (V-V) were preexposed to saline, and animals assigned to group caffeine-caffeine (C-C) were preexposed to caffeine (20 or 30 mg/kg). On day 7, 24 hours after the final preexposure injection, rats were presented with a novel tasting 0.1% (sodium) saccharin solution for 20 minutes given in place of water. Within one minute after completion of the 20- minute saccharin drinking session, animals in groups N-C, V-C and C-C were injected with caffeine (20 or 30 mg/kg) while those animals assigned to group V-V were injected with saline. The doses of caffeine selected have previously been shown to yield reliable CTAs (Brockwell et al., 1991; Smith et al., 1998). The doses of nicotine (0.6, 1.2

and 2 mg/kg) selected have been shown to be within a dose range capable of attenuating an acetaldehyde-induced CTA. A second, third, and fourth pairing of the saccharin solution and the drug or vehicle injections were repeated on days 10, 13 and 16 of this experiment. Days 19, 22, 25 and 28 were drug free test days. On these days, animals were presented with a choice of water and saccharin solution only.

Data Analysis

A saccharin preference ratio (total saccharin consumed/total fluid) was calculated and subjected to analysis. Consistent with a two-bottle test, a CTA was defined as a significant decrease in saccharin preference relative to group V-V. Preference scores were obtained by collapsing the average of two successive test days (i.e., test days 1 and 2 and test days 3 and 4). Saccharin intake data obtained over four saccharin-drug pairings was also subjected to separate analysis.

Separate two-way analyses of variance (ANOVAs) with repeated measures were conducted on saccharin intake and saccharin preference data. Duncan's Post hoc analysis was performed where appropriate for pairwise comparisons and significant interaction effects. An alpha level of .05 was used for all statistical tests.

Results

Nicotine (0.6, 1.2, 2.0 mg/kg) - Caffeine (20 mg/kg)

A two-way (6 X 4) ANOVA conducted across the four pairings revealed no significant effect of group F (5, 29) = 1.686, $p > .05$, a significant effect of day F (3, 87) = 5.096, $p < .05$, and a non significant group x day interaction, F (15, 87) = 1.215, $p > .05$. Table 7 shows that group V-C as well as all other groups failed to decrease their saccharin intake across the four conditioning days ($p > .05$).

Figure 3 shows the attenuating dose-dependent effect of nicotine on a caffeine-induced CTA. The analysis revealed a significant effect of group, F (5, 29) = 4.219, $p < .05$, no significant effect of day, F (1, 29) = 3.454, $p > .05$, and no significant group x day interaction, F (5, 29) = 0.538, $p > .05$. Post hoc analysis using a Duncan's multiple range test revealed that consistent with previously published research (e.g., Steigerwald et al., 1989) caffeine (20 mg/kg) produced a fairly robust CTA across the two test days as revealed by a significant decrease in saccharin preference for group V-C in relation to group V-V ($p < .05$). In addition, preexposure with the lowest dose of nicotine (0.6 mg/kg) attenuated a caffeine-induced CTA as shown by a significant difference in saccharin preference for group V-C compared to group N (0.6)-C (20) across the two test days ($p < .05$). However, group N (1.2)- C (20) and N (0.6)-C (20) failed to differ significantly from group V-C ($p > .05$) suggesting that preexposure with the higher nicotine doses (1.2 mg and 2.0 mg/kg) failed to attenuate a caffeine induced CTA (20 mg/kg). Additionally, group N (0.6) – C (20) did not differ significantly from group C-C and V-V across the two test days ($p > .05$).

Table 7

Effect of Nicotine (0.6 mg/kg) Preexposure on a Caffeine-Induced CTA (20 mg/kg) As Reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-C	15.8 ± 3.9	18.2 ± 3.7	18.0 ± 3.6	16.8 ± 3.4
N (0.6 mg)-C	19.7 ± 2.9	19.5 ± 4.2	21.7 ± 4.8	25.5 ± 2.3
N (1.2 mg)-C	12.3 ± 2.9	16.8 ± 3.2	21.2 ± 1.2	18.3 ± 3.4
N (2.0 mg)-C	22.2 ± 1.4	20.7 ± 2.3	19.8 ± 2.7	20.2 ± 4.2
C (20 mg)-C	15.7 ± 1.7	24.3 ± 1.3	24.0 ± 1.0	29.0 ± 1.4
V-V	14.6 ± 3.9	23.6 ± 1.0	20.8 ± 1.7	22.4 ± 1.0

Note. C = caffeine: N = nicotine: V = vehicle

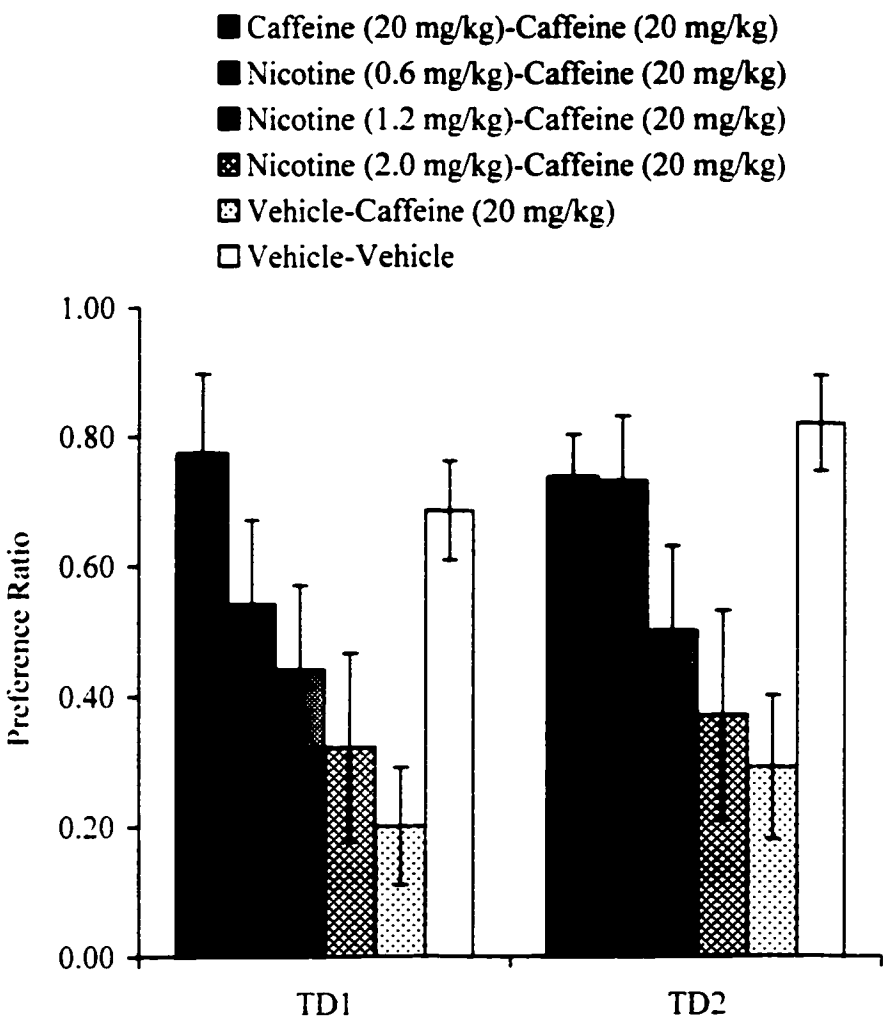


Figure 3. The effect of preexposure with one of three doses of nicotine (0.6. 1.2 and 2.0 mg/kg) on a caffeine-induced conditioned taste aversion (20 mg/kg) as reflected in mean saccharin preference ratio. TD1 is preference collapsed across Test Days 1 and 2. and TD3 is collapsed across Test Days 3 and 4. Vertical lines represent the SEM.

Nicotine (0.6, 1.2, 2.0 mg/kg) - Caffeine (30 mg/kg)

A two-way (6 X 4) ANOVA conducted across the four pairings revealed a significant effect of group F (5, 30) = 18.827, $p < .05$, no significant effect of day F (3, 90) = 1.385, $p > .05$, and a significant group x day interaction F (15, 90) = 2.205, $p < .05$. Table 8 shows that only animals conditioned with caffeine (30 mg/kg) decreased their baseline saccharin intake across the 4 conditioning days ($p < .05$).

Figure 4 shows the attenuating effects of nicotine on a caffeine induced CTA. The analysis conducted on saccharin preference data revealed a significant effect of group F (5, 30) = 9.230, $p < .05$, no significant effect of day F (1, 30) = 0.681, $p > .05$, and no group x day interaction F (5, 30) = 1.372, $p > .05$. Post hoc analysis using a Duncan's multiple range test revealed that caffeine (30 mg/kg) induced a rather robust CTA as revealed by a significant decrease in saccharin preference for group V-C compared to group VV across test days 1 and 2 ($p < .05$). In addition, nicotine (0.6 mg/kg) attenuated CTA resulting from a dose of 30 mg/kg of caffeine as shown by a significant difference in saccharin preference for group V-C compared to group N(0.6)-C(30) across the two test days ($p < .05$). Consistent with the results with the lower caffeine dose, groups N(1.2)-C(30) and N(2.0)-C(30) failed to differ significantly from group V-C across the two test days ($p > .05$). Unlike the results with the lower caffeine dose, group C-C and V-V showed greater saccharin preference compared to group N(0.6)-C(30) across the test days ($p < .05$).

Table 8

Effect of Nicotine (1.2 mg/kg) Preexposure on a Caffeine-Induced CTA (30 mg/kg) As Reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
V-C	12.3 ± 1.8	8.2 ± 1.6	8.5 ± 2.5	4.2 ± 2.2
N (0.6 mg)-C	13.3 ± 3.2	9.5 ± 3.8	18.3 ± 2.6	18.8 ± 4.4
N (1.2 mg)-C	18.0 ± 1.8	16.0 ± 2.7	17.7 ± 2.7	16.5 ± 2.2
N (2.0 mg)-C	17.3 ± 1.0	16.7 ± 3.2	15.5 ± 3.6	17.3 ± 2.8
C (30 mg)-C	18.2 ± 1.4	27.0 ± 1.1	26.7 ± 1.0	26.7 ± 2.0
V-V	21.3 ± 2.5	25.8 ± 1.3	26.5 ± 1.2	28.7 ± 1.0

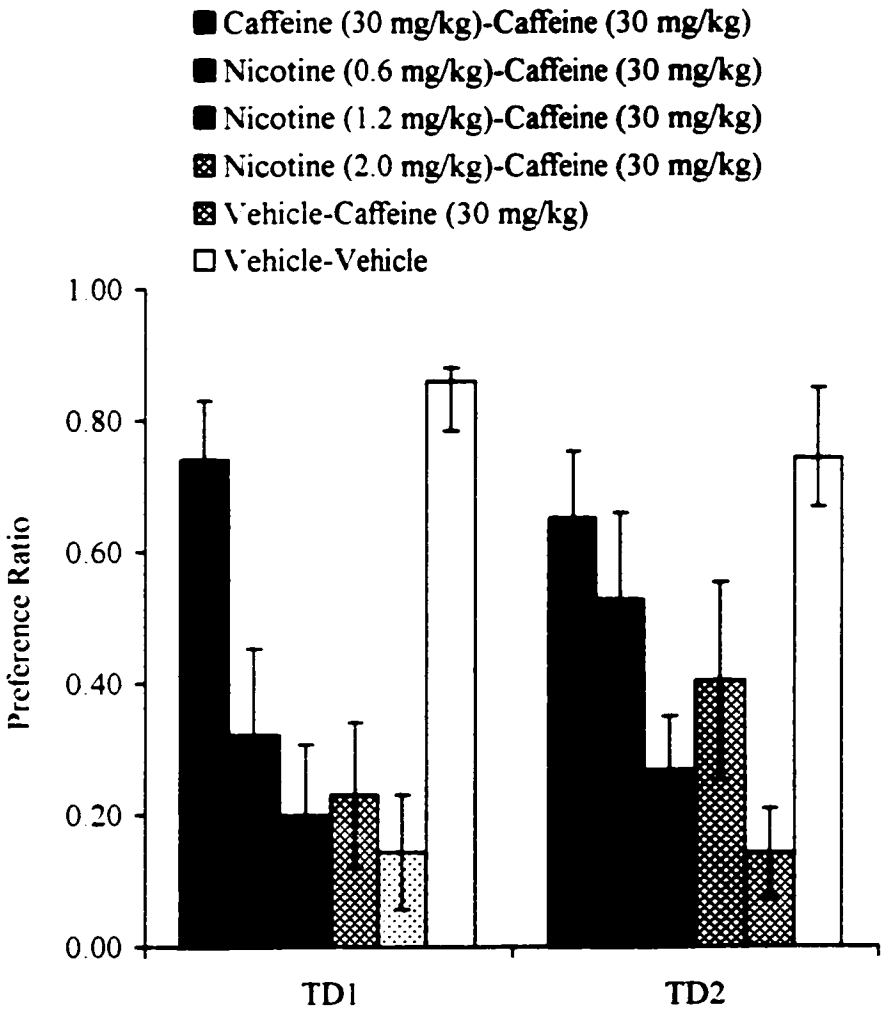


Figure 4. The effect of preexposure with one of three doses of nicotine (0.6, 1.2 and 2.0 mg/kg) on a caffeine-induced conditioned taste aversion (30 mg/kg) as reflected in mean saccharin preference ratio. TD1 is preference collapsed across Test Days 1 and 2, and TD2 is collapsed across Test Days 3 and 4. Vertical lines represent the SEM.

Discussion

The results of Experiment 3 showed a dose-dependent interaction between nicotine and a caffeine-induced CTA with the lowest nicotine dose (0.6 mg/kg) attenuating a caffeine-induced CTA (20 and 30 mg/kg) but the higher nicotine doses (1.2 mg/kg and 2.0 mg/kg) showing no effect on caffeine CTA. These results suggested that caffeine and nicotine share overlapping stimulus properties as reflected in the preexposure CTA paradigm. In fact, so similar were the effects of nicotine (0.6 mg/kg) and caffeine (20 mg/kg) that nicotine's (0.6 mg/kg) ability to block a caffeine-induced CTA (20 mg/kg) was virtually identical to caffeine (20 mg/kg) itself.

It is noteworthy that saccharin intake increased over the course of conditioning with a dose of 20 mg/kg of caffeine, a finding that is in agreement with White and Mason (1985) who reported conditioned taste preference produced by a similar dose of caffeine. In contrast, Steigerwald et al., (1989) showed reductions in saccharin intake following conditioning with a similar caffeine dose. While it is difficult to account for these discrepant findings, it is possible that the use of different strains in these studies may provide an explanation for these conflicting results. Steigerwald et al., (1989) employed Sprague Dawley rats and in the present study Wistar rats were used. Indeed, it has been demonstrated that different rat strains may in fact show differences in sensitivity to the CTA inducing effects of drugs (e.g., Grigson & Freet, 2000).

One question of primary relevance is why the lower dose of nicotine and not the higher ones proved capable of blocking a fairly robust caffeine-induced CTA (20 and 30 mg/kg). One explanation for the lack of dose response relationship between nicotine and a caffeine CTA may have to do with development of aversive effects at the higher

nicotine doses and consequently lack of reinforcing properties (e.g., Parker, 1991). If one assumes that the nature of CTA's to positively reinforcing drugs reflect their rewarding potential (e.g., Hunt & Amit, 1987) then it is conceivable that by raising the dose of nicotine, the strength of its' "aversive" effects increase thereby reducing its similarity to caffeine. The suggestion that higher nicotine doses are more "aversive" than lower nicotine doses would find support in the work of Parker (1991) who demonstrated that rats showed aversive taste reactivity responses when conditioned with nicotine doses that are within a range similar to the higher nicotine doses used in the present set of experiments. It follows that nicotine may have genuine "aversive" properties that resemble LiCl, and this may explain why it's a rather poorly self-administered drug.

In spite of the foregoing, preexposure effects in CTA have also been explained by adopting other concepts such as associative interference effects (Braveman, 1977; Cannon et al., 1977) where the strength of the disruptive effects of drug preexposure on the induction of CTA are thought to be related to the CTA inducing strength of a drug. That is, the greater the CTA inducing potential of a drug, the greater its ability to disrupt CTA when acting as a preexposure agent. The results of the present experiment clearly negate this notion as a dose of nicotine, in itself below the threshold for the induction of CTA (see experiment 2a) was capable of attenuating a robust caffeine-induced CTA (20 and 30 mg/kg). The present results while at odds with an associative explanation suggest that nicotine (0.6 mg/kg) and caffeine (20 and 30 mg/kg) share stimulus properties but that these similarities appear unrelated to their ability to elicit taste aversion as nicotine within a similar dose range was shown to be incapable of supporting CTA (see experiment 2a). As was previously argued in this thesis, the present results underscore the

notion that the interaction between drugs in the preexposure CTA paradigm may be unrelated to their "aversive" potential.

The results of experiment 3 are also at odds with previous findings (e.g., Modrow et al., 1981; Gasoir et al., 1999) reporting that caffeine and nicotine do not have common drug discriminative properties. Gasior et al., (1999) recently reported that caffeine failed to generalize to the nicotine cue in rats trained to discriminate nicotine from saline. The discrepant results in the effects of caffeine and nicotine in the CTA preexposure paradigm vs. drug discrimination (DD) procedure merit attention. It is entirely possible that these discrepant results may reflect obvious procedural differences between these studies. For example, the present study involved fewer experimental sessions (3 preexposure injections and 4 conditioning trials), unlike the Gasior et al., (1999) study, which involved a greater number of experimental sessions (28- 56 training sessions).

Despite obvious procedural differences between the procedures employed in present study and that of Gasior et al., (1999), it is worth noting that the method of caffeine delivery as well as magnitude of drug dose administered differed across studies. For example, in the Gasior et al., (1999) study, caffeine was consumed daily in drinking water for a period of two weeks with an average daily consumption of 135 mg/kg/day. This is in contrast to the present study where caffeine (20 or 30 mg/kg) was delivered acutely in a single bolus injection for merely 4 conditioning trials. In addition, in the Gasior et al., (1999) study, nicotine was administered at slightly lower doses (0.1 and 0.4 mg/kg) compared to the doses of nicotine (0.6-2.0 mg/kg) administered in the present study.

Finally, it is also conceivable that the underlying stimulus properties of caffeine and nicotine as reflected in both procedures (i.e. DD vs. CTA preexposure paradigm) may differ. Indeed, others have reported (e.g., Bienkowski et al., 1998) that the results from operant DD experiments are not always in agreement with the findings from preexposure CTA studies. Together it would appear that while caffeine and nicotine may share common stimulus properties as reflected in the preexposure CTA procedure, they do not share common discriminative properties in the classic drug discrimination procedure.

The following experiment examined whether nicotine's attenuating effect of a caffeine-induced CTA involved nicotine's action at the nicotinic acetylcholine receptor (nAChR), previously shown to mediate the putative positive reinforcing effects of nicotine (Shoaib et al., 1997).

Experiment 4 : Preexposure effects of nicotine on a caffeine-induced conditioned taste aversion: Involvement of the nicotinic acetylcholine receptor

The present experiment examined whether nicotine's attenuating effect on a caffeine-induced CTA involved nicotine's action at the nicotinic acetylcholine receptor (nAChR). It was hypothesized that if nicotine's effect on a caffeine-induced CTA is mediated through the nAChR, previously shown to mediate the putative positive reinforcing effects of nicotine (Shoaib et al., 1997), then, pretreatment with mecamylamine, a non-competitive nicotinic acetylcholine receptor (nAChR) antagonist (Shoaib et al., 1997) would reverse the nicotine-induced effect on caffeine.

Method

Subjects

Subjects were 36 male Wistar rats (Charles River, Quebec), n =6 per group, weighing between 225-250 g at the start of the experiment. The animals were individually housed in stainless steel cages and had free access to lab chow (PMI Nutrition International, Oakville, ON) and water for a 7-day acclimatization period. The animals were maintained in a room regulated for constant temperature and humidity on a 12-hour light-dark cycle. All subjects used in the present set of experiments were treated in accordance with the guidelines of the Canadian Council for Animal Care. In addition, the care and use of, as well as all procedures involving animals in the present study, were approved by the Concordia University Animal Care Committee.

Drugs

Nicotine ditartrate salt (Sigma Chemical Co.) was dissolved in 1ml/kg of 0.9% saline solution and was injected at a dose of 0.6 mg/kg. The dose of nicotine was calculated as the nicotine salt and all injections were administered s.c. Caffeine (Sigma Chemical Co.) was dissolved in 0.9% saline and injected in a volume of 1 ml/kg. The caffeine injections were administered i.p. Mecamylamine HCL (Sigma Chemical Co.), the salt form, was dissolved in 0.9% saline at a concentration of 2mg/ml and injections were administered i.p.

Procedure

The experimental procedures used in the present study were identical to Experiment 3 with the following exceptions. Animals assigned to group mecamylamine-nicotine-caffeine (M-N-C), mecamylamine-vehicle-caffeine (M-V-C) or mecamylamine-vehicle-vehicle (M-V-V) were preexposed to mecamylamine (2.0 mg/kg) administered 30 minutes prior to treatment with nicotine (0.6 mg) or saline and 24-h later conditioned with either caffeine (20 mg) or saline. Animals assigned to group vehicle-nicotine-caffeine (V-N-C), vehicle-vehicle-caffeine (V-V-C) or vehicle-vehicle-vehicle (V-V-V) were preexposed to saline 30 minutes prior to treatment with either nicotine or saline and then 24-h later conditioned with either caffeine (20 mg) or saline. The specific doses of nicotine (0.6 mg/kg) and caffeine (20 mg/kg) were selected because at these doses nicotine appeared to be most effective in attenuating a caffeine-induced CTA in experiment 2. The dose of mecamylamine (2.0 mg) was chosen because it was previously

shown to selectively block behavioral effects of nicotine (Kumar et al., 1983; Shoaib & Stolerman, 1995; Shoaib, et al., 1997; Smith et al., 1999).

Data Analysis

A saccharin preference ratio (total saccharin consumed/total fluid) was calculated and subjected to analysis. Consistent with a two-bottle test, a CTA was defined as a significant decrease in saccharin preference relative to saline control animals (V-V). Preference scores were obtained by collapsing the average of two successive test days (i.e., test days 1 and 2 and test days 3 and 4). Saccharin intake data obtained over 4 saccharin-drug pairings was also subjected to separate analysis.

Separate two-way analyses of variance (ANOVAs) with repeated measures were conducted on saccharin intake and saccharin preference data. Duncan's Post hoc analysis was performed where appropriate for pairwise comparisons and significant interaction effects. An alpha level of .05 was used for all statistical tests.

Results

A two-way (6 X 4) ANOVA conducted across the four pairings revealed a significant effect of group $F(5, 30) = 2.824$, $p < .05$, day $F(3, 90) = 8.479$, $p < .05$, and group x day interaction $F(15, 90) = 1.817$, $p < .05$. Table 9 shows that with the exception of group M-N-C, all groups significantly increased ($p < .05$) their saccharin intake across the 4 conditioning days.

Figure 5 illustrates the effect of preexposure with mecamylamine and nicotine on a caffeine-induced CTA. This analysis revealed a significant effect of group, $F(5, 31) =$

6.970, $p < .05$, day, $F(1, 31) = 4.813$, $p < .05$, and no significant group x day interaction, $F(5, 31) = 0.7971$, $p > .05$. Post hoc analysis on the significant main effect of group revealed that consistent with the results of experiment 3, caffeine (20 mg/kg) induced a fairly robust CTA across the two test days as revealed by a significant decrease in saccharin preference for group V-V-C in relation to group V-V-V ($p < .05$). Additional examination of the data revealed that this effect was blocked by nicotine preexposure as group V-N-C differed significantly from group V-V-C across the test days ($p < .05$). In contrast, group V-N-C did not differ significantly from groups M-V-C and V-V-V across the two test days ($p > .05$).

The results of mecamylamine treatment showed that pretreatment with this agent 30 minutes prior to nicotine preexposure completely reversed nicotine's attenuating effect of a caffeine-induced CTA as shown by a significant decrease in saccharin preference for group M-N-C in relation to group V-N-C across the two test days ($p < .05$). Surprisingly however, mecamylamine alone attenuated the formation of a caffeine induced-CTA in a manner resembling nicotine as groups M-V-C and V-N-C failed to differ from each other across the two test days ($p > .05$).

Table 9

Effect of Mecamylamine (2 mg/kg) and Nicotine (1.2 mg/kg) Preexposure on a Caffeine-Induced CTA (20 mg/kg) as Reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
M-V-V	19.2 ± 0.7	27.8 ± 0.7	27.2 ± 1.9	26.7 ± 1.4
M-V-C	16.5 ± 2.0	18.5 ± 1.9	20.3 ± 1.4	23.2 ± 1.2
V-N-C	14.8 ± 3.6	22.2 ± 2.9	23.2 ± 2.7	23.2 ± 1.2
M-N-C	21.2 ± 1.3	18.9 ± 2.7	19.6 ± 3.5	17.6 ± 3.3
V-V-C	15.3 ± 3.1	16.2 ± 1.1	15.0 ± 3.3	20.5 ± 1.6
V-V-V	15.8 ± 2.3	25.5 ± 1.5	20.0 ± 3.9	24.2 ± 1.1

Note. C = caffeine: M = mecamylamine: N = nicotine: V = vehicle

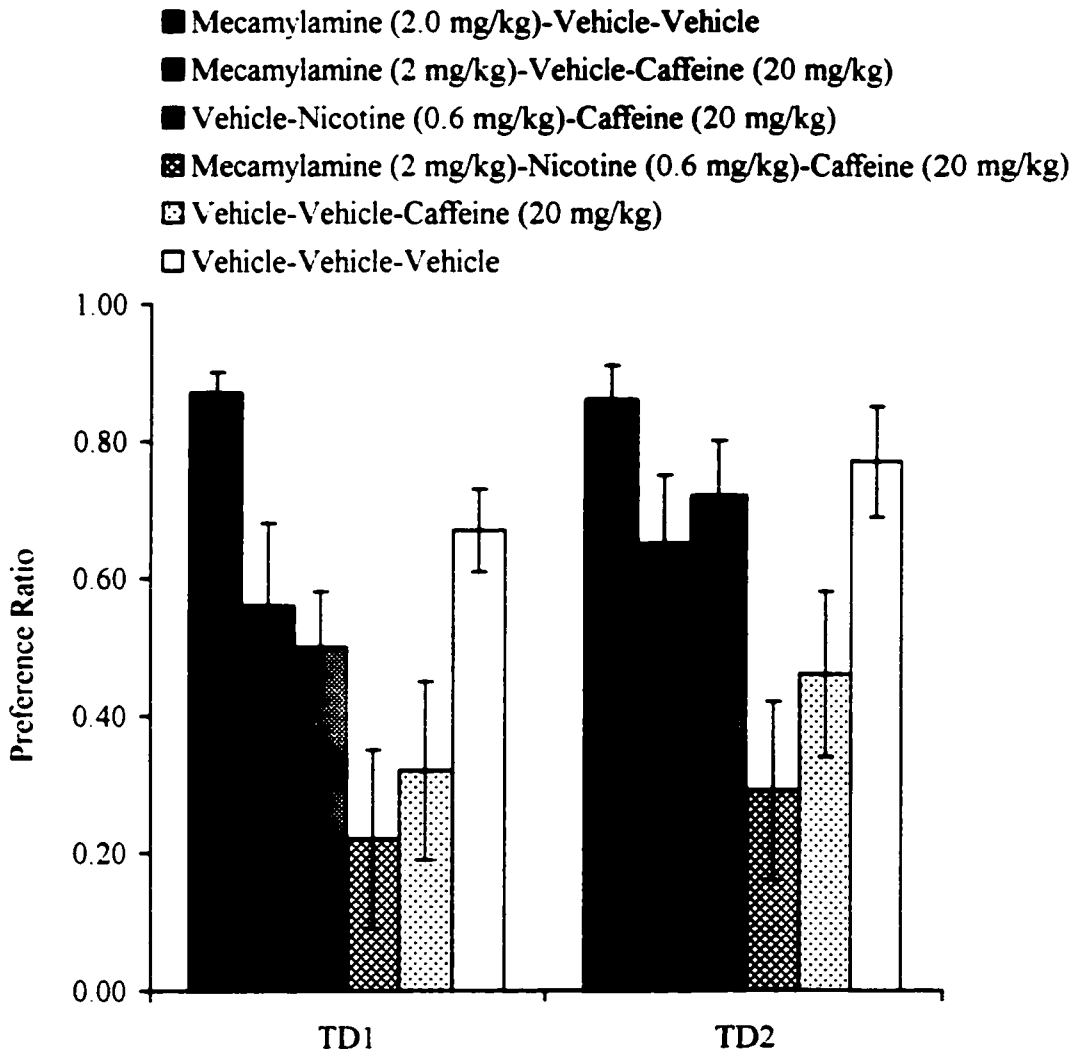


Figure 5. The effect of preexposure with mecamylamine (2.0 mg/kg) and nicotine (0.6 mg/kg) on a caffeine induced conditioned taste aversion (20 mg/kg) as reflected in mean saccharin preference ratio. TD1 is preference collapsed across Test Days 1 and 2, and TD2 is collapsed across Test Days 3 and 4. Vertical lines represent the SEM.

