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**HYPER-REACTIVITY, PAVLOVIAN CONDITIONING, AND ENDOGENOUS OPIOIDS IN  
A RODENT MODEL OF ALCOHOL SELF - ADMINISTRATION**

**by**

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**B.S., University of Pittsburgh, 1985**

**Submitted to the Graduate Faculty of Arts and Sciences in partial  
fulfillment of the requirements for the degree of  
Doctor of Philosophy at the University of Pittsburgh**

**University of Pittsburgh**

**1995**

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University of Pittsburgh, 1995

**ABSTRACT**

An epigenetic theory is proposed and tested with a rodent model to account for both environmental and genetic risk (EGR) features for addiction. Both EGR factors share a common psychobiology: Both EGR factors increase endogenous opioid activity via defensive-autonomic-stress. Both the “**Harshness**” of risk environments (defined as an increase in the aversive event number, intensity and duration) and the “**Hyper-reactivity**” of at-risk temperaments (defined as an increase in the defensive response number, intensity and duration) increase the at-risk individual’s exposure to endogenous opioids (EOs). Increased exposure to EOs causes sensitization to the effects of drugs or endogenous substances which may shift predisposition for conflict, aggression, risk-taking, sensation-seeking, and addiction. This risk model emphasizes the commonalities of defensive-autonomic-stress or “fear” and the effects of addictive psychoactive drugs. Within this framework a risk model involving the Pavlovian conditioning of endogenous opioid activity (CEOA) is proposed. The first Experiment employed CEOA in a 2 x 2 training design in which rats were exposed to one of two levels of foot shock intensity (.8 or 1.6 mA) and duration (1 or 4 seconds). Results indicate that increased US intensity or duration augment the CEOA as measured by hot plate latencies. Experiment 2 & 3 demonstrated a genetic preparedness to CEOA in alcohol preferring M520s but not in the alcohol non-preferring F344s rats. The hypothesis that the M520 line would be more

emotionally reactive to both aversive stimuli and to opioid drugs (morphine/naltrexone) was confirmed both before and after extensive exposure to aversive stimuli that CEOA. CEOA occurred in the M520s but not in the F344s after one conditioning trial. During the extensive aversive conditioning of Experiment 3 the M520s developed CEOA to the transportation cart which preceded the daily exposure to the conditioning chamber and the CS/US. Again demonstrating that the alcohol preferring M520s were genetically prepared to CEOA. A more thorough behavior genetic analysis is proposed using the five pairs of rats that have been genetically selected on the basis of alcohol choice.

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## **Hyper-Reactivity, Pavlovian Conditioning, and Endogenous Opioids in a Rodent Model of Alcohol Self Administration**

Both hyper-reactive temperaments (Pihl et al.1989; Bradizza et al.1994; Finn et al.1992, Harden & Pihl, 1995) and harsh environmental backgrounds (Bry et al.1992; Newcomb et al.1986; Kumpfer & Demarsh; McFall et al.1992) have been associated with risk for drug abuse and the development of alcoholism. Both harsh environments (van der Kolk et al.1985) and hyper-reactive temperaments (Pihl et al.1988) involve increased activity of the autonomic nervous system in response to stressors. Thus, both risk factors similarly involve augmented autonomic response to aversive stimuli. Moreover, it is aversive stimuli that support the Pavlovian conditioning of endogenous opioid activity (Lysle & Fowler 1988). These facts have led to the proposal of a model of the etiology of alcoholism and drug abuse (see Figure 1) whose core mechanism involves the conditioning of endogenous opioid activity (CEOA).

In the CEOA model, the temperaments and environments of individuals at risk are associated with an increase in the conditioning of endogenous opioid activity resulting in a spectrum of psychobiological effects that may directly increase drug seeking behavio, the rewarding effects of drugs, as well as provide operant reinforcement for behavioral risk features. The basic thesis of the CEOA risk model is that three key parameters of Pavlovian conditioning (trial number, US intensity and US duration) are amplified in the risk features for addiction and, in turn, that these risk features augment the conditioning of endogenous opioid activity, providing a core psychobiological mechanism for addiction.

To provide a background for the proposed experiments, this introduction will first briefly review how Pavlovian-conditioning parameters are augmented in high-risk environments. This will be followed by a brief review of how the same three learning

# Common Psychobiology of Genetic and Environmental Risk Factors for Addiction

## Environmental Factors : (Harshness) Increased Density of Aversive Stimulation

- 1) Increased Trials of Aversive Stimulation
- 2) Increased Intensity of Aversive Stimulation
- 3) Decreased Stress Dampening Features = Increased Duration of Aversive Stimulation
  - e.g.:
    - Decreased Social Affiliations/Attachments/Skills
    - Decreased Control over Aversive Stimulation
    - Decreased Predictability of Environment



## Conditioning of Endogenous Opioid Activity



## Risk Related Effects of Increased Opioid Activity

- 1) Sensitization of:
  - a. Stress Response
  - b. Stressor Reward
  - c. Drug Response
  - d. Drug Reward
  - e. Reward Incentive
- 2) Learned Helplessness Effects
  - a. Cognitive Deficits
  - b. Emotional Deficits
  - c. Behavioral Deficits
- 3) Opponent Processes
  - a. Decreased Basal Opioid Activity
  - b. Increased Basal LC Activity/Reactivity
  - c. Craving/Dysphoria
  - d. Conditioned Craving/Dysphoria

## Genetic Factors: (Hyper-Reactivity) Increased Density of Aversive Responding

- 1) Lower Threshold of Aversive Stimulation = Increased Trials of Aversive Stimulation
- 2) Increased Intensity/Strength of Stress Response
- 3) Increased Duration of Stress Response

**Figure 1.**

parameters (trial number, US intensity and US duration) are similarly influenced by high-risk temperaments in the lives of individuals with family histories of alcoholism or drug abuse. The functional equivalence of aversive stimulation and self administered psychoactive drugs will then be reviewed. In this context, a series of experiments will then be proposed to assess the relationships between heritable preparedness for the conditioning of endogenous opioid activity and the self-administration of alcohol in rodents.

#### A. Environmental Risk Factors

Environmental risk for alcohol or drug abuse has been described as a function of the number of risk factors (Bry et al.1982; Newcomb et al.1986), rather than any one, factor. Environmental risk factors include two categories of features that relate to aversive stimuli and stress responding. The first category involves "the harshness" of the high-risk environment, which is defined as an increase in the number, in the intensity, or the duration of exposure to aversive stimuli. This increase in the harshness of exposure to aversive stimuli can provide an increase in the aversive conditioning of endogenous opioid activity. The literature on risk documents environmental risk factors that include aversive stressors, such as trauma, major life events, family violence, poverty, poor family cohesion, family conflict, poor parenting, social isolation, daily hassles and poor school achievement (McFall et al.1992; Plutchik & Plutchick 1988; Baer et al.1987; Kumpfer & DeMarsh 1985; Hesselbrock & Hesselbrock 1990).

The second category of environmental risk factors implicates an absence of the stress dampening features that benefit and protect individuals in low-risk

environments. Stress dampening has been documented in social support-interactions (Kamark et al.1990), in the controllability of aversive stressors through active coping responses (Breier et al.1987; Weiss et al.1991), and through increasing the predictability of aversive stimuli (Arntz et al.1992; Katz & Wykes 1985). Social support, control, and predictability of aversive stimuli function to reduce the intensity and duration of autonomic responses to aversive stimuli (Kamark et al.1990; Weiss, 1991). High-risk environments are characterized by emotional, physical and educational neglect, social isolation, disorderly and unpredictable home environments and poor school achievement (Plutchik & Plutchick 1988; Baer et al.1985; Kumpfer & DeMarsh 1986; Hesselbrock & Hesselbrock 1990). These risk factors present a consistent pattern: risk is increased by an absence of, or a poverty of, stress dampening features. Effectively, these factors increase the intensity and duration of autonomic reactivity to aversive events, and by so doing, support increased Pavlovian conditioning to aversive stimuli.

## B. Behavioral Risk Factors

The temperament factors associated with risk for alcoholism and drug abuse can be summarized by the word "hyper-reactivity." These temperament factors present a similar profile to that of the environmental risk factors in that they tend to: 1) increase the number of aversive events that are responded to with defensive autonomic arousal 2) increased intensity of the defensive-autonomic response and 3) increase the duration of the defensive-autonomic response to aversive stimuli in the lives of individuals at risk for addiction.

High-risk temperaments mirror the environmental risk influences by augmenting the predisposition to respond with defensive-autonomic arousal. The

hyper-reactivity of high-risk temperaments: 1) increases the number of events responded to with autonomic activation, 2) increases the intensity of autonomic activation, and 3) increases the duration of the defensive autonomic response once initiated. These temperament traits of “**hyper-reactivity**” augment the Pavlovian conditioning of emotional responses and endogenous opioid activity just as the “**harshness**” of high-risk environments increase the trial number, the intensity and the duration of exposure to aversive stimuli. Hence, risk temperament influences can be seen as influences on one's preparedness to respond in a mode of “autonomic arousal” or with a defensive “stress response” to aversive environmental stimuli. Lovallo et al. (1990) have shown that measures of reactivity of the autonomic system (e.g., heart rate) covary with stress response reactivity measures of the neuro-endocrine system (e.g., catecholamines).

Behavioral risk factors identified by longitudinal and family studies include difficult temperament (Blackson & Tarter 1994; Blackson, 1994), impulsivity (Cadoret, 1992), negative affect and arousal, (Robins & McEvoy 1990), attention deficits, impulsivity, aggressive, and antisocial behavior (Martin et al. 1994; Sher & Levenson 1982). In sum, the behavioral traits that are associated with risk for alcoholism and drug abuse may be interpreted as increasing the number of aversive conditioning trials in the life paths of the individuals at risk.

Behavioral risk features include a lower threshold for defensive-stress responding, which can be equated with an increased number of defensive autonomic responses in the life path of the genetically at risk individual. In an elegant experimental design, Pihl et al.(1989) found that men with a multi generational history of alcoholism are hyperreactive to a wide range of stimuli and, are autonomically overreacting, i.e., they respond defensively to the presentation of innocuous tone

stimuli. For these genetically at risk, non-alcoholic men, the trait of hyper-reactivity may effectively lower the threshold for aversive stimulation, increasing the number of aversive conditioning trials in the lives of high-risk individuals. Pihl, et al.(1989) found that non-alcohol abusing sons of alcoholics (SOMAs) with a multi generational family history of alcoholism possessed decreased autonomic discrimination between aversive and non-aversive stimuli that result in an increased number of defensive autonomic responses or "autonomic hyper-reactivity."

A lower threshold for aversive stimulation as a risk-temperament factor is consistent with the longitudinal temperament risk studies of Block et al.(1988). They found that risk for early use of drugs (at the age of 12 to 14 years) was correlated with the temperaments of 3 and 4 year olds who were easily upset . Hence, a lower threshold for aversive stimulation may lead to an increase in the number of aversive conditioning events in the life paths of temperamentally at-risk individuals.

Behavioral risk features include an augmented autonomic response to aversive events. This increased intensity of response to aversive stimulation reflects the documented risk features of emotionality (see Tarter et al.1985; Block et al.1988), emotional lability and distress (Swaim et al.1989), and greater cue reactivity (Bradizza et al.1994). Men at risk for alcoholism due to a multi-generational family history have larger skin-conductance orienting responses (Finn et al, 1990), have hyper reactive cardiovascular response when anticipating shock (Finn & Phil 1987; Finn et al.1990), and show larger increases in vasoconstriction to shock (Finn et al.1990).

Behavioral risk features include the temperament trait of an increased duration of defensive responding to a given aversive stimulus or a lack of soothablity. This trait has been identified as a fundamental dimension of heritable temperament by Rowe and Plomin (1979), who labeled this dimension of temperament "soothability." In a review

of temperaments associated with risk for alcoholism in men, Tarter et al.(1985) identified low "soothability" as a risk factor. Similarly, the longitudinal study of Block et al.(1988) found that children who were at risk for early drug use tended to stay upset or remain disturbed long after aversively stimulated. The findings of these studies are consistent with the behavioral-genetic risk assessment of Finn et al.(1990): Shorter latencies and slower habituation rates (i.e., a prolonged defensive autonomic response) occur to tones in men with multi-generational risk for alcoholism.

### C. Risk factors augment Pavlovian conditioning of aversive CSs

Both the environmental risk-features and genetic risk-trait discussed above (see Figure 1) may augment Pavlovian conditioning to aversive stimulation. Aversive stimulation has been shown to provide the basis for both non-opioid and opioid hypoalgesia in a large variety of situations (Mayer, 1988; Maier, 1988). Traditionally, the accepted terminology holds that an analgesic response is said to be opioid if it is reversible by an opioid blocker such as naloxone or naltrexone and non-opioid if not reversible by opioid blocking drugs. Collateral support for the opioid nature of environmentally induced hypoalgesic response is the development of tolerance and cross tolerance with opioid receptor agonists such as morphine (Christie et al. 1982; Katz & Manik, 1984) as well as opioid withdrawal syndromes.

The essential proposition of the “CEOA Risk Model”, as presented in Figure 1, is that both genetic and environmental factors augment the CEOA by enhancing three learning parameters (**trial number, US intensity and US duration**) relevant to the acquisition of a defensive-autonomic-stress (DAS), or fear, response upon which the endogenous opioid hypoalgesic response apparently depends. A review of the

physiological and etiological consequences of increased exposure to endogenous opioids through stress events is presented in an unpublished manuscript by King (1995). The present discussion will address the relationships between risk factors and the CEOA in order to provide a background for the experimental hypotheses of the dissertation.

#### **D. Aversive events, DAS responses, and Pavlovian conditioning**

Environmentally induced stimulation can provide the basis for the Pavlovian conditioning of opioid analgesia (Lysle & Fowler, 1988; Fanselow & Baackes, 1982; Fanselow, 1981; Watkins et al. 1982; Hayes et al. 1978). In a typical Pavlovian conditioning situation a relatively strong stimulus (US) which reliably elicits a response (UR) is presented immediately after the presentation of a relatively mild stimulus (CS). Initially, the CS is said to be neutral with respect to the UR (i.e., prior to conditioning the CS does not elicit the UR). By virtue of the pairing of the CS with the US during acquisition trials the CS, when presented by itself, will elicit an acquired or conditioned response (CR). Pavlovian conditioning of an endogenous opioid analgesic response (CEOA) has been observed in rats (Lysle & Fowler, 1988; Williams et al. 1990; Fanselow, 1984; Fanselow & Baackes, 1982; Hayes et al. 1978) and in humans (Willer & Ernst, 1986; Pitman et al, 1990). Aversive environmental events such as electric shock, exposure to a predator, or the trauma of combat situations are considered from a Pavlovian perspective to be US events that provide for the development of the CR, i.e., activation of endogenous opioid systems.

Concurrently, such aversive events also provide a basis for the development and maintenance of fear responses that CEOA responses are apparently contingent upon. Fanselow & Helmstetter (1988) found that benzodiazepam drugs attenuated both

freezing and the CEOA response, suggesting that CEOA was reduced by dampening the defensive fear response (see also Willer & Ernst, 1986). Similarly, Lysle and Fowler (1988) found that a conditioned fear inhibitor when presented in conjunction with an aversive US had the effect of diminishing stress induced analgesia. A third manipulation that decreases fear is the Pavlovian extinction procedure i.e., the non-reinforced presentation of a fear-conditioned CS. This procedure also attenuates or prevents the CR of opioid analgesia (Williams et al. 1990).

On the other hand, manipulations associated with increased fear, such as augmentation of background stressors or the intensity of an aversive US, are associated with facilitation of opioid analgesia (Levine et al. 1984; Young & Fanselow, 1992). Pharmacological manipulations that increase fear also involve opioid system effects. Injections of mu-opioid receptor blockers, which block the anxiolytic and analgesic actions of opioids, augment the conditioning of fear (Fanselow et al. 1992). Similarly, beta-carboline, a fear and anxiety inducing drug, produces a naloxone reversible analgesia (Helmstetter et al. 1990). Hence, environmentally and pharmacologically induced opioid analgesic responses would appear to be modulated by procedures that modulate fear constructs.

#### E. Correlates and Measures of “Fear”

Both environmental and genetic factors influence the behavioral, physiological and hormonal correlates of fear induction. In humans, the harshness of environmental influences (van der Kolk et al 1985; Pitman et al. 1990) and the trait of autonomic hyper-reactivity (Spence, 1958; Cloninger et al. 1981, 1987; Kagen et al. 1994) are associated with the constructs of unconditioned fear induction and conditioned fear. Responses of

unconditioned or conditioned fear have been associated with behavioral measures of avoidance (Concannon et al., 1980), suppression of appetitive behavior as in the CER or open field drinking paradigm (Skinner & Estes, 1941; Stout & Weiss, 1994), potentiated startle (Davis, 1989), increased freezing (Fanselow, 1986), decreased locomotor activity, increased defecation (Hall, 1934; Broadhurst, 1957); and an increased latency to emerge from an enclosed space (Overstreet et al. 1992). Fear is also associated with physiological correlates of autonomic arousal as measured by heart rate, blood pressure and galvanic skin responses (Spence, 1958; Schacter & Singer, 1962; Gray, 1987), and is associated with and modulated by hormonal stress correlates such as CRF, ACTH, B-endorphin, glucocorticoids, and catecholamines (Weis, 1991; Gray, 1987; Concannon et al 1980; Kalin, et al. 1990; Lee et al. 1994; Van der Kolk et al. 1985; Sapolsky, 1992).

A general conceptualization of fear involves a large set of interactive responses that are highly distributed across behavioral, autonomic and hormonal systems involving a substantial individual variability (Gunnar et al ,1989; Gunnar, 1987) due, in part, to heritable sources (Stevenson et al. 1992; Tellegen et al. 1988; Gray, 1987; Cloninger et al. 1981, 1987). Clearly however, the intensity of the environmental stressor present has been shown to influence the magnitude of GSR conditioning in rats (Levine et al. 1984) and in humans (Spence, 1958) as well as influence the corticosterone concentrations in rats (Song et al, 1994). In sum, autonomic and endocrine measures are thought to generally reflect the innate and conditioned DAS-fear responses ( Lovallo, 1990; Coe et al. 1982; Sapolsky, 1994; Cannon, 1927).

#### **F. Parameters of Pavlovian fear conditioning and CEOA**

The general theory of Pavlovian conditioning presented by Rescorla & Wagner (1972) attributes a primary influence of the strength of the US intensity and trial number

on the acquisition of Pavlovian conditioned responses. Environmental influence on the acquisition of an excitatory Pavlovian response occurs, along with the influence of many other factors, through increases in the 1) intensity of the US; 2) the duration of the US; and 3) number of CS/US trials that accrue a positive expectation of the US onset (Rescorla & Wagner, 1972; Davis & Wright, 1979; Spence 1958).

Hagen & Green (1988) found that increased intensity of an electric shock increased the CEOA in rats. Levine et al. (1984) found that increased intensity of background stress and increased intensity of the US critically facilitated the CEOA response. Fanselow (1984) reported that an increase in the number of shock trials positively covaried with conditioned fear (as measured by freezing) and the conditioning of context induced hypoalgesia. Rossellini et al. (1994) have shown that the number of shock trials increase the CEOA response in the rat. However, a methodical investigation of the influences of shock duration and intensity on CEOA was not found in the literature.

#### **G. Two types of CEOA Elicitors: CS/US or a CS alone (early in training)**

The conditioned opioid hypo-algesic response results from two types of test procedures which have been used following minimal training (less than 3 pairings ) of the CS with the US. A profound hypoalgesia results from the presentation of the CS/US compound (e.g., Lysle & Fowler, 1988) and it would appear that this profound hypoalgesia results regardless of a limited or an extensive number of conditioning trials (Rosellini et al. 1994; Lysle & Fowler, 1988). The other procedure that produces hypoalgesia does so via presentation of the CS-alone after a limited number of CS/US training trials (e.g., Ross, 1985, 1986; Ross & Randich, 1985; Watkins et al., 1982).

**A conditioned hyperalgesic response to a well trained CS-alone**

However, other investigators have found a hyperalgesic effect results from presentations of the CS alone (Davis & Henderson, 1985; Lysle & Fowler, 1988). Lysle & Fowler noted that the different hypo/hyper-algesic outcomes of the CS-alone presentations may be due to the differences in the extent of training used by investigators. They interpret the discrepant results as the development of a temporal discrimination similar to that observed in long delay conditioning. In the long delay CS/US conditioning paradigm different profiles of CRs develop after a few trials or after extensive training trials. Typically, after limited training in a long delay conditioning paradigm the excitatory CR is elicited soon after the onset of the CS. However, after extensive training the excitatory CR appears only immediately before the onset of the US (Macintosh, 1974). Hence, the subject may be said to have acquired a refined temporal discrimination distinguishing early CS from later CS. Lysle and Fowler applied this notion of discrimination learning to the repeated presentation of the CS/US compound noting that an organism which acquired the efficient timing of the onset of opioid system activity with the onset of the US may be more efficient and evolutionarily adaptive.

**Is conditioned hyper-algesia due to a conditioned opioid opponent response?**

Another perspective that builds on Lysle & Fowler's interpretation (1988) of these discrepant hypo/hyper-algesic-CS effects incorporates data relating to opponent process theory. Solomon's opponent process theory (1980) accounted for a large amount of physiological and affective phenomena by applying the fundamental biological principle of homeostasis to psychophysiology. Opponent process theory holds that a particular affective or physiological disturbance (called an "a" process) is elicited by environmental events. The "a" process will activate an opponent response or "b"

process that strengthens with repeated challenges to the organism's equilibrium state by the "a" process. The physiological effects of opioids easily fit into this model of dynamic homeostatic adaptation. It is well known that repeated administrations of exogenous opioids results in tolerance and withdrawal responses (Jaffe & Martin, 1990). Initially opioids in large doses elicit behavioral sedation, heart rate decreases, euphoria, and analgesia. Whereas, repeated administration of opioids results in tolerance to opioid sedative effects and locomotor sensitization effects (Schnur, 1985; Kalivas et al. 1986). Pavlov (1927) recognized that environmental cues that reliably precede drug administration might elicit CRs supported by a drug US. Extensive empirical and theoretical work (Schull, 1979; Siegel 1975, 1976, 1977; Puolos & Cappell 1991; Schwarz-Stevens & Cunningham, 1993) has supported this premise indicating that opponent processes to the effects of morphine and other drugs can be classically conditioned.

Several investigators have attributed a portion of exogenous opioid tolerance and withdrawal effects to Pavlovian conditioning of opponent process mechanisms (Siegel. 1975; O'Brien et al. 1983; Falls & Kersey, 1989; Ehrman et al. 1992; Tiffany et al 1992). The work by Siegel (1975), and others (Siegel et al. 1978) has shown that environmental cues that reliably precede opioid receptor activation (by exogenous opioid injection) can acquire the ability to elicit a conditioned hyper-algesic response.

The effects of conditioned tolerance or conditioned withdrawal to exogenous (injected) opioids may have relevance to endogenous opioid physiology and the hyperalgesic effects of well trained aversive CSs. Schull's conditioned opponent theory (1979) posits that conditioned opponent responses are elicited by CSs that reliably occur prior to the injection of opioid drugs. This schedule of events is very similar to the

schedule of events when cues reliably predict the physiological release of **endogenous** opioids. Aversive environmental events induce tolerance and cross tolerance with morphine (Christie et al. 1982; Chesher & Chan, 1977) as well as provide the physiological basis for opioid withdrawal syndrome (Chrisitie & Chesher, 1982). Hence, in the CEOA an extensively trained, aversive CS/US compound consists of a cue which precedes the activation of endogenous opioid receptor systems that induce hypoalgesia. If presented by itself however, the CS elicits hyperalgesia (Lysle & Fowler, 1988). Perhaps the hyper-algesic effect of the well trained CS-alone presentation results from conditioned homeostatic opponent processes that prepare the autonomic system for the onset of opioid system activity. Essentially, this perspective simply says that regardless of the source of opioid receptor agonist (either exogenous or endogenous), the cues predicting the onset of opioid receptor activation should elicit a homeostatic or opponent process. If this interpretation is correct, then an extensively reinforced aversive CS should, when presented in the absence of the aversive US, diminish the effect of an injection of exogenous opioids.

#### **H. Temperament & Genetic factors in Pavlovian conditioning**

In the CEOA risk model, environmental risk and genetic risk phenomena both augment the psycho-biological stress-reactivity (DAS) of conditioned fear. Genetic factors have long been associated with variability in emotional and autonomic reactivity (Hall, 1934; Broadhurst, 1975; Harrington, 1966). Pavlov categorized his subjects into constitutional “types” in which he considered the “excitable” breed to condition more readily, strongly and stably than the “non-excitable” temperaments (Pavlov, 1957; Eysenck, 1983). More recently trait anxiety has been associated with augmented

conditioning of defensive autonomic responding (Spence, 1956; Becker & Matteson, 1961) as measured by galvanic skin responses.

Broadhurst (1975) and Hall (1951) selectively bred rats to investigate the genetic bases for a construct of emotionality developed by Hall (1934) and others. In America Calvin Hall measured the number of days a rat defecated due to exposure to an initially novel open field environment. Hall called this quantal measurement of habituation the “open field test” and noted that these measures of emotionality strongly correlated with days that rats showed total inhibition of the eating response during the test. In England Broadhurst (1960) extended Hall’s work by selectively breeding rats from Swiss Wistar foundation stock on the basis of measures of high and low emotional defecation. Broadhurst called these strains the Maudsley reactive (MR) and the Maudsley non-reactive (MNR) lines implying a contrast of temperaments across a spectrum fear related constructs.

In validation of the defecation measures as proxy for “emotionality” Broadhurst, (1957) found that increased brightness of lighting increased the amount of defecation in rats. Parker (1939) demonstrated that individual traits of emotionality or “fearfulness” as measured by defecation were reliable trait measures within subjects and generalizable across six different situations that presumably would elicit fear responses such as: novelty of the open field test, presentation of a loud buzzer, dropping suddenly from a height, tilting and sliding down a plane, forced swimming and immobilization. Anderson (1938) found that animals that emerged rapidly from an enclosed (i.e. protected) space into an open space (i.e. unprotected) defecated the least in the open field test whereas rats with long latencies to emerge into the open defecated most in the open field test. The emergence test latencies also consistently reflect the anxiolytic properties of

benzodiazepenes (Shekhar, 1993; Shekhar et al 1993) and anxiogenic treatments (Adamec & McKay, 1993; Lapin, 1993).

As genetic exemplars of “emotionality” MR/MNR have undergone extensive testing that have revealed complex results. The MRs appear to consistently be more easily disrupted from engaging in appetitive behaviors than the MNRs. MRs freeze more than MNRs (Imada, 1972), suppress appetitive behavior more rapidly to punishment than MNRs (Commissaris et al. 1986), and learn to suppress appetitive behavior more rapidly to a conditioned fear stimulus, i.e. CER, (Singh & Eysenck, 1960). However, MRs clearly **do not** learn to avoid shock in a two way shuttle avoidance paradigm as quickly or nearly as often as the less “emotional” MNRs (Joffe, 1964) and this decrease in avoidance responding or increased choice of shock is consistent with findings of hyperemotionality in other strains of rats that perform poorly in shuttlebox avoidance such as the Roman Low Avoidance or the Syracuse Low Avoidance rat lines (Guenaire et al. 1985; Katzev & Mills, 1974; Brush et al., 1985; Brush et al 1979). Both of these rat lines were selectively bred for the behavioral phenotype of not avoiding (or choosing to be exposed to!) foot shock in shuttle box avoidance situations. These low shock avoidance lines are similar to the MR in that they show increased defecation in the open field test (Gentsch et al. 1981, 1982).

The hyper-reactive, “emotional”, non-shock-avoiding MR’s consume more alcohol than the MNR line. This covariation of poor shock avoidance (or shock preference!) behavior, emotionality and alcohol consumption holds true for another often compared pair of rat lines: Fisher 344 and Lewis rats. The Fisher 344 rat exhibits high avoidance behavior (Potts, 1970) and low alcohol consumption (Li & Lumeng, 1984)

while the low avoidance performance Lewis rat (Katzev & Mills, 1974) readily consumes alcohol, morphine, and cocaine (George, 1991).

### I. Rodent Lines Selected for Alcohol Preference

Five pairs of rat lines have been genetically selected for the trait of increased preference in alcohol drinking. The most developed of these rat models is the Preferring (P) and Non-preferring (NP) rats originated by T. K. Li and others (Li & Lumeng et al. 1977). In this genetic selection for alcohol preference the subjects are given access to only alcoholic liquid for 4 days and then given a choice test consisting of alcoholic liquid or plain water. These differences in alcohol consumption were not found to be due to any differences in alcohol metabolism after 8 (Li & Lumeng, 1977) or 20 (Waller et al. 1984) generations of selective breeding. Locomotor activation by alcohol consumption is greater in the P than the NP line (Waller et al. 1984) and locomotor activation is a primary behavioral response to activation of operant reinforcement pathways; Wise, 1987). The alcohol preferring P line rats will bar press for alcohol (Murphy et al. 1989), and will choose to drink enough alcohol to produce withdrawal symptoms during abstinence (Waller et al. 1984).

The heterogenous stock developed by the NIH to provide a carefully maintained outbred line with large genetic variance was used by Li et al. (1988) as the base stock for a replicate of the P/NP lines. These lines, called the high alcohol drinking (HAD) and low alcohol drinking (LAD) lines, have shown similar differences in preference for alcoholic liquids though the mechanisms for differential consumption may have different

biological bases. As with the P/NP lines, the HAD line was found to be more reactive than the LAD line to the locomotor stimulatory effects of a low dose of alcohol (Krimmer & Schecter, 1991).

Sardinian preferring (sP) and Sardinian non-preferring (sNP) rats resulted from the same method of selection by alcohol preference used to develop the P/NP and HAD/LAD lines (Lumeng et al. 1989) though the sP and sNP lines were selected from a foundation stock of Wistar rats. Only two behavioral papers in English language were found on these lines but both reported results consistent with a substantial increase in alcohol consumption in the Sardinian alcohol preferring line (Fadda et al. 1990, Fadda et al. 1989). This difference correlates with a decreased number of D1 and D2 dopamine receptors in the sP line.

