

BEHAVIORAL STUDIES ON THE MECHANISM OF
BUSPIRONE, AN ATYPICAL ANTI-ANXIETY DRUG

1986

MANSBACH

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ABSTRACT

Title of Dissertation: Behavioral Studies on the Mechanism of
Buspirone, an Atypical Antianxiety Drug

Robert S. Mansbach, Doctor of Philosophy, 1986

Dissertation directed by: James E. Barrett, Ph.D., Department of
Psychiatry

Two experiments were conducted to investigate a possible serotonergic mechanism of action for the novel anxiolytic drug, buspirone, a compound known to have effects on several neurotransmitter systems. In the first experiment, a procedure was used in which food-maintained key-pecking in pigeons was punished with electric shock during one component of a multiple schedule. Each thirtieth response during the punishment component produced both food and shock, while in the nonpunishment component, only food was presented. Buspirone (0.1-3.0 mg/kg) and its analog MJ-13805 (0.1-1.0 mg/kg) produced large increases in punished responding while having little effect on or decreasing rates of unpunished responding. When co-administered with doses of the serotonin agonists quipazine (0.1-1.0 mg/kg) or L-5HTP (0.3-3.0 mg/kg), the punishment-increasing effects of buspirone and MJ-13805 were only partially reversed, suggesting that these drugs do not act entirely as serotonin antagonists in producing their antianxiety effects. In the second experiment, the discriminative stimulus properties of buspirone were examined. Pigeons were trained to discriminate injections of buspirone (1.0 mg/kg) from saline in a two-key operant task. A four-component session was employed in which a period of blackout (timeout) preceded each three-minute component of food availability under a fixed-ratio 30 schedule. In training

sessions, pigeons were reinforced for responding on the injection-appropriate key. Cumulative doses of buspirone (1.0-3.0 mg/kg) and MJ-13805 (1.0-3.0 mg/kg) produced in excess of 90% buspirone-appropriate responding in tests of stimulus generalization, while cumulative doses of midazolam (0.03-1.0 mg/kg), haloperidol (0.03-1.7 mg/kg), methysergide (0.1-3.0 mg/kg), apomorphine (0.03-1.0 mg/kg), clozapine (0.1-3.0 mg/kg), and 1-[3-chlorophenyl] piperazine HCl (mCPP) (0.3-10.0 mg/kg) produced little transfer of responding to the buspirone key, even at rate-decreasing doses. However, the putative serotonin 1A ligand 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (0.3-1.0 mg/kg) produced at least 90% buspirone-appropriate responding in all subjects. The results support the designation of buspirone as a nonbenzodiazepine anxiolytic drug, and corroborate behavioral and pharmacologic studies suggesting that buspirone's effects may be mediated via the serotonin 1A binding site, and not by a dopaminergic mechanism.

BEHAVIORAL STUDIES ON THE MECHANISM OF BUSPIRONE, AN
ATYPICAL ANTIANXIETY DRUG

by

Robert S. Mansbach

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I. Introduction

The medical treatment of anxiety has seen many important improvements in the generation since the advent of modern psychopharmacology in the early 1950s. Early practitioners, relying on the sedative drugs at their disposal, generally prescribed barbiturates or bromides in order to ease the sense of apprehension or impending doom from which their patients suffered (Baldessarini, 1985). In the 1960s a new class of anxiolytics, the benzodiazepines, was developed and quickly became enormously popular because of their clinical effectiveness and relative safety. However, these drugs have serious limitations of their own; they produce serious side effects, such as drowsiness, they are habit-forming, and when their use is discontinued following chronic administration they can produce life-threatening withdrawal symptoms (Harvey, 1985). Benzodiazepines are also capable of potentiating the effects of central nervous system (CNS) depressants such as alcohol, with potentially lethal consequences (Harvey, 1985).

Recent research into the biological basis of anxiety and emotional behavior has provided many insights into how the brain regulates these activities. Since the discovery of an endogenous site of action for benzodiazepine anxiolytics, much research has focused on the neuropharmacology of these receptors and their relationship to classical neurotransmitters, principally gamma amino butyric acid (GABA) (Paul, Marangos & Skolnick, 1981). The idea of a functional relationship between GABA and benzodiazepine receptor activity has received much support from experimental findings, leading many to believe that this system represents the key to the study of the etiology and treatment of anxiety disorders (Skolnick & Paul, 1982). Very

recently, however, a new group of compounds has been developed as "anxiolytic" anxiolytics, and have been very effective in preclinical and clinical trials (Wheatley, 1982).

Buspirone (BuSpar[®], Mead Johnson) is far removed in structure and function from any drugs currently used for this purpose, yet shows a remarkable efficacy in decreasing anxiety without inducing any of the typically attendant detrimental side effects (Taylor, Eison, Riblet & VanderMaelen, 1985). At present, buspirone's mechanism of action is unclear. This investigation was an attempt to elucidate buspirone's mechanism of action in the CNS, and, in doing so, investigate further neuropharmacological substrates for the etiology and treatment of anxiety disorders.

Buspirone: A Nonbenzodiazepine Anxiolytic

Clinical trials of buspirone have met with considerable success, with most studies reporting the drug to be equieffective with Valium (diazepam; a benzodiazepine), currently the most widely prescribed anxiolytic on the market (Goldberg & Finnerty, 1979; 1982); however, buspirone shows few other similarities to conventional anxiolytics. While benzodiazepines (BZs) are potent anticonvulsants and sedatives (Harvey, 1980), buspirone shares none of these properties (Taylor et al., 1985). Overuse of BZs results in physical dependence and cases of BZ abuse have occasionally been reported (Hollister, 1981). Animal studies of drug self-administration have shown that while BZ administration is not always well-maintained, buspirone is no better than saline as a response-dependent reinforcer in the rhesus monkey (Balster & Woolverton, 1982), and there is evidence that buspirone and

BZs do not share common subjective effects, as shown in studies of drug discrimination. While discriminative stimuli produced by BZs frequently generalize (that is, animals respond as if they had received a BZ) to other anxiolytics and sedatives, such as barbiturates, buspirone does not seem to share these properties in rats or primates (Ator & Griffiths, 1986; Hendry, Balster & Rosencrans, 1983).

GABA and Anxiolytics

Since the discovery of specific benzodiazepine receptors (Mohler and Okada, 1977; Squires and Braestrup, 1977) much of the research on anxiolytics has centered on their actions on these receptors and their interaction with the GABA neurotransmitter system. The ability of BZ drugs to bind to this receptor is highly correlated both with their clinical effectiveness and with their potency in enhancing GABAergic neurotransmission (Skolnick & Paul, 1982). These findings have led to much investigation of the possible structural and functional relationship between GABA and BZ anxiolytics. The close topographic and pharmacologic relationship between GABA and BZ receptors has led many researchers to speculate that both structures compose a large macromolecular aggregation, known as the GABA-BZ-chloride ionophore complex (Skolnick & Paul, 1982).

Subsequently, studies have shown that seizures caused by GABA antagonists are reversible by benzodiazepines, and that the presence of GABA increases the binding of BZs to their receptor (Paul, Marangos & Skolnick, 1981). These data suggest that changes in GABA activity may be responsible, at least in part, for the effects of BZs on anxiety.

In animal models of anxiety, the potency of BZ drugs in

increasing behavioral responses simultaneously maintained by reinforcers (such as food) and suppressed by aversive events (such as electric shock) was also found to be correlated with that drug's affinity for the benzodiazepine receptor (Sanger, 1985). Drug effects on behavior suppressed by punishment ("conflict behavior") is a major preclinical screen for anxiolytics, and is considered an effective predictor of their effectiveness (Sepinwall & Cook, 1980).

However, compounds that stimulate GABA receptors in the central nervous system normally do not increase behavior suppressed by punishment, nor are GABA antagonists consistently capable of reversing such increases when induced by BZs (Sanger, 1985). Therefore, the question of how anxiolytic compounds function in the brain, particularly with regard to the role of GABA, is an open one. The study of buspirone may help to broaden our perspectives on the neurotransmitters involved in anxiety states.

Pharmacology of Buspirone

Buspirone hydrochloride (8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro [4.5]- decane-7,9 dione) is an azaspirodecanedione derivative. Most studies have indicated that it does not bind to BZ receptors, nor does it interact directly with GABA receptors (Riblet, Taylor, Eison & Stanton, 1982). However, buspirone has been found to enhance the binding of BZ drugs to their receptors (Oakley & Jones, 1983; Weissman, Barrett, Brady, Witkin, Mendelson, Paul & Skolnick, 1984). Buspirone's ability to increase punished responding is not reversible by the BZ receptor antagonist Ro 15-1788, nor does buspirone reverse the convulsive effects of GABA antagonists, a property most

anxiolytics do possess (Barrett, Witkin & Mansbach, 1984; Riblet, Taylor, Eison & Stanton, 1982; Weissman, et al., 1984). Buspirone has little effect at adrenergic, histaminergic and cholinergic receptors as measured by radioligand binding studies (Stanton, Taylor & Riblet, 1981), but has been reported to interact with both serotonergic (5-hydroxytryptamine, or 5-HT) and dopaminergic (DA) receptor sites (see following sections).

Buspirone and Dopamine. Many of buspirone's pharmacologic and behavioral effects suggest an action similar to that of antipsychotic (neuroleptic) drugs. Buspirone increases the concentration of the dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenyl acetic acid (DOPAC) in brain and cerebrospinal fluid, as do the phenothiazines and butyrophenones (Hjorth & Carlsson, 1982; McMillen & McDonald, 1983; Garattini, Caccia & Mennini, 1982). Buspirone also inhibits conditioned avoidance responses (a test believed to reflect antipsychotic potency) (Allen, Ferguson & Cox, 1974), and binds to dopaminergic receptors in the corpus striatum (McMillen, Matthews, Sanghera, Shepard & German, 1983). Buspirone's effects on the concentration of DA metabolites in the brain is thought to result from its ability to bind to presynaptic dopamine autoreceptors, and, acting as an antagonist at this site, elevate the production of DA itself. This increase in synthesis of dopamine is reflected in increases in the activity of tyrosine hydroxylase, an enzyme necessary in the production of this neurotransmitter (McMillen et al., 1983). These effects are similar to those seen with classical antipsychotic drugs, such as chlorpromazine and haloperidol, and reflect the blockade of dopamine receptors (Baldessarini, 1985). However, buspirone does not increase the concentration of another dopamine metabolite, 3-methoxytyramine

(3-MT), as do classical DA antagonists, and in this respect resembles the atypical antipsychotic clozapine (Garattini *et al.*, 1982). Consistent with its role as a dopamine antagonist, buspirone increases the rate of DA cell firing, reverses decreases in such activity induced by the dopamine agonist apomorphine, and also blocks decreases in firing rate induced by iontophoretic infusions of dopamine itself (McMillen *et al.*, 1983). Buspirone also blocks emesis and increases in striatal acetylcholine concentration produced by apomorphine, and has been observed to reverse apomorphine-induced contralateral turning in rats with unilateral 6-hydroxydopamine lesions of the corpus striatum (Allen *et al.*, 1974; McMillen & Mattiace, 1983). These findings are consistent with the classification of buspirone as an antipsychotic dopamine antagonist; however, several lines of evidence distinguish buspirone from classical antipsychotics and suggest a different mechanism of action:

1) Buspirone, unlike most antipsychotic drugs, does not induce catalepsy, a state in which voluntary movement becomes difficult, and limbs take on a character of decreased flexibility (Allen, *et al.*, 1974). Moreover, buspirone reverses the ability of haloperidol to produce this effect (McMillen & McDonald, 1983). It is possible that buspirone's antagonistic effect on DA autoreceptors allows a greater availability of DA at postsynaptic receptors; however, buspirone is capable of blocking haloperidol catalepsy even when available stores of DA are chemically depleted to 5-10% of normal (McMillen & McDonald, 1983). It is still unclear, however, whether presynaptic or postsynaptic receptors are involved since many dopaminergic compounds act at both sites.

