INTERACTIONS OF STRESS AND NICOTINE ON AMPLITUDE, PRE-PULSE INHIBITION AND HABITUATION OF THE ACOUSTIC STARTLE REFLEX

1992

ACRI

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
F. EDWARD HÉBERT SCHOOL OF MEDICINE
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

APPROVAL SHEET

GRADUATE EDUCATION

TEACHING HOSPITALS
WALTER REED ARMY MEDICAL CENTER
NAVAL HOSPITAL, BETHESDA
MALCOLM GROW AIR FORCE MEDICAL CENTER
WILFORD HALL AIR FORCE MEDICAL CENTER

Title of Dissertation: "Interactions of Stress and Nicotine on Amplitude, Pre-pulse Inhibition and Habituation of the Acoustic Startle Reflex"

Name of Candidate: Jane B. Acri
Doctor of Philosophy Degree
September 24, 1992

Dissertation and Abstract Approved:

Committee Chairperson — 24 Sept. 1992 Date

Committee Member — 24 Sept. '92 Date

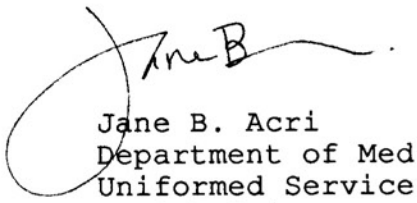
Committee Member — 9/24/92 Date

Committee Member — 9/24/92 Date

The author hereby certifies that the use of any copyrighted material is the thesis manuscript entitled:

"Interactions of Stress and Nicotine on Amplitude, Pre-pulse Inhibition, and Habituation of the Acoustic Startle Reflex"

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violation.



Jane B. Acri
Department of Medical Psychology
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Dissertation: Interactions of Stress and Nicotine on Amplitude, Pre-pulse Inhibition, and Habituation of the Acoustic Startle Reflex

Jane B. Acri, Doctor of Philosophy, 1992

Dissertation directed by: Neil E. Grunberg, Ph.D.
Professor
Department of Medical Psychology

Cigarette smokers report that one reason for smoking is that it helps them cope with stress. There is little evidence that nicotine reduces any of the physiological effects of stress, and instead, nicotine and stress have an additive effect on physiological indices of stress. One way that nicotine may enhance stress-coping in humans is through changes in attention because nicotine enhances vigilance and selective attention in smokers and nonsmokers. Attention is reflected in aspects of the acoustic startle response (ASR), which can be modulated by both stress and nicotine and studied in animals. These experiments used a chronic nicotine administration paradigm and an acute stressor to test for interactions of stress and nicotine on amplitude, pre-pulse inhibition (PPI), and habituation of the ASR during nicotine administration and cessation in rats. ASR amplitude

is a measure of sensorimotor reactivity, and PPI is believed to reflect processes of attention involved in sensory gating and selective attention. In Experiment 1, 12 mg/kg/day, 6 mg/kg/day nicotine, or saline was administered to rats for 11 days. On day 11, rats were stressed by restraint, observation of conspecifics' restraint, or were not stressed for 15-20 minutes prior to startling. Nicotine and stress each increased amplitude and PPI. Nicotine significantly interacted with stress such that nicotine prevented the changes in amplitude and PPI associated with stress or nicotine alone. In Experiment 2, (nicotine cessation), nicotine (12 mg/kg/day) or saline was administered for 11 days prior to explantation. On Day 1 of drug cessation, subjects were stressed by restraint or were not stressed for 15-20 minutes prior to startling. Cessation effects were generally in a direction opposite to effects of nicotine administration, but results were not significant. Median split analysis of baseline reactivity revealed that more reactive animals were primarily responsible for all significant effects related to drug administration or stress. It was concluded that the inverted U-shaped dose-effect curve of nicotine on CNS activation may be shifted to the left by stress. The findings of these experiments are consistent with human smokers' reports of stress-reducing effects of cigarette smoking.

Interactions of Stress and Nicotine on Amplitude, Pre-pulse Inhibition, and Habituation of the Acoustic Startle Reflex

by

Jane B. Acri

Dissertation submitted to the Faculty of the
Department of Medical Psychology
Graduate Program of the Uniformed Services University
of the Health Sciences in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
1992

ACKNOWLEDGMENTS

I wish to thank all of the people who helped with these experiments. Kelly Brown, Sandra Jochum, and Stephanie Nespor assisted with actually running the experiments and were there through those late nights in LAM when the lights were off and there were more pleasant places that they would rather be. I appreciate their willingness to help. Extra thanks to Stephanie for her expertise in the blood collection and for seeing me through the corticosterone assays.

Thanks to the LRC's afternoon and evening computer support staff for their patience and help through Powerpoint new versions of Microsoft Word. They know their programs well and were always willing to share their knowledge.

I appreciate the help of my committee members, John Sarvey, Chris-Ellyn Johanson, Jerry Singer and Neil Grunberg. They improved the experiments and the write up with their suggestions and guidance. They are wonderful people, and having them on my committee made the process a very positive learning experience.

Most of all, I want to thank my advisor, Neil Grunberg. I have benefited immeasurably from his commitment to the success of his students and the time he devotes to teaching. I feel extremely fortunate to have been a part of his laboratory these past few years, and I have learned from him tremendously. Thank you, Neil, for being so generous with your time, ideas, guidance, and criticism. I don't think there are words to express the extent of my gratitude.

TABLE OF CONTENTS

Approval sheet.....i
Copyright statement.....ii
Abstract.....iii
Title page.....v
Acknowledgments.....vi
Table of contents.....viii
List of tables.....x
List of figures.....xii
Introduction.....1
    Nicotine.....3
    Stress.....9
    Stress and Nicotine.....13
    Nicotine Withdrawal.....20
    Acoustic Startle.....22
    Nicotine and Startle.....25
    Pre-pulse Inhibition (PPI) of Startle.....26
    Nicotine and PPI.....27
    Stress and Startle.....28
Overview and Hypotheses.....31
    Overview.....31
    Hypotheses.....33
        Experiment 1.....33
        Experiment 2.....36
Experiment 1.....39
    Overview.....39
    Methods.....40
        Subjects and Housing.....40
        Drug Administration.....41
        Stress.....41
        Acoustic Startle Reflex Testing.....42
        Stress Index.....44
        Procedure.....45

Statistical Analysis.....46
Results.....48
Overview.....48
Stress validation.....50
Multivariate analyses.....50
Amplitude.....51
Pre-pulse inhibition.....54
Habituation.....58
Confirmation of Hypotheses.....59
Discussion.....60
Amplitude.....60
Pre-pulse inhibition.....64
Habituation.....65
Summary.....66
Experiment 2.....67
Overview.....67
Methods.....68
Subjects and Housing.....68
Drug Administration.....68
Stress.....69
Acoustic Startle Reflex Testing.....69
Stress Index.....71
Procedure.....72
Statistical Analysis.....74
Results.....74
Overview.....75
Stress validation.....76
Multivariate analyses.....77
Amplitude.....77
Pre-pulse inhibition.....80
Habituation.....83
Confirmation of Hypotheses.....84
Discussion.....84
Amplitude.....85
Pre-pulse inhibition.....87
Habituation.....88

Summary.....89
General Discussion.....90
Tables.....99
Figures.....114
Figure legends.....114
References.....134

LIST OF TABLES

Table 1.	Experiment 1.	Design of experiment: Effects of nicotine and stress during nicotine administration
Table 2.	Experiment 1.	Timeline of events
Table 3.	Experiment 2.	Design of experiment: Effects of stress during nicotine cessation
Table 4.	Experiment 2.	Timeline of events
Table 5.	Experiment 1.	Hypothesis 5. Predicted dose response effects of nicotine with restraint stress
Table 6.	Experiment 1.	Hypothesis 5. Predicted dose response effects of nicotine with observation stress
Table 7.	Experiment 1.	Weight loss during experiment 1
Table 8.	Experiment 1.	Means and standard deviations of acoustic startle amplitudes using 98 dB, 112 dB and 122 dB stimuli.
Table 9.	Experiment 1.	Significant post hoc comparisons of startle amplitude using 112 dB stimulus.
Table 10.	Experiment 1.	Significant post hoc comparisons of startle amplitude using 122 dB stimulus.
Table 11.	Experiment 1.	Significant post hoc comparisons of response amplitudes (112 dB stimulus) using median split (high/low) of baseline responses as a variable in the analysis.
Table 12.	Experiment 1.	Means and standard deviations of acoustic startle amplitudes using 98 dB, 112 dB, and 122 dB stimuli with pre-pulses.

Table 13. Experiment 1. Significant post hoc comparisons of startle amplitudes using 98 dB stimulus with pre-pulse.

Table 14. Experiment 1. Significant post hoc comparisons of trials using 122 dB stimulus with pre-pulse.

Table 15. Experiment 1. Means and standard deviations for calculated amount of inhibition using 98 dB, 112 dB, and 122 dB stimuli with pre-pulses.

Table 16. Experiment 1. Significant post hoc comparisons of amount of inhibition, 112 dB stimulus with pre-pulse.

Table 17. Experiment 1. Means and standard deviations of calculated percent of inhibition using 98 dB, 112 dB, and 122 dB stimuli.

Table 18. Experiment 1. Significant post hoc comparisons of amount of PPI, (112 dB stimulus) using median split (high/low) of baseline.

Table 19. Experiment 2. Means and standard deviations of acoustic startle amplitudes using 98, 112, and 122 dB stimuli.

Table 20. Experiment 2. Means and standard deviations of amplitude of pre-pulse trials using 98, 112, and 122 dB stimuli with pre-pulses.

Table 21. Experiment 2. Means and standard deviations for calculated amount of inhibition using 98, 112, and 122 dB stimuli with pre-pulses.

Table 22. Experiment 2. Means and standard deviations for calculated percent of inhibition using 98, 112, and 122 dB stimuli with pre-pulses.

LIST OF FIGURES

Figure 1. Experiment 1. Effects of stress on plasma corticosterone concentration (means and standard errors).

Figure 2. Experiment 1. Startle amplitude using 112 dB stimulus (means and standard errors).

Figure 3. Experiment 1. Startle amplitude using 122 dB stimulus (means and standard errors).

Figure 4. Experiment 1. Startle amplitude using 112 dB stimulus and median split of initial reactivity (means and standard errors).

Figure 5. Experiment 1. Amplitude of pre-pulse trials using 98 dB stimulus with pre-pulse (means and standard errors).

Figure 6. Experiment 1. Amount of inhibition using 98 dB with pre-pulse (means and standard errors).

Figure 7. Experiment 1. Amount of inhibition using 112 dB with pre-pulse (means and standard errors).

Figure 8. Experiment 1. Amplitude of pre-pulse trials using 122 dB with pre-pulse (means and standard errors).

Figure 9. Experiment 1. Amount of inhibition using 112 dB with pre-pulse following median split of initial reactivity (means and standard errors).

Figure 10. Experiment 2. Effects of stress on plasma corticosterone concentration (means and standard errors).

Figure 11. Experiment 2. Startle amplitude using 112 dB stimulus (means and standard errors).
Figure 12. Experiment 2. Startle amplitude using 112 dB stimulus following median split of initial reactivity (means and standard errors).
Figure 13. Experiment 2. Amount of inhibition using 98 dB with pre-pulse (means and standard errors).
Figure 14. Experiment 2. Amplitude of pre-pulse trials using 112 dB with pre-pulse (means and standard errors).
Figure 15. Experiment 2. Amount of inhibition using 112 dB with pre-pulse (means and standard errors).
Figure 16. Experiment 2. Amount of inhibition using 122 dB with pre-pulse (means and standard errors).
Figure 17. Experiment 2. Amplitude of pre-pulse trials using 112 dB following median split of initial reactivity (means and standard errors).
Figure 18. Experiment 2. Amount of inhibition using 112 with pre-pulse following median split of initial reactivity (means and standard errors).

INTRODUCTION

"Certainly smoking is one of the diversional activities which, to many people, has proved to be so much more useful than complete rest after exposure to severe stress."

Hans Selye (1973)

Cigarette smoking is the most preventable environmental factor contributing to illness and death in the United States. Smoking is associated with cancers, cardiovascular diseases, bronchopulmonary diseases, and digestive diseases (USDHHS, 1989). Despite these deleterious health consequences, more than 50 million people in this country alone smoke cigarettes or self-administer other nicotine-containing products.

The question of why people who are aware of the health risks of smoking continue to smoke cigarettes does not have a simple answer. It is well established that nicotine, the chief active psychopharmacologic agent in tobacco, is an addictive substance (USDHHS, 1988) and that people who give it up undergo a withdrawal syndrome (Hughes, Hatsukami, Pickens, & Svikis, 1984; Hughes & Hatsukami, 1992; 1986), but there is a great deal of variability in the reported severity of nicotine withdrawal. One commonly described reason for smoking that people report, and that may contribute to the difficulty in quitting, is that smoking helps individuals cope with stress (Shiffman, 1985; Wills & Shiffman, 1985).

How nicotine might interact with stress to relieve some of its negative effects or how it might help people cope remains a mystery. Biochemical interactions of stress and nicotine have been examined, but do not suggest that nicotine reduces the biochemical response to stress (Morse, 1989). Interactions of stress and some of the behavioral effects of

nicotine that may enhance stress-coping responses may yield more useful information.

Stress-coping responses (Lazarus, 1977; Moos & Billings, 1982; Pearlin & Schooler, 1978) can be grouped into categories involving cognitive and behavioral processes (Wills & Shiffman, 1985). Two of the mechanisms within the classification of cognitive coping are efficacy enhancement and distraction (Wills & Shiffman, 1985), both of which may relate to attentional processes.

This dissertation examined interactions of stress and nicotine on three related behaviors during nicotine administration and withdrawal: sensorimotor reactivity or startle amplitude, sensory gating or pre-pulse inhibition, and habituation of the acoustic startle response. These behaviors were selected because they are thought to reflect aspects of attention and effects of nicotine on attention that may be key to the way nicotine interacts with stress and coping. By examining behaviors that are affected by both stress (Leitner, 1989; Davis, 1989; Korn & Moyer, 1965) and nicotine (Acri, Grunberg, & Morse, 1991; Acri, Morse, & Grunberg, 1992), it may be possible to examine some of the ways in which nicotine blocks or ameliorates the effects of stress.

The present experiments assessed the effects of nicotine and stress on attention, as reflected in startle amplitude, pre-pulse inhibition, and habituation using the acoustic startle reflex in rats. The first experiment examined effects during nicotine administration for a period of 11 days; the second experiment evaluated effects on the first day of nicotine cessation following 12 days of administration. It was hypothesized that nicotine would counteract some the deleterious effects of stress on attention as measured by amplitude (reactivity), pre-pulse inhibition (sensory gating), and habituation during nicotine administration. It also was hypothesized that nicotine cessation would exacerbate effects of stress on attention as

measured by these same startle behaviors, which may relate to why episodes of stress are related to smoking relapse among quitters (Shiffman, 1985).

This paper reviews literature relevant to the proposed experiments involving actions of nicotine, stress, acoustic startle, and the known interactions among them. First reviewed are relevant effects of nicotine, including effects on attention, followed by a discussion of stress and alarm substances. Alarm substances released by stressed animals may induce stress responses in non-stressed animals that are exposed to them, and were included in this review because one of the stressors used in these experiments was exposure to other animals that were being stressed by physical restraint. Additionally, relevant papers on stress and nicotine are examined, followed by an examination of the literature on nicotine withdrawal as it relates to stress. The literature on the acoustic startle response is reviewed, including amplitude, pre-pulse inhibition (PPI), and habituation. Finally, the effects of nicotine on startle and PPI are examined, followed by the effects of stress on startle and PPI.

Following these reviews, an overview of the experiments and specific hypotheses are provided. The Methods section details the methods and rationales for the procedures used in the conduct and analysis of the experiments.

Nicotine

Although there is a substantial literature regarding the biochemical and physiological effects of nicotine, the exact mechanisms underlying many of its effects and its addictive properties remain unknown. Nicotine, like other drugs of addiction, enhances mesolimbic dopamine transmission (Imperato, Mulas, & DiChiara, 1986; Clarke, Fu, Jakubovic, & Fibiger, 1988; Mifsud, Hernandez, & Hoebel, 1989). It is

also a cholinergic agonist that stimulates autonomic ganglia, sympathetic effectors, somatic neuromuscular junctions, and results in cortical arousal (Taylor, 1985). In addition, nicotine has a number of behavioral effects that may have reinforcement value, such as muscle relaxation (Domino & von Baumgarten, 1969; USDHHS, 1988; Hall, 1982; Epstein, Dickson, McKenzie, & Russell, 1984), improved concentration (Wesnes & Warburton 1978; 1983; Wesnes 1987), stress reduction (Gilbert & Spielberger, 1987; Pomerleau, Turk, & Fertig, 1984), and body weight changes (Grunberg, 1988; 1990).

Effects of Nicotine on Attention

A number of researchers have reported enhancements in cognitive functions related to attention following nicotine administration (see Warburton & Walters, 1989; Warburton, 1989; Wesnes & Warburton, 1983; for reviews), but many of these studies have been limited by the lack of appropriate non-smoking control groups. That is, many of the reports of improvements in memory and attention have used smokers smoking as compared to deprived smokers, and in such studies, nicotine effects are actually being compared to withdrawal effects in addicted smokers. However, there are several studies that report cognitive enhancements in nonsmokers following nicotine administration.

Wesnes and Warburton (1984a) administered nicotine in tablet form to 12 male non-smoking subjects using a counterbalanced, within-subject design so that each subject received 0 mg, 0.5 mg, 1.0 mg, and 1.5 mg during 4 sessions following 3 training sessions. Subjects were asked to detect specific series or groupings of digits embedded in a visual display of 100 digits per minute. Significant improvements in digit detection were reported for subjects receiving 1.5 mg nicotine as compared to control sessions during the first 10 minutes following administration.

Wesnes, Warburton, and Matz (1983) administered nicotine in tablet form to 36 male and female subjects including 12 smokers of more than 15 cigarettes per day, 12 smokers of less than 5 cigarettes per day, and 12 non-smokers. Subjects received 0 mg, 1.0 mg, and 2.0 mg nicotine in a repeated measures, counterbalanced design. The task involved detection of 50 ms pauses in the continuous movement of a clock hand. Doses of nicotine were administered at 20, 40, and 60 minutes after the start of the session that lasted for 80 minutes. Each subject participated in three sessions in order to receive all doses of nicotine. Results showed a significant main effect for nicotine resulting in greater detection of pauses, regardless of smoking condition (treatment group), and a significant interaction with time as all subjects showed decreased detection over time. The authors interpreted these results to suggest that nicotine prevents decreases in stimulus sensitivity or decreases in the ability to detect pauses that occur over time in placebo conditions for both smokers and non-smokers.

Wesnes and Warburton (1978) reported that nicotine resulted in reductions in the Stroop effect, or distraction produced by irrelevant color names while naming the colors of ink in which words are printed. When subjects are asked to name aloud the ink colors, they take longer to complete the list than they do if asked to simply name patches of color. Performance was compared in six smokers (16-hours deprived) and six non-smokers after receiving 0 mg, 1 mg, and 2 mg nicotine in a counterbalanced, within-subject design. Nicotine reduced the magnitude of the Stroop effect in both smokers and non-smokers in a dose-response fashion. These results suggested that nicotine reduced distraction and argued against the interpretation that nicotine improves performance only by preventing withdrawal.

Many studies of nicotine's effects on performance compared smokers smoking to a control group of smokers not smoking. Wesnes and Warburton (1984b) assessed the effects

of cigarettes of varying nicotine yield on male smokers. Using four nicotine yields and a no-smoking condition, subjects' performance on a digit series detection task was compared during five sessions in a counterbalanced design. The authors reported that nicotine improved performance with a dose-response effect, and prevented performance decrements in both speed and accuracy that occurred over time. However, this experiment used smokers not smoking as a control group, so withdrawal effects provide an alternative interpretation to a nicotine-induced performance enhancement.

Similarly, Andersson and Hockey (1977) compared smokers smoking and smokers not smoking in a between-subjects design. The task involved memory for words presented on a screen. Subjects were instructed to write down the words in the correct order after all eight words had been presented. Words were presented in different corners of the screen, but subjects were not instructed to attend to word placement until a second series of eight words was presented. Results indicated no differences in performance for memory of word order, but when subjects were asked about word location, smokers showed poorer performance in memory for this irrelevant cue when it was not part of the instruction. However, when asked to attend to location of words, performance for word order was poorer for smokers. These results were interpreted to mean that smokers smoking show more limited and focused information processing as compared to smokers not smoking, and a greater ability to block out irrelevant information. However, as in Wesnes and Warburton (1984b), possible withdrawal effects in the comparison group prevent unequivocal conclusions concerning nicotine's effects.

Spilich, June, and Renner (in press) compared smokers smoking, smokers not smoking (1 hour deprived), and nonsmokers in a series of tasks that were graded in complexity. Although these authors reported that smokers in the smoking condition had enhanced performance on simple

visual attention-reaction time tasks and poorer performance on more complex tasks involving short term memory, there are confounding variables in this study. There was no evidence that abstaining subjects were truly deprived smokers after one hour, and because each task was presented to a different subset of subjects within each treatment group, it is not clear that differences in performance were not related to differences in subsets of subjects. Therefore, no meaningful conclusions can be drawn from this study, although if taken at face value, it suggests that smoking enhances simple attentional task performance.

Several other approaches also have been used to examine nicotine's effects on cognitive processes related to attention. Certain cortical evoked potential responses are believed to reflect attentional processes. Nicotine has been reported to enhance specific auditory and visual cortical evoked responses that reflect attentional mechanisms in smokers smoking as compared to smokers not smoking (Knott 1986).

In another approach, several researchers have administered nicotine to patients suffering from dementia of the Alzheimer's type (DAT) and have reported improvements in accuracy and speed of responding in rapid information processing following nicotine as compared to placebo (Sahakian, Jones, Levy, Gray, & Warburton, 1990). There were equal numbers of smokers and nonsmokers in the nicotine and placebo groups. Decreases in intrusion errors in memory tasks following nicotine as compared to placebo also have been reported in nonsmoking patients with DAT (Newhouse, Sunderland, Tariot, Blumhardt, Weingartner, & Mellow, 1988). These effects are believed to reflect nicotinic cholinergic stimulation, which may underlie some or all of nicotine's reported cognitive effects.

In summary, there is evidence that nicotine improves performance in simple cognitive tasks that may relate to attention in nonsmokers as well as smokers. Additionally,

nicotine may enhance cortical evoked potentials related to attention, and may improve cognitive performance related to attention in patients with dementia. These effects may serve to alter some of the cognitive, affective, or behavioral components of stress and coping responses that are reviewed under the heading of "stress." If so, then effects of nicotine on attentional processes may help to explain why people smoke under stress.

Nicotine's Neuroendocrine Effects Related to Stress

It has been hypothesized that one of the reasons that stress and nicotine self-administration are related is because they have similar neuroendocrine effects (Grunberg & Baum, 1985). These neuroendocrine effects are summarized below.

Nicotine has direct and indirect effects on several neuroendocrine and endocrine systems (USDHHS, 1988). In addition to its primary effect as a cholinergic agonist, nicotine affects endogenous opioids (Pomerleau & Pomerleau, 1984; Seyler, Pomerleau, Fertig, Hunt, & Parker, 1986), indoleamines (Benwell & Balfour 1982a; Balfour, Benwell, Graham, & Vale, 1986), and has a range of other biochemical effects (See USDHHS, 1988).

Nicotine also activates the adrenal medulla and the adrenal-pituitary axis. Nicotine can increase peripheral epinephrine and norepinephrine (Grunberg, Popp, Bowen, Nespor, Winders, & Eury, 1988), increase ACTH and cortisol (Newhouse, Sunderland, Narang, Mellow, Fertig, Lawlor, & Murphy, 1990), and alter levels of plasma prolactin (e.g., Sharp, Beyer, Levine, Morley, & McAllen, 1987).

These effects of nicotine on adrenal and pituitary hormones that are also involved in stress underscore the complexity of reported stress-nicotine interactions and highlight the necessity of using more complex and integrated behaviors to evaluate stress-drug interactions.

Stress

Stress is a collective term for an area of study in which environmental demands, internal demands, or both, tax or exceed the adaptive resources of an individual, social system, or tissue system (Monat & Lazarus, 1977). Stress usually involves stimuli or events called stressors, stress responses (physiological, psychological, and behavioral), and mediating variables.

The body responds physiologically to increased demands involved in stress by releasing adrenocorticotropin (ACTH) from the anterior pituitary, glucocorticoids from the adrenal cortex, epinephrine from the adrenal medulla, and norepinephrine from the sympathetic nerves (Axelrod & Reisine, 1984). Variations of this physiological response have been observed in animal stress paradigms involving crowding, immobilization, swimming, and electric shock (Axelrod & Reisine, 1984). Other changes occur involving psychological and behavioral as well as physiological responses to stress that may be specific to the species, type of stressor, appraisal of threat, resources, coping mechanisms, and a host of other mediating variables (Cohen, Horowitz, Lazarus, Moos, Robins, Rose, & Rutter, 1982; Fisher, 1984).

The activation of the peripheral autonomic catecholaminergic systems during stress have long been known (Cannon, 1914). It has only recently been reported that cerebral catecholamine containing neurons also are affected, including norepinephrine (NE) (Glavin, 1985) and dopamine (DA) (Dunn, 1988).

In addition to physiological effects, exposure to stressors often results in cognitive and behavioral coping responses in humans (Wills & Shiffman, 1985). According to these authors, coping responses can be cognitive or behavioral. Cognitive responses include minimization, distraction, downward social comparisons, restructuring,

efficacy enhancement, thoughts of consequences, will power, and acceptance. Behavioral coping responses include decision-making, problem-solving, direct action, withdrawal, assertiveness, social support, relaxation, and pleasure-seeking. Substance use as a coping mechanism is usually framed in terms of effects on mood, although it also seems likely that in the case of smoking, other types of cognitive enhancements are implicated.

If nicotine improves cognitive attentional processes, it could enhance aspects of both cognitive and behavioral coping by increasing efficacy in thought, decision-making, and problem-solving which could be helpful in coping with stress. This possibility is consistent with the archived literature, but has not yet received direct empirical examination of effects of nicotine, stress, and their interaction on attentional processes.

Stress and Alarm Substance

Stressed rats emit odors that can stimulate activity in non-stressed rats (Mackay-Sim & Laing, 1981a). This phenomenon may be the result of pheromones, or more specifically, alarm substances. Pheromones are external chemical secretions having conspecific communication functions. Whereas some of these physiological regulatory functions are related to estrus synchrony in females, other functions are communicative and are classified as sex attractants, alarm substances, trail substances, territoriality, and individual recognition substances (Gleason & Reynierse, 1969).

A study in which odors of urine from a stressed rat, feces from a stressed rat, or the stressed rat itself were present, compared running times in a maze with water reward and running times when the same stimuli from non-stressed rats were available. Mackay-Sim and Laing (1981b) reported that running times were slower when odors from urine and the

body of stressed rats were available, but not odors from feces of stressed rats. These authors concluded that behavior-altering odors are released from the body surface and urine, but not from the feces of stressed rats.

Other experiments have examined the effects of these odors on other animals during free exploration. Mackay-Sim and Laing (1980) reported that animals spent progressively less time on the side of a compartment odorized by rats receiving progressively higher levels of stress. Further, subjects exposed to odors from stressed animals were significantly more active than subjects exposed to odors from non-stressed rats. Odor donors had been either non-stressed, exposed to one 2-sec, 2 mA shock, five 2-sec, 2 mA shocks or five 2-sec, 2 mA shocks for 5 days.

Mackay-Sim and Laing (1981a) reported that activity was increased in animals receiving odors from stressed rats as compared to odors from non-stressed rats. There were greater latencies in time to enter a maze when it was odorized by air passing over the body of a stressed rat, or the blood of a stressed animal. It also was noted that activity was stimulated by odors from rats receiving five 2-sec shocks in 15 minutes, but activity was inhibited by odors from rats receiving 35 minutes of intermittent shock.

Valenta and Rigby (1968) reported that rats trained to discriminate odors from stressed rats and non-stressed rats could reliably discriminate these odors. Air samples were taken from cages of undisturbed rats and rats that had just received several 1 mA shocks to the flanks.

Wasserman and Jensen (1969) reported that detection of odors from stressed rats could disrupt the task performance of non-stressed rats. Experimental rats showed a "pseudo-extinction" effect, or a decrease in running speed in a continuously food-rewarded task after extinction trials of another group on the same apparatus. The apparatus floor was covered with either clean paper, paper traversed by a single reinforced subject, or paper traversed by a single subject

undergoing experimental extinction. Results indicated that the odor trace of a rat undergoing experimental extinction could significantly disrupt the performance of a subsequently run animal that was previously reinforced.

Dunn (1988) reported that, in mice, exposure to stress odors was sufficient to trigger the dopamine (DA) components of the stress response. Mice that were placed in boxes where other mice had received shock (20 x 1 sec at 0.2 mA in 15 min) without cleaning the boxes in between, showed changes in DOPAC:DA (DOPAC is a DA catabolite 3,4-dihydroxyphenylacetic acid) ratios, MHPG(3-methoxy,4-hydroxyphenyleneglycol):NE ratios in prefrontal cortex, hypothalamus and brainstem; and plasma corticosterone similar to those observed in shocked animals. When the boxes were cleaned with a water-ethanol mix and were allowed to dry between animals, the full effect did not occur, suggesting that the effect was mediated by olfactory cues.

Behavioral responses similar to those observed following stress occurred in rats when animals are forced to swim in water in which another animal had been forced to swim. Abel and Bilitzke (1990) studied the duration of immobility when animals were forced to swim. In a forced swim, animals at first swim energetically, but later become immobile, making the minimum number of movements to keep their heads above water. Prior foot shock and noise decreases the duration of the immobility response. Swimming in water in which another animal had been forced to swim also decreased the duration of the immobility response. To control for the possibility that a communicative signal or substance was contained in urine and feces, urine and feces from animals receiving shocks were placed in water in which animals were forced to swim. No decrease in immobility was seen. Abel and Bilitzke (1990) concluded that decreases in immobility occurred due to the presence of an alarm substance that was released during forced swim that was not present in urine and feces of otherwise stressed animals.

It is, therefore, likely that odors and distress calls from stressed animals not only will be perceived by nearby animals, but are capable of altering behavior and may, in fact, induce elements of the stress response itself.

Stress and Nicotine

It has been reported that chronic cigarette smokers smoke more under stress, and smokers self-report that smoking has a calming or relaxing effect (Rose, Ananda & Jarvik, 1983; Wills & Shiffman, 1985; Shiffman, 1985; Barnes & Fishlincki, 1976; Ikard & Thomkins, 1973; Kleinke, Staneski, & Meeker, 1983). It is not known whether people smoke more under stress because smoking decreases the physiological, behavioral, cognitive, or biochemical stress responses, or if people smoke more under stress because stress alters the biochemical availability of nicotine and precipitates a withdrawal response which is in itself stressful. It has been argued that stress affects circulating levels of nicotine through its effects on urinary pH (Schachter, 1978; Schachter, Kozlowski, & Silverstein, 1977), and that stress results in decreased blood levels of nicotine (Winders, 1990). These findings would lead to the hypothesis that stress precipitates withdrawal effects, and that smoking increases under stress in order to prevent withdrawal. On the other hand, there is no evidence that nicotine reduces the neuroendocrine response to stress and, in fact, nicotine increases levels of stress hormones following nicotine administration (Morse, 1989). Both nicotine and stress independently increase blood levels of corticosterone and catecholamines. When nicotine was administered during stress, levels of corticosterone and catecholamines in rabbits were higher than during either nicotine or stress alone (Morse, 1989). This finding would suggest that nicotine has no direct neuroendocrine effect in ameliorating the stress response.

Similarly, Perkins, Epstein, Jennings, and Stiller (1986) and MacDougall, Dembroski, Slaats, Herd, and Eliot (1983) reported that cardiovascular effects of stress and smoking in combination were greater than effects of either stress or smoking alone in humans. Using a video game stress task and aerosol nicotine administration in 12 young, male smokers, Perkins and colleagues (1986) reported that effects of stress and nicotine were additive for heart rate, but less than additive for systolic and diastolic blood pressure.

MacDougall and colleagues (1983) examined cardiovascular reactivity in smokers smoking and smokers sham smoking when exposed to stress. The stressor involved playing a series of difficult video games under challenging instructional conditions. Fifty-one smokers randomly assigned to stress and smoking conditions were compared. Subjects who smoked during stress showed approximately doubled increases in systolic blood pressure, diastolic blood pressure, and heart rate as compared to smokers not exposed to stress or subjects exposed to stress while sham smoking.

Consistent with the view that nicotine exacerbates stress responses, Balfour (1982) has reported that nicotine impairs animals' ability to adapt to repeated stress as measured by corticosterone levels, and reverses the normally positive relationship between hippocampal serotonin and plasma corticosterone. That is, saline-treated animals show decreased plasma corticosterone with stress adaptation, and a positive relationship between hippocampal serotonin and plasma corticosterone after adaptation to stress. In contrast, animals chronically injected with nicotine and exposed to stress show a negative correlation between hippocampal serotonin and plasma corticosterone (Benwell & Balfour, 1979, 1982b). These studies suggest that nicotine does not reduce physiological aspects of the stress response.

However, there is evidence that nicotine may modulate other aspects of the stress response. Yamanaka, Muramatsu, and Kigoshi (1987) reported that long-term administration of

nicotine induces down-regulation of cortical beta adrenoceptors and attenuates the receptor alteration by repeated stress. In this study, nicotine (5-8 mg/kg/day) was administered to rats in drinking water for 4 weeks, and immobilization stress was applied daily for 2 hrs/day for the last 5 days. Both nicotine and stress decreased binding sites for ^{3}H-dihydroalprenolol (DHA), but in combination, failed to further decrease binding sites.