Eriksson (1968) genetically selected two lines of rats referred to as ALKO rats because the selection of these rats based on alcohol preference (AA) and non-preference (ANA) occurred at the Biomedical Research Center of Alko Ltd, in Finland (Eriksson, 1968). His procedure appears to have generally provided the initial behavioral genetic selective breeding model used to select the six lines of rats described above. Eriksson presented rats of the Wistar strain with 10% alcohol solution for 10 days followed by four weeks of choice of the alcohol solution or plain water. The lines were selected based on alcohol choice while avoiding brother/sister mating. Ritz et al. (1986) found operant responding is reinforced in AA lineages by alcohol whereas ANA lineages are not operantly reinforced by alcohol. And similar to the P rats, the AA rats are hyper-reactive to the locomotor stimulatory effects of low doses of alcohol (Hilakivi et al.

1984; Waller et al 1984). Locomotor stimulation is presumed to reflect activation of primary operant reward centers (Wise & Bosarth, 1987).

Rivitalization of the line (by crossing with unrelated rat lines) was employed due to fertility problems after 37 generations (Hyytia et al. 1987). Since revitalization the AA line has shown some evidence of increased freezing and decreased locomotor activity (Fahlke et al. 1993) as well as increased passive immobility before and after defeat in a resident intruder situation (Tuominen et al. 1990). This correlation tends to support the current hypothesis that DAS reactivity covaries with alcohol consumption. However, before revitalization the lines did not differ in open field defecation (Eriksson, 1972) and after revitalization they were not found to differ in corticosterone release (Korpi et al. 1988) or latency to emerge in the plus maze anxiety assessment model (Tuominen et al. 1990).

Mardones (1960), working at the University of Chile, applied selective breeding to white rats based on the drinking of 95% alcohol solution to found the low (UChA) and high (UChB) alcohol consumption lineages in 1948. This led to two lineages of rats (Tampier et al. 1984) in which the high alcohol drinking line consumed more water and less food. Although the metabolic and pharmacological traits of these lines have been studied for decades, behavioral assessments of emotional reactivity were not found in the literature.

### **J. Limbic/Sympathetic hyper-reactivity and alcohol self-administration**

Family, twin, and adoption studies have also provided strong support in establishing a genetic influence in the etiology of alcoholism. Various environmental factors also contribute and interact in the etiology of alcoholism (Bry et al. 1982, Newcomb et al. 1986), thus creating a complicated field for analysis. To simplify this analysis, one might ask what do both the environmental and genetic factors have in common? The CEOA model proposes that a common etiological root of alcoholism is hyper-reactivity of the limbic and sympathetic nervous systems (LC/SNS) to stressors. The genetic component involves an augmented psychobiology or "a preparedness" for limbic and SNS hyper-reactivity while the environmental component is a category of conditions which elicit intense, prolonged, or numerous excitatory reactions of the limbic and SNS stress response. Environmental conditions such as trauma, verbal or physical conflict, deprivation, aversive stimulation, unpredictability, lack of control, novelty, aversive conditioning and task demands are all in the category of stressors that elicit endogenous opioid (EO) responses (Maier, 1988; Mayer, 1988; Bandura et al. 1988, 1987). In the proposed model, environmental risk factors cause increased sympathetic reactivity, increasing the activation of the endogenous opioid (Mayer, 1988) and noradrenergic systems (Weiss, 1991), sensitizing both appetitive/reward (Piazza et al, 1990) and aversive/defense limbic processes (Kalivas et al, 1988; Grau et al, 1981). In this aversive, fear-inducing process, primary operant reward systems may be chronically sensitized (van der Kolk et al. 1985); lowering reinforcement thresholds and augmenting operant reward through sensitization of dopaminergic reward pathways.

Collateral homeostatic and opponent processes simultaneously develop via the noradrenergic pathways in the amygdala and LC lowering the threshold for defensive

and dysphoric motivations. Prior exposure to stress responding has been shown to increase basal electrical activity of the LC (Pavcovich & Ramirez, 1991; Pavcovich et al 1988), to induce a state of hyper-reactivity in the LC neurons to excitatory input (Simpson & Weiss, 1988), and sensitize NE release and synthesis (Nisenbaum & Abercrombie, 1992).

#### **K. The Psychobiology of Stress**

In order to survive, living systems must maintain an adaptive physiological balance in a constantly changing world. The stress response has been preserved through evolutionary pressures for its rapid mobilization of the organism's resources to confront threats to survival such as starvation, predation, intra species competition or other adverse conditions. The stress response inhibits the body's normal maintenance modes of digestion, energy storage, growth, sexual drive, and immune function, while simultaneous actions of stress hormones facilitate fast energy utilization of amino acids, glucose and fatty acids (Sapolsky, 1994) for defense. Intense stress responses also conveniently render the organism analgesic (Mayer, 1988). Hans Selye, who claimed to have borrowed the term "stress" from 19th century physics, intended the term to mean a generalized physiologic response of the body to any demand. Selye partitioned stress responses into "eustress" and "distress" believing that exciting and controllable challenges provide healthy experiences for human development. He considered psychological and physical distress to be an arousal response to uncontrollable and prolonged aversive conditions (Seyle, 1950). Cannon (1929) coined the term "flight or fight" to describe acute and homeostatically disturbing psycho-physiological responses to threat that involve excitation of the sympathetic nervous system.

Stress responding of the SNS involves neuroendocrinological activation of the LC and HPA. A principal modulator of this SNS activation is corticotrophin-releasing factor (CRF). Neurons of the paraventricular nucleus in the anterior hypothalamus release CRF, initiating a cascade of hormonal responses resulting in increased heart rate, blood pressure, blood glucose levels and oxygen consumption. CRF stimulates production of the precursor molecule pro-opiomelanocortin which contains both beta-endorphin (BE) and adrenocorticotropic hormone (ACTH). CRF stimulates the release of ACTH from the pituitary. ACTH, in turn, stimulates the adrenal cortex to release glucocorticoids that provide inhibitory feedback to the HPA through glucocorticoid receptors on the hippocampus (Sapolsky et al 1984; Sapolsky, 1992).

Another major route of sympathetic arousal is the province of direct sympathetic enervation of the adrenal medulla where the catecholamine, norepinephrine (NE), and the opioid mu-receptor agonist, BE are concomitantly released. Direct neuronal stimulation of the sympathetic ganglia and the gut also release NE and endogenous opioids (Grossman & Bouloux, 1984; Chrousos & Gold, 1992). The CNS activity of delta-opioid receptors has been shown to function as an acute inhibitor of the catecholaminergic stress response (Grossman & Bouloux, 1984; Grossman, 1985). In contrast, intraventricular beta-endorphin, a mu-receptor opioid, increases all three catecholamines in blood plasma (Van Loon et al. 1981; Conway et al, 1984).

In humans, opioid analgesia occurs during even mild stressors such as mathematical tasks. Bandura et al. (1988) found that both the mental stress of induced inefficacy at mathematical tasks and post-task self assessment elevated autonomic arousal. Pertinent to the current hypothesis, they found a direct correlation between autonomic variability and opioid analgesia in the induced inefficacy group. Collateral support for the relationship between emotionality to stressors and CEOA is found in

clinical studies as well. The intense affective responses in a case study of phobic anxiety involved endorphin activity (Thyer & Mathews, 1986). Exposure of Vietnam veterans with PTSD to 10 minutes of a combat video (Pitman et al. 1990) also elicits increased EO activity. And hyper-activation or hyper-reactivity of the HPA and CRF system have been documented in depression and anxiety disorders, respectively (Risch et al, 1983; Owens & Nemeroff, 1993).

A seemingly paradoxical finding in the literature is the lower basal levels of beta-endorphin in the blood plasma of joggers (Lobstein et al, 1989), veterans with PTSD (Hoffman, 1989), alcoholics (Vescovie et al 1992) and sons of alcoholics (Gianoulakis et al. 1990) . Because of their increased SNS activity, one might expect to find increased BE levels. However, an interpretation related to Solomon's opponent process theory (Solomon & Corbit, 1974; Solomon, 1980) predicts that intense periodic increases in physiologic processes, such as activation of endogenous opioid systems, collaterally develop adaptive compensatory systems that may increase the central and peripheral catabolism of such opioids, hence reducing the basal levels of EO (Grunberg & Baum, 1985).

#### **L. Homeostasis of SNS arousal via LC/HPA by NE/BE**

The dynamic homeostasis of SNS arousal is modulated tonically and acutely through the reciprocally inhibitory systems of NE and delta-receptor opioids, such as enkephalin (Grossman, 1984). Hypothalamic neurons, pituitary and adrenal cells store NE and BE in vesicles which release them concomitantly during stress (Grossman, 1984). ACTH and BE are co-synthesized in the production of the precursor molecule, pro-opiomelanocortin, and are also co-released during stress responding (Kjaer et al, 1992).

Moderate to large doses of BE inhibit the release of ACTH and NE (De Vries, et al., 1990), acutely dampening the noradrenergic excitation response of the LC/SNS. Conversely, administration of opioid blockers such as naloxone or naltrexone increase the release of catecholamines and ACTH (Van Loon, 1983). Chronic stimulation of LC mu-receptors by chronic stress or daily morphine injection establishes a new homeostatic set point of the LC/SNS and a return to basal electrical functioning is achieved (see Gold, 1993). However, if the chronic opioid inhibition of the LC/SNS is removed by either naltrexone or a decrease in available opioid ligand, the LC/SNS undergoes "withdrawal", an increase in activity which is associated with dysphoria, anxiety, panic, nausea, cold flashes, restlessness, craving, tachycardia and irritability and hyperactivity (Redmond & Krystal, 1984, Roth et al 1982). Hyperactivity of the LC/SNS is associated with powerfully motivating dysphoric emotional states of withdrawal (Redmond & Krystal, 1984; Van der Kolk et al., 1985; 1987). Moreover, relief from dysphoric motivational states is cited most often by the majority of excessive drinkers (Farber et al., 1980).

#### **M. Similar psychobiology of stress and self-administered drugs**

A variety of stressors increase the activity of endogenous opioid systems centrally and/or peripherally e.g., physical exercise, sleep or food deprivation, novelty, defeat, unconditioned and conditioned aversive stimulation, seizure (Mayer, 1988), the stress of mental tasks (Bandura et al. 1988) and affective states (Thyer & Mathews, 1985). In addition, alcohol (Mendelson et al 1992) and other self-administered drugs activate the HPA, as a stressor would. Nicotine, caffeine, alcohol, cocaine, and amphetamine all activate the HPA axis. Opioids in small doses similarly have a psychomotor stimulatory effect (Venzina et al, 1987; Wise & Bozarth, 1987) that

sensitizes with repeated exposures (see Kalivas & Stewart, 1991, for a review), while habituation occurs to the opioid's motor sedating effects (Schnur, 1985). This locomotor stimulation is thought to be a general index of the activation of central operant reward pathways, (Wise, 1987).

The interchange ability of stress and aspects of drug/reward self-administration extend beyond just long term motor activity sensitization effects (Babbini et al., 1975; 1972, Foa et al., 1992). Stressors and self-administered drugs share a common neurobiology. They both activate and interact with endogenous opioid systems. Stressors such as social defeat will produce cross tolerance with opioids (Miczek, 1986; Christie et al 1982) and opioid dependence develops from a schedule of daily stressors such as cold swims (Christie & Chesher, 1982). As noted above, the classic features of opioid withdrawal are expressions of hyper-active and hyper-reactive LC/SNS systems. Stressful or traumatic events often result in persistent symptoms of increased arousal such as difficulty sleeping, irritability, decreased concentration, generalized fear, avoidance, and numbing (Foa et al. 1992); all phenomena that resemble opioid withdrawal or direct opioid effects.

Perhaps one of the most compelling and well known phenomena equating the effects of stress and abused drugs is their common ability to reinstate self administration or relapse (Stewart, 1984; Stewart & Wise, 1992; Marlatt, 1988) or other appetitive behavior such as ICSS (Kornetsky & Porrino, 1992). Mild footshocks (Sadowski et al 1984) have the effect of later increasing food consumption as well as lowering the threshold of intra-cranial self stimulation (ICSS), as do small doses of morphine (Morely et al 1983, Kornetsky & Porrino, 1992). Conditioned fear stimuli also lower the threshold for ICSS and delay the extinction of appetitive responding (Deutsch & Howarth, 1962). These phenomena parallel and complement the priming

effects that small doses of opioids have on the reinstatement of drug self administration behavior (Stewart, 1984).

Stressors and psychoactive drugs activate the same stress/reward pathways.

Nakamura et al (1992) when studying the stress initiating paraventricular nucleus found that of all responsive paraventricular neurons tested, 57 percent did not discriminate between a CS for reward (ICSS or glucose) or a CS for aversive stimulation (foot shock or tail pinch). Similarly, 29 percent of the responsive lateral hypothalamic neurons (a major dopaminergic reward pathway known as the medial forebrain bundle) did not discriminate between aversive and rewarding CSs (Ono et al 1992).

The clinical literature well documents the relapse of drug seeking behavior and craving through exposure to either stressors or re-exposure to drugs (Brown et al, 1990). Indeed, opioid addicts maintained on methadone claim that they subjectively perceive themselves to be in the phenomenologic state of withdrawal when they are under psychosocial stress (Whitehead, 1974).

The similarity of stress and psychomotor stimulant responses to self-administered drugs is more than coincidental. ICSS induces a hormonal stress profile (McHugh, et al., 1966; Uretsky et al., 1966; Terry & Martin, 1978). And in fact, ACTH, a prominent endocrine stress response hormone, supports self-administration responding (Jouhaneau-Bowers & Le Magnen, 1979) as does corticosterone (Piazza et al 1993). Antelman (1979) found manipulations of stress and amphetamine were interchangeable in terms of their sensitization effects on stereotypy and hyperactivity. Both prior stress and prior exposure to amphetamine increase vulnerability to self administration of amphetamine (Piazza et al. 1990). This increased vulnerability to drug self administration by rats covaries with hyper-reactivity of locomotor responses

to novelty. This data provides an elegant animal model of the risk factor of sensation seeking which is correlated with drug use (Dellu et al 1993).

Consistent with the psychobiological commonalities risk model, prior experience with uncontrollable foot shock increases novelty-induced ACTH release while not changing basal levels (van Dijken et al. 1992). Hence, prior exposure to an uncontrollable stressor can induce the risk trait of hyper-reactivity (van Dijken, 1992; Kalivas et al, 1986; Kalivas & Stewart, 1991), vulnerability to drug self administration (Piazza et al 1990; Higley et al 1991), and augment the physiologic release of endogenous substances to novel or stressful stimuli (van Dijken, 1992; see Pittman et al 1990 ) which are known to support self administration (Jouhaneau-Bowers & Le Magnen, 1979).

Pohorecky (1981, 1990, 1991) has proposed that individual differences in stress response directly covary with an increased response to alcohol. The work of Gianoulakis et al. (1989, 1990) and Gianoulakis ( 1993) supports this view. In sum, a biological commonality of stress and substance abuse is found in the common effects of both on limbic/SNS arousal and sensitization. Perhaps the unifying mechanism responsible for the interchange ability of stressors and addictive drug exposure is their common ability to activate dopaminergic and opioidergic systems. Small amounts of opioids increase the reinforcement function of alcohol (Hubbel et al 1986) and other drugs or activities while simultaneously eliciting/augmenting limbic and SNS/HPA activity (Wise, 1987; Narita, et al, 1993; Triana, 1980; Topel, 1988; Pohorecky, 1990).

The interchangeable effects of stressors and drug exposure have bearing on the nature of risk for alcohol and drug abuse through the sensitization of psychobiologic mechanisms. Kalivas and Duffy (1988) found "similar effects of daily cocaine and stress on mesocorticolimbic dopamine" neurons that implicate susceptibility to drug self

administration (Kalivas et al., 1993). In terms of genetic or individual vulnerability factors, Piazza et al. (1989) found individual differences of locomotor reactivity of rodents to novelty (hyper-reactivity) predicted increased rates for drug self administration. Augmented responses isomorphic with this vulnerability trait marker of increased activity response can be induced environmentally by stressors or via drug exposure. Exposure to opioids, alcohol, cocaine, shock, cold/warm water swims, tail pinch, saline injection, and food deprivation all induce sensitization (Antleman, 1988). The psychological stressor of maternal deprivation during infancy and childhood also induces vulnerability to alcohol consumption later in the adult life of monkeys (Higley et al. 1991). Consistent with the notion of stress induced lowering of reinforcement thresholds, these monkeys drink more alcohol during stressful periods possibly due to a priming effect of stress or due to stress activation of the LC similar to opioid withdrawal. Augmented stress in a variety of experimental paradigms is associated with increased self administration of drugs such as alcohol (Volpicelli & Ulm, 1990), opioids (Shaham et al 1991) and cocaine (Taylor et al, 1990) and amphetamine (Maccari et al 1990). Given the aversive nature of risk/vulnerability factors, sensitization of LC-HPA/SNS response by intermittent exposure to a stressor may induce vulnerability to acquisition of alcohol and drug self administration in humans as well.

Repeated exposures to opiates and psychostimulants cause increases in drug reward and/or craving (Robinson & Berridge, 1993; Piazza et al 1989; Jaffe, 1989; Kalivas & Sorg, 1993). Similarly, locomotor sensitization occurs from psychostimulants and opioids (Babbini & Davis, 1972; Kilbey and Ellinwood, 1977, Piazza et al, 1990; Schnur, 1985). The effects of cocaine and morphine "exert common chronic actions" on the dopaminergic brain reward (Beitner-Johnson et al, 1991).

Since the effects of cocaine and morphine are the same during the behavioral sensitization of drug incentive and reward processes, it is likely that endogenous opioid substances induce similar sensitization of craving and drug reward by physiological sequela of repetitive stress responding.

Perhaps one of the strangest lines of argument for the interchangeable nature of stressors and self-administered drugs is the phenomena of self-produced "aversive" events. In a procedure known as response produced shock, monkeys, rats and pigeons will maintain operant responding for an electrical shock up to 10 mA (Barrett & Spealman, 1978; Barrett, 1977; Bacotti, 1978). Such apparent appetite for self-inflicted "aversive" consequences is reminiscent of more normative appetitive risk taking or thrill seeking behaviors such as skydiving, roller coaster-riding or watching horror films. Clinically, the self-injurious behavior (SIB) of self-mutilators and self-batterers has been ascribed to the reinforcing function of endogenous opioids by several investigators and naltrexone reversability has been demonstrated (Barret, et al 1989; Bernstein et al 1987; Sandman et al 1990; Sing et al 1993; Richardson & Zalesky, 1986; Walters et al, 1990).

The category of "appetitive stress" is not trivial. Colloquialisms in our language reflect the presence of "self-produced-aversivenesses" e.g., "itching for a fight", "decompensating", "acting out" and "externalizing." Van der Kolk et al (1985) in their work with war veterans have interpreted the behavioral and physiological symptoms of PTSD as an "addiction to trauma". Support for a neurohormonal correlate of hyper-reactivity in PTSD was found by Hamner & Hitri (1992). They found that compared with controls, PTSD subjects had post exercise, but not basal, elevations of plasma b-endorphin. Nadelson (1992) has discussed the positive hedonia of combat or mortal risk in terms of a normative evolutionary model.

Animal models of response produced shock (RPS) involve prior training with appetitive reinforcers and shock that can maintain escape and avoidance, later leading to self administration of shock (Mello, 1978; Kelleher et al., 1963; Kelleher & Morse, 1968). An important extension of this work is that the effects of amphetamine (Barrett, 1977) and many other psychoactive substances dramatically increase the rate of shock self administration . These data are consistent with the idea that increases in the hormonal "stress profile" of the HPA is associated with an increased reinforcement by "aversive" foot shock. A related phenomenon is the lowering of reinforcement threshold or increasing reward effect of ICSS by opioid injection (Kornetsky & Porrino, 1992). Hence, the presence of opioids or increased reactivity of the opioidergic system may qualitatively change the kind of stimuli that are reinforcing and this effect may be responsible for the aversive behavioral risk features and defensive/aggressive temperament features of at-risk children.

Conversely, the rewarding aspects of a spectrum of abused drugs are inhibited by opioid receptor antagonists including: ethanol (Vereby & Blum, 1979; Altshuler et al., 1980; Hubble et al., 1986; Sandi et al., 1988; Kornet et al., 1991), cocaine (Carroll et al., 1986; Houdi et al., 1989; De Vry et al., 1989; Corrigan & Coen, 1991), amphetamine (Trujillo et al, 1991); benzodiazepines (Cooper, 1984, 1983), pentobarbital (Seeger, 1981) and phencyclidine (Greenberg & Segal, 1986). Exogenous or endogenous opioid withdrawal involves LC/SNS hyperactivity and hyper-reactivity (Redmond & Krystal, 1984; Cox & Werling, 1988; Gold, 1993). Another factor inducing hyper-activity/hyper-reactivity of the LC/SNS involves repeated intermittent, rather than chronic, exposure to a drug or stressor. Increased activity in response to intermittent exposure to a drug or stressor is the hallmark of sensitization (Antelman, 1988). Sensitization of response to drugs by prior exposure to

stressors is a well documented phenomena (Kalivas & Stewart, 1991), as is the sensitization of response to stressors by prior drug exposures (Piazza et al 1989, 1990). The possibility exists that these two processes are in fact both operating via the same mechanisms: through their physiological impact on neuro-endocrinological systems. Augmentation of neuro-endocrinologic stress activation by risk factors may mediate both the environmental and genetic risk factors for alcoholism, drug abuse and addictive behaviors.

#### **N. Objectives.**

The experiments for this dissertation represent a first step in the development of an animal model that relates aversive conditioning of endogenous opioid activity to individual differences in the self-administration of alcohol.

In terms of the behavioral genetics and psychopharmacologic aspects of the model, one of the first questions that might be asked is, "What are the learning factors that influence the conditioning of endogenous opioid activity?" Specifically, is the conditioning of an opioid analgesic response a function of the intensity and duration of the aversive US?

A second type of question that should be asked is, "Do differences in emotionality, defensiveness, and hyper-reactivity covary with a preparedness to condition endogenous opioid activity?" In other words, in a rodent model, do animals that are genetically prepared for CEOA reflect the risk temperament traits of a lower threshold for aversive stimulation and an increased intensity or duration of defensive responding?

A third type of question in the assessment of the CEOA risk model involves assessing whether individual differences in the preparedness for CEOA will covary with the self-administration of psychoactive drugs, such as alcohol.

Because of the prevalence of polydrug use among alcohol and drug abusers (Martin et al, 1993), and the familial resemblance among alcohol and substance abusers (Babor et al, 1992), a fourth relevant question would be: "Do genetic differences in the self-administration of alcohol covary with individual differences in sensitivity to other psychoactive and addictive drugs, such as morphine ?"

A fifth question is of etiologic relevance: "Do genetic differences in preparedness for CEO hypoalgesia covary with sensitivity to psychoactive drugs, such as morphine ?" This question bears on the generalizability of the hyper reactivity trait and as to whether that trait includes reactivity to psychoactive drugs, as well as to aversive events.

A sixth question is whether preparedness to CEOA will enable CEOA to stimuli that are distal both temporally and spatially to the explicit CS/US.

The following methodology was aimed at systematically answering the foregoing questions on the relation of CEOA to environmental and behavioral risk factors.

## II. Method

### A. Subjects.

Male albino rats 80 - 100 days old at experiment initiation, weighing between 200 and 350 gms, of 3 different breeds, were used in this series of experiments. Holtzman Sprague Dawley (Harlan Laboratories Inc.), Marshall 520/NCR , and Fisher F344/NCR inbred rats (Harlan Sprague Dawley Inc.) were received from the Indianapolis facility of

Harlan Sprague Dawley and were kept in a colony room on a 12 hour light - 12 hour dark cycle (lights went on at approximately 0800 hrs). The subjects had continuous access to food and water in their individual home cages, which were situated in colony racks consisting of 60 cages each. All testing was conducted in rooms other than the colony room facility. Upon receipt, all animals were given at least 7 days of acclimation to their new environment before daily handling sessions began. Typically in these handling sessions, subjects were each picked up for 5 seconds each day for 5 - 7 days in order to habituate the subject's defensive and emotional responses to experimenter handling.

Because of different ages of the rats upon arrival (35 to 70 days), all rats were kept in the colony room without handling until they were at least 80 days old and had been given at least 7 days acclimation to the colony room.

## B. Apparatus.

Four rodent chambers (Coulbourn Instruments Model E10-10), each measuring 25 x 30 x 33 cm, served as the conditioning apparatus. The chambers had clear Plexiglas side walls, sheet-metal top and end walls, and a grid floor consisting of 0.24-cm bars, spaced 0.87 cm apart. A 50-ml drinking tube (BRS/LVE Model DR-001), accessible through a 1-cm aperture in the end wall of each chamber, was connected to a contact relay (BRS/LVE Model DR-901/221-05) to allow the recording of licks. An 8.5-cm, 3.5 ohm speaker (Quam Model 3A05), externally mounted on the drinking-tube wall of each chamber, was connected through timer circuitry to an audio generator (BRS/LVE Model AU-902) to provide a CS: a 15 second, 80-dB (re 0.0002 dyne/cm<sup>2</sup>) tone. The grid floor of each chamber was connected through timer circuitry to the output of a shock

generator and scrambler (BRS/LVE Models 903 and SC 902) to provide aversive US foot shock of 1, 4, or 5 seconds in duration and coterminous with the CS. The chambers were individually housed in sound-attenuating cubicles, 50 x 60 x 88 cm, that were located in a room adjacent to the programming equipment. A 100-W, 120-V bulb, recessed behind a frosted plate in the ceiling of each cubicle, was operated at 85 V, ac, to provide diffuse illumination of the chamber. An ambient sound level of about 72 dB was provided by operating the cubicle's ventilating fan at 57 V, ac.

The hot plate was positioned on a movable cart, approximately 1.5 meters from each chamber. It consisted of a 30-cm square copper plate that was affixed to the bottom of a clear Plexiglas cylinder, 26 cm (interior diameter) x 36 cm in height. The plate and its water-tight cylinder were immersed to a water depth that maximally varied from approximately 7 cm to 1 cm during a session in a thermostatically controlled water bath with a water circulator (Fisher Scientific Circulator Model 73) that maintained the surface temperature of the plate at 52.5 degrees Celsius. A Standard Electric timer, adjacent to the hot plate, was used to measure the subject's latency to lick a hind paw or in jumping from the plate. When possible, video recording equipment was used to review the subjects' hot plate testing behaviors.

Alcohol drinking measurement devices were constructed of inverted 50 ml plastic serological pipettes (Falcon 50 ml) whose top ends were sawed off to accommodate rubber stoppers of size 00. The cone tips of these drinking containers were sawed off to match the diameter of stainless steel 4 inch angled Fisher Scientific sipper tubes, each of which contained a ball bearing that minimized leakage. The sipper tube was connected to the serological pipette with a 1.5 inch length of rubber tubing which snugly sleeved over both, providing flexibility to the sipper tube. Each drinking tube was paired with

another by 2 sets of 2 rubber bands, so that each drinking apparatus could provide a subject access to either a 5 percent alcohol solution or tap water. The rubber bands served to support the drinking tubes on the hooks of a peg board where measuring took place before and after each drinking session.

### C. Experiment 1. Design

Sixty-four Sprague Dawley rats were trained in an aversive Pavlovian conditioning procedure in which the US (foot shock) duration and intensity were varied between the four training groups in a 2 X 2 (low or high US intensity X low or high US duration) between subjects factorial design to assess the influence of increasing foot shock duration and increasing foot shock intensity on the conditioning of hypoalgesia. During training the shock intensity was varied at two levels across groups: .8 mA and 1.6 mA. and shock duration was varied at two levels: 1.0 seconds and 4.0 seconds to create four groups of subjects. Subjects underwent CS/US training and were assessed for conditioned hypoalgesia after 1, 3, and 9 trials of conditioning. On test days subjects were exposed to one of three conditions: the tone CS in the conditioning chamber (CS), conditioning chamber, i.e., the conditioning context without the CS (X), and the transportation cart (NTG). No presentations of the US occurred in this first test phase which included the first three hot plate test days.

In the next and final test phase, naloxone injections were used to assess the relative involvement of the endogenous opioids in analgesic responses to both US alone and CS/US compound presentations after all subjects had acquired a total of 10 CS/US conditioning trials over ten days of training. These manipulations were used to assess the following hypotheses: 1) Increasing aversive US intensity increases the conditioned analgesic response to the CS/US compound. 2) Increasing aversive US duration

increases the conditioned analgesic response to the CS/US compound. 3) A component of the conditioned analgesic response is due to opioid receptor activation. 4) After an initial CS/US training trial, presentation of the CS will elicit a hypoalgesic response, i.e., conditioned hypoalgesia (CA). 5) Extensive CS/US training results in a hyperalgesic response to the presentation of the CS.

#### D. Experiment 1. Procedure

Sprague Dawley rats were given habituation to experimenter handling and transportation to the conditioning chamber room. After 7 days of habituation to daily 8 minute exposures to the conditioning chambers, the four US training groups (1 sec. X .8 mA; 4 sec. X .8 mA; 1 sec X 1.6 mA; and 4 sec. X 1.6 mA) began daily CS/US training involving one conditioning trial per day. Subjects were tested after acquiring 1, 3 and 9 CS/US training events. On test days the subjects were exposed to one of three conditions: the chamber and the conditioning stimulus (CS), the conditioning context without the CS (X), or the transportation cart which is called the "nothing" group or "nominal transportation" group (NTG). No US presentations occurred on test days for any subjects in the first phase of Experiment 1.

Before each hot plate latency test, subjects were exposed to the cold plate in order to habituate them to the testing apparatus. The day after each hot plate latency test subjects were also exposed to the unheated testing apparatus ("cold plate exposure") to reduce aversive conditioning to the testing apparatus.

After all subjects had received 10 CS/US conditioning trials, a final test phase began to assess the opioid nature of the conditioned hypoalgesia. Subjects were either given a naloxone injection or no injection and either exposed to the CS/US compound or simply the US alone.

