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RELATIONSHIP OF AMYGDALOID 5-HYDROXYTRYPTAMINE-CONTAINING NEURONS TO ANTICONFLICT EFFECTS OF BENZODIAZEPINES IN THE RAT

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Clinton Donald Kilts

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RELATIONSHIP OF AMYGDALOID 5-HYDROXYTRYPTAMINE-CONTAINING NEURONS TO ANTICONFLICT EFFECTS OF BENZODIAZEPINES IN THE RAT

By

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ABSTRACT

Relationship of Amygdaloid 5-Hydroxytryptamine-Containing Neurons to Anticonflict Effects of Benzodiazepines in the Rat

by

Clinton Donald Kilts

Despite widespread clinical use of the benzodiazepines, the mechanisms by which these drugs affect central neurons remain obscure. Determining how benzodiazepines produce their anxiolytic actions may afford insights into the neuronal substrates of clinically-defined anxiety. However, the concept that the anxiolytic effect is a specific action on a given neuronal system appears to be an oversimplification, as studies of these drug effects have implicated virtually every known putative neurotransmitter as well as the cyclic nucleotides.

A conflict procedure was developed based on intermittent electrification of a drinking tube set in a box housing a thirsted rat. This method allowed for rapid acquisition of stable baseline performance, a high output and identification of side effects; it also yielded anticonflict profiles for the benzodiazepines correlated with their relative clinical antianxiety potency. Past studies have associated anticonflict effects with disruption of brain 5-hydroxytryptamine functions. If reduced central 5-hydroxytryptamine activity is causally related to anticonflict efficacy, other pharmacological manipulations that decrease 5-hydroxytryptamine activity should influence conflict behavior.

However, in the shocked drinking tube procedure 5-hydroxytryptamine antagonists showed poor anticonflict activity and failed to modify the anticonflict effect of diazepam.

Elevation of brain 5-hydroxytryptamine following 5-hydroxytryptophan (with a peripheral decarboxylase inhibitor), potentiated the anticonflict effect of a submaximal dose of diazepam in the shocked drinking tube paradigm. Amitriptyline, which alone had no effect on suppression of drinking, potentiated the anticonflict effect of a submaximal dose of diazepam. p-Chlorophenylalanine yielded an equivocal anticonflict effect on the drinking behavior not well related to 5-hydroxytryptamine depletion. Depletion of brain 5-hydroxytryptamine by intracerebroventricular or intra-amygdaloid 5,7-dihydroxytryptamine produced a significant increase in punished responding, but only in the modified Geller-Seifter procedure using food reinforcement. Thus, while changes in brain 5-hydroxytryptamine mechanisms may influence some types of conditioned suppression, reduction of brain 5-hydroxytryptamine activity is probably not the major mechanism of the anticonflict effect. Previous anticonflict studies using food reinforcement may have been misleading in this regard.

The effects of acute and repeated doses of diazepam on brain 5-hydroxytryptamine activity were estimated from the rate of decline of 5-hydroxyindoleacetic acid following monoamine oxidase inhibition by pargyline. Five consecutive daily doses of 1.8 mg/kg diazepam, but not an acute dose, reduced 5-hydroxytryptamine activity in the amygdaloid complex and anterior hypothalamus. A larger acute dose of diazepam (5.6 mg/kg) similarly reduced 5-hydroxytryptamine turnover, suggesting that chronic diazepam may cause an accumulation of the drug and/or

active metabolites. Anticonflict activity of diazepam may be associated with reduced 5-hydroxytryptaminergic input to the amygdala and hypothalamus, but perhaps not as a direct influence of the drug. It may be indirectly mediated, compensatory to a drug-induced enhancement of activity of postsynaptic tryptaminergic neurons.

Both single and repeated doses of diazepam reduced dopamine turnover, as measured by decline of the amine levels following α-methyltyrosine in some amygdaloid nuclei. This effect was less pronounced than that observed for 5-hydroxytryptamine and insufficiently characterized to attempt a rigid correlation with behavioral effects. Nevertheless, the effect on dopamine turnover was less after chronic, as compared to acute, drug treatment, suggesting the development of tolerance. Since the anticonflict effect became more prominent during the chronic drug regimen, the effects of diazepam on brain dopamine systems and on behavior appear to be poorly associated.

It can be concluded that effects of benzodiazepines on brain 5-hydroxytryptamine neurons are not related causally to anticonflict efficacy. Effects in the shocked drinking tube paradigm indicated that increased activity in brain 5-hydroxytryptamine neurons may actually release punished responding. Since benzodiazepines release and d-amphetamine enhances conditioned suppression similarly in the Geller-Seifter, Estes-Skinner (conditioned emotional response) and shocked drinking tube procedures, the latter method appears to be a valid measure of drug effects on conflict behavior.

ACKNOWLEDGEMENTS

I would like to acknowledge the contribution of my wife, Susan, to this effort. Her encouragement and compassion made this dissertation possible. It is with considerable respect and pride that I would like to thank my parents who gave me the ideals and encouragement to undertake this work. I would also like to express my gratitude to the members of my committee: Drs. Brody, Moore and Bennett with special thanks to Dr. Rech for giving me the opportunity to pursue and develop my research interests and to Dr. Rickert for the unselfish gift of his time and experience.

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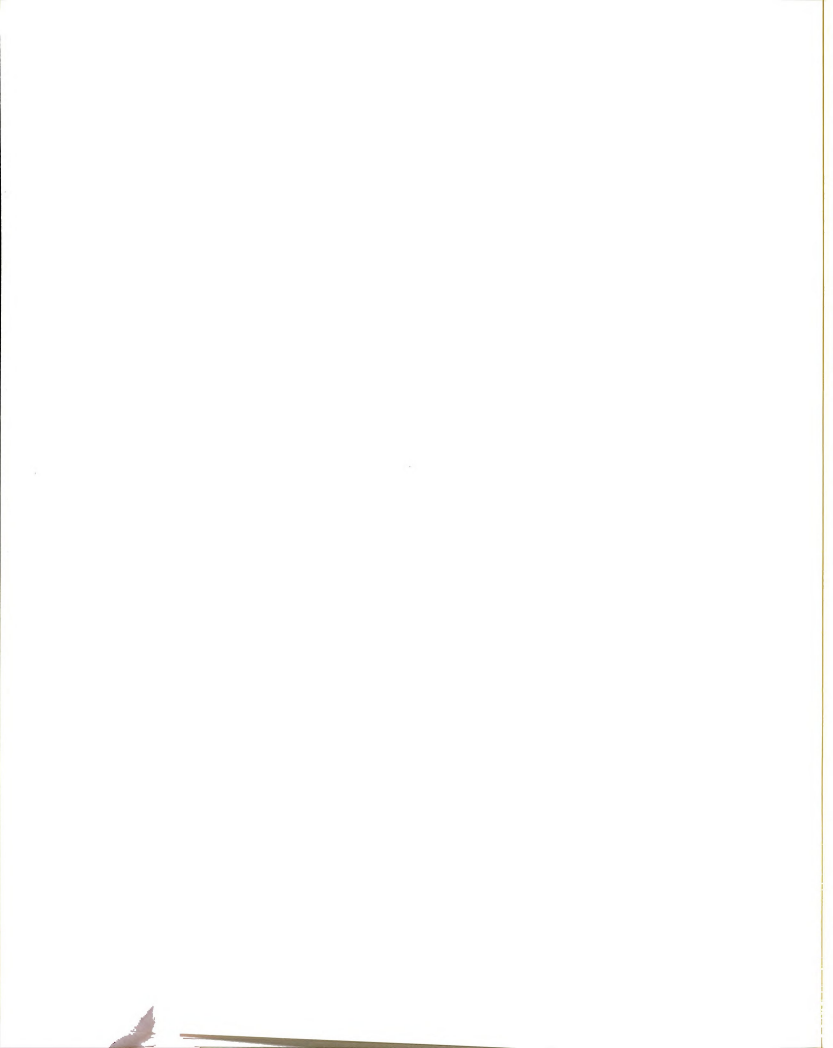

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INTRODUCTION

A. Objectives

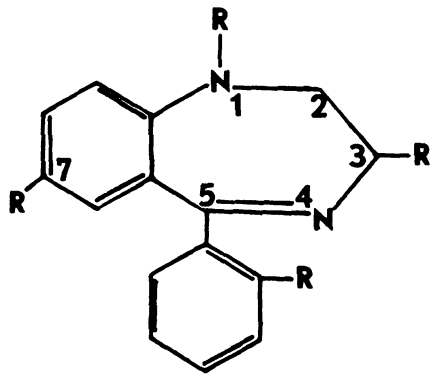
Derivatives of the 1,4-benzodiazepine nucleus (Figure 1) are among the most widely used drugs in clinical medicine in the United States. More than 57 million prescriptions and refills for diazepam, the most frequently prescribed member of this class of compounds, were filled in the twelve month period extending from March, 1976, to April, 1977. Until 1960 the pharmacotherapy of distressing anxiety symptoms consisted of little more than nonspecific sedation. It was with the introduction of the benzodiazepines (Randall et al., 1960; Randall, 1960) that an effective means of attenuating anxiety which was reasonably devoid of sedative liabilities became available. The mechanisms by which the benzodiazepines exert their antianxiety activity have been sought, with dubious success, by a number of research scientists. This is understandable in light of our lack of knowledge of the cellular basis of anxiety and the basis for its therapeutic management. Elucidation of the processes involved in mediating the anxiolytic actions of the benzodiazepines would greatly improve our concept of emotional states in general and afford additional insight in the areas of diagnosis and pharmacological manipulation.

The objectives of this research were two-fold, both of which related to the actions of diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one; Valium^{R}) in the rat. The

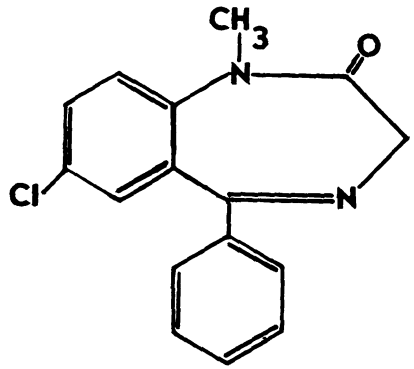
structural formula of this 1,4-benzodiazepine derivative is illustrated in Figure 1. The first objective was to examine the effects of benzodiazepines on a behavioral model of anxiety and to assess the value of such a model in establishing relevant pharmacological correlates of clinical antianxiety activity. As a second objective the potential of diazepam to alter the activity of neuronal systems utilizing several putative neurotransmitters was studied in an attempt to define the role of these transmitters in the mechanism of action of the benzodiazepines. To accomplish this, biochemical estimates of the effects of diazepam on the functional activity of selected neurotransmitters were utilized. Salient variables, i.e., doses and pretreatment intervals, were drawn from the time course and dose-effect relationship of the previously determined diazepam-induced behavioral alterations.

An important corollary in attempts to identify the neurochemical substrate(s) of a drug's action on the central nervous system is the identification of its anatomical substrate as well. The effects of diazepam on the concentrations and dynamics of the neurotransmitters 5-hydroxytryptamine (serotonin) and dopamine (3,4-dihydroxyphenylethylamine) in the amygdala of the rat was examined. This collection of telencephalic nuclear groups was selected in light of the reported electrophysiological effects of the benzodiazepines on the amygdala (Schallek et al., 1964; Chou and Wang, 1977; Haefely, 1978) as well as data regarding the interaction between selective lesions of this structure and conditioned behavior (Kellicutt and Schwartzbaum, 1963; Spevack et al., 1975).

The experimental data presented in this dissertation support a diazepam-induced depression in the functional activity of dopamine- and

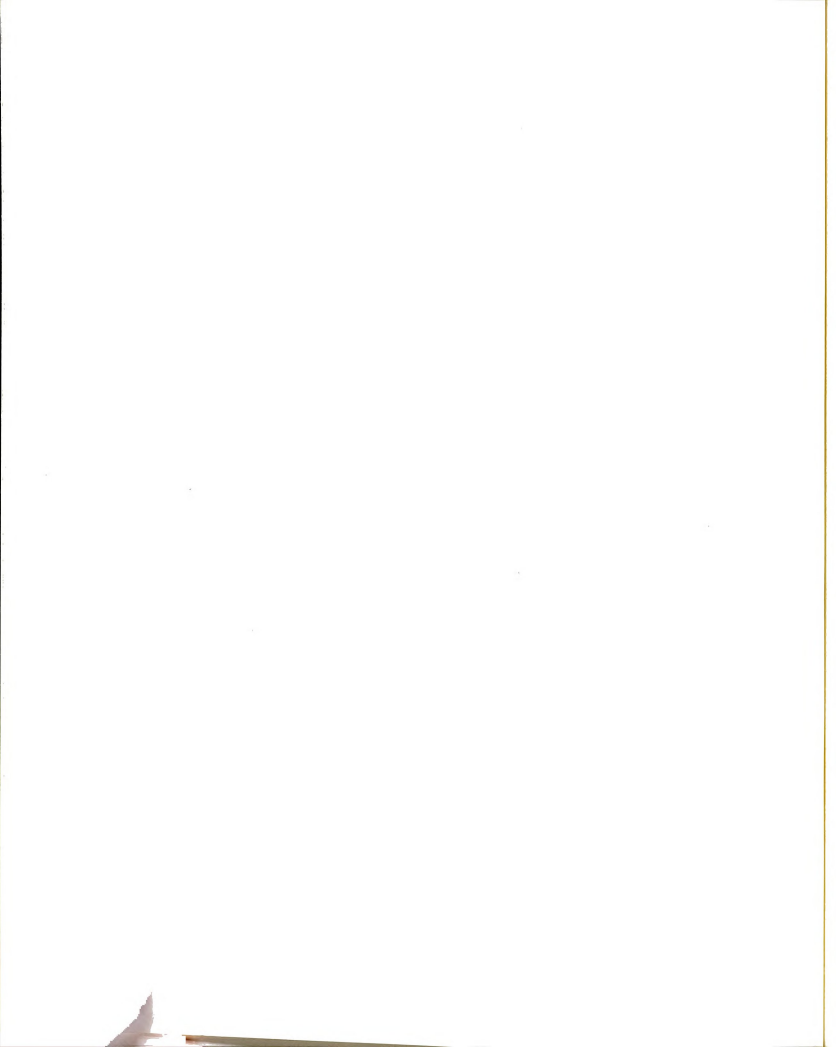


1,4-BENZODIAZEPINE NUCLEUS



DIAZEPAM

Figure 1. Structural formula of the 1,4-benzodiazepine nucleus and a derivative, diazepam.



5-hydroxytryptamine-containing neurons with terminals in the amygdala as well as in other areas receiving a moderate to rich input. Whether the observed depression is the result of a direct action on presynaptic receptors or represents a compensatory or reflex response to an action on postsynaptic sites cannot be discerned from the present evidence alone. The relationship between the benzodiazepines and 5-hydroxytryptamine has been interpreted heretofore as being an interference with 5-hydroxytryptamine-mediated synaptic mechanisms by the drug. However, over the course of these behavioral investigations into the effects of relatively selective 5-hydroxytryptaminergic agents and their interactions with diazepam, it became clear that a re-evaluation of this relationship may be in order.

B. Anxiety: Problems in Quantitation

Available methods for quantitation of clinical anxiety have serious limitations that make the accurate clinical assessment of drug effects on anxiety difficult. It is ironic that anxiety neurosis, by far the most common indication for benzodiazepine pharmacotherapy, is the disorder for which their clinical efficacy is most difficult to demonstrate. An understanding of this problem is apparent when one considers the nature of this emotional state. Anxiety is an elusive syndrome that defies definition and quantitation. It describes a phasic or episodic psychophysiological response resembling fear, though inappropriate to the reality of any perceived threat. Direct measurement of the effects of psychotropic agents on anxiety is not possible due to the fact that no single satisfactory method for quantifying anxiety has been devised. Results from numerous controlled

and uncontrolled clinical trials using physician-rated and/or patient self-rated scales testify to the difficulties involved in unequivocally delineating the relative effectiveness of an agent in ameliorating anxiety. However, the fact remains that no other antianxiety agents, regardless of how new or exotic, have proven superior to the benzodiazepines (Greenblatt and Shader, 1974,1978).

The use of animal behavioral models of clinical anxiety offers several indispensable advantages in evaluating theories regarding the mechanism of anxiolytic action of the benzodiazepines as well as of anxiety itself. First, ethical reasons preclude the invasive study of the physical and psychological make-up of the human subject. The selection and regulation of experimental parameters, so important to the formation of sound empirical conclusions, is difficult or often not permissible in the clinical setting. Second, animal models may prove useful because the phenomenon under study (anxiety) is simply too complex in the clinical setting for the experimental and conceptual tools at hand. Clinical diagnosis relevant to anxiety suffers from a reliance on verbal reports of feelings. While such feelings do indeed exist, the problem relates, as Skinner (1975) has suggested, to the lack of a suitable language for reliably conveying such an emotional state.

It can be argued with some vigor that anxiety represents a uniquely human condition and therefore no direct animal model is possible. However, animal behavioral models are not necessarily true models of human psychological or emotional disorders (Dews, 1976). Such procedures are tools that provide data correlating with the therapeutic as

well as the undesirable effects of drugs in man (Cook and Davidson, 1973). These models are best viewed as experimental compromises in which a possibly simpler and available system is used to gain insight into the more complex and less readily accessible system. Attempts to readily extrapolate from the animal pharmacology of the benzodiazepines to their effects on human anxiety neurosis result in behavioral interpretations that may be more in the eye of the beholder than in the brain of the animal. Heroic acts of faith should not be necessary in accepting underlying assumptions or interpretations of data if one adheres to the true value of the animal model as a predictor of clinical antianxiety activity. Tedeschi (1969) has proposed that such predictive value depends on the fulfillment of necessary but not sufficient criteria:

1. Tests should be selective enough to differentiate false positives and to distinguish therapeutic activity from side effects.
2. The relative potency of reference agents in the animal test should compare favorably with their relative potency in man.
3. Tolerance should not develop to the measure presumed to reflect therapeutic activity.
4. Tests should be sensitive enough to detect the activity of reference agents within a reasonable dose range.

A point worth reiterating is that animal behavioral models are useful tools when used to establish relevant pharmacological correlates of the clinical properties of the benzodiazepines. Their utility does not imply the ability to precipitate and/or identify anxiety in animals. With this orientation in mind the following represents a brief

review of the literature concerning the most consistent alterations in central nervous system (CNS) physiology and pharmacology elicited by the benzodiazepines. The behavioral effects of selective lesions of various limbic structures will be reviewed to illustrate potential functional similarities to the effects of systemic administration of the benzodiazepines.

C. Effects of Benzodiazepines on Avoidance and Suppressed Behavior

1. Avoidance Behavior

Experiments in which subjects have to refrain from responding to avoid punishment (passive avoidance) may be influenced by benzodiazepines. Response latency or passive avoidance percentage is generally taken as an indirect measure of emotionality in passive avoidance situations, an increased latency and percentage of witheld responses being indicative of an ongoing "fear" reaction. Pretreatment with benzodiazepines produces a significant decrease in both parameters (Oishi et al., 1972; Fuller, 1970). Rats can also be taught to associate the termination of a previously neutral conditioned stimulus with footshock so that they actively avoid the shock by climbing a pole, jumping a hurdle or entering an alternate chamber on presentation of the conditioned stimulus (Greenblatt and Shader, 1974). Treatment with benzodiazepines prior to testing reduces the active avoidance responses evoked by presentation of the conditioned stimulus in trained rats, although the deficit is less consistent and requires a higher dose than that necessary for disinhibition of passive avoidance (Greenblatt and Shader, 1974). Some authors (Cannizzaro et al., 1972; Delini-Stula, 1971; Molinengo and Gamalero, 1969) claim that active avoidance

inhibition is a resultant of nonspecific sedation and neuromuscular impairment rather than fear attenuation. However, the benzodiazepines have been shown to produce active avoidance deficits at doses well below those which produce significant central nervous system depression (Cole and Wolf, 1962; Chittal and Sheth, 1963; Heise and Boff, 1962). The degree of the response deficit for any given benzodiazepine is, in part at least, a function of the manual difficulty involved in the avoidance response. Results from active avoidance studies are complicated by a number of factors which modulate the effects of benzodiazepines, e.g., strain (Kuribara et al., 1976) and species differences and rates of baseline shock acceptance (Dantzer, 1977). Additional variability arises from the uncertain role conditioned fear plays from a motivational point of view and its postulated attenuation by benzodiazepines (Haefely, 1978).

2. Suppressed Responding: Response-contingent Punishment (Conflict) and Conditioned Suppression

The restoration of behavior suppressed by punishment (anti-conflict or disinhibition effect) is the most consistently observed sequel of benzodiazepine treatment. The virtually unanimous finding that antianxiety agents attenuate the behaviorally suppressant effects of punishment in an immensely diverse array of experimental designs is testimony to the remarkable predictability of this effect. Typically, behavior reinforced to a high frequency of occurrence is subsequently suppressed by experimenter-introduced manipulation. The great majority of experimental approaches utilize a multiple schedule format. Geller and Seifter (1960) introduced the first "multiple schedule" procedure for which the most salient feature was a two component, repetitive

cycle: a punishment component signalled by the onset of a previously neutral conditioned stimulus used to assay selective drug effects, i.e., potential anxiolytic activity, and an unpunished segment used to evaluate nonspecific drug effects, such as general depressant activity.

In experimental designs in which signalled periods of responding are simultaneously rewarded (food or water) and punished (electrical shock), interposed on a background of appetitive behavior, the benzodiazepines consistently increase the rate of punished responding. Such procedures utilizing response-contingent punishment, referred to as conflict situations, have their basis in the prototype described by Geller and Seifter (1960). The alternation of periods of positively rewarded behavior with signalled periods of punishment-induced suppression of that behavior is considered to represent a conflict situation since the animal's response tendency is controlled by the consequences of both positive and negative reinforcement. The behavioral baseline often takes the form of conditioned operant responses that are positively reinforced on a variety of schedules although the use of unconditioned, innate responses such as food or water intake yields similarly stable baselines (Bertsch, 1976). Benzodiazepines appear to effectively attenuate the suppressive effects of punishment on consummatory as well as on operant behavior (Miczek, 1973a; Vogel et al., 1971).

The specific benzodiazepine-induced diminution of the suppressant effects of response-contingent punishment (anticonflict effect) is characteristically accompanied by an unchanged rate of unpunished responding (Goldberg and Ciofalo, 1969; Miczek, 1973a;

Geller et al., 1962; Geller, 1964; Blum, 1970; Ts'o and Chenoweth, 1976; Robichaud et al., 1973), for chlordiazepoxide. Equivalent anti-conflict effects were obtained for chlordiazepoxide when the intensity of the conflict situation was progressively increased over successive trials by increasing the intensity of shock resulting from incorrect lever presses and by making the discrimination (light intensity) between punished and nonpunished sessions more difficult (Bremner et al., 1970). A significant restoration of punishment-suppressed responding has been reported for diazepam (Geller, 1964) and oxazepam (Babbini, 1975; Geller, 1964) at doses producing no appreciable nonspecific debilitation.

In a modification of the conflict paradigms utilizing response-contingent punishment cited above, chlordiazepoxide has been shown to increase responding on an intermittent punishment schedule (fixed ratio) over a wide range of doses (2.2 to 35.6 mg/kg i.p., Cook and Davidson, 1973; 1.25 to 40 mg/kg p.o., Cook and Sepinwall, 1975). Decreases in the rate of unpunished behavior in the nonshocked segment (variable interval of reinforcement) of the two-schedule design was seen only at the higher doses tested. Such a disinhibition of punishment-suppressed responding by chlordiazepoxide was corroborated by Miczek (1973a) in a similar concurrent variable interval food reinforcement/fixed ratio punishment schedule. Diazepam exhibits a greater and oxazepam a lesser potency relative to chlordiazepoxide in releasing behavior suppressed by intermittent punishment (Cook and Davidson, 1973). While the aforementioned anticonflict effects of the benzodiazepines have dealt exclusively with rats, these effects appear to

generalize across a number of species including squirrel monkeys (Miczek, 1973a; Sepinwall et al., 1978), pigeons (McMillan, 1973), and pigs (Dantzer and Roca, 1974).

Operant responses for a food reward may be suppressed during the presentation of a conditioned stimulus associated with and preceding an aversive unavoidable stimulus (shock), even if the shock is not contingent upon response (Estes and Skinner, 1941). The suppression of responding during the conditioned stimulus presentations is interpreted by Millenson and Leslie (1974) to represent an indirect measure of a conditioned emotional response (CER) which has seen considerable, though highly controversial, use as a behavioral baseline for the identification and characterization of antianxiety drugs. Pretreatment with chlordiazepoxide (Lauener, 1963; Miczek, 1973b; Bainbridge, 1968) attenuates this response suppression, again at doses producing insignificant effects on the rate of unpunished behavior.

The findings with antianxiety drugs in conditioned emotional response paradigms appear less consistent than with response-contingent shock. In an attempt to define the role played by the relationship between the animal's behavior and the shock delivery, Huppert and Iversen (1975) found that response-contingent shock produced greater suppression of the behavior than did noncontingent shock. More significantly, they showed that chlordiazepoxide was more effective in releasing behavior from suppression when shock was response-contingent (controllable) than when shock was uncontrollable. The reported variability in the effects of benzodiazepines on the conditioned emotional response may be attributed to methodological inconsistencies

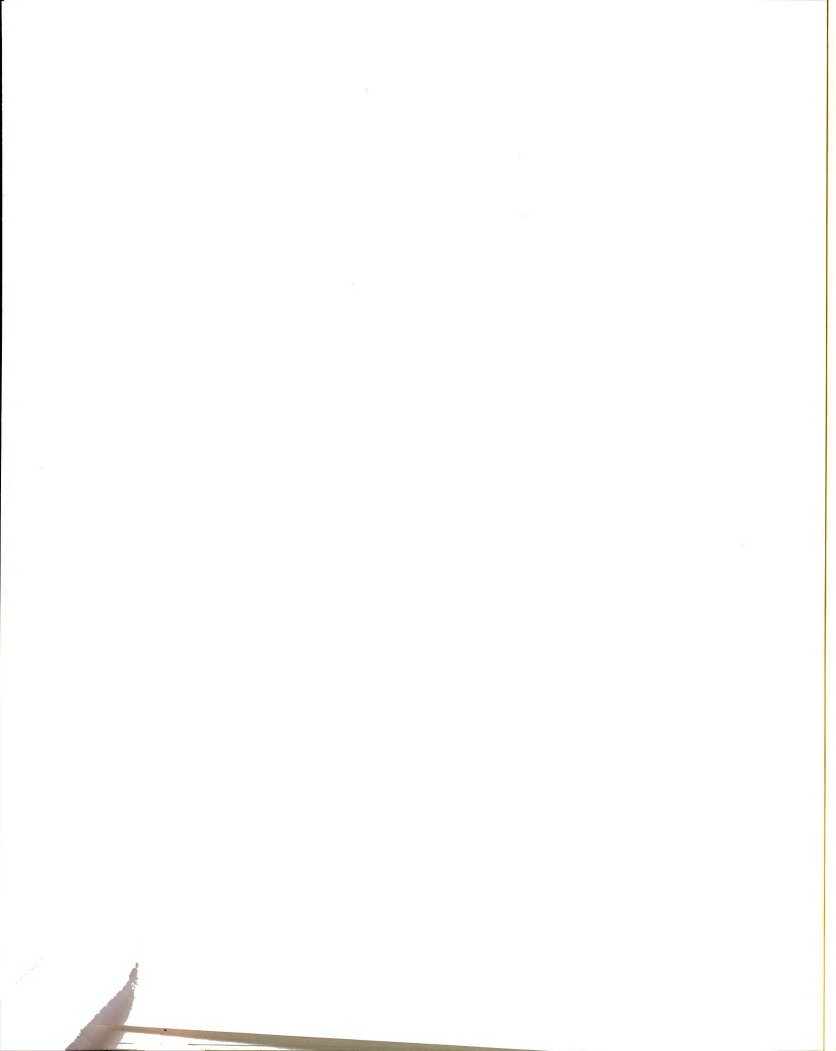
as well (Millenson and Leslie, 1974). For whatever the reasons, it would appear that conflict procedures (response-contingent punishment) exhibit certain advantages in terms of flexibility of design and unanimity of findings over methods using noncontingent shock (e.g., conditioned emotional response) as reliable methods capable of yielding relevant pharmacological correlates of clinical antianxiety activity.

In terms of qualitative discriminations, conflict procedures seem to be quite accurate in distinguishing minor tranquilizers from other classes of compounds. For instance, punished and unpunished behavior are not appreciably altered by neuroleptics until high dose levels are reached, at which point both are depressed simultaneously (Cook and Sepinwall, 1975; Schallek et al., 1972; Geller et al., 1962). While an alleviation of suppression is seen for various barbiturates at low doses, the therapeutic index (median behavioral debilitating dose to median conflict-attenuating dose ratio) is characteristically much larger for the benzodiazepines (Blum, 1970). Ethanol significantly increased the rate of punished responding at a dose (1000 mg/kg, p.o.) which minimally affected the rate of unpunished responding (Cook and Davidson, 1973). Amphetamine (Geller, 1960; Wilson, 1977) and morphine (Geller et al., 1963; Cook and Davidson, 1973) appeared to be ineffective in restoring punishment-suppressed responding over a wide range of doses. In fact, d-amphetamine is well known to enhance this suppression. Conflict methods have the added advantage of being able to detect qualitatively different profiles within the class of benzodiazepines. Different benzodiazepines can be seen to differ in the magnitude and dose range of the anticonflict effect as well as the dose

at which significant depression of unpunished responding first occurs (Cook and Sepinwall, 1975).

3. Chronic vs. Acute Benzodiazepine Administration: The "initial treatment phenomenon"

All of the behavioral results described in the previous discussion were obtained after the animals had been exposed to the benzodiazepines on several prior occasions, i.e., after the animals were already drug-experienced. Margules and Stein (1968) observed that the degree and even the direction of the modification induced in the punishment-suppressed behavior by antianxiety drugs could vary depending on whether the animals had a prior history of drug exposure or were being given the agent for the first time ("drug-naive"). Upon initial administration to conflict-trained, drug-naive animals, the benzodiazepines often produce a marked nonspecific depression of behavior, visible in a reduction of unpunished behavior with either decreased or relatively unchanged rates of punished responding. With additional exposure to the drugs at daily or longer intervals characteristic increases in punished behavior occur while unpunished behavior returns to predrug control rates (Margules and Stein, 1968). A qualitatively similar development of tolerance to the depressant effects and simultaneous unmasking or development of their antipunishment effects has been reported for chlordiazepoxide (Cook and Sepinwall, 1975; Sepinwall et al., 1978; Goldberg et al., 1967), diazepam (Sepinwall et al., 1978), flurazepam (Cannizzaro et al., 1972), and oxazepam (Margules and Stein, 1968).



Sepinwall et al. (1978) also found the effects of benzodiazepines on unpunished and punished behavior to follow different courses with their repeated administration in squirrel monkeys. These effects were found to be dose-related and to reflect individual differences in drug sensitivity. A possible clinical correlate to the "initial treatment phenomenon" defined in animals may exist in that after benzodiazepines have been administered to humans it has been reported that sedative effects predominate at first but then diminish in intensity and disappear after a few days of treatment in anxious patients (Warner, 1965) as well as normal volunteers (Hillestad et al., 1974). The initial sedation was replaced by feelings of relaxation and well-being after several daily treatments. An additional similarity between the animal and clinical findings is that the effect of diazepam in drug-naive humans also appears to be dose-related, i.e., marked sedative effects occur initially at a daily dose level of 30 but not 15 mg (Hillestad et al., 1974).

D. Effects of Benzodiazepines on the Dynamic Equilibrium of Putative Neurotransmitters in the Central Nervous System

Various drugs acting on the central nervous system appear to do so either by regulating the synthesis, storage, release or metabolism of a neurotransmitter or by facilitating or inhibiting the action of a transmitter at postsynaptic receptors. Such a simplified rationale has not been shown to exist for the benzodiazepines or central nervous system depressants in general and there is no unified theory capable of correlating their pharmacological effects with a specific action on a given neuronal system. To the contrary, the collective findings of a

recent symposium (Costa and Greengard, 1975) devoted to the study of the benzodiazepines appear to implicate virtually every known chemical family of neurotransmitters as well as the cyclic nucleotides. The following section is a brief review of attempts that have been made to relate some of the pharmacological actions of the benzodiazepines to their effects on various possible neurochemical substrates. It is in keeping with the overall theme of this dissertation that certain neurochemical influences of the benzodiazepines, perhaps localized to a specific area of the brain, underlie their efficacy in the treatment of anxiety. Therefore, this review will be primarily limited to their anxiolytic activity as predicted by effects of these drugs on suppressed responding in animal models. The scope of this review will be further restricted to those neurotransmitters that appear to be the most promising candidates in light of the current state of the art, i.e., catecholamines, 5-hydroxytryptamine and γ-aminobutyric acid.

1. Catecholamines

Neither chlordiazepoxide (Corrodi et al., 1967) nor diazepam (Consolo et al., 1975) have been shown to consistently alter the whole brain content of norepinephrine or dopamine. However, static tissue concentrations, especially whole brain values, do not necessarily reflect the effects of drugs on neurotransmitter function. Estimates of transmitter turnover rate (i.e., the rate at which the renovation of the pool of a transmitter in a given area proceeds) have supplanted static measurements as viable approximations of neuronal activity.

Using both biochemical and histochemical techniques Lidbrink et al. (1973) reported a decrease in whole brain dopamine turnover by

benzodiazepines and barbiturates in animals pretreated with α-methyltyrosine. The doses used were associated with a clear-cut depression and, in the case of barbiturates, a hypnotic action. More significantly, Fuxe et al. (1975) have shown a similar retardation in the rate of disappearance of dopamine fluorescence following α-methyltyrosine. This was found in the nucleus accumbens, olfactory tubercle and head of the caudate nucleus by lower, behaviorally effective anticonflict doses of diazepam and chlordiazepoxide (1 and 10 mg/kg, respectively). The actions of diazepam and chlordiazepoxide on dopamine turnover appear to be more definitive in the limbic forebrain as compared to the caudate due to the high variability in the response in the latter.

In an attempt to characterize the interaction between benzodiazepines and neuroleptics at central dopamine neurons, Fuxe et al. (1975) demonstrated the ability of diazepam (1 and 5 mg/kg) to selectively counteract the pimozide-induced increase in dopamine turnover in the "limbic forebrain" (nucleus accumbens and olfactory tubercle) but not in the neostriatum. Keller et al. (1976) have reported the reduction by diazepam (10 mg/kg) of the haloperidol or chlorpromazine-induced increase in 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA) to be similar in striatum and limbic forebrain. Diazepam (10 mg/kg) also reduces DA turnover in the entorhinal cortex (Fuxe et al., 1975), a terminal of the mesocortical dopaminergic projections (Hökfelt et al., 1974). Furthermore, chlordiazepoxide and diazepam decrease striatal dopamine turnover as estimated from the rate of decline of the specific activity of ^{3}H-dopamine following its intraventricular administration, i.e., labelling of the endogenous stores of catecholamines (Taylor and Laverty, 1973).

High doses of diazepam and chlordiazepoxide (10 and 25 mg/kg, respectively) reduce cortical norepinephrine turnover, an observation attributed to a reduction of neuronal activity in the ascending cerulocortical noradrenergic pathway (Corrodi et al., 1971). Norepinephrine turnover in subcortical structures appears to be relatively unaffected. However, chlordiazepoxide (Corrodi et al., 1967) and diazepam can counteract the stress-induced increase in norepinephrine turnover in all parts of the rat brain (Lidbrink et al., 1973). Thus, when activity in noradrenergic pathways is generally increased, selectivity is lost. The significance of such an effect of the benzodiazepines in regard to their antianxiety action is not supported by the inability of lower, nonsedative doses (at least in the cortex) to attenuate the stress-induced increase in norepinephrine turnover (Fuxe et al., 1975).