Discussion

The results of the present experiment demonstrated that mecamylamine, an nAChR antagonist, reversed the nicotine-induced attenuation of a caffeine CTA. These results suggested that blockade of the nAChR directly mediates the nicotine-induced attenuation of a caffeine CTA. It is also notable that mecamylamine itself attenuated a caffeine-induced CTA in a manner resembling nicotine suggesting that the expression of a caffeine-induced CTA may be related to action at the nicotinic-cholinergic receptor. This may suggest that nicotine, caffeine and mecamylamine may share common stimulus properties as reflected in the preexposure CTA procedure.

The following experiment sought to examine a possible interaction between caffeine and ethanol with the goal of assessing whether caffeine pretreatment will alter ethanol intake.

Experiment 5a : The effect of caffeine on the acquisition of voluntary ethanol intake

While there is evidence for a correlation between the use of alcohol and caffeine in the form of coffee (e.g., Istvan & Matarazzo, 1984), only a few studies have shown that caffeine pretreatment will alter voluntary ethanol intake (e.g., Gilbert, 1976). Unfortunately, the studies that have shown effects of caffeine pretreatment on voluntary ethanol ingestion have been either confounded by states of food deprivation (Gilbert, 1976; Gilbert, 1979; Hederra et al., 1975) or have used very high doses of caffeine (e.g., Dietze & Kulkosky, 1991). Thus, the present experiment examined the effect of caffeine exposure, at low to moderate doses, on the acquisition of ethanol drinking behavior in free-feeding, ethanol naive rats.

Method

Subjects

Twenty-one male Wistar rats (Charles River Canada, Inc) weighing between 275 and 325 g at the start of the experiment were used. Rats were individually housed in hanging stainless steel wire cages with standard lab chow (PMI Nutrition International, Oakville, ON) and water freely available ad libitum at all times throughout the experiment, except during a 1-h limited access ethanol session. The animals were maintained on a 12-h on/ 12-h off light dark cycle in a room regulated for constant temperature and humidity. All subjects used in this and the following experiments were treated in accordance with the guidelines of the Canadian Council for Animal Care. In

addition, the care and use of, as well as all procedures involving animals in the present study, were approved by the Concordia University Animal Care Committee.

Drugs

Caffeine (Sigma Chemical Co.) was dissolved in 0.9% saline and injected in a volume of 1ml/kg. Injections were administered i.p. Ethanol solutions were prepared through a dilution of a 95% stock solution with tap water and was presented to animals in a 12 ml graduated drinking tube fitted with one-holed rubber stopper with a stainless steel ball-bearing spouts.

Procedure

Following 7 days of acclimatization to the colony room conditions rats were subjected to a limited access ethanol drinking acquisition regimen. The rats remained in their home cages at all times throughout the study but had their water bottles removed during a 1-h ethanol presentation period. During this period, rats received two drinking tubes with a choice between water and ethanol. Ethanol was presented to the rats in ascending concentrations beginning with 2% v/v presented for 6 consecutive days followed by an additional 6 days of 5% ethanol, 4 days of 8% culminating with 4 days of 10% ethanol for a total of 20 days (Smith et al., 1999). Prior to the first ethanol exposure session, animals were randomly assigned to one of three groups (n=7). One group received saline solution at a volume of 1 ml/kg, while the other two groups received injections of caffeine. (5 mg/kg and 10 mg/kg respectively). Injections were administered

daily, 30 min prior to ethanol exposure sessions for the 20-day duration of the experiment. Ethanol intake was measured to the nearest 0.1 ml.

Results

Figure 6 (upper panel) shows ethanol intake (g/kg) across 20 ethanol presentation days for the caffeine and saline treated animals. A two-way ANOVA with a repeated measure across presentation days for absolute ethanol intake (g/kg) revealed significant effects of group $\underline{F}$ (2, 18) = 1.444, $\underline{p} < 0.05$, days $\underline{F}$ (19, 323) = 0.315, $\underline{p} < 0.01$ and group x days interaction $\underline{F}$ (38, 342) = 0.123, $\underline{p} < 0.01$. Test of simple effects followed by test of simple comparisons (alpha level =0.05) revealed that the caffeine (5 mg/kg) treated animals increased their ethanol intake relative to the saline and caffeine (10 mg/kg) treated animals beginning on the third presentation of 8% ethanol and continuing to the final presentation of 10% ethanol. In contrast, animals treated with the higher dose of caffeine (10 mg/kg) did not differ from saline-treated rats.

Figure 6 (bottom panel) shows water intake expressed as ml/kg across the 20 ethanol presentation days for animals pretreated with either caffeine or saline. A two-way ANOVA with a repeated measure across presentation days for water intake showed no significant effects of group $\underline{F}$ (2, 18) = 0.899, $\underline{p} > 0.05$, days $\underline{F}$ (19, 342) = 1.192, $\underline{p} > 0.05$ and group x days interaction $\underline{F}$ (38, 342) = 0.841, $\underline{p} > 0.01$. These results suggested that there were no systematic differences in water consumption between the groups across the 20 days.

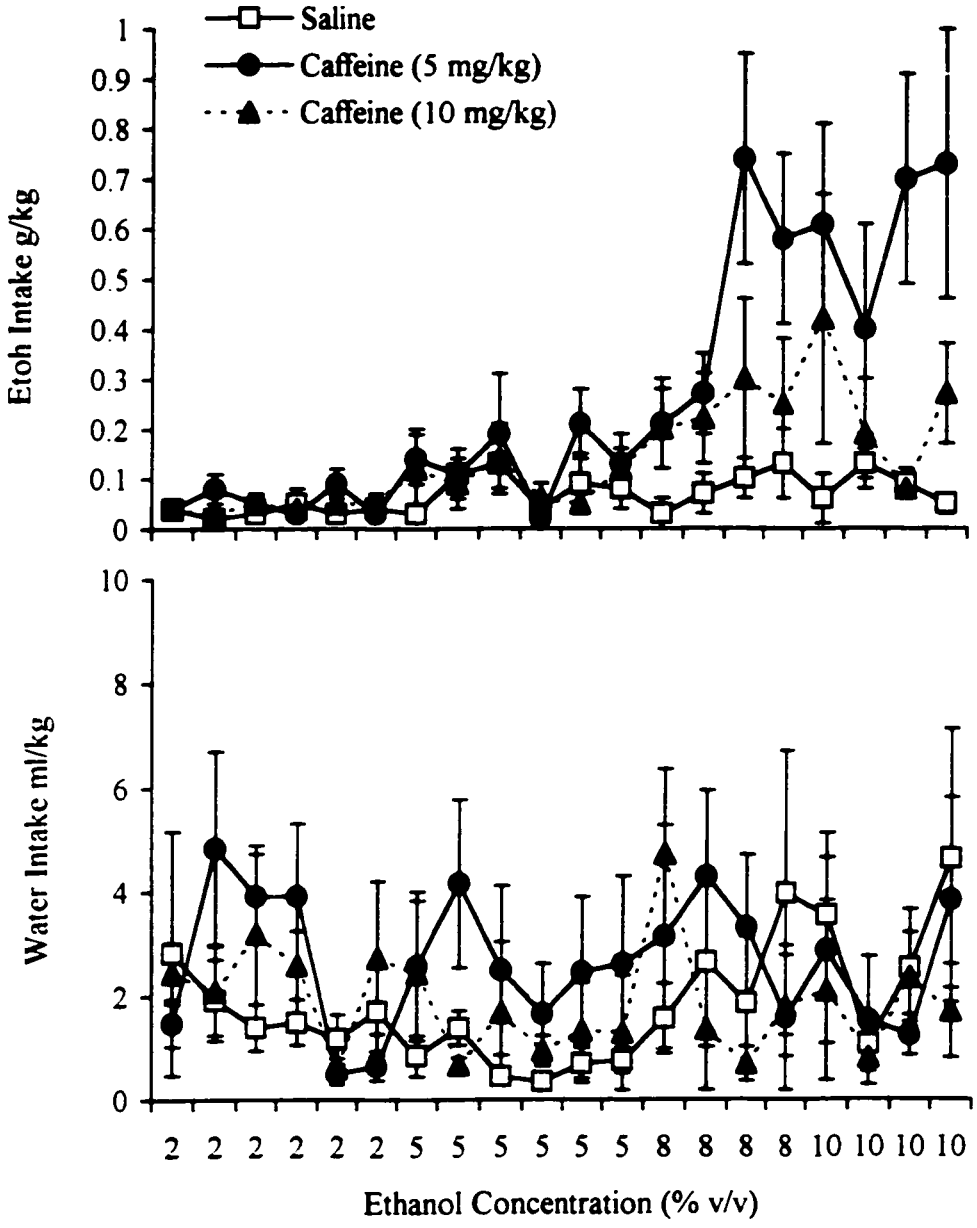


Figure 6. The effect of caffeine (5 and 10 mg/kg) on ethanol consumed (g ethanol/kg body weight, upper panel) and water intake (expressed as ml fluid/kg body weight, lower panel). Vertical lines represent the SEM.

A two-way ANOVA with a repeated measure across presentation days for total fluid data revealed significant effects of group F (2, 18) = 5.107, $p < 0.05$, days F (19, 342) = 2.521, $p < 0.01$ and group x days interaction F (38, 342) = 1.526, $p < 0.05$. Subsequent analysis revealed that caffeine- (5 mg/kg) treated animals increased their total fluid intake relative to saline- and caffeine- (10 mg/kg) treated animals on the third presentation of 8% ethanol and the final two presentations of 10% ethanol.

Discussion

Since observations in humans suggest that the use of caffeine and alcohol are positively correlated (e.g., Istvan & Matarazzo, 1984), it was of some interest to assess the direct effects of exposure to caffeine on the acquisition of ethanol consumption in free feeding, ethanol naïve laboratory rats. The results of the present experiment revealed that pretreatment with caffeine at a dose of 5 mg/kg but not 10 mg/kg facilitated the ingestion of ethanol solutions at 8% and 10% ethanol concentrations. Moreover, the effects of caffeine appeared to be specific to ethanol and not due to generalized increases in fluid intake as no accompanying increase in water intake was observed during the choice presentation of ethanol and water.

Given the schedule of ethanol presentation in the present experiment, the caffeine - induced elevation in ethanol drinking occurred after roughly 12 caffeine injections, during presentations of 8% and then 10% ethanol solutions. On the basis of the results of the present experiment, it was argued that caffeine might have enhanced the ability of the animals to initiate ethanol-drinking behavior as a response to the impact of ethanol's post-ingestive rewarding effects. Such a view is consistent with the observation that

caffeine injected animals increased their ethanol drinking at the higher ethanol concentrations where ethanol is believed to be consumed for its post-ingestive pharmacological effects (Boyle, Smith, Spivak, & Amit, 1994).

One question of primary relevance is why only the lower dose of caffeine was capable of promoting ethanol drinking. Experiment 5b served to further test this issue.

Experiment 5b: The effect of caffeine on the maintenance of voluntary ethanol intake

The results of Experiment 5a demonstrated that caffeine (5 mg/kg) facilitated the acquisition of ethanol drinking in ethanol naïve rats. It was argued that caffeine may have enhanced the ability of the animals to initiate ethanol-drinking behavior as a response to the impact of ethanol's post-ingestive rewarding effects. Experiment 5b. was designed to determine the extent of caffeine's action on ethanol drinking behavior with the intent of examining caffeine's effect on ongoing ethanol drinking behavior in ethanol-experienced animals. The first objective of this experiment was to determine whether the effect of caffeine on ethanol consumption was specifically related to the acquisition of ethanol drinking. Presumably, if caffeine can augment ethanol drinking in rats with a pre-established history of ethanol drinking, then the effects of a caffeine-induced enhancement are not uniquely related to the facilitation in the learning to drink ethanol per se but to a direct pharmacological interaction between these agents.

A second objective of this study was to further test the notion that caffeine may have enhanced the ability of the animals to initiate ethanol-drinking behavior as a response to the impact of ethanol's post-ingestive rewarding effects. It was hypothesized that if caffeine (5 mg/kg) enhanced the ability of the animals to initiate ethanol-drinking behavior as a response to the impact of ethanol's post-ingestive rewarding effects, then raising or lowering the caffeine dose above or below this optimal dose of 5 mg/kg should exert no effect on ethanol drinking.

Method

Subjects

Forty male Wistar rats (Charles River Canada, Inc) weighing between 275 and 325 g were used in the present experiment. Housing conditions were identical to those outlined in Experiment 5a.

Procedure

Following 7 days of acclimatization to the colony room conditions animals were subjected to a limited access ethanol drinking acquisition regimen. Animals were first exposed to a free choice between increasing concentrations of ethanol (2-10%) and water for a period of 20 days in a manner identical to the previous experiment but with no drug pretreatment. Following this acclimatization period, animals were presented with ethanol (10%) for an additional 6 days, constituting the baseline phase. During the baseline-drinking phase, all animals were given i.p saline injections once daily, 30 minutes prior to choice sessions between ethanol and water. These saline injections served to habituate animals to the injection regimen. Following the 6 days of baseline, animals were assigned to one of four treatment conditions based on their mean levels of ethanol intake (g/kg) across the baseline phase, which resulted in similar mean ethanol intake among the four groups. Animals were then given one of three doses of caffeine (2.5, 5 or 10 mg/kg) or saline depending on their group assignment (n=10 per group) for an additional 6 consecutive days. Injections were administered 30 minutes prior to ethanol and water presentation. The final 6 days comprised the post-treatment phase, where all animals were administered saline injections, 30 minutes prior to choice sessions.

Results

Figure 7 (upper panel) shows the effect of caffeine or saline treatment on the maintenance of ethanol intake (g/kg) across baseline, treatment and post-treatment phases. A two-way ANOVA with a repeated measure across presentation days for absolute ethanol intake (g/kg) revealed significant effects of group F (3, 34) = 4.527, p < 0.01, days F (17, 578) = 2.114, p < 0.01 and group x days interaction F (51, 578) = 2.123, p < 0.01. Test of simple effects followed by test of simple comparisons (p = 0.05) revealed that the caffeine- (5 mg/kg) treated rats increased their ethanol intake relative to the saline and caffeine- (2.5 and 10 mg/kg) treated rats beginning on the fourth treatment day and continuing throughout the post-treatment phase with the exception of day 4. Moreover, rats treated with caffeine (2.5 and 10 mg/kg) did not differ from saline treated rats on any day.

Figure 7 (lower panel) shows water intake expressed as ml/kg across baseline, treatment and post-treatment phases for rats treated with caffeine or saline. A two-way ANOVA with a repeated measure across presentation days for water intake showed no significant effects of group F (3, 34) = 0.887, p > 0.05, days F (17, 578) = 1.329, p > 0.05 and group x days interaction F (51, 578) = 0.857, p > 0.05. These results suggested that there were no systematic differences in water consumption between the groups across baseline, treatment and post-treatment phases of the experiment.

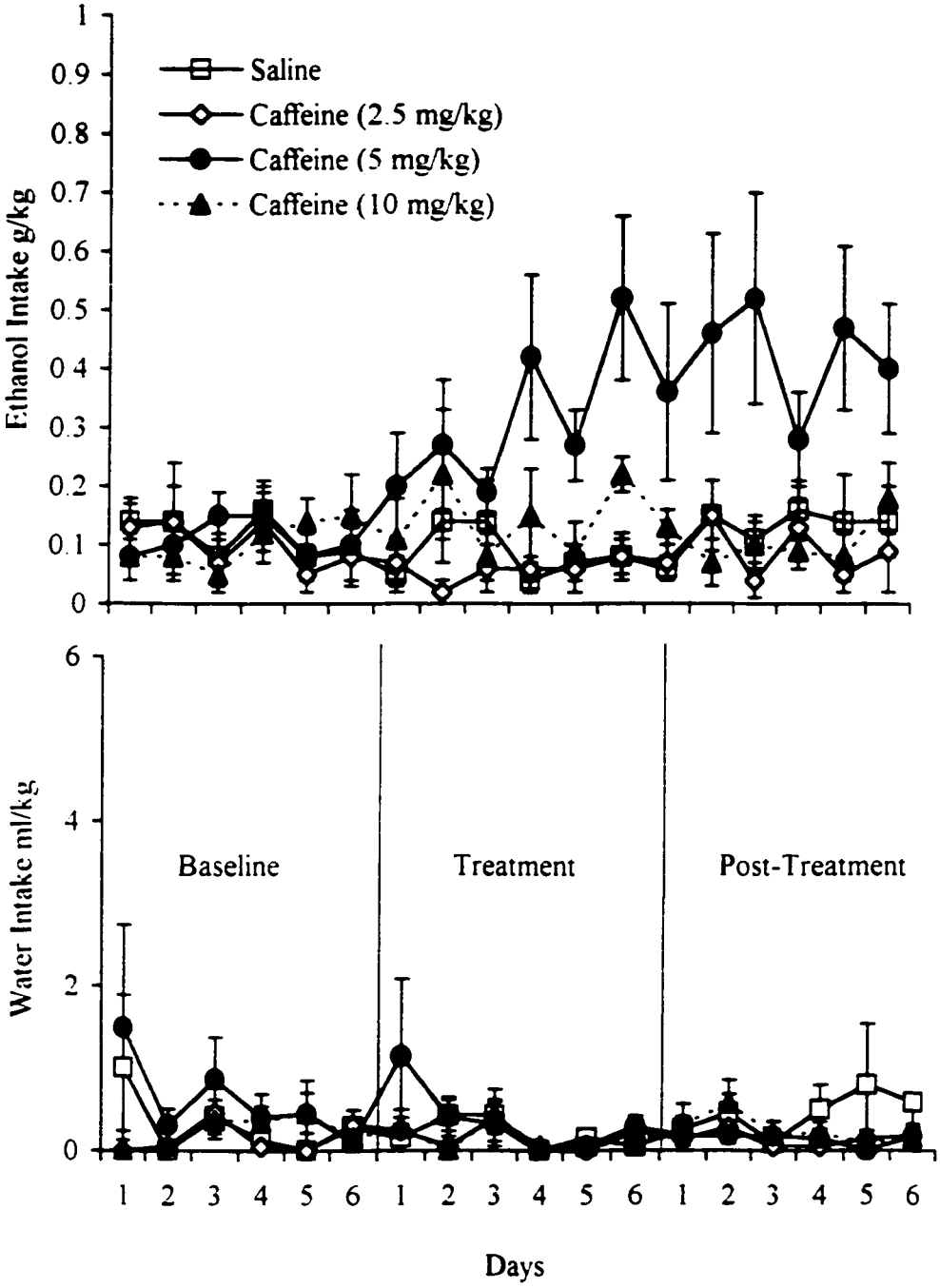


Figure 7. The effect of caffeine (2.5, 5 and 10 mg/kg) or saline on the maintenance of ethanol intake (g ethanol/body weight, upper panel) and water intake (expressed as ml fluid/kg body weight, lower panel). Vertical lines represent the SEM.

A two-way ANOVA with a repeated measure across presentation days for total fluid data revealed significant effects of group F (3, 17) = 5.248, $p < 0.01$, days F (17, 578) = 2.252, $p < 0.01$ and group x days interaction F (51, 578) = 1.526, $p < 0.01$. Subsequent analysis revealed that caffeine- (5 mg/kg) treated rats increased their total fluid intake relative to saline- and caffeine- (2.5 and 10 mg/kg) treated rats beginning on the fourth treatment day and continuing throughout most of the post-treatment phase.

Discussion

The results of Experiment 5b demonstrated a caffeine-induced facilitation in ethanol drinking in animals exposed to an ethanol acquisition regimen. The effects of caffeine on ethanol intake followed an inverted-U curve progression where 5 mg/kg of caffeine augmented ethanol drinking by as much as 400% but doses of 2.5 and 10 mg/kg of caffeine failed to alter ethanol drinking. It is worth noting that caffeine- (5 mg/kg) injected animals increased their ethanol drinking after merely four caffeine injections suggesting that acute caffeine- (5 mg/kg) treatment is sufficient to augment ethanol consumption. Furthermore, these animals maintained an elevated drinking pattern throughout the post-treatment phase, despite cessation of caffeine treatment.

The present findings suggested a very narrow dose range of caffeine that can result in elevation in ethanol drinking. As was argued previously, it is conceivable that at a dose of 5 mg/kg, caffeine may have enhanced the ability of the animals to initiate ethanol-drinking behavior as a response to the impact of ethanol's post-ingestive rewarding effects. In contrast, caffeine at a dose of 2.5 mg/kg may have been below threshold such that it failed to enhance the ability of the animals to initiate ethanol-drinking behavior to

the impact of ethanol's post-ingestive rewarding effects. Caffeine at a dose of 10 mg/kg may have been outside the optimal range capable of producing an enhancement in ethanol drinking. It is possible that the high dose of caffeine may have interfered with the enhancement in ethanol drinking that appeared to result from exposure to the more moderate dose of caffeine because at this dose caffeine may become aversive and thus fail to support positive motivation. It is notable that other manipulations have been shown to effect behavior in a fashion resembling an inverted-U shaped curve (e.g., Galina, Sutherland, & Amit, 1983).

The present findings are in disagreement with previous observations that caffeine administration failed to alter ethanol intake (Potthoff et al., 1983). One explanation for these discrepant results could be related to differences in caffeine delivery (i.e., slow-release pellet vs. an acute i.p injection of caffeine). That is, unlike an i.p injection, with its fairly rapid rate of absorption, under conditions of slow pellet release, the drug slowly diffuses into the body over an extended period of time. The present findings are also at odds with reports that caffeine reduced ethanol consumption in rats (Dietze & Kulkosky, 1991; Hedera et al., 1975). However, these studies used high doses of caffeine that were within a range producing toxic effects (Dietze & Kulkosky, 1991). The present findings suggest a very narrow dose range of caffeine that results in an elevation in ethanol drinking. It is possible that the dose exposure of caffeine in previous studies may have been outside this narrow effective dose and that doses of, say, 3.5 mg/kg and 6.5 mg/kg may also show a different type of result.

One explanation for the apparent caffeine-induced enhancement in ethanol intake may be related to self-medication (Potthoff et al., 1983), where one drug antagonizes the

effects of a second drug. Indeed, others have demonstrated that increases in ethanol intake may result from treatment with nicotine (Blomqvist et al., 1996; Potthoff et al., 1983; Smith et al., 1999), and amphetamine (Potthoff et al., 1983). In the present study, it is shown that caffeine, a drug with stimulant properties, will also augment ethanol intake. It is conceivable that continuous stimulant administration may have induced hyperactivity such that animals may consume more ethanol in order to antagonize this effect (Potthoff et al., 1983). However, a self-medication explanation cannot account for the observation that caffeine- (5 mg/kg) treated animals maintained high levels of ethanol drinking after caffeine injections had been terminated. As was previously mentioned, at 5 mg/kg, caffeine may have enhanced the ability of the animals to initiate ethanol-drinking behavior as a response to the impact of ethanol's post-ingestive rewarding effects thus producing elevations in ethanol drinking even upon cessation of caffeine treatment, a finding that would not find support in a self-medication hypothesis.

Experiment 5c: The effect of caffeine on blood ethanol levels

The results of Experiments 5a and 5b demonstrated that caffeine exposure enhanced the acquisition and maintenance of voluntary ethanol intake, respectively. While, the mechanism by which caffeine produces enhancement in ethanol drinking remains unclear, it is conceivable that caffeine may have altered the overall rates of ethanol metabolism and this may in turn have influenced ethanol intake. Experiment 5c examined the effect of caffeine on the overall rates of ethanol metabolism.

Method

Subjects

Twenty-four male Wistar rats (Charles River Canada) weighing between 275 and 325 g were used in the present experiment. Housing conditions were identical to those outlined in Experiment 5a.

Procedure

After 7 days of adaptation to the laboratory housing conditions, animals were randomly assigned to one of four groups (n=6). Animals assigned to group caffeine-ethanol and saline-ethanol were injected with caffeine (5 mg/kg) or saline (1ml/kg) 30 minutes prior to injections with ethanol (0.8 g/kg; 20% v/v) and sacrificed by decapitation at 15 or 30 minutes post ethanol injection. The dose of 5 mg/kg of caffeine was chosen since it produced the behavioral effect seen in Experiments 5a and 5b. The

dose of ethanol selected has been shown to yield blood ethanol levels within a similar range to that achieved by animals that voluntarily consume ethanol (Gill et al., 1986). Immediately after its collection. trunk blood was kept on ice in heparinized (100 U) tubes. A volume of 1 ml of heparinized blood was mixed with 0.5 ml of a PCA (perchloric acid) reagent (1 N PCA. 20 mM thiourea). The samples were centrifuged and the supernatant (0.5 ml) was sealed in glass vials for headspace analysis using Perkin-Elemer HS-6B Headspace sampler. Control samples were prepared by replacing blood with an equal volume of saline mixed with alcohol from 0.2 mg/ml to 1.0 mg/ml. The vial samples were stored at -20° C overnight and then subject to analysis the following day. The procedure was based on that of Whitmire and Whitmire (1995).

Results

Table 10 shows the effect of caffeine on blood ethanol levels at different time intervals after ethanol injections. A two-way ANOVA (group x time) conducted on whole blood levels revealed no significant effects of group F (2, 18) = 0.899, $p > 0.05$, time F (19, 342) = 1.192, $p = 0.06$ and group x time interaction F (38, 342) = 0.841, $p > 0.05$. These results suggested there was no significant alteration in blood ethanol levels resulting from caffeine pretreatment within the time period used for the ethanol drinking session.

Table 10

Effect of Caffeine (5 mg/kg) on Blood Ethanol Levels After Administration of Ethanol (0.8 g/kg ip)

	Time after ethanol administration	
Treatment Condition	15 minutes	30 minutes
Caffeine – ethanol	59.10 ± 5.50	51.720 ± 3.76
Saline – ethanol	61.10 ± 6.75	49.52 ± 2.41

Note. Blood ethanol levels were measured in milligrams per 100 ml of whole blood

Discussion

The results of this experiment showed that caffeine, at the dose tested, failed to alter blood levels of ethanol following ethanol treatment. This observation suggests that the nature of the interaction between caffeine and ethanol was not the result of a simple change in blood ethanol levels. As such, it is unlikely that the alteration in ethanol drinking behavior was the result of a caffeine-induced change in ethanol metabolism.