### E. Experiment 2. Design

In order to assess if genetic differences in preparedness to condition opioid hypoalgesia covary with genetic predisposition to consume alcohol a 2 (genetic line of rodent) x 2 (injection type) x 2 (stimulus type) design was employed. Two different strains of male albino rats (F344 and M520s) were chosen due to the strong contrast in their propensity to consume alcohol (Li & Lumeng, 1984). All subjects were exposed to very few trials of identical protocols for Pavlovian conditioning with an aversive CS/US compound. On test days subjects were given either a naltrexone or a saline injection to assess for the involvement of opioid activity in the modulation of algesic responses. Each of three hot plate tests sampled all subject's responses under the same test conditions: for hot plate one (HP1) all subjects were exposed to a novel context; for hot plate two (HP2) all subjects were exposed to a familiar context; for hot plate three (HP3) all subjects were exposed to a Pavlovian conditioned aversive CS. These successive hot plate assessments were then combined or contrasted to assess for differences in algesic latencies due to novelty or CS effects. For example, the CS effect after one training trial was assessed by contrasting the hot plate latencies after exposure to the CS in HP3 with the hot plate latencies after exposure to the NTG condition in HP2.

These manipulations were employed to assess the following hypotheses: 1) The alcohol consuming M520 genetic line would respond to novelty with an opioid analgesic response. 2) The non-alcohol consuming F344 line would not respond to novelty with an analgesic response. 3) The amount of conditioning of endogenous opioid hypoalgesia between rat genetic lines would positively covary with the reported genetic predisposition to consume alcohol across these rodent strains. 4) Emotional

reactivity (as measured by boli counts) would positively covary with both measures of alcohol consumption as well as with measures of the CEOA.

#### **F. Experiment 2. Procedure**

Twenty five M520/NCR and 25 Fisher 344/NCR male albino rat subjects were received into the colony room at approximately 5 - 6 weeks of age. Food and water were available on an ad lib schedule. The subjects rested and were not removed from their home cages for the next 30 days. All experimental manipulations were begun at approximately 1 PM and were finished before 6 PM. After 30 days, the subjects underwent a handling acclimation procedure over a span of 6 days in which they were picked up by the tail for a period of 5 seconds for each of the next 3 days, then the next day weighed, the next day picked up by the tail, and on the final handling acclimation day placed in the transportation cart for 5 seconds. During this process and most all subsequent handling procedures, a record of subjects' bolus counts and squeaks were recorded as a measure of emotional reactivity.

Habituation to these handling procedures was followed by daily exposure to the conditioning chambers for 8 minutes on each of the next four days. On the fifth day subjects were similarly transported to the chamber room but were individually exposed for two minutes to the unheated analgesia testing apparatus without being exposed to the conditioning chamber context. Two more days of conditioning context exposure (X) followed this "cold plate" (CP) exposure. On the next day one trial of Pavlovian CS/US conditioning occurred for all subjects. During the third minute on this 8th day of procedures involving transportation to the conditioning chamber room, a 15 second tone (CS) was presented with a 5 second coterminous 2.0 mA scrambled electric floor grid shock.

All subjects were then given daily exposure for eight minutes to the conditioning chamber context on each of the next ten days. The testing of algesia sensitivity (hot plate testing) began after appreciable attenuation of fear to the context had occurred. This reduction of fear to the conditioning context was operationally defined as a return to baseline levels of emotional defecation in both M520 and F344 subjects. Beginning with the first hot plate algesia test (HP1) subjects were split into staggered treatment groups such that one group received the exact experimental schedule as the other had on the preceding day. Partitioning the subjects into staggered groups decreased the daily experimental running time, minimizing the variation of the subjects' circadian rhythm phases during testing, as well as serving to reduce the variation in stress effects due to extended colony room disturbances on test days and an additional exposure to the unheated test apparatus (cold plate day).

HP1 was designed to assess for endogenous opioid involvement in the hypoalgesic response to novelty. Thirty minutes prior to HP1 subjects were injected with either saline (1ml/kg) or naltrexone (2 mg/kg) and placed into a novel holding rack in a novel environment for 30 minutes and then tested for latency to either a hind paw lick response or a jumping response. A two minute cold plate exposure followed on the day subsequent to the hot plate test. This cold plate exposure was employed to dissipate fear conditioning to the testing apparatus. Five more days of exposure to the "X" context occurred prior to the next hot plate test, HP2.

HP2 was designed to assess for opioid involvement in pain sensitivity under conditions that were familiar to the subjects. Thirty minutes prior to HP2 subjects were given injections of either saline (1ml/kg) or naltrexone (7 mg/kg) that were counterbalanced with the subjects' injection conditions in HP1. All subjects were transported to the chamber room and placed into the conditioning chamber for 2 mins

prior to hot plate testing. The day following HP2 all subjects received a cold plate exposure which was followed by an "X" context exposure on the subsequent day.

HP3 occurred on the following day and was designed to assess for differences in the CEOA. All subjects' injection conditions were counterbalanced with those of HP2 though the dose of naltrexone was reduced to 2 mg/kg to minimize any potentially analgesic effect due to a high dose of naltrexone. After two minutes of "X" context exposure, all subjects were presented with the excitatory CS alone (which had only received 1 conditioning trial 2 weeks earlier). Immediately upon termination of the CS the subjects were taken from the conditioning chamber and placed upon the hot plate.

#### **G. Experiment 3. Design.**

The first two hot plate tests of Experiment 3 (HP1A & HP1B) created a 2 x 2 counterbalanced design which was used to assess hot plate latency differences in response to morphine and saline injections in two different strains of rats. The experimental conditions of the next hot plate test, HP2, were identical to those of HP1B with the exception that all subjects had undergone 11 days of CS/US training procedures, that were documented in Experiment 1 to activate endogenous opioid analgesic systems. These identical test conditions of HP2A were designed to assess for changes in opioid analgesic reactivity due to extensive exposure to aversive conditioning. Specifically, augmentation of opioid tolerance and/or opioid opponent processes were predicted on the theory that repetitive CEOA would elicit similar physiological adaptations as repetitive injections of an opioid analgesic drug. All subjects were exposed to procedures which presented subjects with a minimal level of experimental stimulation. This minimal stimulation procedure involves the subjects' receiving injections in their colony room 30 minutes prior to transportation to the

conditioning chamber room where they are taken from the transportation cart and tested. This procedure is called a “nothing” or “NTG” procedure as it is the nominal transportation group, or control condition, that is typically employed in our hot plate testing procedures.

HP3A, the next hot plate test, used a 2 x 2 design to assess if a conditioned opponent process to opioid analgesic activity had developed during the aversive CS conditioning. All subjects of both genetic strains received an injection of morphine (5 mg/kg), as well as an exposure to either the transportation cart (NTG) or the CS in the conditioning chamber context. This was done to assess if: 1) Exposure to a well trained aversive CS would elicit an opponent-process to the analgesic effect of morphine; and if 2) Genetic differences exist in preparedness to condition this opponent process that reduces or opposes opioid receptor agonist effects such as morphine hypoalgesia.

HP3B, the next hot plate test, was identical to HP3A except that all subjects were placed in stimulus conditions that were counterbalanced with the prior test. This test was designed to assess for differences in preparedness to condition a hyperalgesic response between the F344 and the M520 rodent lines. All subjects received 5 mg/kg morphine.

HP4 replicated HP3B with all subjects receiving saline injections. Again, all subjects were placed in counterbalanced stimulus conditions relative to the prior test, HP3B.

HP5, the final hot plate test, was employed to assess if: 3) genetic differences exist in the preparedness to condition endogenous opioid hypoalgesia to the transportation cart. Both inbred rodent strains were injected with either naltrexone or saline after transportation to the conditioning chamber room (NTG) to assess opioid involvement in hot plate latency scores.

### H. Experiment 3. Procedure.

Twelve F344s and twelve M520s were received into the colony room and underwent typical colony room acclimation and handling protocols as in the prior experiments. A hot plate latency assessment (HP1A) of all subjects was made 30 minutes after a morphine injection (1 mg/kg) or a 1 ml/kg injection of isotonic saline. This was followed one week later by a similar assessment (HP1B) in which a 5 mg/kg morphine injection was used. Subjects were placed into the opposite injection group in order to counterbalance the treatments for all subjects.

This was followed by 10 days of aversive CS/US training. All subjects were given daily exposure to the conditioning chambers for eight minutes in which the subjects were exposed to a 15 second tone coterminous with a 1.6 mA shock that lasted for 5 seconds on a random variable time schedule. The CS/US presentation occurred for all subjects at the same time on a given training day some time after the first and before the last minute of each daily eight minute session.

This CS/US training was followed by 2 days of baseline locomotor activity measures in the open field box. Subjects were placed into the activity box and videotaped for a period of 2.5 minutes. After a CS/US training refresher day, another hot plate test (HP2A) followed in which all subjects were transported to the conditioning chamber room (NTG) 30 minutes after being injected intramuscularly with either saline (1ml/kg) or morphine (5 mg/kg). This manipulation was designed to assess for development of an opponent-process (habituation) to the analgesic effects of opioid injection through the repetitive exposure to aversive conditioning procedures that were documented in Experiment 1 to activate endogenous opioid analgesic systems.

Twelve more CS/US training days followed before a 2 x 2 design assessed for a hyperalgesic response to a CS alone presentation (HP3A). On the HP3A test day both inbred strains of subjects were exposed to either just the transportation cart (NTG) or to the CS in the conditioning chamber context. All subjects received a morphine injection (5 ml/kg) and were tested 30 minutes later.

This was followed by three days of CS/US refresher training in which the running schedule was staggered into two sets of subjects, as was done in Experiment 2, such that half of the subjects were run on the same experimental schedule a day before the second half of the subjects were run. After a cold plate exposure day, another hot plate test (HP3B) occurred which was identical to the HP3A test conditions with the exception that all subjects were placed into the stimulus conditions that were counterbalanced with those of HP3A. These manipulations were designed to assess genetic differences in preparedness to condition the hyperalgesic response to a well trained CS alone. After a cold plate exposure day and three more refresher days of CS/US training, HP4 was employed to replicate the findings of HP3B with all subjects receiving saline injections and stimulus conditions that were counterbalanced with their assignments in HP3B.

Three more refresher days of CS/US conditioning then preceded an ancillary assessment 2 (F344 or M520) x 2 (saline or naltrexone injection) of a conditioned opioid analgesic response to the transportation cart which had been indicated as a possibility from the results of the prior 2 hot plate tests. All subjects were transported to the chamber room in the transportation cart 30 minutes after receiving either a naltrexone injection (2 mg/kg) or a saline injection. All subjects remained in the cart for 1 minute before being tested for hot plate response latencies. This manipulation was to assess the hypothesis that genetic line differences exist in the preparedness to

condition endogenous opioids to temporally distal contextual stimuli (e.g., exposure to the transportation cart) that precede the explicit conditioning context and stimuli.

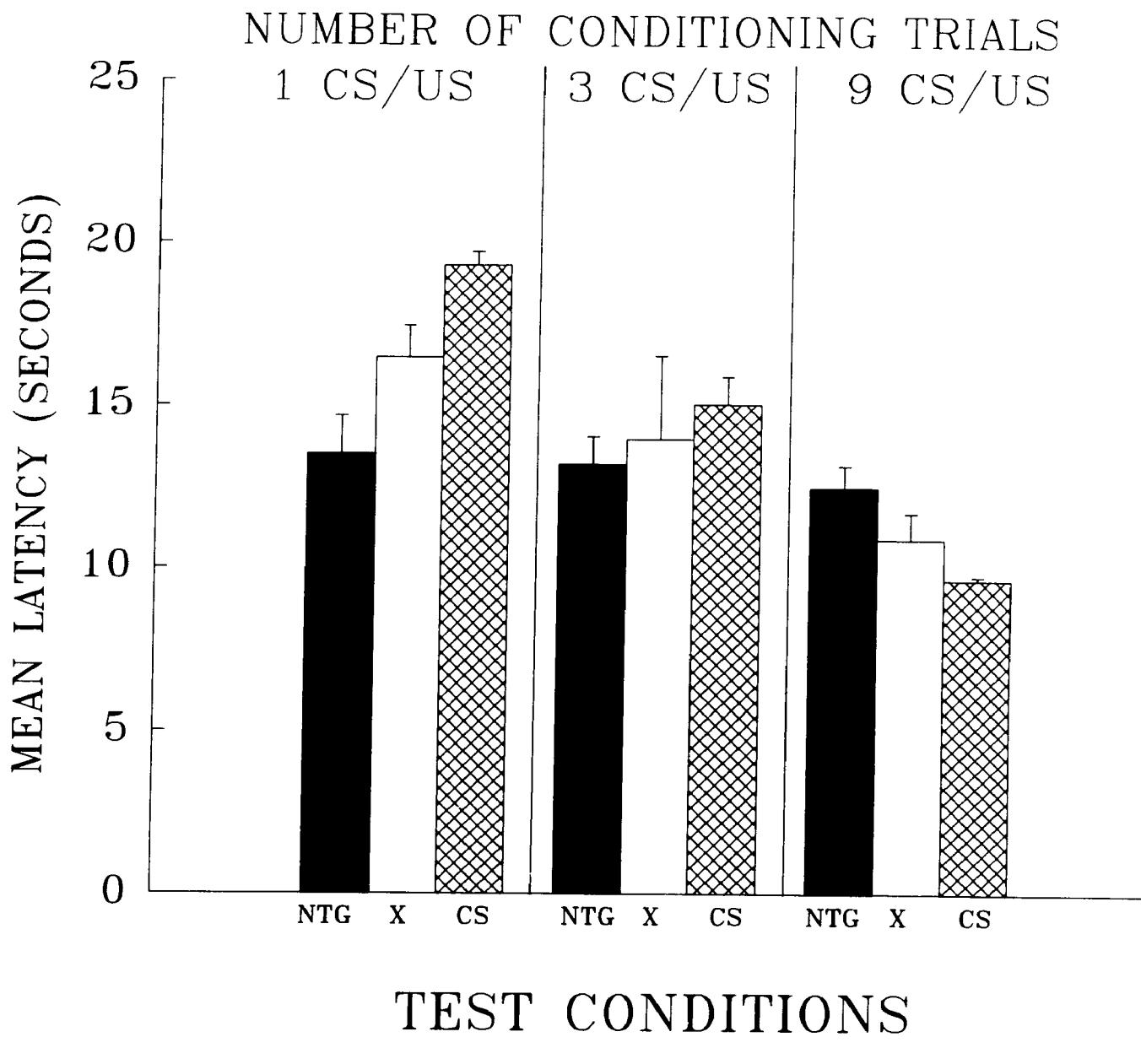
Finally, an alcohol/water two-bottle preference test assessed the propensities of these inbred rodent lines to consume alcohol. Subjects were transferred from their home cages to similar individual cages on a rack facing their home cages in the colony room. These test cages contained more Wayne rodent chow than the subjects could consume given the 20 minute duration of the test. Subjects were given ad lib access to both a 5% alcohol solution and tap water for 20 minutes before being returned to their home cages.

### **III. Results and Discussion**

#### **A. Experiment 1.**

Figure 2 represents the mean latency to produce a coping response in the three test conditions of the hot plate algesia measure during the first phase of Experiment 1. Subjects' latencies to emit coping responses (either jumping from the hot plate or licking a hind paw) were measured after exposure to one of three test conditions: exposure to the CS in the conditioning chamber context (CS), exposure to the conditioning chamber context (X), or exposure to the transportation cart and the room in which the conditioning chambers and the hot plate testing apparatus were located (NTG).

A  $4 \times 4$  ANOVA was applied to the each of the three sets of hot plate test data. Each ANOVA consists of two factors. One factor is comprised of four levels representing the 4 US groups (low shock & low duration, low shock & high duration, high shock & low duration, and high shock & high duration). The other ANOVA factor consists of four levels in which the CS condition is represented twice; as n for the



**Figure 2.** Mean hot-plate latencies after one, three, and nine conditioning trials in Experiment 1 for four US intensity-duration groups given no treatment (NTG) or an exposure to the conditioning context (X) or the CS in that context immediately prior to the test. Aversive footshock (US) was only presented on conditioning days and not during testing.

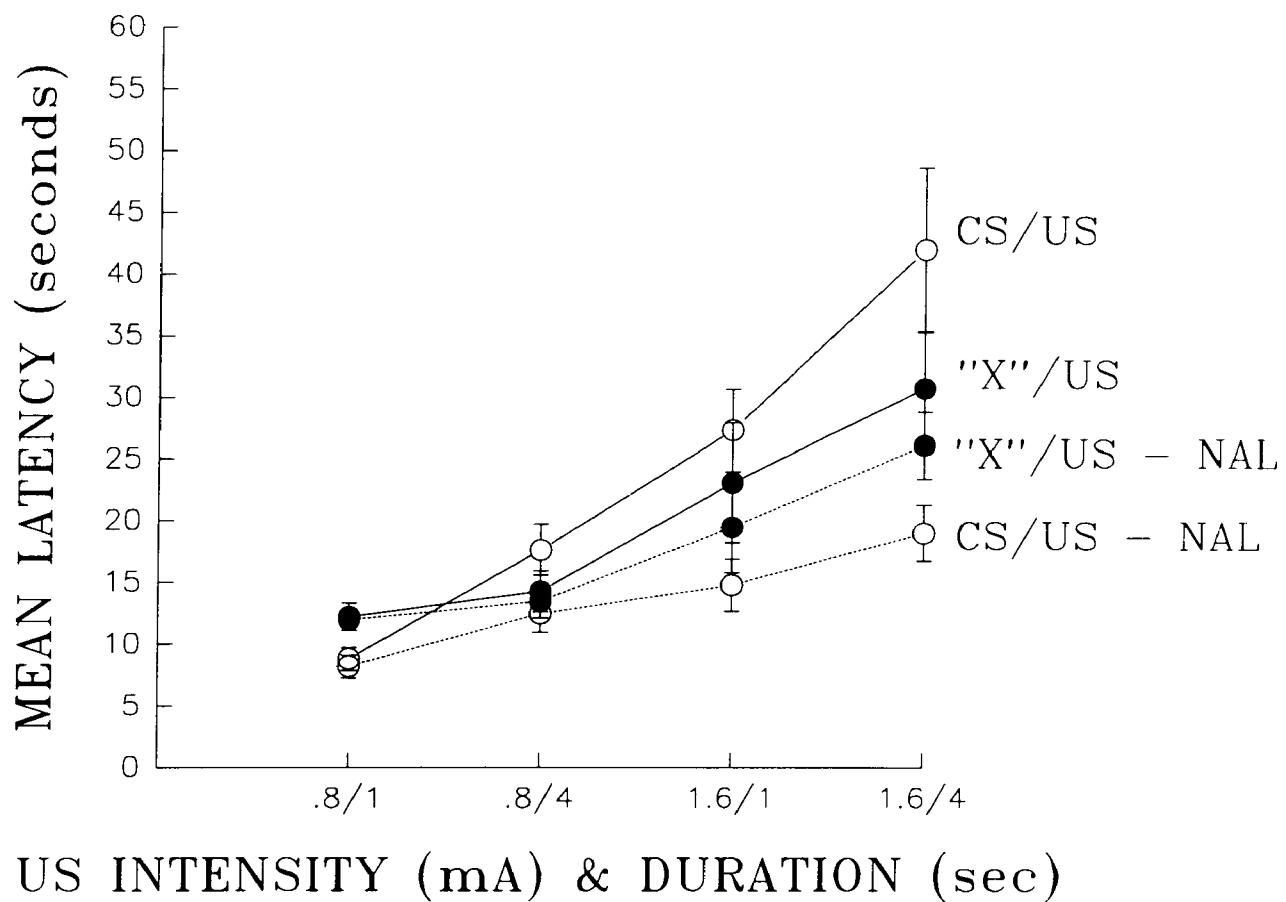


Figure 3. Mean hot-plate latencies after 10 conditioning trials in Exp.1 for four US intensity-duration groups that were exposed to either US alone or the CS/US compound immediately prior to the test. NAL indicates that an injection of naloxone was given 15 mins prior to test.

CS condition is twice as large as both the NTG or the "X" condition. Hence, the 3 X 4 experimental design was analyzed with a 4 X 4 ANOVA (see Table 1).

The left panel of Figure 2 shows the mean hot plate (HP) latencies for the three test conditions after one CS/US training trial. A 4 X 4 ANOVA indicated a significant difference between the conditions after one conditioning trial,  $F(3,57) = 3.21, P < .03$  (see Table 1). ANOVA contrasts revealed that subjects in the CS condition were significantly more analgesic than those of the NTG condition,  $t(1,57) = 2.99, P < .005$  as well as more analgesic than the NTG and X conditions combined,  $t(1,57) = 2.72, P < .01$  (Table 2C). However, no differences were detected between the "CS" and "X" test conditions. No differences were found between the high and low US-intensity training groups or between long and short duration of US training groups.

The middle panel of Figure 2 represents the mean hot plate latencies after 3 CS/US conditioning trials. There were no differences between the US groups or test conditions, (see Table 3).

The right hand panel of Figure 2 (see Table 5A) shows mean hot plate latencies for the three test conditions after nine CS/US conditioning trials. An ANOVA contrast found, once again, a difference between the conditions of CS and NTG exposure,  $t(1,59) = 3.67, P < .0006$ , as well as a difference between the CS condition contrasted with the NTG and X conditions combined  $t(1,59) = 3.24, P < .003$ . The differences, however, indicate that the CS alone condition now produced a hyperalgesic response relative to the NTG control (see Tables 5) in contrast to the hypoalgesia elicited after 1 CS/US training trial.

Once again, no differences were detected between the CS and the X conditions. Again, no differences were found between the high and low US intensity groups, nor

between the long and short duration US groups in either the "CS" or the "CS & X" test conditions (see Table 5A).

Figure 3 represents mean hot plate latency to produce a coping response of the four US intensity and duration groups after 10 conditioning trials. Subjects were tested after exposure to their respective group CS/US compound or to their respective USs after receiving either no injection or an injection of naloxone. A 4 (US groups=4) X 2 (Nal Injection or no Injection) X 2 (CS/US or US-alone) ANOVA was used to analyze the data in this fourth and final hot plate test (see Tables 6 through 10).

ANOVA contrasts (see Tables 9 & 10) indicated that hypoalgesia increased with an increase in shock duration,  $t(1,122) = 4.28$ ,  $P < .0001$  and increased with increasing shock intensity  $t(1,122) = 8.94$ ,  $P < .0001$ . Over all training groups and test stimuli conditions collectively, naloxone injections decreased hot plate test hypoalgesia,  $t(1,122) = 4.28$ ,  $P < .0001$ . There was an effect of naloxone on the response to the CS for the higher US intensity groups  $t(1,122) = 10.15$ ,  $P < .0001$ . There was no significant effect of naloxone injection on the hypoalgesia of subjects exposed to the test condition of US-alone.

The primary hypotheses of Experiment 1 were confirmed: Increasing shock duration or shock intensity was associated with increased conditioning of hypoalgesia when tested following exposure to CS/US compounds. This hypoalgesia was reversible by a naloxone injection for the three US intensity/duration groups with appreciable conditioned hypoalgesia.

A consistent absence of differences between the "CS" and "X" conditions and the consistent median latency score of the "X" group between the CS and NTG groups is noteworthy as it is consistent with empirical and theoretical work of other investigators indicating that conditioning occurs to contextual stimuli.

The general model of Pavlovian conditioning by Rescorla and Wagner (1972) accounts for the conditioning process in terms of the predictiveness or informativeness of stimuli. According to this theory, during early training, the conditioning context (e.g., the chamber) provides implicit background cues that after one training trial accrues a modicum of informativeness regarding the US event. Hence, the conditioning chamber may accrue a moderate amount of Pavlovian conditioning early in training while after many trials a salient and discrete CS immediately preceding the US would be maximally informative and hence maximally supportive of Pavlovian conditioning.

Empirically this pattern of differences is consistent with observations of conditioned hypoalgesia to contextual stimuli (Fanselow & Baackes, 1982; Hayes et al. 1978; MacLennan et al. 1980; Sherman et al. 1984; Watkins et al. 1982) indicating that analgesic conditioning or analgesic potentiation occurs to both discrete CSs and to the conditioning context in which they were trained.

This conception of contextual cues as subtle conditioning stimuli is consistent with the Experiment 1 data from the left and right hand panels of Figure 2. In both of these Figure panels the latencies of the subjects exposed to the context are intermediate to the CS and NTG conditions. Moreover the "X", or context, condition was not significantly different from the latencies of the "CS" condition subjects as one may expect if conditioning were indeed accruing to both "X" and "CS".

The central panel in Figure 2 shows the mean hot plate latencies of subjects in the three test conditions. This panel is essentially a snapshot of the transition process from a conditioned hypo-algesia after one conditioning trial to the conditioned hyperalgesia after nine conditioning trials. The absence of any difference between any training group or between any test condition reflects the large variance of individual response

found between subjects within the same groups and conditions during a very transitory state of learning.

## B. Experiment 2. Results and Discussion

Three time periods of bolus droppings were used as measures of "emotionality": during transportation to the conditioning chamber room (B1), during exposure to the conditioning chambers (B2), and during transportation back to the colony room (B3), see Tables 11a - 11i. Figure 8 shows that the M520 rodent strain, in terms of total bolus responding, was more emotionally reactive than the F344s on days of exposure to "X". Similarly, the M520s produced significantly more vocalizations during handling procedures on the second day of exposure to the conditioning chambers  $F(1,46) = 7.96$ ,  $P<.007$ , see Table 12b. Figure 4b presents the partial measure of emotional bolus production (B1) that occurred daily as the subjects were being transported to the conditioning chamber context, "X". Figure 4c shows the partial measure of emotional bolus production (B2) by subjects during daily "X" conditioning chamber, or "context" exposures. Figure 4d shows the emotional bolus production of subjects during transportation back to the colony room home cages after "X" exposure. The fifth day of transportation to the conditioning chamber room involved a cold plate exposure without "X" context exposure and hence data for that day are not included in Figures 4a-d because they present the data for "Days of exposure to the conditioning chamber". On the seventh day of transportation to the conditioning chamber room all subjects received a single trial of aversive CS/US conditioning. The substantial influence of this fear conditioning event is reflected in the increased boli production of both the F344s and the M520s (an increase of 4.12 and 1.52, respectively; see Tables 11c -d). For both genetic lines a substantial increase in boli production was followed

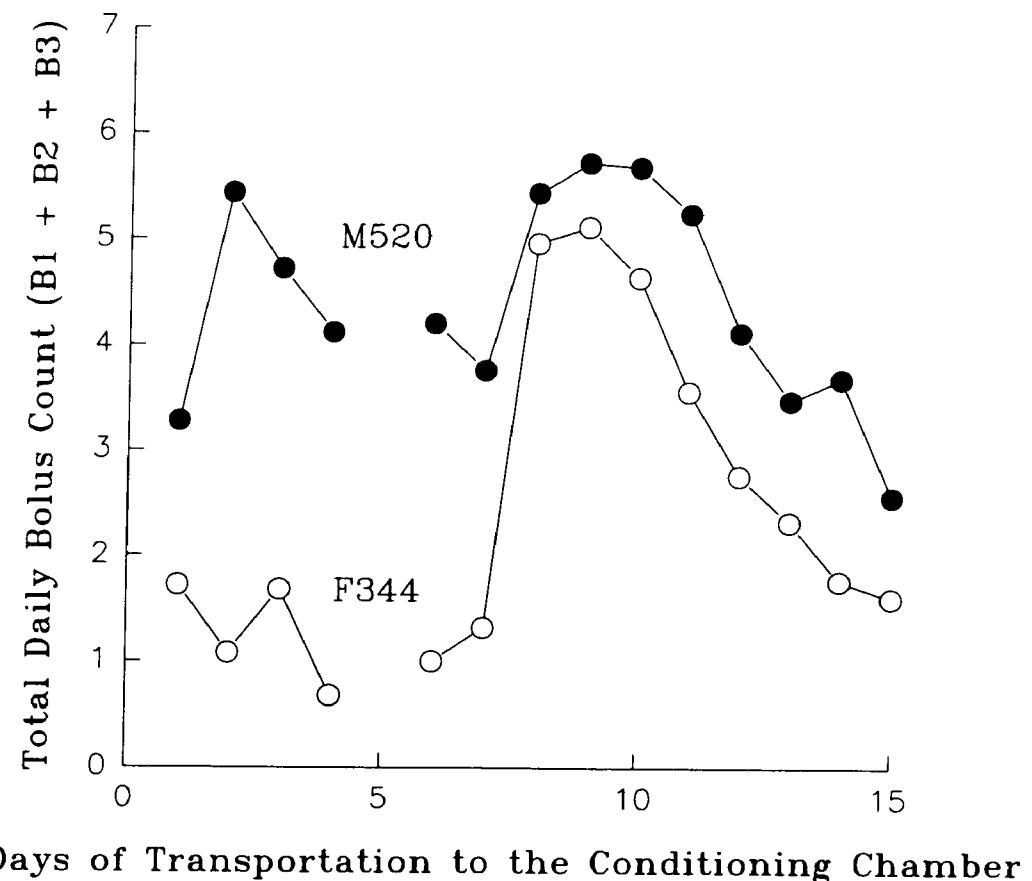


Figure 4a. Daily average bolus counts of two strains of rats including boli produced during transportation to conditioning chambers (B1), while in the conditioning chambers (B2), and during transportation from the conditioning chamber (B3) to the homecage colony room. Filled circles represent the average boli of 25 M520 subjects while the open circles represent the average boli of 25 F344 subjects.

