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Dedication

To Jeff...Until we meet again

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Abstract

Compared to other drugs of abuse, studies of the effects of nicotine on intracranial self-stimulation (ICSS) are few in number. Since the effect of nicotine on reward is not well established, one goal of the present study was to clarify the characteristics of nicotine-induced changes in brain stimulation reward using a rigorously validated, rate-free and reward-selective procedure. Once established, the second goal was to assess the ability of specific dopamine (sulpiride), serotonin (MDL 26,508), and mixed dopamine/serotonin (MDL 28,133A) antagonists to modify nicotine's effects.

Results showed that nicotine (0.1 to 1.0 mg/kg) produced a dose-dependent lowering of ICSS thresholds and that this effect did not tolerate with repeated administration. The effectiveness of the antagonists in attenuating or blocking nicotine's effect ranked in the order: MDL 28,133A > sulpiride > MDL 26,508. Although MDL 26,508 produced no observable effects alone, it appears that the presence of a specific 5-HT_2 antagonism contributes to the reversal of nicotine's effect by MDL 28,133A. These results suggest that drugs with mixed D_2/5-HT_2 antagonist properties may be effective pharmacotherapies for nicotine dependence. However, conclusive statements about dopaminergic and serotonergic mediation of nicotine reward will require additional neuropharmacological and electrophysiological studies.

List of Tables

List of Figures

Table of Contents

Nicotine is a naturally occurring alkaloid in tobacco leaves (Gilman, Goodman, Rall & Murad, 1985), and was first isolated from the plant Nicotiana tabacum in 1828 by Posselet and Reiman (Taylor, 1985). The oldest cited evidence of tobacco use appears on a Mayan stone carving dated AD 629 (Corti, 1931), which shows a Mayan priest smoking a cigar or reed pipe. At that time, tobacco was smoked and chewed for a variety of narcotic, stimulant, medicinal, social and religious purposes (Mangan & Golding, 1984).

At the time of Columbus, tobacco usage was prevalent throughout the Americas. In the 16th century, tobacco was brought from the New World to Europe by explorers such as Sir Walter Raleigh, Andre Thevet and Jean Nicot (from whom the tobacco plant was named), and its usage gained rapid popularity in Europe and other parts of the world. In the 16th and 17th centuries pipe smoking was the most common form of use. In the 18th century the "snuffing" of finely chopped tobacco became prevalent, and, in the 19th century the chewing of tobacco and cigar smoking rose in popularity. In the late 1800's improvements in plant culture, leafcuring and the introduction of cigarette-making machines provided favorable conditions for a massive growth in tobacco consumption. Then, as today, the positive and negative effects of tobacco products were being debated and, as early as 1920, some investigators were concluding that nicotine was responsible for the compulsive use of tobacco products (Armstrong-Jones, 1927; Dorsey, 1936).

Despite a strong anti-tobacco lobby, the percentage of people in the United States who used tobacco products continued to increase until the mid-1960's when, in 1964,

the U.S. Surgeon General's report concluded that cigarette smoking is a cause of lung cancer in men and a suspected cause of lung cancer in women, and that smoking increased the risk of dying from pulmonary emphysema (US Public Health Service, 1964). Since the 1964 US Surgeon General's Report linking cigarette smoking with lung cancer, subsequent reports have shown that smoking causes coronary and peripheral vascular disease (US Department of Health and Human Services, 1983), chronic obstructive lung disease (US Department of Health and Human Services, 1984), peptic ulcer disease, and reproductive disturbances. The National Health Institute Surveys (1965 to 1985) have indicated a steady decline in smoking prevalence beginning in the late 1960's from 42.4% in adults 20 years of age and older, to 30.4% in 1985 (US Department of Health and Human Services, 1987). This decrease was present in all age, sex, and race categories with the exception of women age 65 years and older.

Nicotine as a Reinforcer

Due in part to the health hazards associated with smoking, along with declining social acceptability of smoking, the proportion of smokers who would like to quit increased from 66% in 1977 to 77% in 1987 (Gallup, 1987). However, human smoking behavior is a complex process with both psychological and pharmacological components. It is assumed that most people smoke tobacco to obtain nicotine as evidenced by recent studies demonstrating that animals can learn to self-administer this substance (Goldberg, Spealman & Goldberg, 1981; Nelson & Cox, 1982).

Although the pharmacology of nicotine has been extensively studied, little is known about the neurobiological mechanisms that are responsible for its reinforcing properties.

The primary mechanism by which addictive drugs maintain drug use is by functioning as positive reinforcers. Whether or not a drug is reinforcing depends upon the dose of the drug, previous exposure of the subject to that or other drugs, the behavioral history of the subject, and contingencies relating responses and subsequent injections of the drug (Brady,1991). In habitual smokers, nicotine clearly acts as a reinforcer, i.e., (1) nicotine is self-administered by human volunteers deprived of cigarettes (Henningfield & Goldberg, 1983), (2) nicotine polacrilex gum shows evidence of being an adequate substitute for tobacco and helps alleviate withdrawal symptoms associated with smoking cessation (Hughes, Hatsukami, Pickens, Krahn, Malin & Luknic, 1984) and, (3) pretreatment with mecamylamine (a central nicotinic antagonist) in humans results in a decrease in smoking behavior (Tennant, Tarver & Rawson, 1983).

Early animal studies employing intravenous (IV) injections found that nicotine was ineffective or marginally effective as a reinforcer. Until recently, most experiments on nicotine self-administration involved continuous reinforcement schedules in which each response by an individual subject resulted in an IV injection of nicotine [see Henningfield & Goldberg (1983) for review]. Under continuous reinforcement schedules, (1) rates of responding were low (ranging from about 0.008 to 0.005 response per second), (2) changes in nicotine dose produced only small and

inconsistent changes in rate of responding, (3) rate of responding was relatively insensitive to pretreatment with mecamylamine (a nicotinic antagonist), (4) differences in rates of responding maintained by nicotine compared with saline were generally small and, (5) marked intersubject differences in self-administration of nicotine were often reported.

Although initial studies of nicotine IV self-administration resulted in low, but significant, rates of responding, later research showed that, under intermittent schedules of reinforcement (fixed ratio and fixed interval schedules), nicotine is an effective reinforcer that maintains persistent drug-seeking behavior in both animals and humans (Corrigall & Coen, 1989; Goldberg & Henningfield, 1988). It appears that intermittent schedules more closely approximate the patterns of human cigarette smoking behavior where nicotine is administered in intermittent doses (i.e., puffs) and with a larger interval between dosing times as a result of time between cigarettes (Henningfield, 1984). Intermittent schedules also allow animals to adjust their levels to any preferred value, just as humans adjust their smoking behavior to regulate what appears to be an individually determined range of nicotine levels in their body (Benowitz & Jacob, 1984).

Physiological Changes Associated with Nicotine

Nicotine is a potent, psychoactive drug with diverse effects. Depending on dose, history of nicotine exposure and the particular biological systems under study, nicotine produces a complex array of stimulant and depressant effects involving

central and peripheral, cardiovascular, endocrine, gastrointestinal, reproductive, pulmonary, and skeletal motor systems (US Department of Health and Human Services, 1988).

Nicotine has both a stimulant and a depressant phase of action (Gilman et al., 1985). In the peripheral nervous system, nicotine produces a transient stimulation followed by a depression of all autonomic ganglia function. Small doses of nicotine directly stimulate ganglion cells and directly facilitate transmission of impulses, while with larger doses, the initial stimulation is rapidly followed by a blockade of transmission. Also within the peripheral nervous system, nicotine causes the release of catecholamines in isolated organs, which results in a sympathetic response to nicotine.

The effect of nicotine in the central nervous system is primarily that of a stimulant. At the appropriate doses, nicotine will produce tremors in humans and laboratory animals and, with larger doses (40 to 60 mg in humans: Goldfrank, Melinek & Blum, 1980) the tremor is followed by convulsions and frequently death. At high doses, the initial stimulation of the central nervous system is followed by depression (occurring in the medulla oblongata), and death results from failure of respiration due to both central paralysis and peripheral blockade of respiratory muscles (Gilman et al., 1985). In animals, nicotine administration is accompanied by electroencephalogram (EEG) activation (Domino, 1967). However, at the same time, there is decreased skeletal muscle tone, decreased amplitude in the electromyogram, and a decrease in deep tendon reflexes, due primarily to stimulation of Renshaw cells

in the spinal cord (Domino, 1973).

The cardiovascular responses to nicotine are primarily due to the stimulation of sympathetic ganglia and the adrenal medulla, along with the release of catecholamines from sympathetic nerve endings (Gilman et al., 1985). Activation of chemoreceptors in the aortic and carotid bodies results in vasoconstriction, tachycardia and elevated blood pressure (Grunberg & Baum, 1985).

In the gastrointestinal tract, the effects of nicotine are largely due to parasympathetic stimulation (Thompson & George, 1972). The combined activation of parasympathetic ganglia and cholinergic nerve endings results in increased tone and motor activity of the bowel and systemic absorption of nicotine is followed by nausea, vomiting and diarrhea (Mangan & Golding, 1984). In animals, nicotine potentiates peptic ulcer formation induced by histamine or pentagastrin (both of which stimulate the secretion of gastric acid) (Konturek, Radecki, Thor, Dembinski & Jacobson, 1971).

In humans, cigarette smoking during pregnancy increases the risk of low birth weight, prematurity, spontaneous abortion and perinatal mortality, which has been referred to as the fetal tobacco syndrome (Nieburg, Marks, McLaren & Remington, 1985). Divers, Wilkes, Babaknia & Yen (1981) have suggested that nicotine contributes to the adverse effects of cigarette smoking on reproduction by acting on utero-placental circulation.

Cigarette smoking is the major cause of chronic obstructive lung disease (US Department of Health and Human Services, 1984). Nicotine may directly or

indirectly contribute to the development of emphysema in smokers (Lai & Diamond, 1987). Nicotine can also worsen pulmonary function in smokers who already have lung disease. Acute exposure to nicotine causes constriction of both central and peripheral airways (Yamatake, Sasagawa & Yanaura, 1978). The increase in airway resistance produced by nicotine involves vagal reflexes and stimulation of parasympathetic ganglia in the bronchial wall (Nakamura, Haga, Miyano, Sasaki & Takishima, 1986).

Thus, the effects of acute and chronic nicotine administration are widespread throughout both the central and peripheral nervous systems. In humans, nicotine has been reported to relieve stress and anxiety, enhance cognitive performance, and reduce weight (Jarvik, 1991). Therefore, both the depressant and stimulant effects of nicotine may, in part, contribute to the reinforcing aspects of tobacco use.

The Influence of Nicotine on Neurotransmitter Systems

Investigators have shown that nicotine induces neurotransmitter release from a variety of brain sites [see Balfour (1982, 1989) for reviews]. In vitro studies have demonstrated an ability of nicotine, or nicotinic agonists, to enhance the release of dopamine (DA), noradrenaline (NA), and 5-hydroxytryptamine (5-HT) (Balfour, 1973; Goodman, 1974; Giorguieff-Chesselet, Kemel, Wandscheer & Glowinski, 1979; Hall & Turner, 1972; Yoshida, Kato & Imura, 1980). In looking at specific brain sites affected by nicotine, Mitchell, Brazell, Joseph, Alavijeh & Gray (1989) found that acute administration of nicotine (0.4 and 0.8 mg/kg s.c.) produced a

regionally specific increase in the rate of catecholamine synthesis in the rat nucleus accumbens, hypothalamus, and hippocampus. In vivo studies using microdialysis (Brazell, Mitchell & Gray, 1991; Imperato, Mulas & DiChiara, 1986; Mifsud, Hernandez & Hoebel, 1989) have shown that nicotine increases extracellular levels of NA, DA & 5-HT in the hippocampus and that nicotine preferentially stimulates release of DA in the nucleus accumbens. However, chronic nicotine administration to rats causes a regionally selective decrease in the concentration and synthesis of 5-HT in the hippocampus (Benwell & Balfour, 1982). Postmortem studies on chronic cigarette smokers (Benwell, Balfour & Anderson, 1990) showed that smoking was associated with decreases in concentration of 5-hydroxyindoleacetic acid (a serotonin metabolite) in the hippocampal neocortex, hippocampal formation and the median raphe nuclei and there was a significant reduction in the level of 5-HT in the hippocampal formation. Benwell et al. (1990) suggest that these effects may be associated with the development of nicotine dependence.

Nicotine and the Mesolimbic Dopamine System

It has recently been suggested that activation of mesolimbic dopaminergic neurons may contribute to the rewarding effects of nicotine (DiChiara & Imperato, 1988).

Autoradiographic studies have shown that there are nicotinic binding sites on mesolimbic neurons (Clarke & Pert, 1985; Clarke, Pert & Pert, 1984; Clarke, Schwartz, Paul, Pert & Pert, 1985) and that these nicotinic receptors are both at the level of the dopaminergic cell bodies (within the ventral tegmental area) and terminals

(nucleus accumbens and olfactory tubercle). Electrophysiological studies have shown that systemic administration of nicotine can increase the rate of firing of dopaminergic neurons in the ventral tegmental area (Grenhoff, Aston-Jones & Svensson, 1986; Mereu, Yoon, Boi, Gessa, Naes & Westfall, 1987), apparently by direct depolarization of the dopaminergic cells themselves (Calabresi, Lacey & North, 1989). Nicotine can also stimulate the release of dopamine from the nucleus accumbens both in vitro (Rowell, Carr & Garner, 1987) and in vivo (Imperato et al., 1986; Mifsud et al., 1989). Fung (1989) has reported an increase in release of newly synthesized [^{3}H] dopamine from rat nucleus accumbens slices, indicating that nicotine has a direct action on terminals of the mesolimbic system. In vivo experiments using microdialysis have shown that nicotine stimulates release of dopamine in both the mesolimbic and nigrostriatal systems (Imperato et al., 1986). However, it appears that the mesolimbic system is more sensitive to nicotine than the nigrostriatal system since the ED_{50} for the effect on dopamine release in the nucleus accumbens was lower than the ED_{50} for its effects on striatal release, suggesting that nicotine's primary influence is on the mesolimbic dopamine system rather than the nigrostriatal dopamine system. Dopamine turnover following acute nicotine is also greater in the nucleus accumbens than in other brain regions investigated (Lapin, Maker, Sershen, Hurd & Lajtha, 1987). Recently, Izenwasser, Jacocks, Rosenberger & Cox (1991) have demonstrated that nicotine is also able to block dopamine reuptake in the striatum. This ability to increase extracellular levels of dopamine is a characteristic nicotine shares with other drugs of abuse such as cocaine, opiates and alcohol, and it is

hypothesized that it is the action of nicotine on mesolimbic neurons which underlies its reinforcing properties (DiChiara & Imperato, 1988).

When compared to other psychomotor stimulants (i.e., d-amphetamine and cocaine), nicotine produces a behavioral profile which is consistent with the hypothesis that nicotine stimulates the mesolimbic dopaminergic system: (1) nicotine stimulates locomotor activity (Clarke & Kumar, 1983), (2) nicotine, both systemic and intracerebral, produces a conditioned place preference (Fudala & Iwamoto, 1986; Fudala, Teoh & Iwamoto, 1985; Iwamoto, 1990); (3) nicotine enhances the rewarding effects of electrical brain stimulation (Clarke & Kumar, 1984; Manderscheid & Frank, 1989; Olds & Domino, 1969; Pradhan & Bowling, 1971); and (4) nicotine is self-administered by both animals and humans (Corrigall & Coen, 1989; Goldberg & Henningfield, 1988).

Further evidence that dopaminergic mechanisms are involved in the reinforcing effects of nicotine comes from a recent study by Corrigall & Coen (1991), in which the selective D1 antagonist, SCH 23390, and the selective D2 antagonist, spiperone, both produced a dose-dependent decrease in nicotine self-administration. It is important to note that in that study nicotine self-administration was reduced primarily in the latter part of the test session after the subjects had sampled nicotine, thus demonstrating that the effect was not due to motor impairment.

Nicotine and the Serotonergic System

Although there is much evidence to suggest that nicotine alters 5-hydroxytryptamine (5-HT) function in the brain (Balfour & Benwell, 1980; Benwell & Balfour, 1979, 1982; Fuxe, Everett & Hokfelt, 1979; Mitchell et al., 1989), the effects are complex and depend on many factors. These factors include the initial physiological state of the organism, previous exposure to the drug, and the brain region studied (Balfour, 1982).

For example, Balfour, Khullar & Longden (1975) found that acute nicotine administration to unstressed rats produced a decrease in 5-HT concentration in the hippocampus 45 min post-injection whereas at 60 min post-injection , there was an increase in 5-HT concentration. However, these changes were not seen in rats exposed to mild stress. Using in vivo microdialysis, File, Curle, Baldwin & Neal (1987) have shown a positive correlation between reduced 5-HT turnover in the hippocampus and increased anxiety, suggesting that nicotine may have anxiolytic properties and that the reduced 5-HT turnover in the hippocampus of nicotine-withdrawn rats could be associated with anxiety symptoms.