Experiment 5d: Augmentation of corticosterone release by means of a caffeine-ethanol interaction

On the basis of results obtained from a number of studies (e.g., Fahlke, Engel, Eriksson, Hard, & Soderpalm 1994a), investigators have suggested that treatment with corticosterone (CORT) may play a modulating role in the self-administration of ethanol. For example, adrenalectomy was shown to reduce ethanol intake in rats, and the level of intake was restored by administration of CORT in drinking water (Fahlke, et al., 1994a) or by subcutaneous implantation of CORT pellets (Fahlke, Hard, Eriksson, Engel, & Hansen, 1995). Also, the CORT synthesis inhibitor metyrapone was shown to suppress ethanol drinking, and this effect was partially blocked by prior treatment with CORT (Fahlke, Hard, Thomasson, Engel, & Hansen, 1994b).

Given that both caffeine and CORT have been shown to promote ethanol drinking, the present experiment was designed to assess whether acute treatment with caffeine delivered 30 minutes before an ethanol injection would elevate plasma CORT levels. In addition, the effect of caffeine on alterations in blood ethanol levels was assessed in order to determine whether the putative enhancement in CORT was related to changes in ethanol metabolism.

Method

Subjects

Seventy-two male Wistar rats (Charles River Canada, Inc. Quebec) weighing between 250 and 300 g at the start of the experiment were used. Housing conditions were identical to those outlined in Experiment 5a.

Procedure

After 10 days of adaptation to the laboratory housing conditions, animals were randomly assigned to 1 of 8 groups (n=6). Animals assigned to groups caffeine-ethanol (CE), caffeine-vehicle (CV), vehicle-ethanol (VE) or vehicle-vehicle (VV) were injected with caffeine (5 mg/kg in 1 ml kg of 0.9% saline i.p) or 0.9% saline (1 ml/kg) 30 minutes before injections with ethanol (0.8 g/kg: 20% v/v, i.p) or 0.9% saline. A dose of 5 mg/kg of caffeine was selected because it was shown to augment ethanol drinking in both the acquisition and a maintenance ethanol-drinking procedure. In addition, the dose of ethanol (0.8 g/kg) selected was within a range previously shown to yield blood ethanol levels that are similar to those achieved by animals that voluntarily consume ethanol within a limited-access regimen (Gill et al., 1986). All injections were administered in the animals' home cages. Fifteen or 30 minutes after the second injection, animals were transported to an adjacent room where they were killed by decapitation. Immediately after decapitation, trunk blood was collected and kept on ice in heparinized (100 U) tubes. One millilitre of heparinized blood was mixed with ice cold 0.5 ml of a PCA (1 N perchloric acid) reagent and 20 mM thiourea. The samples were centrifuged at 4°C and the supernatant (0.5 ml) was sealed in glass vials for headspace analysis by using a

a Perkin-Elemer HS-6B Headspace sampler (Whitmire & Whitmire, 1995). Control samples were prepared by replacing blood with an equal volume of saline mixed with alcohol from 0.2 mg/ml to 1.0 mg/ml. The vial samples were stored at –20°C overnight and then subjected to analysis the following day. Trunk blood was then centrifuged (3000 rpm for 5 minutes), and 200 µl of plasma was transferred into 500 µl Epindorf tubes and stored at –70°C for later measurement of corticosterone with the use of radioimmunoassay (Krey, Lu, Bulter, Hotchkiss, Pivan, & Knobil, 1975). Ten microliters of each plasma sample was extracted with 1 ml of ethanol (100%) and centrifuged. Then 100 µl sample extracts were assayed in duplicate alongside extracts of corticosterone standards ranging from 7.8/1000 pg prepared in ethanol (100%). The dried extracts were incubated overnight with antibody to corticosterone (Endocrine Sciences, Calabasas Hills, CA) and [3H] corticosterone (New England Nuclear, Boston, MA). Bound corticosterone was separated from free corticosterone and the bound corticosterone was quantified.

Data analysis

Corticosterone data were analyzed by means of a two-way factorial analysis of variance (ANOVA). The first factor was group and included four levels (VE, CE, CV, VV), while the second factor was time and included two levels (15 and 30 minutes). Blood ethanol data were analyzed with a two-way ANOVA. The factors were group with two levels (VE and CE) and time with two levels (15 and 30 minutes). Post hoc analysis was conducted by using Tukey's HSD. Significance was set at $p < .05$.

Results

Corticosterone Determinations

A two-way ANOVA (group x time) conducted on plasma corticosterone levels revealed a significant effect of group F (3, 39) = 5.465, $p < 0.01$, no significant effect of time F (1, 39) = 3.004, $p > 0.05$, and no significant group x time interaction F (3, 39) = 1.101, $p > 0.05$. Post hoc Tukey's analysis revealed that animals in group CE had significantly elevated plasma CORT levels relative to animals in group's VE, CV, and VV. None of the other groups differed significantly from one another. Together it appears that although acute treatment with caffeine or ethanol singly failed to elevate plasma CORT levels beyond those of saline in control animals, treatment with caffeine before the delivery of ethanol resulted in elevated plasma CORT release (see Figure 8).

Blood Ethanol Determinations

Table 11 shows the effect of caffeine on blood ethanol levels at 15- and 30-minute intervals after an acute ethanol injection. A two-way ANOVA (group x time) conducted on whole blood levels revealed no significant effects of group F (1, 19) = 0.046, $p > 0.05$, time F (1, 19) = 3.936, $p = 0.06$, and group x time interaction F (1, 19) = 0.891, $p > 0.05$.

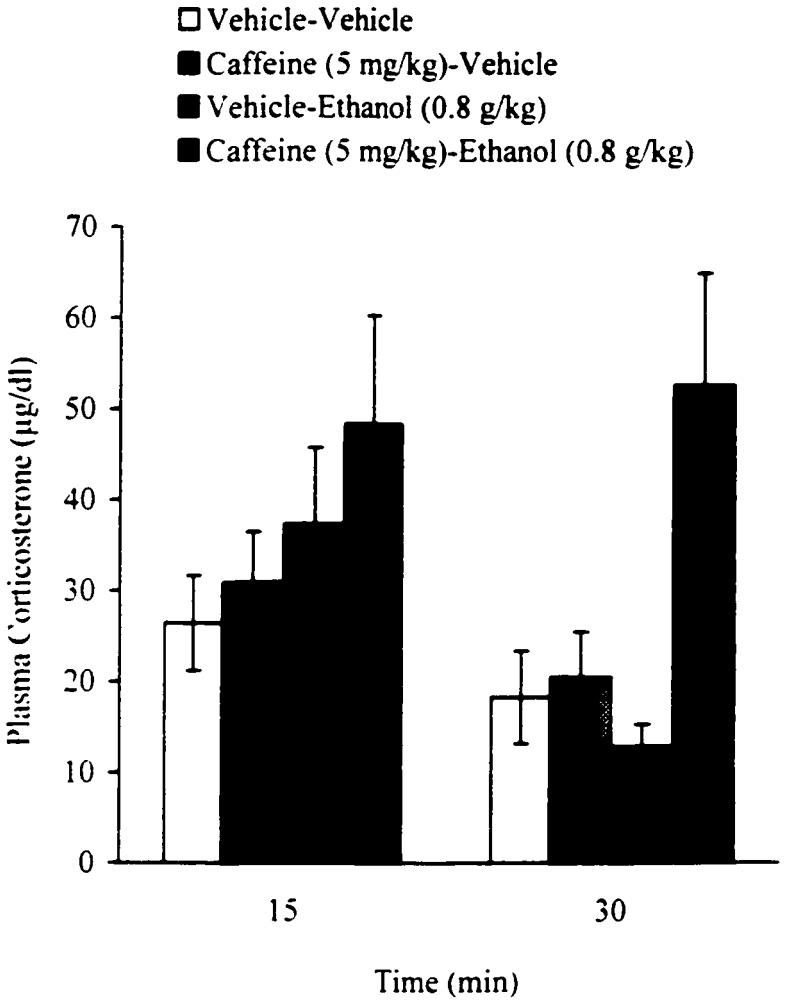


Figure 8. The effect of caffeine (5 mg/kg) or saline pretreatment on mean bound plasma corticosterone levels at 15 and 30 minutes after a challenge with an acute dose ethanol (0.8 g/kg: 20% v/v). Vertical lines reflect the SEM

Table 11

Effect of Caffeine (5 mg/kg) on Blood Ethanol Levels After Administration of Ethanol (0.8 g/kg i.p)

	Time after ethanol administration	
Treatment Condition	15 minutes	30 minutes
Caffeine – ethanol	66.59 ± 3.21	52.07 ± 3.73
Saline – ethanol	62.63 ± 7.28	57.81 ± 3.70

Note: Blood ethanol levels were measured in milligrams per 100 millilitres of whole blood.

Discussion

The present experiment was designed to assess whether acute caffeine pretreatment delivered before an ethanol injection and at a dose shown to augment ethanol intake would also elevate plasma CORT levels. Caffeine and ethanol administered to animals in close temporal proximity displayed elevated plasma CORT levels, whereas both caffeine and ethanol delivered singly failed to augment CORT levels beyond those observed after saline administration in control animals. This finding is not surprising given the low doses of each drug tested. Previous findings from other laboratories have shown that substantially higher doses of each drug will be needed to elevate plasma CORT levels on their own (Brick & Pohorecky, 1984, 1985; Aragon, Nicoletti, Rogan, & Amit, 1987; Nicholson, 1989; Concas, Porcu, Sogliano, Serra, Purdy, & Biggio, 2000). Still, it is interesting to note that in combination, these drugs, at doses that failed to augment plasma CORT levels on their own, together acted to enhance plasma CORT levels, which persisted across the 30 minutes tested.

Although, the nature of this interaction remains to be determined, it seems to be unrelated to changes in ethanol metabolism as caffeine failed to alter blood ethanol levels. It is plausible that the present elevations in plasma CORT levels resulting from the co-administration of caffeine and ethanol may be involved in the mediation of the caffeine-induced elevations in ethanol drinking previously observed. The latter suggestion seems possible in light of a growing body of evidence showing that CORT may directly modulate ethanol drinking (Fahlke et al., 1994a, Fahlke et al., 1994b, Fahlke et al., 1995, Fahlke et al., 1996; Lamblin & De Witte, 1996).

In summary, if infusions of CORT are capable of stimulating ethanol drinking, and if elevated plasma CORT levels are associated with elevated ethanol drinking then it is conceivable that the present elevations in plasma CORT levels observed in animals co-administered caffeine and ethanol may play a role in the caffeine-induced enhancement of ethanol drinking previously observed.

Experiment 6a: The effect of caffeine pretreatment on conditioned taste aversion to a low dose of ethanol

The results of Experiments 5a and 5b provided data to suggest that caffeine augmented ethanol intake, presumably by means of a facilitation in ethanol's positively reinforcing effects. If one assumes that the ability of positively reinforcing drugs to support CTA is related to their well-documented positive reinforcing effects (e.g., Hunt & Amit, 1987) and if caffeine promotes ethanol drinking by increasing the reinforcing efficacy of ethanol, then caffeine should also promote an ethanol-induced CTA.

Conversely, it is possible that caffeine's ability to promote ethanol drinking may involve the antagonism of ethanol's aversive effects. In fact, it has been argued that the combined use of specific drugs in humans may reflect a form of "self-medication", where one drug neutralizes or ameliorates the aversive effects of a second drug (Potthoff et al. 1983; Zacny, 1990). It follows that if CTA induced by positively reinforcing drugs reflect their aversive nature, and if caffeine attenuates ethanol's aversive effects, then caffeine should also attenuate an ethanol-induced CTA.

In light of the above comments, it was hypothesized that if CTA induced by positively reinforcing drugs reflects aversion per se, and if caffeine's facilitation of ethanol drinking reflected an attenuation of ethanol's aversive effects, then caffeine should block an ethanol-induced CTA. Conversely, if CTA induced by positive reinforcing agents reflects the their rewarding effects and if caffeine promoted ethanol drinking by increasing the reinforcing efficacy of ethanol, then caffeine should enhance an ethanol-induced CTA in the pretreatment CTA procedure.

Method

Subjects

Thirty male Wistar rats (Charles River Canada, Inc, Quebec) weighing between 250 and 300 g at the start of the experiment were used. Rats were individually housed in hanging stainless-steel wire cages with standard laboratory chow (PMI Nutrition International. Oakville. ON) and water available ad libitum prior to start of the experiment. The animals were maintained on a 12-h on 12-h off light/dark cycle in a room regulated for constant temperature and humidity. All animals used in the present study were treated in accordance with the guidelines of the Canadian Council for Animal Care. In addition. the care and use of. as well as all procedures involving animals in the present study. were approved by the Concordia University Animal Care Committee.

Drugs

Caffeine (Sigma Chemical Co.) was dissolved in 0.9% saline and injected in a volume of 1 ml/kg. All caffeine injections were administered i.p and injection volumes were constant across doses (2.5. 5. and 10 mg/kg). Ethanol solution was prepared by diluting a 95% ethanol stock in saline and was injected at doses of 1.0 and 1.5 g/kg (20% v/v). All ethanol injections were delivered i.p Saccharin (0.1% Sodium Saccharin. Sigma) was prepared as a 1g/l solution diluted in tap water.

Procedure

Following one week of acclimatization to the laboratory housing conditions. rats were placed on a 23 –h and 40-min water-deprivation schedule. Tap water was presented

to the animals in stoppered plastic tubes fitted with stainless steel ball bearing spouts for 20 min beginning at noon each day. The spouts were inserted through the fronts of the home cages and were presented in this manner for 6 consecutive days. Fluid was measured to the nearest millilitre.

On day 7, following 6 days of exposure to the water deprivation regimen, rats were randomly assigned to one of five groups (n=6). Animals assigned to group vehicle-vehicle (VV) received an injection of saline 10 minutes prior to the presentation of a novel saccharin solution given in place of tap water. Within a minute following this 20-minute saccharin exposure session these animals were again injected with saline. Animals assigned to group vehicle-ethanol (VE) were injected with saline 10 minutes prior to the saccharin session and then injected with ethanol (1.0 g/kg) immediately following the 20-minute session. Animals assigned to group caffeine-ethanol (C-E) were injected with one of three doses of caffeine (2.5, 5 and 10 mg/kg) 10 minutes prior to the saccharin drinking session and were then injected with ethanol (1.0 g/kg) immediately following the 20-minute drinking session. This dose of ethanol has previously been reported to produce CTA (Froehlich, Harts, Lumeng, & Li, 1988; Stewart, McBride, Lumeng, Li, & Murphy, 1991). In addition, because caffeine administered 30 minutes prior to an ethanol drinking session has been shown to promote ethanol drinking (Experiments 5a and 5b), the same temporal parameter was adopted in the present study. A second, third and fourth pairing of the saccharin solution and drug or vehicle was carried out on days 10, 13 and 16. Days 19, 22, 25 and 28 comprised drug free test days.

Data Analysis

A saccharin preference ratio (total saccharin consumed/total fluid) was calculated and subjected to analysis. Consistent with the usual procedure in a 2 bottle- test, a CTA was defined as a significant decrease in saccharin preference relative to the saline control group. Preference scores were obtained by collapsing the average of 2 successive test days (i.e., test days 1 and 2 and test days 3 and 4). In addition, saccharin intake data obtained over 4 saccharin-drug pairings was also subjected to separate analysis.

Separate two-way analyses of variance (ANOVAs) with repeated measures were conducted on saccharin intake and saccharin preference data, which constituted the dependant variables. Post hoc Tukey tests and tests of simple effects were performed where appropriate for pairwise comparisons and significant interaction effects. An alpha level of .05 was used for all statistical tests.

Results

A two-way (5 X 4) ANOVA conducted on saccharin intake across the 4 pairing days revealed no significant effect of group F (4, 25) = 2.714, $p > .05$, day F (3, 75) = 4.211, $p > .05$, or group x day interaction F (12, 75) = 1.218, $p > .05$. This analysis revealed no significant effect of caffeine pretreatment on ethanol induced CTA across the 4 pairing days (Table 12).

The analysis conducted on saccharin preference data revealed a significant effect of group F (4, 25) = 3.002, $p < .05$, no significant effect of day F (1, 25) = 0.453, $p > .05$, and a significant group x day interaction F (4, 25) = 2.907, $p < .05$. Test of simple effects revealed that while on its own, ethanol (1.0 g/kg) failed to induce CTA ($p > .05$), a

finding that is at odds with previously published reports (Froehlich et al. 1988: Stewart et al.. 1991) animals pretreated with caffeine (2.5 and 10 mg/kg) and then conditioned with ethanol (1.0 g/kg) showed significant CTAs on TD1 exclusively ($p < .05$). Figure 9 illustrates that caffeine at doses of 2.5 and 10 mg/kg- promoted an ethanol-induced CTA (1.0 g/kg) in a sensitive two-bottle choice presentation.

Table 12.

Effect of Caffeine Pretreatment (2.5, 5 and 10 mg/kg) on an Ethanol-Induced CTA (1.0 g/kg) as reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4.

	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
C(2.5 mg)E	18.5 ± 1.6	22.0 ± 1.8	22.0 ± 1.7	16.3 ± 2.0
C(5 mg)E	17.0 ± 3.0	21.5 ± 1.6	17.0 ± 2.3	23.5 ± 1.3
C(10 mg)E	15.3 ± 2.0	17.7 ± 1.8	20.0 ± 1.5	25.3 ± 2.2
VE(1.0 g/kg)	22.0 ± 2.1	21.5 ± 2.1	22.8 ± 2.1	19.5 ± 1.4
VV	13.5 ± 1.8	16.7 ± 2.6	21.2 ± 1.2	15.5 ± 1.9

Note. C = caffeine; E = ethanol; V = vehicle

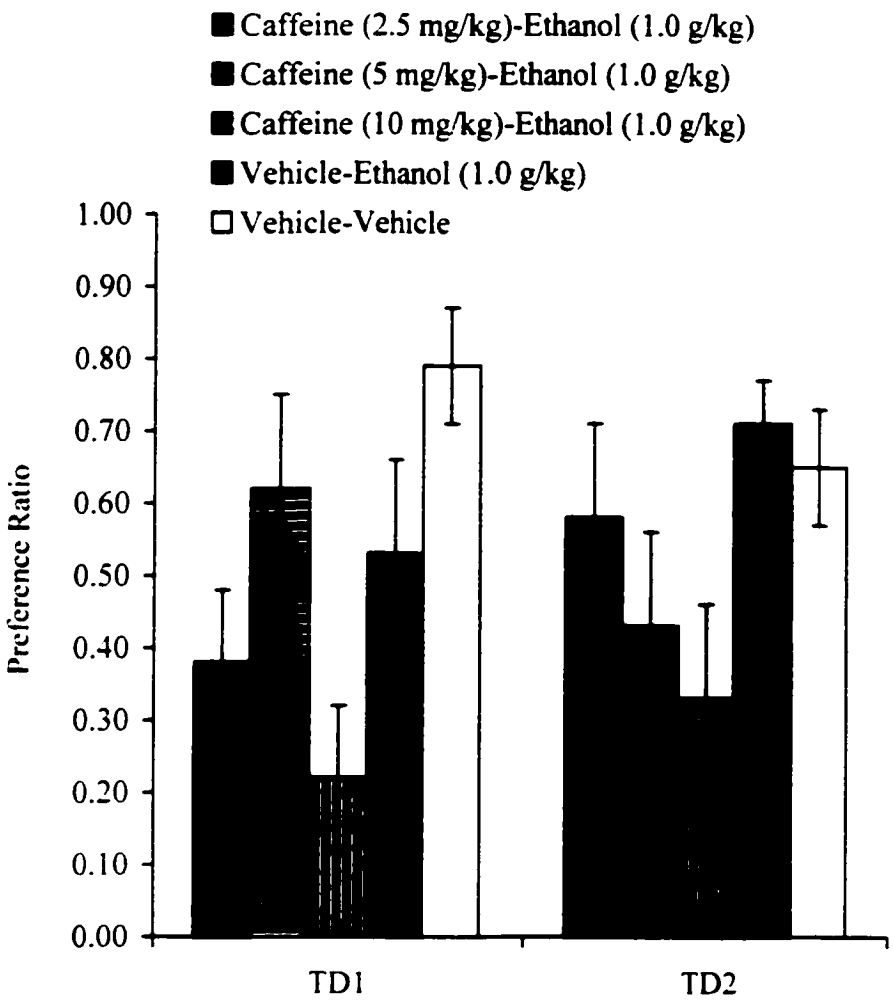


Figure 9. The effect of pretreatment with caffeine (2.5, 5 and 10 mg/kg) on conditioned taste aversion induced by ethanol (1.0 g/kg) as reflected in mean saccharin preference ratio. TD1 is preference collapsed across Test Days 1 and 2, and TD2 is collapsed across Test Days 3 and 4. Vertical lines represent the SEM.

Discussion

Rather than attenuate an ethanol-induced CTA, the results of the present experiment demonstrated that caffeine augmented an ethanol-induced CTA. However, this finding does not negate the possibility that caffeine may attenuate an ethanol-induced CTA at higher ethanol doses. It is notable that a dose of 1.0 g/kg of ethanol failed to induce CTA, a finding that is at odds with some previously published reports (Froehlich et al., 1988; Stewart et al., 1991). One explanation for this discrepancy may have to do with the fact that these reports employed 5 conditioning days as compared to the 4 conditioning days used in the present study. It is conceivable that an additional conditioning day is required in order to observe ethanol CTA at a dose of 1.0 g/kg. Alternatively, the discrepancy in results may be related to the strain of rats used. Specifically, the former studies tested female rats that were genetically selected for ethanol drinking as compared to the present study that employed a genetically unselected strain of male rats. As was argued previously in this thesis, different rats may also show differences in sensitivity to the CTA inducing effects of positive reinforcing drugs (Grigson & Freet, 2000).

One possible explanation for the finding that caffeine augmented an ethanol-induced CTA may be related to the possibility that the avoidance producing properties of caffeine and ethanol summate to produce greater avoidance than that produced by ethanol alone. The difficulty with such an interpretation is that the dose of ethanol (1.0 g/kg) tested was without the ability to promote avoidance.

In spite of the foregoing, some have interpreted drug pretreatment effects in terms of general interference effects (Domjan, 1980). That is, prior drug exposure results in a malaise thereby disrupting the subsequent association between the saccharin-drug

pairing. In view of caffeine's facilitation of an ethanol-induced CTA, the present findings cannot be explained in terms of general interference effects (Domjan, 1980). Indeed, it has been previously argued that the pretreatment CTA procedure may serve as a useful tool to reveal pharmacologically specific interactions between drugs (Kunin, et al, 1999b).

Experiment 6b: The effect of caffeine pretreatment on conditioned taste aversion to a high dose of ethanol

The present experiment examined the effect of pretreatment with caffeine (2.5, 5 and 10 mg/kg) on a CTA induced by a higher dose of ethanol (1.5 g/kg). Because in the previous study ethanol (1.0 g/kg) failed to elicit CTA, we raised the dose to 1.5 g/kg as this dose has previously been shown to yield a fairly robust CTA (Bienkowski, et al., 1998) in the same rat strain.

Method

Subjects

Forty-eight male Wistar rats (Charles River Canada, Inc. Quebec) weighing between 250 and 300 g at the start of the experiment were used. Housing conditions were identical to those outlined in Experiment 6a.

Procedure

The procedure used in the following experiment was identical to Experiment 5a with the following exceptions. The present experiment assessed the effect of pretreatment with caffeine (2.5, 5 and 10 mg/kg) on a CTA induced by a higher dose of ethanol (1.5 g/kg). In addition, 3, caffeine –vehicle (CV) control groups were added to the standard 5 treatment groups described in Experiment 6a. Animals assigned to group caffeine-vehicle (CV) received an injection of caffeine (2.5, 5 or 10 mg/kg) 10 min prior to presentation of a novel saccharin solution, presented for 20 minutes, and immediately following the 20-

minute saccharin exposure session were injected with saline. These control groups were included in order to rule out the possibility that caffeine pretreatment may by itself attenuate saccharin intake across conditioning days (i.e., backward conditioning).

Results

A two-way (8 X 4) ANOVA conducted on saccharin intake data revealed a significant effect of group F (7, 39) = 2.317, $p < .05$, day F (3, 117) = 5.876, $p < .05$, and group x day interaction F (21, 117) = 5.992, $p < .05$. Test of simple main effects revealed that animals pretreated with caffeine (2.5, 5 and 10 mg/kg) and then conditioned with ethanol (1.5 g/kg) significantly decreased their baseline saccharin intake across the conditioning days ($p < .05$). This was in contrast to control animals that increased their baseline saccharin intake across the conditioning days ($p < .05$) (Table 13).

The analysis conducted on saccharin preference data revealed a significant effect of group F (7, 39) = 10.020, $p < .05$, no significant effect of day F (1, 39) = 2.254, $p > .05$, and no significant group x day interaction F (7, 39) = 0.197, $p > .05$. Post hoc Tukeys conducted on the significant main effect of group revealed that caffeine (2.5, 5 and 10 mg/kg) pretreatment was unable to attenuate an ethanol-induced CTA ($p > .05$). Figure 10 illustrates that none of the caffeine doses were capable of attenuating an ethanol-induced CTA (1.5 g/kg) across the two-bottle choice test days.

Table 13

Effect of Caffeine Pretreatment (2.5, 5 and 10 mg/kg) on an Ethanol-Induced CTA (1.5 g/kg) as Reflected in Mean Saccharin Consumption (Milliliters) for Pairing Days 1-4.