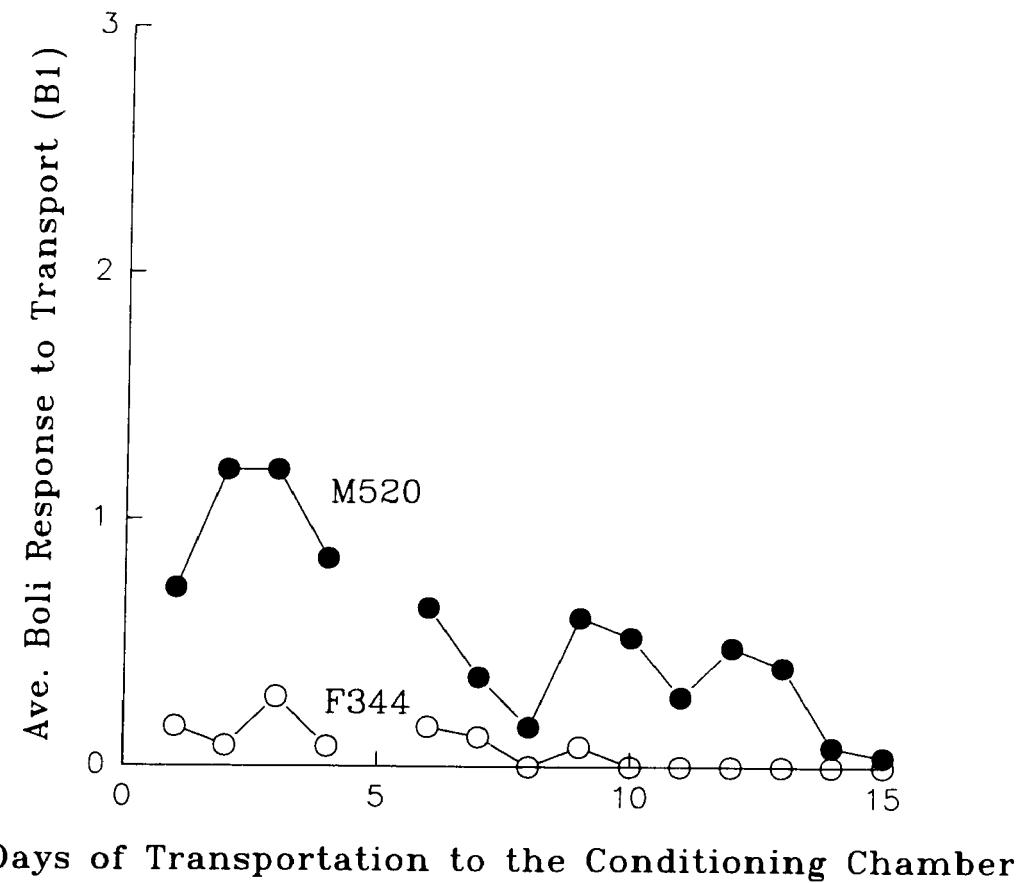


Figure 4b. Daily average bolus counts of two strains of rats during transportation to the conditioning chamber room, (B1). Filled circles represent the average boli of 25 M520 subjects while the open circles represent the average boli of 25 F344 subjects.

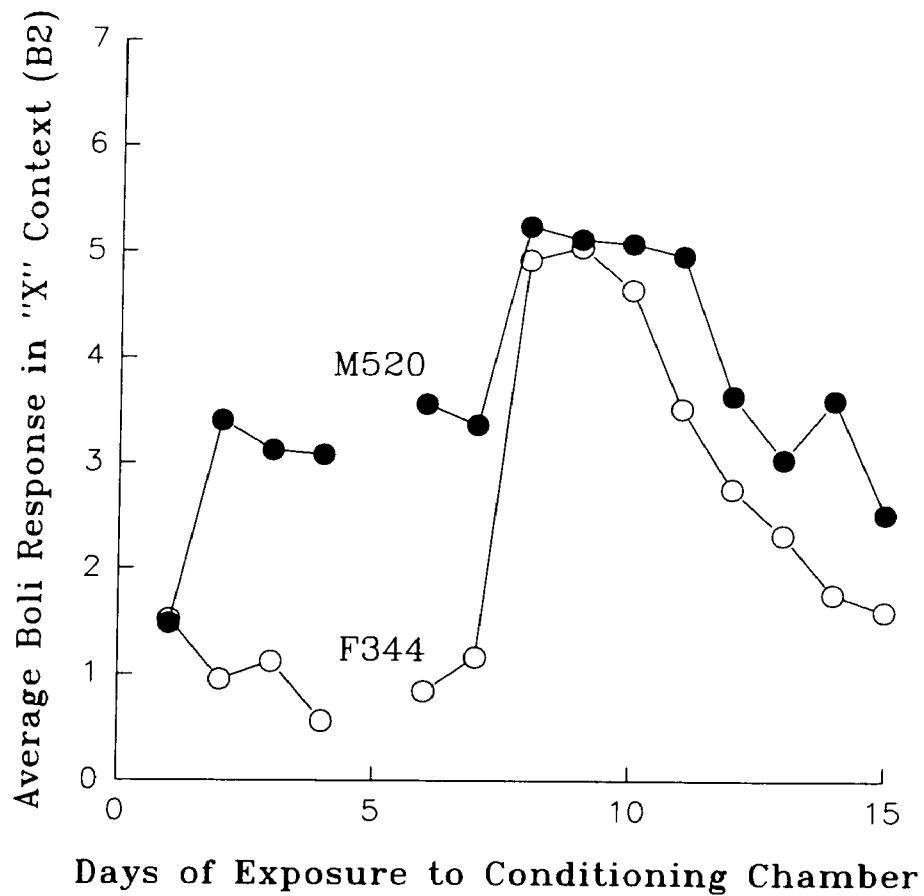


Figure 4c. Daily average bolus counts of two strains of rats during 8 minute exposure to the conditioning chambers, (B2). Filled circles represent the daily average boli of the 25 M520 subjects while the open circles represent the average boli of 25 F344 subjects. Day 5 involved a cold plate exposure without "X" exposure. Day 8 was a CS\US conditioning day.

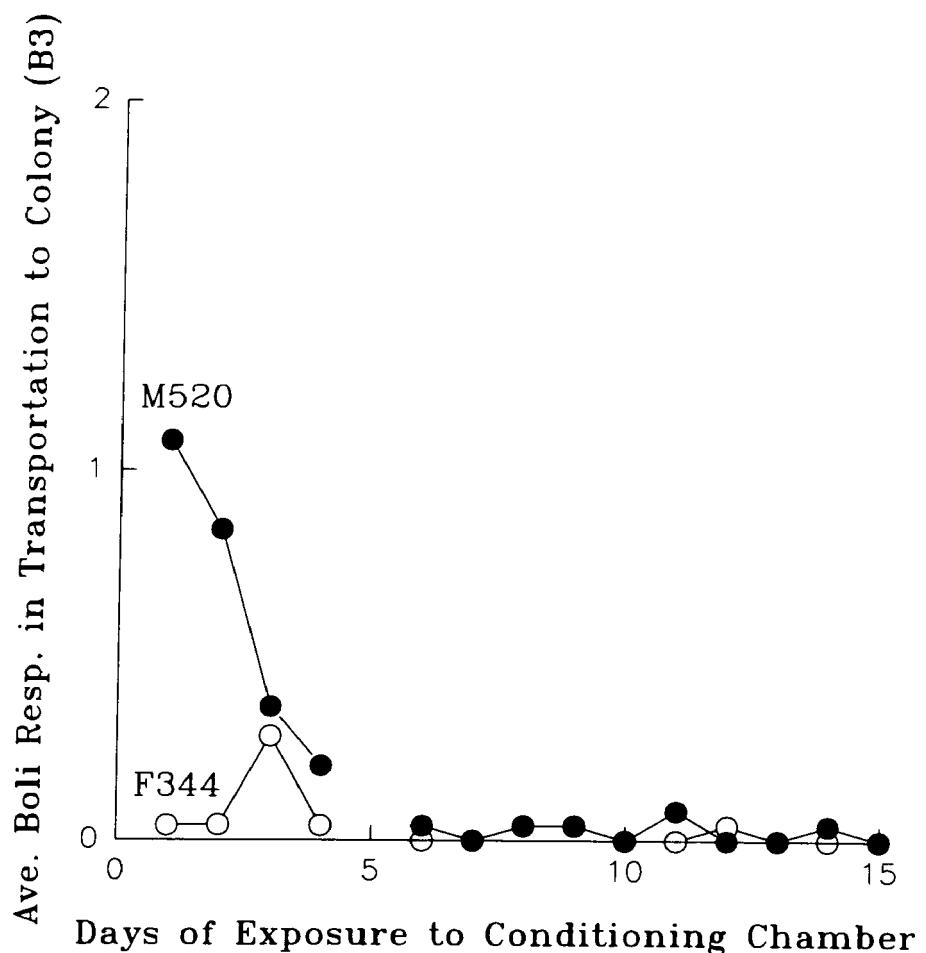


Figure 4d. Daily average bolus counts of two strains of rats during transportation from the conditioning chamber, (B3). Filled circles represent the daily average boli of the 25 M520 subjects while the open circles represent the average boli of 25 F344 subjects. Day 5 subjects were exposed to the cold plate rather than the "X" context.

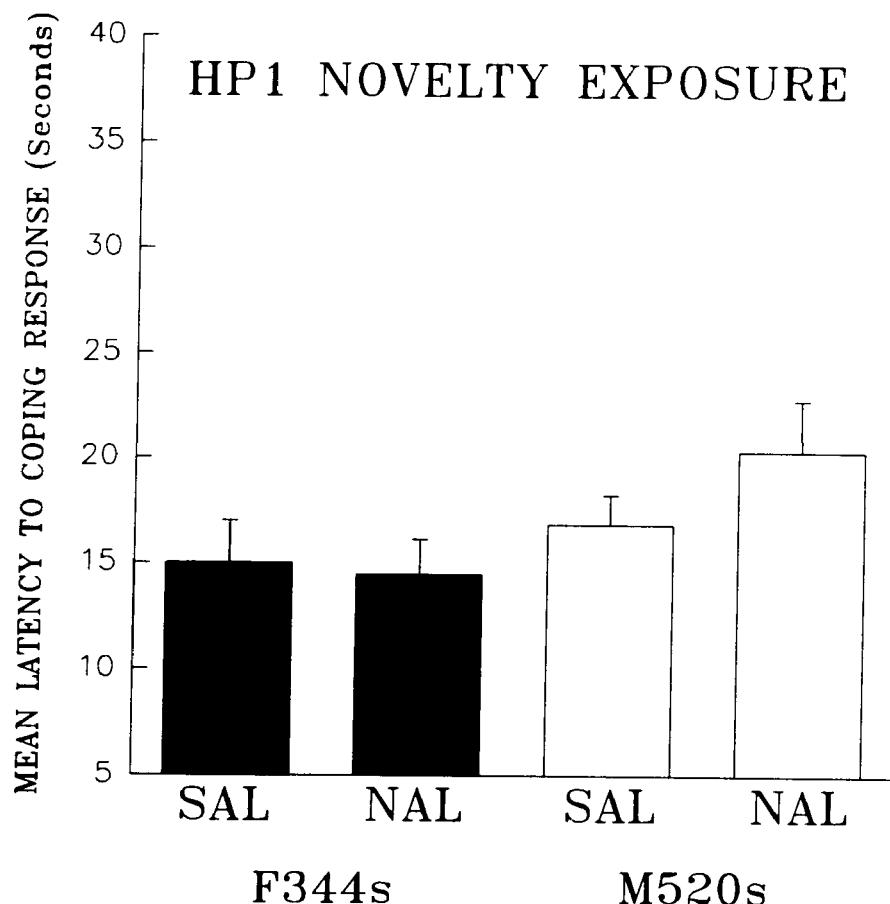
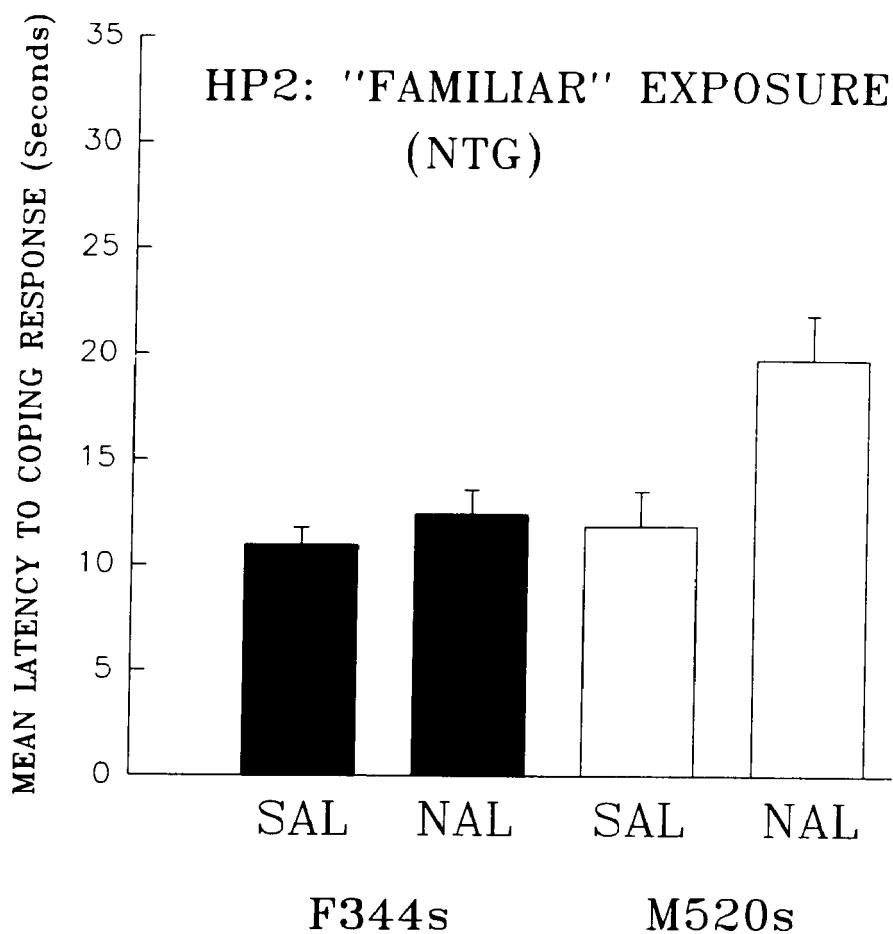
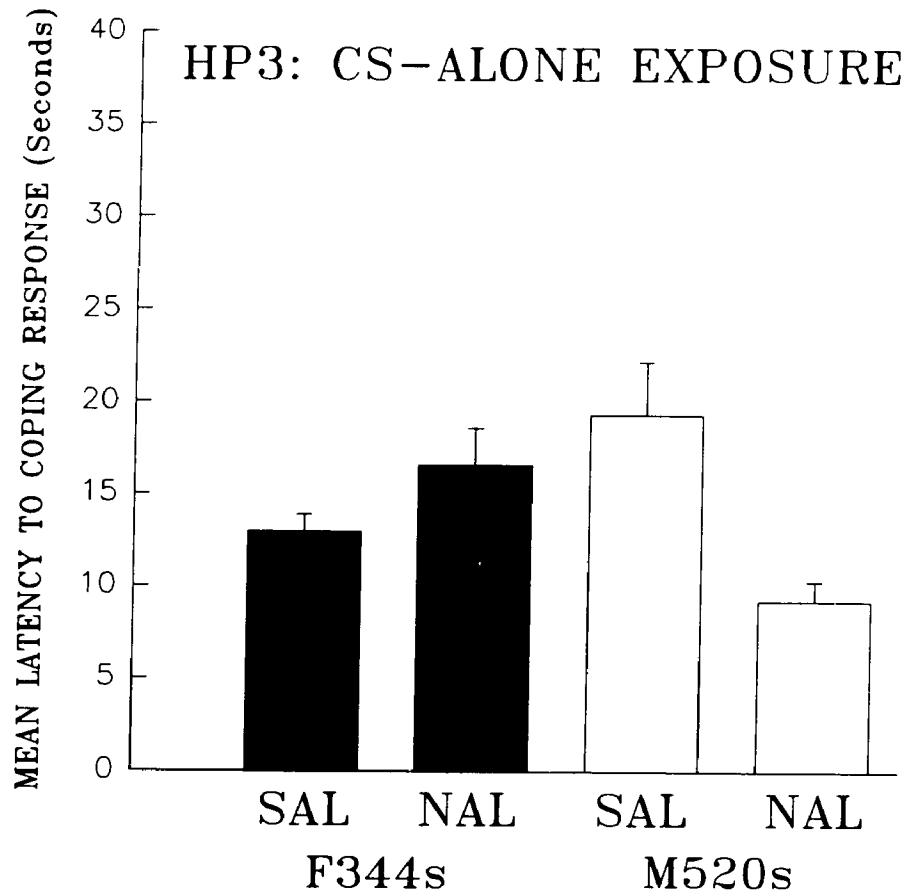


Figure 5. The first hot plate test of Experiment 2 compares the latency to coping response by male F344 ( $n=25$ ) and M520 ( $n=25$ ) rats. Subjects were injected wth either saline (1 ml/kg) or naltrexone (2 mg/kg) and placed in a novel context for 30 minutes prior to test.



**Figure 6.** The second hot plate test of Experiment 2 compares the latency to produce a coping response by F344 ( $n=25$ ) and M520 ( $n=25$ ) rats. Subjects were injected with either saline (1 ml/kg) or naltrexone (7 mg/kg) 30 minutes prior to placement into the familiar "X" conditioning context for 2 minutes prior to test.



**Figure 7.** The third hot plate test of Experiment 2 compares the latency to produce a coping response between F344 and M520 rats. Subjects were injected with either saline (1 ml/kg) or naltrexone (7 mg/kg) 30 minutes prior to placement into the conditioning context, "X". After 2 mins of "X" exposure, subjects were presented with the tone CS which had received only one conditioning trial.

by a gradual decline back to levels of defecation that were at, or below, baseline levels after eight more days of "X" exposure. This pattern of boli responding is consistent with the operationalization of "emotionality" or fear as increased bolus production. Continued exposure of both M520s and F344s eventually resulted in near zero bolus production. In terms of Pavlovian conditioning processes, presentation of the CS/US compound should increase fear to the conditioning alone context, while repeated exposure to the unreinforced conditioning context should result in extinction of fear responding to the conditioning chamber. The predictions of Pavlovian conditioning theory are reflected in the bolus data over the 15 day period shown.

Figure 5 shows the results of the first hot plate test in which both the F344 and the M520 strain were exposed to novelty after being injected with either saline or naltrexone. An ANOVA contrast of these data (see Table 13b) showed no effect of naloxone on hypoalgesia though a marginal difference was present between strains,  $F(1,46) = 4.15, P<.05$ .

The principal hypothesis of HP1 was not supported: The M520 data presents no indication of an endogenous opioid analgesic response to novelty. In fact, the average hot plate latencies of the M520 naltrexone group was significantly greater than the other three groups  $F(1,46)= 5.02, P<.03$ . This hypo-algesic reactivity of the M520s/NAL group is consistent with the genetic trait for emotional hyper-reactivity shown above. Figure 4a indicates the emotional hyper reactivity of the M520s to environmental events. Similarly, Figures 5 and 6 indicate that the M520s are pharmacologically hyper-reactive to naltrexone. This hypoalgesic response to naltrexone is consistent with several reports of hypoalgesia to another opioid receptor blocking drug, naloxone, as well as findings that naloxone enhances hypoalgesia to novelty (Rochford et al 1993; Rochford & Dawes 1992; Rochford & Stewart, 1992). These effects are increased by the noradrenergic

receptor antagonist yohimbine and inhibited by the noradrenergic alpha-2 agonist clonidine. Hence it appears that both the hypoalgesia induced by novelty and that induced by opioid blockers are regulated through the noradrenergic system. It appears that the hypoalgesic system of M520 rats is hyper-reactive to both novelty and naltrexone, and this may be due to the hyper-reactivity of the M520 noradrenergic system by both novelty and naltrexone.

An important consideration for the evaluation of the CEOA model is whether the M520s and F344s are genetically predisposed to differ in unconditioned pain sensitivity or if the differences emerge under conditions of substantial stress reactivity. This issue is addressed by comparison of the average HP latency scores for the F344/SAL and the M520/SAL groups. These groups were not statistically different, though the M520/SAL group was elevated almost 2 seconds.

Figure 6 shows the results of the second hot plate test in which both strains were again injected with either saline or naltrexone prior to the hot plate test. During this second hot plate test however, all subjects were exposed to the habitual daily experimental cues such as the transportation cart (NTG) and chamber room. An ANOVA contrast (see Table 14) showed a highly significant difference of genetic line  $F(1,44)=8.00, P<.007$  and injection type  $F(1,44)=9.56, P<.004$ , which was primarily due to an unexpected analgesic effect of the 7 mg/kg naltrexone injection in the M520 strain,  $F(1,44)=12.74, P<.0009$ . The saline injected M520s did not show any trend toward greater hypoalgesia than the F344s,  $F(1,44)=.06, P=.8$ , nor did the naltrexone injected F344s show any hypoalgesia relative to their saline injected counterparts,  $F(1,44)=.65, P=.42$ . Moreover, the absence of a difference between the saline injected F344s and saline injected M520s during familiar conditions underscores that the distinctions between these strains may be primarily a function of reactivity rather than

basal pain sensitivity. This pattern of reactivity to drug is consistent across both HP1 and HP2.

Figure 7 shows the latencies for hot plate test three (HP3) in which all subjects were presented with the CS by itself 23 days after a single CS/US training session. ANOVA contrasts (see Table 15b) confirmed that there was no main effect of genetic line on hotplate latencies and only a marginal trend toward a main effect of injection,  $F(1,44)=3.98$ ,  $P=.053$ . However, a large effect of naltrexone injection was present within the M520 rodents,  $F(1,44) = 19.3$ ,  $P<.0001$ , indicating a substantial opioid component was present in the hypoalgesic response of the M520/SAL group.

Moreover, **the F344s data gave no indication of an opioid analgesic response to the CS**. In fact, a weak trend of augmented hypo-algesia was induced in the F344s by naltrexone. Relative to the saline control groups, the increased latency in the F344/NAL group contrasted with the decreased latency of the M520/NAL group resulting in a significant genetic difference between the naltrexone groups in HP1,  $F(1,40)= 10.27$ ,  $P<.003$ . This is to say that genetic line influenced the direction of the naltrexone effect.

ANOVA contrasts between the "NTG" exposure condition of HP2 and the "CS" exposure condition of HP3 presented an opportunity assess the effects of the CS (see Table 16b). The saline injected, F344/NTG group was not different from the saline injected F344/CS group. However, a strong CS analgesic effect was present in the saline injected M520s,  $F(1,87) = 5.16$ ,  $P<.025$ . The increased latency of the M520/SAL group in HP was naltrexone reversible in HP3  $F(1,44) = 19.30$ ,  $P<.0001$ , presenting an interaction of injection type with the genetic line of rodent.

In sum, the high alcohol consuming M520s are hyper-reactive to environmental stressors and the effects of opioid blocking drugs relative to the low alcohol consuming

F344s. Most importantly, the high alcohol consuming M520s conditioned endogenous opioid activity when the low alcohol consuming F344s do not.

### C. Experiment 3. Results and Discussion

Genetic variability in analgesic response to morphine was assessed in the first and second hot plate tests of Experiment 3 (HP1A & HP1B). The results of these tests are presented in Figure 8 and Figure 9. Tables 17 and 18 contain the results of the first hot plate latency test, HP1A. ANOVA contrasts confirmed a main effect of genetic line on the HP1A latency scores. The increased latency of 11 seconds in the M520 line relative to the F344s in HP1A was a significant effect of genetics,  $F(1,20) = 9.81, P<.006$ .

Type of injection (1 mg/kg morphine or saline) produced only a marginal trend in the M520 line,  $F(1,20) = 3.63, P=.07$ . Most surprising, the direction of this trend of the 1 mg/kg morphine injections relative to the saline injections was toward a hyperalgesic effect!

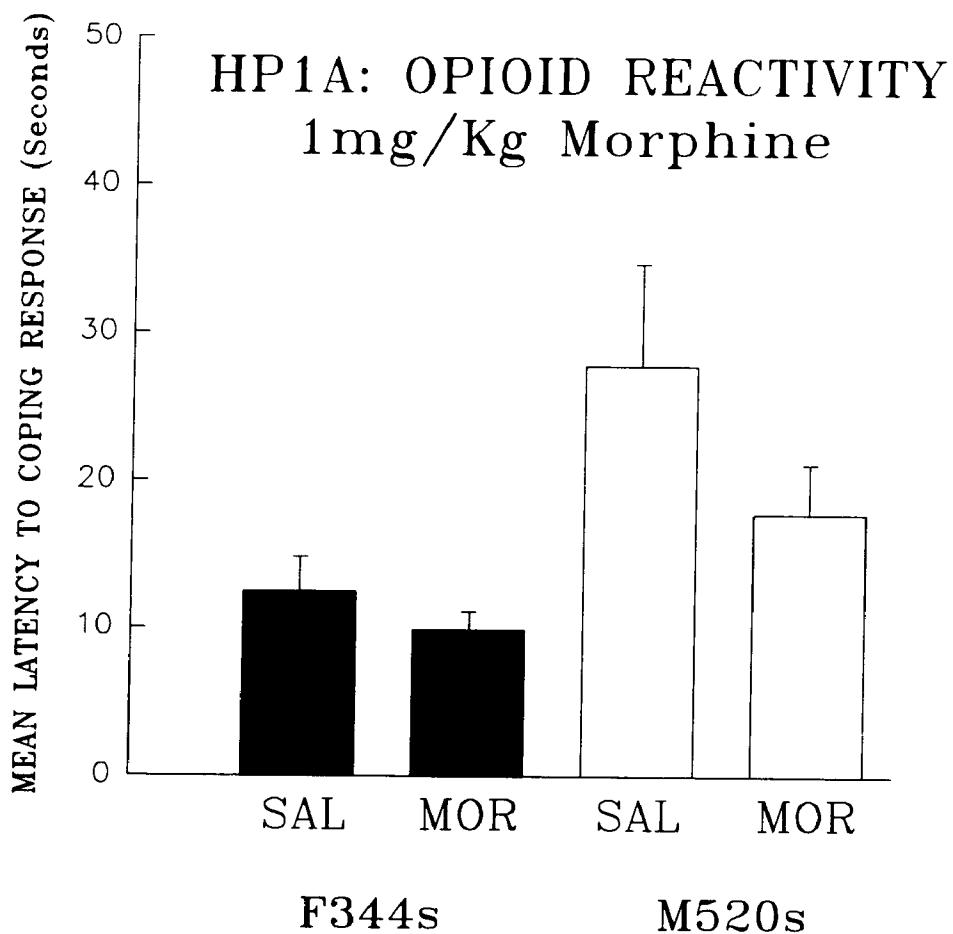
This hyperalgesic action of morphine is not without empirical precedent or theoretical support. Researchers have found that application of low doses of peripheral morphine induces an hyperalgesic effect (Van der Kooy & Nagy, 1985; Hughes 1992). Moreover, hot plate assessments in novel contexts in Experiment 3 and Experiment 2 found opioid blockers did not decrease latency measures of pain sensitivity. Hence it would appear likely that some other analgesic system may be primarily activated during these challenging stressors of novelty (Rochford & Dawes, 1993 ;Lictman & Faneslow, 1991). It has been reported that opioid analgesic and serotonergic analgesic systems may be mutually inhibitory though others report the mutual dependence of these systems (Xu. et al, 1994; Matos et al, 1992). Hence, a small dose of opioid, such as 1

mg/kg in a rat model may possibly block some other analgesic system should it be activated during the hot plate test.

Alternatively, a small dose of morphine may, as an anxiolytic, decrease DAS-fear responding. In effect, a morphine anxiolytic effect may induce a decrease in the activity of the autonomic and neuro-endocrine stress response systems that activate hypoalgesic response systems that may be of either an opioid or non-opioid nature. This anti-analgesic effect of anxiolytics has been demonstrated by Fanselow & Helmstetter (1988). In essence, there are two alternative explanations for the relative hyperalgesia of the M520 morphine group vis a vis the M520 saline group. First, the morphine may have interfered with the nonopioid analgesic systems in a purely pharmacologic sense that did not impact the DAS fear responsiveness. Alternatively, the morphine injections may have decreased the intensity of the unconditioned DAS fear response - effectively decreasing the activation of any opioid or non-opioid hypoalgesic systems.

These data, while surprising, are still consistent with an interpretation of hyper-reactivity in the alcohol self administering (M520) strain. Morphine did not substantially decrease hot plate latencies in the F344 rodents while it had a marginal trend toward significance,  $F(1,20) = 3.63$ ,  $P = .07$ , in the M520 strain, see Table 18. prompted a replication HP test using an increased dose of morphine (5 mg/kg) in the following test.

Figure 9 shows the mean HP1B latencies of the M520 and F344 rodents following morphine (5 mg/kg) or saline injections 30 minutes prior to test. ANOVA contrasts (see Tables 19 -20) revealed a significant effect of genetic line  $F(1,20) = 9.66$ ,  $P < .006$  and a trend for an effect of injection type,  $F(1,20) = 3.55$ ,  $P = .0741$ .



**Figure 8.** The first hot plate test of Experiment 3 compares the latency to produce a coping response between F344 ( $n=12$ ) and M520 ( $n=12$ ) rats after an injection either of saline (1 ml/Kg) or of morphine (1 mg/Kg) 30 minutes prior to test.

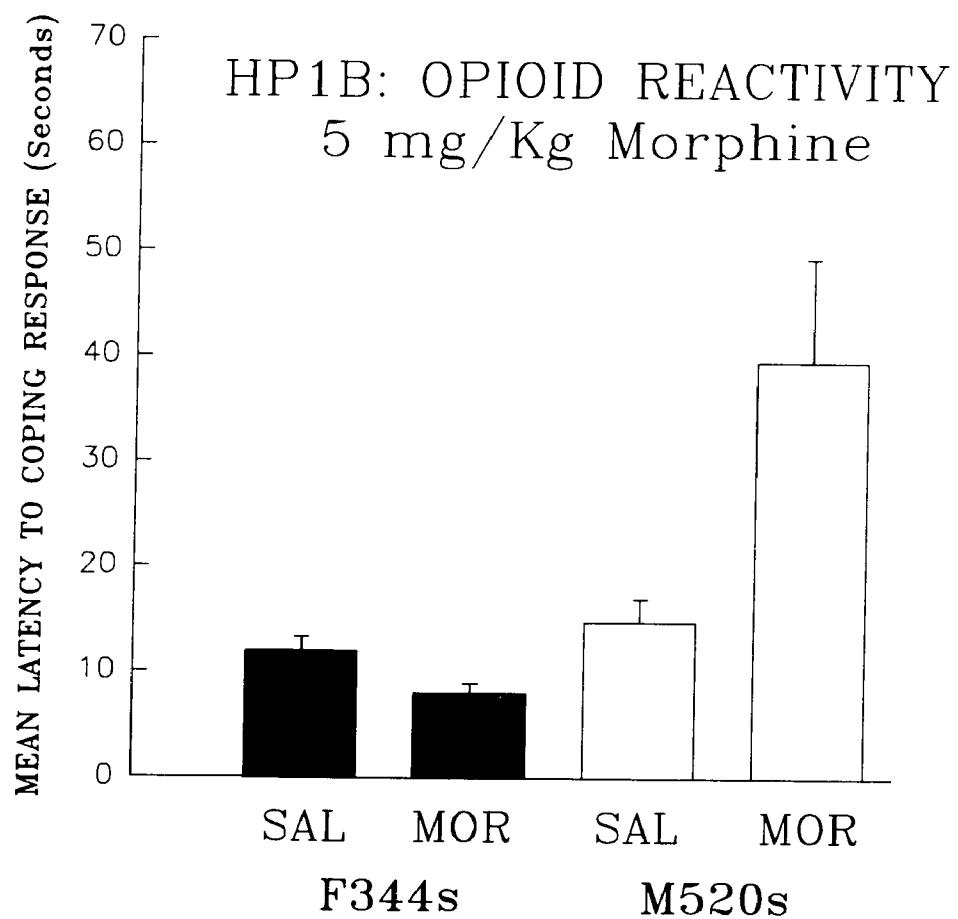


Figure 9. The second hot plate test of Experiment 3 compares the latency to produce a coping response in F344 and M520 rats after an injection of saline (1 ml/Kg) or morphine (5 mg/Kg) 30 minutes prior to test.

