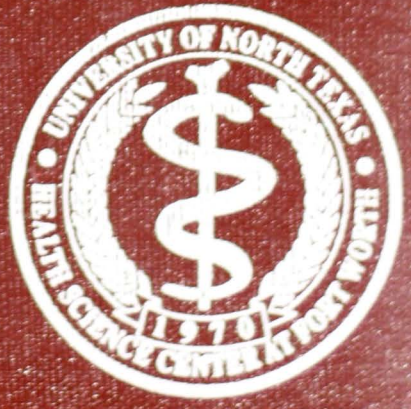
UNIVERSITY OF NORTH TEXAS
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The administration of PTZ or mCPP produces anxiety-like behavior as measured by an increase in the percentage of entries into the open arms and the time spent on the open arms of the elevated plus maze (Prunell *et al.*, 1994). Reportedly, PTZ and mCPP substitute for each other in the drug discrimination paradigm (Wallis and Lal, 1998). It is therefore suggested that a commonality exists among anxiogenic drugs as perceived by trained animals. Andrews and Stephen (1990) suggested that this overall parallelism is an indication that anxiogenic agents may possess similar properties. Therefore, the question posed is as follows: Is there a common denominator of anxiety? The global hypothesis is that the core component of anxiety, produced by anxiogenic agents or processes, involves stimulation of the HPA axis to release CRF, ACTH and/or CORT.

Long Evans rats were trained to discriminate either mCPP (1.4 mg/kg) or PTZ (16 mg/kg) from saline in a two-lever choice procedure (FR10) which is food reinforced. Animals were pretreated with CRF, α-helical CRF (a CRF antagonist), two steroid synthesis inhibitors (ketoconazole, KETZ and aminoglutethimide, AMG), CORT or underwent an adrenalectomy prior to behavioral testing in order to test the hypothesis that the release of CRF and/or CORT are components of the discriminative stimulus of mCPP and/or PTZ.

Pretreatment with CRF, KETZ, AMG and an adrenalectomy facilitated mCPP lever selection. However in the absence of mCPP neither drug nor adrenalectomy produced drug lever selection. In addition CORT did not alter the mCPP dose response curve. However, CORT replacement therapy returned the dose response curve to baseline in adrenalectomized animals. Alpha-helical CRF did not block mCPP discrimination.

Unlike mCPP-trained animals, KETZ and AMG decreased PTZ-lever selection in PTZ-trained animals. In addition, CORT enhanced and partially substituted for the discriminative stimulus of PTZ. However, adrenalectomy completely abolished drug lever selection in PTZ animals. To compare the discriminative stimulus effects of mCPP and PTZ, PTZ-trained animals were injected with cumulative doses of mCPP. mCPP-trained animals were injected with cumulative doses of PTZ. mCPP and PTZ minimally substituted for each other.

The results suggested that neither CRF nor CORT are components of the discriminative stimulus of mCPP and that the role of the HPA axis in mCPP discrimination maybe be a modulator of the stress response. However, CORT is a component of the discriminative stimulus of PTZ such that CORT is necessary for drug lever selection in PTZ trained animals.

CORTICOTROPIN-RELEASING FACTOR AND CORTICOSTERONE MODULATE THE ANXIOGENIC-LIKE EFFECTS OF mCPP

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DISSERTATION

Presented to the Graduate Council of the
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University of North Texas Health Science Center at Fort Worth
in Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Jennifer A. Jenkins, B.S., M.S.
Fort Worth, Texas
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CHAPTER 1

INTRODUCTION

Behavioral health problems affect approximately 30% (one-third) of the United States population each year. The most common of these behavioral health problems is anxiety disorders (including panic and phobia disorders) which effect 17% of the population (SAMHSA, 1997). Because of the increased incidence of anxiety disorders there is a need for better understanding of the mechanism of anxiety in order to provide appropriate treatment and prevention regimes. The development of animal models which represent human anxiety can aid in this process.

Anxiety is regulated by the sympathetic nervous system and the hypothalamo-pituitary adrenal (HPA) axis (Badgy *et al.*, 1989). Anxiety is implicated in the initiation and the continuation of drug use (Littleton and Little, 1994). Withdrawal from drugs such as cocaine, diazepam, morphine and ethanol produces anxiety in rodents as measured by drug discrimination and the elevated plus maze (EPM) paradigm (Vargas *et al.*, 1992, Emmett-Oglesby *et al.*, 1988, Harris *et al.*, 1986, Wood *et al.*, 1989 and Sarnyai *et al.*, 1995).

On the neurotransmitter receptor level, serotonin and GABA (γ-amino-n-butyric acid) modulate the development of anxiety. For several years benzodiazepines, which act on the $GABA_A$ receptor (Haefely, 1985), have been the drugs of choice in the treatment of anxiety (Lader, 1989). The administration of alcohol, opiates and

barbiturates are capable of producing tolerance to each other in rodents (Pugh *et al.*, 1992 and Lal *et al.*, 1988) which suggests a similarity of effects between the drugs belonging to these drug classes. Antidepressants (Kahn *et al.*, 1987) and monamine oxidase inhibitors (Shader and Greenblatt, 1983) are also used in the treatment of anxiety. More recently, the serotonin receptor is implicated in the modulation of anxiety (Eison and Eison, 1994). Although drugs from these various drug classes relieve anxiety, the anxiolytic effects are produced through different mechanisms.

Inasmuch as there are anxiolytic agents which act at different receptors and through different mechanisms to relieve anxiety, the same can be stated for agents or processes which produce anxiety. Pentylenetetrazole (PTZ) and m-chlorophenylpiperazine (mCPP) interact at different receptors, (GABA and serotonin, respectively) yet these two drugs produce anxiety in both humans and rodents (Mueller *et al.*, 1985, Charney *et al.*, 1987, Kennett *et al.*, 1989, Rodin and Calhoun, 1970). Ethanol withdrawal produces anxiety which involves a variety of receptors (Pandey *et al.*, 1996, Devaud *et al.*, 1996 and Davidson *et al.*, 1995). Lal *et al.* (1988) and Wallis and Lal (1998) reported that the anxiogenic stimulus produced by ethanol withdrawal substitutes for the anxiogenic discriminative stimulus of PTZ and mCPP. Likewise, the discriminative stimulus effects of mCPP and PTZ substitute for each other (Wallis and Lal, 1998). The different receptors involved in the production of anxiety induced by ethanol withdrawal or the administration of anxiogenic agents, such as PTZ or mCPP, implicate the involvement of different mechanisms of action which elicit anxiogenic effects. Therefore, it is suggested that the anxiety-like behavior produced in animals by these different paradigms

shares a common physiological pathway. One possible pathway is the release of corticosterone (CORT) from the hypothalamo-pituitary-adrenal (HPA) axis.

Anxiety stimulates the HPA axis to release corticotropin-releasing factor (CRF), adrenocorticotropin (ACTH) and CORT. Britton *et al.* (1986) demonstrated that central administration of CRF produces anxiety in rodents. CORT increases the severity of ethanol withdrawal in mice (Roberts *et al.*, 1994). An increase of these HPA-released hormones is observed during anxiety induced by ethanol withdrawal or the administration of mCPP or PTZ (Rassnick *et al.*, 1993 and Curzon and Kennett, 1990, Hamon, 1994). As such, it is possible that the release of CRF, ACTH and/or CORT is a component of the discriminative stimulus of ethanol withdrawal, PTZ and/or mCPP.

Drug Discrimination.

The drug discrimination paradigm enables the identification of distinct interoceptive or internal states produced by drugs as perceived by trained animals. To obtain food (or avoid shock), animals are trained in a two-choice task to press one lever in the presence of one drug, and to press the other lever when administered a second drug or vehicle. The training drug is alternated with the vehicle randomly in a series of training sessions until the animal can consistently select the correct lever. The training drug is used as a reference stimulus for a different drug. Selection of the drug-associated lever versus the vehicle-associated lever is controlled by resemblance of the test drug stimulus to that of the training drug stimulus (Emmett-Oglesby *et al.*, 1990 and Emmett-Oglesby and Rowan, 1991).

Two basic types of experiments are possible using drug discrimination: 1)

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Two basic types of experiments are possible using drug discrimination: 1)

substitution testing - experiments testing the potential of drugs to substitute for the training drug stimulus, and 2) antagonism - experiments testing the potential of drugs to antagonize the training drug stimulus. Substitution testing is the replacement of a test drug for the training drug and the generation of a dose-response curve to determine how and at what dose the test drug substitutes for the training drug. Antagonism is the administration of a test drug before or after the training drug and the generation of a dose response curve to observe attenuation of drug appropriate responding or selection (Andrews and Stephens, 1990).

In an attempt to develop an appropriate animal model of anxiety, several studies investigated the discriminative stimulus effects of anxiogenic agents. Lal and Emmett-Oglesby (1983) demonstrated the utilization of PTZ discrimination as an animal model of anxiety. Withdrawal from benzodiazepines or ethanol fully substituted for the discriminative stimulus effects of PTZ (Emmett-Oglesby and Mathis, 1989). In addition, withdrawal from cocaine, morphine and nicotine partially substituted for PTZ (Harris *et al.*, 1986, Wood *et al.*, 1989 and Emmett-Oglesby *et al.*, 1988). Because of the anxiogenic effects produced by the administration of mCPP, as demonstrated by the EPM (Gibson *et al.*, 1994), the discrimination of mCPP has also been proposed as a model of anxiety.

Anxiety, Adrenal Hormones and Neuropeptides

Anxiety causes the stimulation of the HPA axis which leads to an increase in CRF, ACTH and glucocorticoids (GC), including CORT (Herman *et al.*, 1996). GCs are released to relieve anxiety and stress-induced mechanisms (Keller-Wood and Dallman,

1984). Acute and chronic stressors alter CRF concentrations, receptors and messenger RNA levels in several areas of the brain, including the hypothalamus (Imaki *et al.*, 1991 and Chappell *et al.*, 1986). CRF (a neuropeptide) is a major physiological regulator of endocrine and visceral responses to stress via the HPA axis (Menzaghi *et al.*, 1994).

Administration of CRF to rodents produces a variety of physiological and behavioral responses such as decreased food intake, suppressed exploration of a novel environment and disruption of sexual behavior (Heinrichs *et al.*, 1995). Gargiulo and Donoso (1996) reported an increase of anxiety-like patterns such as grooming in rats after central administration of CRF. Chlorodiazepoxide (an anxiolytic) decreased the response suppression induced by CRF in the conflict test, an animal model of anxiety (Britton *et al.*, 1985). Britton *et al.* (1986) also reported a reversal of the anxiogenic effects of CRF after the administration of the CRF antagonist, α-helical CRF. Likewise, α-helical CRF reversed the anxiogenic-like response of ethanol withdrawal in rats (Baldwin *et al.*, 1991). In addition, α-helical CRF blocked the enhancement of stress induced behavior (stereotypy and increased locomotor activity) after restraint stress followed by the administration of amphetamine (Cole *et al.*, 1990). These results further suggested an involvement of the activation of the HPA axis in anxiety-like behavior.

Steroid Synthesis Inhibitors

Steroid synthesis inhibitors are compounds reported to prevent adrenal and/or gonadal steroid production (Shaw *et al.*, 1988). Aminoglutethimide (AMG) and ketoconazole (KETZ) are steroid synthesis inhibitors which decrease circulating (basal) levels of CORT (Hughes and Burley, 1970) and/or inhibit stimulated secretion of CORT

(Pont *et al.*, 1982), respectively. Such steroidogenesis inhibition decreases CORT levels and therefore increases CRF levels due to lack of negative feedback by CORT (Worgul *et al.*, 1981).

The indication of KETZ was initially as an antifungal agent and treatment of prostate cancer (Irsy and Koranyi, 1990). However, steroid synthesis inhibition by KETZ in the testes and adrenal glands produced side effects in humans, such as gynecomastia, impotence or adrenal insufficiency (Irsy and Koranyi, 1990). In the steroid synthesis pathway, KETZ inhibits the enzyme activity of the cholesterol side-chain cleavage enzyme, 17-hydroxylase, 11-hydroxylase and mostly the 17-20 desmolase activity (Kowal, 1983; Loose *et al.*, 1983 and Couch *et al.*, 1987) Basal aldosterone and cortisol levels are not altered by KETZ because the inhibitory process is partial (Irsy and Koranyi, 1990). On the other hand, androgen steroid production is more sensitive to KETZ suppression of steroid synthesis (Irsy and Koranyi, 1990).

AMG is an anticonvulsant therapeutically used for estrogen dependent breast carcinoma, prostate tumors and hypercortisolemic disorders (Schteingart and Conn, 1967; Shaw *et al.*, 1988 and Santen *et al.*, 1990). Like KETZ, AMG inhibits several enzymes involved in the synthesis of glucocorticoids as well as the aromatase enzyme which converts androgens to estrogens (Fishman *et al.*, 1967 and Dexter *et al.*, 1967). However, AMG primarily blocks 20-22 adrenal desmolase which converts cholesterol to pregnenolone (Goldman, 1970).

Both KETZ and AMG have been used in tests to determine the effect of pharmacological adrenalectomy on behavior. Ambrahamsen and Carr (1996) reported

that AMG produced an increase in maximal response rates of lateral hypothalamic self-stimulation in food restricted rats. Minnick and Wehner (1992) studied the effects of AMG on ethanol absorption in mice. KETZ has been investigated for its use as an antidepressant for endocrine manifestations of depression (Wolkowitz *et al.*, 1993 and Thakore *et al.*, 1995). Goeders and Guerin (1997) reported that KETZ decreased cocaine self-administration. The results of these studies indicate a role of CORT in animal behavior.

PTZ

Pentylenetetrazole (PTZ, GABA antagonist) produces anxiogenic effects that have been demonstrated in humans (Rodin and Calhoun, 1970) and animals (Lal and Shearman, 1980). In animals, PTZ generates anxiogenic effects as evidenced by increased defecation and vocalization, decreased locomotor activity and decreased social interaction (File and Lister, 1984). The discrimination of PTZ is used as an animal model of anxiety (Emmett-Oglesby *et al.*, 1988 and Andrews and Stephens, 1990).

GABA inhibits hypothalamic interneurons and/or afferent pathways involved in the regulation of ACTH release (Makara and Stark, 1974) and produces a decrease of CORT. On the other hand, GABA antagonists such as picrotoxin and PTZ inhibit the actions of GABA and produce an increase of CRF, ACTH and CORT (Ixart *et al.*, 1983). It is therefore possible that the release of CORT is a component of the discriminative stimulus effects of PTZ.

Serotonin (5-HT)

The anxiogenic effect seen in animals undergoing withdrawal is modulated by serotonin (5-HT). For example, el-Kadi and Sharif (1995) demonstrated an attenuation of withdrawal symptoms after chronic opioid treatment by the administration of 5HT antagonists, methysergide and cyproheptadine, suggesting that the 5-HT receptors are involved in the expression of withdrawal symptoms. Baumann *et al.* (1995) reported deficits in pre-synaptic 5-HT function after withdrawal from chronic cocaine. Likewise, Parsons *et al.* (1995) stated that deficient 5-HT neurotransmission may be a significant factor in cocaine withdrawal symptomology.

5-HT is associated with the neural mechanisms underlying anxiogenic and anxiolytic actions (Iverson, 1984). 5-HT_{1A} receptor agonists, such as buspirone, and 5-HT_2 receptor antagonists, such as mianserin, have anxiolytic effects (Traber *et al.*, 1984 and Meert and Janssen, 1989) whereas 5-HT_{2C} receptor agonists, such as mCPP, have anxiogenic effects (Kennett *et al.*, 1989).

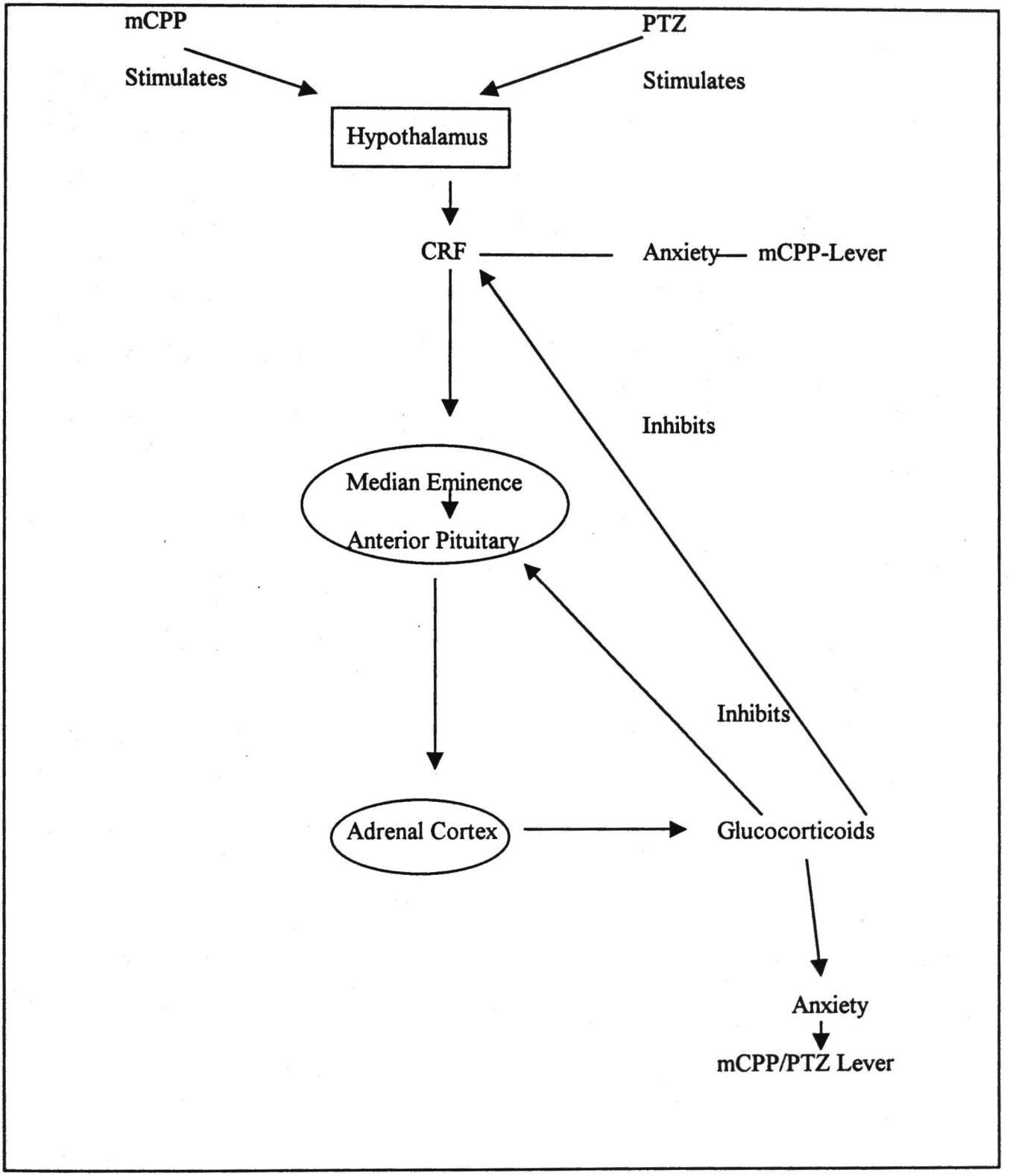
mCPP

mCPP, (1-(3-chlorophenyl)piperazine), is classified as a partial 5-HT agonist which acts at the 5-HT_{1B} and 5-HT_{2C} receptor sites (Callahan and Cunningham, 1994). The anxiogenic-like effects of mCPP are demonstrated in humans (Charney *et al.*, 1987) and animals (Kennett *et al.*, 1989). In rodents, mCPP produces anxiogenic effects, such as decreased open arm activity on the elevated plus maze (Gibson *et al.*, 1994), decreased locomotor activity, decreased food intake and decreased social interaction (Curzon and Kennett, 1990), that are similar to those effects observed after PTZ and CRF

administration. Previous studies have shown that mCPP produces a discriminative stimulus (Winter and Rabin, 1993 and Callahan and Cunningham, 1994) that substitutes completely in a drug discrimination paradigm for other $5HT_{1B}$ and $5HT_{2C}$ agonists . As previously stated, the anxiogenic and discriminative stimulus effects of mCPP are useful properties that may contribute to the use of mCPP as an animal model for anxiety.

Ethanol withdrawal or the administration of PTZ or mCPP produces anxiety-like behavior as measured by an increase in the percentage of entries into the open arms and the time spent on the open arms of the elevated plus maze (Prunell *et al.*, 1994). Because ethanol withdrawal or PTZ or mCPP administration substitutes for each other in the drug discrimination paradigm, it is suggested that a commonality exists among them as perceived by trained animals. Andrews and Stephen (1990) suggested that this overall parallelism is an indication that different anxiogenic agents may possess similar properties. Therefore, the question posed is as follows: Is there a common denominator of anxiety? The global hypothesis is that the core component of anxiety, produced by anxiogenic agents or processes, involves stimulation of the HPA axis to release CRF, ACTH and/or CORT. More specifically, animals were pretreated with CRF, α-helical CRF (a CRF antagonist), two steroid synthesis inhibitors (KETZ and AMG) or CORT or underwent an adrenalectomy in order to test the hypothesis that the release of CRF and/or CORT are components of the anxiogenic discriminative stimulus of mCPP and/or PTZ (Fig 1).

Fig 1. Flow diagram of the hypothesis that release of CRF and/or CORT is a component of the discriminative stimulus effects of mCPP and/or PTZ. Both mCPP and PTZ stimulate the release of CRF from the hypothalamus. It is hypothesized that the CRF-induced anxiety is reflected by drug lever selection in trained animals. CRF causes the release of ACTH which causes the release of glucocorticoids. Therefore, it is also hypothesized that the release of glucocorticoids will also produce drug lever selection in trained animals.


mCPP
Stimulates
PTZ
Stimulates
Hypothalamus
CRF
Anxiety
mCPP-Lever
Inhibits
Median Eminence
Anterior Pituitary
Inhibits
Adrenal Cortex
Glucocorticoids
Anxiety
mCPP/PTZ Lever

Abbreviations

PTZ, pentylenetetrazole; mCPP, (1-(3-chlorophenyl)piperazine); ACTH, adrenocorticotropin; CORT, CORT; AMG, aminoglutethimide; CRF, corticotropin-releasing factor; 5HT, 5-hydroxytryptamine (serotonin); GABA, γ-amino-n-butyric acid; ADX, adrenalectomy; HPA, hypothalamo-pituitary adrenal; i.c.v., intracerebroventricular; i.p., intraperitoneal; s.c., subcutaneous; EPM, elevated plus maze; ketoconazole, KETZ; GC, glucocorticoid; EPM, elevated plus maze

CHAPTER 2
METHODS

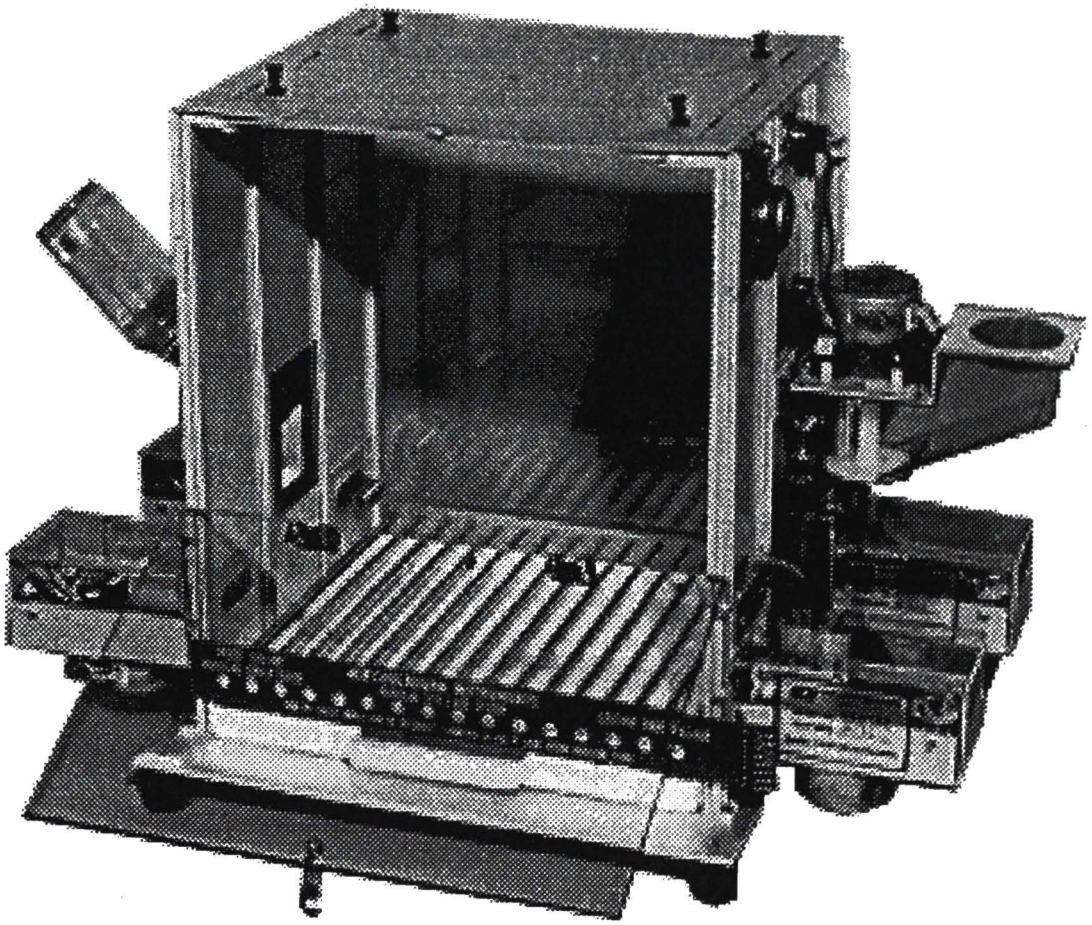
Animals

Two hundred and two male Long Evans rats (Harlan Laboratories, Indianapolis, IN) weighing 300-350 g were randomly divided into three groups. One group (n=88) was trained to discriminate mCPP from saline. A second group (n=51) was trained to discriminate PTZ from saline and the third group (n=63) was put on an ethanol diet. Animals were maintained at 80% of their free-feeding body weights by restricting daily access to food. Food was rationed to 16-18 g/day and was provided after each training session. Water was available, ad libitum, except during periods of training and testing (approximately 1 hour and 30 minutes). Animals were maintained on a 12:12 light:dark cycle (7:30 a.m. - 7:30 p.m.).