Benwell & Balfour (1979) examined the effect of chronic nicotine administration (0.4 mg/kg nicotine for a maximum of 40 days) on 5-HT and 5-HIAA levels in the rat hippocampus, hypothalamus and residual brain area (i.e., remainder of the brain that was pooled together). They found that nicotine consistently reduced 5-HT concentration in the hippocampus and that this effect was apparent after only one day of nicotine treatment. However, at 20 days of nicotine treatment, 5-HIAA levels were

significantly increased in the withdrawal group.

In contrast, hypothalamic 5-HT and 5-HIAA levels did not change in chronically treated rats (Benwell & Balfour, 1979). Also, levels of 5-hydroxyindoles in residual brain were not affected by nicotine administration until day 20, when there was a significant reduction in 5-HT concentration in chronically treated animals. Benwell & Balfour (1979, 1982) have suggested that this regionally selective decrease in the concentration and biosynthesis of 5-HT in the hippocampus, after chronic nicotine administration, may be associated with the development of dependence upon nicotine.

More recently, Benwell, Balfour & Anderson (1990) found significant reductions in the concentration of 5-HIAA in the hippocampal neocortex, hippocampal formation and in the median raphe nuclei in post-mortem tissue samples from chronic smokers. These observations are similar to those seen in rats chronically treated with nicotine and, indicate that smoking (and thus nicotine) may exert regionally selective effects on brain 5-HT systems.

Overall, the effect of chronic nicotine administration on hippocampal 5-HT seems well established (i.e., nicotine produces a decrease in concentration and synthesis of 5-HT), whereas the effect of acutely administered nicotine remains a controversial area (Mitchell et al., 1989). This may be due, in part, to a lack of studies on the effect of nicotine on 5-HT function in various brain areas.

Recently, it has been shown that two specific 5-HT_3 receptor antagonists, ICS 205-930 and MDL 72,222, significantly reduce nicotine- and morphine-induced place preference (Carboni, Acquas, Leone & DiChiara, 1989), suggesting that serotonergic

mechanisms may be involved in nicotine's rewarding effects. The serotonergic projection from the dorsal and median raphe nuclei to the ventral tegmental area, nucleus accumbens and caudate nucleus (Steinbusch, 1981) may prove to be an important component in nicotine reward. In fact, there is much evidence in support of an inhibitory effect of serotonin in the nucleus accumbens (Costall, Naylor, Marsden & Pycock, 1976; Jenner, Sheehy & Marsden, 1983). For example, 5-HT injections into the nucleus accumbens reduces dopamine-induced hyperactivity and, lesions of the median raphe nucleus increase the hyperactivity response to dopamine (Costall et al., 1976). Also, Herve, Pickel, Joh & Beuadet (1987) have shown that serotonergic terminals make direct synaptic contact with A10 dopamine cell bodies in the ventral tegmental area. Therefore, it is possible that nicotine's effect may be modulated indirectly via an inhibitory serotonergic effect on the mesolimbic dopamine system.

Pharmacological Treatment Strategies for Nicotine Dependence

Pharmacologic interventions for the treatment of drug dependence are those which are designed primarily to suppress the effects of withdrawal from an addictive substance. Pharmacologic therapy may be an important component in preventing relapse. For example, Gottleib, Killen, Marlatt & Taylor (1987) found that both physical and psychological withdrawal symptoms predicted early relapse. Studies generally support the notion that combinations of treatment strategies (i.e., psychological and pharmacological) generally result in greater abstinence rates than

any single treatment alone (Fagerstrom, 1982; Lando, 1982).

Four pharmacologically-based approaches for the treatment of drug dependence can be identified; (1) replacement or substitution therapy, (2) blockade therapy, (3) nonspecific pharmacotherapy, and (4) deterrent therapy. All of these strategies have been used to treat nicotine dependence with varying degrees of success (US Surgeon General's Report, 1988).

The general principle of replacement therapy is to present the patient with a safer and more therapeutically manageable form of the drug that directly alleviates signs and symptoms of withdrawal and craving (Jaffe, 1985). The assumption underlying this approach is that nicotine-specific withdrawal symptoms interfere with smoking cessation and that these withdrawal symptoms can be alleviated by nicotine replacement, thereby facilitating cessation.

Several nicotine delivery systems have been developed; (1) nicotine polacrilex chewing gum, (2) a transdermal patch for delivery of nicotine through the skin, (3) a nasal nicotine solution, and (4) a nicotine vapor inhaler. These different forms of nicotine replacement allow for variations in delivery (e.g., dose and speed) which may influence effectiveness, relief of withdrawal, patience acceptance, and outcome (US Surgeon General, 1988).

Nicotine replacement therapy has been shown to be effective for relieving symptoms of withdrawal and as an aid to smoking cessation when used in conjunction with a smoking cessation program (Hughes et al., 1984; Russell, Jarvis, Sutherland & Feyerabend, 1987). Although nicotine gum has been shown to reduce acute

withdrawal symptoms and to serve as a temporary substitute for cigarettes (Hughes et al., 1984), it is often associated with adverse side effects such as nausea and irritation of the mouth and throat (Hughes & Miller, 1984). A nicotine nasal solution has been developed to provide for a more rapid and efficient absorption of nicotine than is possible with nicotine gum (Jarvis, 1986). Preliminary studies (Jarvis, 1986) report a decrease in craving for cigarettes and an encouraging abstinence rate at a 1-year follow-up. Faster absorption and higher plasma nicotine levels obtained with the nasal solution, as opposed to nicotine gum, suggest that the nasal solution may be more effective and better accepted by smokers as a replacement for cigarettes (West, Jarvis, Russell & Feyerabend, 1984).

More recently, attention has focused on the nicotine transdermal patch as a pharmacologic adjunct for smoking cessation (Abelin, Muller, Buchler, Vesanen & Imhof, 1989; Buchkremer, Bents, Horstmann, Opitz & Tolle, 1989). A transdermal patch can eliminate some of the compliance and chewing problems associated with nicotine gum use (Hughes et al., 1984). One of the most important advantages of transdermal delivery is that it provides continuous absorption of the drug and, therefore, has potential for reducing the craving and withdrawal symptoms associated with smoking cessation (Bannon, Corish, Corrigan, Devane, Kavanagh & Mulligan, 1989). Recent reports on a transdermal nicotine patch show a significantly higher short-term rate of smoking cessation among users who received an active patch than among those who received a placebo patch (Abelin et al., 1989; Buchkremer et al., 1989). Also, when used as an adjunct to low-intervention counseling, it was found

that transdermal nicotine therapy enhanced both short-term and long-term smoking cessation rates (Daughton, Heatley, Prendergast, Causey, Knowles, Rolf, Cheney, Hatelid, Thompson & Rennard, 1991).

Nonspecific pharmacotherapies are those in which the patient is treated symptomatically. Because nicotine produces a number of neurohormonal and physiological effects that may be critical in the maintenance and relapse of cigarette smoking, it is assumed that treatments that address those factors will aid in cessation.

The signs and symptoms of nicotine withdrawal listed in the DSM-III-R (American Psychiatric Association, 1987) are craving for nicotine,irritability, anxiety, difficulty concentrating, restlessness, decreased heart rate and, an increased appetite or weight gain. Additional symptoms may include lightheadedness, headache, dizziness, tremor, sleep disturbances, nausea and, constipation (Jarvik, 1979). Therefore, pharmacotherapies that address these symptoms may aid in cessation by alleviating some of the physical discomfort that may lead to relapse. For example, because nicotine withdrawal in some ways mimics some symptoms of early depression, some investigators have used antidepressants as an adjunct to smoking cessation (Edwards, Murphy, & Downs, 1989). Some of the earliest attempts to treat nicotine dependence involved the use of stimulants like amphetamine (Ross, 1967) and tranquilizers (Schwartz & Dubinsky, 1968). However, there is little evidence that these approaches are particularly effective (Gritz & Jarvik, 1977). In fact, Henningfield & Griffiths (1981) reported that d-amphetamine administration to smokers actually enhances the pleasure gained by smoking and increases the rate of smoking.

More recently, attention has focused on the alpha$_2$-noradrenergic antagonist clonidine, since it has been found to be effective in reducing withdrawal symptoms among opiate and alcohol abusers (Baumgartner & Rowen, 1987; Jasinsky, Johnson & Kocher, 1985). Several studies (Glassman, Jackson, Walsh & Roose, 1984; Glassman, Stetner, Walsh, Razman, Fleiss, Cooper & Covey, 1988) have supported clonidine's usefulness in diminishing cigarette craving and the following nicotine withdrawal symptoms: anxiety, tension, irritability and restlessness. Clinical observations (Sees & Stalcup, 1989) suggest that by combining nicotine chewing gum with clonidine, the rate of nicotine withdrawal and the extent to which withdrawal symptoms are treated can be controlled.

Preliminary studies also show that buspirone (a non-benzodiazepine anxiolytic) and doxepin (a tricyclic antidepressant) may be useful pharmacologic adjuncts to smoking cessation (Edwards et al., 1989; West, Hajek & McNeill, 1991). In the study by West et al. (1991) subjects received buspirone (a 5-HT$_{1A}$ and D$_2$ partial agonist) for a 6-week treatment period (2 weeks prior to the quit date and for 4 weeks post-cessation) along with weekly group counseling. At the end of treatment (4 weeks post-cessation) 47% of those using buspirone were reported as being sustained non-smokers throughout the 4-week period, compared with 16% of those receiving placebo. Because there is some similarity in symptoms of nicotine withdrawal and depression (e.g., drowsiness, irritability, concentration problems), Edwards et al. (1989) assessed the ability of doxepin as an aid to smoking cessation. Subjects (N=9) were treated with doxepin for 7 weeks (with the smoking quit date at week 3). Two

months after the quit date, 7 of the 9 subjects (78%) treated with doxepin reported abstinence (versus only 1 out of 10 of the placebo subjects). Unfortunately neither expired air carbon monoxide levels nor cotinine levels were measured for an objective verification of non-smoking status. Therefore, although buspirone and doxepin show promise as adjuncts to smoking cessation, more larger-scale clinical trials are needed to substantiate their utility in treating nicotine dependence.

In deterrent therapy, pretreatment with a substance results in aversive effects when the abused drug is subsequently taken (Jaffe, 1985). The most common example of this therapy is the use of disulfiram in the treatment of alcoholism (Jaffe & Ciraulo, 1985). With regard to cigarette smoking, the main analog to disulfiram treatment is the administration of silver acetate (Malcom, Currey, Mitchell & Keil, 1986). The physiological basis of this approach is that, when silver acetate comes into contact with the sulfides in tobacco smoke, distasteful sulfide salts are produced. Malcom et al. (1986) found that silver acetate chewing gum demonstrated a modest benefit over placebo as a smoking deterrent. More recently, Jensen, Schmidt, Pedersen & Dahl (1991) looked at the effect of silver acetate, nicotine and ordinary chewing gum on smoking cessation. They found that when smoking history is taken into consideration, silver acetate chewing gum produces a higher rate of abstinence in light smokers, while nicotine chewing gum was more effective in heavier smokers.

Whereas the goal of replacement and symptomatic treatment is to relieve withdrawal by mimicking the effects of the drug from which the person is attempting to abstain, blockade therapy does not provide any potentially reinforcing effect. In

blockade therapy, the goal is to reduce or eliminate any rewarding pharmacological effects by pretreatment with an antagonist, should a person attempt to resume drug use. With respect to nicotine dependence, smokers attempting to quit by this method would be administered a centrally active nicotinic receptor antagonist and then be encouraged to smoke. This type of treatment should produce an effect that is analogous to extinction. Nicotine blockade therapy may also be useful in reducing craving (i.e., the desire or urge to smoke), since it appears that craving may occur as a result of classical conditioning (Clarke, 1991). According to the principles of classical conditioning, repeated presentation of cues (either internal or environmental) without the contingent reinforcement of the drug, should result in extinction of the conditioned withdrawal or craving response.

Mecamylamine, a nicotinic receptor antagonist which acts both centrally and peripherally (Gilman et al., 1985), has shown potential as a possible pharmacotherapy for treatment of cigarette smoking (Henningfield, 1984). Pretreatment with mecamylamine (1) produces a dose-related blockade of the ability of animals and humans to discriminate nicotine from a placebo (Rosencrans & Meltzer, 1981), (2) reduces the reinforcing effect of IV nicotine administration in animals (Goldberg, Spealman, Risner & Henningfield, 1983) and (3) increases various measures of cigarette smoking behavior and tobacco smoke intake when subjects are allowed to freely smoke (Pomerleau, Pomerleau & Majchrzak, 1987; Stolerman, Goldfarb, Fink & Jarvik, 1973). Rose, Sampson, Levin & Henningfield (1989) have found that low doses of mecamylamine decrease the perceived strength and harshness of cigarette

smoke and reduce the effect of smoking on subsequent desire for a cigarette. However, one main obstacle to this treatment approach is the ganglionic blocking and antihypertensive effects of mecamylamine (Gilman et al., 1985). Also, although an antagonist may be effective in blocking the centrally mediated effects of nicotine, conditioned and non-nicotine-mediated reinforcers of tobacco use may be powerful enough to sustain urges to smoke even when they are no longer associated with the pharmacological effects of nicotine. Therefore, more controlled studies of chronic mecamylamine administration need to be conducted to assess its clinical potential as an adjunct in smoking cessation treatment.

Animal Models for Assessing Reward

To develop effective treatment strategies for substance abuse it is necessary to understand the properties that underlie a substance's abuse liability. As a result, a considerable amount of research effort has focused on the study of the rewarding properties of abused drugs. Since the study of addictive substances in humans is often limited by ethical and practical constraints, researchers have turned to the use of animals to (1) determine brain mechanisms involved in drug abuse, (2) screen new compounds for potential abuse liability, (3) study basic motivational processes and, (4) develop treatments for drug abuse (Bozarth, 1987). More importantly, there is converging evidence for the generalizability of results obtained from animal models to observations concerning drug abuse liability in humans (Brady, 1991).

A number of methods have been developed within the animal model to assess the

rewarding properties of drugs. These include drug self-administration (Katz, 1898; Wolverton & Nader, 1990), conditioned place preference (Carr, Fibiger & Phillips, 1989), drug discrimination (Overton, 1987), and intracranial self-stimulation (Kornetsky & Bain, 1990; Stellar & Rice, 1989).

In drug self-administration studies, one typically determines whether animals will acquire and maintain behavior that leads to intravenous or intracerebral drug injections [see Johanson & Shuster (1991) for review]. Many studies have demonstrated that there is a strong relationship between the range of substances self-injected by laboratory animals and those abused by humans (Yokel, 1987). Self-administration of a substance by experimental animals can be viewed as an operant behavior in which the response (usually a lever press) is followed by a drug injection. Therefore, in operational terms, the self-administration of a drug by an animal can be viewed as a direct measure of the reinforcing properties of a drug (i.e., if the drug increases the probability of the response it is assumed to be acting as a reinforcer).

Conditioned place preference (CPP) has also been used to characterize the rewarding properties of drugs. The ability of CPP to measure reinforcing or aversive effects depends on the association of the internal state produced by the unconditioned stimulus (i.e., the drug) with an external stimulus to be conditioned (e.g., a particular chamber of the CPP apparatus). In this procedure one compartment of a shuttle box is repeatedly paired with the administration of a drug, and it is assumed that the rewarding properties of the drug become associated with distinctive environmental cues (e.g., tactile, visual, and/or olfactory) present in the drug-paired environment.

The animal is later tested (in a drug-free state) to determine if there is an increase in the amount of time spent in the compartment associated with the drug injection. If so, this is presumed to occur as a result of the drug's rewarding properties.

Drug discrimination procedures are used to assess the stimulus properties of drugs that underlie their abuse liability. In a drug discrimination experiment an animal is trained to discriminate between an injection of a specific drug and an injection of saline. In a typical experiment, animals are initially food deprived and then trained to press one of two levers according to a fixed ratio schedule, which results in food delivery to the deprived animal (Overton, 1987). Prior to a test session animals are injected with either the drug under study or saline. Once in the testing chamber only one of the two levers is operative so that, for example, after a drug injection pressing the operative lever will result in food delivery, while pressing the alternate lever has no consequence. Drug and saline sessions are randomly ordered until the animal has reached an arbitrary criterion of correctly responding on the "drug lever" after a drug injection or on the "saline lever" after a saline injection. Once that animal has acquired this discrimination, tests of stimulus generalization are conducted in which an animal is injected with a novel drug and its operant responding is recorded. If the novel drug produces responding on the "drug lever," then stimulus generalization with the training drug has occurred, and it is inferred that the novel drug produced subjective effects which were similar to the training drug.