Treatment	Days			
Groups	PD1	PD2	PD3	PD4
	M	M	M	M
C(2.5 mg)V	22.3 ± 1.8	24.3 ± 1.0	26.7 ± 1.2	27.8 ± 2.2
C(5 mg)V	6.2 ± 1.8	16.3 ± 3.3	22.2 ± 2.7	22.2 ± 3.3
C(10 mg)V	12.3 ± 2.1	18.0 ± 1.7	21.7 ± 1.7	23.0 ± 2.6
C(2.5 mg)E	20.8 ± 0.8	19.0 ± 1.5	17.8 ± 1.8	12.7 ± 3.5
C(5 mg)E	21.0 ± 1.1	21.0 ± 2.1	18.2 ± 2.5	14.5 ± 2.7
C(10 mg)E	18.8 ± 1.7	17.8 ± 3.6	17.3 ± 3.4	16.2 ± 3.5
VE(1.5g/kg)	19.0 ± 1.8	20.8 ± 1.2	14.8 ± 3.7	13.6 ± 4.0
VV	18.0 ± 1.3	21.2 ± 1.5	24.0 ± 1.2	19.3 ± 0.7

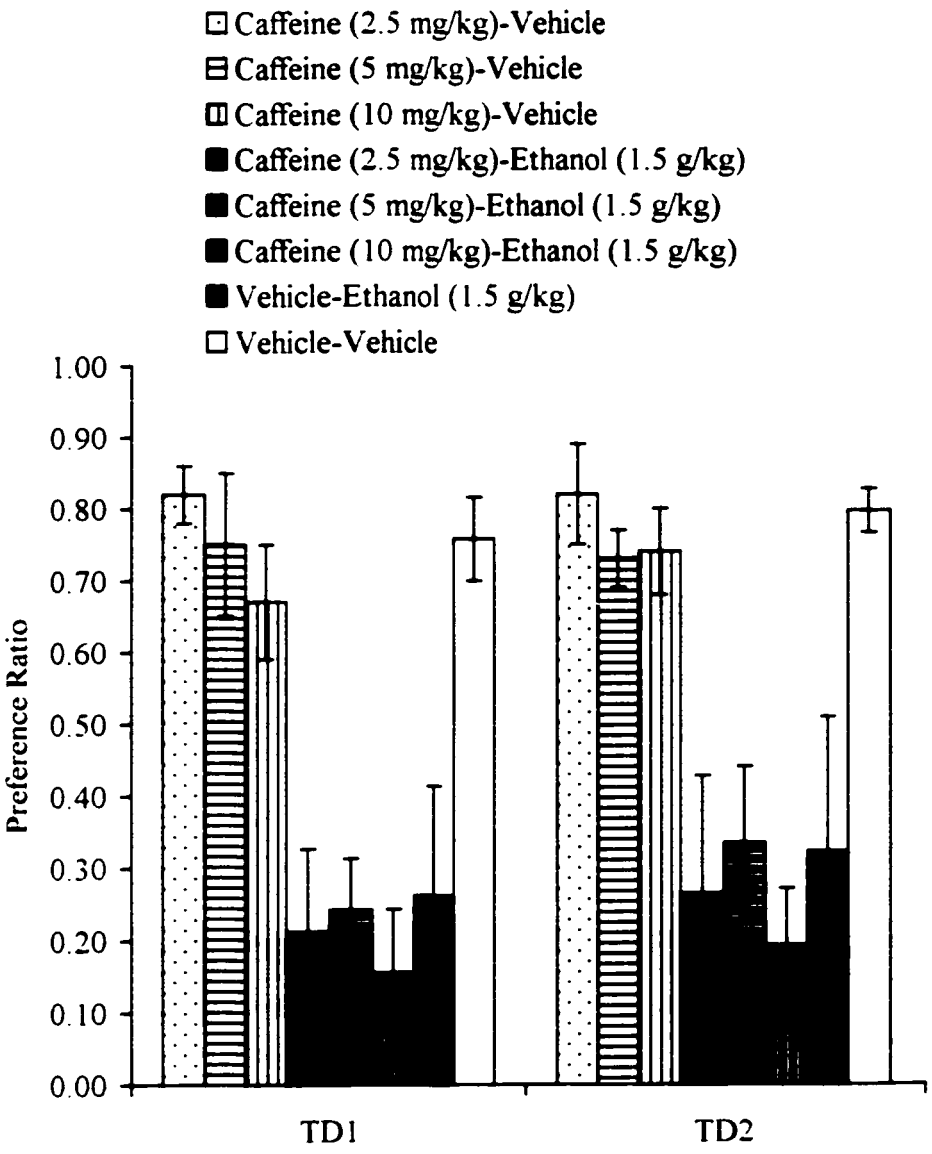


Figure 10. The effect of pretreatment with caffeine (2.5, 5 and 10 mg/kg) on conditioned taste aversion induced by ethanol (1.5 g/kg) as reflected in mean saccharin preference ratio. TD1 is preference collapsed across Test Days 1 and 2, and TD2 is collapsed across Test Days 3 and 4. Vertical lines represent the SEM.

Discussion

Despite the appearance of a rather robust ethanol-induced CTA, caffeine pretreatment failed to attenuate the effect. This finding is at odds with the hypothesis that caffeine's ability to promote ethanol drinking may involve the antagonism of ethanol's aversive effects. However, the present results also seem incompatible with the notion that CTA to positive reinforcing drugs reflect their rewarding properties as caffeine failed to augment an ethanol-induced CTA. Still, it is reasonable to assume that the failure to observe a caffeine-induced enhancement of ethanol CTA may have been due to a floor effect. In other words, if caffeine enhances an ethanol-induced CTA, there is little room for this effect to manifest itself with a dose of 1.5 g/kg of ethanol as animals conditioned with this dose exhibit almost complete avoidance of the saccharin-flavoured fluid.

Experiment 6c: The effect of caffeine pretreatment on levels of blood ethanol

Experiment 6c was designed to assess whether the caffeine-induced facilitation of an ethanol CTA observed in experiment 6a was a result of an alteration in the overall rates of ethanol metabolism.

Method

Subjects

Twenty-four male Wistar rats (Charles River Canada, Inc. Quebec) weighing between 250 and 300 g at the start of the experiment were used. Housing conditions were identical to those outlined in Experiment 6a.

Procedure

After 7 days of adaptation to the laboratory housing conditions, rats were randomly assigned to one of four groups (n=6). Rats assigned to groups caffeine-ethanol and saline-ethanol were injected with caffeine (10 mg/kg in 1 ml of 0.9% saline i.p) or 0.9% saline (1 ml/kg) 30 minutes prior to injections with ethanol (1.0 g/kg; 20% v/v, i.p) and sacrificed by decapitation at 15 or 30 minutes following the ethanol injection. The doses of caffeine (10 mg/kg) and ethanol (1.0 g/kg) were selected as they were shown to optimally interact in Experiment 5a. Immediately after its collection, trunk blood was kept on ice in heparinized (100 U) tubes. One milliliter of heparinized blood was mixed with 0.5 ml of a PCA (perchloric acid) reagent and 20 mM thiourea. The samples were

centrifuged and the supernatant (0.5 ml) was sealed in glass vials for headspace analysis using Perkin-Elemer HS-6B Headspace sampler. Control samples were prepared by replacing blood in an equal volume of saline mixed with alcohol from 0.2 mg/ml to 1.0 mg/ml. The vial samples were stored at -20°C overnight and then subjected to analysis the following day. The procedure was based on that of Whitmire and Whitmire (1995).

Results and Discussion

Table 14 shows the effect of caffeine on blood ethanol levels at different time intervals after ethanol injections. A two-way (2 X 2) ANOVA conducted on whole blood levels revealed no significant effect of group F (1, 20) = 0.001, $p > .05$, time F (1, 20) = 3.746, $p > .05$ and group x time interaction F (1, 20) = 0.183, $p > .05$. These results suggested there was no significant alteration in blood ethanol levels resulting from caffeine pretreatment.

Table 14

Effect of Caffeine (10 mg/kg) on Blood Ethanol Levels After Administration of Ethanol (1.0 g/kg i.p)

	Time after ethanol administration	
Treatment Condition	15 minutes	30 minutes
Caffeine – ethanol	67.31 ± 6.33	70.77 ± 2.20
Saline – ethanol	71.70 ± 1.88	62.73 ± 3.68

Note. Blood ethanol levels were measured in milligrams per 100 ml of whole blood

Experiment 7a: Differences in locomotion response to an inescapable novel environment predicts sensitivity to amphetamine-induced conditioned taste aversion

As was discussed earlier in this thesis, an accumulated body of data has pointed to a functional relationship between the neurochemical systems mediating the positively reinforcing and CTA inducing properties of a variety of drugs. It has been demonstrated that while the neurochemical systems sub serving the CTA inducing and positively reinforcing properties of self-administered drugs overlap, they are also distinct from the mechanisms sub serving CTA induced by drugs that do not possess positively reinforcing properties (Goudie et al., 1975; Grupp, 1977; Hunt et al., 1985; Sklar & Amit, 1977; Wagner, Foltin, Seiden, & Schuster 1981).

There are, in addition, a number of behavioural variables that also appear to be involved in the expression of both positive reinforcement and CTA to a variety of drugs. For example, a number of stress variables including isolation housing, food deprivation and shock have all been shown to modify both CTA to and self-administration of a variety of positive reinforcing drugs (Bell, Thiele, Seeley, Bernstein, & Woods 1998; Bowers, Gingras, & Amit 1996; Bozarth, Murray, & Wise 1989; Carroll, France, & Meische 1979; de la Garza, Bergman, & Hartel 1981; Goeders & Guerin, 1994; Mark-Kaufman & Lewis, 1984; Schenk, Hunt, Klukowski, & Amit, 1987; Smith, Neill, & Costall, 1998). The involvement of similar mechanisms, both behavioural and neurochemical, in the mediation of CTA and self-administration to a variety of positive

reinforcing drugs has provided support for the notion that these apparently dichotomous behaviours are somehow interrelated.

Piazza, Deminiere, LeMoal, and Simon (1989) demonstrated that locomotion response to an inescapable novel environment, predicted the tendency of rats to acquire amphetamine self-administration. Rats displaying high activity response to an inescapable novel environment (HR) compared to low activity responders (LR) rapidly acquired amphetamine self-administration. A similar relationship between novelty-induced locomotion and drug-induced activity was elucidated (Piazza et al., 1989). That is, rats exhibiting a higher locomotion response to an inescapable novel environment showed greater drug-induced activity (e.g., Deroche, Piazza, Le Moal, & Simon, 1993; Hooks, Jones, Liem, & Justice, 1992; Hooks, Jones, Neill, & Justice, 1991; Piazza et al., 1989). Together, these findings suggested that locomotion response to an inescapable novel environment predicted sensitivity to drug reward and drug-induced activity. While there is considerable evidence that locomotion response to an inescapable novel environment is associated with the proclivity towards drug self-administration (e.g., Gingras & Cools, 1995; Piazza, Deminiere, LeMoal, & Simon 1990; Piazza et al., 1989), it does not appear to predict conditioned place preference (Erb & Parker, 1994; Gong, Neill, & Justice, 1996).

Since the positively reinforcing and CTA inducing properties of self-administered drugs are functionally related and mediated by overlapping neural substrates, it follows that the variables that predict sensitivity to the positively reinforcing properties of self-administered drugs may also serve to predict sensitivity to the CTA inducing properties of the same drugs. In view of the above, the present study was designed to examine a

possible relationship between locomotion responses to an inescapable novel environment and CTA to amphetamine. Previous research has shown that rats exhibiting high locomotion response to an inescapable novel environment compared to those displaying a lower locomotion response showed greater sensitivity to the positively reinforcing properties of amphetamine (Piazza et al., 1989; Piazza et al., 1990). As such, it was hypothesized that rats displaying high activity response to an inescapable novel environment compared to those exhibiting a low activity response would display greater sensitivity to CTA induced by amphetamine.

Method

Subjects

The subjects were 80 male Sprague Dawley rats (Charles River, Quebec), weighing between 275-325 g at the start of the experiment. Animals were individually housed in hanging stainless steel wire cages with standard lab chow (PMI Nutrition International, Oakville, ON) and water freely available prior to the onset of the experiment. The animals were maintained on a 12-h on/ 12-h off light dark cycle, with the lights on at 0800 h and the lights off at 2000 h, in a room regulated for constant temperature and humidity. All subjects used in this experiment were treated in accordance with the guidelines of the Canadian Council for Animal Care. In addition, the care and use of, as well as all procedures involving animals in the present study, were approved by the Concordia University Animal Care Committee.

Apparatus

All fluid consumption testing was conducted in the home cages by introducing 100 ml plastic drinking tubes with stainless steel ball-bearing spouts inserted through the front of the cage. Locomotor responses to an inescapable novel environment were assessed in a novel wooden chamber (1 m x 1 m) with two photocell beams on each wall, 2 cm above the floor, equally spaced. A locomotion count was registered on counters in an adjacent room each time a photocell beam was interrupted. The open field chambers were illuminated by lights (40 watts) situated above each chamber.

Drugs

Dextroamphetamine Sulfate (*d*-amphetamine sulfate) purchased from Smith, Kline & French, Canada Ltd, expressed as the salt, was dissolved in 1ml/kg of 0.9% saline solution and was injected at doses of 1.5, 2 and 3 mg/kg. All injections were delivered i.p. Sodium saccharin was dissolved in tap water in a concentration of 0.1%.

Procedure

After 10 days of acclimatization to the laboratory housing conditions, animals were tested for their locomotor response to an inescapable novel environment. Each animal was placed individually in the open field chamber and its activity was monitored for a period of 120 minutes. All testing was conducted between 1200 and 1800 h. On the basis of their cumulative activity count over 120 minutes, animals were classified as high or low responders depending on whether their cumulative count was above or below the median for the total sample of animals tested in the novel environment (Erb & Parker,

1994; Piazza et al., 1989). Seven days after the animals were tested in the novel environment their water bottles were removed. A water deprivation regimen consisting of a 20-minute daily exposure to water presented to the animals in single stoppered plastic tubes fitted with stainless steel ball bearing spouts was instituted. All fluids were presented to the animals in their home cages beginning at noon each day.

After seven days of adaptation to the water deprivation schedule animals were assigned to one of eight conditions (n =10 per group): High Responding (HR) saline or amphetamine (1.5, 2.0 and 3.0 mg/kg), and Low Responding (LR) saline or amphetamine (1.5, 2.0 and 3.0 mg/kg). On this day (day 8), which constituted pairing day 1, animals were presented with a novel saccharin solution (0.1%) instead of water for a period of 20 minutes. Immediately following the 20-minute saccharin presentation, rats were injected with either saline or amphetamine (1.5, 2.0 and 3.0 mg/kg). These drug doses were selected because they have all previously been shown to produce reliable CTAs (Bell et al., 1998; Bowers et al., 1996; Smith et al., 1998). In addition, the doses of amphetamine used were within the range used in other studies assessing the relationship between novelty-induced locomotion and sensitivity to amphetamine's effects (e.g., Erb & Parker, 1994; Klebaur & Bardo, 1999).

On the following day (day 9), all animals were given 20-minutes access to water. The conditioning procedure (pairing day) that took place on day 8 was repeated again on day 10. Days 12, 14, 16, 18, 20 and 22 constituted drug free test days, where animals were presented with saccharin for 20 minutes without any drug treatment. On all intervening days, animals were presented with water for 20 minutes. All fluid consumption was measured to the nearest millilitre.

Data Analysis

Locomotion counts obtained during the response to an inescapable novel environment were subjected to a two-way ANOVA with response to an inescapable novel environment (HR/LR) as the between subjects factor and time as the within-subjects factor. CTA data were subjected to a three-way ANOVA with two between subject factors: locomotion and drug, and one within-subjects factor: days. In view of the single-bottle test used in the present study, a CTA was defined as a significant decrease in saccharin consumption of a given drug treatment group relative to its own baseline saccharin consumption. All post hoc comparisons were conducted using the Newman-Keuls test. Statistical significance was set at $p < 0.05$.

Results

Locomotion Response to an Inescapable Novel Environment

A two-way ANOVA conducted on locomotion counts for animals used in the present experiment revealed significant effects of group $F(1,71) = 52.036$, $p < 0.01$, time $F(11,781) = 286.782$, $p < 0.01$ and group x time interaction $F(11,781) = 7.119$, $p < 0.01$. Figure 11 illustrates that HR animals showed a slower rate of habituation to the novel environment compared to LR animals.

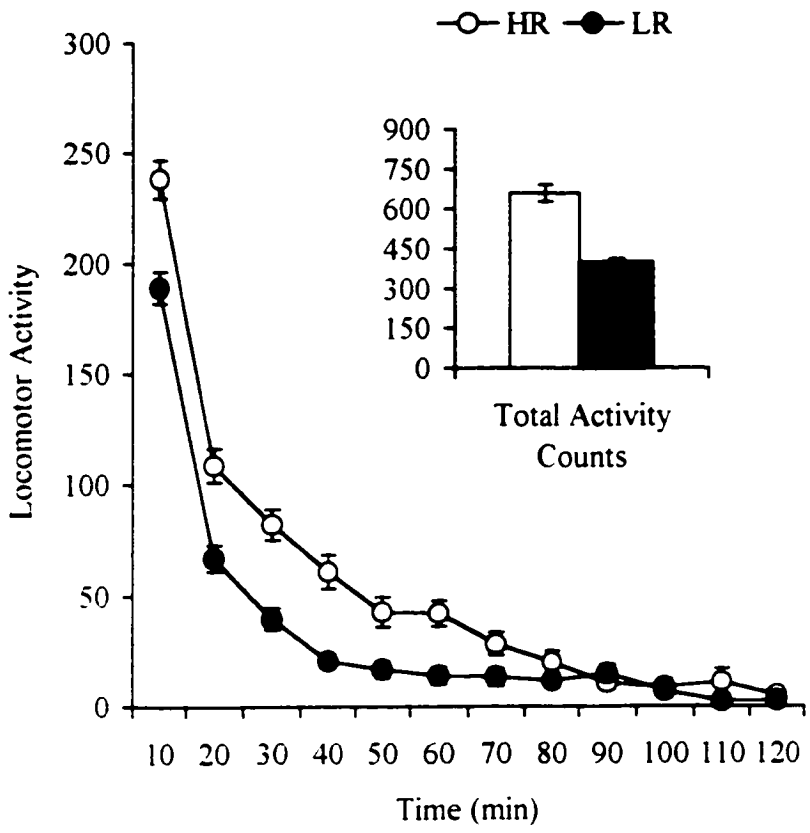


Figure 11. Mean locomotor counts obtained during the response to novelty screen over a 2-h period. Column graph shows the mean 2-h total activity counts obtained for high responding (HR) and low reponding (LR) rats subsequently conditioned with amphetamine.

Amphetamine-Induced CTA

A three-way ANOVA with repeated measures conducted on saccharin intake data showed a significant three-way interaction (locomotion x drug x day). F (21.455) = 2.603, $p < 0.01$. Post hoc Newman-Keuls revealed that LR but not HR rats conditioned with amphetamine (1.5 mg/kg) significantly decreased their baseline saccharin intake on PD2 and TD1. In addition, LR rats consumed significantly less saccharin than HR rats on these days. No significant differences were observed between HR and LR rats conditioned with saline. Figure 12a shows that LR rats appeared more sensitive than HR rats to the CTA inducing effects of amphetamine.

For animals conditioned with amphetamine (2.0 mg/kg) both HR and LR rats decreased their baseline saccharin intake on PD2 and TD1. However, LR rats consumed significantly less saccharin relative to HR rats on TD3-TD5 inclusive, suggesting that LR rats recovered more slowly from the CTA. Figure 12b shows that while HR and LR rats both acquired CTA, LR rats recovered more slowly from the CTA. Finally, when conditioned with amphetamine (3.0 mg/kg), LR rats decreased their baseline saccharin intake on PD2 and TD1, whereas HR rats decreased their baseline saccharin intake only on TD1. In addition, LR rats consumed significantly less saccharin than HR rats on PD2 and TD1. Figure 12c shows that LR compared to HR rats showed greater initial sensitivity to the acquisition of amphetamine CTA.

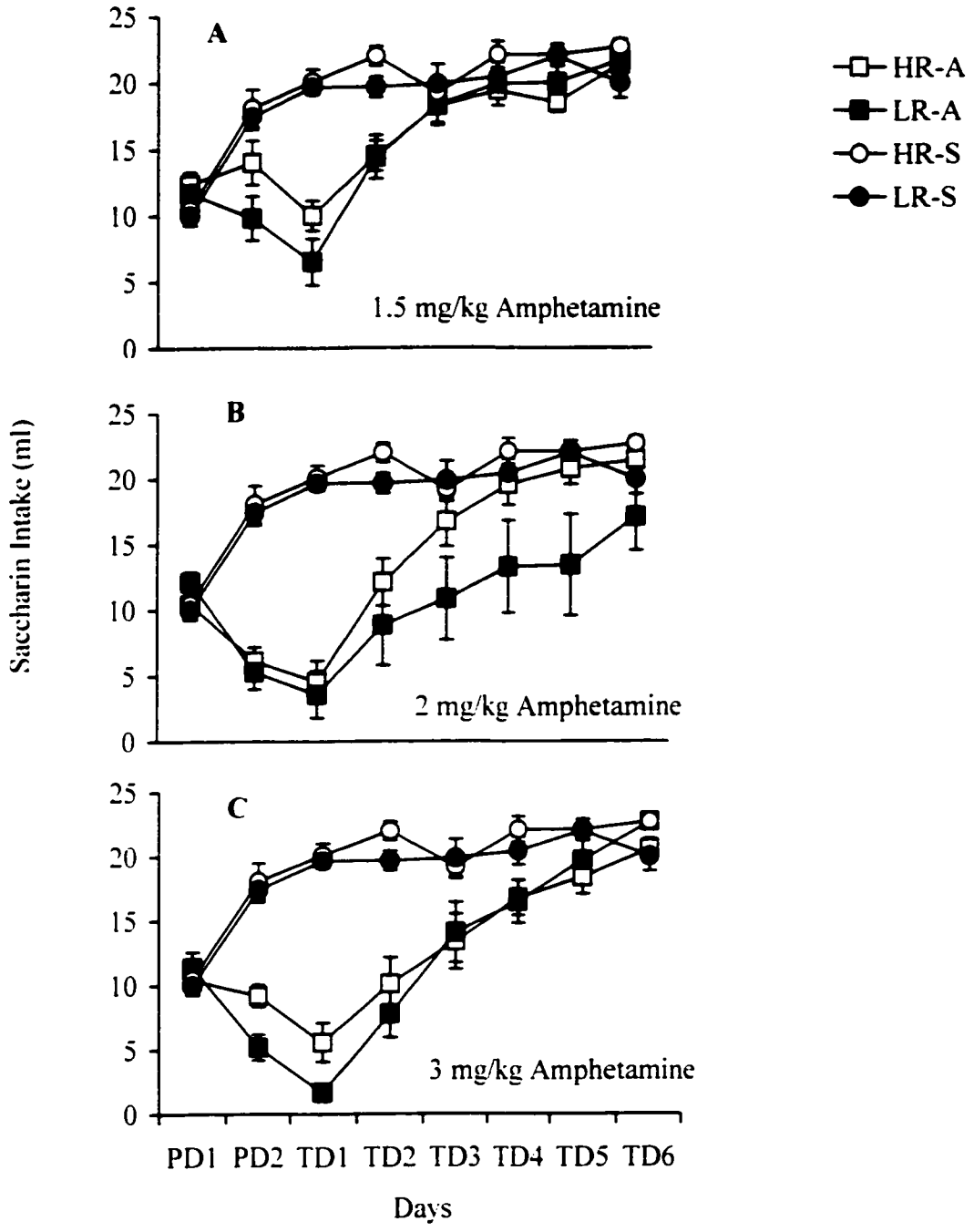


Figure 12. Mean saccharin intake (ml) across pairing and test days for high responding (HR) or low responding (LR) rats conditioned with amphetamine: 1.5 mg/kg (A): 2 mg/kg: (B) and 3 mg/kg (c). Vertical lines represent the SEM.

Discussion

The present study examined whether differences in locomotion response to an inescapable novel environment would predict differences in sensitivity to CTA with amphetamine. It was hypothesized that HR compared to LR rats would show greater sensitivity to CTA with amphetamine. Contrary to the present prediction, the results with amphetamine revealed that LR rats compared to HR rats showed greater sensitivity to CTA. Specifically, at 1.5 mg/kg, while LR rats showed a CTA to amphetamine, HR rats failed to acquire one. At 2.0 mg/kg, LR rats showed greater resistance to extinction from the CTA. The results with 3.0 mg/kg demonstrated that LR rats acquired CTA after merely a single saccharin-amphetamine pairing while HR rats required an additional saccharin-amphetamine pairing, suggesting less initial sensitivity to the CTA inducing effects of amphetamine. The present results suggested a dose-dependent expression of an amphetamine-induced CTA, where the intermediate dose of amphetamine differentiates between HR and LR rats during the extinction phase, while the low and higher amphetamine doses differentiate HR and LR rats during the acquisition phase.

Although at odds with the prediction that HR rats compared to LR rats would show greater sensitivity to amphetamine CTA, the present finding, that LR animals compared to HR rats showed greater sensitivity to amphetamine CTA can be explained in a number of ways. First, if one considers the fact that amphetamine is strongly psychotomimetic (mimics psychosis) (Robinson & Becker, 1986) and that this effect may be related to amphetamine's ability to elicit CTA, then it is conceivable that rats that are more emotional as reflected in their diminished activity in response to the open field (LR

animals) may also be more sensitive to amphetamine's psychotomimetic effects and thereby demonstrate greater sensitivity to amphetamine CTA.

Alternatively, it is possible that differences between LR and HR rats in sensitivity to amphetamine CTA may be a function of the relationship between emotionality as reflected in the open field and amphetamine's ability to produce stereotypy (repetitive movements such as head bobbing, sniffing and locomotion activity) (Hooks et al., 1991; Piazza et al., 1989) where rats who are more sensitive to amphetamine's locomotor activating effects are more emotional. If one assumes that amphetamine's ability to support CTA is positively related it's ability to induce stereotypy and that the open field is a measure of emotionality (Walsh & Cummins, 1976), where animals exhibiting less activity in response to an inescapable novel environment (LR animals) are more emotional, then it is conceivable that LR animals may in fact be those that are most affected by amphetamine-induced stereotypy and thereby more sensitive to amphetamine induced CTA.

While these latter explanations regarding differences between HR and LR animals in sensitivity to amphetamine CTA are speculative in nature, in the last section of the thesis I will attempt to propose some avenues for future research that may help to resolve this issue.

Experiment 7b: Differences in locomotion response to an inescapable novel environment predicts sensitivity to morphine-induced conditioned taste aversion

The results of experiment 7a demonstrated that differences in locomotion response to an inescapable novel environment predicted sensitivity to amphetamine CTA, with LR rats displaying greater sensitivity to amphetamine CTA as compared to HR rats. The present experiment sought to examine whether morphine, a drug that can support self-administration (Ambrosio, Goldberg & Elmer, 1995) and CTA (Cappell et al., 1973) but is pharmacologically different from amphetamine will show a different pattern of results than had been observed in experiment 7a. It was hypothesized that HR rats compared to LR rats would display greater sensitivity to a morphine-induced CTA.

Method

Subjects

The subjects were 80 male Sprague Dawley rats (Charles River, Quebec), weighing between 275-325 g at the start of the experiment. Animals were individually housed in hanging stainless steel wire cages with standard lab chow (PMI Nutrition International, Oakville, ON) and water freely available prior to the onset of the experiment. The animals were maintained on a 12-h on/ 12-h off light dark cycle, with the lights on at 0800 h and the lights off at 2000 h, in a room regulated for constant temperature and humidity. In addition, the care and use of, as well as all procedures involving, animals in the present study were approved by the Concordia University Animal Care Committee.

Drugs

Morphine hydrochloride (May & Baker. Ltd.) expressed as the salt. was dissolved in 1 ml/kg of 0.9% saline solution and was injected at doses of 10. 12.5 and 15 mg/kg. All injections were delivered i.p. Sodium saccharin was dissolved in tap water in a concentration of 0.1%.

Procedure

The procedures were identical to Experiment 7a with the following exceptions. The drug used to induce conditioned taste aversion was morphine hydrochloride. Animals were assigned to groups High Responding saline or morphine (10. 12.5 and 15 mg/kg) and Low Responding saline or morphine (10. 12.5 and 15 mg/kg). The following doses of morphine were selected since they have been shown to be within an effective range capable of producing CTA (Farber. Gorman. & Reid. 1976: Schenk et al.. 1987).

Results

Locomotion Response to an Inescapable Novel Environment

A two-way ANOVA conducted on locomotion counts for animals used in Experiment 7b revealed significant effects of group F (1.71) = 52.036. $p < 0.01$. time F (11.781) = 286.782. $p < 0.01$ and group x time interaction F (11.781) = 7.119. $p < 0.01$. Figure 13 illustrates that HR rats showed a slower rate of habituation to the novel environment compared to LR animals.