Behaviorally, intracerebroventricular (Stein et al., 1975) and systemic (Robichaud et al., 1973; Sepinwall et al., 1973) administration of propranolol, a β-adrenergic receptor antagonist, failed to release punishment-suppressed behavior in the rat conflict test. Phentolamine, an α-adrenergic receptor antagonist, was similarly inactive. Intracerebroventricular injections of l-norepinephrine increased rather than decreased the punishment-lessening activity of systemically administered benzodiazepines (Stein et al., 1973). These findings contradict the hypotheses that the anxiety-reducing activity of the benzodiazepines depends on a reduction of transmitter function at noradrenergic synapses. The inactivity of the dopamine receptor blocking agents in conflict procedures similarly weakens any role that the benzodiazepine-induced decrease in dopaminergic activity (if the

decreased turnover is truly reflecting this) may play in their anti-conflict effects. Furthermore, the central catecholamine destruction produced by the intraventricular administration of 6-hydroxydopamine (6-OHDA) neither produces an anticonflict effect nor interferes with the anticonflict activity of diazepam (Lippa et al., 1977a).

Intraventricular l-norepinephrine antagonized the depressant effect of oxazepam (10 mg/kg) on nonpunished responding in the rat conflict test (Stein et al., 1973), suggesting that the generalized depressant activity of benzodiazepines may be mediated by a reduction of noradrenergic activity. These same authors proposed that the initial decrease in unpunished behavior on the first exposure of conflict-trained, drug-naive animals to benzodiazepines ("initial treatment phenomenon") is related to a transient benzodiazepine-induced decrease in norepinephrine turnover. They have subsequently reported that a decrease in norepinephrine turnover in the midbrain-hindbrain region, as estimated by the reduced rate of decline of ^{3}H-norepinephrine following its intraventricular administration, was no longer detectable after six daily doses of oxazepam (20 mg/kg, i.p.). While this correlates with the disappearance of the generalized depressant action of this dose of oxazepam on unpunished responding in the conflict test following six daily injections, Cook and Sepinwall (1975) have found the correlation to be much less clearcut.

2. 5-Hydroxytryptamine

Studies with various benzodiazepines have yielded ambiguous results concerning their effects on brain 5-hydroxytryptamine concentration. Lidbrink et al. (1974) and Consolo et al. (1975) have

found chlordiazepoxide and diazepam to produce no reproducible changes in cortical or whole brain steady state 5-hydroxytryptamine values. In contrast, a significant increase in whole brain 5-hydroxyindoles and tryptophan has been reported for diazepam (Jenner et al., 1975). However, the observed increase could be due, at least in part, to the influence of benzodiazepines to competitively inhibit the binding of tryptophan onto serum albumin, thus increasing the concentration of free serum tryptophan and thereby increasing the amount available to the brain (Bourgoin et al., 1975).

Whereas a reduction of brain 5-hydroxytryptamine turnover associated with the acute administration of benzodiazepines is generally agreed upon, considerable variability exists in terms of the benzodiazepine studied, dose, site of maximum effect and technique used to estimate turnover. Little or no work has been done in investigating the time course of this effect. Using radioisotopic tracer techniques, a decreased rate of decline of injected radiolabelled 5-hydroxytryptamine has been reported for chlordiazepoxide (20 mg/kg; Lippmann and Pugsley, 1974), oxazepam (Wise et al., 1972) and diazepam (Chase et al., 1970). Furthermore, diazepam (5 and 10 mg/kg) and chlordiazepoxide (20 mg/kg) slow the rate of formation of ^{3}H-5-hydroxytryptamine from its radiolabelled precursor (^{3}H-tryptophan) following the intravenous injection of the latter (Dominic et al., 1975). Sedative doses of chlordiazepoxide and diazepam decrease the rate of decline of cortical 5-hydroxytryptamine following the inhibition of the rate-limiting enzyme involved in its synthesis (Lidbrink et al., 1974). A single injection of diazepam (10 mg/kg) decreased in vitro midbrain tryptophan hydroxylase activity (Rastogi et al., 1977). Although the

benzodiazepines appear to decrease 5-hydroxytryptamine activity, little is known concerning the level of regulation at which this effect is exerted or even whether it is perhaps secondary or compensatory to a drug-induced increase in serotonergic activity.

If the anxiety-reducing activity of benzodiazepines is related to their ability to reduce brain serotonin turnover then agents that reduce serotonergic activity should also counteract the suppressive effects of punishment. This contention (Stein et al., 1975) has been the impetus for a large number of studies dealing with the interaction between putative antagonists of 5-hydroxytryptamine or inhibitors of its synthesis and conflict behavior or other forms of conditioned suppression. Increases in the rate of punished responding have been reported following the administration of the peripheral 5-hydroxytryptamine antagonists methysergide (Stein et al., 1973; Graeff, 1974; Cook and Sepinwall, 1975), cinanserin (Geller et al., 1974; Cook and Sepinwall, 1975) and cyproheptadine (Graeff, 1974; Schoenfeld, 1976). Miczek and Luttinger (1978) have reported methysergide to be ineffective in attenuating the behaviorally suppressive effects of noncontingent punishment (conditioned suppression or conditioned emotional response). The utility of these peripheral antagonists as blockers of central 5-hydroxytryptamine receptors is compromised by what appears to be an uncertain relationship with 5-hydroxytryptamine receptors in the brain (Haigler and Aghajanian, 1974; Jacoby et al., 1978; Bürki et al., 1978).

p-Chlorophenylalanine (p-CPA) selectively depletes 5-hydroxytryptamine consequent to the inhibition of tryptophan hydroxylase, the

rate-limiting enzyme involved in 5-hydroxytryptamine synthesis (Jequier et al., 1967). The administration of p-chlorophenylalanine has been associated with a significant anticonflict effect (Robichaud and Sledge, 1969; Geller and Blum, 1970; Stein et al., 1973) as well as an "attenuation of a conditioned suppression of the conditioned emotional response type" (Hartmann and Geller, 1971). However, Blakely and Parker (1973) and Cook and Sepinwall (1975) have been unable to obtain consistent anticonflict effects with p-chlorophenylalanine. The intracerebroventricular or intracerebral injection of the neurotoxic derivatives of 5-hydroxytryptamine, i.e., 5,6- and 5,7-dihydroxytryptamine (5,6- and 5,7-DHT), have been observed to produce a marked reduction in brain 5-hydroxytryptamine. When 5,6-dihydroxytryptamine was administered by an intracerebroventricular route to conflict-trained rats a profound increase in punished responding was observed (Stein et al., 1975; Lippa et al., 1977b). This anticonflict effect reportedly paralleled the pharmacological time course of 5-hydroxytryptamine depletion, while the rate of unpunished responding was little affected. Bilateral injection of 5,7-dihydroxytryptamine into the ventral medial tegmentum, the main pathway taken by ascending serotonergic fibers, prevented the acquisition of behavioral suppression in a conflict paradigm and released punished behavior in previously trained animals (Tye et al., 1977). In addition, the behaviorally suppressive effects of intracerebroventricular 5-hydroxytryptamine or chemical stimulation of the dorsal raphe nucleus (concentration of 5-hydroxytryptamine-containing cell bodies in the midbrain that supply the serotonergic innervation to areas of the forebrain and diencephalon) by carbachol can be reversed by oxazepam (Stein et al., 1973).

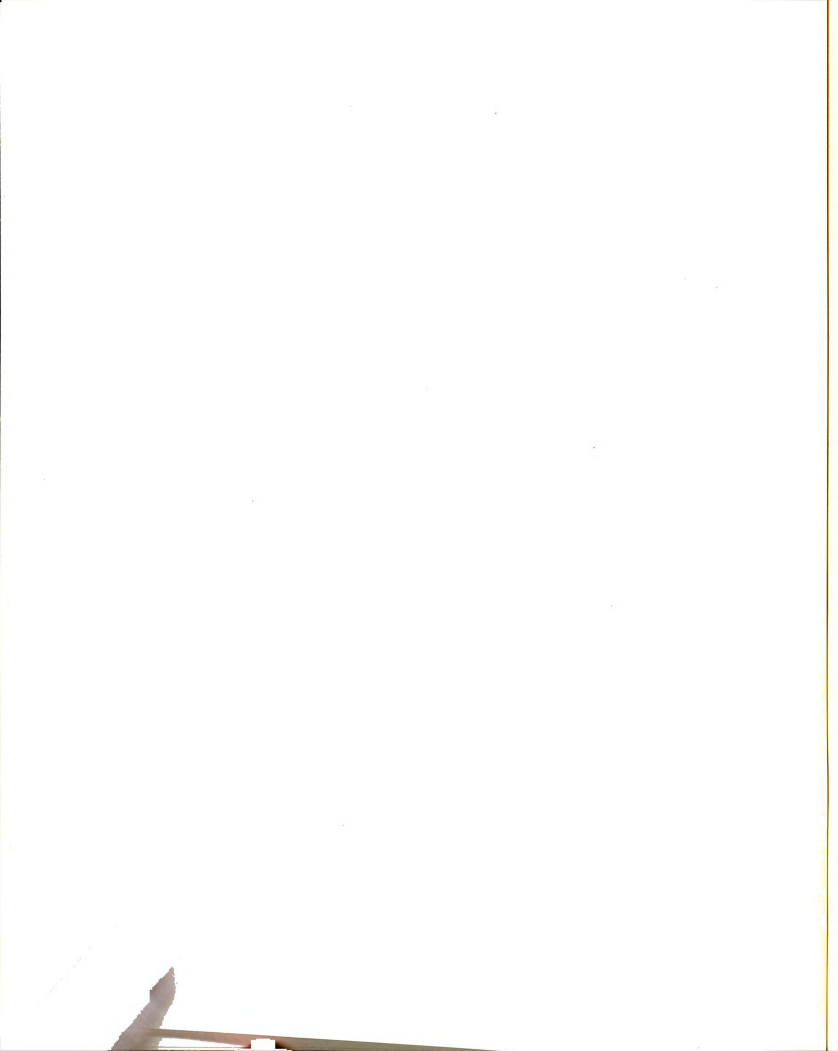
These findings, together with those obtained from neurochemical studies, have led to the suggestion that a benzodiazepine-induced decrease in functional 5-hydroxytryptamine activity may be causally related to their anxiety-reducing actions. However, the relationship between the benzodiazepines and central 5-hydroxytryptamine activity may be less simplistic than this. The activation of brain 5-hydroxytryptamine receptors by 5-hydroxytryptamine precursors or by 5-hydroxytryptamine agonists has been found to induce head twitches (Jacobs, 1976; Corne et al., 1963). Nakamura and Fukushima (1977,1978) have found the head twitches induced by intracerebroventricular 5-hydroxytryptamine or the systemic administration of 5-hydroxytryptamine precursors and agonists to be potentiated by benzodiazepines. Thus, gross neurological imbalances in volitional motor control may be induced by the increase in central serotonergic activity, which may then indirectly influence the pattern of punished responding. It should also be emphasized that the proposed role of 5-hydroxytryptamine as the neurotransmitter implicated in conditioned suppression was developed from results involving conflict tests utilizing food reinforcement. Since brain 5-hydroxytryptamine pathways appear to be implicated in the motivation of feeding behavior (Samanin et al., 1977), an indirect effect on the pattern of conflict behavior may be exerted via this mechanism.

3. γ-Aminobutyric Acid and Glycine

Various data suggest that some of the biochemical (Fuxe et al., 1975; Biggio et al., 1977; Mao et al., 1975; Costa et al., 1975), pharmacological (Haefely et al., 1975; Costa et al., 1975) and

behavioral (Soubrie et al., 1976; Soubrie and Simon, 1977) effects of benzodiazepines result from an as yet undefined interaction with γ-aminobutyric acid mechanisms. Electrophysiological studies of the nature of this interaction have yielded conflicting results since a facilitation of γ-aminobutyric acid-mediated synaptic transmission (Polc et al., 1974; Haefely et al., 1975; Polc and Haefely, 1976; Costa et al., 1975) as well as an antagonism of γ-aminobutyric acid-mediated inhibition (Steiner and Felix, 1976; Gahwiler, 1976; Curtis et al., 1976; MacDonald and Barker, 1978) have been reported for the benzodiazepines. The relationship of these findings to the pharmacological profile of the benzodiazepines has led to the suggestion that several actions of the benzodiazepines (muscle relaxation, ataxia, anticonvulsant effects) may be mediated by an enhanced function of γ-aminobutyric acid-containing neurons. Costa et al. (1975) suggest a similar mechanism to be involved in their antianxiety effects based on a concept of functional economy or efficiency. However, when one associates the main therapeutic actions and side effects of benzodiazepines with accepted or presumed functions of γ-aminobutyric acid, an involvement of γ-aminobutyric acid in their anxiolytic action is not presently apparent (Haefely et al., 1975). Along the same line of investigation, quite obvious connections for γ-aminobutyric acid-mediated pathways with the muscle relaxant, ataxic and anticonvulsant effects of the benzodiazepines appear to be substantiated.

Studies of the central neurotransmitter role of γ-aminobutyric acid and effects of benzodiazepines have led to 2 major proposals: 1) The depolarization of afferent terminals by the putative transmitter



and the enhancement of this process of presynaptic inhibition by the benzodiazepines may be of significance for the muscle relaxant properties of these drugs (Polc et al., 1974). 2) The postsynaptic hyperpolarization of γ-aminobutyric acid in cerebellar inhibitory processes may be facilitated by benzodiazepines and may account in some part for the ataxia and motor incoordination elicited by this drug class (Costa et al., 1975; Haefely et al., 1975). A similar enhancement of postsynaptic inhibition at higher levels of the neuraxis has been reported for benzodiazepines, i.e., the medulla (Dray and Straughan, 1976), substantia nigra (Haefely et al., 1975) and cerebral cortex (Zakusov et al., 1975).

It has been suggested that information concerning the participation of γ-aminobutyric acid in mediating some of the central actions of the benzodiazepines may be obtained through a study of the selectivity and potency of antagonism against convulsions elicited by perturbations of γ-aminobutyric acid function (Costa et al., 1975). Benzodiazepines preferentially and specifically antagonize the seizures associated with a reduction in the biosynthesis of γ-aminobutyric acid elicited by isoniazid (Costa et al., 1975), thiosemicarbazide and 3-mercaptopropionic acid (Haefely et al., 1975). Their ED_{50} anticonvulsant dose was approximately 7- to 10-fold higher against seizures elicited by γ-aminobutyric acid receptor blocking agents, i.e., picrotoxin and bicuculline (Mao et al., 1975). Barbiturates and diphenylhydantoin weakly and equipotently antagonized the convulsions induced by γ-aminobutyric acid synthesis inhibition or receptor blockade. The action of aminooxyacetic acid, a compound that increases the concentration of γ-aminobutyric acid in the central nervous system by

inhibiting γ-aminobutyric acid-transaminase, parallels that of the benzodiazepines on convulsions induced by inhibition of γ-aminobutyric acid synthesis (Haefely et al., 1975). Various γ-aminobutyric acid-mediated systems appear to be affected in a similar fashion by the benzodiazepines, aminooxyacetic acid and exogenous γ-aminobutyric acid: enhancement of presynaptic inhibition in the spinal cord (Polc et al., 1974) and cuneate nucleus (Polc and Haefely, 1976), increase in firing rate of ponto-geniculo-occipital waves induced by depletion of brain 5-hydroxytryptamine (Ruch-Monachon et al., 1976), and enhancement of the cataleptic effect of neuroleptics (Keller et al., 1976).

It should be mentioned that the majority of studies concerning the interaction between γ-aminobutyric acid-mediated systems and benzodiazepines have so designated such functions based upon the ability of picrotoxin and/or bicuculline to antagonize them. Therefore, conclusions drawn from such studies are only as valid as the selectivity and specificity of these agents for γ-aminobutyric acid receptors. Similar caution should be exercised when using inhibitors of the synthesis or catabolism of γ-aminobutyric acid.

γ-Aminobutyric acid content in the lumbosacral cord of spinal cats was significantly elevated by diazepam (3 mg/kg, i.v.). Saad (1972) reported a significant increase in mouse cerebral hemisphere γ-aminobutyric acid content following doses of 5 and 10 mg/kg diazepam. Higher doses (30 mg/kg, i.p.) were required to increase mouse and rat whole brain concentrations (Haefely et al., 1975). Studies concerning the effect of diazepam on the estimated γ-aminobutyric acid turnover rate in various brain regions suggest that the increased concentrations

of γ-aminobutyric acid following diazepam are not due to an acceleration of its synthesis. Diazepam decreased the turnover rate of γ-aminobutyric acid in rat whole brain (Fuxe et al., 1975), various cortical regions (Pericic et al., 1977) and the cerebellum (Guidotti, 1978), as well as the nucleus accumbens and caudate nucleus (Mao et al., 1977). The γ-aminobutyric acid receptor agonist, muscimol (Krogsgaard-Larsen et al., 1975), similarly decreases γ-aminobutyric acid turnover (Guidotti, 1978; Mao et al., 1977). This fact further supports a positive influence of the benzodiazepines on γ-aminobutyric acid activity and suggests that the observed decrease in γ-aminobutyric acid turnover elicited by diazepam may reflect a reflex or compensatory decrease in γ-aminobutyric acid synthesis in response to an enhanced γ-aminobutyric acid receptor activity. The molecular mechanisms by which benzodiazepines enhance γ-aminobutyric acid-mediated inhibition is the subject of much debate (Haefely, 1977), the scope of which exceeds the intent of this review.

Behavioral evaluation of the effects of manipulations in central γ-aminobutyric acid neuronal activity on punishment-suppressed responding (conflict) generally yield negative results. An evaluation of aminooxyacetic acid for anticonflict activity revealed no increase in the rate of punished responding in doses ranging from 2.5 to 25 mg/kg, i.p., whether given 40 or 240 minutes prior to a session (Cook and Sepinwall, 1975). Furthermore, the response to a subthreshold anticonflict dose of diazepam was unaffected by aminooxyacetic acid pretreatment. The elevation of brain γ-aminobutyric acid produced by another inhibitor of its catabolism, ethanolamine-O-sulphate, was

similarly ineffective (File and Hyde, 1977). The γ-aminobutyric acid receptor agonist, muscimol, exhibited no anticonflict activity and did not alter the anticonflict activity of diazepam (Sullivan et al., 1978). Unpunished responding was dose-relatedly depressed.

Considerable controversy exists concerning the effects of γ-aminobutyric acid receptor blockade on the anticonflict profile of the benzodiazepines. The anticonflict effects of oxazepam (Stein et al., 1978) and chlordiazepoxide (Billingsley and Kubena, 1978) were antagonized by picrotoxin in a dose-related manner without appreciably affecting the rate of unpunished responding. However, Cook and Davidson (1978) have found picrotoxin to be ineffective in this regard. Moreover, Lippa et al. (1977b) have shown the effects of picrotoxin on the ataxic and anticonflict properties of chlordiazepoxide to be dissociable. Subconvulsant doses of picrotoxin produced a dose-related reversal of the ataxic effects of chlordiazepoxide while failing to appreciably alter its anticonflict effects in untrained rats. Thus, the γ-aminobutyric acid-mediated properties of the benzodiazepines may play a role in the sedative and motor-incoordinating effects of the benzodiazepines whereas the evidence for a similar role in their anticonflict (antianxiety) activity is much less convincing. Further support for this contention is suggested by the antagonism exhibited by γ-aminobutyric acid receptor blockers of the behaviorally depressant effects of diazepam (Soubrie and Simon, 1978) and chlordiazepoxide (Billingsley and Kubena, 1978). Furthermore, chronic diazepam administration resulted in the development of tolerance to the γ-aminobutyric acid-mediated properties of this benzodiazepine, as measured by the

ability of diazepam to protect against the convulsions produced by the γ-aminobutyric acid receptor blocker, bicuculline (Lippa and Regan, 1977). A similar tolerance development is not characteristic of the anxiolytic actions of benzodiazepines and thus minimizes the importance of γ-aminobutyric acid mechanisms in mediating this effect.

In addition to γ-aminobutyric acid, glycine is a prominent inhibitory neurotransmitter in the central nervous system. The distribution of glycine is predominantly in the brainstem and spinal cord and, like γ-aminobutyric acid, this transmitter hyperpolarizes the neuronal membrane of postsynaptic cells. However, unlike γ-aminobutyric acid, it does not depolarize presynaptic terminals, i.e., does not evoke presynaptic inhibition (Costa et al., 1975). Young et al. (1974) have proposed that benzodiazepines may exert their antianxiety and muscle-relaxing effects by mimicking glycine at its postsynaptic receptor sites. ^{3}H-Strychnine, a potent glycine antagonist, binds to synaptic membrane preparations of spinal cord and brain stem in a selective fashion, indicating an interaction with postsynaptic glycine receptors (Young and Snyder, 1973). The regional localization of strychnine binding in the central nervous system correlates with endogenous glycine concentrations, i.e., spinal cord > medulla > midbrain. Additionally, the displacement of strychnine by glycine and other amino acids parallels their glycine-like neurophysiologic activity.

Benzodiazepines have been shown to be very potent inhibitors of the in vitro specific binding of labelled strychnine to glycine receptors. Moreover, the potency of a series of 21 benzodiazepines as inhibitors of ^{3}H-strychnine binding correlates closely with their

potency in pharmacological and behavioral tests considered relevant to clinical efficacy (Young et al., 1974). While the above findings would suggest an interaction of benzodiazepines with the neurotransmitter glycine at its central nervous system receptor sites, Hunt and Raynaud (1977) have not found the in vitro receptor binding activity of the benzodiazepines to be reflective of differences in in vivo pharmacological activity and suggest that differences in their liposolubility may well be an important factor in the displacement of ^{3}H-strychnine binding. Evidence from electrophysiological investigations further weakens the significance of an interaction between benzodiazepines and glycine receptors, as diazepam neither induced inhibitory phenomena characteristic of glycine nor blocked inhibitory processes in which glycine is claimed to be involved (Curtis et al., 1976). Furthermore, the rank-order potency of 10 benzodiazepines in displacing ^{3}H-strychnine and in producing anticonflict effects did not correlate significantly (Cook and Sepinwall, 1975). The disinhibitory effect of oxazepam on punished behavior (anticonflict effect) was not selectively antagonized by strychnine (Stein et al., 1975). Finally, doubt concerning the possible role of glycine involvement derives from the observation that the protection afforded by benzodiazepines against convulsions induced by strychnine is relatively weak (Costa et al., 1975) and tolerates out with repeated diazepam administration (Lippa and Regan, 1977).

4. Acetylcholine and Cyclic Nucleotides

Acetylcholine (ACh) is unique among neurotransmitters in that the steady state concentration of brain acetylcholine is consistently

increased by diazepam (Ladinsky et al., 1973; Cheney et al., 1973; Consolo et al., 1975). The concentrations of choline as well as choline acetyltransferase and acetylcholinesterase activities in vitro were unaffected (Consolo et al., 1974). Based on these findings it has been suggested that diazepam may be acting centrally by inhibiting the presynaptic release of acetylcholine. Although this hypothesis would also explain the decreased turnover of brain acetylcholine by diazepam (Cheney et al., 1973), a similar decrease can be obtained upon administration of dopamine or γ-aminobutyric acid agonists, e.g., apomorphine and muscimol, respectively (Zsilla et al., 1976). Therefore, these findings do not exclude the possibility that the observed effects on cholinergic activity may be indirectly mediated.

In the rat the increase in acetylcholine produced by diazepam (5 mg/kg, i.v.) was limited to hemispheric structures, i.e., the striatum, hippocampus, and hemispheric residuum after removal of the hippocampus and striatum (Consolo et al., 1975). The increase attained a maximum within 15 minutes and waned to control values by 60 minutes. Among diazepam's effects, the anticonflict, anticonvulsant or hypothermic actions cannot be temporally correlated with the increase in hemispheric acetylcholine. However, study of more specific areas of the brain or the use of more specific measures of cholinergic activity may indicate a stronger correlation between the biochemical effect on acetylcholine mechanisms and the pharmacological actions of benzodiazepines.

A variety of evidence has been amassed which implicates the cyclic nucleotides in translating the release of neurotransmitters into

a postsynaptic response (Daly, 1976). If the central actions of the benzodiazepines can be attributed to an alteration of neurotransmitter function, then their activity may be reflected in changes in cyclic nucleotide concentrations or the activity of the enzyme systems controlling the steady state concentrations. Decreases in rat cerebellar 3',5'-cyclic guanosine monophosphate (cGMP) (Costa et al., 1975), increases in 3',5'-cyclic adenosine monophosphate (cAMP) in brain slices (Hess et al., 1975) and inhibition of in vitro phosphodiesterase activity (Beer et al., 1972) have been reported for the benzodiazepines. The possibility of a dopaminergic or cholinergic link is suggested by the ability of dopamine and acetylcholine receptor agonists or blockers to alter rat cerebellar 3',5'-cyclic guanosine monophosphate content (Burkard et al., 1976). Alternatively, diazepam can prevent the increase of cerebellar 3',5'-cyclic guanosine monophosphate content elicited by a decreased γ-aminobutyric acid synthesis or a blockade of γ-aminobutyric acid receptors (Costa et al., 1975; Biggio et al., 1977). The consistency of the relationship between cerebellar 3',5'-cyclic guanosine monophosphate concentrations and γ-aminobutyric acid inhibitory transmission has prompted the measurement of concentrations of cerebellar 3',5'-cyclic guanosine monophosphate to monitor in vivo drug interactions with γ-aminobutyric acid inhibitory mechanisms (Mao et al., 1975). The concentrations of γ-aminobutyric acid and 3',5'-cyclic adenosine monophosphate in the rat cerebellum were unaltered by diazepam pretreatment. However, while the cerebellum may play a significant role in some actions of the benzodiazepines, i.e., ataxia and motor incoordination, it is doubtful that it functions in

their antianxiety effects. The effects of benzodiazepines on non-cerebellar cyclic nucleotides are poorly characterized. In contrast to the findings of Beer et al. (1972), an evaluation of potent phosphodiesterase inhibitors, i.e., caffeine and theophylline, for anticonflict activity showed them to be inactive; nor did theophylline alter the effect of a subthreshold dose of chlordiazepoxide (Cook and Sepinwall, 1975). Moreover, anticonflict potency and in vitro phosphodiesterase inhibitory activity for a series of benzodiazepines appeared to be unrelated, further weakening the possible participation of the phosphodiesterase inhibitory activity of many benzodiazepines in their antianxiety effects.

E. "Site of Action" of Antianxiety Drugs: The Limbic System

An important corollary in attempting to associate benzodiazepine-induced behavioral alterations with their biochemical effects is the elucidation of the anatomical substrate in the central nervous system which, upon interaction with benzodiazepines, produces the response characteristically seen in the whole organism. Our primitive understanding of the cellular basis of anxiety greatly complicates attempts to relate structure to function. However, the limbic system has for years been implicated by neurophysiologists as the seat of emotion and its behavioral, autonomic and endocrinological sequelae. For the sake of brevity, the "limbic" system will be considered to represent a functionally interconnected composite of subcortical nuclear groups, i.e., the amygdala, septum, hippocampus and hypothalamus. However, in its broadest definition, based on the numerous projections and interconnections of this core, the limbic system contains cortical and olfactory

components in addition to other subcortical structures (Isaacson, 1974). A concept involving anxiety as an emotional manifestation of limbic system function and its subsequent attenuation by a benzodiazepine-induced alteration in the activity of limbic neurons is plausible. Such an association is no less warranted than attempts to correlate ataxic or sedative effects of these drugs with alterations in motor function of the cerebellum or sensory processing in the spinal cord, respectively. Moreover, the term "chemical amygdalectomy" has been used to describe electrophysiological evidence which focuses the action of various benzodiazepines on the limbic system, particularly the amygdala (Schallek et al., 1964; Morillo, 1962; Chou and Wang, 1977; Haefely, 1978).

The placement of relatively specific, circumscribed lesions in a particular structure has seen widespread usage in an attempt to gain information concerning the function of the area in the expression of a behavioral pattern. When considering the effects of lesions of the amygdala, septum or hippocampus on behavioral paradigms previously shown to be affected in a consistent fashion by benzodiazepines, the behavioral effects of amygdaloid lesions more closely parallel those seen following benzodiazepine administration. The following is a brief review of the effects of bilateral lesions of the amygdala, septum and hippocampus on avoidance behavior and suppressed responding.

1. Effects of Limbic Structure Lesions on Avoidance Behavior

Bilateral limbic lesions, whether septal, hippocampal or amygdaloid, are generally associated with poor passive avoidance performance. The deficit obtained with amygdaloid lesions is characteristic for a number of qualitatively different experimental designs

(see below), all relying on the animal's suppression of a prepotent motor response as an indication of passive avoidance. The characteristic pattern seen with bilateral ablation of the amygdala is a decreased latency, when compared to sham-operated controls, in performing a response previously associated with an aversive stimulus. Amygdaloid lesions have been reported to disrupt passive avoidance, i.e., decreased latency to respond and increased rate of shock-contingent responding, in a shocked drinking tube procedure (Pellegrino, 1968; Kemble and Tapp, 1968) or shock prod (Blanchard and Blanchard, 1972). Similar results were obtained when measuring latency in entering a shocked second compartment of a two-compartment design (Suboski et al., 1970; Gaston and Freed, 1969) or in stepping down onto a shocked grid floor (Russo et al., 1976). Although lesions of the corticomedial division of the amygdala impair passive avoidance performance to some extent, the basolateral lesions produce a more severe deficit (Kemble and Tapp, 1968; Pellegrino, 1968).

While passive avoidance deficits produced by amygdaloid lesions are not inconsistent with a lesion-induced inability to associate fear or anxiety reactions with neutral stimuli paired with pain, Blanchard and Blanchard (1972) have reported a similar deficit for rats in the passive avoidance of unconditioned threatening stimuli (an immobile cat). The decreased freezing and crouching and increased approach behavior parallel that seen in response to a conditioned stimulus, i.e., shock prod. Moreover, the decreased avoidance and enhanced approach tendencies to both unconditioned and conditioned threat stimuli do not support a lesion-induced increase in general motor activity or an alteration in response perseveration as

explaining the behavioral effects. No appreciable difference between amygdaloid-lesioned and control animals was seen for post-operative, home cage, food and water consumption (Dacey and Grossman, 1977) or in bar-pressing rate on either a continuous reinforcement schedule or gradually increasing fixed ratio schedule for a food reward (Pellegrino, 1968). As flinch-jump and galvanic skin responses shock thresholds are not increased by amygdaloid lesions, impairments in shock motivated behavior are probably not due to elevated pain thresholds (Blanchard and Blanchard, 1972).

The effects of bilateral amygdaloid lesions in passive avoidance behavior must be interpreted in terms of the size and location of the lesion in the amygdala and the nature of the inhibited response. The inclusion of extra-amygdaloid structures, i.e., internal capsule, caudate-putamen, pyriform cortex, does not appear to be a significant factor in the lesion-induced impairment of passive avoidance (Kemble and Tapp, 1968; Blanchard and Blanchard, 1972). The passive avoidance deficits appear to generalize to a variety of inhibited responses and aversive stimuli (Nagel and Kemble, 1976).

The most consistent behavioral manifestation of amygdaloid lesions is a taming effect; placidity is increased and responsiveness to normally noxious stimuli is markedly reduced. Amygdaloid lesions can even produce a calm demeanor in animals made rageful and hyperreactive by lesions in the septal area. Slotnick (1973) found that animals with amygdaloid lesions, in contrast to controls, continue to show shock-contingent exploratory behavior and little or no "fear" behavior (freezing). Such a deficit in fear behavior is reflected by a

reduction in species-specific defensive reactions and an altered reactivity to fearful stimuli (Blanchard and Blanchard, 1972) and has been attributed to a lesion-produced decrement in "fear or anxiety arousal".

Bilateral lesions of selected limbic structures have been repeatedly shown to alter performance in a variety of active avoidance tasks. Rats with bilateral septal (Schwartzbaum et al., 1967; Krieckhaus et al., 1964) or hippocampal (Isaacson et al., 1961; Copobianco et al., 1977) lesions are facilitated in the acquisition of a conditioned avoidance response. These findings are in good agreement with a postulated role of septal and hippocampal areas in response inhibition (Pellegrino, 1968) and are further supported by the poor passive avoidance performance seen in animals similarly lesioned. In contrast, rats with bilateral amygdaloid lesions are generally deficient in acquiring conditioned active avoidance responses. Bilateral amygdalectomy produces deficits in the acquisition of a shuttle-box conditioned avoidance response (Bush et al., 1973; Suboski et al., 1970). In addition to a retarded acquisition of an active avoidance response, Robinson (1963) reports a lesion-induced impairment in the acquisition of a new response (wheel turning) motivated by the acquired fear rather than shock itself. Similar deficits appear to exist for active avoidance responses other than avoidance performance in a shuttle-box: avoiding an approaching shock prod (Blanchard and Blanchard, 1972) and bar-pressing (Campenot, 1969). While the unconditioned stimulus is usually in the form of electric shock, the conditioned stimulus has taken many forms, e.g., tones of varying intensity, light, different colored compartments, and a combination of a tone and a change in

illumination. A similar lesion-produced deficit in responding signalled by a diverse array of conditioned stimuli argues against a lesion-induced alteration in any specific sensory modality involved in the recognition of a fearful stimulus.

Lesion-induced deficits in the performance of active avoidance tasks are consistent with amygdalectomy curtailing the registration of habituation to the conditioned stimulus. If such were the case, the unconditioned suppressive quality of the conditioned stimulus would not habituate out with repeated presentation and the response rates would remain low in subsequent conditioning, i.e., conditioned stimulus-unconditioned stimulus pairings. However, Spevack et al. (1975) have failed to demonstrate any reliable effects of amygdalectomy on habituation.

The dual findings of poor passive avoidance but enhanced or unaltered active avoidance learning with septal and hippocampal lesions is consistent with a lesion-induced impairment in response inhibition. The poor performance of amygdalectomized animals in both types of avoidance tasks weakens the suggestion that destruction of this latter structure interferes with the ability to withhold responding. While the amygdala in the rat may play a significant role in the animals' response to aversive situations, extrapolation of an analogous function to the amygdaloid complex of humans is tenuous. However, the decreased emotionality resulting from temporal lobe lesions (Kluver-Bucy syndrome) and the feelings of fear or anger reported by conscious patients undergoing stimulation of the amygdaloid complex, which often occurs at the start of an epileptic seizure emanating from the temporal

lobe, implicate limbic structures as the neurological substrate of emotions in humans (DeGroot, 1975).

2. Effects of Limbic Structure Lesions on Suppressed Behavior

Evaluation of lesion-induced alterations in the postoperative conditioning of a conditioned emotional response (CER) affords further differentiation between the effects of specific limbic lesions. The conditioned emotional response was previously mentioned in terms of its disinhibition by various benzodiazepines and can be defined as a behavioral pattern, elicited by the presentation of a conditioned stimulus predicting onset of an unavoidable aversive stimulus, which is incompatible with previously learned operant responding for food reward. Responding during the conditioned stimulus is not shock-contingent: termination of the conditioned stimulus is associated with the unconditioned stimulus, usually footshock, regardless of the subject's response. In studying the effects of lesions located in circumscribed areas of the hippocampus, Nadel (1968) found the acquisition of a conditioned emotional response to be unaffected or slightly enhanced by ventral or dorsal hippocampal lesions, respectively. Similarly, rats with bilateral septal lesions show levels of response suppression (conditioned emotional response) during the conditioned stimulus not unlike that seen in control animals (Hobbs, 1976).

Lesions of the amygdala characteristically disrupt the formation of conditioned emotional responses, i.e., a lack of response suppression with successive conditioned-stimulus-unconditioned stimulus pairings. The consistent and reproducible nature of the lesion-induced impairment has led Kellicutt and Schwartzbaum (1963) to postulate the

utilization of the conditioned emotional response technique as a sensitive index of amygdaloid damage. While sham-operated controls require only 2-3 daily sessions to attain a reasonable level of response suppression during the conditioned stimulus presentation, amygdalectomized animals failed to meet an identical criterion of suppression within the preset limit of 15 sessions (Kellicutt and Schwartzbaum, 1963). Moreover, the lesioned group as a whole exhibited no pattern of suppression exceeding that attributable to chance. Doubling the intensity or duration of the shock proved ineffective in promoting the suppression of bar-pressing during the conditioned stimulus. Similar deficits in a qualitatively similar conditioned emotional response paradigm have been reported for bilateral amygdaloid lesions by Spevack et al. (1975). The licking response of water-deprived amygdalectomized rats was relatively unaffected by successive presentations of a compound conditioned stimulus, the termination of which was associated with footshock. In sham-operated and unoperated controls the conditioned stimulus presentations initiated a significant conditioned suppression of licking behavior.

The slight adipsia reported for amygdalectomized animals makes unlikely the possibility that the lesion-produced conditioned emotional response deficits can be attributed to their inability to inhibit drinking. Spevack et al. (1975) conclude that the observed deficits in response suppression are reflective of an interference with the arousal of fear rather than lesion-produced deficits in habituation or response inhibition.