2) It is well known that dopamine antagonists are, in general,

not capable of increasing punished responding in laboratory species and are not especially effective as anxiolytics (Seiden & Dykstra, 1977). Buspirone has been observed to increase punished responding in rats (Riblet, Eison, Eison, Taylor, Temple & VanderMaelen, 1984; Porter, Johnson, & Jackson, 1985), monkeys (Geller & Hartmann, 1982; Weissman et al., 1984) and pigeons (Barrett et al., 1984; Barrett, Witkin, Mansbach, Skolnick & Weissman, in press), though some reports have shown no such increase (Sanger, Joly & Zivkovic, 1985; Sullivan, Keim & Sepinwall, 1983). Other preclinical screens of anxiolytic activity have, however, yielded positive results (Tompkins, Clemento, Taylor & Perhach, 1980; Riblet et al., 1982; Riblet, Eison, Eison, Newton, Taylor & Temple, 1983).

Large increases in punished responding have been found in the pigeon following administration of buspirone (Barrett et al., 1984; Barrett, et al., in press). Doses that had minimal effects on unpunished responding produced increases in punished responding comparable to those of the benzodiazepine, chlordiazepoxide. However, unlike the benzodiazepines, effects of buspirone were not reversible by the benzodiazepine antagonist Ro 15-1788.

Using an identical behavioral baseline in pigeons, Witkin & Barrett (1986) administered punishment-increasing doses of buspirone in combination with doses of haloperidol or apomorphine, which, when given alone, had no effect on responding. No notable antagonism or potentiation of buspirone's effects was seen when co-administered with these compounds. However, when doses of apomorphine which produced profound decreases in unpunished responding were given in conjunction with moderate doses of buspirone, response rates in this component were restored to approximately normal. These findings indicate that although

dopaminergic changes may well contribute to buspirone's behavioral effects, the mechanism of its ability to increase behavior suppressed by punishment remains uncertain and cannot be ascribed to dopamine receptor antagonism alone.

3) Chronically-administered buspirone does not markedly alter its binding to DA receptors (Hyslop, Becker, Crane, Riblet & Taylor, 1983; McMillen, 1983), and its removal does not block DA-mediated adenylate cyclase, actions typical of most dopamine antagonists (Cimino, Ponzio, Achilli, Vantini, Perego, Algeri & Garattini, 1983). The preferential action of buspirone on DA autoreceptors may in part be responsible for its unique pharmacologic actions.

4) Unlike most clinically-used dopamine antagonists, buspirone is ineffective in the treatment of psychoses. Sathananthan, Sanghvi, Phillips & Gershon (1975) administered buspirone to a group of severely schizophrenic patients and found that the drug produced either small, transient improvements or no effect at all.

Buspirone and Serotonin. While the effects of buspirone on dopaminergic systems have been extensively documented, it is becoming increasingly clear that the serotonergic system may also play a role in the effects of buspirone on punished responding. A number of investigators have proposed that compounds which bind to serotonin receptors in the CNS may be effective in preclinical screens for antianxiety action. Several serotonin (5-HT) antagonists including methysergide, metergoline, cyproheptadine, and cinanserin have been shown to increase punished responding in laboratory animals (Graeff & Schoenfeld, 1970; Geller, Hartmann & Croy, 1974; Brady & Barrett, 1985), while 5HT agonists have either no effect or further decrease rates of punished responding (Aprison & Ferster, 1961; Graeff & Schoenfeld,

1970). These findings suggest that 5-HT systems may be important in anxiolysis.

Despite early reports to the contrary (Stanton *et al.*, 1981), it has recently been determined that buspirone binds to central 5HT receptors in some species. Riblet *et al.* (1982) reported that buspirone was inactive at inhibiting the binding of [³H]-5HT in the rat, but did inhibit the binding of [³H]-spiperone, which is considered a ligand for the 5HT₂ receptor. In support of this finding, Eison, VanderMaelen, Matheson, Eison & Taylor (1983) found that, using [³H]-spiperone as the radioactive ligand, buspirone had selective affinity for 5HT₂ receptors. However, Glaser & Traber (1983) reported that buspirone displaced the binding of [³H]-5HT from receptors in the calf hippocampus, indicating possible 5HT₁ action. Skolnick, Paul & Weissman (1984) found that while buspirone had a low affinity for the 5HT₂ receptor as labeled by [³H]-ketanserin in rats, 5HT₁ receptor binding was seen in frontal cortex and hippocampus. 1-PP, a buspirone metabolite, was inactive at 5HT₁ and 5HT₂ receptors in this study. Taylor, Becker, Crane, Hyslop, Riblet & Temple (1983) found that upon prolonged administration of buspirone, binding to 5HT₂ receptors was reduced, suggesting an agonist action on serotonin neurons.

Using a new compound, 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide), thought to be selective for the putative 5HT_{1A} receptor (Gozlan, El Mestikawy, Pichat, Glowinski & Hamon, 1983), several investigators have reported that while buspirone has a relatively high affinity for this receptor subtype, it had a low affinity for 5HT_{1B} receptors, which were labeled by [³H]-5HT (Hiner,

Ison & Peroutka, 1985; Peroutka, 1985). These preliminary findings further suggest a role for serotonin in buspirone's pharmacologic and behavioral actions.

Electrophysiological studies have also supported a functional role for buspirone in 5HT systems. VanderMaelen & Wilderman (1984) and Mauk, Peroutka & Kocsis (1985) reported that iontophoretically-administered buspirone decreases cell firing rate in the raphe nucleus. Effects of buspirone on 5-HIAA (5-hydroxyindole acetic acid), a serotonin metabolite, have been variously reported, with some studies indicating no change (Cimino et al., 1983) while in others decreases have been noted (Hjorth & Carlsson, 1982). Decreases in a serotonin precursor, 5-hydroxytryptophan, have also been reported (Hjorth & Carlsson, 1982).

The development and testing of an analog of buspirone, MJ 13805, has provided additional supporting evidence for the role of serotonin in buspirone's actions. MJ 13805 is reportedly inactive in displacing radiolabeled dopamine compounds from their receptors, and produces minimal change in the accumulation of dopamine metabolites in the rat (McMillen & Mattiace, 1983). However, MJ 13805 is highly active in increasing punished responding, especially so in the pigeon (Barrett et al., in press), and relatively potent at displacing [³H]-5-HT compounds from their receptors, showing particularly high affinities for 5HT_{1A} and 5HT₂ receptors in the rat (Yocca & Maayani, 1985). As with buspirone, the effects of MJ 13805 on punished responding are not antagonizable by Ro 15-1788, the BZ antagonist (Barrett et al., in press), but MJ 13805 is as effective as buspirone in the reversal of neuroleptic-induced catalepsy (McMillen & Mattiace, 1983), despite its relative

ineffectiveness at the classical dopaminergic receptor site (Eison, Taylor, Riblet, New, Temple & Yevich, 1982). Taken together, this information suggests that buspirone's actions on catalepsy and punished responding may be completely separate, or perhaps focus on sites efferent to dopaminergic nerve terminals.

In behavioral tests with pigeons (unpublished data), either buspirone or MJ-13805 was co-administered with high doses of the serotonin agonists 5-HTP and quipazine; these doses routinely produce a number of profound motor dysfunctions, most notably an increase in lateral head shakes. When doses of buspirone or MJ 13805 were given in combination with these agonists, head shakes were markedly reduced. These data appear to suggest that the behavioral effects of buspirone or MJ 13805 may, at least in part, be mediated through an occupation of central serotonin receptors.

It is unclear at this point whether buspirone and MJ 13805 act as serotonin agonists or antagonists. While the headshake data suggest an antagonist role, the biochemical and electrophysiologic evidence would indicate that these compounds behave most like agonists. It is possible, however, that these drugs may be acting as partial agonists at selective receptor subpopulations; a report by Engle, Hjorth, Svensson, Carlsson & Liljequist (1984) indicates that 8-OH-DPAT, the putative 5HT_{1A} agonist, is capable of increasing punished responding in naive rats, but when punished responding was increased by the chemical depletion of serotonin in other subjects, 8-OH-DPAT reversed the effect. These data suggest that compounds which act as agonists but have little intrinsic activity may be differentially sensitive to ongoing behavior, depending on the number and state of the receptor population.

Objectives of these experiments

The purpose of the present investigation was to extend our knowledge of the behavioral actions of buspirone and to help elucidate its pharmacologic and behavioral mechanisms of action. Specifically, recent reports emphasizing the effects of buspirone on serotonergic neurotransmission have provided a strong basis for investigating the drug's effects on this system. It now seems clear that dopaminergic actions alone cannot account for all of buspirone's effects, and the absence of an indisputable antagonist of those effects suggest that buspirone has complex influences over several neurotransmitter systems, leading some to refer to the drug as a "midbrain modulator." As mentioned earlier, 5HT systems have been implicated in the release of punished responding and as such represent an appropriate focus for the investigation of buspirone's subjective and anxiolytic actions.

Experiment 1. This study examined the functional importance of 5HT activity in the punishment-attenuating effects of buspirone and MJ 13805. Specifically, pigeons were trained on an operant schedule by which punished and unpunished responding was monitored on a daily basis. Preliminary data has shown that both drugs reliably increase punished responding at doses which have relatively little effect on unpunished responding. If buspirone and MJ 13805 are producing these behavioral changes by acting as serotonergic antagonists, then the coadministration of 5HT agonists would have been expected to reverse the increases in punished responding. Both buspirone and MJ 13805 are active at 5HT receptors in the rat brain, but only buspirone has measurable activity at DA receptors; if 5HT agonists were to reverse the punishment-attenuating effects of both compounds, a crucial role for 5HT

in their effects on anxiety is implied; however, if only MJ 13805's actions were to be reversed, then a role for DA in buspirone's effects cannot be ruled out.