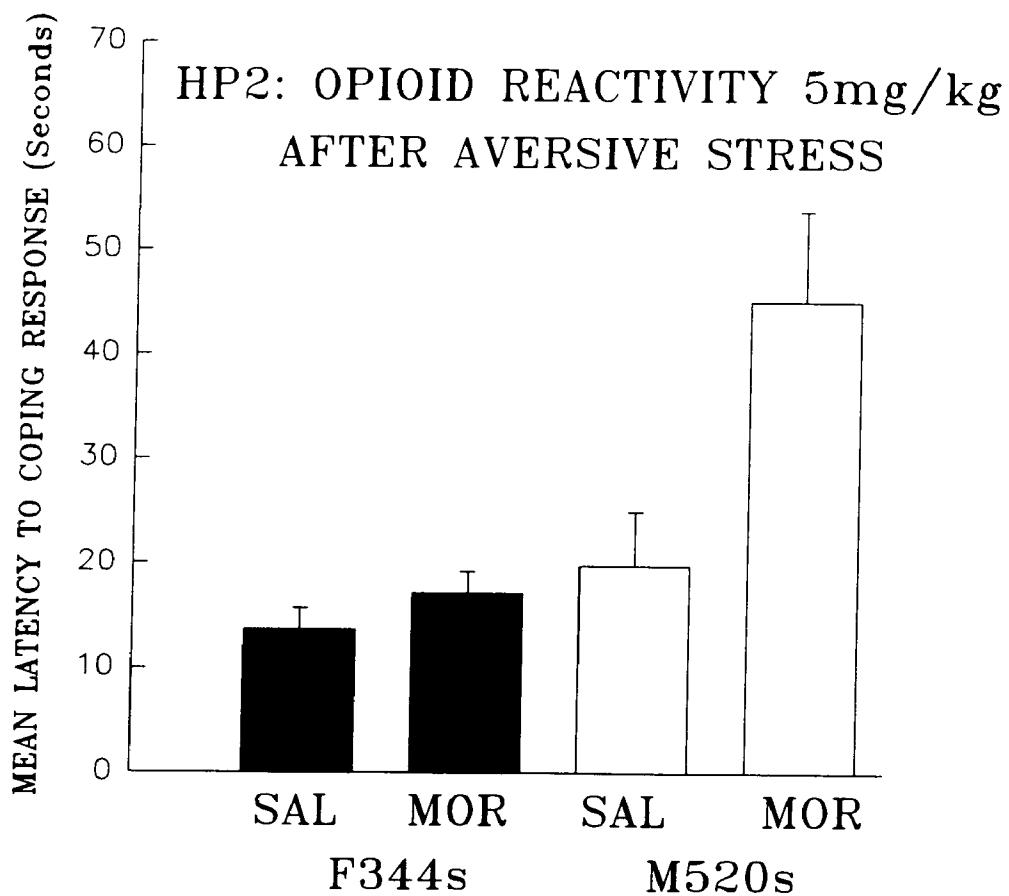


Figure 10. HP2 of Experiment 3 compares the hot plate test latencies of F344 and M520 rats after injection with either saline or morphine (5 mg/kg) 30 minutes prior to test. All subjects were transported to the chamber room (NTG) but were not placed into the conditioning chamber context (X). HP2 differed from HP1B only in that prior to HP2 all subjects had been exposed to the extensive stress exposure of aversive CS/US conditioning over the prior two week period.

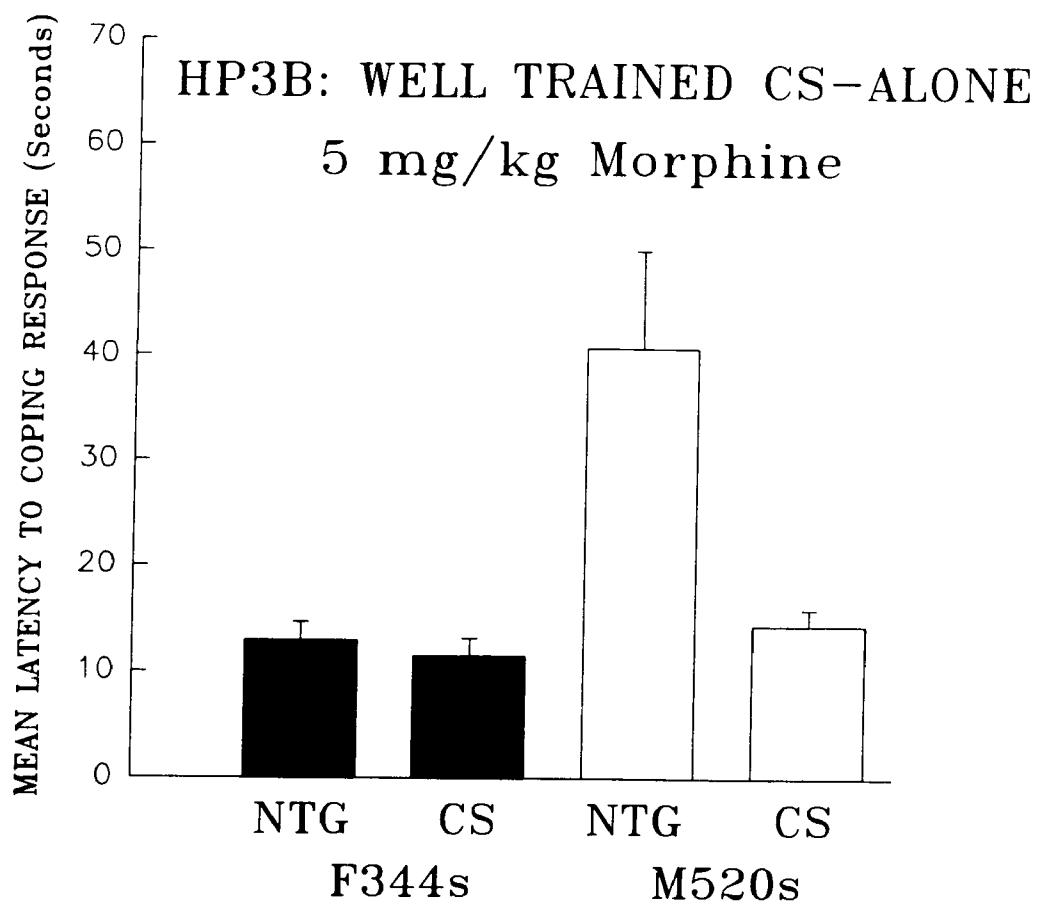


Figure 11. HP3B of Experiment 3 compares the hot plate test latencies of F344 and M520 rats after exposure to either the transportation cart (NTG) or a well conditioned aversive CS. All subjects were injected with 5 mg/kg morphine 30 minutes prior to hot plate testing.

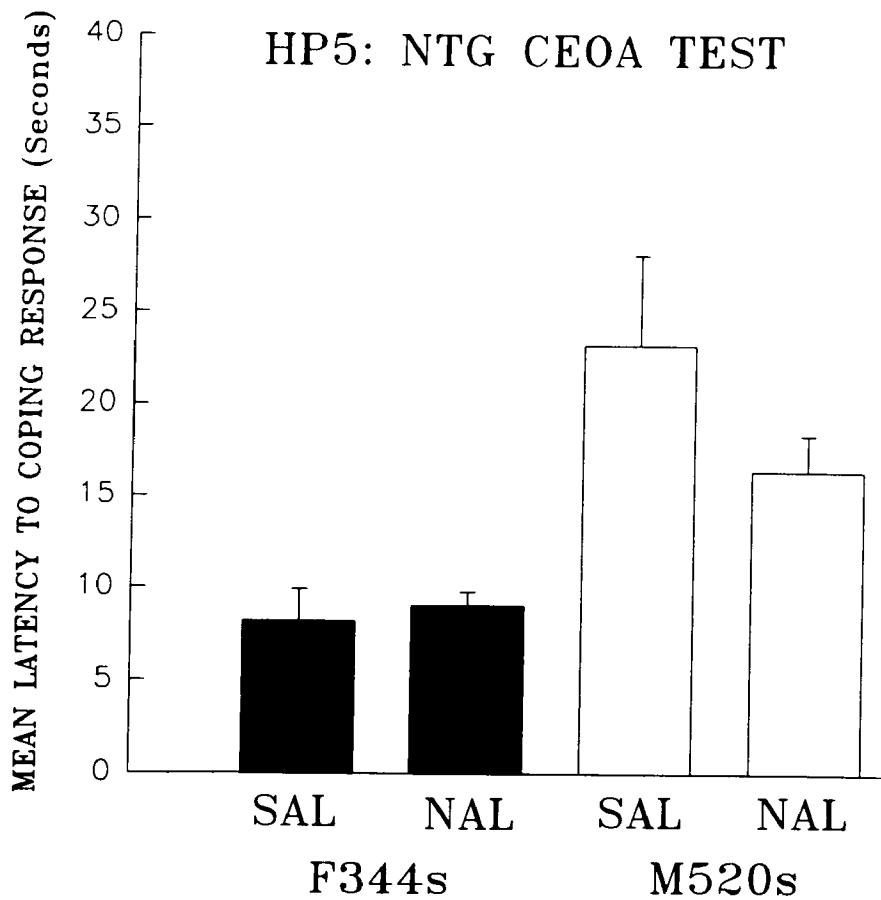


Figure 12. HP5 of Experiment 3 compares the hot plate test latencies of F344 and M520 rats after injection with either saline or naltrexone (7 mg/kg) 30 minutes prior to test. All subjects were transported to the chamber room (NTG) but were not placed into the conditioning chamber context (X) where explicit aversive CS/US conditioning had occurred.

The morphine injections increased the mean HP1B latencies of the M520 rodents by more than 24 seconds,  $F(1,20) = 10.06$ ,  $P<.005$ , while not significantly influencing the mean latencies of the F344s. This interaction presented a highly significant contrast of the M520/morphine group compared with the other three groups,  $F(1,20) = 19.22$ ,  $P<.0004$ .

Particularly worth noting is the hyper-reactive analgesic systems of the saline injected M520s as compared with the saline injected F344s in HP1A. In contrast to the extensive habituation of Experiment 2 prior to testing, the protocol of Experiment 3 involved **no habituation** to transportation, to the conditioning chamber room or to the colony room disturbances and handling of test day protocols. In essence, the first hot plate test of experiment 3 exposed the subjects to the most intense "novelty condition" of all the hot plate tests reported on here. Hence, the profound analgesia of the M520/SAL group in HP1A (27.75 seconds) should be contrasted with the more normative latencies (12 - 17 seconds) of the M520/NTG groups in Experiments 1 & 2, as well as HP1B in Experiment 3. Thus the M520/SAL groups are profoundly **more reactive** to the effects of environmental stressors than the F344s. This significant difference between the saline injected F344s and M520s ( $F=8.55$ ,  $P<.009$ , see Table 18) dissipated by HP1B, (see Figures 13 - 14 and Table 20). This decreased latency of the saline injected M520 groups from HP1A to HP1B is attributed to habituation to transportation and the HP test procedure that was novel in HP1A but not in HP1B.

Figure 10, Table 21a, and Table 21b all show the mean hot plate test latencies of the next hot plate test, HP2, which followed HP1B after 11 days of aversive CS/US conditioning. Both the M520 and F344 rodents were given either morphine injections or saline injections and tested for HP latencies following exposure to just the transportation cart control condition designated as "NTG". In this test, a reduction or

habituation of analgesic response relative to that found in HP1B was anticipated. This development of opioid tolerance was expected as a result of the repetitive activation of opioid analgesic systems by the prior 11 aversive CS/US conditioning trials. This expectation of a reduction in the morphine induced hypoalgesic latencies of HP2 relative to those of HP1B was based on the theory that repetitive events CEOA should function to produce tolerance and opponent processes just as repetitive injection of morphine or any systemic mu-opioid receptor agonist would.

The data, however, tend to indicate instead that a sensitization to the psycho-pharmacological effects of morphine may have occurred and perhaps sensitization to the effects of the stressful testing procedures may have occurred as well. Compared with the saline group latencies from HP1B, the saline group latencies in HP2 (after exposure to daily aversive stress) increased an average of 3 seconds. Similarly, the morphine groups of both lines increased almost an average of 10 seconds after the 11 days of aversive CS/US conditioning. This increase in the morphine groups' hot plate test latencies was due primarily to the increase in the F344s sensitivity to the analgesic effects of morphine after the 11 days of aversive conditioning though the mean latencies of the M520s also increased. These sensitization effects to events and drugs are consistent with the sensitization of response to psychoactive substances in the CEOA risk model.

ANOVA contrasts (see Table 21b) of the HP2 data confirmed a main effect of inbred genetic rodent line  $F(1,21)= 8.85$ ,  $P<.008$ , and a main effect of injection type  $F(1,21) = 13.15$ ,  $P<.002$ . However, an induction of opioid tolerance by repetitive events of CEOA was not observed. The effect of primary interest, a putative conditioned opioid opponent process, would have been evident in a reduction of hot plate latency in HP2 relative to those of HP1B. This effect was clearly not present and

in fact, the mean latencies of all groups increased. However, the latency of coping responses over the course of several hot plate tests are influenced by operant learning factors as well. The coping responses are operantly reinforced by the removal of the aversive thermal pain. Over the course of successive hot plate tests the subjects learn to produce these operant responses more rapidly, decreasing the hot plate latencies. However, the large hiatus between HP1B and HP2 may have induced a "forgetting" or increased latency to produce a negatively reinforced operant or coping response. However, sensitization of the hypoalgesic systems to the action of opioid agonists would be an interpretation consistent with many reports of sensitization to drugs following exposure to aversive stimuli as reviewed in the introduction.

The data of HP2 (see Table 21b) once again presents a hyper-reactive psychogenetic profile of the M520 line. The M520/morphine group was significantly different from the M520/saline group. In contrast, the F344/morphine group was not significantly different from the F344/saline group. This difference can be ascribed to the differential reactivities of the F344 and M520 genetic lines to the drug, as the baseline controls, the M520 and F344 saline groups, were not different in HP2,  $F(1,21)=.83$ ,  $P=.3719$ . In contrast the F344 and M520 morphine groups showed a substantial difference,  $F(1,21)= 10.85$ ,  $P<.004$ .

Tables 22a and 22b present the results of the next hot plate test, HP3A, which was designed to see if a well trained CS-alone presentation could elicit a hyperalgesic response that could reduce the hypoalgesia induced by morphine injection. HP3A produced no significant differences between any groups although a marginal trend was found for effect of genetic type,  $F(1,21)= 4.25$ ,  $P<.06$  and the NTG/M520 group showed a trend toward being more hypoalgesic than all other groups,  $F=3.67$ ,  $P=.070$ .

Figure 11, Tables 23a, and 23b present the results of HP3B which replicated the experimental conditions of HP3A with the exception that the injection conditions were counterbalanced with those of HP3A. ANOVA contrasts confirmed the highly significant effects of genetic line differences  $F(1,21)=11.96$ ,  $P<.003$  that were due primarily to the unique hypoalgesia of the M520/NTG group  $F(1,21) = 29.54$ ,  $P<.0001$ . A strong effect of reduced latency was present in the CS group of the M520 rodent line  $F(1,21) = 17.73$ ,  $P = .0004$ ; while no relative decrease was present in the CS group of the F344s,  $F(1,21) = .2$ ,  $P=.65$ . The work of Wiertelak et al. (1994) indicates that this CS anti-analgesic effect may be due to cholecystokinin activity at the spinal level.

However, this decreased latency of the M520/CS group relative to the M520/NTG does not represent hyperalgesia. Rather the M520/CS score is similar to the normative values of prior NTG test data from several sources (e.g., see Experiment 1, Figure 2). Nor was the M520/CS group different from the F344s. However, the M520/NTG group mean latency score of 40 seconds indicated a profound morphine induced hypoalgesia relative to the normal range of NTG (control) latencies between 11 to 16 seconds in saline or no injection preparations (Figure 2 from Experiment 1, see Lysle, 1986; Lysle & Fowler, 1986). In sum, the M520/CS group was not different from F344s  $F(1,21)= .15$ ,  $P=.7$ , and in the range of normative NTG control scores indicating that the CS had reversed the analgesia induced by the morphine injections.

The effect of a well trained CS on saline injected rats was assessed in the next test, HP4, in which all subjects were placed in counterbalanced stimulus conditions relative to HP3B. These data are presented in Tables 24a & 24b. A profound effect of genetic line emerged  $F(1,17)= 22.24$ ,  $P<.0003$ , as did a marginal main effect of stimulus condition. This stimulus effect was due primarily to the difference between the

M520/NTG and M520/CS groups,  $F(17,1) = 5.28$ ,  $P < .04$ ; no similar difference was found between the CS and NTG groups of the F344S.

Figure 12 presents the results of a final hot plate test that exposed all subjects to the transportation cart (NTG) for 2 mins prior to testing. Subjects received either a naltrexone or a saline injection 30 minutes prior to test. This procedure was designed to assess the opioid component of the M520/NTG analgesic effect. ANOVA contrasts (see Table 25a - 25b) revealed a strong effect of genetics on hot plate latency  $F(1,21) = 18.78$ ,  $P = .0003$ , but only a trend toward naltrexone reversibility of the M520 transportation cart induced analgesic effect  $F(1,21) = 3.51$ ,  $P = .075$ .

Data from an M520 subject, which had heretofore not been included in the data analysis because it was an hermaphrodite, was included in an ancillary analysis (see Tables 26 a-b). This subject had run in parallel with the other subjects throughout the experiment. Inclusion of this subject into the ANOVA resulted in a significant effect indicating that the M520s had indeed conditioned endogenous opioid analgesia to the transportation cart  $F(1,23) = 5.44$ ,  $P < .03$ . In contrast, the F344s showed no indication of such conditioning  $F(1,23) = .05$ ,  $P = .82$ . M520/SAL group was uniquely analgesic  $F(1,23) = 18.85$ ,  $P < .0003$  and this analgesia was naltrexone reversible.

Finally, results of the alcohol self administration test can be found on Table 27 and Table 28 confirming the reports in the literature that the M520 genetic line prefers alcohol more than the F344 line,  $F(1,18) = 11.77$ ,  $P < .004$ .

#### IV. General Discussion.

In the current work support for the CEOA model was provided. For example Experiment 1 presents data showing that increases in the intensity and the duration of

the US were found to increase the CEOA. Given that environmental risk features for drug and alcohol use and addiction include a long array of aversive stressors (see Introduction and King 1995) the findings of Experiment 1 would suggest that environmental risk features enhance the CEOA in the lives of at-risk individuals.

Experiment 2 supported the CEOA risk model in the finding that the high alcohol consuming M520 line was indeed temperamentally hyper-reactive to environmental stressors such as novelty and aversive foot shock as measured by bolus and hot plate latencies. The M520s also were hyper-reactive to the analgesic effect of the naltrexone injections in both the “novel” and “familiar” contexts. Essential for the “hyper-reactivity” interpretation is the equivalence of the M520s and the F344s under the “NTG”/saline condition which demonstrates that the differences between the genetic lines of rodents result as a function of exposure to stressors though these genetic differences are not apparent under baseline or non-stressed conditions. This interpretation is consistent with the performance of the saline groups in Experiment 3 as well: The behavioral genetic differences were due to the stress of novel conditions and were not apparent in HP1b of Experiment 3. This result indicating differential reactivity of the M520 and F344 lines to environmental stress can be found by a comparison of the saline groups in Figure 5 with 6 and in comparison of Figure 8 with 9 which represent the first and second hot plate measures of Experiments 2 and 3. The subjects in Experiment 2 were habituated to the handling and transportation procedures over the 14 days prior to their first hot plate test (HP1) while the subjects in Experiment 3 did not undergo acclimation to experimental transportation protocols. The extensive exposure to the conditioning chamber room and transportation protocols of Experiment 2 resulted in no significant differences between the F344 and M520 saline injected groups during the first two hot plate tests. In contrast, the subjects in Experiment 3

received no exposure to the transportation protocols or to the conditioning chamber room prior to hot plate test HP1A and this test resulted in a substantial difference between the saline injected F344s and M520s. This hyper-reactivity of the M520s had substantially habituated by the next hotplate test, HP1B, see Figure 9 and Table 20a. Hence, the difference between the hot plate latencies of the F344s and the M520s appears not to be due to innate differences in activity of algesic systems but due to innate differences in the activation of hypoalgesic systems to environmental stressors or drug effects. It is the differential **reactivities** of the M520s and F344s to drug or environmental stimuli that account for the differences in hot plate latencies.

Experiment 2 & 3 showed that M520s were prepared to CEOA to both explicit CSs and contexts (NTG) that F344s did not. Experiment 3 also demonstrated that the M520 line, relative to the F344 line was genetically hyper-reactive to morphine injection, as well as hyper-reactive to naltrexone injection. Again, a pattern of hyper-reactivity to drug or an aversive environmental event was associated with the high alcohol preference M520 rodent line. This pattern of data supports the notion that the trait of hyper-reactivity to aversive events is based upon the same neurobiological substrates that support hyper-reactivity to addictive drugs.

This work represents a first step in the assessment of the validity of a CEOA risk model for alcoholism and drug addiction. The strongest test of the model would involve two sorts of assessments. One strong type of test for the model would involve assessment of the CEOA in all six pairs of rat lines that have been selectively bred for differences in propensity to consume alcohol. Perfect concordance of preparedness to CEOA with the predisposition for alcohol consumption would provide strong support for the model. The strongest support for the model would be rendered if positive

covariation of the phenotypes of CEOA with the phenotype of alcohol preference was maintained in the offspring of crosses of the high and low alcohol consuming lines.

Another strong test for the model would involve assessment of individual propensities of outbred rats (e.g., NIH/rats) which are maintained by breeding practices that ensure large genetic variation between individuals of the line. An assessment of the covariation of the phenotype of alcohol preference with preparedness for CEOA would similarly be supportive of the model.

Observations from the drug and alcoholism literature which are consistent with the CEOA model are worth noting. The epidemiological data of Phil et al. (1989, 1990) indicate that hyper-reactivity of autonomic defensive arousal systems are heightened in males who possess a multi-generational family history of alcoholism. They possess a lower threshold for defensive arousal, cardiovascularly respond more intensely, and have a longer duration of defensive autonomic response than those not at risk (see Introduction and Unpublished Manuscript). This risk trait of soothability should be assessed in the rodent model through assessment of the duration of suppression of appetitive behavior by an aversive US. The CEOA risk model would predict that a preparedness to CEOA and preference for alcohol should covary with the perseveration of the effects of an aversive stimulus on the disruption of appetitive behavior.

In the daily variation of human experiences, these traits of **Hyper-reactivity** may effectively result in increasing Pavlovian learning parameters that condition endogenous opioid activity: trial number, the intensity of the effects of the US and the duration of the effects of the US. Similarly, environmental risk factors of **Harshness** increase the number, intensity or duration of aversive events that condition endogenous opioid activity. The repetitive activation of DAS-fear responses by exposure to aversive events has been shown to cause sensitization to the effects of abused drugs

(Kalivas & Stewart, 1991). This sensitization effect is similar to that induced by repetitive exposure to opioids (Kalivas et al 1986; see King 1995). Hence, environmental risk factors and genetic risk factors that increase hyper-reactivity or prevent coping with harsh aversive stressors may provide psychobiological steps, each step increasing risk for the etiology of drug abuse and alcoholism.

One benefit of the CEOA risk model is that it provides a coherent model for accounting for the variety of genetic and environmental risk features for addiction. Another benefit of the CEOA risk model is the clarity of the implications for prevention efforts: the intervention efforts should be directed toward reducing stress caused by the risk features. Intervention efforts should be directed toward the development of coping skills and the skills essential for the development of coping resources (e.g., social networks) to increase stress dampening features. Cultural and developmental interventions should be directed toward at minimizing the aversive realities in the lives of children at risk while at the same time increasing their social resources, their coping skills and their skills for building stronger, more stable and goal directed lives.

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**VI. APPENDIX A: STATISTICAL TABLES**

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1)	<u>US INTENSITY x DURATION</u>
PHASE I	
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PHASE II	
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2)	<u>GENETIC DIFFERENCES I</u>
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16	ANOVA Composite of HP2 & HP3
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24a - b	HP4 NTG_CS
25a - b	HP5 Sal_Nal (all subj. NTG, n=25)
26a	HP5 Add reserve subj. n=26
27 - 28	Alcohol Preference test M520/F344

**Table 1**  
**SEE FIGURE 2.**

EXPERIMENT 1: FIRST HOTPLATE TEST, "CS ALONE LATENCY ACROSS TRIALS"  
 HOT PLATE TEST AFTER 1 CS/US TRAINING TRIAL

ANALYSIS OF VARIANCE TABLE FOR HP89\_1

SOURCE	DF	SS	MS	F	P
COND (A)	3	385.627	128.542	3.21	0.0291
GROUP (B)	3	34.8906	11.6302	0.29	0.8330
SUBJ (C)					
A*B*C	57	2279.35	39.9886		
TOTAL	63	2699.87			
GRAND AVERAGE	1	18783.7			
GRAND MEAN		17.132	SE	0.7905	

**Table 2A  
FIGURE 2.**

**EXPERIMENT 1: FIRST HOTPLATE TEST, "CS ALONE LATENCY ACROSS TRIALS".  
HOT PLATE TEST AFTER 1 CS/US TRAINING TRIAL**

**MEANS OF HP89\_1 FOR TEST COND**

TEST COND	MEAN	SS (MEAN)
1	20.054	1233.0
2	18.504	512.36
3	16.477	227.40
4	13.492	341.52

OBSERVATIONS PER CELL 16  
 STD ERROR OF AN AVERAGE 1.5809  
 STD ERROR (DIFF OF 2 AVE'S) 2.2357  
 ERROR TERM USED: COND\*GROUP\*SUBJ, 57 DF

**MEANS OF HP89\_1 FOR GROUP**

US GROUP	MEAN	SS (MEAN)
1	16.237	725.75
2	17.826	845.82
3	16.569	504.35
4	17.895	589.06

OBSERVATIONS PER CELL 16  
 STD ERROR OF AN AVERAGE 1.5809  
 STD ERROR (DIFF OF 2 AVE'S) 2.2357  
 ERROR TERM USED: COND\*GROUP\*SUBJ, 57 DF

HP89\_1 = Hot plate latency scores after 1 CS+ training trial.

**TEST CONDITION CODES:**

- 1 = CS alone (same as group 2)
- 2 = CS alone (same as group 1)
- 3 = X = exposure to context only (Context)
- 4 = N = exposure to transportation cart (Nothing)

**US PARAMETERS**

GROUP CODES:	mA	X	SECONDS
1 =	.8	X	1.0
2 =	.8	X	4.0
3 =	1.6	X	1.0
4 =	1.6	X	4.0

Table 2B

**EXPERIMENT 1: FIRST HOTPLATE TEST, "CS ALONE LATENCY ACROSS TRIALS".  
HOT PLATE TEST AFTER 1 CS/US TRAINING TRIAL**

MEANS OF HP89_1 FOR TEST COND*GROUP			
TEST COND	US GROUP	MEAN	SS (MEAN)
1	1	19.343	361.54
1	2	19.965	298.37
1	3	19.767	313.55
1	4	21.142	252.37
2	1	17.058	129.40
2	2	20.738	233.67
2	3	16.707	44.008
2	4	19.512	59.974
3	1	14.832	77.361
3	2	16.925	60.460
3	3	15.930	28.663
3	4	18.220	35.946
4	1	13.718	82.888
4	2	13.675	128.94
4	3	13.870	46.353
4	4	12.705	79.950

OBSERVATIONS PER CELL <sup>4</sup>  
 STD ERROR OF AN AVERAGE 3.1618  
 STD ERROR (DIFF OF 2 AVE'S) 4.4715  
 ERROR TERM USED: COND\*GROUP\*SUBJ, 57 DF

TEST CONDITION CODE GUIDE:

- 1 = CS alone (same as group 2)
- 2 = CS alone (same as group 1)
- 3 = X = exposure to context only (Context)
- 4 = N = exposure to transportation cart (Nothing)

HP89\_1 = Hot plate latency scores after 1 CS+ training trial.

Table 2C  
SEE FIGURE 2.

**EXPERIMENT 1: FIRST HOTPLATE TEST, "CS ALONE LATENCY ACROSS TRIALS".  
HOT PLATE TEST AFTER 1 CS/US TRAINING TRIAL  
ANOVA CONTRASTS OF HP89\_1 BY COND\*GROUP**

CONTRAST NUMBER 1 (CS VS "NTG" TEST CONDITION)  
 CONTRAST COEFFICIENTS: 1 1 1 1 1 1 1 0 0 0 0 -2 -2 -2 -2  
 CONTRAST 46.297 SS (CONTRAST) 357.24  
 SCHEFFE'S F 0.99 P (SCHEFFE'S F) 0.4568  
 T-STATISTIC 2.99 P (T-STATISTIC) 0.0041  
 SE (CONTRAST) 15.490

CONTRAST NUMBER 2 (HIGH vs LOW SHOCK GROUPS)  
 CONTRAST COEFFICIENTS: 1 1 -1 -1 1 1 -1 -1 1 1 -1 -1 1 1 -1 -1  
 CONTRAST -1.6025 SS (CONTRAST) 0.6420  
 SCHEFFE'S F 0.00 P (SCHEFFE'S F) 1.0000  
 T-STATISTIC -0.13 P (T-STATISTIC) 0.8996  
 SE (CONTRAST) 12.647

CONTRAST NUMBER 3 (LONG vs SHORT SHOCK DURATION GROUPS)  
 CONTRAST COEFFICIENTS: 1 -1 1 -1 1 -1 1 -1 1 -1 1 -1 1 -1 1 -1  
 CONTRAST -11.657 SS (CONTRAST) 33.974  
 SCHEFFE'S F 0.09 P (SCHEFFE'S F) 0.9995  
 T-STATISTIC -0.92 P (T-STATISTIC) 0.3606  
 SE (CONTRAST) 12.647

Table 3  
SEE FIGURE 2.