Similarities in the behavioral effects of bilateral amygdaloid lesions and the systemic administration of benzodiazepines suggest that a benzodiazepine-induced decrease in amygdaloid function may be somehow related to their actions observed in the whole animal. Evidence in support of such a "pharmacological amygdalectomy" comes from electrophysiological studies which report a benzodiazepine-induced reduction in the electrical activity recorded from the amygdala and an alteration by low doses in the influence of this structure on various other neuronally connected systems.

Diazepam and chlordiazepoxide suppress the spontaneous single unit activity of the amygdala and hippocampus in a dose-related manner (Chou and Wang, 1977). Umemoto and Olds (1975) found that these same two benzodiazepines reduce both the background rate of discharge and the neuronal responses correlated with the presentation of a conditioned stimulus signalling aversive stimulation (conditioned emotional response paradigm), but only in the amygdala. Benzodiazepines exert a profound inhibitory action upon the hippocampal response to low frequency stimulation of the ipsilateral amygdala (Morillo et al., 1962). Moreover, the hippocampal potential evoked by amygdaloid stimulation in the curarized cat is reduced by antianxiety drugs with an order of potency reflecting therapeutically effective doses, whereas other psychoactive agents, such as tricyclic antidepressants and neuroleptics, are either inactive or modify the induced activity in the opposite direction (Haefely, 1978). Tsuchiya and Kitagawa (1976) have extended the influence of the benzodiazepines (using evoked potentials) to include the various neuronal connections of the intra-limbic as well as

midbrain-limbic systems. It should be emphasized that an alteration of spontaneous or evoked electrical events in specific regions of neuronal organization by a psychoactive drug does not necessarily prove that the therapeutic action of the drug in humans is mediated through this particular brain area. A more direct indication for a limited site of action may be obtained by intracerebral injections of drugs. Thus, diazepam injected in minute amounts into the amygdala, but not the hippocampus, was found to produce changes in electroencephalographic activity (EEG) and to inhibit the carbachol-induced hypothalamic rage reaction in a manner similar to systemic administration (Nagy and Decsi, 1973).

F. The Amygdaloid Complex: Anatomical, Neurochemical and Electrophysiological Aspects

1. Anatomical Aspects of the Amygdala

The amygdala is a collection of nuclei found in the anterior portions of the temporal lobes in the brains of primates. Subdivision of the amygdaloid nuclei into groups has produced counts of from 5 to 22 in various species and by various authors. The amygdaloid complex of the rat is generally accepted to be composed of eight major dissectable nuclei: the central, lateral, medial, cortical, basal, medial posterior, basal posterior and posterior (Figure 2). The basal nucleus is further subdivided into medial and lateral parts by some authors (Isaacson, 1974). A consensus shows that the amygdala is phylogenetically, physiologically and anatomically divided into a corticomedial and basolateral division. The corticomedial complex is generally thought to be composed of the cortical, medial and central nuclei and

the nucleus of the lateral olfactory tract and, at least in the rat, is associated with olfactory structures. The basolateral division is considered to be made up of the basal, lateral and accessory basal nuclei. This group is associated with neocortical systems and is the most prominent nuclear group of the amygdala in the human.

The projections from the amygdala have not been completely mapped but two main efferent systems are recognized: the stria terminalis and ventral amygdalofugal pathways. The stria terminalis presumably receives the majority of its fibers from the corticomedial division and a lesser contribution from the basolateral nuclei. DeOlmos (1972) further subdivides the stria terminalis into dorsal, ventral and commissural components and includes among their terminations the lateral septal nucleus, nucleus accumbens, olfactory tubercle, anterior olfactory nucleus, preoptic area, ventromedial nucleus of the hypothalamus, premammillary area, habenula and the bed nucleus of the stria terminalis. The ventral amygdalofugal pathways are thought to receive input from both nuclear divisions and a considerable contribution from periamygdaloid cortex. In addition to innervating many of the structures receiving input from the stria terminalis, the ventral pathway fibers project to the prepyriform, pyriform and entorhinal cortex, the caudate-putamen, and the thalamus. In light of the interconnections between cortex and amygdala, Kemble and Tapp (1968) have argued that the behavioral manifestation of amygdaloid lesions may reflect the destruction of fibers that originate in the periamygdaloid cortex and course through or end in the amygdaloid cortex. They have subsequently shown a similar impairment of passive avoidance responding

to result from bilateral lesions of the pyriform cortex or basolateral amygdaloid nuclei.

Afferents to the amygdala arise from many sources. The mesencephalon, diencephalon and telencephalon are all known to directly or indirectly supply input to the amygdala (Veening, 1978a,b).

2. Neurochemical Aspects of the Amygdala

The monoamines, acetylcholine and γaminobutyric acid are well represented in the amygdala. Drug-induced changes in the concentration of these putative transmitters in the amygdala *in toto* offers little insight into the potential functional importance of these changes. This is due to their uneven distribution among the nuclei which make up the amygdala. Table 1 summarizes the available information concerning the distribution of dopamine, norepinephrine, 5-hydroxytryptamine and acetylcholine in the amygdaloid nuclei of male rats. The *in vitro* activity of L-glutamate decarboxylase, the rate-limiting enzyme in the synthesis of γ-aminobutyric acid from glutamate, is included as a marker of γ-aminobutyric acid-containing neurons. The olfactory tubercle and nucleus accumbens are included as representatives of the rostral limbic nuclei. The caudate nucleus is included as a reference structure.

Dopamine is well represented and unevenly distributed among the amygdaloid nuclei of the rat (Table 1), in agreement with the density and distribution of catecholaminergic fluorescent nerve terminals in the rat amygdala (Jacobowitz and Palkovits, 1974). The central and lateral nuclei contain the most dopamine; the medial, medial posterior and posterior nuclei contain the least. The high dopamine

TABLE 1

Distribution of Neurotransmitters Among the Amygdaloid Nuclei

Brain Nuclei	DA	NE	5-HT	ACh	GAD
Amygdaloid Nuclei					
Anterior amygdaloid area	45.9± 5.1	9.5±0.9	19.0±1.9	54.0± 7.6	---
Central	16.9± 2.8	13.4±2.5	16.8±2.2	---	422±40
Lateral	22.0± 3.3	12.0±1.0	20.7±2.7	23.4± 1.6	333±38
Basal	8.6± 0.5	9.1±0.9	17.5±1.6	45.3± 6.4	341±34
Basal posterior	5.4± 1.1	6.3±0.9	23.0±2.1	---	---
Medialposterior	1.6± 0.4	4.3±1.0	17.6±2.1	---	---
Posterior	1.9± 0.3	2.5±0.5	15.9±1.6	---	---
Cortical	5.1± 1.0	7.5±1.7	17.3±1.3	71.5±14.2	349±33
Medial	3.6± 0.6	11.4±1.2	19.9±4.0	48.2± 6.3	310±15
Rostral limbic Nuclei					
Nucleus accumbens	67.5± 4.0	14.1±1.5	14.7±1.2	87.6± 8.9	574±70
Olfactory Tubercle	114.4±15.8	7.2±0.9	26.1±2.6	29.2± 2.5	526±28
Caudate nucleus	96.6± 6.5	1.1±0.2	5.8±0.5	87.6± 4.7	270±10

Dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) concentrations are expressed as ng/mg protein ± S.E.M. (Brownstein et al., 1974; Saavedra et al., 1974; Demarest et al., 1979; W. Lyness, personal communication). Acetylcholine (ACh) concentrations are expressed as ng/mg protein ± S.E.M. (Cheney et al., 1975). L-Glutamate decarboxylase (GAD) activity is expressed in pmole CO_2 produced/ g protein/hr ± S.E.M. (Tappaz et al., 1976).

content of the central and lateral amygdaloid nuclei is consistent with a reported pathway from midbrain dopaminergic cell bodies terminating in the central (Ungerstedt, 1971) and lateral (Kizer, 1976) amygdaloid nuclei. Moreover, Hökfelt et al. (1974) described the presence of dopaminergic terminals in the amygdala, with the lateral and basal areas exhibiting a particularly dense localization. Tyrosine hydroxylase, the enzyme that catalyzes the rate-limiting step in the synthesis of dopamine, also shows a high content and uneven distribution among the amygdaloid nuclei (Saavedra and Zivin, 1976). A similar pattern is seen for both isoenzymes of monoamine oxidase (type A and B), a major enzyme in the catabolism of dopamine, norepinephrine and 5-hydroxytryptamine (Hirano et al., 1978).

Microiontophoretically applied dopamine has been reported to depress the firing rate of spontaneously active and glutamate-excited amygdaloid cells of the cat (Ben-Ari and Kelly, 1976; Straughan and Legge, 1967) and rat (McCrea et al., 1973). Moreover, the inhibitory action of dopamine was antagonized by iontophoretic applications of the neuroleptic α-flupenthixol (Ben-Ari and Kelly, 1976). A dopamine-sensitive adenylate cyclase in the amygdala (Clement-Cormier and Robison, 1977), the stimulation of which is inhibited by neuroleptics, may mediate the inhibitory effects of dopamine receptor stimulation. Thus, various lines of investigation suggest that dopamine may function as a neurotransmitter or modulator of the total synaptic response in the amygdala as has been demonstrated for the better characterized dopaminergic systems.

5-Hydroxytryptamine exists in fairly high concentrations, and, unlike dopamine, is rather uniformly distributed among the amygdaloid nuclei (Table 1). The relatively moderate concentrations of tryptophan hydroxylase in the amygdaloid nuclei (Saavedra, 1977) suggests that this structure has some capacity to synthesize 5-hydroxytryptamine from its precursors. The amygdala receives a dense, uniform 5-hydroxytryptamine input from the midbrain raphé (Aghajanian et al., 1973). Both microiontophoretically applied 5-hydroxytryptamine and electrical stimulation of the dorsal raphé nucleus markedly inhibit the spontaneous firing rate of amygdaloid cells (Wang and Aghajanian, 1977). The inhibitory influence of 5-hydroxytryptamine on the amygdaloid cells appears to be of a tonic nature as the depletion of brain 5-hydroxytryptamine by 5,7-dihydroxytryptamine or p-chlorophenylalanine results in a significantly increased discharge rate in the amygdala in addition to preventing the inhibitory effects of dorsal raphé nucleus stimulation.

Norepinephrine exhibits less regional differences among the amygdaloid nuclei than dopamine. In general, the more basal and caudal nuclei are poorer in norepinephrine than the more dorsal and rostral ones.

The concentrations of acetylcholine exhibit considerable variability among the amygdaloid nuclei (Table 1). The iontophoretic application of acetylcholine produces an excitatory effect on amygdaloid firing rate (Wang and Aghajanian, 1977). L-glutamate decarboxylase activity is rather evenly distributed throughout the amygdaloid nuclei, with the greatest activity in the central nucleus (Table 1). Ben-Ari et al. (1976) report that L-glutamate decarboxylase activity is

higher in the rostral than in the caudal part of the central nucleus. Spontaneously active and glutamate-excited amygdaloid cells are inhibited by the iontophoretic application of γ-aminobutyric acid (Ben-Ari and Kelly, 1976).

In summary, the amygdala is biochemically as well as anatomically heterogeneous. Any one nucleus receives a multiplicity of inputs.

STATEMENT OF PURPOSE

The validity of studies attempting to correlate drug-induced behavioral alterations with their biochemical effects requires that these effects share similar dose-effect and temporal relationships. Although the concept of the benzodiazepines as potent anxiety-alleviating agents has received extensive behavioral consideration, the great majority of the biochemical studies, whether by design or interpretation, has revolved around properties other than that of an anxiolytic, i.e., as anticonvulsants, muscle relaxants and sedatives. The doses of the benzodiazepines characteristically used in studying their effects on the dynamics of putative neurotransmitters in the central nervous system are usually of sufficient magnitude to elicit pronounced sedation and muscle relaxation and are well in excess of doses that are effective as anticonflict (antipunishment) agents in animal models. While it has been argued that biochemical determinations are generally less sensitive than functional tests to reveal changes in neuronal activity, it is in most cases improper to attempt to correlate low-dose behavioral effects with biochemical effects that are obtained with relatively much higher doses.

This investigation characterized the effects of diazepam on a quantitative behavioral index of drug action (experimentally induced conflict), the relevance of which to clinical anxiety has been generally accepted by the foremost investigators in this area of

experimental pharmacology. The salient characteristics of diazepam administration so derived (e.g., dose- and time-effect relationships and differences resulting from acute vs. chronic dosing regimens) were then applied to a study of the effects of diazepam on biochemical measures seeking putative neurotransmitter correlates in limbic and extralimbic structures of the anticonflict activity.

MATERIALS AND METHODS

A. Animals

Male Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) weighing 200-320 g were maintained in air conditioned rooms with room-lights alternated on a 12-hour dark-light cycle. Subjects used in all aspects of this research were group-housed three or four to a cage, except during periods of training, testing or controlled feeding or drinking. Animals classified as "food-deprived" were gradually reduced to 75-80 percent of their original body weight and maintained at these weights by limited feeding after each testing session. "Water-deprived" animals were denied access to water for a 48-hour period prior to conditioning and were subsequently limited to water obtained during daily experimental sessions.

B. Body Temperature Recordings

The rectal temperature of male rats was measured by inserting a thermistor probe 6 cm into the rectum and displayed on a telethermometer (Model 41TA, Yellow Springs Instrument Co.). Temperature readings for diazepam and vehicle-injected control subjects were made at 15-minute intervals for 60 minutes post-injection.

C. Behavioral Procedures

1. Suppressed Responding: Response-contingent Shock (Conflict)

Two forms of conflict behavior were used which differed in the nature of the response used to generate the behavioral baseline. In the first case, the behavior took the form of an unconditioned, consummatory response (tube licking) and in the second a conditioned, instrumental response (bar pressing) was employed. The response suppression consequent to the punishment of consummatory behavior, here termed the conditioned suppression of drinking, will be described first.

The experimental chamber used in the conditioned suppression of drinking conflict procedure consisted of a rectangular box (30x56x28 cm) with plexiglass sides and a stainless steel floor and ceiling. A 6.5 cm drinking tube protruded through the center of one wall at an angle of approximately 75 degrees to the wall plane and at a height of 15 cm from the floor. A speaker attached to a tone generator was affixed to the ceiling. Contact between the floor and drinking tube by the rat completed a circuit during the latter period of tone presentation resulting in an electric shock being delivered through the tube, with each shock automatically recorded. The drinking tube was coated with Insl-X insulating paint up to the tip so as to limit the completion of the circuit to contact with the floor and the water in the tube. A calibrated polyethylene tube was attached to the drinking tube to allow for monitoring the volume of water consumed.

Water-deprived rats were allowed two consecutive daily 10-minute periods of unpunished access to the drinking tube. Subsequent placement in the chamber initiated a 10-minute daily session with

alternating periods of unpunished and punished consummatory responding distinguished by the presence of a tone. A 7-second tone was introduced at varying intervals of time ($\bar{X}$ = 21 sec) which signalled the delivery of a shock on tube contact during the last 5 seconds of the tone. Tube contact was not punished during the initial 2 seconds of the tone and during the silent intervals. A shock intensity of 30 μamps was found to produce a low, stable baseline of shocks received following approximately two weeks of daily sessions. More significantly, an intensity of 30 μamps produced a level of suppression amenable to rate-decreasing as well as rate-enhancing effects of drugs. The measurable parameters in the punished and unpunished components, i.e., number of shocks received and water consumption, respectively, were analyzed separately. The method has been used in another study, the results of which have been published (Ford et al., 1979).

The second form of experimentally-induced conflict behavior represents a modification of the conflict procedure of Geller and Seifter (1960). The apparatus consisted of standard sound insulated environmental chambers, each containing a lever, an automatic feeder for the delivery of food reinforcement (45 mg Noyes food pellets), a loudspeaker to provide auditory stimuli, and a grid floor which could be electrified. Electro-mechanical programming equipment was used to control experimental contingencies and record data.

Food-deprived rats were trained to respond on a fixed ratio schedule of 40 lever presses for each food pellet reinforcement (FR-40). Following stabilization of this operant response a non-aversive tone of 17 seconds duration was randomly presented on an average of once every three minutes (habituation trials). When performance had

again stabilized a punishment contingency was added in which every response during the last 15 seconds of the tone was simultaneously rewarded (continuous reinforcement) and punished (scrambled footshock). An important corollary concerning the effect of benzodiazepine administration on suppressed responding is that the magnitude of the disinhibition may be dependent on the level of baseline or control suppression, which in turn is a function of the intensity of the punishing stimulus (McMillan, 1973), magnitude of deprivation and inter-animal differences. To normalize the inherent differences between animals and to maximize the drug related differences, a similar level of response suppression in each animal was maintained by titrating with varying shock intensities (0.4-1.1 mA, 25 msec duration). After training (40-60 days), subjects made very few punished responses while the tone was on. Testing sessions were of one hour duration with total responses during the alternating silent, unpunished period (fixed ratio-40) and tone-signalled, punished (continuous reinforcement) components collected and analyzed separately.

2. Suppressed Responding: Noncontingent Shock (Conditioned Emotional Response)

In contrast to the response-contingent (controllable) nature of shock presentation characteristic of conflict procedures, the response suppression engendered by noncontingent shock is characterized by the uncontrollable quality of the aversive stimulus, i.e., the animal is shocked irrespective of its own actions. The "conditioned suppression" or "conditioned emotional response" paradigm employed was a modification of that reported by Estes and Skinner (1941).

The experimental apparatus was the same as that used in the above-mentioned bar-press conflict procedure. Additional commonalities shared by both methods include the shaping of the operant response (bar pressing) to a stable performance on a fixed ratio-40 schedule of reinforcement and the subsequent habituation trials to remove the unconditioned effects of the tone. However, the conditioned emotional response paradigm differed from the bar-press conflict method in two important ways: 1) the fixed ratio-40 reinforcement schedule was maintained throughout the alternating signalled and unsignalled periods; and 2) the termination of the tone (15 sec in duration with a variable intertrial interval averaging 3 min) was associated with an unavoidable shock delivered to the grid floor through a shock scrambler. Testing sessions were of one hour duration with total responses during the silent intervals and the tone-signalled periods being collected and analyzed separately.

These three behavioral models share several features essential to their utility. First, all three procedures utilize conditioned stimuli which forewarn of shocks, as well as silent intervals or "safe periods" in which shocks never occur. Drug-induced increases in the rate of suppressed responding are interpreted as an index of anti-anxiety activity. Comparison of behavior in the safe periods before and after the introduction of the drug provides a within-sessions control for nonspecific side effects on locomotor activity, sensory function and overall motivation. Secondly, a selective manipulation of parameters is used to produce a partial, but not total, suppression of the conditioned or unconditioned behavior. Thirdly, experimental sessions in all three procedures were conducted 7 days a week with drug

testing sessions conducted at 5-day intervals. In evaluating drug effects each subject was used as its own control. The average number of responses in each component on the 3 days immediately preceding drug treatment were compared to the number on the treatment day.

D. Chemical Lesioning Techniques

Past efforts to develop agents which exhibited neurotoxic specificity towards indoleamine-containing neurons in the central nervous system culminated in the synthesis of the 5-hydroxytryptamine derivatives 5,6-dihydroxytryptamine and 5,7-dihydroxytryptamine. The spectrum of biochemical effects attributed to these dihydroxylated tryptamines (Baumgarten et al., 1973a,b) is considered to result from the degeneration of serotonergic preterminal axons and axon terminals. The cytotoxic effect of 5,7-dihydroxytryptamine on 5-hydroxytryptamine-containing neurons was used to investigate the role of 5-hydroxytryptamine in the expression of conflict behavior in rats. 5,7-Dihydroxytryptamine was selected over its 5,6-dihydroxylated congener in light of the reported advantages in terms of stability, potency and toxicity (Sanders-Bush and Massari, 1977).

In the present study intracerebral injections of 5,7-dihydroxytryptamine were used to produce selective lesions of the amygdala, an area which receives a dense and uniform serotonergic input from the midbrain raphé (Aghajanian et al., 1973). Rats weighing 270-320 g were anesthetized with equithesin (an anesthetic mixture containing chloral hydrate, pentobarbital, magnesium sulfate, propylene glycol, and ethyl alcohol) and mounted in a Kopf stereotaxic instrument with the

incisor bar set 5.0 mm above the interaural plane. The creatinine sulfate salt of 5,7-dihydroxytryptamine (Regis Chemical Co.) was dissolved in 0.9% NaCl with ascorbic acid added (0.2 mg/ml) to protect against autooxidation. Stereotaxic injections were made through a 31 gauge stainless steel cannula connected to a 10 µl Hamilton syringe. The coordinates (Pellegrino and Cushman, 1967) were for the amygdala: 1.0 mm posterior to bregma, ±4.5 mm lateral to midline, 8.0 mm ventral to the surface of the cortex. The 5,7-dihydroxytryptamine (calculated as the free base) was injected bilaterally in the amygdala (7 µg in 3.5 µl on each side of the brain). The injection speed was 0.75 µl/min and the cannula remained in the brain for an additional 4 minutes to permit the drug to diffuse away from the injection site. Subjects were pretreated with desmethylimipramine (25 mg/kg, i.p.) 45 minutes before surgery to antagonize the cytotoxic effect of 5,7-dihydroxytryptamine on noradrenergic neurons (Breese and Cooper, 1975).

In addition to these intracerebral injections 5,7-dihydroxytryptamine (100 µg in 20 µl) was injected into the lateral ventricle via a stereotaxically guided 31-gauge cannula. The injection site was 1.2 mm posterior to bregma, 3.0 mm lateral to midline and 3.0 mm below the dorsal surface of the brain.

Control rats were treated in an identical manner as the 5,7-dihydroxytryptamine rats, except that only the vehicle was injected into either amygdala (3.5 µl, bilaterally) or lateral ventricle (20 µl).

E. Biochemical Procedures

1. Dissections:

After decapitation rat brains were rapidly collected and the hindbrain removed by a midcollicular cut. The remainder of the brain was placed base-down on the stage of an AO microtome (model 880, AO Instrument Co.) and frozen using a Histo-freeze (Scientific Products). Consecutive 500 µm thick frontal sections were sliced and transferred to glass slides on dry ice. With the aid of a stereomicroscope, using the decussation of the anterior commissure as a point of reference, the desired brain nuclei were bilaterally punched out from the unfixed brain slices (Palkovits, 1973) using stainless steel tubing (0.8 or 1.0 mm i.d.). The atlas of König and Klippel (1963) served as a general guide, although a different cutting angle from that of the atlas was used. Figure 2 represents a schematic drawing (modified from König and Klippel, 1963) of the amygdaloid nuclei as seen in frontal sections of the rat brain at different distances (in µm) from the anterior commissure. The circles represent the size and location of tissue pellets removed by punch. Palkovits et al. (1974) afforded additional aid in the dissection of extra-amygdaloid areas as well the amygdaloid nuclei.

Tissue samples were homogenized in 0.5 ml glass homogenizers (Kontes Glass Co.) in 150 µl of 0.1 M HCl containing 50 µM ascorbate. The resulting homogenates were transferred to microcentrifuge tubes and centrifuged for 3 minutes in a Beckman Microfuge at 4°C. The clear supernatants were transferred to silanized glass vials while the homogenate pellet was assayed for protein content according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Figure 2. Schematic drawing of frontal sections of the rat brain 4620 and 3750 μM anterior to the frontal zero plane according to the stereotaxic atlas of Konig and Klippel (1963). The amygdaloid nuclei and hippocampus are designated by numbers as follows: 1 = central amygdaloid nucleus; 2 = lateral amygdaloid nucleus; 3 = medial amygdaloid nucleus; 4 = basal amygdaloid nucleus; 5 = cortical amygdaloid nucleus; 6 = medial posterior amygdaloid nucleus; 7 = basal posterior amygdaloid nucleus; 8 = posterior amygdaloid nucleus; 9 = hippocampus. Abbreviations: cp = caudate putamen, cl = claustrum, cai = capsula interna, mfb = medial forebrain bundle; vt = ventral thalamus; cae = capsula externa.

2. Methods of Quantitation: Dopamine

Supernatants from tissue homogenates were analyzed for endogenous dopamine content by selected ion monitoring (SIM) (Koslow et al., 1972; Ko et al., 1974; Kilts et al., 1977) with computer control. Selected ion monitoring, or mass fragmentography, consists of the alternate focusing of the mass spectrometer on identified fragments of the compound of interest and on the corresponding fragments of a stable isotopically labelled or structural analog as they are eluted from a gas chromatographic column.

An internal reference standard of deuterated dopamine (75 pmole) was added to all samples to be assayed prior to homogenization. Trideutero-3,4-dihydroxyphenylethylamine (d_3-dopamine) was prepared from dopamine by an acid catalyzed exchange reaction (Lindstrom et al., 1974), the aromatic hydrogen atoms at positions 2, 5 and 6 having been replaced by deuterium atoms. Mass spectral analysis of the deuterated dopamine demonstrated that contamination by the protium form was less than 1% with a typical deuterium isotope enrichment, calculated from the pentafluoropropionyl derivative of d_3-dopamine to be <1% d_0, 2% d_1, 13% d_2 and 84% d_3. Following homogenization and centrifugation the resulting supernatants were evaporated to dryness under a stream of nitrogen. The formation of nonpolar, volatile derivatives, suitable for gas chromatographic separation, was accomplished by the addition of 40 µl of pentafluoropropionic anhydride (Regis Chemical Co.) and ethyl acetate (10 µl) to the residue. The vials were then sealed and heated for 20 minutes at 75°C. This derivatization procedure resulted in the introduction of three pentafluoropropionyl groups into the endogenous

dopamine and the added d_3-dopamine (Figure 3). The samples were then cooled, dried under nitrogen and the residue was redissolved in 20 µl of a 1% solution of pentafluoropropionic anhydride in ethyl acetate. One to 5 µl were then injected into the gas chromatograph-mass spectrometer (GC-MS).

Blank-corrected standard curves for the quantitation of endogenous dopamine were prepared by analyzing a series of standard solutions containing a fixed amount of d_3-dopamine (75 pmole) and varying amounts of dopamine (0-105 pmole). The ions selected for monitoring were obtained from the mass spectrum of the derivatized compounds (Figure 3). The electron impact mass spectra of the pentafluoropropionyl derivative of dopamine demonstrated several major fragmentation patterns. The ion at m/e 428 results from the cleavage of the bond between the α-carbon and the nitrogen, with the positive charge retained on the moiety containing the aromatic ring and the α- and β-carbons of the ethylamine side chain. A similar α-carbon-nitrogen cleavage plus loss of COC_2F_5 from the 4 position of the aromatic ring yields an ion at m/e 281. Mass spectral analysis of ring-deuterated d_3-dopamine (Figure 3) revealed a shift of three atomic mass units (amu) for those fragments which retain the aromatic ring, e.g., m/e 431 and 284. The dopamine concentrations of the samples were determined mathematically from the ion current ratios generated by monitoring the ions characteristic for dopamine/d_3-dopamine, i.e., 428/431 and 281/284, using the slope and intercept of the standard curve.

The analysis was performed on a Finnigan 3200 gas chromatograph-mass spectrometer system interfaced with a System Industries

Figure 3. Partial mass spectra of dopamine (upper) and dopamine-2,5,6-d_3 (lower) as pentafluoropropionyl (PFP) derivatives with the proposed fragmentation patterns. Minor fragments (<5% relative abundance) have been omitted. Fragmentation of 500 ng of each derivative was accomplished by electron impact and spectra obtained at a scanning rate of 20 a.m.u.s.$^{-1}$. The retention time for elution of dopamine-PFP and dopamine-d_3-PFP was 2.1 min. See text for details of derivatization and instrument conditions.

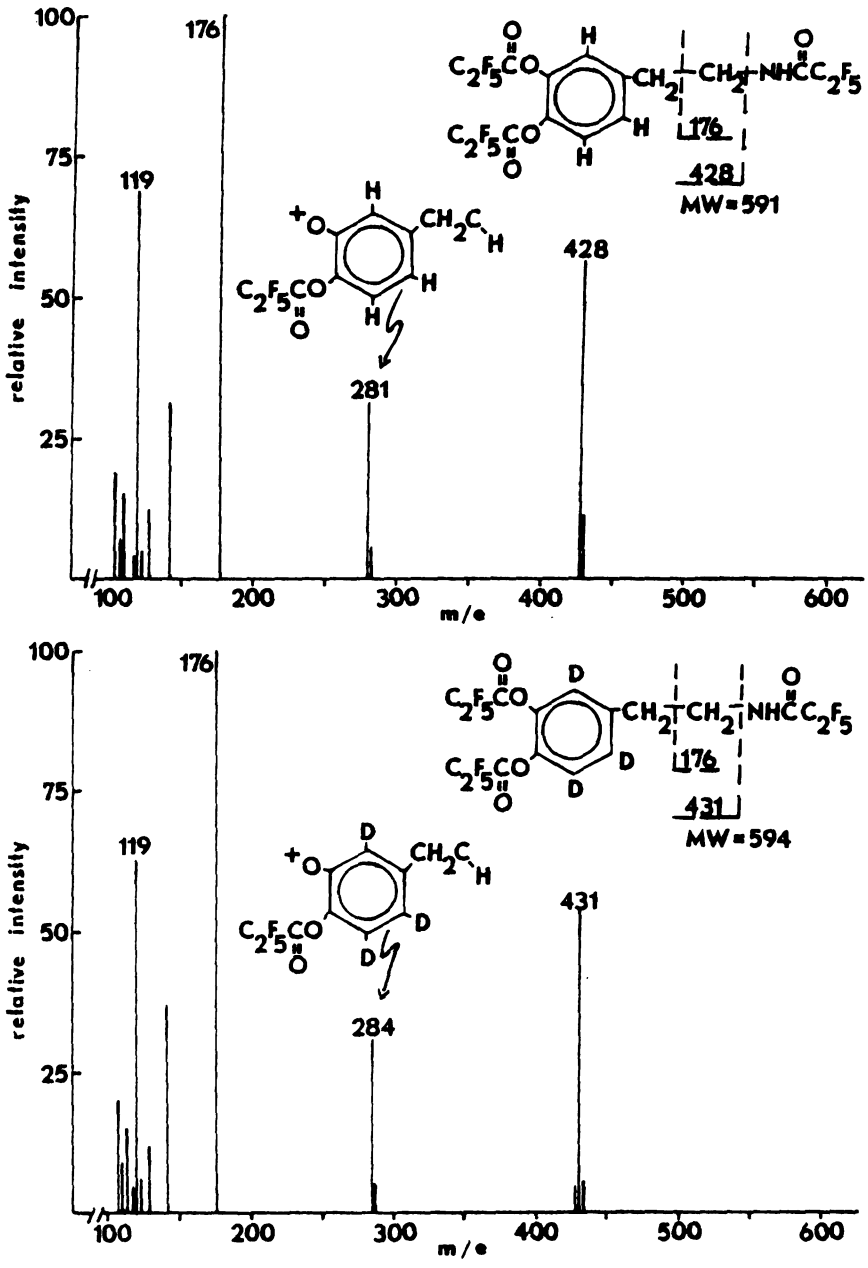

100
75
50
25
relative intensity
176
119
428
281
431
284
100
200
300
400
500
600
m/e
176
428
MW=591
431
MW=594

Figure 3

System/150 data system. A 1.6 m x 2 mm (i.d.) silanized glass column packed with 3% SP2250 on Gas Chrom Q was used for separation. The carrier gas (helium) was maintained at a flow rate of 10 ml/min. Temperatures were: column 160°C (isothermal), injector port 250°C, separator 250°C. Fragmentation was accomplished by electron impact at 70 eV and 500 μamp. Integration times were varied between 100 and 200 msec to maximize those ions of lower relative abundance.

3. Methods of Quantitation: 5-Hydroxytryptamine and 5-Hydroxy-Indoleacetic Acid

Supernatants from microdissected tissue homogenates were assayed for 5-hydroxytryptamine content by a mass fragmentographic technique (Cattabeni et al., 1972; Beck et al., 1977). An internal reference standard of α-d_2,β-d_2-5-hydroxytryptamine (d_4-5-HT; Merck, Sharp and Dohme) was added (85 pmoles) to the supernatant to control for variabilities in sample preparation and derivatization. The small volume of tissue assayed (<1 mg) precluded the use of solvent extractions necessary when examining larger amounts of tissue (Beck et al., 1977). Therefore, the aqueous supernatant was evaporated to dryness under nitrogen and used directly for the determination of 5-hydroxytryptamine.

The formation of stable, volatile perfluoroacylated derivatives of 5-hydroxytryptamine was accomplished by the addition of 40 μl of pentafluoropropionic anhydride and 10 μl of dry acetonitrile to the residue. However, the secondary amine group in the indole nucleus was found to be relatively unreactive and, in agreement with Gelpi et al. (1974), the heating time of the reaction with pentafluoropropionic anhydride and acetonitrile was increased to improve the ratio of tri- to

dipentafluoropropionyl 5-hydroxytryptamine. Vials were sealed and heated overnight (12-16 hrs) at 75°C. The samples were then cooled, dried under nitrogen and the residue was redissolved in 20 µl of a 1% solution of pentafluoropropionic anhydride in ethyl acetate. One to 5 µl were then injected into the gas chromatograph-mass spectrometer.

Quantitation of 5-hydroxytryptamine was performed in a manner similar to that described for dopamine, with calibration curves generated using solutions containing a constant amount of d_4-5-hydroxytryptamine (85 pmole) and varying amounts of 5-hydroxytryptamine (0-115 pmole). From the mass spectra of 5-hydroxytryptamine and its deuterated analogue the ions at m/e 451 and 454 and the fragments at m/e 438 and 440 were chosen for mass fragmentographic analysis (Figure 4). The ion at m/e 451 (and 454) is the product of a cleavage of the bond between the α-carbon and the nitrogen of the ethylamine side chain with a hydrogen (or deuterium) atom on the α-carbon transferred onto the neutral moiety. Cleavage of the bond between the α- and β-carbons yields an ion at m/e 438 (and 440).

Instrument conditions were as follows. The gas chromatograph was equipped with a 3% SP-2250 column (1.6 m x 2 mm i.d.) with a helium flow rate of 20 ml/min. Temperatures were: column 190° (isothermal), injector port 250°C, separator 250°C. Electron energy was 70 eV with an emission current of 500 µamps.

Telencephalic 5-hydroxytryptamine concentrations were determined by the fluorometric method of Curzon and Green (1970).

The determination of supernatant 5-hydroxyindoleacetic acid was performed by a modification of the mass fragmentographic method of

Figure 4. Partial mass spectra of 5-hydroxytryptamine (5-HT) (upper) and α-d_2,β-d_2-5-HT (lower) as pentafluoropropionyl (PFP) derivatives with the proposed fragmentation patterns. Minor fragments (<5% relative abundance) have been omitted. Fragmentation of 500 ng of each derivative was accomplished by electron impact and spectra obtained at a scanning rate of 20 a.m.u.s.$^{-1}$. The retention time for elution of PFP-5-HT and α-d_2,β-d_2-5-HT was 2.4 min. See text for details of derivatization and instrument conditions.