Two 5HT agonists were employed in this study. 5HTP (L-5-hydroxytryptophan) is the metabolic precursor of 5HT and its administration raises the available concentration of serotonin at central 5HT receptors. Its pharmacologic profile reflects a dominant role at 5HT₁ receptors, and was used to investigate the importance of this receptor population (Peroutka & Snyder, 1979). Accordingly, a putative 5HT₂ agonist, quipazine, was employed to examine the functional importance of this receptor subtype in the anxiolytic action of buspirone (Friedman, Barrett & Sanders-Bush, 1984).

Experiment 2. This study investigated the role of serotonin in the stimulus properties of buspirone using a drug discrimination procedure in the pigeon. Studies of buspirone as a discriminative stimulus have so far met with limited success. Hendry *et al.* (1983) found that rats only poorly discriminate buspirone from saline. Interestingly, buspirone is relatively less effective in increasing punished responding in the rat than it is in the pigeon. It therefore seems appropriate to use this animal in studying buspirone as a discriminative stimulus.

Pigeons were trained to discriminate buspirone from vehicle and then tests of stimulus control were conducted with MJ 13805, metergoline (a 5HT antagonist known to increase punished responding in pigeons; Graeff & Schoenfeld, 1970), clozapine, an atypical antipsychotic with neuropharmacologic properties similar to buspirone (Stanton *et al.*, 1981), and midazolam, a benzodiazepine with antipunishment activity

(Witkin & Barrett, 1985). These tests were designed to determine whether or not buspirone shares stimulus properties with other compounds that increase punished responding. One way in which drugs may alter behavior is by causing a change in the stimulus conditions normally present under nondrug conditions (Schuster & Balster, 1977). Drugs which share stimulus properties may be considered to have similar behavioral mechanisms of action. Drug discrimination experiments are often useful in determining the role of specific neurotransmitter systems in the behavioral effects of drugs, and in distinguishing between drug classes (Schuster & Balster, 1977). In one study, baboons trained to discriminate lorazepam, a BZ, from saline did not generalize this discrimination to buspirone; in other words, buspirone did not produce effects that could be recognized as "benzodiazepine-like" even though the two drugs have similar effects in preclinical screens of anxiety (Ator & Griffiths 1986).

If the discriminative cues produced by buspirone were to generalize to MJ 13805 and metergoline, then the hypothesis that buspirone's anxiolytic effects are mediated through serotonergic mechanisms is supported. Clozapine, which is classified as an antipsychotic, possesses antidopaminergic properties and, like buspirone, does not induce catalepsy (Stanton et al., 1981). Recently, we have determined that clozapine also increases punished responding in pigeons (unpublished data). Therefore, it was of interest to determine whether this drug shares discriminative properties with buspirone. Midazolam was included as a control for benzodiazepine anxiolytic activity; it was not expected that buspirone-appropriate responding would generalize to this drug.

II. Methods

Subjects. Fifteen white male Carneaux pigeons (Palmetto Pigeon Plant, Sumter, SC) were individually housed in a temperature- and light-controlled animal facility. Tap water and crushed oyster shell grit were continuously available in the home cages. Prior to any experimental manipulations, subjects were reduced to 80% of their free-feeding weights. During the experiments, these weights were maintained through post-session supplemental feeding (Purina Pigeon checkers).

Apparatus. Subjects were tested in 28 x 28 x 23 cm Plexiglas operant chambers surrounded by ventilated, sound-attenuating enclosures. Responses were recorded when the pigeon pecked a circular translucent Plexiglas key, which was mounted on the chamber's aluminum front wall. In experiment 1, one such key was mounted 22 cm above the floor of the chamber and equidistant from each side. In experiment 2, two such keys were present, both mounted 22 cm above the floor and exactly 2.25 inches from the center of the panel. Each key, transilluminated by 7W colored lights, registered a response when pecked with a force exceeding 20g (0.2N) and produced the audible click of a feedback relay mounted behind the front wall.

A rectangular opening, 5 cm from the floor of the front panel, provided periodic access to mixed grain, located in a solenoid-activated food hopper. Food availability was signaled by the illumination of the food hopper and by extinguishing the keylight. Electric shock (120 volts AC, 60 Hz) was delivered to the pigeon through stainless steel wire electrodes implanted around the pubis bones (Azrin, 1959), and wired through series resistance. The electrode assembly was held in

place by a cloth jacket, worn continuously by the pigeon, and connected to the ceiling of the chamber with an electrical plug and coiled extension cord. The shock, which was used to suppress responding in experiment 1, was of adjustable intensity and manipulated separately for each animal. Reinforcement schedules were programmed on electromechanical and solid-state equipment located in a separate room. Cumulative response recorders (Ralph Gerbrands, Arlington, MA) provided detailed summaries of each daily performance.

Preliminary procedure. Following food deprivation, key pecking was established according to the method of successive approximations. Initially each response was reinforced with grain in a single key chamber; the requirement was gradually raised to a fixed-ratio schedule in which each thirtieth response was reinforced by the presentation of food (FR 30).

Experimental procedure: Experiment 1. In a single-key chamber, a schedule of reinforcement was presented in which 3-min periods (components) of white and red keylight alternated for a total of 5 cycles (10 components). A 30-sec period of darkness separated each component from the next. Initially, responding was reinforced on the fixed-ratio 30 schedule for both keylights; there was no limitation on the number of foods the animal could obtain in each 3-min period. When responding stabilized, the punishment schedule was instituted during red key-light periods. In addition to the presentation of food (2.1-2.7 sec in duration, depending on the animal), each thirtieth response simultaneously produced a brief electric shock (shock intensities ranged from 2.5-7.0 mA). This procedure, termed a multiple schedule, has been used successfully in studies of buspirone's antipunishment effects (Barrett, et al., in press).

Five pigeons (P4273, P1727, P1971, P431 and P3808) were used to study the antipunishment effects of buspirone and an additional four (P1072, P596, P463 and P3583) were used in the study of MJ 13805. All subjects were experimentally naive except P4273 and P1727; these subjects were pharmacologically and behaviorally experienced, but had received no drugs at least two weeks prior to training. After determining dose-effect curves for each compound under the multiple schedule, doses of these drugs which increased punished responding were coadministered with doses of 5HTP and quipazine which, when given alone, had no effect on responding. In this way, dose-effect curves of the interactions between buspirone and the 5HT compounds were determined in one group, while the interactions between MJ 13805 and the 5HT compounds were established in the other group.

Overall response rates were collected for each component of the multiple schedule and expressed as the percentage of control rates, which were computed from nondrug performances. Animals were tested five days per week (M-F) with Tuesdays and Fridays serving as drug days; subjects were drugged when responding stabilized under the schedule. Drug effects that deviated more than 2 S.D. from control measures were considered significant.

Experimental procedure: Experiment 2: Four pigeons (P1076, P3579, P3755 and P1057) were used to study the effects of buspirone as a discriminative stimulus. Of these, only P1076 had been used in previous experiments. Subjects were trained to respond under the FR 30 schedule of food presentation on each key of the two-key chamber. Both keys were transilluminated with white lights. Each session consisted of four discrete components, each preceded by a seven-minute blackout period (timeout). During each component, which was 3 min in duration,

food was available on an FR 30 schedule. A maximum of five food presentations were available during each component; the period of food presentation was 4 sec in duration. If all presentations were obtained before the 3 minutes had elapsed, both keys were turned off and the remainder of that component spent in timeout.

After the subject acquired this task, and reliably obtained all available food presentations within the time allowed, one of the keys was designated as the buspirone key and the other as the non-drug key. Subjects then underwent training sessions for establishing the discrimination between saline (or sham injection) and buspirone under the schedule of food reinforcement. Initially, saline and buspirone were given on alternate days; a maximum of 40 food presentations were available in a 20 min period on the appropriate key. Injections were given 7 min prior to the session. When the pigeons made at least 90% of their responses on the correct key, the training procedure was modified and returned to the original four-component structure. In this procedure, pigeons were injected with saline (or sham injection) before each training session. Responding was reinforced under the FR 30 schedule in the first component, but only on the nondrug key. Incorrect responses reset the requirement on the injection-appropriate key. Immediately following the end of the first component, the training dose of buspirone (1.0 mg/kg) was injected and responding thereafter reinforced on the buspirone key only. Occasionally, two nondrug components were given before buspirone administration. The percent of correct responses were recorded for each component, as well as the overall response rate (both keys included) for each component. Before testing could begin, subjects must have had shown at least 90% injection-appropriate responding in each component for five consecutive

days. Saline-only sessions were interspersed with training sessions in order to discourage the development of tolerance. Sessions were conducted 6-7 days/week.

Once training criteria were met, testing sessions were conducted. Testing sessions were performed under conditions of cumulative dosing; that is, the test drug was injected in increasing doses before each component to approximate cumulative doses of 1/4 or 1/2 log units. This procedure allows the determination of a complete dose-effect curve in one session (Bertalmio, Herling, Hampton, Winger & Woods, 1982; France & Woods, 1985). The first injection was given immediately before the session, and each subsequent injection given at the beginning of the timeout period; interinjection intervals were always constant at 10 min. Thirty consecutive responses on either key were reinforced on the FR 30 schedule on test days; key switching reset the requirement on both keys. Saline sessions were interposed between training and testing sessions (*i.e.*, sal, train, sal, test, etc.). Tests of stimulus generalization were conducted 1-2 times under the cumulative-dosing procedure for MJ 13805, methysergide, midazolam, clozapine, and for buspirone itself. On occasion additional drugs were studied under this procedure because of their unique pharmacologic properties (see table 2 for results). Doses were selected from previous data, and included a range from noneffective to rate-decreasing. Occasionally, an extra test component was included to test a higher dose range. Drugs were given in a mixed order.

The data were expressed as average percent buspirone key responding \pm 1 S.E.M. and response rates (in resp/sec) presented individually for each component. Drugs were considered to share stimulus properties with buspirone if they occasioned 90%

bupirone-appropriate responding in any given component.