**EXPERIMENT 1: 2ND HOTPLATE TEST, "CS ALONE LATENCY ACROSS TRIALS"  
HOT PLATE TEST AFTER 3 CS/US TRAINING TRIALS**

ANALYSIS OF VARIANCE TABLE FOR HP89\_2

SOURCE	DF	SS	MS	F	P
TEST COND (A)	3	36.206	12.069	0.26	0.8524
GROUPS (B)	3	50.852	16.951	.37	0.7765
A*B	9	497.88	55.32	1.20	0.3166
SUBJ (C)					
A*B*C	48	2211.3	46.069		
TOTAL	63	2796.2			
GRAND AVERAGE	1	1.3078E+4			

MEANS OF HP89\_2 FOR TEST CONDITIONS

TEST COND	MEAN	SS (MEAN)
1	15.64	339.15
2	14.41	646.7
3	13.96	1.584E+03
4	13.18	175.6

OBSERVATIONS PER CELL 16  
STD ERROR OF AN AVERAGE 1.697  
STD ERROR (DIFF OF 2 AVE'S) 2.400  
ERROR TERM USED: COND\*GROUPS\*SUBJ, 48 DF

MEANS OF HP89\_2 FOR US TRAINING GROUPS

US GROUP	MEAN	SS (MEAN)
1	13.40	507.0
2	14.18	328.9
3	14.11	637.6
4	15.49	1.287E+03
GRAND MEAN	14.29	SE 8.484E-01

OBSERVATIONS PER CELL 16  
STD ERROR OF AN AVERAGE 1.697  
STD ERROR (DIFF OF 2 AVE'S) 2.400  
ERROR TERM USED: COND\*GROUPS\*SUBJ, 48 DF

Table 4  
SEE FIGURE 2.

**EXPERIMENT 1: 3RD HOTPLATE TEST, "CS ALONE LATENCY ACROSS TRIALS".  
HOT PLATE TEST AFTER 3 CS/US TRAINING TRIAL**

ANALYSIS OF VARIANCE TABLE FOR HP89\_3

SOURCE	DF	SS	MS	F	P
TEST COND (A)	3	115.201	38.4004	4.83	0.0046
GROUPS (B)	3	13.7772	4.59240	0.58	0.6356
SUBJ (C)					
A*B*C	59	468.666	7.94350		
TOTAL	65	597.645			
GRAND AVERAGE	1	9068.63			

MEANS OF HP89\_3 FOR TEST COND

TEST COND	MEAN	SS (MEAN)
1	10.047	136.77
2	9.2075	73.090
3	10.876	158.03
4	12.457	114.56

OBSERVATIONS PER CELL 20  
STD ERROR OF AN AVERAGE 0.6302  
STD ERROR (DIFF OF 2 AVE'S) 0.8913  
ERROR TERM USED: COND\*GROUPS\*SUBJ, 59 DF

MEANS OF HP89\_3 FOR GROUPS

US GROUPS	MEAN	SS (MEAN)
1	10.104	106.05
2	11.110	119.74
3	10.984	132.09
4	10.391	225.99

OBSERVATIONS PER CELL 20  
STD ERROR OF AN AVERAGE 0.6302  
STD ERROR (DIFF OF 2 AVE'S) 0.8913  
ERROR TERM USED: COND\*GROUPS\*SUBJ, 59 DF

Table 5A  
SEE FIGURE 2.

**EXPERIMENT 1: 3RD HOTPLATE TEST, "CS ALONE LATENCY ACROSS TRIALS".  
AOV CONTRASTS OF HP89\_3 BY TEST COND\*GROUPS**

CONTRAST NUMBER 1 (CS VS "X" TEST CONDITIONS)  
 CONTRAST COEFFICIENTS: 1 1 1 1 1 1 1 1 -2 -2 -2 -2 0 0 0 0  
 CONTRAST -9.9910 SS (CONTRAST) 20.796  
 SCHEFFE'S F 0.29 P (SCHEFFE'S F) 0.9739  
 T-STATISTIC -1.62 P (T-STATISTIC) 0.1110  
 SE (CONTRAST) 6.1749

CONTRAST NUMBER 2 (CS VS "NTG" TEST CONDITIONS)  
 CONTRAST COEFFICIENTS: 1 1 1 1 1 1 1 0 0 0 0 -2 -2 -2 -2  
 CONTRAST -22.636 SS (CONTRAST) 106.75  
 SCHEFFE'S F 1.49 P (SCHEFFE'S F) 0.1712  
 T-STATISTIC -3.67 P (T-STATISTIC) 0.0005  
 SE (CONTRAST) 6.1749

AOV CONTRASTS OF HP89\_3 BY COND\*GROUPS  
 CONTRAST NUMBER 1 (HIGH VS LOW US TRAINING INTENSITIES)  
 CONTRAST COEFFICIENTS: -1 -1 1 1 -1 -1 1 1 -1 -1 1 1 -1 -1 1 1  
 CONTRAST 0.6435 SS (CONTRAST) 0.1294  
 SCHEFFE'S F 0.00 P (SCHEFFE'S F) 1.0000  
 T-STATISTIC 0.13 P (T-STATISTIC) 0.8989  
 SE (CONTRAST) 5.0417

CONTRAST NUMBER 4 (SHORT VS LONG US TRAINING DURATIONS)  
 CONTRAST COEFFICIENTS: 1 -1 1 -1 1 -1 1 -1 1 -1 1 -1 1 -1 1 -1  
 CONTRAST -1.6534 SS (CONTRAST) 0.8543  
 SCHEFFE'S F 0.01 P (SCHEFFE'S F) 1.0000  
 T-STATISTIC -0.33 P (T-STATISTIC) 0.7441  
 SE (CONTRAST) 5.0417

CONTRAST NUMBER 5 ("X" VS NTG TEST CONDITIONS)  
 CONTRAST COEFFICIENTS: 0 0 0 0 0 0 0 1 1 1 1 -1 -1 -1 -1  
 CONTRAST -6.3225 SS (CONTRAST) 24.984  
 SCHEFFE'S F 0.35 P (SCHEFFE'S F) 0.9534  
 T-STATISTIC -1.77 P (T-STATISTIC) 0.0813  
 SE (CONTRAST) 3.5651

ERROR TERM USED: COND\*GROUPS\*SUBJ, 59 DF

Table 6  
SEE FIGURE 3.

EXPERIMENT 1: 4th HOTPLATE TEST  
HOT PLATE TEST AFTER 10 CS/US TRAINING TRIALS  
CONDITIONED ANALGESIA PARAMETERS OF US INTENSITY & DURATION

ANALYSIS OF VARIANCE TABLE FOR HP89\_T65A

SOURCE	DF	SS	MS	F	P
INJ (A)	1	1423.17	1423.17	18.32	0.0000
STIM (B)	1	0.38725	0.38725	0.00	0.9438
GROUP (C)	3	7778.86	2592.95	33.38	0.0000
SUBJ (D)					
A*B*C*D	122	9476.41	77.6755		
TOTAL	127	18678.8			
GRAND AVERAGE	1	51844.4			

CODE GUIDE FOR TABLES 6-10:

INJECTION (INJ) :

- 1= NO INJECTION
- 2= NALOXONE INJECTION

TEST STIMULUS:

- 1= CS/US EXPOSURE IMMEDIATELY BEFORE HP TEST
- 2= "X"/US EXPOSURE IMMEDIATELY BEFORE HP TEST

US TRAINING GROUP:

INTENSITY x DURATION

- |          |   |       |
|----------|---|-------|
| 1 = LOW  | X | SHORT |
| 2 = LOW  | X | LONG  |
| 3 = HIGH | X | SHORT |
| 4 = HIGH | X | LONG  |

Table 7  
SEE FIGURE 3.

EXPERIMENT 1: 4th HOTPLATE TEST  
HOT PLATE TEST AFTER 10 CS/US TRAINING TRIALS  
CONDITIONED ANALGESIA PARAMETERS OF US INTENSITY & DURATION

GRAND MEAN 18.974 SE 0.7344

MEANS OF HP89\_T65A FOR INJ  
INJ MEAN SS (MEAN)

1	22.118	1.27E+04	NONE
2	15.831	4589.3	NALOXONE

MEANS OF HP89\_T65A FOR US GROUP  
US GROUP MEAN SS (MEAN)

1	10.291	347.52	LOW X SHORT (L-S)
2	14.525	800.00	LOW X LONG (L-L)
3	21.378	3500.8	HIGH X SHORT (H-S)
4	29.703	6251.6	HIGH X LONG (H-L)

MEANS OF HP89\_T65A FOR STIM  
STIM MEAN SS (MEAN)

1	18.923	1.18E+04	CS/US
2	19.026	6864.7	"X"/US

MEANS OF HP89\_T65A FOR INJ\*STIM  
INJ STIM MEAN SS (MEAN)

NONE	1	CS/US 1	23.793
	1	"X"/US 2	20.443
NAL	2	1	14.052
	2	2	17.610

MEANS OF HP89\_T65A FOR STIM\*GROUP  
STIM US GROUP MEAN SS (MEAN)

NONE	1	L-S 1	8.6927
	1	L-L 2	14.981
	1	H-S 3	21.235
	1	H-L 4	30.781
NAL	2	1	11.890
	2	2	14.069
	2	3	21.521
	2	4	28.625

Table 8  
SEE FIGURE 3.

EXPERIMENT 1: 4th HOTPLATE TEST  
HOT PLATE TEST AFTER 10 CS/US TRAINING TRIALS  
CONDITIONED ANALGESIA PARAMETERS OF US INTENSITY & DURATION

MEANS OF HP89_T65A FOR INJ*STIM*GROUP				MEAN	SS (MEAN)
INJ	STIM	US GROUP			
NONE	1	CS/US	L-S	9.3103	67.628
	1		L-L	17.634	240.06
	1		H-S	27.313	797.36
	1		H-L	40.916	2534.8
	1	"X"/US	2	12.359	71.785
	1		2	14.663	165.10
	1		3	23.568	716.15
	1		4	31.182	877.23
	2	1	1	8.0751	49.099
	2	1	2	12.329	124.03
NAL	2	1	3	15.157	257.45
	2	1	4	20.646	305.08
	2	2	1	11.421	56.194
	2	2	2	13.474	130.29
	2	2	3	19.474	988.72
	2	2	4	26.069	526.11

CODE GUIDE FOR TABLES 6-10:

INJECTION (INJ):

- 1= NO INJECTION
- 2= NALOXONE INJECTION

TEST STIMULUS:

- 1= CS/US EXPOSURE IMMEDIATELY BEFORE HP TEST
- 2= "X"/US EXPOSURE IMMEDIATELY BEFORE HP TEST

US TRAINING GROUP:

INTENSITY x DURATION

- |          |   |           |     |
|----------|---|-----------|-----|
| 1 = LOW  | X | SHORT L-S |     |
| 2 = LOW  | X | LONG      | L-L |
| 3 = HIGH | X | SHORT H-S |     |
| 4 = HIGH | X | LONG      | H-L |

Table 9  
SEE FIGURE 3.

EXPERIMENT 1: 4th HOTPLATE TEST  
HOT PLATE TEST AFTER 10 CS/US TRAINING TRIALS  
CONDITIONED ANALGESIA PARAMETERS OF US INTENSITY & DURATION

AOV CONTRASTS OF HP89\_T65A BY INJ\*STIM\*GROUP

CONTRAST NUMBER 1 (EFFECT OF INCREASED SHOCK DURATION)  
 CONTRAST COEFFICIENTS: 1 -1 1 1 -1 1 -1 1 -1 1 -1 1 -1 1 -1  
 CONTRAST -50.236 SS (CONTRAST) 1419.6  
 SCHEFFE'S F 6.09 P (SCHEFFE'S F) 0.0008  
 T-STATISTIC -4.28 P (T-STATISTIC) 0.0000  
 SE (CONTRAST) 11.751

CONTRAST NUMBER 2 (EFFECT OF INCREASED SHOCK INTENSITY)

CONTRAST COEFFICIENTS: -1 -1 1 1 -1 -1 1 1 -1 -1 1 1 -1 -1 1 1  
 CONTRAST 105.06 SS (CONTRAST) 6208.6  
 SCHEFFE'S F 26.64 P (SCHEFFE'S F) 0.0000  
 T-STATISTIC 8.94 P (T-STATISTIC) 0.0000  
 SE (CONTRAST) 11.751

CONTRAST NUMBER 3 (NALOXONE EFFECT)

CONTRAST COEFFICIENTS: 1 1 1 1 1 1 1 -1 -1 -1 -1 -1 -1 -1 -1 -1  
 CONTRAST 50.300 SS (CONTRAST) 1423.2  
 SCHEFFE'S F 6.11 P (SCHEFFE'S F) 0.0008  
 T-STATISTIC 4.28 P (T-STATISTIC) 0.0000  
 SE (CONTRAST) 11.751

CONTRAST NUMBER 4 (CS+ POTENTIATION OF ANALGESIA  
IN 3 HARSHEST US TRAINING GROUPS)

CONTRAST COEFFICIENTS: 0 1 1 1 0 -1 -1 -1 0 0 0 0 0 0 0 0 0  
 CONTRAST 16.450 SS (CONTRAST) 405.92  
 SCHEFFE'S F 1.74 P (SCHEFFE'S F) 0.1603  
 T-STATISTIC 2.29 P (T-STATISTIC) 0.0240  
 SE (CONTRAST) 7.1961

CONTRAST NUMBER 5 (CS+ POTENTIATION OF ANALGESIA  
IN HARSHEST US TRAINING GROUP)

CONTRAST COEFFICIENTS: 0 0 0 1 0 0 0 -1 0 0 0 0 0 0 0 0 0  
 CONTRAST 9.7342 SS (CONTRAST) 426.39  
 SCHEFFE'S F 1.83 P (SCHEFFE'S F) 0.1436  
 T-STATISTIC 2.34 P (T-STATISTIC) 0.0208  
 SE (CONTRAST) 4.1547

CONTRAST NUMBER 5 (CS+ POTENTIATION OF ANALGESIA)

CONTRAST COEFFICIENTS: 1 1 1 1 -1 -1 -1 -1 0 0 0 0 0 0 0 0 0  
 CONTRAST 13.402 SS (CONTRAST) 202.07  
 SCHEFFE'S F 0.87 P (SCHEFFE'S F) 0.4625  
 T-STATISTIC 1.61 P (T-STATISTIC) 0.1094  
 SE (CONTRAST) 8.3093

Table 10  
SEE FIGURE 2.  
EXPERIMENT 1: 4th HOTPLATE TEST

CONDITIONED ANALGESIA PARAMETERS OF US INTENSITY & DURATION  
HOT PLATE TEST AFTER 10 CS/US TRAINING TRIALS  
ANCILLARY CONTRASTS

(NALOXONE EFFECT ON CS+ ANALGESIA  
IN HIGH SHOCK GROUPS)

CONTRAST COEFFICIENTS:	0 0 1 1	0 0 0 0	0 0 -1 -1	0 0 0 0
CONTRAST	32.426	SS (CONTRAST)	2365.8	
SCHIFFE'S F	10.15	P (SCHIFFE'S F)	0.0000	
T-STATISTIC	5.52	P (T-STATISTIC)	0.0000	
SE (CONTRAST)	5.8756			

(NALOXONE EFFECT ON US-ALONE ANALGESIA)

CONTRAST COEFFICIENTS:	0 0 0 0 1 1 1 0 0 0 0 -1 -1 -1 -1		
CONTRAST	11.333	SS (CONTRAST)	144.49
SCHIFFE'S F	0.62	P (SCHIFFE'S F)	0.6073
T-STATISTIC	1.36	P (T-STATISTIC)	0.1751
SE (CONTRAST)	8.3093		

(NALOXONE EFFECT ON US-ALONE ANALGESIA  
IN HIGH SHOCK TRAINING GROUPS)

CONTRAST COEFFICIENTS:	0 0 0 0	0 0 1 1	0 0 0 0	0 0 -1 -1
CONTRAST	9.2066	SS (CONTRAST)	190.71	
SCHIFFE'S F	0.82	P (SCHIFFE'S F)	0.4888	
T-STATISTIC	1.57	P (T-STATISTIC)	0.1197	
SE (CONTRAST)	5.8756			

ERROR TERM USED: INJ\*STIM\*GROUP\*SUBJ, 122 DF

**Table 11a (SEE FIGURES 4a - 4d)**  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

strain diffs "X" day1, bolus count, SD 7 21 92

DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_721	25	0.1600	0.1108	0.0000
B2_721	25	1.5200	0.2951	1.0000
B3_721	25	0.0400	0.0400	0.0000
BTOT_721	25	1.7200	0.3190	1.0000

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_721	25	0.7200	0.1873	0.0000
B2_721	25	1.4800	0.2894	1.0000
B3_721	25	1.0800	0.2939	0.0000
BTOT_721	25	3.2800	0.3980	4.0000

bolus and squeak count for day 2 of "X", SD 7 22 92"

DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_722	25	0.0800	0.0800	0.0000
B2_722	25	1.0800	0.3412	0.0000
B3_722	25	0.0400	0.0400	0.0000
BTOT_722	25	1.2000	0.3559	0.0000
S1_722	25	1.8400	0.3987	2.0000
S2_722	25	0.9200	0.2375	0.0000
S3_722	25	0.5200	0.2245	0.0000
STOT_722	25	3.2800	0.4779	3.0000

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_722	25	1.2400	0.2104	1.0000
B2_722	25	3.3200	0.4030	3.0000
B3_722	25	0.8800	0.3018	0.0000
BTOT_722	25	5.4400	0.5918	5.0000
S1_722	25	2.9600	0.4881	2.0000
S2_722	25	2.6400	0.5624	1.0000
S3_722	25	0.7600	0.3124	0.0000
STOT_722	25	6.3600	0.9813	5.0000

**Table 11b (SEE Figure 4a & 9A, 9B, 9C)**  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

**BOLUS COUNT AND SQUEAKS FOR DAY 3 OF "X"** 7 23 92

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_723	25	0.2800	0.1583	0.0000
B2_723	25	1.1200	0.3929	0.0000
B3_723	25	0.2800	0.1685	0.0000
BTOT_723	25	1.6800	0.4572	0.0000
S1_723	25	2.4800	0.4402	3.0000
S2_723	25	0.6000	0.1732	0.0000
S3_723	25	0.2800	0.1474	0.0000
STOT_723	25	3.3600	0.4757	3.0000

**DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520**

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_723	25	1.2000	0.2887	0.0000
B2_723	25	3.1200	0.4055	3.0000
B3_723	25	0.3600	0.1720	0.0000
BTOT_723	25	4.7200	0.4984	5.0000
S1_723	25	3.4800	0.5660	3.0000
S2_723	25	1.2000	0.2887	1.0000
S3_723	25	0.5200	0.2010	0.0000
STOT_723	25	5.0800	0.7787	4.0000

**bolus and squeak count for day 4 of "X"**

SD 7 24 92

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_724	25	0.0800	0.0800	0.0000
B2_724	25	0.5600	0.2166	0.0000
B3_724	25	0.0400	0.0400	0.0000
BTOT_724	25	0.6800	0.2215	0.0000
S1_724	25	2.6400	0.5288	2.0000
S2_724	25	0.8000	0.1915	0.0000
S3_724	25	0.2800	0.1356	0.0000
STOT_724	25	3.7200	0.6312	4.0000

**DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520**

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_724	25	0.8400	0.2212	0.0000
B2_724	25	3.0800	0.6555	2.0000
B3_724	25	0.2000	0.1633	0.0000
BTOT_724	25	4.1200	0.6790	4.0000
S1_724	25	2.2800	0.4144	2.0000
S2_724	25	1.4000	0.4546	1.0000
S3_724	25	0.4800	0.1541	0.0000
STOT_724	25	4.1600	0.8920	3.0000

Table 11c  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

bolus and squeak count for day 1 of cold plate				7 25 92
DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_725	25	0.4400	0.2242	0.0000
B2_725	25	3.5600	0.4126	4.0000
B3_725	25	0.0000	0.0000	0.0000
BTOT_725	25	4.0000	0.4546	4.0000
S1_725	25	4.0800	0.5857	3.0000
S2_725	25	1.9200	0.3508	2.0000
S3_725	25	0.5200	0.1925	0.0000
STOT_725	25	6.5200	0.8208	5.0000
DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				7 27 92
VARIABLE	N	MEAN	SE MEAN	
B1_725	25	1.6400	0.3553	1.0000
B2_725	25	2.4800	0.3062	3.0000
B3_725	25	0.0400	0.0400	0.0000
BTOT_725	25	4.1600	0.3945	4.0000
S1_725	25	4.2400	0.5867	4.0000
S2_725	25	2.7200	0.5523	2.0000
S3_725	25	0.7600	0.3229	0.0000
STOT_725	25	7.7200	0.9047	9.0000
bolus and squeak for day 5 of "X"				7 27 92
DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_727	25	0.1600	0.1600	0.0000
B2_727	25	0.8400	0.3859	0.0000
B3_727	25	0.0000	0.0000	0.0000
BTOT_727	25	1.0000	0.4041	0.0000
STOT_727	25	4.4800	0.8065	4.0000
DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				7 27 92
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_727	25	0.6400	0.1815	0.0000
B2_727	25	3.5600	0.4126	4.0000
B3_727	25	0.0000	0.0000	0.0000
BTOT_727	25	4.2000	0.4726	4.0000
STOT_727	25	3.2800	0.8176	3.0000

Table 11d  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

bolus/squeak, day1, tone/shock - 7/28/92 - q	7 28 92
<b>DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344</b>	
VARIABLE	N MEAN SE MEAN MEDIAN
B1_728	25 0.0000 0.0000 0.0000
B2_728	25 4.9200 0.3997 5.0000
B3_728	25 0.0400 0.0400 0.0000
BTOT_728	25 4.9600 0.4061 5.0000
STOT_728	25 3.1200 0.4666 3.0000
 <b>DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520</b>	
VARIABLE	N MEAN SE MEAN MEDIAN
B1_728	25 0.1600 0.0945 0.0000
B2_728	25 5.2400 0.3928 5.0000
B3_728	25 0.0400 0.0400 0.0000
BTOT_728	25 5.4400 0.3833 6.0000
STOT_728	25 3.1200 0.5869 2.0000
 bolus and squeak for day 7 of "X"	
<b>DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344</b>	
VARIABLE	N MEAN SE MEAN MEDIAN
B1_729	25 0.0800 0.0800 0.0000
B2_729	25 5.0400 0.4183 5.0000
B3_729	25 0.0000 0.0000 0.0000
BTOT_729	25 5.1200 0.4096 5.0000
STOT_729	25 3.9200 0.6270 3.0000
 <b>DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520</b>	
VARIABLE	N MEAN SE MEAN MEDIAN
B1_729	25 0.6000 0.2160 0.0000
B2_729	25 5.1200 0.3887 5.0000
B3_729	25 0.0000 0.0000 0.0000
BTOT_729	25 5.7200 0.3489 6.0000
STOT_729	25 1.1200 0.2962 0.0000

Table 11e  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

bolus and squeak count for day 8 of "X"				7 30 92
DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_730	25	0.0000	0.0000	0.0000
B2_730	25	4.6400	0.5095	5.0000
B3_730	25	0.0000	0.0000	0.0000
BTOT_730	25	4.6400	0.5095	5.0000
STOT_730	25	3.1200	0.4841	2.0000

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				7 31 92
DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_730	25	0.5200	0.2894	0.0000
B2_730	25	5.0800	0.3363	5.0000
B3_730	25	0.0800	0.0800	0.0000
BTOT_730	25	5.6800	0.3946	5.0000
STOT_730	25	0.7200	0.2123	0.0000

bolus and squeak for day 9 for "X"				7 31 92
DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_731	25	0.0000	0.0000	0.0000
B2_731	25	3.5200	0.5004	4.0000
B3_731	25	0.0400	0.0400	0.0000
BTOT_731	25	3.5600	0.5069	4.0000
STOT_731	25	2.4000	0.5000	2.0000

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				7 31 92
DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_731	25	0.2800	0.1781	0.0000
B2_731	25	4.9600	0.3936	5.0000
B3_731	25	0.0000	0.0000	0.0000
BTOT_731	25	5.2400	0.4445	5.0000
STOT_731	25	0.2800	0.1083	0.0000

Table 11f  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

**bolus and squeak for day 10 of "X"**

DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				8 1 92
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_81	25	0.0000	0.0000	0.0000
B2_81	25	2.7600	0.6740	2.0000
B3_81	25	0.0000	0.0000	0.0000
BTOT_81	25	2.7600	0.6740	2.0000
STOT_81	25	2.1600	0.3591	2.0000

**DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520**

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				8 1 92
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_81	25	0.4800	0.2653	0.0000
B2_81	25	3.6400	0.4277	4.0000
B3_81	25	0.0000	0.0000	0.0000
BTOT_81	25	4.1200	0.4409	4.0000
STOT_81	25	0.2800	0.2043	0.0000

**bolus and squeak for day 11 of "X"**

DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344				8 2 92
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_82	25	0.0000	0.0000	0.0000
B2_82	25	2.3200	0.4889	2.0000
B3_82	25	0.0000	0.0000	0.0000
BTOT_82	25	2.3200	0.4889	2.0000
STOT_82	25	2.5200	0.4363	2.0000

**DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520**

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				8 2 92
VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_82	25	0.4000	0.1826	0.0000
B2_82	25	3.0400	0.4672	3.0000
B3_82	25	0.0400	0.0400	0.0000
BTOT_82	25	3.4800	0.5660	3.0000
STOT_82	25	0.2000	0.1291	0.0000

Table 11g  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

bolus and squeak for day 12 of "X"				8 3 92
<b>DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344</b>				
<b>VARIABLE</b>	<b>N</b>	<b>MEAN</b>	<b>SE MEAN</b>	<b>MEDIAN</b>
B1_83	25	0.0000	0.0000	0.0000
B2_83	25	1.7600	0.3664	2.0000
B3_83	25	0.0000	0.0000	0.0000
BTOT_83	25	1.7600	0.3664	2.0000
STOT_83	25	2.6400	0.5256	2.0000
 DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				
<b>VARIABLE</b>	<b>N</b>	<b>MEAN</b>	<b>SE MEAN</b>	<b>MEDIAN</b>
B1_83	25	0.0800	0.0800	0.0000
B2_83	25	3.6000	0.5033	3.0000
B3_83	25	0.0000	0.0000	0.0000
BTOT_83	25	3.6800	0.4855	3.0000
STOT_83	25	0.1200	0.0663	0.0000
 bolus and squeak for cold plate #2				8 4 92
<b>DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344</b>				
<b>VARIABLE</b>	<b>N</b>	<b>MEAN</b>	<b>SE MEAN</b>	<b>MEDIAN</b>
B1_87	25	0.0000	0.0000	0.0000
B2_87	25	2.3600	0.3311	2.0000
B3_87	25	0.0000	0.0000	0.0000
BTOT_87	25	2.3600	0.3311	2.0000
STOT_87	25	1.1600	0.2428	1.0000
 DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520				
<b>VARIABLE</b>	<b>N</b>	<b>MEAN</b>	<b>SE MEAN</b>	<b>MEDIAN</b>
B1_87	25	0.0400	0.0400	0.0000
B2_87	25	3.2800	0.2678	3.0000
B3_87	25	0.0800	0.0554	0.0000
BTOT_87	25	3.4000	0.2708	4.0000
STOT_87	25	0.3200	0.1604	0.0000

Table 11h

**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CREA, & ALCOHOL CHOICE**

BOLUS AND SQUEAK COUNT FOR 1ST DAY OF "X" AFTER HP TEST                    8 8 92  
 DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_88	25	0.0800	0.0554	0.0000
B2_88	25	2.2800	0.4950	2.0000
B3_88	25	0.0000	0.0000	0.0000
BTOT_88	25	2.3600	0.5095	2.0000
STOT_88	25	1.1600	0.2868	1.0000

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_88	25	0.6800	0.1977	0.0000
B2_88	25	3.4800	0.3422	4.0000
B3_88	25	0.0800	0.0800	0.0000
BTOT_88	25	4.2400	0.4175	4.0000
STOT_88	25	0.2000	0.1000	0.0000

bolus and squeak count for day 2 of "X" after HP test

8 10 92

DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_810	25	0.1600	0.1249	0.0000
B2_810	25	2.4800	0.4623	2.0000
B3_810	25	0.0000	0.0000	0.0000
BTOT_810	25	2.6400	0.4792	2.0000
STOT_810	25	0.5200	0.1837	0.0000

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520

VARIABLE	N	MEAN	SE MEAN	MEDIAN
B1_810	25	0.0000	0.0000	0.0000
B2_810	25	2.1600	0.3682	2.0000
B3_810	25	0.0000	0.0000	0.0000
BTOT_810	25	2.1600	0.3682	2.0000
STOT_810	25	0.0000	0.0000	0.0000

Table 11i  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

bolus and squeak for day 3 of "X" after HP test  
 DESCRIPTIVE STATISTICS FOR GENETIC LINE = F344 8 12 92  
 VARIABLE N MEAN SE MEAN MEDIAN  
 B1\_812 25 0.0800 0.0800 0.0000  
 B2\_812 25 1.8800 0.4772 0.0000  
 B3\_812 25 0.0000 0.0000 0.0000  
 BTOT\_812 25 1.9600 0.4707 1.0000  
 STOT\_812 25 0.8800 0.1943 1.0000