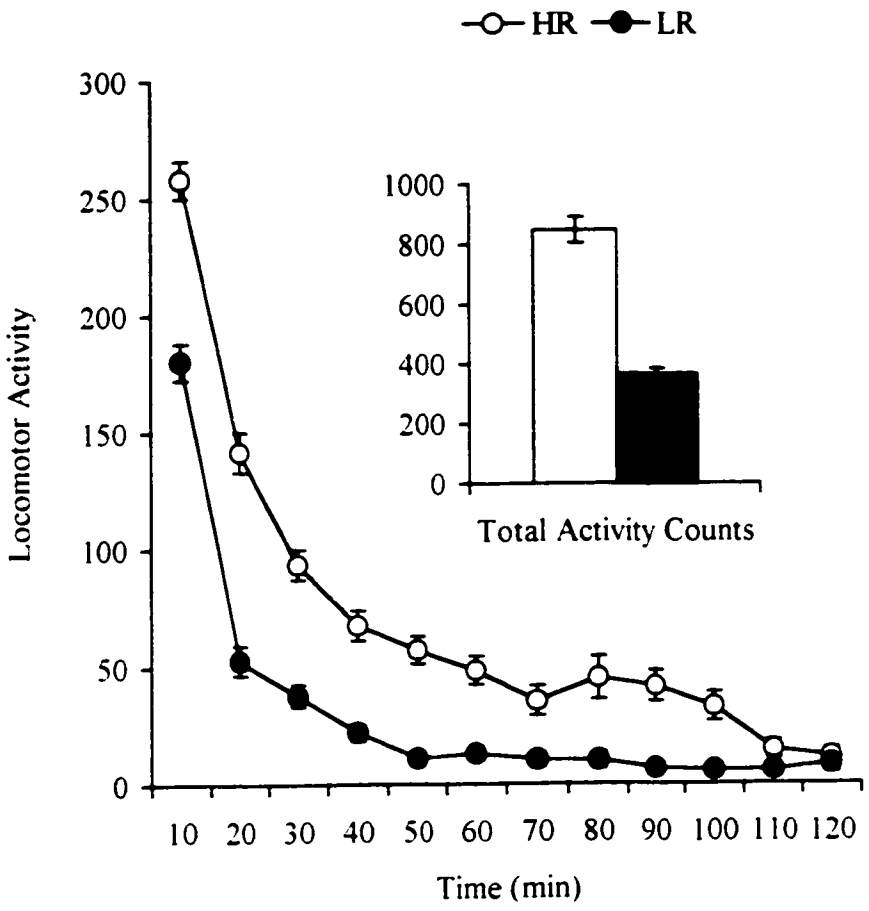


Figure 13. Mean locomotor activity counts obtained during the response to novelty screening over a 2-h period. Column graph shows the mean 2-h total activity counts obtained for high responding (HR) and low responding (LR) rats subsequently conditioned with morphine.

Morphine-Induced CTA

A three-way ANOVA with repeated measures conducted on saccharin intake data revealed a significant three-way interaction (locomotion x drug x day) F (21.476) = 2.042, $p < 0.01$. Post hoc Newman-Keuls revealed that HR but not LR rats conditioned with morphine (10 mg/kg) significantly decreased their baseline saccharin intake on PD2 and TD1 (Figure 14a). In addition, HR rats consumed significantly less saccharin than LR rats on TD2-TD5 inclusive. No significant differences were observed between HR and LR rats conditioned with saline. These results suggested that at a dose of 10 mg of morphine, HR demonstrated greater sensitivity to a morphine-induced CTA compared to LR rats.

Post hoc Newman-Keuls revealed no significant differences between HR and LR animals conditioned with 12.5 mg (Figure 14b) or 15 mg of morphine (Figure 14c).

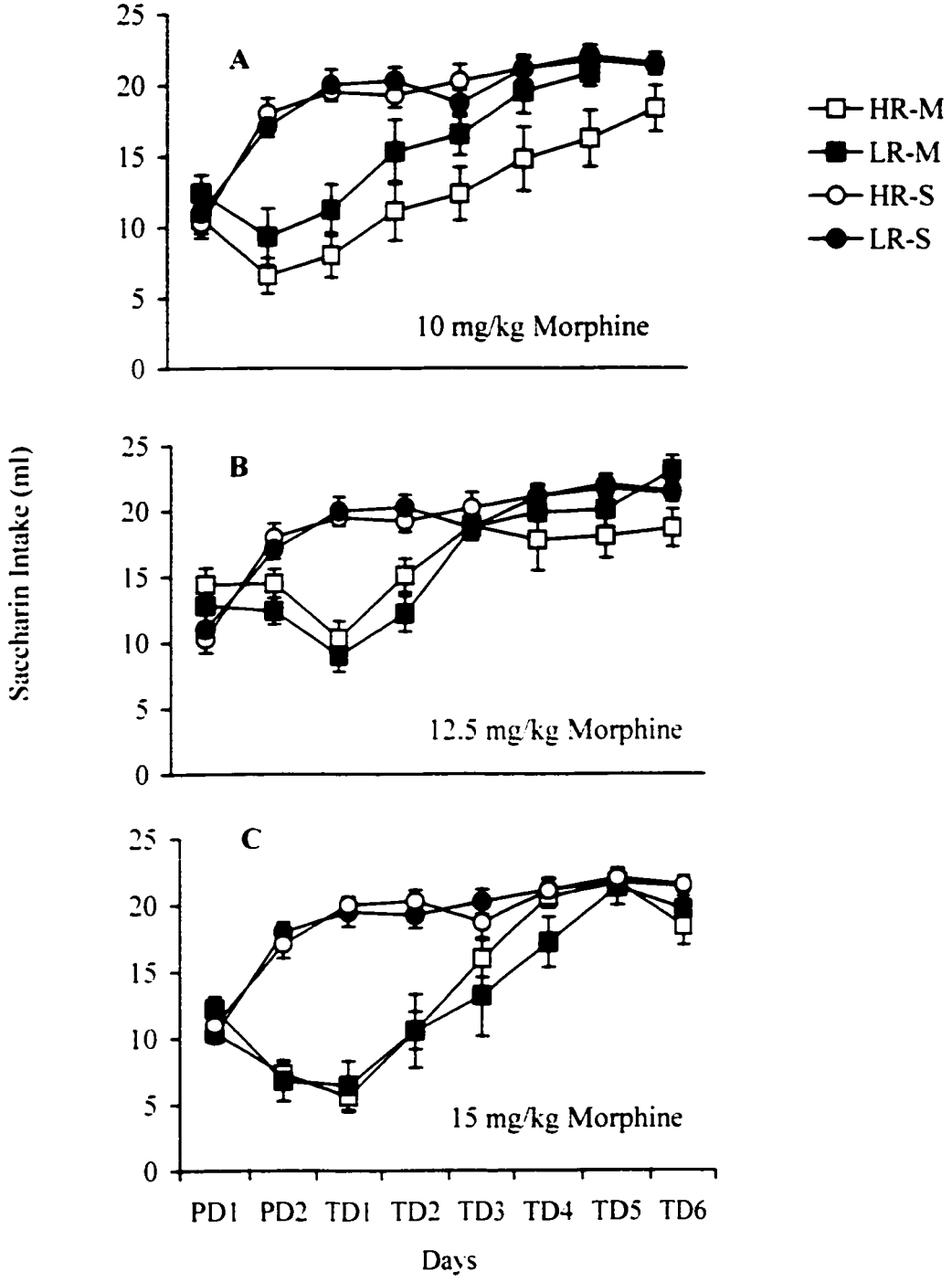


Figure 14. Mean saccharin intake (ml) across pairing and test days for high responding (HR) or low responding (LR) rats conditioned with morphine: 10 mg/kg (A); 12.5 mg/kg (B); and 15 mg/kg (C). Vertical lines represent the SEM

Discussion

When conditioned with a dose of 10 mg/kg of morphine, only HR rats displayed a CTA. However, merely raising the morphine dose by 25% eliminated the observed differences between HR and LR rats. These results suggested that HR compared to LR rats appeared more sensitive to the CTA inducing effects of morphine but only at a narrow range around 10 mg/kg.

The finding that HR animals relative to LR rats showed greater sensitivity to a morphine induced CTA could be explained in terms of the relationship between the emotional state of the animals', as reflected in locomotion response to the open field, and the specific properties of morphine. If the capacity of morphine to induce CTA is positively related to its' positive reinforcing properties (White et al., 1977), and if one assumes that motor depression is related to positive reinforcement, then it is conceivable that the capacity of morphine to induce CTA is related to its' psychomotor depressant action where animals exhibiting greater activity in response to an inescapable novel environment (HR animals) are also less emotional. It follows that HR animals may in fact be those that are more sensitive to morphine's psychomotor depressant effects and thereby more sensitive to a morphine-induced CTA. It is possible that higher morphine doses may have produced excessive sedation in all animals thus overriding any differential sensitivity in the perception of morphine's effects.

Alternatively, it is possible that HR rats with higher baseline activity levels, are more sensitive to the depressant action and psychomotor impairment resulting from a dose of 10 mg/kg of morphine (Babbini & Davis, 1972) and therefore show greater sensitivity to a morphine induced CTA. It is conceivable that the higher morphine doses

may have generated motor depression in all rats thereby overriding any differential sensitivity to CTA between HR and LR rats.

The latter explanations while somewhat speculative at this point in time could be tested in a number of ways that will be elaborated on in the general discussion.

Experiment 7c: Differences in locomotion response to an inescapable novel environment fail to predict sensitivity to LiCl-induced conditioned taste aversion

Experiments 7a and 7b demonstrated a relationship between locomotion responses to novel environment and sensitivity to CTA induced by amphetamine and morphine. Experiment 7c was designed to assess the relationship between locomotion response to an inescapable novel environment and CTA to LiCl. It was hypothesized that if differences in locomotion response to an inescapable novel environment can predict differences in sensitivity to CTA to positively reinforcing drugs then there should be no relationship between locomotion response and a LiCl-induced CTA, as LiCl is a non self-administered emetic drug possessing no properties of positive reinforcement.

Method

Subjects

The subjects were 60 male Sprague Dawley rats (Charles River, Quebec), weighing between 275-325 g at the start of the experiment. Animals were individually housed in hanging stainless steel wire cages with standard lab chow (PMI Nutrition International, Oakville, ON) and water freely available prior to the onset of the experiment. The animals were maintained on a 12-h on/ 12-h off light dark cycle, with the lights on at 0800 h and the lights off at 2000 h, in a room regulated for constant temperature and humidity. In addition, the care and use of, as well as all procedures involving, animals in the present study were approved by the Concordia University Animal Care Committee.

Drugs

Lithium Chloride (Abbott Laboratories) was dissolved in distilled water to make a 0.15 M solution. The doses of LiCl administered were 0.6 mEq/kg (4 ml/kg of a 0.15 M solution) and 1.2 mEq/kg (8 ml/kg of a 0.15 M solution). All injections were delivered i.p. Sodium saccharin was dissolved in tap water in a concentration of 0.1%.

Procedure

The procedures were identical to Experiment 7a with the following exceptions. Animals were assigned to groups High Responding saline or LiCl (0.6 mEq. 1.2 mEq) and Low Responding saline or LiCl (0.6 mEq. 1.2 mEq). The doses of LiCl selected have previously been shown to produce reliable CTAs (e.g., Nachman & Ashe. 1973b).

Results

Locomotion Response to an Inescapable Novel Environment

A two-way ANOVA conducted on locomotion counts for animals used in the present experiment revealed significant effects of group $F(1,56) = 57.084$, $p < 0.01$, time $F(11,616) = 259.986$, $p < 0.01$ and group x time interaction $F(11,616) = 4.803$, $p < 0.01$ effect. Figure 15 shows a slower rate of habituation to the novel environment in the HR group compared to the LR group

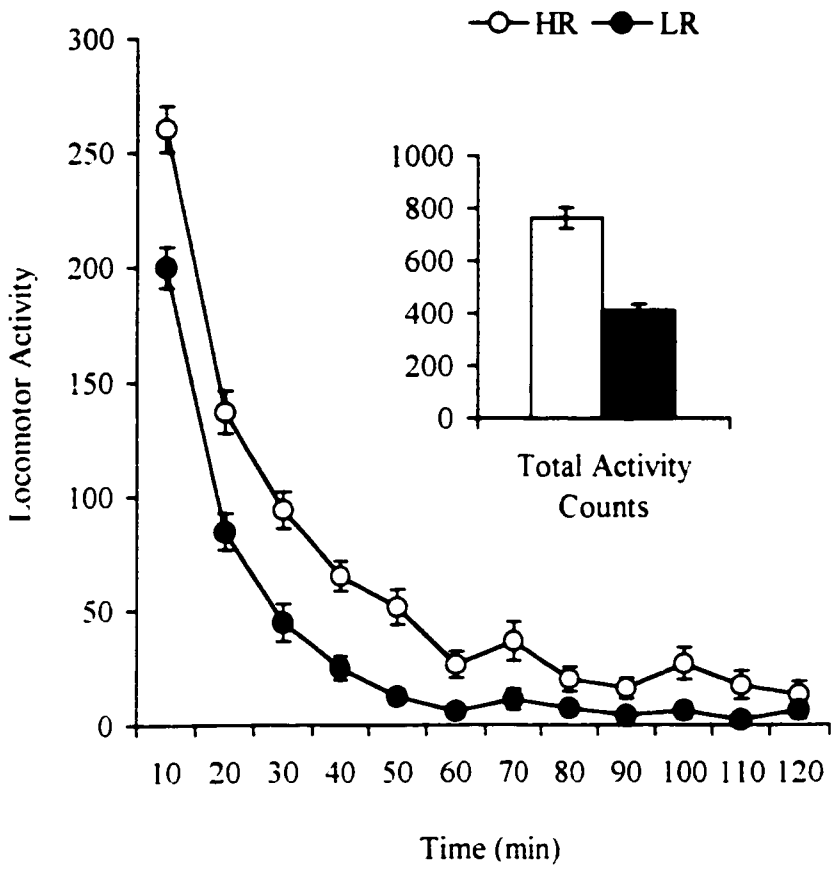


Figure 15. Mean locomotor activity counts obtained during the response to novelty screening over a 2-h period. Column graph shows the mean 2-h total activity counts obtained for high responding (HR) and low responding (LR) rats subsequently conditioned with LiCl.

LiCl-Induced CTA

A three-way ANOVA with repeated measures conducted on saccharin intake data showed no significant locomotion x drug F (2,52) = 0.080, $p > 0.05$, locomotion x day F (7,364) = 0.150, $p > 0.05$ or locomotion x drug x days F (7,364) = 0.416, $p > 0.05$ interaction effect. However, there was a significant drug x day interaction F (14,364) = 19.496, $p < 0.01$. Consistent with previously published reports (e.g., Nachman & Ashe, 1973b) LiCl produced a CTA. However, HR and LR rats showed no differential sensitivity to LiCl-induced CTA at doses of 0.6 mEq (Figure 16a) and 1.2 mEq (Figure 16b).

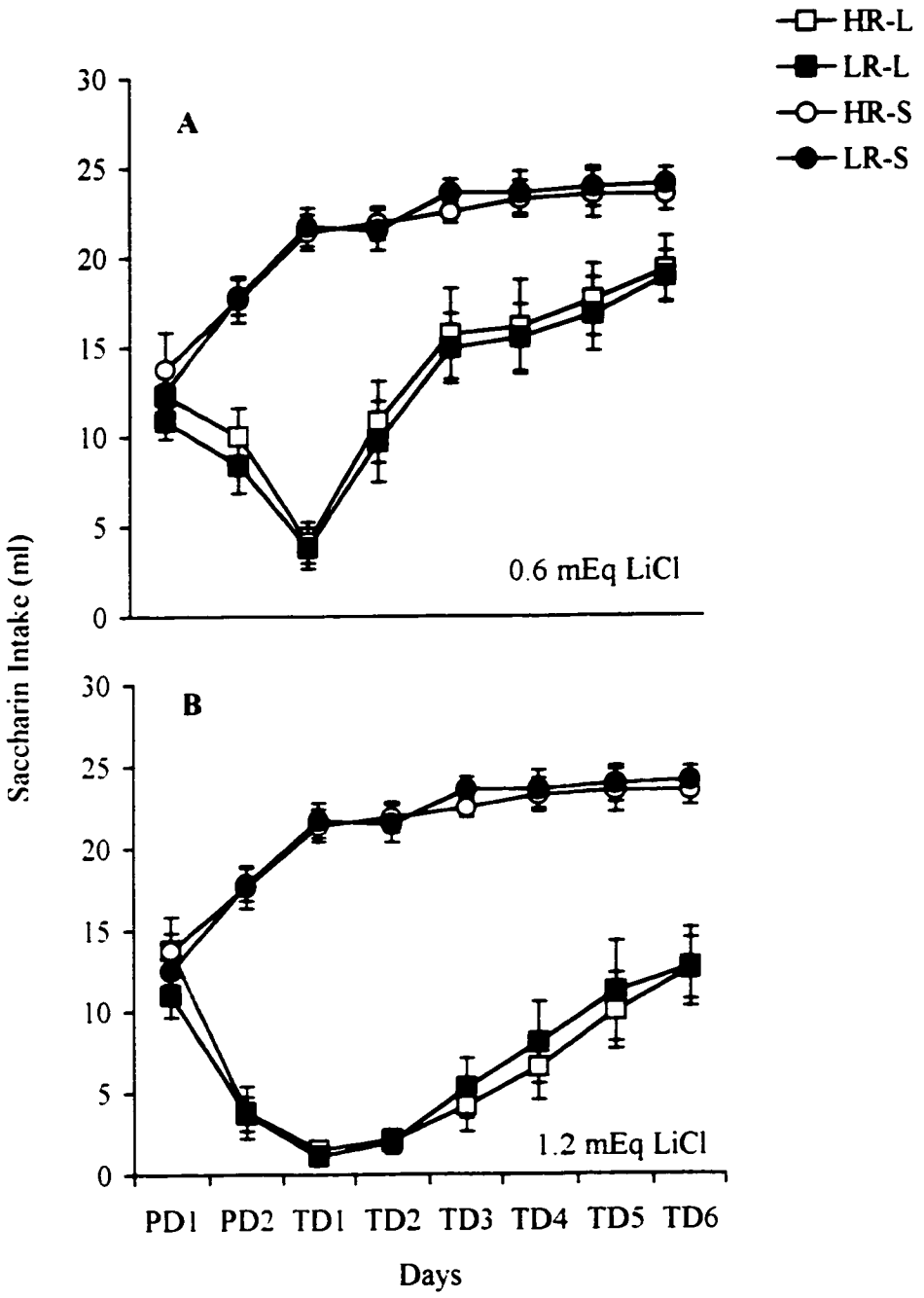


Figure 16. Mean saccharin intake (ml) across pairing and test days for high responding (HR) or low responding (LR) rats conditioned with LiCl: 0.6 mEq (A) and 1.2 mEq (B). Vertical lines represent the SEM

Discussion

The present results with LiCl demonstrated that HR and LR animals did not differ in their sensitivity to a lithium-induced CTA. These results suggested that the locomotion response to an inescapable novel environment and the aversive properties of LiCl reflected in CTA do not appear to be related. The latter finding is not particularly surprising given that LiCl is an emetic drug with no known properties of positive reinforcement.

The fact that an animal's locomotor response to an inescapable novel environment served to predict sensitivity to CTA to amphetamine or morphine but not to LiCl is an important piece of data that helps to advance our understanding of the nature of CTA's to positive reinforcing drugs. The CTA literature contains evidence of the existence of two qualitatively distinct types of CTA, one mediated by emetic agents and the other by positively reinforcing drugs (Hunt & Amit, 1987). As was alluded to earlier in this thesis, the underlying mechanisms mediating the positive reinforcing and CTA inducing properties of self-administered drugs are similar to one another yet different from the underlying neural mechanism mediating CTA's induced by illness-inducing drugs such as LiCl (e.g., Sklar & Amit, 1977). It follows then that the factors that may give rise to the positive reinforcing effects of drugs may also give rise to CTA's to these same drugs. This line of reasoning is consistent with the argument that has been advanced in this thesis that the nature of CTAs to positive reinforcing drugs may reflect their rewarding capacity and not their aversive effects.

Experiment 8: The US Preexposure effect in taste aversion learning: The role of the Area postrema and the Nucleus of the solitary tract

One variant of the CTA paradigm is the US preexposure effect. As predicted from the characteristics of this variant of the classical CTA paradigm, CTA should be attenuated by prior exposure with the US that will subsequently serve as the conditioning agent. The US preexposure effect has been shown with a wide variety of agents including amphetamine (Goudie, Taylor & Atherton, 1975) and LiCl (Cannon, Berman, Baker & Atkinson, 1975). Of some significance, it has been demonstrated that CTA's induced by positively reinforcing drugs are more sensitive to CTA disruption compared with drugs not endowed with these properties (Elsmore & Fletcher, 1972; Switzman et al., 1981). A number of explanations have been offered to account for the US preexposure phenomenon and those have variously emphasized the importance of habituation to novelty or drug state (Gamzu, 1977), drug tolerance (Dacanay & Riley, 1982), and or associative blocking (Braveman, 1977). Although there is little consensus concerning the mechanism mediating this effect, the procedure has been employed by researchers to examine the basis of CTA.

During the past several years, researchers have attempted to elucidate the neural mechanisms mediating CTA. For the most part, interest has focused on brainstem sites, particularly the Nucleus of the Solitary Tract (NTS) and the Parabrachial Nucleus (PBN), the region where taste and visceral information are thought to converge (Reilly et al., 1993; Spector, Nogren & Grill, 1992). An additional area, of particular importance in the mediation of CTA in the brainstem, is the Area Postrema (AP), the site of

chemoreceptors for detection of circulating toxins and for integration of emetic reflexes (Bernstein, 1999). Recently, a number of studies have also implicated the amygdala (AMYG) and the Insular Cortex (IC) as critical to CTA learning (Schafe & Bernstein, 1996; Schafe & Bernstein, 1998).

Most of the research elucidating the neural mechanisms underlying CTA has employed conventional lesion methodologies. One alternative approach to defining the underlying neural structures of CTA is by use of the immunohistochemical staining technique for the expression of the immediate-early gene *c-fos*, an endogenous marker of activated neurons in the central nervous system (Sager, Sharp, & Curran, 1988). Previous research has demonstrated that CTA induces FLI (*c-Fos*-like immunoreactivity) in different brain regions including the NTS, PBN, AP, AMYG and the IC (Schafe & Bernstein, 1996; Schafe & Bernstein, 1998).

While previous studies have succeeded in elucidating some of the mechanisms involved in mediating the expression of CTA (e.g., Schafe & Bernstein, 1996), to date no studies have attempted to identify the underlying neural mechanisms involved in the US preexposure effect. Thus, the present study was designed to examine whether FLI is expressed in the NTS and AP following CTA induced by amphetamine and LiCl using a standard one-bottle CTA procedure for both the conditioning and test day. Second, the present experiment examined whether preexposure with amphetamine and or LiCl would block the expression of CTA induced by these drugs and the resulting elevations in FLI in these brain regions. It was predicted that LiCl-induced CTA, whose effects are mediated through the AP, would elicit greater elevations in FLI in this region compared with amphetamine CTA, which does not depend on the integrity of this structure (Berger,

Wise, & Stein, 1973). It was also predicted that while amphetamine and LiCl would elicit CTA's of equal magnitude, there would not be differences in the expression of FLI in the NTS, as this structure appears critical for CTA conditioning with both these drugs (Swank, Schafe, & Bernstein, 1995). Finally, in view of research showing that self-administered drugs are more succeptible to the attenuating effects of drug preexposure (e.g., Elsemore & Fletcher, 1972; Switzman et al., 1981) it was predicted that amphetamine preexposure as compared with LiCl preexposure would more readily attenuate CTA induced to the same drug and correspondingly show greater attenuation of FLI in the NTS.

Method

Subjects

Male Long-Evans rats (Charles River, Quebec), weighing between 300-400 g were individually housed in hanging stainless steel wire cages with standard lab chow (PMI Nutrition International, Oakville, ON) and water freely available prior to the onset of the experiment. The animals were maintained on a 12:12-h light/dark cycle in a room regulated for constant temperature and humidity. All animals used in the present study were treated in accordance with the guidelines of the Canadian Council for Animal Care. In addition, the care and use of, as well as all procedures involving, animals in the present study were approved by the Concordia University Animal Care Committee. Because this experiment was based on the work of Swank et al., 1995 we decided to employ the same strain of rat.

Drugs

Dextroamphetamine Sulfate (*d*-amphetamine sulfate) purchased from Smith, Kline & French, Canada Ltd. expressed as the salt, was dissolved in 1ml/kg of 0.9% saline solution and was injected at a dose of 3 mg/kg. Lithium Chloride (Abbott Laboratories) was dissolved in distilled water to make a 0.15 M solution. The dose of LiCl administered was 1.2 mEq/kg (8 ml/kg of a 0.15 M solution). All injections were delivered i.p. Sodium saccharin was dissolved in tap water in a concentration of 0.1%.

Procedure

Following one week of acclimatization to the laboratory housing conditions, rats were placed on a 23 hour and 40 minute water deprivation schedule. Tap water was presented to the animals in stoppered plastic tubes fitted with stainless steel ball bearing spouts for 20 minutes beginning at noon each day. The spouts were inserted through the wire mesh in front of the home cages. Water was presented in this manner at the same time daily and was measured to the nearest mililiter. A one bottle drinking procedure was used for all drinking sessions.

After 3 days of adaptation to the water deprivation schedule animals were randomly assigned to one of four treatment groups. The preexposure injections were administered on days 4, 5 and 6, following the 20-minute water session. Rats assigned to groups Vehicle-Amphetamine (V-A), Vehicle-LiCl (V-L) and Vehicle-Vehicle (V-V) were preexposed to 0.9% saline (20 ml/kg, i.p) while rats assigned to groups Amphetamine-Vehicle (A-V), Amphetamine-Amphetamine (A-A), LiCl-Vehicle (L-V) and LiCl-LiCl (L-L) were preexposed to either *d*-amphetamine sulfate (3mg/kg) or LiCl (0.15 M; 20

ml/kg). On day 7, rats received water for 20-minutes in their home cages. On day 8. 48 h after the last pre-exposure injections. rats were presented with a novel tasting 0.1% (w/v) sodium saccharin solution for 20 minutes. Within one minute after completion of the saccharin drinking session. rats assigned to groups V-A (n=3). A-A (n=3). V-L and or L-L (n=3) were administered a single injection of either *d*-amphetamine sulfate (3mg/kg. i.p). or LiCl (0.15 M: 20 ml/kg. i.p) while rats in groups A-V (n=3). L-V (n=3) and V-V (n=3) were injected with 0.9% saline solution (20 ml/kg). The drug doses selected have previously been shown to yield saccharin aversions of comparable strengths (Parker. 1982). On day 9. animals again received water for 20 minutes.

On day 10. 48 h after conditioning trials. rats were presented with a saccharin solution for 20 minutes but with no corresponding drug treatment in order to assess for the presence of CTA. One hour after testing. rats were anesthetized with sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde for 4 hours and then stored overnight in a 30% sucrose solution at 4°C. The following day. brain sections of 30 μm thickness were cut on a microtome in the horizontal plane with every third section being kept and processed. Slices were rinsed (3 x 5 minutes) in 0.9% Trizma Buffered Saline (TBS) and then incubated in Hydrogen Peroxide (30%) for 30 minutes. Sections were then rinsed (3 x 5 minutes in TBS). incubated in a preblocking solution for 90 minutes and then transferred without rinsing to the primary c-fos antibody solution (1:75.000). which consisted of c-*fos* polyclonol rabbit IgG (Santa Cruz Biotechnology. Santa Cruz. CA). After a 72 h incubation period at 4°C. slices were rinsed (3 x 5 minutes in TBS) and processed using the standard ABC method (Vector

Laboratories, Burlingame, CA). Briefly, slices were rinsed (3 x 5 minutes in TBS) and incubated for 1h in a 2° antibody (1:200; Vector Laboratories). Slices were then transferred to a solution containing biotinylated anti-rabbit IgG and avidin-biotin peroxidase for 2 h, rinsed (3 x 5 minutes in TBS, then 50 mM Tris buffer for 10 minutes) and developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB) (10 minutes) and then with nickel chloride intensified DAB (10 minutes). Following development, slices were rinsed in TBS (3 x 10 minutes), mounted on slides, and coverslipped with Permount.