Drugs. Buspirone hydrochloride (Bristol-Myers, Evansville IN), MJ 13805 hydrochloride (Bristol-Myers), midazolam maleate (Hoffmann LaRoche, Nutley, NJ), 5-hydroxytryptophan (Aldrich Chemical Co., Milwaukee, WI), pentobarbital sodium (Sigma Chemical, St. Louis, MO), doxepin hydrochloride (Pennwalt Corp., Rochester, NY), chloroimipramine hydrochloride (gift of P. Skolnick), bromocriptine substance (Sandoz Pharmaceuticals, East Hanover, NJ), mCPP (1-[3-chlorophenyl] piperazine hydrochloride) (RBI, inc., Wayland MA), 8-OH-DPAT (RBI, inc.), methysergide maleate (Sandoz) and quipazine maleate (Miles Laboratories, Naperville, IL) were dissolved into 0.9% sterile saline. Clozapine base (Sandoz) was first dissolved into a 1N solution of acetic acid in a concentration of 10 mg/ml. Dilutions were then made with the appropriate volume of 0.9% saline. Mazindol substance (Sandoz) and spiperone substance (Janssen Pharmaceutica, Beerse, Belgium) were placed into a solution of 90% distilled water and 10% Emulphor (GAF, inc.) to which 1 drop dilute lactic acid per ml had been added, and gently sonicated. TVX Q 7821 (synthesized at NIH) was placed into saline to which 1 drop dilute lactic acid per ml had been added, and sonicated. Cyproheptadine hydrochloride (Merck, Sharp and Dohme, Rahway, NJ) was placed in sterile water and heated slightly. Apomorphine hydrochloride (Sigma) was placed into distilled water and heated slightly. Haloperidol lactate (McNeil Pharmaceutical, Spring House PA) was diluted with distilled water from a commercial stock solution of 5 mg/ml. All injections were administered into the pectoral muscle in a volume of 1.0 ml/kg. Doses are expressed in terms of the prepared forms of the compounds, as indicated. In experiment 1, buspirone and MJ 13805 were administered immediately prior to the session; 5-HTP was given 10 min

before the session and quipazine 5 min beforehand. Table 1 shows the putative actions and doses of the principal compounds used in these experiments.

TABLE 1. Putative functions of compounds used in these experiments

<u>COMPOUND</u>	<u>PUTATIVE ROLE</u>	<u>DOSES USED (mg/kg)</u>
BUSPIRONE	?	0.1-3.0
MJ 13805	?	0.03-3.0
MIDAZOLAM	BENZODIAZEPINE AGONIST	0.03-1.0
METHYSERGIDE	NONSELECTIVE 5-HT ANTAGONIST	0.1-3.0
QUIPAZINE	5-HT ₂ AGONIST	0.1-1.0
5HTP	5-HT ₁ AGONIST	0.3-3.0
CLOZAPINE	DOPAMINE ANTAGONIST 5-HT ANTAGONIST	0.1-3.0
APOMORPHINE	DOPAMINE AGONIST	0.03-1.0
HALOPERIDOL	DOPAMINE ANTAGONIST	0.03-1.7
8-OH-DPAT	5-HT _{1A} LIGAND	0.03-1.0
mCPP	5-HT _{1B} LIGAND	0.3-10.0

III. Results (Experiment 1)

Control performance. During control sessions, response rates under the punishment condition were reduced to approximately 5-10% of unpunished rates. Average control rates for individual subjects, along with shock intensity values, are presented in Table 2.

Effects of buspirone and MJ 13805 on punished responding. Administration of buspirone and MJ 13805 resulted in large increases in punished responding at doses that had little effect in the unpunished components. Figure 1 shows representative cumulative records of responding under control conditions and following the injection of buspirone or MJ 13805. Both drugs produced sustained, sizable increases in response rate during the punishment component and in the number of shocks administered.

Buspirone (figures 2,3) and MJ 13805 (figures 4,5) significantly increased rates of punished responding in all subjects at 2 or more doses. Dose-response curves took the shape of an inverted-U, with the highest doses producing lesser increases in punished responding and frequently producing decreases in rate during the nonpunishment component.

MJ 13805 significantly decreased unpunished responding at the 3.0 mg/kg dose, while the 3.0 mg/kg dose of buspirone resulted in significant rate decreases in the nonpunishment component.

Interactions of buspirone with serotonin agonists. Co-administration of buspirone with 0.1 mg/kg quipazine, a dose with little effect when given alone (figure 2), resulted in a flattening of the dose-effect curve, with smaller increases in punished responding. Only at the 0.1 mg/kg dose of buspirone, however, were punishment rates

TABLE 2. CONTROL PERFORMANCES UNDER THE PUNISHMENT PROCEDURE*

SUBJECT	RESPONSE RATE (RESP/SEC)		SHOCK INTENSITY
	UNPUNISHED	PUNISHED	
P4273 (QUIP)	2.90 [0.17]	0.12 [0.08]	3.0
P4273 (5HTP)	2.62 [0.15]	0.14 [0.05]	3.0
P1727 (QUIP)	2.34 [0.10]	0.12 [0.05]	6.0
P1727 (5HTP)	2.26 [0.16]	0.15 [0.09]	6.0
P1971 (QUIP)	1.86 [0.20]	0.19 [0.09]	2.5
P3808 (QUIP)	1.77 [0.23]	0.15 [0.08]	4.0
P431 (5HTP)	2.24 [0.17]	0.07 [0.07]	6.0
P463 (QUIP)	2.24 [0.43]	0.25 [0.16]	5.0
P463 (5HTP)	1.20 [0.29]	0.14 [0.03]	7.0
P3583 (QUIP)	2.97 [0.57]	0.06 [0.03]	5.0
P3583 (5HTP)	2.94 [0.52]	0.15 [0.07]	5.0
P1072 (QUIP)	3.10 [0.38]	0.30 [0.11]	6.0
P1072 (5HTP)	4.57 [0.49]	0.26 [0.21]	5.0
P596 (QUIP)	2.13 [0.25]	0.08 [0.03]	5.0
P596 (5HTP)	2.33 [0.12]	0.11 [0.08]	4.0

*Numbers in brackets represent ± 1 S.D.

Control rates were calculated separately for each drug-interaction series

Figure 1. Cumulative records of responding under the multiple schedule of punished and unpunished responding. The left panel shows performances of subject P1072 under control conditions (top) and following the administration of 1.0 mg/kg of MJ 13805 (bottom). The right panel illustrates control responding and performance following 0.3 mg/kg buspirone in pigeon P1727. During each three-minute component every response incremented the response pen one step; this pen reset at the end of the component. A diagonal slash on the response record indicates the delivery of food. The lower (event) pen recorded the component under which the animal responded; when the pen was deflected upward, the nonpunishment schedule was in effect, and when the pen was deflected downward the punishment schedule was operating. Small slashes on the event record during the punishment component indicate the delivery of electric shock.

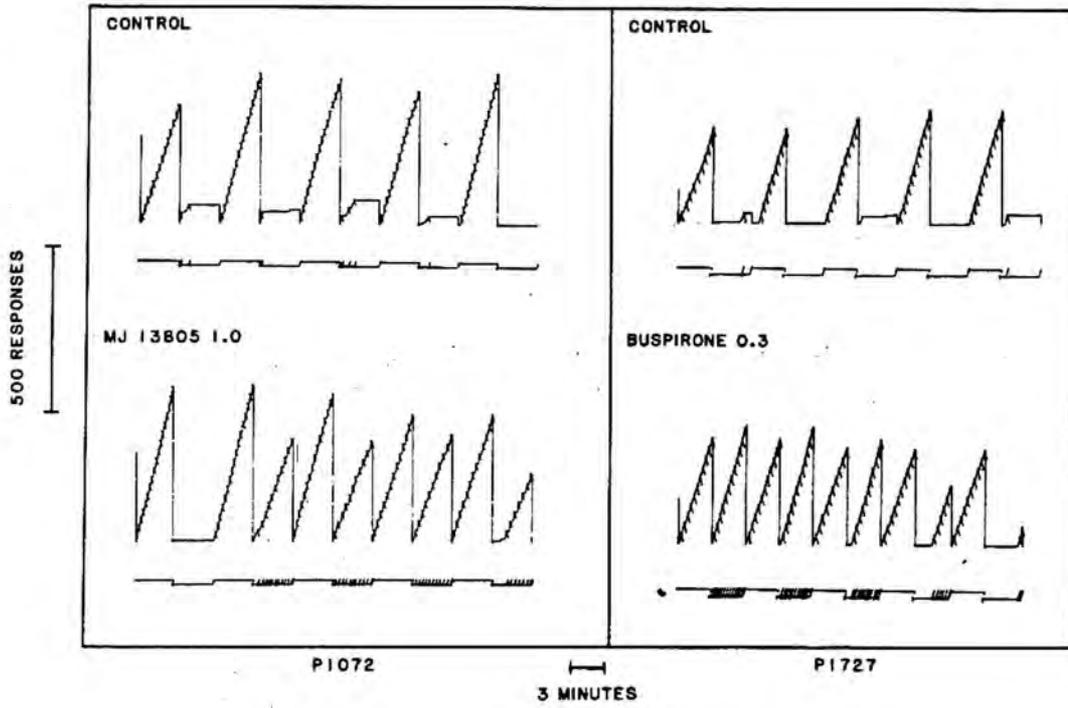


Figure 2. Dose-response curves for buspirone (open circles), quipazine, and their co-administration on responding under nonpunishment (left) and punishment (right) conditions. The open circle on the left of each panel shows control performance ± 1 S.D. The other unconnected points represent effects of various doses of quipazine when given alone. Drug effects falling outside ± 2 S.D. of control performance were considered significant. Individual points represent the mean of 1-2 determinations in each of 3-4 subjects.

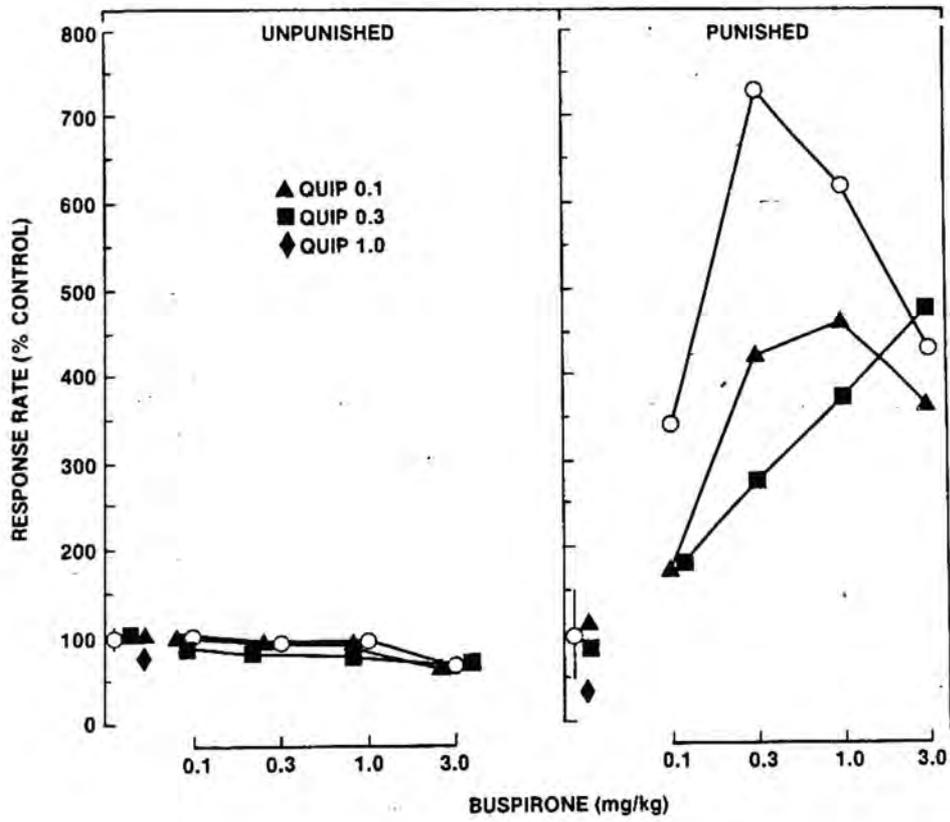


Figure 3. Dose-response curves for buspirone (open circles), 5-HTP, and their co-administration on responding under nonpunishment and punishment conditions. See figure 2 for other details.