Sharp and colleagues (1987) reported that single doses of nicotine (0.75 - 3.0 mg/kg) significantly decrease the elevations in plasma prolactin (PRL) due to restraint stress administered 60 minutes later in rats. Although single doses of nicotine without stress elevate adrenocorticotropin (ACTH) levels and have biphasic effects on plasma PRL in rats, this stimulatory effect desensitizes. Chronic nicotine administration for 7 days also does not affect PRL responses to restraint stress. These results suggest that single doses of nicotine reduce the PRL component of the stress response, but that chronic nicotine administration does not affect this aspect of the stress response.

Kiritsy-Roy, Mousa, Appel, and Van Loon (1990) reported that systemic or intraventricular administration of nicotine produced dose-related increases in the concentration of epinephrine in plasma. Increases in norepinephrine occurred only with systemic administration of nicotine. Rats showed tolerance to nicotine effects after a single intraventricular injection, but required injections every 30 minutes in order to show tolerance to systemically administered nicotine. Rats that were tolerant to systemic administration of nicotine were cross tolerant to stress with regard to sympathoadrenal stimulation, but cross tolerance was not detected in rats following intraventricular administration. These results suggest that nicotinic receptors in the brain modulate the sympathetic outflow and adapt readily to nicotine stimulation, but probably are not involved in sympathoadrenal stress responses.

Collins, Bhat, Pauly, and Marks (1990) reported that chronic corticosterone (CCS) treatment resulted in decreases in the number of brain nicotinic receptors measured by ^{125}I-bungarotoxin binding in vivo. Additionally, in vitro addition of CCS inhibited binding to nicotinic receptors. These authors propose that changes in numbers of receptors and altered steroid interactions with nicotinic receptors are involved in tolerance to nicotine, but they may also be involved in the stress response.

Overstreet, Janowsky, Gillin, Shiromani, and Sutin (1986) studied the effects of stress and cholinergic antagonists on several rat strains. The Finders Sensitive Line (FSL) of rats, known to be more sensitive to cholinergic agonists, was found to show greatest immobility in response to forced swim. These results, in light of those of Collins et al. (1990) described above, are consistent with stress-ameliorating effects of nicotine.

In summary, there is evidence that nicotine administration may influence aspects of the stress response under specific conditions, through complex interactions of central nicotinic receptors and the secretory activity of the hypothalamic-hypophyseal-adrenal axis.

Other explanations for increased smoking during stress focus on affective and cognitive changes. It has been suggested that nicotine's effects on subjective experience of stress are related to nicotine's effects to reduce negative affect in deprived smokers (Hughes, Hatsukami, Pickens, Krahn, Malin, & Luknic, 1984; Shiffman, 1985). Few examples of mood elevation in response to nicotine in non-deprived smokers have been found, so it may be that nicotine simply averts dysphoric withdrawal effects. Alternatively, smoking may serve as a psychological tool (Ashton & Stepney, 1982) by offering an alternate focus of attention or by changing attentional processes such that smokers are less aware of negative somatic experiences. That is, smokers may have

altered awareness of negative affect or negative somatic experiences, as described in the following experiments.

Woodson, Buzzi, Nil, and Battig (1986) reported that smoking prevented increases in self-reported stress reactivity to noise bursts in female smokers smoking as compared to female smokers sham smoking. Smokers also experienced suppression of noise-induced tachycardia and partial inhibition of noise-induced vasoconstriction.

Nesbitt (1973) and Silverstein (1982) reported that smoking increases pain tolerance for shock, again suggesting an altered awareness of or attention to somatic experience. Pomerleau and colleagues (1984) reported smoking-related reductions in anxiety levels when smokers were exposed to difficult anagrams or pain using the cold pressor test. Smokers of nicotine-containing cigarettes reported reductions in pain and anxiety as compared to smokers of cigarettes containing no nicotine (Pomerleau, Turk & Fertig, 1984).

In contrast, Hatch, Bierner, and Fisher (1983) did not find nicotine-induced reductions in emotional behavior (peripheral autonomic, electrocortical, self-report, and observer ratings of overt motor behavior reflecting anxiety) of subjects preparing for a speaking task. All subjects were smokers ($\geq$20 cigarettes/day for $\geq$ one year) asked to either smoke a high- or low-nicotine cigarette or no cigarette at all while preparing for public speaking. Groups did not differ on any of the four dimensions of emotional behaviors measured in this experiment, despite greater desire to smoke during stress.

Similarly, Gilbert and Hagen (1980) reported no difference in self-reported emotion between smokers given high (1.3 mg) or low (0.2 mg) nicotine cigarettes and exposed to emotionally arousing videotapes. Subjects in the high nicotine condition reduced self-reports of perceived muscular tension and startle, but did not perceive increased heart activity in spite of actual significant increases. These authors concluded that decreases in perceived muscle tension

and failure to detect increased heart activity in the high nicotine condition is consistent with an increased perceptual threshold model of nicotine's affect-reducing properties. In other words, nicotine may raise the threshold of perception of affective and somatic changes.

Other studies have been less clear cut in terms of nicotine's stress reduction. Jarvik, Caskey, Rose, Herskovik, and Sadeghpour (1989) reported that smoking reduced anticipatory anxiety in an anagram task, but smokers and deprived smokers reported equal anxiety following noise stress on an auditory vigilance task.

A possible explanation has been offered by Eysenck (1973) for some of the inconsistent effects of nicotine on stress reduction. Eysenck (1973) argued that discordant effects can be accounted for when the effects of the organism's initial state of arousal are taken into consideration. In this view, extraverts, who tend to smoke more (Eysenck, 1965) are characterized by lower cortical and autonomic arousal, whereas introverts are characterized by higher cortical and autonomic arousal. However, extraverts prefer higher arousal and introverts prefer lower arousal such that a low level of stimulation will have a positive hedonic tone for introverts, and a negative hedonic tone for extraverts. Thus smoking, with its arousal effect on the electroecephalogram (EEG) (Armitage, Hall, & Morrison, 1968), will be reinforcing for extraverts or those preferring higher levels of arousal. However, at higher doses, nicotine may have the opposite effect (Armitage et al., 1968) and may thus be reinforcing to introverts or those preferring lower levels of arousal. Smoking could, therefore, be used during stress to modulate arousal to a more desirable level.

Similarly, Nelsen (1978) proposed that nicotine influences behavior via its modification of the activities of, and the interactions between, certain neural systems involved in arousal (cortical, limbic, and reticular formation). If reticular formation (RF) and limbic systems

are mutually inhibitory, possibly the over-arousal or hyperstimulated state resulting from increased RF activation could be counteracted by increased limbic activation. Nelsen proposed that chronic nicotine results in a predominance of limbic-mediated arousal. A study was conducted to test the efficacy of nicotine as an antagonist of the behavioral disruption resulting from RF stimulation (cortical arousal). Nicotine significantly reduced the amount of "freezing" time or behavioral disruption caused by RF stimulation in rats. Nelsen also used a "cat stressor" with rats and found that nicotine treatment afforded protection from the behaviorally disruptive influence of the cat, and animals originally categorized as more highly emotional were preferentially protected by nicotine in terms of the relative disruption of specific elements of behavior (freezing). Nelsen hypothesized that this effect is mediated by shifting the hippocampus toward a less synchronized state as it is during the spontaneous focusing of attention. She concluded that nicotine induces a behavioral state which is less susceptible to disruption by intrusive environmental or emotional challenges.

The studies described in this section suggest that nicotine does not have a direct effect to reduce plasma corticosterone or catecholamines during stress, and does not reduce cardiovascular effects of stress. However, nicotine may modulate adrenocortical activity through changes in central nicotine receptors and may modulate sympathetic outflow, as plasma corticosterone may be involved in mechanisms of nicotine tolerance. There may, however, be more direct and immediate effects of nicotine on cognitive processes that allow an individual to be less aware of somatic sensation, to modulate arousal, and to induce a behavioral state which is less susceptible to intrusions. Through changes in attentional mechanisms, nicotine may decrease perceptions of negative affective and somatic experience; an effect that may be directly stress-reducing.

Nicotine may alter attentional mechanisms to allow for more effective coping by decreasing the intrusiveness of stressful stimuli.

Nicotine Withdrawal

The tobacco withdrawal syndrome has been well documented in humans and consists of symptoms of craving for tobacco, irritability, anxiety, difficulty concentrating, restlessness, bradycardia, impatience, somatic complaints, insomnia, increased hunger, and increased eating (Hughes et al., 1984b; Hughes & Hatsukami, 1986; Hatsukami, Dahlgren, Zimmerman, & Hughes, 1988; APA, 1987; West & Russell, 1988). These symptoms peak between 1-3 days following smoking cessation. Withdrawal symptoms from giving up cigarettes are generally believed to be associated with relapse but there is a known association in only about 45% of cases (Shiffman, 1985). This relationship has been found to be stronger for female than for male smokers (Guilford, 1966). There does, however, seem to be some evidence that stress and negative affect situations are related to relapse in general.

Studies linking stress and negative affect with relapse appear in human clinical settings. Shiffman (1982) analyzed data on relapse crises of 183 ex-smokers calling a relapse-counseling hotline. Most relapse crises were associated with negative affects, particularly anxiety, anger, or depression. Abrams, Monti, Pinto, Elder, Brown, and Jacobus (1987) studied coping skills in male and female cigarette quitters and relapsers in four situational contexts. Successful quitters coped better than relapsers with intrapersonal negative mood smoking specific-situations (scenarios previously determined to be high risk situations for smoking relapse because of the presence of stress, tension, anger/frustration, boredom, loneliness, etc.). Successful quitters also had lower anxiety scores. Women self-reported more stress and less confidence in their

ability to cope with stress than did men. Groups did not differ in responses to general social competence and social anxiety measures. Livson and Leino (1988) reported that smokers generally describe different motivations for smoking, with women smokers more likely to report smoking for pleasure and reduction of negative affect. These authors interpret these findings as reflecting attempts to cope with stress. Other reasons involve habit, addiction, stimulation, and sensorimotor manipulation.

Animal studies also have examined withdrawal effects in light of stress. Morrison (1974) examined the effects of nicotine and withdrawal on rats' performance on signaled and unsignaled avoidance trials. Twenty-five pairs of rats were trained on an unsignaled avoidance paradigm and nine pairs of rats were trained on signaled shock avoidance trials. One rat from each pair received 0.4 mg nicotine/kg subcutaneously immediately prior to each training trial. The other rat from each pair received an equal volume of saline. Rats that had received nicotine prior to training received fewer shocks than saline treated animals on unsignaled avoidance trials. However, when saline was substituted for nicotine (withdrawal), there was a significant deterioration in performance for rats that had previously received nicotine, such that they received significantly more shocks than animals previously treated with saline. For signaled shock avoidance, nicotine-treated rats took significantly fewer shocks than did saline-treated rats, but there were no significant differences between the two groups when saline was substituted for nicotine (withdrawal). Morrison (1974) concluded that effects of nicotine withdrawal are determined by stressfulness of the task, such that nicotine withdrawal only disrupted performance when stress was involved in unsignaled shock avoidance.

In tasks where there is no stressor, withdrawal effects may not be seen. Levin, Lee, Rose, Reyes, Ellison, Jarvik, and Gritz (1990) assessed performance of rats in a

radial-arm maze during and after chronic nicotine administration. Nineteen rats received either 0 mg or 3.4 mg nicotine per day via subcutaneously implanted glass and Silastic pellets. Pellets were removed after three weeks. Results indicated improved choice accuracy during and for two weeks following nicotine administration. The authors interpreted these results to suggest that nicotine improved performance on a spatial memory task both during administration and for two weeks after nicotine cessation.

Overall, these results suggest that nicotine withdrawal effects may be exacerbated by stress. Effects of nicotine cessation may not be detected unless a stressor is involved in the behavioral paradigm used. Therefore, it is advantageous to use behavioral paradigms in which effects of both drugs and stressors can be measured. The acoustic startle response meets these criteria and is related to the proposed mechanism, attention.

Acoustic Startle

The acoustic startle reflex (ASR) is a sensitive index of reactivity to external stimulation (Davis, 1984). It is a relatively simple behavior that occurs naturally in mammals and is affected by a variety of experimental treatments. The ASR consists of a series of rapid movements beginning at the head causing contraction and extension of major muscle groups in response to auditory stimuli with a rapid onset, or rise time. Responses are graded in amplitude in relation to stimulus intensity, and may show pre-pulse inhibition, habituation, and sensitization. These phenomena are related to interstimulus interval, number of stimuli, and signal-to-noise ratio.

The intrinsic neural pathway underlying the startle response includes the ventral cochlear nucleus, the ventral nucleus of the lateral lemniscus, the nucleus reticularis pontis caudalis, and motor neurons in the facial motor

nucleus and spinal cord. Startle responses can be elicited electrically from each of these areas in the rat (Davis, 1986).

Although the primary or intrinsic neural pathway of the startle reflex is contained within the brainstem, a number of extrinsic neural structures are capable of modulating the response. Lesions of the olfactory bulbs (van Riezen, Schneider, & Wren, 1977), hippocampus (Coover & Levine, 1972), septum (Gage, 1978), periaquaductal gray (Blair, Liran, Cytryniak, Shizgal, & Amit, 1978), and median raphe all increase startle, whereas lesions of the auditory cortex and inferior colliculus decrease startle amplitude (Davis, 1984).

Although the startle response is a basic, defensive reflex, it is of interest because it can be modulated by higher level processes such as attention (Anthony & Graham, 1983; Simons & Zelson, 1985; Anthony, 1990) and emotion (Vrana & Lang, 1990; Bradley, Cuthbert & Lang, 1990), and it provides an index of the influence of these variables on sensorimotor reactivity. Additionally, the phenomenon of pre-pulse inhibition, which occurs when a weaker, non-startle-eliciting stimulus precedes the startle-eliciting stimulus by a specific interval, is thought to be a model of sensory gating or an aspect of selective attention (Swerdlow, Braff, & Geyer, 1986; Swerdlow, Koob, Geyer, Mansbach, & Braff, 1988; Peng, Mansbach, Braff, & Geyer, 1990). Habituation of the startle response, or decrements in amplitude when stimuli are presented with a fixed interstimulus interval, have been used as a model of behavioral plasticity or a rudimentary form of learning (Groves & Thompson, 1970; Thompson & Spencer, 1966). It can also be viewed as a decrement in reactivity related to decreased attention. The startle response, therefore, provides measures of general reactivity, sensory gating, and behavioral plasticity that might be influenced by behavioral state and pharmacologic manipulation.

Many studies have reported that startle amplitude can be modified by administration of drugs that alter neurochemical transmission and startle has been used as a paradigm to determine sites and mechanisms of drug action. With regard to drugs of addiction, the amplitude of startle is increased by acute doses of d-amphetamine (Kokkinidis & Anisman 1978; Davis, Svensson, & Aghajanian, 1975). Chronic amphetamine treatment with 10 mg/kg i.p. injection for five consecutive days increased startle amplitude compared to saline controls, but this enhanced startle decreased each day (Kokkinidis & Anisman, 1978). Further, there was no amphetamine withdrawal effect on acoustic startle measured 24 hours after 10 days of amphetamine administration (Kokkinidis, Zacharko, & Anisman, 1986). A dose of 10 mg/kg cocaine injected i.p. increased startle (Harty & Davis, 1985), but cocaine's effects on startle amplitude could be blocked by pre-treatment with the DA antagonist, haloperidol (Davis, 1985). These results are consistent with the interpretation that DA is involved in startle modulation. Pohorecky, Cagan, Brick, and Jaffe (1976) reported that a dose of 1 g/kg ethanol reduced startle amplitude, and Mansbach, Gold, and Harris (1991) reported that naloxone injection decreased startle amplitude in rats treated with morphine as compared to naloxone injected rats treated with placebo.

Habituation is a relatively transient form of behavioral plasticity that occurs in most members of the animal kingdom. Drugs affecting the cholinergic system were used by Overstreet (1977) to determine if this neurochemical system may specifically mediate habituation of the acoustic startle response in rats. The author reported that acute administration of diisopropyl fluorophosphate (DPF), an irreversible anticholinesterase agent, significantly reduced the rate of habituation, but DPF was the only drug affecting the cholinergic system that did so and its effects were transient. There was a tendency for physostigmine

(cholinesterase inhibitor) and pilocarpine (muscarinic agonist) to depress the startle amplitude and for atropine to increase it, but these drugs did not interfere with habituation, suggesting that the cholinergic system may modulate the startle response level, but does not generally appear to play a role in habituation. Drugs that raise levels of acetylcholine (ACh) or mimic its actions lower the response amplitude, whereas those that block the muscarinic action of ACh increase the response level (Overstreet, 1977).

Warburton and Groves (1969) found that scopolamine, a muscarinic cholinergic antagonist, increased the amplitude of startle response, but did not alter the rate of habituation in rats. Pavlasek (1989) reported that microinjection of norepinephrine (NE) into the pontomedullary reticular formation (RF) resulted in increased startle amplitude. These results suggest that NE may play a neuromodulatory role with RF neurons to increase the amplitude of startle. Davis, Cedarbaum, Aghajanian, and Gendelman (1977) reported that the alpha-adrenergic agonist clonidine reduced startle amplitude by increasing within-session habituation in rats. This effect was postulated to be mediated not by increases in NE, but stimulation of epinephrine.

Acoustic startle amplitude and habituation, therefore, are modifiable by neurotransmitter systems involved in both stress and nicotine administration.

Nicotine and Startle

Collins and colleagues (1986, 1988) reported effects of i.p. injection of nicotine on the amplitude of acoustic startle in mice. Collins, Evans, Miner, and Marks (1986) reported data for two strains of mice, and of these, C3H mice showed significant increases in amplitude and the DBA strain showed significant amplitude decreases. Collins, Miner, and Marks (1988) again reported differences according to strain,

with C3H mice again showing increases and C57BL mice showing no change.

Recently, Acri, Grunberg, and Morse (1991) reported that chronic nicotine administration to rats at a dose of 12 mg/kg/day significantly increased the amplitude of the acoustic startle reflex. This effect was present after 1 day of nicotine administration and rats showed no evidence of tolerance to this effect over 10 days of chronic administration. Acri and colleagues (1991) concluded that increases in startle amplitude reflect nicotine's actions to enhance attention.

Helton, Tizzano, Modlin, and Rasmussen (1991) administered either 0, 10, or 20 mg/kg/day nicotine for 12 days using osmotic minipumps. Changes in startle amplitude during nicotine administration were not reported, but startle amplitude increased during days 1-5 of nicotine withdrawal in rats. These results are in contrast with Acri and colleagues (1991), who reported that startle amplitudes returned to baseline and were not different from saline responses during nicotine withdrawal.

Gilbert and Hagan (1980) studied the effect of nicotine on self-report of startle in humans in order to test the hypotheses that nicotine reduced emotional reactions. Smokers viewed videotapes of emotion-producing scenes following smoking a high (1.3 mg) or low (0.2 mg) nicotine cigarette. Nicotine reduced self-report of muscular tension and self-report of startle, but did not cause a perception of increased heart rate despite its occurrence. The actual startle response, eyeblink, or other aspects of attention were not measured in this experiment.

Pre-pulse Inhibition of Startle

Pre-pulse inhibition (PPI) of the startle reflex, like startle amplitude, also may reflect attentional processes. PPI occurs when a weak (non-startle-eliciting), pre-stimulus

precedes the startle eliciting stimulus by 20-500 msec and results in an attenuation of the amplitude of the startle reflex. This effect is believed to reflect an active inhibitory process that develops independently of startle itself (Parisi & Ison, 1979) and can be altered by lesions of various brain structures (Leitner, Powers, Stitt, & Hoffman, 1981; Leitner & Cohen 1985; 1988). Deficits in pre-pulse inhibition of the startle reflex occur in schizophrenic patients (Braff, Stone, Callaway, Geyer, Glick, & Bali, 1978), and PPI has been offered as a model for the study of specific time-dependent information processing and sensory gating disturbances that have been identified in schizophrenics (Swerdlow et al., 1986). This disruption has been shown to occur in rats following apomorphine (dopamine agonist) stimulation of denervated dopamine receptors within the nucleus accumbens (Swerdlow et al., 1986). Disruption of pre-pulse inhibition by apomorphine has been debated in the literature, but has been identified specifically in Wistar rats (Rigdon, 1990) and in Sprague-Dawley rats only under conditions in which a low intensity pre-pulse is used or when the signal-to-noise ratio is decreased (Davis, Mansbach, Swerdlow, Campeau, Braff, & Geyer, 1990). Dopamine agonists have generally been found to increase the amplitude of ASR without affecting PPI (Davis, 1988).

Nicotine and PPI

Nicotine also is known to affect dopamine transmission in the nucleus accumbens (Imperato et al., 1986; Clarke et al., 1988; Mifsud et al., 1989), but unlike other dopamine agonists, preliminary studies have shown that acute administration of nicotine enhances PPI (Acri, Morse, & Grunberg, 1992). This effect also has been interpreted in light of nicotine's effects on attentional processes, although whether these effects are mediated by increased mesolimbic dopamine or increased cholinergic transmission is

unknown. Nicotine's actions on memory and attention in demented populations are believed to be mediated by cholinergic mechanisms (Newhouse et al., 1988; 1990), which may be responsible for startle amplitude and PPI effects as well.

Stress and Startle

The effects of stress on startle are not clear, although a number of experiments have been done. Leitner (1989) found that cold and warm swim stressors, in which rats were forced to swim for 3.5, minutes produced slight decreases in startle amplitude with decreases in pre-pulse inhibition. Cold stress produced the least PPI relative to baseline. The author interpreted this finding in terms of a reduction in sensitivity to environmental stimuli following stress, mediated by deficits in attention.

Korn and Moyer (1965) reported that electric shock increased startle amplitude tested immediately after the shock, but that this effect declined relative to control if rats were startled 24 hours after shock administration. The same effect occurred as a consequence of handling the animals as compared to leaving them in home cages.

Tail-pinch was found by Sorenson and Swerdlow (1982) to significantly depress the amplitude of the ASR. This reduction in startle amplitude during tail pinch was not found in animals with bilateral nucleus accumbens lesions. Results were interpreted in terms of the role of the mesolimbic dopamine systems actions as filters to regulate levels of reactivity.

Footshock has been found to significantly increase the amplitude of ASR when startle is tested 2-4 minutes after shock, peaking at 10 minutes and dissipating over the following 40 minutes (Davis, 1989). Stronger shocks resulted in delayed onset of startle enhancement. Davis and Astrachan (1978) reported that, in the potentiated startle paradigm in

which shock is paired with light, the increase in startle amplitude in the presence of a light was dependent upon the intensity of the shock with which it was paired, with low and high shock levels producing less potentiation of startle amplitude than intermediate levels. The authors concluded that shock, like fear, may affect subsequent behavior in a non-monotonic inverted U-shaped function.

Kokkinidis and MacNeill (1982) studied the effects of isolation stress and inescapable shock on startle in combination with amphetamine administration in mice. Isolation and shock had no effect on ASR, whereas amphetamine caused increases in amplitude of the ASR. When isolation or shock was administered in combination with amphetamine, both isolation and shock increased the amplitude of ASR as compared to amphetamine alone, no isolation, or no shock conditions. This result suggests that while a stressor may have no effect in itself, it may potentiate amphetamine effects of increasing ASR amplitude.

Effects of stress on ASR have been approached from another direction in the study of habituation. Stern (1971) reported that rats sleep-deprived for five days and those subjected to cold water immersion for one hour on five days habituated to startle stimuli significantly faster than did control rats that were not stressed or sleep-deprived. Davis and Zolovick (1972) reported that adrenalectomized rats were compared with non-operated and sham operated rats to determine if habituation to startle differed among these groups. Results showed no differences, suggesting that the adrenal glands are not important in the habituation of startle and that, therefore, habituation to startle is the product of a different mechanism than that responsible for habituation to stress.

Adrenalectomized mice have been reported to have increased sensitivity to nicotine effects (Pauly, Ullman, & Collins, 1988). In this study, nicotine administered intraperitoneally increased startle response in mice, and

adrenalectomy further increased sensitivity to nicotine. Adrenalectomy itself had no effect on startle, but effects on response to nicotine could be reversed by administration of corticosterone (CCS) or dexamethasone. Related to this finding, Swerdlow, Geyer, Vale, and Koob (1986) reported that intracerebroventricular administration of corticotropin releasing factor (CRF) significantly increased the amplitude of the acoustic startle response. CRF is an endogenous neuropeptide that mobilizes the normal hypothalamo-pitituary-adrenal axis response to stress.

Experiments described in this section suggest that stress can both increase and decrease startle amplitude depending upon both the stressor and elapsed time following the stressor. Davis (1989) has reported that shock initially increases, and then decreases startle amplitudes over a period of 30 minutes. Changes in ASR amplitudes do not appear to be mediated by stress-induced changes in CCS as suggested by Pauly and colleagues (1988), but such changes may interact with drugs to either increase drug effects as in the case of amphetamine (Kokkinidis & MacNeill, 1982) or decrease drug effects as in the case of nicotine (Pauly et al., 1988). However, CRF, which is also involved in stress responses, may have independent effects to increase startle amplitude (Swerdlow et al., 1986), but it is not known how this hormone interacts with nicotine in a stressed animal. Therefore, it is of interest to evaluate the interactions of stress and nicotine on startle amplitude, PPI, and habituation in the intact animal.

OVERVIEW AND HYPOTHESES

Overview

These experiments were designed to examine the interactions of stress and nicotine on amplitude, pre-pulse inhibition, and habituation of the ASR in male Sprague-Dawley rats during nicotine administration and cessation. This design tested the hypothesis that nicotine and stress interact in their effects on aspects of startle behavior that can be modulated by both nicotine and stress. It was hypothesized that the mechanisms through which nicotine affects startle and stress-induced changes in startle behavior are cognitive functions involving attention, sensory gating, and habituation that are reflected in startle behavior. Although there is anecdotal evidence from human smokers that smoking has a calming effect or in some way ameliorates the stress response, there is no previous experimental evidence to support this reported phenomenon.

These experiments investigated effects of stress and nicotine on startle behavior, including amplitude, PPI, and habituation. Experiment 1 examined effects of stress and nicotine after 11 days of chronic nicotine administration, and Experiment 2 examined effects of nicotine cessation and stress following 12 days of chronic nicotine administration.

Subjects in Experiment 1 were 76 male Sprague-Dawley rats. Each rat received one of three doses of nicotine (0, 6, or 12 mg/kg/day) administered by osmotic minipump for 11 days. Animals were tested for startle amplitude prior to drug administration and several times during drug administration. Animals received one of three levels of stress exposure on day 11 of drug administration (see Table 1). Immediately prior to startle testing on day 11 of nicotine administration, each animal received twenty minutes of one of three levels of stress. (See Table 2 for

timeline.) Individuals from one group were restrained in a finger-like restraining device for a period of 15-20 minutes. Animals from the "observation-stress" condition remained in home cages but were in the presence of restrained animals. The no stress control group remained in home cages for the same period of time. Each animal was transported to the startle chamber within five minutes after the termination of stress, and was tested for startle amplitude, PPI, and habituation for a period of 22 minutes. Four animals were tested simultaneously. Within five minutes after the termination of startle testing, each animal was sacrificed for blood collection and later measurement of plasma corticosterone to validate the effectiveness of the stressors.

Subjects in Experiment 2 were 32 male Sprague-Dawley rats. Each rat received one of two doses of nicotine (0 or 12 mg/kg/day) administered by osmotic minipump. Animals were tested for startle amplitude prior to and during drug administration. Minipumps were explanted on day 12 of drug administration to insure nicotine cessation. On day 1 of nicotine cessation, animals received one of two levels of stress (see Table 3). Immediately prior to startle testing on day 1 of nicotine cessation, each animal received one of two levels of stress. (See Table 4 for timeline). Animals from the stress group were restrained in a finger-like restraint device for a period of 15-20 minutes, and animals from the stress control group remained in home cages in the colony room. Each animal was transported to the startle chamber within five minutes after the termination of stress, and was tested for startle amplitude, PPI, and habituation for a period of 22 minutes. Four animals were tested simultaneously. Within five minutes after the termination of startle testing, each animal was sacrificed for blood collection and later measurement of plasma corticosterone to validate the effectiveness of the stressor.

Hypotheses

Experiment 1

Hypothesis 1: It was hypothesized that nicotine administration would increase startle amplitude in a positive dose-dependent fashion (ASR amplitude: 12 mg/kg nic > 6 mg/kg nic > saline).

Rationale: A previous study (Acri et al., 1991) found significant increases in acoustic startle amplitude for animals receiving 12 mg/kg/day nicotine as compared to animals receiving 0 or 6 mg/kg/day nicotine during the period of nicotine administration.

Hypothesis 2: It was hypothesized that nicotine administration would increase pre-pulse inhibition, with greater inhibition for higher nicotine dose (PPI: 12 mg/kg nic > 6 mg/kg nic > saline).

Rationale: A previous study using SC injected nicotine and female Sprague-Dawley rats found increases in percent of pre-pulse inhibition at doses of 0.001 and 0.01 mg/kg nicotine (Acri, Morse, & Grunberg, 1992). Nicotine is known to improve attentional performance in humans (Wesnes & Warburton, 1978; 1984a; Wesnes et al., 1983,) and PPI is sensitive to changes in sensory gating, believed to underlie certain aspects of attention (Braff et al., 1978; Swerdlow et al., 1986).

Hypothesis 3: It was hypothesized that nicotine administration would decrease habituation to startle measured within-sessions, in a dose-response fashion (Habituation: Saline > 6 mg/kg nic > 12 mg/kg nic).

Rationale: Nicotine is known to improve performance on vigilance tasks in humans (Wesnes & Warburton, 1978; 1984a; Wesnes et al., 1983). Such tasks involve responses to repetitive stimulus presentations and habituation to stimuli would be incompatible with improved performance.

Hypothesis 4: It was hypothesized that stress would increase sensorimotor reactivity through increased amplitude of acoustic startle in a dose-response fashion (ASR amplitude: Restraint > observation > no stress).

Rationale: Previous studies using stressors have found increases in reactivity with increased stressor intensity (Davis & Astrachan, 1978), and humans suffering from post-traumatic stress disorder have been reported to have increased startle amplitudes (Butler, Braff, Rausch, Jenkins, Sprock, & Geyer, 1990). Stressors used in this experiment were not thought to be extreme enough to show decreases in responsivity associated with an inverted U, but instead were predicted to show only the rising arm of the U. Pilot studies in this lab (Acri & Grunberg, unpublished data) had shown that 20-30 minutes of restraint stress increased startle amplitudes measured immediately afterward.

Hypothesis 5: It was hypothesized that restraint stress would interact with nicotine administration to produce smaller increases in sensorimotor reactivity as measured by startle amplitude than increases associated with either nicotine or restraint alone (ASR amplitude: Nicotine ≥ Stress > Nicotine + Stress). See Table 5. Using restraint stress, this effect was predicted to show a inverse dose-response pattern with 12 mg/kg nicotine resulting in less increase than 6 mg/kg (ASR amplitude: Restraint + 6 mg/kg Nicotine > Restraint + 12 mg/kg Nicotine). See Table 6. The observation stressor was hypothesized to result in lower startle amplitudes than restraint (ASR amplitude: Restraint > Alarm Sub), and because the stress effect should be less than restraint, this stressor was predicted to interact in a positive dose-response pattern with nicotine, such that 12 mg/kg nicotine resulted in greater amplitude increases than 6 mg/kg nicotine (ASR amplitude: Alarm Sub + 12 mg/kg Nicotine > Alarm Sub + 6 mg/kg Nicotine). See Table 7.

Rationale: Although stress and nicotine both act independently to increase startle reactivity when

administered alone, in combination, a lower dose of nicotine may increase reactivity and attentional mechanisms, whereas a higher one may decrease reactivity in an animal already highly aroused by stress. This is consistent with Eysenck's (1973) explanation of nicotine's discordant effects. A higher dose of nicotine may be stress-reducing by preventing a hyperarousal in highly stressed animals, but a lower dose may increase reactivity in highly stressed animals. In animals receiving lower levels of stress, both doses of nicotine may increase reactivity above that of the stressor alone.

Hypothesis 6: Stress was hypothesized to decrease pre-pulse inhibition by disrupting attentional mechanisms in a dose response fashion (PPI: No stress > observe > restraint).

Rationale: Humans exposed to stressors show deficits in pre-pulse inhibition. Ornitz and Pynoos (1989) reported that children with post-traumatic stress disorder also show significant deficits in PPI and hypothesized that the deficits might reflect cortically mediated attentional dysfunction. Leitner (1989) reported decreases in PPI in rats exposed to swim stress.

Hypothesis 7: Nicotine was hypothesized to interact with stress to reduce the disruptive effect of stress on pre-pulse inhibition (PPI: Nicotine + Stress > Stress).

Rationale: Nicotine will increase attentional and selective attentional abilities that are disrupted by stress through increases in cholinergic transmission (Newhouse et al., 1990). As described above, stress decreases PPI (Leitner, 1989) and nicotine increases PPI (Acri et al., 1992). Changes in PPI may reflect the underlying attentional mechanisms through which nicotine attenuates perceptions of stress such that humans report that nicotine relieves stress effects.

Hypothesis 8: It was predicted that stress would increase habituation to stimuli (Habituation: Stress > No stress).

Rationale: Stern (1971) reported that rats that had been stressed by either cold water immersion or sleep deprivation habituated to startle stimuli significantly faster did than non-stressed control rats.

Hypothesis 9: It was hypothesized that nicotine would interact with stress to reduce within-session habituation (Habituation: Stress > Nicotine + Stress ≥ No stress).