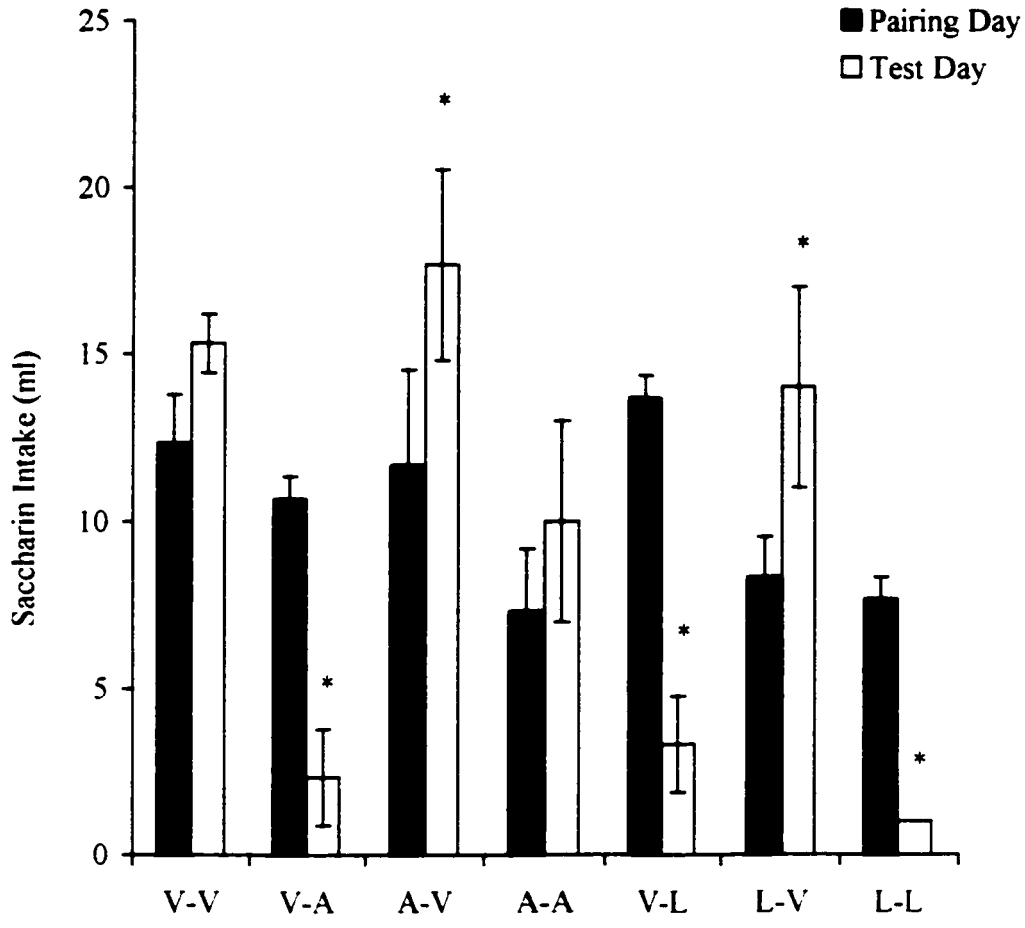
Data Analysis

FLI counts were analyzed as the sum of both sides within the different subnuclei of the NTS. FLI-positive neurons were defined as cells with nuclei containing reaction product that was black throughout the nucleus. CTA was defined as a significant decrease in saccharin consumption of a given group relative to its own baseline saccharin consumption. Alpha levels were set at .05 for all statistical analysis.

Results

Figure 17 shows the effect of preexposure with either amphetamine, LiCl or saline on CTA induced by these same drugs. A 2 x 2 ANOVA (with group between subjects factor and day within-subjects factor) was conducted for the behavioral CTA data. This analysis revealed a significant effect of group F (6,14) = 4.950, $p < 0.01$, no significant effect of day F (1,14) = 4.397, $p > 0.05$ and a significant group x day interaction F (6,14) = 23.705, $p < 0.01$. Test of simple effects revealed that there were no significant group

differences in baseline saccharin intake (PD1). However, consistent with the present definition of CTA, groups VA, VL and LL decreased their baseline saccharin intake on TD1 ($p < 0.05$). In contrast, groups VV and AA maintained their baseline saccharin consumption on TD1, while groups AV and LV increased theirs ($p < 0.05$).



* significant at $p < 0.05$

Figure 17. Mean saccharin intake (ml) for pairing and test days for rats preexposed with amphetamine (A), LiCl (L) or vehile (V) and subsequently conditioned with these drugs. Vertical lines represent the SEM.

Mean number of FLI positive nuclei in the AP are depicted in Figure 18 (upper panel) for the various conditioning groups. A 2 x 2 ANOVA (with drug a between subjects factor and conditioning group a second between subjects factor) was conducted for the FLI data in the AP. This analysis revealed no significant effect of drug $\underline{F}$ (1,3) = 0.332, $\underline{p} > 0.05$, conditioning group $\underline{F}$ (3,13) = 2.566, $\underline{p} > 0.05$ or drug x conditioning group interaction $\underline{F}$ (3,13) = 0.42, $\underline{p} > 0.05$. These results demonstrated that there were no significant group differences in FLI in the AP.

Mean number of cFLI positive nuclei in the NTS are depicted in Figure 18 (lower panel) for the various conditioning groups. A 2 x 2 ANOVA was conducted for the FLI data in the NTS. This analysis revealed no significant effect of drug $\underline{F}$ (1,3) = 2.880, $\underline{p} > 0.05$, a significant effect of conditioning group $\underline{F}$ (3,15) = 5.018, $\underline{p} < 0.05$ and no significant drug x conditioning group interaction $\underline{F}$ (3,15) = 2.757, $\underline{p} > 0.05$. Post hoc Newman-Keuls conducted on the significant main effect of conditioning group revealed that animals preexposed to either amphetamine or LiCl and then subsequently conditioned with saline, showed greater FLI in the NTS compared to all other groups ($\underline{p} < 0.05$).

A Pearson Product-Moment correlation was calculated between the amount of saccharin consumed on TD1 and FLI expression in the NTS for all animals serving in this experiment. Contrary to our expectation, the amount of saccharin consumed on TD1 was positively correlated with the amount FLI in the NTS ($\underline{r} = .54$; $\underline{p} < 0.05$).

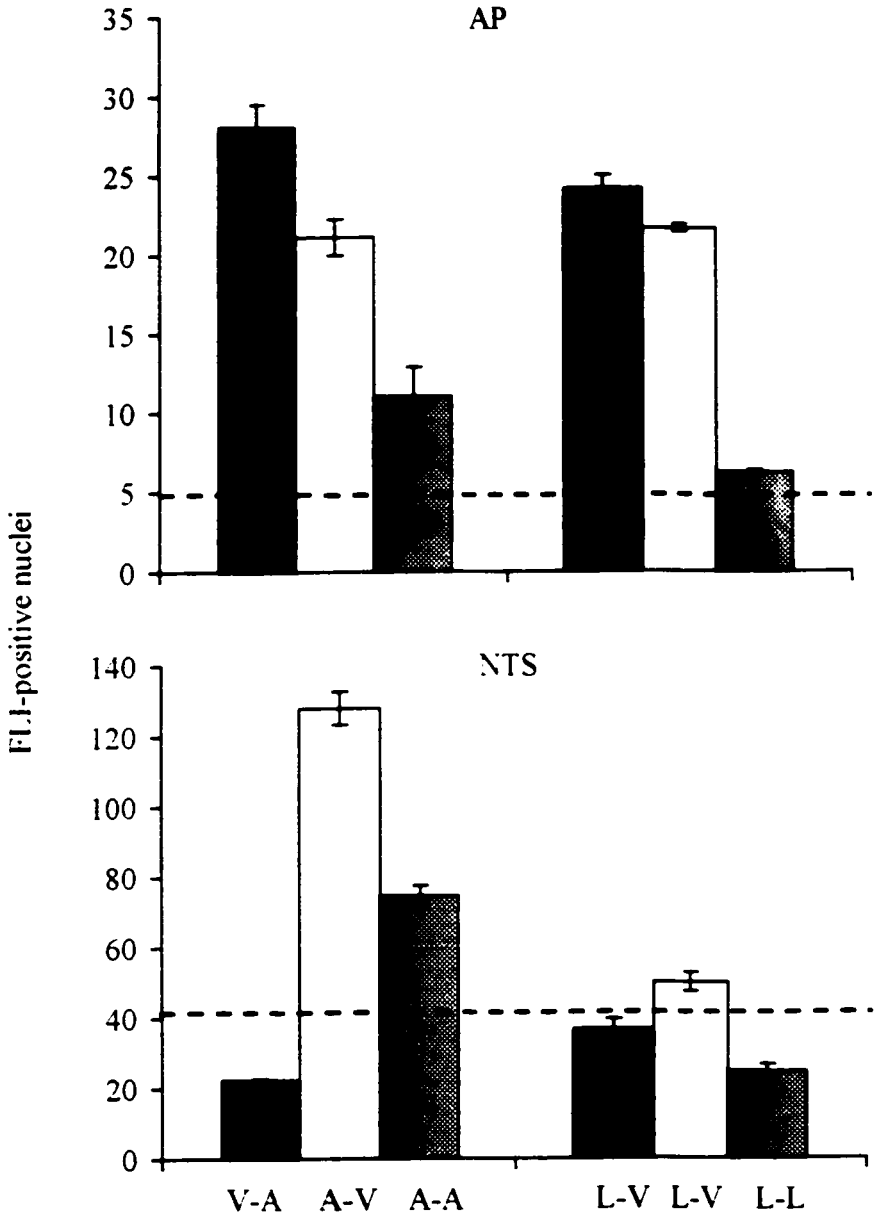


Figure 18. Mean number of nuclei positive for FLI in the Area Postrema (AP) [upper panel] and the Nucleus of the Solitary Tract (NTS) [lower panel] for the various conditioning groups. Dashed horizontal lines represent FLI for group V-V. Vertical lines represent the SEM

Discussion

The present results demonstrated that while amphetamine and LiCl produced CTAs of equal magnitude, only amphetamine preexposure proved capable of attenuating an amphetamine-induced CTA. This observation is consistent with the notion that positively reinforcing drugs are more sensitive to CTA disruption compared to drugs not endowed with positive reinforcing effects (Switzman et al., 1981). However, contrary to our prediction, the degree of CTA attenuation by drug preexposure did not predict the level of FLI attenuation in either the AP or the NTS.

Consistent with previously published reports (Swank et al., 1995), the present results showed no relationship between the magnitude of CTA and FLI expression in the AP. This was confirmed by the finding that there were no significant group differences in FLI in the AP. However, contrary to previously published reports that FLI elevation in the NTS reliably predicts CTA with both amphetamine and LiCl (Swank et al., 1995), examination of the present data would reveal conflicting results. That is, only unpaired control animals preexposed with either amphetamine or LiCl and then subsequently conditioned with saline showed significant elevations in FLI in the NTS. Interestingly, these unpaired control groups also showed a conditioned increase in baseline saccharin intake.

Further analysis of this data revealed a significant positive correlation between the amount of saccharin consumed on the test day for all animals, regardless of their conditioning group, and the amount of FLI in the NTS. That is, animals that consumed the greatest amount of saccharin on the test day also showed the greatest amount of FLI

activation in the NTS. Contrary to previously published reports (Swank et al., 1995), the present results suggested that rather than predicting CTA, activation of FLI in the NTS appears to be related to saccharin preference.

General Discussion

The "paradox of self-administered drugs" (Goudie, 1979) stipulates that drugs that are endowed with positive reinforcing properties can also support the induction of taste aversion; a contradiction of the more intuitive prediction that these drugs that are endowed with positive reinforcing properties should elicit conditioned taste preference exclusively. While some have suggested that the ability of positively reinforcing drugs to support CTA is reflective of their aversive and or peripherally toxic effects (e.g., Riley & Tuck, 1985) others have argued that the nature of CTA to positively reinforcing drugs reflect their well-known rewarding effects (e.g., Hunt & Amit, 1987). In fact, a wide array of data reviewed in this thesis has provided support for the view that rather than being discrete, dichotomous and mutually exclusive, the positive reinforcing and CTA inducing stimulus properties of self-administered drugs are inextricably linked.

The goal of this thesis was to examine the relationship between the positive reinforcing and CTA-inducing properties of a variety of commonly used drugs such as alcohol, caffeine and nicotine, and in so doing, to further elucidate the nature of the relationship between the phenomena of positive reinforcement and CTA. It was argued in this thesis that the study of drug induced CTA's may provide clues to the mechanisms involved in the mediation of their positive reinforcing effects.

Experiments 1a-c and 2a-c, which examined the interactive effects of nicotine and acetaldehyde in the preexposure CTA, revealed that the stimulus effects of nicotine generalized to acetaldehyde, the primary reinforcing metabolite of ethanol, thereby disrupting the development of its CTA.

As was mentioned earlier in this thesis, preexposure effects on CTA have been explained by adopting other concepts such as associative interference effects (Braveman, 1977; Cannon et al., 1977) where the strength of the disruptive effects of drug preexposure on the induction of CTA are thought to be related to the CTA inducing strength of a drug. In accordance with an associative account of the drug preexposure phenomenon, the greater the CTA inducing potential of a drug, the greater its ability to disrupt CTA when acting as a preexposure agent. The results of experiments 1a-c and 2a-c clearly negate this notion as a dose of nicotine, in itself below the threshold for the induction of CTA and thus presumably without significant "aversive" properties, was capable of attenuating a robust acetaldehyde induced CTA. It is also notable that this dose of nicotine is within a range previously claimed to be self-administered (Shoaib et al., 1997). These results while at odds with an associative explanation of the preexposure procedure suggest that the stimulus properties of the nicotine preexposure share specific discriminative similarities with a high dose of acetaldehyde which was capable of eliciting a robust CTA. It follows that the nature of CTA induced by acetaldehyde, the putative reinforcing metabolite of ethanol (Smith et al., 1997), rather than simply reflecting the peripherally noxious component of the drug's action, may also include those stimulus properties that are related to positive reinforcement.

The results of experiment 3 showed that nicotine and caffeine share common stimulus characteristics as the effects of nicotine preexposure generalized to caffeine thereby disrupting its CTA. This interaction was dose-dependent with the lowest nicotine dose (0.6 mg/kg) showing the greatest blockade of a caffeine induced CTA (20 and 30 mg/kg) and the higher nicotine doses (1.2 mg/kg and 2.0 mg/kg) showing no effect. It is

notable that the lowest dose of nicotine (0.6 mg/kg), below the threshold for the induction of CTA, effectively abolished the caffeine induced CTA. These results paralleled the results of experiment 1c, and suggested that CTA to this class of drugs may not be reflective of the aversive stimuli resulting from their administration. One explanation for why the lower nicotine dose was more effective than the higher nicotine doses in blocking a caffeine-induced CTA may be related to the development of genuine aversive effects in the higher nicotine doses (e.g., Parker, 1991), and consequently the lack of reinforcing properties at these doses. That is, by increasing the dose of nicotine, the strength of its' "aversive" effects increase thereby reducing its similarity to caffeine.

Experiment 4 assessed whether nicotine's effects in attenuating a caffeine-induced CTA involved nicotine's action at the nicotinic acetylcholine receptor (nAChR). The results of this experiment demonstrated that mecamylamine, a centrally acting nAChR antagonist (Shoaib et al., 1997), reversed the nicotine-induced attenuation of caffeine CTA. These results suggested that nicotine's ability to block the formation of a caffeine-induced CTA appeared to be related to nicotine's action at the nicotinic-cholinergic receptor, a receptor site shown to mediate nicotine's putative positively reinforcing effects. Suprisingly, mecamylamine also attenuated a caffeine-induced CTA on a magnitude similar to nicotine suggesting that mecamylamine is acting like caffeine in the preexposure CTA paradigm.

While previous studies assessing the effect of caffeine on voluntary ethanol intake have been confounded by states of food deprivation (Gilbert, 1976; Gilbert, 1979; Hederra et al., 1975) or have used very large doses of caffeine (Dietze & Kulkosky, 1991), experiment 5a sought to correct this state of affairs by assessing whether caffeine

at low to moderate doses would directly influence the acquisition of ethanol intake in free feeding ethanol naïve rats. The results of this experiment provided evidence to suggest that caffeine in fact facilitated the acquisition of ethanol drinking in free feeding ethanol naïve animals.

Experiment 5b assessed whether the effects of caffeine on ethanol consumption observed in experiment 5a was specifically related to the acquisition of ethanol drinking or a result of a direct pharmacological interaction between these agents. It was argued that if caffeine augmented ethanol drinking in rats with a pre-established history of ethanol drinking, then the effects of caffeine on ethanol drinking are not uniquely related to the facilitation of the learning to drink ethanol per se but rather to a direct pharmacological interaction between these agents. The results of experiment 5b suggested that caffeine's effects on ethanol drinking followed an inverted-U shape function with the low and high caffeine doses exerting no effect on ethanol drinking but the intermediate caffeine dose enhancing ethanol drinking in rats with a pre-established history of ethanol drinking. These results revealed a dose specific pharmacological interaction between the effects of caffeine and ethanol and suggested that caffeine's effects (at the intermediate dose level of 5 mg/kg) on ethanol drinking may have reflected an enhancement in the association between ethanol's sensory properties and it's post-ingestive rewarding effects.

Experiment 5c ruled out the likelihood that caffeine's action on ethanol drinking occurred by means of an alteration in ethanol metabolism because caffeine pretreatment failed to alter blood ethanol levels within the time period used for the ethanol drinking session. Experiment 5d demonstrated that the combination of caffeine and ethanol

produced elevations in plasma corticosterone levels beyond that of either drug administered singly. It was argued that the increase in plasma corticosterone levels resulting from the co-administration of these drugs may have played a role in the caffeine-induced elevations in ethanol drinking observed in experiments 5a and 5b. Indeed there is a growing body of evidence demonstrating that corticosterone may at least in part modulate the intake of ethanol in rats (e.g., Fahlke et al., 1994; Fahlke et al., 1995; Fahlke et al., 1996). For example, Fahlke et al., (1996) showed that intracerebroventricular infusions of corticosterone facilitate voluntary ethanol consumption in laboratory rats. In addition, adrenalectomy (Fahlke et al., 1994a) or treatment with the coticosterone synthesis inhibitor metyrapone (Fahlke et al., 1994b) decreases alcohol intake in alcohol preferring rats, restoring them to the level of non-alcohol preferring rats. Of some significance, the positive reinforcing effects of psychostimulants (e.g., amphetamine, cocaine) are also enhanced by corticosterone. For example, administration of corticosterone facilitates the acquisition and maintenance of amphetamine self-administration (Piazza, Maccari, Deminiere, Le Moal, Mormede & Simon, 1991).

Although somewhat speculative, one mechanism by which corticosterone may facilitate ethanol consumption is by means of enhanced sensitivity of the positively reinforcing properties of ethanol (Fahlke et al., 1994b; Fahlke et al., 1996). As had been suggested for psychostimulants such as amphetamine (e.g., Piazza et al., 1991), corticosterone may enhance the positively reinforcing effects of this drug by stimulating dopamine release in the nucleus accumbens and sensitivity at postsynaptic dopaminergic receptors thereby increasing amphetamine self-administration (Piazza et al., 1989; Piazza

et al., 1991; Piazza & Le Moal, 1996). It is possible that by stimulating dopamine release, corticosterone may also indirectly promote ethanol intake. This latter notion assumes that dopamine plays a role in governing ethanol intake. However, support for dopamine's involvement in the mediation of voluntary ethanol intake would appear to be tenuous at best (e.g., Amit, Levitan, & Lindros, 1976; Amit & Brown, 1982; Davis, Smith & Werner, 1978).

In view of the numerous neurochemical actions of corticosterone and ethanol, there are other ways in which corticosterone secretions may influence ethanol drinking. For example, corticosterone may facilitate ethanol drinking by stimulating $GABA_A$ receptors previously shown to modulate ethanol intake in rats (Fahlke, Hard & Hansen, 1996; Hyytia & Koob, 1995). Ultimately, the mechanism whereby corticosterone promotes ethanol drinking remains to be elucidated.

Experiment 6a examined whether caffeine pretreatment, within a dose range shown to promote ethanol drinking, would also alter an ethanol-induced CTA. It was hypothesized that if CTA's induced by positively reinforcing drugs reflect aversion per se (Riley & Tuck, 1985), and if caffeine's facilitation of ethanol drinking reflected an attenuation of ethanol's aversive effects, then caffeine should block an ethanol-induced CTA. Conversely, if one assumes a functional relationship between the rewarding properties and CTA inducing properties of positively reinforcing drugs (e.g., Hunt and Amit, 1987) then caffeine pretreatment, within a dose range shown to facilitate voluntary ethanol drinking, would also promote an ethanol-induced CTA.

The results of experiments 6a and 6b revealed an interaction between caffeine and an ethanol-induced CTA that was dose-dependent in nature. Specifically, at the lower

ethanol dose (1.0 g/kg), which was below threshold for induction of CTA, caffeine (2.5 and 10 mg/kg) pretreatment facilitated the CTA resulting from this dose, which was not the case for caffeine at a dose of 5 mg/kg. The fact that caffeine exerts its' effects on ethanol CTA only through some doses and not others merits attention. One explanation for this latter finding may be related to the possibility that caffeine's psychopharmacological properties (at the specific doses employed) change in a U shaped fashion, thereby affecting its' interaction with an ethanol CTA in a dose-dependant manner.

While it is difficult to account for this dose-specific caffeine induced augmentation of an ethanol induced CTA, the observation that caffeine augments CTA to a low dose rather than a high ethanol dose also merits attention. It has previously been suggested that the mechanisms mediating CTAs induced by high vs. low ethanol doses differed (Brown, Amit, Smith, & Rockman, 1978; Hunt & Amit, 1987). While CTAs to low and moderate ethanol doses may be mediated by effects related to ethanol's positively reinforcing properties, CTAs induced by high ethanol doses may be mediated by ethanol's aversive effects related to peripheral toxicosis (Brown et al., 1978). Indeed Parker (1988) showed that high doses of ethanol (e.g., 3.0 g/kg) produced aversive orofacial rejection responses that are typical of emetic agents such as LiCl. It follows that if CTA's to low and moderate ethanol doses reflect ethanol's positively reinforcing effects, the results of experiment 6a support the conclusion that caffeine may have acted to enhance ethanol's positively reinforcing effects.

This latter point is critical as it is most commonly assumed that the behavioral effects of drugs, as reflected in their capacity to induce CTA, is indicative exclusively of

a drug's aversive effects (e.g., Riley & Tuck, 1985). As was argued in this thesis, there is a growing body of evidence supporting the notion that rather than reflecting the peripherally toxic actions of these drugs, CTA's induced by positively reinforcing drugs actually reflect their rewarding nature (e.g., Hunt & Amit, 1987; Grigson, 1997; Parker, 1995). If true, then by studying the CTA inducing properties of positively reinforcing drugs we may be tapping into the same underlying motivational properties and neural substrates governing their rewarding effects.

The results obtained in experiment 6c ruled out the likelihood that caffeine's effects on an ethanol induced CTA occurred by means of an alteration in blood ethanol levels. On the other hand, it is conceivable however, that elevations in plasma corticosterone levels resulting from the co-administration of caffeine and ethanol may also be involved in the mediation of the present caffeine-induced facilitation of an ethanol-induced CTA. Indeed, if caffeine's ability to facilitate ethanol CTA reflects an increase in the reinforcing efficacy of ethanol, then the latter suggestion seems possible in light of emerging evidence showing that corticosterone may enhance ethanol self-administration (Fahlke et al., 1996), a direct measure of this drug's positive reinforcing effects. Although speculative in nature, one mechanism by which corticosterone may enhance an ethanol CTA is by facilitation in dopamine release.

Experiments 7a and 7b were designed to assess whether differences in sensitivity to a novel environment as reflected in novelty-induced locomotion, previously shown to predict sensitivity to drug self-administration (e.g., Piazza et al., 1989) would also predict sensitivity to the expression of CTA. If one assumes (e.g., Hunt & Amit, 1987) a functional relationship between the positive reinforcing and CTA inducing properties of

self-administered drugs then it could be predicted that differences in novelty-induced locomotion should mediate the expression of these seemingly paradoxical drug effects. The results of experiment 7a and 7b provided data to indicate that locomotion responses to an inescapable novel environment appeared to be differentially related to the development of CTAs to amphetamine and morphine in rats. It was argued that the disparate findings for amphetamine and morphine may be related to the interaction between emotionality as reflected in locomotion response to an open field and sensitivity to CTA to drugs that share the capacity to induce positive reinforcement, but are nevertheless endowed with very different pharmacological profiles.

In contrast to the prediction that High responders (HR) compared to Low responders (LR) would show greater sensitivity to amphetamine-induced CTA, the results of experiments 7a demonstrated that LR rats compared to HR rats appeared more sensitive to amphetamine CTA. A number of explanations were proposed to account for this finding. First, it was argued that the relationship between novelty induced locomotion and sensitivity to amphetamine CTA may have reflected a relationship between emotionality and sensitivity to amphetamine's psychotomimetic effects. In other words, the more emotional LR rats showed greater sensitivity to amphetamine CTA because they may have also been more sensitive to amphetamine's psychotomimetic effects. This latter notion would necessarily suggest that amphetamine's psychotomimetic effects are related to its ability to support CTA. Indeed, amphetamine's psychotomimetic effects are related to amphetamine's positive reinforcing properties (Cloninger, 1987). Second, it was argued that the relationship between novelty induced locomotion and amphetamine induced CTA may have reflected a relationship between emotionality and greater

sensitivity to amphetamine-induced stereotypy. In other words, the more emotional LR rats may have showed greater sensitivity to amphetamine CTA because they are also more sensitive to amphetamine induced stereotypy. Again, this latter notion would suggest that amphetamine-induced stereotypy is related to its ability to support CTA. Indeed, amphetamine-induced stereotypy is related to amphetamine's positively reinforcing properties (Piazza et al., 1991).

A third possible explanation for the finding that LR rats compared to HR rats exhibited greater sensitivity to amphetamine CTA may simply be related to the interaction between the psychostimulant effects of amphetamine and inherent baseline activity levels. It is conceivable that LR rats are more sensitive to amphetamine CTA because they are also more sensitive to the psychostimulant effects of amphetamine. It is possible that additional stimulation produced by amphetamine may have generated greater CTA in animals with lower baseline activity levels.

One simple method to determine which of the aforementioned explanations can account for LR animals' greater sensitivity to amphetamine CTA would be to use an alternate stimulant drug, one without psychotomimetic properties and with no ability to elicit stereotypy. One such drug is nicotine. If LR rats compared to HR rats demonstrate greater sensitivity to a nicotine-induced CTA then it could be argued that the relationship between novelty induced locomotion and sensitivity to CTA to stimulant type drugs may reflect a relationship between emotionality and sensitivity to psychostimulant effects as nicotine is not known to have psychotomimetic effects and or the ability to elicit stereotypy.

The results of experiment 7b demonstrated that, when conditioned with morphine, HR rats showed greater sensitivity to CTA compared to LR rats but only when conditioned with a dose of 10 mg/kg. It was argued that the capacity of morphine to induce CTA was also related to its' psychomotor depressant action where animals exhibiting greater activity in response to an inescapable novel environment (HR animals) were more sensitive to morphine's psychomotor depressant effects and thereby more sensitive to a morphine-induced CTA. The failure of higher morphine doses to differentiate HR from LR rats may have reflected the fact that higher morphine doses produced uniform sedation in all animals thus overriding any differential sensitivity in the perception of morphine's effects.

Alternatively, a dose of 10 mg/kg of morphine may have yielded greater CTA in HR rats with higher baseline activity levels. It is conceivable that the higher morphine doses, while producing depressant action, may have generated uniform aversion in all rats (similar to a LiCl type CTA) and were thus unrelated to novelty locomotion.