Apparatus

Twenty-four two-lever operant chambers (30-cm x 26-cm x 24-cm; Coulburn Instruments, Lehigh Valley, PA) were connected to three IBM-compatible PCs (8 chambers/computer) which were programmed with OPN software (Spencer and Emmett-Oglesby, 1985) to control food pellet delivery and record operant responding via LVB interfaces (Med Associates, East Fairfield, VT). Each chamber (Fig 2) was equipped with a food dispenser mounted equidistant between two response levers on one wall and housed in a light- and sound-attenuating shell. The levers were 2.5 cm in width, protruded

Fig 2. Operant chamber (30-cm x 26-cm x 24-cm) for drug discrimination was connected to an IBM-compatible PC which was programmed with OPN software to control food pellet delivery and record operant responding via LVB interfaces. Chamber was equipped with a food dispenser mounted equidistant between two response levers on one wall and housed in a light- and sound-attenuating shell. Illumination was provided by a 28-V house light; ventilation and masking noise were supplied by ventilation fan.



3.0 cm from the wall and were located 2.0 cm above the grid floor. Illumination was provided by a 28-V house light; ventilation and masking noise were supplied by ventilation fan. Four dependent measures were collected for each chamber at the end of a session: 1) total number of responses on each of two levers, 2) latency to the first response, 3) number of responses made on each lever prior to the first reinforcement, and 4) latency until the first reinforcement was obtained. The criterion for determining lever selection was the completion of 10 responses on a lever.

Drug-Saline Discrimination Training

Animals were trained on a two-lever choice procedure to press a lever under a fixed ratio 10 (FR10) schedule of food reinforcement such that every ten presses on the correct lever resulted in delivery of a food pellet (Noyes 45mg). Drug- and saline-appropriate levers were assigned among animals such that the drug lever was to the left of the food cup for half the animals and to the right of the food cup for the other half. Fifteen minutes before each session, animals were injected i.p. with either mCPP (1.4 mg/kg), PTZ (16 mg/kg) or saline. Saline was given in a volume of 1ml/kg. The doses of drugs were chosen because they produced the most stable discriminative stimulus without severe behavioral disruption or convulsions.

Ten-minute training sessions were conducted daily according to a random sequence of vehicle and/or drug sessions. An accelerated training method (Emmett-Oglesby and Herz, 1987) was used to adjust the animals to a cumulative dosing procedure such that three to five sessions were performed on one day. Drug sessions were only performed once per day. Animals were considered to be trained when they selected the

correct lever on 10 consecutive random sessions. Only trained animals that selected the correct lever at least four of the five (≥ 80%) preceding sessions were eligible for test sessions. The session prior to each test session was saline.

Cumulative Dose Testing

Animals were first injected with saline and placed in the chamber for 5 minutes or until 40 lever presses were completed. Both levers were activated during testing. Animals were then injected with drug as described in Table 1 (mCPP) and Table 2 (PTZ). After each injection the animals underwent a wait period of 15 minutes before being placed in the chambers. All doses were given on one day unless full substitution for the discriminative stimulus occurred, more than 50% of the animals did not select a lever during the 5 minute test period or the animals had been tested five times on that day.

Stimulus Control Tests

The day following all dose response tests, the ability of the animals to recognize the discriminative stimulus of the drug when compared to vehicle was determined. After the administration of vehicle and drug, animals which selected the saline and drug-appropriate lever, respectively, were considered to be trained and eligible for use in the next test session. Animals that were not eligible were continuously trained until criteria was met.

Intracerebroventricular (ICV) Surgery

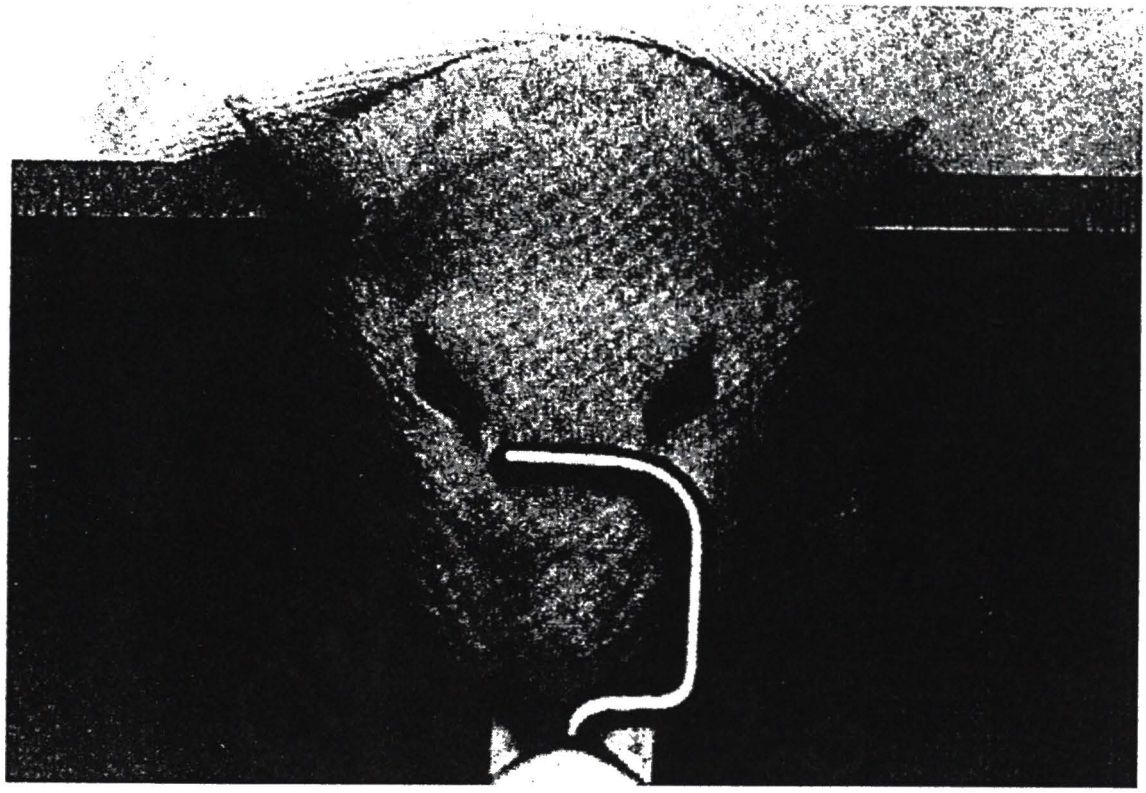
Nineteen rats were trained to discriminate mCPP from saline. After the majority of animals (93%) met the training criteria, animals were anesthetized with sodium pentobarbital (50 mg/kg, Sigma) and stereotaxically implanted with a stainless steel guide

cannula (23-gauge, 7-mm) placed in the lateral ventricle. Stereotaxic coordinates were based on the atlas of Paxinos and Watson (1997). The coordinates were: anterior-posterior, -0.6 from bregma; lateral, +2.0 mm from midline; and dorsal-ventral, -3.2 mm from the skull surface, with the incisor bar set 5 mm above the interaural line (Fig 3). The cannula was fastened to the skull with dental cement and sealed with a 7-mm stylet wire. Animals were allowed a minimum of seven days to recover before re-training for drug discrimination. Animals were trained prior to surgery in order to increase the endurance of the cannula and the longevity of the animals. To verify cannula placement the animals were euthanized with an overdose of sodium pentobarbital (100 mg/kg) and injected i.c.v. with 2 µl methylene blue dye. Animals were decapitated and the brains were removed from the skull and thinly sliced with a new razor blade and examined to ensure that the methylene blue dye had spread through the ventricular system (Cole and Koob, 1994).

Adrenalectomy

Animals were anaesthetized with sodium pentobarbital (50 mg/kg, Sigma). Adrenals were removed via bilateral flank incisions. SHAM-operated animals were treated in the same manner, except the adrenals were left *in situ*. The incisions were closed with wound clips. Animals were initially provided with continuous access to a salt pellet (99.99% sodium chloride) in their home cages but were later switched to saline as drinking water in order to ensure constant access to salt and to compensate for salt loss caused by adrenalectomy. Animals were allowed a maximum of four days to recover before being tested for mCPP or PTZ discrimination.

Fig 3. Stereotaxic implantation of guide cannula in rat. Rats were anesthetized with sodium pentobarbital and place in the stereotaxic apparatus with the coordinates of anterior-posterior, -0.6 from bregma; lateral, +2.0 mm from midline; and dorsal-ventral, -3.2 mm from the skull surface, with the incisor bar set 5 mm above the interaural line. After recovery from surgery and re-training for drug discrimination, animals were administered CRF or α-helical CRF intracerebroventricularly.



Drug Administration for Drug Discrimination

For training animals, PTZ (16 mg/kg, Sigma) and mCPP (1.4 mg/kg, RBI) were dissolved in saline. For dose response testing, mCPP was given in cumulative doses of 0, 0.26, 0.46, 0.8, 1.4 mg/kg (Table 1). PTZ was given cumulatively to equal doses of 0, 2, 4, 8 and 16 mg/kg (Table 2).

AMG (steroid synthesis inhibitor, Sigma) was prepared in peanut oil (20 mg/ml) by heating in a Pyrex beaker at 37°C until homogenous solution was obtained The solution was then cooled to room temperature and administered in doses of 5, 10, 20 or 40 mg/kg for mCPP-trained animals and 5 or 20 mg/kg for PTZ- trained animals. Animals were injected subcutaneously with AMG 30 minutes and 12 hours prior to testing for mCPP or PTZ dose response. The steroid synthesis inhibitor KETZ (1.09-17.5 mg/kg, RBI) was dissolved in 20 microliters of acetic acid (glacial), 0.1M sodium acetate and distilled water with a final pH of 5.5. Animals were injected with KETZ at 30 minutes and 12 hours prior to testing mCPP or PTZ dose response. CORT (5, 10, 20 mg/kg, Sigma) was suspended in sesame seed oil. Animals were injected subcutaneously with CORT 1 hour prior to mCPP or PTZ dose response testing. The doses of drugs and times of administration chosen for these experiments were in accord with previous behavioral studies indicating significant behavioral effects. Dose response testing after 30 minute pretreatment with KETZ was chosen after observations of increased behavior in animals by the experimenter.

CRF was dissolved in sterile isotonic saline whereas α-helical CRF was dissolved in distilled water that was buffered to pH 6.7 with acetic acid. Both drugs were freshly

Table 1. Actual and Cumulative Doses Injected for mCPP. Animals were trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. After training criteria was met such that animals selected the correct lever on ten consecutive sessions and had selected the correct lever four of the five preceding sessions, a cumulative dose effect curve was performed. Doses injected were in log steps of 0.57 from the first injection of mCPP.

Injection	Dose Injected (mg/kg)	Cumulative Dose (mg/kg)
1st	0 (vehicle)	0 (vehicle)
2nd	0.26	0.26
3rd	0.20	0.46
4th	0.34	0.80
5th	0.60	1.4

Table 2. Actual and Cumulative Doses Injected for PTZ. Animals were trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. After training criteria was met such that animals selected the correct lever on ten consecutive sessions and had selected the correct lever four of the five preceding sessions, a cumulative dose effect curve was performed. Doses injected were in log steps of 2.0 of the first injection of PTZ.

Injection	Dose Injected (mg/kg)	Cumulative Dose (mg/kg)
1st	0 (vehicle)	0 (vehicle)
2nd	2	2
3rd	2	4
4th	4	8
5th	8	16

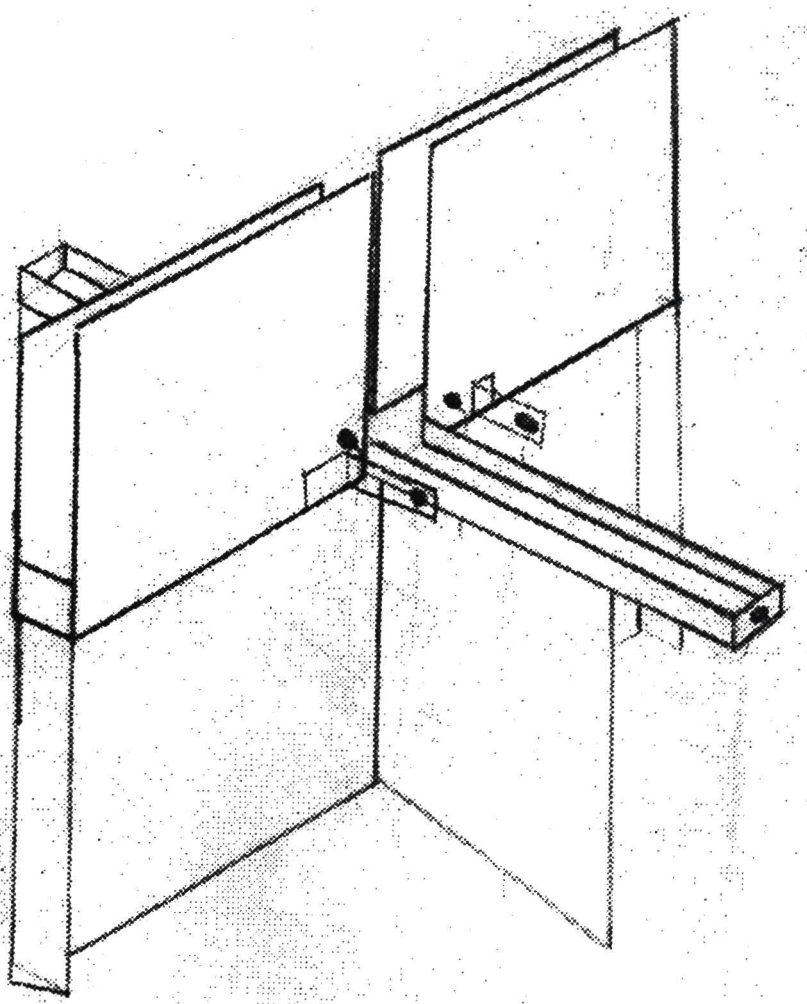
prepared before administration. For ICV injections of CRF and α-helical CRF, the stylet was removed from the guide cannula and an 8-mm injector connected to approximately 70 cm of calibrated PE 10 tubing was inserted into the cannula. One microliter of CRF was infused under gravity by lifting the tubing above the head of the animal. For ICV injections of α-helical CRF, the injector was connected by PE 10 tubing to a 10 µl Hamilton syringe and inserted into the cannula. Two microliters of α-helical CRF were infused over approximately 60 seconds. In both procedures the injector was left in place for 30 seconds after the injection to prevent backflow before the stylet was replaced in the guide cannula.

For CORT replacement therapy, adrenalectomized and SHAM-operated animals were injected daily with CORT (5 mg/kg) or sesame seed oil (1ml/kg). Animals were injected subcutaneously with CORT for four days and 1 hour prior to mCPP or PTZ dose response testing.

Elevated Plus Maze

The white Plexiglas maze had four arms (10 x 50 cm) at right angles to each other and was elevated 50 cm from the floor (Fig 4). Two of the arms had 40 cm high walls (the enclosed arms) and two arms had 2 cm walls (the open arms). The central square was 10 cm on a side. The entry into each arm was monitored by photo cells connected to a PC computer through a Med Associates' interface using software developed in this laboratory. Animals were tested in a sound attenuated dimly lit room. Animals were given 30 minutes to adjust to the test room, then injected with the test drug. After the specified wait period, the animals were removed from the transfer cage and placed in the

Fig 4. Elevated Plus Maze. White Plexiglas maze with four arms (10 x 50 cm) at right angles to each other, elevated 50 cm from the floor. Two of the arms had 40 cm high walls (closed arms) and two arms had 2 cm walls (open arms). Entry into each arm was monitored by photo cells connected to a PC computer through a Med Associates' interface. The number of entries into the open and closed arms and the time spent in each arm was recorded automatically during a 5 min observation period. Between tests the maze was wiped clean with a damp sponge.



center portion of the elevated plus-maze (EPM). The number of entries into the open and closed arms and the time spent in each arm was recorded automatically during a 5 min observation period. Between tests the maze was wiped clean with a damp sponge.

Ethanol Withdrawal

Animals were given an ethanol liquid diet for 10 days. Twelve hours after the last dose of ethanol, animals were tested for overt signs and symptoms of ethanol withdrawal. Scoring of ethanol withdrawal was as follows:

Withdrawal Signs,	Points
1. Vocalization- spontaneous/on handling	1
2. Urination- on handling	1
3. Defecation-on handling	1
4. Caudal Posture	0-3

a) 0 point(s) for limp or normal tail, b) 1 point(s) for stiff, curls around finger, c) 2 point(s) for stiff, curls around finger, stays elevated after released, d) 3 point(s) for spontaneous abnormal posture of tail such as severe deviation or lift above back, stiff, curls around finger, and stays elevated after released.

5. Tremor 0-3

a) 0 point(s) for no tremor, b) 1 point(s) for mild tremor in one portion of body (for example, face), c) 2 point(s) for generalized occasional tremor, d) 3 point(s) for constant generalized tremor.

6. Startle 0-3

a) 0 point(s) for none, b) 1 point(s) for twitch, c) 2 point(s) for jump or freeze,

d) 3 point(s) for exaggerated jump or freeze.

7. Convulsions (handling) 0-3

a) 0 point(s) for none, b) 1 point(s) short duration clonic, c) 2 point(s) for multiple clonic, d) 3 point(s) for tonic-clonic.

8. Death. 10

After scoring for withdrawal animals were placed on the EPM for 5 minutes.

Drug Administration for the Elevated Plus Maze

KETZ (1.09, 4.38 and 8.75 mg/kg, i.p.) was dissolved in 20 µl of acetic acid (glacial), 0.1M sodium acetate and distilled water with a final pH of 5.5. Animals were injected with ketoconazole 30 minutes prior to being placed on EPM.

Liquid Ethanol Diet

A palatable and nutritionally balanced ethanol diet was fed to rats in graduated tubes to allow determination of the amount of liquid consumed and calculation of the ethanol dose. Each liter of ethanol diet contained an aqueous suspension of pulverized casein (42 g), l-methionine (0.6 g), AIN vitamin mixture (2.1g), AIN mineral mixture (7.3 g), sucrose (25g), xanthum gum (3g), choline bitartrate (0.4g), Celufil cellulose (1 g), corn oil (10.5 g), and ethanol and dextrin. Dextrin and ethanol varied in combination to provide the same caloric content in all formulations. Saccharin (215.6 mg) was added to mask the taste of ethanol. One hundred ml of this diet (6.5% ethanol) was placed in each home cage daily for nine days followed by a 50 ml aliquot on the morning of the final day of chronic treatment. Ten hr later animals were gavaged with a final dose of ethanol (10

ml of 3 g/kg) in the same liquid diet. Body weights and volume of liquid diet consumed were monitored at the time of placing the daily ration of diet into the home cages.

Data Analysis

The data collected in the drug discrimination sessions were quantitative and quantal measurements. The quantitative measurement is the number of presses upon the drug-appropriate lever divided by the total number of presses upon both the drug- and the vehicle-appropriate levers at the end of a test session, this fraction is expressed as a percentage (QM=# presses on drug-appropriate lever/total # of presses x 100%). If an animal failed to select a lever during a test, that animal was not used to calculate lever selection data. Full drug lever selection was defined, using quantal measurement, as 80% or greater of subjects completing the first FR 10 on the drug lever. As standard in the lab, partial substitution was considered to occur when average drug-lever selection was between 20 and 80%. Similarly, antagonism was defined as no more than 20% drug-lever selection after pretreatment with at least one dose of an antagonist. Significance of dose effect curves was determined using quantitative measure of drug lever selection with two-way analysis of variance (ANOVA; Systat version 7.0 for the IBM-compatible computer).

The effects of dose on the rate of responding (responses per second) were analyzed using repeated measures analysis of variance (ANOVA; Systat version 7.0 for the IBM-compatible computer) and Student's t test.

Elevated plus maze data was presented as mean ± SEM and analyzed with Student's t test or by two-way analysis of variance (3 doses of 1.09, 4.38, 8.75 mg/kg of

KETZ). Significance was determined at the 0.05 probability level calculated by the above programs.

CHAPTER 3
RESULTS

mCPP versus saline discrimination was acquired in approximately 30 sessions (Fig 5). PTZ discrimination was acquired in approximately 45 sessions (Fig 6). According to testing criteria, animals were trained (as described in Methods) and used for behavioral testing.

The Effect of CORT on mCPP Drug Discrimination

Long Evans rats were pretreated with CORT (5, 10, and 20 mg/kg, s.c.) or sesame seed oil (vehicle, 1ml/kg, s.c.) one hour prior to behavioral testing. As shown in Fig. 7 (upper panel), CORT pretreatment did not alter mCPP lever selection when compared to vehicle. The doses of 5 (n=10) and 20 mg/kg of CORT(n=10) significantly increased response rates when compared to vehicle [$F(1,90)=35.72$, $p<0.001$ and $F(1,90)=13.51$, $p<0.001$] (Fig 7, lower panel). The dose of 10 mg/kg CORT (n=8) did not effect the rate of response [$F(1,70)=0.377$, $p=0.541$).

The Effect of Ketoconazole on mCPP Drug Discrimination

Ten male Long Evans rats were pretreated (30 min) with ketoconazole (KETZ, 1.09, 4.38, 8.75 and 17.5 mg/kg, i.p.) or distilled water (vehicle, i.p.) prior to behavioral testing. As shown in Fig. 8 (upper panel), KETZ (4.38, 8.75 and 17.5 mg/kg) produced dose dependent facilitation of mCPP lever selection that was significantly different when compared to control [$F(1,90)=5.72$, $p<0.05$, $F(1,64)=34.31$, $p<0.001$ and $F(1,64)=77.70$, $p<0.001$, respectively]. Fig. 8 (lower panel) shows that KETZ decreased the response rates dose dependently [F(4,

Fig 5. Acquisition of mCPP discrimination. Animals were trained to discriminate mCPP (1.4 mg/kg) from saline (1 ml/kg) as described in Methods. Training data was calculated by dividing the number of drug lever selection by the total number of lever selection and multiplied by 100%. After approximately 30 sessions of drug and vehicle, animals show approximately 90% mCPP lever selection after the administration of drug and 6% mCPP lever selection after the administration of saline. The X-axis represents the number of training sessions. The Y-axis represents the percentage of animals selecting the mCPP lever.

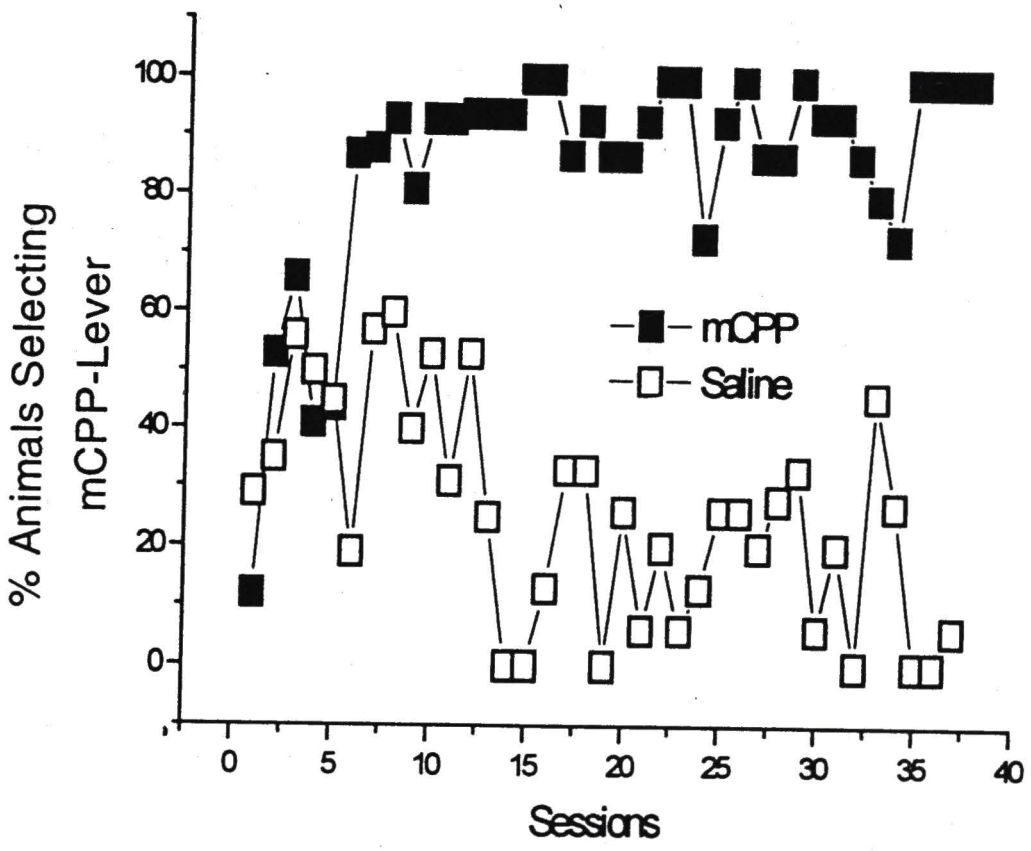

% Animals Selecting
mCPP-Lever
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Sessions
mCPP
Saline

Fig 6. Acquisition of PTZ discrimination. Animals were trained to discrimination PTZ (16 mg/kg) from saline (1 ml/kg) as described in Methods. Training data was calculated by dividing the number of drug lever selection by the total number of lever selection and multiplied by 100%. After approximately 45 sessions of drug and vehicle administration, animals showed approximately 95% PTZ lever selection after the administration of drug and 10% PTZ lever selection after the administration of saline. The X-axis represents the number of training sessions. The Y-axis represents the percentage of animals selecting the PTZ lever.