Rationale: Nicotine improves performance on vigilance tasks in humans (Wesnes et al., 1983), suggesting that nicotine interferes with processes of habituation by maintaining stimulus sensitivity.

Experiment 2

Hypothesis 10: Nicotine withdrawal was hypothesized to have no effect on sensorimotor reactivity during cessation (ASR amplitude: Nicotine cessation = saline cessation).

Rationale: Although Helton, Tizzano, Modlin, and Rasmussen (1991) recently reported increased ASR amplitude during the period of nicotine cessation, Acri and colleagues (1991) reported return to baseline rather than a withdrawal effect. Both findings are generally consistent with theories of opponent processes that would predict cessation effects in a direction opposite to those of drug effects (Solomon, 1977; Himmelsbach, 1943; Koob & Bloom, 1988).

Hypothesis 11: It was hypothesized that pre-pulse inhibition would be decreased during nicotine cessation as compared to saline (PPI: Saline cessation > nicotine cessation).

Rationale: Nicotine has been reported to increase PPI during administration, so decreases in PPI during cessation would be consistent with theories of opponent processes or rebound effects that are thought to apply at behavioral,

cellular, and molecular levels following exposure to drugs (Koob & Bloom, 1988). Additionally, nicotine withdrawal is known to cause difficulty concentrating in humans (Hughes et al., 1984; APA, 1987) that may involve attentional mechanisms.

Hypothesis 12: Nicotine withdrawal was hypothesized to increase habituation to startle measured within-sessions (Habituation: Nicotine cessation > Saline cessation).

Rationale: Nicotine withdrawal effects involve rebound decreases in concentration and vigilance (Wesnes & Warburton, 1984B; Andersson & Hockey, 1977; Hughes et al., 1984, APA, 1987). This reduction in concentration and vigilance may result in greater habituation because there is less attention to stimuli.

Hypothesis 13: It was hypothesized that stress would interact with nicotine cessation to produce greater increases in sensorimotor reactivity as measured by startle amplitude than amplitude levels associated with either nicotine cessation or stress. This interaction was hypothesized to result in an increase in ASR amplitude as compared to baseline (ASR amplitude: Nicotine cessation + Stress > Saline cessation + Stress > Nicotine cessation = Saline cessation).

Rationale: Unlike nicotine's interaction with stress that decreases reactivity during administration, nicotine cessation will potentiate the stress effect to increase reactivity. This hypothesis is consistent with opponent processes theory that predicts effects opposite those of drug administration (Solomon, 1977, 1980; Himmelsbach, 1943).

Hypothesis 14: It was hypothesized that stress would decrease pre-pulse inhibition during nicotine cessation (PPI: Saline cessation = Nicotine cessation > Saline cessation + Stress > Nicotine cessation + Stress).

Rationale: Humans exposed to stressors show deficits in pre-pulse inhibition. Ornitz and Pynoos (1989) reported that children with post-traumatic stress disorder also show significant deficits in PPI and hypothesized that the

deficits might reflect cortically mediated attentional dysfunction. Leitner (1989) reported decreases in PPI in rats exposed to swim stress. Humans in nicotine withdrawal are known to have difficulty concentrating (Hughes et al., 1984; APA, 1987). This effect may reflect within-systems opponent processes as described by Koob and Bloom (1988) involving increased cholinergic transmission during nicotine administration (Newhouse et al., 1990).

Hypothesis 15: Stress was hypothesized to increase habituation during nicotine cessation (Habituation: Nicotine cessation + Stress > Saline cessation + Stress > Nicotine cessation = Saline cessation).

Rationale: Stress and nicotine cessation are both associated with decreased attention and concentration (Ornitz & Pynoos, 1989; Hughes et al., 1984; APA, 1987) which should result in lack of stimulus sensitivity or habituation. Therefore, stress and cessation were hypothesized to result in greater habituation than either one alone.

EXPERIMENT 1

Overview

This experiment was designed to examine the interactions of stress and nicotine on amplitude, pre-pulse inhibition, and habituation of the ASR in male Sprague-Dawley rats during nicotine administration. This experiment tested the hypothesis that nicotine and stress interact in their effects on aspects of startle behavior that can be modulated by both nicotine and stress. It was hypothesized that the mechanisms through which nicotine affects startle and stress-induced changes in startle behavior involve attention, sensory gating, and habituation that are reflected in startle behavior. Although there is anecdotal evidence from human smokers that smoking has a calming effect or in some way ameliorates the stress response, there is no convincing experimental evidence to support this reported phenomenon.

This experiment investigated effects of stress and nicotine on startle behavior, including amplitude, PPI, and habituation. The experiment examined effects of stress and nicotine after 11 days of chronic nicotine administration.

Subjects were 76 male Sprague-Dawley rats. Each rat received one of three doses of nicotine (0, 6, or 12 mg/kg/day) administered by osmotic minipump for 11 days. Animals were tested for startle amplitude prior to drug administration and several times during drug administration. Animals were exposed to one of three levels of stress on day 11 of drug administration (see Table 1), immediately prior to startle testing (see Table 2 for timeline). Individuals from the restraint group were restrained in a finger-like restraining device for a period of 15-20 minutes. Animals from the observation-stress condition remained in home cages but were in the presence of restrained animals. The no stress control group remained in home cages for the same

period of time. Each animal was transported to the startle chamber within five minutes after the termination of stress, and was tested for startle amplitude, PPI, and habituation for a period of 22 minutes. Four animals were tested simultaneously. Within five minutes after the termination of startle testing, each animal was sacrificed for blood collection and later measurement of plasma corticosterone to validate the effectiveness of the stressors.

Methods

Subjects and Housing

Subjects were 76 male Sprague-Dawley rats (225-250g) obtained from the Charles River Laboratories. Animals were individually housed in standard polypropylene cages (35.56 cm x 15.24 cm x 20.32 cm) with absorbent Pine-Dri bedding and metal grill lids. Animals were maintained in a room with a 12 hr light/dark cycle (dark 7 pm to 7 am) at approximately 72 degrees F and 50% humidity. Standard pellet rat chow and water were continuously available, and cages were changed twice weekly.

An animal model was chosen for this experiment because of ethical issues involved in administering nicotine on a chronic basis to nonsmoking humans. Rats were chosen as the appropriate species for the current study because rats have been used extensively in nicotine, stress, and startle experiments and much related data are already available. Sprague-Dawley rats were selected because they are neither the most, nor the least responsive rat strain in previous startle experiments (Acri, Saah, & Grunberg, unpublished data) and, therefore, were likely to provide the greatest generalizability. Male rats were used because there was no a priori evidence of gender differences in cognitive effects of nicotine and the use of both male and females would have doubled the size and costs of the experiment.

Drug administration

Nicotine or saline was administered using Alzet mini osmotic pumps (Model 2002, Alza Corp., CA). Each minipump was filled with a saline or nicotine solution made from nicotine dihydrochloride administered at a rate of approximately 0.5 µl/hr (Theeuwes & Yum, 1977). Doses were calculated based on body weight such that each animal received a dose of either 12 mg/kg/day, 6 mg/kg/day, or 0 mg/kg/day. Minipumps were used to maintain a constant rate of nicotine administration without the trauma of repeated injections. Pumps were implanted under sterile conditions using methoxyflurane anesthesia. These drug dosages were selected based on previous studies in which behavioral effects in rats approximate those of humans (Grunberg, Bowen, & Morse, 1984).

Stress

Animals were restrained in commercially available finger-like restraint cages (Centrap Cage, Fisher Scientific) for a period of 15-20 minutes. Upon the animal's entry into the restraining cage, the "fingers" were tightened to restrict the animals' movements but were not tightened enough to induce pinching or pain. Restraint stress has been used in previous studies and has been shown to produce elevations in ACTH, beta-endorphin, and corticosterone (Flores et al., 1990), increased plasma renin, prolactin levels, and corticosterone (Paris, Lorens, Van de Kar, Urban, Richardson-Mortin, & Bethea, 1987) as well as serotonin and NE in the brain (Adell, Garcia-Marquez, Armario, & Gelpi, 1988) consistent with a stress response. Restraint stress has been reported to increase the amplitude of sensory evoked potentials in rats (Casada & Dafny, 1990) and has increased

the amplitude of ASR in a pilot study (Acri & Grunberg, unpublished data).

An equal number of animals were left in home cages but were in close proximity to animals being restrained, such that these animals were able to detect sounds and odors emanating from the stressed animals from a distance of 8-24 inches. This manipulation was thought to represent another, possibly less intense stressor (Dunn, 1988). Control non-stressed animals remained in home cages in the colony room during this time.

Acoustic Startle Reflex Testing

Acoustic startle reflex amplitudes, pre-pulse inhibition, and habituation were measured in a Coulbourn Instruments Acoustic Response Test System. Each rat was individually placed in a 8 x 8 x 16 cm open air cage that restricted locomotion but did not immobilize or restrain the movements of the animal, and was placed on one of four platforms within a sound-attenuating chamber. Platforms were arranged radially around central speakers in the floor and ceiling of the chamber. A ventilating fan provided an ambient noise level of 50 dB SPL. All stimulus intensity levels are described in terms of sound pressure level or SPL (re: 0.0002 dynes/cm^2), meaning that an unweighted measurement scale was used. There was a 3 minute quiet adaptation period following the placement of four animals within the chamber.

Startle stimuli consisted of 98, 112, or 122 dB SPL noise bursts sometimes preceded 100 ms by 68 dB, 1 kHz pre-pulses. These stimulus intensity levels were verified with a GenRad Type 1982 sound level meter with microphone placement in the position of a subject's head. There were six types of stimulus trials and two types of control trials. There were 8 trials of a 98 dB noise burst, 8 trials of a 98 dB noise burst preceded by a 1 kHz, 68 dB pre-pulse, 8 trials of a 112 dB noise burst, 8 trials of a 112 noise bursts preceded by a

1 kHz pre-pulse, 8 trials of a 122 dB noise burst, 8 trials of a 122 noise burst preceded by a 68 dB 1 kHz pre-pulse, 4 pre-pulse alone control trials, and 4 no-stimulus control trials. There was a total of 56 of these trials, with each stimulus trial type presented once every seven trials in a block randomized fashion. Each stimulus had a 2 ms rise-fall time such that onset and offset were abrupt. Sudden onset of an intense stimulus is a primary criterion for elicitation of startle. Interstimulus intervals ranged from 10-39 seconds, and were varied randomly. Habituation also was measured using 112 dB SPL noise bursts at a fixed interstimulus interval of 10 seconds at the end of the session. Ten habituation trials were presented, bringing the total number of trials to 66. No-stimulus trials and pre-pulse alone trials were presented as controls for random movement and to insure that pre-pulses did not elicit startle responses.

Following presentation of each stimulus, movement on each platform was measured for a period of 200 ms by coupling through a sensor pin connected to a strain gauge. Four platforms were simultaneously measured by an interfaced microcomputer that controlled both the stimulus presentation schedule and measurement of responses. Changes in voltage underwent analog to digital conversion, and were fed into calibration equations derived for each platform to equate voltage to grams of weight. Amplitudes were recorded as the maximum response occurring within 200 ms following stimulus presentation, and were calculated as grams of weight change. Responses were averaged within trial types which were presented in block-randomized order, as described above. Each animal's response to each stimulus, therefore, represents an average of 8 stimulus presentations. Pre-pulse inhibition, measured as amount or percent, is the difference in amplitude between trials involving a startle stimulus alone and a startle stimulus preceded by a pre-pulse. These parameters have been used effectively in previous studies of

nicotine (Acri et al., 1991; 1992) and strain (Acri, Saah, & Grunberg, unpublished data) effects.

Rats were tested for startle behavior four at a time. Stressed animals were not run prior to, or simultaneously with, unstressed animals to avoid the influence of alarm substances. Each animal was tested during the beginning of the activity cycle because there is evidence that startle amplitudes are more stable at that time (Davis & Sollberger, 1971).

Stress Index

The stressfulness of the procedure was evaluated by measurement of plasma corticosterone (CCS) in all groups. Blood was collected within 5 minutes after the termination of startle testing, and within 30 minutes after the termination of stress. Plasma corticosterone is an indicator of adrenal cortical activity and increases following restraint stress (Paris et al., 1987; Flores et al., 1990) as well as nicotine administration (Morse, 1989; Newhouse et al., 1990). Corticosterone has a longer half life than catecholamines and is less sensitive to rapid environmental changes (Baum et al., 1982). It is, therefore, more likely to show effects of prior stress during the previous hour, rather than the more immediate effects of decapitation for blood collection. Rats were sacrificed by decapitation, and trunk blood was collected in 14 ml polypropylene tubes containing 50 µl of 10,000 IU/ml heparin and centrifuged. The resulting plasma was frozen in a -70 degree C freezer for later corticosterone assay. Assays were performed on thawed plasma using ^{125}I-labeled corticosterone and a specific anti-corticosterone antiserum to determine the corticosterone concentrations in specimens using the double antibody technique (Rattner, Gruenau, & Altland, 1979). Reagents for this radioimmunoassay were purchased from ICN Biomedicals, Costa Mesa, CA.

Procedure

For logistical reasons, start days in Experiment 1 were staggered over three days because of the number of animals and the need to maintain constant time-of-day effects. Experimental "days," as described below, are relative to start day for each animal and the sequence of events within the experiments was the same for all animals within the experiment. Experimental treatment groups were equally distributed over the three staggered groups.

Careful attention was given to time-of-day consistency in procedures. All handling and surgical procedures occurred in the last 4 hours of the animal's sleep cycle (light), and all startling and stressing occurred in the last hour of the sleep cycle (light) and the first 2 hours of the activity cycle (dark).

Rats were gentled by handling daily during the baseline period. Each animal was weighed three times during the baseline period, and baseline startle amplitudes were obtained on days 2, 5, and 8 relative to start day. Animals were quasi-randomly assigned to drug groups according to baseline startle amplitude. Subjects from each drug group were then further divided in a quasi-random fashion into stress conditions, again with regard to baseline startle amplitude. The last of the three baseline startle testing days was used for this purpose. There were 9 groups (3 x 3) within the nicotine administration study (8-9 animals per group). See Table 1 for experimental design.

Three days following baseline procedures, each animal was anesthetized with methoxyflurane and a minipump containing the appropriate drug solution was implanted subcutaneously. An incision of approximately 3 cm was made between the shoulder blades and a pump was implanted under the skin. The incision was closed with 9 mm stainless steel

wound clips. Following surgery, each animal was returned to the home cage.

Startle testing was performed on drug administration days 1, 4, 7, and 11; that is, 1, 4, 7, and 11 days after implantation. On day 11, stress was applied prior to startle testing (see Table 2 for timeline). Unstressed animals were run first, to control for the effects of odors and alarm substances, although cages thoroughly washed between animals For the no-stress condition, four animals at a time were transferred to the startle treatment room, startled, and following the end of the startle testing period, were transferred to the necropsy room and decapitated within 5 minutes. Trunk blood was collected for later assay. Following startling all animals from the no-stress control condition, stressed animals were run as follows. Two animals to be restrained and two to observe, or to be in the presence of restrained animals, were taken to a treatment room in which two animals were placed in restrainers and two animals were left in home cages, but were in close proximity to restrained animals. Observing rats could move freely within their cages, and distances from restrained animals ranged from 8 - 24 inches. After 15-20 minutes of stress, animals were transferred to startle cages and were placed in the startle chamber within 5 minutes. Following the startle testing period of 22 minutes, animals were decapitated within 5 minutes and blood was collected for later assay.

Statistical Analysis

An initial Multivariate Analysis of Variance (MANOVA) was performed on the nine dependent measures from day 11 of drug administration. Dependent measures included startle amplitude (mean peak response - mean average no stimulus response), amplitude of pre-pulse inhibition trials (mean peak response of PPI trials - mean average no stimulus response), and amount of inhibition ([mean peak response -

mean average no stimulus response]- [mean peak response of PPI trials - mean average no stimulus response]). The MANOVA included drug (saline, 6 mg/kg/day nicotine, or 12 mg/kg/day nicotine) and stress (no stress, observation, or restraint) as between-subject factors, and stimulus intensity (98, 112, or 122 dB) and type (with or without pre-pulse) as within-subject factors. Following this analysis, 3 x 3 factorial ANOVAs were used to examine effects of nicotine and stress on each individual dependent measure. Dependent measures included startle amplitude, amplitude of PPI trials, and amount of pre-pulse inhibition. Percent of pre-pulse inhibition ([mean peak response - mean average no stimulus response]- [mean peak response of PPI trials - mean average no stimulus response])/ [mean peak response - mean average no stimulus response] was not included in the MANOVA, but was analyzed by separate ANOVA because it was statistically related to the other measures.

Habituation data were analyzed using repeated measures ANOVA to determine within-session habituation. This analysis was a repeated measures analysis of changes in response to 10 habituation trials, using between-subject factors of drug and stress.

Group size of 8-9 animals per group was based on previous studies of this type in which group size of eight was sufficient to yield significant differences in response to drug (Acri et al., 1991). A significance level of 0.05 was used for all statistical tests.

Post hoc t-tests were used to determine which groups differed when significant differences were found on ANOVAs. Fisher PLSD tests were used, where appropriate.

A post hoc analysis of nicotine effects was done for measures using the 112 dB stimulus to determine if nicotine's effects were dependent upon initial level of arousal. That is, this analysis was done to determine if more reactive animals responded differently to stress, nicotine, or the combination. Consequently, separate ANOVAs following median-

split of baseline startle amplitudes within the nicotine and saline groups were run for the principle measures of amplitude, pre-pulse inhibition, and habituation.

Data from the biochemical assay of plasma corticosterone concentration was analyzed by ANOVA (3 dose x 3 stress). Post hoc t-tests were done to determine which groups differed.

Results

Overview

Some subjects were inadvertently deprived of water during the experiment because animal caretakers in the Laboratory Animal Medicine (LAM) department overfilled water bottles which created a vacuum such that water would not flow through the tube to the rat in the cage below. By visual inspection, all animals had access to an abundance of food and water, but 12 subjects from this experiment lost from 1 - 92 grams, depending upon the period of water deprivation. The eight animals most severely affected by the problem lost between 37 - 92 grams and were excluded from all analyses because food or water deprivation increases the amplitude of the acoustic startle response (Melgren, 1969; Meryman, 1952), so inclusion of these subjects in the analyses would confound experimental results. The four animals that lost between 1 - 10 grams were included in the analyses, because their weight loss was considerably less and occurred over a shorter period of time. See Table 7 for data on weight loss.

All rats of this age and with continuous access to food and water normally gain weight. Animals treated with nicotine gain less weight than saline-treated animals, but do not typically lose weight as a result of nicotine administration. Fully half of the present cases of weight loss began during the baseline period before any drug was administered and, further, the most severe cases of weight

loss occurred in saline-treated animals. Therefore, it is extremely unlikely that any cases of weight loss were the result of experimental treatments.

All acoustic startle measures were derived by subtraction of raw data of no-stimulus, or "catch" trials, from stimulus trials. This process controls for differences in random movement and activity. Stimulus trials of the same type were averaged for each animal. An overall multivariate analysis of variance (MANOVA) was conducted, in which drug (saline, 6 mg/kg/day nicotine, or 12 mg/kg/day nicotine) and stress (no stress, observation, or restraint) were between-subject factors, and stimulus intensity (98 dB, 112 dB or 122 dB) and type (with or without 68 dB 1 kHz pre-pulse) were within-subject variables in an analysis of differences in response amplitude, or grams of weight change. Amplitude data for stimuli of 98 dB, 112 dB, and 122 dB were then analyzed in separate analyses of variance followed by post-hoc tests when significant main effects or interactions were found.

Pre-pulse inhibition data were reported in three ways. First, as in the MANOVA, the amplitude of trials in which a pre-pulse precedes the startle-eliciting stimulus was reported. Second, the amount of inhibition was derived by subtraction of pre-pulse trial amplitudes from amplitudes of trials using the same startle-eliciting stimulus without a pre-pulse, or the amount of the response that was inhibited. Third, the percent of inhibition was derived by dividing the amount of inhibition by the amplitude of non pre-pulse trials of the same stimulus to determine the percent of the response that was inhibited. (See Davis [1988] for a discussion of methods for deriving pre-pulse inhibition.)

Habituation trials consisted of 10 presentations of 112 dB separated by a fixed inter-stimulus interval of 10 seconds. These results were analyzed by a separate repeated measures analysis of variance.

All results were based on a two-tailed test of significance. The alpha level for all analyses was set at 0.05.

As a post hoc analysis, results of all measures using 112 dB stimuli were further analyzed by dividing groups in half according to initial reactivity (median split), to determine whether initial level of reactivity was an important determinant of stress and drug effects.

Stress Validation

Plasma Corticosterone Levels

Exposure to stressful events results in a number of neuroendocrine responses, and one of the most robust of these responses in rats is the release of corticosterone from the adrenal cortex. To determine the effectiveness of restraint and the effects of being in the presence of conspecifics that are being restrained as stressors, plasma corticosterone (CCS) levels were measured 30-35 minutes following exposure to these stressful events. Figure 1 presents the mean plasma CCS concentration for each stress condition, collapsing across drug treatment conditions. Restraint stress resulted in CCS levels significantly greater than both observation (Fisher PLSD = 59.4, $p < 0.05$) and no stress (Fisher PLSD = 60.6, $p < 0.05$). Observation stress also resulted in CCS levels that were significantly greater (Fisher PLSD = 60.5, $p < 0.05$) than non-stressed controls. This main effect of stress was significant [F (2,59) = 15.21, $p < 0.05$]. Drug treatment condition had no effect on CCS levels.

Multivariate Analysis

The multivariate analysis of variance (MANOVA) was conducted, in which drug (saline, 6 mg/kg/day nicotine, or 12 mg/kg/day nicotine) and stress (no stress, observation, or

restraint) were between-subject factors, and stimulus intensity (98 dB, 112 dB, or 122 dB) and type (with or without 68 dB 1 kHz pre-pulse) were within-subject factors in an analysis of differences in response amplitude, or grams of weight change. This MANOVA was done to insure that significant findings on any univariate analysis were not the result of chance, due to the number of analyses conducted. Both reactivity (amplitude) and gating (PPI) trials were included in this analysis. There was a significant main effect of stimulus intensity [Hotellings F (2,58) = 112.67, $p < 0.05$] and post hoc analysis of the effect of intensity by planned contrasts indicated that responses to the three stimulus intensities (98 dB vs. 112 dB, and 112 dB vs. 122 dB) were significantly different [F (1,59) = 227.93, $p < 0.05$; F (1,59) = 181.74, $p < 0.05$], with greater amplitude responses to more intense stimuli as previously reported (Acri et al., 1991). There was also a significant intensity by type interaction [Hotellings F (2,58) = 90.16, $p < 0.05$], suggesting that the significant inhibition in response amplitude usually seen when pre-pulses are used may have differed across the stimulus intensity levels used in the experiment. Additionally, there was a significant main effect of stress [F (2,59) = 4.37, $p < 0.05$], and a significant stress by intensity interaction [Hotellings F (4,114) = 2.39, $p < 0.05$]. Univariate tests were then conducted to examine effects of stress and drug in relation to specific hypotheses.

Reactivity (Startle Amplitudes)

Reactivity to external stimulation is reflected in the amplitude of the acoustic startle response. Startle amplitudes were measured using 98 dB, 112 dB, and 122 dB noise stimuli. Using a stimulus level of 98 dB, there were no significant differences by drug or stress condition. An overall mean amplitude of 10.68 grams of weight change was

measured, indicating a very low level of response to this stimulus. Mean responses to this stimulus for each drug and stress condition are presented in Table 8.

Using a stimulus level of 112 dB, there was a significant drug by stress interaction [F (4,59) = 2.89, p < 0.05], with saline- and 6 mg/kg/day nicotine-treated animals exhibiting progressively greater startle amplitudes following observation and restraint stress respectively, as predicted. Animals treated with 12 mg/kg/day nicotine had progressively lower startle amplitudes with increasing stress, as predicted (see Table 8 and Figure 2). Without stress, animals treated with 12 mg/kg nicotine were significantly more reactive than animals treated with 6 mg/kg nicotine, but neither group differed significantly from saline. In contrast, under conditions of restraint stress, animals treated with 12 mg/kg nicotine were significantly less reactive than animals treated with 6 mg/kg nicotine. For the 6 mg/kg nicotine dose, increased levels of stress resulted in amplitudes that were significantly greater than those of unstressed animals receiving 6 mg/kg/day nicotine. Significant post hoc comparisons are listed in Table 9. These results partially confirm Hypothesis 5, which predicted differential effects of nicotine dose on reactivity, with the 12 mg/kg nicotine dose resulting in smaller amplitudes following restraint stress than observation stress, and the 6 mg/kg nicotine dose yielding a positive dose-response pattern with increased stress. Hypothesis 4 was confirmed, in that increased stress resulted in increased startle amplitude in saline-treated animals, although the trend was not significant. Hypothesis 1, that predicted increased startle amplitude with nicotine, was partially confirmed in that a dose of 12 mg/kg/day increased amplitude, but dose-response effects were not seen. There were no significant main effects for nicotine or stress.

Using a stimulus level of 122 dB, there was a main effect of stress [F (2,59) = 4.28, p< 0.05] which

significantly increased the amplitude of the acoustic startle response for the observation stressor only. There were no main effects or interactions with nicotine. However, as can be seen in Figure 3, increasing doses of nicotine reduced the amplitude of startle relative to saline for stressed animals but not for unstressed animals, with identical trends for both stressors. An inverted U-shaped amplitude function is suggested, with the lesser, observation stress resulting in higher amplitudes than the more severe, restraint stressor, and 12 mg/kg/day nicotine producing lower amplitude responses with stress than either saline or 6 mg/kg/day nicotine. Means and significant post hoc comparisons are listed in Tables 8 and 10, respectively. These results are partially consistent with predictions of Hypothesis 5, in that there were inverse dose-effects of nicotine with increased stress.

A post hoc analysis in which each group was split according to initial level of reactivity during baseline was conducted for the 112 dB stimulus amplitude trials. To clarify, the analysis was performed in order to examine the differential effects of baseline reactivity, rather than to remove the effects from the analysis. Each treatment group was divided into high and low reactors, according to baseline amplitudes (M = 27 grams), and a 3-way ANOVA was conducted (Stress x Drug x Reactivity). A significant main effect for initial reactivity was found [F (1,50) = 7.05, $p < 0.05$], with an additionally significant drug by stress interaction [F (4,50) = 3.11, $p < 0.05$]. In Figure 4 these results are presented without data for the 6 mg/kg/day nicotine groups for simplicity, although animals treated with 6 mg/kg/day nicotine were included in the analysis (see Table 11 for all significant post hoc comparisons).

Results from the high reactors (see Figure 4) accentuate the differences found in earlier analyses (see Figure 2) of amplitude results for this stimulus. Overall, results of this analysis highlight the differential effects of nicotine according to individual differences in baseline

state of reactivity. That is, highly reactive animals have greater stimulus amplitudes in response to restraint stress and to nicotine, but when both are administered, amplitude is reduced over that of stress alone or nicotine alone. For the less reactive animals, nicotine and stress administered separately or together have little effect.

In summary, results obtained using the 112 dB stimulus supported hypotheses that nicotine would counteract the effects of restraint stress on reactivity as measured by the amplitude of the acoustic startle response. Post-hoc analyses by median split of initial levels of reactivity suggested that animals initially classified as highly reactive are responsible for all significant effects of nicotine and stress, and that less responsive animals are much less affected by either nicotine treatment or stress condition.

Sensory Gating (Pre-pulse Inhibition)

Sensory gating, or the screening out of irrelevant stimuli, is a process related to selective attention. Pre-pulse inhibition of the startle reflex, or the amplitude reduction that occurs when a non-startling sound precedes the startle-eliciting stimulus is thought to reflect this attentional process. Pre-pulse inhibition was analyzed in several ways in this experiment, using amplitudes of pre-pulse trials, amount, and percent of inhibition as variables. Each stimulus intensity was examined separately.

Using a 98 dB startle-eliciting stimulus preceded by a 1 kHz pre-pulse of 68 dB, amplitudes were measured as presented in Table 12. Startle amplitudes were so minimal when using the 98 dB stimulus (with and without pre-pulses) that it was not clear that pre-pulse inhibition had, in fact, occurred. However, comparison of trials with and without pre-pulses indicated significant amplitude reductions with pre-pulses, [F (1,65) = 12.46, $p < 0.05$], indicating that pre-

pulse inhibition did occur. There were no significant main effects of drug or stress, but there was a significant drug by stress interaction [F (4,59) = 3.57, $p < 0.05$]. See Figure 5 for a clear and significant trend in which amplitude of pre-pulse trials decreased for saline-treated animals with increasing levels of stress. This finding is contrary to Hypothesis 6, which predicted less inhibition with increasing stress rather than more. That is, previous literature upon which hypotheses were based suggested that stress reduces, rather than increases PPI. There was no apparent trend for drug-treated animals. Post hoc analyses indicated that saline-treated restrained animals have significantly lower amplitudes than saline- and 6-mg/kg/day nicotine-treated no stress groups, saline and 12 mg/kg/day nicotine-treated observer groups, and 6 mg/kg/day nicotine-treated restrained animals. Additionally, 6 mg/kg/day nicotine observing animals had significantly lower amplitudes than saline no stress and 6 mg/kg/day nicotine restrained animals (see Table 13 for a listing of these significant differences).

The meaning of the above finding of decreased amplitudes of pre-pulse trials for the saline-treated group is not clear, because there were no significant treatment group differences in amount or percent of inhibition using the 98 dB stimulus. However, there are trends for increasing amount and percent of inhibition with increasing stress for saline-treated rats, and the opposite trend of decreasing amount and percent of inhibition with increasing stress for the 12 mg/kg/day nicotine-treated rats. It would, therefore, appear that nicotine does counteract the effects of stress on PPI as predicted in Hypothesis 7, but the direction of effects is the opposite of those predicted in Hypotheses 6 and 7 (see Figure 6 for illustration of these non significant trends).

Using a 112 dB startle-eliciting stimulus preceded by a 1 kHz 68 dB pre-pulse, amplitudes were measured as shown in Table 12. These amplitudes are significantly lower than

amplitudes for non pre-pulse trials, as expected [F (1,65) = 60.95, $p < 0.05$], indicating that significant pre-pulse inhibition occurred. There was a significant effect of drug [F (2,59) = 3.13, $p < 0.05$] with no significant stress effects or interactions. Amplitudes for the 6 mg/kg/day nicotine-treated group were always greater than the saline and 12 mg/kg/day nicotine groups, especially for the observation stressor. This effect resulted in significant post hoc comparisons with all other treatment groups (except saline observe) for this measure. There do not appear to be any trends with regard to stress or drug, and the meaning of these findings is not clear.

In the analysis of amount of inhibition using the 112 dB stimulus with pre-pulse, there were no significant main effects for drug or stress, but there was a significant drug x stress interaction [F (4,59) = 3.55, $p < 0.05$]. Figure 7 illustrates clear and significant trends for increased amount of inhibition with increasing stress for the 6-mg/kg/day nicotine-treated animals, and decreased amount of inhibition with increasing stress for the 12 mg/kg/day nicotine-treated animals. A very slight trend for increasing inhibition with increased stress was evident for the saline-treated animals (see Tables 15 and 16 for treatment group means and significant post hoc comparisons, respectively). These results are similar to those reported for the 98 dB stimulus, with effects of stress and nicotine opposite in direction of effects predicted by Hypotheses 6, but in accordance with Hypothesis 7, there is some indication that nicotine and stress together result in attenuation of the effects of either one alone.

Analysis of data for percent of inhibition was not significant for stress, drug, or interactions using the 112 dB stimulus with pre-pulse (see Table 17 for means). However, there was a non-significant trend for increasing percent of inhibition with increasing stress for the 6-mg/kg/day nicotine-treated group, consistent with findings of

amount of inhibition. No trends are evident for the saline or 12 mg/kg/day nicotine groups.

Using the 122 dB startle stimulus with a 68 dB 1 kHz pre-pulse, there was significant amplitude reduction or pre-pulse inhibition [F (1,65) = 160.12, $p < 0.05$] compared with non pre-pulse trials. In these trials, the observing stressor groups had higher amplitude responses than did the no stress groups and animals receiving restraint stress. This finding is consistent with amplitude trials in that it shows the same inverted U shape. There was a significant effect of stress [F (2,59) = 3.88, $p < 0.05$] with no significant drug or interaction effects (see Figure 8). Post hoc comparisons indicated that the stress observation groups were all significantly different from the 6 mg/kg/day no stress group, in terms of the amplitude of the inhibited response to the 122 dB stimulus (see Tables 12 for means and 14 for significant post hoc comparisons, respectively).

For amount and percent of inhibition using the 122 dB stimulus, there were no significant drug or stress effects, and there were no significant interactions. Means of treatment groups are listed in Tables 15 and 17. There are no clear trends for these data, other than a slightly decreasing percent of inhibition for the 6 mg/kg/day nicotine-treated group with increasing stress.

Additional analysis of pre-pulse inhibition using a 112 dB stimulus intensity level with 1 kHz 68 dB pre-pulse was also done using a median split of baseline response amplitudes, in order to determine if initial reactivity was an important determinant of the effects of nicotine and stress on pre-pulse inhibition. To clarify, the analysis was performed in order to examine the differential effects of baseline reactivity, rather than to remove the effects from the analysis. Following median split, there was a significant main effect of initial reactivity [F (1,50) = 5.62, $p < 0.05$] and a significant interaction of drug and stress [F (4,50) = 3.86, $p < 0.05$] on amount of inhibition.