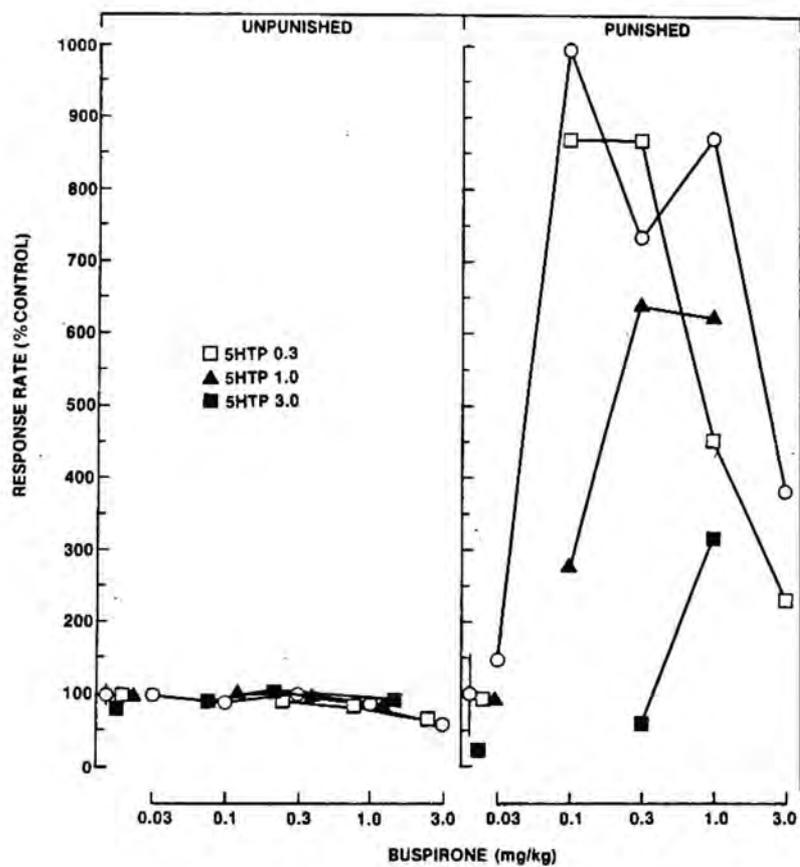


Figure 4. Dose-response curves for MJ-13805 (open circles), quipazine, and their co-administration on responding under nonpunishment and punished conditions. See figure 2 for other details.

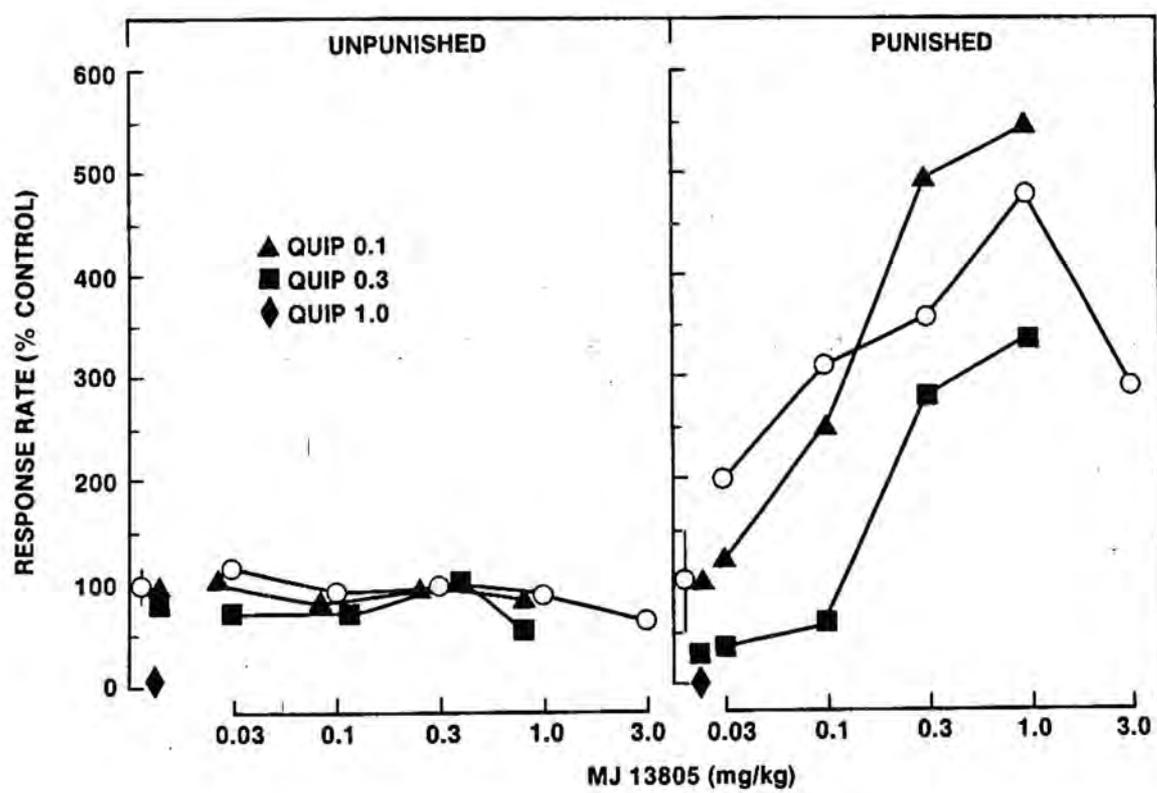
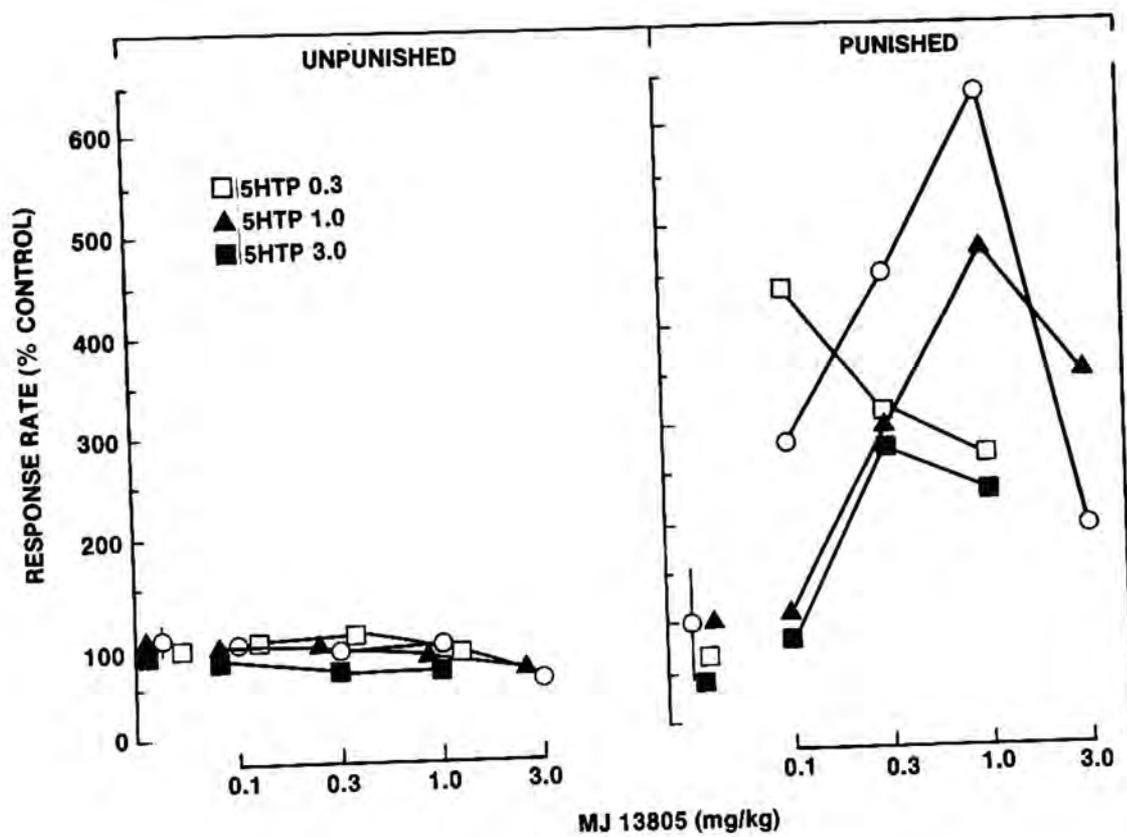


Figure 5. Dose-response curves for MJ-13805 (open circles), 5HTP, and their co-administration on responding under nonpunishment and punished conditions. See figure 2 for other details.



reduced to within 2 S.D. of control performance. Co-administration of the 0.3 mg/kg dose of quipazine, which produced some rate decreases when given alone, with buspirone resulted in a further flattening of the dose-effect curve. Again, only at the 0.1 mg/kg dose of buspirone was punished responding reduced to a level not significantly above that of control performance. Quipazine, alone or in combination with buspirone, had little effect on unpunished responding. The highest dose of quipazine, 1.0 mg/kg, decreased both unpunished and punished responding.

Figure 3 shows interactions between buspirone and 5HTP. The 0.3 mg/kg dose of 5HTP had little effect when given alone, and when co-administered with 0.1-0.3 mg/kg of buspirone, generated a dose-effect curve roughly parallel to that of buspirone alone. Only at the 3.0 mg/kg dose of buspirone did 0.3 mg/kg 5HTP reduce punished responding to a level not significantly above control; however, this point lies on the descending limb of the buspirone curve. Decreases in unpunished responding at the same dose were not reversed by 5HTP.

Interactions with 1.0 mg/kg 5HTP, a dose with little intrinsic effect, resulted in a slightly flattened dose-effect curve. When buspirone was co-administered with 3.0 mg/kg 5HTP, a dose that decreased both unpunished and punished responding, the response to buspirone was greatly reduced.

Interactions of MJ 13805 with serotonin agonists. The combined administration of MJ 13805 and quipazine is illustrated in figure 4. The 0.1 mg/kg dose of quipazine had little effect on the dose-effect curve, with some small increases in punished responding that were beyond those of buspirone alone. The dose of 0.3 mg/kg quipazine, which decreased both punished and unpunished responding, was not capable of returning punished responding to control levels at the 0.3 or 1.0 mg/kg

doses of MJ 13805. Similarly, decreases in unpunished responding induced by quipazine were not reversed by ineffective doses of MJ 13805 (left panel).