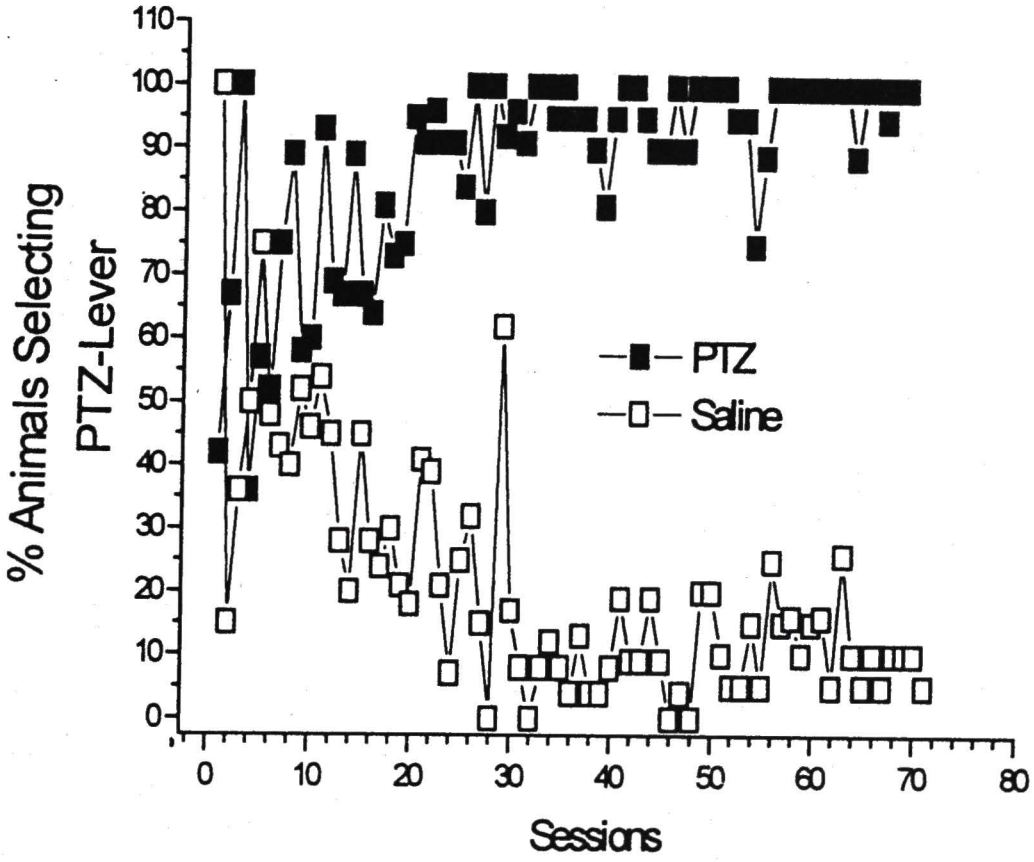

% Animals Selecting
PTZ-Lever
110
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
Sessions
PTZ
Saline

Fig 7. Effect of CORT (5 mg/kg,n=10; 10 mg/kg, n=8; 20 mg/kg; n=10) in rats trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Pretreatment with CORT did not alter mCPP discrimination. Lower panel shows the response rates. Doses of 5 and 20 mg/kg of CORT increased the response rates ($p<0.001$). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever. In the lower panel, the Y-axis represents the response rates (res/sec).

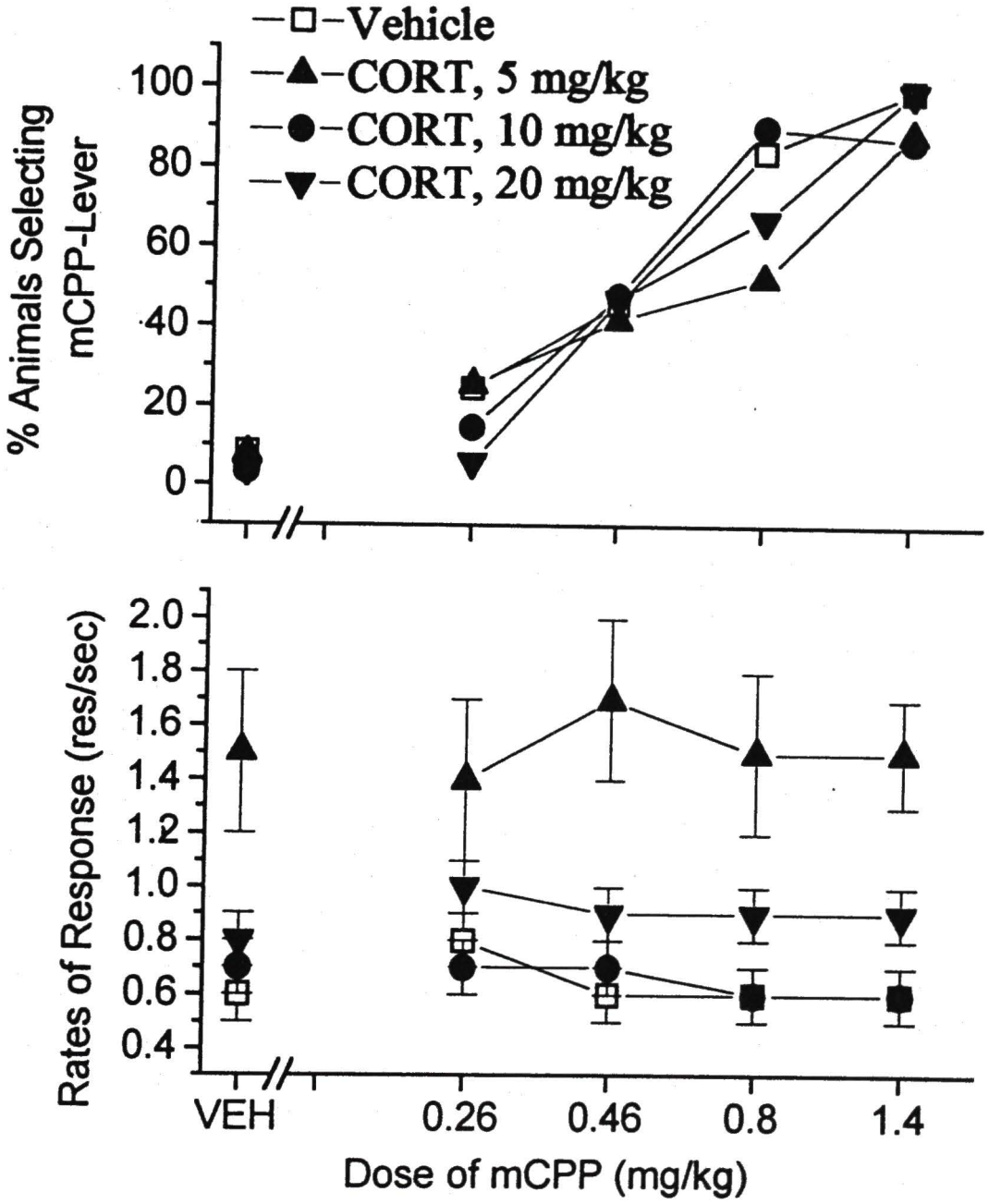
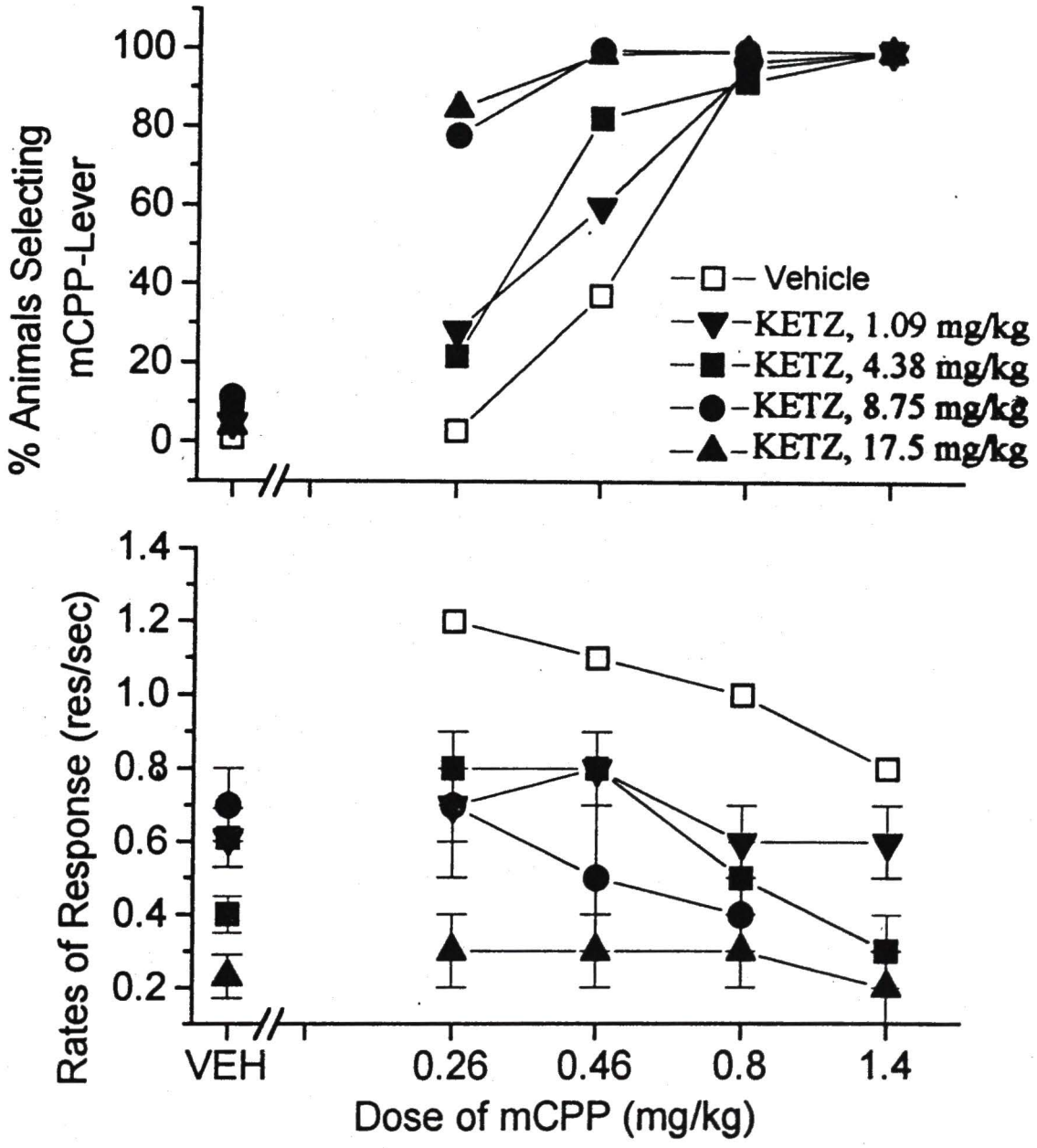

Vehicle
CORT, 5 mg/kg
CORT, 10 mg/kg
CORT, 20 mg/kg
% Animals Selecting mCPP-Lever
100
80
60
40
20
0
Rates of Response (res/sec)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

Fig 8. Effect of KETZ (n=10) in rats trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Thirty minute pretreatment with KETZ (1.09, 4.38, 8.75, 17.5 mg/kg) dose dependently facilitated mCPP lever selection. Lower panel shows the response rates. KETZ dose dependently decreased the response rates ($p<0.001$). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever. In the lower panel, the Y-axis represents the response rates (res/sec).


% Animals Selecting mCPP-Lever
100
80
60
40
20
0
Vehicle
KETZ, 1.09 mg/kg
KETZ, 4.38 mg/kg
KETZ, 8.75 mg/kg
KETZ, 17.5 mg/kg
Rates of Response (res/sec)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

200)=10.36, $p<0.001$] with significant interaction (KETZ x mCPP) [F(16, 200)=3.58, $p<0.001$]. KETZ (4.38, 8.75 and 17.5 mg/kg, i.p., n=10) pretreatment (12 hours) did not produce any significant shifts of the dose effect curve [$F(1,90)=0.167$, $p=0.683$, $F(1,90)=0.188$, $p=0.665$ and $F(1,90)=0.007$, $p=0.932$] (Fig. 9, upper panel). The highest dose of KETZ (17.5mg/kg) produced a significant decrease in the response rates [$F(1,90)=13.48$, $p<0.001$] (Fig 9, upper panel).

The Effect of Aminoglutethimide on mCPP Drug Discrimination

Twelve male Long Evans rats were pretreated with aminoglutethimide (AMG, 5, 10, 20 and 40 mg/kg) or peanut oil (vehicle, s.c.). AMG (5 and 10 mg/kg) after thirty minutes facilitated mCPP lever selection (Fig. 10, upper panel). The administration of higher doses of AMG (20 and 40 mg/kg) did not significantly effect mCPP lever selection [20 mg/kg, $F(1,110)=0.576$, $p>0.05$; 40 mg/kg, $F(1, 110)=0.065$, $p>0.5$]. After pretreatment with the highest dose of AMG (40 mg/kg), the maximal drug lever selection was 80% at the highest cumulative dose of mCPP (1.4 mg/kg). There was no significant difference in the response rates produced by any dose of AMG [5 mg/kg, $F(1,110)=1.35$, $p>0.05$; 10 mg/kg, $F(1,110)=3.70$, $p>0.05$ and 20 mg/kg, $F(1,110)=1.22$, $p>0.05$ and 40 mg/kg, $F(1,110)=1.22$, $p>0.05$] (Fig. 10. lower panel).

Pretreatment with AMG (5 mg/kg) after twelve hours facilitated mCPP lever selection. The 10 mg/kg dose of AMG also facilitated mCPP lever selection, but not significantly [$F(1,110)=3.18$, $p=0.077$] (Fig. 11, upper panel). AMG did not significantly alter the response rates [$F(1,110)=1.09$, $p>0.05$; 10 mg/kg, $F(1,110)=0.596$,

Fig 9. Effect of KETZ (n=10) on the discriminative stimulus of mCPP in rats trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Twelve hour pretreatment with KETZ (1.09, 4.38, 8.75, 17.5 mg/kg) did not alter mCPP discrimination. Lower panel shows the response rates. The highest dose of KETZ (17.5 mg/kg) significantly decreased the response rates ($p<0.001$). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever. In the lower panel, the Y-axis represents the response rates (res/sec).

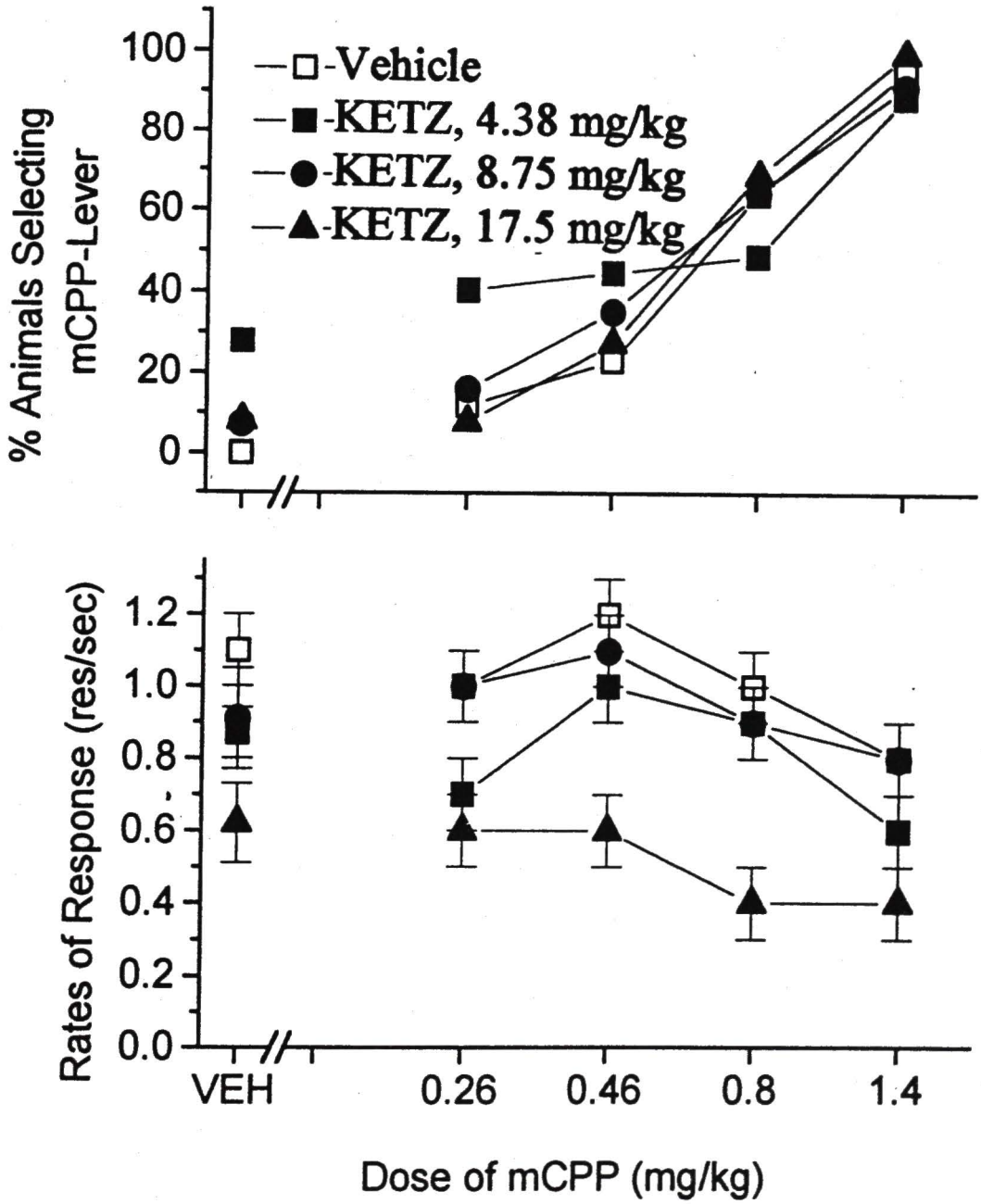

% Animals Selecting mCPP-Lever
100
80
60
40
20
0
—□-Vehicle
—■-KETZ, 4.38 mg/kg
—●-KETZ, 8.75 mg/kg
—▲-KETZ, 17.5 mg/kg
Rates of Response (res/sec)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

Fig 10. Effect of AMG (n=12) on the discriminative stimulus of mCPP in animals trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Thirty minute pretreatment with AMG (5 and 10 mg/kg) facilitated mCPP lever selection. The highest dose of AMG (40 mg/kg) produced a maximal drug lever selection of 80%. Lower panel shows the response rates. AMG had no significant effect on the response rates ($p>0.05$). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever. In the lower panel, the Y-axis represents the response rates (res/sec).

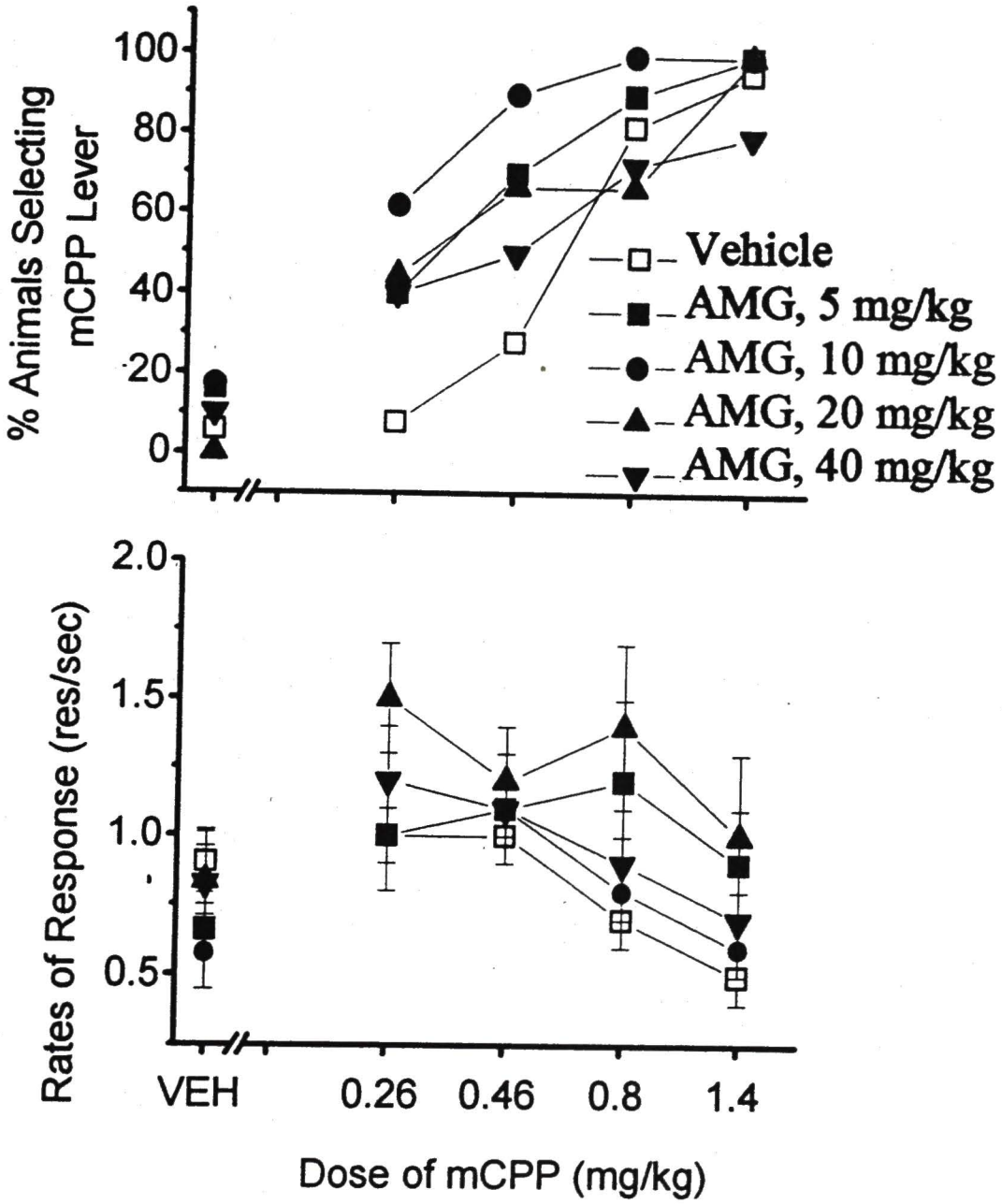
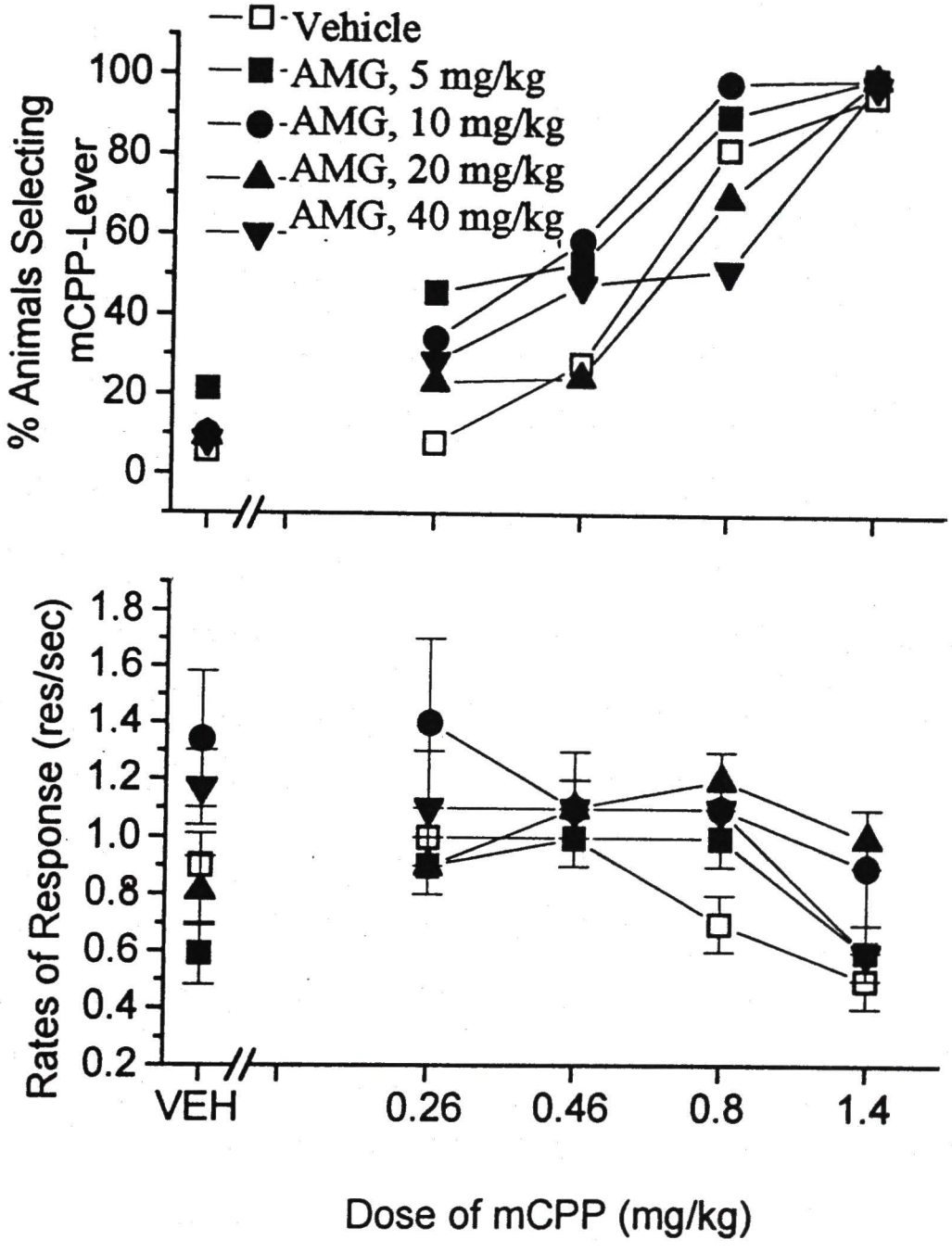

% Animals Selecting mCPP Lever
100
80
60
40
20
0
Vehicle
AMG, 5 mg/kg
AMG, 10 mg/kg
AMG, 20 mg/kg
AMG, 40 mg/kg
Rates of Response (res/sec)
2.0
1.5
1.0
0.5
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

Fig 11. Effect of AMG (n=12) on the discriminative stimulus of mCPP in animals trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Twelve hour pretreatment with AMG (5 and 10 mg/kg) facilitated mCPP lever selection. Lower panel shows the response rates. AMG had no significant effect on the response rates ($p>0.05$). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever. In the lower panel, the Y-axis represents the response rates (res/sec).