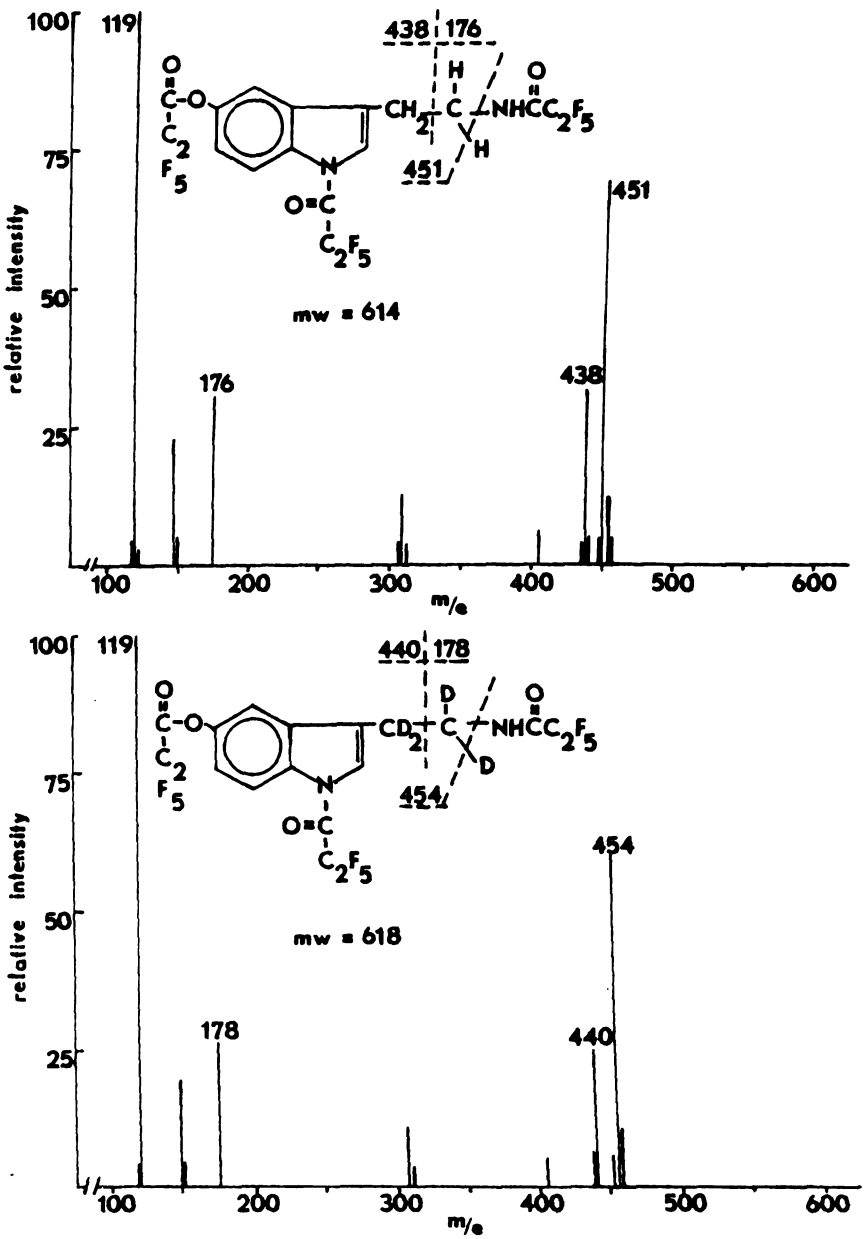

100
119
438
176
451
75
50
25
relative intensity
mw = 614
176
438
451
100
200
300
400
500
600
m/e
440
178
454
mw = 618
178
440
454

Figure 4

Swahn et al. (1976) using α-d_2-5-hydroxyindole-3-dideuteroacetic acid (d_2-5-HIAA; Merck, Sharp and Dohme) as an internal standard. The aqueous supernatant was acidified to pH 1 with 3 N HCl (50 µl), 52 pmole of d_2-5-hydroxyindoleacetic acid was added and the mixture saturated with sodium chloride. The 5-hydroxyindoleacetic acid was extracted into redistilled diethyl ether (2x500 µl) by shaking and centrifuging (500 rpm) for 2 and 5 minute periods, respectively. The organic extract containing 5-hydroxyindoleacetic acid was removed and evaporated to dryness under nitrogen. The pentafluoropropyl ester of 1,5-dipentafluoropropionyl-5-hydroxyindoleacetic acid (PFP_3-5-HIAA) was formed by adding 40 µl of pentafluoropropionic anhydride and 10 µl of pentafluoro-N-propanol (Regis Chemical Co.) to the residue. However, using the reaction conditions described by Watson et al. (1974) resulted in the majority (>75%) of the perfluoroacylated 5-hydroxyindoleacetic acid in the form of the partially reacted monopentafluoropropionyl derivative (PFP_2-5-HIAA) which, upon mass spectral analysis, revealed a parent ion at m/e 469. The fully derivatized product (PFP_3-5-HIAA) showed a parent ion at m/e 615. Study of the time course of the reaction between 5-hydroxyindoleacetic acid and pentafluoropropionic anhydride-pentafluoro-N-propanol revealed that the formation of the fully derivatized product had reached a steady state after approximately 4 hours at 75°C (Figure 5). Thereafter, vials were sealed and heated overnight at 75°C. The samples were then cooled, dried and redissolved in 1% pentafluoropropionic anhydride in ethyl acetate (20 µl) prior to injection into the gas chromatograph-mass spectrometer.

Figure 5. Time course of the formation of the products of 5-hydroxyindoleacetic acid (5-HIAA) reacted with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH). Forty ng of 5-HIAA was reacted with 40 µl of PFPA and 10 µl of PFPOH at 75°C and the reaction was terminated at varying times by evaporation of the reagents under nitrogen. The parent ion of the pentafluoropropyl ester of 1,5-dipentafluoropropionyl-5-HIAA (PFP_3-5-HIAA) at m/e 615 and that of the pentafluoropropyl ester of 5-monopentafluoropropionyl-5-HIAA (PFP_2-5-HIAA) at m/e 469 were identified by selected ion monitoring. m/e 615, ● ; m/e 469, ○ . Values represent means and vertical lines ± 1 S.E.M. of 5 determinations.

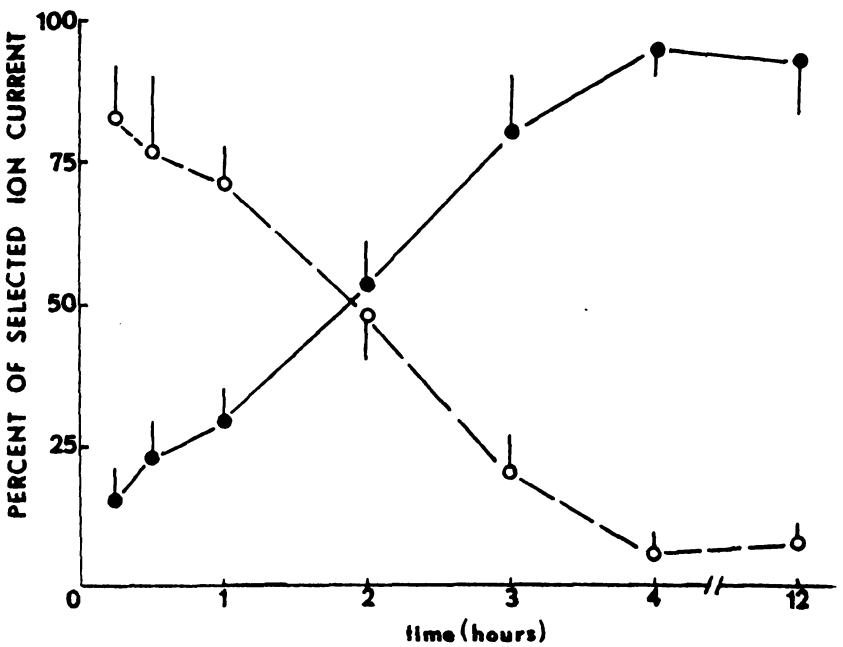

PERCENT OF SELECTED ION CURRENT
100
75
50
25
0
1
2
3
4
12
time (hours)

Figure 5

Quantitation of 5-hydroxyindoleacetic acid was as described for dopamine using solutions containing a fixed amount of d_2-5-hydroxyindoleacetic acid (52 pmole) and varying amounts of 5-hydroxyindoleacetic acid (0-104 pmole). For routine analysis the fragments m/e 438 and 615 and m/e 440 and 617 were monitored (Figure 6). The ion at m/e 438 (and 440) results from cleavage between the α-carbon and the esterified carboxyl group. The ion at m/e 615 (and 617) represents the parent or molecular ion (loss of an electron) of the pentafluoropropionyl derivative of 5-hydroxyindoleacetic acid (and d_2-5-hydroxyindoleacetic acid).

Instrument conditions were as described for 5-hydroxytryptamine except that gas chromatrographic separation was achieved at an isothermal column temperature of 165°C with a carrier gas (helium) flow rate of 15 ml/min.

F. Estimation of Dopamine and 5-Hydroxytryptamine Turnover

Nonsteady state methods were used to estimate the relative rates of turnover of both dopamine and 5-hydroxytryptamine. While these techniques have proven to be of considerable empirical value, the number of substantiated as well as unsubstantiated assumptions intrinsic to this methodology limits their utility to a purely comparative application (Weiner, 1974).

The turnover rate of dopamine in the present study was estimated by following the rate of decline of the endogenous dopamine concentration subsequent to synthesis inhibition with α-methyltyrosine (Brodie et al., 1966). Dopamine concentrations were determined immediately

Figure 6. Partial mass spectra of 5-hydroxyindoleacetic acid (5-HIAA) (upper) and α-d_2-5-HIAA (lower) as pentafluoropropyl ester pentafluoropropionyl derivatives with the proposed fragmentation patterns. Minor fragments (<5% relative abundance) have been omitted. Fragmentation of 500 ng of each derivative was accomplished by electron impact and spectra obtained at a scanning rate of 20 a.m.u.s.$^{-1}$. The retention time for elution of PFP-5-HIAA and PFP-α-d_2-5-HIAA was 1.9 min. See text for details of derivatization and instrument conditions.

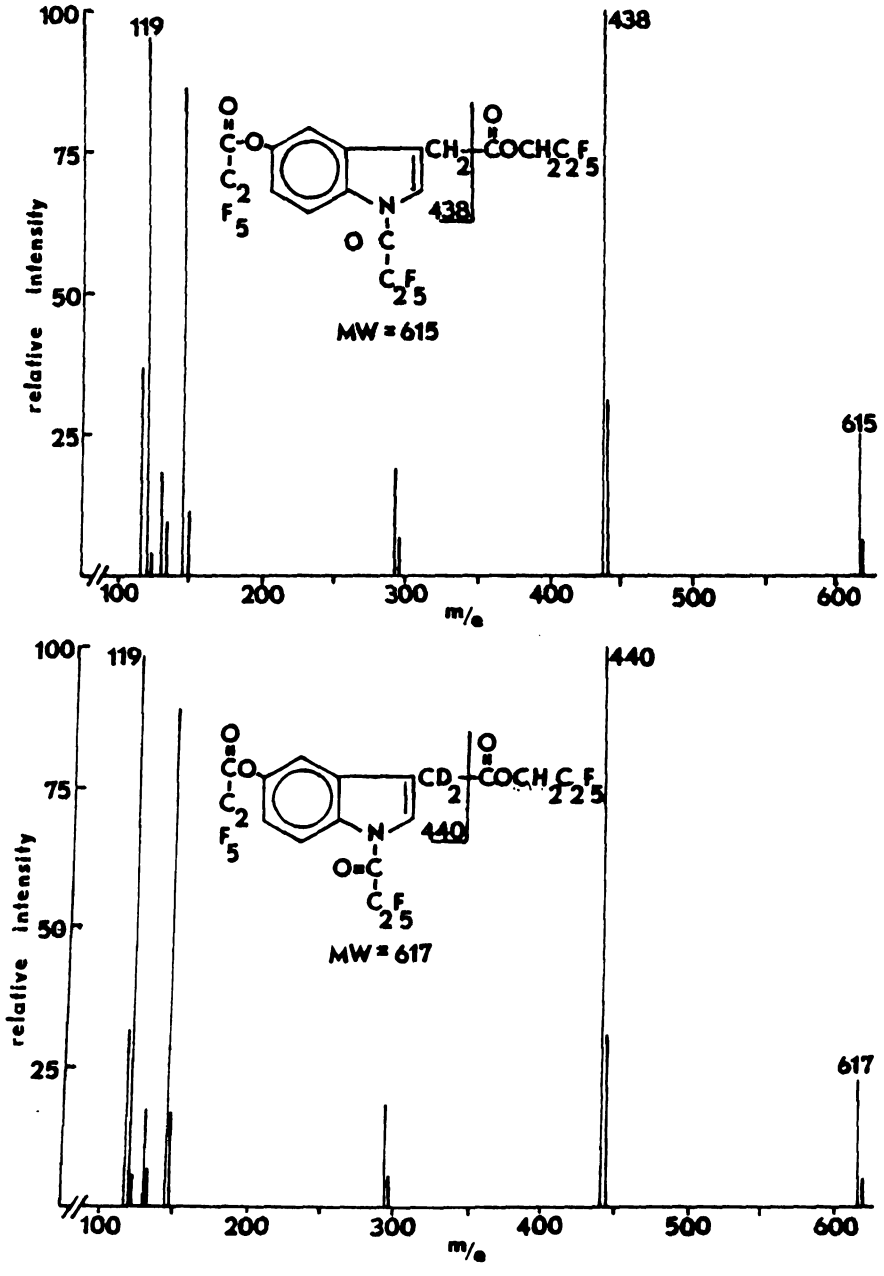

100
75
50
25
relative intensity
119
438
615
MW = 615
100
200
300
400
500
600
m/e
100
75
50
25
relative intensity
119
440
617
MW = 617
100
200
300
400
500
600
m/e

Figure 6

before (zero time) or 45 and 90 minutes after the administration of α-methyltyrosine methyl ester HCl (300 mg/kg of free amino acid, i.p.). The calculated rate constants for the decline of dopamine concentrations were compared among groups to determine treatment differences. As an alternative index of dopamine turnover, the extent of dopamine depletion was determined at one time point, 90 minutes, following α-methyltyrosine. Differences in turnover were determined by comparing the subsequently depleted dopamine concentrations in various treatment groups.

5-Hydroxytryptamine turnover was estimated by observing the rate of decline of endogenous 5-hydroxyindoleacetic acid concentration following the inhibition of its synthesis with the monoamine oxidase inhibitor pargyline (Tozer et al., 1966; Morot-Gaudry et al., 1974). 5-Hydroxyindoleacetic acid concentrations were determined immediately before (zero time) or 20 and 40 minutes after the administration of pargyline HCl (75 mg/kg of the free base, i.p.). Treatment differences were determined by comparing the calculated rate constants for the decline of 5-hydroxyindoleacetic acid concentrations. Alternatively, the extent of 5-hydroxyindoleacetic acid depletion at one time point, 40 minutes, after pargyline was used as an index of 5-hydroxytryptamine turnover. Treatment differences were determined from a comparison of the percent depletion at this time.

G. Drugs

The following drugs were administered as a suspension in 0.5% methylcellulose: diazepam (Ro 5-2807), flunitrazepam (Ro 5-4200) and

desmethyldiazepam (Ro 5-2180) were obtained through the courtesy of Dr. W.E. Scott, Hoffmann-LaRoche Inc., Nutley, N.J.; oxazepam, obtained from Wyeth Laboratories, Philadelphia, Pa.; D,L-5-hydroxytryptophan, purchased from Regis Chemical Co., Morton Grove, Ill.; β-(3,4-dihydroxyphenyl)-α-hydrazine-α-methyl-DL-propionic acid (MK-486), obtained from Merck, Sharp and Dohme Research Laboratories, West Point, Pa.; methaqualone, obtained from W.H. Rorer, Inc., Fort Washington, Pa.

The following drugs were dissolved in saline: methysergide maleate, obtained through the courtesy of Sandoz Pharmaceuticals, E. Hanover, N.J.; cinanserin HCl, obtained through the courtesy of E.R. Squibb and Sons, Inc., Princeton, N.J.; p-chlorophenylalanine methylester HCl and pargyline HCl purchased from Regis Chemical Co., Morton Grove, Ill.; D,L-α-methyltyrosine methylester HCl, purchased from Aldrich Chemical Co., Milwaukee, Wis.; chlorpromazine HCl, obtained from Smith Kline and French Laboratories, Philadelphia, Pa.; caffeine citrate, obtained from K and K Laboratories, Plainview, N.Y.; desipramine HCl, obtained from Merrell National Laboratories, Cincinnati, Ohio; amitriptyline HCl, obtained from Merck, Sharp and Dohme; sodium barbital, purchased from Sigma Chemical Co., St. Louis, Mo.; sodium amobarbital and sodium pentobarbital purchased from Ganes Chemical Co. Picrotoxin (purchased from Sigma Chemical Co., St. Louis, Mo.) was dissolved in a hot saline solution.

Cyproheptadine HCl (obtained from Merck, Sharp and Dohme Research, West Point, Pa.) and d-lysergic acid diethylamide bitartrate (d-LSD; obtained through the courtesy of Sandoz Pharmaceuticals, E. Hanover, N.J.) were dissolved in distilled water.

Drug solutions of varying concentrations were prepared to allow the administration of a constant volume (1 ml/kg). All drugs were administered by the intraperitoneal route.

5,7-Dihydroxytryptamine creatinine sulfate (purchased from Regis Chemical Co., Morton Grove, Ill.) was dissolved in 0.9% saline with 0.2 mg/ml ascorbic acid immediately prior to intracerebral or intraventricular injection.

H. Statistics

Statistical analysis of the behavioral effects of each given treatment was performed using a two-way analysis of variance (ANOVA; Sokal and Rohlf, 1969) with subjects as rows and days as columns. Data from 4 days were used for each ANOVA: the 3 control days before a treatment plus the treatment day. The lowest tested dose of a given compound that produced a significant treatment F ratio was designated the minimum effective dose (MED). The highest tested dose which produced a significant antipunishment effect without significantly altering unpunished responding was termed the highest effective dose (HED).

The significance of the differences in the behavioral effects of diazepam alone and in combination with various agents was tested by a Mann-Whitney U-test. Student's t-test was used to examine the behavioral effects of chronic p-chlorophenylalanine administration and of 5,7-dihydroxytryptamine injections. The effects of diazepam on body temperature were also tested by Student's t-test.

The significance of the differences in the fractional rate constants, obtained by a least-squares regression analysis (Sokal and Rohlf, 1969), was examined by Student's t-test. The effects of diazepam on the pargyline-induced depletion of 5-hydroxyindoleacetic acid and the α-methyltyrosine-induced reduction of dopamine in selected brain regions were analyzed using Student's t-test (dopamine) or a Mann-Whitney U-test (5-hydroxyindoleacetic acid).

RESULTS

A. Effects of Benzodiazepines on Suppressed Responding

The effects of benzodiazepines on behavior are dominated by their striking ability to release conditioned and unconditioned behaviors previously suppressed by punishment. Of the three behavioral procedures employed, the use of an unconditioned, consummatory response (tube-licking) to establish the behavioral baseline offered several noticeable advantages (see Discussion). Obvious advantages such as the relatively short time necessary to attain stable baseline performance (14 days) and the substantial number of animals that can be tested on a daily basis led to the almost exclusive use of this procedure for the evaluation of anticonflict (antipunishment) activity.

Figure 7 illustrates the dose-effect profile of diazepam on punished responding and background water consumption. Treatment effects are expressed as a percentage of the mean control baseline. The actual levels of response in control trained subjects ranged from 15-25 shocks taken per session and 13-17 ml of water consumed per session. Diazepam produced graded, dose-related increases in punished responding with the effect becoming mixed and diminished with higher doses. This "inverted U-shaped" dose-effect curve is characteristic of behaviorally active benzodiazepines and closely resembles that obtained with methods using more complex, operantly conditioned responses (Cook and Sepinwall, 1975). The minimum effective dose and the highest

Figure 7. Dose-effect curve for diazepam on punished ( ) and unpunished ( ) water consumption in terms of the number of shocks taken and the volume of water consumed, respectively. Doses of diazepam were administered intraperitoneally as a suspension in 0.5% methylcellulose 15 minutes prior to testing. Ordinate: Effect on treatment days is expressed as a percentage of the mean value for the 3 control days preceding each treatment (designated as 100%). Symbols represent the means and vertical lines ± 1 S.E.M. for 3 to 11 separate determinations. Where no S.E.M. is indicated the value is less than the radius of the symbol. * indicates those values which differ significantly ($p<0.05$) from control.

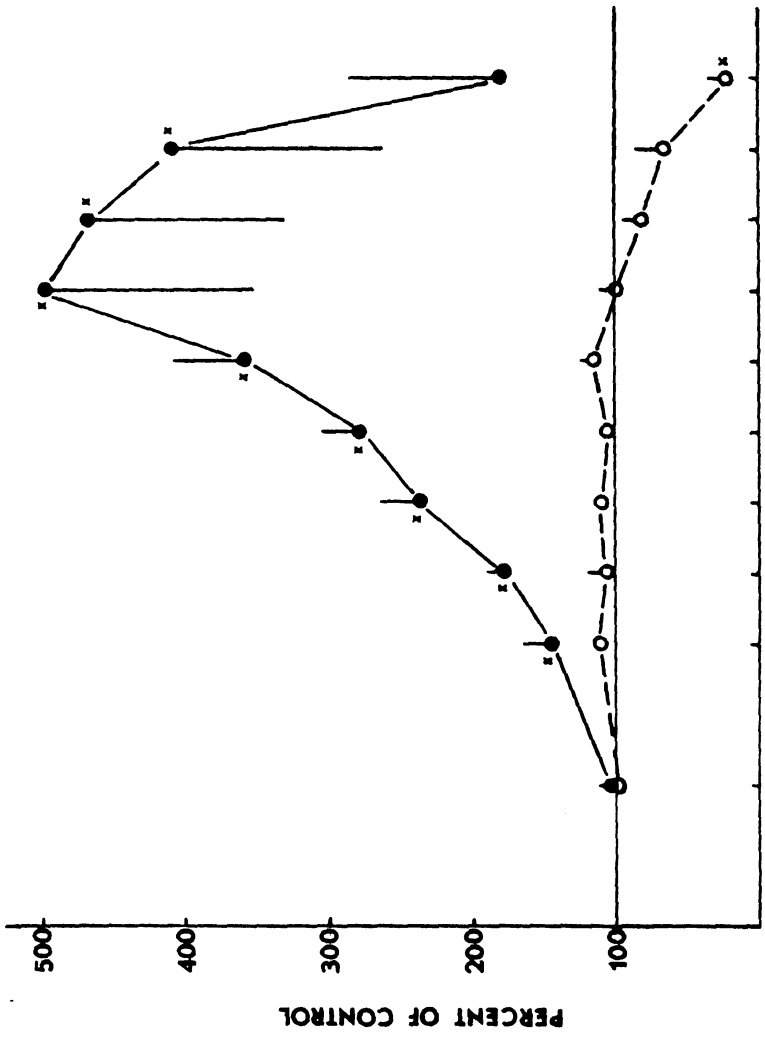

PERCENT OF CONTROL
500
400
300
200
100
0.1
0.3
0.56
1.0
1.8
3.0
5.6
10
18
30
mg/kg i.p.

Figure 7

effective dose of diazepam that increased punished responding were 0.3 and 18.0 mg/kg, respectively. The highest effective dose refers to the largest tested dose which significantly increased the number of shocks accepted without altering the volume of water consumed (unpunished responding) relative to control values. Unpunished responding (water consumption) was significantly decreased (23% of control) at a dose of 30 mg/kg. Such a decrease in unpunished responding is generally interpreted as an index of the dose at which nonspecific sedative activity occurs (Geller and Seifter, 1960).

The relatively short duration (10 min) of each experimental session of this conflict procedure permits an assessment of the time course of drug effects and, more importantly, the time of peak anti-punishment effect. Evaluation of the time course of the antipunishment effect of a submaximal dose (1.8 mg/kg) of diazepam devoid of appreciable effects on unpunished responding as indicated in Figure 7 shows a peak antipunishment effect (275% of control) 15 minutes following its administration (Figure 8). The rate of punished responding had waned to control values 60 minutes following diazepam injection.

The conditioned suppression of drinking paradigm, as with more elaborate conflict techniques, appears able to detect qualitatively different profiles within the class of benzodiazepines. Flunitrazepam, the 7-nitro, 5-phenyl ortho fluorine congener of diazepam, exhibits a greater anticonflict potency than diazepam over a narrower range of doses (Table 2). The minimum and maximum doses of flunitrazepam that increased punished responding were 0.1 mg/kg and 1.0 mg/kg, respectively. An obvious criterion to be fulfilled by animal tests to have predictive value as models for a particular human condition is that

Figure 8. Time course of the effects of a submaximal dose (1.8 mg/kg) of diazepam on punished (●) and unpunished (○) water consumption. Diazepam was administered intraperitoneally as a methylcellulose suspension at varying times prior to the initiation of behavioral testing. Each time point represents the mean ± 1 S.E.M. of 3 to 11 separate determinations. Ordinate: Same as in Figure 7. Abscissa: Interval between time of drug administration and time of testing. * indicates those values which differ significantly ($p<0.05$) from control.

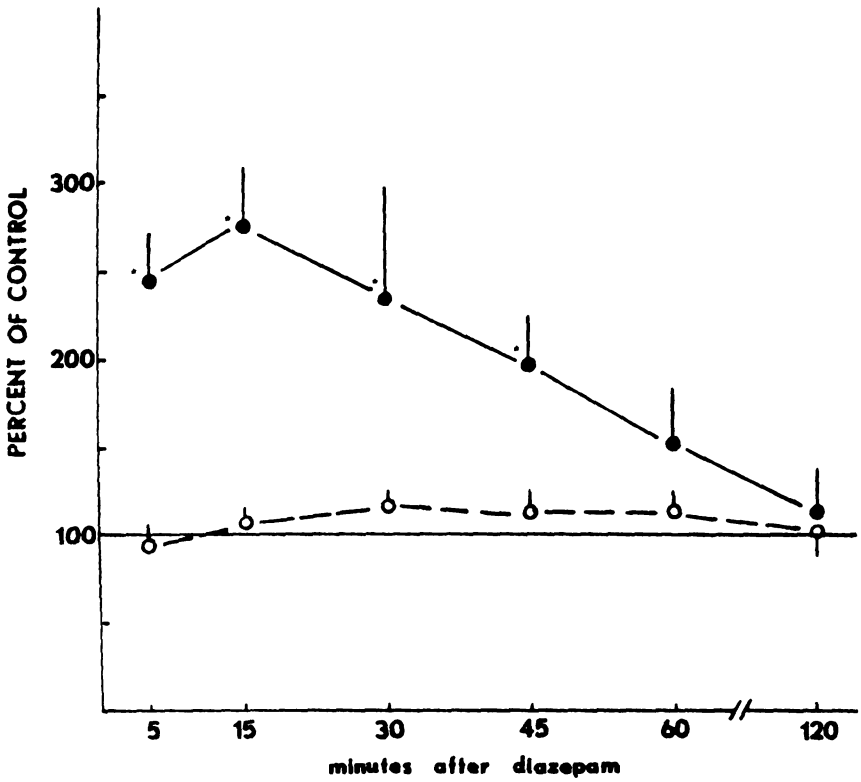

PERCENT OF CONTROL
300
200
100
5
15
30
45
60
120
minutes after diazepam

Figure 8

TABLE 2

Effects of various drugs on an experimentally-induced conflict situation involving the punished suppression of water consumption

Treatment	Range of doses*	N	Pretreatment time (min)	Increase in Punished Responding*		Decrease in Unpunished Responding*
				MED	HED	MED
Active Compounds						
diazepam	0.1-30.0	3-16	15	0.3	18.0	30.0
flunitrazepam	0.03-3.0	3-5	15	0.1	1.0	1.8
N-desmethyldiazepam	0.3-30.0	3-7	15	1.0	18.0	----
oxazepam	3.0-56.0	3-7	15	5.6	30.0	56
pentobarbital	0.3-18.0	4-7	10	1.0	10.0	18
amobarbital	1.0-30.0	7	10	3.0	10.0	18
phenobarbital	6.25-50	7	10	12.5	35.0	50
barbital	25-160	3	30	40.0	63.5	100
methaqualone	4.4-18.5	3-8	10	6.0	10.0	14.3
Inactive Compounds						
imipramine	1.0-10.0	3-5	30	----	----	10.0
amitriptyline	1.0-10.0	3-7	30	----	----	5.6
chlorpromazine	0.1-3.0	3-6	30	----	----	3.0
caffeine	3.0-56.0	3-4	30	----	----	56.0
mescaline	5.6-30.0	4	10	----	----	18.0
ethanol	30.0-2000	4-7	10	----	----	1000.0

MED or minimum effective dose indicates the lowest tested dose of a given compound that produced a significant increase in the number of shocks taken (punished responding) or a decrease in the volume of water consumed (unpunished responding) as determined by analysis of variance. HED or highest effective dose indicates the largest tested dose of a given compound that significantly increased punished responding without appreciably altering unpunished responding. Where no value is indicated (----) the compound tested failed to significantly alter responding over the range of doses examined. N = number of animals tested per dose. *Doses expressed as mg/kg, i.p.

they be able to distinguish therapeutically effective agents from other classes of compounds. In agreement with the findings of Schallek et al. (1972), the phenothiazine-type neuroleptic, chlorpromazine, was found to be devoid of anticonflict activity (Figure 9). In fact, chlorpromazine significantly enhanced the behavioral decrement produced by response-contingent shock.

Table 2 summarizes the effects of various agents on a conflict situation involving the punishment of a consummatory response (tube-licking). This procedure, conditioned suppression of drinking, appears sensitive to differential drug actions and selective for clinically-defined antianxiety agents. All of the benzodiazepine derivatives examined increased the rate of punished responding at doses lacking any measurable nonspecific sedative actions. Barbiturates, long the drug of choice for the treatment of distressing anxiety symptoms (Berger, 1963) also exhibit significant antipunishment activity (Table 2). However, the difference between the minimum effective dose which increases punished responding and that which decreases unpunished responding is considerably less than that seen with the benzodiazepines, an observation that agrees quite well with the findings of Blum (1972). The anticonflict activity of the oxoquinazoline derivative, methaqualone, in this conflict procedure may be indicative of an anxiolytic component of activity which has been reported in clinical studies (Cromwell, 1968; Duchastel, 1962).

The dose-response data summarized in Table 2 were obtained after the rats had been exposed to various benzodiazepines on several occasions, i.e., after they were "drug-experienced". An additional

Figure 9. Evaluation of chlorpromazine for anticonflict activity in conditioned suppression of drinking. Punished responding (●); unpunished responding (○). Doses of chlorpromazine HCl (expressed as the salt) were administered intraperitoneally in saline 30 min prior to testing. Symbols represent the mean ± 1 S.E.M. of 3-6 separate determinations. Ordinate: Same as in Figure 7. * indicates a significant ($p<0.05$) decrease in responding compared to control values.

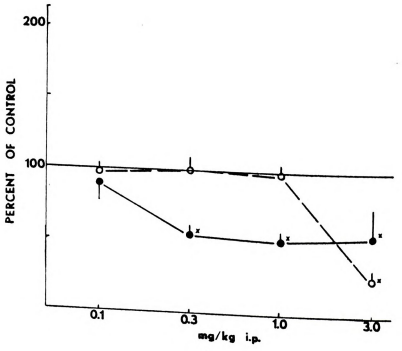


Figure 9

characteristic of the interaction between benzodiazepines and conflict behavior, termed the "initial treatment phenomenon", is seen when these drugs are administered for the first time to conflict-trained, drug-naive animals. Initial treatment with benzodiazepines produces a qualitatively different effect, compared to that seen in drug-experienced subjects, which changes over the course of the first several drug administrations (Margules and Stein, 1968; Cook and Sepinwall, 1975). Characteristically, upon first exposure to 5.6 mg/kg of diazepam, rats trained to a stable baseline of response suppression in the conditioned suppression of drinking conflict paradigm exhibited a significant decrease in unpunished water consumption while punished responding was submaximally increased (Figure 10). During two additional treatments the anticonflict effect further increased and reached asymptote while background water consumption returned to predrug values. Apparently the general depressant or nonspecific sedative effects, represented by the initial decrease in unpunished responding, rapidly undergoes tolerance while the anticonflict activity fails to show tolerance. The fact that a similar pattern was seen when the same dose of diazepam was administered at 5-day intervals (Figure 10, right-hand panel) rather than daily (Figure 10, left-hand panel) argues against an explanation of this phenomenon involving an accumulation of drug and/or active metabolites. Figure 11 depicts the same patterns when testing a lower dose (1.8 mg/kg) of diazepam. However, unpunished water consumption was unaltered from pre-drug control values at any time after treatment with the 1.8 mg/kg dose. Interanimal differences in drug sensitivity are reflected in the variability of the responses to

Figure 10. Initial treatment effects of diazepam (5.6 mg/kg) on punished (●) and unpunished (O) responding in conflict-trained, drug-naive rats (conditioned suppression of drinking). Diazepam was administered intraperitoneally as a methylcellulose suspension either daily (left) or at 5 day intervals (right). Ordinate: Details as in Figure 7. Abscissa: C represents vehicle control treatments (1 ml/kg). Groups of 4 rats were tested at each dosing interval. * indicates those values which differ significantly ($p<0.05$) from vehicle injected controls.

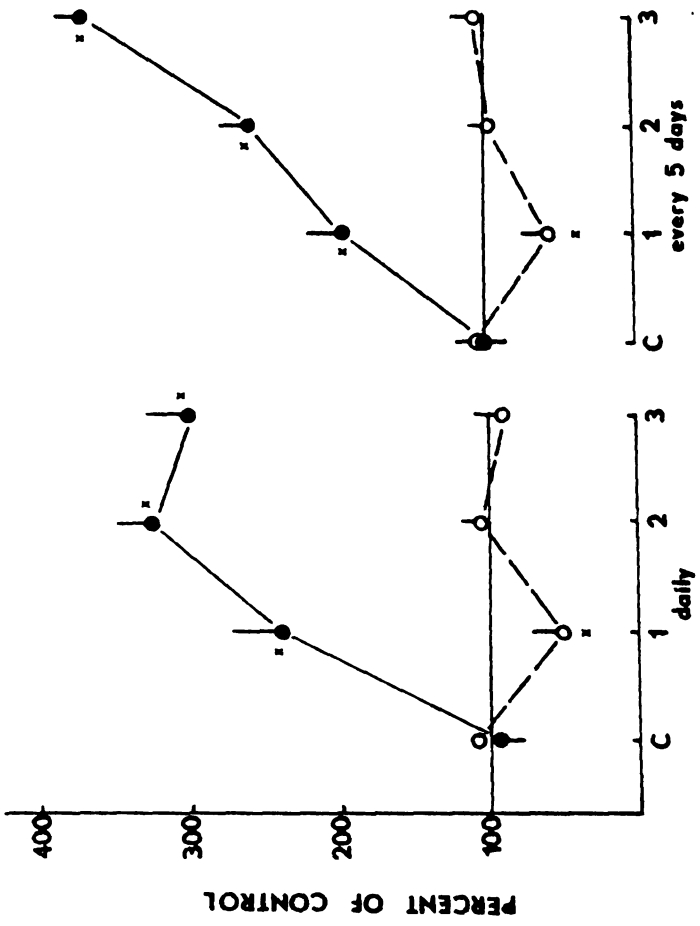

PERCENT OF CONTROL
400
300
200
100
C
1
daily
2
3
C
1
every 5 days
2
3
Treatment

Figure 10

Figure 11. Initial treatment effects of diazepam (1.8 mg/kg) on punished (●) and unpunished (○) responding in conflict-trained, drug-naive rats (conditioned suppression of drinking). Diazepam was administered intraperitoneally as a methylcellulose suspension either daily (left) or at 5 day intervals (right). Each value represents the mean ± 1 S.E.M. of 3 (daily treatments) or 4 (treatment at 5 day intervals) separate determinations. Ordinate: Details as in Figure 7. Abscissa: C represents vehicle control treatments (1 ml/kg). * indicates a significant ($p<0.05$) increase in the number of shocks taken compared to vehicle injected controls.

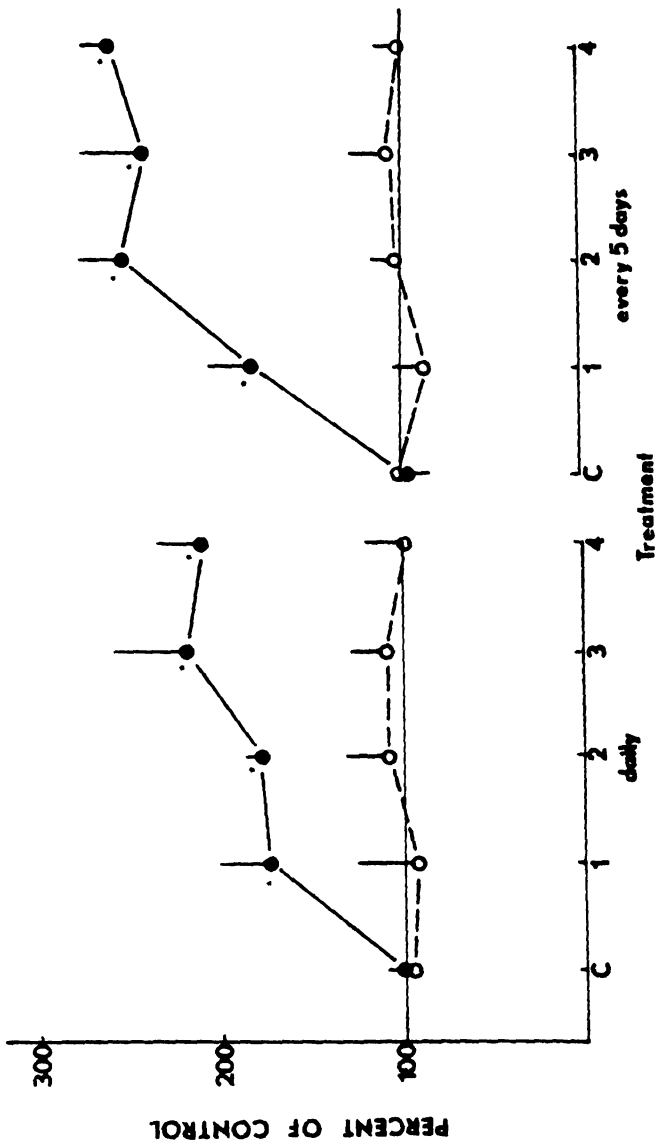

PERCENT OF CONTROL
300
200
100
C
1
2
3
4
daily
every 5 days
Treatment

Figure 11

both single and repeated diazepam administration (Figures 10 and 11). Thus, in agreement with the findings of Sepinwall et al. (1978) in squirrel monkeys, it is possible in drug-naive rats, at certain doses, to obtain a pattern of anticonflict activity similar to that seen in rats with a prior history of benzodiazepine exposure.