Figure 9 has been simplified by showing only saline and 12 mg/kg/day nicotine groups by median split, although the high reactors from 6 mg/kg/day nicotine-treated animals were very similar to the saline-treated animals, meaning that inhibition increased with increasing levels of stress. Table 18 presents significant treatment group comparisons including the 6 mg/kg/day nicotine-treated animals. As in the previous analysis by median split, it is clear that the high reactors are responsible for the greatest differences in the effects of nicotine and stress, with nicotine-treated high reactors decreasing in amount of pre-pulse inhibition with increasing stress, and saline-treated high reactors increasing in amount of pre-pulse inhibition with increasing stress. Again, these effects are opposite of those predicted by Hypotheses 6 because stress appears to enhance, rather than decrease PPI, but as predicted in Hypothesis 7, nicotine appears to attenuate the effects of stress on PPI, or alternatively, stress attenuates the effects of nicotine on PPI in the high reactors.

In summary, there is evidence that both nicotine and stress increase sensory gating as measured by pre-pulse inhibition of the acoustic startle reflex. Effects of nicotine are as predicted, but the effects of stress on PPI are in a direction opposite of those predicted by hypotheses. There was, however, the predicted interactive effect such that nicotine and stress in combination did not increase PPI, but instead resembled no stress saline controls.

Habituation

Habituation data were subjected to a repeated-measures analysis of variance with time as a within-subject factor and stress and drug as between-subject factors. There was a significant effect of time [F (9,531) = 2.49, $p < 0.05$], meaning that all groups habituated to some extent to the ten habituation stimuli of 112 dB presented at a fixed inter-

stimulus interval. However, there were no significant main effects for drug or stress, and there were no significant interactions. These results do not confirm Hypotheses 8 and 9, because both stress and nicotine had no significant effects on habituation. Analysis following median split by initial amplitudes revealed no differences in habituation between the high and low reactivity groups.

In summary, there were no effects of nicotine or stress on habituation using the acoustic startle reflex. This finding suggests that reflex habituation as measured in this paradigm is not sensitive to the previously reported effects of nicotine on vigilance, sustained attention, and concentration.

Confirmation of Hypotheses

Hypothesis 1. The hypothesis that nicotine would increase sensorimotor reactivity (startle amplitude) in a positive dose-response manner was not confirmed, although 12 mg/kg/day nicotine did increase reactivity significantly as compared to 6 mg/kg/day.

Hypothesis 2. A nicotine dose of 12 mg/kg/day significantly increased PPI relative to 6 mg/kg/day, partially confirming Hypothesis 2.

Hypothesis 3. The hypothesis that nicotine would increase habituation was not confirmed.

Hypothesis 4. The hypothesis that stress would increase startle amplitude in a dose-response fashion was confirmed by a non-significant trend.

Hypothesis 5. The hypothesis that nicotine and stress would interact to produce less increase in ASR amplitude than

either alone was confirmed by a significant statistical interaction.

Hypothesis 6. The hypothesis that stress would decrease PPI in a dose-response fashion was not confirmed, and there was a non-significant trend for stress to increase PPI.

Hypothesis 7. Stress and nicotine significantly interacted such that nicotine blocked the effects of stress on PPI, confirming this hypothesis.

Hypothesis 8. Stress did not increase ASR habituation, disconfirming this hypothesis.

Hypothesis 9. Stress and nicotine did not interact to reduce habituation, disconfirming this hypothesis.

Discussion

This experiment evaluated interactions of stress and nicotine on reactivity (startle amplitude), sensory gating (pre-pulse inhibition), and habituation of the acoustic startle reflex, in order to determine if nicotine counteracted any of the effects of stress on these variables. This question was of interest because smokers report that cigarette smoking has a calming effect and helps them cope with stress, despite evidence that nicotine does not reduce the physiological indices of stress. The acoustic startle response can be modulated by both stress and nicotine, and therefore was expected to be a good model to test this interaction.

Reactivity (Startle amplitude)

Reactivity to external stimulation was measured as the amplitude of the acoustic startle response. As predicted,

there were significant interactions of stress and nicotine on the amplitude of the startle reflex using a stimulus level of 112 dB. Animals receiving 12 mg/kg/day nicotine or restraint stress were more reactive than controls, whereas animals receiving both stress and nicotine were indistinguishable from controls. For the lower dose of nicotine, stress effects on reactivity were enhanced. Specifically, for animals receiving either saline or the lower dose of nicotine, increasing levels of stress resulted in increases in the amplitude of ASR. In contrast, animals receiving the higher dose decreased amplitude with increased stress. The higher dose of nicotine may prevent the increased amplitude associated with stress, and may account for some of the reported stress-reducing effects of nicotine and stress-induced smoking in human smokers (Rose, Ananda, & Jarvik, 1983; Wills & Shiffman, 1985; Shiffman, 1985; Barnes & Fishlincki, 1976; Ikard & Thomkins, 1973; Kleinke, Staneski, & Meeker, 1983).

Additionally, analysis by median split of baseline levels of reactivity indicate that experimental differences are attributable to the more reactive subjects in each treatment condition. That is, the high reactors in the saline group reacted significantly more to restraint stress than did the low reactors, and the high reactors in the nicotine group reacted more to nicotine. This analysis suggests that high reactors in both groups are primarily responsible for all of the differences measured, and is consistent with findings of Nelsen (1978) and theories of Eysenck (1973). Nelsen (1978) reported that rats were more protected by nicotine from behavioral disruption in the presence of a stressor when they had been originally categorized as highly emotional. In the present experiment, animals were protected from stress-induced increases in amplitude if they were initially more reactive and had been administered 12 mg/kg/day nicotine. Eysenck (1973) theorized that nicotine has different effects in individuals according

to their initial level of arousal. In this view, individuals with low levels of arousal will experience stimulant effects of nicotine and individuals with high levels of arousal will experience tranquilization. In this experiment, it was only the high reactors that were responsive to nicotine-induced reduction in amplitude when animals were stressed.

The significance of this finding is two-fold. First, it may help to explain why heavy smokers smoke more during stress and report that it has a calming effect, despite increases in sympathetic nervous system activity (Morse, 1989; Kiritsy-Roy et al., 1990). These individuals may be similar to the more reactive animals in that they may react more to stress, and may prevent increased reactivity in the presence of stress by smoking or increasing their nicotine dose. Second, it lends support to Eysenck's (1973) theory that nicotine interacts with level of arousal to produce different effects in different individuals depending upon baseline arousal level. That is, less reactive individuals may not experience a protective effect of nicotine because they are less responsive to effects of both stress and nicotine as compared to high reactors. In this experiment, arousal was manipulated by the stressors that were applied, and it also was inferred through the individual differences in startle amplitude (reactivity) at baseline. It appears that both are important determinants of nicotine's effects on reactivity.

It had been hypothesized that decreases in amplitude would not be measured in the 12 mg/kg/day nicotine group following observation stress. However, if one considers the inverted U-shaped function that generally describes nicotine effects, it is evident from Figure 2 that the 12 mg/kg/day group is at the apex of the inverted U in the no-stress condition, and increasing stress can only result in amplitude decreases, for both observation stress and to a greater degree, restraint. In contrast, saline and 6 mg/kg/day nicotine groups are on the rising arm of the inverted U in

the no stress condition, and increasing levels of stress bring these groups higher on the rising arm in terms of amplitude. However, with increased stimulus intensity which itself increases amplitude, a clearly inverted U-shaped pattern is evident for all drug groups in Figure 3. While stress effects are significant for the observation stressor, drug effects are masked by the higher intensity stimulus.

The pattern of these figures suggests that stress shifts the dose effect curve of nicotine to the left, such that 12 mg/kg/day nicotine produces increased amplitude without stress, but that after exposure to stress, amplitude is reduced. In contrast, stress increases amplitude for saline and the 6 mg/kg/day nicotine dose except with a higher stimulus intensity, where the inverted U pattern is evident for both doses of nicotine.

Although the results of this experiment do not allow the unequivocal conclusion that stress changes the dose-response curve of nicotine or the converse, that nicotine changes the dose-response pattern of stress, the evidence that these results are specific to nicotine rather than being attributable to simple increases in arousal can be found in two ways. First, Davis and Astrachan (1978) reported that in the potentiated startle paradigm, the increase in startle amplitude was dependent upon the intensity of the shock used in training, with low and high shock levels producing less increase in startle amplitude than intermediate levels. The authors concluded that shock, like fear, may affect behavior in a non-monotonic inverted U-shaped function. If restrained animals are simply at the apex of the inverted U of stress effects, then both doses of nicotine would have decreased the amplitude of the reflex and not shown the differential effects of Figure 2. Secondly, Kokkinidis and MacNeill (1982) reported that in mice, while prior stress did not increase amplitude, it resulted in increased drug effects. The dose effect pattern was positive, with greater increases for higher doses of drug in their experiment. In this

experiment, using 112 dB stimulus, the observation stressor did not significantly increase amplitude (as in Kokkinidis and MacNeill, 1982), but amplitude was increased by this stressor in combination with the 6 mg/kg/day dose of nicotine more than for the 12 mg/kg/day dose. The positive dose effect curve reported by Kokkinidis and MacNeill (1982) for stress and amphetamine was not observed, probably because nicotine's inverted U dose effect pattern differs from amphetamine. The present experiment thus suggests that stress may intensify nicotine's effects, or shift the dose response pattern to the left. Because of nicotine's inverted U shaped function, this decreases amplitude rather than increasing it, as with amphetamine. Most importantly, it is consistent with the reports of human smokers that nicotine's effects can be tranquilizing when under stress, at doses that would be expected to be stimulating.

Sensory Gating (Pre-pulse Inhibition)

Sensory gating, or the ability to screen out unwanted stimuli, is indexed through the magnitude of pre-pulse inhibition, with greater inhibition signifying better screening or selective attention. As previously reported (Acri et al., 1992) and as predicted, nicotine increased PPI as compared to saline. This effect is consistent with reports that nicotine enhances selective attention and concentration. However, with stress, PPI increased for saline-treated animals and 6 mg/kg/day nicotine, and decreased for the higher dose of nicotine. This finding is contrary to reports of decreased PPI in humans (Ornitz & Pynoos, 1989) or in rats exposed to swim stress (Leitner, 1989). Although these effects of nicotine were significant, as was the drug by stress interaction, results were not as predicted.

These findings are not consistent with those in the literature which have reported decreased PPI in humans

(Ornitz & Pynoos, 1989) or in rats exposed to swim stress (Leitner, 1989). They also are inconsistent with previously reported findings with acute nicotine doses, in which the lowest acute doses of nicotine resulted in the greatest increases in startle amplitude and PPI reported as percent (Acri et al., 1992).

If indeed PPI reflects changes in attentional mechanisms, it may be that the types of stressors used resulted in increased attention or possibly a hypervigilance effect. Nicotine did interact with stress but not in the predicted direction, in that 12 mg/kg/day nicotine reduced sensory gating as reflected in PPI of stressed rats. Although the direction of effects of stress in the saline group were not as predicted in that stress increased PPI instead of decreasing it, there were still significant interactions of stress and nicotine on PPI that indicate a cancellation of effects when both are administered, such that PPI in restrained, 12 mg/kg/day nicotine-treated animals was indistinguishable from saline no stress controls.

As with the reactivity data, median split by initial startle amplitudes revealed that high reactors in both the saline and nicotine groups were chiefly responsible for the differences in sensory gating with nicotine or stress, and that initial level of reactivity is an important determinant of the effects of nicotine and stress (see Figure 9). That is, sensory gating in low reactors was less affected by drug treatment or stress treatment than was gating in high reactors. Further, under conditions of increased reactivity or amplitude attributable to high stimulus intensity, differences in treatment groups according to drug or stress group were not evident.

Habituation

Habituation was measured using 10 trials consisting of 112 dB stimuli and a fixed interstimulus interval. Decreases

in reactivity over the ten stimulus presentation was hypothesized to index changes in vigilance or sustained attention. Although there was a significant effect of time, indicating decreases in response amplitude consistent with habituation, there were no differences according to drug or stress, and no apparent trends. Analysis by median split did not result in significant differences, and Hypotheses 3, 8, and 9 were not confirmed. Results were inconsistent with Stern (1971) who reported that stress increased habituation in rats, but were consistent with Overstreet (1977) who postulated the cholinergic system was not involved in habituation. Results suggest that the present stress and drug treatment conditions do not change habituation or stimulus sensitivity as measured in this paradigm.

Summary

Results of this experiment suggest that nicotine does counteract some of the effects of stress that increase reactivity (startle amplitude) and sensory gating (PPI). Further, a close examination of the dose effect patterns of both nicotine and stress suggest that stress may shift the inverted U shaped function describing effects of nicotine to the left. This interaction may be the mechanism through which cigarette smokers experience a calming effect of nicotine.

EXPERIMENT 2

Overview

This experiment was designed to examine the interactions of stress and nicotine cessation on amplitude, pre-pulse inhibition, and habituation of the ASR in male Sprague-Dawley rats. This experiment tested the hypothesis that nicotine and stress interact in their effects on aspects of startle behavior that can be modulated by both nicotine and stress, and that nicotine cessation would exacerbate effects of stress on startle. It was hypothesized that the mechanisms through which nicotine cessation affects startle and stress-induced changes in startle behavior involve attention, sensory gating, and habituation that are reflected in startle behavior. The experiment examined effects of nicotine cessation and stress following 11 days of chronic nicotine administration.

Subjects were 32 male Sprague-Dawley rats. Each rat received one of two doses of nicotine (0 or 12 mg/kg/day) administered by osmotic minipump. Animals were tested for startle amplitude prior to and during drug administration. Minipumps were explanted on day 12 of drug administration to insure nicotine cessation. On day 1 of nicotine cessation, animals received one of two levels of stress (see Table 3) immediately prior to startle testing (see Table 4 for timeline). Animals from the stress group were restrained in a finger-like restraint device for a period of 15-20 minutes, and animals from the no stress control group remained in home cages in the colony room. Each animal was transported to the startle chamber within five minutes after the termination of stress, and was tested for startle amplitude, PPI, and habituation for a period of 22 minutes. Four animals were tested simultaneously. Within five minutes after the termination of startle testing, each animal was sacrificed for blood collection and later measurement of plasma corticosterone to validate the effectiveness of the stressor.

Methods

Subjects and Housing

Subjects were 32 male Sprague-Dawley rats (225-250g) obtained from the Charles River Laboratories. Animals were individually housed in standard polypropylene cages (35.56 cm x 15.24 cm x 20.32 cm) with absorbent Pine-Dri bedding and metal grill lids. Animals were maintained in a room with a 12 hr light/dark cycle (dark 7 pm to 7 am) at approximately 72 degrees F and 50% humidity. Standard pellet rat chow and water were continuously available, and cages were changed twice weekly.

An animal model was chosen for this experiment because of ethical issues involved in administering nicotine on a chronic basis to nonsmoking humans. Rats were chosen as the appropriate species for the current study because rats have been used extensively in nicotine, stress, and startle experiments and much related data are already available. Sprague-Dawley rats were selected because they are neither the most, nor the least responsive rat strain in previous startle experiments (Acri, Saah, & Grunberg, unpublished data) and, therefore, were likely to provide the greatest generalizability. Male rats were used because there was no a priori evidence of gender differences in cognitive effects of nicotine and the use of both male and females would have doubled the size and costs of the experiment.

Drug administration

Nicotine or saline was administered using Alzet mini osmotic pumps (Model 2002, Alza Corp., CA). Each minipump was filled with a saline or nicotine solution made from nicotine dihydrochloride administered at a rate of approximately 0.5 µl/hr (Theeuwes & Yum, 1977). Doses were calculated based on body weight such that each animal

received a dose of either 12 mg/kg/day or 0 mg/kg/day. Minipumps were used to maintain a constant rate of nicotine administration without the trauma of repeated injections. Pumps were implanted (and explanted twelve days later) under sterile conditions using methoxyflurane anesthesia. These drug dosages were selected based on previous studies in which behavioral effects in rats approximate those of humans (Grunberg et al., 1984).

Stress

Animals were restrained in commercially available finger-like restraint cages (Centrap Cage, Fisher Scientific) for a period of 15-20 minutes. Upon the animal's entry into the restraining cage, the "fingers" were tightened to restrict the animals' movements but were not tightened enough to induce pinching or pain. Restraint stress has been used in previous studies and has been shown to produce elevations in ACTH, beta-endorphin, and corticosterone (Flores et al., 1990), increased plasma renin, prolactin levels, and corticosterone (Paris, Lorens, Van de Kar, Urban, Richardson-Mortin, & Bethea, 1987) as well as serotonin and NE in the brain (Adell, Garcia-Marquez, Armario, & Gelpi, 1988) consistent with a stress response. Restraint stress has been reported to increase the amplitude of sensory evoked potentials in rats (Casada & Dafny, 1990) and has increased the amplitude of ASR in a pilot study (Acri & Grunberg, unpublished data). Control non-stressed animals remained in home cages in the colony room during this time.

Acoustic Startle Reflex Testing

Acoustic startle reflex amplitudes, pre-pulse inhibition, and habituation were measured in a Coulbourn Instruments Acoustic Response Test System. Each rat was individually placed in a 8 x 8 x 16 cm open air cage that

restricted locomotion but did not immobilize or restrain the movements of the animal, and was placed on one of four platforms within a sound-attenuating chamber. Platforms were arranged radially around central speakers in the floor and ceiling of the chamber. A ventilating fan provided an ambient noise level of 50 dB SPL. All stimulus intensity levels are described in terms of sound pressure level or SPL (re: 0.0002 dynes/cm^2), meaning that an unweighted measurement scale was used. There was a 3 minute quiet adaptation period following the placement of four animals within the chamber.

Startle stimuli consisted of 98, 112, or 122 dB SPL noise bursts sometimes preceded 100 ms by 68 dB, 1 kHz pre-pulses. These stimulus intensity levels were verified with a GenRad Type 1982 sound level meter with microphone placement in the position of a subject's head. There were six types of stimulus trials and two types of control trials. There were 8 trials of a 98 dB noise burst, 8 trials of a 98 dB noise burst preceded by a 1 kHz, 68 dB pre-pulse, 8 trials of a 112 dB noise burst, 8 trials of a 112 noise bursts preceded by a 1 kHz pre-pulse, 8 trials of a 122 dB noise burst, 8 trials of a 122 noise burst preceded by a 68 dB 1 kHz pre-pulse, 4 pre-pulse alone control trials, and 4 no-stimulus control trials. There were a total of 56 of these trials, with each stimulus trial type presented once every seven trials in a block randomized fashion. Each stimulus had a 2 ms rise-fall time such that onset and offset were abrupt. Sudden onset of an intense stimulus is a primary criterion for elicitation of startle. Interstimulus intervals ranged from 10-39 seconds, and were varied randomly. Habituation also was measured using 112 dB SPL noise bursts at a fixed interstimulus interval of 10 seconds at the end of the session. Ten habituation trials were presented, bringing the total number of trials to 66. No-stimulus trials and pre-pulse alone trials were presented as controls for random movement and to insure that pre-pulses did not elicit startle responses.

Following presentation of each stimulus, movement on each platform was measured for a period of 200 ms by coupling through a sensor pin connected to a strain gauge. Four platforms were simultaneously measured by an interfaced microcomputer that controlled both the stimulus presentation schedule and measurement of responses. Changes in voltage underwent analog to digital conversion, and were fed into calibration equations derived for each platform to equate voltage to grams of weight. Amplitudes were recorded as the maximum response occurring within 200 ms following stimulus presentation, and were calculated as grams of weight change. Responses were averaged within trial types which were presented in block-randomized order, as described above. Each animal's response to each stimulus, therefore, represents an average of 8 stimulus presentations. Pre-pulse inhibition, measured as amount or percent, is the difference in amplitude between trials involving a startle stimulus alone and a startle stimulus preceded by a pre-pulse. These parameters have been used effectively in previous studies of nicotine (Acri et al., 1991; 1992) and strain (Acri, Saah, & Grunberg, unpublished data) effects.

Rats were tested for startle behavior four at a time. Stressed animals were not run prior to, or simultaneously with, unstressed animals to avoid the influence of alarm substances. Each animal was tested during the beginning of the activity cycle because there is evidence that startle amplitudes are more stable at that time (Davis & Sollberger, 1971).

Stress Index

The stressfulness of the procedure was evaluated by measurement of plasma corticosterone (CCS) in all groups. Blood was collected within 5 minutes after the termination of startle testing, and within 30 minutes after the termination of stress. Plasma corticosterone is an indicator of adrenal

cortical activity and increases following restraint stress (Paris et al., 1987; Flores et al., 1990) as well as nicotine administration (Morse, 1989; Newhouse et al., 1990). Corticosterone has a longer half life than catecholamines and is less sensitive to rapid environmental changes (Baum et al., 1982). It is, therefore, more likely to show effects of prior stress during the previous hour, rather than the more immediate effects of decapitation for blood collection. Rats were sacrificed by decapitation, and trunk blood was collected in 14 ml polypropylene tubes containing 50 µl of 10,000 IU/ml heparin and centrifuged. The resulting plasma was frozen in a -70 degree C freezer for later corticosterone assay. Assays were performed on thawed plasma using ^{125}I-labeled corticosterone and a specific anti-corticosterone antiserum to determine the corticosterone concentrations in specimens using the double antibody technique (Rattner, Gruenau, & Altland, 1979). Reagents for this radioimmunoassay were purchased from ICN Biomedicals, Costa Mesa, CA.

Procedure

Experiment 2 was run after Experiment 1. All animals in Experiment 2 were run on the same day. Experimental "days," as described below, are relative to start day for and the sequence of events within the experiment was the same for all animals. Careful attention was given to time-of-day consistency in procedures. All handling and surgical procedures occurred in the last 4 hours of the animal's sleep cycle (light), and all startling and stressing occurred in the last hour of the sleep cycle (light) and the first 2 hours of the activity cycle (dark).

Rats were gentled by handling daily during the baseline period. Each animal was weighed three times during the baseline period, and baseline startle amplitudes were obtained on days 2, 5, and 8 relative to start day. Animals

were quasi-randomly assigned to drug groups according to baseline startle amplitude. Subjects from each drug group were then further divided in a quasi-random fashion into stress conditions, again with regard to baseline startle amplitude. The last of the three baseline startle testing days was used for this purpose. There were 4 groups (2 x 2) with 8 animals per group, for a total 32 animals. See Table 3 for experimental design.

Three days following baseline procedures, each animal was anesthetized with methoxyflurane and a minipump containing the appropriate drug solution was implanted subcutaneously. An incision of approximately 3 cm was made between the shoulder blades and a pump was implanted under the skin. The incision was closed with 9 mm stainless steel wound clips. Following surgery, each animal was returned to the home cage.

Startle testing was performed on drug administration days 2, 5, and 8; that is, 2, 5, and 8 days after implantation (see Table 4 for timeline). On day 12, animals were anesthetized and pumps were explanted to insure drug cessation. All surgical procedures were identical to those described for implantation, except that pumps were removed. Twenty-four hours later, or on day 1 of drug cessation, animals were stressed, startled, and sacrificed. Unstressed animals were run first, to control for odors and alarm substances. For the no-stress condition, four animals at a time were transferred to the startle treatment room, startled, and following the end of the startle testing period, were transferred to the necropsy room and decapitated within 5 minutes. Trunk blood was collected for later assay. Following startling of all animals from the no-stress control condition, stressed animals were run. Four animals to be restrained were taken to a treatment room and were placed in restrainers for 15-20 minutes. Animals were then transferred to startle cages and were placed in the startle chamber within 5 minutes. Following the startle testing period of 22

minutes, animals were transfered to the necropsy room and decapitated within 5 minutes. Trunk blood was collected for later assay.

Statistical Analysis

An initial Multivariate Analysis of Variance (MANOVA) was performed on the nine dependent measures from day 1 of drug cessation. Dependent measures included startle amplitude (mean peak response - mean average no stimulus response), amplitude of pre-pulse inhibition trials (mean peak response of PPI trials - mean average no stimulus response), and amount of inhibition ([mean peak response - mean average no stimulus response]- [mean peak response of PPI trials - mean average no stimulus response]). The MANOVA included drug (saline or 12 mg/kg/day nicotine) and stress (no stress or restraint) as between-subject factors, and stimulus intensity (98, 112, or 122 dB) and type (with or without pre-pulse) as within-subject factors. Following this analysis, 2 x 2 factorial ANOVAs were used to examine effects of nicotine and stress on each individual dependent measure from cessation day 1. Dependent measures included startle amplitude, amplitude of PPI trials, and amount of pre-pulse inhibition. Percent of pre-pulse inhibition ([mean peak response - mean average no stimulus response]- [mean peak response of PPI trials - mean average no stimulus response])/ [mean peak response - mean average no stimulus response] was not included in the MANOVA because it was statistically related to the other measures, but was analyzed by separate ANOVA.

Habituation data were analyzed using repeated measures ANOVA to determine within-session habituation. This analysis was a repeated measures analysis of changes in response to 10 habituation trials, using between-subject factors of drug cessation and stress.

Group size of 8 animals per group was based on previous studies of this type in which this group size was sufficient to yield significant differences in response to drug (Acri et al., 1991). A significance level of 0.05 was used for all statistical tests.

Post hoc t-tests were used to determine which groups differed when significant differences were found on ANOVAs. Fisher PLSD tests were used, where appropriate.

A post hoc analysis of nicotine effects was performed for measures using the 112 dB stimulus to determine if nicotine cessation effects were dependent upon initial level of arousal. Separate ANOVAs following median-split of baseline startle amplitudes within the nicotine and saline cessation groups were run for the principle measures of amplitude, pre-pulse inhibition, and habituation. This analysis was done to determine if more reactive animals responded differently to stress, nicotine cessation, or the combination.

Results

Overview

An overall multivariate analysis of variance (MANOVA) was conducted, in which drug (saline or 12 mg/kg/day nicotine) and stress (no stress or restraint) were between-subject factors, and amplitude (98 dB, 112 dB or 122 dB) and type (with or without 68 dB 1 kHz pre-pulse) were within-subject variables in an analysis of differences in response amplitude, or grams of weight change. Amplitude data for stimuli of 98 dB, 112 dB, and 122 dB were then analyzed in separate analyses of variance followed by post hoc tests when significant main effects or interactions were found.

Pre-pulse inhibition data were reported in three ways. First, the amplitude of trials in which a pre-pulse precedes the startle-eliciting stimulus was reported. Second, the

amount of inhibition was derived by subtraction of pre-pulse trial amplitudes from amplitudes of trials using the same startle-eliciting stimulus without a pre-pulse, or the amount of the response that was inhibited. Third, the percent of inhibition was derived by dividing the amount of inhibition by the amplitude of non pre-pulse trials of the same stimulus to determine the percent of the response that was inhibited.

Habituation trials consisted of 10 presentations of 112 dB separated by a fixed inter-stimulus interval of 10 seconds. These results were analyzed by repeated measures analysis of variance.

All results were based on a two-tailed test of significance. The alpha level for all analyses was set at 0.05.

Stress Validation

Plasma Corticosterone Levels

To determine the effectiveness of restraint as a stressor, plasma corticosterone (CCS) levels were measured 30-35 minutes following exposure to restraint. Figure 10 presents the mean plasma CCS concentration for each stress and drug treatment-condition. Restraint stress resulted in CCS levels that were significantly higher for animals in the stress condition than for those in the no stress condition. This main effect for stress was significant [F (1,28) = 21.16, $p < 0.05$]. There were no significant main effects for drug condition, and there were no significant interactions with stress and drug. Post hoc tests reveal that both saline- and nicotine-cessation groups that were stressed had significantly higher levels of plasma corticosterone than both saline- and nicotine-cessation groups that were not stressed (Fisher PLSD = 153.83, $p < 0.05$).

Multivariate Analysis

The multivariate analysis of variance (MANOVA) was conducted, in which drug (saline or 12 mg/kg/day nicotine) and stress (no stress or restraint) were between-subject factors, and stimulus intensity (98 dB, 112, dB or 122 dB) and type (with or without 68 dB 1 kHz pre-pulse) were within-subject variables in an analysis of differences in response amplitude, or grams of weight change. This analysis was done to insure that significant findings on any univariate analysis were not the result of chance, due to the number of analyses conducted. Both reactivity (amplitude) and gating (PPI) trials were included in this analysis.

There was a significant main effect of stimulus intensity [Hotellings F (2,27) = 4.50, $p < 0.05$], and post hoc analysis of the effect of intensity by planned contrasts indicated that responses to the three stimulus intensities (98 dB vs 112 dB, and 112 dB vs 122 dB) were significantly different [F (1,28) = 125.64, $p < 0.05$; F (1,28) = 110.09, $p < 0.05$], with greater amplitude responses to more intense stimuli as previously reported (Acri et al., 1991). There was also a main effect of trial type [Hotellings F (2,27) = 4.48, $p < 0.05$], with significant response inhibition for trials in which the pre-pulse was present, as extensively reported in the literature. There was a significant drug by stress by trial type interaction [F (1,28) = 4.91, $p < 0.05$], and a significant drug by stress by intensity by type interaction [F (2,56) = 3.51, $p < 0.05$]. In order to examine these differences, univariate tests were then conducted to examine these significant interactions and effects of stress and drug cessation in relation to hypotheses.

Reactivity (Amplitude)

Reactivity to external stimulation is reflected in the amplitude of the acoustic startle response. Acoustic startle

amplitude was measured using 98 dB, 112 dB, and 122 dB noise stimuli. Using these stimuli, there were no significant differences by drug cessation or stress condition for any of the stimuli. Table 19 presents responses of subjects from the two drug and stress conditions to each of these stimuli. Although there were no significant effects, closer examination of responses to the 112 dB stimulus are warranted.

In the previous experiment, the 112 dB stimulus was the most effective of the three stimulus intensities in eliciting reflex amplitudes that were modulated by nicotine and stress, and in reflecting the nicotine-stress interaction during nicotine administration. Although the effects are not statistically significant during nicotine cessation, all nicotine cessation effects for the 112 dB stimulus are in a direction opposite to those seen during nicotine administration, and the same interaction pattern observed during nicotine administration is evident (see Figure 11). For no stress conditions, animals in the nicotine cessation condition have lower amplitudes than saline controls, and with restraint stress, the pattern reverses so that restrained animals in the nicotine cessation condition have higher amplitudes than restrained saline cessation controls. The same trend is evident to a slightly lesser degree for the 122 dB stimulus. These trends are consistent with Hypothesis 10, which predicted that nicotine cessation would not affect startle amplitude. However, it was predicted in Hypothesis 13 that stress would increase startle relative to no stress, which it did not do for saline- or nicotine-cessation animals. It is not known why stress did not increase startle amplitude in this experiment.

An additional analysis of responses to the 112 dB stimulus level following a median split of baseline amplitudes was done to determine if individual differences in baseline reactivity are an important determinant of nicotine cessation and stress effects. To clarify, this analysis was

done in order to examine the differential effects of baseline reactivity rather than to remove baseline effects. Each treatment group was divided into high and low reactors, according to baseline amplitudes (M = 30 grams), and a 3-way ANOVA was conducted (stress x drug cessation x high/low reactivity). In this analysis, there was a significant effect of stress [F (1,24) = 4.47, $p < 0.05$], a significant effect of baseline reactivity [F (1,24) = 7.27, $p < 0.05$], and a significant stress x reactivity interaction [F (1,24) = 7.85, $p < 0.05$]. These results are presented in Figure 12, in which it is clear that stress reduces startle amplitude only in the high reactors for both nicotine and saline cessation. Differences are significant for the saline cessation high reactors. There is a trend for nicotine cessation to reduce the amplitude of startle in nicotine cessation group as compared to the saline cessation group, but the trend is evident only for the high reactors.

As in Experiment 1, the median split analysis accentuates the differences among nicotine- and saline-treated animals, and indicates that it is the high reactors in both categories that are responsible for these differences. Results again confirm Hypothesis 10, that nicotine cessation would have no effect on startle amplitude although there is a trend for reduced reactivity (amplitude). Hypothesis 13, stating that stress would increase reactivity was not confirmed, in that stress clearly reduced amplitudes in all drug cessation groups with a significant main effect, although there was much greater reduction in the high reactors and a slight increase in the low reactors. Interestingly, high reactors in the saline cessation group decreased startle amplitude with stress, whereas high reactors in the saline administration group in Experiment 1 increased amplitudes with stress. It is not known what effects of saline cessation were responsible for differences between saline administration and saline cessation. Nicotine cessation resulted in the same direction of effects for high

reactors as did nicotine administration in Experiment 1, and again, it is not known why responses of animals in nicotine-cessation would resemble those of animals during nicotine administration. Compare Figure 12 with Figure 4 from Experiment 1.

In summary, there were no significant effects of nicotine cessation or stress on reactivity or amplitude in this experiment. However, when analyzed with reference to baseline reactivity, stress reduced amplitude in both saline- and nicotine-cessation groups that were high reactors. These results are contrary to expectations based on hypotheses and literature, and an obvious interpretation is not evident. Possible explanations are presented in the discussion section.

Sensory Gating (Pre-pulse Inhibition)

Sensory gating, or the screening out of irrelevant stimuli, is a process related to selective attention. Pre-pulse inhibition of the startle reflex, or the amplitude reduction that occurs when a non-startling sound precedes the startle-eliciting stimulus, is thought to reflect this attentional process as previously described. MANOVA results presented earlier indicated significant effects of trial type, indicating that significant pre-pulse inhibition occurred for all stimulus intensity levels. Pre-pulse inhibition was analyzed in several ways in this experiment, using amplitudes of pre-pulse trials, amount, and percent of inhibition as variables. Each stimulus intensity was examined separately.

Using a 98 dB startle-eliciting stimulus preceded by a 1 kHz pre-pulse of 68 dB, amplitudes were measured as shown in Table 20. There were no significant differences in amplitudes of pre-pulse trials for 98 dB, and no apparent trends. There also were no significant differences in amount or percent of inhibition for this stimulus (Tables 21 and 22,

respectively). One slight trend is a reduction in both amount and percent of inhibition for animals in the nicotine cessation condition as compared to saline, as predicted by Hypothesis 11, but overall results obtained with this stimulus suggest that either nicotine cessation and stress have no effect on PPI, or that this stimulus level was not optimal for detection of stress and cessation effects (see Figure 13).