The co-administration of MJ 13805 and 5HTP is shown in figure 5. Interaction curves were generally shifted downward, resulting in lesser peak increases in punished responding. Doses of 0.3 and 3.0 mg/kg 5HTP considerably reduced rates of punished responding induced by 1.0 mg/kg MJ 13805, but these rates still remained significantly above control levels. Only at the lowest dose of MJ 13805 did co-administration of the two compounds result in near-control performance.

IV. Discussion (Experiment 1)

Consistent with earlier work (Barrett, Witkin & Mansbach, 1984; Barrett et al., in press), buspirone and MJ 13805 produced large increases in punished responding. These increases, often in excess of 500 percent of control, are characteristic of buspirone's effects in the pigeon and stand in sharp contrast with smaller increases found in other species (Porter et al., 1985; Weissman et al., 1984).

The purpose of the present study was to assess the contribution, if any, of serotonergic neurotransmission to the anxiolytic effects of buspirone. Serotonin antagonists have often been noted to increase punished responding, while agonists rarely do so (Iversen, 1984); if buspirone and MJ 13805 work through this mechanism, it would be expected that the serotonin agonists quipazine and 5HTP would modify these rate increases. Co-administration of buspirone and MJ 13805 with the two agonists generally resulted in flattened dose-effect curves, and decreased maximal increases in punished responding. However, neither of the agonists was able to restore punished response rates to control levels across a range of doses, and even doses of 5HTP and quipazine that resulted in large rate decreases when given alone were often incapable of returning punishment response rates to control levels. This partial antagonism of the effects of buspirone and MJ 13805 may reflect the nonselective nature of their pharmacologic actions, and underscore their well-deserved reputation as "midbrain modulators" (Eison & Eison, 1984).

It cannot be determined conclusively from these results whether 5HT₁ or 5HT₂ receptor actions were responsible for the reduction in punished responding; each of the agonists produced some alteration in

punishment curves, but neither showed a pronounced superiority over the other. However, it should be noted that neither drug was able, apart from isolated instances, to restore response rate decreases in the unpunished component. Recent experimentation has shown that rate decreases in unpunished behavior produced by 5HT agonists is readily reversed by 5HT antagonists (Mansbach & Barrett, 1986) and similar decreases induced by benzodiazepines are easily reversed by BZ antagonists (Witkin & Barrett, 1985).

Although buspirone and the selective 5HT antagonists, such as methysergide and metergoline, share the ability to increase punished responding (Brady & Barrett, 1985), buspirone's effects are, at least in the pigeon, far greater; it appears likely that if buspirone's anxiolytic effects are tied to a serotonergic final common pathway, they are mediated indirectly or via regionally-specific receptor subpopulations.

Recently, Witkin, Barrett, Bolger, Skolnick & Weissman (in preparation) determined that buspirone and MJ 13805 were relatively inactive at 5HT₁ (labeled by [³H]-5HT) and 5HT₂ (labeled by [³H]-ketanserin) receptors in pigeon brain. However, buspirone did bind with relatively high affinity to 5HT_{1A} receptors labeled by 8-OH-DPAT in the rat (Gozlan *et al.*, 1983). We have recently found that 8-OH-DPAT produces large increases in punished responding in the pigeon (unpublished results); this latter finding is quite novel, not only for the magnitude of the effect, but for the particular specificity of this compound. Of the serotonergic compounds that increase punished responding, most are selective for 5HT₂ receptors. 5HT₁ receptors, presumed to be presynaptic, are not normally associated with the release

of punished responding (Iversen, 1984). From the relatively little neurochemical evidence available on 8-OH-DPAT, it appears that this compound may be a presynaptic agonist, since it decreased 5HTP and 5-HIAA formation, and depressed 5HT turnover in vivo, while having relatively poor affinity for 5HT₂ receptors (Mason, Marsh, Perry, Snoddy & Fuller, 1983). It should be noted that both the 5HT₁ agonist 5HTP and 5HT₂ agonist quipazine have, in recent reports, been tentatively classified as 5HT_{1B} receptor ligands, but these designations have been made with respect to very specific brain areas (Sills, Wolfe & Frazer, 1984; Peroutka, 1985). Nevertheless, it is not altogether surprising that these compounds would not be completely effective in reversing the effects of buspirone on punished responding.

Results reported in the second experiment of this investigation suggest a marked dichotomy in the relevance of the two 5HT₁ receptor subtypes in the behavioral effects of buspirone. Further studies employing specific 5HT_{1A} and 5HT_{1B} receptor ligands are needed in order to partial out their importance in the antipunishment effects of buspirone and MJ 13805.

V. Results (Experiment 2)

Control Performance. During saline control sessions, pigeons responded at an average of 2.24-2.28 responses per second, making close to 100 percent of their key-pecks on the saline-appropriate key (Table 3 [A]). The rate of responding in control sessions during which buspirone (1.0 mg/kg) was administered was slightly lower than that of saline control sessions (2.03-2.24 responses per second); however, average accuracy on the injection-appropriate key remained above 99% in the three-session sequences which met criteria for testing (see Table 3 [B]). Response rates for individual subjects are displayed in Table 3 [C-F].

Effects of drugs on performance under the discrimination procedure. Cumulative doses of buspirone from 0.03-3.0 mg/kg occasioned responding on the buspirone-appropriate key in all subjects, with the key transfer typically occurring at the 0.3 or 1.0 mg/kg dose (Figure 6). Complete transfer of responding to the buspirone key occurred in seven out of eight test sessions. Figure 7 presents representative cumulative records from control and test sessions. Some decreases in response rate were noted at the higher doses.

MJ 13805, the buspirone analog, occasioned a complete transfer of key-pecking to the buspirone key in all subjects at doses (0.03-1.0 mg/kg) which had little or no effect on overall response rate (Figure 6, second panel).

Cumulative doses of haloperidol (0.03-1.0 mg/kg) failed to occasion a substantial transfer of responding to the buspirone-designated key. Response rates at the highest doses, however, were not decreased as greatly as was expected. When higher cumulative doses were administered (3.0-5.6 mg/kg), occasional key-peck transfers in excess of 50% were

TABLE 3. CONTROL PERFORMANCES UNDER THE DRUG DISCRIMINATION PROCEDURE

A. SALINE TRAINING (ALL SUBJECTS)

	<u>RATE (RESP/SEC)</u>				<u>PERCENT CORRECT</u>			
	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>
MEAN	2.28	2.28	2.24	2.24	99.90	99.65	99.61	99.74
S.D.	0.53	0.55	0.58	0.56	0.62	1.23	1.34	1.38

B. BUSPIRONE TRAINING (ALL SUBJECTS)

	<u>RATE (RESP/SEC)</u>				<u>PERCENT CORRECT</u>			
	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>
MEAN	2.24	2.03	2.06	2.03	99.80	99.66	99.82	99.94
S.D.	0.53	0.36	0.26	0.27	0.77	1.35	0.92	0.35

C. SALINE TRAINING (1057)

	<u>RATE (RESP/SEC)</u>				<u>PERCENT CORRECT</u>			
	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>
MEAN	3.11	3.09	3.07	3.01	99.75	99.5	99.29	99.88
S.D.	0.27	0.20	0.24	0.26	0.68	0.83	1.63	0.34

D. SALINE TRAINING (1076)

	<u>RATE (RESP/SEC)</u>				<u>PERCENT CORRECT</u>			
	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>
MEAN	2.34	2.45	2.40	2.42	100.0	100.0	100.0	100.0
S.D.	0.31	0.35	0.39	0.33	0.0	0.0	0.0	0.0

E. SALINE TRAINING (3579)

	<u>RATE (RESP/SEC)</u>				<u>PERCENT CORRECT</u>			
	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>
MEAN	1.81	1.83	1.76	1.72	99.77	99.55	99.26	99.03
S.D.	0.21	0.31	0.38	0.39	1.09	1.29	1.88	2.66

F. SALINE TRAINING (3755)

	<u>RATE (RESP/SEC)</u>				<u>PERCENT CORRECT</u>			
	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>	<u>C1</u>	<u>C2</u>	<u>C3</u>	<u>C4</u>
MEAN	2.03	1.88	1.86	1.91	100.0	99.4	99.7	100.0
S.D.	0.34	0.23	0.23	0.21	0.0	1.99	1.21	0.0

Note: C=Component

Figure 6. Transfer tests for cumulative doses of buspirone, MJ 13805, midazolam, and haloperidol on the drug discrimination procedure. The average percent buspirone-appropriate responding in each component (± 1 S. E.) is shown in the top panel; the lower panel shows average response rate (± 1 S. E.). Each point represents the mean of 1-2 determinations in 4 pigeons.

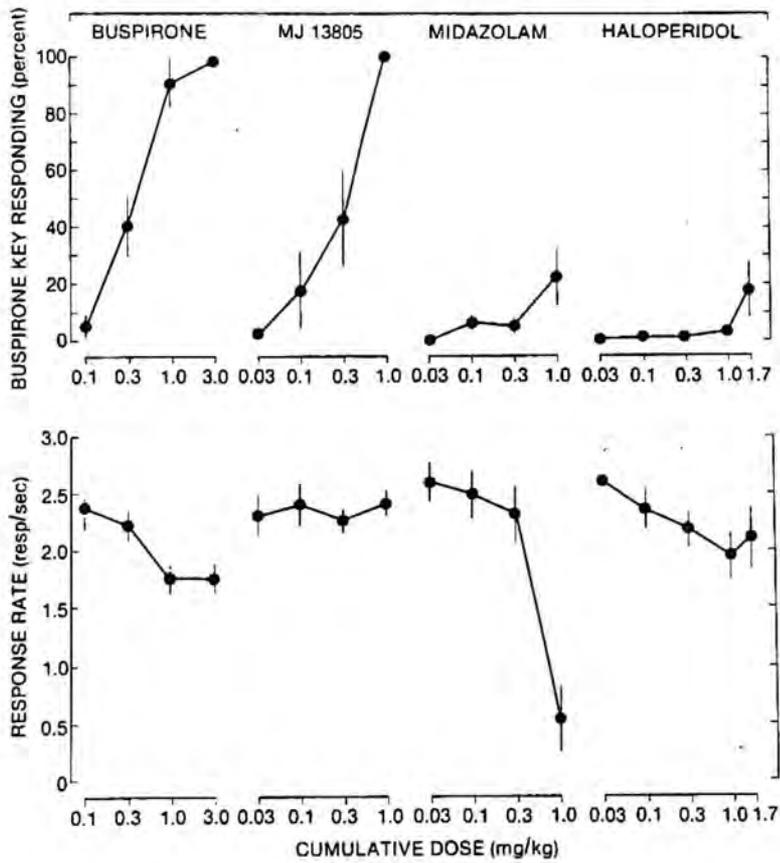


Figure 7. Schedule-controlled performances under the drug discrimination procedure. The cumulative records illustrate performances on left (left panel) and right (right panel) keys during representative sessions. The top set of records shows responding during a buspirone training session. Each component (event marker up) of the session is preceded by a seven-minute timeout (event marker down); at the beginning of each timeout an injection was given (shown above left-key records). The training session shows that following the injection of buspirone, the animal switched from the right to the left key. Slashes on the response record indicate the delivery of food. The second and third sets of records show responses to cumulative doses (mg/kg) of buspirone and 8-OH-DPAT, respectively. Note that for each drug the animal switched from the saline-appropriate (right) to the buspirone-appropriate (left) key.