Vehicle
AMG, 5 mg/kg
AMG, 10 mg/kg
AMG, 20 mg/kg
AMG, 40 mg/kg
% Animals Selecting mCPP-Lever
100
80
60
40
20
0
Rates of Response (res/sec)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

$p>0.05$, 20 mg/kg, $F(1,110)=3.10$, $p>0.05$ and 40 mg/kg, $F(1,110)=0.09$, $p>0.5$] (Fig. 11, lower panel).

The Effect of CORT on PTZ Drug Discrimination

Male Long Evans rats (n=8) were pretreated with CORT (5, 10, and 20 mg/kg, s.c.) or sesame seed oil (vehicle, 1ml/kg, s.c.) one hour prior to behavioral testing. As shown in Fig. 12 (upper panel), CORT produced a significant facilitation of PTZ lever selection [5mg/kg, $F(1,70)=16.78$, $p<0.001$; 10mg/kg, $F(1,70)=12.60$, $p<0.001$; 20mg/kg, $F(1, 70)=10.01$, $p<0.001$]. In the absence of the training drug, CORT (5, 10, 20 mg/kg) produced 18, 53 and 45% PTZ lever selection, respectively. CORT did not significantly alter the response rates when compared to vehicle [5mg/kg, $F(1,70)=1.74$, $p=0.149$; 10mg/kg, $F(1, 70)=0.534$, $p=0.711$; 20mg/kg, $F(1,70)=1.02$, $p=0.403$] (Fig 12, lower panel).

The Effect of Ketoconazole on PTZ Drug Discrimination

Eight male Long Evans rats were pretreated thirty minutes and twelve hours with ketoconazole (KETZ, 1.09, 4.38 and 8.75 mg/kg, i.p.) or distilled water (vehicle, i.p.) prior to behavioral testing. As shown in Fig.13 and Fig. 14 (upper panel), KETZ attenuated PTZ lever selection. Fig. 13 and Fig. 14 (lower panel) show that KETZ did not significantly alter the response rates of PTZ at thirty minutes [$F(1, 60)=0.166$, $p=0.685$] nor twelve hours [$F(1, 60)=0.043$, $p=0.835$].

The Effect of Aminoglutethimide on PTZ Drug Discrimination

Eight male Long Evans rats were pretreated with aminoglutethimide (AMG, 5, and 20 mg/kg) or peanut oil (vehicle, s.c.) prior to behavioral testing. AMG at thirty minutes and twelve hours attenuated PTZ lever selection (Fig. 14 and Fig. 16, upper panel). There was no

Fig 12. Effect of CORT (n=8) on the discriminative stimulus of PTZ in animals trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the PTZ lever. CORT facilitated PTZ lever selection. In addition, CORT partially substituted for the anxiogenic discriminative stimulus of PTZ. Lower panel shows the response rates. CORT did not produce a significant effect on the response rates ($p>0.05$). X-axis represents the dose of PTZ (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the PTZ lever. In the lower panel, the Y-axis represents the response rates (res/sec).

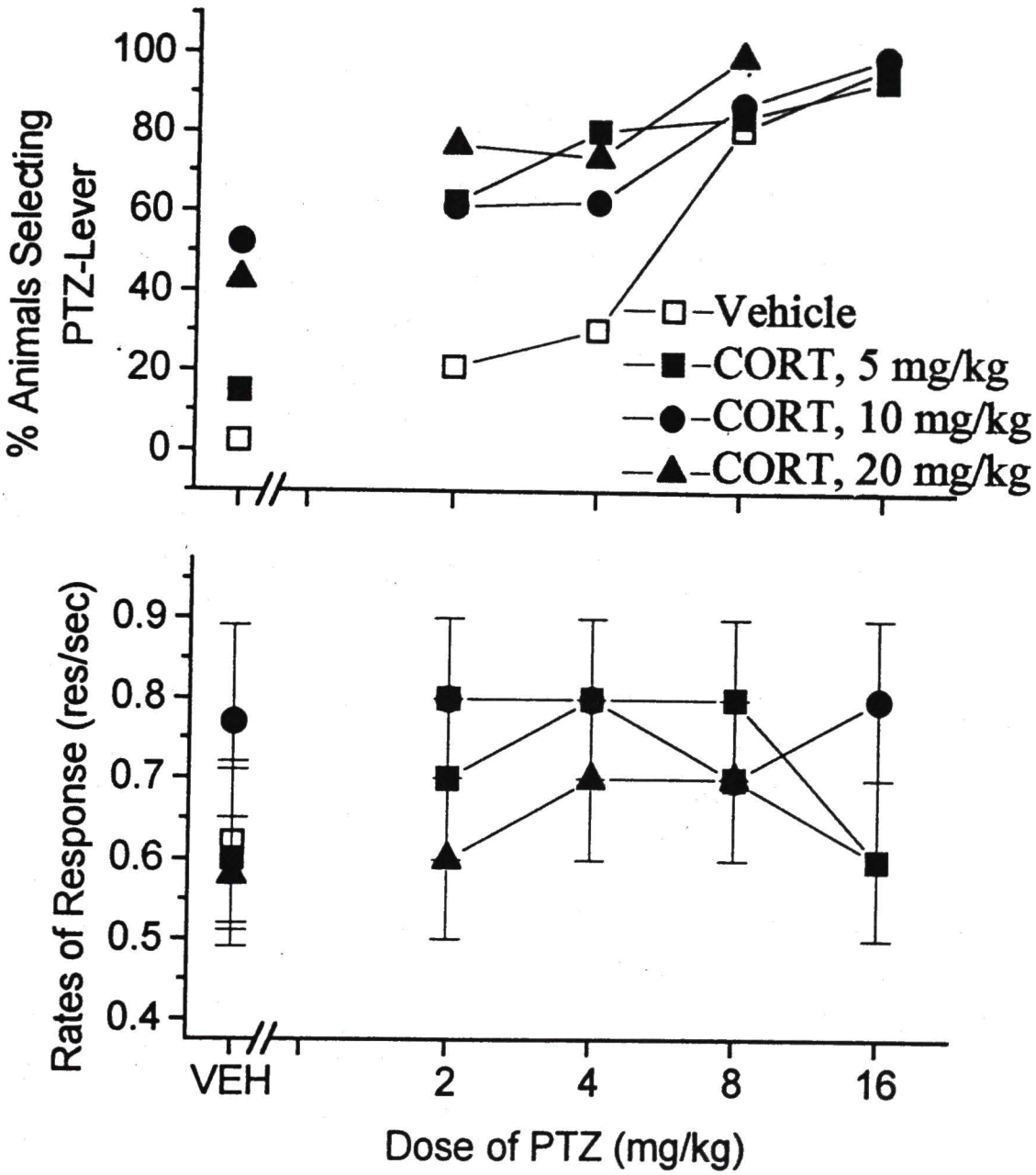

% Animals Selecting PTZ-Lever
100
80
60
40
20
0
Vehicle
CORT, 5 mg/kg
CORT, 10 mg/kg
CORT, 20 mg/kg
Rates of Response (res/sec)
0.9
0.8
0.7
0.6
0.5
0.4
VEH
2
4
8
16
Dose of PTZ (mg/kg)

Fig 13. Effect of KETZ (n=8) on the discriminative stimulus of PTZ in animals trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the PTZ lever. Thirty minute pretreatment with KETZ decreased the percent of animals selecting the PTZ lever. Lower panel shows the response rates. KETZ did not significantly alter the response rates ($p>0.05$). X-axis represents the dose of KETZ (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the PTZ lever. In the lower panel, the Y-axis represents the response rates (res/sec).

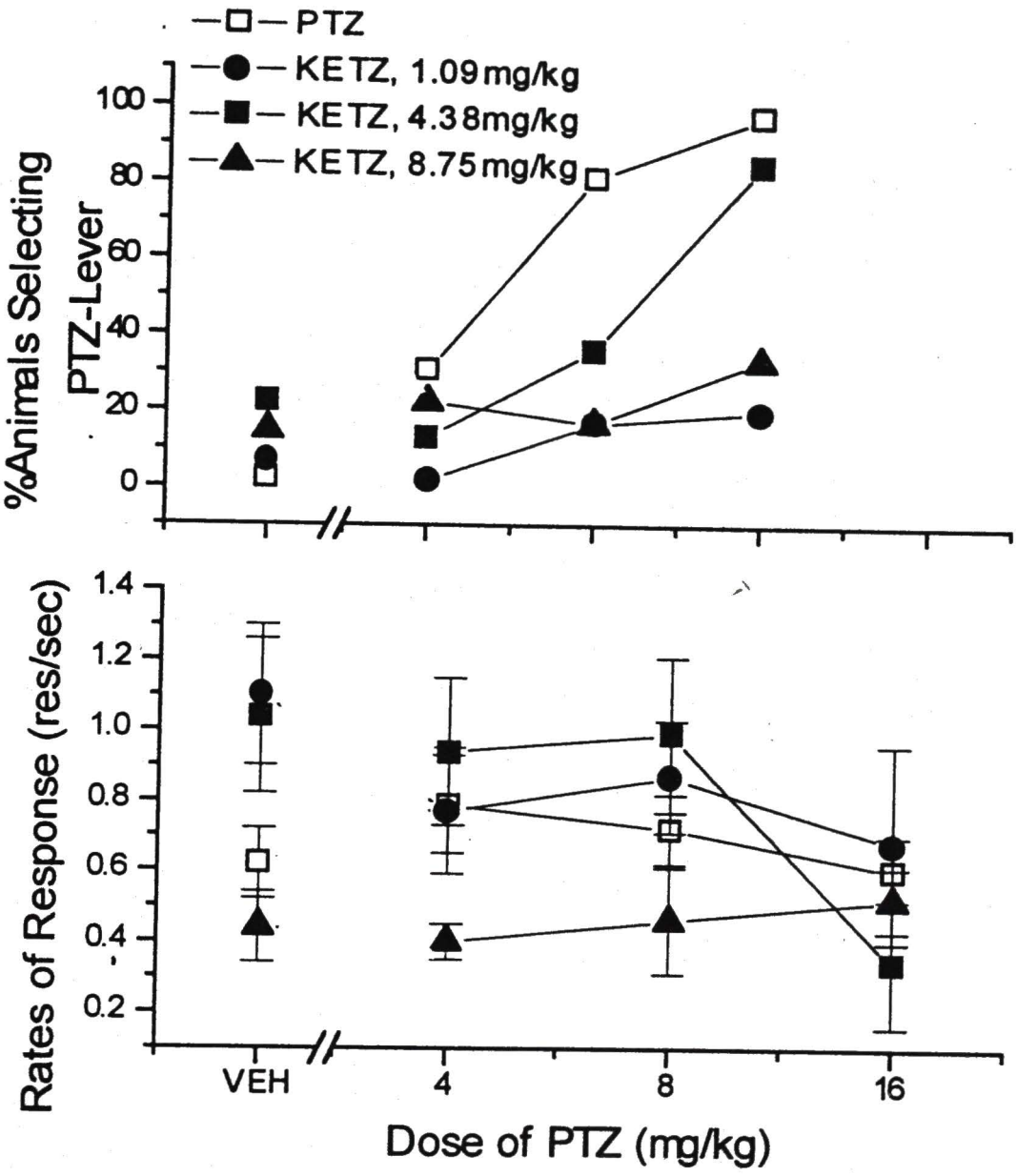

PTZ
KETZ, 1.09mg/kg
KETZ, 4.38mg/kg
KETZ, 8.75mg/kg
%Animals Selecting PTZ-Lever
100
80
60
40
20
0
Rates of Response (res/sec)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
VEH
4
8
16
Dose of PTZ (mg/kg)

Fig 14. Effect of KETZ (n=8) on the discriminative stimulus of PTZ in animals trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the PTZ lever. Twelve hour pretreatment with KETZ decreased the percent of animals selecting the PTZ lever. Lower panel shows the response rates. KETZ did not significantly alter the response rates ($p>0.05$). X-axis represents the dose of KETZ (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the PTZ lever. In the lower panel, the Y-axis represents the response rates (res/sec).

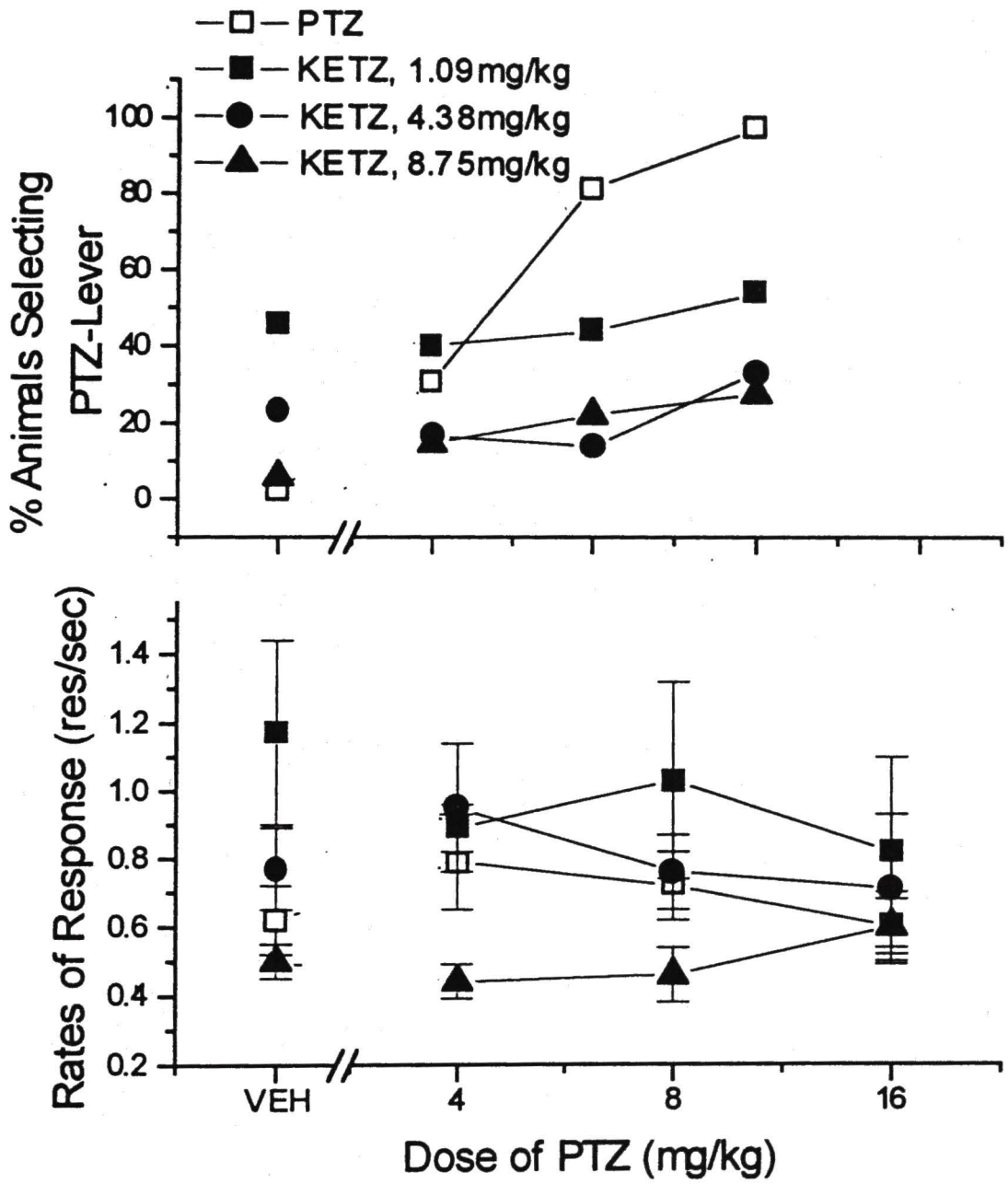

PTZ
KETZ, 1.09mg/kg
KETZ, 4.38mg/kg
KETZ, 8.75mg/kg
% Animals Selecting PTZ-Lever
100
80
60
40
20
0
Rates of Response (res/sec)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
VEH
4
8
16
Dose of PTZ (mg/kg)

Fig 15. Effect of AMG (n=8) on the discriminative stimulus of PTZ in animals trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the PTZ lever. Thirty minute pretreatment with AMG decreased the percent of animals selecting the PTZ lever. Lower panel shows the response rates. AMG did not significantly alter the response rates ($p>0.05$). X-axis represents the dose of AMG (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the PTZ lever selection. In the lower panel, the Y-axis represents the response rates (res/sec).

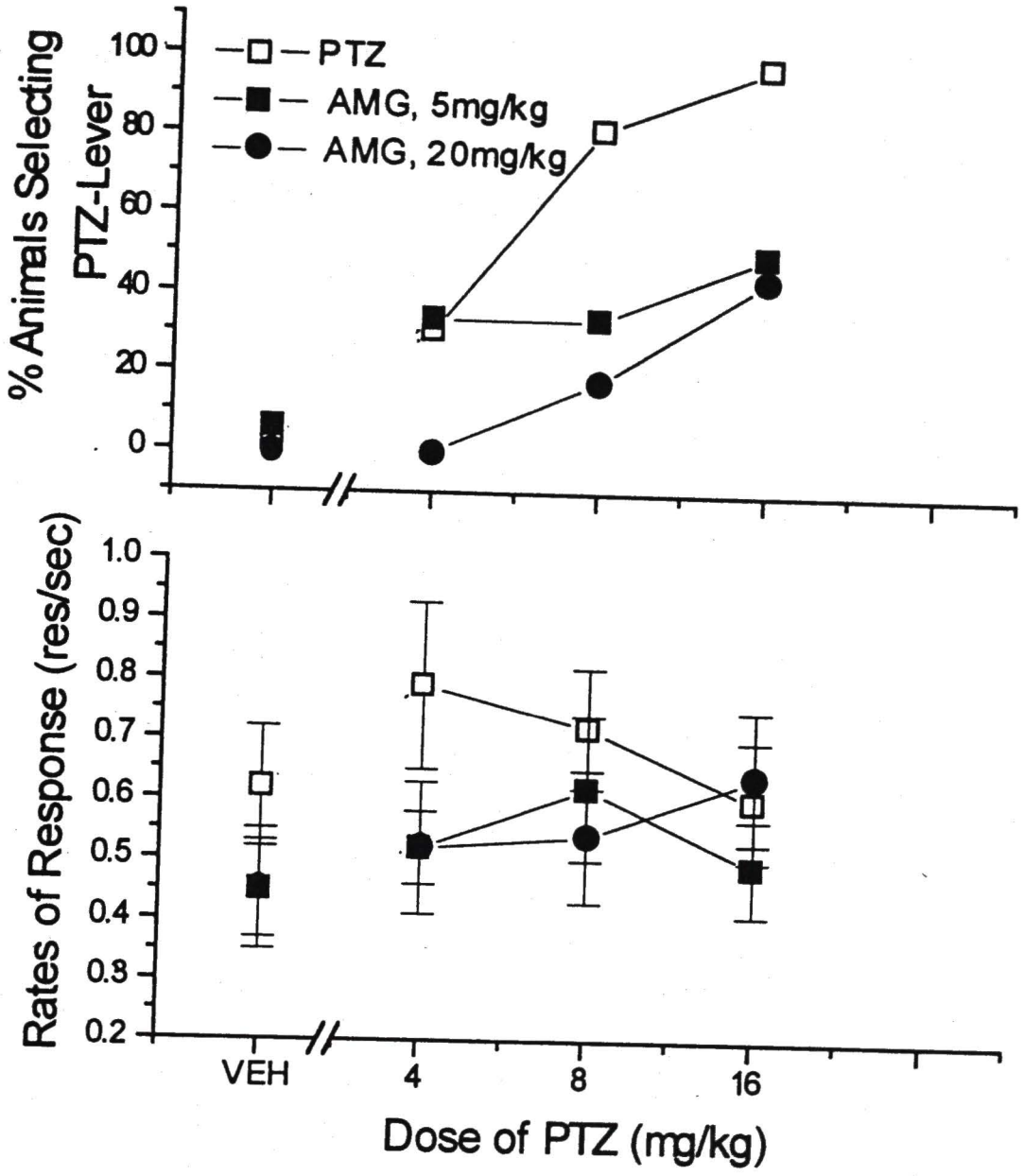
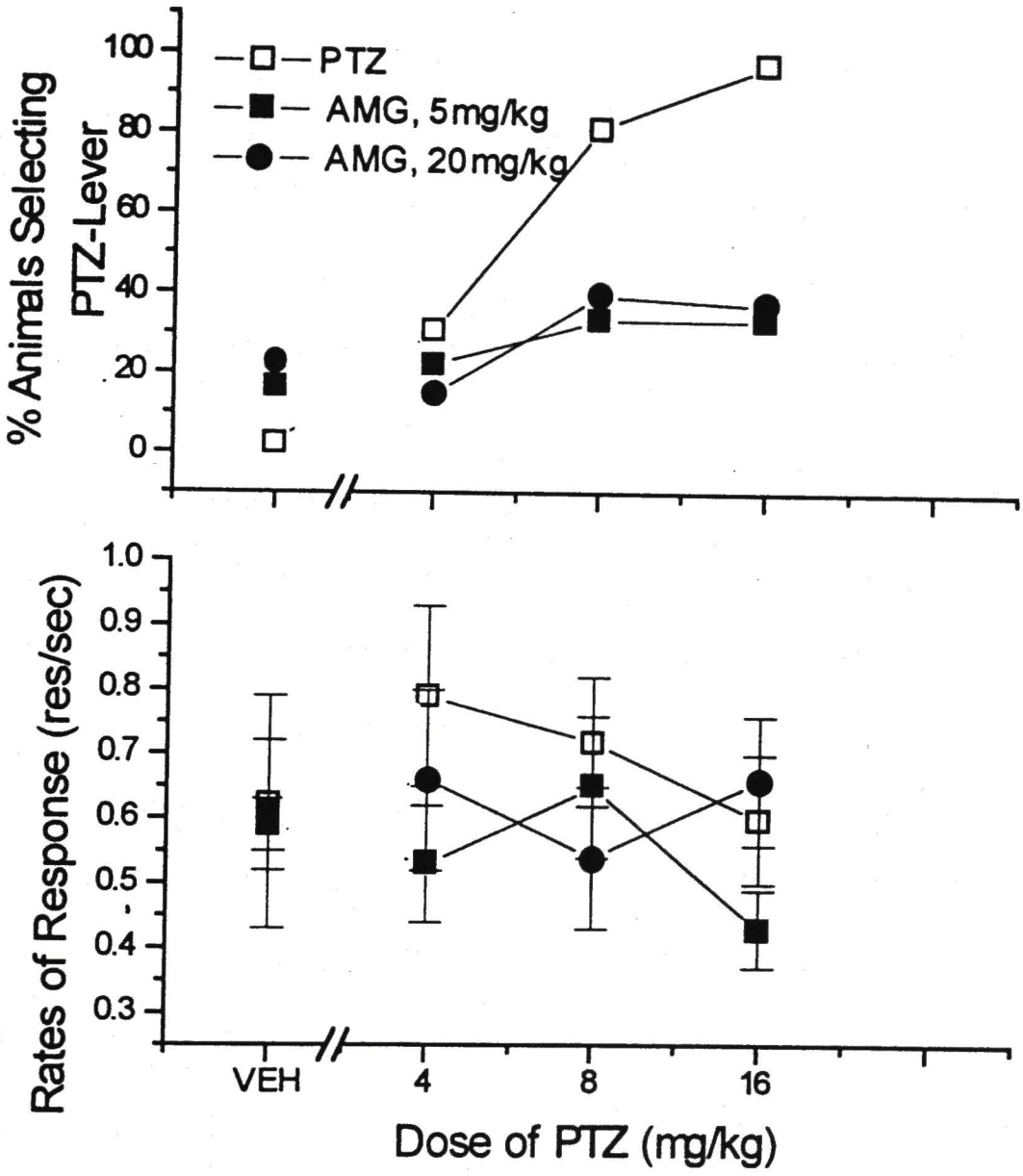

% Animals Selecting PTZ-Lever
100
80
60
40
20
0
PTZ
AMG, 5mg/kg
AMG, 20mg/kg
Rates of Response (res/sec)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
VEH
4
8
16
Dose of PTZ (mg/kg)

Fig 16. Effect of AMG (n=8) on the discriminative stimulus of PTZ in animals trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the PTZ lever. Twelve hour pretreatment with AMG decreased the percent of animals selecting the PTZ lever. Lower panel shows the response rates. AMG did not significantly alter the response rates ($p>0.05$). X-axis represents the dose of AMG (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the PTZ lever selection. In the lower panel, the Y-axis represents the response rates (res/sec).


% Animals Selecting PTZ-Lever
100
80
60
40
20
0
PTZ
AMG, 5mg/kg
AMG, 20mg/kg
Rates of Response (res/sec)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
VEH
4
8
16
Dose of PTZ (mg/kg)

significant difference in the response rates produced at either time interval [30 min, $F(1,70)=1.3, p>0.05$; 12 hr, $F(1,70)=0.18, p>0.05$] (Fig. 15 and Fig. 16, lower panel).

Effect of Corticotropin-Releasing Factor on mCPP Drug Discrimination

Nineteen male Long Evans rats underwent intracerebroventricular surgery and cannula placement. All animals were continuously trained. Only those animals that met training and testing criteria (as described in Methods) were used for i.c.v. administration of drugs. Fourteen animals had correct cannula placement after histological examination. It should be noted that the procedure of i.c.v administration produced mCPP lever selection. Initial injections of distilled water and isotonic saline produced 71% and 33%, respectively, of animals selecting the drug lever (Fig. 17). Animals underwent mock injections for 2 weeks to habituate them to the type of handling for drug administration. Animals (n=10) were intracerebroventricularly administered 0.1 mg/kg, 0.5 or 1.25 μg/μl CRF or isotonic saline (vehicle) thirty minutes prior to behavioral testing. However, three animals in the groups administered 0.1 and 0.5 μg/μl CRF and 2 animals that were administered 1.25 μg/μl CRF did not have correct cannula placement. Also, one animal in the group administered 0.5 μg/μl tore his cannula from his skull when the cannula was caught in his home cage. This animal was euthanized without histological examination.

As shown in Fig. 18 (upper panel), the highest dose of CRF (1.25 mg/ml) produced a. significant effect [$F(1,56)=10.40, p<0.005$] of the mCPP dose effect curve without any rate depressing effects $F(1,56)=2.23, p>0.05$] (Fig 18, lower panel).