DESCRIPTIVE STATISTICS FOR GENETIC LINE = M520  
 VARIABLE N MEAN SE MEAN MEDIAN  
 B1\_812 25 0.1200 0.1200 0.0000  
 B2\_812 25 1.5200 0.4835 0.0000  
 B3\_812 25 0.0000 0.0000 0.0000  
 BTOT\_812 25 1.6400 0.5029 0.0000  
 STOT\_812 25 0.0000 0.0000 0.0000

bolus and squeak count for day 4 of "X"  
 ONE-WAY AOV FOR BTOT 724 BY STRAIN  
 SOURCE DF SS MS F P  
 -----  
 BETWEEN 1 147.920 147.920 23.20 0.0000  
 WITHIN 48 306.080 6.37667  
 TOTAL 49 454.000  
 SAMPLE GROUP  
 STRAIN MEAN SIZE STD DEV  
 -----  
 1 0.6800 25 1.1075  
 2 4.1200 25 3.3951  
 TOTAL 2.4000 50 2.5252  
 CASES INCLUDED 50 MISSING CASES 0

bolus and squeak count for day 4 of "X"  
 ONE-WAY AOV FOR STOT 724 BY STRAIN  
 SOURCE DF SS MS F P  
 -----  
 BETWEEN 1 2.42000 2.42000 0.16 0.6890  
 WITHIN 48 716.400 14.9250  
 TOTAL 49 718.820  
 SAMPLE GROUP  
 STRAIN MEAN SIZE STD DEV  
 -----  
 1 3.7200 25 3.1559  
 2 4.1600 25 4.4598  
 TOTAL 3.9400 50 3.8633  
 CASES INCLUDED 50 MISSING CASES 0

Table 12a  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

strain diffs "X" day1, total bolus count, sd72192  
 ONE-WAY AOV FOR BTOT\_721 BY STRAIN

SOURCE	DF	SS	MS	F	P
BETWEEN	1	30.4200	30.4200	9.36	0.0036
WITHIN	48	156.080	3.25167		
TOTAL	49	186.500			

STRAIN	MEAN	SAMPLE		GROUP STD DEV
		SIZE	STD DEV	
1	1.7200	25	1.5948	
2	3.2800	25	1.9900	
TOTAL	2.5000	50	1.8032	
CASES INCLUDED	50	MISSING CASES	6	

Table 12b

**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**

bolus and squeak count for day 2 of "X", "SD72292"  
 ONE-WAY AOV FOR BTOT\_722 BY STRAIN

SOURCE	DF	SS	MS	F	P
BETWEEN	1	224.720	224.720	37.69	0.0000
WITHIN	48	286.160	5.96167		
TOTAL	49	510.880			

STRAIN	MEAN	SAMPLE SIZE	GROUP STD DEV
1	1.2000	25	1.7795
2	5.4400	25	2.9592
TOTAL	3.3200	50	2.4417

CASES INCLUDED 50 MISSING CASES 0

bolus and squeak count for day 2 of "X", "SD72292"  
 ONE-WAY AOV FOR STOT\_722 BY STRAIN

SOURCE	DF	SS	MS	F	P
BETWEEN	1	118.580	118.580	7.96	0.0069
WITHIN	48	714.800	14.8917		
TOTAL	49	833.380			

STRAIN	MEAN	SAMPLE SIZE	GROUP STD DEV
1	3.2800	25	2.3896
2	6.3600	25	4.9065
TOTAL	4.8200	50	3.8590

CASES INCLUDED 50 MISSING CASES 0

Table 13a  
**Experiment 2: M520 & F344S, GENETICS, TEMPERAMENT, CEOA & ALCOHOL CHOICE**  
**HP1 (ALL SUBJs "NOVELTY" EXPOSURE: SAL/NAL X F344/M520)**

SOURCE	DF	SS	MS	(NOVELTY EXPOSURE)	
				F	P
STRAIN (A)	1	193.434	193.434	4.15	0.0473
INJ1 (B)	1	28.1272	28.1272	0.60	0.4410
A*B	1	52.7880	52.7880	1.13	0.2926
SUBJ (C)					
A*B*C	46	2142.13	46.5680		
TOTAL	49	2416.48			
GRAND AVERAGE	1	14392.4			
CASES INCLUDED	50	MISSING CASES 2			
GRAND MEAN	16.637	SE 0.9463			

MEANS OF HP1 FOR STRAIN		
STRAIN	MEAN	SS (MEAN)
F344 1	14.708	1012.8
M520 2	18.565	1210.2

MEANS OF HP1 FOR INJ1		
INJ1	MEAN	SS (MEAN)
SAL 1	15.901	970.21
NAL 2	17.372	1418.1

STRAIN	INJ1	MEAN	(NOVELTY EXPOSURE)	
			SS (MEAN)	
F344 1	SAL 1	14.980	632.28	
F344 1	NAL 2	14.436	378.61	
M520 2	SAL 1	16.822	315.86	
M520 2	NAL 2	20.308	815.37	

Table 13b

**Experiment 2: M520 & F344s, GENETICS, TEMPERAMENT, CEOA & ALCOHOL CHOICE**

**HP1 (ALL SUBJs "NOVELTY" EXPOSURE: SAL/NAL X F344/M520)**

AOV CONTRASTS OF HP1 BY STRAIN*INJ1		(NOVELTY EXPOSURE - NTG)	
CONTRAST NUMBER 1		<b>(MAIN EFFECT OF GENETICS)</b>	
CONTRAST COEFFICIENTS: 1 1 -1 -1			
CONTRAST	-7.7148	SS (CONTRAST)	193.43
SCHEFFE'S F	4.15	P (SCHEFFE'S F)	0.0473
T-STATISTIC	-2.04	P (T-STATISTIC)	0.0473
SE (CONTRAST)	3.7853		
CONTRAST NUMBER 2		<b>(MAIN EFFECT OF INJECTION)</b>	
CONTRAST COEFFICIENTS: 1 -1 1 -1			
CONTRAST	-2.9419	SS (CONTRAST)	28.127
SCHEFFE'S F	0.60	P (SCHEFFE'S F)	0.4410
T-STATISTIC	-0.78	P (T-STATISTIC)	0.4410
SE (CONTRAST)	3.7853		
CONTRAST NUMBER 3		<b>(EFFECT OF INJ ON F344s)</b>	
CONTRAST COEFFICIENTS: 1 -1 0 0			
CONTRAST	0.5442	SS (CONTRAST)	1.9248
SCHEFFE'S F	0.04	P (SCHEFFE'S F)	0.8398
T-STATISTIC	0.20	P (T-STATISTIC)	0.8398
SE (CONTRAST)	2.6766		
CONTRAST NUMBER 4		<b>(EFFECT OF INJ ON M520s)</b>	
CONTRAST COEFFICIENTS: 0 0 1 -1			
CONTRAST	-3.4860	SS (CONTRAST)	78.990
SCHEFFE'S F	1.70	P (SCHEFFE'S F)	0.1993
T-STATISTIC	-1.30	P (T-STATISTIC)	0.1993
SE (CONTRAST)	2.6766		
CONTRAST NUMBER 5		<b>(NO DIFF BTWN SAL GROUPS)</b>	
CONTRAST COEFFICIENTS: 1 0 -1 0		(SAL/F344 VS SAL/M520)	
CONTRAST	-1.8423	SS (CONTRAST)	22.062
SCHEFFE'S F	0.47	P (SCHEFFE'S F)	0.4947
T-STATISTIC	-0.69	P (T-STATISTIC)	0.4947
SE (CONTRAST)	2.6766		
CONTRAST NUMBER 6		<b>(DIFFERENCE BTWN NAL GROUPS)</b>	
CONTRAST COEFFICIENTS: 0 1 0 -1		(NAL/F344 VS NAL/M520)	
CONTRAST	-5.8725	SS (CONTRAST)	224.16
SCHEFFE'S F	4.81	P (SCHEFFE'S F)	0.0333
T-STATISTIC	-2.19	P (T-STATISTIC)	0.0333
SE (CONTRAST)	2.6766		
CONTRAST NUMBER 7		<b>(UNIQUENESS OF THE M520/NAL GROUP)</b>	
CONTRAST COEFFICIENTS: 1 1 1 -3			
CONTRAST	-14.687	SS (CONTRAST)	233.68
SCHEFFE'S F	5.02	P (SCHEFFE'S F)	0.0300
T-STATISTIC	-2.24	P (T-STATISTIC)	0.0300
SE (CONTRAST)	6.5564		

Table 14a  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**  
**HP2 : (ALL SUBJs "NTG" EXPOSURE: SAL/NAL X F344/M520)**

ANALYSIS OF VARIANCE TABLE FOR HP2 (ALL SUBJs : NTG CONDITION)

SOURCE	DF	SS	MS	F	P
STNHP2 (A)	1	222.933	222.933	8.35	0.0060
INJHP2 (B)	1	284.598	284.598	10.66	0.0021
A*B	1	135.326	135.326	5.07	0.0295
SUBHP2 (C)					
A*B*C	43	1147.48	26.6857		
TOTAL	46	1790.34			
GRAND AVERAGE	1	9800.48			
CASES INCLUDED	47	MISSING CASES 5			
GRAND MEAN		13.728	SE 0.7164		

MEANS OF HP2 FOR STNHP2

STNHP2	MEAN	SS (MEAN)
F344 1	11.658	286.14
M520 2	15.799	1281.3

MEANS OF HP2 FOR INJHP2

INJHP2	MEAN	SS (MEAN)
SAL 1	11.389	397.45
NAL 2	16.068	1108.3

MEANS OF HP2 FOR STNHP2\*INJHP2  
**CONDITION)** (ALL SUBJ = "NTG")

STNHP2	INJHP2	MEAN	SS (MEAN)
F344 1	SAL 1	10.932	90.402
F344 1	NAL 2	12.384	182.02
M520 2	SAL 1	11.846	301.61
M520 2	NAL 2	19.752	573.46

hot plate 2 (f344/m520 X saline/nal)

Table 14b

**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL  
CHOICE**  
**HP2: (NTG EXPOSURE: SAL/NAL X F344/M520)**

AOV CONTRASTS OF HP2 BY STNHP2\*INJHP2 (ALL SUBJs: NTG CONDITION)

CONTRAST NUMBER 1				(EFFECT OF GENETICS)		
CONTRAST COEFFICIENTS: 1 1 -1 -1						
CONTRAST	-8.2822			SS (CONTRAST)	222.93	
SCHEFFE'S F	8.35			P (SCHEFFE'S F)	0.0060	
T-STATISTIC	-2.89			P (T-STATISTIC)	0.0060	
CONTRAST NUMBER 2				(EFFECT OF INJECTION)		
CONTRAST COEFFICIENTS: 1 -1 1 -1						
CONTRAST	-9.3578			SS (CONTRAST)	284.60	
SCHEFFE'S F	10.66			P (SCHEFFE'S F)	0.0021	
T-STATISTIC	-3.27			P (T-STATISTIC)	0.0021	
CONTRAST NUMBER 3				(INJ ON F344s)		
CONTRAST COEFFICIENTS: 1 -1 0 0						
CONTRAST	-1.4525			SS (CONTRAST)	13.713	
SCHEFFE'S F	0.51			P (SCHEFFE'S F)	0.4773	
T-STATISTIC	-0.72			P (T-STATISTIC)	0.4773	
CONTRAST NUMBER 4				(INJ ON M520s)		
CONTRAST COEFFICIENTS: 0 0 1 -1						
CONTRAST	-7.9053			SS (CONTRAST)	406.21	
SCHEFFE'S F	15.22			P (SCHEFFE'S F)	0.0003	
T-STATISTIC	-3.90			P (T-STATISTIC)	0.0003	
CONTRAST NUMBER 5				(DIFF BTWN SAL GROUPS)		
CONTRAST COEFFICIENTS: 1 0 -1 0						
CONTRAST	-0.9147			SS (CONTRAST)	5.4384	
SCHEFFE'S F	0.20			P (SCHEFFE'S F)	0.6539	
T-STATISTIC	-0.45			P (T-STATISTIC)	0.6539	
CONTRAST NUMBER 6				(DIFF BTWN NAL GROUPS)		
CONTRAST COEFFICIENTS: 0 1 0 -1						
CONTRAST	-7.3675			SS (CONTRAST)	352.82	
SCHEFFE'S F	13.22			P (SCHEFFE'S F)	0.0007	
T-STATISTIC	-3.64			P (T-STATISTIC)	0.0007	
CONTRAST NUMBER 7				(UNIQUENESS OF M520/NAL GROUP)		
CONTRAST COEFFICIENTS: 1 1 1 -3						
CONTRAST	-24.093			SS (CONTRAST)	628.84	
SCHEFFE'S F	23.56			P (SCHEFFE'S F)	0.0000	
T-STATISTIC	-4.85			P (T-STATISTIC)	0.0000	
CONTRAST NUMBER 8				(M520/SAL NOT DIFF FROM F344s)		
CONTRAST COEFFICIENTS: 1 1 -2 0						
CONTRAST	-0.3769			SS (CONTRAST)	0.3078	
SCHEFFE'S F	0.01			P (SCHEFFE'S F)	0.9150	
T-STATISTIC	-0.11			P (T-STATISTIC)	0.9150	

ERROR TERM USED: STNHP2\*INJHP2\*SUBHP2, 43 DF

Table 15a  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL  
 CHOICE**  
**HP3 : ("CS" EXPOSURE: SAL/NAL X F344/M520)**

NAL (7mg/kg)

(CS EXPOSURE: SAL/NAL X

**F344/M520)**

**ANALYSIS OF VARIANCE TABLE FOR HP3**

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	3.35743	3.35743	0.10	0.7550
INJ3 (B)	1	135.243	135.243	3.98	0.0530
A*B	1	605.099	605.099	17.79	0.0001
SUBJ (C)					
A*B*C	40	1360.27	34.0067		
TOTAL	43	2103.97			
GRAND AVERAGE	1	10901.4			
CASES INCLUDED	44	MISSING CASES	8		
GRAND MEAN	14.479	SE	0.8087		

**MEANS OF HP3 FOR STRAIN**

STRAIN	MEAN	SS (MEAN)
<b>F344 1</b>	14.733	745.33
<b>M520 2</b>	14.225	1355.3

**MEANS OF HP3 FOR INJ3**

INJ3	MEAN	SS (MEAN)
<b>SAL 1</b>	16.092	1000.6
<b>NAL 2</b>	12.866	968.13

**MEANS OF HP3 FOR STRAIN\*INJ3**

STRAIN	INJ3	MEAN	SS (MEAN)
<b>F344 1</b>	1	12.935	142.19
<b>F344 1</b>	2	16.532	519.03
<b>M520 2</b>	1	19.249	599.25
<b>M520 2</b>	2	9.2010	99.795

Table 15b

**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CECO, & ALCOHOL  
CHOICE**

**HP3 (CS-ALONE TEST) 6.19.92-6.20.92 SAL/NAL 7mg/kg**

AOV CONTRASTS OF HP3 BY STRAIN\*INJ3

CONTRAST NUMBER 1

CONTRAST COEFFICIENTS:	1 1 -1 -1	<b>MAIN EFFECT OF GENETICS</b>
CONTRAST	1.0164	SS (CONTRAST) 3.3574
SCHEFFE'S F	0.10	P (SCHEFFE'S F) 0.7550
T-STATISTIC	0.31	P (T-STATISTIC) 0.7550
SE (CONTRAST)	3.2347	

CONTRAST NUMBER 2

CONTRAST COEFFICIENTS:	1 -1 1 -1	<b>MAIN EFFECT OF INJECTION</b>
CONTRAST	6.4508	SS (CONTRAST) 135.24
SCHEFFE'S F	3.98	P (SCHEFFE'S F) 0.0530
T-STATISTIC	1.99	P (T-STATISTIC) 0.0530
SE (CONTRAST)	3.2347	

CONTRAST NUMBER 3

CONTRAST COEFFICIENTS:	1 -1 0 0	<b>EFFECT OF INJECTION ON F344s</b>
CONTRAST	-3.5971	SS (CONTRAST) 84.102
SCHEFFE'S F	2.47	P (SCHEFFE'S F) 0.1237
T-STATISTIC	-1.57	P (T-STATISTIC) 0.1237
SE (CONTRAST)	2.2873	

CONTRAST NUMBER 4

CONTRAST COEFFICIENTS:	0 0 1 -1	<b>EFFECT OF INJECTION ON M520s</b>
CONTRAST	10.048	SS (CONTRAST) 656.24
SCHEFFE'S F	19.30	P (SCHEFFE'S F) 0.0001
T-STATISTIC	4.39	P (T-STATISTIC) 0.0001
SE (CONTRAST)	2.2873	

CONTRAST NUMBER 5

CONTRAST COEFFICIENTS:	1 1 1 -3	<b>UNIQUENESS of M520/NAL</b>
CONTRAST	21.112	SS (CONTRAST) 482.87
SCHEFFE'S F	14.20	P (SCHEFFE'S F) 0.0005
T-STATISTIC	3.77	P (T-STATISTIC) 0.0005
SE (CONTRAST)	5.6028	

CONTRAST NUMBER 6

CONTRAST COEFFICIENTS:	0 1 0 -1	<b>DIFF BTWN NAL GROUPS</b>
CONTRAST	7.3307	S (CONTRAST) 349.30
SCHEFFE'S F	10.27	P (SCHEFFE'S F) 0.0027
T-STATISTIC	3.20	P (T-STATISTIC) 0.0027
SE (CONTRAST)	2.2873	

CONTRAST NUMBER 7

CONTRAST COEFFICIENTS:	1 0 -1 0	<b>DIFF BTWN SAL GROUPS</b>
CONTRAST	-6.3143	SS (CONTRAST) 259.16
SCHEFFE'S F	7.62	P (SCHEFFE'S F) 0.0087
T-STATISTIC	-2.76	P (T-STATISTIC) 0.0087
SE (CONTRAST)	2.2873	

ERROR TERM USED: STRAIN\*INJ3\*SUBJ, 40 DF

Table 16a  
**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CECOA, & ALCOHOL CHOICE**  
**HP2 & HP3 (CS VS NTG CONDITIONS: COMPOSITE OF HP2 & HP3)**

ANALYSIS OF VARIANCE TABLE FOR **HP2\_3** (ANOVA COMPOSITE OF HP2\_&HP3)

SOURCE	DF	SS	MS	F	P
NTG_CS (A)	1	13.1420	13.1420	0.32	0.5715
STNHP2_3 (B)	1	90.9246	90.9246	2.23	0.1388
INJHP2_3 (C)	1	40.1759	40.1759	0.99	0.3234
SUBHP2_3 (D)					
A*B*C*D	87	3544.33	40.7394		
TOTAL	90	3688.57			
GRAND AVERAGE	1	20766.3			
CASES INCLUDED	91	MISSING CASES 13			

MEANS OF HP2\_3 FOR NTG\_CS

NTG_CS	MEAN	SS (MEAN)
NTG 1	13.775	1742.4
CS 2	14.486	1933.0

MEANS OF HP2\_3 FOR STNHP2\_3

STNHP2_3	MEAN	SS (MEAN)
F344 1	13.196	1139.5
M520 2	15.066	2458.2

MEANS OF HP2\_3 FOR INJHP2\_3

INJHP2_3	MEAN	SS (MEAN)
SAL 1	13.509	1553.4
NAL 2	14.752	2095.0

MEANS OF HP2\_3 FOR NTG\_CS\*STNHP2\_3\*INJHP2\_3

NTG_CS	STNHP2_3	INJHP2_3	MEAN	SS (MEAN)
1= NTG	1= F344	1= SAL	11.031	91.931
1	1	2= NAL	12.467	183.09
1	2= M520	1	12.191	310.11
1	2	2	19.412	591.49
2= CS	1	1	12.935	142.19
2	1	2	16.350	524.17
2	2	1	17.880	654.07
2	2	2	10.780	207.82

Table 16b

**Experiment 2: M520s & F344s, GENETICS, TEMPERAMENT, CEOA, & ALCOHOL CHOICE**  
**HOT PLATES 2 & 3 COMBINED (NTG vs. CS: f344/m520 X saline/nal)**

AOV CONTRASTS OF HP2\_3 BY NTG\_CS\*STNHP2\_3\*INJHP2\_3

CONTRAST NUMBER 1		<b>EFFECT OF CS ON SAL/M520</b>	
CONTRAST COEFFICIENTS: 0 0 1 0 0 0 -1 0			
CONTRAST	-5.6886	SS (CONTRAST)	210.34
SCHEFFE'S F	5.16	P (SCHEFFE'S F)	0.0255
T-STATISTIC	-2.27	P (T-STATISTIC)	0.0255
SE (CONTRAST)	2.5035		
CONTRAST NUMBER 2		<b>EFFECT OF CS ON NAL/M520</b>	
CONTRAST COEFFICIENTS: 0 0 0 1 0 0 0 -1			
CONTRAST	8.6318	SS (CONTRAST)	484.30
SCHEFFE'S F	11.89	P (SCHEFFE'S F)	0.0009
T-STATISTIC	3.45	P (T-STATISTIC)	0.0009
SE (CONTRAST)	2.5035		
CONTRAST NUMBER 3		<b>EFFECT OF CS ON saline F344</b>	
CONTRAST COEFFICIENTS: 1 0 0 0 -1 0 0 0			
CONTRAST	-1.9040	SS (CONTRAST)	23.563
SCHEFFE'S F	0.58	P (SCHEFFE'S F)	0.4490
T-STATISTIC	-0.76	P (T-STATISTIC)	0.4490
SE (CONTRAST)	2.5035		
CONTRAST NUMBER 4		<b>EFFECT OF CS ON NAL F344</b>	
CONTRAST COEFFICIENTS: 0 1 0 0 0 -1 0 0			
CONTRAST	-3.8832	SS (CONTRAST)	98.013
SCHEFFE'S F	2.41	P (SCHEFFE'S F)	0.1245
T-STATISTIC	-1.55	P (T-STATISTIC)	0.1245
SE (CONTRAST)	2.5035		

ERROR TERM USED: NTG\_CS\*STNHP2\_3\*INJHP2\_3\*SUBHP2\_3, 87 DF

Table 17  
**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE TRAINING  
 HP1A (1 mg/kg morphine, no habituation to transportation)**

**ANALYSIS OF VARIANCE TABLE FOR HP1A**

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	948.920	948.920	9.81	0.0052
INJ1A (B)	1	277.416	277.416	2.87	0.1058
A*B	1	97.0090	97.0090	1.00	0.3285
SUBJ1A (C)					
A*B*C	20	1933.68	96.6838		
TOTAL	23	3257.02			
GRAND AVERAGE	1	8015.53			
CASES INCLUDED	24	MISSING CASES	4		
GRAND MEAN	16.919	SE	1.8582		

**MEANS OF HP1A FOR STRAIN**

STRAIN	MEAN	SS (MEAN)
<b>M520</b> 0	22.741	1999.9
<b>F344</b> 1	11.098	308.22

**MEANS OF HP1A FOR INJ1A**

INJ1A	MEAN	SS (MEAN)
<b>SAL</b> 0	20.067	2488.5
<b>MOR</b> 1	13.772	491.15

**MEANS OF HP1A FOR STRAIN\*INJ1A**

STRAIN	INJ1A	MEAN	SS (MEAN)
<b>M520</b> 0	<b>SAL</b> 0	27.750	1422.7
	<b>MOR</b> 1	17.732	225.97
<b>F344</b> 1	0	12.384	239.44
	1	9.8117	45.623

Table 18

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
HP1A (1 mg/kg morphine, no habituation to transportation)  
(Before exposure to aversive conditioning)**

AOV CONTRASTS OF HP1A BY STRAIN\*INJ1A

		<b>EFFECT OF GENETICS</b>	
CONTRAST NUMBER 1		<b>EFFECT OF INJECTION</b>	
CONTRAST COEFFICIENTS:	1 1 -1 -1	SS (CONTRAST)	948.92
CONTRAST	23.286	P (SCHEFFE'S F)	0.0052
SCHEFFE'S F	9.81	P (T-STATISTIC)	0.0052
T-STATISTIC	3.13		
SE (CONTRAST)	7.4329		
CONTRAST NUMBER 2		<b>EFFECT OF INJ ON F344</b>	
CONTRAST COEFFICIENTS:	1 -1 1 -1	SS (CONTRAST)	277.42
CONTRAST	12.591	P (SCHEFFE'S F)	0.1058
SCHEFFE'S F	2.87	P (T-STATISTIC)	0.1058
T-STATISTIC	1.69		
SE (CONTRAST)	7.4329		
CONTRAST NUMBER 3		<b>EFFECT OF INJ ON M520</b>	
CONTRAST COEFFICIENTS:	0 0 1 -1	SS (CONTRAST)	23.164
CONTRAST	2.5726	P (SCHEFFE'S F)	0.6298
SCHEFFE'S F	0.24	P (T-STATISTIC)	0.6298
T-STATISTIC	0.49		
SE (CONTRAST)	5.2558		
CONTRAST NUMBER 4		<b>UNIQUENESS OF F344/SAL</b>	
CONTRAST COEFFICIENTS:	1 -1 0 0	SS (CONTRAST)	351.26
CONTRAST	10.018	P (SCHEFFE'S F)	0.0711
SCHEFFE'S F	3.63	P (T-STATISTIC)	0.0711
T-STATISTIC	1.91		
SE (CONTRAST)	5.2558		
CONTRAST NUMBER 5		<b>DIFF BTWN SALINE GROUPS</b>	
CONTRAST COEFFICIENTS:	1 1 -3 1	SS (CONTRAST)	191.97
CONTRAST	18.141	P (SCHEFFE'S F)	0.1742
SCHEFFE'S F	1.99	P (T-STATISTIC)	0.1742
T-STATISTIC	1.41		
SE (CONTRAST)	12.874		
CONTRAST NUMBER 6		<b>DIFF BTWN NALTREXONE GROUPS</b>	
CONTRAST COEFFICIENTS:	1 0 -1 0	SS (CONTRAST)	826.37
CONTRAST	15.366	P (SCHEFFE'S F)	0.0084
SCHEFFE'S F	8.55	P (T-STATISTIC)	0.0084
T-STATISTIC	2.92		
SE (CONTRAST)	5.2558		
CONTRAST NUMBER 7		<b>ERROR TERM USED: STRAIN*INJ1A*SUBJ1A, 20 DF</b>	
CONTRAST COEFFICIENTS:	0 1 0 -1	SS (CONTRAST)	219.56
CONTRAST	7.9203	P (SCHEFFE'S F)	0.1475
SCHEFFE'S F	2.27	P (T-STATISTIC)	0.1475
T-STATISTIC	1.51		
SE (CONTRAST)	5.2558		

Table 19  
**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
 HP1B (5 mg/kg morphine before exposure to aversive conditioning)**

**ANALYSIS OF VARIANCE TABLE FOR HP1B**

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	2047.55	2047.55	9.66	0.0055
INJ1B (B)	1	752.931	752.931	3.55	0.0741
A*B	1	1434.01	1434.01	6.76	0.0171
SUBJHP1B (C)					
A*B*C	20	4239.69	211.984		
TOTAL	23	8474.18			
GRAND AVERAGE	1	9616.45			
CASES INCLUDED	24	MISSING CASES 4			

**MEANS OF HP1B FOR STRAIN**

STRAIN	MEAN	SS (MEAN)
<b>M520</b> 1	27.084	6300.6
<b>F344</b> 2	9.9808	126.03

**MEANS OF HP1B FOR INJ1B**

INJ1B	MEAN	SS (MEAN)
<b>SAL</b> 0	13.347	229.36
<b>MOR</b> 1	23.718	7491.9

**MEANS OF HP1B FOR STRAIN\*INJ1B**

STRAIN	INJ1B	MEAN	SS (MEAN)
<b>M520</b> 1	<b>SAL</b> 0	14.742	147.09
	<b>MOR</b> 1	39.426	4020.9
<b>F344</b> 2	0	11.952	55.029
	1	8.0100	16.620

Table 20

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
HP1B (5 mg/kg morphine before aversive conditioning, "NTG COND")**

## AOV CONTRASTS OF HP1B BY STRAIN\*INJ1B

## CONTRAST NUMBER 1

CONTRAST COEFFICIENTS: 1 -3 1 1		UNIQUENESS OF M520/MOR	
CONTRAST	-83.574	SS (CONTRAST)	4074.3
SCHEFFE'S F	19.22	P (SCHEFFE'S F)	0.0003
T-STATISTIC	-4.38	P (T-STATISTIC)	0.0003
SE (CONTRAST)	19.063		

## CONTRAST NUMBER 2

CONTRAST COEFFICIENTS: 1 -1 0 0		EFFECT OF INJ ON M520	
CONTRAST	-24.684	SS (CONTRAST)	2132.6
SCHEFFE'S F	10.06	P (SCHEFFE'S F)	0.0048
T-STATISTIC	-3.17	P (T-STATISTIC)	0.0048
SE (CONTRAST)	7.7825		

## CONTRAST NUMBER 3

CONTRAST COEFFICIENTS: 0 0 1 -1		EFFECT OF INJ ON F344	
CONTRAST	3.9417	SS (CONTRAST)	54.379
SCHEFFE'S F	0.26	P (SCHEFFE'S F)	0.6181
T-STATISTIC	0.51	P (T-STATISTIC)	0.6181
SE (CONTRAST)	7.7825		

## CONTRAST NUMBER 4

CONTRAST COEFFICIENTS: 1 1 -1 -1		EFFECT OF GENETICS	
CONTRAST	34.206	SS (CONTRAST)	2047.6
SCHEFFE'S F	9.66	P (SCHEFFE'S F)	0.0055
T-STATISTIC	3.11	P (T-STATISTIC)	0.0055
SE (CONTRAST)	11.006		

## CONTRAST NUMBER 5

CONTRAST COEFFICIENTS: 1 -1 1 -1		EFFECT OF INJECTION	
CONTRAST	-20.742	SS (CONTRAST)	752.93
SCHEFFE'S F	3.55	P (SCHEFFE'S F)	0.0741
T-STATISTIC	-1.88	P (T-STATISTIC)	0.0741
SE (CONTRAST)	11.006		

## CONTRAST NUMBER 6

CONTRAST COEFFICIENTS: 0 1 0 -1		DIFFERENCE BTWN MOR GROUPS	
CONTRAST	15.366	SS (CONTRAST)	826.37
SCHEFFE'S F	8.55	P (SCHEFFE'S F)	0.0084
T-STATISTIC	2.92	P (T-STATISTIC)	0.0084
SE (CONTRAST)	5.2558		

## CONTRAST NUMBER 7

CONTRAST COEFFICIENTS: 1 0 1 0		DIFFERENCE BTWN SAL GROUPS	
CONTRAST	7.9203	SS (CONTRAST)	219.56
SCHEFFE'S F	2.27	P (SCHEFFE'S F)	0.1475
T-STATISTIC	1.51	P (T-STATISTIC)	0.1475
SE (CONTRAST)	5.2558		