The rewarding and/or incentive-motivational properties of drugs can also be assessed by the method of intracranial self-stimulation (ICSS). This method involves

training animals to work (i.e., perform an operant task) for electrical brain stimulation and then assessing the effect of a drug on brain stimulation reward. ICSS has been used to determine whether a substance enhances reward (Gallistel & Karras, 1984; Kornetsky, 1985; Lorens & Mitchell, 1973; Stein & Ray, 1960) or blocks reward (Fouriezos, Hanson & Wise, 1978; Gallistel & Karras, 1984; Zarevics & Setler, 1979). The observation made with this approach is that drugs with rewarding effects (as demonstrated, for example, by an increased rate of response for fixed intensity brain stimulation or as a lowering of current thresholds for brain stimulation) augment activity in the neural systems that mediate BSR and thus increase the subject's willingness to respond for the brain stimulation. The magnitude of the drug-induced euphoria is indexed by the degree to which a drug facilitates BSR (see Hall, Bloom & Olds, 1977; Kornetsky, 1985 for reviews). The opposite argument is made for pharmacological agents that block BSR. This argument is supported by the observation that most addictive substances facilitate BSR, while substances that antagonize dopamine receptors attenuate BSR (Wise & Bozarth, 1987).

All of the above-mentioned methods have been employed in an effort to characterize the rewarding properties of nicotine (see Clarke, 1987 for a review), and each method has specific advantages depending upon the question under investigation. For example, if one were interested in studying patterns of drug intake, the intravenous self-administration technique would be appropriate, whereas the drug-discrimination technique would be more appropriate for investigating the structure-activity relationship of drugs. Although the ICSS approach involves an indirect

method for assessing the rewarding effects of drugs, it is useful in determining dose and time-after-injection parameters (Bozarth, 1987). This information can then be used, for example, to evaluate the effectiveness of pharmacological interventions and to provide insight into the neural basis of drug reward.

Using BSR to Assess Nicotine Reward

When compared to other drugs of abuse, studies of the effects of nicotine on intracranial self-stimulation (ICSS) have been few in number. In early studies which used simple measures based on changes in rate of responding, nicotine was reported to both increase and decrease rate of response (Olds & Domino, 1969; Pradhan & Bowling, 1967). For example, Olds & Domino (1969) found that in rats, nicotine (0.025 - 0.6 mg/kg) produced an initial decrease followed by an increase in response rates, but noted that depressant effects predominated. Pradhan & Bowling (1971) reported that the effects of nicotine were rate-dependent: facilitation of responding for brain stimulation reward (BSR) was observed with high doses of nicotine in rats with low response rates. In rats with high response rates nicotine had very little effect and, in some cases, caused a reduction in response rate. Similar results were reported by Newman (1972), who used a variable-interval rather than a continuous reinforcement schedule (CRF). Schaefer & Michael (1986) found that on a CRF, nicotine did not alter response rate except at a dose of 1.0 mg/kg, which produced a decrease in the rate of responding. However, when animals were tested in a fixed-ratio-15 paradigm, nicotine (0.03 - 1.0 mg/kg) produced a biphasic dose-response

curve: at 0.1 mg/kg, response rates increased to approximately 60% above baseline, while at 1.0 mg/kg, response rates decreased to approximately 90% below baseline (Schaefer & Michael, 1986). These results may be due in part to nicotine's effect on the peripheral nervous system and time of testing after nicotine administration. For example, the primary effect of nicotine on the peripheral nervous system consists initially in transient stimulation and subsequently in a more persistent depression of all autonomic ganglia (Goodman et al., 1985). Small doses of nicotine stimulate the ganglion cells directly and facilitate the transmission of impulses, whereas with larger doses, the initial stimulation is followed very quickly by a blockade of transmission. Other autonomic effects produced by nicotine which tolerate after chronic exposure to the drug are decreased body temperature (Lapin et al.,1987; Marks & Collins, 1985), increased respiration (Marks & Collins, 1985), decreased heart rate (Aceto, Tucker, Ferguson & Hinson, 1986; Marks & Collins, 1985), and increased blood pressure (Aceto et al., 1986). Therefore, the initial depressant effects produced by high doses of nicotine on self-stimulation rates may be due to blockade of autonomic neurotransmission since the autonomic ganglia blocker, mecamylamine, can prevent the ataxia and locomotor depressant effects of nicotine in non-tolerant rats (Clarke & Kumar, 1983).

The preceding studies provide evidence that response rate is not an adequate measure of brain stimulation reward since (1) it is easily influenced by drugs which affect motor performance (Liebman, 1983), and (2) fixed values of stimulation parameters are typically used, which may limit an animal's response capacity, i.e.,

create floor or ceiling effects (Valenstein, 1964).

With the use of rate-free measures of ICSS (i.e., those that allow the experimenter to assess the effects of a drug on BSR independently of the drug's influence on motor performance), nicotine has been shown either to enhance BSR (Clarke & Kumar, 1984) or produce little or no effect (Clarke & Kumar, 1983a; Schaefer & Michael, 1986).

Clarke & Kumar (1983a,1984), using two "rate-free" methods to measure the magnitude of rewarding brain stimulation, have obtained contradictory results regarding the effect of nicotine on ICSS. In a Y-maze choice procedure (Clarke & Kumar, 1983a), nicotine (0.025-0.4 mg/kg) affected response rates without measurably altering the magnitude of brain stimulation reward, as measured by the animal's ability to discriminate different current intensities. In this study, two arms of a Y-maze provided BSR and the rat was required to shuttle back and forth between the two "on" arms. The first ten responses that the animal made were used to measure "accuracy," i.e., the percentage of rewarded responses. In acute testing, nicotine depressed rewarded responding in a dose-related way during the first 20 min of the test session. From 40 to 80 min, the actions of nicotine were predominantly stimulatory. However, accuracy did not differ significantly from baseline levels. With chronic treatment (0.4 mg/kg nicotine, daily), there was still no improvement in accuracy. Therefore, although nicotine exerted a behavioral stimulant action (i.e., increased rates of responding), its failure to increase accuracy of discrimination suggested that it did not enhance the rewarding strength of electrical stimulation of the

medial forebrain bundle.

However, in another choice procedure employing a shuttle box (Clarke & Kumar, 1984), nicotine (0.4 mg/kg) enhanced the rewarding effect of electrical stimulation in a manner similar to d-amphetamine. In these studies, the dependent variable consisted of total time during which rewarding brain stimulation was delivered. Clarke & Kumar (1984) found that nicotine initially reduced self-stimulation time (SST) in the first third of the test (the first 26 min), while in the last two-thirds of the session nicotine enhanced SST. However, by the last nicotine test (day 10 of daily nicotine injections) tolerance had developed to the initial reduction in SST, with the SST of the last two-thirds of the test session remaining unchanged. Therefore, in the shuttle box procedure, nicotine appeared to enhance the rewarding properties of median forebrain bundle stimulation. Although both of the studies by Clarke & Kumar (1983a, 1984) used procedures that are presumably rate-independent measures of BSR, the conflicting results suggest a need for a more rigorous validation of those procedures.

Threshold techniques have been developed to differentiate between reward and performance effects (Liebman, 1983). These techniques are used to determine the minimum current intensity, duration, or frequency for which an animal will maintain some specified level of performance. One method of measuring BSR that has been validated for reward-selectivity is the rate-frequency, curve-shift method developed by Edmonds & Gallistel (1974). In this method, brain stimulation reinforcement is given as a burst composed of discrete pulses, and the pulse frequency is varied from trial to

trial within a session. Plotting self-stimulation responding against the various pulse frequencies yields a sigmoidal rate-frequency curve (see Figure 1). Shifts in the lateral position of the curve are believed to be a selective measure of stimulation-produced reward, while shifts in the behavioral asymptote (i.e., maximal response rate) are thought to reflect the performance capacity of the animal (Edmonds & Gallistel, 1974; Stellar et al., 1988).

Frank, Markou & Wiggins (1987) developed and validated the train-duration method, which is similar to the rate-frequency method of Edmonds & Gallistel (1974). In this method, the train duration of the stimulating current (i.e., the length of time the current is available) is varied from trial to trial, while other parameters (e.g., frequency) are held constant. Since stimulation frequency is held constant, changes in train duration result in changes in the number of stimulating pulses that an animal receives in each train of stimulation. A train-duration response function, analogous to the rate-frequency curve obtained by Edmonds & Gallistel (1974), is generated by plotting response rate against train duration (see Figure 2). Two measures of an animal's response to the stimulation can be calculated from these functions; (1) maximal response rate and (2) train-duration threshold (Frank et al., 1987). Maximal response rate is defined as the highest average rate of responding produced by the stimulation and serves as an index of the performance capacity of the animal. The train-duration threshold is defined as the shortest train duration that supports 50% of the maximal response rate. Thresholds provide an index of the incentive or reward value of the stimulation. The selectivity of the threshold measure

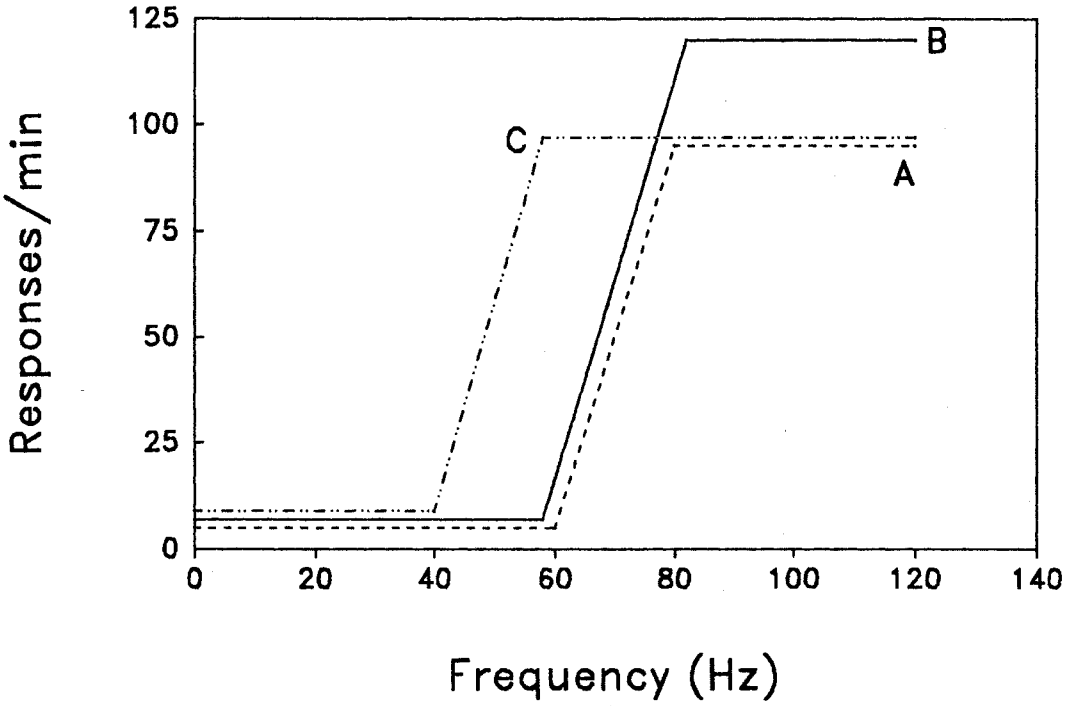

Figure 1. Hypothetical rate-frequency curve-shift function in which response rate is plotted against the frequency of a burst of electrical stimulation. Curve A represents baseline response, Curve B represents a vertical shift (e.g., an increase in response rate due to a change in performance capacity) and, Curve C represents a lateral shift (e.g., a change in the reward value of the stimulation).

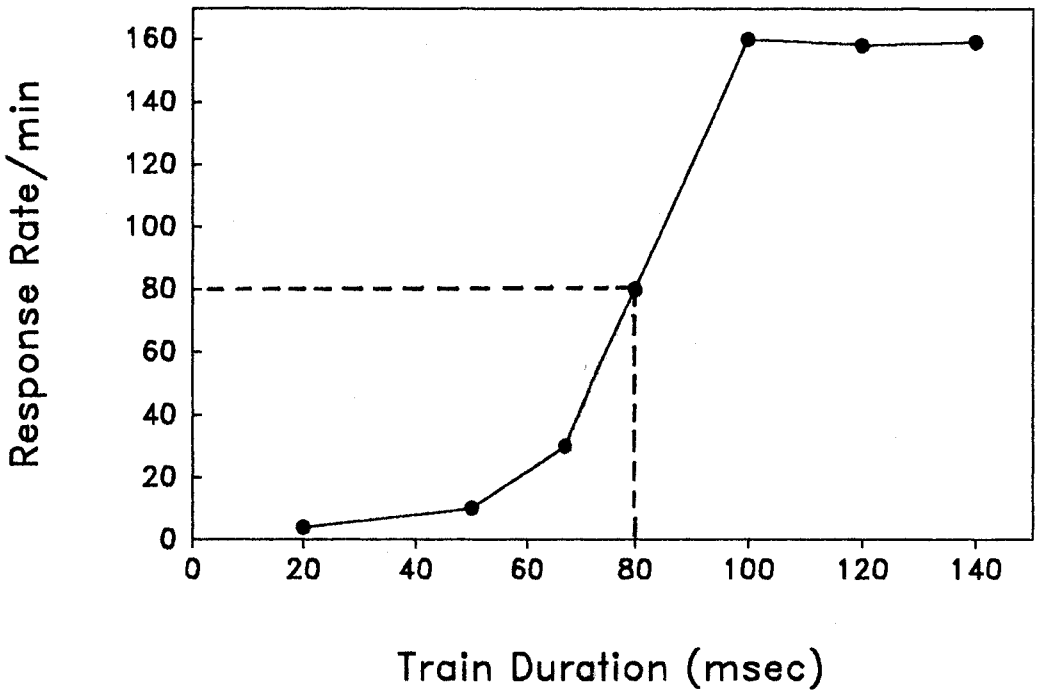

Figure 2. Hypothetical response rate/train-duration function with a threshold of 80 msec.

is supported by the results of Frank et al. (1987), who found that (1) performance manipulations, while having significant effects on maximum response rates, do not significantly affect the position of the train-duration response function, and (2) reward manipulations produce significant shifts in thresholds.

Because the effect of nicotine on brain stimulation reward is not well established, one goal of the present study was to clarify the characteristics of nicotine-induced changes in BSR using a rigorously validated, rate-free and reward-selective procedure (Frank et al., 1987) and to extend the findings of Manderscheid & Frank (1989) which demonstrated that nicotine (0.4 mg/kg) significantly enhances BSR in the train-duration paradigm. Both the acute and chronic effects of nicotine were assessed as a function of dose, time of testing, and previous number of injections. These experiments also allowed for the assessment of tolerance or any dysphoric reaction resulting from chronic administration of nicotine. Tolerance would be evidenced by a diminished response to the drug over repeated days of testing (i.e., a decrease in nicotine-induced changes in train-duration thresholds over time), whereas withdrawal-induced dysphoria would be evidenced by an increase in train-duration thresholds above baseline values following the discontinuation of drug treatment.

Evaluation of Pharmacotherapies Using BSR

There are not as yet any clear indications of which pharmacotherapies are most effective for treating stimulant abuse. Since the neural basis for drug-reinforced behavior is not well understood, behavioral approaches that are able to assess the

abuse liability of drugs may also be useful for examining the effects of specific pharmacological treatments. Since euphorigenic drugs decrease self-stimulation thresholds, and drugs that blunt reward increase thresholds (Gallistel & Karras, 1984), it is possible to evaluate whether a drug's ability to reduce or block the reinforcing effects of nicotine is due to a drug-induced reversal of nicotine's effects, the deactivation of the reward substrate, or the drug's own euphorigenic effects. Therefore, self-stimulation threshold experiments can also provide information concerning the mechanism(s) by which a compound modifies the rewarding effect of a drug (Frank, Pommering & Nitz, 1988).

To date, pharmacological treatment of nicotine dependence has been only moderately successful. Recently, Panicker, Manderscheid, Frank, Kehne, Schmidt & Sorensen (1991) have shown that the mixed 5-HT_2/D_2 antagonist MDL 28,133A is able to block both amphetamine- and cocaine-induced facilitation of ICSS. Preliminary data suggest that this compound is also able to block nicotine-induced facilitation of ICSS. These results indicate that both dopaminergic and serotonergic mechanisms may be involved in stimulant-induced reward and that drugs affecting these neurotransmitter systems may be useful for the treatment of drug abuse.

Therefore, once the basic profile of nicotine's effect on BSR was established, preliminary studies assessing the ability of a selective 5-HT_2 (MDL 26,508), D_2 (sulpiride) and a mixed 5-HT_2/D_2 (MDL 28,133A) antagonist to modify nicotine-induced enhancement of BSR were conducted. Specifically, these compounds were assessed for their ability to reverse nicotine-induced lowering of self-stimulation

thresholds. A comparison of the effects of MDL 26,508 and sulpiride to MDL 28,133A should provide insight about the relative importance of the serotonergic and dopaminergic components of MDL 28,133A in its ability to attenuate nicotine's effects.