Fig 17. Effect of i.c.v. administration of vehicle on the discriminative stimulus of mCPP in animals trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Thirty minute pretreatment with distilled water produced 71% mCPP - lever selection. Isotonic saline pretreatment produced 30% mCPP lever selection. Animals were habituated to the i.c.v. procedure until a normal baseline curve was obtained. The X-axis represents the dose of mCPP. The Y-axis is the percentage of animals selecting the mCPP lever.

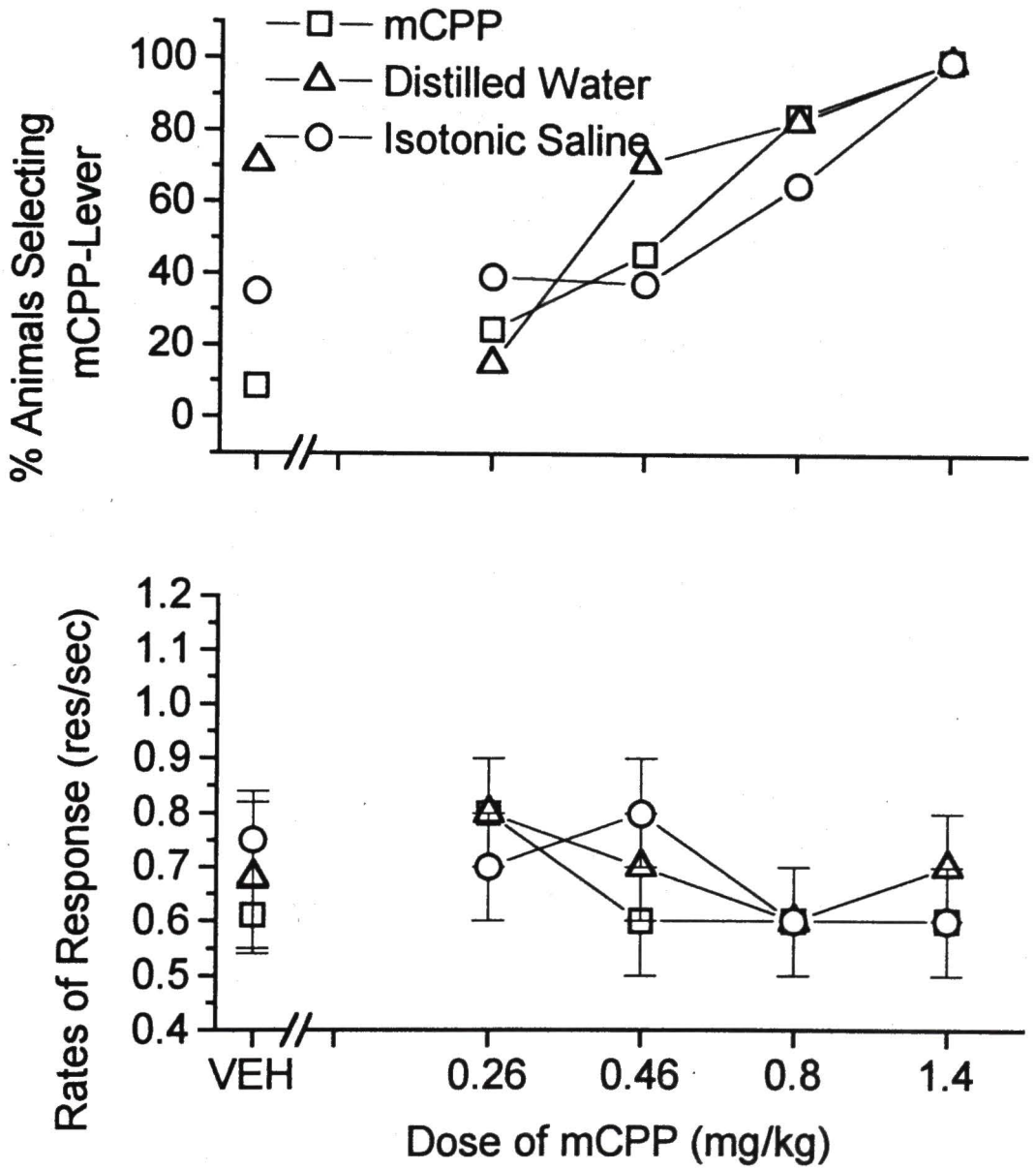
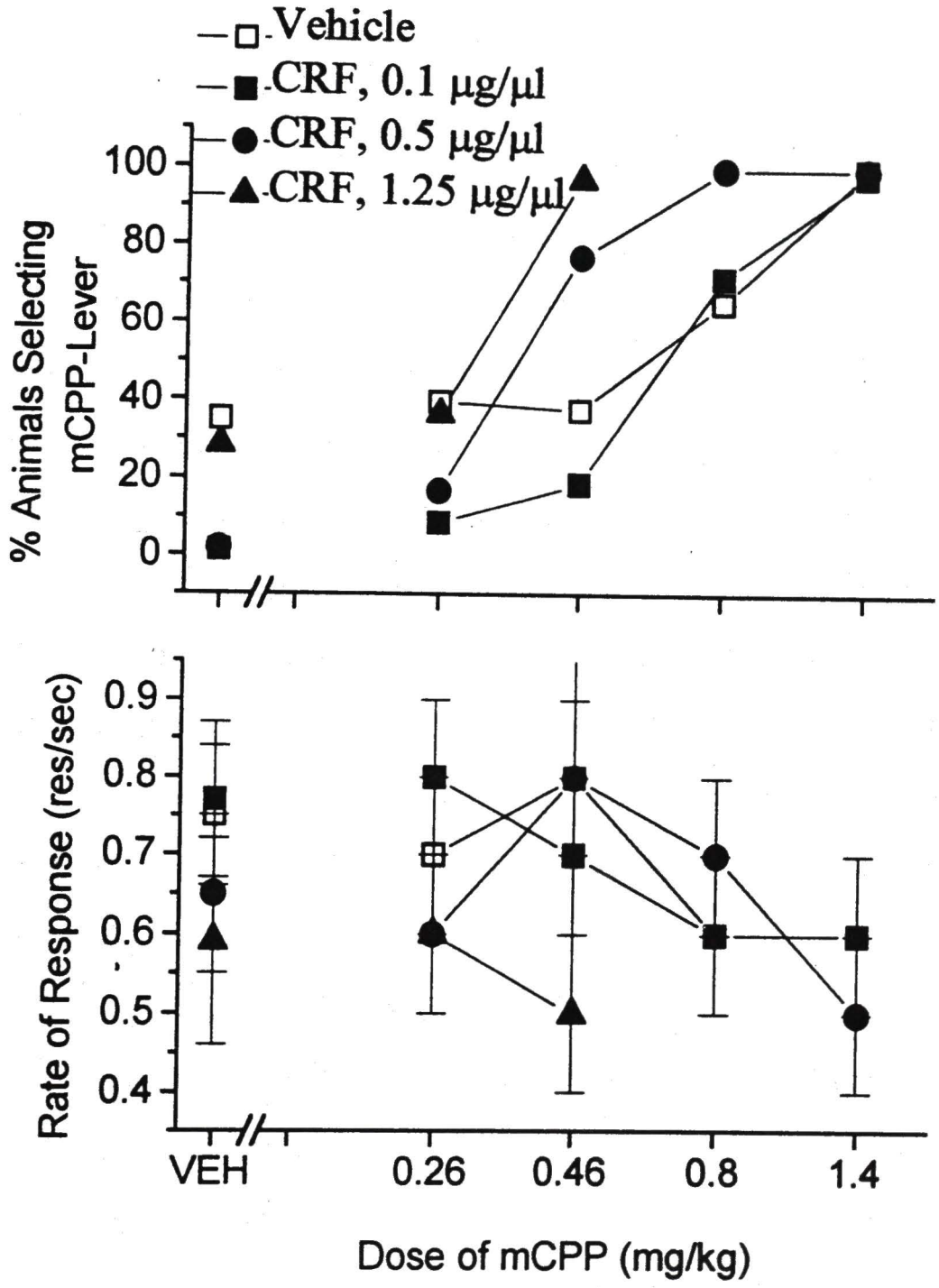

mCPP
Distilled Water
Isotonic Saline
% Animals Selecting mCPP-Lever
100
80
60
40
20
0
Rates of Response (res/sec)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

Fig 18. Effect of CRF on the discriminative stimulus of mCPP in animals trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Thirty minute pretreatment with CRF shifted the mCPP dose effect curve to the left. Lower panel shows the response rates. CRF did not significantly alter the response rates ($p>0.05$). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever. In the lower panel, the Y-axis represents the response rates (res/sec).


Vehicle
CRF, 0.1 µg/µl
CRF, 0.5 µg/µl
CRF, 1.25 µg/µl
% Animals Selecting mCPP-Lever
100
80
60
40
20
0
Rate of Response (res/sec)
0.9
0.8
0.7
0.6
0.5
0.4
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

Effect of α-helical Corticotropin-Releasing Factor on mCPP Drug Discrimination

Ten male Long Evans rats were intracerebroventricularly administered α-helical CRF (1, 5 and 10 μg/μl) or distilled water (vehicle, 2μl) thirty minutes prior to behavioral testing. Alpha-helical CRF did not produce significant attenuation of the anxiogenic discriminative stimulus of mCPP (Fig. 19, upper panel). The highest dose of α-helical CRF (10μg/μl) produced rate-depressing effects[$F(1,63)=2.23$, $p<0.05$, n=8] (Fig. 19, lower panel).

Effect of Adrenalectomy on mCPP Discrimination

Long Evans rats (n=10) were adrenalectomized. Following recovery from surgery, mCPP dose effect testing was performed. As shown in Fig 20 (upper panel), adrenalectomy produced significant facilitation of mCPP lever selection when compared to SHAM-operated animals [$F(1, 72)=15.65$, $p<0.001$]. The response rates were significantly decreased by adrenalectomy when compared to SHAM-operated animals [$F(1, 72)=6.62$, $p<0.01$] (Fig 20, lower panel).

Effect of CORT Replacement on mCPP Discrimination

Nine male adrenalectomized Long Evans rats were treated for four days with CORT (5 mg/kg). On the fourth, day mCPP dose effect testing was performed. As shown in Fig 20 (upper panel), CORT replacement in adrenalectomized animals returned the mCPP dose effect curve to baseline. CORT replacement had no effect on the mCPP dose effect curve of SHAM-operated animals. The response rates were not significantly altered by CORT replacement in ADX animals [$F(1, 80)=0.94$, $p=0.34$] (Fig 20, lower panel).

Fig 19. Effect of α-helical CRF on the discriminative stimulus of mCPP in animals trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Thirty-minute pretreatment with α-helical CRF did not significantly attenuate the percent of animals selecting the mCPP lever. Lower panel shows the response rates. The highest dose of α-helical CRF reduced the response rates (p>0.05). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever selection. In the lower panel, the Y-axis represents the response rates (res/sec).

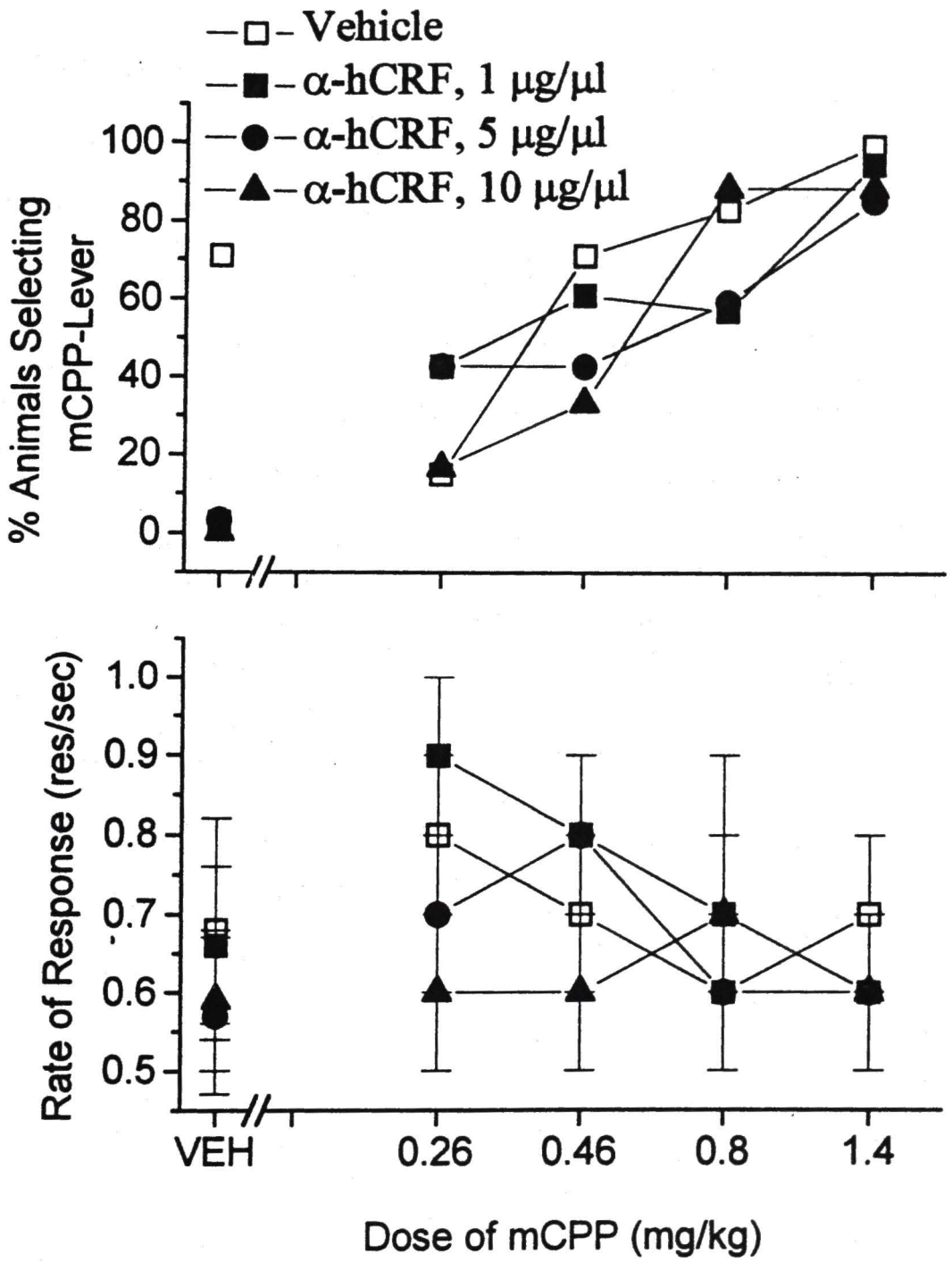
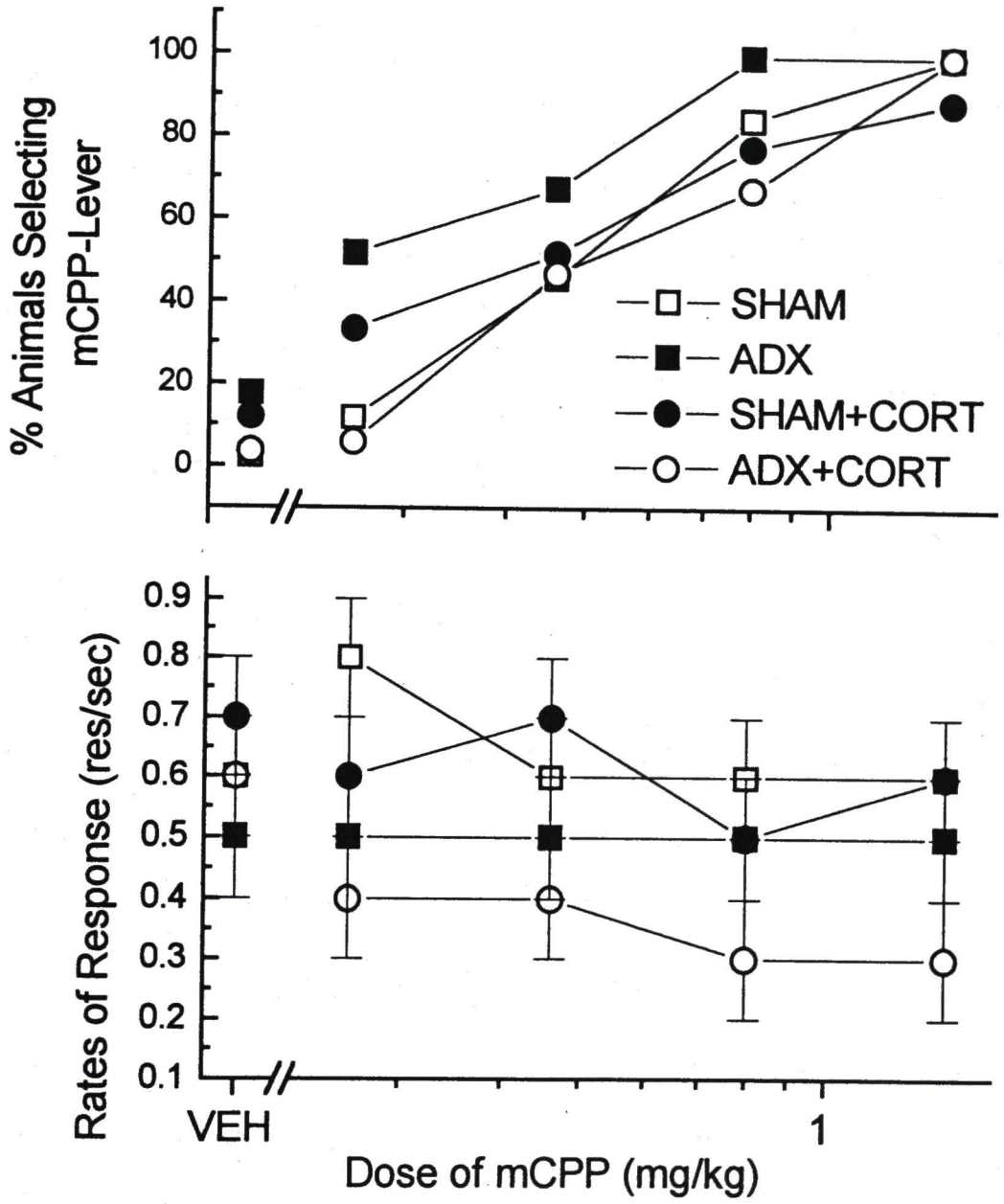

Vehicle
α-hCRF, 1 μg/μl
α-hCRF, 5 μg/μl
α-hCRF, 10 μg/μl
% Animals Selecting mCPP-Lever
100
80
60
40
20
0
Rate of Response (res/sec)
1.0
0.9
0.8
0.7
0.6
0.5
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

Fig 20. Effect of an adrenalectomy on the discriminative stimulus of mCPP in animals trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. Upper panel shows the percentage of animals that selected the mCPP lever. Four days after recovery from surgery, animals (n=10) were tested for mCPP discrimination. Adrenalectomy produced a significant shift of the dose effect curve to the left ($p<0.001$). Four days of CORT replacement returned the mCPP dose effect curve of adrenalectomized (ADX) animals (n=9) to that of SHAM-operated animals (n=10). Lower panel shows the response rates. The response rates were significantly decreased by an adrenalectomy ($p<0.01$) but were not altered by CORT replacement when compared to SHAM-operated animals ($p>0.05$). X-axis represents the dose of mCPP (mg/kg). In the upper panel, the Y-axis is the percentage of animals selecting the mCPP lever selection. In the lower panel, the Y-axis represents the response rates (res/sec).


% Animals Selecting mCPP-Lever
100
80
60
40
20
0
SHAM
ADX
SHAM+CORT
ADX+CORT
Rates of Response (res/sec)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
VEH
1
Dose of mCPP (mg/kg)

Substitution of PTZ for mCPP Discrimination

Male Long Evans rats (n=10) trained to discriminate mCPP from saline were administered cumulative doses of PTZ to determine the amount of PTZ substitution for the mCPP discriminative stimulus. As shown in Fig.21, PTZ partially substituted for the discriminative stimulus of mCPP with 52% maximum mCPP-lever selection. Pretreatment with KETZ (8.75 mg/kg) attenuated the partial substitution of PTZ for mCPP and produced 0% mCPP-lever selection at the highest dose of PTZ (16 mg/kg) [$F(1,90)=12.60$, $p<0.01$]. However, four animals did not respond at this dose.

Two days after KETZ pretreatment PTZ did not substitute for mCPP and produced a maximum of 10% mCPP-lever selection. Pre-KETZ PTZ dose response curve was significantly different from post-KETZ PTZ dose response curve [$F(1,90)=10.76$, $p=0.001$] As shown in Fig.22, the response rates were not significantly altered by PTZ or KETZ and PTZ [$F(1,90)=7.1E-05$, $p>0.05$].

Substitution of mCPP for PTZ Discrimination

Male Long Evans rats (n=10) trained to discriminate PTZ from saline were administered cumulative doses of mCPP to determine the amount of mCPP substitution for the PTZ discriminative stimulus. As shown in Fig.23, the administration of mCPP to PTZ trained animals produced a maximum of 28% of the animals selecting the PTZ lever. Fig 24 demonstrates a dose-dependent decrease in the rate of response after mCPP administration.

Fig 21. Substitution of PTZ in animals (n=10) trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. PTZ partially substituted for the discriminative stimulus of mCPP. Ketoconazole (8.75 mg/kg) attenuated the partial substitution of PTZ for mCPP. The top X-axis represents the dose of mCPP (mg/kg). The bottom X-axis represents the dose of PTZ (mg/kg). The Y-axis is the percentage of animals selecting the mCPP lever.

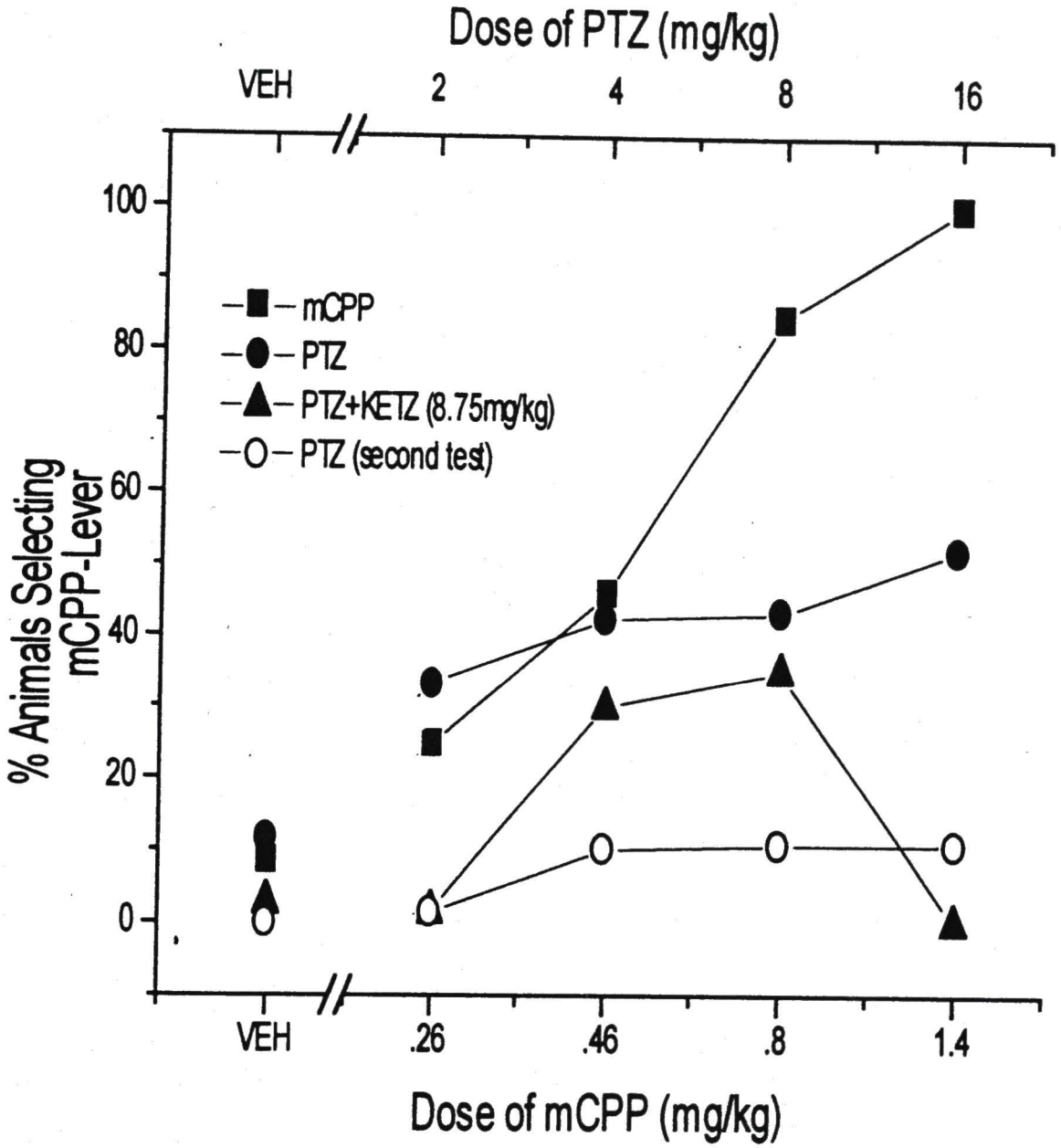

Dose of PTZ (mg/kg)
VEH
2
4
8
16
100
80
60
40
20
0
% Animals Selecting mCPP-Lever
mCPP
PTZ
PTZ+KETZ (8.75mg/kg)
PTZ (second test)
VEH
.26
.46
.8
1.4
Dose of mCPP (mg/kg)

Fig 22. Effect of PTZ and KETZ on the response rates in animals (n=10) trained to discriminate mCPP (1.4 mg/kg) from saline as described in Methods. KETZ did not significantly alter the response rates of mCPP-trained animals administered PTZ ($p>0.05$). The top X-axis represents the dose of mCPP (mg/kg). The bottom X-axis represents the dose of PTZ (mg/kg). The Y-axis is the response rates (res/sec).