The fact that the antipunishment (anticonflict) and general depressant actions of the benzodiazepines follow different courses during their repeated administration, with the anticonflict effects failing to show tolerance and even increasing with repeated doses, may be of value in evaluating possible synaptic mechanisms involved in the anxiety-reducing action of benzodiazepines. Thus, one may assume that a change in neurotransmitter dynamics that persists following the prolonged administration of diazepam may reflect synaptic mechanisms related to its antianxiety action. Similarly, those changes elicited by diazepam which show tolerance may be unrelated to its antianxiety actions or perhaps related to the general depressant effects of the drug. The validity of this assumption obviously rests on the power of this conflict procedure for predicting the therapeutically desirable properties of benzodiazepines in the treatment of human anxiety.

While response-contingent shock has been extensively used to generate low rates of suppressed responding, the relative intensity of the suppression and, by inference, its subsequent attenuation by benzodiazepines, may be dependent on the nature of the response, i.e., unconditioned consummatory responding vs. operantly conditioned, instrumental responding (Bertsch, 1976). Table 3 summarizes the effects of various agents on a conflict procedure utilizing an operant

TABLE 3

Effects of various drugs on an experimentally-induced conflict situation involving the punished suppression of an operantly conditioned response (modified Geller-Seifter)

Treatment	Range of doses*	N	Pretreatment time (min)	Increase in Punished Responding*		Decrease in Unpunished Responding*
				MED	HED	MED
Active Compounds						
diazepam	0.6-18	4-9	10	1.0	10.0	18.0
oxazepam	3.0-56.0	3-8	10	5.6	30.0	56.00
pentobarbital	1.0-18.0	3-8	10	3.0	10.0	18.00
Inactive Compounds						
amitriptyline	1.0-10.0	3-6	20	---	----	5.6
imipramine	1.0-10.0	3-6	20	---	----	5.6
cinanserin	10.0-56.0	4-7	60	---	----	30.0
methysergide	1.0-18.0	3-5	30	---	----	18.0

MED or minimum effective dose indicates the lowest tested dose of a given compound that produced a significant increase in the number of punished responses (bar presses) or a decrease in the number of unpunished responses on the intermediate schedule of reinforcement (FR-40). HED or highest effective dose indicates the largest tested dose of a given compound that significantly increased punished responding without altering unpunished responding. Where no value is indicated (---) the compound tested failed to significantly increase the number of punished responses at any dose examined. N = number of animals tested per dose. *Doses expressed as mg/kg, i.p.

response (bar pressing) for food maintained on either a fixed ratio 40 (unpunished component) or continuous reinforcement (punished component) schedule. This is the modified Geller-Seifter procedure (1960). While subtle differences exist in terms of the magnitude and breadth of the anticonflict effect, the dose-effect profiles are qualitatively similar to those obtained with the conditioned suppression of drinking method despite obvious differences in the nature of the response and type of reinforcer. Although benzodiazepines and barbiturates appear to effectively attenuate the suppressive effects of punishment on consummatory responses as well as complex operant responding, the formidable methodological problems encountered in comparing consummatory and instrumental behavior (Bertsch, 1976) limits comparisons of relative drug effects on these behaviors.

Another factor influencing the intensity of the response suppression engendered by electric shock is the relationship between the shock and the animal's behavior, i.e., response-contingent vs. noncontingent shock (Huppert and Iversen, 1975). The decrease in operant response rate, referred to as the conditioned emotional response, obtained by the repeated pairing of a signal with unavoidable (noncontingent) shock is considered to represent a behavioral analogue of anxiety (Millenson and Leslie, 1974). The effects of various agents on responding in this behavioral paradigm are summarized in Table 4. The disinhibitory effect of benzodiazepines on suppressed responding appears to generalize to experimental designs in which the subject is shocked irrespective of response it makes. The use of the conditioned emotional response as a behavioral baseline susceptible to alteration by

TABLE 4

Effects of various agents on response suppression resulting from noncontingent shock (conditioned emotional response)

Treatment	Range of doses*	N	Pretreatment time (min)	Increase in Suppressed Responding*		Decrease in Baseline FR-40 Responding*
				MED	HED	MED
Active Compounds						
diazepam	0.18-10.0	6-8	15	0.56	3.0	5.0
flunitrazepam	0.03-3.0	3-6	15	0.10	0.56	1.0
oxazepam	2.0-40.0	3-5	15	5.0	10.0	20.0
pentobarbital	1.0-30.0	3-6	15	3.0	10.0	18.0
Inactive Compounds						
phenobarbital	2.5-40.0	3-5	15	----	-----	20.0
amphetamine	0.3-3.0	4-5	15	----	-----	1.8
methysergide	1.0-10.0	4-6	30	----	-----	----
diphenylhydantoin	3.0-56.0	3-9	15	----	-----	30.0

MED or minimum effective dose indicates the lowest dose of a given compound that produced a significant increase in responding suppressed by noncontingent shock or a decrease in the number of responses made in that portion of the behavioral baseline (FR-40) unassociated with footshock. The HED or highest effective dose indicates the largest tested dose of a given compound that significantly increased responding suppressed by noncontingent shock without altering responding in the silent periods. Values for the MED and HED were determined by an analysis of variance using subjects as their own controls. Where no value is indicated (----) the compound tested failed to significantly increase the rate of suppressed responding or to decrease the number of responses in the nonshocked periods (e.g., methysergide) over the range of doses tested. N = number of animals tested per dose. *Doses expressed as mg/kg, i.p.

benzodiazepines proved to be a fragile design dependent upon rigorous control of the temporal and incremental aspects of the variables (i.e., conditioned stimulus duration, shock intensity, degree of food deprivation, schedule of reinforcement) which interact to produce the conditioned suppression. The effects of benzodiazepines on responding in the conditioned emotional response procedure were considerably more variable than that in the conflict tests involving response-contingent punishment.

B. Effects of Drug-induced Alterations in Brain 5-Hydroxytryptamine Activity on Conflict Behavior

A seemingly convincing body of evidence obtained from animal studies implicates 5-hydroxytryptamine systems in the anxiety-reducing actions of benzodiazepines (see Introduction and Stein et al., 1977). The peripheral 5-hydroxytryptamine antagonists methysergide (Gyermek, 1961), cinanserin (Rubin et al., 1964) and cyproheptadine (Stone et al., 1961; van Riezen, 1972) were evaluated for anticonflict activity using the conditioned suppression of drinking conflict procedure. Methysergide had no significant effect on punished responding whether administered one or 30 minutes prior to behavioral testing (Figure 12). The combination of a submaximal anticonflict dose of diazepam (1.8 mg/kg) with a dose of methysergide (3.0 mg/kg, one minute pretreatment, which alone had no significant anticonflict effect) produced a greater anticonflict effect than that of diazepam alone (Figure 13). The injection of various doses of cyproheptadine, 30 minutes prior to testing, also failed to significantly alter the rate of punished responding (Figure 14). Pretreatment with cyproheptadine (1 and 3 mg/kg) did not significantly alter the anticonflict effect of diazepam

Figure 12. Evaluation of methysergide for anticonflict activity (conditioned suppression of drinking). Punished responding (●); unpunished responding (○). Doses of methysergide maleate (expressed as the salt) were administered intraperitoneally in saline either 1 min (left) or 30 min (right) prior to testing. Symbols represent the mean ± 1 S.E.M. of 3-8 separate determinations. Ordinate: Details as in Figure 7. * indicates those values which differ significantly ($p<0.05$) from control. Unpunished responding was significantly decreased only at 1 min after the largest dose, 18 mg/kg, of methysergide.

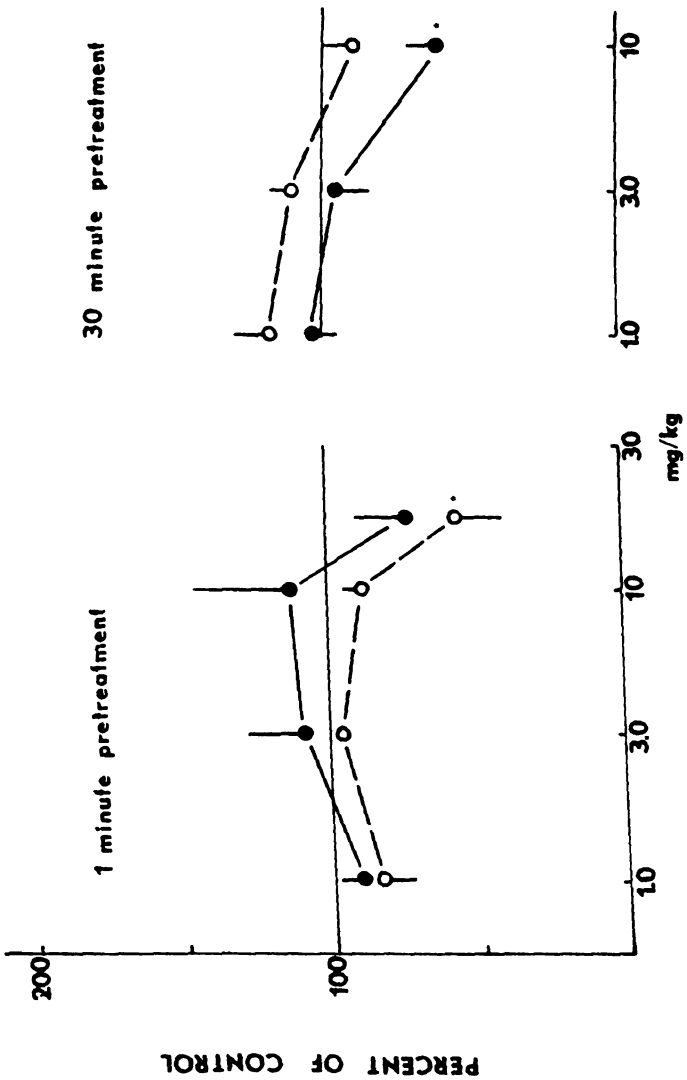

PERCENT OF CONTROL
200
100
1 minute pretreatment
30 minute pretreatment
1.0
3.0
10
30
mg/kg

Figure 12

Figure 13. Effects of peripheral 5-hydroxytryptamine antagonists on the anticonflict activity (conditioned suppression of drinking) of a submaximal dose of diazepam (1.8 mg/kg). Punished responding, open bars; unpunished responding, striped bars. Methysergide maleate was administered 1 min, cyproheptadine HCl 30 min and cinanserin HCl 60 min prior to testing. Doses are expressed as the salt and were administered by the intraperitoneal route. Diazepam was administered 15 min prior to testing. Each value represents the mean and vertical lines ± 1 S.E.M. of 4-9 separate determinations. Ordinate: Details as in Figure 7. * indicates significant ($p<0.05$) decreases in unpunished water consumption compared to diazepam alone.

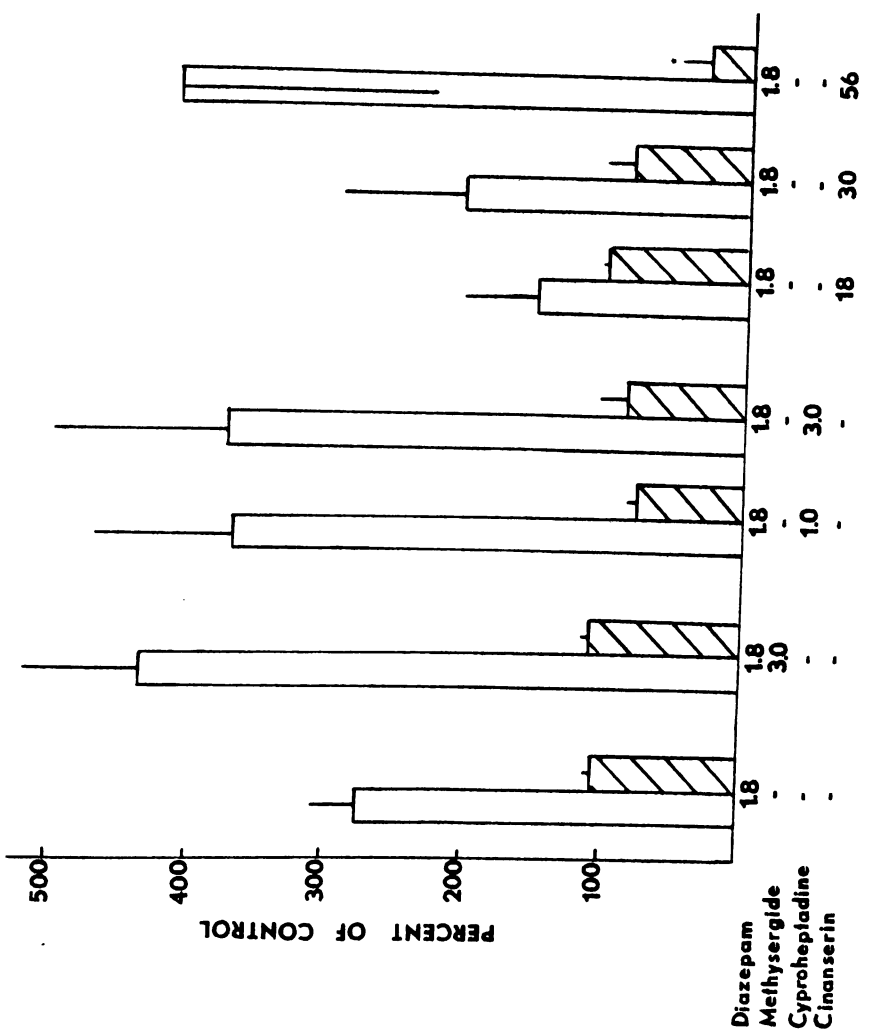

PERCENT OF CONTROL
500
400
300
200
100
Diazepam
Methysergide
Cyproheptadine
Cinanserin
1.8 - - -
1.8 3.0 - -
1.8 - 1.0 -
1.8 - 3.0 -
1.8 - - 18
1.8 - - 30
1.8 - - 56

Figure 13

Figure 13. Effects of peripheral 5-hydroxytryptamine antagonists on the anticonflict activity (conditioned suppression of drinking) of a submaximal dose of diazepam (1.8 mg/kg). Punished responding, open bars; unpunished responding, striped bars. Methysergide maleate was administered 1 min, cyproheptadine HCl 30 min and cinanserin HCl 60 min prior to testing. Doses are expressed as the salt and were administered by the intraperitoneal route. Diazepam was administered 15 min prior to testing. Each value represents the mean and vertical lines ± 1 S.E.M. of 4-9 separate determinations. Ordinate: Details as in Figure 7. * indicates significant ($p<0.05$) decreases in unpunished water consumption compared to diazepam alone.

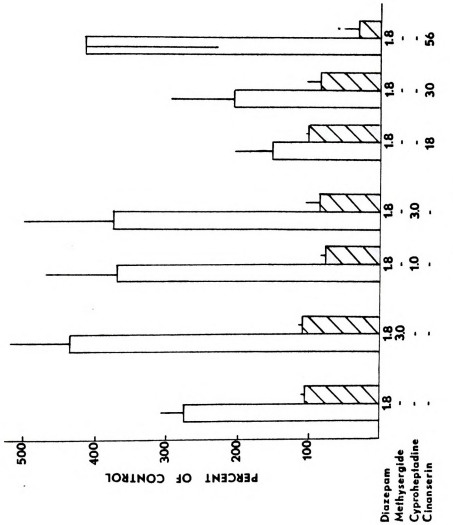

PERCENT OF CONTROL
500
400
300
200
100
Diazepam
Methysergide
Cyproheptadine
Cinanserin
1.8 - - -
1.8 3.0 - -
1.8 - 1.0 -
1.8 - 3.0 -
1.8 - - 18
1.8 - - 30
1.8 - - 56

Figure 13

Figure 14. Evaluation of cyproheptadine for anticonflict activity. Punished responding (●); unpunished responding (○). Doses of cyproheptadine HCl (expressed as the salt) were administered intraperitoneally in distilled water 30 min prior to testing. Each value represents the mean ± 1 S.E.M. of 3-7 separate determinations. Ordinate: Details as in Figure 7. * indicates a significant ($p<0.05$) decrease in unpunished water consumption.

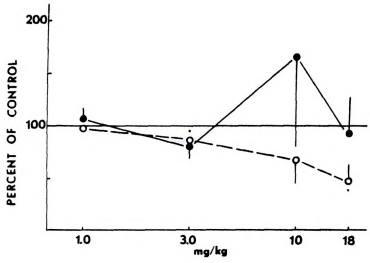


Figure 14

(1.8 mg/kg; Figure 13). In agreement with Geller et al. (1974), cinanserin increased punished responding, but only at a single dose (56 mg/kg) which was associated with a significantly decreased water consumption (Figure 15). However, in contrast to the findings of Cook and Sepinwall (1975), no anticonflict activity was observed with lower doses of cinanserin. The effect of various doses of cinanserin on the anticonflict activity of diazepam was examined (Figure 13). Pretreatment with either 18 or 30 mg/kg cinanserin did not appear to change the response to diazepam (1.8 mg/kg). Combination of the active dose of cinanserin (56 mg/kg) with diazepam (1.8 mg/kg) yielded an anticonflict effect, the magnitude of which suggested the interaction to be of an additive nature. However, the response to this combination was mixed and was accompanied by a large decrease in unpunished water consumption.

The following study was undertaken to investigate the effects of an enhanced 5-hydroxytryptamine-mediated activity on the anticonflict effects of diazepam in the conditioned suppression of drinking. The brain concentration of 5-hydroxytryptamine was elevated by the peripheral administration of the immediate precursor of 5-hydroxytryptamine, 5-hydroxytryptophan (Moir and Eccleston, 1968). In order to minimize any peripheral side effects of the 5-hydroxytryptamine formed from the exogenously administered 5-hydroxytryptophan, a peripherally acting L-aromatic amino acid decarboxylase inhibitor (MK-486, 60 mg/kg, i.p.) was administered 30 minutes prior to 5-hydroxytryptophan. The 5-hydroxytryptophan, given in conjunction with the decarboxylase inhibitor, produced a dose-related increase in whole brain 5-hydroxytryptamine (Table 5). When administered without prior MK-486 treatment,

Figure 15. Evaluation of cinanserin for anticonflict activity (conditioned suppression of drinking). Punished responding (●); unpunished responding (O). Doses of cinanserin HCl (expressed as the salt) were administered intraperitoneally in saline 60 min prior to testing. Symbols represent the means and vertical lines indicate ± 1 S.E.M. as determined from 3-6 separate determinations. Ordinate: Details as in Figure 7. *Significantly different ($p<0.05$) from control.

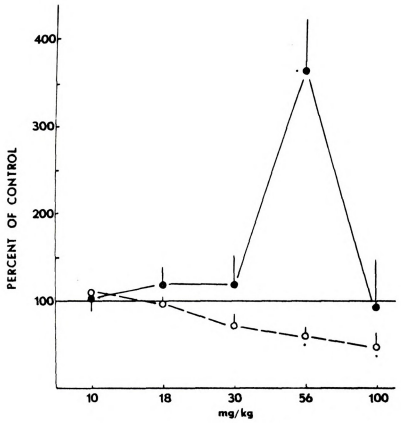


Figure 15

TABLE 5

Brain 5-Hydroxytryptamine Concentrations Following 5-Hydroxytryptophan, 5,7-Dihydroxytryptamine or p-Chlorophenylalanine Administration

Treatment	N	5-HT µg/g ± S.E.M.	% of Control
Control	3	0.53±0.02	100
5-HTP (18) + MK-486 (60)	3	2.13±0.03	402.7
5-HTP (30) + MK-486 (60)	3	2.77±0.10	524.0
intravent. 5,7-DHT	3	0.39±0.02	73.8
acute p-CPA	3	0.30±0.03	57.5
chronic p-CPA	3	0.21±0.01	40.2

Telencephalic 5-hydroxytryptamine (5-HT) concentrations were determined by fluorometric detection after o-phthalaldehyde reaction (Curzon and Green, 1970). Excitation and emission wave-lengths were 360 and 470 nm respectively. Standard solutions containing 25-1250 ng 5-HT and tissue samples were assayed in duplicate. Blank solutions were run in triplicates. Animals receiving 5-hydroxytryptophan (5-HTP) were pretreated with the peripheral aromatic amino acid decarboxylase inhibitor MK-486 (60 mg/kg, i.p.) 30 minutes prior to 5-HTP treatment and were sacrificed 60 minutes later. p-Chlorophenylalanine (p-CPA) treated animals were sacrificed either 5 days following a single injection of 400 mg/kg of p-CPA ("acute p-CPA") or 14 days from the initiation of a regimen of 200 or 300 mg/kg p-CPA every 2nd or 3rd day respectively ("chronic p-CPA"). The assay of brain 5-HT content of rats receiving 5,7-dihydroxytryptamine (5,7-DHT) (100 µg) intracerebroventricularly was performed 10 days after the injections.

Numbers in parentheses designate doses expressed as mg/kg, i.p.

5-hydroxytryptophan (18 mg/kg) produced a significant decrease in punished responding and a trend to decrease, although not significant, the anticonflict effect of a single dose of diazepam (1.8 mg/kg, Figure 16). This enhancement of the behaviorally suppressant effects of response-contingent shock by 5-hydroxytryptophan administration was no longer observed when the animals were pretreated with the peripheral decarboxylase inhibitor. Surprisingly, the anticonflict effect of diazepam (1.8 mg/kg) was significantly increased in animals pretreated with 5-hydroxytryptophan and MK-486 (Figure 16).

Inhibition of the major mechanism involved in the termination of the synaptic actions of 5-hydroxytryptamine, neuronal reuptake from the synaptic cleft, enhances 5-hydroxytryptamine-mediated synaptic transmission by prolonging the effect of 5-hydroxytryptamine at the synapse. The clinically useful tertiary amine tricyclic antidepressants have been reported to inhibit the reuptake of 5-hydroxytryptamine at nerve endings *in vivo* (Carlsson *et al.*, 1969; Von Voigtlander and Losey, 1976). As already mentioned, the tertiary amines imipramine and amitriptyline exhibited no measureable anticonflict activity (Table 2). Additionally, a reportedly specific blocker of neuronal 5-hydroxytryptamine uptake mechanisms, fluoxetine (3 mg/kg, 120 minute pretreatment; Fuller *et al.*, 1975), was found to be similarly ineffective in increasing the rate of punished responding. However, amitriptyline (5.6 mg/kg) enhanced the anticonflict effect of diazepam (1.8 mg/kg) in the drinking procedure; doses of 3 and 10 mg/kg amitriptyline were ineffective in this regard (Figure 17) . While this observation is in general agreement with the findings of Babbini *et al.* (1976), the

Figure 16. Effects of 5-hydroxytryptophan, alone or in combination with a peripheral decarboxylase inhibitor (MK-486), on the anticonflict activity (conditioned suppression of drinking) of a submaximal dose of diazepam (Diaz.; 1.8 mg/kg). Punished responding, open bars; unpunished responding, striped bars. 5-Hydroxytryptophan was administered 60 min, MK-486 90 min and diazepam 15 min prior to testing. Where no treatment dose is indicated the subjects received injections (1 ml/kg) of the vehicle, 0.5% methylcellulose. Each value represents the mean ± S.E.M. of 3-10 determinations. Ordinate: Details as in Figure 7. *Significant ($P<0.05$) decrease in punished responding compared to methylcellulose injected controls. °Significant ($P<0.05$) increase in punished responding compared to subjects receiving diazepam alone.

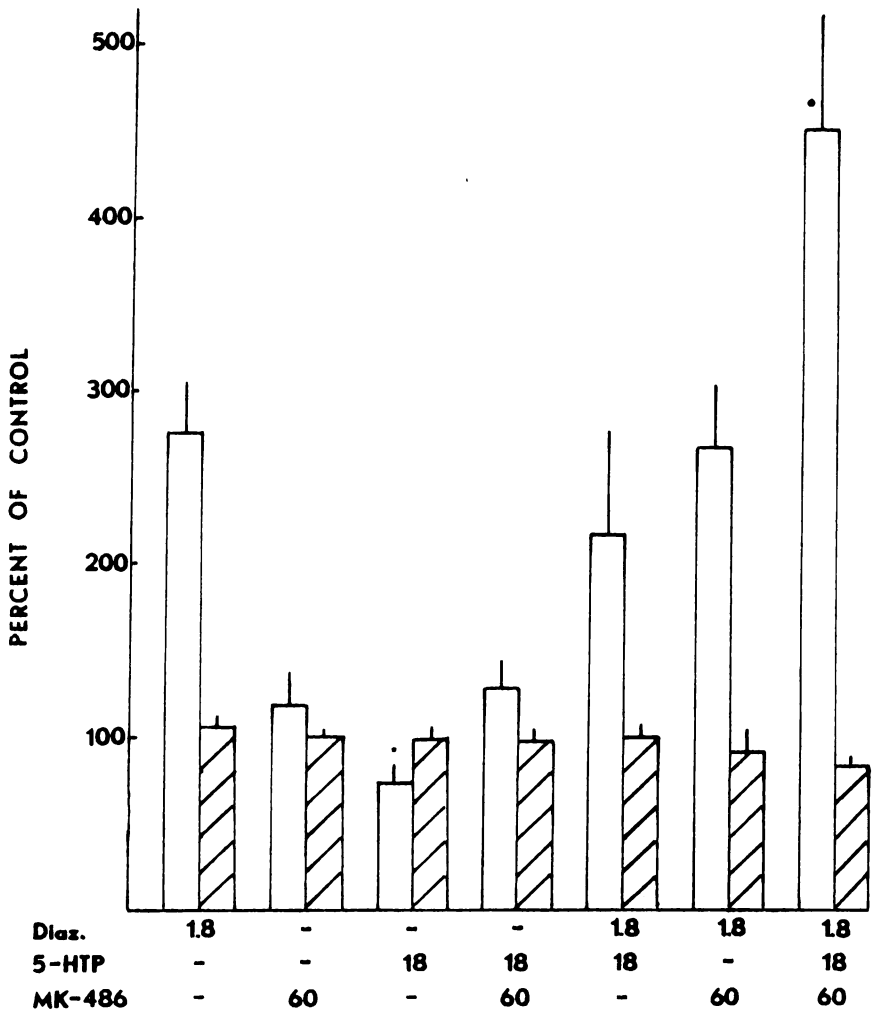

PERCENT OF CONTROL
500
400
300
200
100
Diaz. 1.8 - - - 1.8 1.8 1.8
5-HTP - - 18 18 18 - 18
MK-486 - 60 - 60 - 60 60

Figure 16

Figure 17. Effects of amitriptyline on the anticonflict activity (conditioned suppression of drinking) of a submaximal dose of diazepam (1.8 mg/kg). Punished responding, open bars; unpunished responding, striped bars. Doses of amitriptyline HCl (expressed as the salt) were administered intraperitoneally 30 min prior to testing. Diazepam was administered 15 min prior to testing. Where no treatment dose is indicated the subjects received injections of the appropriate vehicle: Saline or 0.5% methylcellulose in lieu of amitriptyline or diazepam, respectively. Each value represents the mean ± S.E.M. of 3-6 determinations. Ordinate: Details as in Figure 7. *Significant ($P<0.05$) decrease in punished or unpunished responding compared to saline injected controls. °Significant ($P<0.05$) increase in punished responding compared to subjects receiving diazepam alone.

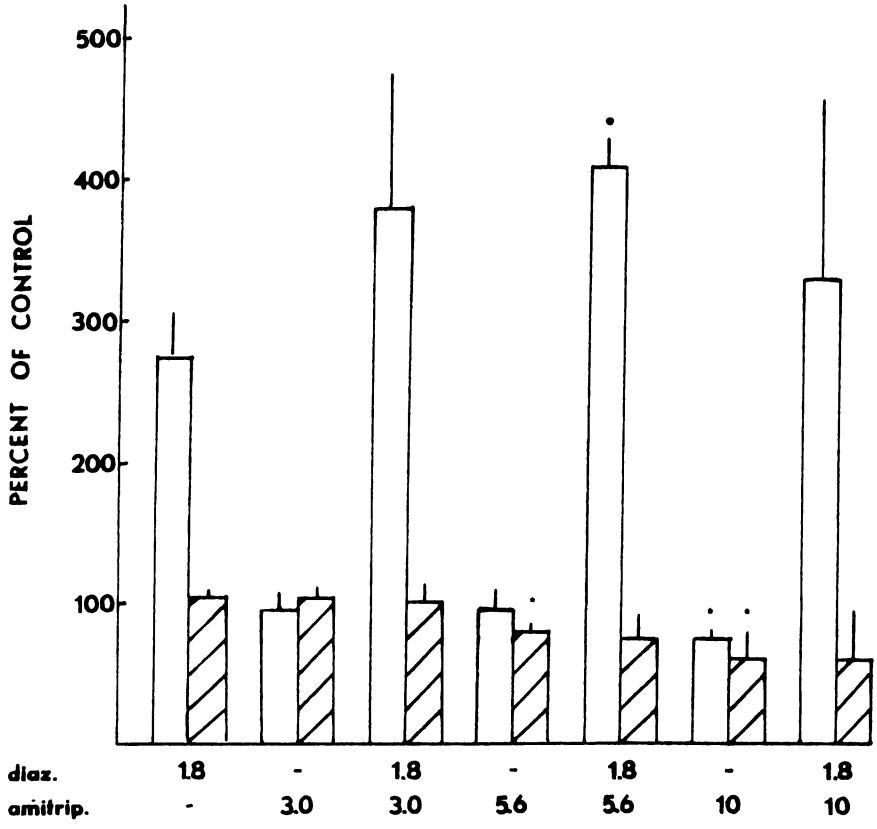

500
400
300
200
100
PERCENT OF CONTROL
diaz.
amitrip.
1.8
-
-
3.0
1.8
3.0
-
5.6
1.8
5.6
-
10
1.8
10

Figure 17

significance of this interaction is obscured by the uncertain relationship between the tricyclic antidepressants and the synaptic activity of 5-hydroxytryptamine (Fuxe et al., 1977).

The systemic administration of very small doses (10-20 µg/kg) of d-lysergic acid diethylamide (LSD) produces a marked, reversible inhibition of activity of 5-hydroxytryptamine neurons (Aghajanian et al., 1968) presumably as a consequence of a direct inhibitory effect on cell bodies comprising the raphé nuclei. An evaluation of the effects of lysergic acid diethylamide on conflict behavior revealed no significant anticonflict activity whether the hallucinogen was administered one or 30 minutes prior to behavioral testing (Figure 18). This finding is in seeming contradiction with that of Schoenfeld (1976), who reported that lysergic acid diethylamide significantly attenuated the suppressive effect of punishment on licking behavior in untrained rats. It must be emphasized, however, that important methodological differences exist between the two paradigms. In fact, lysergic acid diethylamide (10, 30 and 100 µg/kg) significantly decreased the rate of punished responding when given one minute prior to testing (Figure 18). A one-minute pretreatment time was employed in light of the very rapid short-lived depressant effect of lysergic acid diethylamide on 5-hydroxytryptamine-containing midbrain raphé neurons (Aghajanian et al., 1968).

C. Effects of 5-Hydroxytryptamine Depletion on Conflict Behavior

A decrease in brain 5-hydroxytryptamine content was effected by an inhibition of 5-hydroxytryptamine synthesis by p-chlorophenylalanine or by the administration of 5,7-dihydroxytryptamine, which has a neurotoxic

Figure 18. Effects of d-lysergic acid diethylamide (d-LSD) on punished (●) and unpunished (O) responding in conditioned suppression of drinking. Doses of d-LSD bitartrate (expressed as the free base) were administered either 1 min (right) or 30 min (left) prior to the initiation of behavioral testing. Symbols represent means and vertical lines ± 1 S.E.M. as calculated from 4-12 separate determinations. Ordinate: Details as in Figure 7. * indicates a significant decrease in the number of shocks taken or volume of water consumed compared to control values.

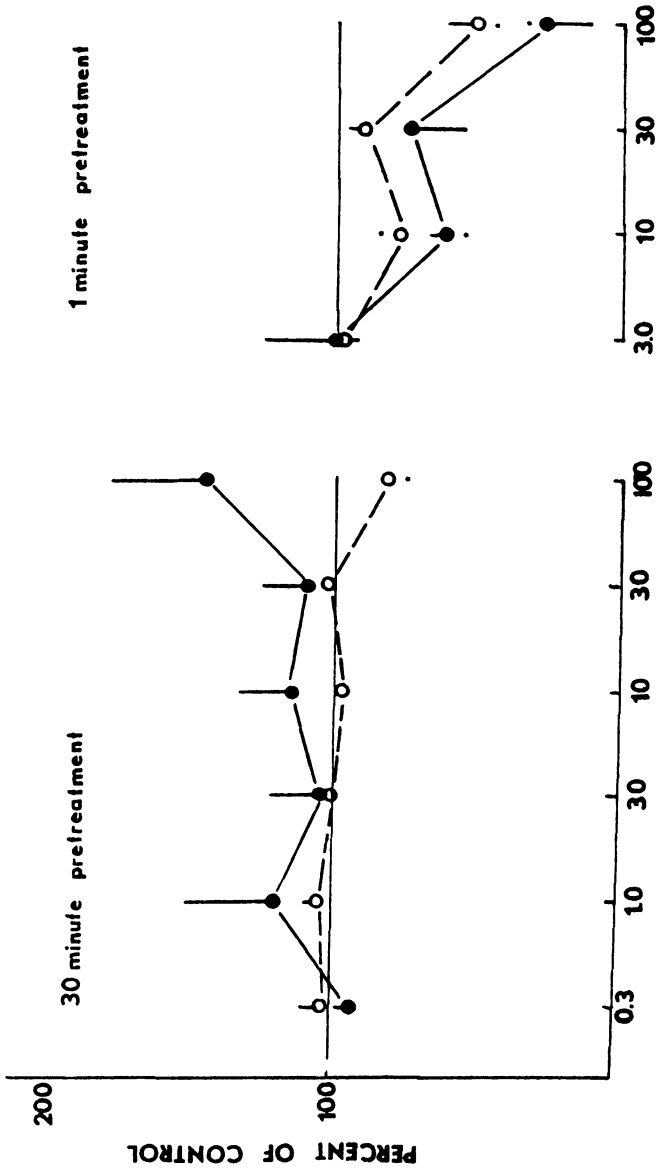

1 minute pretreatment
30 minute pretreatment
PERCENT OF CONTROL
200
100
0.3
1.0
3.0
10
30
100
µg/kg

Figure 18

effect on 5-hydroxytryptamine-containing neurons (Table 5). In agreement with the findings of Koe and Weissman (1966), pretreatment with 400 mg/kg p-chlorophenylalanine, i.p. (120 hours) caused a considerable (57.5 percent of control) depletion of whole brain 5-hydroxytryptamine (Table 5). The effects of this single dose of p-chlorophenylalanine on experimentally-induced conflict behavior are shown in Figure 19. Punished responding was significantly increased 48 hours (day 2) following p-chlorophenylalanine administration, actually decreased significantly below control levels on the next day, and attained control values by day 5. Unpunished water consumption was significantly decreased on all testing days subsequent to p-chlorphenylalanine administration. As the effects of p-chlorophenylalanine on brain 5-hydroxytryptamine and tryptophan hydroxylase activity are reversible (Koe and Weissman, 1966), p-chlorophenylalanine was chronically administered (200 or 300 mg/kg i.p. every 2 or 3 days, respectively, for a total of 14 days) to another group of conflict-trained rats to effect a more uniform decrease in 5-hydroxytryptamine concentrations and enzyme activity. Although the chronic administration of p-chlorophenylalanine appeared to increase the rate of punished responding, only the increase on days 5 and 7 attained statistical significance ($p<.05$; Figure 19). Unpunished water consumption tended to be decreased, reaching significance on several days, throughout the course of drug administration. Subjects sacrificed upon termination of the study exhibited a marked decrease (to 40 percent of control) in whole brain 5-hydroxytryptamine (Table 5). In agreement with Blakely and Parker (1973), the findings of this experiment fail to support the contention of Geller and Blum

Figure 19. Effects of single and repeated administrations of p-chlorophenylalanine (p-CPA) on experimentally-induced conflict behavior (conditioned suppression of drinking). An acute injection of p-CPA (400 mg/kg; left) was administered immediately following the final control session and the effects on punished and unpunished responding were evaluated for 5 days. Chronic p-CPA administration (200 or 300 mg/kg; right) was similarly initiated following the final control session and repeated following testing on days 2, 4, 6, 8 and 11. Symbols represent means and vertical lines ± 1 S.E.M. as determined from groups of 4 (acute) and 5 (chronic) animals. Ordinate: Details as in Figure 7. Abscissa: Days of testing and doses (in mg/kg, i.p.) of p-CPA administered. *Significantly different ($P<0.05$) from controls.