Although there is much evidence to indicate that dopamine plays a major role in the rewarding aspects of psychomotor stimulant drugs, the role of serotonin is not as well established (Stellar & Rice, 1989). For example, p-chlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, or a 5-HT neurotoxic lesion using 5,7-dihydroxytryptamine (5,7-DHT) increased self-stimulation in some studies and decreased it in others (Gibson, McGeer & McGeer, 1970; Lorens, 1971). Recently Nakahara, Ozaki, Miura, Miura & Nagatsu (1989) found that self-stimulation of the medial forebrain bundle in rats produced increases in both dopamine and serotonin metabolites in the nucleus accumbens, but that the increase in serotonin metabolites had a different time course from that of dopamine. Therefore, Nakahara et al. (1989) have suggested that serotonin may play a role in maintaining self-stimulation of the medial forebrain bundle. Also, recent studies indicate that serotonin may have a role in stimulant self-administration. For example, 5,7-DHT lesions of the medial forebrain bundle produced significant increases in amphetamine (Leccese & Lyness, 1984) and cocaine (Loh & Roberts, 1990) self-administration. Schmidt & Kehne (1990) and Schmidt et al. (1991) have also demonstrated that selective 5-HT_2 receptor antagonists can block MDMA-stimulated dopamine release. Since nicotine has been shown to stimulate dopamine release both in vitro and in vivo (Imperato et al., 1986;

Rowell et al., 1987), a specific 5-HT_2 antagonist (e.g., MDL 26,508) may be effective in reversing nicotine's effect on BSR. If serotonin antagonists directly modulate dopamine functioning, it was predicted that MDL 26,508 would attenuate or block the effects of nicotine.

Recently, Corrigall & Coen (1991) found that pretreating animals with spiperone, a selective D2 antagonist, decreased nicotine self-administration without impairing motor functioning [however, it should be noted that although spiperone is classified as a selective D_2 antagonist, it also has a high affinity for 5-HT_2 receptors (Meltzer, 1987)]. Therefore, if dopamine is the primary substrate involved in the mediation of nicotine's effects, it was expected that sulpiride would produce an attenuation or blockade of nicotine's effects. The racemic form of sulpiride was chosen since it appears that this mixture of enantiomers does not induce extrapyramidal side effects (Fuxe, Anderson, Agnati & Orgen 1979). Clinically, sulpiride exhibits antipsychotic properties without producing overt signs of Parkinsonism or dystonia (Bjerkenstedt, Harnryd & Sedvall, 1979). A careful analysis of the effect of sulpiride on train-duration thresholds is indicated, since an antagonist capable of reversing the effect of nicotine without increasing self-stimulation thresholds when administered alone should have great promise as a pharmacotherapy for nicotine abuse.

The effect of a mixed D_2/5-HT_2 antagonist (MDL 28,133A) on nicotine-induced enhancement of BSR was also tested so that the results of the studies with MDL 26,508 and sulpiride could be evaluated against this compound. Comparisons of this nature would help to further define the contribution of dopamine and serotonin to

nicotine reward. Also, a comparison of these results to those produced when MDL 28,133A is administered in combination with amphetamine and cocaine (Panicker et al., 1991) may provide some insight into the mechanism(s) by which nicotine produces its rewarding effects.

In summary, the purpose of the following experiments was to (1) provide a more complete characterization of the effects of nicotine on BSR and (2) use pharmacologic manipulations to modify nicotine's effects on BSR so as to gain insight into the relative contributions of dopaminergic and serotonergic mechanisms in nicotine reinforcement. These results can then be used to identify compounds which demonstrate promise as pharmacotherapies for nicotine dependence and elucidate the basic pharmacology of nicotine reward.

General Experimental Procedure

Method

Subjects. Experimentally naive male Long-Evans rats (Harlan Industries, Indianapolis,IN) weighing between 300-400 g (at the time of surgery) served as subjects. The animals were housed in individual stainless steel wire hanging cages and maintained on ad lib food and water. The lighting in the colony was regulated automatically to operate on a 12-hr reverse light/dark cycle with lights on at 1800 hr. All behavioral testing was performed during the dark cycle. Ambient temperature in the colony was maintained at a temperature of 70° F.

Stereotaxic Surgery. All rats were anesthetized with sodium pentobarbital (60 mg/kg) and bipolar platinum electrodes (Plastics One Co., Roanoke, VA: diameter = 0.25 mm) were implanted unilaterally into the ventral tegmental area according to the following coordinates: 4.5 mm posterior from bregma, 1.5 mm lateral from the midline, and 8.5 mm ventral from the skull surface, with the skull positioned level between lambda and bregma. The electrodes were permanently affixed to the surface of the skull with acrylic cement and stainless steel screws. Following surgery, 7 to 10 days were allowed for the animals to recover before self-stimulation testing began.

Apparatus. Training and testing took place in twelve metal and Plexiglas chambers (23 x 21 x 19 cm) with a floor constructed of aluminum rods spaced 1.0 cm apart. One wall of the chamber had a 3.5 cm square hole, positioned 5.0 cm above the floor, which opened into a 5 x 5 x 4 cm chamber containing a photocell beam. A

1.0 cm excursion of an object (such as a rat's nose) into the chamber initiated a signal pulse that was registered as a response by an Advanced Logic Research 80286 computer. A mercury swivel commutator and a bipolar electrode lead allowed the animal to move freely within the box while connected to the stimulation circuit.

Brain stimulation was delivered by Grass SD9 square-wave stimulators. These stimulators were part of a system that delivered capacitance-coupled, constant-current biphasic stimulation through a high-impedance stimulation circuit. Stimulation frequency and pulse widths were set at 100 Hz and 5.0 ms, respectively. Currents were selected such that train-duration response functions were centered around 70 - 100 ms (see below). Train-duration was timed with Advanced Logic Research 80286 computers. The computers also controlled the house lights and all other timing and logic functions.

Behavioral Testing. Animals were screened for self-stimulation following a 10-day, post-operative recovery period. The screening procedure involved shaping the subjects to poke their nose in the hole found in each operant chamber. This was accomplished by manually delivering electrical stimulation to an animal after successive approximations of the operant. During this screening/shaping period, train durations were fixed at 150 msec, with current levels adjusted to minimize stimulation-induced motor responses which sometimes accompany stimulation. The rats were screened for self-stimulation during seven 1-hr sessions, and those animals that averaged 30 responses/min during at least one session were retained for further training. Once the subjects were screened, they were trained to discriminate between

stimulation testing and time-out periods. In those sessions, 150 ms train durations were available for 60 sec alternating with 15-sec time-out periods, during which a houselight was illuminated and no stimulation was available. Forty-five test and time-out trials were run in each session during this phase of the training. Once responding during the time-out periods was eliminated, the next phase of training began. During the next phase, the train duration available on each trial was varied randomly from 0 to 140 ms (in 10 msec intervals) so that over a block of 15 trials, animals were tested at train durations which ranged from 0 msec to 140 msec. Test trials began with a 15-sec warm-up followed by a 60 sec test. A 15-sec time-out followed each test period. Each session consisted of three blocks of trials, with 15 trials in each block. Nose-poking responses made during the warm-up, stimulation test, and time-out period were recorded. Each testing session required 67.5 min to complete.

Following each testing session, the median response rate at each train duration was used to generate a train-duration response function for each animal. Medians were used because this measure of central tendency better reflects the step-like character of train-duration response functions (Frank et al., 1987). The train-duration threshold of each rat was determined by calculating the lowest train-duration that supported 50% of the median maximal rate. Over a period of six to eight test sessions, current levels were adjusted so that each rat's train-duration threshold was stabilized in a range of 70-100 msec. This interval was selected so that any subsequent drug-induced shift in threshold could be easily assessed.

Drugs. (-)Nicotine was obtained from Sigma (St. Louis, MO) in free base form

(98-100% purity) and was prepared in saline with an injection volume of 1.0 ml/kg.

MDL 28,133A (1-(4-fluorophenyl)-2-[4-[(4-methanesulfon-amido-phenyl)carbonyl]-1-piperidinyl]-ethanone hydrochloride) and MDL 26,508 (4-piperidinemethanol, alpha-(2,3-dimethoxyphenyl)-1-(2-phenylethyl)-, hydrochloride) were obtained from Marion Merrell Dow Research Institute (Cincinnati, OH) in crystalline form and were dissolved in acetic acid and saline, with an injection volume of 1.0 ml/kg. The pH was titrated to 7.0 $\pm$ 0.2 with the addition of 1M NaOH.

($\pm$)Sulpiride was obtained from Sigma (St. Louis, MO) in crystalline form and was dissolved in acetic acid and saline. The pH was titrated to 7.0 $\pm$ 0.2 with the addition of 1M NaOH.

Histology. At the end of each experiment, a representative sample of animals was anesthetized with sodium pentobarbital (65 mg/kg) and perfused through the heart with isotonic saline, followed by a 10% formal-saline solution. The brains were removed, frozen, and cut at 50 u for histological verification of electrode placement.

Experiment 1

The purpose of Experiment 1 was to assess the time course of nicotine-induced shifts in threshold as a function of dose, time of testing, and previous number of drug injections. Preliminary studies (Manderscheid & Frank, 1989) showed that 0.4 mg/kg IP nicotine produces a significant reduction in thresholds from saline baseline, and that this effect is still evident 80 min post-injection.

Previous studies (Olds & Domino, 1969; Pradhan & Bowling, 1971) have shown

that nicotine can stimulate or depress ICSS lever responding in non-tolerant animals. Typically, response rates are reduced for several minutes after systemic administration, especially at higher doses and if pre-drug response rates are high, whereas responding is stimulated at longer intervals after injection, especially if the baseline rate is low (Newman, 1972; Olds & Domino, 1969; Pradhan & Bowling, 1971; Wanner & Battig, 1966).

Clarke & Kumar (1983, 1983b, 1984) have also described a biphasic depressant and stimulant effect of nicotine with respect to response rate. A depressant action is most likely to occur shortly after the administration of a high dose of nicotine (2 to 15 min post-injection). Conversely, a stimulant action is commonly seen at longer intervals (60 to 80 min) after the injection of lower doses. With repeated administration, however, the depressant action diminishes, and in tolerant subjects, nicotine exerts a robust stimulant action (Clarke & Kumar, 1983b, 1983c). Because of this unique biphasic effect of nicotine, a time course analysis of the threshold shifts that follow chronic nicotine treatment should elucidate the temporal pattern of nicotine-induced changes in reward.

Two preliminary dose-response experiments were conducted to (1) determine the effects of chronic injections of nicotine on ICSS and (2) to determine a dose of nicotine that would produce an effect where interactions with other drugs could be easily detected.

Method

Procedure.

Chronic Nicotine. Thirty animals were screened and trained as described in the General Procedure Section. Once trained, self-stimulation testing sessions were conducted for three days following IP injections of isotonic saline (0.25 ml). Animals were then randomly divided into three groups (n=10) and injected with one of three doses of nicotine (0.1, 0.5 or 1.0 mg/kg) for nine days to assess the effects of acute and chronic administration. Following drug testing, three days of saline testing were carried out to determine if chronic drug administration had altered baseline levels of responding. Testing was conducted at 15 min post-saline or post-nicotine injection.

Acute Nicotine. Eighteen animals from the chronic study were randomly selected as subjects for the acute study. There was a two week wash-out period between experiments in order to ensure that there were no drug carry-over effects. After three days of saline testing, animals received either 0.2, 0.4 or 0.8 mg/kg nicotine, the order of drug administration being counterbalanced within subjects. Following three days of nicotine testing, animals were again tested with saline during a 3-day, post-drug period.

Results and Discussion

Chronic Nicotine. The first 15 sec of each stimulation trial were considered a warm-up and sampling period, and response rates obtained during this time were not analyzed in detail. Only data from the 60-sec test period on each trial were used to

calculate train-duration thresholds and maximal median response rates. A daily, median response rate was determined for each animal. The median response rate was then used to calculate the train-duration threshold for each animal. Mean thresholds for each condition, across all animals, were then calculated.

A repeated-measures t-test was performed on the pre-drug and post-drug saline conditions for each group. The mean thresholds across animals for the pre- and post-drug conditions were not significantly different within groups. Therefore, data from these two conditions were collapsed to obtain a single, saline baseline. All subsequent saline comparisons used this baseline.

Data from the 9 days of nicotine administration at all dosages were analyzed by means of a one-between, one-within ANOVA with repeated measures over time. The results of the analysis showed that across days, the effect of nicotine did not change significantly [$F(8,216)=1.15$, $p>0.33$]. Therefore, the data were collapsed to produce a single, nicotine baseline for each dose. When compared to saline, nicotine produced a significant, dose-dependent reduction in thresholds [$t(9)=3.32$, $p<.01$; $t(9)=2.98$, $p<.05$; $t(9)=4.93$, $p<.01$ for 0.1, 0.5 and 1.0 mg/kg nicotine, respectively] (see Figure 3).

Due to nicotine's biphasic effect (i.e., initial response depression followed by response stimulation), data were further analyzed by breaking down the 60 min of testing into three 15-trial blocks with T1, T2 and T3 representing the first, second and third blocks. Each block represented 22.5 min of the total testing time. Data were collapsed over 3-day blocks, and repeated-measures ANOVA's showed that

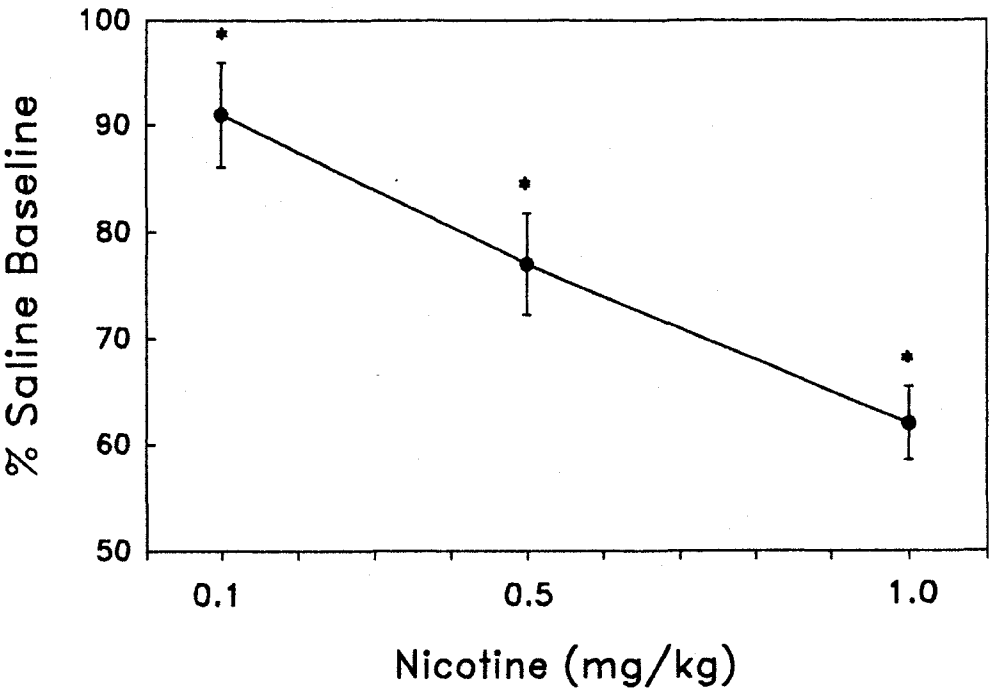

Figure 3. Dose-dependent reduction in train-duration thresholds produced by nicotine. Each point represents the effect of daily nicotine administration collapsed over 9 days of testing (comparison to saline, $^{*}p < .05$). Error bars represent one standard error of the mean.

there were no significant differences in nicotine thresholds across days at any of the time blocks [$F(2,18)=0.77$, n.s.; $F(2,16)=1.88$, n.s.; $F(2,18)=0.69$, n.s. for 0.1, 0.5 and 1.0 mg/kg nicotine, respectively], so data from the blocks were collapsed across days to yield a single, mean nicotine threshold for each time period. The data for the three testing times are shown in Figure 4.

Repeated-measures t-tests showed that at all doses, nicotine produced a threshold-lowering effect that was evident 15 min post-injection [$t(9)=2.42$, $p<.05$, $t(8)=4.04$, $p<.01$, $t(9)=3.31$, $p<.01$ for 0.1, 0.5 and 1.0 mg/kg, respectively].

By T3 (60.5-82.5 min post-injection), repeated measures t-tests showed that for 0.1 and 0.5 mg/kg nicotine, thresholds did not differ significantly from saline baseline [$t(9)=0.95$, n.s. and $t(8)=2.29$, n.s., respectively]. However, the effect of 1.0 mg/kg nicotine was still significant at this time period [$t(9)=4.36$, $p<.01$].

Acute Nicotine. Although in the chronic administration study all doses of nicotine produced a significant reduction in self-stimulation thresholds, a second dose-response study was conducted using additional doses because (1) even though 0.1 mg/kg nicotine produced a significant reduction in thresholds when compared to saline [$F(1,9)=11.03$, $p<.01$], the effect size was very small (61.8 msec saline versus 56.0 msec nicotine) and (2) periodic observation of the rats revealed some adverse effects (e.g, seizures) at the highest dose (1.0 mg/kg).

A repeated-measures t-test was performed on the pre- and post-drug saline conditions for each group in the acute study. The mean thresholds across animals for the pre- and post-drug conditions were not significantly different within groups.