Using a 112 dB startle-eliciting stimulus preceded by a 1 kHz 68 dB pre-pulse, amplitudes were measured as shown in Table 20. There was a significant effect of stress [F (1,28) = 7.07, $p < 0.05$], with stressed animals having consistently lower amplitudes than non-stressed animals (see Figure 14). Post hoc analyses indicated that the saline no stress group differed significantly from the nicotine-cessation restrained group (Fisher PLSD = 4.75, $p < 0.05$). There were no significant effects of drug cessation or interactions, although the slight reduction in PPI for the nicotine cessation animals was again evident in both stress conditions. For amount and percent of inhibition using the 112 dB stimulus with pre-pulse, there were no significant main effects for drug or stress, and there were no significant interactions. Tables 21 and 22 include these results. Slight reductions in amount and percent of inhibition are seen in the no stress nicotine cessation animals, and the pattern appears to reverse with restraint stress (see Figure 15 for amount of inhibition). As with the 98 dB stimulus, nicotine cessation appears to slightly reduce PPI compared to saline cessation as predicted by Hypothesis 11, but results with restraint stress were not as predicted. It is not known why nicotine cessation and restraint resulted in more PPI than saline cessation and restraint.

Using the 122 dB startle stimulus with a 68 dB 1 kHz pre-pulse, there were no significant main effects for stress or drug cessation, and there were no interactions. However, when analyzed as amount of inhibition, there was a

significant drug cessation x stress interaction [F (1,28) = 4.74, $p < 0.05$]. Post hoc comparisons revealed that the saline no stress and saline restrained groups differed (Fisher PLSD = 48.8, $p < 0.05$), with more PPI in the saline no stress group. These results are presented in Figure 16, and means are included in Table 21. These results are consistent with predictions of Hypothesis 14 for the saline animals, in that there was significantly less sensory gating or PPI following stress. However, it was predicted that nicotine cessation would intensify the stress effect by further reducing PPI, but this did not happen. There were no significant main effects of drug or stress. When analyzed as percent of inhibition, the same trends are seen without significant effects for drug, stress or interaction. The trend of decreased PPI for the nicotine no stress animals as compared to saline was again consistent with Hypothesis 11. These means are included in Table 22.

Results obtained using the 112 dB stimulus with pre-pulses also were analyzed following a median split of baseline response amplitudes to determine if initial reactivity is an important determinant of effects of drug cessation and stress on sensory gating. For amplitude of pre-pulse trials, there was a significant effect of stress [F (1,24) = 7.06, $p < 0.05$] and these results are presented in Figure 17, in which it is clear that restraint decreased amplitude of pre-pulse trials overall, and significantly for the nicotine-cessation high reactors. For amount of inhibition, there was a significant effect of initial reactivity [F (1,24) = 9.07, $p < 0.05$] and a significant stress x initial reactivity interaction [F (1,24) = 6.82, $p < 0.05$]. These results are presented in Figure 18, in which high reactors show more sensory gating or PPI without stress, but with restraint stress, PPI is reduced in high reactors and increased slightly in low reactors. With no stress, both high and low reactors in the nicotine cessation groups show less PPI than saline high reactors. When analyzed as percent

of inhibition, there was a significant effect of initial reactivity [F (1,24) = 7.71, $p < 0.05$], and the same pattern as in amount of inhibition is present. That is, high reactors show decreased percent of PPI with stress, and low reactors show increased percent of PPI with stress, slightly more so for nicotine-cessation as compared to saline cessation. It is interesting to note that as compared to Experiment 1, nicotine cessation high reactors show stress effects in the same direction as nicotine-administration high reactors in terms of amount of inhibition. However, saline cessation high reactors show opposite effects of stress on amount of inhibition as compared to saline administration. It is not known why animals in saline cessation would react differently to restraint stress as compared to animals in the saline administration group (cf. Figures 18 and 9).

Habituation

Habituation data were subjected to a repeated-measures analysis of variance with time as a within-subject factor and both stress and drug as between-subject factors. There were no significant effects of time, although this measure approached significance [F (9,252) = 1.86, $p < 0.058$], meaning that all groups habituated somewhat to the ten habituation trials of 112 dB presented at a fixed inter-stimulus interval. Results are inconsistent with predictions of Hypothesis 15, in that there were no significant stress or drug effects on habituation.

These data were also analyzed following median split of baseline amplitudes, and there were no significant effects of drug, stress, initial reactivity, or time, and there were no significant interactions.

In summary, there were no effects of nicotine cessation or stress on habituation using the acoustic startle reflex. This finding suggests that reflex habituation as measured in this paradigm is not sensitive to the previously

reported effects of nicotine on vigilance, sustained attention, and concentration.

Confirmation of Hypotheses

Hypothesis 10. Nicotine cessation had no effect on startle amplitude as predicted, confirming this hypothesis.

Hypothesis 11. Nicotine cessation decreased pre-pulse relative to saline, consistent with this hypothesis although the trend was not significant.

Hypothesis 12. Nicotine cessation did not increase habituation relative to saline as predicted, disconfirming this hypothesis.

Hypothesis 13. Stress did not interact with nicotine cessation to produce greater amplitude increases than nicotine cessation or stress alone, so this hypothesis was not confirmed.

Hypothesis 14. Stress did not decrease PPI during nicotine cessation except for a non-significant trend for the high reactors, disconfirming this hypothesis.

Hypothesis 15. Stress did not increase PPI during nicotine cessation, and this hypothesis was not confirmed.

Discussion

In Experiment 2, measures of reactivity, gating, and habituation were taken 24 hours after explantation of minipumps containing 12 mg/kg/day nicotine or saline, and it was reasonable to believe that this timing was appropriate to obtain drug cessation effects. Previous studies found no significant withdrawal effect for nicotine at 1, 2, and 3

days post explant (Acri et al., 1991); however, it was hypothesized that exposure to stress would reveal cessation effects that would not be obvious in the absence of stress, as in Morrison (1974). Morrison (1974) used a signalled and unsignalled shock avoidance tasks with rats and reported that nicotine withdrawal only disrupted performance in the more stressful, unsignalled shock avoidance task as compared to saline controls.

Reactivity (Amplitude)

It was hypothesized that nicotine cessation effects compared with saline cessation would be opposite in direction to nicotine administration effects compared to saline administration. (All comparisons are in reference with the saline control group for the same experiment.) While administration of nicotine in Experiment 1 resulted in increased amplitude without stress and decreased amplitude with stress, in contrast, nicotine cessation in Experiment 2 resulted in slightly lower amplitude without stress, and slightly increased amplitude with stress as predicted in Hypothesis 13. Taken at face value, this finding could be interpreted as evidence of a cessation effect. It is not interpreted as a rebound or return to baseline, because this experiment used a between- rather than a within-subject design.

As predicted, these effects were opposite in direction to nicotine administration effects, consistent with opponent process theories (Solomon, 1977; Himmelsbach, 1943; Koob & Bloom, 1988). That is, if nicotine enhanced amplitude during administration, amplitude should decrease during cessation. This change in amplitude or reactivity may reflect some of the same processes that make human smokers restless, irritable, anxious, or impatient in withdrawal (Hughes and Hatsukami, 1992; Hughes et al., 1984b; Hughes & Hatsukami,

1986; Hatsukami et al., 1988; APA, 1987; West & Russell, 1988).

From analysis of amplitude data in Experiment 2 by median split to determine the influence of individual differences in baseline reactivity on drug cessation and stress, it was again evident, as in Experiment 1, that most of the difference in amplitude between saline- and nicotine-cessation groups could be accounted for by high reactors. Low reactors varied little as to drug treatment or stress condition, whereas high reactors in both drug cessation conditions consistently were less reactive following restraint. There was slightly less amplitude reduction following restraint in the nicotine-cessation condition. Although a clear and significant nicotine cessation effect was not measured with or without stress, in high or low reactors, these results again highlight the importance of individual differences in assessment of stress and drug cessation effects. Animals classified as high or low reactors may be functioning at different points on the inverted U shaped function for arousal. High reactors may be near the apex of the function and decrease amplitude with stress, whereas low reactors may be on the rising arm of the inverted U, and therefore increase in response to stress.

These differences in response to drug cessation are roughly opposite of those seen during administration, and highlight the importance of individual difference variables in reactivity as a determinant of stress and drug effects. This initial reactivity difference variable may provide a partial explanation of the differences experienced by human smokers when they quit. That is, some smokers report few, or mild withdrawal effects, whereas others report a conglomerate of severe symptoms that include drowsiness, irritability, difficulty concentrating, etc. (Hughes and Hatsukami, 1992; Hughes et al., 1984b; Hughes & Hatsukami, 1986; Hatsukami et al., 1988; APA, 1987; West & Russell, 1988).

Sensory Gating (Pre-pulse Inhibition)

For animals in drug cessation, there were significant effects of stress that decreased pre-pulse inhibition or sensory gating in saline-cessation animals, and slightly increased or did not change inhibition in nicotine-cessation animals. Without stress, nicotine cessation, as compared to saline cessation, resulted in slightly decreased PPI, as predicted by Hypothesis 11. However, Hypothesis 14 predicted that stress would decrease PPI for both drug cessation conditions with nicotine cessation effects greater than saline-cessation effects, and this hypothesis was not confirmed. In contrast, PPI was slightly increased by stress for animals in nicotine cessation, but it was decreased by stress for animals in saline cessation. The magnitude of the effect was small, but the interaction was significant for the 122 dB stimulus.

For the saline group, these effects of stress on sensory gating are consistent with those reported in the literature of decreased PPI in humans (Ornitz & Pynoos, 1989) or in rats exposed to swim stress (Leitner, 1989). However, it was not predicted, and it is not clear why nicotine cessation as compared to saline cessation would increase PPI in stressed animals. When analyzed following median split, it is evident that without stress, individual differences in baseline reactivity account for much of the difference in drug cessation conditions with high reactors showing significant differences in the no stress condition. However, with stress, all low reactors increase amount of PPI slightly as compared to no stress, and all high reactors decrease slightly as compared to no stress, and the interaction of stress and baseline reactivity was significant. So, on day 1 of drug cessation, individual differences in reactivity were a more important determinant of stress effects than was nicotine.

Both high reactors in saline and nicotine cessation had decreased PPI with stress. High reactors in the nicotine cessation group were affected by stress in a pattern similar to animals during nicotine administration in Experiment 1, as in amplitude findings. However, the high reactors in the saline-cessation group responded to restraint stress in a pattern that was opposite of the pattern during drug administration in Experiment 1. That is, amount of PPI or sensory gating in the saline high reactors diminishes with stress as predicted in this experiment, whereas in Experiment 1, PPI increased with increasing stress for saline-treated animals. The predicted rebound, or withdrawal effect that is opposite in direction to that of drug administration did not occur following stress for the nicotine-cessation animals, but was evident in the absence of stress. It could be that a cessation effect was masked rather than enhanced by the intensity of the stressor.

Habituation

Habituation was measured using 10 trials consisting of 112 dB stimuli and a fixed interstimulus interval. Decreases in reactivity over the ten stimulus presentation was used to index changes in vigilance or sustained attention. Although there was a significant effect of time, indicating decreases in response amplitude consistent with habituation, there were no differences according to drug or stress, and no apparent trends. Analysis by median split did not result in significant differences. Hypotheses 12 and 15 were not confirmed, and results suggest that the present stress and drug cessation treatment conditions do not modulate habituation as measured in this paradigm.

Summary

Results of this experiment suggest that nicotine cessation effects on startle behavior are subtle and may be masked by intense stress. Further, effects of stress in saline-cessation animals were to decrease amplitude and gating (PPI), whereas effects of stress in saline administration animals were to increase amplitude and gating. Because all nicotine comparisons were to the appropriate saline control group and because it was not anticipated that saline cessation effects would differ from saline administration effects, results of Experiment 2 are equivocal. However, it appears that nicotine cessation effects are in roughly the opposite direction as administration effects.

GENERAL DISCUSSION

These experiments addressed the possibility of interactions of stress and nicotine on aspects of the acoustic startle reflex, namely reactivity (amplitude), sensory gating (pre-pulse inhibition), and habituation that may be relevant to the reported stress-reducing effects of nicotine administration by cigarette smoking in humans. Experiment 1 examined these effects during day 11 of nicotine administration, and used three levels of drug (saline, 6 mg/kg/day nicotine, and 12 mg/kg/day nicotine) and three levels of stress (no stress, observation of restraint, and restraint stress). Experiment 2 examined effects on day 1 of cessation following 11 days of administration, and used two levels of drug (saline and 12 mg/kg/day nicotine) and two levels of stress (no stress and restraint).

These studies confirmed that nicotine is capable of counteracting stress effects on reactivity and sensory gating, although the direction of stress effects on gating or PPI were not as predicted. Although a higher dose of nicotine increases reactivity in non-stressed subjects, it prevents increases in reactivity or amplitude associated with stress, such that nicotine treated, stressed subjects resemble saline controls. Similarly, while nicotine enhances sensory gating in the absence of stress, it prevents increases in gating associated with stress. This effect may be related to the stress reducing effects of nicotine reported by human smokers.

Nicotine cessation effects as compared to saline were generally in the opposite direction from those observed during nicotine administration. However, this effect may have occurred not because of opponent processes of nicotine administration, but may have been the result of comparisons of nicotine cessation with saline cessation. Unexpectedly, animals in the saline cessation group reacted to stress in a

manner opposite that of animals administered saline, which influenced all of the nicotine cessation vs. saline cessation comparisons. Why this effect occurred is unknown. It may have been an unanticipated result of explant surgery and anesthesia only 24 hours earlier, but these experimental effects should have been identical in all drug treatment conditions. If viewed without reference to saline, stressed animals in nicotine cessation were slightly less reactive as compared with no stress, as they were during nicotine administration. However, stress slightly increased PPI for animals in nicotine cessation, as opposed to decreasing it, so it is not likely that the results can be interpreted as lasting effects of drug. Therefore, cessation effects of nicotine and stress are equivocal when using a between-subject design.

Dose-response effects of nicotine administration with restraint stress were generally not observed, as predicted by Hypothesis 5. The 6 mg/kg/day nicotine dose increased amplitude and PPI, as compared to saline-treated subjects, rather than decreasing amplitude and PPI as in the interaction pattern of the 12 mg/kg/day nicotine dose. That is, stress and 6 mg/kg/day nicotine may have had an additive effect to increase amplitude and PPI, whereas the 12 mg/kg/day dose did not (see Figures 2 and 7). The reasons for the lack of dose-response effects may be related to the fact that nicotine dose effects show an inverted U shaped function in terms of CNS activation, with low doses resulting in stimulation, and high doses in depression (Armitage et al., 1968). Whereas the 12 mg/kg/day nicotine dose is usually not high enough to result in depression of amplitude or gating (PPI) (see Figures 2 and 7, no stress condition), manipulation of stress levels and concomitant sympathetic activation may have shifted the nicotine dose-response curve to the left, such that highly stressed animals receiving 12 mg/kg/day nicotine did not show the changes in amplitude or gating that are associated with stress, whereas the subjects

receiving either lower doses of nicotine or a lesser dose of stress did show these effects. This interaction, or shift, may help account for differences in stress-reducing effects reported in the literature, in which smoking sometimes reduces stress (Woodson et al., 1986; Nesbitt, 1973; Silverstein, 1982) and sometimes has no effect (Hatch et al., 1983; Gilbert & Hagen, 1980; Jarvik et al., 1989).

Another factor that may have contributed to arousal and further shifted the dose-response curves of both stress and nicotine to the left is stimulus intensity. In Figure 3, it is evident with the 122 stimulus intensity level, maximal responses were observed in the saline observer group, whereas increases in nicotine dose, or stress, or both resulted in predictable decreases, until the 12 mg/kg/day nicotine restrained animals showed amplitudes similar to the saline no stress group. In a previous experiment, amplitudes measured using a similar stimulus intensity level were reported to show ceiling effects for the 12 mg/kg/day nicotine dose without stress (Acri et al., 1991); however, if ceiling effects were the only operational factor preventing increased amplitude in this experiment, the clear, inverted U shape in Figure 3 would show a plateau, rather than a descending pattern with increased stress. This pattern again suggests that increased arousal caused by stress or stimulus intensity can shift the dose effect curve of nicotine to the left.

This experiment validated the usefulness of the observation stressor as a stressful experience that was less intense than restraint itself. The observation stressor occurred when a rat was in close proximity to a restrained rat. Restrained rats typically urinate, defecate, and occasionally emit audible sounds. Based on the literature, it is presumed that some alarm substance is also emitted from the restrained animal, but the channel of communication whether visual, auditory, olfactory, or through some as yet undetermined channel is not known. The mechanism through which this communication induces a stress response in the

observing animal is also unknown. However, corticosterone levels confirmed that the observation stressor was indeed stressful; the corticosterone levels for animals experiencing the observation stressor were significantly higher than levels of animals in the no stress condition, and were significantly lower than in animals following 15-20 minutes of restraint. Additionally, use of this stressor permitted illustration of dose-response effects of stress on startle, the less intense stressor resulting in less increase in amplitude (Figure 2) or PPI (Figure 7). Although corticosterone increases were used to validate or index the stressfulness of the observation manipulation as well as restraint, it was not hypothesized the corticosterone levels mediate effects of stress on startle (Davis & Zolovick, 1972).

Additionally, there were no significant effects of drug administration or cessation on corticosterone levels in either experiment. It has been previously reported that nicotine administration and cessation both increase corticosterone (Morse, 1989; Newhouse et al., 1990) but nicotine-induced increases were not seen in this experiment. It may also be noted that plasma CCS concentrations were above 400 ng/ml which appears high; however, it should be noted that blood was collected 30-35 minutes after the stress manipulation, and several hours into the activity cycle, at a time when circadian variations in plasma CCS concentration in the rat are elevated. It is likely that the stressors induced such marked effects that smaller differences attributable to drug were not detected. Alternatively, in may be that effects of drug on corticosterone were not measured because, in contrast to Morse (1989) and Newhouse and colleagues (1990), these experiments used a chronic rather than acute dose of nicotine.

One of the most interesting findings of these experiments was the influence of individual differences in reactivity on drug and stress effects. That is, reactivity

prior to drug administration or cessation was found to be an important determinant of both drug and stress effects on amplitude and PPI. Low initial reactors had little response to drug administration, stress or drug cessation. In contrast, high initial reactors appeared to be responsible for all experimental differences reported in these experiments. This finding suggests that for nicotine, very different experimental effects may be derived from different subject populations or from different subjects within the same population. Further, this effect may account for some of the disparate effects of stress and nicotine reported in the literature, because not all subjects respond in similar ways, and treatment effects may not be statistically significant as a result of the magnitude of the variance. Assuming that high responders are randomly distributed through out treatment groups, these initial differences could not be appropriately analyzed by analysis of covariance, but must be treated as separate variable. Further, greater amplitudes in initially high responders is not predicted by the law of initial values (Wilder, 1967), which would predict the less experimental reactivity in initially high responders rather than more. However, the law of initial values has been criticized (Jin, 1992; Myrtek & Foerster, 1986) and may not hold for all subjects and all response systems (Stern & Sison, 1990). Jin (1992) argues the law of initial values should be revised to say that the higher the initial value, the greater the organism's subsequent reactivity, although reversed responses may occur when the initial value reaches its upper limits. In order to test the law of initial values with regard to nicotine, stress, and ASR, a repeated measure design is necessary and should be considered in future studies.

Effects of initial reactivity in this experiment are interesting in light of other studies in which reactivity has been found to be correlated with drug self-administration. The Maudsley high and low reactive strains of Sprague-Dawley

rats are characterized by differences in their responses to the open-field test. The high reactors defecate more and are less active than low reactors, but do not differ in corticosterone responses (Abel, 1991). However, the Maudsley high reactors are more likely to develop a preference for ethanol than are the Maudsley low reactors (Adams, Shihabi, & Blizard, 1991). Startle amplitudes in these rats, however, have not have not been reported to differ, although the high reactors have been found to habituate to startle stimuli significantly faster than low reactors (Commissaris, Harrington, Baginski, & Altman, 1988). Recent work by Piazza and colleagues (Piazza, Deminiere, Maccari, Le Moal, Mormede & Simon, 1991; Piazza, Deminiere, Maccari, Mormede, Le Moal & Simon, 1990; Piazza, Deminiere, Le Moal, & Simon, 1989) also has reported that the locomotor response of drug-naive rats to novel environments is predictive of amphetamine self-administration. High responders to the novel environment were those that had greater locomotor activity as determined by a median split, and these animals were more likely to develop amphetamine self-administration (Piazza et al., 1989). High responders also had enhanced corticosterone responses to the novel environment at 120 minutes, but not at 30 minutes as in the present experiment (Piazza et al., 1991). High levels of initial reactivity is clearly an important variable to consider in future experiments.

These experiments also raise doubts concerning the functions thought to be reflected in PPI, or possibly the calculations used to measure it. Davis (1988) suggested that amount of inhibition is usually constant for any stimulus intensity level, and that reductions in percent of inhibition will be found for manipulations that increases startle amplitude. However, the present findings indicate clear differences in amount of inhibition following both nicotine administration or stress, suggesting that amount of inhibition can be modulated by manipulations other than stimulus intensity. Further, nicotine has been reported to

increase percent of inhibition at doses that also increase startle amplitude relative to control (Acri, et al., 1992), contrary to Davis' prediction that drugs that increase amplitude will decrease percent of inhibition. Therefore, amount and percent of PPI can be modulated more than has previously been reported. Although it was expected that nicotine would increase PPI, it was not expected that through both calculation methods there would be corroborating data that PPI is increased, rather than decreased, by stress.

If PPI reflects processes related to selective attention and the screening out of unwanted stimuli, it is not known why stress would increase this function, in Experiment 1, unless it represents a mechanism in which environmental stimuli are blocked out. Alternatively, stress could produce an extreme attentiveness, or a hypervigilance effect. Previous studies in which PPI was reported to decrease after stress (Leitner, 1989; Ornitz & Pynoos, 1989) also reported greatly decreased ASR amplitudes such that floor effects may have been operating. Ornitz and Pynoos (1989) concluded that there may have been a behavioral "shutdown" in their subjects as a result of PTSD. Therefore, the results Experiment 1 in which clear increases in PPI occurred following acute stress suggests that the stressful events increase the attentional processes underlying PPI in rats.

The strongest line of evidence suggesting that PPI reflects processes of sensory gating has been derived from studies of schizophrenic patients in whom PPI has been found to be deficient (Braff et al., 1978). From these data, it has been hypothesized that deficits in PPI reflect the more widely reported cognitive, information processing, and attentional deficits of schizophrenics (Nuechterlein & Dawson, 1984). However, a major confound of Braff and colleagues (1978) and other studies of ASR abnormalities in schizophrenics (Geyer & Braff 1982; Braff, Grillon, & Geyer, 1992) is that the majority of schizophrenic subjects were

receiving psychotropic medication, such that effects of schizophrenia and medication used to treat it were confounded. It may be that schizophrenics who are not medicated might show increased amplitude and PPI, as do the stressed subjects in these experiments. Studies of ASR and PPI have yet to be done in unmedicated schizophrenics, or in those without a history of medication and, therefore, conclusions concerning the function of PPI based on this literature are not useful for interpreting the present findings of increased PPI following stress.

Future studies should attempt to relate PPI to human cognitive function to better understand the mechanism through which PPI occurs and also its functional significance. Both ASR and PPI are clearly related to attention, but what human cognitive functions, if any, that they subserve or reflect, must be better understood in order to draw meaningful conclusions about their modulation by drugs or stress. The ASR paradigm in animals is of interest because it occurs in both animals and humans in similar forms and is modulated by a variety of experimental treatments; however, its ultimate usefulness must await studies in which PPI is blocked or enhanced and concomitant behavioral/cognitive effects are assessed to determine the functional relevance of PPI to the behaving organism.

Future studies also should address the importance of individual differences in reactivity, as measured by ASR amplitude, as a marker of sensitivity to both nicotine and stress effects. These experiments suggest that only more reactive individuals respond to nicotine and possibly other drugs; less reactive individuals experience little effect. It seems likely that more reactive individuals would, therefore, be more sensitive to drug effects and more likely to self-administer drugs to gain these effects. Additionally, only highly reactive individuals will experience drug and stress interactions or shifts in the dose-effect curve of the drug. If these individual

differences were better understood in terms of their endogenous mechanisms or genetic markers, prevention of drug abuse in humans could be more effectively targeted to individuals at greater risk. Further, treatments for drug cessation could include interventions that address the symptoms and needs of highly reactive individuals with the recognition that they are fundamentally different in their responses to stress and drug states than less reactive individuals. Future studies of the stress-reducing effects of nicotine should consider these individual differences, and more complete dose-effect curves for both stress and nicotine for each of these groups should be generated.

Although we have derived the dose-effect curve of acute doses of nicotine on ASR and PPI (Acri et al., 1992), it would be useful to document the dose-effect curve of nicotine on startle and PPI using several levels of stress and different reactivity groups. It may be that present day smokers are more reactive individuals, and that stress-reducing effects of nicotine in smokers are very different from stress-reducing effects of nicotine in a laboratory study in which nicotine is administered to possibly less reactive non smokers. It is likely that failure to consider these individual difference may account for some of the disparate results in studies of nicotine's effects.

In conclusion, there were significant interactions of stress and nicotine on reactivity and sensory gating (PPI) as measured by acoustic startle reflex amplitudes. Nicotine prevented stress-induced changes in amplitude and PPI, possibly because stress shifted the dose-response curve of nicotine. These findings may relate to the mechanisms through which cigarette smokers experience relief from stress through smoking.

TABLES

Table 1. Experiment 1. Methods: Design of Experiment: Effects of nicotine and stress during nicotine administration.

		Drug Condition	
	Saline	6 mg/kg/day Nicotine	12 mg/kg/day Nicotine
Stress Condition			
No Stress	8	8	8
Observe	8	8-9	8-9
Restraint	8-9	8	8-9

Table 2. Experiment 1. Timeline of events.

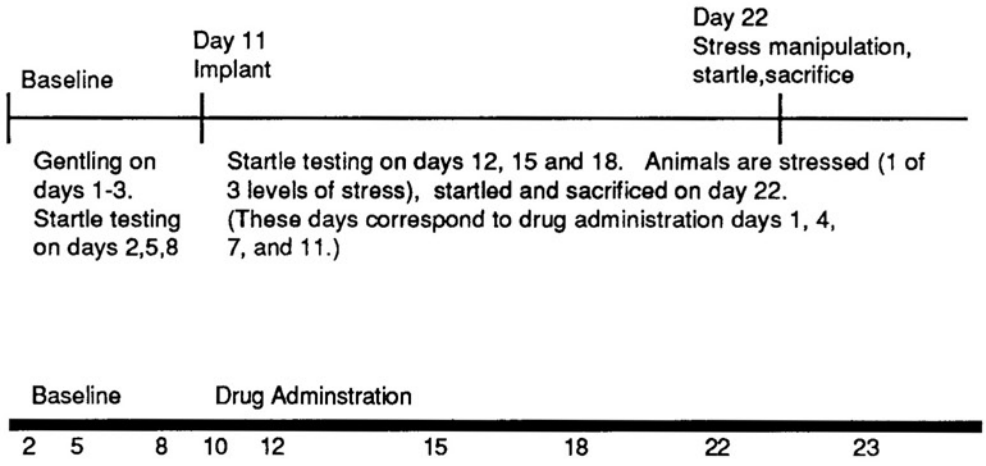


Table 3. Experiment 2. Methods: Design of Experiment. Effects of stress during nicotine cessation.

	Drug Condition	
	Saline	12mg/kg/day Nicotine
Stress Condition		
No Stress	8	8
Restraint Stress	8	8

Table 4. Experiment 2. Timeline of events.

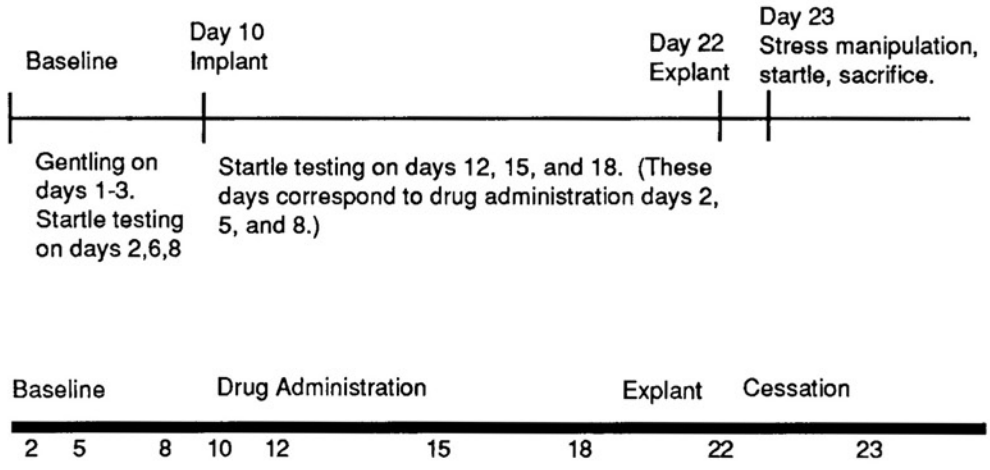


Table 5. Experiment 1. Methods: Hypothesis 5. Predicted dose response effects of nicotine with restraint stress.

Drug Condition			
	Saline	6mg/kg/day Nicotine	12mg/kg/day Nicotine
Stress Condition			
No Stress	+	++	+++
Restraint Stress	+++	++	+

Table 6. Experiment 1. Methods: Hypothesis 5. Predicted dose response effects of nicotine with observation stress

Drug Condition			
	Saline	6mg/kg/day Nicotine	12mg/kg/day Nicotine
Stress Condition			
No Stress	*	**	***
Observation Stress	**	***	****

Table 7. Weight loss during Experiment 1.

Animal number	Grams of weight loss	Duration (days)	Drug Condition	Inclusion or Exclusion
7	1	3	12 mg/kg	included
18	74	10	6 mg/kg	excluded
21	10	3	12 mg/kg	included
26	83	4	saline	excluded
33	2	3	12 mg/kg	included
49	66	7	saline	excluded
50	54	7	saline	excluded
51	37	4	saline	excluded
54	46	7	12 mg/kg	excluded
56	92	10	saline	excluded
67	6	3	6 mg/kg	included
70	88	10	saline	excluded

Table 8. Means and standard deviations of acoustic startle amplitudes using 98 dB, 112 dB, and 122 dB stimuli.

Stimulus intensity	98 dB			112 dB			122 dB		
Drug	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg
Stress									
No stress	10.67 sd=2.81 n = 6	10.13 sd=3.09 n = 8	12.43 sd=7.21 n = 7	29.17 sd=17.99 n = 6	24.0 sd=8.07 n = 8	52.86 sd=36.33 n = 7	152.17 sd=101.3 n = 6	127.75 sd=57.02 n = 8	188.57 sd=106.7 n = 7
Observe	11.67 sd=5.09 n = 6	11.25 sd=6.14 n = 8	12.22 sd=5.36 n = 9	32.33 sd=13.82 n = 6	52.13 sd=38.14 n = 8	38.67 sd=19.27 n = 9	245.17 135.09 n = 6	238 sd=46.29 n = 8	206.33 sd=114.0 n = 9
Restraint	7.0 sd=1.92 n = 7	12.50 sd=4.78 n = 8	8.33 sd=3.43 n = 9	41.14 sd=38.05 n = 7	60.86 sd=35.02 n = 8	26.56 sd=14.49 n = 9	179.29 sd=89.83 n = 7	171.38 sd=59.62 n = 8	141 sd=96.3 n = 9

Table 9. Significant post hoc comparsons of startle amplitude using 112 dB stimulus.

Comparison Groups	Fisher PLSD; $p < 0.05$
12 mg no stress vs 6 mg no stress	29.98
Saline no stress vs 6 mg restraint	29.19
12 mg restraint vs 6 mg restraint	26.27
6 mg no stress vs 6 mg restraint	27.03
6 mg no stress vs 6 mg observe	27.03

Table 10. Significant post hoc comparisons of startle amplitude using 122 dB stimulus.

Comparison Groups	Fisher PLSD; $p < 0.05$
12 mg restraint vs saline observe	97.11
12 mg restraint vs 6 mg observe	89.53
6 mg no stress vs saline observe	99.50
6 mg no stress vs 6 mg observe	92.12

Table 11. Significant post hoc comparisons of response amplitudes (112 dB stimulus) using median split (high/low) of baseline responses as a variable in the analysis.

Comparison Groups	Fisher PLSD, $p < 0.05$
12 mg no stress (low) vs saline restraint (high)	39.09
12 mg no stress (high) vs saline no stress (low)	46.72
12 mg no stress (high) vs saline restraint (low)	44.32
12 mg no stress (high) vs 12 mg restraint (low)	44.32
12 mg no stress (high) vs 6 mg no stress (low)	44.32
12 mg no stress (high) vs 6 mg no stress (high)	44.32
Saline no stress (low) vs saline restraint (high)	41.79
Saline no stress (low) vs 6 mg restraint (low)	39.09
Saline no stress (low) vs 6 mg observe (high)	37.38
Saline restraint (low) vs saline restraint (high)	39.09
Saline restraint (low) vs 6 mg restraint (low)	36.19
Saline restraint (low) vs 6 mg restraint (high)	36.19
Saline restraint (low) vs 6 mg observe (high)	34.33
Saline restraint (high) vs 12 mg restraint (high)	39.09
Saline restraint (high) vs 12 mg restraint (high)	37.38
Saline restraint (high) vs 6 mg no stress (low)	39.09
Saline restraint (high) vs 6 mg no stress (high)	39.09
Saline restriant (low) vs saline observe (low)	41.79
12 mg restraint (low) vs 6 mg restraint (low)	36.19
12 mg restraint (low) vs 6 mg restraint (high)	36.19
12 mg restraint (low) vs 6 mg observe (high)	34.33
6 mg no stress (low) vs 6 mg restraint (low)	36.19
6 mg no stress (low) vs 6 mg observe (high)	34.33
6 mg no stress (high) vs 6 mg restraint (low)	36.19
6 mg no stress (high) vs 6 mg observe (high)	34.33

Table 12. Experiment 1. Means and standard deviations of acoustic startle amplitudes using 98 dB, 112 dB, and 122 dB stimuli with pre-pulses.