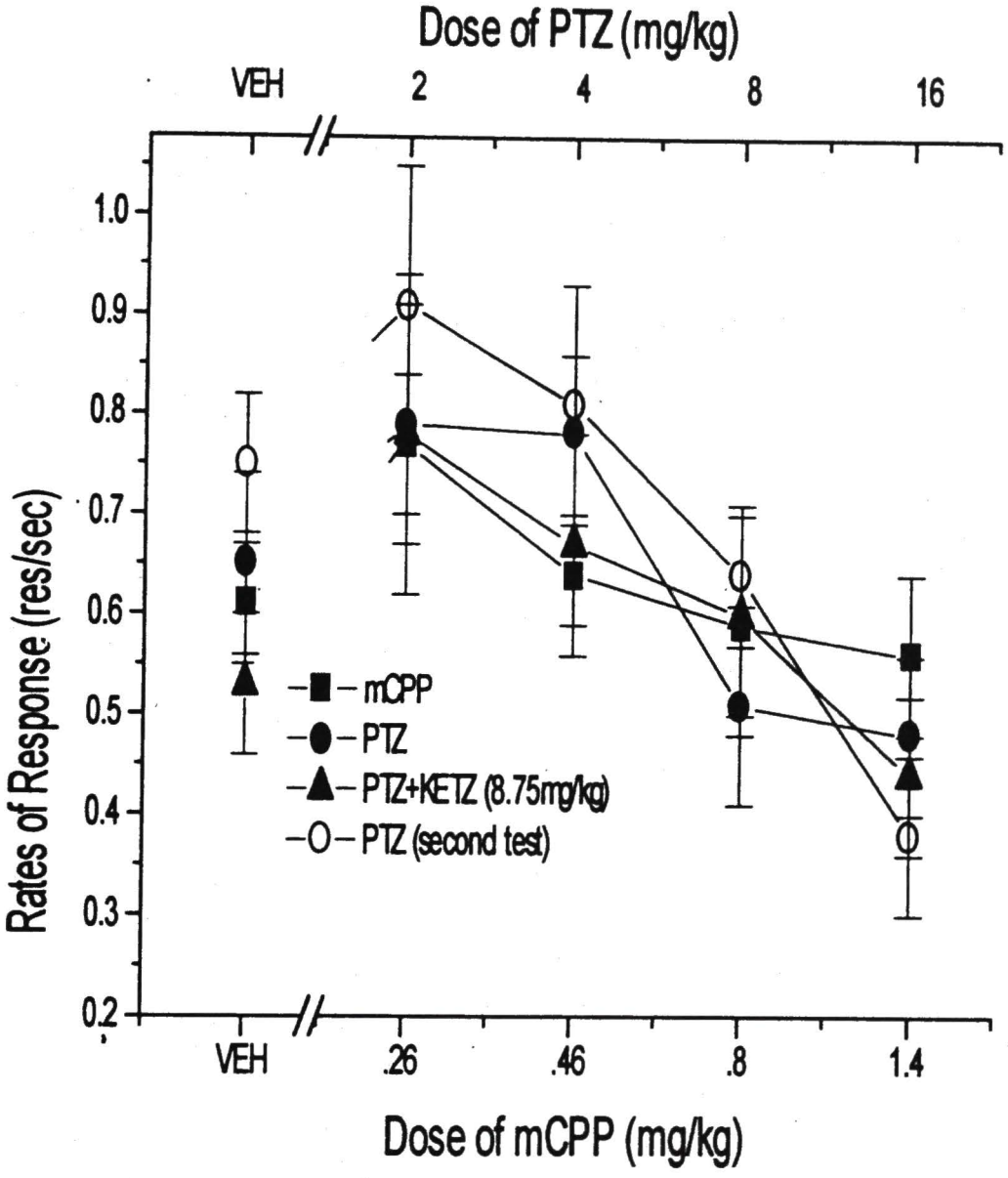

Dose of PTZ (mg/kg)
VEH
2
4
8
16
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Rates of Response (res/sec)
mCPP
PTZ
PTZ+KETZ (8.75mg/kg)
PTZ (second test)
VEH
.26
.46
.8
1.4
Dose of mCPP (mg/kg)

Fig 23. Substitution of mCPP in animals (n=10) trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. mCPP produced 28% of animals selecting the PTZ animals. Full substitution did not occur. The top X-axis represents the dose of mCPP (mg/kg). The bottom X-axis represents the dose of PTZ (mg/kg). The Y-axis is the percent of animals selecting the PTZ lever.

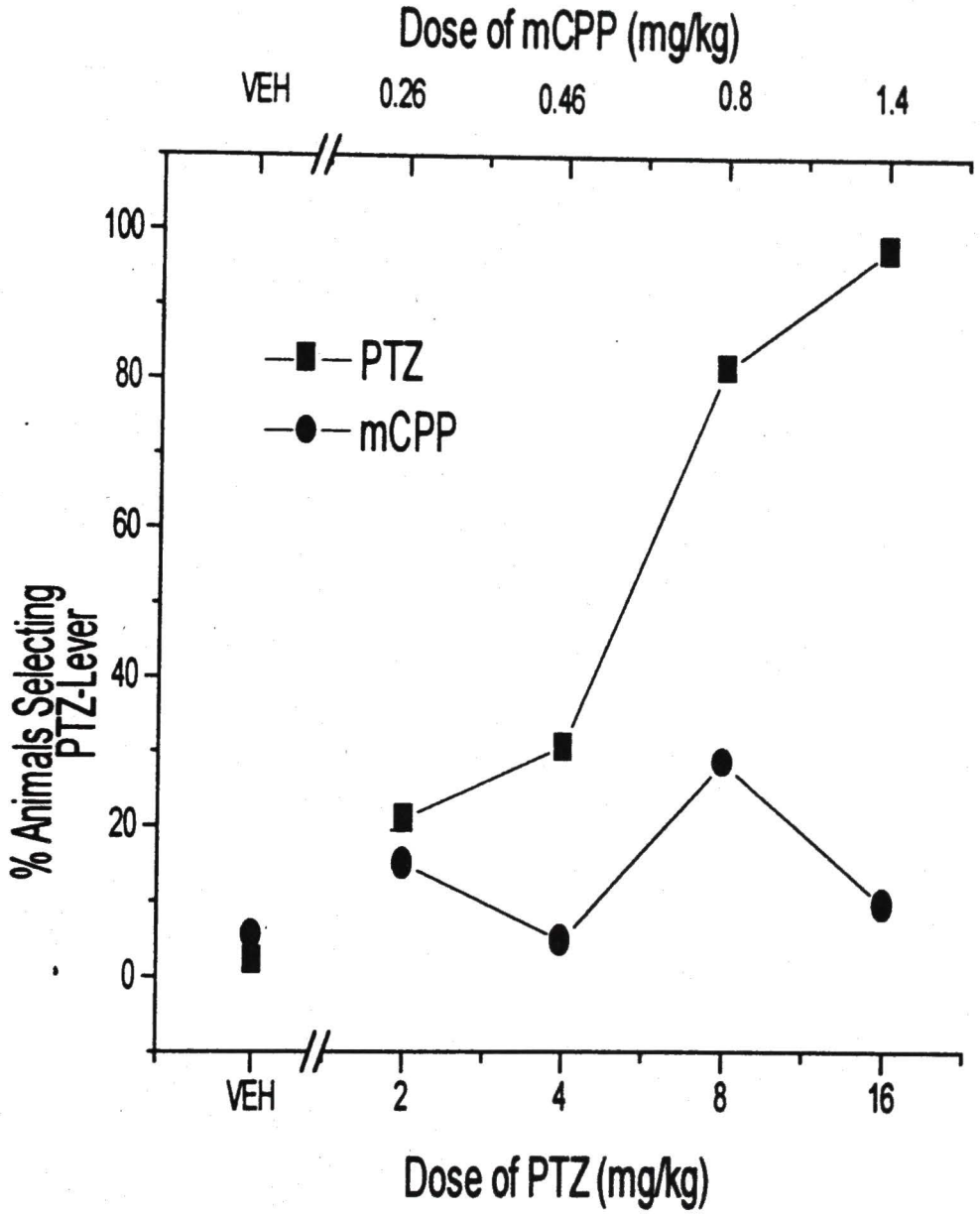
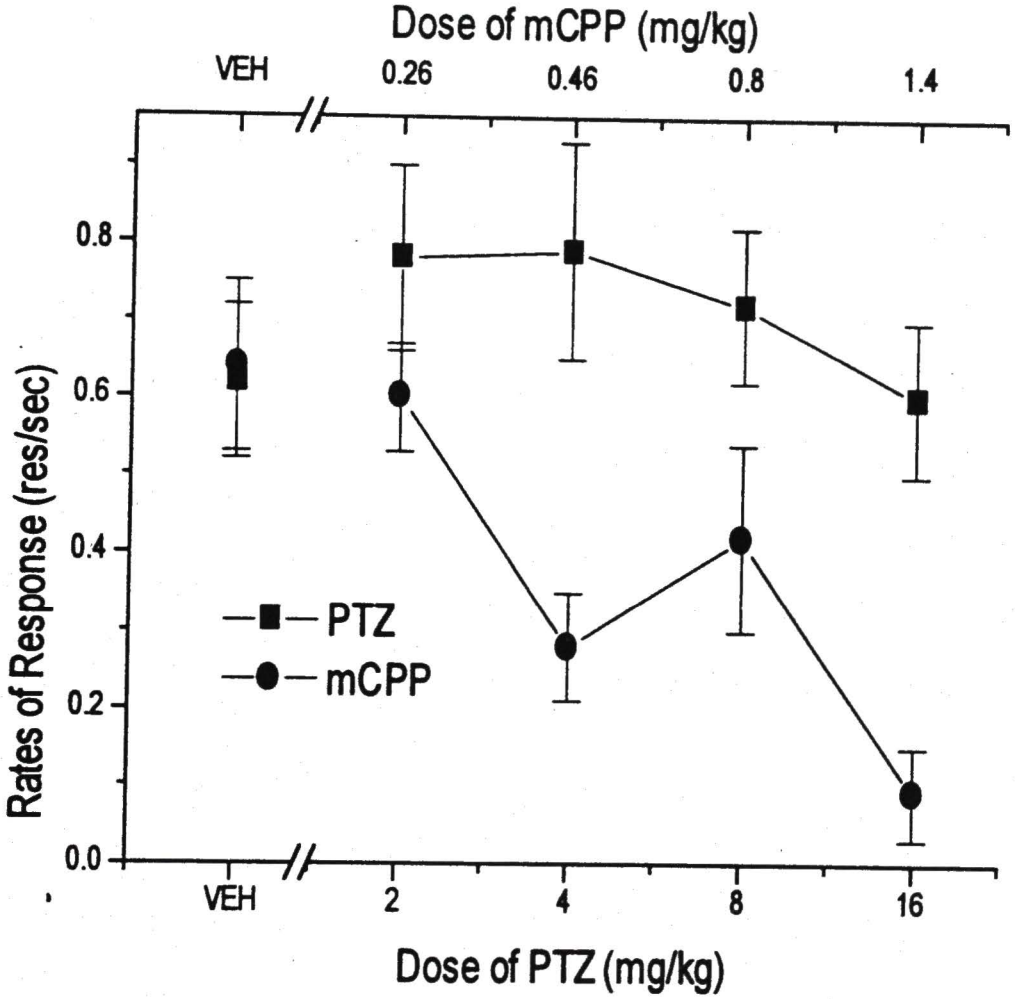

Dose of mCPP (mg/kg)
VEH
0.26
0.46
0.8
1.4
100
80
60
40
20
0
PTZ
mCPP
% Animals Selecting PTZ-Lever
VEH
2
4
8
16
Dose of PTZ (mg/kg)

Fig 24. Effect of mCPP on the response rates in animals (n=10) trained to discriminate PTZ (16 mg/kg) from saline as described in Methods. mCPP produced lower rates than PTZ in PTZ trained animals ($p<0.05$). The top X-axis represents the dose of mCPP (mg/kg). The bottom X-axis represents the dose of PTZ (mg/kg). The Y-axis is the response rates (res/sec).


Dose of mCPP (mg/kg)
VEH
0.26
0.46
0.8
1.4
0.8
0.6
0.4
0.2
0.0
Rates of Response (res/sec)
PTZ
mCPP
VEH
2
4
8
16
Dose of PTZ (mg/kg)

Effect of Adrenalectomy on PTZ Discrimination

Nine male Long Evans rats were adrenalectomized. Following recovery, PTZ dose effect testing was performed. Removal of the adrenal glands produced behavioral disruption in PTZ animals.[1] Only four animals responded to saline administration. These four animals selected the saline lever. Only one animal responded to PTZ administration. This animal selected the saline lever.

Effect of CORT Replacement on PTZ discrimination

After 4 days of CORT replacement, only 2 animals responded to saline. These animals selected the saline lever. Due to the lack of responding after saline administration, PTZ was not administered. This experiment was terminated because of the lethal effect adrenalectomy had on the animals even in the presence of CORT replacement. Four of the animals died of unknown causes.

Effect of KETZ on Ethanol Withdrawal

To determine the effect of KETZ on ethanol withdrawal four groups (seven animals per group) were administered KETZ (vehicle, 1.09, 4.38, or 8.75 mg/kg) 30 minutes prior to being placed on the EPM. The fifth group (DEX-K) was the dextrin control with 8.75 mg/kg KETZ. The sixth group (DEX) was dextrin control with vehicle. KETZ did not effect the percent of open arm entries when compared to controls [$F(1,60)=1.48$, $p>0.05$] (Fig 25). Administration of KETZ produced a dose dependent decrease in the percent of time on the open arms when compared to control [$F(2,20)=1.20$, $p<0.05$] (Fig 26).

[1] Time was increased to 60 minutes in operant chambers.

Fig 25. Effect of KETZ on ethanol withdrawal (n=7/group). Animals were fed 10 days of 6.5% liquid ethanol diet or dextrin diet. On the tenth day ethanol was removed and 12 hrs later the animals were scored for ethanol withdrawal signs. Animals were then placed on the elevated plus maze for five minutes to determine the effects of KETZ (1.09, 4.38 or 8.75 mg/kg) on the percent of entries onto the open arms of the elevated plus maze. KETZ did not effect the percent of entries onto the open arms ($p>0.05$). DEX = dextrin, EW = ethanol withdrawal, DEX-K = dextrin and ketoconazole (8.75 mg/kg). The X-axis represents the treatment. The Y-axis represents the percent of entries onto the open arms.

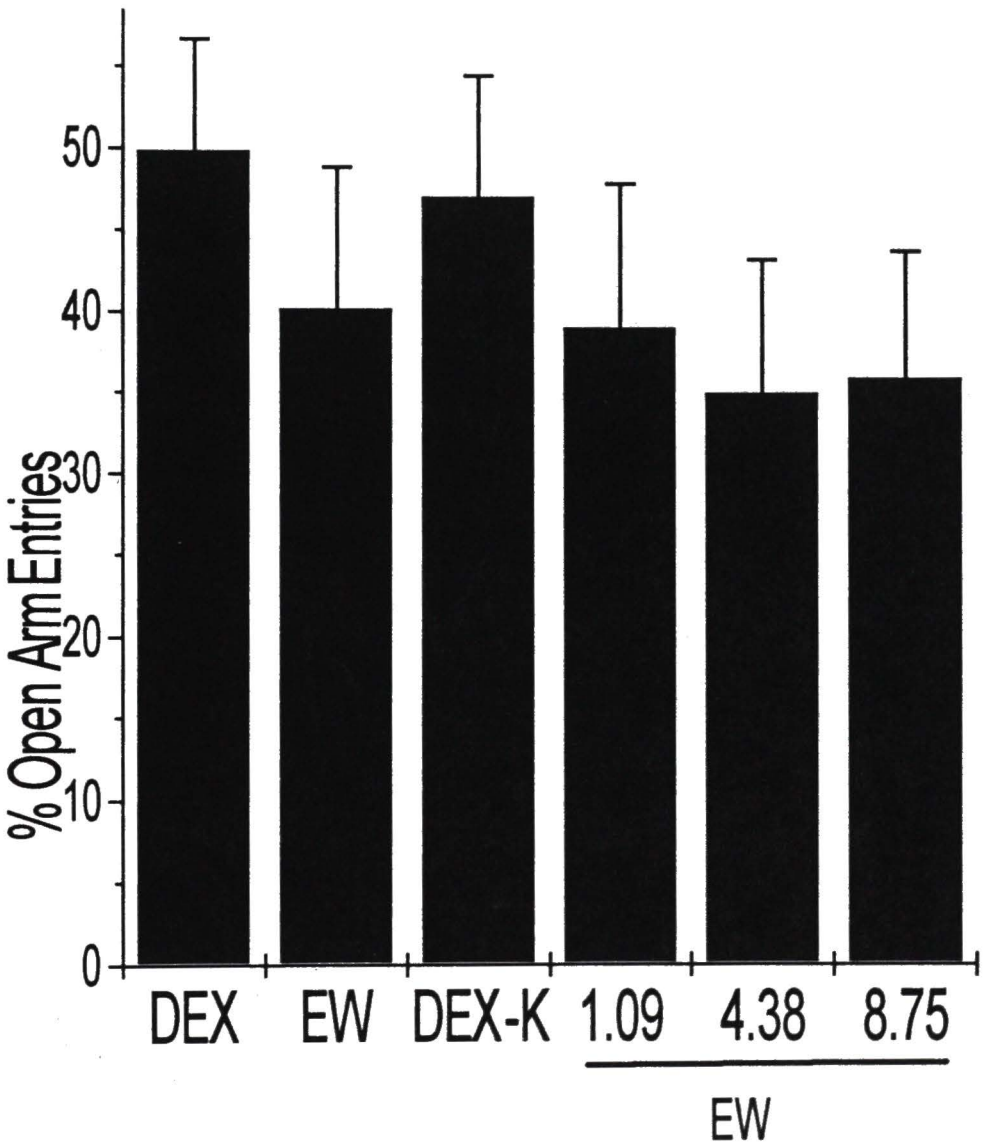
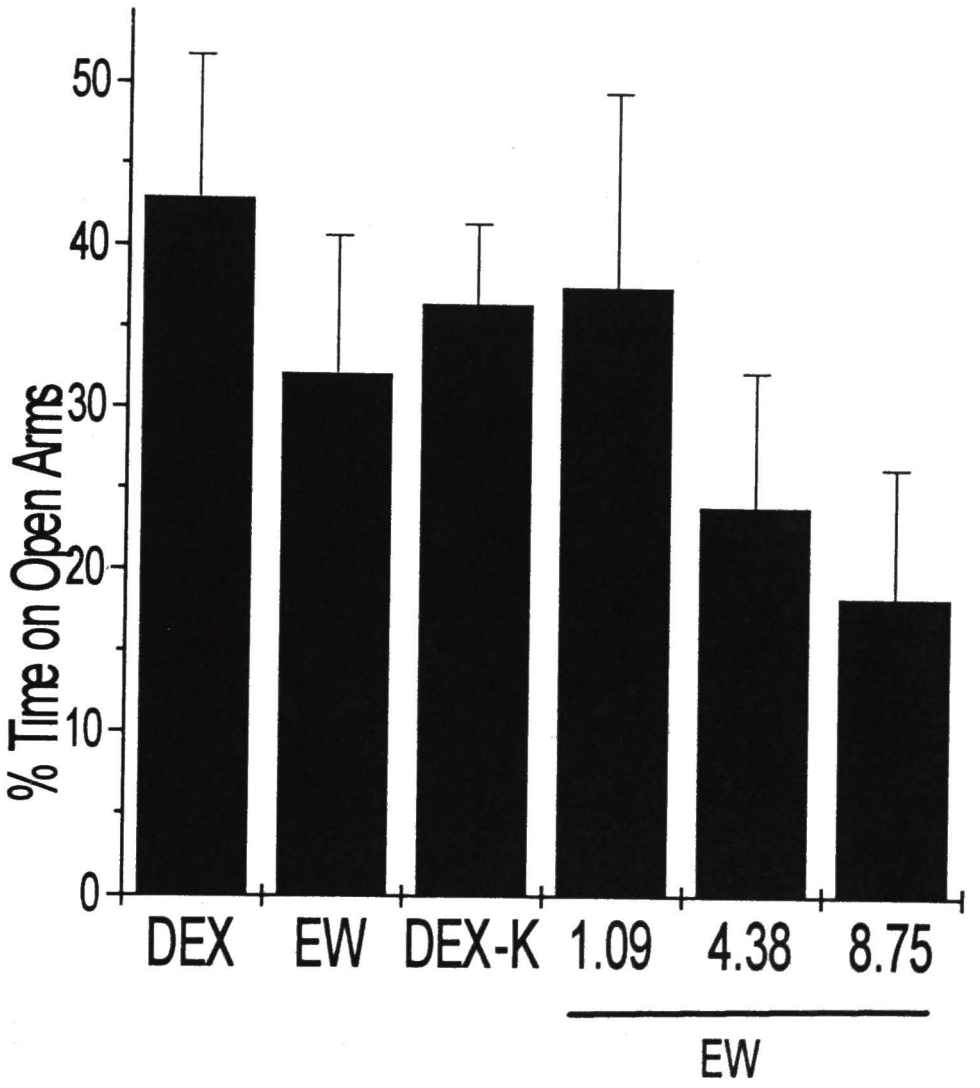

% Open Arm Entries
0
10
20
30
40
50
DEX
EW
DEX-K
1.09
4.38
8.75
EW

Fig 26. Effect of KETZ on ethanol withdrawal (n=7/group). Animals were fed 10 days of 6.5% liquid ethanol diet or dextrin diet. On the tenth day ethanol was removed and 12 hrs later the animals were scored for ethanol withdrawal signs. Animals were then placed on the elevated plus maze for five minutes to determine the effects of KETZ (1.09, 4.38 or 8.75 mg/kg) on the percent of time on the open arms of the elevated plus maze. KETZ did not produce significant decrease in the percent of time spent on open arms ($p<0.05$). DEX = dextrin, EW = ethanol withdrawal, DEX-K = dextrin and ketoconazole (8.75 mg/kg). The X-axis represents the treatment. The Y-axis represents the percent of time spent on the open arms.


% Time on Open Arms
0
10
20
30
40
50
DEX
EW
DEX-K
1.09
4.38
8.75
EW

CHAPTER 3
DISCUSSION

It was hypothesized that the core component of anxiety produced by anxiogenic agents or processes involved the stimulation of the HPA axis. Specifically it was hypothesized that the release of CORT was a component of the discriminative stimulus of mCPP and PTZ. Two possibilities of the effect of CORT on the discriminative stimulus effects of mCPP and PTZ were expected. Firstly, as an anxiolytic, CORT would block the discriminative stimulus of the drug. Secondly, CORT was part of the stimulus and would substitute for the drug. However, in mCPP-trained animals the results differed from both possibilities. If CORT was a component of mCPP discriminative stimulus, inhibition of CORT synthesis should have produced a predominately saline-lever selection whereas exogenous CORT administration would have produced facilitation of mCPP lever selection.

In animals trained to discriminate mCPP from saline, the administration of CORT did not alter the discriminative stimulus of mCPP. On the other hand, the increase of response rates observed after the administration of CORT may indicate an anxiolytic-like effect or an attenuation of the behavioral disruption that is often observed in rats administered mCPP. It is of interest that anxiolytic effects of CORT were not shown in drug lever selection of mCPP. A possible explanation for the lack of effect is that basal levels of CORT may be sufficient to maintain the mCPP discriminative stimulus. As such, exogenous CORT administration would not produce an effect on mCPP discrimination but may prevent behavioral disruption which

enhances the response rates. It is also possible that the effect of CORT administration on mCPP discrimination occurred at a time interval outside of those tested.

Dallman and Yates (1969) proposed the idea of rate sensitive feedback mechanisms such that injection of corticosteroids in rats prevented the CORT response to histamine if the injection preceded histamine administration by 15 seconds or 5 minutes but not if CORT was injected 15 minutes before or 2 minutes after the administration of histamine suggesting a rapid inhibitory effect of CORT that occurs while the plasma concentration of CORT are increasing. Saphier and Feldman (1990) demonstrated a rapid inhibition of PVN neurons after a local injection of glucocorticoids. The rapid feedback inhibition by CORT on stress responses implicates actions at the cellular membrane (Herman *et al.*, 1996). Still, cellular mechanisms for this process are unclear because membrane receptors for CORT have only recently been identified (Orchinik *et al*, 1994).

Dallman and Yates (1969) also proposed a delayed feedback mechanism of CORT such that administration of CORT 120 minutes prior to histamine injection inhibited the CORT response to histamine, however infusions beginning at 45 minutes prior to histamine administration did not inhibit the response, suggesting a feedback effect of CORT that was independent of circulating CORT levels at the time of stress and requires at least 45 min but less than 120 minutes to develop. However, in the present study, neither 30 nor 120 minutes administration of CORT produced an effect on mCPP discrimination. This could possibly be due to the route of administration or the stress response being blocked. Pharmacokinetic principles such as absorption, distribution, metabolism of CORT may be responsible for lack of effect of CORT on mCPP discrimination.

Using a dose range which decreased plasma CORT levels (Abrahamsen and Carr, 1996; Minnick and Wehner, 1992 and Goeders and Guerin, 1997), animals were pretreated with KETZ and AMG in order to test the effects of basal levels of CORT and/or stimulated and secreted CORT on mCPP discrimination. Pretreatment with KETZ thirty minutes prior to behavioral testing produced a dose dependent facilitation of mCPP lever selection. However, this effect was not seen after 12 hours. Response rates were dose-dependently decreased after 30 minute-pretreatment, indicating that KETZ may enhance the disruption of behavior produced by mCPP. Rate-depressing effects had disappeared at all but the highest dose of KETZ (17.5 mg/kg) after 12 hour-pretreatment. On the other hand, AMG at doses of 5 and 10 mg/kg facilitated mCPP lever selection at both 30 minutes and twelve hours.

The highest doses (20 and 40 mg/kg) of AMG had no effect on the mCPP dose effect curve. In addition, although thirty-minute pretreatment with 40 mg/kg of AMG facilitated mCPP lever selection, maximal response of 100% was not achieved. Instead pretreatment with 40 mg/kg AMG produced only 80% mCPP-lever selection at the highest dose of mCPP. While this percentage still met the criteria of full substitution, these animals achieved 100% drug lever selection after twelve hours. Interestingly, unlike KETZ, AMG had no significant effects on the rate of response at either 30 minute or twelve-hour pretreatment schedules.