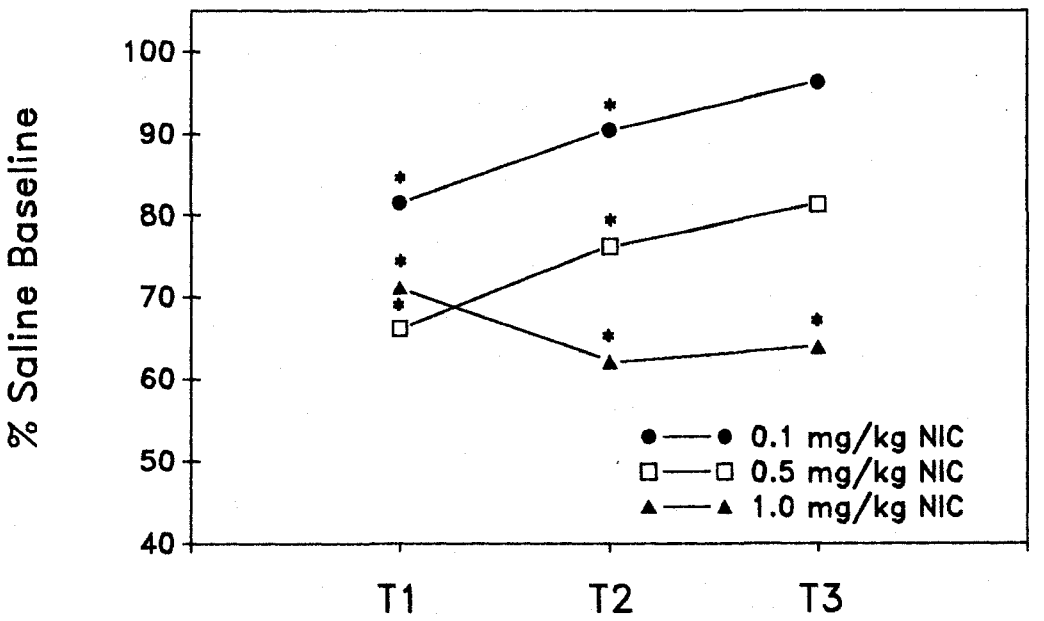

Figure 4. The time-course of nicotine (NIC) during test sessions (45 trials each) broken down into 15-trial blocks and collapsed over 9 days. T1=0-22.5 min post-injection, T2=22.5-45 min post-injection and, T3=45-67.5 min post-injection.

Therefore, data from these two conditions were collapsed to obtain a single, saline baseline measure. All subsequent saline comparisons used this baseline.

Repeated-measures t-tests showed that at all doses tested, nicotine produced a significant reduction in ICSS thresholds when compared to saline [$t(17)=2.89$, $p<.01$, $t(17)=3.05$, $p<.01$ and $t(17)=7.89$, $p<.001$ for 0.2, 0.4 and 0.8 mg/kg nicotine, respectively] (see Figure 5).

The following findings and conclusions may be drawn from the chronic and acute experiments. Nicotine produced a dose-dependent lowering of thresholds at all doses tested (0.1-1.0 mg/kg). At 0.1 and 0.5 mg/kg, nicotine's effect was evident 15 min post-injection. However, by the end of the test session, the effect of 0.1 mg/kg and 0.5 mg/kg was absent, while the effect of 1.0 mg/kg was still present.

Due to the small effect size produced by 0.1 mg/kg nicotine and some observed motor impairment at 1.0 mg/kg nicotine, it was determined that a dose range of 0.2 to 0.8 mg/kg nicotine would be suitable to use in the subsequent interaction studies.

Experiment 2

The purpose of Experiment 2 was to (1) assess the effect of MDL 28,133A, a mixed D_2/5-HT_2 antagonist, on ICSS thresholds and (2) determine if MDL 28,133A reliably blocks or attenuates nicotine-induced facilitation of ICSS. MDL 28,133A was chosen based on the recent report by Panicker et al. (1991), who found that pretreating animals with 5.0 mg/kg MDL 28,133A significantly attenuated the threshold-lowering effects of amphetamine and cocaine, with the effect on

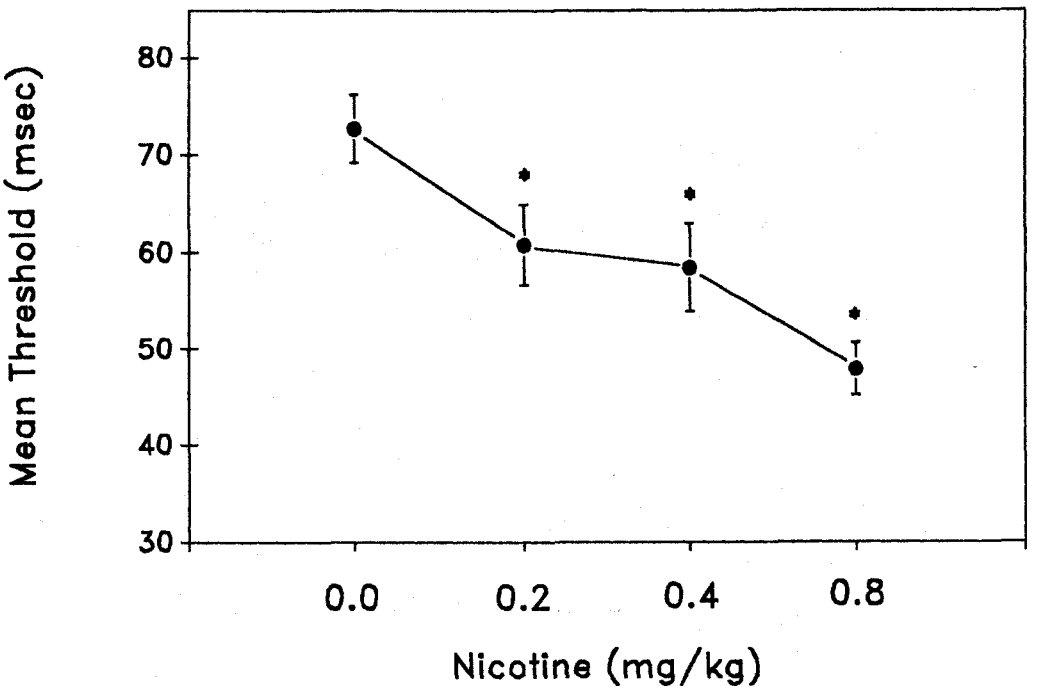

Figure 5. Dose-dependent reduction in train-duration thresholds produced by acute nicotine administration (data points represent the mean, 3-day median thresholds). Saline is represented as 0.0 mg/kg. $^{*}p < .01$, comparison to saline. Error bars represent one standard error of the mean.

amphetamine being more pronounced. These results suggest that this compound may have potential as a pharmacotherapy for stimulant abuse. However, in that study, MDL 28,133A produced a significant elevation in ICSS thresholds when administered alone, making data interpretation difficult. Therefore, one objective of this experiment was to determine if there is a dose of MDL 28,133A that produces no significant effect on self-stimulation thresholds when administered alone, but when co-administered with nicotine, blocks or attenuates nicotine's threshold-lowering effect.

The ability of MDL 28,133A to block or attenuate nicotine's effect on self-stimulation thresholds is presumed to be based on the common effects of psychomotor stimulants on mesolimbic dopamine neurotransmission (Wise & Bozarth, 1987). Wise & Bozarth (1987) have suggested that nicotine has amphetamine-like properties. Like amphetamine, nicotine increases stimulated dopamine release (Imperato et al., 1986). Like cocaine, nicotine also blocks dopamine reuptake (Izenwasser et al., 1990). Since nicotine, like amphetamine and cocaine, facilitates mesolimbic dopamine neurotransmission, it was assumed that MDL 28,133A would affect nicotine-induced changes in BSR in a manner similar to MDL 28,133A's effect on both cocaine and amphetamine-induced threshold changes. A comparison of the effects of MDL 28,133A on nicotine, amphetamine and cocaine-induced lowering of thresholds will provide information about nicotine's similarity to other psychomotor stimulants.

A preliminary study [in which animals were pretreated with MDL 28,133A (5 mg/kg IP, 1 hr prior to testing) and administered 0.4 mg/kg nicotine 15 min prior to testing] showed that MDL 28,133A was able to effectively block the threshold-

lowering effect of nicotine (see Figure 6). Nicotine by itself produced a significant reduction in thresholds when compared to saline [75.6 msec for saline versus 56.8 msec for nicotine, $t(19)=5.82$, $p<.001$]. This effect was completely blocked by MDL 28,133A (75.6 msec for saline versus 76.8 msec for MDL+nicotine). In this pilot study, the effect of MDL 28,133A alone on self-stimulation thresholds was not assessed. Therefore, the purpose of Experiment 2 was to assess the effects of MDL 28,133A on nicotine-induced decreases in self-stimulation thresholds using multiple doses of both nicotine and MDL 28,133A both alone and in combination.

Method

Procedure. Animals were screened and trained as described in the General Experimental Procedures Section. Once the rats were trained, self-stimulation testing sessions were conducted for 3 days following IP injections of isotonic saline (0.25 ml). Following pre-drug saline testing, animals ($n=10$) received either 0.2, 0.4 or 0.8 mg/kg nicotine with the order of drug administration being counterbalanced within subjects (i.e., a different dose of nicotine was given to each animal over a 3 day period). After a 2-day wash-out period, animals were injected (IP) with either 1.0, 3.0, or 6.0 mg/kg MDL 28,133A 30 min prior to testing. During the next three days, animals were pretreated with the same dose of MDL 28,133A as on day 1 and, 15 min prior to testing, were injected with either 0.2, 0.4, or 0.8 mg/kg nicotine IP. As in the nicotine alone condition, each animal received a different dose of nicotine over the 3 days with the dose of MDL 28,133A remaining constant within animals.

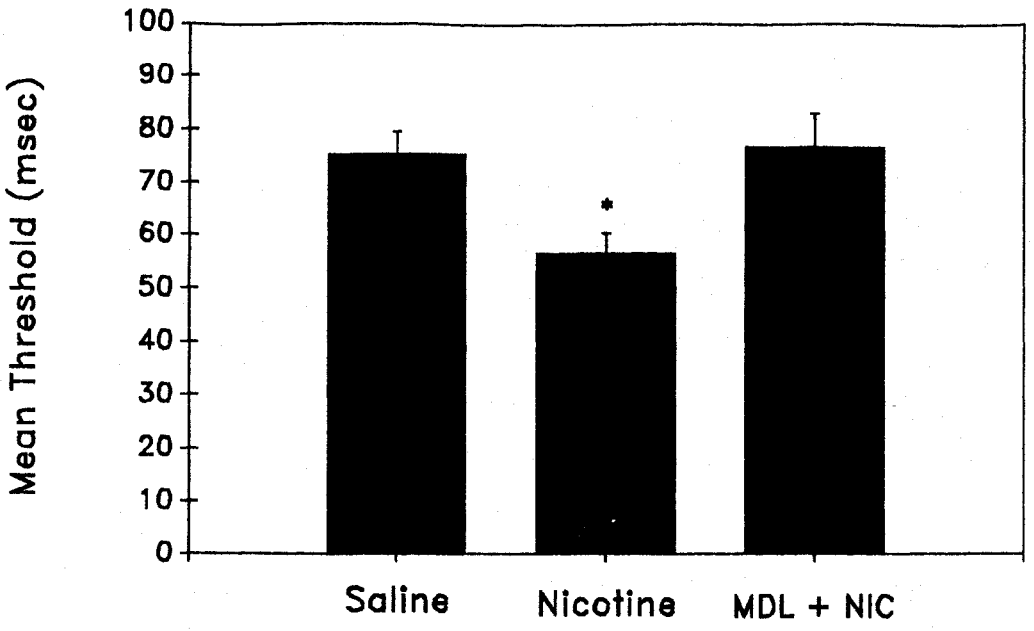

Figure 6. Attenuation of the threshold-lowering effect of 0.4 mg/kg nicotine (NIC) by 5.0 mg/kg MDL 28,133A (MDL). *$p < .01$, comparison to saline. Error bars represent one standard error of the mean.

After the 3 days of the MDL/Nicotine co-administration, animals were again tested with the dose of the antagonist used on Day 1. This procedure of a 5-day block of testing was used for three weeks (each 5-day block was separated by a 2-day washout period), with MDL 28,133A dose being a between-week factor and nicotine dose a within-week factor. Three days of post-drug, saline testing followed the completion of drug testing to ensure that animals were still responding at pre-drug, baseline levels.

Results and Discussion

A repeated-measures t-test was performed on the pre-drug and post-drug saline conditions to determine if drug administration had caused any shift in baseline responding. The mean thresholds across animals for the saline conditions were not significantly different [$t(9)=0.20$, n.s.]. Therefore, data were collapsed to obtain a single, saline baseline.

One-way repeated measures ANOVA's were used to assess the effects of nicotine and MDL 28,133 on ICSS thresholds. Nicotine treatment had a significant effect on thresholds [$F(3,27)=10.66$, $p<.0001$], while the effect of MDL 28,133A on thresholds was not significantly different from saline [$F(3,27)=1.6$, n.s.]. The latter effect is important since, to make inferences about the effect of MDL 28,133A on nicotine-induced lowering of thresholds, MDL 28,133A alone should not produce any changes in baseline responding. Table 1 shows the breakdown of doses of nicotine alone and MDL 28,133A alone compared to saline using repeated-measures t-tests.

Table 1

Mean Thresholds (msec) for Nicotine and MDL 28,133A

Nicotine (mg/kg)		MD 28,133A (mg/kg)	
Dose	Threshold	Dose	Threshold
0.2	68.6	1.0	74.0
0.4	51.5*	3.0	81.7
0.8	51.0*	6.0	86.6

Note: * $p < .01$ when compared to saline threshold of 78.3 msec.

The mean threshold for 0.2 mg/kg nicotine was not significantly different from saline baseline. A significant threshold-lowering effect was produced with 0.4 and 0.8 mg/kg nicotine [$t(9)=5.42$, $p<.001$ and $t(9)=4.57$, $p<.01$, respectively]. However, the effects of 0.4 and 0.8 mg/kg nicotine were not significantly different from each other [$t(9)=0.13$, n.s.].

A two-way, repeated-measures ANOVA was used to assess the effect of MDL 28,133A on nicotine-induced lowering of thresholds. The effects of MDL 28,133A on nicotine were assessed by analyzing the nicotine alone and nicotine + MDL 28,133A conditions. Results of the ANOVA showed a significant main effect for nicotine [$F(3,81)=13.63$, $p<.0001$] and for MDL 28,133A + nicotine [$F(3,81)=5.62$, $p<.001$]. There was also a significant interaction between dose of nicotine and dose of MDL 28,133A + nicotine [$F(9,81)=3.0$, $p<.01$]. Post-hoc, repeated-measures t-tests comparing nicotine (collapsed across doses) to all doses of MDL 28,133A + nicotine revealed that pretreatment with both 3.0 and 6.0 mg/kg MDL 28,133A significantly attenuated the effect of nicotine [$t(9)=2.37$, $p<.05$ and $t(9)=3.96$, $p<.01$, respectively], while pretreatment with 1.0 mg/kg MDL 28,133A did not produce any significant change in thresholds from nicotine baseline [$t(9)=0.55$, n.s.] (see Figure 7). Table 2 summarizes the effect of MDL 28,133A on nicotine's threshold-lowering effect. MDL 28,133A alone, at all doses, did not produce any significant changes in ICSS thresholds. However, when animals were pretreated with MDL 28,133A (1.0, 3.0 and 6.0 mg/kg), the threshold-lowering effect of 0.4 mg/kg nicotine was significantly attenuated by 1.0 and 6.0 mg/kg MDL

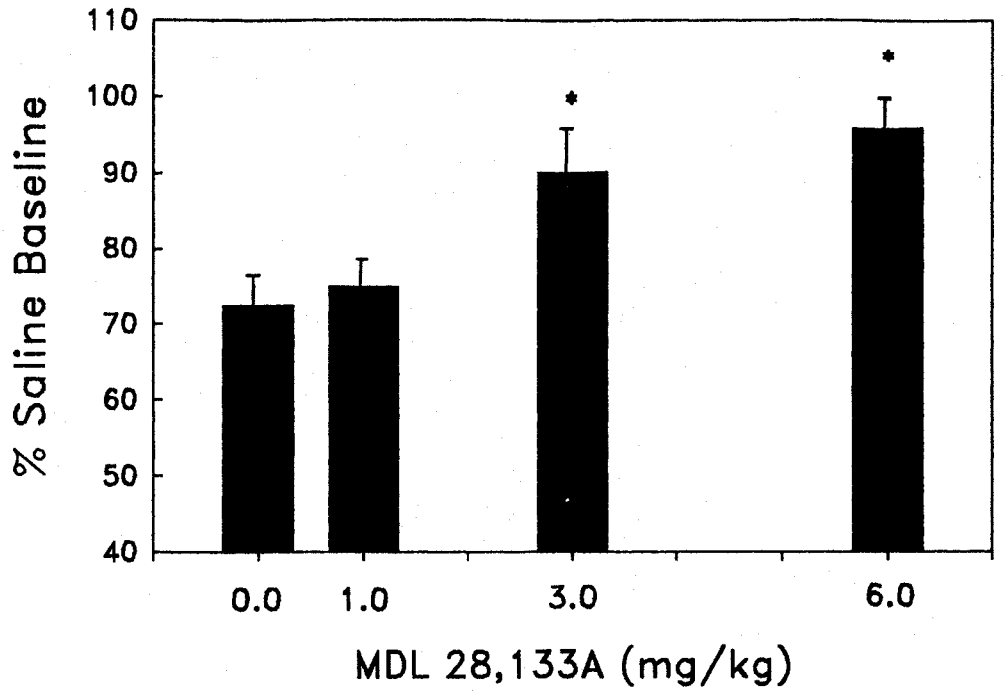

Figure 7. MDL 28,133A attenuation of nicotine-induced threshold lowering. Nicotine doses are collapsed. $^{*}p < .05$ compared to nicotine (0.0 mg/kg). Error bars represent one standard error of the mean.