ERROR TERM USED: STRAIN\*INJ1B\*SUBJHP1B, 20 DF

Table 21a  
**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
 HP2 (5 mg/kg morphine after 11 daily aversive CS/US TRIALS)**

**ANALYSIS OF VARIANCE TABLE FOR HP2**

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	1400.40	1400.40	8.85	0.0072
INJHP2 (B)	1	2082.33	2082.33	13.15	0.0016
A*B	1	448.840	448.840	2.84	0.1070
SUBJ (C)					
A*B*C	21	3324.27	158.299		
TOTAL	24	7255.85			
GRAND AVERAGE	1	17968.0			
CASES INCLUDED	25	MISSING CASES	3		
GRAND MEAN	25.332	SE	2.3777		

**MEANS OF HP2 FOR STRAIN**

STRAIN	MEAN	SS (MEAN)
M520 1	32.404	5328.0
F344 2	18.260	527.41

**MEANS OF HP2 FOR INJHP2**

INJHP2	MEAN	SS (MEAN)
SAL 1	16.708	1112.9
MOR 2	33.956	4060.6

**MEANS OF HP2 FOR STRAIN\*INJHP2**

STRAIN	INJHP2	MEAN	SS (MEAN)
M520 1	SAL 1	19.777	812.74
	MOR 2	45.032	2282.9
F344 2	1	13.640	168.36
	2	22.880	60.227

Table 21b

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
HP2 (5 mg/kg morphine after 11 days of aversive CS/US training)**

## AOV CONTRASTS OF HP2 BY STRAIN\*INJHP2

## CONTRAST NUMBER 1

## EFFECT OF GENETICS

CONTRAST COEFFICIENTS: 1 1 -1 -1  
 CONTRAST 28.288  
 SCHEFFE'S F 8.85  
 T-STATISTIC 2.97  
 SE (CONTRAST) 9.5109

SS (CONTRAST) 1400.4  
 P (SCHEFFE'S F) 0.0072  
 P (T-STATISTIC) 0.0072

## CONTRAST NUMBER 2

## EFFECT OF INJECTION

CONTRAST COEFFICIENTS: 1 -1 1 -1  
 CONTRAST -34.495  
 SCHEFFE'S F 13.15  
 T-STATISTIC -3.63  
 SE (CONTRAST) 9.5109

SS (CONTRAST) 2082.3  
 P (SCHEFFE'S F) 0.0016  
 P (T-STATISTIC) 0.0016

## CONTRAST NUMBER 3

## EFFECT OF INJ ON F344

CONTRAST COEFFICIENTS: 0 0 1 -1  
 CONTRAST -9.2400  
 SCHEFFE'S F 1.89  
 T-STATISTIC -1.37  
 SE (CONTRAST) 6.7252

SS (CONTRAST) 298.82  
 P (SCHEFFE'S F) 0.1839  
 P (T-STATISTIC) 0.1839

## CONTRAST NUMBER 4

## EFFECT OF INJ ON M520

CONTRAST COEFFICIENTS: 1 -1 0 0  
 CONTRAST -25.255  
 SCHEFFE'S F 14.10  
 T-STATISTIC -3.76  
 SE (CONTRAST) 6.7252

SS (CONTRAST) 2232.4  
 P (SCHEFFE'S F) 0.0012  
 P (T-STATISTIC) 0.0012

## CONTRAST NUMBER 5

## DIFF BTWN SALINE GROUPS

CONTRAST COEFFICIENTS: 1 0 -1 0  
 CONTRAST 6.1367  
 SCHEFFE'S F 0.83  
 T-STATISTIC .91  
 SE (CONTRAST) 6.7252

SS (CONTRAST) 131.81  
 P (SCHEFFE'S F) 0.3719  
 P (T-STATISTIC) 0.3719

## CONTRAST NUMBER 6

## DIFF BTWN MORPHINE GROUPS

CONTRAST COEFFICIENTS: 0 1 0 -1  
 CONTRAST 22.152  
 SCHEFFE'S F 10.85  
 T-STATISTIC 3.29  
 SE (CONTRAST) 6.7252

SS (CONTRAST) 1717.4  
 P (SCHEFFE'S F) 0.0035  
 P (T-STATISTIC) 0.0035

ERROR TERM USED: STRAIN\*INJHP2A\*SUBJ, 21 DF

Table 22a

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**  
**HP3A: ALL SUBJs 5 mg/kg morphine after 23 days of av. CS/US training**

## ANALYSIS OF VARIANCE TABLE FOR HP3A

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	1472.51	1472.51	4.25	0.0524
NTG_CS (B)	1	172.646	172.646	0.50	0.4883
A*B	1	103.958	103.958	0.30	0.5898
SUBJ (C)					
A*B*C	20	6926.01	346.301		
TOTAL	23	8675.13			
GRAND AVERAGE	1	19057.0			
GRAND MEAN	28.179	SE 3.7986			

## MEANS OF HP3A FOR STRAIN

STRAIN	MEAN	SS (MEAN)
M520 1	36.012	4890.2
F344 2	20.346	2312.5

## MEANS OF HP3A FOR NTG\_CS

NTG_CS	MEAN	SS (MEAN)
NTG 1	30.861	4845.2
CS 2	25.497	3657.2

## MEANS OF HP3A FOR STRAIN\*NTG\_CS

STRAIN	NTG_CS	MEAN	SS (MEAN)
1	1	40.775	1723.0
1	2	31.248	2894.9
2	1	20.947	1942.7
2	2	19.745	365.37

Table 22b

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**  
**HP3A: ALL SUBJs = 5 mg/kg morphine (F344/M520 X NTG/CS)**  
 after 23 days of av. CS/US training

## AOV CONTRASTS OF HP3A BY STRAIN\*NTG\_CS

## CONTRAST NUMBER 1

## EFFECT OF GENETICS

CONTRAST COEFFICIENTS: 1 1 -1 -1

CONTRAST	31.332	SS (CONTRAST)	1472.5
SCHEFFE'S F	4.25	P (SCHEFFE'S F)	0.0524
T-STATISTIC	2.06	P (T-STATISTIC)	0.0524
SE (CONTRAST)	15.194		

## CONTRAST NUMBER 2

## EFFECT OF NTG\_CS STIM

CONTRAST COEFFICIENTS: 1 -1 1 -1

CONTRAST	10.728	SS (CONTRAST)	172.65
SCHEFFE'S F	0.50	P (SCHEFFE'S F)	0.4883
T-STATISTIC	0.71	P (T-STATISTIC)	0.4883
SE (CONTRAST)	15.194		

## CONTRAST NUMBER 3

## EFFECT OF STIM ON F344

CONTRAST COEFFICIENTS: 0 0 1 -1

CONTRAST	1.2017	SS (CONTRAST)	4.3320
SCHEFFE'S F	0.01	P (SCHEFFE'S F)	0.9121
T-STATISTIC	0.11	P (T-STATISTIC)	0.9121
SE (CONTRAST)	10.744		

## CONTRAST NUMBER 4

## EFFECT OF STIM ON M520

CONTRAST COEFFICIENTS: 1 -1 0 0

CONTRAST	9.5267	SS (CONTRAST)	272.27
SCHEFFE'S F	0.79	P (SCHEFFE'S F)	0.3858
T-STATISTIC	0.89	P (T-STATISTIC)	0.3858
SE (CONTRAST)	10.744		

Table 23a

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
 HP3B: ALL SUBJs = 5 mg/kg morphine (F344/M520 X NTG/CS)  
 AFTER 26 DAYS OF CS/US TRAINING**

## ANALYSIS OF VARIANCE TABLE FOR HP3B

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	1630.86	1630.86	11.96	0.0023
CS_NTG (B)	2	2444.93	1222.47	8.97	0.0015
A*B	21	2862.69	136.319		
TOTAL	24	6938.49			
GRAND AVERAGE	1	11063.0			
CASES INCLUDED	25	MISSING CASES	3		
GRAND MEAN	19.877	SE	2.2065		

## MEANS OF HP3B FOR STRAIN

STRAIN	MEAN	SS (MEAN)
M520 1	27.509	5073.1
F344 2	12.245	234.48

## MEANS OF HP3B FOR CS\_NTG

CS_NTG	MEAN	SS (MEAN)
NTG 1	27.147	5281.4
CS 2	12.607	177.29

## MEANS OF HP3B FOR STRAIN\*CS\_NTG

STRAIN	CS_NTG	MEAN	SS (MEAN)
M520 1	NTG 1	40.650	2587.9
1	CS 2	14.368	67.715
F344 2	1	13.644	140.92
2	2	10.847	66.167

Table 23b

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
ALL SUBJs 5 mg/kg MORPHINE, 26 DAYS CS/US TRAINING**

AOV CONTRASTS OF HP3B BY STRAIN*CS_NTG			
<b>CONTRAST NUMBER 1</b>			<b>EFFECT OF GENETICS</b>
CONTRAST COEFFICIENTS:	1 1 -1 -1		
CONTRAST	30.527	SS (CONTRAST)	1630.9
SCHIFFE'S F	11.96	P (SCHIFFE'S F)	0.0023
T-STATISTIC	3.46	P (T-STATISTIC)	0.0023
SE (CONTRAST)	8.8259		
<b>CONTRAST NUMBER 2</b>			<b>EFFECT OF STIM</b>
CONTRAST COEFFICIENTS:	1 -1 1 -1		
CONTRAST	29.079	SS (CONTRAST)	1479.8
SCHIFFE'S F	10.86	P (SCHIFFE'S F)	0.0035
T-STATISTIC	3.29	P (T-STATISTIC)	0.0035
SE (CONTRAST)	8.8259		
<b>CONTRAST NUMBER 3</b>			<b>EFFECT OF STIM ON F344</b>
CONTRAST COEFFICIENTS:	0 0 1 -1		
CONTRAST	2.7976	SS (CONTRAST)	27.393
SCHIFFE'S F	0.20	P (SCHIFFE'S F)	0.6585
T-STATISTIC	0.45	P (T-STATISTIC)	0.6585
SE (CONTRAST)	6.2408		
<b>CONTRAST NUMBER 4</b>			<b>EFFECT OF STIM ON M520</b>
CONTRAST COEFFICIENTS:	1 -1 0 0		
CONTRAST	26.282	SS (CONTRAST)	2417.5
SCHIFFE'S F	17.73	P (SCHIFFE'S F)	0.0004
T-STATISTIC	4.21	P (T-STATISTIC)	0.0004
SE (CONTRAST)	6.2408		
<b>CONTRAST NUMBER 5</b>			<b>UNIQUENESS OF NTG/M520</b>
CONTRAST COEFFICIENTS:	3 -1 -1 -1		
CONTRAST	83.091	SS (CONTRAST)	4027.4
SCHIFFE'S F	29.54	P (SCHIFFE'S F)	0.0000
T-STATISTIC	5.44	P (T-STATISTIC)	0.0000
SE (CONTRAST)	15.287		
<b>CONTRAST NUMBER 6</b>			<b>CS/M520 = F344</b>
CONTRAST COEFFICIENTS:	0 2 -1 -1		
CONTRAST	4.2457	SS (CONTRAST)	21.030
SCHIFFE'S F	0.15	P (SCHIFFE'S F)	0.6984
T-STATISTIC	0.39	P (T-STATISTIC)	0.6984
SE (CONTRAST)	10.809		

ERROR TERM USED: STRAIN\*CS\_NTG\*SUBJ, 21 DF

Table 23c

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**  
**ALL SUBJs 5 mg/kg MORPHINE, 26 DAYS CS/US TRAINING**

<b>CONTRAST NUMBER 7</b>		<b>M520/NTG VS F344/NTG</b>	
CONTRAST COEFFICIENTS: 1 0 -1 0			
CONTRAST	27.06	SS (CONTRAST)	2552.6
SCHEFFE'S F	18.73	P (SCHEFFE'S F)	0.0003
T-STATISTIC	4.33	P (T-STATISTIC)	0.0003
SE (CONTRAST)	6.2408		
<b>CONTRAST NUMBER 8</b>		<b>CS/M520 = F344</b>	
CONTRAST COEFFICIENTS: 0 1 0 -1			
CONTRAST	3.5217	SS (CONTRAST)	43.407
SCHEFFE'S F	0.32	P (SCHEFFE'S F)	0.5785
T-STATISTIC	0.56	P (T-STATISTIC)	0.5785
SE (CONTRAST)	6.2408		

ERROR TERM USED: STRAIN\*CS\_NTG\*SUBJ, 21 DF

Table 24a

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**  
 ALL SUBJs SALINE INJECTION AFTER 29 DAYS OF CS/US TRAINING  
 HP4: (NTG/CS x M520/F344)

## ANALYSIS OF VARIANCE TABLE FOR HP4

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	353.730	353.730	22.24	0.0002
CS_NTG (B)	1	78.6055	78.6055	4.94	0.0401
A*B	1	16.7592	16.7592	1.05	0.3190
SUBJ (C)					
A*B*C	17	270.382	15.9048		
TOTAL	20	719.477			
GRAND AVERAGE	1	4017.35			
CASES INCLUDED	21	MISSING CASES	7		
GRAND MEAN	11.978	SE	0.7537		

## MEANS OF HP4 FOR STRAIN

STRAIN	MEAN	SS (MEAN)
M520 1	15.533	267.27
F344 2	8.4239	98.473

## MEANS OF HP4 FOR STRAIN

STRAIN	MEAN	SS (MEAN)
M520 1	15.533	267.27
F344 2	8.4239	98.473

## MEANS OF HP4 FOR STRAIN\*CS\_NTG

STRAIN	CS_NTG	MEAN	SS (MEAN)
M520 1	NTG 1	17.982	141.61
	CS 2	13.083	41.689
F344 2	1	9.3257	76.708
	2	7.5220	10.379

Table 24b

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**  
**HP4: (NTG/CS x M520/F344)**  
**ALL SUBJs: SALINE INJECTION AFTER 29 DAYS OF CS/US TRAINING**

**AOV CONTRASTS OF HP4 BY STRAIN\*CS\_NTG**

				<b>EFFECT OF GENETICS</b>
<b>CONTRAST NUMBER 1</b>				
<b>CONTRAST COEFFICIENTS: 1 1 -1 -1</b>				
CONTRAST	14.217	SS (CONTRAST)	353.73	
SCHEFFE'S F	22.24	P (SCHEFFE'S F)	0.0002	
T-STATISTIC	4.72	P (T-STATISTIC)	0.0002	
SE (CONTRAST)	3.0147			

**CONTRAST NUMBER 2**

				<b>EFFECT OF STIM</b>
<b>CONTRAST COEFFICIENTS: 1 -1 1 -1</b>				
CONTRAST	6.7020	SS (CONTRAST)	78.606	
SCHEFFE'S F	4.94	P (SCHEFFE'S F)	0.0401	
T-STATISTIC	2.22	P (T-STATISTIC)	0.0401	
SE (CONTRAST)	3.0147			

**CONTRAST NUMBER 3**

				<b>EFFECT OF STIM ON F344</b>
<b>CONTRAST COEFFICIENTS: 0 0 1 -1</b>				
CONTRAST	1.8037	SS (CONTRAST)	11.387	
SCHEFFE'S F	0.72	P (SCHEFFE'S F)	0.4092	
T-STATISTIC	0.85	P (T-STATISTIC)	0.4092	
SE (CONTRAST)	2.1317			

**CONTRAST NUMBER 4**

				<b>EFFECT OF STIM ON M520</b>
<b>CONTRAST COEFFICIENTS: 1 -1 0 0</b>				
CONTRAST	4.8983	SS (CONTRAST)	83.978	
SCHEFFE'S F	5.28	P (SCHEFFE'S F)	0.0345	
T-STATISTIC	2.30	P (T-STATISTIC)	0.0345	
SE (CONTRAST)	2.1317			

**CONTRAST NUMBER 5**

				<b>UNIQUENESS OF NTG/M520</b>
<b>CONTRAST COEFFICIENTS: 3 -1 -1 -1</b>				
CONTRAST	24.014	SS (CONTRAST)	336.39	
SCHEFFE'S F	21.15	P (SCHEFFE'S F)	0.0003	
T-STATISTIC	4.60	P (T-STATISTIC)	0.0003	
SE (CONTRAST)	5.2216			

**CONTRAST NUMBER 6**

				<b>M520/CS NOT DIFF F344s</b>
<b>CONTRAST COEFFICIENTS: 0 2 -1 -1</b>				
CONTRAST	9.3190	SS (CONTRAST)	101.32	
SCHEFFE'S F	6.37	P (SCHEFFE'S F)	0.0218	
T-STATISTIC	2.52	P (T-STATISTIC)	0.0218	
SE (CONTRAST)	3.6923			

ERROR TERM USED: STRAIN\*CS\_NTG\*SUBJ, 17 DF

Table 24c

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
ALL SUBJs SALINE INJECTION AFTER 29 DAYS OF CS/US TRAINING  
HP4: (NTG/CS x M520/F344)**

CONTRAST NUMBER 7

CONTRAST COEFFICIENTS: 1 0 -1 0

CONTRAST	8.656	SS (CONTRAST)	262.24
SCHEFFE'S F	16.49	P (SCHEFFE'S F)	0.0008
T-STATISTIC	4.06	P (T-STATISTIC)	0.0008
SE (CONTRAST)	2.1317		

**M520/NTG vs. F344/NTG**

CONTRAST NUMBER 8

CONTRAST COEFFICIENTS: 0 1 0 -1

CONTRAST	5.5613	SS (CONTRAST)	108.25
SCHEFFE'S F	6.81	P (SCHEFFE'S F)	0.0183
T-STATISTIC	2.61	P (T-STATISTIC)	0.0183
SE (CONTRAST)	2.1317		

**M520/CS vs. F344/CS**

ERROR TERM USED: STRAIN\*CS\_NTG\*SUBJ, 17 DF

TABLE 25a  
**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**  
**HP5: (SAL/NAL x M520/F344)**  
**ALL SUBJ's NTG TEST CONDITION AFTER 32 DAYS OF CS/US TRAINING**

TO TEST FOR CEOA TO THE TRANSPORTATION PROTOCOLS

ANALYSIS OF VARIANCE TABLE FOR HP5

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	870.871	870.871	18.78	0.0003
INJ (B)	1	62.6256	62.6256	1.35	0.2583
A*B	1	102.446	102.446	2.21	0.1521
SUBJ (C)					
A*B*C	21	973.914	46.3768		
TOTAL	24	2009.86			
GRAND AVERAGE	1	5607.31			
CASES INCLUDED	25	MISSING CASES	3		
GRAND MEAN	14.151	SE	1.2870		

MEANS OF HP5 FOR STRAIN

STRAIN	MEAN	SS (MEAN)
M520 1	19.728	993.33
F344 2	8.5744	145.65

MEANS OF HP5 FOR INJ

INJ	MEAN	SS (MEAN)
SAL 1	15.647	1625.8
NAL 2	12.656	321.43

MEANS OF HP5 FOR STRAIN\*INJ

STRAIN	INJ	MEAN	SS (MEAN)
M520 1	SAL 1	23.137	715.01
1	NAL 2	16.320	115.68
F344 2	1	8.1571	125.44
2	2	8.9917	17.779

TABLE 25b

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
HP5:NTG (SAL/NAL x M520/F344) AFTER 32 DAYS OF CS/US TRAINING  
TO TEST FOR CEOA TO THE TRANSPORTATION PROTOCOLS**

## AOV CONTRASTS OF HP5 BY STRAIN\*INJ

## CONTRAST NUMBER 1

**EFFECT OF GENETICS**

CONTRAST COEFFICIENTS: 1 1 -1 -1

CONTRAST	22.308	SS (CONTRAST)	870.87
SCHEFFE'S F	18.78	P (SCHEFFE'S F)	0.0003
T-STATISTIC	4.33	P (T-STATISTIC)	0.0003
SE (CONTRAST)	5.1479		

## CONTRAST NUMBER 2

**EFFECT OF INJ(SAL/NAL)**

CONTRAST COEFFICIENTS: 1 -1 1 -1

CONTRAST	5.9821	SS (CONTRAST)	62.626
SCHEFFE'S F	1.35	P (SCHEFFE'S F)	0.2583
T-STATISTIC	1.16	P (T-STATISTIC)	0.2583
SE (CONTRAST)	5.1479		

## CONTRAST NUMBER 3

**EFFECT OF INJ IN F344**

CONTRAST COEFFICIENTS: 0 0 1 -1

CONTRAST	-0.8345	SS (CONTRAST)	2.4375
SCHEFFE'S F	0.05	P (SCHEFFE'S F)	0.8209
T-STATISTIC	-0.23	P (T-STATISTIC)	0.8209
SE (CONTRAST)	3.6401		

## CONTRAST NUMBER 4

**EFFECT OF INJ IN M520**

CONTRAST COEFFICIENTS: 1 -1 0 0

CONTRAST	6.8167	SS (CONTRAST)	162.63
SCHEFFE'S F	3.51	P (SCHEFFE'S F)	0.0751
T-STATISTIC	1.87	P (T-STATISTIC)	0.0751
SE (CONTRAST)	3.6401		

## CONTRAST NUMBER 5

**UNIQUENESS OF SAL/M520**

CONTRAST COEFFICIENTS: 3 -1 -1 -1

CONTRAST	35.941	SS (CONTRAST)	753.53
SCHEFFE'S F	16.25	P (SCHEFFE'S F)	0.0006
T-STATISTIC	4.03	P (T-STATISTIC)	0.0006
SE (CONTRAST)	8.9165		

## CONTRAST NUMBER 6

**M520/SAL vs. F344/SAL**

CONTRAST COEFFICIENTS: 1 0 -1 0

CONTRAST	14.980	SS (CONTRAST)	785.35
SCHEFFE'S F	16.93	P (SCHEFFE'S F)	0.0005
T-STATISTIC	4.12	P (T-STATISTIC)	0.0005
SE (CONTRAST)	3.6401		

## CONTRAST NUMBER 7

**M520/NAL vs. F344/SAL**

CONTRAST COEFFICIENTS: 0 1 0 -1

CONTRAST	7.3283	SS (CONTRAST)	187.97
SCHEFFE'S F	4.05	P (SCHEFFE'S F)	0.0571
T-STATISTIC	2.01	P (T-STATISTIC)	0.0571
SE (CONTRAST)	3.6401		

ERROR TERM USED: STRAIN\*INJ\*SUBJ, 21 DF

Table 26a

**SAME HP5 ANALYSIS ADDING 1 RESERVE SUBJECT TO M520/NAL GROUP  
 Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
 HP5:NTG (SAL/NAL x M520/F344) AFTER 32 DAYS OF CS/US CONDITIONING**

TO TEST FOR CEOA TO THE TRANSPORTATION PROTOCOLS

**ANALYSIS OF VARIANCE TABLE FOR HP5**

SOURCE	DF	SS	MS	F	P
STRAIN (A)	1	806.234	806.234	17.82	0.0003
INJ (B)	1	99.8236	99.8236	2.21	0.1510
A*B	1	148.818	148.818	3.29	0.0828
SUBJ (C)					
A*B*C	23	1040.66	45.2461		
TOTAL	26	2095.54			
GRAND AVERAGE	1	5441.39			
CASES INCLUDED	27	MISSING CASES	1		
GRAND MEAN	13.940	SE	1.2712		

**MEANS OF HP5 FOR STRAIN**

STRAIN	MEAN	SS (MEAN)
<b>M520</b> 1	19.306	1143.6
<b>F344</b> 2	8.5744	145.65

**MEANS OF HP5 FOR INJ**

INJ	MEAN	SS (MEAN)
<b>SAL</b> 1	15.829	1669.9
<b>NAL</b> 2	12.052	325.81

**MEANS OF HP5 FOR STRAIN\*INJ**

STRAIN	INJ	MEAN	SS (MEAN)
<b>M520</b> 1	1	23.500	720.56
	2	15.113	176.89
<b>F344</b> 2	1	8.1571	125.44
	2	8.9917	17.779

Table 26b  
**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING  
 HP5:NTG (SAL/NAL x M520/F344) AFTER 32 DAYS OF CS/US TRAINING  
 TO TEST FOR CEOA TO THE TRANSPORTATION PROTOCOLS**

AOV CONTRASTS OF HP5 BY STRAIN*INJ			
CONTRAST NUMBER 1		EFFECT OF GENETICS	
CONTRAST COEFFICIENTS:	1 1 -1 -1		
CONTRAST	21.464	SS (CONTRAST)	806.23
SCHIFFE'S F	17.82	P (SCHIFFE'S F)	0.0003
T-STATISTIC	4.22	P (T-STATISTIC)	0.0003
SE (CONTRAST)	5.0848		
CONTRAST NUMBER 2		EFFECT OF INJECTION	
CONTRAST COEFFICIENTS:	1 -1 1 -1		
CONTRAST	7.5526	SS (CONTRAST)	99.824
SCHIFFE'S F	2.21	P (SCHIFFE'S F)	0.1510
T-STATISTIC	1.49	P (T-STATISTIC)	0.1510
SE (CONTRAST)	5.0848		
CONTRAST NUMBER 3		EFFECT OF INJ ON F344	
CONTRAST COEFFICIENTS:	0 0 1 -1		
CONTRAST	-0.8345	SS (CONTRAST)	2.4375
SCHIFFE'S F	0.05	P (SCHIFFE'S F)	0.8185
T-STATISTIC	-0.23	P (T-STATISTIC)	0.8185
SE (CONTRAST)	3.5955		
CONTRAST NUMBER 4		EFFECT OF INJ ON M520	
CONTRAST COEFFICIENTS:	1 -1 0 0		
CONTRAST	8.3871	SS (CONTRAST)	246.20
SCHIFFE'S F	5.44	P (SCHIFFE'S F)	0.0288
T-STATISTIC	2.33	P (T-STATISTIC)	0.0288
SE (CONTRAST)	3.5955		
CONTRAST NUMBER 5		M520/SAL vs F344/SAL	
CONTRAST COEFFICIENTS:	1 0 -1 0		
CONTRAST	15.343	SS (CONTRAST)	823.91
SCHIFFE'S F	18.21	P (SCHIFFE'S F)	0.0003
T-STATISTIC	4.27	P (T-STATISTIC)	0.0003
SE (CONTRAST)	3.5955		
CONTRAST NUMBER 6		F344/NAL vs F344/NAL	
CONTRAST COEFFICIENTS:	0 -1 0 1		
CONTRAST	6.1212	SS (CONTRAST)	131.14
SCHIFFE'S F	2.90	P (SCHIFFE'S F)	0.1021
T-STATISTIC	1.70	P (T-STATISTIC)	0.1021
SE (CONTRAST)	3.5955		
CONTRAST NUMBER 7		UNIQUENESS OF M520/SAL	
CONTRAST COEFFICIENTS:	3 -1 -1 -1		
CONTRAST	38.238	SS (CONTRAST)	852.93
SCHIFFE'S F	18.85	P (SCHIFFE'S F)	0.0002
T-STATISTIC	4.34	P (T-STATISTIC)	0.0002
SE (CONTRAST)	8.8071		
ERROR TERM USED: STRAIN*INJ*SUBJ, 23 DF			

Table 27

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**

## DESCRIPTIVE STATISTICS FOR STRAIN = 1

VARIABLE	N	MEAN	SE MEAN	MEDIAN
P810	10	64.663	11.038	66.071
P811	9	82.552	5.6517	88.060
P812	9	78.583	5.8560	89.286
P813	9	64.407	3.5719	62.500
P814	9	68.578	4.8006	73.913
P815	9	67.206	5.7017	71.739
P816	10	79.370	4.0032	85.418
P817	10	74.172	7.2188	81.977
P818	9	65.606	7.5830	67.500
PSUMRY	9	660.97	033.206	709.69
T810	10	3.1100	0.7062	2.8500
T811	9	5.1444	0.5937	4.9000
T812	9	5.7556	1.0000	5.1000
T813	9	4.4444	0.4534	4.6000
T814	9	5.5444	0.6044	5.1000
T815	9	5.6333	0.4673	6.3000
T816	10	6.9300	0.7556	7.0500
T817	10	4.8500	0.5216	4.7500
T818	9	2.4333	0.3109	2.7000

## DESCRIPTIVE STATISTICS FOR STRAIN = 2

VARIABLE	N	MEAN	SE MEAN	MEDIAN
P810	11	57.291	6.0932	60.000
P811	11	60.139	6.1811	58.889
P812	10	58.136	7.5041	57.327
P813	11	47.254	10.095	38.806
P814	11	43.507	7.1115	45.238
P815	11	43.471	7.2542	35.821
P816	11	67.581	5.7816	71.429
P817	10	48.998	7.5899	52.679
P818	11	47.267	10.398	38.667
PSUMRY	11	471.35	41.864	470.64
T810	11	2.6636	0.7394	1.6000
T811	11	4.6182	0.8674	3.5000
T812	10	5.5400	1.1895	3.9500
T813	11	3.1455	0.8578	2.6000
T814	11	3.1727	0.7316	2.8000
T815	11	2.9091	0.5542	2.7000
T816	11	5.0727	0.9657	4.2000
T817	10	2.2300	0.4487	1.8500
T818	11	2.4000	0.5652	2.7000

Table 28

**Experiment 3: M520s & F344s, EFFECTS OF EXTENSIVE CS/US TRAINING**

ONE-WAY AOV FOR PSUMRY BY GENETIC LINE OF RODENT  
 PSUMRY = THE AVERAGE PERCENTAGE OF ALCOHOL PREFERENCE OVER 9 DAYS

SOURCE	DF	SS	MS	F	P
BETWEEN	1	1.780E+05	1.780E+05	11.77	<b>0.0030</b>
WITHIN	18	2.722E+05	15120.7		
TOTAL	19	4.502E+05			

BARTLETT'S TEST OF EQUAL VARIANCES	CHI-SQ	DF	P
	0.89	1	0.3456

COCHRAN'S Q - 0.6602  
 LARGEST VAR / SMALLEST VAR 1.9426

COMPONENT OF VARIANCE FOR BETWEEN GROUPS 16450.4  
 EFFECTIVE CELL SIZE 9.9

GENETICS	MEAN	SAMPLE SIZE	GROUP STD DEV
1	660.97	9	99.619
2	471.35	11	138.85
TOTAL	556.68	20	122.97

CASES INCLUDED 20 MISSING CASES 1

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