Activation of serotonergic receptors in the hypothalamus stimulates the HPA axis to release CRF and CORT (Liposits *et al.*, 1987). CRF release is partially controlled by glucocorticoids through a negative feedback mechanism (Keller-Wood and Dallman, 1984). The administration of steroid synthesis inhibitors (KETZ and AMG) prevents the negative control of CRF release by corticosteroids (Worgul *et al.*, 1981 and Ricciardi *et al.*, 1992) and

thus may cause the anxiogenic-like effects shown in these studies. The time-dependent and dose-dependent differences between the effects of the two steroid synthesis inhibitors on mCPP discrimination may be due to the different mechanisms by which KETZ and AMG decrease CORT levels.

The rapid effect seen after 30 minute pretreatment of KETZ and AMG suggested that these steroid synthesis inhibitors may act as antagonists at glucocorticoid receptors. Abrahamsen and Carr (1996) reported a significant decrease of CORT plasma levels in food-restricted animals between 30 minutes and 2 hours after the administration of AMG. Fishman *et al.*, (1967) reported that the initial inhibition of CORT synthesis is overcome by a compensatory increase in ACTH. On the other hand, because AMG also produced effects after twelve hours, this compensatory action of ACTH may be time dependent. Studies have reported that the half-life of AMG is 7-12 hours indicating that the drug is present long after the initial decrease of CORT (Murray *et al.*, 1979 and Worgul *et al.*, 1981). In addition, Plotsky and Sawchenko (1987) reported a decrease of plasma levels 72 hours after the administration of a combination of metyrapone and AMG. This suggested that steroid synthesis inhibition by AMG may also produce facilitation of the discriminative stimulus of mCPP after 12 hours.

It has been reported that KETZ decreases CORT levels between 4 and 8 hours (Pont *et al.*, 1984) or up to twelve hours (Peltier, personal communication) after its administration. However, after 30 minute pretreatment, KETZ produced a significant effect on mCPP discrimination. Therefore, it is possible that a different mechanism of the effects of KETZ other than steroid synthesis inhibition produce facilitation of drug lever selection during mCPP

discrimination. Data from previous studies indicated that KETZ exhibits glucocorticoid antagonist activity (Loose *et al.*, 1983 and Svec, 1988). Loose *et al.* (1983) demonstrated that KETZ was competitive and specific for the glucocorticoid receptor. As stated earlier, it is possible that the fast feedback mechanism of CORT may be due to its interaction with receptors within the cellular membrane (Orchinik *et al.*, 1994) and therefore it is possible that the rapid effect of KETZ is also caused by its interaction with these receptors.

On the other hand, KETZ did not produce an effect after twelve hours suggesting that its role as a steroid synthesis inhibitor is not important in the discriminative stimulus of mCPP. Several steps of steroid synthesis are inhibited by KETZ and AMG (Shaw *et al.*, 1988). However, AMG is a more potent inhibitor of the rate-limiting step of pregnenolone synthesis than KETZ (Mason *et al.*, 1985; Shaw *et al.*, 1988 and Goldman, 1970). Therefore, it is possible that KETZ does not fully prevent the formation of CORT precursors which may possess anxiolytic or CORT-like effects.

Stressful conditions produce an increase in basal levels of CRF and CORT (Herman *et al.*, 1996). KETZ and AMG prevent stimulatory secretion of CORT and/or attenuate basal CORT levels reducing the stress response (Shaw *et al.*, 1988). This effect could result in a reduction of peripheral feedback which may produce facilitation of stressor effects. In addition, CORT maintains tonic inhibition of serotonergic neurons (Chaouloff, 1993). Removal of this inhibition via chemical or physiological adrenalectomy could result in disinhibition and therefore facilitation of the anxiogenic effects of mCPP. This is also likely because administration of CORT alone to intact animals did not enhance nor substitute for the mCPP discriminative stimulus. As stated earlier, it is also possible that basal levels of CORT

are sufficient to maintain mCPP discrimination. Finally, unlike AMG, the half-life of KETZ is 2-3 hours. It is possible that even at the highest dose of KETZ, after 12 hours there is not enough drug present in the plasma to induce inhibition of steroidogenesis. Interestingly, these results were not observed in animals trained to discriminate PTZ from saline.

CORT partially substituted for the discriminative stimulus of PTZ without an effect on the response rates. In the present experiment drug lever selection by the animals did not illustrate an anxiolytic effect of CORT as described by others (File *et al.*, 1979). Instead, CORT not only partially generalized but also facilitated the discriminative stimulus of PTZ suggesting a potential role of CORT as a component of PTZ discrimination.

Animals were pretreated with the steroid synthesis inhibitors KETZ and AMG to determine the effects of basal levels of CORT versus stimulated and secreted CORT on PTZ discrimination. Pretreatment with KETZ thirty minutes and 12 hours prior to PTZ discrimination decreased PTZ-lever selection. The maximum drug lever selection was 35% (30 min) and 54% (12 hours). Response rates were not different at either time period. In addition, AMG decreased drug lever selection at both 30 minutes and twelve hours pretreatment times producing maximum drug lever selection of 50% and 39%, respectively. Like KETZ, the response rates after the administration of AMG were not different from vehicle at either time period.

Activation of GABA-ergic interneurons and/or receptors in the hypothalamus inhibit the HPA axis from releasing corticotropin-releasing factor and CORT (Manev and Pericic, 1983). PTZ prevents this inhibition of GABA and therefore produces an increase of CRF and CORT (Makara and Stark, 1974). The administration of steroid synthesis inhibitors (KETZ

and AMG) prevents the release of corticosteroids and thus may attenuate PTZ discrimination by removing a component of the discriminative stimulus. However, there seemed to be a time difference, although not significant, between the effect of KETZ and AMG such that KETZ was more effective decreasing PTZ lever selection at 30 minutes whereas AMG was more effective after 12 hours. As observed in mCPP trained animals, this difference could be due to the mechanistic actions of KETZ and AMG.

In order to partially control stress mechanisms, GABA inhibits the adrenocorticotropic system (Ixart *et al.*, 1983). CORT inhibits GABA-ergic neurons which would prevent the inhibitory effect of GABA on the HPA axis and allow the release of CORT (Herman *et al.*, 1996). This property of CORT may explain its partial substitution for the discriminative stimulus of PTZ. Similarly, PTZ suppresses the chronic inhibition by GABA on the HPA axis which causes an increase of CORT release (Makara and Stark, 1974). The administration of AMG and KETZ prevent basal and/or stimulatory levels of CORT. Therefore, response to stress is reduced or abolished. If the release of CORT (due to a stress response) is a component of the discriminative stimulus of PTZ, the administration of KETZ and AMG would produce inhibition of PTZ discrimination.

As in mCPP animals KETZ and AMG produced effects after 30 minutes in PTZ-trained animals. Unlike mCPP discrimination, the steroid synthesis inhibitors produced a marked decrease of PTZ lever selection after twelve hours. However, neither steroid synthesis inhibitor produced complete blockade of PTZ discrimination. It is possible that CORT precursors are formed from the partial action of steroidogenesis inhibition of KETZ and therefore produce a partial PTZ effect after 12 hours. However, the actions of KETZ do

not explain the partial substitution of AMG and suggest that CORT is only one component of the PTZ discriminative stimulus and that other factors may be involved.

Intracerebroventricular administration of CRF produced facilitation of mCPP lever selection. However, the administration of a CRF antagonist (α-helical CRF) did not attenuate the anxiogenic stimulus of mCPP. Alpha-helical CRF has been shown to relieve the anxiety-like effects of CRF and ethanol withdrawal (Baldwin *et al.*, 1991 and Britton *et al.*, 1986) as demonstrated in rodents on the elevated plus maze. However, although α-helical produced rate-depressing effects, the anxiolytic properties of α-helical CRF were not demonstrated in the drug lever selection of mCPP. The rate-depressing effect, however, was not surprising in that several anxiolytics such as diazepam have been shown to depress response rates (Egilmez *et al.*, 1997). Therefore, the decrease in response rates observed after α-helical CRF administration could be due to the anxiolytic effect of the drug.

Both mCPP and CRF produce anxiogenic-like behavior in animals as measured by the EPM (Gibson *et al.*, 1994 and Baldwin *et al.*, 1991). Administration of CRF produces a variety of physiological and behavioral responses which are very similar to those produced by mCPP administration such as decreased food intake, suppressed exploration of a novel environment and disruption of sexual behavior (Heinrichs *et al.*; 1995 , 1984 and Curzon and Kennett, 1990). mCPP interacts with serotonergic receptors on the paraventricular nucleus of the hypothalamus to produce an increase in the release of CRF, ACTH and CORT (Liposits *et al.*, 1987). Therefore, it was hypothesized that the release of CRF was a component of the discriminative stimulus of mCPP. It was observed in these studies that although CRF

produced facilitation of the discriminative stimulus of mCPP, in the absence of mCPP, CRF produced a predominately saline lever selection. Therefore, CRF did not substitute for the mCPP discriminative stimulus. In addition, α-helical CRF did not inhibit the discriminative stimulus of mCPP. It is possible that the release of CRF is not involved in the discriminative stimulus of mCPP because the antagonist of CRF did not attenuate the discriminative stimulus. However, since the combined administration of CRF and its agonist were not administered, that statement can not yet be justified. It is also possible that the different results of the anxiolytic activity of α-helical CRF found in this study versus those found in others (Baldwin *et al.*, 1991, Britton *et al.*, 1986, Menzaghi *et al.*, 1994) reflect different measurements of anxiety.

The discriminative stimulus of mCPP (measured by drug discrimination) may differ from the anxiety-like behavior observed on the EPM. Drug discrimination measured the interoceptive stimulus and responding of an animal whereas EPM measured the "natural" behavior of animals based on approach-avoidance conflict manifested by fear of open/bright areas and a natural drive to explore a new environment (Gibson *et al.*, 1994). Therefore, different paradigms may result in different results after the administration of CRF and the CRF antagonist. This was also shown in work by Baldwin *et al.* (1991) such that anxiety-like behavior in rats on the elevated plus maze was abolished by α-helical CRF while anxiety-like behavior demonstrated in operant responding tasks was not (Cole and Koob, 1994).

Adrenalectomy of mCPP-trained facilitated mCPP lever selection. After CORT replacement therapy, the mCPP dose effect curve of ADX animals returned to baseline.

However, adrenalectomy of PTZ-trained animals produced complete disruption of behavior. Four of nine PTZ trained animals selected the saline lever after saline administration whereas the other five animals did not respond. After PTZ administration, only one animal selected the saline lever whereas the other eight animals did not respond. Although the wait period and program time was increased from 15 minutes to 60 minutes, animals did not respond. CORT replacement did not restore responding on either the saline or PTZ lever in PTZ trained animals. The experiment was terminated because 4 animals died of unknown causes. It should be noted that all PTZ trained animals lost weight and were fed mash diet placed directly into the home cages because food intake of rat chow was zero. Therefore, the complete removal of the adrenal glands had a detrimental effect on animals treated long-term with PTZ.

File *et al.* (1979) reported that adrenalectomy produced anxiety-like effects in rodents whereas CORT replacement removed this effect. Likewise, Pacak *et al.* (1993) stated that adrenalectomy enhanced stress response while CORT replacement abolished this effect. Similar results were demonstrated in the present study in mCPP trained animals. Adrenalectomy enhanced the discriminative stimulus of mCPP, however in the absence of mCPP, ADX was not perceived as anxiogenic by the animals as reflected in the drug discrimination paradigm. However, CORT replacement removed the effect of ADX on mCPP discrimination. On the other hand, ADX produced a loss of response in PTZ-trained animals. This observation in PTZ-animals was similar to that observed after administration of the steroid synthesis inhibitor KETZ. Twenty-four hours after the administration of low doses of KETZ (1.09 and 4.38 mg/kg) PTZ-trained animals had a loss of responding for approximately

1 month. After which, the animals responded on a lever but were not able to discriminate between saline and PTZ for approximately three months. This effect was not seen in mCPP animals which required only ten days to return to a normal baseline dose effect curve.

PTZ and other GABA antagonists enhance memory and retention in rodents (McGaugh, 1973; Kumar *et al.*, 1988; and Lal *et al.*, 1988). CORT may be part of the memory enhancing or learning component of PTZ discrimination, therefore it is possible that the removal of CORT by adrenalectomy or the administration of steroid synthesis inhibitors also removes some of the memory enhancement and retention properties after long-term administration of PTZ. It should be noted that both mCPP and PTZ-trained animals were 98 and 95 percent trained prior to KETZ administration and were less than 50% trained after administration of KETZ.

In comparing the proposed model of anxiety (mCPP discrimination) to that of a more established model (PTZ discrimination) the effects of CORT administration were opposite. Because the basis of this study was formed on prior information that mCPP and PTZ possessed similar discriminative properties, it was then logical to compare the discriminative stimulus of mCPP and PTZ. Initially, PTZ partially substituted for the discriminative stimulus of mCPP. In order to determine if CORT was involved in the partial substitution of PTZ for mCPP, mCPP-trained animals were administered 8.75mg/kg of KETZ 15 minutes prior to PTZ (16 mg/kg) administration. KETZ blocked the partial substitution of PTZ for mCPP discriminative stimulus. After stimulus control was established animals were tested again for PTZ substitution for mCPP. PTZ produced a maximum of 10% mCPP-lever selection and therefore did not substitute for mCPP. However, subsequent tests did not replicate the partial

substitution initially found in the same group of animals or a new group. It is possible that external variables such as malfunction of the timer for the light cycle or cage changing induced drug lever selection in the initial testing of PTZ substitution in mCPP animals. Likewise, mCPP only produced 26% of animals selecting the PTZ lever, suggesting that the discriminative stimuli of PTZ and mCPP are perceived differently by these animals.

Contrary to previous reports of similar discriminative stimuli between mCPP and PTZ (Wallis and Lal, 1998), these drugs at most only partially substituted for each other in the present study. However, several differences can be noted between the present and previous studies. Firstly, the breed of animal and supplier were different. This study used viral free Long Evans rats from Harlan Industries whereas the previous study used non-viral free Long Evans rats from Charles Rivers. The previous study used up to a dose of 2.5 mg/kg of mCPP whereas this study used only 1.4 mg/kg. This dose was chosen because it produced the most stable discriminative stimulus without a large disruption in behavior. A dose of 2.5 mg/kg, produced severe disruption in behavior and therefore caused a wait time of one hour and 15 minutes before the animals selected a lever. In addition, during the present experiment, for approximately a year, there was construction in the animal care facility and on several occasions there were problems with the timer for the light control.

Environmental stressors also activate the stress response within an individual (Koob *et al.*, 1993). It is therefore reasonable to suggest that environmental stressors played a role in the discriminative stimulus of mCPP and/or PTZ. Finally, it is possible that the interaction of these drugs at other receptors also plays a role in the differences of their discriminative stimuli. mCPP not only interacts with $5HT_{2C}$ but also at other serotonin receptors including,

$5HT_{1B}$ receptors in addition to adrenergic sites (Gibson *et al.*, 1994). These receptor interactions could play a role in its anxiogenic effects as related to the activation of the HPA axis since mCPP simultaneously activates the HPA and the sympathetic nervous system (Bagdy *et al.*, 1989). Likewise, PTZ is not only a GABA antagonist but also a central nervous system stimulant. Therefore the various receptor interactions or mechanisms of mCPP and PTZ may provide these two drugs with different discriminative stimulus effects.

Ethanol withdrawal produces a discriminative stimulus similar to PTZ (Lal *et al.*, 1988). It was therefore of interest to investigate if KETZ would produce a similar attenuation of anxiety-like behavior in rats during ethanol withdrawal. Roberts *et al.* (1994) reported that the administration of CORT increased the severity of withdrawal in animals. KETZ had no effect on the percent of open arm entries. There was a significantly lower percent of time spent on the open arm when compared controls possibly demonstrating an anxiogenic effect of KETZ.

Roberts *et al.* (1994) reported that CORT decreased open arm activity in mice undergoing ethanol withdrawal therefore it was expected that KETZ would decrease the anxiety of ethanol withdrawal. The results reported may differ from expectations because of the dose of KETZ administered. However, the dose of KETZ (8.75 mg/kg) was chosen because this dose produced effects on the mCPP discriminative stimulus without disrupting behavior. A second possibility is that the effect of KETZ on steroid synthesis inhibition is partial (Irsy and Koranyi. 1990). As stated earlier, several CORT precursors remain after steroid synthesis inhibition by KETZ which may be capable of CORT-like activity. However, the actions of KETZ observed after 30 minutes do not constitute steroid synthesis inhibition

but rather an interaction with glucocorticoid receptors. The exact mechanism of antagonistic actions of KETZ on glucocorticoid receptors is unknown (Svec, 1988), therefore a third possibility is that the mechanistic actions of KETZ are not sufficient to offset the enhancement of withdrawal. This explanation however does not explain why KETZ in dextrin-fed animals produced anxiety. A fourth possibility is that pretreatment time of 30 minutes of KETZ is not effective. Perhaps the steroid synthesis inhibition mechanisms would produce different results after a longer time period. However, longer time intervals were not possible due to the short half-life of KETZ and the measurement of acute ethanol withdrawal which occurs approximately 10-12 hours after the removal of ethanol diet.

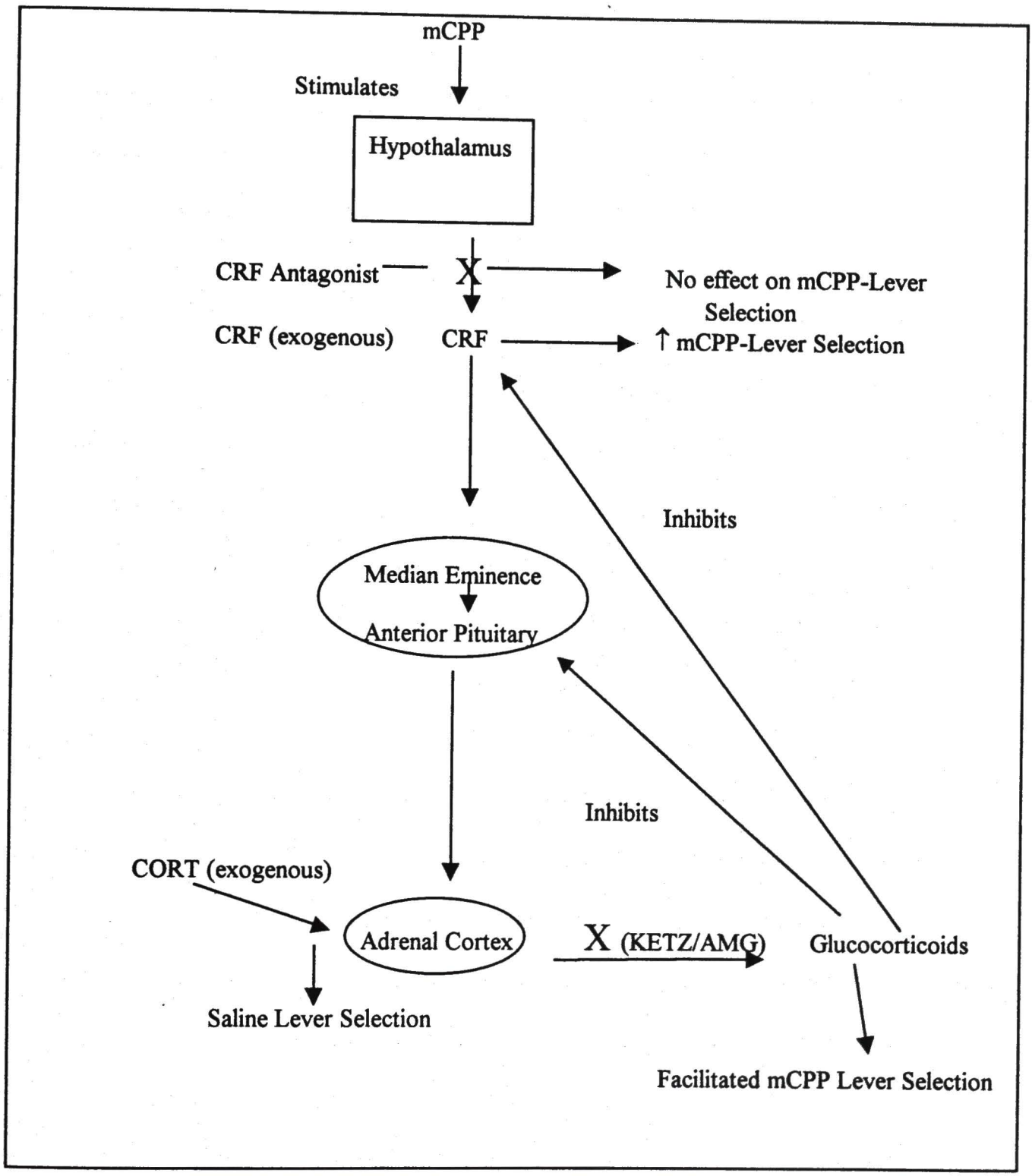
In a comparison of anxiogenic agents or processes it would appear that ethanol withdrawal is pharmacologically similar to both mCPP and PTZ. KETZ increased the discriminative stimulus of mCPP and the anxiogenic symptoms of ethanol withdrawal as demonstrated on the elevated plus maze (that is, facilitated anxiety). However, anxiety induced by ethanol withdrawal did not substitute for mCPP (Appendix A). On the other hand, Sze (1977) reported that during ethanol withdrawal the number of audiogenic seizures increased and were decreased by adrenalectomy and recovered by CORT replacement. In this instance ethanol withdrawal was more similar to PTZ such that PTZ animals were more susceptible to audiogenic seizures and chemical adrenalectomy. Adrenalectomy decreased the perceived anxiety in PTZ trained animals. Likewise, KETZ blocked the discriminative stimulus of PTZ discrimination and produced a predominately saline-lever selection. It has also been demonstrated that anxiety induced by ethanol withdrawal substituted for the discriminative stimulus effect of PTZ (Lal *et al.*, 1988).

In summary, Liposits *et al.* (1987) reported that serotonergic neurons excite the PVN of the hypothalamus to cause the secretion of CRF. Ixart *et al.* (1983) stated that GABA antagonists remove the inhibition of GABA on the secretion of CRF and therefore produce CRF release from the HPA axis. The administration of mCPP and of PTZ produced anxiety-like effects that can be used as a discriminative stimulus which may involve activation of the HPA axis and an increase release of CRF and/or the secretion of CORT. The results obtained from the present experiment demonstrated that pretreatment with CORT did not change the mCPP dose effect curve suggesting that the rise in CORT levels after i.p. injection with CORT did not mimic the salient cues for mCPP discrimination (Fig. 27).

Although actual plasma levels of CORT were not taken during any of the tests performed in this study, the doses of steroid synthesis inhibitors used in these experiments were comparable to doses in the literature that decreased CORT synthesis. Pretreatment with KETZ and AMG produced facilitation of the discriminative stimulus of mCPP. It is speculated that this is due to either the direct antagonism of CORT receptors or steroid synthesis inhibition. Therefore, these data suggested that the release of CORT was not a component of the anxiogenic discriminative stimulus of mCPP.

On the other hand, pretreatment with KETZ and AMG blocked the discriminative stimulus of PTZ, probably due to antagonism of CORT receptors or steroid

Fig 27. Flow diagram of the results of testing the hypothesis that the release of CRF and/or CORT is a component of the discriminative stimulus effects of mCPP. CRF facilitated mCPP lever Selection but did not substitute for mCPP. Alpha-helical CRF did not block mCPP discrimination. KETZ and AMG facilitated mCPP discrimination. CORT had not effect of the discriminative stimulus of mCPP but may play a permissive role in the stress response of mCPP.


mCPP
Stimulates
Hypothalamus
CRF Antagonist
X
No effect on mCPP-Lever Selection
CRF (exogenous)
CRF
↑ mCPP-Lever Selection
Inhibits
Median Eminence
Anterior Pituitary
Inhibits
CORT (exogenous)
Adrenal Cortex
X (KETZ/AMG)
Glucocorticoids
Saline Lever Selection
Facilitated mCPP Lever Selection

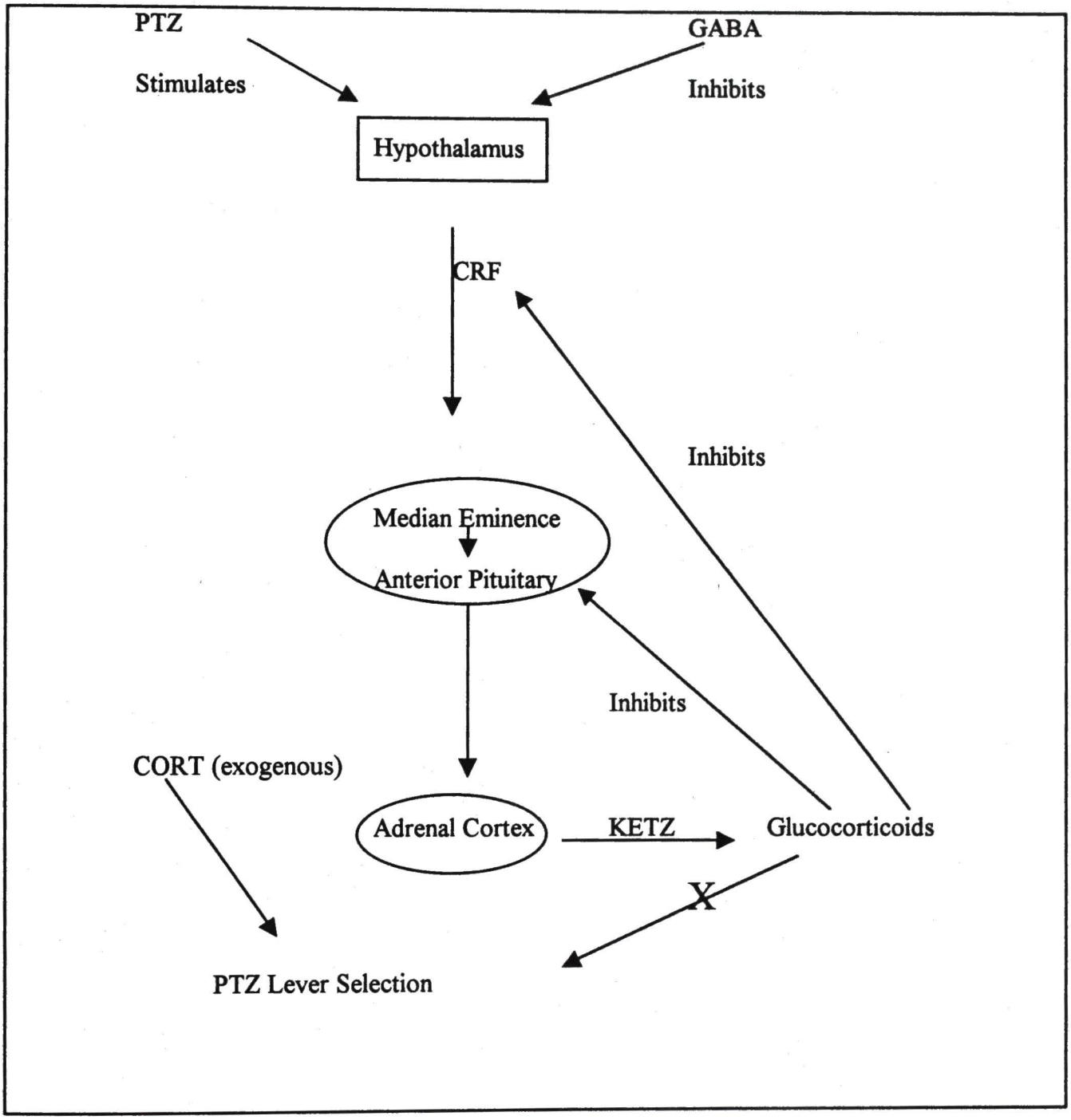
synthesis inhibition (Fig. 28). Pretreatment with CORT produced facilitation to and partially substituted for the discriminative stimulus of PTZ, suggesting that the rise in CORT levels after i.p. injection with CORT mimicked the salient cues for PTZ discrimination. Therefore, these data suggested that the release of CORT was a component of the discriminative stimulus of PTZ.