Table 2

Effect of Nicotine, MDL 28,133A and Nicotine + MDL 28,133A on BSR Thresholds

		MDL 28,133A (mg/kg)			
		0.0	**1.0**	**3.0**	**6.0**
Nicotine (mg/kg)	**0.0**	78.3	74.0	81.7	85.6
	0.2	68.6	61.6	70.6	70.8
	0.4	51.5	65.6	72.3*	60.9
	0.8	51.0	50.8	68.6	92.0*

Note: * $p < .01$ when compared to nicotine collapsed across levels (collapsed nicotine threshold = 57.1 msec).

28,133A and completely blocked by 3.0 mg/kg MDL 28,133A. The threshold lowering effect of 0.8 mg/kg nicotine was completely blocked by 6.0 mg/kg MDL 28,133A.

The pattern of results seen in this experiment suggest that MDL 28,133A affects nicotine's lowering of thresholds much in the same way as it does that of amphetamine (Panicker et al., 1991). However, in this experiment, MDL 28,133A alone did not significantly elevate self-stimulation thresholds as in the Panicker et al. (1991) study. This difference could be attributed to the different dose range of MDL 28,133A used in these two studies and, to the observation that at higher doses, there is a non-specific disruption of behavior which increases variability in the data.

The observation that MDL 28,133A alone did not significantly increase self-stimulation thresholds suggests that the 5-HT_2 component of the compound may be modulating dopaminergic function since, typically, D_2 antagonists produce an elevation in self-stimulation thresholds (Gallistel & Davis, 1983). Recently, Schmidt, Fayadel, Sullivan & Taylor (1992) have suggested that the regulation of dopamine synthesis mediated by 5-HT_2 receptors is likely to be a phasic rather than a tonic effect, which becomes significant only during states of high serotonergic and dopaminergic transmission. In vivo studies have shown that nicotine stimulates release of dopamine in both the mesolimbic and nigrostriatal systems (Imperato et al., 1986). Therefore, it is possible that this phasic increase in dopamine release is being antagonized by the 5-HT_2 component of MDL 28,133A and is responsible for its anti-nicotine effect. In general, drugs of abuse increase extracellular levels of dopamine

(DiChiara & Imperato, 1988). Therefore, drugs with antiserotonergic activity may be useful as anti-abuse agents. Meltzer (1989) has shown that drugs with mixed D_2/5-HT_2 antagonist properties exhibit the capacity to modulate dopaminergic neurotransmission without producing extrapyramidal side effects. For example, the improved efficacy of clozapine (an atypical neuroleptic with a high affinity for 5-HT_2 receptors) over typical antipsychotic agents (e.g., haloperidol) has been attributed to its antagonism of 5-HT_2 receptors (Meltzer, 1989). To determine the relative contribution of the serotonergic component to the efficacy of MDL 28,133A's anti-nicotine effect, a study assessing the effect of a specific 5-HT_2 antagonist (MDL 26,508) was conducted.

Experiment 3

The purpose of Experiment 3 was to determine if the specific 5-HT_2 antagonist MDL 26,508 could attenuate or block the reinforcing effect of nicotine.

It is well known that serotonin systems interact with mesolimbic neurons (Chesselet, 1984; Steinbusch, 1981). It has been shown that selective 5-HT_2 receptor antagonists can block dopamine-dependent neurochemical alterations produced by MDMA, an amphetamine analogue (Schmidt & Kehne, 1990; Schmidt et al., 1991). Also, 5-HT_2 antagonists have been shown to reverse amphetamine-induced slowing of A10 neurons caused by stimulated dopamine synthesis (Sorensen et al., 1991). These observations provide a rationale for using substances with a high degree of anti-

serotonergic activity in the treatment of conditions that are dependent upon dopamine release, an effect which is produced by nicotine as well as other psychomotor stimulants.

Method

Procedure. Animals were screened and trained as described in the General Procedures Section. Once the rats were trained, self-stimulation testing sessions were conducted for 3 days following IP injections of isotonic saline (0.25 ml). Following pre-drug saline testing, animals (n=8) were injected with 0.4 mg/kg nicotine (IP) for three days. The next phase of testing consisted of 2 blocks of 5 days (separated by a 2-day washout) in which on days 1 and 5 of the first block, half of the animals were injected with either 1.0 or 5.0 mg/kg MDL 26,508 30 min prior to testing. Preliminary data showed that 5.0 mg/kg MDL 26,508 produced a 21 msec increase in self-stimulation thresholds above baseline, and that this increase approached significance. Therefore 5.0 mg/kg MDL 26,508 was used as the cut-off point. Animals that received 1.0 mg/kg MDL 26,508 during block 1 received 5.0 mg/kg during block 2 and vice versa. On days 2-4 of the blocks of testing, animals were injected with the same dose of MDL used on day 1 plus nicotine (0.4 mg/kg, 15 min post-antagonist) and were tested 15 min later. Three days of post-drug saline testing followed the completion of drug testing to ensure that animals were still responding at pre-drug, baseline levels.

Results and Discussion

Data were analyzed using repeated-measures t-tests. Due to the death of three animals prior to the post-drug saline testing, the comparison between pre-drug saline and post-drug saline was based on the five animals remaining at the end of the experiment. The comparison between pre- and post-drug saline did not reveal any significant difference [$t(4)=1.98$, n.s.). Therefore, all subsequent comparisons to saline were based on the pre-drug condition.[1]

Repeated-measures t-tests showed that, as in the previous experiments, 0.4 mg/kg nicotine produced a significant lowering of thresholds when compared to saline [$t(7)=2.90$, $p<.05$] (Table 3). Also, the thresholds for the animals receiving the antagonist alone (1.0 and 5.0 mg/kg) were not significantly different from saline baseline [$t(7)=1.02$, n.s. and $t(7)=0.81$, n.s., respectively].

Thresholds for the co-administration of MDL 26,508 (1.0 and 5.0 mg/kg) plus nicotine (0.4 mg/kg) were significantly different from saline [$t(7)=2.99$, $p<.05$ and $t(7)=2.76$, $p<.05$, respectively], but were not significantly different from the nicotine alone condition (see Figure 8).

Therefore, at the doses tested, MDL 26,508 was not effective in attenuating or blocking the threshold-lowering effect of nicotine. This suggests that antagonism of 5-HT_2 receptors alone is not sufficient to suppress the reinforcing effects of nicotine.

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[1] A possible cause for the death of the three animals may be related to repeated administration of the drug and order of dose, since the deaths occurred after the final administration of 5.0 mg/ml MDL 26,508 which, for these animals, was the final day of drug testing.

Table 3

Mean Thresholds (msec) for MDL 26,508 and Nicotine + MDL 26,508

		MDL 26,508 (mg/kg)		
		0.0	**1.0**	**5.0**
Nicotine (mg/kg)	**0.0**	84.2	78.0	81.1
	0.4	61.3*	64.3*	61.9*

Note: * $p < .05$ when compared to saline baseline.

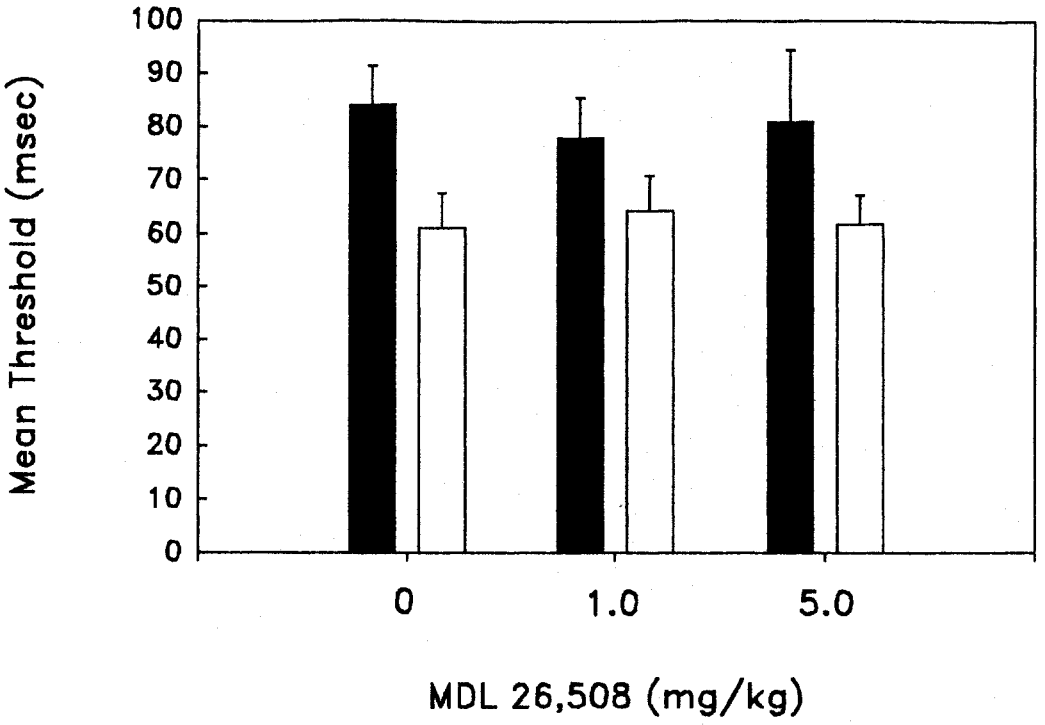

Figure 8. The effect of MDL 26,508 on nicotine-induced lowering of train duration thresholds. Solid bars represent doses of MDL 26,508 (0.0 mg/kg=saline). Open bars represent nicotine (0.4 mg/kg) in combination with MDL 26,508 (0.0 mg/kg=0.4 mg/kg nicotine). Error bars represent one standard error of the mean.

When compared to the effect of MDL 28,133A, these results suggest that blockade of the D_2 receptor is the critical factor in the ability of MDL 28,133A to block nicotine-induced lowering of thresholds. Therefore, Experiment 4 was conducted to determine if a specific D_2 antagonist would attenuate the effect of nicotine to a greater or lesser extent than the mixed antagonist MDL 28,133A.

Experiment 4

The attenuation of BSR following administration of dopamine antagonists is considered to be evidence that catecholamine-releasing neurons either (1) carry the reward signal or (2) modulate the reinforcing effect of that signal (Crow, 1972; German et al., 1974). For example, Gallistel & Karras (1984) demonstrated that pimozide (a D_2 antagonist) attenuated the rewarding effect of BSR and that this effect was reversed by amphetamine. These results are consistent with the hypothesis that blockade of dopamine receptors attenuates reward, while substances that enhance dopaminergic transmission facilitate reward.

Although blockade of dopamine receptors, in general, has an inhibitory effect on self-stimulation behavior, there is still controversy over whether this inhibition is due to an attenuation in the rewarding value of brain stimulation or to a deficit in performance capacity. Many studies conducted with neuroleptics, using rate-free measures of BSR, have shown that systemic administration of neuroleptics attenuates medial forebrain bundle self-stimulation (Stellar & Rice, 1989). However, some studies also suggest that neuroleptics produce motor/performance effects that may be

independent of the rewarding value of the drug. For example, Ettenberg, Koob & Bloom (1981) suggested that the suppression of self-stimulation behavior produced by neuroleptics may be due in part to the response requirements in the experimental paradigm. In their experiment, a comparison of the effect of alpha-flupenthixol on lever-pressing and nose-poking for BSR showed that alpha-flupenthixol produced a dose-dependent reduction in lever-pressing response rates. However, when the requirement was changed to nose-poking there was little attenuation in performance when compared to a saline baseline measure. Ettenberg et al. (1981) concluded that there is a principal effect of neuroleptics on motor performance since motorically, nose-poking is an easier task.

While there is general agreement that dopaminergic systems are critically involved in reward produced by stimulation of the medial forebrain bundle, results of studies using DA antagonists emphasize the importance of using reward-specific behavioral measures. Gallistel & Davis (1983) have reported that the ability of a neuroleptic to attenuate BSR correlates strongly with its affinity for the D_2 receptor site, but not with its affinity for D_1, D_3, alpha-adrenergic, 5-HT_1, or 5-HT_2 receptors. Over the past decade this line of research has been advanced by the development of antagonists that are specific (i.e., demonstrate a high affinity) for D_2 receptors. One such group of substances is the substituted benzamides (Jenner & Marsden, 1979), which are characterized by a substituent on the nitrogen atom of the benzamide molecule. These substances are also referred to as atypical neuroleptics because, although they are similar to the "classical neuroleptics" (e.g., haloperidol) in their ability to antagonize

dopamine receptors, unlike the classical neuroleptics, they possess a low potential for causing extrapyramidal side effects and tardive dyskinesia (White & Wang, 1983).

Sulpiride is a substituted benzamide which, when compared to more typical neuroleptics (e.g., haloperidol, trifluoperazine, pimozide & thioridazine) has a low affinity for 5-HT, histamine, noradrenaline, acetylcholine, or gamma-aminobutyric receptor sites (Jenner & Marsden, 1981). Its ability to displace spiperone (a D_2 antagonist) from striatal binding sites is indicative of its D_2 receptor affinity. Sulpiride also differs from the typical neuroleptics by exhibiting an inability to block the effects of dopamine on dopamine-sensitive adenylate cyclase, further indicating its selectivity as an antagonist of D_2 receptors (O'Connor & Brown, 1982).

Since the ability of a neuroleptic to attenuate BSR is related to its affinity for the D_2 receptor (Gallistel & Davis, 1983), it was hypothesized that if nicotine produces its rewarding effect (either directly or indirectly) via stimulation of D_2 receptors, sulpiride should either block or attenuate the threshold-lowering effect of nicotine on BSR.

Therefore, the purpose of Experiment 4 was to assess the relative contribution of 5-HT_2 and D_2 receptors to the anti-nicotine effects of MDL 28,133A.

Method

Procedure.

Sulpiride Dose-Response. Animals were screened and trained as described in the General Procedures Section. Once the rats were trained, self-stimulation testing sessions were conducted for three days following IP injections of isotonic saline (0.25 mg/kg). Animals (n=5) were then injected with either 25.0, 50.0 or 100.0 mg/kg sulpiride 1 hr prior to testing, with the order of drug administration being randomized within subjects. Following three days of sulpiride testing, animals were again tested with saline during a 3-day, post-drug period.

Sulpiride and Nicotine Co-administration. An additional group of animals was screened and trained as described in the General Procedures Section. Once the rats were trained, self-stimulation testing sessions were conducted for three days following IP injections of isotonic saline (0.25 ml). Once this pre-drug, saline baseline was completed, animals (n=10) were injected with one of three doses of nicotine (0.2, 0.4 or 0.8 mg/kg) on three consecutive days, with doses being counterbalanced across animals and over days. Testing began 15 min post-injection. Following a 2-day washout period, animals were injected with either 25.0 (week 1) or 50.0 (week 2) mg/kg sulpiride (SUL) 1 hr prior to testing. During the next three days, animals were pretreated with the same dose of sulpiride as on day 1 and were injected with either 0.2, 0.4 or 0.8 mg/kg nicotine (NIC) IP 15 min prior to testing. After the 3 days of the SUL/NIC co-administration, animals were again tested with the dose of sulpiride used on day 1. This procedure of a 5-day block of testing was used for two

weeks (each 5-day block was separated by a 2-day washout period), with sulpiride being a between-week factor and nicotine dose a within-week factor. Three days of nicotine testing followed the last 5-day block, and after a 2-day washout period, three days of post-drug, saline tested followed to ensure that animals were still responding at pre-drug baseline levels.

Results and Discussion

Data from the sulpiride dose-response experiment were analyzed with a one-way, repeated-measures ANOVA. Sulpiride, at all doses tested, did not produce a significant change in self-stimulation thresholds when compared to saline baseline [$F(3,12)=2.66$, n.s.]. However, there was a trend towards a significant increase in thresholds at the 100.0 mg/kg dose (92.4 msec saline versus 125.7 msec sulpiride). Therefore, only 25.0 and 50.0 mg/kg sulpiride were used in the co-administration study.