Experiment 7c examined whether differences in novelty locomotion would also predict differences in sensitivity to a LiCl induced CTA, a drug possessing no known positive reinforcing properties. The results of this experiment demonstrated that when conditioned with lithium chloride (LiCl), HR and LR rats showed no differential sensitivity to CTA. If one assumes (e.g., Hunt & Amit, 1987) that the underlying neural substrate mediating CTA's to positively reinforcing vs. emetic drugs differ, then it is of little surprise that novelty locomotion failed to predict differences in sensitivity to LiCl CTA as this agent does not possess properties of positive reinforcement.

Taken together. the results of experiments 7a -c add a significant dimension to an emerging body of literature demonstrating a relationship between locomotion response to a novel environment and sensitivity to psychoactive compounds in a variety of different paradigms (Deroche et al., 1993; Hooks et al., 1991; Jodogne. Marinelli. LeMoal & Piazza. 1994: Klebaur & Bardo, 1999; Piazza et al., 1989). The present findings provide support for the notion that factors such as emotionality as reflected in locomotion response to an open field. that may serve to predict sensitivity to the positive reinforcing properties of self-administered drugs may also serve to predict sensitivity to CTA to the very same drugs. These results are consistent with available evidence that while the positively reinforcing and CTA inducing properties of self-administered drugs are functionally related and probably mediated by a shared substrate they are also different from the underlying substrate mediating the expression of CTAs induced by emetic agents. These findings also underscore the unique capacity of the CTA paradigm as a sensitive tool capable of discriminating specific properties and relationships specifically related to positive reinforcing drugs.

In contrast to studies showing that c-Fos-like immunoreactivity (FLI) elevations in the Nucleus of the Solitary Tract (NTS) accompany taste aversion conditioning using the intraoral (I/O) conditioning method. the findings of experiment 8 revealed little if any FLI expression in the NTS as a function of CTA's induced by either amphetamine or LiCl. Instead. the results of this experiment demonstrated that FLI activation in the NTS predicted conditioned saccharin preference. That is. animals that consumed the greatest amount of saccharin on the test day also showed greatest FLI expression in the NTS.

These results may suggest that FLI may be more reactive to positive rather than aversive stimuli within this particular paradigm.

The present results with the NTS raise a question as to the nature of the discrepancy between our findings and those of others (Swank et al., 1995), reporting that FLI in the NTS is a cellular correlate of CTA expression. One explanation may be related to the method of taste delivery. Most studies examining the underlying cellular correlate of CTA employ the I/O method where taste stimuli are infused into the oral cavity (Swank & Bernstein, 1994; Swank et al., 1995), a method that contrasts with the present study that used a single bottle procedure requiring the animal to voluntarily approach and consume the fluid. These two procedures differ fundamentally in that the I/O method conforms most closely to a classical conditioning paradigm, one where there is essentially no response requirement. In contrast, a "bottle presentation" is characterized by instrumental learning where the conditioning and test day protocol necessitate a response.

It is notable that these two procedures are also thought to differ in that CTA's resulting from the I/O method reflect nausea and aversion characterized by an altered affective reaction to the CS taste (from positive to aversive) (Balleine & Dickinson, 1991; Limebeer & Parker, 2000). In contrast, CTA's resulting from a bottle conditioning method reflect a form of signal learning in which a taste signals a novel change in the physiological state of the animal thereby resulting in avoidance of the taste stimuli (Hunt & Amit, 1987; Balleine & Dickinson, 1991; Limebeer & Parker, 2000). Interestingly, it has recently been shown (Spray, Halsell, & Bernstein, 2000) that the I/O method but not the bottle conditioning method induces strong FLI in the NTS. It follows that these two

different methods of conditioning may reflect distinct underlying processes, a point argued elsewhere (Limebeer & Parker, 2000).

Conclusions

The present thesis examined the relationship between the positive reinforcing and CTA-inducing properties of a variety of commonly used drugs such as alcohol, caffeine and nicotine in order to further elucidate the nature of the relationship between the phenomena of positive reinforcement and CTA. The results of the studies reported here reveal a number of important findings regarding the nature of the relationship between the positive reinforcing and CTA inducing properties of alcohol, caffeine and nicotine. First, it was reported that the putative reinforcing metabolite of ethanol, acetaldehyde (Smith et al., 1997), may share common stimulus properties with nicotine and as such may mediate the previously observed (e.g., Kunin et al., 1999b) interaction between nicotine and ethanol in the preexposure CTA paradigm. In view of the fact that acetaldehyde plays an important role in mediating the reinforcing properties of ethanol (Smith et al., 1997), one may speculate that the interaction between acetaldehyde and nicotine may reflect an overlap in the reinforcing properties of these two classes of drugs. Moreover, if one assumes a functional relationship between the positively reinforcing and CTA inducing properties of self-administered drugs (e.g., Hunt & Amit, 1987) then it is conceivable that acetaldehyde may mediate the previously observed nicotine-induced elevations in ethanol drinking (e.g., Smith et al., 1999).

The results of experiment 3 showed that caffeine and nicotine shared common stimulus properties in the preexposure CTA procedure. If it is argued that the CTA

inducing and positively reinforcing stimulus properties of self-administered drugs are functionally related and mediated by overlapping neural mechanisms (e.g., Hunt & Amit, 1987) then it may be assumed that nicotine preexposure may also alter the psychopharmacological effects of ingested caffeine.

It is noteworthy, that caffeine's behavioral effects appear to be mostly mediated by blockade of adenosine A_1 and A_{2a} receptors (Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999). It has been suggested (Ferre, Fredholm, Morelli, Popoli & Fuxe, 1997; Nehlig, 1999) that by reducing the inhibitory effects of adenosine on dopaminergic receptors, caffeine may facilitate dopamine neurotransmission. Of some significance, nicotine also increases dopamine release (Fung & Lau, 1986; Rowell, Carr & Garner, 1987). In view of the fact that both caffeine and nicotine appear to stimulate dopamine neurotransmission, it is possible that action at the dopamine receptor may be partly responsible for the present interaction between nicotine and caffeine in the preexposure CTA paradigm. Further research will be required to verify a role for dopamine receptors in the mediation of the present nicotine-caffeine interaction.

The results of experiment 4 demonstrated that mecamylamine, a centrally acting nAChR antagonist (Shoaib et al., 1997), reversed the nicotine-induced attenuation of caffeine CTA. Furthermore, mecamylamine blocked the formation of a caffeine induced CTA suggesting that like caffeine and nicotine, caffeine and mecamylamine share common stimulus properties. Although mecamylamine has been considered to be a selective antagonist at central as well as peripheral nACh receptors, there are reports that this agent may also block neurotransmission at the NMDA receptor complex (e.g., O'dell & Christensen, 1988). In view of mecamylamine's possible lack of pharmacological

specificity, the question then arises whether in the present study mecamylamine's action occurs specifically at nACh receptors. One way in which to examine this issue would be to use a more selective nACh receptor antagonist such as dihydro-β-erythroidine (DHβE) in place of mecamylamine and determine whether it would attenuate a caffeine-induced CTA. Similarly, one could assess whether DHβE could reverse nicotine's blockade of a caffeine-induced CTA.

Additional findings reported in this thesis were that a moderate caffeine dose, within a range ingested by regular coffee drinkers (e.g., Nehlig, 1999), augmented ethanol intake. This effect while unrelated to alteration in blood ethanol may have been related to an increase in corticosterone resulting from the co-administration of both caffeine and ethanol. It was argued that one mechanism by which corticosterone may have facilitated ethanol consumption was by means of enhanced sensitivity of the positively reinforcing properties via stimulation of dopamine receptors, a point meriting further research.

In a parallel study it was demonstrated that caffeine also augmented an ethanol-induced CTA within a dose range shown to augment voluntary ethanol intake. This finding, while underscoring the functional relationship between the positive reinforcing and CTA inducing properties of self-administered drugs, suggested that rather than attenuating ethanol's "aversive" effects, caffeine's ability to promote an ethanol-induced CTA reflected an increase in ethanol's rewarding effects caused by elevations in plasma corticosterone resulting from the co-administration of caffeine and ethanol.

It was also reported in this thesis that emotionality as reflected in locomotion response to an open field, which predicts sensitivity to drug self-administration also

predicts sensitivity to CTA to drugs that share the capacity to induce positive reinforcement, but are nevertheless endowed with very different pharmacological profiles. However, the results with lithium chloride (LiCl), an emetic agent, showed no relationship between the development of CTA to this drug and novelty locomotion. In view of the fact that the underlying neural substrate mediating CTA's to positively reinforcing vs. emetic drugs differ it was not surprising that novelty locomotion failed to predict sensitivity to LiCl CTA as LiCl does not possess properties of positive reinforcement. It also follows that CTA to any drug given in aversive (LiCl like) doses should not be related to novelty locomotion.

Future research may consider examination of whether the relationship between novelty-induced locomotion and sensitivity to amphetamine's effects truly reflects an interaction between emotionality and sensitivity to amphetamine's effects. The simplest way to address this issue would be to determine whether differences in locomotion response to a familiar vs. unfamiliar environment also predict sensitivity to amphetamine-induced conditioned taste aversion.

Finally, it was reported that the mechanisms mediating CTA's using a conventional "bottle method" do not appear to involve the same neural structures as CTA's using the I/O method. It was argued that methodological differences in studies that purport to measure CTA may actually represent two distinct processes. Future studies should examine the nature of these two forms of taste conditioning and determine whether they can also be dissociated in other brain regions believed to be critical for CTA learning. Such investigations would seem merited as they may broaden our conceptualization of a phenomenon that is still rather poorly understood.

In summary, the present results have broadened our understanding of the mechanisms, both behavioral and biochemical, governing the relationship between commonly used drugs such as alcohol, caffeine and nicotine. In addition, they have revealed important information about the nature of the relationship between the positive reinforcing and CTA inducing stimulus properties of drugs that are self-administered. Together the present results have important implications for our understanding of drug-motivated behavior.

References

Ambrosio, E., Goldberg, S.R., & Elmer, G.I. (1995). Behavior genetic investigation of the relationship between spontaneous locomotor activity and the acquisition of morphine self-administration behavior. Behavioural Pharmacology, 6, 229-237.

Amit, Z., Baum, M. (1970). Comment on the increased resistance-to-extinction of an avoidance response induced by certain drugs. Psychological Reports, 27, 310.

Amit, Z., Brown, Z. W (1982). Actions of drugs of abuse on brain reward systems: A reconsideration with specific attention to alcohol. Pharmacology, Biochemistry and Behavior, 17, 233-238.

Amit, Z., Levitan, D.E., & Lindros, K.O. (1976). Suppression of ethanol intake following administration of dopamine-beta-hydroxylase inhibitors in rats. Archives internationales de pharmacodynamie et de therapie, 223, 114-9.

Aragon, C.M.G., Abitbol, M., Amit, Z (1986). Acetaldehyde may mediate reinforcement and aversion produced by ethanol: an examination using the conditioned taste aversion paradigm. Neuropharmacology, 25, 79-83.

Aragon, C.M.G., Abitbol, M., Amit, Z. (1991). Ethanol-induced CTA mediated by acetaldehyde through central catecholamine activity. Psychopharmacology, 103, 74-77.

Aragon, C.M., Nicoletti, S., Rogan, F., & Amit, Z. (1987). Corticosterone response to an acute dose of ethanol in naive and ethanol experienced rats. Alcohol and Alcoholism Suppl 1, 345-349.

Azcona, O., Barbanoj, M.J., Torrent, J., & Jane, F. (1995). Evaluation of the central effects of alcohol and caffeine interaction. British Journal of Clinical Pharmacology, 40, 393-400.

Babbini, M., & Davis, W.E. (1972). Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. British journal of Pharmacology, 46, 213-224.

Balleine, B.W., & Dickinson, A. (1991). Instrumental performance following reinforcer devaluation depends upon incentive learning. The Quarterly Journal of Experimental Psychology, 43B, 279-296.

Bardo, M.T., & Bevins, R.A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology, 2000, 31-43.

Batel, P., Pessione, C., Maitre, C., & Rueff, B. (1995) Relationship between alcohol and tobacco dependencies among alcoholics who smoke. Addiction, 90, 977-980.

Bell, M.S., Thiele, T.E., Seeley, R.J., Bernstein, I.L., & Woods, S.C. (1998). Effects of food deprivation on conditioned taste aversions in rats. Pharmacology Biochemistry & Behavior, 60, 459-466.

Belzung, C., & Barreau, S. (2000). Differences in drug-induced place conditioning between BALB/c and C57B1/6 mice. Pharmacology Biochemistry & Behavior, 65, 419-423.

Berger, B.D. (1972). Conditioning of food aversions by injections of psychoactive drugs. Journal of Comparitive and Physiological Psychology, 81, 21-26.

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

Berman, R.F., & Cannon, D.S (1974). The effect of prior ethanol experience on ethanol-induced saccharin aversions. Physiology and Behavior, 12, 1041-1044.

Bermudez-Rattoni, F. & McGaugh, J.L. (1991). Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. Brain research, 549, 165-170.

Bernstein, I.L. (1999). Taste aversion learning : A contemporary perspective. Nutrition, 15, 229-234.

Berridge, K., Grill, H.J., & Nogren, R. (1981). Relation of consumatory responses and preabsorptive insulin release to palatability and learned aversions. Journal of Comparative and Physiological Psychology, 95, 363-382.

Bienkowski, P., Piasecki, J., Koros, E., Stefanski, R., & Kostowski, W. (1998). Studies on the role of nicotinic acetylcholine receptors in the discriminative and aversive stimulus properties of ethanol in the rat. European Neuropharmacology, 8, 79-87.

Blomqvist, O., Ericson, M., Engel, J., & Soderpalm, B. (1997). Accumbal dopamine overflow after ethanol: Localization of the antagonizing effect of mecamylamine. European Journal of Pharmacology, 334, 149-156.

Blomqvist, O., Ericson, M., Johnson, D. H., Engel, J. A. & Soderpalm, B. (1996) Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. European Journal of Pharmacology, 314, 257-267.

Blomqvist, O., Soderpalm, B. & Engel, J.A. (1992). Ethanol-induced locomotor activity: Involvement of central nicotinic acetylcholine receptors? Brain Research Bulletin, 29, 173-178.

Borison, H.L., & Wang, S.C. (1953). Physiology and Pharmacology of Vomiting. Pharmacological reviews, 5, 193-230.

Bowers, W.J., Gingras, M.A., & Amit, Z. (1996). Time-dependent exacerbation of amphetamine induced taste aversions following exposure to footshock. Psychopharmacology, 125, 43-49.

Boyle, A.E.L., Smith, B.R., Spivak, K.K., & Amit, Z. (1994). Voluntary ethanol consumption in rats: the importance of the exposure paradigm in determining final intake outcome. Behavioural Pharmacology, 4, 502-512.

Bozarth, M.A., Murray, A., & Wise, R.A. (1989). Influence of housing conditions on the acquisition of intravenous heroin and cocaine self-administration in rats. Pharmacology Biochemistry & Behavior, 33, 903-907.

Braveman, N. S. (1977). What studies on pre-exposure to pharmacological agents tell us about the nature of the aversion-inducing agents. In L.M. Barker, M.R. Best & M. Domjan (Eds.) Learning Mechanisms in Food Selection (pp. 511-530). Waco, Texas: Baylor University.

Brick, J., & Pohorecky, L.A. (1984). Role of noradrenergic neurons in ethanol-induced elevation of free fatty acids and corticosterone. Life Sciences, 35, 207-212.

Brick, J., & Pohorecky, L.A. (1985). Monoamines and the effect of ethanol on corticosterone and non-esterified fatty acids. Alcohol, 2, 261-265.

Brockwell, N. T., Eikelboom, R., & Beninger, R. J. (1991). Caffeine-induced place and taste conditioning: Production of dose-dependent preference and aversion. Pharmacology Biochemistry & Behavior, 38, 513-517.

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

Brownson, R.C., & Chang, J.C. (1987). Exposure to alcohol and tobacco and the risk of laryngeal cancer. Archives of Environmental Health, 42, 192-196.

Budney, A.J., Higgins, S.T., Hughes, J.R., & Bickel, W.K. (1993). Nicotine and caffeine use in cocaine-dependent individuals. Journal of Substance Abuse, 5, 117-130.

Burch, J. B., deFiebre, C. M., Marks, M. J. & Collins, A. C. (1988). Chronic ethanol or nicotine treatment results in partial cross-tolerance between these agents. Psychopharmacology, 95, 452-458.

Buresova, O., & Bures, J. (1977). The effect of anesthesia on acquisition and extinction of conditioned taste aversion. Behavioural Biology, 20, 41-50.

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

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

Cappell, H., & LeBlanc, A.E. (1971). Conditioned aversion to saccharin by a single administration of mescaline or amphetamine. Psychopharmacologia, 22, 352-356.

Cappell, H., & LeBlanc, A.E. (1973). Punishment of saccharin drinking by amphetamine in rats and its reversal by chlordiazepoxide. Journal of comparative and Physiological Psychology, 85, 97-104.

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

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

Carmody, T.P., Brischetter, C.S., Matarazzo, J.D., O'Donnell, R.P., & Connor, W.E. (1985). Co-occurent use of cigarettes, alcohol and coffee in healthy community living men and women. Health Psychology, 4, 323-335.

Carr, G.D., & White, N.M. (1986). Anatomical disassociation of amphetamine's rewarding and aversive effects: An intracranial microinjection study. Psychopharmacology, 89, 340-346.

Carroll, M.E., France C.P., Meische, R.A. (1979). Food deprivation increases oral and intravenous drug intake in rats. Science, 205, 319-321.

Carroll, M.E., & Meisch, R.A. (1984). Increased drug self-administration due to food deprivation. In T. Thompson, P.B. Dews, J.E. Barrett (Eds.) Advances in behavioral pharmacology (pp. 47-88). New York: Academic Press.

Chait, L.D., & Griffiths, R.R. (1983). Effects of caffeine on cigarette smoking and subjective response. Clinical Pharmacology and Therapeutics, 34, 7612-7622.

Clarke, P.B.S., & Kumar, R. (1983). Characterization of the locomotor stimulant action of nicotine in tolerant rats. British Journal of Pharmacology, 80, 587-594.

Cloninger, C.R. (1987). Recent advances in the genetics of anxiety and somatoform disorders. In. H. Meltzer (Ed.) Psychopharmacology: The Third Generation of Progress (pp. 955-965). New York: Raven Press.

Cohen, C., Welzl, H., Battig, K. (1991). Effects of nicotine, caffeine and their combination on locomotor activity in rats. Pharmacology, Biochemistry and Behavior, 40, 121-123.

Coil, J.D., & Nogren, R. (1981). Tatse aversions conditioned with intravenous copper sulfate : Attenuation by ablation of the area postrema. Brain Research, 212, 425-433.

Coil, J.D., Rogers, R.C., Garcia, J., & Novin, D. (1978). Conditioned taste aversions : Vagal and circulatory mediation of the toxic unconditioned stimulus. Behavioral Biology, 24, 509-519.

Collins, A. C., Burch, J. B., deFiebre, C. M. & Marks, M. J. (1988). Tolerance to and cross tolerance between ethanol and nicotine. Pharmacology, Biochemistry & Behavior, 29, 365-373.

Concas, A., Porcu, P., Sogliano, C., Serra, M., Purdy, R.H., & Biggio, G. (2000). Caffeine-induced increases in the brain and plasma concentrations of neuroactive steroids in the rat. Pharmacology, Biochemistry and Behavior, 66, 39-45.

Cubero, I., Thiele, T.E., & Bernstein, I.L. (1999). Insular cortex lesions and taste aversion learning : Effects of conditioning method and timing of lesion. Brain Research, 839, 323-330.

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

Dar, M. (1988). The biphasic effects of centrally and peripherally administered caffeine on ethanol-induced motor in coordination in mice. The Journal of Pharmacy and Pharmacology, 40, 482-487.

Dar, M.S., Jones, M., Close, G., Mustafa, S.J., & Wooles, W.R. (1987). Behavioral interactions of ethanol and methylxanthines. Psychopharmacology, 91, 1-4.

Dar, M.S., Li, C., & Bowman, E.R. (1993). Central behavioral interactions between ethanol, (-)-nicotine, and (-)-cotinine in mice. Brain Research Bulletin, 32, 23-28.

Davis, W.M., & Smith, S.G. (1975). Central cholinergic influence on self-administration of morphine and amphetamine. Life Sciences, 16, 237-246.

Davis, W.M., Smith, S.G., & Werner, T.E. (1978). Noradrenergic role in self-administration of ethanol. Pharmacology, Biochemistry and Behavior, 9, 369-374.

De Beun, R., Rijk, H.W., & Broekkamp, C.L.E. (1993). Cross-familiarization conditioned taste aversion procedure as a method to reveal stimulus resemblance between drugs : studies on the 5-HT_{1A} agonist 8-OHDPAT. Psychopharmacology, 112, 121-128.

De Beun, R., Rijk, H.W., & Broekkamp, C.L.E. (1996). Ethanol intake educing effects of isapirone in rats are not due to simple stimulus substitution. Pharmacology, Biochemistry and Behavior, 53, 891-898.

de Fiebre, C.M., & Collins, A.C. (1993). A comparison of the development of tolerance to ethanol and cross-tolerance to nicotine after chronic ethanol treatment in long-

and short-sleep mice. Journal of Pharmacology and Experimental Therapeutics, 266, 1398-1406.

de la Garza, R., Bergman, J., & Hartel, C.R. (1981). Food deprivation and cocaine self-administration. Pharmacology, Biochemistry and Behavior, 15, 141-144.

Deroche, V., Piazza, P.V., Le Moal, M., Simon, H. (1993). Individual differences in the psychomotor effects of morphine are predicted by reactivity to novelty and influenced by corticosterone secretion. Brain Research, 623, 341-4.

Dietze, M.A., & Kulkosky, P.J. (1991). Effects of caffeine and bombesin on ethanol and food intake. Life Sciences, 48, 1837-44.

DiFranza, J.R., & Guerrera, P. (1990). Alcoholism and smoking. The Journal of Studies on Alcohol, 51, 130-135.

D'Mello, G.D., Goldberg, D.M., Goldberg, S.R., & Stolerman, I.P. (1981). Conditioned taste aversion and operant behavior in rats: Effects of cocaine, apomorphine and some long activating derivatives. Journal of Pharmacology and Experimental Therapeutics, 219, 60-68.

Domjan, M. (1980). Ingestional aversion learning: unique and general processes. In: Advances in the Study of Behavior, Vol II. (pp. 275-336). New York: Academic Press.

Donny, E.C., Caggiula, A.R., Knopf, S., & Brown, C. (1995). Nicotine self-administration in rats. Psychopharmacology, 122, 390-394.

Dragoin, W., McCleary, G.E., & McCleary, P. (1971). A comparison of two methods of measuring conditioned taste aversion. Behavior Research Methods and Instruments, 3 309-310.

Dreher, K.F., & Fraser, J.G. (1967). Smoking habits of alcoholic outpatients. I. The International Journal of Addictions, 2, 259-270.

Dunn, L.T., & Everitt, B.J. (1988). Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion. Behavioral Neuroscience, 102, 3-23.

Elsmore, T.F., & Fletcher, G.V. (1972). Delta-4-9-tetrahydrocannabinol: Aversive effects in rats at high doses. Science, 175, 911-912.

Emurian, H.H., Nellis, M.J., Brady, J.V., & Ray, R.L. (1982). Event time series relationship between cigarette smoking and coffee drinking. Addictive Behaviors, 7, 441-444.

Erb, S.M., & Parker, L.A. (1994). Individual differences in novelty-induced activity do not predict strength of amphetamine-induced place conditioning. Pharmacology, Biochemistry and Behavior, 60, 459-466.

Ericson, M., Engel, J.A., & Soderpalm, B. (2000). Peripheral involvement in nicotine-induced enhancement of ethanol intake. Alcohol, 21, 37-47.

Etkind, S.A., Fantegrossi, W.E., & Riley, A.L. (1998). Cocaine and alcohol synergism in taste aversion learning. Pharmacology, Biochemistry & Behavior, 59, 649-655.

Etscorn, F., Moore, G. A., Scott, E. P., Hagen, L. S., Caton, T. M., Sanders, D. L. & Divine, K. K. (1987). Conditioned saccharin aversions in rats as a result of cutaneous nicotine or intraperitoneal nicotine administered in divided doses. Pharmacology, Biochemistry & Behavior, 28, 495-502.

Etscorn, F., & Stephens, R. (1973). Establishment of conditioned taste aversion with a 24-hr CS-US interval. Physiological Psychology, 1, 251-253.

Exner, M., & Clark, D. (1993). Behavior in the novel environment predicts responsiveness to *d*-amphetamine in the rat. Behavioural Pharmacology, 4, 47-56.

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

Fahlke, C., Hard, E., Eriksson, C.J., Engel, J.A., & Hansen, S. (1995). 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 antagonists. Psychopharmacology, 117, 216-224.

Fahlke, C., Hard, E., & Hansen, S. (1996). Facilitation of ethanol consumption by intracerebroventricular infusions of corticosterone. Psychopharmacology, 127, 133-139.

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

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

Ferre S., Fredholm B.B., Morelli, M., Popoli, P., & Fuxe, K. (1997). Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends in Neuroscience, 20, 482-487.

Franks, H.M., Hagedorn, H., Hensley, V.R., Hensley, W.J., & Starmer, G.A. (1981). The effect of caffeine on human performance alone and in combination with ethanol. Psychopharmacology, 45, 171-181.

Fredholm, B.B., Battig, K., Holmen, J., Nehlig, A., Zvartau, E.E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to widespread use. Pharmacological Reviews, 51, 83-133.

Friedman, G.D., Siegelaub, A.B., & Seltzer, C.C. (1974). Cigarettes, alcohol, coffee and peptic ulcer. New England Journal of Medicine, 290, 469-473.

Froehlich, J.C., Harts, J., Lumeng, L., & Li, T.K. (1988). Differences in response to the aversive properties of ethanol in rats selectively bred for oral ethanol preference. Pharmacology Biochemistry & Behavior, 31, 215-222.

Fudin, R., & Nicastro, R. (1988). Can caffeine antagonize alcohol-induced performance decrements in humans? Perceptual and Motor Skills, 67, 375-391.

Fung, Y.K., & Lau, Y.S. (1986). Acute effect of nicotine on the striatal dopaminergic system in the rat. The Journal of Pharmacy and Pharmacology, 38, 920-922.

Gaiardi, M., Bartoletti, M., Bacchi, A., Gubellini, C., Costa, M., & Babbini, M. (1991). Role of repeated exposure to morphine in determining its affective properties: Place and taste conditioning studies in rats. Psychopharmacology, 103, 183-186.

Galaverna, O.G., Seeley, R.J., Berridge, K.C., Grill, H.J., Epstein, A.N., & Schulkin, J. (1993). Lesions of the central nucleus of the amygdala. I: Effects on taste reactivity, taste aversion learning and sodium appetite. Behavioral Brain Research, 59, 11-17.

Galina, Z.H., Sutherland, C.J., & Amit, Z. (1983). Effects of heat-stress on behavior and the pituitary adrenal axis in rats. Pharmacology, Biochemistry and Behavior, 19, 251-256.

Gallo, M., Roldan, G., & Bures, J. (1992). Differential involvement of gustatory insular cortex and amygdala in the acquisition and retrieval of conditioned taste aversion in rats. Behavioral Brain Research, 52, 91-97.

Gamzu, E. (1977). The multifaceted nature of taste aversion-inducing agents: is there a single common factor ? In L.M. Barker, M.R. Best & M. Domjan (Eds.) Learning Mechanisms in Food Selection (pp. 511-530). Waco, Texas: Baylor University.