Stimulus intensity		98 dB			112 dB			122 dB		
Stress	Drug	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg
No stress		10.83 sd=3.13 n = 6	10.25 sd=4.71 n = 8	7.57 sd=1.51 n = 7	14.0 sd=7.46 n = 6	16.25 sd=6.92 n = 8	12 sd=4.47 n = 7	72.5 sd=51.77 n = 6	52.63 sd=27.29 n = 8	82.71 sd=61.28 n = 7
Observe		9.17 sd=3.19 n = 6	7.13 sd=1.73 n = 8	9.22 sd=4.41 n = 9	15 sd=4.34 n = 6	22.88 sd=17.16 n = 8	12.44 sd=3.78 n = 9	125.83 sd=55.34 n = 6	109.13 sd=48.01 n = 8	111.67 sd=94.52 n = 9
Restraint		5.0 sd=1.73 n = 7	10.50 sd=5.04 n = 8	7.89 sd=1.69 n = 9	10 sd=3.83 n = 7	12.75 sd=4.71 n = 8	11.78 sd=5.31 n = 9	93.43 sd=48.09 n = 7	98.63 sd=39.63 n = 8	56.11 sd=49.02 n = 9

Table 13. Experiment 1. Signficant post hoc comparisons of startle amplitudes using 98 dB stimulus with pre-pulse.

Comparison Groups	Fisher PLSD; $p < 0.05$
Saline restraint vs 6 mg restraint	3.38
Saline restraint vs 12 mg observe	3.38
Saline restraint vs saline no stress	3.74
Saline no stress vs 6 mg observe	3.63
Saline restraint vs 6 mg no stress	3.48
Saline restraint vs saline observe	3.74
6 mg restraint vs 6 mg observe	3.36

Table 14. Experiment 1. Signficant post hoc comparisons of trials using 122 dB stimulus with pre-pulse.

Comparison Groups	Fisher PLSD; $p < 0.05$
12 mg restraint vs saline observe	59.56
6 mg no stress vs saline observe	61.03
6 mg no stress vs 6 mg observe	56.50
6 mg no stress vs 12 mg observe	54.91

Table 15. Experiment 1. Means and standard deviations for calculated amount of inhibition using 112 dB, and 122 dB stimuli with pre-pulses.

Stimulus intensity	98 dB			112 dB			122 dB		
Drug	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg
Stress									
No stress	-0.17 sd=4.83 n = 6	-0.13 sd=2.90 n = 8	4.86 sd=6.12 n = 7	15.17 sd=12.71 n = 6	7.75 sd=12.67 n = 8	40.86 sd=36.72 n = 7	79.67 sd=54.98 n = 6	75.13 sd=37.55 n = 8	105.86 sd=54.33 n = 7
Observe	2.5 sd=6.57 n = 6	4.13 sd=5.38 n = 8	3.0 sd=4.92 n = 9	17.33 sd=13.78 n = 6	29.25 sd=30.39 n = 8	26.22 sd=20.49 n = 9	119.33 sd=85.98 n = 6	128.88 sd=54.17 n = 8	94.67 sd=64.44 n = 9
Restraint	2.0 sd=3.0 n = 7	2.0 sd=4.87 n = 8	0.44 sd=3.81 n = 9	31.14 sd=37.56 n = 7	48.13 sd=32.67 n = 8	14.78 sd=11.44 n = 9	85.86 sd=66.22 n = 7	72.75 sd=65.52 n = 8	84.89 sd=60.11 n = 9

Table 16. Experiment 1. Significant post hoc comparisons of amount of inhibition using 112 dB stimulus with pre-pulse.

Comparison Groups	Fisher PLSD; $p < 0.05$
12 mg no stress vs 12 mg restraint	25.52
12 mg no stress vs 6 mg no stress	26.20
6 mg restraint vs saline no stress	27.34
6 mg restraint vs 12 mg restraint	24.60
6 mg restraint vs 6 mg no stress	25.32
6 mg restraint vs saline observe	27.34

Table 17. Experiment 1. Means and standard deviations of calculated percent of inhibition (amount of inhibition/amplitude of trial without pre-pulse) using 98 dB, 112 dB, and 122 dB stimuli.

Stimulus intensity	98 dB			112 dB			122 dB		
Drug / Stress	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg	Saline	6 mg/kg	12 mg/kg
No stress	-10.4 sd=10.4 n = 6	0.5 sd=28.9 n = 8	26.4 sd=31.0 n = 7	48.9 sd=21.6 n = 6	19.9 sd=47.8 n = 8	59.3 sd=41.0 n = 7	52.1 sd=9.8 n = 6	59.3 sd=13.6 n = 8	59.7 sd=12.8 n = 7
Observe	6.5 sd=47.9 n = 6	26.9 sd=29.9 n = 8	18.8 sd=33.1 n = 9	45.6 sd=31.6 n = 6	45.7 sd=39.3 n = 8	56.1 sd=33.3 n = 9	42.5 sd=18.4 n = 6	53.4 sd=19.2 n = 8	49.0 sd=24.0 n = 9
Restraint	21.7 sd=42.0 n = 7	6.8 sd=43.8 n = 8	-13.2 ad=56.5 n = 9	44.3 sd=60.7 n = 7	72.6 sd=20.1 n = 8	48.3 sd=30.6 n = 9	43.8 sd=33.4 n = 7	34.4 sd=33.8 n = 8	59.8 sd=16.1 n = 9

Table 18. Experiment 1. Significant post hoc comparisons of amount of PPI (112 dBH stimulus) using median split (high/low) of baseline responses as a variable in the analysis.

Comparison Groups	Fisher PLSD, $p < 0.05$
12 mg no stress (low) vs saline restraint (high)	37.29
12 mg no stress (high) vs saline no stress (low)	44.57
12 mg no stress (high) vs saline restraint (low)	42.29
12 mg no stress (high) vs 12 mg restraint (low)	42.29
12 mg no stress (high) vs 6 mg no stress (low)	42.29
12 mg no stress (high) vs 6 mg no stress (high)	42.29
Saline no stress (low) vs saline restraint (high)	39.87
Saline no stress (low) vs 6 mg restraint (low)	37.29
Saline no stress (high) vs saline restraint (high)	39.87
Saline restraint (low) vs saline restraint (high)	37.29
Saline restraint (low) vs 6 mg restraint (low)	34.57
Saline restraint (low) vs 6 mg restraint (high)	34.53
Saline restraint (high) vs 12 mg restraint (low)	37.29
Saline restraint (high) vs 12 mg restraint (high)	35.66
Saline restraint (high) vs 6 mg restraint (low)	37.29
Saline restraint (high) vs 6 mg no stress (high)	37.29
Saline restriant (high) vs saline observe (low)	39.87
Saline restraint (high) vs saline observe (high)	39.87
Saline restraint (high) vs 6 mg observe (low)	39.87
12 mg restraint (low) vs 6 mg restraint (low)	34.54
12 mg restraint (low) vs 6 mg restraint (high)	34.53
6 mg no stress (low) vs 6 mg restraint (low)	34.53
6 mg no stress (high) vs 6 mg restraint (low)	34.53
6 mg no stress (high) vs 6 mg observe (high)	34.53
Saline observe (low) vs 6 mg restraint (low)	37.29

Table 19. Experiment 2. Means and standard deviations of acoustic startle amplitudes using 98 dB, 112 dB, and 122 dB stimuli.

	Stimulus intensity					
	98 dB		112 dB		122 dB	
Drug	Saline	12 mg/kg	Saline	12 mg/kg	Saline	12 mg/kg
Stress						
No stress	10.25 sd=4.53 n = 8	8.0 sd=0.76 n = 8	50.13 sd=40.41 n = 8	36.38 sd=21.51 n = 8	197.88 sd=71.30 n = 8	185.88 sd=85.12 n = 8
Restraint	10.25 sd=4.62 n = 8	9.25 sd=3.81 n = 8	23.38 sd=10.60 n = 8	33.13 sd=13.04 n = 8	130.38 sd=51.12 n = 8	168.88 sd=55.34 n = 8

Table 20. Experiment 2. Means and standard deviations of acoustic startle amplitudes using 98 dB, 112 dB, and 122 dB stimuli with pre-pulses.

	Stimulus intensity					
	98 dB		112 dB		122 dB	
Drug	Saline	12 mg/kg	Saline	12 mg/kg	Saline	12 mg/kg
Stress						
No stress	8.0 sd=3.07 n = 8	8.5 sd=1.93 n = 8	14.63 sd=5.93 n = 8	14.13 sd=4.16 n = 8	75.5 sd=65.73 n = 8	87.5 sd=68.78 n = 8
Restraint	8.5 sd=4.34 n = 8	8.5 sd=4.99 n = 8	10.63 sd=2.62 n = 8	9.88 sd=4.22 n = 8	75.63 sd=56.31 n = 8	64.75 sd=26.35 n = 8

Table 21. Experiment 2. Means and standard deviations of calculated amount of inhibition using 98 dB, 112 dB, and 122 dB stimuli with pre-pulses.

Stimulus intensity	98 dB		112 dB		122 dB	
Drug	Saline	12 mg/kg	Saline	12 mg/kg	Saline	12 mg/kg
Stress						
No stress	2.25 sd=2.55 n = 8	-0.5 sd=2.0 n = 8	35.5 sd=40.94 n = 8	22.25 sd=19.34 n = 8	122.38 sd=66.64 n = 8	98.38 sd=34.99 n = 8
Restraint	1.75 sd=3.73 n = 8	0.75 sd=2.92 n = 8	12.75 sd=10.10 n = 8	23.25 sd=13.47 n = 8	54.75 sd=37.46 n = 8	104.13 sd=44.85 n = 8

Table 22. Experiment 2. Means and standard deviations of percent of inhibition using 98 dB, 112 dB, and 122 dB stimuli with pre-pulses.

Stimulus intensity	98 dB		112 dB		122 dB	
Drug	Saline	12 mg/kg	Saline	12 mg/kg	Saline	12 mg/kg
Stress						
No stress	16.7 sd=24.2 n = 8	-7.0 sd=26.5 n = 8	48.0 sd=56.1 n = 8	46.7 sd=36.6 n = 8	63.5 sd=23.9 n = 8	58.6 sd=21.4 n = 8
Restraint	13.4 sd=24.5 n = 8	10.9 sd=28.2 n = 8	46.4 sd=29.9 n = 8	64.4 sd=26 n = 8	47.1 sd=29.7 n = 8	60.5 sd=13.7 n = 8

FIGURES

Figure Legends

For all figures, "no stress" refers to the control group that remained in home cages prior to startle, "observe" refers to the animals that were in the presence of other rats that were being restrained, and "restraint" refers to animals that were restrained for 15-20 minutes in restraint devices.

The following notation applies to Figures 2-3, 5-8, 10, and 14-16.

1 Denotes a significant difference from saline no stress control ($p < 0.05$)

2 Denotes a significant difference from 6 mg/kg/day nicotine no stress condition ($p < 0.05$)

3 Denotes a significant difference from 12 mg/kg/day nicotine no stress ($p < 0.05$)

4 Denotes a significant difference from the saline observation stress group ($p < 0.05$)

5 Denotes a significant difference from the 6 mg/kg/day nicotine-treated observation stress group ($p < 0.05$)

6 Denotes a significant difference from the 12 mg/kg/day nicotine-treated observation stress group ($p < 0.05$)

7 Denotes a significant difference from the saline restraint stress group ($p < 0.05$)

8 Denotes a significant difference from the 6 mg/kg/day nicotine-treated restraint stress group ($p < 0.05$)

9 Denotes a significant difference from the 12 mg/kg/day nicotine-treated restraint stress group ($p < 0.05$)

The following notation applies to Figures 4, 9, 12, and 17-18.

a Denotes a significant difference from saline no stress low reactors ($p < 0.05$)

b Denotes a significant difference from saline no stress high reactors ($p < 0.05$)

c Denotes a significant difference from 12 mg/kg/day nicotine no stress high reactors ($p < 0.05$)

d Denotes a significant difference from 12 mg/kg/day nicotine no stress high reactors ($p < 0.05$)

e Denotes a significant difference from saline observation stress low reactors ($p < 0.05$)

f Denotes a significant difference from saline observation stress high reactors ($p < 0.05$)

g Denotes a significant difference from 12 mg/kg/day nicotine observation stress low reactors ($p < 0.05$)

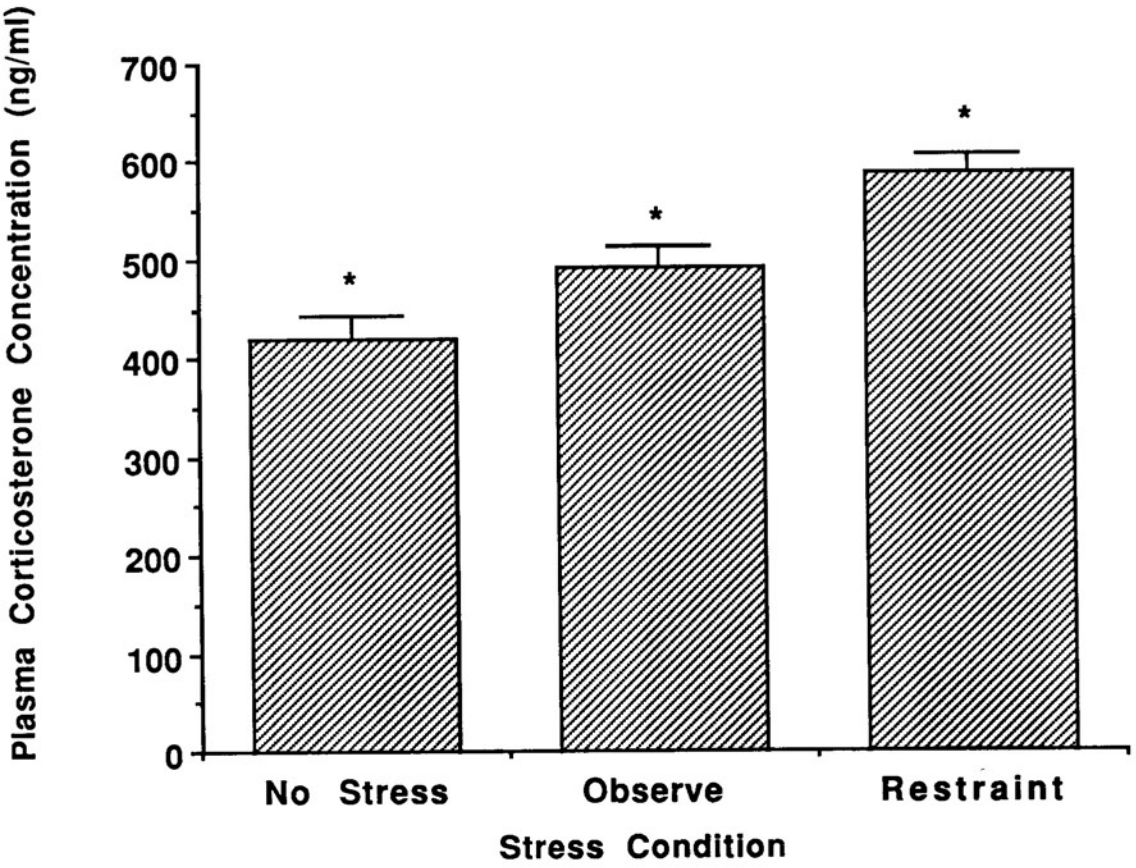
h Denotes a significant difference from 12 mg/kg/day nicotine observation stress high reactors ($p < 0.05$)

i Denotes a significant difference from saline restraint stress low reactors ($p < 0.05$)

j Denotes a significant difference from saline restraint high reactors ($p < 0.05$)

k Denotes a significant difference from 12 mg/kg/day nicotine restraint stress low reactors ($p < 0.05$)

l Denotes a significant difference from 12 mg/kg/day nicotine restraint stress high reactors ($p < 0.05$)



* Differs from both other groups at $p < 0.05$

Figure 1.
Experiment 1. Effects of stress on plasma corticosterone concentration (means and standard errors).

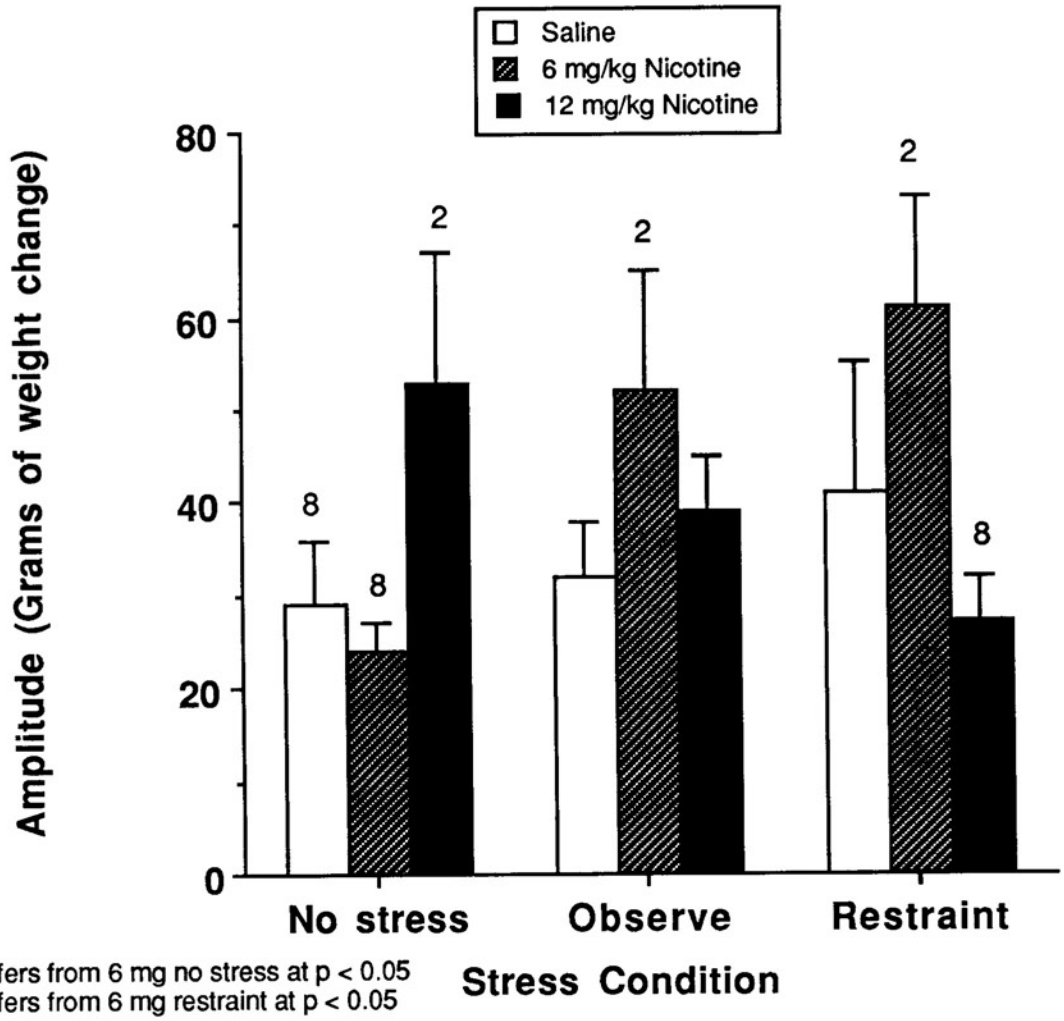


Figure 2.
Experiment 1. Startle amplitude using 112 dB stimulus (means and standard errors).

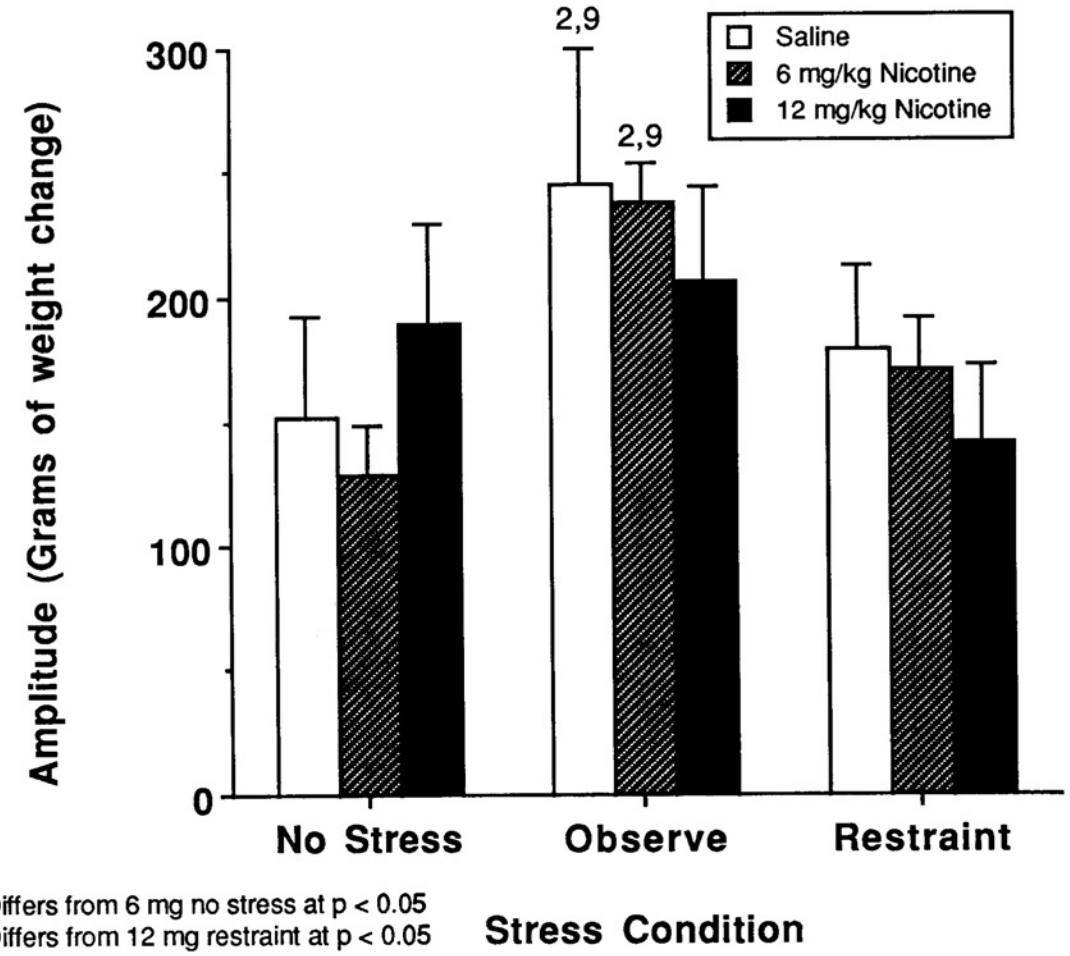


Figure 3.
Experiment 1. Startle amplitude using 122 dB stimulus (means and standard errors).

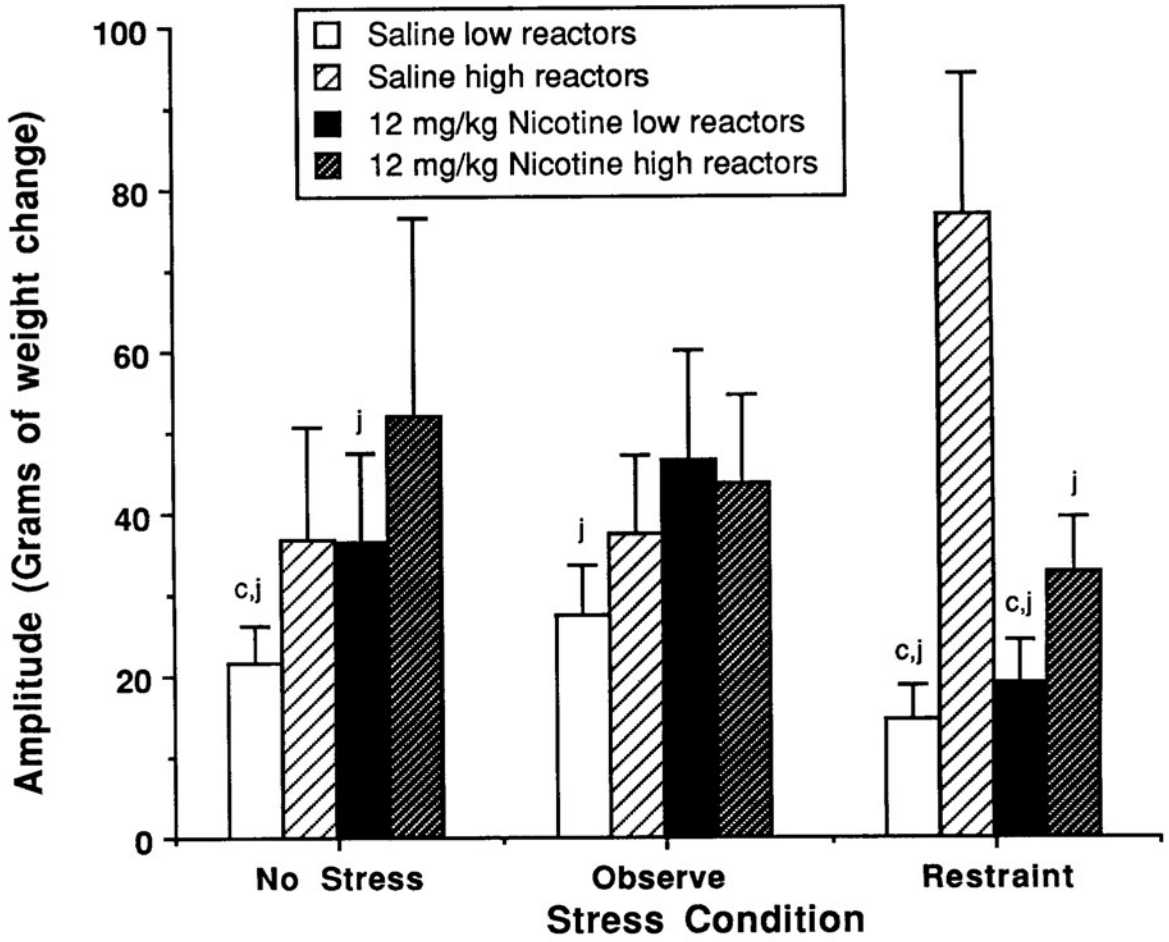
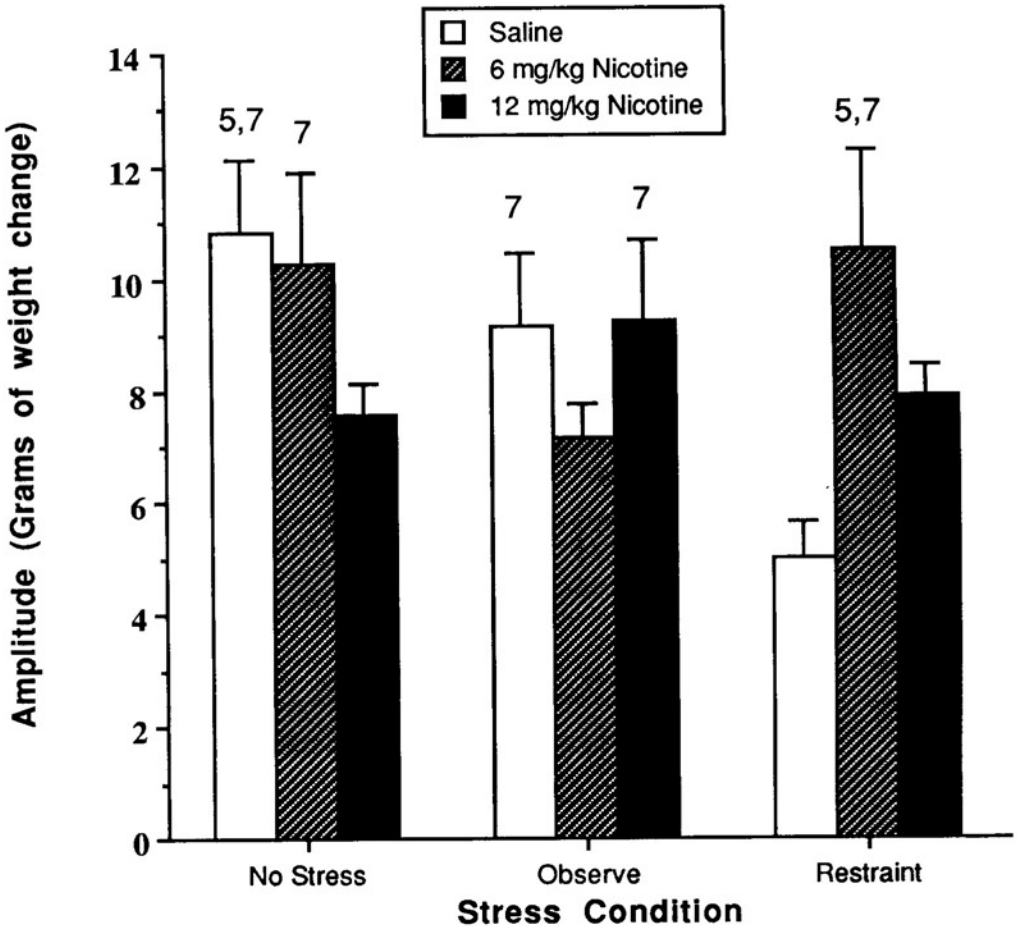


Figure 4.
Experiment 1. Startle amplitude using 112 dB stimulus and median split of initial reactivity (means and standard errors).



5 Differs from 6 mg/kg observe at $p < 0.05$

7 Differs from saline restraint at $p < 0.05$

Figure 5.
Experiment 1. Amplitude of pre-pulse trials using 98 dB stimulus with pre-pulse (means and standard errors).

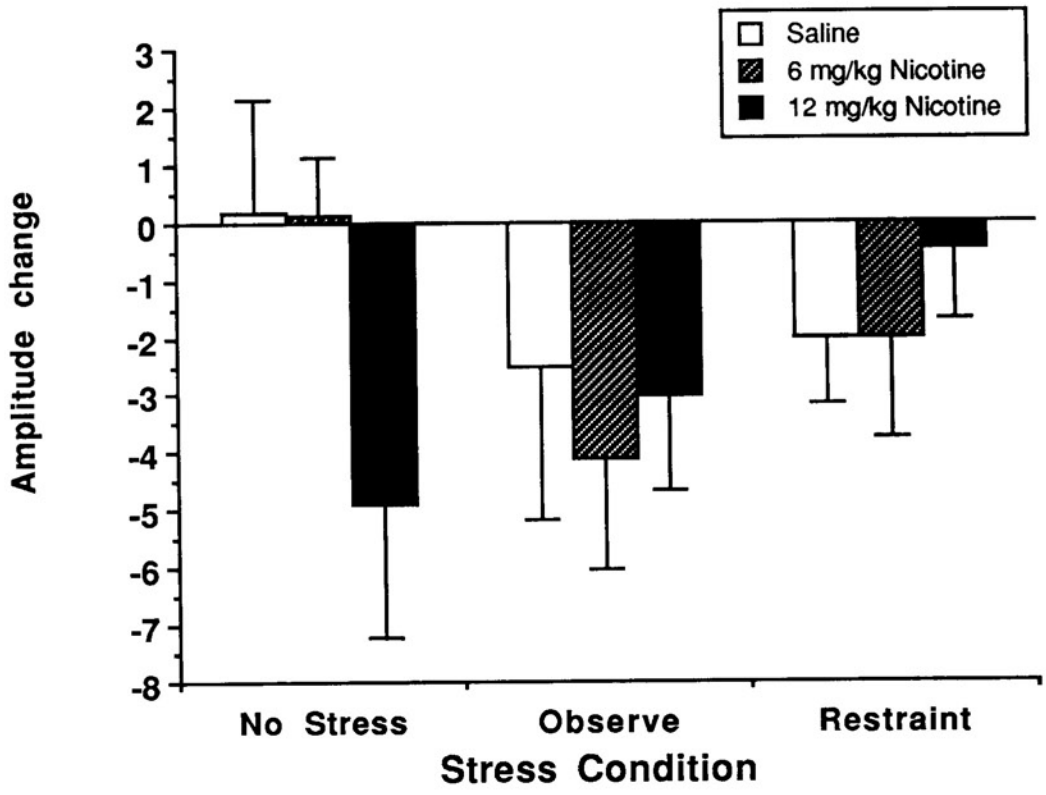


Figure 6.
Experiment 1. Amount of inhibition using 98 dB with pre-pulse (means and standard errors).