Pre- and post-drug saline data from the co-administration study were analyzed using a repeated-measures t-test. The mean thresholds across animals for the saline conditions were not significantly different. Therefore, data were collapsed to obtain a single, saline baseline.

Repeated-measures t-tests comparing pre- and post-drug nicotine thresholds revealed no significant change in nicotine baseline responding across doses of nicotine. Therefore, pre- and post-nicotine thresholds were collapsed to produce a baseline nicotine threshold for each dose.

Sulpiride data from Days 1 and 5 of the 5-day blocks were analyzed using repeated-measures t-tests. Results of the analysis showed that there was no significant change in response to sulpiride at either 25.0 or 50.0 mg/kg. Therefore, data were collapsed to obtain a single, baseline threshold for each dose.

When compared to saline baseline, repeated-measures t-tests showed that nicotine produced a significant lowering of self-stimulation thresholds at all doses tested [$t(9)=4.14$, $p<.01$ for 0.2 mg/kg nicotine; $t(9)=6.35$, $p<.001$ for 0.4 mg/kg nicotine; $t(9)=4.71$, $p<.01$ for 0.8 mg/kg nicotine]. However, a repeated-measures ANOVA showed that there was not a significant difference in effect between the doses of nicotine [$F(2,18)=0.94$, n.s.]. The effects of 25.0 mg/kg sulpiride and 50.0 mg/kg sulpiride alone were not significantly different from saline. Figure 9 shows the effect of nicotine and sulpiride (both collapsed across doses) on train duration thresholds when compared to saline.

A two-way,repeated-measures ANOVA was used to assess the effect of sulpiride on nicotine-induced lowering of thresholds, comparing the nicotine alone (collapsed for each dose level) and nicotine + sulpiride conditions. Attenuation of the effect of nicotine would be inferred if the sulpiride + nicotine thresholds were significantly higher than the nicotine-alone thresholds. Overall, there were significant main effects for both nicotine [$F(3,27)=10.08$, $p<.001$] and sulpiride [$F(2,18)=4.3$, $p<.05$]. The interaction effect was not significant [$F(6,54)=0.33$, n.s.].

Repeated measures t-tests comparing nicotine (collapsed across all dose levels) to all doses of sulpiride + nicotine (levels of nicotine collapsed at each level of

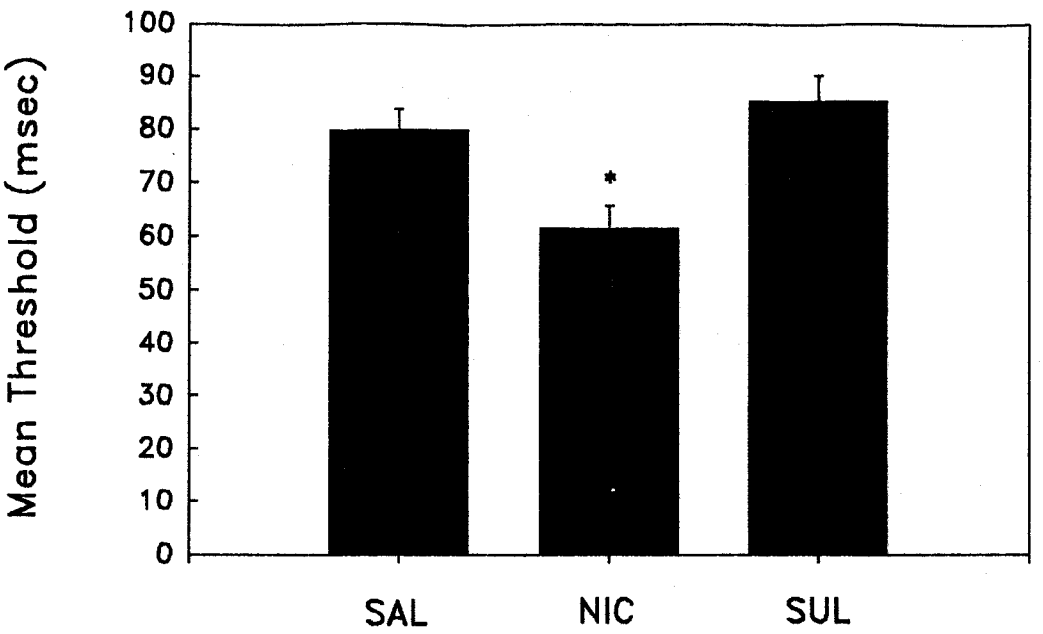

Figure 9. The effect of nicotine collapsed across doses (NIC) and sulpiride collapsed across doses (SUL) on train-duration thresholds. $^{*}p < .01$ compared to saline. Error bars represent one standard error of the mean.

sulpiride) revealed that 50 mg/kg sulpiride significantly attenuated the effect of nicotine [sulpiride + nicotine threshold = 72.1 msec versus collapsed nicotine threshold = 61.8 msec; $t(9) = 2.93$, $p < .05$] on self-stimulation thresholds. Pretreatment with 25 mg/kg sulpiride did not produce any significant change in threshold from nicotine baseline (see Figure 10). Table 4 shows the effect of nicotine, sulpiride and nicotine + sulpiride on self-stimulation thresholds for each dose of nicotine and sulpiride tested.

Neither dose of sulpiride tested produced a significant change in self-stimulation thresholds when compared to saline. Although the mean threshold value for 50 mg/kg sulpiride was slightly elevated compared to saline (sulpiride threshold = 90.2 msec versus saline threshold = 80.1), this difference was not statistically significant. At 25 mg/kg, sulpiride did not affect nicotine's effect on thresholds. However, pretreatment with 50 mg/kg sulpiride significantly attenuated the threshold-lowering effect of nicotine, suggesting that intact functioning of D_2 receptors are necessary for the expression of nicotine's rewarding effects on BSR.

Histological Results

Histological analyses were performed on a representative sample of animals (n = 12) to verify the placement of the electrode tip. Only a subset of the implanted animals was included in the histological analyses since the results obtained in these experiments were similar to those obtained in previous studies in which the same coordinates were used. Sections were examined microscopically and results show that

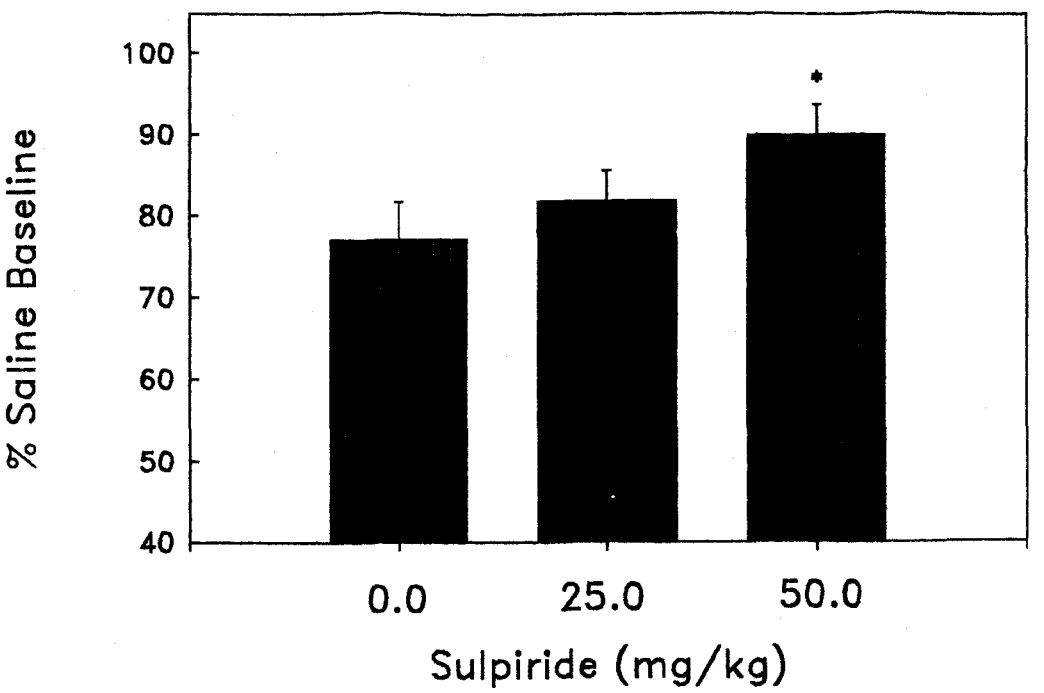

Figure 10. Sulpiride attenuation of nicotine-induced threshold lowering. Nicotine doses are collapsed across levels of sulpiride. $^{*}p < .05$ when compared to collapsed nicotine baseline (0.0 mg/kg). Error bars represent one standard error of the mean.

Table 4

Mean Thresholds (msec) for Sulpiride and Nicotine + Sulpiride

		Sulpiride (mg/kg)		
		0.0	**25.0**	**50.0**
	0.0	80.1	80.6	90.2
Nicotine (mg/kg)	**0.2**	64.3[a]	66.0	77.5*
	0.4	61.8[a]	69.7[a]	68.6
	0.8	59.3[a]	63.9[a]	70.1[a]

Note: * $p < .05$ when compared to collapsed nicotine threshold.
a = significantly different from saline.

the electrode tips were located along the course of the medial forebrain bundle from the level of the ventral tegmental area to the posterior hypothalamus (see Figure 11). The distribution of loci was essentially identical to those reported previously (e.g., Frank et al., 1987).

General Discussion

Nicotine is voluntarily self-administered by humans (Henningfield & Goldberg, 1983) and by laboratory animals (Goldberg, Spealman & Goldberg, 1981), but the neural mechanisms that underlie the drug's reinforcing action are not well understood. One model used to assess the rewarding properties of abused substances is the brain stimulation reward (BSR) paradigm. Using this paradigm, various investigators have found that nicotine can either enhance BSR or produce little or no effect (Clarke & Kumar, 1983a; Clarke & Kumar, 1984; Schaefer & Michael, 1986). Therefore, the purpose of Experiment 1 was to clarify the characteristics of nicotine-induced changes in BSR by using a rigorously validated, rate-free and reward-selective procedure (Frank et al., 1987). A second goal was to assess the ability of dopaminergic and serotonergic antagonists to modify nicotine's effects, thus providing a better understanding of the mechanisms underlying nicotine's reward-altering properties.

Experiment 1 demonstrated that nicotine (0.1 to 1.0 mg/kg) produced a dose-dependent lowering of BSR thresholds and that this effect did not tolerate over 9 days of administration. In the chronic study, nicotine doses of 0.1, 0.5 & 1.0 mg/kg reduced thresholds by 10%, 16% and 38%, respectively. A similar pattern was seen

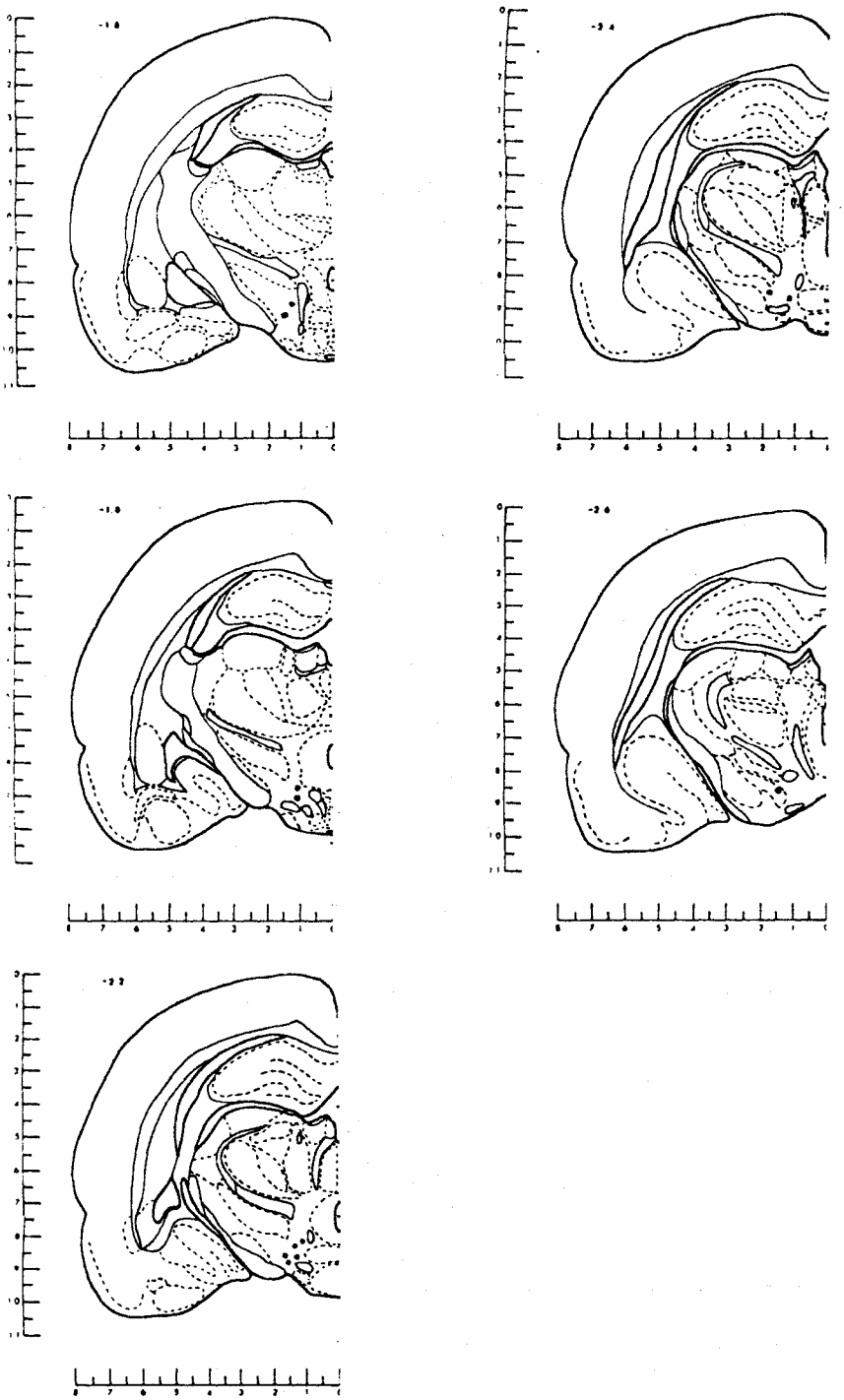

Figure 11. Location of electrode tips from a representative sample of animals used in the study. (The plates are based on Pellegrino, Pellegrino & Cushman, 1979).

with acute administration, where nicotine doses of 0.2, 0.4 & 0.8 mg/kg reduced thresholds by 16%, 20% and 34%, respectively. These results are in agreement with those of Newman (1972), who found that nicotine (0.1 to 0.8 mg/kg) facilitated intracranial self-stimulation (ICSS) and with those of Clarke & Kumar (1984), who found that nicotine-induced facilitation of ICSS (0.4 mg/kg nicotine) did not tolerate with repeated, daily administration over 15 days. When compared to other stimulants, the effect of nicotine on BSR thresholds is similar to that seen with both amphetamine and cocaine (Frank et al., 1987; Frank, Manderscheid, Panicker, Williams & Kokoris, 1992). For example, amphetamine (0.3-0.9 mg/kg) lowered BSR thresholds approximately 15-40% and cocaine (15-30 mg/kg) produced a 25-35% reduction in thresholds. It is worth noting that, like amphetamine, nicotine appears to release dopamine from newly synthesized cytoplasmic pools of transmitter both in the nucleus accumbens and caudate-putamen (Clarke, 1990). Similar to cocaine, nicotine can also inhibit the re-uptake of dopamine, but its effectiveness is approximately one-half that of cocaine's (Izenwasser et al., 1991).

At the two highest doses tested (0.8 and 1.0 mg/kg), nicotine produced some observable motor impairment. This effect has also been described by other investigators (Clarke & Kumar, 1983; Morrison, 1967). In general, the animals became flaccid and ataxic within about 2 min post-injection and subsequently were prostrate, with a transient loss of righting reflex. These symptoms dissipated between 5 and 15 min after injection and did not appear to affect the animal's ability to respond during the test session.

The results of Experiment 1 indicate that nicotine can reliably, and in a dose-dependent manner, enhance BSR. The mechanism by which this occurs is not known. However, as with other psychomotor stimulants, it is hypothesized that nicotine produces its rewarding effect by enhancing dopaminergic transmission in the mesolimbic dopamine system (Wise & Bozarth, 1987). Electrophysiological and neurochemical studies are consistent with this hypothesis. For example, nicotine increases the firing rate of mesolimbic dopamine neurons (Grenhoff et al., 1986) and stimulates dopamine release in the nucleus accumbens, both in vitro and in vivo (Imperato et al., 1986; Rowell et al., 1987). Also, 6-hydroxydopamine lesions of the nucleus accumbens attenuate nicotine self-administration in rats (Singer, Wallace & Hall, 1982), suggesting that an intact mesolimbic pathway is necessary for nicotine to produce its reinforcing effects.