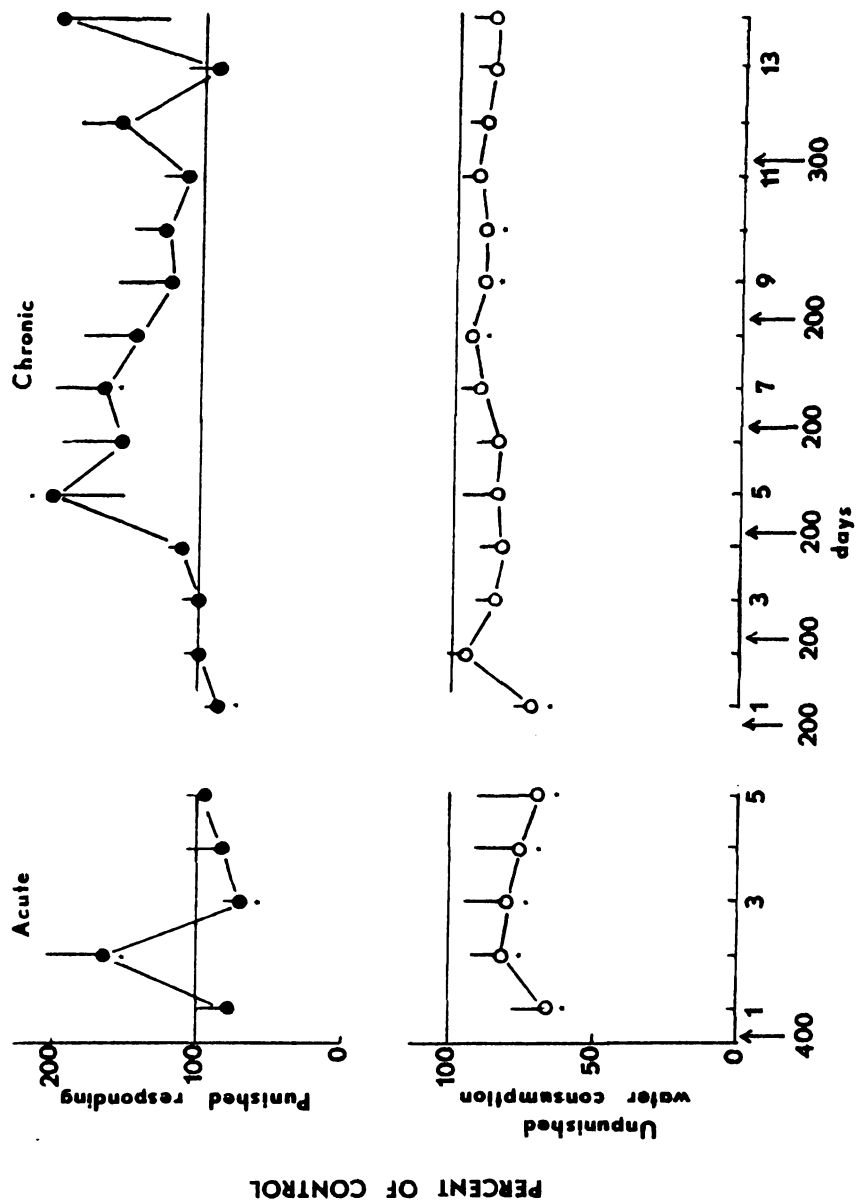

Chronic
Acute
Punished responding
Unpunished water consumption
PERCENT OF CONTROL
days
200
100
0
400
50
1
3
5
7
9
11
13
200
300

Figure 19

(1970) and Robichaud and Sledge (1969) that depletion of brain 5-hydroxytryptamine by p-chlorophenylalanine produces an attenuation of the behaviorally suppressive effects of punishment qualitatively similar to that reported for various antianxiety agents. However, numerous methodological differences in the paradigms limit the number of meaningful comparisons.

The intracerebroventricular injection of 5,7-dihydroxytryptamine (100 µg) produced a transitory release of punishment-suppressed behavior in the modified Geller-Seifter procedure (Figure 20). Qualitatively similar, though less long-lasting, anticonflict effects have been reported by Stein et al. (1975) following the injection of 5,6-dihydroxytryptamine (100 µg) by the same route. Unpunished responding regained control rates within 5 days of 5,7-dihydroxytryptamine injection but was significantly depressed prior to this time (Figure 20). Vehicle injected controls exhibited a similar initial depression, suggesting that this effect may be the resultant of the after-effects of the anesthesia and/or the trauma of the surgical procedure. Intracerebroventricularly injected 5,7-dihydroxytryptamine produced a 26% decrease in whole brain 5-hydroxytryptamine compared to controls in animals sacrificed 10 days following the injection of the neurotoxin or vehicle (Table 5).

Bilateral injections of 5,7-dihydroxytryptamine (7 µg) into the amygdala produced a profound, transitory anticonflict effect in the Geller-Seifter paradigm of more rapid onset and shorter duration than that observed following intracerebroventricular injection (Figure 20). Rates of unpunished responding had reached control values by day 3. The 5-hydroxytryptamine content of the amygdala (dissected free in toto

Figure 20. Effects of intraventricular and intraamygdaloid administration of 5,7-dihydroxytryptamine (5,7-DHT) on experimentally-induced conflict behavior (modified Geller-Seifter procedure). Punished responding (●); unpunished responding (○). 5,7-DHT was injected into the lateral ventricle (100 µg; left) and the amygdala (7 µg, bilaterally; right) of conflict-trained rats and behavioral testing initiated the following day (day 1). See text for details of surgery and drug injection. Symbols represent the means and vertical lines ± 1 S.E.M. for 3 separate determinations. Ordinate: Details as in Figure 7. *Significantly different ($p<0.05$) from preinjection control values.

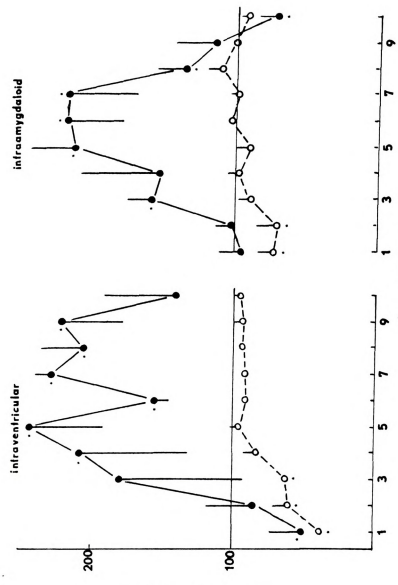

intraventricular
intraamygdaloid
200
100
PERCENT OF CONTROL
1
3
5
7
9
days post surgery

Figure 20

on ice) was decreased by 27% (1.20±0.17 μg/g) relative to controls (1.66±0.20 μg/g) 10 days following treatment with the neurotoxin. Neither intra-amygdaloid nor intracerebroventricular injections of the vehicle yielded consistent effects on conflict activity (data not shown). Thus, interference with normal 5-hydroxytryptamine functioning can produce an effect on conflict behavior quantitatively similar to that observed with benzodiazepines. However, the manifestation of this effect does not appear to be directly related to the degree to which whole brain 5-hydroxytryptamine content is depleted, as the depletion produced by p-chlorophenylalanine was substantially greater than that produced by intracerebroventricular 5,7-dihydroxytryptamine. Rather, the results suggest that the discrepancy between the behavioral effects of p-chlorophenylalanine and 5,7-dihydroxytryptamine-induced 5-hydroxytryptamine depletion may be related to a differential effect on the integrity of 5-hydroxytryptamine-containing nerve terminals. These are destroyed by 5,7-dihydroxytryptamine but remain structurally intact following p-chlorophenylalanine treatment. Moreover, Sloviter et al. (1978) have reported that p-chlorophenylethylamine (p-CPEA), a decarboxylation product of p-chlorophenylalanine, displaces 5-hydroxytryptamine from intact terminals. Alternatively, the measurement of 5-hydroxytryptamine concentrations in more circumscribed areas of the brain may reveal a closer correlation between the degree of depletion and effects on punishment suppressed responding.

These experiments involving the administration of 5,7-dihydroxytryptamine yielded results in apparent contradiction to the findings depicted in Figures 16, 17 and 18. That is, reducing activity of brain

5-hydroxytryptamine pathways by treating with the neurotoxin showed a clear anticonflict effect in the Geller-Seifter test. However, a presumed reduction in forebrain serotonergic tone by LSD caused an increased suppression in the drinking procedure, while enhanced 5-hydroxytryptamine activity by combining 5-hydroxytryptophan or amitriptyline with diazepam actually increased the anticonflict effect. It must be emphasized that the Geller-Seifter procedure utilized food reinforcement, whereas the conditioned suppression of drinking utilized the motivation of thirst. These different reinforcements did not seem to affect the anticonflict spectrum of the benzodiazepines alone. On the other hand, the alteration in brain serotonergic function may be expected to influence food-motivated behaviors on the basis of the reinforcement since increased 5-hydroxytryptamine activity is anorectic and decreased activity tends to increase food consumption (Samanin et al., 1977). This topic will be expanded in the Discussion. In addition, the administration of 5-hydroxytryptophan is not necessarily selective in producing changes in brain 5-hydroxytryptamine. The amino acid may also enter brain catecholamine neurons and influence levels of these neurotransmitters.

D. Effects of Single and Repeated Administrations of Diazepam on Body Temperature

Bartholini et al. (1973) have suggested that the effects of high doses of diazepam (10 mg/kg) on the dynamics of various neurotransmitters (e.g., dopamine) in the central nervous system may be secondary to a hypothermic effect of this benzodiazepine. It was thus of interest to determine whether diazepam, at an effective anticonflict dose (1.8 mg/kg, i.p.), devoid of measurable nonspecific sedative effects

(Figure 7), similarly decreased the rectal temperature of rats when given acutely or following repeated administrations. In agreement with the findings of Carpenter et al. (1977) a single injection of diazepam (1.8 mg/kg) failed to significantly alter the rectal temperature of rats (Table 6). Furthermore, when chronically administered (1.8 mg/kg/day for 5 consecutive days), diazepam produced no significant effects on rectal temperature compared to vehicle injected controls (Table 6). It would thus appear unlikely that the effects of diazepam on neuronal activity as related to the anticonflict effect, at least at this dosing regiment, are secondary to alterations in body temperature. In support of this contention Fuxe et al. (1975) have found the benzodiazepine-induced alterations in rat brain dopamine turnover to be unchanged when the animals' body temperature is maintained by an elevated environmental temperature.

E. Effects of Diazepam on Dopaminergic Neuronal Pathways

The steady state dopamine concentrations and average protein content of the amygdaloid nuclei, nucleus accumbens, olfactory tubercle and caudate nucleus are listed in Table 7. The anterior amygdaloid area, while representing the most rostral aspect of the amygdala, contains a number of fibers and cell bodies which subserve neuronal systems other than those involving the amygdala (Palkovits et al., 1974). The relatively uniform protein content values attest to the reproducibility of the microdissection techniques. The dopamine concentration of the amygdaloid nuclei are essentially the same as, though somewhat higher than, those reported by Brownstein et al. (1974). Corresponding values for the caudate nucleus, nucleus accumbens and

TABLE 6

Effect of Diazepam (1.8 mg/kg) on Rectal Temperature (°C) in Rats

Day	Treatment	Time After Injection (min) 15	30	45	60
1-5	vehicle	38.6±0.2	38.3±0.3	38.3±0.2	38.2±0.2
1	diazepam	38.7±0.3	38.5±0.4	38.5±0.2	38.5±0.2
2	diazepam	38.5±0.1	38.4±0.2	38.5±0.1	38.4±0.1
3	diazepam	38.7±0.1	38.6±0.1	38.5±0.1	38.5±0.1
4	diazepam	38.5±0.2	38.3±0.2	38.3±0.2	38.3±0.1
5	diazepam	38.4±0.2	38.0±0.1	37.9±0.2	37.9±0.2

Diazepam or vehicle (0.5% methylcellulose) was administered i.p. Temperature recordings for any given time point in vehicle-injected controls were not significantly altered by repeated vehicle administrations and were subsequently combined. Each value represents the mean ± 1 S.E.M. of 15 (vehicle-injected controls) or 5 (diazepam) separate determinations.

TABLE 7

Dopamine Concentrations in Various Nuclei in the Rat Brain

Brain Nuclei	N	µg of Protein per sample	Dopamine ng/mg protein
amygdaloid nuclei			
anterior amygdaloid area	13	62± 3	39.7±4.1
Central	17	99±11	26.3±5.2
Lateral	18	113± 7	17.9±2.7
Basal	15	96±10	10.1±1.9
Cortical	16	77± 9	7.2±0.8
Medial	16	89± 6	4.4±0.7
Posterior	19	40± 6	2.2±0.6
Basal posterior	19	61± 3	6.6±0.9
Medial posterior	18	42± 5	1.8±0.5
rostral limbic nuclei			
nucleus accumbens	11	108± 8	79.7±7.3
olfactory tubercle	12	79± 9	53.9±4.0
Caudate nucleus	11	87± 2	83.6±4.3

Values represent means ± 1 S.E.M. N = number of samples assayed.

olfactory tubercle are similar to those reported by other investigators (Koslow et al., 1974; Cheney et al., 1975).

The endogenous dopamine concentration of the brain is a reflection of the finely regulated processes of synthesis and release at a steady state. The characteristically consistent maintenance of this steady state ensures a relatively unchanging endogenous dopamine concentration in spite of physiologically- and pharmacologically-induced alterations in dopaminergic neuronal activities. Hence, the study of the steady state dopamine content of various brain regions is of little value by itself when estimating the activity of neuronal pathways involving these areas. The function of the central nervous system involves a dynamic interaction within and between neurons. Therefore, function, both basal and drug-induced alterations thereof, may be best described in terms of biochemical correlates by studying the dynamics of the neurotransmitters in various brain nuclei in terms of their estimated rate of turnover. Similar arguments pertain to 5-hydroxytryptamine and other neurotransmitters as well.

1. Effects of Acute Diazepam Administration on Dopamine Turnover in the Amygdala, Olfactory Tubercle, Nucleus Accumbens and Caudate Nucleus

With the exception of the medial posterior amygdaloid nucleus and the posterior amygdaloid nucleus, the concentrations of dopamine declined exponentially after administration of α-methyltyrosine (300 mg/kg, i.p.) in all brain areas examined. Representative examples of the logarithmic nature of the α-methyltyrosine-induced depletion of dopamine are illustrated in Figure 21. The failure of the two aforementioned amygdaloid nuclei to exhibit a similar pattern most probably

Figure 21. The logarithmic decline of dopamine concentrations in various brain nuclei following the administration of α-methyltyrosine (300 mg/kg, i.p.). Animals were sacrificed immediately before (0 time) or 45 and 90 minutes after treatment with α-methyltyrosine. Symbols represent means and vertical lines ± 1 S.E.M. as determined from 5-8 animals.

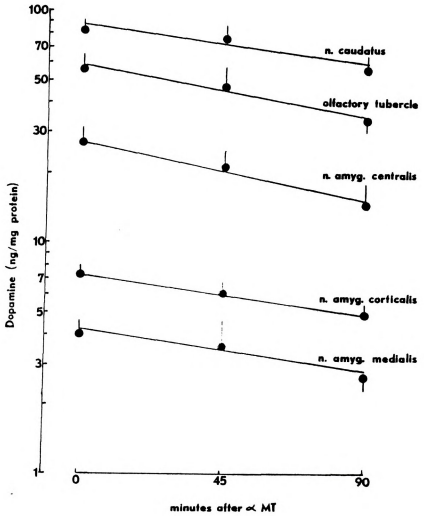

100
70
50
30
10
7
5
3
1
Dopamine (ng/mg protein)
n. caudatus
olfactory tubercle
n. amyg. centralis
n. amyg. corticalis
n. amyg. medialis
0
45
90
minutes after α MT

Figure 21

reflects methodological difficulties involved in reproducibly measuring depleted dopamine concentrations in tissue samples with a calculated approximate wet weight of 400 µg (assuming protein content represents 10-12% of the wet weight) and possessing a relatively low intrinsic dopamine content.

Diazepam (1.8 mg/kg, 15 minutes prior to sacrifice) failed to alter the steady state dopamine concentration in any of the brain areas examined before the administration of α-methyltyrosine (i.e., zero time concentration). However, when given in conjunction with α-methyltyrosine, a single dose of diazepam (1.8 mg/kg, i.p.) reduced the rate constants for the α-methyltyrosine-induced decline of dopamine in amygdaloid nuclei: the lateral, central, cortical and basal posterior, as well as in the olfactory tubercle (Table 8). Acute diazepam administration failed to significantly alter the rate constant for the α-methyltyrosine-induced decline of dopamine in the basal and medial amygdaloid nuclei or the nucleus accumbens and caudate nucleus.

As the clinical (Warner, 1965) and animal behavioral (Margules and Stein, 1968; Sepinwall et al., 1978) effects of the benzodiazepines appear to undergo significant qualitative and quantitative changes with their repeated administration, it was of interest to determine whether the diazepam-induced change in dopamine turnover exhibits a similar alteration over the course of repeated administrations.

2. Effects of Repeated Diazepam Administrations on Dopamine Turnover in the Amygdala, Olfactory Tubercle, Nucleus Accumbens and Caudate Nucleus

Rats were injected with diazepam (1.8 mg/kg, i.p.) daily for 5 days. The last dose was given 15 minutes before the administration

TABLE 8

Effects of Acute Diazepam Administration on the α-Methyltyrosine-Induced Depletion of Dopamine in Forebrain Areas

Nuclei (N)	Steady State Concentration	Fractional Rate Constant	Calculated Rate of Formation
	ng/mg protein ± S.E.M.	h^{-1} ± S.E.M.	ng/mg protein/h
amygdaloid nuclei			
Lateral			
vehicle (15)	16.6±1.1	0.28±0.05	4.6
diazepam (23)	17.7±2.0	0.15±0.03*	2.6
Central			
vehicle (15)	28.0±3.7	0.39±0.05	10.9
diazepam (26)	25.5±2.9	0.27±0.06*	6.8
Cortical			
vehicle (14)	7.0±1.0	0.26±0.02	1.8
diazepam (22)	7.7±0.6	0.20±0.03*	1.5
Basal			
vehicle (15)	8.8±1.3	0.26±0.04	2.2
diazepam (22)	9.2±0.9	0.22±0.03	2.0
Medial			
vehicle (15)	3.9±0.7	0.28±0.02	1.1
diazepam (21)	4.6±0.8	0.27±0.06	1.2
Basal Posterior			
vehicle (16)	6.9±0.8	0.30±0.03	2.1
diazepam (21)	2.7±1.2	0.21±0.03*	1.2
rostral limbic nuclei			
Olfactory tubercle			
vehicle (13)	59.2±3.8	0.32±0.03	19.1
diazepam (17)	61.1±4.4	0.21±0.04*	12.8
Nucleus accumbens			
vehicle (14)	84.4±2.9	0.29±0.01	24.3
diazepam (16)	82.3±3.7	0.26±0.03	21.4
Caudate nucleus			
vehicle (14)	80.6±4.2	0.25±0.03	20.1
diazepam (16)	88.8±6.3	0.22±0.03	19.3

Diazepam (1.8 mg/kg) or vehicle (1 ml/kg) was injected 15 minutes before α-methyltyrosine (300 mg/kg, i.p.). Groups of 4-11 rats were sacrificed at 0, 45 and 90 minutes after α-methyltyrosine. Rate of formation of dopamine was estimated by multiplying the fractional rate constant of the decline of dopamine concentrations times the steady state dopamine content in each brain region. Steady state values for subjects receiving vehicle or diazepam were determined 90 minutes after injection of saline (N = 6-8).

*Indicates those values of the diazepam-pretreated animals which differ significantly ($p<.05$) from the vehicle-pretreated animals.

of α-methyltyrosine. The steady state dopamine concentrations of the brain nuclei examined were similar in animals chronically pretreated with diazepam or vehicle (Table 9). The rate constant of dopamine loss following synthesis inhibition was decreased following prolonged diazepam treatment in only two of the amygdaloid nuclei: the lateral and basal amygdaloid nuclei (Table 9). The rate of dopamine formation was similarly decreased in the olfactory tubercle and nucleus accumbens. These results suggest that, at least in the amygdala, some degree of tolerance may develop to the effects of diazepam on dopamine turnover over the course of its repeated administration. The rate-decreasing effect of chronic diazepam treatment on dopamine turnover in the olfactory tubercle and nucleus accumbens, but not in the caudate nucleus, closely resembles the results obtained by Fuxe et al. (1975) in these brain regions with higher doses of diazepam (5 and 10 mg/kg). Thus, the differential effects of acute vs. chronic diazepam administration on dopamine turnover in these latter areas may be partly the result of an accumulation of diazepam or its active metabolites with repeated injections.

Whether the observed effects of acute diazepam administration on dopamine turnover rates represent a direct action of the drug on dopaminergic neurons or are secondary to actions on other neurotransmitter systems cannot be discerned from the present evidence.

3. Effects of Picrotoxin on Diazepam-induced Changes in Dopamine Turnover in the Amygdala and Olfactory Tubercle

A number of investigators have postulated that benzodiazepines may exert a primary action on γ-aminobutyric acid-containing

TABLE 9

Effects of Repeated Diazepam Administration on the α-Methyltyrosine-Induced Depletion of Dopamine in Forebrain Areas

Nuclei (N)	Steady State Concentration	Fractional Rate Constant	Calculated Rate of Formation
	ng/mg protein ± S.E.M.	h^{-1} ± S.E.M.	ng/mg protein/h
amygdaloid nuclei			
Lateral			
vehicle (12)	15.3±1.6	0.31±0.05	4.8
diazepam (17)	15.9±1.1	0.19±0.03*	3.1
Central			
vehicle (14)	23.7±2.9	0.38±0.04	8.9
diazepam (22)	25.0±3.3	0.32±0.06	7.9
Cortical			
vehicle (13)	6.7±0.9	0.28±0.04	1.9
diazepam (19)	6.7±0.5	0.26±0.03	1.7
Basal			
vehicle (14)	8.1±0.9	0.25±0.02	2.0
diazepam (19)	8.3±1.2	0.17±0.04*	1.4
Medial			
vehicle (13)	3.7±0.3	0.26±0.05	1.0
diazepam (20)	4.1±0.7	0.31±0.05	1.3
Basal Posterior			
vehicle (13)	5.8±0.8	0.29±0.04	1.7
diazepam (18)	6.3±1.7	0.27±0.05	1.7
rostral limbic nuclei			
Olfactory tubercle			
vehicle (12)	52.6±4.1	0.29±0.02	15.1
diazepam (16)	59.1±5.4	0.18±0.03*	10.8
Nucleus accumbens			
vehicle (13)	90.2±7.0	0.32±0.02	29.0
diazepam (16)	88.8±3.8	0.26±0.01*	23.1
Caudate nucleus			
vehicle (13)	86.6±6.2	0.27±0.04	22.9
diazepam (15)	77.9±8.3	0.23±0.04	18.1

Diazepam (1.8 mg/kg) or vehicle was injected i.p. daily for 5 consecutive days. α-Methyltyrosine(300 mg/kg, i.p.) was administered 15 minutes following the last injection. Groups of 3-9 rats were sacrificed at 0, 45 and 90 min later. Other details of the calculations are the same as for Table 8 (N=7-9).

*Indicates those values from the chronic diazepam-pretreated animals which differ significantly ($p<0.05$) from their vehicle-pretreated controls.

neurons which in turn modulate the activity of other neuronal pathways utilizing different neurotransmitters (Costa et al., 1975; Haefely, 1977). If the diazepam-induced reduction in dopamine turnover is secondary to an enhanced γ-aminobutyric acid-mediated transmission, then the administration of a γ-aminobutyric acid receptor blocking agent, such as picrotoxin (Curtis and Johnston, 1974), should antagonize the effects of diazepam on dopamine turnover. Diazepam (1.8 mg/kg, i.p.) was administered 15 minutes, and picrotoxin (1 or 2 mg/kg, i.p.) 5 minutes, prior to α-methyltyrosine or saline. Animals were sacrificed 90 minutes after the administration of α-methyltyrosine and dopamine content was measured in those brain nuclei which exhibited a significant reduction in the rate constant for the decline of dopamine and, consequently, the calculated rate of formation of dopamine following acute diazepam treatment (Table 8). Ninety minutes after α-methyltyrosine treatment the dopamine concentration in the amygdaloid nuclei and olfactory tubercle of vehicle-pretreated rats was reduced to approximately 63% of the steady state value. A single injection of diazepam (1.8 mg/kg, i.p.) attenuated this α-methyltyrosine-induced depletion of dopamine concentrations to approximately 78% of the steady state value.

Picrotoxin, at a dose of 1 mg/kg, did not significantly alter the effect of diazepam on the α-methyltyrosine-induced depletion of dopamine concentrations in the central, lateral, cortical and basal posterior amygdaloid nuclei or the olfactory tubercle. However, a doubling of the dose of picrotoxin was found to counteract the depressant effect of diazepam on the α-methyltyrosine-induced reduction

of dopamine concentrations in most of these brain regions (Table 10). That is, the dopamine concentrations 90 minutes after α-methyltyrosine treatment were the same in animals receiving either vehicle or the combination of diazepam and picrotoxin. Neither dose of picrotoxin appreciably altered the steady state dopamine concentration in any region examined. The concentration of dopamine in the cortical amygdaloid nucleus after α-methyltyrosine administration was not significantly increased in diazepam-pretreated animals compared to those receiving vehicle.

The results suggest that a picrotoxin-sensitive mechanism may mediate the effects of acute diazepam administration on dopamine turnover in the amygdala and olfactory tubercle. However, although the steady state dopamine concentrations in these brain regions were unaltered by this large dose of picrotoxin, the direct effect of picrotoxin on the dopamine depletion evoked by α-methyltyrosine was not examined. Nevertheless, both the behavioral (Waddington and Longden, 1977) and biochemical (Keller et al., 1976) expression of the interaction between benzodiazepines and dopaminergic systems have been shown to be negatively influenced by the γ-aminobutyric acid receptor blocking agents. Moreover, a low dose of diazepam (1 mg/kg) has been reported to decrease γ-aminobutyric acid turnover in subcortical nuclei (Mao et al., 1977) and the limbic cortex (Pericic et al., 1977). The amygdaloid nuclei are rich in γ-aminobutyric acid and its synthesizing enzyme glutamate decarboxylase (Ben-Ari et al., 1976; Tappaz et al., 1976). Thus, a diazepam-induced facilitation of γ-aminobutyric acid-mediated transmission could potentially influence the activity of dopamine-containing neurons terminating in the amygdala by a presynaptic action

TABLE 10

Effects of Picrotoxin on the Diazepam-induced Attenuation of the α-Methyltyrosine-induced Depletion of Dopamine in the Amygdala and Olfactory Tubercle

Nuclei Pretreatment	N	Treatment	
		Saline[a]	α-Methyltyrosine
		ng/mg protein ± S.E.M.	
Lateral amygdala			
vehicle	5		11.4±1.2 (66)
diazepam	7	17.2±1.3	14.2±0.9 (83)*
diazepam + picrotoxin	6		12.7±1.6 (74)
Central amygdala			
vehicle	5		15.1±1.1 (56)
diazepam	8	27.2±3.6	19.5±1.8 (72)*
diazepam + picrotoxin	6		16.0±1.7 (59)
Cortical amygdala			
vehicle	5		4.7±0.6 (65)
diazepam	8	7.2±0.9	5.7±0.8 (79)
diazepam + picrotoxin	6		4.5±0.8 (63)
Basal posterior amygdala			
vehicle	5		4.0±0.5 (64)
diazepam	8	6.3±0.6	5.4±0.6 (85)*
diazepam + picrotoxin	6		3.7±0.3 (59)
Olfactory tubercle			
vehicle	5		34.6±3.9 (61)
diazepam	5	56.6±4.6	46.3±5.5 (82)*
diazepam + picrotoxin	6		40.2±6.1 (71)

Animals received vehicle (0.5% methylcellulose) or diazepam (1.8 mg/kg) intraperitoneally 105 minutes prior to sacrifice. An additional group of rats received picrotoxin (2 mg/kg, i.p.) 10 minutes following diazepam treatment (95 minutes before sacrifice). Ninety minutes before sacrifice, half of the animals in each of the three groups received α-methyltyrosine (300 mg/kg, i.p.) and the other half received saline (1 ml/kg).

Numbers in parentheses represent the α-methyltyrosine values expressed as a percentage of the combined pretreatment values obtained in saline injected animals for each brain region.

[a]As the dopamine concentrations in the various brain nuclei of rats administered saline were similar regardless of the pretreatment these values were combined.

*Designates those values which differ significantly ($p<.05$) from vehicle-injected controls. N = number of samples assayed.

on midbrain cell bodies or via pre- and postsynaptic inhibitory mechanisms at the terminals.

F. Effects of Diazepam on 5-Hydroxytryptamine Neuronal Pathways

The effects of diazepam on the steady state concentration of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid were determined in the amygdaloid nuclei as well as other nuclei which have been shown by histochemical fluorescent mapping techniques to contain a major serotonergic input (Aghajanian et al., 1973). The microdissected hippocampal tissue samples contained all the layers of the hippocampus. Hypothalamic samples represent tissue collected from the region of the anterior hypothalamic nucleus. 5-Hydroxytryptamine and 5-hydroxyindoleacetic acid were simultaneously assayed in each brain region examined. The effects of single (1.8 mg/kg) and repeated (1.8 mg/kg/day for 5 days) intraperitoneal diazepam injections were evaluated since several actions of the benzodiazepines deemed relevant to their properties as anxiolytics undergo appreciable change over the course of repeated administration (see Introduction).

1. Effects of Acute and Repeated Diazepam Administration on Steady State 5-Hydroxytryptamine and 5-Hydroxyindoleacetic Acid Concentrations in the Amygdala, Hypothalamus and Hippocampus

The endogenous 5-hydroxytryptamine concentrations of the amygdaloid nuclei, hippocampus and hypothalamus from animals receiving vehicle or either acute (1.8 mg/kg) or chronic (1.8 mg/kg/day for 5 days) diazepam administration are listed in Table 11. Animals (n=8) in each of the three groups were sacrificed 30 minutes following the last pretreatment and tissue samples of pairs of rats in each group were

TABLE 11

Effects of Acute and Chronic Diazepam Administration on the Steady State Concentration of 5-Hydroxytryptamine (5-HT) in the Amygdala, Hippocampus and Hypothalamus

Nuclei	Treatment		
	Vehicle	Acute Diazepam	Chronic Diazepam
	ng 5-HT/mg protein		
Amygdaloid nuclei			
lateral	18.8±2.0	18.5±2.1	21.7±3.0
central	15.9±1.8	16.8±1.8	21.3±2.8
cortical	17.0±1.1	16.1±0.9	24.1±2.9*
basal	14.3±1.5	16.7±1.4	17.2±1.5
medial	18.6±2.3	17.7±2.0	21.0±2.6
basal posterior	18.2±2.0	20.6±3.1	20.2±3.8
medial posterior	17.1±3.1	16.9±2.6	17.9±2.3
posterior	13.9±2.2	12.8±1.9	16.1±2.5
hippocampus	4.2±0.6	3.6±0.7	4.6±0.7
hypothalamus	19.2±1.7	18.3±2.1	23.6±3.3

Animals received vehicle (0.5% methylcellulose) or diazepam (1.8 mg/kg for one or 5 consecutive days) 30 minutes prior to sacrifice. Tissue samples collected from pairs of animals were pooled. Values represent the means ± 1 SEM for 4 separate determinations.

*Indicates a significant increase ($p<0.05$) in 5-HT concentrations compared to vehicle injected controls.

pooled prior to assay. The 5-hydroxytryptamine values for the amygdaloid nuclei and hippocampus of vehicle-injected rats are in good agreement with those reported by Saavedra (1977). The value for the anterior hypothalamic nucleus is somewhat greater than that found by this same author (10.2±2.3 ng 5-HT/mg protein). A single injection of diazepam (1.8 mg/kg, i.p.) yielded no consistent effect on 5-hydroxytryptamine concentrations in any of the brain regions examined (Table 11). Upon repeated administration, diazepam produced a slight increase in the endogenous 5-hydroxytryptamine content, with the increase being statistically significant in the cortical amygdaloid nucleus.

Table 12 illustrates the steady state 5-hydroxyindoleacetic acid concentrations of the amygdaloid nuclei, hippocampus and hypothalamus from animals receiving vehicle or a single diazepam administration. Comparative data concerning the endogenous 5-hydroxyindoleacetic acid content in the amygdaloid nuclei was not found, although the value obtained in the hypothalamus is essentially the same as that reported by Curzon and Green (1970). Acute diazepam administration (1.8 mg/kg, i.p.) did not appreciably influence the 5-hydroxyindoleacetic acid concentrations in any of the brain regions examined. However, 30 minutes following the last of five consecutive daily diazepam treatments steady state 5-hydroxyindoleacetic acid concentrations were decreased, significantly so in the lateral, central, cortical and medial amydaloid nuclei and the anterior hypothalamus (Table 13). This finding is in contrast to the enhanced 5-hydroxyindoleacetic acid content of various brain regions reported by Rastogi et al. (1977) using a large acute dose of diazepam (10 mg/kg) and may reflect

TABLE 12

The Effects of Acute Diazepam on the Rate of Decline of the 5-Hydroxyindoleacetic Acid (5-HIAA) in Forebrain Areas of Pargyline-treated Rats

Nuclei (N)	Steady State Concentration	Fractional Rate Constant	Calculated Rate of Formation of 5-HT
	ng/mg protein ± S.E.M.	h^{-1} ± S.E.M.	ng/mg protein/h
amygdaloid nuclei			
Lateral			
vehicle (14)	15.2±1.1	0.76±0.09	11.6
diazepam (17)	15.0±1.7	0.81±0.11	12.2
Central			
vehicle (14)	13.7±1.6	0.73±0.07	10.0
diazepam (18)	14.6±1.9	0.73±0.09	10.7
Cortical			
vehicle (13)	14.3±1.0	0.82±0.10	11.7
diazepam (17)	16.2±2.3	0.76±0.11	12.3
Basal			
vehicle (13)	12.6±1.3	0.77±0.08	9.7
diazepam (17)	13.9±1.1	0.83±0.10	11.5
Medial			
vehicle (13	13.8±1.7	0.87±0.10	12.0
diazepam (17)	14.4±1.5	0.80±0.12	11.5
Basal Posterior			
vehicle (14)	11.6±1.3	0.68±0.09	7.9
diazepam (18)	11.7±1.8	0.73±0.06	8.5
Medial Posterior			
vehicle (13)	15.0±2.8	0.66±0.08	9.9
diazepam (16)	14.6±1.9	0.69±0.11	10.0
Posterior			
vehicle (14)	11.7±1.2	0.70±0.07	8.2
diazepam (18)	11.8±1.6	0.75±0.08	8.9
Hippocampus			
vehicle (16)	4.3±0.6	0.62±0.08	2.7
diazepam (20)	3.9±0.5	0.64±0.11	2.5
Hypothalamus			
vehicle (14)	11.7±1.9	0.74±0.08	8.7
diazepam (18)	12.9±1.2	0.73±0.06	9.4

Diazepam (1.8 mg/kg) or vehicle (0.5% methylcellulose, 1 ml/kg) was injected i.p. 15 minutes before pargyline. Groups of 3-7 rats were sacrificed at 0, 20 and 40 min after pargyline administration (75 mg/kg).