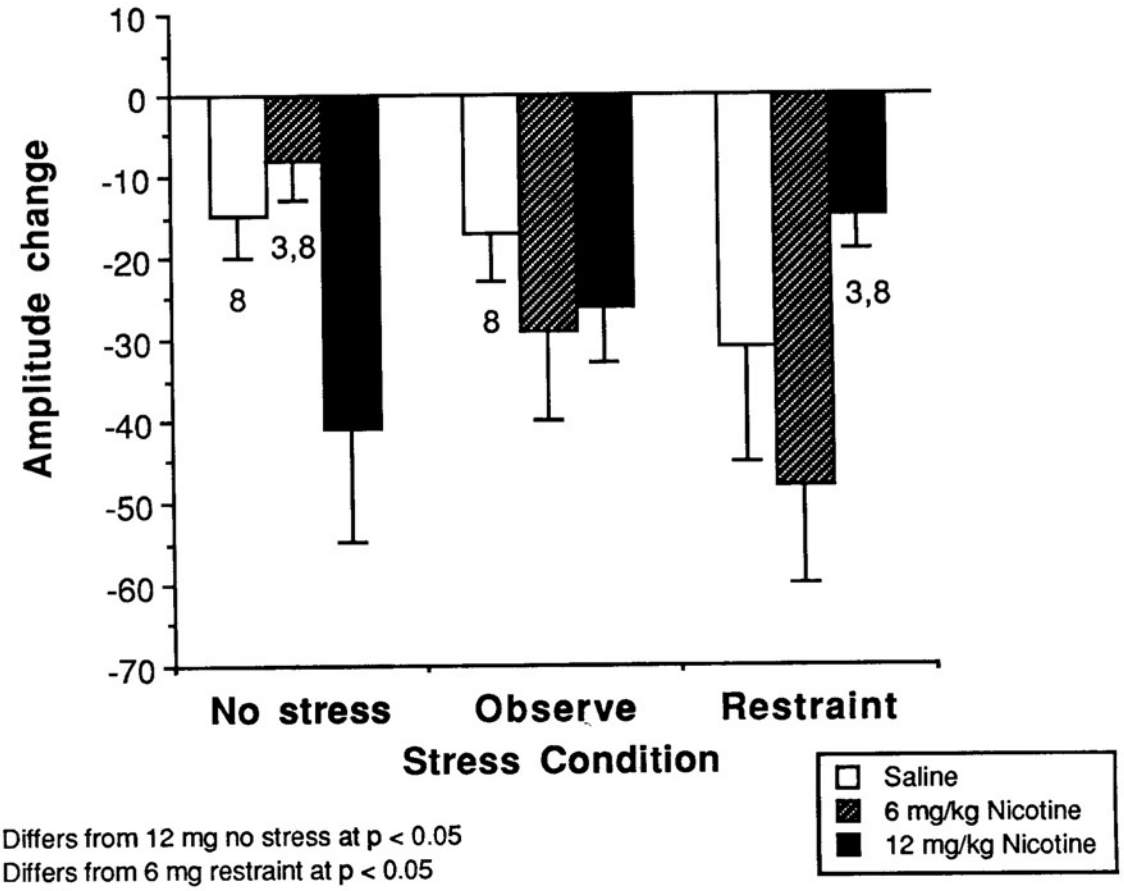
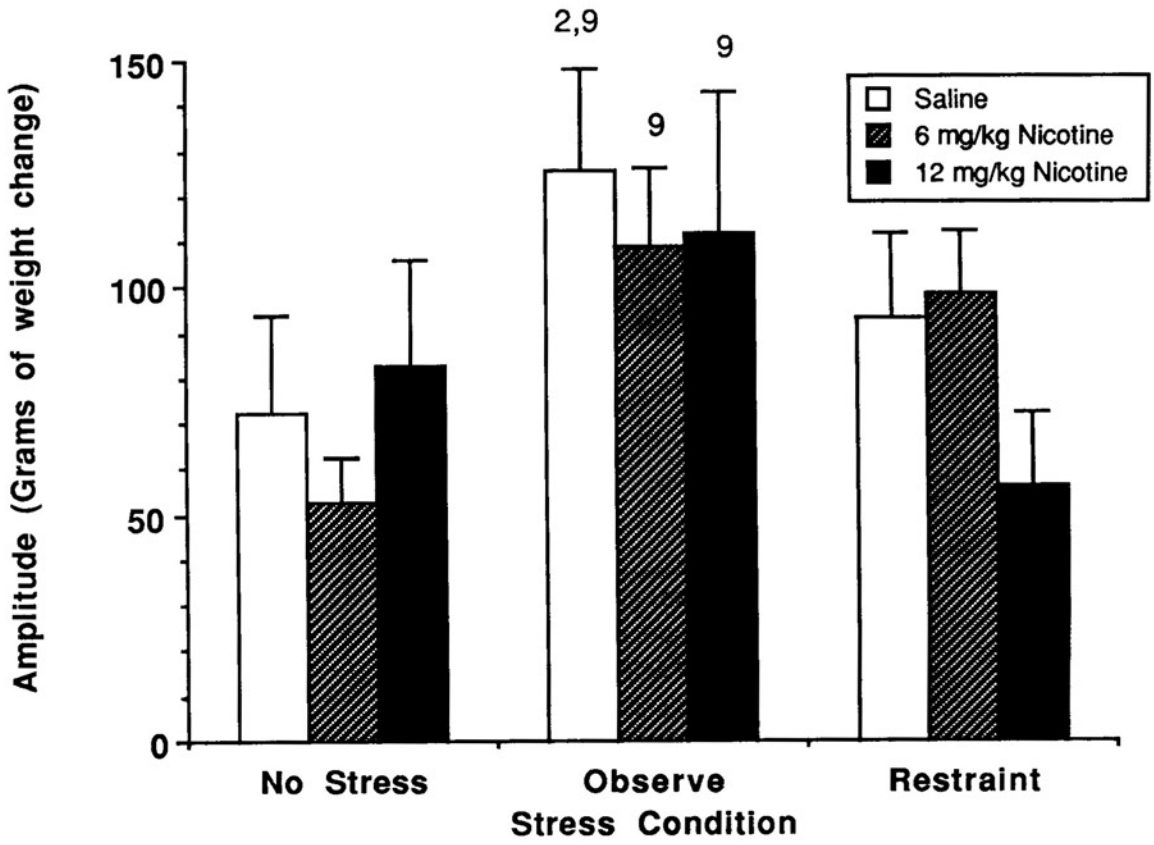
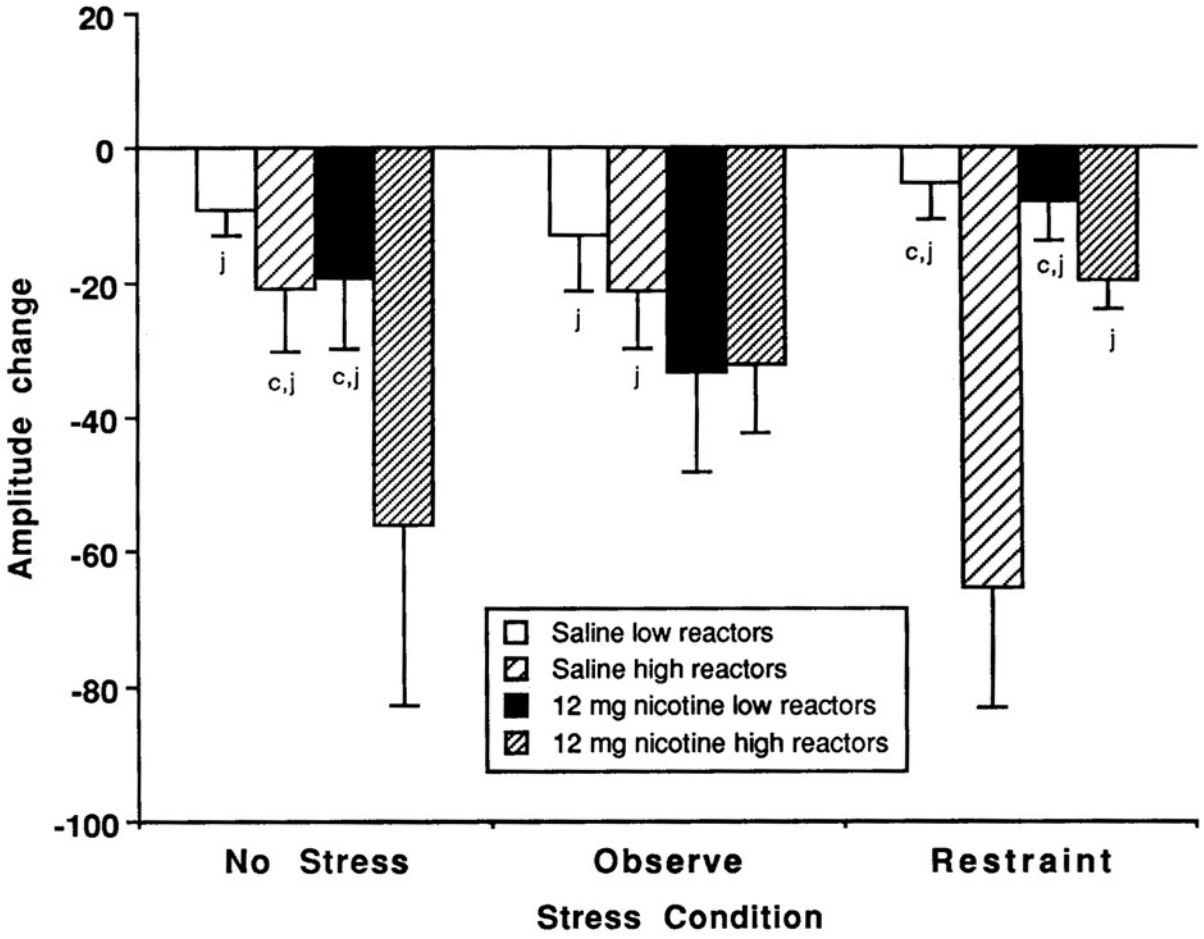


Figure 7.
Experiment 1. Amount of inhibition using 112 dB with pre-pulse (means and standard errors).



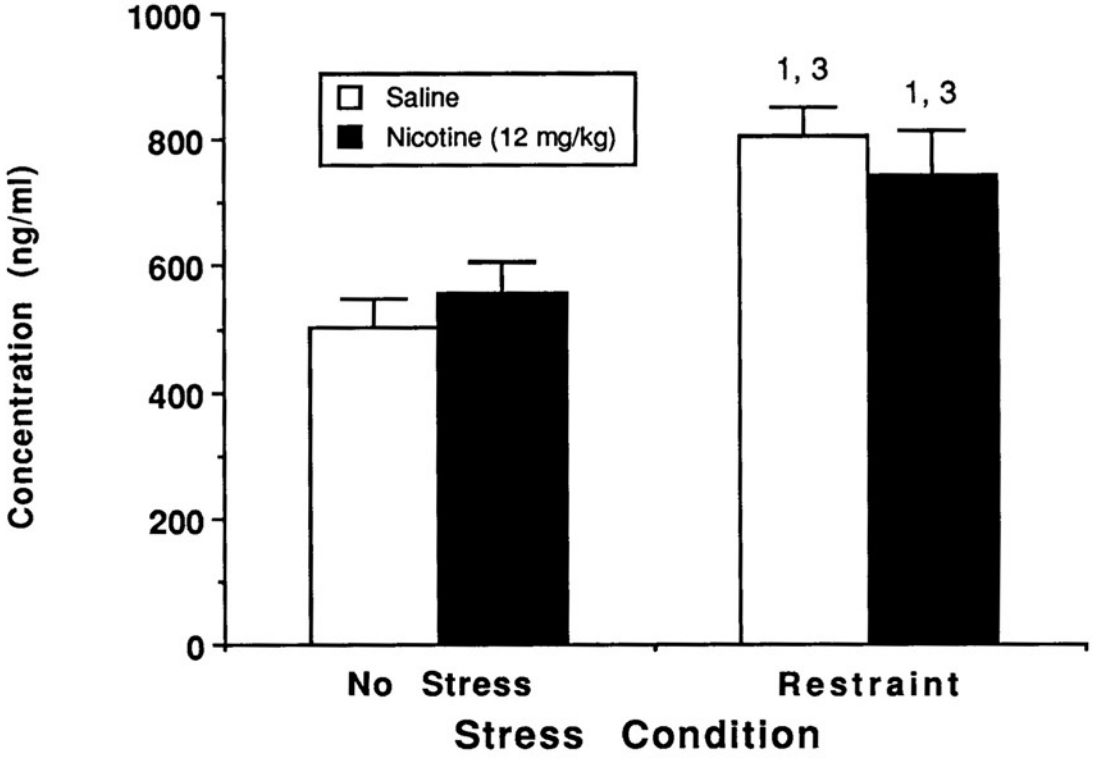
2 Differs from 6 mg/kg no stress at $p < 0.05$
9 Differs from 12 mg/kg restraint at $p < 0.05$

Figure 8.
Experiment 1. Amplitude of pre-pulse trials using 122 dB with pre-pulse (means and standard errors).



c Differs significantly from 12 mg/kg nicotine no stress (high reactors) at $p < 0.05$
j Differs significantly from saline restraint (high reactors) at $p < 0.05$

Figure 9.
Experiment 1. Amount of inhibition using 112 dB with pre-pulse following median split of initial reactivity (means and standard errors).



1 Differs from saline, no stress control, $p<0.05$
3 Differs from nicotine, no stress, $p<0.05$

Figure 10.
Experiment 2.
Effects of stress on plasma corticosterone concentration (means and standard errors).

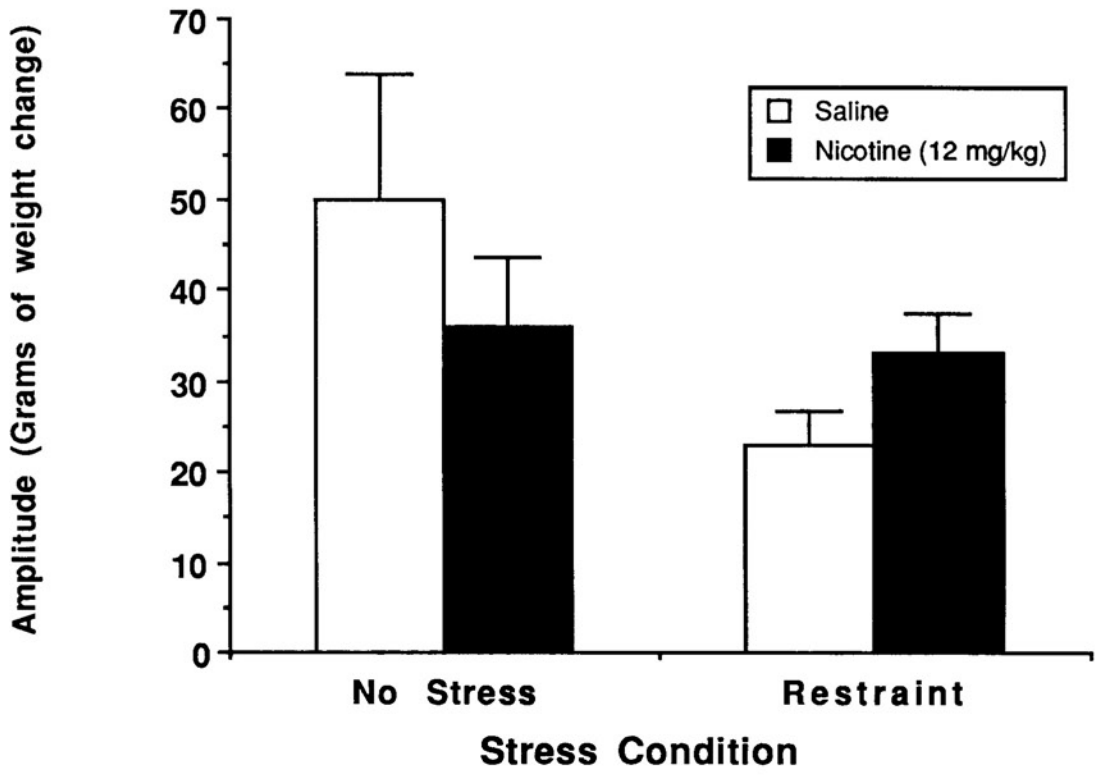
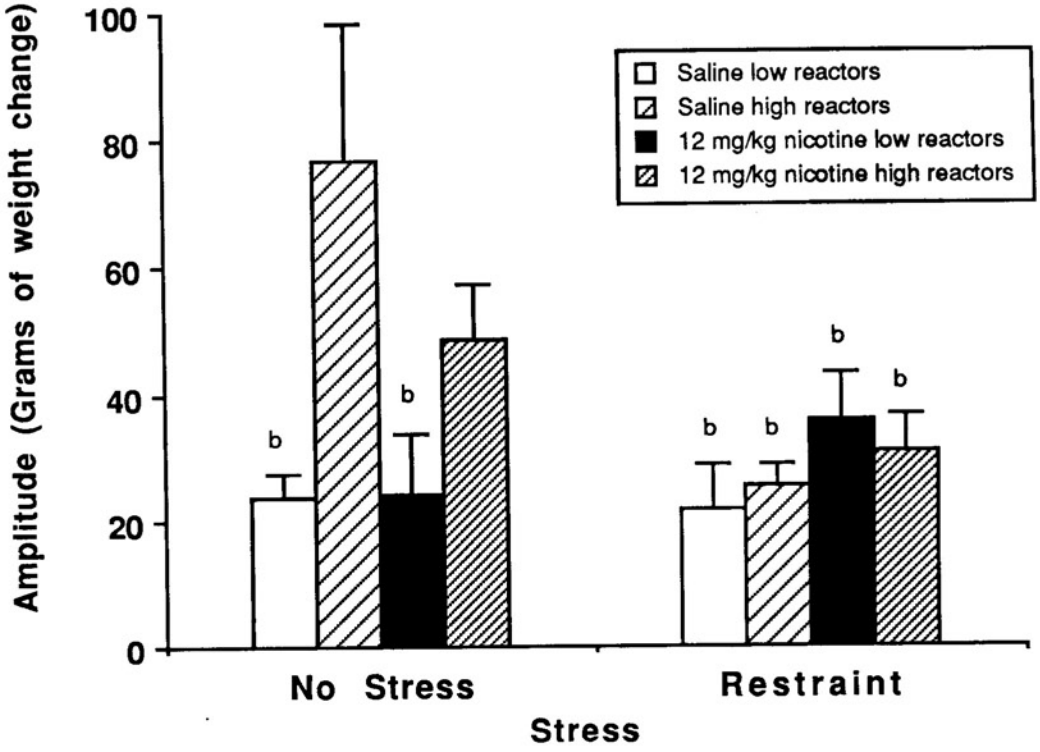


Figure 11.
Experiment 2.
Startle amplitude using 112 dB stimulus (means and standard errors).



b Differs from saline no stress (high reactors) at $p < 0.05$

Figure 12.
Experiment 2. Startle amplitude using 112 dB stimulus following median split of initial reactivity (means and standard errors).

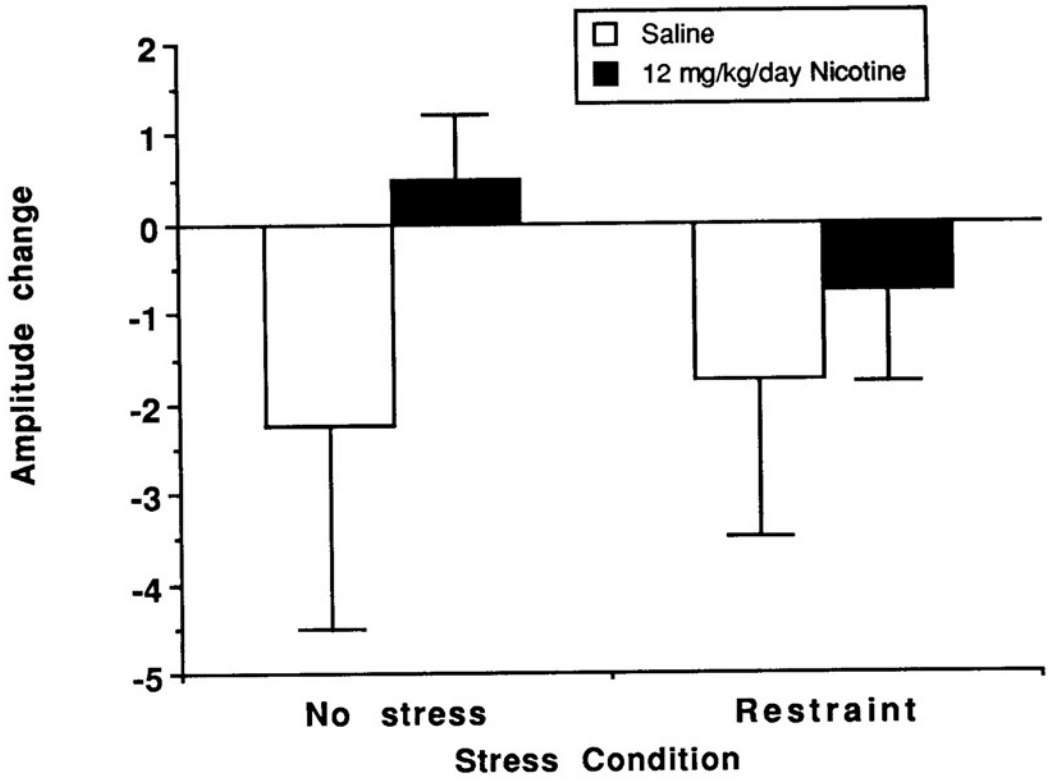
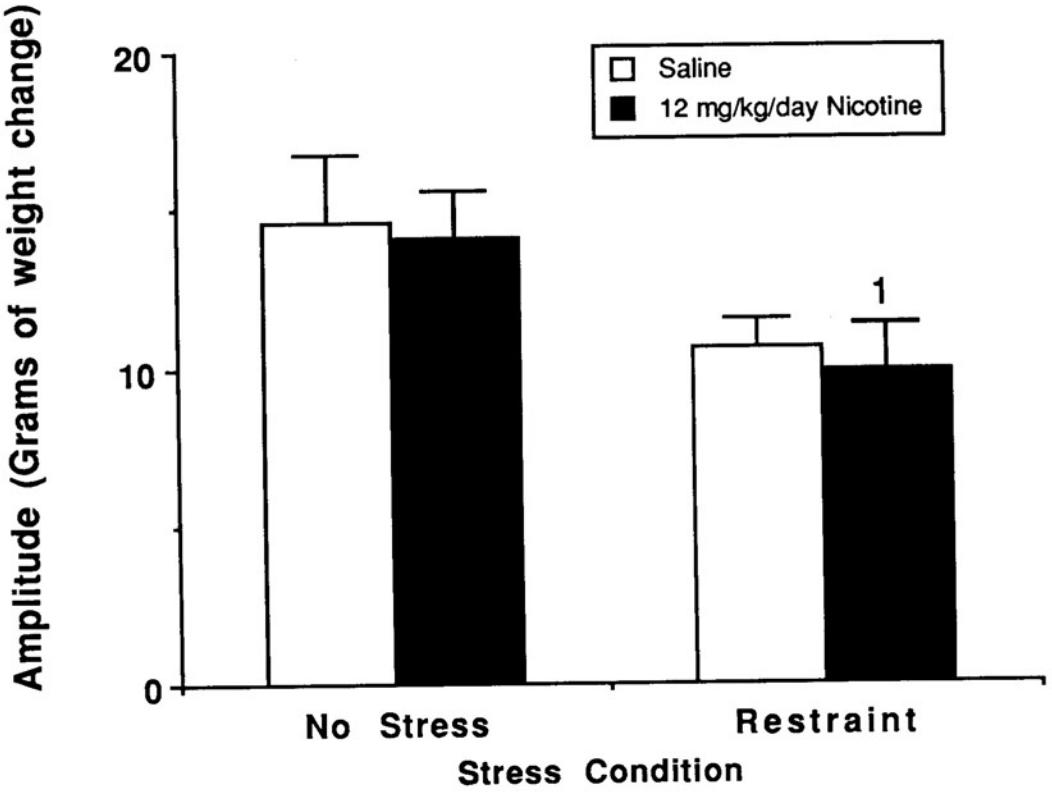


Figure 13.
Experiment 2. Amount of inhibition using 98 dB with prepulse (means and standard errors).



1 Differs from saline no stress at $p < 0.05$

Figure 14.
Experiment 2. Amplitude of pre-pulse trials using 112 dB with pre-pulse (means and standard errors).

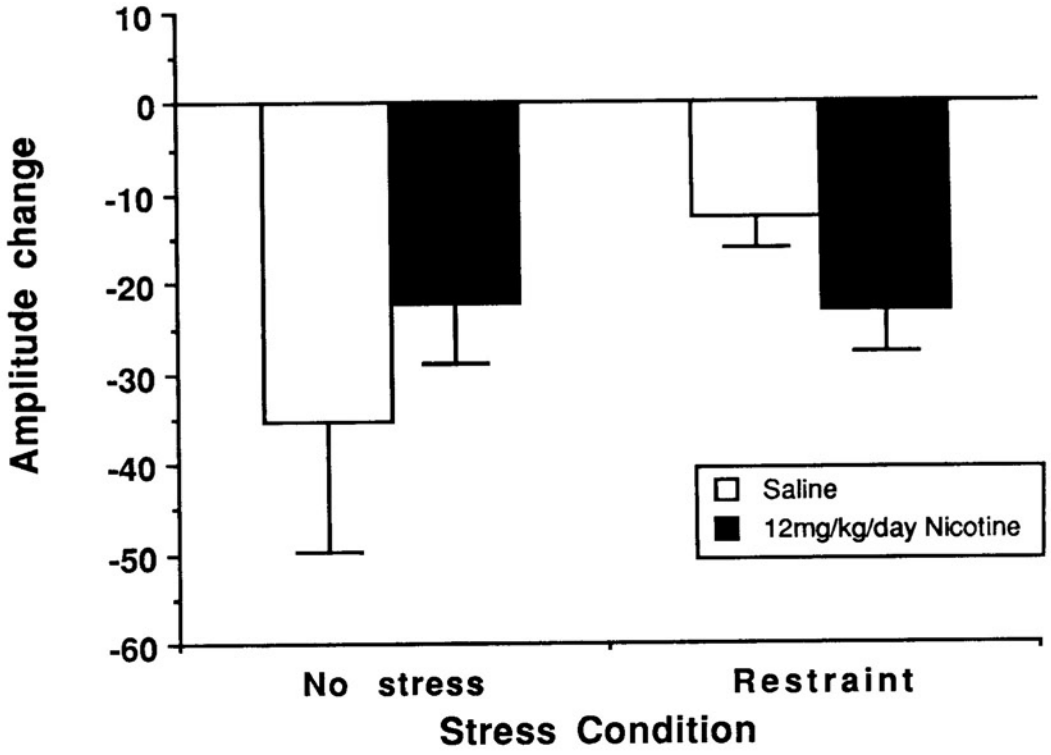
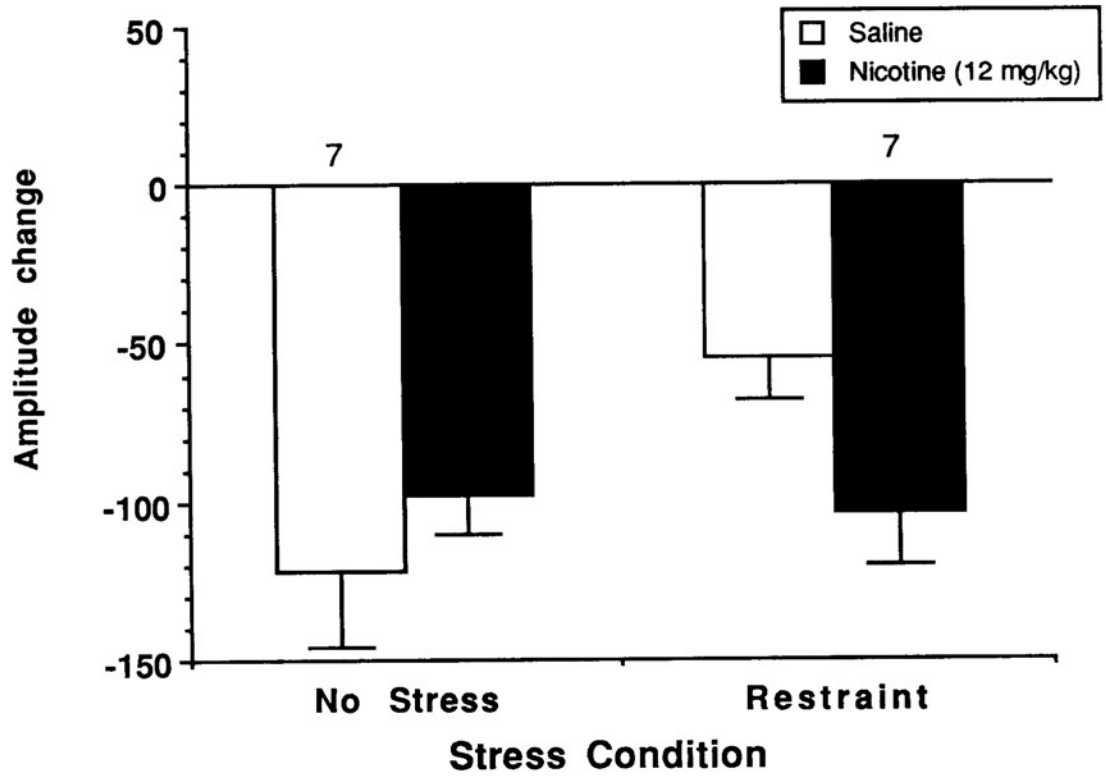
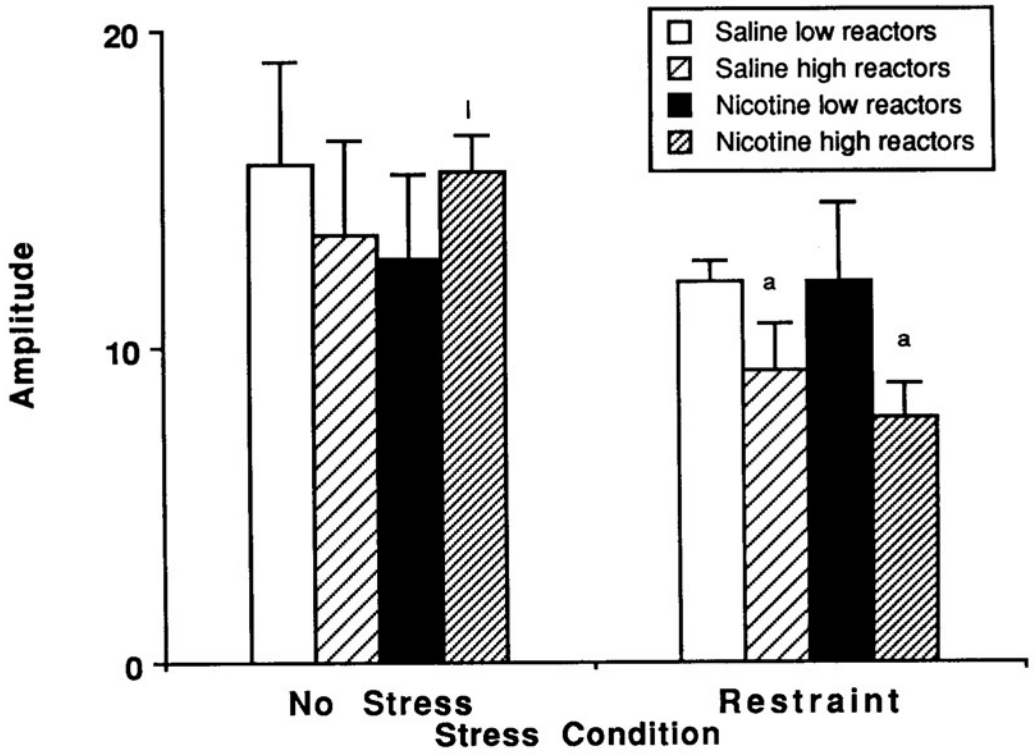


Figure 15.
Experiment 2. Amount of inhibition using 112 dB with prepulse (means and standard errors).



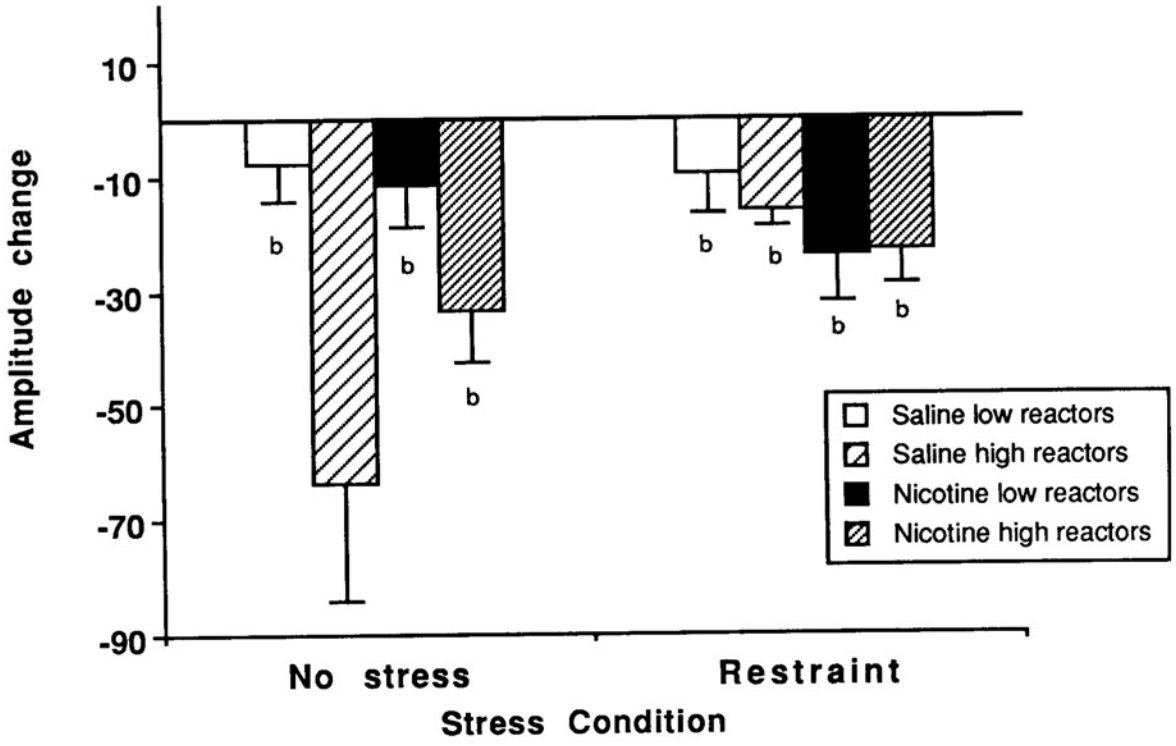
7 Differs from saline restraint, $p < 0.05$

Figure 16.
Experiment 2. Amount of inhibition using 122 dB with pre-pulse (means and standard errors).



a Differs from saline no stress (low reactors) at $p < 0.05$
I Differs from nicotine restraint (high reactors) at $p < 0.05$

Figure 17.
Experiment 2. Amplitude of pre-pulse trials using 112 dB following median split of initial reactivity (means and standard errors).



b Differs from saline no stress (high reactors) at $p < 0.05$

Figure 18.
Experiment 2. Amount of inhibition using 112 dB with pre-pulse following median split of initial reactivity (means and standard errors).