However, there is evidence that the initial depressant effect of nicotine may be produced by muscarinic cholinergic activation, since this effect is potentiated by the muscarinic cholinergic agonist physostigmine (Morrison, Goodyear & Sellers, 1969) and blocked by the muscarinic antagonist atropine (Olds & Domino, 1969). That the facilitory effect of nicotine on ICSS is produced by activation of nicotinic cholinergic receptors is supported by the observation that atropine does not alter the stimulatory effect of nicotine (Morrison et al., 1969). More recently, Huston-Lyons & Kornetsky (1992) demonstrated that the threshold-lowering effect of nicotine could be blocked by the nicotinic cholinergic antagonist mecamylamine. Additional evidence that nicotine's effects on BSR are primarily nicotinic in nature comes from studies

examining the effect of nicotinic and muscarinic agonists and antagonists on stimulated [^{3}H] dopamine release in the nucleus accumbens. When compared to nicotine, the muscarinic agonists arecoline and oxotremorine, along with acetylcholine (which functions as the neurotransmitter at peripheral nicotinic receptors), showed only a marginal ability to release [^{3}H] dopamine in the nucleus accumbens (Rowell et al., 1987). While the nicotinic antagonists reduced nicotine-induced release by approximately 50%, the muscarinic antagonist atropine was without effect (Rowell et al., 1987).

Experiment 2 assessed the ability of MDL 28,133A (a mixed 5-HT_2/D_2 antagonist) to attenuate or block nicotine-induced facilitation of ICSS. Based on the results of Experiment 1, it was determined that a dose range of 0.2 to 0.8 mg/kg nicotine would be suitable to use in this interaction study. To make any inference about the effect of MDL 28,133A on nicotine-induced threshold lowering, it was important that MDL 28,133A, by itself, did not produce any change in baseline ICSS thresholds. MDL 28,133A (1.0, 3.0 & 6.0 mg/kg) alone did not produce any significant changes in ICSS thresholds although there was a slight (but not statistically significant) elevation in thresholds at 6.0 mg/kg. When animals were pretreated with 3.0 and 6.0 mg/kg MDL 28,133A 1.0 hr prior to nicotine administration, MDL 28,133A significantly attenuated the threshold lowering effect of nicotine. The observation that MDL 28,133A alone did not elevate self-stimulation thresholds is interesting since compounds with D_2 antagonist properties typically produce an elevation in self-stimulation thresholds (Gallistel & Davis, 1983). These results suggest that the 5-HT_2

component of the compound may, in some way, be modulating dopaminergic function. Also, it has been shown that serotonin receptor antagonists can diminish neuroleptic-induced catalepsy (Waldmeier & Delini-Stula, 1979), suggesting that diminishing serotonergic activity can decrease the inhibitory effects of neuroleptics on dopaminergic output.

To determine the relative contribution of the serotonergic component to the efficacy of MDL 28,133A's anti-nicotine effect, Experiment 3 assessed the effect of a specific 5-HT_2 antagonist (MDL 26,508) on BSR and nicotine-induced threshold lowering. MDL 26,508 alone did not produce any significant changes in ICSS thresholds, nor did it alter nicotine's threshold-lowering effect. These results suggest that antagonism of 5-HT_2 receptors alone is not sufficient to suppress the rewarding effects of nicotine. Recently, Schmidt et al. (1992) reported that the selective 5-HT_2 antagonist MDL 100,907 inhibits MDMA-stimulated dopamine release. It has also been demonstrated that nicotine enhances the release of newly synthesized dopamine (Fung & Lau, 1991). However, Schmidt et al. (1991) also noted that MDL 100,907 did not affect the basal rate of dopamine synthesis. Therefore, if MDL 26,508 produces effects similar to MDL 100,907, the facilitation of dopaminergic activity produced by self-stimulation alone (Murrin & Roth, 1976) may have produced an upward shift in the basal rate of dopamine synthesis, which may have compensated for any inhibition of nicotine-stimulated dopamine release produced by MDL 26,508.

Since MDL 26,508 produced no significant changes in ICSS thresholds, Experiment 4 assessed the ability of sulpiride, a specific D_2 antagonist, to modify

nicotine's effect on BSR. Sulpiride alone (25.0 and 50.0 mg/kg) did not produce any significant changes in ICSS thresholds. However, when co-administered with nicotine, sulpiride (50.0 mg/kg) effectively attenuated the threshold-lowering effect of nicotine so that animals were responding at levels similar to saline baseline levels of response [threshold for 50 mg/kg sulpiride + nicotine (collapsed across doses) = 72.07 msec versus saline threshold = 80.1 msec: $t(9)=1.89$, n.s.].

A comparison of the results of the antagonist experiments reveals the following pattern (Table 5). Nicotine alone reduced ICSS thresholds an average of 20 msec. MDL 26,508 plus nicotine reduced nicotine's effect 3% to 13%, indicating no significant effect of a specific 5-HT_2 antagonist on nicotine-induced enhancement of BSR. MDL 28,133A plus nicotine reduced nicotine's effect from 10% to 83%, which was greater than the magnitude of the effects observed for the sulpiride and nicotine combinations (26% to 56%). These results are interesting if one looks at the selectivity of sulpiride and MDL 28,133A for D_2 receptors. Data from receptor binding assays show that sulpiride, in vitro, is over two times as selective for the D_2 receptor as MDL 28,133A (Jenner & Marsden, 1981). Therefore, if the ability of a substance to block D_2 receptors is the primary mechanism through which stimulant-induced facilitation of BSR is attenuated, one would predict that sulpiride would have had a larger affect on the attenuation of BSR than MDL 28,133A. Recently, Schmidt, Black & Taylor (1990) have shown that 5-HT_2 antagonists can block the stimulation of dopamine synthesis that normally occurs in response to MDMA-induced dopamine release. This effect has been attributed to blockade of the

Table 5

Percent Reduction in Nicotine's Effect Produced by Each Antagonist

Antagonist	% Reduction
MDL 28,133A	
1 mg/kg	10
3 mg/kg	63
6 mg/kg	83
MDL 26,508	
1 mg/kg	3
5 mg/kg	13
SULPIRIDE	
25 mg/kg	26
50 mg/kg	56

activation of tyrosine hydroxylase, since alpha-methyl-para-tyrosine (a dopamine synthesis inhibitor) antagonized MDMA-induced dopamine release and the serotonergic deficits produced by MDMA (Schmidt et al., 1990). Inhibition of new dopamine synthesis would result in a decrease in the concentration of intracellular dopamine and subsequently, a reduction in dopamine release. Therefore, the blockade of D_2 receptors by the D_2 antagonist component of MDL 28,133A along with the inhibition of dopamine synthesis produced by the 5-HT_2 antagonist component may be more effective in inhibiting dopaminergic transmission (and therefore inhibiting nicotine-induced facilitation of BSR) than either a specific D_2 or 5-HT_2 antagonist alone. This explanation accounts for the greater magnitude of effect of MDL 28,133A on nicotine-induced enhancement of BSR compared to that of either sulpiride or MDL 26,508.

Conclusion

MDL 28,133A is a mixed 5-HT_2/D_2 antagonist which may combine components that act both pre- and post-synaptically. Although the results of this series of experiments suggest that the addition of a 5-HT_2 antagonist component to a D_2 antagonist is more effective in blocking the rewarding effects of nicotine than a D_2 antagonist alone, they do not provide any direct information concerning the mechanism by which these compounds modify nicotine's actions. Conclusive statements about the dopaminergic and serotonergic mediation of nicotine reward will require additional studies using both neurochemical (e.g., microdialysis) and

electrophysiological (e.g., intra- or extracellular recording) methods.

The use of in vivo microdialysis and extracellular recording will help determine whether the anti-nicotine effect of MDL 28,133A and sulpiride are related to stimulant-induced alterations in mesolimbic dopamine activity. It will also be important to include specific 5-HT_2 compounds in these studies since alterations in transmitter levels may not always be reflected in an animal's behavior.

Even though MDL 26,508 produced no overt behavioral changes, if microdialysis studies show alterations in nicotine-induced dopamine release, two possible conclusions could be drawn. For example, if MDL 26,508 inhibited nicotine-induced dopamine release, it could be concluded that 5-HT_2 antagonists modulate mesolimbic dopamine activity, possibly by blocking the activity of tyrosine hydroxylase (Sorensen et al., 1991). This inhibition could also be produced by 5-HT activation via nicotinic receptors located on serotonergic nerve terminals (Westfall, Grant & Perry, 1983). The inability of MDL 26,508 to affect nicotine-induced dopamine release could be related to overall level of nicotine-stimulated dopamine release, since Schmidt et al. (1992) suggest that the regulation of dopamine synthesis mediated by 5-HT_2 receptors becomes significant only during states of high serotonergic and dopaminergic transmission. For example, the amount of nicotine-stimulated dopamine release may not be sufficiently high to activate the serotonergic system, and, therefore, extracellular levels would remain unchanged (compared to nicotine alone) in the presence of the antagonist. Also, an inability of MDL 26,508 to inhibit nicotine-induced dopamine release could be related to its lower specificity for 5-HT_2 receptors,

since MDL 100,907 (which has a subnanomolar affinity for the 5-HT_2 receptor) significantly inhibits MDMA-induced increases in extracellular concentrations of dopamine (Schmidt et al., 1992).

However, since MDL 28,133A was more effective than either sulpiride or MDL 26,508 at reversing nicotine's effect on self-stimulation, it would be predicted that if there were a direct relationship between synaptic dopamine and reward, there would be a decrease in nicotine-induced dopamine release. Since substances with D_2 antagonist properties typically increase dopamine release (Carboni et al., 1989), a decrease in synaptic dopamine would imply that serotonergic and dopaminergic mechanisms interact to limit the responsiveness of mesolimbic neurons to the effects of nicotine. One possible mechanism by which this may occur is that the activity of the 5-HT_2 antagonist component may be inhibiting tyrosine hydroxylase, which would cause a decrease in dopamine synthesis (Schmidt et al., 1991) while at the same time, the D_2 component may be acting pre-synaptically to block pre-synaptic D_2 dopamine autoreceptors which could result in a decrease in dopamine release due to depolarization inactivation of A10 dopamine neurons (Chiodo & Bunney, 1983). This explanation would account for pre-synaptic actions of MDL 28,133A on nicotine's effects (e.g., the increase in tyrosine hydroxylase activity and increase in dopamine release produced by nicotine). However, nicotine may be producing its effects post-synaptically or at sites other than the mesolimbic system. Therefore, a comparison of the magnitudes of dopamine release (using both intracellular and extracellular methods) associated with administration of nicotine and sulpiride and MDL 26,508 in

the presence of nicotine would serve as an important baseline condition for explaining the effect of MDL 28,133A. Differences in the magnitudes of dopamine release may provide insight into the mechanisms by which antagonist drugs produce their anti-stimulant effects.

Nicotine is known to stimulate the release of dopamine in the mesolimbic system (Imperato et al., 1986). This is a characteristic it shares with other drugs of abuse (Carboni et al., 1989; DiChiara & Imperato, 1988; Petit & Justice, 1989). Therefore, substances that either directly (e.g., mecamylamine) or indirectly (e.g., dopamine antagonists) block the effect of nicotine may be useful pharmacotherapies for nicotine dependence. However, it could be argued that substances that block the effects of nicotine should precipitate withdrawal and, therefore, would not be indicated for acute smoking cessation. A preliminary clinical trial was conducted by Tennant et al., (1984) to determine if mecamylamine (a nicotinic antagonist) could be safely and efficaciously used to treat cigarette smoking. Mecamylamine was given to a population of heavy cigarette smokers in conjunction with counseling to quit smoking. Tennant et al. (1984) found that mecamylamine reduced tobacco craving in 13 of 14 subjects, and that half of the subjects quit smoking within 2 weeks of initiation of mecamylamine treatment. Also, four subjects reported a reduced consumption of cigarettes to under 5 per day, and subjects reported only minor symptoms of nicotine withdrawal. One possible explanation for these results is that the beneficial effects of mecamylamine were due to its sedating effect. One advantage of using mecamylamine is that it does not produce subjective effects and is

not known to be a positive reinforcer. However, Nemeth-Coslett, Henningfield, O'Keeffe & Griffiths (1986) found that mecamylamine produced dose-related increases in smoking, which is consistent with the hypothesis that mecamylamine blocks the central effects of nicotine that is delivered in cigarette smoke, thus resulting in compensatory increases in cigarette smoking.

Drugs with mixed antagonist properties may be beneficial for the treatment of substance abuse, since several neurotransmitter systems are affected by stimulants (Kosten, 1990). For example, stimulants enhance dopaminergic transmission (Wise & Bozarth, 1987) and, chronic stimulant use appears to deplete serotonin (Benwell & Balfour, 1979; Kosten & Kosten, 1991). While an increase in dopamine is associated with the positively reinforcing aspects of stimulant abuse (Koob & Bloom, 1988), a decrease in serotonin has been associated with negatively reinforcing effects (Aghajanian, Sprouse, & Rasmussen, 1987). Therefore, an ideal blocking agent would be one that blocked both the positive effects associated with drug use (i.e., euphoria) and the negative, or withdrawal effects (i.e., craving) associated with drug abstinence (Kosten & Kosten, 1991).

There is substantial evidence to indicate that nicotine produces its rewarding effect via its interaction with the mesolimbic dopamine system and that the rewarding effects of other psychomotor stimulants can be attenuated by substances with dopamine antagonist properties (Gallistel & Karras, 1984; Sands & Ciraulo, 1992; Yokel & Wise, 1976). Recently, Corrigall & Coen (1991) have shown that the dopamine antagonists spiperone, SCH 23390 and haloperidol reduce nicotine self-administration

in doses that do not impair motor capacity. These results suggest that low doses of dopamine antagonists may be useful in reducing the rewarding effects of nicotine without producing extrapyramidal effects typically seen with the classic neuroleptics. The classic neuroleptics block dopamine receptors in both the mesolimbic and nigrostriatal dopamine systems. It is to the latter effect that the production of extrapyramidal side effects is attributed (Meltzer & Stahl, 1976). In contrast to the classic neuroleptics, atypical neuroleptics demonstrate a relative selectivity for functional mesolimbic D_2 receptor blockade and, as such, could prove to be more clinically effective as pharmacotherapies for stimulant abuse.

The predominantly antidopaminergic properties of sulpiride classify it as an atypical neuroleptic. Atypical neuroleptics are also distinguished from the classic neuroleptics by their increased antiserotonergic and antidopaminergic potencies (Tamminga & Gerlach, 1987). The results of the antagonist studies suggest that mixed 5-HT_2/D_2 antagonists may be effective as pharmacotherapies for nicotine dependence since their antiserotonergic properties, relative to their antidopaminergic properties, may account for its lack of extrapyramidal side effects. Also, substances with 5-HT_2 antagonist properties have been shown to increase serotonin release (Csernansky, 1993) and, as a consequence, may be effective in blocking the negative reinforcing effects of stimulants, since sertraline, a serotonin reuptake inhibitor, has been shown to reduce craving for cocaine (Kosten & Kosten, 1991).

Further experimentation with mixed antagonists may also help elucidate the role of smoking and nicotine in various psychiatric disorders. For example, when compared

to a population-based control group, Hughes, Hatsukami, Mitchell & Dahlgren (1986) found that the prevalence of smoking in a group of psychiatric outpatients (N=217) was 1.6 times higher than that of the population and particularly prevalent (88%) among patients with schizophrenia. Smoking was also prevalent in patients with depression (49%). Hughes et al. (1986) have provided several hypotheses as to why psychiatric patients are more likely to smoke. They (1) have neurotransmitter deficiencies that are increased by smoking, (2) are bored, (3) have problems with aggression, concentration, or relaxation which can be alleviated by smoking, (4) have higher levels of extroversion, impulsivity or other personality traits that are associated with smoking or, (5) use smoking to offset the sedative effects of other drugs like benzodiazepines and neuroleptics.

There is evidence to indicate that some symptoms of schizophrenia may be caused by an imbalance of serotonergic and dopaminergic activity (Meltzer, 1989). Glassman (1993) has proposed that the negative symptoms associated with schizophrenia (e.g., apathy and lack of motivation) are a result of hypoactivity in the prefrontal cortex, which in turn may result in decreased dopamine activity in the ventral tegmental area and nucleus accumbens. Nicotine's ability to augment dopamine release could be seen as a potential physiological basis for the high frequency of smoking in schizophrenics. Clozapine (an atypical neuroleptic with both D_2 and 5-HT_2 antagonist properties) produces elevation in dopamine and serotonin turnover in the nucleus accumbens, while haloperidol (a classic neuroleptic) showed only an increase in dopamine turnover (Csernansky, 1993). Meltzer (1989) has

attributed the increased efficacy of clozapine over classic neuroleptics to its 5-HT_2 antagonist properties. Therefore, investigation of the relationship of psychiatric disorders to nicotine use may help identify neural mechanisms involved in nicotine dependence and provide information that can be used to develop more effective pharmacotherapies for smoking cessation and stimulant abuse in general.

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