Rate of formation of 5-hydroxytryptamine (5-HT) was estimated by multiplying the fractional rate of 5-HIAA loss by the endogenous 5-HIAA concentration in the corresponding controls. Steady state values for subjects receiving vehicle or diazepam were determined 30 min following an i.p. injection of saline (N=8).

TABLE 13

The Effects of Repeated Diazepam Administration on the Rate of Decline of the 5-Hydroxyindoleacetic Acid in Forebrain Areas of Pargyline-treated Rats

Nuclei (N)	Steady State Concentration	Fractional Rate Constant	Calculated Rate of Formation of 5-HT
	ng/mg protein ± S.E.M.	h^{-1} ± S.E.M.	ng/mg protein/h
amygdaloid nuclei			
Lateral			
vehicle (14)	15.2±1.1	0.73±0.08	11.1
diazepam (18)	11.6±2.0*	0.50±0.10*	5.8
Central			
vehicle (14)	13.7±1.6	0.70±0.11	9.6
diazepam (21)	9.8±1.1*	0.41±0.12*	4.1
Cortical			
vehicle (13)	14.3±1.0	0.79±0.07	11.3
diazepam (19	9.6±1.3*	0.39±0.08*	3.8
Basal			
vehicle (13)	12.6±1.3	0.69±0.09	8.7
diazepam (20)	11.9±1.2	0.42±0.11*	5.0
Medial			
vehicle (13)	13.8±1.7	0.81±0.10	11.2
diazepam (18)	9.3±1.0*	0.46±0.08*	4.3
Basal Posterior			
vehicle (14)	11.6±1.3	0.71±0.09	8.2
diazepam (20)	10.3±1.0	0.46±0.07*	4.7
Medial Posterior			
vehicle (13)	15.0±2.8	0.65±0.07	9.8
diazepam (20)	12.6±1.8	0.43±0.07*	5.4
Posterior			
vehicle (14)	11.7±1.2	0.68±0.05	8.0
diazepam (20)	10.9±1.3	0.56±0.08	6.1
Hippocampus			
vehicle (16)	4.3±0.6	0.65±0.09	2.8
diazepam (22)	3.8±0.6	0.61±0.07	2.3
Hypothalamus			
vehicle (16)	11.7±1.3	0.70±0.07	8.2
diazepam (21)	8.6±0.8*	0.44±0.07*	3.9

Diazepam (1.8 mg/kg) or vehicle (1 ml/kg) was administered daily for 5 consecutive days. Pargyline was administered 15 min after the last injection of diazepam or vehicle and groups of 3-8 rats were sacrificed at 0, 20 and 40 min later. Other details of the analysis were the same as indicated for Table 12.

*Indicates those values from the chronic diazepam-pretreated animals which differ significantly ($p<0.05$) from their vehicle-pretreated controls.

dose-related differences in interfering with the normal processes for transport of 5-hydroxyindoleacetic acid from the brain (Chase et al., 1970) or methodological differences (e.g., dissimilar pretreatment times).

It thus would appear that subtle differences may exist between the effects of acute vs. chronic diazepam administration on 5-hydroxytryptamine-containing neurons. A comparison of the effects of acute vs. chronic treatment on the estimated rate of 5-hydroxytryptamine turnover in the aforementioned brain nuclei was performed to more critically assess this possibility.

2. Effects of Acute Diazepam Administration on 5-Hydroxytryptamine Turnover in the Amygdala, Hippocampus and Hypothalamus

The initial rate of loss of the biogenic amine metabolites after blocking their formation has proven to be a reliable estimate of the rate of formation of the amine at a steady state in neuronal tissue (Wiesel et al., 1973; Wilk et al., 1975; Tozer et al., 1966). The method is most appropriate for estimates of 5-hydroxytryptamine turnover, as the major pathway of metabolism of this amine is via deamination to 5-hydroxyindoleacetic acid. While minute amounts of 5-methoxytryptamine have been identified in various regions of the rat brain (Green et al., 1973), O-methylation is not considered to contribute significantly to the normal metabolism of 5-hydroxytryptamine.

Inhibition of the oxidative deamination of 5-hydroxytryptamine subsequent to the administration of the monoamine oxidase inhibitor pargyline (75 mg/kg, i.p.) produced an exponential decline of 5-hydroxyindoleacetic acid in all brain nuclei examined. Representative examples are illustrated in Figure 22. The effects of a single dose of diazepam (1.8 mg/kg, i.p.) on the rate constants for the

Figure 22. The logarithmic decline of 5-hydroxyindoleacetic acid (5-HIAA) concentrations in various brain nuclei following the administration of pargyline (75 mg/kg, i.p.). Animals were sacrificed immediately before (0 time) or 20 and 40 minutes after treatment with pargyline. Symbols represent means and vertical lines ± 1 S.E.M. as determined from 5-7 animals.

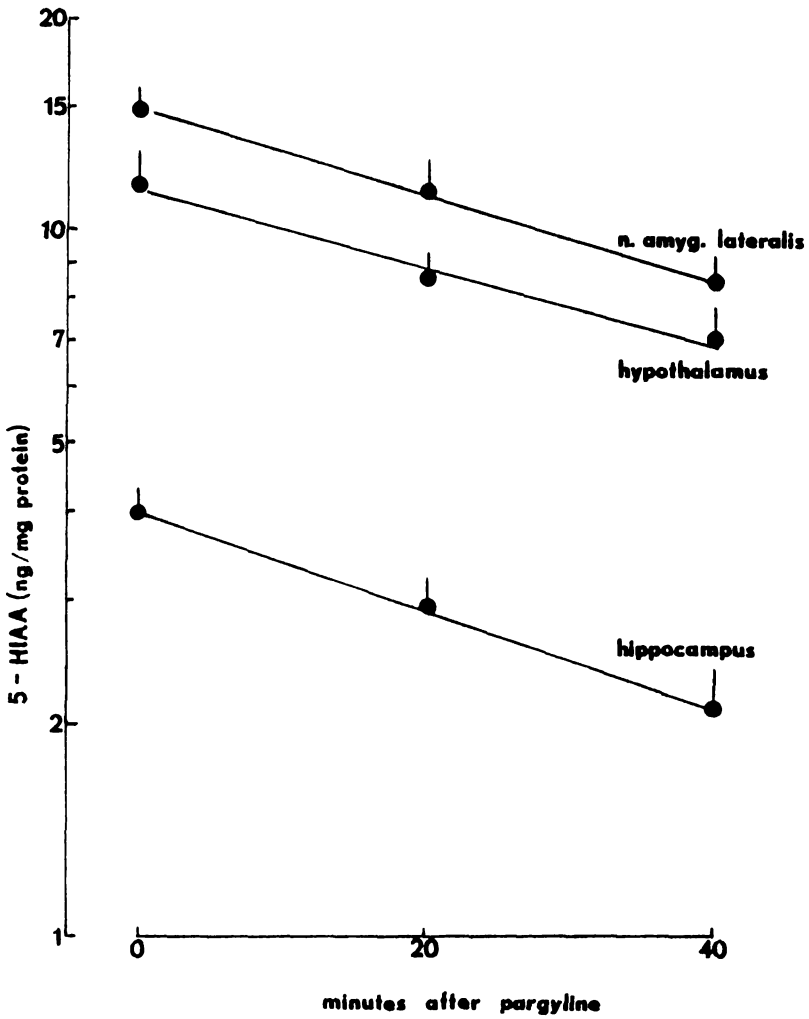

20
15
10
7
5
2
1
5 - HIAA (ng/mg protein)
n. amyg. lateralis
hypothalamus
hippocampus
0
20
40
minutes after pargyline

Figure 22

pargyline-induced decline of 5-hydroxyindoleacetic acid in the amygdaloid nuclei, hippocampus and hypothalamus are listed in Table 12. Acute diazepam administration produced no consistent effect on the rate constant for the decline of 5-hydroxyindoleacetic acid concentrations and, assuming steady state kinetics, the calculated rate of formation of 5-hydroxytryptamine in any brain region studied. The values for the fractional rate of 5-hydroxyindoleacetic acid loss evoked by pargyline in vehicle-pretreated rats are relatively constant between 5-hydroxytryptamine regions and are essentially the same as those reported by Tozer et al. (1966) in rat whole brain. The apparent inability of acute diazepam treatment to alter 5-hydroxytryptamine turnover fails to corroborate the findings of several investigators (Wise et al., 1972; Stein et al., 1975; Lippmann and Pugsley, 1974; Dominic et al., 1975) using radioisotopic tracer techniques. This discrepancy may relate to differences in methods employed to estimate turnover, type and dose of benzodiazepine studied or anatomical level of dissection (i.e., discrete nuclei vs. whole brain determinations).

3. Effects of Repeated Diazepam Administration on 5-Hydroxytryptamine Turnover in the Amygdala, Hippocampus and Hypothalamus

The chronic administration of diazepam (1.8 mg/kg/day for 5 days) evoked a qualitatively different pattern of effects on 5-hydroxytryptamine turnover in the amygdala, hippocampus and hypothalamus than that seen following acute administration (Table 13). With the single exception of the posterior amygdaloid nucleus, prolonged diazepam treatment significantly decreased the rate constant for the pargyline-induced decline of 5-hydroxyindoleacetic acid in the amygdaloid nuclei.

The rate constant for the depletion of the 5-hydroxyindoleacetic acid concentration in the anterior hypothalamus and, consequently, the estimated rate of 5-hydroxyindoleacetic acid formation were similarly decreased (Table 13). The diazepam-induced decrease in the synthesis rate of 5-hydroxyindoleacetic acid in these brain nuclei also reflects the variable reduction (5-28%) in endogenous 5-hydroxyindoleacetic acid concentrations produced by prolonged diazepam treatment (Table 13). In contrast to the amygdala and anterior hypothalamus, the rate constant for the pargyline-induced depletion of hippocampal 5-hydroxyindoleacetic acid content was unaffected by repeated diazepam administration. The various amygdaloid nuclei were influenced in a similar manner by repeated diazepam treatment with no differential response observed for the corticomedial and basolateral subdivisions.

These results obtained in the amygdala and anterior hypothalamus following repeated diazepam administration differ from those observed after the single injection of diazepam. The possibility exists that the decrease in 5-hydroxytryptamine turnover rate in these brain regions seen after prolonged diazepam administration may be due to the accumulation of diazepam and/or active metabolites. To evaluate this possibility the effects of a single dose of 5.6 mg/kg of diazepam on the pargyline-induced depletion of 5-hydroxyindoleacetic acid in the more rostral amygdaloid nuclei (i.e., the lateral central, basal, cortical and medial amygdaloid nuclei) and the anterior hypothalamus were examined. Acute administration of this relatively large dose of diazepam did not increase the endogenous 5-hydroxyindoleacetic acid concentration in the amygdaloid and anterior hypothalamic nuclei (Table 14). Thus, the effect of a larger dose of diazepam on the steady state

TABLE 14

Effects of Diazepam (5.6 mg/kg) on the Pargyline-induced Depletion of 5-Hydroxyindoleacetic Acid in the Rostral Amygdala and Anterior Hypothalamus

Nuclei Pretreatment	N	Treatment Saline	Pargyline
		ng/mg protein	
Amygdaloid nuclei			
Lateral			
vehicle	7	14.7±1.2	8.6±1.0 (59)
diazepam	8	16.3±1.7	11.0±1.9 (68)
Central			
vehicle	7	13.9±1.1	7.2±1.1 (52)
diazepam	11	16.0±2.0	11.2±1.4 (70)*
Cortical			
vehicle	7	12.9±0.7	7.0±0.8 (54)
diazepam	9	15.1±1.8	11.3±1.3 (75)*
Basal			
vehicle	7	13.6±0.9	8.1±0.9 (60)
diazepam	9	14.0±1.2	10.8±0.8 (77)*
Medial			
vehicle	7	13.1±1.4	7.6±0.9 (58)
diazepam	8	13.9±1.6	9.5±0.8 (72)*
Hypothalamus			
vehicle	6	12.6±1.0	7.7±0.6 (61)
diazepam	9	14.3±1.8	10.3±1.3 (72)*

Rats received diazepam (5.6 mg/kg, i.p.) or vehicle 55 min prior to sacrifice. Half of the animals in each group received saline and the other half received pargyline (75 mg/kg, i.p.) 40 minutes before sacrifice. Each value represents the mean ± 1 S.E.M. of 6-11 determinations.

Numbers in parentheses represent the pargyline values of vehicle and diazepam-pretreated animals expressed as a percent of the saline values of the corresponding controls.

*Indicates those values of the percent depletion in diazepam-pretreated animals which are significantly different from vehicle-pretreated animals ($p<0.05$). N = number of animals receiving either saline or pargyline.

5-hydroxyindoleacetic acid concentrations in these brain regions is opposite to that seen with repeated administration of a lesser dose (Table 13). Nevertheless, an acute dose of 5.6 mg/kg attenuated the depletion of 5-hydroxyindoleacetic acid concentrations evoked by pargyline in the central, cortical, basal and medial amygdaloid nuclei as well as in the anterior hypothalamic nuclei. Although the pargyline-induced decline of 5-hydroxyindoleacetic acid in the lateral amygdaloid nucleus was similarly decreased, the difference was not significant.

The finding of significantly more 5-hydroxyindoleacetic acid in the amygdala and anterior hypothalamus of pargyline-treated rats following either multiple injections of diazepam (1.8 mg/kg) or an acute dose of 5.6 mg/kg suggests that the apparent decrease in 5-hydroxytryptamine turnover elicited by this chronic regimen may be attributed to an accumulation of the drug and/or active metabolites. However, the mechanism underlying the reduced decline of 5-hydroxyindoleacetic acid following monoamine oxidase inhibition may be different for the two dosing situations. This possibility is strengthened by the observation that, unlike repeated diazepam administration, a larger dose given acutely variably increased endogenous 5-hydroxyindoleacetic acid concentrations. Such an elevation may reflect an interference with an acid transport system which removes 5-hydroxyindoleacetic acid from the brain (Neff et al., 1967). If so, the administration of 5.6 mg/kg of diazepam in conjunction with monoamine oxidase inhibition would tend to overestimate the depressant effect, if any, of this dose on the rate of 5-hydroxytryptamine synthesis as estimated by this technique.

DISCUSSION

A. Utility of Conflict (Punishment) Procedures in Establishing Relevant Correlates of the Therapeutic Action of Antianxiety Drugs

Information regarding the mechanism of the therapeutic action of a drug is typically predicated on the use of an animal correlate of this action which possesses measurable behavioral, biochemical or physiological parameters. Obviously, the amount and value of the information so derived depends upon the predictive reliability of the animal model utilized. Predetermined limitations to this approach which must be considered include differences in the mechanism and rate of drug metabolism between man and animal as well as differential sensitivities of physiological and psychic functions to drug-induced alterations. As technical and ethical implications preclude the direct measurement of human anxiety, animal models have been devised which attempt to define this emotional state in a behavioral framework. The behavioral action which seems to be the most relevant for benzodiazepine antianxiety agents is the remarkable ability of this class of drugs to reinstate behavior, either operantly conditioned or innate, previously suppressed by punishment. Operationally, the utility of this indirect estimation is based on the supposition that the emotional state (anxiety) can be evaluated objectively and quantitatively if it is found to inhibit in conditioned animals a recordable activity which they have been trained to perform.

From the large number of descriptive accounts of the disinhibitory effects of benzodiazepines on the suppression of positively reinforced behavior produced by aversive stimuli has evolved a proportionately large number of interpretations of the mechanism of this effect. Wuttke and Kelleher (1970) proposed that the ability of benzodiazepines to release punishment-suppressed responding was not a unique property but merely represented an example of how these agents can increase all low rates of responding, irrespective of how these low rates were generated. However, an analogous degree of response suppression which has been shown to develop during stimuli that precede the non-contingent presentation of food ("positive conditioned suppression") was unaffected by diazepam and chlordiazepoxide (Miczek, 1973; Poling et al., 1977). From a motivational point of view, the response suppression engendered by response-contingent punishment can be considered to represent a balance between the motivational strengths of the positive and negative reinforcers which additively interact to determine the frequency of occurrence of the recorded response (hence the term "conflict behavior"). The antipunishment activity of the benzodiazepines can therefore be interpreted as the result of a drug-induced shift in food or water motivation and/or the motivational properties of the aversive stimulus. Effects of benzodiazepines on water consumption are equivocal: no effect (Falk and Burnide, 1970) as well as significant enhancements of drinking (Knowler and Ukena, 1973) have been described. Moreover, in those studies which report a benzodiazepine-induced increase in water consumption, it is not possible to ascribe this change to a direct effect on regulatory centers or as the result

of a drug-induced reduction of the inhibitions associated with drinking behavior within the confines of the experimental design.

Several observations argue against a benzodiazepine-induced alteration in pain thresholds being causally related to their antipunishment effects. Benzodiazepines are not very effective analgesics, and morphine, a potent antinocioceptive agent in animal studies, is without effect on behavior suppressed by electric shock (Geller et al., 1963). Furthermore, the facilitatory effects of benzodiazepines on suppressed responding appear to generalize to situations in which the aversive stimulus is in some form other than electric shock, i.e., nonreinforcement (Heise et al., 1970) or aversively adulterated drinking solutions (Falk and Burnidge, 1970). In addition, although both shock removal and benzodiazepine treatment increase the response rate in conflict situations, profound qualitative differences exist in terms of the rate at which the increase develops, i.e., gradual vs. abrupt, respectively (Ts'o and Chenoweth, 1976).

While a number of alternative explanations have been advanced to account for the effects of benzodiazepines on the behaviorally suppressive effects of punishment, such as drug-induced alterations in learning and memory, arousal and response perseveration, none have reasonably withstood closer scrutiny.

A frequently mentioned criticism of the use of this animal model in predicting antianxiety activity is that the suppression of positively rewarded behavior bears no obvious relationship to anxiety. The demonstration of components of anxiety inherent to the numerous behavioral paradigms in which anti-punishment or disinhibitory effects of the benzodiazepines have been demonstrated is a task which perhaps

can best be addressed by psychologists. It can be alternatively proposed that the demonstration of an apparent relevance between the effects of benzodiazepines on conflict procedures and their use in the pharmacotherapy of anxiety is not a necessary requirement and may in fact place too much strain on this model. Rather, the merit or power of this model for the pharmacologist may be best expressed in pharmacological terms: how strong is the correlation between the potency of members of the class of benzodiazepines and other psychotropic agents in alleviating the behaviorally suppressive effects of punishment in the laboratory and their therapeutic potency in the clinic? In an attempt to determine the relevance of pharmacological activity in the rat conflict-punishment procedure to their clinical application, Cook and Davidson (1973) compared the minimum effective anticonflict dose of various compounds to their average daily dose found effective in the clinical treatment of psychoneurotic disorders. Their relative clinical potency was shown to correlate very closely with their potency in this animal model. Although the reliability of this correlation is tempered by inherent difficulties in the clinical evaluation of anti-anxiety agents, the use of conflict-punishment procedures appears to have some validity in performing the transition from the subjective to the quantitative. Therefore, the value of this animal model lies not in whether the behavioral effect of a drug is phenomenologically similar in animal and man but is based on a concept of relative differential potencies of various classes of psychoactive drugs.

B. Effects of Benzodiazepines on Experimentally-induced Conflict Behavior

As the evaluation of drug effects on punishment-suppressed responding (conflict behavior) was performed almost exclusively with the use of an unconditioned consummatory response (tube-licking) to generate the behavioral baseline, the question arises as to how the data obtained with this experimental design compare to those obtained with more complex paradigms utilizing operantly conditioned behaviors. The relative potency of the limited number of benzodiazepines examined in effecting an increase in punishment-suppressed responding is in complete agreement with the rank order values established with an operant response for a food reward (Cook and Sepinwall, 1975; Cook and Davidson, 1973; Geller, 1964). The sensitivity of the procedure employed in this dissertation, rats trained to suppress the unconditioned consummatory behavior of imbibing water, was comparable to methods using operantly conditioned behaviors (Cook and Sepinwall, 1975) and greater than that of procedures utilizing untrained animals (Vogel et al., 1971; Lippa et al., 1977). As regards the estimation of the dose at which nonspecific sedative activity occurs (i.e., the dose at which significant depression of unpunished water consumption first occurs), the values obtained with the benzodiazepines examined (Table 2) are in general agreement with values obtained with more complex behavioral procedures (Cook and Sepinwall, 1975).

The reliability of the conditioned suppression of drinking paradigm in evaluating mechanism of action hypotheses regarding the benzodiazepines is dependent upon the ability of this conflict procedure to

detect and differentiate compounds shown to be devoid of clinical antianxiety activity, yet yield false positives when subjected to behavioral screening procedures. By means of example, Beer et al. (1972) proposed that the anxiolytic activity of the benzodiazepines may be a manifestation of their ability to inhibit the activity of cyclic adenosine monophosphate phosphodiesterase, the enzyme that hydrolyzes this cyclic nucleotide. Their hypothesis was partially based on the finding of anticonflict activity associated with the administration of various methylxanthines, which are potent in vitro phosphodiesterase inhibitors. However, the findings of Beer et al. (1972), using the suppression of an unconditioned behavior in untrained (i.e., experimentally naive) animals, was not substantiated by Cook and Sepinwall (1975) using a more complex conflict procedure. Similarly, caffeine, a methylxanthine with phosphodiesterase inhibiting properties, was without any significant anticonflict activity in the present study (Table 2). Therefore, unlike the benzodiazepines, the purported anticonflict activity of the methylxanthines fails to generalize to other conflict methods which develop stable baseline rates of punished and unpunished responding and suggests that this effect may be unique to the experimental design of Beer et al. (1972).

It would thus appear that, at least on the surface, the conditioned suppression of drinking paradigm satisfies the criteria of Tedeschi (1969) in assessing the value of an animal model in predicting potential antianxiety activity in that it appears to be sensitive and selective and is capable of distinguishing side effects. Furthermore, the anticonflict profiles of the benzodiazepines fail to show tolerance

development in this test and correlate favorably with their relative potency as antianxiety agents in the clinic.

C. Feasibility of Pharmacological Manipulation of Brain 5-Hydroxytryptamine Activity; Effects on Conflict Behavior

The administration of agents reputed to affect 5-hydroxytryptamine mechanisms in conjunction with experimentally-induced conflict situations has generated a considerable body of evidence which theoretically implicates 5-hydroxytryptamine systems in the anxiety-reducing actions of benzodiazepines. The usual interpretation of these findings centers around a reduction of the functional activity of 5-hydroxytryptamine-containing neurons by the benzodiazepines as being central to their antipunishment (anticonflict) effects. Thus, this hypothesis has been derived largely from the reported anticonflict activity of the peripheral 5-hydroxytryptamine antagonists (Graeff and Schoenfeld, 1970; Geller et al., 1974; Cook and Sepinwall, 1975; Stein et al., 1975), the purported antagonism of 5-hydroxytryptamine actions at postsynaptic receptor sites by these agents reducing activity of 5-hydroxytryptamine at these same sites. Therefore, benzodiazepines and 5-hydroxytryptamine antagonists appeared to exert similar effects on conflict behavior. However, an evaluation of three peripheral 5-hydroxytryptamine antagonists for anticonflict activity in the conditioned suppression of drinking yielded no clear-cut anticonflict activity. Methysergide, which reportedly increases punished responding when administered one minute or less before testing (although no evidence exists in support of such a short time to peak anti-5-hydroxytryptamine activity) was found to be without significant anticonflict activity when tested at this pretreatment interval (Figure 12). When given 30 minutes prior to

testing, methysergide was similarly without effect on behavior suppressed by response contingent (Figure 12) as well as noncontingent (Table 4) shock, the latter observation being in agreement with the findings of Miczek and Luttinger (1978). Cyproheptadine was also devoid of significant anticonflict activity (Figure 14) while cinanserin enhanced punished responding only in doses that affected unpunished water consumption (Figure 15).

The negative findings obtained with the peripheral 5-hydroxytryptamine antagonists in conditioned suppression of drinking does not necessarily exclude an anticonflict effect of benzodiazepines as being mediated by decreased activity of 5-hydroxytryptamine systems in the central nervous system. The possibility exists that the physiologically relevant 5-hydroxytryptamine receptors at serotonergic synapses in the central nervous system are not equivalent to 5-hydroxytryptamine receptors located in target organs in the periphery. The technique considered by some to be most appropriate for the assessment of potential transmitter agonists or antagonists is that of microiontophoresis (Padjen and Bloom, 1975). Neither systemic administration nor microiontophoretic application of these putative 5-hydroxytryptamine antagonists was found to antagonize the depressant effects of iontophoretically applied 5-hydroxytryptamine on the spontaneous electrical activity of neurons in areas with a dense and uniform 5-hydroxytryptamine input (Haigler and Aghajanian, 1974; Sharma, 1977). Studies concerning the neurochemical effects of cyproheptadine and methysergide on 5-hydroxytryptamine systems in the central nervous system indicate little or no effect on biochemical estimates of the dynamics of

5-hydroxytryptamine-containing neurons (D'amico et al., 1976; Jacoby et al., 1978) until higher doses are reached, at which point the observed effects (Bürki et al., 1978; Kehr, 1977) are similar to those observed with 5-hydroxytryptamine agonists and reuptake blockers (Hamon et al., 1974, 1976). The selectivity of the putative 5-hydroxytryptamine antagonists, especially at high doses, for 5-hydroxytryptamine mediated systems is tenuous at best in light of the reported antidopaminergic, anticholinergic and antihistaminic activity of members of this group of compounds (van Riezen, 1972; Oppizzi et al., 1977; Stone et al., 1961). Still, data obtained from behavioral studies (Clineschmidt and Lotti, 1974; von Riezen, 1972) and attempts to elaborate mechanisms of anorectics (Samanin et al., 1977) provide some support for an antagonism of the actions of 5-hydroxytryptamine in the rat brain by the peripheral 5-hydroxytryptamine antagonists. Apparently, peripheral 5-hydroxytryptamine receptors do not appear to be valid models for the study of 5-hydroxytryptamine receptors in the brain and therefore these agents should be employed with caution as selective 5-hydroxytryptamine blocking agents in behavioral studies.

Schoenfeld (1976) theorized that, if the anticonflict activity of the benzodiazepines was causally related to a reduction in the functional activity of 5-hydroxytryptamine, the inhibition of serotonergic neuronal activity by lysergic acid diethylamide (Aghajanian et al., 1968) should also exert anticonflict effects. Indeed, when administered 30 minutes prior to behavioral testing lysergic acid diethylamide (1 and 3 μg/kg) was found to significantly increase punished responding in a conflict procedure similar to that of Beer et al.

(1972) using untrained, experimentally naive animals (Schoenfeld, 1976). As the half-life of lysergic acid diethylamide in the rat is very short and the depressant effect of systemically administered lysergic acid diethylamide on raphé neurons is very rapid and short-lived (Aghajanian et al., 1968), it would appear that the anticonflict effect of these small doses of lysergic acid diethylamide, measured 30 minutes following drug administration, may reflect drug-induced alterations in central nervous system physiology and pharmacology other than depression of the spontaneous discharge rate of raphé neurons. Moreover, when administered one minute prior to testing, lysergic acid diethylamide exhibited no anticonflict activity and, in fact, appeared to increase the suppressive effects of punishment on water consumption (Figure 18). Thus, the question arises as to whether or not the reported anticonflict activity for lysergic acid diethylamide (Schoenfeld, 1976), like caffeine (Beer et al., 1972), may be unique to the experimental design of the conflict procedure used in both cases.

The use of precursors to increase the tissue concentrations of specific neurotransmitters is a technique widely used in neurobiology to elucidate the importance of specific populations of neurons in brain function. The administration of the 5-hydroxytryptamine precursor, 5-hydroxytryptophan, has been used to further define the role of 5-hydroxytryptamine in conflict behavior. The intraperitoneal administration of 5-hydroxytryptophan reportedly reversed the anticonflict effects of the peripheral 5-hydroxytryptamine antagonist cinanserin (Geller et al., 1974) as well as that seen following 5-hydroxytryptamine depletion by p-chlorophenylalanine (Geller and Blum, 1970).

However, no mention was made of the fact that in both cases the rate of unpunished responding was significantly depressed following 5-hydroxytryptophan administration, suggesting a direct or indirect nonspecific depressant action of 5-hydroxytryptophan as operating to reinstate the suppressed responding. A source of indirect effects here relates to the use of food reinforcement in the Geller procedure, since increased activity in brain serotonin pathways reduces motivation for food. This point will be elaborated later.

An examination of the effects of 5-hydroxytryptophan administration on the rate of punished responding showed the 5-hydroxytryptamine precursor to further reduce responding suppressed by response-contingent shock (Figure 16). The high extracerebral aromatic acid decarboxylase activity makes it probable that the decarboxylation of systemically administered 5-hydroxytryptophan to 5-hydroxytryptamine occurs primarily in the periphery with only small amounts reaching the brain to contribute to endogenous 5-hydroxytryptamine concentrations. Consistent with this interpretation is the finding that endothelial cells throughout the brain appear highly fluorescent upon histochemical fluorescence analysis of 5-hydroxytryptamine following the administration of 5-hydroxytryptophan, an effect not seen when this precursor is given to animals pretreated with a peripheral decarboxylase inhibitor (Fuxe et al., 1971). Furthermore, the administration of a peripheral decarboxylase inhibitor antagonizes the rate-decreasing effect of 5-hydroxytryptophan administration on operant responding for water reinforcement, suggesting the involvement of peripheral rather than central mechanisms in the behaviorally depressant effects of 5-hydroxytryptophan (Carter et al., 1978). When administered to rats so pretreated,

5-hydroxytryptophan significantly elevated brain 5-hydroxytryptamine concentrations (Table 5) and enhanced the anticonflict effect of a submaximal dose of diazepam in the conditioned suppression of drinking (Figure 16). The significantly enhanced release of suppressed behavior by combined 5-hydroxytryptophan and diazepam (with inhibition of peripheral decarboxylase) compared to diazepam alone would appear to argue against a benzodiazepine-induced decrease in central 5-hydroxytryptamine-mediated activity as being causally related to its anticonflict effect. However, precursors of a specific neurotransmitter may have effects upon other neurotransmitter systems which could modulate the response to 5-hydroxytryptophan. For instance, 5-hydroxytryptophan elevates endogenous norepinephrine concentrations (Smith et al., 1977) and causes intraneuronal displacement of dopamine from vesicle storage sites (Andrews et al., 1978).

The interactive effects of 5-hydroxytryptophan and diazepam to facilitate anticonflict effects was not the only indication that increased activity in 5-hydroxytryptamine pathways may actually tend to reverse the suppression of drinking. The tricyclic tertiary amine amitriptyline, which does not increase overall brain 5-hydroxytryptamine concentration and exhibits no anticonflict activity of itself, also showed some potentiation of the anticonflict effect of a submaximal dose of diazepam (Figure 17). The presumed relationship between amitriptyline and synaptic 5-hydroxytryptamine activity varies from a facilitated neurotransmission via inhibition of reuptake mechanisms at the neuronal membrane (Carlsson et al., 1969) to receptor blocking activity (Fuxe et al., 1977), so that interpretations of the interaction between amitriptyline and diazepam must be cautious.

Still, the fact that these two treatments presumed to increase brain serotonergic activity appear to enhance anticonflict effect rather than antagonize it tends to weaken the hypothesis that brain 5-hydroxytryptamine pathways mediate punished suppression.

The different effects of 5-hydroxytryptamine depletion produced by pchlorophenylalanine (Figure 20) and 5,7-dihydroxytryptamine (Figure 21) on experimentally-induced conflict in drinking behavior may prove informative as regards the role of 5-hydroxytryptamine in punishment-suppressed responding. These differences in behavioral effects may reflect differences in the selectivity of the two agents for 5-hydroxytryptamine systems, as p-chlorophenylalanine induces significant reductions in brain norepinephrine and dopamine as well as 5-hydroxytryptamine, an effect not observed with 5,7-dihydroxytryptamine in animals pretreated with a catecholamine uptake inhibitor (Trulson et al., 1976). Alternatively, the discrepancy may reflect regional differences in 5-hydroxytryptamine depletion induced by the two agents (Aghajanian et al., 1973; Sanders-Bush and Massari, 1977).

An inviting comparison exists between the postulated mechanisms by which p-chlorophenylalanine and 5,7-dihydroxytryptamine deplete brain 5-hydroxytryptamine and the resulting concomitants. Trulson et al. (1976) reported that the intracerebroventricular injection of 5,7-dihydroxytryptamine resulted in a marked supersensitivity (i.e., a shift to the left in the dose-response relationship) to 5-hydroxytryptamine precursors and agonists using a behavioral syndrome thought to specifically reflect the activity of central 5-hydroxytryptamine receptors. Chronic p-chlorophenylalanine administration failed to

similarly induce behavioral supersensitivity although producing a depletion of brain 5-hydroxytryptamine greater than that produced by 5,7-dihydroxytryptamine. More definitively, the response of amygdaloid and ventral lateral geniculate neurons to the microiontophoretic application of 5-hydroxytryptamine agonists was found to be supersensitive in animals similarly pretreated with 5,7-dihydroxytryptamine (de Montigny and Aghajanian, 1977). Furthermore, the time course for the onset of the behavioral index of 5-hydroxytryptamine receptor supersensitivity (2-4 days, Trulson et al., 1976) parallels the latency associated with the observed anticonflict effect of intracerebroventricular and intraamygdaloid injections of 5,7-dihydroxytryptamine (Figure 20). The similarity of the anticonflict effects of 5,7-dihydroxytryptamine injected into the lateral ventricles or the amygdala implicates 5-hydroxytryptamine and the amygdala as the neurochemical and anatomical substrates, respectively, functioning in the expression of conflict behavior. However, the anticonflict effects associated with intraamygdaloid 5,7-dihydroxytryptamine injections may reflect a cytotoxic effect on fiber systems coursing en passant through the amygdala producing an altered neuronal function in some area other than the amygdala. The popular interpretation of these effects of dihydroxylated tryptamines on conflict behavior would be that they relate to the time course of the depletion and recovery of brain 5-hydroxytryptamine (Stein et al., 1975; Lippa et al., 1977a). However, the inability to demonstrate consistent anticonflict activity with p-chlorophenylalanine (Figure 19; Blakely and Parker, 1973; Cook and Sepinwall, 1975) suggests that some mechanism other than 5-hydroxytryptamine depletion may operate in producing the effects of these

neurotoxins on conflict behavior (e.g., denervation supersensitivity resulting from the destruction of presynaptic terminals).

One very important issue must be stressed in regard to the effects of the neurotoxic indole derivative on conflict behavior. These experiments were done in the modified Geller-Seifter procedure, which used food reinforcement. The majority of the other drug treatments were done with the shocked drinking tube procedure, which used water reinforcement in animals deprived of water. The two conflict procedures appeared to be equivalent in terms of the specificity of benzodiazepines as anticonflict agents (Tables 2 and 3). Nevertheless, they may not be equivalent with reference to brain 5-hydroxytryptamine changes. Brain serotonin pathways have profound effects on food motivation, presumably through a satiating mechanism, whereas the influence of this neurotransmitter on thirst mechanisms appears to be slight (Samanin et al., 1977; Garattini and Samanin, 1978). Depletion of forebrain 5-hydroxytryptamine by the neurotoxins in the Geller procedure may have increased the appetitive drive for food, thus offseting the suppressant effects of footshock. Such a mechanism would not be operative in the shocked drinking tube procedure. To assist in resolving this problem, preliminary experiments have been carried out, testing rats pretreated with intraventricular 5,7-dihydroxytryptamine in the conditioned suppression of drinking. In this case, the treatment did not exert an anticonflict effect as was seen in the Geller procedure. This suggests, then, that this destruction of brain 5-hydroxytryptamine does not affect conflict behavior directly, but may reduce conflict in those procedures utilizing food reinforcement.

Since the great majority of previous experiments dealing with neurotransmitter influences on conflict behavior have used food-reinforced procedures, the interpretations from these past results are open to the same criticism.