REFERENCES

Abel, E.L. (1991). Behavior and corticosteroid response of Maudsley reactive and nonreactive rats in the open field and forced swimming tests. Physiology and Behavior, 50(1), 151-153.

Abel, E.L., & Bilitzke, P.J. (1990). A possible alarm substance in the forced swimming test. Physiology and Behavior, 48, 233-239.

Abrams, D.B., Monti, P.M., Pinto, R.P., Elder, J.P., Brown, R.A., & Jacobus, S.I. (1987). Psychosocial stress in smokers who relapse or quit. Health Psychology, 6(4), 289-303.

Acri, J.B., Grunberg, N.E., & Morse, D.E. (1991). Effects of nicotine on the acoustic startle response in rats. Psychopharmacology, 104(2), 244-249.

Acri, J.B., Morse, D.E., & Grunberg, N.E. (1992). Nicotine increases pre-pulse inhibition of acoustic startle reflex in rats. Paper presented at the annual meeting of the American Psychological Association, Washington, D. C.

Adams, N., Shihabi, Z.K., & Blizard, D.A. (1991). Ethanol preference in the Harrington derivation of the Maudsley reactive and non-reactive strains. Alcoholism, Clinical and Experimental Research, 15(2), 170-174.

Adell, A., Garcia-Marquez, C., Armario, A., & Gelpi, E. (1988). Chronic stress increases serotonin and noradrenalin in rat brain and sensitizes their responses to a further acute stress. Journal of Neurochemistry, 50(6), 1678-1681.

American Psychiatric Association. (1987). Diagnostic and statistical manual of mental disorders (3rd ed., rev.) Washington, D.C.: Author.

Andersson, K., & Hockey, G.R.J. (1977). Effects of cigarette smoking on incidental memory. Psychopharmacology, 52, 223-226.

Anthony, B.J. (1990). Blink modulation in attention-deficit disorder [Abstract] Psychophysiology 27(4A), S6.

Anthony, B.J., & Graham, F.K. (1983). Evidence for sensory-selective set in young infants. Science, 220, 742-743.

Armitage, A.K., Hall, G.H., & Morrison, C.F. (1968). Pharmacological basis for the tobacco smoking habit. Nature, 217, 331-334.

Ashton, H., & Stepney, R. (1982). Smoking: Psychology and pharmacology. London: Tavistock.

Axelrod, J., & Reisine, T.D. (1984). Stress hormones: Their interaction and regulation. Science, 224, 452-459.

Balfour, D.J.K. (1982). The pharmacology of nicotine dependence: a working hypothesis. Pharmacology & Therapeutics 15, 239-250.

Balfour, D.J.K., Benwell, M.E.M., Graham, C.A., & Vale, A.L. (1986). Behavioural and adrenocortical responses to nicotine measured in rats with selective lesions of the 5-hydroxytryptaminergic fibres innervating the hippocampus. British Journal of Pharmacology, 89, 341-347.

Barnes, G.E., & Fishlincki, M. (1976). Stimulus intensity modulation, smoking and craving for cigarettes. Addictive Diseases: An International Journal, 2(3), 479-484.

Baum, A., Grunberg, N.E., & Singer, J.E. (1982). The use of psychological and neuroendocrinological measurements in the study of stress. Health Psychology 1, 217-236.

Benwell, M.E.M., & Balfour, D.J.K. (1979). The effects of nicotine administration and its withdrawal on plasma corticosterone and brain 5-hydroxyindoles. Psychopharmacology, 63,7-11.

Benwell, M.E.M., & Balfour, D.J.K. (1982a). The effects of nicotine administration on 5-HT uptake and biosynthesis in rat brain. European Journal of Pharmacology, 84,71-77.

Benwell, M.E.M., & Balfour, D.J.K. (1982b). Effects of chronic nicotine administration on the response and adaptation to stress. Psychopharmacology, 76, 160-162.

Blair, R., Liran, J., Cytryniak, H., Shizgal, P., & Amit, Z. (1978). Explosive motor behavior, rigidity and periaqueductal gray lesions. Neuropharmacology, 17, 205-210.

Bradley, M.M., Cuthbert, B.N., & Lang, P.J. (1990). Startle reflex modification: Emotion or attention? Psychophysiology, 27(5), 513-522.

Braff, D.L., Grillon, C., & Geyer, M.A. (1992). Gating and habituation of the startle reflex in schizophrenic patients. Archives of General Psychiatry, 49, 206-215.

Braff, D.L., Stone, C., Callaway, E., Geyer, M.A., Glick, I.D., & Bali, L. (1978). Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology, 15, 339-343.

Butler, R.W., Braff, D.L., Rausch, J.L, Jenkins, M., Sprock, J., & Geyer, M.A. (1990). Physiologic Evidence of exaggerated startle response in a subgroup of Vietnam veterans with combat-related PTSD. American Journal of Psychiatry, 147(10), 1308-1312.

Cannon, W.B. (1914). The emergency function of the adrenal medulla in pain and the major emotions. American Journal of Physiology, 33, 356-372.

Casada, J.H., & Dafny, N. (1990). Restraint stress modulates sensory evoked potentials. International Journal of Neuroscience, 53(2-4), 265-274.

Clarke, P.B.S., Fu D., Jakubovic, A., & Fibiger, H.C. (1988). Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. Journal of Pharmacology and Experimental Therapeutics, 246, 701-708.

Cohen, F., Horowitz, M.J., Lazarus, R.S., Moos, R.H., Robins, L.N., Rose, R.M., & Rutter, M. (1982). Panel report on psychosocial modifiers of stress. In G.R. Eliott & C. Eisendorfer (Eds.) Stress and Human Health. New York: Springer Publishing Co.

Collins, A.C., Bhat, R.V., Pauly, J.R., & Marks, M.J. (1990). Modulation of nicotine receptors by chronic exposure to nicotinic agonists and antagonists. Ciba Foundation Symposium 152, 68-82.

Collins, A.C., Evans, C.B., Miner, L.L., & Marks, M.J. (1986). Mecamylamine blockade of nicotine responses: evidence for two brain nicotinic receptors. Pharmacology Biochemistry and Behavior, 24, 1767-1773.

Collins, A.C., Miner, L.L., & Marks, M.J. (1988). Genetic influences on acute responses to nicotine and nicotine tolerance in the mouse. Pharmacology Biochemistry and Behavior, 30, 269-278.

Commissaris, R.L., Harrington, G.M., Baginski, T.J., & Altman, H.J. (1988). MR/Har and MNRA/Har Maudsley rat strains: differences in acoustic startle habituation. Behavior Genetics, 18(6), 663-669.

Coover, G.D., & Levine, S. (1972). Auditory startle response of hippocampectomized rats. Physiology and Behavior, 9, 75-78.

Davis, M. (1984). The mammalian startle response. In R. Eaton (Ed.) Neural mechanisms of startle behavior. New York: Plenum Press, 287-351.

Davis, M. (1985). Cocaine: Excitatory effects on sensorimotor reactivity measured with acoustic startle. Psychopharmacology, 86, 31-36.

Davis, M. (1986). Pharmacological and anatomical analysis of fear conditioning using the fear-potentiated startle paradigm. Behavioral Neuroscience, 100, 814-824.

Davis, M. (1988). Apomorphine, *d*-amphetamine, strychnine and yohimbine do not alter prepulse inhibition of the acoustic startle reflex. Psychopharmacology, 95, 151-156.

Davis, M. (1989). Sensitization of the acoustic startle reflex by footshock. Behavioral Neuroscience, 103, 495-503.

Davis, M., & Astrachan, D.I. (1978). Conditioned fear and startle magnitude: Effects of different footshock or backshock intensities used in training. Journal of Experimental Psychology, Animal Behavior Processes, 4, 95-103.

Davis, M., & Sollberger, A. (1971). Twenty-four hour periodicity of the startle response in rats. Psychonomic Science 25:37-39.

Davis, M., & Zolovick, A.J. (1972). Habituation of the startle response in adrenalectomized rats. Physiology and Behavior, 8, 579-584.

Davis, M., Mansbach, R.S., Swerdlow, N.R., Campeau, S., Braff, D.L., & Geyer, M.A. (1990). Apomorphine disrupts the inhibition of acoustic startle induced by weak prepulses in rats. Psychopharmacology, 102, 1-4.

Davis, M., Svensson, T.H., & Aghajanian, G.K. (1975). Effects of *d*- and *l*- amphetamine on habituation and sensitization of the acoustic startle response in rats. Psychopharmacology, 43, 1-11.

Davis, M., Cedarbaum, J.M., Aghajanian, G.K., & Gendelman, D.S. (1977). Effects of clonidine on habituation and sensitization of acoustic startle in normal, decerebrate and locus coeruleus lesioned rats. Psychopharmacology, 51, 243-253.

Domino, E.F., & von Baumgarten, A.M. (1969). Tobacco cigarette smoking and patellar reflex depression. Clinical Pharmacology & Therapeutics, 10, 72-79.

Dunn, A.J. (1988). Stress-related activation of cerebral dopaminergic systems. In P. W. Kalivas & C. B. Nemeroff, (Eds.), The mesocorticolimbic dopamine system, Annals of the New York Academy of Sciences, 537. New York: New York Academy of Sciences.

Epstein, L.H., Dickson, B.E., McKenzie, S., & Russell, P.O. (1984). The effect of smoking on perception of muscle tension. Psychopharmacology, 83, 107-113.

Eysenck, H.J. (1965). Smoking, health and personality. New York: Basic Books.

Eysenck, H.J. (1973). Personality and the maintenance of the smoking habit. In W.L. Dunn (Ed.), Smoking behavior: Motives and incentives. Washington, D. C.: V.H. Winston & Sons, 113-146.

Fisher, S. (1984). Stress and the perception of control. Hillsdale: Lawrence Erlbaum, 89-119.

Flores, C.M., Hernandez, M.C., Hargreaves, K. M., & Bayer, B. M. (1990). Restraint stress-induced elevations in plasma corticosterone and beta-endorphin are not accompanied by alterations in immune function. Journal of Neuroimmunology, 28(3), 219-225.

Gage, F.H. (1978). Septal hyperreactivity: a multivariate analysis of neuroanatomical correlates. Physiological Psychology, 6, 314-318.

Geyer, M.A., & Braff, D.L. (1982). Habituation of the blink reflex in normals and schizophrenic patients. Psychophysiology, 19, 1-6.

Gilbert, D.G., & Hagen, R.L. (1980). The effects of nicotine and extraversion on self-report, skin conductance, electromyographic and heart responses to emotional stimuli. Addictive Behaviors, 5, 247-257.

Gilbert, D.G., & Spielberger, C.D. (1987). Effects of smoking on heart rate, anxiety, and feelings of success during social interaction. Journal of Behavioral Medicine, 10, 629-638.

Glavin, G.B. (1985). Stress and brain noradrenalin: A review. Neuroscience and Biobehavioral Reviews, 9, 233-243.

Gleason, K.K., & Reynierse, J. H. (1969). The behavioral significance of pheromones in vertebrates. Psychological Bulletin, 71(1), 58-73.

Groves, P.M., & Thompson, R.F. (1970). Habituation: A dual-process theory. Psychological Review, 77, 415-450.

Grunberg, N.E. (1988). Nicotine and body weight: behavioral and biological mechanisms. In M. Rand & K. Thurau (Eds.), The pharmacology of nicotine: ICSU symposium series. Washington, D.C.: IRL Press, 97-110.

Grunberg, N.E. (1990). The inverse relationship between tobacco use and body weight. In L.T. Kozlowski, H.M. Annis, H.D. Cappell, F.B. Glaser, M.S. Goodstadt, Y. Israel, H. Kalant, E.M. Sellers, & E.R. Vingilis, (Eds.) Research advances in alcohol and drug problems, 9, New York: Plenum Press, 273-315.

Grunberg, N.E., & Baum, A. (1985). Biological commonalties of stress and substance abuse. In S. Shiffman & T. Wills, (Eds.) Coping and substance use. New York: Academic Press, 25-62.

Grunberg, N.E., Bowen, D.J., & Morse, D.E. (1984). Effects of nicotine on body weight and food consumption in rats. Psychopharmacology, 83, 93-98.

Grunberg, N.E., Popp, K.A., Bowen, D.J., Nespor, S.M., Winders, S.E., & Eury, S.E. (1988). Effects of chronic nicotine administration on insulin, glucose, epinephrine and norepinephrine. Life Sciences, 42, 161-170.

Guilford, J.S. (1966). Factors related to successful abstinence from smoking. Pittsburgh PA: American Institutes for Research.

Hall, G.H. (1982). Pharmacological responses to the intracerebral administration of nicotine. Pharmacology & Therapeutics, 15, 223-238.

Harty, T.P., & Davis, M. (1985). Cocaine: effects on acoustic startle and startle elicited electrically from the cochlear nucleus. Psychopharmacology, 87, 396-399.

Hatch, J.P., Bierner, S.M., & Fisher, J.G.(1983). Effects of smoking and cigarette nicotine content on smokers' preparation and performance of a psychosocially stressful task. Journal of Behavioral Medicine, 6(2), 207-216.

Hatsukami, D. K., Dahlgren, L., Zimmerman, R., & Hughes, J. R. (1988). Symptoms of tobacco withdrawal from total cigarette cessation versus partial cigarette reduction. Psychopharmacology, 94, 242-247.

Helton, D., Tizzano, J., Modlin, D., & Rasmussen, K. (1991). Nicotine tolerance and dependence: A behavioral assessment using schedule controlled responding, locomotor activity and sensorimotor reactivity. Society for Neuroscience Abstracts, 17, 331.

Hughes, J.R., & Hatsukami, D.K. (1986). Signs and symptoms of tobacco withdrawal. Archives of General Psychiatry, 43, 289-294.

Hughes, J.R., & Hatsukami, D.K. (1992). The nicotine withdrawal syndrome: a brief review and update. International Journal of Smoking Cessation, 1, 21-26.

Hughes, J.R., Hatsukami, D.K., Pickens, R.W., & Svikis, D.S. (1984a). Consistency of the tobacco withdrawal syndrome. Addictive Behaviors, 9, 409-412.

Hughes, J.R., Hatsukami, D.K., Pickens, R. W., Krahn, D., Malin, S., & Luknic, A. (1984b). Effect of nicotine on the tobacco withdrawal syndrome. Psychopharmacology, 83, 82-87.

Ikard, F.F., & Tomkins, S. (1973). The experience of affect as a determinant of smoking behavior: A series of validity studies. Journal of Abnormal Psychology, 81(2), 172-181.

Imperato, A., Mulas, A., & DiChiara, G. (1986). Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. European Journal of Pharmacology, 132, 337-338.

Jarvik, M.E., Caskey, N.H., Rose, J.E., Herskovik, J.E., & Sadeghpour, M. (1989). Anxiolytic effects of smoking associated with four stressors. Addictive Behaviors, 14, 379-386.

Jin, P. (1992). Toward a reconceptualization of the law of initial value. Psychological Bulletin, 3, 176-184.

Kiritsy-Roy, J.A., Mousa, S.A., Appel, N.M., & Van Loon, G.R. (1990). Tolerance to nicotine-induced sympathoadrenal stimulation and cross-tolerance to stress: differential central and peripheral mechanisms in rats. Neuropharmacology, 29(6), 579-589.

Kleinke, C.L., Staneski, R.A., & Meeker, F.B. (1983). Attributions for smoking behavior: Comparing smokers with nonsmokers and predicting smokers' cigarette consumption. Journal of Research in Personality, 17, 242-255.

Knott, V.J. (1986). Tobacco effects on cortical evoked potentials to task stimuli. Addictive Behaviors, 11, 219-223.

Kokkinidis, L., & Anisman, H. (1978). Involvement of norepinephrine in startle arousal after acute and chronic d-amphetamine administration. Psychopharmacology, 59, 285-292.

Kokkinidis, L., & MacNeill, E.P. (1982). Stress-induced facilitation of acoustic startle after d-amphetamine administration. Pharmacology Biochemistry and Behavior, 17, 413-417.

Kokkinidis, L., Zacharko, R.M., & Anisman, H. (1986). Amphetamine withdrawal: a behavioral evaluation. Life Sciences, 38, 1617-1623.

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

Korn, J.H., & Moyer, K.E. (1965). The effects of pre-shock and handling on the startle response in the rat. Psychonomic Science, 3, 409-410.

Lazarus, R.S. (1977). Cognitive and coping processes in emotion. In A. Monat & R.S. Lazarus (Eds.) Stress and coping: An anthology. New York: Columbia University Press.

Leitner, D.S., & Cohen, M.E. (1985). Role of the inferior colliculus in the inhibition of acoustic startle in the rat. Physiology and Behavior, 34, 65-70.

Leitner, D.S. (1989). Multisensory deficits in rats produced by exposure to cold swim stress. Behavioral Neuroscience, 103(1), 151-157.

Leitner, D.S., & Cohen, M.E. (1988). Tectal modulation of acoustic startle in the rat. Society for Neuroscience Abstracts, 14, 1100.

Leitner, D.S., Powers, A.S., Stitt, C.L., & Hoffman, H.S. (1981). Midbrain reticular formation involvement in the inhibition of acoustic startle. Physiology and Behavior, 26, 259-268.

Levin, E.L., Lee, C., Rose, J.E., Reyes, A., Ellison, G., Jarvik, M., & Gritz, E. (1990). Chronic nicotine and withdrawal effects on radial-arm maze performance in rats. Behavioral and Neural Biology, 53, 269-276.

Livson, N., & Leino, E.V. (1988). Cigarette smoking motives: Factorial structure and gender differences in a longitudinal study. The International Journal of the Addictions, 23(6), 535-544.

MacDougall, J.M., Dembroski, T.M., Slaats, S., Herd, J.A., & Eliot, R.S. (1983). Selective cardiovascular effects of stress and cigarette smoking. Journal of Human Stress, 9(3), 13-21.

Mackay-Sim, A., & Laing, D.G. (1980). Discrimination of odors from stressed rats by non-stressed rats. Physiology and Behavior, 24, 699-704.

Mackay-Sim, A., & Laing, D.G. (1981a). Rats' responses to blood and body odors of stressed and non-stressed conspecifics. Physiology and Behavior, 27, 503-510.

Mackay-Sim, A., & Laing, D.G. (1981b). The sources of odors from stressed rats. Physiology and Behavior, 27, 511-513.

Mansbach, R.S., Gold, L.H., & Harris, L.S. (1991). The acoustic startle response as a measure of behavioral dependence in rats. Neuroscience Abstracts, 17, 331.

Mellgren, R.L. (1969). Magnitude of the startle response and drive level. Psychological Reports, 25, 187-193.

Meryman, J.J. (1952). Magnitude of startle response as a function of hunger and fear. Unpublished master's thesis, University of Iowa.

Mifsud, J.C., Hernandez, L., & Hoebel, B.G. (1989). Nicotine infused directly into the nucleus accumbens increases synaptic dopamine as measured by in vivo microdialysis. Brain Research, 478, 365-367.

Monat, A., & Lazarus, R.S. (1977). Introduction, In A. Monat & R.S. Lazarus (Eds.) Stress and coping: An anthology. New York: Columbia University Press, 1-11.

Moos, R.H., & Billings, A.G. (1982). Conceptualizing and measuring coping resources and processes. In L. Goldberger & S. Breznitz (Eds.), Handbook of stress: theoretical and clinical aspects. New York: MacMillan.

Morse, D.E. (1989). Neuroendocrine responses to nicotine and stress: enhancement of peripheral stress responses by the administration of nicotine. Psychopharmacology, 98, 539-543.

Morrison, C.F. (1974). Effects of nicotine and its withdrawal on the performance of rats on signalled and unsignalled avoidance schedules. Psychopharmacologia, 38, 25-35.

Myrtek, M. & Foerster, F. (1986). The law of initial value: A rare exception. Biological Psychology, 22, 227-237.

Nelsen, J.M. (1978). Psychological consequences of chronic nicotinization: A focus on arousal. In K.Battig (Ed.), Behavioural effects of nicotine. Basel: S. Karger, 1-17.

Nesbitt, P.D. (1973). Smoking, physiological arousal, and emotional response. Journal of Personality and Social Psychology, 25, 137-144.

Nuechterlein, K., & Dawson, M. (1984). Information processing and attentional functioning in the developmental course of schizophrenic disorders. Schizophrenia Bulletin, 10, 160-202.

Newhouse, P.A., Sunderland, T., Tariot, P.N., Blumhardt, C.L., Weingartner, H., & Mellow, A. (1988). Intravenous nicotine in Alzheimer's disease: A pilot study. Psychopharmacology, 95, 171-175.

Newhouse, P.A., Sunderland, T., Narang, P.K., Mellow, A.M., Fertig, J.B., Lawlor, B.A., & Murphy, D.L. (1990). Neuroendocrine, physiologic and behavioral responses following intravenous nicotine in nonsmoking healthy volunteers and in patients with Alzheimer's Disease. Psychoneuroendocrinology, 15(5-6), 471-484.

Ornitz, E. M. & Pynoos, R.S. (1989). Startle modulation in children with posttraumatic stress disorder. American Journal of Psychiatry, 146(7), 866-870.

Overstreet, D.H. (1977). Pharmacological approaches to habituation of the acoustic startle response in rats. Physiological Psychology, 5(2), 230-238.

Overstreet, D.H., Janowsky, D.S., Gillin, J.C., Shiromani, P.J., & Sutin, E.L. (1986). Stress-induced immobility in rats with cholinergic supersensitivity. Biological Psychiatry, 21(7), 657-664.

Paris, J.M., Lorens, S. A., Van de Kar, L.D., Urban, J.H., Richardson-Mortin, K.D., & Bethea, C.L. (1987). A comparison of acute stress paradigms: Hormonal responses and hypothalamic serotonin. Physiology and Behavior, 39, 33-43.

Parisi, T., & Ison, J.R. (1979). Development of the acoustic startle response in the rat: Ontogenetic changes in the magnitude of inhibition by prepulse stimulation. Developmental Psychobiology, 12, 219-230.

Pauly, J.R., Ullman, E.A., & Collins, A.C. (1988). Adrenocortical hormone regulation of nicotine sensitivity in mice. Physiology and Behavior, 44, 109-116.

Pavlasek, J. (1989). The startle reaction to an acoustic stimulus in the rat: The effect of noradrenaline administered by microinjection into the pontomedullary reticular formation. Physiologia Bohemoslovaca, 38, 81-91.

Pearlin, L.I., & Schooler, C. (1978). The structure of coping. Journal of Health and Social Behavior, 19, 2-21.

Peng, R.Y., Mansbach, R.S., Braff, D.L., & Geyer, M.A. (1990). A D2 dopamine receptor agonist disrupts sensorimotor gating in rats. Neuropsychopharmacology, 3(3), 211-218.

Perkins, K.A., Epstein, L.H., Jennings, J.R., & Stiller, R. (1986). The cardiovascular effects of nicotine during stress Psychopharmacology, 90(3), 373-8.

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

Piazza, P.V., Deminiere, J-M., Maccari, S., Le Moal, M., Mormede, P. & Simon, H. (1991). Individual vulnerability to drug self-administration: action of corticosterone on dopaminergic systems as a possible pathophysiological mechanism. In P. Willner & J. Scheel-Kruger (Eds.), The mesolimbic dopamine system: from motivation to action, Chichester: Wiley & Sons, 473-495.

Piazza, P.V., Deminiere, J-M., Maccari, S., Mormede, P., Le Moal, M., and Simon, H. (1990). Individual reactivity to novelty predicts probability of amphetamine self-administration. Behavioural Pharmacology, 1, 339-345.

Pohorecky, L.A., Cagan, M., Brick, J., & Jaffe, L.S. (1976). Startle response in rats: effects of ethanol. Pharmacology Biochemistry and Behavior, 4, 311-316.

Pomerleau, O.F., & Pomerleau, C.S. (1984). Neuroregulators and the reinforcement of smoking: towards a biobehavioral explanation. Neuroscience and Biobehavioral Reviews, 8, 503-513.

Pomerleau, O.F., Turk, D.C., & Fertig, J.B. (1984). The effects of cigarette smoking on pain and anxiety. Addictive Behaviors, 9, 265-271.

Rattner, B.A., Gruenau, S.P. & Atland, P.D. (1979). Crossadaptive effects of cold, hypoxia, or physical training on decompression sickness in mice. Journal of Applied Physiology, 47, 412.

Rigdon, G.C. (1990). Differential effects of apomorphine on prepulse inhibition of acoustic startle reflex in two rat strains. Psychopharmacology, 102, 419-421.

Rose, J.E, Ananda, S., & Jarvik, M.E. (1983). Cigarette smoking during anxiety-provoking and monotonous tasks. Addictive Behaviors 8, 353-359.

Sahakian, B.J., Jones, G.M.M., Levy, R., Gray, J.A., & Warburton, D.M. (1990). Nicotine and human cognition In Effects of Nicotine on Biological Systems, Proceedings of the International Symposium on Nicotine, Hamburg, FRG: German Research Council.

Schachter, S. (1978). Pharmacological and Psychological Determinants of Smoking. Annals of Internal Medicine, 88, 104-114.

Schachter, S., Kozlowski, L.T., & Silverstein, B. (1977). Effects of urinary pH on cigarette smoking. Journal of Experimental Psychology, General, 106(1), 13-19.

Selye, H. (1973). Some introductory remarks. In W.L.Dunn (Ed.), Smoking Behavior: Motives and incentives. Washington, D. C.: V.H. Winston & Sons, 1-3.

Seyler, L.E., Pomerleau, O.F., Fertig, J., Hunt, D., & Parker, K. (1986). Pituitary hormone response to cigarette smoking. Pharmacology Biochemistry and Behavior, 24, 159-162.

Sharp, B.M., Beyer, H.S., Levine, A.S., Morley, J.E., & McAllen, K.M. (1987). Attenuation of the plasma prolactin response to restraint stress after acute and chronic administration of nicotine in rats. Journal of Pharmacology and Experimental Therapeutics, 241(2), 438-42.

Shiffman, S. (1982). Relapse following smoking cessation: A situational analysis. Journal of Consulting and Clinical Psychology, 50(1), 71-86.

Shiffman, S. (1985). Coping with temptations to smoke. In S. Shiffman & T.A. Wills (Eds.), Coping and substance use, New York: Academic Press, 223-240.

Silverstein, B. (1982). Cigarette smoking, nicotine addiction, and relaxation. Journal of Personality and Social Psychology, 42, 946-950.

Simons, R.F., & Zelson, M.F. (1985). Engaging visual stimuli and reflex blink modulation. Psychophysiology, 22(1), 44-49.

Solomon, R.L. (1977). An opponent-process theory of acquired motivation IV: the affective dynamics of addiction. In J. Masur & M. Seligman (Eds.), Psychopathology: Experimental Models. San Francisco: W.H. Freeman, 66-103.

Solomon, R.L. (1980). An opponent-process theory of acquired motivation: the costs of pleasure and the benefits of pain. American Psychologist, 35(8), 691-712.

Sorenson, C.A., & Swerdlow, N.R. (1982). The effect of tail pinch on the acoustic startle response in rats. Brain Research, 247, 105-113.

Spilich, G., June, L., & Renner, J. (in press). Cigarette smoking and cognition: Performance up in smoke? British Journal of Addiction.

Stern, W. (1971). Effects of desynchronized sleep deprivation upon startle response habituation in rats. Psychonomic Science, 23, 31-32.

Stern, R.M. & Sison, C.E. (1990). Response patterning. In J. T. Cacioppo & L.G. Tassinary (Eds.) Principles of Psychophysiology. New York: Cambridge University Press, 193-215.

Swerdlow, N.R., Braff, D.L., Geyer, M.A., & Koob, G.F. (1986). Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. Biological Psychiatry, 21, 23-33.

Swerdlow, N.R., Geyer, M.A., Vale, W.W., & Koob, G.F. (1986). Corticotropin releasing factor potentiates acoustic startle in rats: blockade by chlordiazepoxide. Psychopharmacology, 88, 147-152.

Swerdlow, N.R., Koob, G.F., Geyer, M.A., Mansbach, R., & Braff, D.L. (1988). A cross-species model of psychosis. Animal Models of Psychiatric Disorders, 2, Basel: Karger, 1-19.

Taylor, P. (1985). Ganglionic stimulating and blocking agents. In A.G. Gilman, L.S. Goodman, T.W. Rall, & F. Murad (Eds.), The pharmacologic basis of therapeutics. New York: MacMillan Publishing Co., 215-221.

Theeuwes, F., & Yum, S.I. (1977). Principles of the design and operation of generic osmotic pumps for the delivery of semisolid or liquid drug formulations. Annals of Biomedical Engineering, 4, 343-353.

Thompson, R.F., & Spencer, W.A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychological Review, 73, 16-43.

USDHHS (1988). The health consequences of smoking: nicotine addiction. A report of the Surgeon General. DHHS Pub. No. (CDC) 88-8406, Washington, D. C.: U.S. Government Printing Office, 75-143, 375-456.

USDHHS (1989). Reducing the health consequences of smoking: 25 years of progress. A report of the Surgeon General. DHHS Pub. No. (CDC) 89-8411, Washington, D. C.: U.S. Government Printing Office.

Valenta, J.G., & Rigby, M.K. (1968). Discrimination of the odor of stressed rats. Science, 161, 599-601.

van Riezen, H., Schneider, H., & Wren, A.F. (1977). Olfactory bulb ablation in the rat: behavioural changes and their reversal by antidepressant drugs. British Journal of Pharmacology, 60, 521-528.

Vrana, S.R., & Lang, P.J. (1990). Fear imagery and the startle-probe reflex. Journal of Abnormal Psychology, 99(2), 189-197.

Warburton, D.M. (1989). Nicotine: An addictive substance or a therapeutic agent? Progress in Drug Research, 33, 9-41.

Warburton, D.M., & Groves, P.M. (1969). The effects of scopolamine on habituation of acoustic startle in rats. Communications in Behavioral Biology, 3, 289-293.

Warburton, D.M., & Walters, A.C. (1989). Attentional processing In T. Ney, T. & A. Gade (Eds.), Smoking and Human Behavior. Chichester: Wiley & Sons.

Wasserman, E.A., & Jensen, D.D. (1969). Olfactory stimuli and the "pseudo-extinction" effect. Science, 166, 1307-1309.

Wesnes, K. (1987). Nicotine increases mental efficiency: But how? In W.R. Martin, G.R. Van Loon, E.T. Iwamoto, & D.L. Davis (Eds.) Tobacco smoke and nicotine. A neurobiological approach. New York: Plenum Press, 63-79.

Wesnes, K., & Warburton, D.M. (1978). The effects of cigarette smoking and nicotine tablets upon human attention. In R.E. Thornton (Ed.), Smoking behavior: physiological and psychological influences, London: Churchill-Livingston, 131-147.

Wesnes, K., & Warburton, D.M. (1983). Smoking, nicotine and human performance. Pharmacology & Therapeutics, 21, 189-208.

Wesnes, K., & Warburton, D.M. (1984a). Effects of scopolamine and nicotine on human rapid information processing performance. Psychopharmacology, 82, 147-150.

Wesnes, K., & Warburton, D.M. (1984b). The effects of cigarettes of varying yield on rapid information processing performance. Psychopharmacology, 82, 338-342.

Wesnes, K., Warburton, D.M., & Matz, B. (1983). Effects of nicotine on stimulus sensitivity and response bias in a visual vigilance task. Neuropsychobiology, 9, 41-44.

West, R., & Russell, M.A.H. (1988). Loss of acute nicotine tolerance and severity of cigarette withdrawal. Psychopharmacology, 94, 563-565.

Wilder, J. (1967). Stimulus and response: the law of initial values. Bristol: Wright.

Wills, T.A., & Shiffman, S. (1985). Coping and substance use: A conceptual framework. In S. Shiffman & T.A. Wills, (Eds.) Coping and substance use, New York: Academic Press, 3-21.

Winders, S. (1990, unpublished doctoral dissertation). The effects of stress on levels of nicotine in the rat. Bethesda, MD: Uniformed Services University of the Health Sciences.

Woodson, P.P., Buzzi, R., Nil, R., & Battig, K. (1986). Effects of smoking on vegetative reactivity to noise in women. Psychophysiology, 23(3), 272.

Yamanaka, K., Muramatsu, I., & Kigoshi, S. (1987). Effect of chronic nicotine treatment against repeated immobilization stress. Pharmacology Biochemistry and Behavior, 26(2), 259-63.