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DRUG DISCRIMINATION

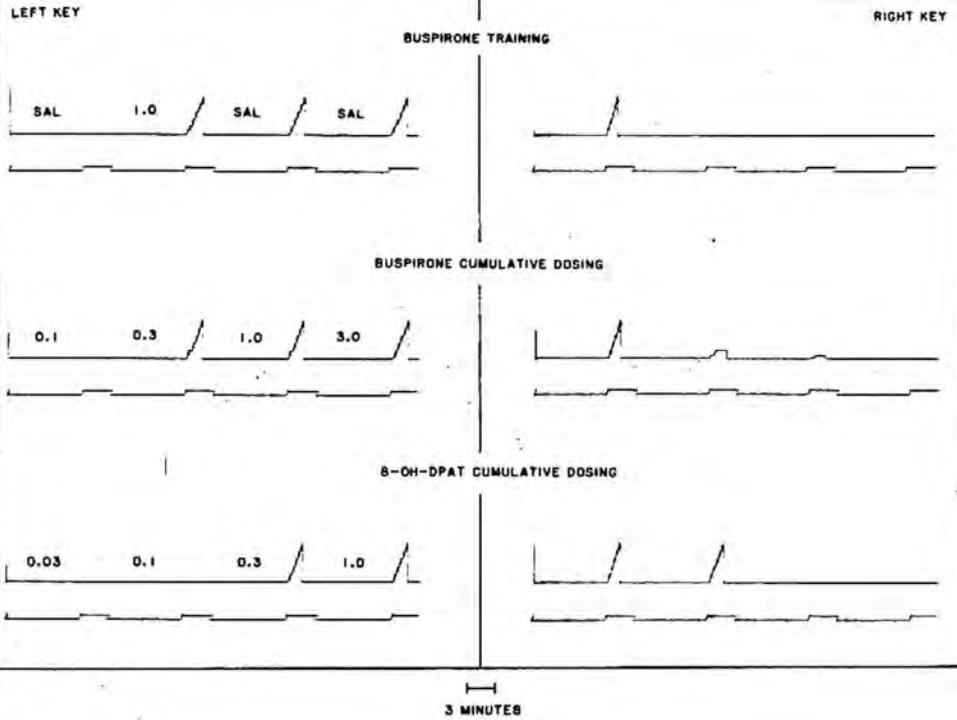


Figure 8. Transfer tests for methysergide, apomorphine, and clozapine on the drug discrimination procedure. Other details are the same as in figure 6.

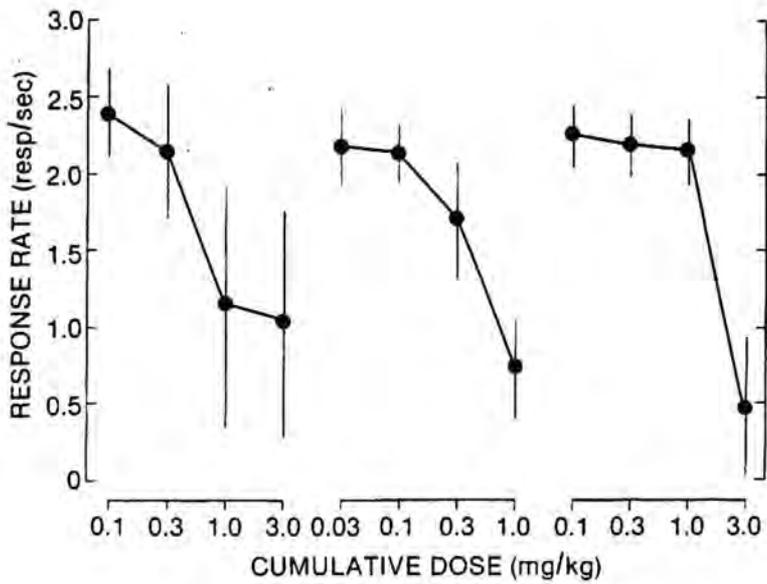
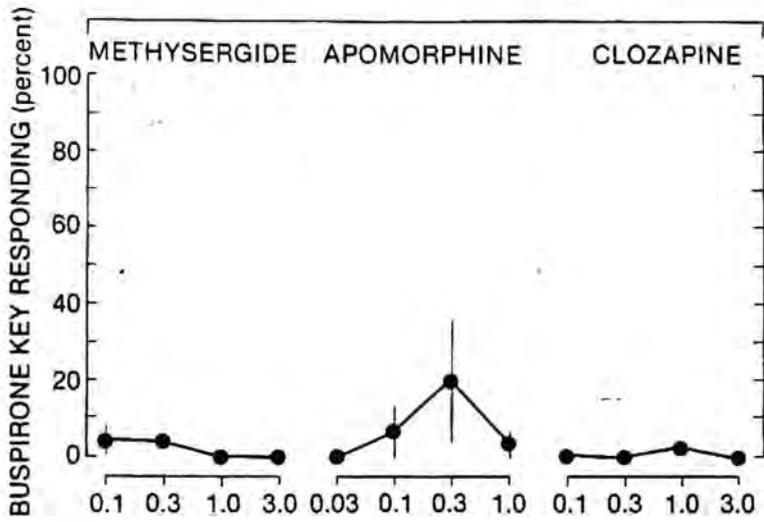


Figure 9. Transfer test results for cumulative doses of 8-OH-DPAT and mCPP on the drug discrimination procedure. For 8-OH-DPAT, data from individual subjects are presented (see legend). Each data point represents the mean of 2 determinations. The right panel presents data averaged across all subjects ± 1 S. E. See figure 6 for other details.

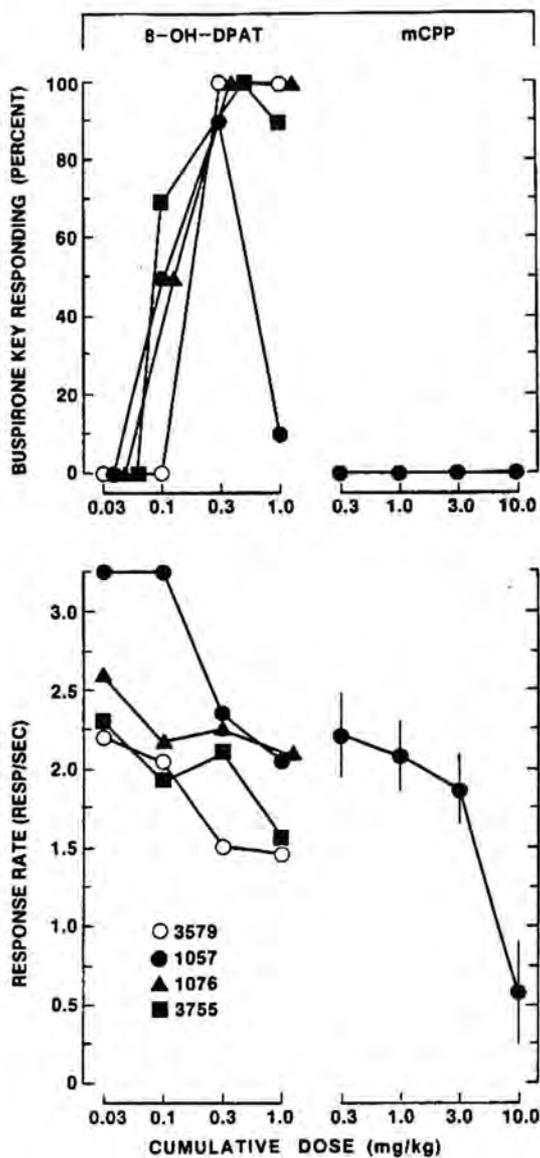


TABLE 4. TRANSFER TEST RESULTS FOR DOSES OF VARIOUS DRUGS ON THE
BUSPIRONE DISCRIMINATION TASK

<u>CUMULATIVE DOSE</u> <u>(MG/KG)</u>	<u>% RESPONDING ON</u> <u>BUSPIRONE KEY</u>	<u>RESPONSE RATE</u> <u>(RESP/SEC)</u>
BROMOCRIPTINE (1076)		
0.03	0	2.59
0.1	0	2.73
0.3	0	2.24
1.0	0	2.68
1.7	0	2.50
CYPROHEPTADINE (1076)		
0.1	0	1.56
0.3	0	2.21
1.0	20	2.24
1.7	0*	0.00
MAZINDOL (1076)		
0.1	0	1.72
0.3	0	0.03
DOXEPIN (1076)		
1.0	3	1.42
3.0	2	1.50
5.6	0	0.01
SPIPERONE (1076)		
0.03	0	2.14
0.1	0	2.11
0.3	0	1.88
1.0	0	1.58
PENTOBARBITAL (3579)		
0.3	0	2.24
1.0	0	1.92
3.0	0	1.88
10.0	31	0.67
CHLOROIMIPRAMINE (1076)		
0.1	0	2.59
0.3	0	2.34
1.0	0	2.42
3.0	0	2.24
5.6	0	0.03

*Note that no responding occurred at this dose

observed, though these were normally associated with response rate decreases (data not shown).

Administration of midazolam (Fig. 6), methysergide, apomorphine, and clozapine (Fig. 8) did not result in substantial transference to the buspirone key (top panels); these compounds produced sizable decreases in overall response rate. Large variability in response rate occurred when individuals showed differing sensitivities to the rate-decreasing effects of these compounds (Fig. 8).

Effects of other drugs. The putative 5HT_{1A} agonist 8-OH-DPAT produced in excess of 90% responding on the buspirone key at 1 or more doses in all subjects. Administration of mCPP, a 5HT_{1B} agonist, did not produce buspirone-appropriate responding up to those doses which severely depressed response rate (Fig. 9).

The effects of bromocriptine, cyproheptadine, mazindol, doxepin, spiperone, pentobarbital, and chloroimipramine under the discrimination procedure are shown in Table 4. None of these compounds produced a sizable transfer of responding to the buspirone-appropriate key. In preliminary tests, the novel anxiolytic TVXQ 7821 (2-(4-[4-(2-(2-pyrimidinyl)-1-piperazinyl] butyl)-1, 2-benzisothiazol-3(2H)one-1,1-dioxide hydrochloride) produced a complete transfer of responding to the buspirone key at doses of 1.0 mg/kg or lower in 2 of 3 subjects tested (data not shown).