Steroid synthesis inhibitors enhanced the anxiogenic effects of mCPP while CORT had no effect. Sze (1977) described a permissive role of glucocorticoids in the development of tolerance and dependence to ethanol. CORT seemed to have a similar permissive role in the discriminative stimulus of mCPP such that in the absence of CORT there is a lack of control of stress response to mCPP. That is, normal response to the regulatory signals fails unless adrenal corticosteroids are present. The steroids are needed for a reaction to occur but do not initiate these actions themselves.

The anxiety produced by ethanol withdrawal appeared to be similar to both mCPP and PTZ however, the level at which this similarity of anxiogenic effects occurs was also not shown in this study. CORT inhibition produced facilitation of the discriminative stimulus of mCPP and suggested that perhaps CRF release was involved in the discriminative stimulus of mCPP. CRF also produced facilitation of the discriminative effects of mCPP. However, in the absence of mCPP, neither steroid synthesis inhibitors nor CRF substituted for the mCPP discriminative stimulus. Therefore, these results suggest that neither CRF nor CORT release are components of mCPP discrimination. However, CORT is a potential component of the discriminative stimulus of PTZ.

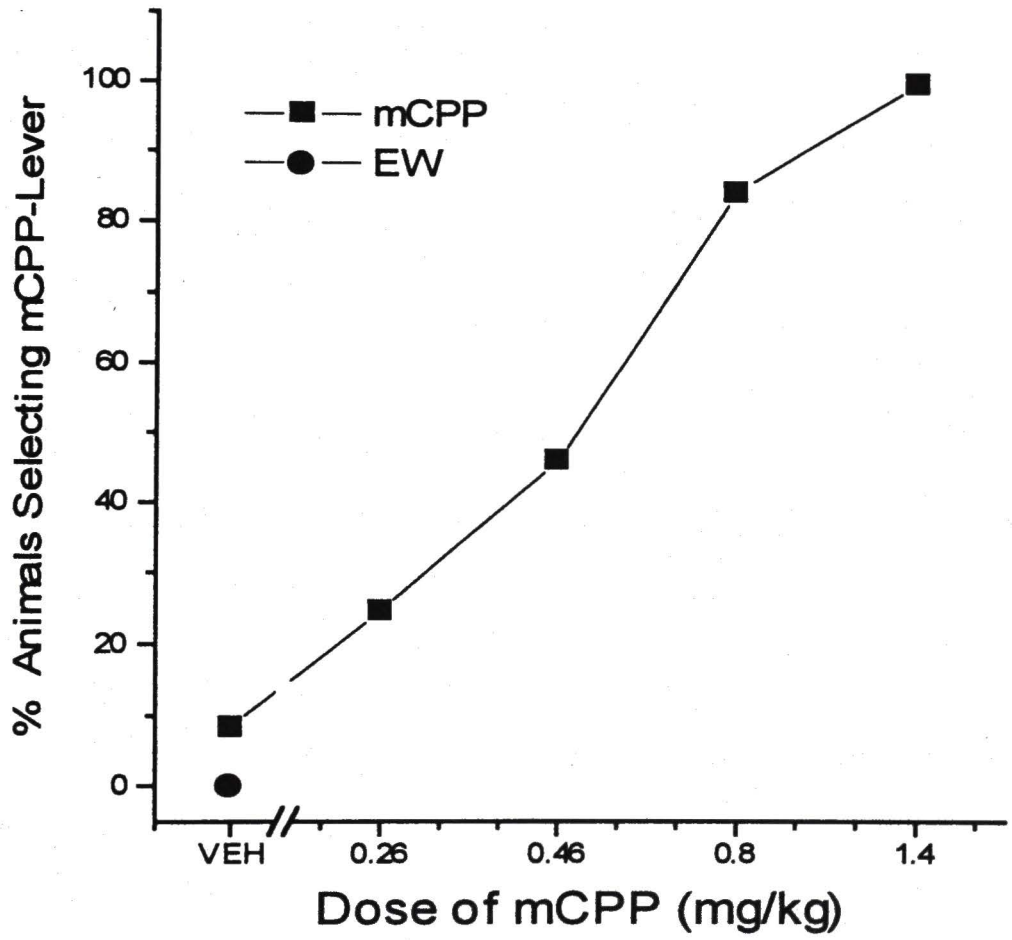
In conclusion, the anxiogenic effects of mCPP and PTZ are regulated through different receptors (serotonin and GABA, respectively). Although a small degree of substitution occurred between mCPP and PTZ the results reported in this study do not support the hypothesis that the similarity of interoceptive cues stems from activation of the hypothalamo-pituitary-adrenal axis and the release of CORT. Is the use of mCPP or PTZ drug discrimination still valid as models of anxiety although differences between the discriminative stimuli have been demonstrated? Both paradigms can be used as models of anxiety because all anxiety disorders are not the same, nor do they have the same effect on different individuals. For example, Korte (1995) demonstrated a clear difference between fear and anxiety and the interaction of glucocorticoid and mineralocorticoid receptors. The lack of full substitution shown in the present study does not remove the validity for either mCPP or PTZ discrimination as a model of anxiety. In fact, the partial substitution shown between mCPP and PTZ support the validity of the discrimination of these two drugs as an model for anxiety such that some component of anxiety is similar but there are still other components that differ and may represent other types of anxiety and stress responses. These results therefore add to the development of new and better treatments of anxiety and other mental disorders.

Fig 28. Flow diagram of the results of testing the hypothesis that the release of CORT is a component of the discriminative stimulus effects of PTZ. KETZ and AMG decreased PTZ lever selection. Neither steroid synthesis inhibitory completely blocked PTZ discrimination. CORT not only facilitated the PTZ lever selection but also partially substituted for PTZ in PTZ trained animals indicating that CORT is only one component of the discriminative stimulus of PTZ.


PTZ
Stimulates
GABA
Inhibits
Hypothalamus
CRF
Inhibits
Median Eminence
Anterior Pituitary
Inhibits
CORT (exogenous)
Adrenal Cortex
KETZ
Glucocorticoids
X
PTZ Lever Selection

APPENDIX

Fig 29. Substitution of anxiety induced by ethanol withdrawal in animals (n=12) trained to discriminate mCPP (1.4 mg/kg) from saline. Twelve hours after removal of ethanol diet, animals did not select mCPP lever. The X-axis represents the dose of mCPP (mg/kg). The Y-axis is the percent of animals selecting the mCPP lever.


% Animals Selecting mCPP-Lever
100
80
60
40
20
0
mCPP
EW
VEH
0.26
0.46
0.8
1.4
Dose of mCPP (mg/kg)

REFERENCES

Abrahamsen GC and Carr KD (1996) Effects of corticosteroid synthesis inhibitors on the sensitization of reward by food restriction. *Brain Res* **726**:39-48.

Andrews JS and Stephens DN (1990) Drug discrimination models in anxiety and depression, (Review). *Pharmacol Ther* **47**:267-280.

Baldwin HA, Rassnick S, Rivier J, Koob GF and Britton KT (1991) CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. *Psychopharm* **103**:227-232.

Badgy G, Calogero AE, Murphy DL and Szemeredi K (1989) Serotonin agonists cause parallel activation of sympathoadrenomedullary system and the hypothalamo-pituitary-adrenocortical axis in conscious rats. *Endocrin* **125**:2664-2669.

Baumann MH, Mash DC and Staley JK (1995) The serotonin agonist m-chlorophenylpiperazine (mCPP) binds to serotonin transporter sites in human brain. *Neuroreport* **6**:2150-2152.

Britton KT, Lee G, Vale W, Rivier J and Koob GF (1986) Corticotropin releasing factor (CRF) receptor antagonist blocks activating and 'anxiogenic' actions of CRF in rats. *Brain Res* **369**:303-306.

Britton KT, Morgan J, Rivier J, Vale W and Koob GF (1985) Chlorodiazepoxide attenuates response suppression induced by corticotropin-releasing factor in the conflict test. *Psychopharm* **86**:170-174.

Callahan PM and Cunningham KA (1994) Involvement of 5-HT_{2C} receptors in mediating the discriminative stimulus properties of m-chlorophenylpiperazine (mCPP). *Eur J Pharmacol* **257**:27-38.

Chappell PB, Smith MA, Kilts CD, Bissette G, Ritchie J, Anderson C and Nemeroff CB (1986) Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat brain regions after acute and chronic stress. *J Neuroscience* **6**:2908-2914.

Charney DS, Woods SW, Goodman WK and Heninger GR (1987) Serotonin function in anxiety II. Effects of the serotonin agonist mCPP in panic disorder patients and healthy subjects. *Psychopharm* **92**:14-24.

Chaouloff F (1993) Physiopharmacological interactions between stress hormones and central serotonergic systems. *Brain Res* **18**:1-32.

Cole BJ, Cador M, Stinus L, Rivier J, Vale W, Koob GF and Moal ML (1990) Central administration of a CRF antagonist blocks the development of stress-induced behavioral sensitization. *Brain Res* **512**:343-346.

Cole BJ and Koob GF (1994) Corticvotropin-releasing factor and schedule-induced polydipsia. *Pharm Biochem Behav* **47**:393-398.

Couch RM, Muller J, Perry YS and Winter JSD (1987) Kinetic analysis of inhibition of human adrenal steroidogenesis by ketoconazole. *J Clin Endocrinol Metab* **65**:551-554.

Curzon G and Kennett GA (1990) m-CPP: a tool for studying behavioral responses associated with 5-HT_{1C} receptors. [Review] *Trends Pharmacol Sci* **11**:181-182.

Dallman MF and Yates FE (1969) Dynamic asymetries in the corticosteroid feedback path and distribution-metabolism-binding elements of the adrenocortical system. *Ann NY Acad Sci* **156**:696-699.

Davidson M, Shanley B and Wilce JP (1995) Increased NMDA-induced excitability during EW: a behavioral and histological study. *Brain Res* **67**:91-96.

Devaud LL, Purdyn DA and Morrow AL (1996) Sensitization of gamma-aminobutyric acidA receptor to neuroactive steroids in rates during ethanol withdrawal. *J Pharmacol Exp Ther* **278**:510-517.

Dexter RN, Fishman LM, Ney RL and Liddle GW (1967) Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: studies of the mechanism of action. *J Clin Endocr* **27**:473-480.

Egilmez-Harrison Y, Jenkins JA, Rocha BA, Lytle DA, Jung ME and Oglesby MW (1998) Discriminative stimulus effects of diazepam, ketamine and their mixture: ethanol substitution patterns. *Beh Pharm* (in press).

Eison, AS. and Eison, MS. (1994) Serotonergic mechanisms in anxiety. *Prog Neuro-Psychopharm Biol Psychiat* **18**:47-62.

el-Kadi AO and Sharif SI (1995) The role of 5-HT in the expression of morphine withdrawal in mice. *Life Sci* **57**:511-6.

Emmett-Oglesby MW and Herz A (1987) Opioid modulation of the discriminative stimulus produced by pentylenetetrazol. *Psychopharm* **92**:313-319.

Emmett-Oglesby MW, Mathis DA, Harris CM, Idemudia SO and Lal H (1988) Withdrawal from diazepam substitutes for the discriminative stimulus properties of pentylenetetrazol. *J Pharmacol Exp Ther* **244**:892-897.

Emmett-Oglesby MW and Mathis DA (1989) Withdrawal from benzodiazepine dependence as a discriminative stimulus. *NIDA Research Monograph* **95**:284.

Emmett-Oglesby MW, Mathis DA, Moon RT and Lal H (1990) Animal models of drug withdrawal symptoms [Review]. *Psychopharm* **101**:292-309.

Emmett-Oglesby MW and Rowan GA (1991) Drug Discrimination used to study drug withdrawal. [Review]. *NIDA Research Monograph.* **116**:337-357, 1991.

File SE, Vellucci SV and Wendlandt S (1979) CORT-an anxiogenic or an anxiolytic agent? *J Pharm Pharmacol* **31**:300-305.

File SE and Lister RG (1984) Do the reductions in social interaction produced by picrotoxin and pentylenetetrazole indicate anxiogenic actions? *Neuropharmy* **23**:793-796.

Fishman LM, Liddle GW, Island DP, Fleischer N and Kuchel O (1967) Effects of amino-glutethimide on adrenal function in man. *J Clin Endocr* **27**: 481-490.

Gargiulo PA and Donoso AO (1996) Distinct grooming patterns induced by intracerebroventricular injection of CRH, TRH and LHRH in male rats. *Brazilian J of Med Bio Res* **29**:375-379.

Gibson EL, Barnfield AM and Curzon G (1994) Evidence that mCPP-induced anxiety in the plus-maze is mediated by postsynaptic 5-HT_{2C} receptors but not by sympathomimetic effects. *Neuropharm* **33**:457-465.

Goeders NE and Guerin JG (1997) Effects of ketoconazole on intravenous cocaine self-administration in rats. ed. L.S. Harris. NIDA Research Monograph, Problems of Drug Dependence (in press).

Goldman A (1970) Production of congenital adrenal hyperplasia in rats and inhibition of cholesterol side chain-cleavage. *Endocrin* **86**:1245-1251.

Hamon M (1994) Neuropharmacology of anxiety: perspectives and prospects. [Review] *Trends Pharmacol Sci* **15**:36-39.

Harris CM, Emmett-Oglesby MW, Robinson NG, Lal H (1986) Withdrawal from chronic nicotine substitutes partially for the interoceptive stimulus produced by pentylenetetrazole (PTZ). *Psychopharm* **90**:85-89.

Heinrichs SC, Menzaghi F, Merlo Pich E, Britton KT and Koob GF (1995) The role of CRF in behavioural aspects to stress [Review]. *Ann NY Acad Sci* **771**:92-104.

Herman JP, Prewitt CMF and Cullinan WE (1996) Neuronal cirucit regualtion of the hypothalamo-pituitary-adrenocortical stress axis. *Crit Rev Neurobio* **10**:371-394.

Hughes SWM and Burley DM (1970) Aminoglutethimide: a side effect turned to therapeutic advantage. *Postgrad Med J* **46**:409-416.

Imaki J, Imaki T, Vale W and Sawchenko PE (1991) Distribution of corticotropin-releasing factor mRNA and immunoreactivity in the central auditory system of the rat. *Brain Res* **547**:28-36.

Irsy G and Koranyi L (1990) Neuroendocrinological effect of ketoconazole in rats. *Acta Endocrin (Copenh)* **122**:409-413.

Iversen SD (1984) Serotonin and anxiety. *Neuropharm* **23**:1553-1560.

Ixart G, Cryssogelou H, Szafarczyk A, Malaval,F and Assenmacher I (1983) Acute and delayed effects of picrotoxin on the adrenocorticotropic system of rats. *Neuroscience Letters* **43**: 235-240.

Kahn RJ, McNair DM and Frankenthaler LM (1987) Tricyclic treatment of generalized anxiety disorder. *J Affective Disorders* **13**:145-151.

Keller-Wood ME and Dallman MF (1984) Corticosteroid inhibition of ACTH secretion. *Endocrin Rev* **5**:1-4.

Kennett GA, Whitton P, Shah K and Curzon G (1989) Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. *Eur J Pharmacol* **164**:445-454.

Koob GF and Weiss F (1992) Neuropharmacology of cocaine and ethanol dependence. [Review] *Rec Dev Alcoh* **10**:201-233.

Koob GF, Heinrichs SC, Pich EM, Menzaghi F, Baldwin H, Miczek K and Britton KT (1993) the role of corticotropin releasing factor in behavioural responses to stress [Review]. *CIBA Foundation Symposium* **172**:277-289.

Korte SM, DeBoer Sf, DeKloet ER and Bohus B (1995) Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus maze. *Psychoneuroendocrin* 20:385-394.

Kowal J (1983) The effect of ketoconazole on steroidogenesis in cultured mouse adrenal cortex tumor cells. *Endocrin* **112**:1541-1543.

Kumar BA, Forster MJ and Lal H (1988) CGS 8216, a benzodiazrpine antagonist, enhances learning and memory in mice. *Brain Res* **460**:195-198.

Lader M (1989) Drug treatment of anxiety: implications for animal models. *Behav Pharm* **1**:95-100.

Lal H and Shearman GT (1980) Interoceptive discriminative stimuli in the development of CNS drugs and a case of an animal model of anxiety. *Ann Rep Med Chem* **15**:51-58.

Lal H and Emmett-Oglesby MW (1983) Behavioral analogs of anxiety: animal models. *Neuropharm* **22**:1423-1441.

Lal H, Harris CM, Benjamin D, Springfield AC, Bhadra S and Emmett-Oglesby MW (1988) Characterization of a pentylenetetrazole-like interoceptive stimulus produced by ethanol withdrawal. *J Pharmacol Exp Ther* **247**:508-518.

Lal H, Kumar B and Forster MJ (1988) Enhancement of learning and memory in mice by a benzodiazepine antagonist. *FASEB* **2**:2707-2711.

Littleton J and Little H (1994) Current concepts of ethanol dependence. [Review] *Addiction* **89**:1397-1412.

Liposits Z. Phelix C.and Paull WK (1987) Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. *Histochemistry* **86**:541-549

Loose DS, Kan PB, Hirst MA, Marcsa RA and Felmans D (1983) Ketoconazole blocks adrenal steroidogenesis by inhibiting P450 dependent enzymes. *J Clin Invest* **71**:1495-1499.

Makara GB and Stark E (1974) Effect of gamma-aminobutyric acid (GABA) and GABA antagonist drugs on ACTH release. *Neuroendocrin* **16**:178-190.

Manev H and Pericic D (1983) Hypothalamic GABA system and plasma CORT in ether stressed rats. *Pharm Biochem Behav* **18**:847-850.

Mason JI, Murry BA, Olcott M and Sheofs JJ (1985) Imidazole antimycotics: inhibitors of steroid aromatase. *Biochem Pharm* **34**:1087-1092.

McGaugh JL (1973) Drug facilitation of learning and memory. *Annu Rev Pharm* **13:**229-241.

Meert TF and Janssen PA (1989) Psychopharmacology of ritanserin: comparison with chlordiazepoxide. *Drug Dev Res* **18**:119-144.

Menzaghi F, Rassnick, S, Heinrichs S, Baldwin H, Pich EM, Weiss F and Koob GF (1994) The role of corticotropin-releasing factor in the anxiogenic effects of ethanol withdrawal. [Review] *Ann NY Acad of Sci* **739**:176-84, 1994.

Minnick SA and Wehner JM (1992) The rate of ethanol absorption is influenced by CORT in long sleep and short sleep mice. *Alcoh Clin Exp Res* **16**:460-465.

Mueller EA, Murphy DL and Sunderland, T (1985) Neuroendocrine effects of m-chlorophenylpiperazine, a serotonin agonist in humans. *J Clin Endocrin Metab* **61**:1179-1184.

Murray FT, Santner S, Samajlik E, and Santen RJ (1979) Serum aminoglutethimide levels: studies of serum half-life, clearance and patient compliance. *J Clin Pharm* **19**:704-708.

Orchinik M, Moore FL and Rose JD (1994) Mechanistic and functional studies of rapid corticosteroied actions. *Ann NY Acad Sci* **746**:101-114.

Pacak K, Kvetnansky R, Palkovits M, Fukuhara k, Yadid G, Kopin IJ and Goldstein S (1993) Adrenalectomy augments in vivo release of norepinephrine in the paraventricular nucleus during immobilization stress. *Endocrin* 1404-1410.

Pandey SC, Piano MR and Pandey GN (1996) Alterations in serotonin 1B ($5HT_{1B}$) receptors subtypes in the brain of ethanol-treated rats. *Drug Alcoh Depe* **41**:219-225.

Parsons L, Koob GF and Weiss F (1995) Serotonin dysfunction in the nucleus accumbens of rats during withdrawal after unlimited access to intravenous cocaine *J Pharmacol Exp Ther* **274**:1182-1191.

Paxinos G and Watson C (1997) The rats brain in stereotaxic coordinates. Academic Press. San Diego.

Pellow S, Chopin P, File DE and Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* **14**:149-167.

Plotsky PM and Sawchenko PE (1987) Hypophysial-portal plasma levels, median eminence content and immunohistochemical staining of corticotropin-releasing factor, arginine vasopressin and oxytocin after pharmacological adrenalectomy. *Endocrin* **120**:1361-1369.

Pont A, Graybill JR, Cravan PC (1984) High dose ketoconazole therapy and adrenal and testicular function in humans. *Arch Int Med* 144:-2150-2153.

Pont A, Williams PL, Loose DS, Feldman D, Reitz RE, Bochra C and Stevens DA (1982) Ketoconazole blocks adrenal steroid synthesis. *Ann Inter Med* **97**:370-372.

Prunnell M, Escorihuela RM, Fernadez-Teruel A, Nunez JF and Tobena (1994) Anxiolytic profiles of alprazolam and ethanol in the elevated plus-maze test and the early acquisition of shuttlebox avoidance. *Pharmacol Res* **29**:37-46.

Pugh SL, Boone MS, and Emmett-Oglesby MW (1992) Tolerance, cross tolerance and withdrawal in rats made dependent on diazepam. *J Pharm Exp Ther* **262**:751-758, 1992.

Rassnick S, Heinrichs SC, Britton KT and Koob GF (1993) Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res* **605**:25-32.

Ricciardi MP, Pellegrini A, Grieco M, Marroni P and Materazzi F (1992) Effects of stress on adrenal steroidogenesis blockade by aminoglutethimide. *In vivo* **6**:103-106.

Roberts AJ, Crabbe JC and Keith LD (1994) CORT increases severity of acute withdrawal from ethanol, pentobarbital and diazepam in mice. *Psychopharm* **115**:278-284.

Rodin EA and Calhoun HD (1970) Metrazol tolerance in a "normal" volunteer population. *J Psychiat Res* **13**:65-76.

Santen RJ, Lipton A and Harvey H (1990) Endocrine treatment of breast cancer in women. *Endocr Rev* **11**:221:265.

Saphier D and Feldman S (1990) Iontophoresis of cortisol inhibits responses of identified paraventricular nucleus neurones to sciatic nerve stimulation. *Brain Res* **535**:159-162.

Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J and Telegdy G (1995) Brain corticotropin-releasing factor mediates 'anxiety-like' behavior induced by cocaine withdrawal in rats. *Brain Res* **675**:89-97.

Schteingart DE and Conn JW (1967) Effects of aminoglutethimide upon adrenal function and cortisol metabolism in Cushing syndrome. *J Clin Metab* **27**:1657-1666.

Shader RI and Greenblatt DJ (1983) Some current treatment options for symptoms of anxiety. *J Clin Psychiat* **44**:21-29.

Shaw MA, Nicholls PJ and Smith HJ (1988) Aminoglutethimde and ketoconazole: historical perspectives and future prospects. *J Steroid Biochem* 31:137-146.

Spencer D and Emmett-Oglesby MW (1985) Parallel processing strategies in the application of microcomputers to the behavioral laboratory. *Behav Res Methods Instrument Comp* **17**:294-300.

Svec F (1988) Differences in the interaction of RU486 and ketoconazole with the second binding site of the glucocorticoid receptor. *Endocrin* **123**:1902-1906.

Sze PY (1977) The permissive role of glucocorticoids in the development of ethanol dependence and tolerance. *Drug Alchol Dep* **2**:381-396.

Traber J, Davies MA, Dompert WV, Glaser T, Schuurman T and Seidel PR (1984) Brain Serotonin receptors as a target for the putative anxiolytic TVX0 7821. *Brain Res. Bull* **12**:741-44.

Thakore JH and Dinan TG (1995) Cortisol synthesis inhibition: a new treatment strategy for the clinical and endocrine manifestations of depression. *Biol Psychiat* **37**:364-368.

Vargas MA, Bissette G, Owen MJ, Ehlers CI and Nemeroff CB (1992) Effects of chronic ethanol and benzodiazepine treatment and withdrawal on corticotropin-releasing factor reward systems. *Ann NY Acad of Sci* **654**:145-52.

Wallis CJ and Lal H (1998) A discriminiative stimulus produced by 1-(3-chlorophenyl)-piperazine (mCPP) as a putative animal model of anxiety. *Prog Neuro-Psychopharm Biol Psychiat* **22**:547-565.

Winter JC and Rabin RA (1993) Discriminative stimulus properties of m-chlorophenylpiperazine. *Pharmacol Biochem Behav* **45**:221-223.

Wolkowitz OM, Reus VI, Manfredi F, Ingbar J, Brizendine L and Weingartner H (1993) Ketoconazol administration in hypercortisolemic depression. *Am J Psychiat* **150**:810-812.

Wood DM, Laraby PR, Lal H A pentylenetetrazol-like stimulus during cocaine withdrawal: blockade by diazepam and not haloperidol. *Drug Dev Res* **16**:269-276.

Worgul TJ, Kendall J and Santen RJ (1981) Recovery of hypothalamic-pituitary-adrenal function after long term suppression by aminoglutethimide and hydrocortisone. *J Clin Endocrin Metab* **53**: 879-882.