Garcia, J., & Ervin, F.R. (1968). Gustatory-visceral and telereceptor-cutaneous conditioning-adaptation in internal and external milieus. Communications in Behavioral Biology, 1, 398-415.

Garcia, J., & Hankins, W.J. (1977). On the origins of food aversion paradigms. In L.M. Barker, M.R. Best & M. Domjan (Eds.) Learning Mechanisms in Food Selection (pp. 511-530). Waco, Texas: Baylor University.

Garcia, J., Hankins, W.G., & Rusiniak, K.W. (1974). Behavioral regulation of the milieu intern in man and rat. Science, 185, 823-831.

Garcia, J., Kimmeldorf, D. J., & Hunt, E.L. (1957). Spatial avoidance behavior in the rat as a result of exposure to ionizing radiation. British journal of Radiology, 30, 318-320.

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

Garcia, J., & Koelling, R.A. (1966). Relation of cue to consequence in avoidance learning. Psychonomic Science, 5, 121-122.

Garrett, B.E., & Griffiths, R.R. (2001). Intravenous nicotine and caffeine: Subjective and physiological effects in cocaine abusers. The Journal of Pharmacology and Experimental Therapeutics, 296, 486-494.

Gasior, M.J., Shoaib, M., Yasar, S., Jaszyna, M., & Goldberg, S.R. (1999). Acquisition of nicotine discrimination and discriminative stimulus effects of nicotine in rats chronically exposed to caffeine. The Journal of Pharmacology and Experimental Therapeutics, 288, 253-273.

Gilbert, R.M. (1976). Dietary caffeine and EtOH consumption by rats. The Journal of Studies on Alcohol, 37, 11-18.

Gilbert, R.M. (1979). Augmentation of EtOH-consumption by caffeine in malnourished rats. Journal of Studies Alcohol, 40, 19-27.

Gill, K., France, C., & Amit, Z. (1986). Voluntary ethanol consumption in rats: an examination of blood/brain ethanol levels and behavior. Alcoholism Clinical and Experimental Research, 10, 457-462.

Gingras, M.A., & Cools, A.R. (1995). Differential ethanol intake in high or low responders to novelty. Behavioural Pharmacology, 6, 718-723.

Glautier, S., Clements, K., White, J.A.W., Taylor, C., & Stolerman, I.P. (1996). Alcohol and the reward value of cigarette smoking. Behavioural Pharmacology, 7, 144-154.

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

Gong, W., Neill, D.B., & Justice, J.B Jr. (1996). Locomotor response to novelty does not predict cocaine place preference conditioning in rats. Pharmacology, Biochemistry and Behavior, 53, 191-196.

Gordon, T.L., Meehan, S.M., & Schechter, M.D. (1993). P and NP rats respond differentially to the discriminative stimulus effects of nicotine. Pharmacology, Biochemistry and Behavior, 45, 305-308.

Goudie, A. J. (1979). Aversive stimulus properties of drugs. Neuropharmacology, 18, 971-979.

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

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

Goudie, A.J., Thornton, E.W., & Wheatley, J. (1975). Attenuation by alpha-methyltyrosine of amphetamine induced conditioned taste aversions in rats. Psychopharmacologia, 45, 119-123.

Goudie, A.J., Thornton, E.W., & Wheeler, T.J. (1976). Drug pretreatment effects in drug induced taste aversions: Effects of dose and duration of pretreatment. Pharmacology, Biochemistry and Behavior, 4, 629-633.

Grant, V.L. (1987). Do conditioned taste aversions result from activation of emetic mechanisms? Psychopharmacology, 93, 405-415.

Griffiths, R. R., Bigelow, G. E. & Liebson, I. (1976) Facilitation of human tobacco administration by ethanol: a behavioral analysis. Journal of the Experimental Analysis of Behavior, 25, 279-292.

Grigson, P. S. (1997). Conditioned taste aversions and drugs of abuse: A reinterpretation. Behavioral Neuroscience, 111, 129-136.

Grigson, P.S., & Freet, C.S. (2000). The suppressive effects of sucrose and cocaine, but not lithium chloride, are greater in Lewis than in Fisher rats: Evidence for the reward comparison hypothesis. Behavioral neuroscience, 114, 353-363.

Grigson, P.S., Shimura, T., & Nogren, R. (1997). Brainstem lesions and gustatory function: II. The role of the nucleus of the solitary tract in NA+ appetite, conditioned taste aversion and conditioned odor aversion in rats. Behavioral Neuroscience, 111, 169-179.

Grill, H.J., & Norgren, R. (1978). The taste reactivity test. I: Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research, 143, 263-279.

Grote, F.W., & Dickins, D.W. (1971). Conditioned taste aversions: two-stimulus tests are more sensitive than one-stimulus tests. Behavior Research Methods and Instruments, 3, 311-312.

Grupp, L.A. (1977). Effects of pimozide on the acquisition, maintenance and extinction of amphetamine-induced taste aversion. Psychopharmacology, 53, 225-242.

Hederra, A., Aldunate, J., Segovia-Riquelme, N., & Mardones, J. (1975). Effects of caffeine on the voluntary alcohol intake of rats. In J.D Sinclair, K. Kiianmaa (Eds.). The effects of centrally active drugs on voluntary alcohol consumption (vol. 24, pp.9-13). Helsinki: Finnish Foundation for Alcohol Studies.

Hooks, M.S., Jones, G.H., Liem, B.J., Justice, J.B Jr. (1992). Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intracranial amphetamine injections. Pharmacology Biochemistry and Behavior, 43, 815-823.

Hooks, M.S., Jones, G.H., Neill, D.B., & Justice, J.B Jr. (1991). Individual differences in amphetamine sensitization: dose-dependent effects. Pharmacology, Biochemistry and Behavior, 41, 203-210.

Hunt, T., & Amit, Z. (1987). Conditioned taste aversion induced by self-administered drugs: paradox revisited. Neuroscience and Biobehavioral Reviews, 11, 107-130.

Hunt, T., Segal, & Amit, Z. (1987). Differential involvement of central cholinergic mechanisms in the aversive stimulus properties of morphine and amphetamine. Pharmacology, Biochemistry & Behavior, 28, 335-339.

Hunt, T., Spivak, K., & Amit, Z. (1985). Aversive stimulus properties of morphine: Evaluation using the drug preexposure conditioned taste aversion paradigm. Behavioral and Neural Biology, 44, 60-73.

Hunt, T., Switzman, L., & Amit, Z. (1985). Involvement of dopamine in the aversive stimulus properties of cocaine in rats. Pharmacology, Biochemistry and Behavior, 22, 945-948.

Hyytia, P., & Koob, G.F. (1995). $GABA_A$ receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. European Journal of Pharmacology, 283, 151-159.

Ionescu, E., & Buresova, O. (1977). Failure to elicit CTA by sever poisoning. Pharmacology, Biochemistry and Behavior, 6, 251-254.

Istvan, J., & Matarazzo, J.D. (1984). Tobacco, alcohol, and caffeine use: A review of their interrelationships. Psychological Bulletin, 95, 301-326.

Iwamoto, E.T., & Williamson, E.C. (1984). Nicotine-induced taste aversion: characterization and preexposure effects in rats. Pharmacology, Biochemistry and Behavior, 21, 527-532.

Jaszyna, M., Gasior, M., Shoaib, M., Yasar, S., & Goldberg, S. (1998). Behavioral effects of nicotine, amphetamine and cocaine under a fixed-interval schedule of food reinforcement in rats chronically exposed to caffeine. Psychopharmacology, 140, 257-271.

Jodogne, C., Marinelli, M., Le Moal, M.L., & Piazza, P. (1994). Animals predisposed to develop amphetamine self-administration show higher susceptibility to develop contextual conditioning of both amphetamine-induced hyperlocomotion and sensitization. Brain Research, 657, 236-244.

Jornby, D.E., Steinpreis, R.E., Sherman, J.E. & Baker, T.B. (1990). Aversion instead of preference learning indicated by nicotine place conditioning in rats. Psychopharmacology, 101, 533-538.

Kirby, L.G., Rowan, G.A., Smith, R.L., & Lucki, I. (1994). Discriminative stimulus properties of the benzodiazepine receptor inverse agonist methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM). Psychopharmacology, 113, 351-360.

Klatsky, A.L., Friedman, G.D., Siegelaub, A.B., & Gerard, M.J. (1977). Alcohol consumption among white, black, or oriental men and women: Kaiser-Permanente multiphasic health examination data. American Journal of Epidemiology, 105, 311-323.

Klebaur, J.E., & Bardo, M.T. (1999). Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. Pharmacology, Biochemistry and Behavior, 63, 131-136.

Kozlowski, L.T., Henningfield, J.E., Keenan, R.M., Lei, H., Leigh, G., Jelinek, L.C., Pope, M.A., & Haertzen, C.A. (1993). Patterns of alcohol, cigarette and caffeine and other drug use in two drug abusing populations. Journal of Substance Abuse Treatment, 10, 171-179.

Kreek, M. J. (1987). Multiple drug abuse patterns and medical consequences. In H. Meltzer (Ed.) Psychopharmacology: The Third Generation of Progress (pp. 1597-1604). New York: Raven Press.

Krey, L.C., Lu, K. H., Bulter, W.R., Hotchkiss, J., Piva, F., & Knobil, E. (1975). Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. II. GH and cortisol secretion. Endocrinology, 96, 1088-1093.

Kumar, R., Pratt, J.A., & Stolerman, I.P. (1983). Characteristics of conditioned taste aversion produced by nicotine in rats. British Journal of Pharmacology, 179, 245-253.

Kunin D., Smith, B.R., & Amit, Z. (1999a). Cocaine and ethanol interaction in the conditioned taste aversion paradigm. Physiology and Behavior, 67, 627-630.

Kunin D., Smith, B.R., & Amit, Z. (1999b). Nicotine and ethanol interaction on conditioned taste aversions induced by both drugs. Pharmacology, Biochemistry and Behavior, 62, 215-221.

Lamblin, F., & De Witte, P (1996). Adrenalectomy prevents the development of alcohol preference in male rats. Alcohol, 13, 233-238.

Lasiter, P.S., & Glanzman, D.L. (1985). Cortical substrates of taste aversion learning: involvement of dorsolateral amygdaloid nuclei and temporal neocortex in taste aversion. Behavioral Neuroscience, 99, 257-276.

Leigh, G. (1982) The combined effects of alcohol consumption and cigarette smoking on critical flicker frequency. Addictive Behavior, 7, 251-259.

Leigh, G. & Tong, J. E. (1976) Effects of ethanol and tobacco on time judgment. Perceptual and Motor Skills, 43, 899-903.

Limebeer, C.L., & Parker, L.A. (2000). The antiemetic drug ondansetron interferes with lithium induced conditioned rejection reactions, but not lithium-induced taste avoidance in rats Journal of Experimental Psychology: Animal Behavior Processes, 4,371-384.

Madden, P.A., Heath, A.C., Starmer, G.A., Whitfield, J.B., & Martin, N.G. (1995). Alcohol sensitivity and smoking history in men and women. Alcoholism, Clinical and Experimental Research, 19, 1111-20.

Mark-Kaufman, R., & Lewis, M.J. (1984). Early housing experience modifies morphine self-administration and physical dependence in adult rats. Addictive Behaviors , 9, 235-243.

Marshall, W.R., Green, S.B., Epstein, L.H., Rogers, C.M., & McCoy, J.F. (1980). Coffee drinking and cigarette smoking II. Coffee, urinary pH and cigarette smoking behavior. Addictive Behaviors , 5, 395-400.

Martin, J.R., Cheng, F.Y., & Novin, D. (1978). Acquisition of learned taste aversion following bilateral subdiaphragmatic vagotomy in rats. Physiology and Behavior, 21, 13-17.

McGlone, J.J., Ritter, S., & Kelley, K.W. (1980). The anti-aggressive effect of lithium is abolished by area postrema lesion. Physiology and Behavior, 24, 1095-1100.

Meische, R.A., & Kliner, D.J. (1979). Etonitazene as a reinforcer for rats: Increased etonitazene-reinforced behavior due to food-deprivation. Psychopharmacology, 63, 97-98.

Mitchell, W.D., de Wit, H., & Zacny, J.P. (1995). Effects of varying ethanol dose on cigarette consumption in healthy normal volunteers. Behavioural Pharmacology, 6, 359-365.

Modrow, H.E., Holloway, F.A., & Carney, J.M. (1981). Caffeine discrimination in the rat. Pharmacology, Biochemistry and Behavior, 14, 683-688.

Nachman, M., & Ashe, J.H. (1973a). Effects of basolateral amygdala lesions on neophobia, learned taste aversions, and sodium appetite in rats. Journal of Comparative and Physiological Psychology, 87, 622-643.

Nachman, M., & Ashe, J.H. (1973b). Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. Physiology and Behavior, 10, 73-78.

Nachman, M. & Hartley, P. L. (1975). Role of illness in producing learned taste aversions in rats: A comparison of several rodenticides. Journal of Comparative and Physiological Psychology, 89, 1010--1018.

Naor, C., & Dudai, Y. (1996). Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. Behavioral Brain Research, 79, 61-67.

Nehlig, A. (1999). Are we dependent on coffee and caffeine? A review of human and animal data. Neuroscience Biobehavioral Reviews, 23, 563-576.

Ng Cheong Ton, M., & Amit, Z. (1984). Attenuation of ethanol-induced conditioned taste aversion by naloxazone: Behavioral evidence for an opiate-receptor mediated morphine-ethanol interaction. Neuroscience letters, 48, 127-132.

Ng Cheong Ton, M., & Amit, Z. (1985). Receptor stereospecificity in opiate-ethanol interaction using the preexposure-conditioned taste aversion (CTA) paradigm. Phamacology, Biochemistry and Behavior, 22, 255-259.

Nicholson, S.A. (1989). Stimulatory effect of caffeine on the hypothalamo-pituitary-adrenocortical axis in the rat. Journal of Endocrinology, 122, 535-543.

Nil, R., Buzzi, R., & Battig, K. (1984). Effects of single doses of alcohol and caffeine on cigarette smoke puffing behavior. Phamacology, Biochemistry and Behavior, 20, 583-590.

O'dell, T.J., & Christensen, B.N. (1988). Mecamylamine is a selective non-competitive antagonist of N-methyl-D-aspartate- and aspartate-induced currents in horizontal cells dissociated from the catfish retina. Neuroscience Letters, 94, 93-98.

Osborne, D.J., & Rogers, Y. (1983). Interactions of alcohol and caffeine on human reaction time. Aviation Space and Environmental Medicine, 54, 528-534.

Parker, L.A. (1982). Nonconsummatory and consummatory behavioral CRs elicited by lithium–and amphetamine–paired flavors. Learning and Motivation, 13, 281-303.

Parker, L.A. (1988). Positively reinforcing drugs may produce a different kind of CTA than drugs which are not positively reinforcing. Learning and Motivation, 19, 207-220.

Parker, L.A. (1991). Taste reactivity responses elicited by reinforcing drugs: A dose-response analysis. Behavioral Neuroscience, 105, 955-964.

Parker, L.A. (1993). Taste reactivity responses elicited by cocaine, phencyclidine and methamphetamine-paired sucrose solutions. Behavioral Neuroscience, 107, 118-127.

Parker, L.A. (1995). Rewarding drugs produce taste avoidance, but not taste aversion. Neuroscience and Biobehavioral Reviews, 19, 143-151.

Parker, L.A., & Carvell, T. (1986). Orofacial and somatic responses elicited by lithium-, nicotine- and amphetamine-paired sucrose solution. Pharmacology Biochemistry and Behavior, 24, 883-887.

Parsons, W.D., & Neims, A.H. (1978). Effect of smoking on caffeine clearance. Clinical Pharmacology and Therapeutics, 24, 45-50.

Patten, C.A., Martin, J.E., & Owen, N. (1996). Can psychiatric and chemical dependency treatment units be smoke free? Journal of Substance Abuse Treatment, 13, 107-118.

Phillips, A.G. (1984). Brain reward circuitry: A case for separate systems. Brain Research Bulletin, 12, 195-201.

Piazza, P.V., Deminiere, J.M., LeMoal, M., & Simon, H. (1989). Factors that predict individual vulnerability to amphetamine self-administration. Science, 245, 1511-1513.

Piazza, P.V., Deminiere, J.M., LeMoal, 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., Maccari, S., Deminiere, J.M., LeMoal, M., Mormede, P., & Simon, H. (1991). Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proceedings of the National Academy of Sciences of the United States of America, 88, 2088-92.

Piazza, P.V., & Le Moal, M. (1996). Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annual review of Pharmacology and Toxicology, 36, 359-78.

Potthoff, A.D., Ellison, G., & Nelson, L. (1983). Ethanol intake increases during continuous administration of amphetamine and nicotine, but not several other drugs. Pharmacology, Biochemistry & Behavior, 18, 489-493.

Rabin, B.M. (1996). Free radical and taste aversion learning in the rat: Nitric oxide, radiation and dopamine. Progress in Neuro psychopharmacology and Biological Psychiatry, 20, 691-707.

Rabin, B.M., Hunt, W.A., & Lee, J. (1983). Attenuation of radiation-and drug-induced conditioned taste aversions following area postrema lesions in the rat. Radiation Research, 93, 388-394.

Rabin, B.M., Hunt, W.A., & Lee, J. (1985). Intragastric copper sulfate produces a more reliable conditioned taste aversion in vagotomized rats than in intact rats. Behavioral and Neural Biology, 44, 364-373.

Rabin, B.M., Hunt, W.A., & Lee, J. (1988). Attenuation and cross-attenuation in taste aversion learning in the rat: Studies with ionizing radiation, lithium chloride and ethanol. Pharmacology, Biochemistry and Behavior, 31, 909-918.

Rabin, B.M., Hunt, W.A., Chedester, A.L., & Lee, J. (1986). Role of the area postrema in radiation-induced taste aversion learning and emesis in cats. Physiology and Behavior, 37, 815-818.

Redila, V.A., Smith, B.R., & Amit, Z. (2000). The effects of aminotriazole and acetaldehyde on ethanol drug discrimination with a conditioned taste aversion procedure. Alcohol, 21, 279-285.

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

Reilly, S., Grigson, P.S., & Nogren, R. (1993). Parabrachial nucleus lesions and conditioned taste aversion: evidence supporting an associative deficit. Behavioral Neuroscience, 107, 1005-1017.

Revusky, S., & Garcia, J. (1970). Learned associations over long delays. In G.H Bower, J. Spence (Eds.). Psychology of Learning and Motivation: Advances in Research and Theory (vol. 4). New York: Academic Press.

Revusky, S., & Reilly, S. (1989). Attenuation of conditioned taste aversion by external stressors. Pharmacology Biochemistry and Behavior, 33, 219-226.

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

Riley, A. L., Jacobs, W. J. & Lolordo, V. M. (1978). Morphine-induced taste aversions: A consideration of parameters. Physiological Psychology, 6, 96-100.

Riley, A.L., & Tuck, D.L. (1985). Conditioned taste aversions: A behavioral index of toxicity. Annals of the New York Academy of Sciences, 443, 272-292.

Risinger, F.O., & Brown, M.M. (1996). Genetic difference in nicotine-induced conditioned taste aversion. Life Sciences, 58, 223-229.

Ritter, S., McGlone, J.J., & Kelly, K.W. (1980). Absence of lithium-induced taste aversion after area postrema lesion. Brain Research, 201, 501-506.

Roberts, D.C., & Fibiger, H.C. (1975). Attenuation of amphetamine-induced conditioned taste aversion following intraventricular 6-hydroxydopamine. Neuroscience Letters, 1, 343-347.

Robinson, T.E., & Becker, J.B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine: A review and evaluation of animal models of amphetamine psychosis. Brain Research Reviews, 11, 157-198.

Roll, D.L., & Smith, J.C. (1972). Conditioned taste aversion in anesthetized rats. In M.E.P. Seligman, J.L Hager (Eds.), Biological Bounderies in Learning (pp.98-102). New York: Appleton-Century-Crofts.

Rowell, P.P., Carr, L.A., & Garner, A.C. (1987). Stimulation of [^{3}H]dopamine release by nicotine in rat nucleus accumbens. Journal of Neurochemistry, 49, 1449-54.

Sager, S.M., Sharp, F.R., & Curran, T. (1988). Expression of *C*-Fos protein in brain: metabolic mapping at the cellular level. Science, 240, 1328-1331.

Schafe, G.E., & Bernstein, I.L. (1996). Forebrain contribution to the induction of a brainstem correlate of conditioned taste aversion: I. The amygdala. Brain Research, 741, 109-116.

Schafe, G.E., & Bernstein, I.L. (1998). Forebrain contribution to the induction of a brainstem correlate of conditioned taste aversion: II. Insular (gustatory) cortex. Brain Research, 800, 40-47.

Schaefer, G.J., & Michael, R.P. (1992). Interactions between alcohol and nicotine on intracranial self-stimulation and locomotor activity in rats. Drug and Alcohol Dependence, 30, 37-47.

Schenk, S., Hunt, T., Klukowski, G., & Amit, Z. (1987). Isolation housing decreases the effectiveness of morphine in the conditioned taste aversion paradigm. Psychopharmacology , 92, 48-51.

Schenk, S., Valades, A., Horger, B.A., Snow, S., & Wellman, P.J. (1994). Interactions between caffeine and cocaine in tests of self-administration. Behavioural Pharmacology, 5, 153-158.

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

Shi, D., Nikodijevic, O., Jacobson, K.A., & Daly, J.W. (1993). Chronic caffeine alters the density of adenosine, adrenergic, cholinergic, GABA, and serotonin receptors and calcium channels in mouse brain. Cellular and Molecular Neurobiology, 13, 247-261.

Shoaib, M., Schindler, C.W., & Goldberg, S.R. (1997). Nicotine self-administration in rats: strain and nicotine preexposure effects on acquisition. Psychopharmacology, 129, 35-43.

Shoaib, M., & Stolerman, I.P. (1995). Conditioned taste aversions in rats after intracerebral administration of nicotine. Behavioral Pharmacology, 6, 375-385.

Shoaib, M., Swanner, L.S., Yasar, S., & Goldberg, S. (1999). Chronic caffeine exposure potentiates nicotine self-administration in rats. Psychopharmacology, 142, 327-333.

Signs. S. A. & Schecter. M. D. (1986) Nicotine-induced potentiation of ethanol discrimination. Pharmacology. Biochemistry & Behavior, 24, 769-771.

Simbayi, L, C., Boakes. R.A.. & Burton. M.J. (1986). Effects of basolateral amygdala lesions on taste aversion produced by lactose and lithium chloride in the rat. Behavioral Neuroscience, 100. 455-465.

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

Smith, B.R.. Aragon. C.M.G.. & Amit. Z. (1997). Catalase and the production of acetaldehyde: a possible mediator of the psychopharmacological effects of ethanol. Addiction Biology. 2277-289.

Smith. B.R.. Horan. J.T.. Gaskin. S.. & Amit. Z. (1999). Exposure to nicotine enhances acquisition of ethanol drinking by laboratory rats in a limited access paradigm. Psychopharmacology. 142.408-412.

Smith. J.K.. Neill. J.C.. & Costall. B. (1998). The influence of postweaning housing conditions on drug-induced conditioned taste aversion. Pharmacology Biochemistry and Behavior. 59. 379-386.

Spector. A.C.. Nogren. R.. & Grill. H.J. (1992). Parabrachial gustatory lesions impair taste aversion learning in rats. Behavioral Neuroscience, 106. 147-161.

Spray. K.J.. Halsell. C.B.. & Bernstein. I.L. (2000). *c-Fos* induction in response to saccharin after taste aversion learning depends on conditioning method. Brain Research, 852. 225-227.

Steigerwald, E.S., Rusiniak. K.W., Eckel. D.L., & O'regan, M.H. (1989). Aversive conditioning properties of caffeine in rats. Pharmacology, Biochemistry and Behavior, 31, 579-584.

Stewart, J. & Eikelbloom, R. (1978). Pre-exposure to morphine and the attenuation of conditioned taste aversion in rats. Pharmacology, Biochemistry and Behavior, 9, 639-645.

Stewart. R.B., McBride. W.J., Lumeng, L., Li. T.K., & Murphy, J.M. (1991). Chronic alcohol consumption in alcohol-preferring P rats attenuates subsequent conditioned taste aversion produced by ethanol injections. Psychopharmacology, 105, 530-534.

Stolerman. I.P., & D'Mello. G.D. (1978). Amphetamine-induced taste aversion demonstrated with operant behavior. . Pharmacology, Biochemistry and Behavior. 8, 107-111.

Swank. M. W., Schafe. G., & Bernstein. I.L. (1995). *c*-Fos induction in response to taste stimuli previously paired with amphetamine or LiCl during taste aversion learning. Brain Research. 673, 251-261.

Swanson. J.A., Lee. J.W., & Hopp. J.W. (1994). Caffeine and nicotine: A review of their joint use and possible interactive effects in tobacco withdrawal. Addictive Behaviors, 19, 229-256.

Switzman. L., Amit. Z., White. N., Fishman, B. (1978). Novel tasting food enhances morphine discriminability in rats. In F. C. Colpaert, & J. A. Rosecrans (Eds.). Stimulus Properties of drugs: Ten Years of Progress, (pp. 199-207). Amsterdam: Elsevier.

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

Switzman, L., Hunt, T., & Amit, Z. (1981). Heroin and morphine: Aversive and analgesic effects in rats. Pharmacology, Biochemistry & Behavior, 15, 755-759.

Tong, J.E., Knott, V. J., McGraw, D. J. & Leigh, G. (1974) Alcohol, visual discrimination and heart rate, effects of dose, activation and tobacco. Quarterly Journal of Studies on Alcoholism, 35, 1003-1022.

Van Der Kooy, D. (1984). Area postrema: Site where cholecystokinin acts to decrease food intake. Brain Research, 295, 345-347.

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

Vogel, J. R. & Nathan, B. A. (1976). Reduction of learned taste aversions by pre-exposure to drugs. Psychopharmacology, 49, 167-172.

Wagner, G.C. Foltin, R.W., Seiden, L.S., & Schuster, C.R. (1981). Dopamine depletion by 6-hydroxydopamine prevents CTA induced by methylamphetamine but not LiCl. Pharmacology Biochemistry and Behavior, 14, 85-88.

Waldeck, B. (1974). Ethanol and caffeine: A complex interaction with respect to locomotor activity and central catecholamines. Psychopharmacologia, 36, 209-220.

Walsh, R.N., & Cummins, R.A. (1976). The open-field test: A critical review. Psychological Bulletin, 83, 482-504.

Weeks, J.R., & Collins, R.J. (1964). Factors affecting voluntary morphine intake in self-maintained addicted rats. Psychopharmacologia, 6, 267-279.

White, J.M. (1988). Behavioral interactions between nicotine and caffeine. Pharmacology, Biochemistry and Behavior, 29, 63-66.

White, B.C., & Mason, F.D. (1985). Caffeine-induced taste aversion and mimetic responses. Pharmacology, Biochemistry and Behavior, 23, 515-518.

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

Whitmire, D., & Whitmire, P.S. (1995). Analysis of ethanol and acetaldehyde recovery from perchloric acid-treated blood. Alcohol & Alcoholism, 5, 623-628.

Wise, R.A. (1980). Action of drugs of abuse on brain reward system. Pharmacology, Biochemistry & Behavior, 13, 213-223.

Wise, R.A., Yokel, R.A., & DeWit, H. (1976). Both positive reinforcement and conditioned aversion from amphetamine and apomorphine in rats. Science, 191, 1273-1274.

Zacny, J. P. (1990). Behavioral aspects of alcohol-tobacco interactions. In M. Galanter (Ed.) Recent Developments in Alcoholism (pp. 205-219). New York: Plenum.