VI. Discussion (Experiment 2)

The results of this experiment show that buspirone exerts stimulus properties in the pigeon. The range of doses used were the same as those which produce large increases in punished responding in this species, and were capable in all cases of producing complete discriminative control under training conditions, and drug-appropriate transfer under test conditions. These findings are at variance with those of Hendry et al. (1983), who reported that buspirone produced a poor discrimination from saline in rats. Their finding that buspirone generalizes poorly to lever pressing on which oxazepam- or pentobarbital-appropriate responding had been trained is consistent with the classification of buspirone as a novel anxiolytic. Similarly, Ator and Griffiths (1986) reported that buspirone did not generalize in rodents and primates trained using other anxiolytics. It is interesting that buspirone does not serve as a discriminative stimulus in rodents or primates and is relatively poor in elevating responding suppressed by punishment. In the pigeon, however, buspirone produces both large increases in punished responding and serves as a discriminative stimulus. It is interesting to note, nevertheless, that although buspirone has been shown to possess stimulus properties in the pigeon, these properties are not shared by the benzodiazepine anxiolytic, midazolam. It has been generally found in other species that compounds which share the ability to increase punished responding also are interchangeable on drug discrimination tasks, though exceptions have been reported (Spealman, 1985). That buspirone does not share stimulus properties with midazolam (Fig. 6) or pentobarbital (Table 4) indicates an underlying difference in mechanism of action for buspirone, as well as a contrast in the

subjective effects of these agents. Most available evidence indicates that buspirone does not interact with the GABA-BZ ionophore complex (Riblet *et al.*, 1982), suggesting that traditional benzodiazepine receptor mechanisms are not involved in the drug's effectiveness in treating anxiety. The notable lack of behavioral evidence to support GABA as the primary neurotransmitter system in anxiolysis (*e.g.*, Sanger, 1985) may suggest that both buspirone and the BZs act through a common, but as yet unspecified, mechanism.

Generalization tests conducted with the dopaminergic antagonist haloperidol and agonist apomorphine indicated that buspirone's stimulus properties are not mediated via dopamine receptors. These results support recent behavioral data (Witkin and Barrett, 1986) showing a lack of reversal of buspirone's effects by these compounds on punished responding. Though occasional transfers to the buspirone key were noted with haloperidol, these effects appeared only at doses (1.7-5.6 mg/kg) far above those which normally eliminate food-maintained responding in the pigeon. Several factors may account for these unusual findings; repeated exposure to other drugs, including buspirone and haloperidol itself, may have produced pharmacological or behavioral tolerance, precluding the typical response rate decreases. This may have in turn resulted in the expression of behavioral effects normally masked by the decrease in key pecking.

Clozapine, an antidopaminergic compound with antipunishment activity (Spealman & Katz, 1980) and neurochemical similarities to buspirone (Stanton *et al.*, 1981), did not generalize to the buspirone stimulus cue. Since clozapine has been shown to possess marked serotonergic actions as well (Ruch, Asper & Burki, 1976), these data suggest that perhaps neither drug increases punished responding by a

dopaminergic mechanism.

The numerous pharmacological (e.g., McMillen et al., 1983) and biochemical (e.g., McMillen & McDonald, 1983) reports implicating dopaminergic activity in the anxiolytic effects of buspirone have not thus far been supported by behavioral data. The importance of buspirone's interaction with dopamine receptors remains to be defined, though it seems probable that these effects are of indirect or secondary importance to the clinical effectiveness of this compound against anxiety.

It appears equally unlikely that buspirone's mechanism of action is similar to those of classical antidepressant agents. Though buspirone shows considerable promise as an antidepressant in human subjects (Goldberg & Finnerty, 1982), it possesses little activity at noradrenergic receptors (Stanton et al., 1981) and based on preliminary data presented in Table 4 apparently does not share stimulus properties with the amine uptake blockers mazindol, doxepin or chloroimipramine.

Buspirone does not share stimulus properties with the serotonin antagonist, methysergide (Fig. 8) or cyproheptadine (Table 4). Though these data would presumably suggest that buspirone does not produce its behavioral effects by acting as a 5-HT antagonist, they do not eliminate serotonin as a possible mechanism of action. 8-OH-DPAT, the 5HT_{1A} ligand, produced a transfer of responding to the buspirone key in all subjects, while the 5HT_{1B} compound mCPP did not produce any responding on the buspirone-appropriate key in any subject over a wide range of doses (figure 9). These data provide encouraging support for assigning a role for the 5HT_{1A} receptor in buspirone's anxiolytic actions, especially since buspirone and 8-OH-DPAT have distinct chemical structures. Early

indications from data collected on the 5HT_{1A} compound TVXQ 7821 suggest that it too shares stimulus properties with buspirone (see results) and increases punished responding in the rat (Engle *et al.*, 1984).

It is difficult to classify buspirone's pharmacologic action in terms of the current 5-HT₁-5-HT₂ nomenclature. Mixed results have been reported in rats (Eison *et al.*, 1983, Skolnick *et al.*, 1984) while recent findings (Witkin, *et al.*, in preparation) suggest that though high affinity sites for [³H] 5-HT and [³H] ketanserin binding do exist in the pigeon brain, buspirone and MJ 13805 were essentially inactive in displacing these radioactive ligands. Since the pigeon appears to be the most suitable species for the evaluation of buspirone as an anxiolytic, further pharmacologic studies in this species will hopefully help clarify an otherwise confusing state of affairs.

Physiologic studies, however, do support 5-HT as a possible final common pathway for anxiolytics. Both buspirone and the BZs inhibit cell firing in the raphe nucleus (VanderMaelen & Wilderman, 1984), in which 5-HT receptors show a considerable presence (Marcinkiewicz, Verge, Gozlan, Pichat & Harmon, 1984), and chemical depletion of serotonin has been reported to produce increases in punished responding (Iversen, 1984). BZs have also been reported to decrease serotonin turnover and release in the brain (Saner & Plescher, 1979). Though it is still unclear what effect buspirone has on these physiologic functions, 8-OH-DPAT parallels the benzodiazepines in this respect (Mason *et al.*, 1983).

Taken together, the results of this experiment show that buspirone is an anxiolytic agent distinct from benzodiazepines in its chemical structure, pharmacologic properties, and subjective effects.

The novelty of buspirone's stimulus properties is supported in animal (Ator & Griffiths, 1986) and human (Cole, Orzack, Beake, Bird & Bar-Tal, 1982) experiments, and suggests the possible presence of multiple systems in the brain for regulating anxiety.

VII. General Discussion

This investigation was an attempt to elucidate the mechanisms by which buspirone exerts its anxiolytic effects. In experiment 1, the possibility of an antiserotonergic role for buspirone was explored by co-administering the drug with doses of 5HT agonists. The results showed that although serotonin was clearly involved in the drug's antipunishment effects, it could not be concluded that buspirone was producing its effects purely as a serotonin antagonist. Experiment 2 confirmed and extended these findings by showing that buspirone did not share stimulus properties with methysergide, a 5HT antagonist known to increase punished responding, and supported recent suggestions that buspirone and several related compounds may produce anxiolytic effects by acting as 5HT agonists. The finding that buspirone's stimulus effects generalized to 8-OH-DPAT and not to mCPP lent further parsimony to these claims and strongly supported data collected from receptor binding studies. Finally, the experiment highlighted the basic underlying differences between buspirone and traditional antianxiety agents, by demonstrating no transfer of stimulus properties to the benzodiazepine, midazolam.

Recent developments and future directions

Originally, all drugs used in the treatment of anxiety had a sedative component to their effects, a feature once considered central to their success. These drugs, such as ethanol and pentobarbital, are regarded as relatively nonspecific in their CNS actions (Paul & Skolnick 1981). The development of benzodiazepines represented a radical

departure in the treatment of anxiety disorders; though they did have a sedative component to their actions, they were effective at doses that produced only minor sedation, and they were safe to use. Later studies showed BZs to have specific, localized areas of action in the brain, with large numbers of binding sites in the cerebral cortex (Paul & Skolnick, 1981).

The discovery of discrete binding sites for BZs in the brain (Mohler & Okada, 1977) unleashed a flurry of speculation that perhaps an internal pharmacologic system existed in the CNS for the regulation of anxiety. Further studies, using specific antagonists to BZ receptors, demonstrated inverse agonist, or "anxiogenic" effects of these compounds (Ninan, Insel, Cohen, Cook, Skolnick & Paul, 1982). These findings led many to believe that an endogenous ligand for the BZ receptor must exist, in order to regulate the system. Part of the thrust of the investigation of this idea was directed toward manipulation of the GABA-chloride ionophore complex, since numerous experiments had linked it with several benzodiazepine actions (Marangos, Paul & Goodwin, 1979). It became increasingly clear that GABA drugs, though clearly effective in modifying BZ effects, were not themselves effective against anxiety (Sanger, 1985). The true importance of GABA in anxiety remains in doubt, especially with the development of non-BZ, non-GABA compounds such as buspirone.

Buspirone, and other novel anxiolytics such as TVXQ 7821, have forced a rethinking of how all anti-anxiety drugs work. Since buspirone has so little in common with BZs, researchers began concentrating on its most salient pharmacologic and neurochemical properties. Initially, dopamine showed a great deal of promise but recent experiments, including the present ones, have shown it to be a kind of chemical red

herring. Dopamine must not be ignored, however, and its role in buspirone's anxiolytic effects may yet be exhumed.

Most recently, much of the research on anxiolysis has concentrated on the importance of serotonin (Sepinwall, 1983). Now, with the finding of specific, regionally localized 5HT₁ receptor subtypes in the raphe and hippocampus, and behavioral changes associated with the manipulation of serotonin, the possible role of this neurotransmitter in the effects of novel anxiolytics such as buspirone has enjoyed firm foundation. The interactions between BZs and serotonin systems dovetail with some of these findings, and interconnections between the raphe nucleus, hippocampus, and septal nuclei (all areas thought to be important in emotion) appear to be potential targets of anxiolytic drugs (Sepinwall, 1983).

One of the most troubling questions surrounding the search for serotonin receptor subtypes is the assignment of meaningful functional correlates to them. Up until now, researchers have mainly focused on subtle differences in behavioral "syndromes" produced by 5HT₁ and 5HT₂ agonists (Peroutka, 1984; Bradley, 1984). While these distinctions have been of some use in drug interaction studies, they are of little explanatory power in discussing the purposes of the neuronal systems involved. Findings relating these specific receptor subtypes to important behavioral processes such as reinforcement and punishment, especially at physiologically-relevant doses, are certainly moving to fill this gap in understanding. Additional studies that closely relate results from binding studies, neurochemical studies, physiological experiments, and behavioral procedures will doubtless yield handsome dividends in the investigation of psychopharmacologic phenomena.

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