An examination of the effects of systemic administration of the benzodiazepines and iontophoretically applied 5-hydroxytryptamine on the spontaneous firing rate of terminals of the projections from the raphé nuclei reveals a potential inconsistency with the contention that a benzodiazepine-induced decrease in functional 5-hydroxytryptamine activity is causally related to their anticonflict efficacy. Studies involving neurons receiving an identified 5-hydroxytryptamine input indicate that raphé neurons have a tonic inhibitory influence on their postsynaptic cells: raphé neurons fire tonically and 5-hydroxytryptamine is inhibitory on the postsynaptic neurons (Aghajanian et al., 1975). Therefore, one would anticipate that agents effecting a decrease in the activity of 5-hydroxytryptamine-containing neurons would produce a release of postsynaptic cells from tonic inhibitory influence, i.e., an acceleration of the firing rate. However, the systemic administration of the benzodiazepines has been found to produce exclusively depressant effects on the spontaneous electrical activity recorded from cortical and subcortical areas as well as from cerebellar purkinje cells (Schallek et al., 1962; Olds and Olds, 1969; Guerrero-Figueroa et al., 1973; Chou and Wang, 1977).

D. Methodological Considerations Relevant to the Estimation of Dopamine and 5-Hydroxytryptamine Turnover

A major stimulus for the development of methods that estimate brain monoamine turnover rate in vivo has been the belief that drug actions on central monoaminergic mechanisms can be described in terms of, and are directly related to, their capacity to alter the rate of turnover. Empirical support for this belief is derived from the finding that electrical stimulation of monoamine-containing cell bodies increases the estimated turnover rate in brain areas receiving terminal projections (Korf et al., 1973). The initial rate of decline of dopamine subsequent to inhibition of tyrosine hydroxylase by α-methyltyrosine has been used to estimate the rate of dopamine turnover in the brain and other tissues. This technique, as with all the available nonsteady state methods employed in estimating turnover rates, is based on inherent assumptions and incurs both methodological and theoretical disadvantages. The assumption of a complete inhibition of tyrosine hydroxylase activity is reportedly satisfied if large doses of α-methyltyrosine are administered, with repeated doses required when measuring depletion over periods greater than 3 hours (Kizer et al., 1975). This procedure also involves the additional assumption that dopamine is stored in a single metabolic compartment, the content of which reflects a dynamic equilibrium between the processes of synthesis and degradation. Furthermore, as diazepam was studied in conjunction with α-methyltyrosine-induced dopamine depletion, it must be assumed that the synthesis inhibition is not appreciably altered by the administration of diazepam. The validity of these assumptions is strengthened by the finding that the decline of dopamine in amygdaloid

nuclei, olfactory tubercle, nucleus accumbens and striatum of both vehicle and diazepam pretreated animals after α-methyltyrosine followed first-order kinetics.

Obvious limitations to the use of dopamine decline following synthesis inhibition to estimate the rate of turnover are apparent when considering the gross perturbation of the finely regulated system affected by this technique. There can be little arguement to the point that one is examining drug effects on a severely compromised system: the intraneuronal concentration of dopamine available for release is progressively decreased and obviously no information can be obtained concerning effects on the turnover of newly synthesized dopamine. Nevertheless, the finding of nearly identical rates of catecholamine turnover using steady state radioisotopic tracer techniques and non-steady state methods involving synthesis inhibitors (Brodie et al., 1966) may represent the most meaningful common denominator. Thus, the nonsteady state method of observing the decline of endogenous dopamine concentration following synthesis inhibition with α-methyltyrosine may be of value in evaluating drug effects on estimated dopamine turnover and, by implication, dopaminergic neuronal activity. However, the estimation is probably of a comparative rather than an absolute nature (Weiner, 1974).

5-Hydroxytryptamine turnover was estimated using the nonsteady state method of following the decline of endogenous 5-hydroxyindole-acetic acid concentration after inhibition of monoamine oxidase activity with pargyline. Basic assumptions inherent to this method include the requirement that pargyline rapidly and completely inhibits monoamine oxidase activity and that neither pargyline nor diazepam alters

the normal processes for transport of 5-hydroxyindoleacetic acid from the brain. The rapid and complete inhibition of the formation of 5-hydroxyindoleacetic acid by pargyline seems to be approximated in experimental situations utilizing a dose of the magnitude employed in this dissertation (75 mg/kg, i.p., Goridis and Neff, 1971; Karoum et al., 1977). As the endogenous 5-hydroxyindoleacetic acid concentration of the brain areas examined was either unchanged or decreased by acute and chronic diazepam treatment (1.8 mg/kg), respectively, it appears unlikely that diazepam, at this dose, interferes with 5-hydroxyindoleacetic acid transport mechanisms. The elevated 5-hydroxyindoleacetic acid concentrations seen following a larger dose (5.6 mg/kg) of diazepam suggests that the processes involved in the removal of this acid from the brain may be impaired at this dose. The most obvious shortcoming of this method is that the inhibition of monoamine oxidase results in an accumulation of 5-hydroxytryptamine, as well as dopamine and norepinephrine, which influence 5-hydroxytryptamine synthesis by intraneuronal and/or interneuronal mechanisms (Carlsson et al., 1976; Mandell, 1978). For this reason the effects of diazepam on the short term (40 minute) decline of 5-hydroxyindoleacetic acid induced by pargyline were examined. In agreement with findings obtained with whole brain (Tozer et al., 1966) and brain areas containing predominantly 5-hydroxytryptamine terminals or cell bodies (Neckers and Meek, 1976), the pargyline-induced decline of the endogenous 5-hydroxyindoleacetic acid content of the amygdaloid nuclei, anterior hypothalamus and hippocampus was linear for at least 40 minutes and followed first order kinetics.

The value of this nonsteady state method in reliably estimating 5-hydroxytryptamine turnover is dependent on the assumption that 5-hydroxytryptamine is metabolized solely and entirely to 5-hydroxyindoleacetic acid and that 5-hydroxytryptamine is the single precursor in the formation of 5-hydroxyindoleacetic acid. While it is generally agreed upon that this relationship very nearly represents that involved in serotonergic function in the central nervous system, the exact nature of the relationship between endogenous 5-hydroxyindoleacetic acid concentrations in the brain and the neuronal processes such as release and reuptake which determine the functional activity of 5-hydroxytryptamine is still a subject of debate. In other words, does 5-hydroxyindoleacetic acid derived from 5-hydroxytryptamine reflect the oxidative deamination of 5-hydroxytryptamine within 5-hydroxytryptamine-containing neurons without prior release into synapses or does this deamination take place in these neurons subsequent to release and presynaptic reuptake? The answer to this question is of obvious importance in using this methodology to estimate 5-hydroxytryptamine turnover, as well as interpreting this turnover measure as a reflection of 5-hydroxytryptamine synthesis and release at postsynaptic receptor sites. Reinhard and Wurtman (1977) suggest that changes in brain 5-hydroxyindoleacetic acid represent physiological or drug-induced alterations in the release and reuptake of 5-hydroxytryptamine rather than intraneuronal catabolism without prior release into synapses.

In any study on the effects of drugs on 5-hydroxytryptamine turnover in whole brain or discrete nuclei, a minimum of two methods should be used to minimize the pharmacological or analytical shortcomings of a given technique. Initial attempts to examine the effects of diazepam

on functional 5-hydroxytryptamine activity involved the estimation of 5-hydroxytryptamine turnover by following the rate of accumulation of 5-hydroxyindoleacetic acid after inhibition of its transport with probenecid (Neff et al., 1967). However, the accumulation of 5-hydroxyindoleacetic acid after probenecid (200 mg/kg, i.p.) was not consistently linear in the brain nuclei examined when groups of rats were sacrificed either immediately before or 30 and 60 minutes following probenecid treatment.

E. Effects of Acute and Repeated Diazepam Administration on Dopamine Turnover in the Amygdala, Olfactory Tubercle, Nucleus Accumbens and Caudate Nucleus

A single dose of diazepam (1.8 mg/kg, i.p.) significantly reduced the rate constants for the α-methyltyrosine-induced decline of dopamine in the central and lateral amygdaloid nuclei, areas containing terminal projections of cell bodies in the A8, A9 and A10 areas (according to Dahlström and Fuxe, 1965) of the substantia nigra, as well as in the cortical and basal posterior amygdaloid nuclei (Table 8). The fractional rate constant was similarly reduced in the olfactory tubercle. Since the fractional rate constant represents the fraction of the dopamine pool being synthesized or metabolized (by definition a steady state) per unit of time, assuming that neuronal activity relates to rates of transmitter metabolism, it can be inferred that, since the fractional rate constants of these nuclei are lower in diazepam pretreated animals than in vehicle injected controls, dopaminergic neuronal activity in these nuclei is decreased by diazepam. The diazepam-induced decrease in dopamine turnover in the olfactory tubercle and lack of effect in the caudate nucleus are in good agreement with the

findings of Fuxe et al. (1975) following a similar low dose of diazepam (1 mg/kg, i.p.).

Repeated daily administration of diazepam (1.8 mg/kg/day for 5 consecutive days) also influenced amygdaloid dopamine turnover (Table 9), and, as was observed following acute administration, the amygdala was not uniformly influenced. Only the lateral and basal nuclei of the amygdala demonstrated a significant diazepam-induced decrease in the fractional rate constant of dopamine depletion following α-methyl-tyrosine. The lesser number of amygdaloid nuclei which exhibit a decreased dopamine turnover in response to prolonged diazepam treatment as compared to acute administration is suggestive of some degree of tolerance development over the course of treatment. It is feasible that the decrease in dopaminergic neuronal activity in the lateral and basal amygdaloid nuclei may reflect the relevant synaptic events causally related to the behavioral effects associated with this dosing regimen. More meaningful attempts to correlate the anticonflict and nonspecific sedative effects of diazepam with biochemical estimates of the activity of dopaminergic neurons terminating in the amygdala would require a much more rigorous extrapolation of the dose- and time-effect relationships obtained from the behavioral studies to the experimental design of the turnover studies. For instance, the effects of the repeated administration of a larger dose of diazepam (e.g., 5.6 mg/kg) on estimated dopamine turnover may yield more significant correlations with behavioral effects, as the effects of this dose of diazepam undergo significant qualitative and quantitative changes with repeated administrations (Figure 10), a phenomenon which is less readily

apparent with lower doses (Figure 11). Characterization of the time course and time to peak effect of diazepam-induced alterations in amygdaloid dopamine turnover is probably not possible, given the rigid time constraints of this nonsteady state technique utilized to estimate turnover. The preferential action of repeated diazepam administration on the mesolimbic dopaminergic system, without affecting the nigrostriatal neurons, is similar to the findings of Fuxe et al. (1975). They employed a single, larger dose of diazepam (5 mg/kg, i.p.), which raises the possibility that an accumulation of diazepam and/or its active metabolites may be in some part responsible for the observed effects of this chronic regimen on dopaminergic neuronal systems.

F. Effects of Acute and Repeated Diazepam Administration on 5-Hydroxytryptamine Turnover in the Amygdala, Hypothalamus and Hippocampus

Early attempts to correlate the antipunishment activity of the benzodiazepines with drug-induced alterations in the turnover of brain 5-hydroxytryptamine commonly estimated turnover from the slope of the decline in the specific activity of radiolabelled intracerebroventricular 5-hydroxytryptamine administration (Wise et al., 1972; Lippmann and Pugsley, 1974; Stein et al., 1973; Cook and Sepinwall, 1975). However, the assumption that radiolabelled 5-hydroxytryptamine so administered is specifically taken up and bound in endogenous 5-hydroxytryptamine storage sites does not appear to be adequately satisfied (Gallager et al., 1975). In light of this obvious limitation the effects of diazepam on 5-hydroxytryptamine turnover were re-examined in the present study using the nonsteady state method of observing the

decline of endogenous 5-hydroxyindoleacetic acid concentration after monoamine oxidase inhibition with pargyline.

A single dose of diazepam (1.8 mg/kg, i.p.) was without effect on 5-hydroxytryptamine activity in the amygdala, hippocampus and anterior hypothalamus, as indicated by a lack of effect on estimated 5-hydroxytryptamine turnover in these areas and unchanged endogenous concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Cook and Sepinwall (1975) also report a similar lack of effect of a single chlordiazepoxide treatment (10 mg/kg, p.o.) on whole brain 5-hydroxytryptamine turnover as estimated by a radioactive steady state procedure. The possibility that the anticonflict activity of diazepam reflects alterations in the functional activity of 5-hydroxytryptamine in these brain regions is weakened by the fact that initial treatment with 1.8 mg/kg of diazepam is associated with a significant, albeit submaximal, anticonflict effect (Figure 11), while 5-hydroxytryptamine turnover appears unaffected. However, the diazepam-induced alteration in neuronal activity may be of a subtle nature, of insufficient duration, or confined to a more circumscribed neuronal system in which the neurochemical changes are obscured by analysis of too gross an area.

The repeated administration of diazepam (1.8 mg/kg/day for 5 consecutive days) significantly decreased the rate constant for the pargyline-induced decline of 5-hydroxyindoleacetic acid in the amygdaloid nuclei, with the single exception of the posterior amygdaloid nucleus. Turnover as estimated by nonisotopic techniques is defined by and utilizes principles of steady state kinetics. Repeated diazepam treatment appears to disturb the dynamic equilibrium between the

processes of synthesis and loss, as the endogenous 5-hydroxyindoleacetic acid content of the more rostral amygdaloid nuclei and the hypothalamus was significantly decreased by this treatment regimen. Thus, the requirement of an unaltered steady state 5-hydroxyindoleacetic acid concentration in diazepam-pretreated animals, a tenet basic to the use of this methodology in estimating turnover rate, is apparently not satisfied in half of the brain regions examined. While this may represent a serious limitation to the data obtained with this technique and any conclusions drawn therefrom, it is notable that a similar response to prolonged diazepam treatment (a decreased rate constant of 5-hydroxyindoleacetic acid decline) was observed in nuclei exhibiting either a decreased or unaltered steady state concentration of 5-hydroxyindoleacetic acid in response to diazepam.

The depressant effect of diazepam on serotonergic neuronal activity exhibits some selectivity, as neither the steady state 5-hydroxyindoleacetic acid content nor the estimated rate of 5-hydroxytryptamine turnover in the hippocampus was significantly influenced by diazepam, at least at the dose examined. This observation is counter to the notion of Weiner (1974) that drug effects on the turnover rate of a specific neurotransmitter are demonstrable in all the neurons which utilize that particular neurotransmitter, irrespective of the functions mediated by the neural systems. However, no solid evidence exists in support of this contention and it can be alternatively suggested that the differential effects of chronic diazepam treatment on the serotonergic neuronal activity of the rostral amygdala, hypothalamus and

hippocampus may represent real differences which relate to the anti-punishment (anticonflict) effects of benzodiazepines.

While the finding of a benzodiazepine-induced decrease in amygdaloid and hypothalamic 5-hydroxytryptamine turnover is in agreement with data from radioactive steady state procedures utilizing whole brain, it cannot be discerned from the available evidence whether this effect reflects a direct, depressant effect of the benzodiazepines or a compensatory response to an enhanced activity at 5-hydroxytryptamine-mediated synapses. While a consensus favors a benzodiazepine-induced reduction in the activity at 5-hydroxytryptamine-mediated synapses to underlie their anticonflict properties, this contention is derived mainly from studies involving food-reinforced conflict behavior and effects of agents purported to affect 5-hydroxytryptamine mechanisms. However, the research findings presented in this dissertation generally fail to corroborate this relationship, particularly in reference to the data derived from conditioned suppression of drinking. A re-evaluation of this contention appears to be needed in light of the dubious value of currently available agents as selective agonists or antagonists of 5-hydroxytryptamine. Indirect evidence exists in support of an involvement of negative neuronal feedback mechanisms, secondary to a facilitated action at 5-hydroxytryptamine synapses, as being operative in the reduction of 5-hydroxytryptamine turnover evoked by diazepam. This depressant effect of diazepam on estimated 5-hydroxytryptamine turnover is similar to that observed with presumed direct and indirect acting 5-hydroxytryptamine agonists (Hamon et al., 1974,1976; Svensson, 1978). Gallagher (1978) reports that neither

systemic administration nor microiontophoretic application of benzodiazepines appears to alter the spontaneous firing rate of dorsal raphé cell bodies, but Dalsass et al. (1976) report that low intravenous doses of chlordiazepoxide reversibly depress raphé firing rate. Therefore, important questions which remain to be answered concerning the interaction between benzodiazepines and 5-hydroxytryptamine neuronal systems include determination of the level of organization (presynaptic vs. postsynaptic) or of regulation (release, synthesis, reuptake, intraneuronal metabolism) at which the system is influenced. A point to be emphasized is that the use of conflict procedures to investigate the anticonflict mechanism of action of the benzodiazepines is only as valid as the pharmacological tools used to manipulate the behavior and particular neurotransmitter-mediated systems.

Many centrally acting drugs are assumed to combine with specific membrane receptors, which may be identified biochemically in binding studies with synaptic membranes using the labelled drug as ligand. Direct binding studies using [^{3}H]diazepam have indicated a specific high affinity binding site for the central nervous system, which may be relevant to the pharmacological actions of benzodiazepines in brain (Squires and Braestrup, 1977). The binding is stereospecific (Mohler and Okada, 1977; Waddington and Owen, 1978), the density of the binding sites shows an uneven distribution in rat (Squires and Braestrup, 1977) and human (Speth et al., 1978) brain and the estimated affinity of benzodiazepines for this "receptor" parallels their pharmacological potency in animal test systems presumably predictive of anxiolytic activity and correlates closely with their therapeutic potency as

anxiolytics and hypnotics (Mohler and Okada, 1977,1978). As the physiological significance of the benzodiazepine binding site is at present unknown, the contribution of these *in vitro* binding studies to the elucidation of the molecular mechanism of action of this class of compounds remains undetermined. However, information concerning the regional density of these binding sites may help to identify the site of central action of the benzodiazepines. In this regard, Mohler and Okada (1978) report a 24-fold variation in the density of the binding sites in different brain areas, with the highest density in cortical regions and areas of the limbic system, particularly the amygdala.

The characterization of the mechanisms involved in producing the well documented clinical properties of the benzodiazepines will undoubtedly reflect the collective findings of neurochemical, behavioral and electrophysiological studies.

SUMMARY AND CONCLUSIONS

1) A behavioral paradigm (conditioned suppression of drinking) was developed to assess the effects of various agents on this experimentally-induced conflict situation. This procedure offered significant advantages over conventional conflict methods which utilize operantly conditioned responses to generate the behavioral baseline.

2) Agents purported to influence 5-hydroxytryptamine mechanisms generally did not interact with diazepam to support a postulated diazepam-induced decrease in the functional activity of 5-hydroxytryptamine-containing neurons as being causally related to the anticonflict (antipunishment) activity of this drug. However, the validity of such a conclusion is dependent upon the reputed selectivity of these other agents for central 5-hydroxytryptamine mechanisms. Another important factor appears to be the type of positive reinforcement used, this being water in thirsted rats in the present case. Previous conflict studies supporting 5-hydroxytryptamine as the "anxiety" transmitter have used food reinforcement, which may have been misleading since 5-hydroxytryptamine agonists and antagonists affect appetite for food directly.

3) A submaximal anticonflict dose of diazepam decreased biochemical estimates of the functional activity of dopaminergic neurons terminating in the amygdala and in terminals of the mesolimbic

dopaminergic neurons. On chronic dosing with the drug, a degree of tolerance developed to this effect. However, time constraints and technical limitations precluded more complete characterization of this effect necessary to formulate meaningful conclusions regarding its relevance to diazepam-induced effects on conflict behavior.

4) Effective anticonflict doses of diazepam decrease the estimated activity of 5-hydroxytryptamine-containing neurons terminating in the amygdala and anterior hypothalamus. However, this influence was only observed following the repeated administration of a submaximal or maximal anticonflict dose. Diazepam-induced alterations in 5-hydroxytryptamine turnover in the amygdala and anterior hypothalamus would appear to share similar dose- and time-effect relationships with the effects of this benzodiazepine derivative on punished responding. The significance of this correlation is weakened by an apparent disparity between the behavioral and neurochemical alterations as related to dosing regimen. However, it has been argued that biochemical determinations are generally less sensitive than functional tests to reveal drug-induced changes in neuronal activity. Thus, altered levels of brain 5-hydroxytryptamine activity may still have a role in the anticonflict efficacy of benzodiazepines, but the precise nature of this role is not as simplistic as previously postulated.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aghajanian, G.K., Foote, W.E. and Sheard, M.H.: Lysergic acid diethylamide: Sensitive neuronal units in the midbrain raphe. Science 161: 706-708, 1968.

Aghajanian, G.K., Kuhar, M.J. and Roth, R.H.: Serotonin-containing neuronal perikarya and terminals: Differential effects of p-chlorophenylalanine. Brain Res. 54: 85-101, 1973.

Aghajanian, G.K., Haigler, H.J. and Bennett, J.L.: Amine receptors in CNS. III. 5-Hydroxytryptamine in brain. In: Handbook of Psychopharmacology, ed. by L.L. Iversen, S.D. Iversen and S.H. Snyder, Plenum, New York, pp. 63-96, 1975.

Andrews, D.W., Patrick, R.L. and Barchas, J.D.: The effects of 5-hydroxytryptophan and 5-hydroxytryptamine on dopamine synthesis and release in rat brain striatal synaptosomes. J. Neurochem. 30: 465-470, 1978.

Babbini, M., Gaiardi, M., Bartoletti, M., Torrielli, M.V. and De Marchi, F.: The conflict behavior in rats for the evaluation of a homogeneous series of 3-hydroxybenzodiazepines: structure-activity relationships. Pharmacol. Res. Comm. 7: 337-346, 1975.

Babbini, M., Gaiardi, M. and Bartoletti, M.: Effects of combined treatment with nortriptiline and lorazepam on conflict behavior and motility of rats. Psychopharmacology 48: 251-254, 1976.

Bainbridge, J.G.: The effect of psychotropic drugs on food-reinforced behavior and on food consumption. Psychopharmacologia 12: 204-213, 1968.

Bartholini, G., Keller, H., Pieri, L. and Pletscher, A.: The effect of diazepam on the turnover of cerebral dopamine. In: The Benzodiazepines, ed. by S. Garattini, E. Mussini and L.O. Randall, Raven Press, New York, pp. 235-240, 1973.

Baumgarten, H.G., Björklund, A., Lachenmayer, L. and Nobin, A.: Evaluation of the effects of 5,7-dihydroxytryptamine on serotonin and catecholamine neurons in the rat CNS. Acta Physiol. Scand. 391: 1-19, 1973a.

Baumgarten, H.G., Victor, S.J. and Lovenberg, W.: Effect of intraventricular injection of 5,7-dihydroxytryptamine on regional tryptophan hydroxylase of rat brain. J. Neurochem. 21: 251-253, 1973b.

Beck, O., Wiesel, F-A. and Sedvall, G.: Mass fragmentographic determination of 5-hydroxytryptamine and 5-hydroxyindole-3-acetic acid in brain tissue using deuterated internal standards. J. Chromatogr. 134: 407-414, 1977.

Beer, B., Chasin, M., Clody, D.E., Vogel, J.R. and Horovitz, Z.P.: Cyclic adenosine monophosphate phosphodiesterase in brain: Effect on anxiety. Science 176: 428-430, 1972.

Ben-Ari, Y. and Kelly, J.S.: Dopamine evoked inhibition of single cells of the feline putamen and basolateral amygdala. J. Physiol. 256: 1-21, 1976.

Ben-Ari, Y., Kanazawa, I. and Zigmond, R.E.: Regional distribution of glutamate decarboxylase and GABA within the amygdaloid complex and stria terminalis system of the rat. J. Neurochem. 26: 1279-1283, 1976.

Berger, T.M.: The similarities and differences between meprobamate and barbiturates. Clin. Pharmacol. Ther. 4: 209-231, 1963.

Bertsch, G.J.: Punishment of consummatory and instrumental behavior: A review. Psychol. Rec. 26: 13-32, 1976.

Biggio, G., Brodie, B.B., Costa, E. and Guidotti, A.: Mechanisms by which diazepam, muscimol, and other drugs change the content of cGMP in cerebellar cortex. Proc. Natl. Acad. Sci. 74: 3592-3596, 1977.

Billingsley, M.L. and Kubena, R.K.: The effects of naloxone and picrotoxin on the sedative and anticonflict effects of benzodiazepines. Life Sci. 22: 897-906, 1978.

Blakely, T.A. and Parker, L.F.: The effects of parachlorophenylalanine on experimentally induced conflict behavior. Pharmacol. Biochem. Behav. 1: 609-613, 1973.

Blanchard, D.C. and Blanchard, R.J.: Innate and conditioned reaction to threat in rats with amygdaloid lesions. J. Comp. Physiol. Psychol. 81: 281-290, 1972.

Blum, K.: Effects of chlordiazepoxide and pentobarbital on conflict behavior in rats. Psychopharmacologia 17: 391-398, 1970.

Bourgoin, S., Hery, F., Ternaux, J.P. and Hamon, M.: Effects of benzodiazepines on the binding of tryptophan in serum. Consequences of 5-hydroxyindole concentrations in the rat brain. Psychopharmacol. Comm. 1: 209-216, 1975.

Breese, G.R. and Cooper, B.R.: Behavioral and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered intracisternally to adult and developing rats. Brain Res. 98: 517-527, 1975.

Bremner, F.J., Cobb, H.W. and Hahn, W.C.: The effect of chlordiazepoxide on the behavior of rats in a conflict situation. Psychopharmacologia 17: 275-282, 1970.

Brodie, B.B., Costa, E., Dlabac, A., Neff, N.H. and Smookler, H.H.: Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. J. Pharmacol. Exp. Ther. 154: 493-498, 1966.

Brownstein, M., Saavedra, J.M. and Palkovits, M.: Norepinephrine and dopamine in the limbic system of the rat. Brain Res. 79: 431-436, 1974.

Burkard, W.P., Pieri, L. and Haefely, W.: In vivo changes in guanosine 3',5'-cyclic phosphate in rat cerebellum by dopaminergic mechanisms. J. Neurochem. 27: 297-298, 1976.

Bürki, H.R., Asper, H., Ruch, W. and Zuger, P.E.: Bromocriptine, dihydroergotoxine, methysergide, d-LSD, CF25-397, and 29-712: Effects on the metabolism of the biogenic amines in the brain of the rat. Psychopharmacology 57: 227-237, 1978.

Bush, D.F., Lovely, R.H. and Pagano, R.R.: Injection of ACTH induces recovery from shuttle-box avoidance deficits in rats with amygdaloid lesions. J. Comp. Physiol. Psychol. 83: 168-172, 1973.

Campenot, R.B.: Effect of amygdaloid lesions upon active avoidance acquisition and anticipatory responding in rats. J. Comp. Physiol. Psychol. 69: 492-497, 1969.

Cannizzaro, G., Nigoto, S., Provenzano, P.M. and Vitikova, T.: Modification of depressant and disinhibitory action of flurazepam during short term treatment in the rat. Psychopharmacologia 26: 173-184, 1972.

Carlsson, A., Corrodi, H., Fuxe, K. and Hökfelt, T.: Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-α-ethyl-metatyramine. Eur. J. Pharmacol. 5: 357-366, 1969.

Carlsson, A., Kehr, W. and Lindqvist, M.: The role of intraneuronal amine levels in the feedback control of dopamine, noradrenaline and 5-hydroxytryptamine synthesis in rat brain. J. Neural Transmission 39: 1-19, 1976.

Carpenter, D.C., Hrdina, P.D. and Beaubien, A.R.: Effects of phenobarbital and diazepam on imipramine-induced changes in blood pressure, heart rate and rectal temperature of rats. Res. Comm. Chem. Path. Pharmacol. 18: 613-625, 1977.

Carter, R.B., Dykstra, L.A., Leander, J.D. and Appel, J.B.: Role of peripheral mechanisms in the behavioral effects of 5-hydroxytryptophan. Pharmacol. Biochem. Behav. 9: 249-253, 1978.

Cattabeni, F., Koslow, S.H. and Costa, E.: Gas chromatographic-mass spectrometric assay of four indole alkylamines of rat pineal. Science 178: 166-168, 1972.

Chase, T.N., Katz, R.I. and Kopin, I.J.: Effect of diazepam on fate of intracisternally injected serotonin-C^{14}. Neuropharmacology 9: 103-108, 1970.

Cheney, D.L., Trabucchi, M., Hanin, I. and Costa, E.: Effect of several benzodiazepines on concentrations and specific activities of choline and acetylcholine in mouse brain. Pharmacologist 15: 162, 1973.

Cheney, D.L., LeFevre, H.F. and Racagni, G.: Choline acetyltransferase activity and mass fragmentographic measurement of acetylcholine in specific nuclei and tracts of rat brain. Neuropharmacology 14: 801-809, 1975.

Chittal, S.M. and Sheth, U.K.: Effects of drugs on conditioned avoidance response in rats. Arch. Int. Pharmacodyn. Ther. 114: 471-480, 1963.

Chou, D.T. and Wang, S.C.: Unit activity of amygdala and hippocampal neurons: Effects of morphine and benzodiazepines. Brain Res. 126: 427-440, 1977.

Clement-Cormier, Y.C. and Robison, G.A.: Adenylate cyclase from various dopaminergic areas of the brain and the action of antipsychotic drugs. Biochem. Pharmacol. 26: 1719-1722, 1977.

Clineschmidt, B.V. and Lotti, V.J.: Indoleamine antagonists: Relative potencies as inhibitors of tryptamine- and 5-hydroxytryptophan-evoked responses. Brit. J. Pharmacol. 50: 311-313, 1974.

Cole, H.F. and Wolf, H.H.: The effects of some psychotic drugs on conditioned avoidance and aggressive behaviors. Psychopharmacologia 3: 264-282, 1962.

Consolo, S., Ladinsky, H., Peri, G. and Garattini, S.: Effect of diazepam on mouse whole brain and brain area acetylcholine and choline levels. Eur. J. Pharmacol. 27: 266-268, 1974.

Consolo, S., Garattini, S. and Ladinsky, H.: Action of the benzodiazepines on the cholinergic system. In: Mechanism of Action of Benzodiazepines, ed. by E. Costa and P. Greengard, Raven Press, New York, pp. 63-80, 1975.

Cook, L. and Davidson, A.B.: Effects of behaviorally active drugs in a conflict-punishment procedure in rats. In: The Benzodiazepines, ed. by S. Garattini, E. Mussini and L.O. Randall, Raven Press, New York, pp. 327-345, 1973.

Cook, L. and Sepinwall, J.: Behavioral analysis of the effects and mechanism of action of benzodiazepines. In: Mechanism of Action of Benzodiazepines, ed. by E. Costa and P. Greengard, Raven Press, New York, pp. 1-28, 1975.

Cook, L. and Davidson, A.B.: Behavioral pharmacology: Animal models involving aversive control of behavior. In: Psychopharmacology: A Generation of Progress, ed. by M.A. Lipton, A. DiMascio and K.F. Killam, Raven Press, New York, pp. 563-567, 1978.

Copobianco, S., MacDougall, J.M. and Foster, S.M.: Direct neurobehavioral comparisons within the septohippocampal system. Physiol. Psychol. 5: 215-220, 1977.

Corne, S.J., Pickering, R.W. and Warner, B.T.: A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. Brit. J. Pharmacol. 20: 106-120, 1963.

Corrodi, H., Fuxe, K. and Hökfelt, T.: The effects of some psychoactive drugs on central monoamine neurons. Eur. J. Pharmacol. 1: 363-368, 1967.

Corrodi, H., Fuxe, K., Lidbrink, P. and Olson, L.: Minor tranquilizers, stress and central catecholamine neurons. Brain Res. 29: 1-16, 1971.

Costa, E. and Greengard, P. (eds.): Mechanism of action of benzodiazepines. Advances in Biochemical Psychopharmacology. Vol. 14, Raven Press, New York, 1975.

Costa, E., Guidotti, A., Mao, C.C. and Suria, A.: New concepts on the mechanism of action of benzodiazepines. Life Sci. 17: 167-186, 1975.

Cromwell, H.A.: Methaqualone--a sedative or minor tranquilizer for geriatric patients. J. Amer. Geriat. Soc. 16: 814-817, 1968.

Curtis, D.R. and Johnston, G.A.R.: Amino acid transmitters in the mammalian central nervous system. Rev. Physiol. Biochem. Exp. Pharmacol. 69: 97-188, 1974.

Curtis, D.R., Game, C.G.A. and Lodge, D.: Benzodiazepines and central glycine receptors. Brit. J. Pharmacol. 56: 307-311, 1976.

Curzon, G. and Green, A.R.: Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Brit. J. Pharmacol. 39: 653-655, 1970.

Dacey, D.M. and Grossman, S.P.: Aphagia, adipsia and sensory-motor deficits produced by amygdala lesions: A function of extra-amygdaloid damage. Physiol. Behav. 19: 389-395, 1977.

Dahlström, A. and Fuxe, K.: Evidence for the existence of monoamine-containing neurons in the cell bodies of brain stem neurons. Acta Physiol. Scand. 62(Suppl. 232): 1-55, 1965.

Dalsass, M., Schoenfeld, R. and Stern, W.C.: Effects of chlordiazepoxide on spontaneous raphe unit activity in the anesthetized rat. Neurosci. Abstracts 2: 865, 1976.

Daly, J.W.: The nature of receptors regulating the formation of cyclic AMP in brain tissue. Life Sci. 18: 1349-1358, 1976.

D'amico, D.J., Patel, B.C. and Klawans, H.L.: The effect of methysergide on 5-hydroxytryptamine turnover in whole brain. J. Pharm. Pharmacol. 28: 454-465, 1976.

Dantzer, R. and Roca, M.: Tranquilizing effects of diazepam in pigs subjected to a punishment procedure. Psychopharmacologia 40: 235-240, 1974.

Dantzer, R.: Behavioral Effects of Benzodiazepines: A Review. Biobehav. Rev. 1: 71-86, 1977.

DeGroot, J.: The limbic system: An anatomical and functional orientation. In: American Handbook of Psychiatry, Vol. 6. Basic Books, New York, pp. 89-106, 1975.

Delini-Stula, A.: Drug-induced suppression of conditioned hyperthermic and conditioned avoidance behavior response in rats. Psychopharmacologia 20: 153-159, 1971.

Demarest, K.T., Alper, R.H. and Moore, K.E.: DOPA accumulation as a measure of dopamine synthesis in the median eminence and posterior pituitary. J. Neural Transmission, in press, 1979.

DeMontigny, C. and Aghajanian, G.K.: Preferential action of 5-methoxytryptamine and 5-methoxydimethyltryptamine on presynaptic serotonin receptors: A comparative iontophoretic study with LSD and serotonin. Neuropharmacology 16: 811-818, 1977.

DeOlmos, J. and Ingram, W.R.: The projection field of the stria terminalis in the rat brain: An experimental study. J. Comp. Neurol. 146: 303-334, 1972.

Dews, P.B.: Symposium on pharmacology of emotional behavior-closing remarks. In: Proc. 6th Int. Congr. Pharmacology, vol. 3, CNS and Behavioral Pharmacology, ed. by M. Airaksinen, Pergamon Press, New York, p. 238, 1976.

Dominic, J.A., Sinha, A.K. and Barchas, J.D.: Effect of benzodiazepine compounds on brain amine metabolism. Eur. J. Pharmacol. 32: 124-127, 1975.

Dray, A. and Straughan, D.W.: Benzodiazepines: GABA and glycine receptors on single neurons in the rat medulla. J. Pharm. Pharmacol. 28: 314-315, 1976.

Duchastel, Y.: Methaqualone: A synthetic nonbarbiturate hypnotic: a "double-blind" clinical evaluation of 100 cases. Can. Med. Assn. J. 86: 586-587, 1962.

Estes, W.K. and Skinner, B.F.: Some quantitative properties of anxiety. J. Exp. Psychol. 29: 390-400, 1941.

Falk, J.L. and Burnidge, G.K.: Fluid intake and punishment-attenuating drugs. Physiol. Behav. 5: 199-202, 1970.

File, S.E. and Hyde, J.R.G.: The effects of p-chlorophenylalanine and ethanolamine-o-sulphate in an animal test of anxiety. J. Pharm. Pharmacol. 29: 735-738, 1977.

Ford, R.D., Rech, R.H., Commissaris, R.L. and Meyer, L.Y.: Effects of acute and chronic interactions of diazepam and d-amphetamine on punished behavior of rats. Psychopharmacology, in press, 1979.

Fuller, J.L.: Strain differences in the effects of chlorpromazine and chlordiazepoxide upon active and passive avoidance in mice. Psychopharmacologia 16: 261-271, 1970.

Fuller, R.W., Perry, K.W. and Molloy, B.B.: Effect of 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. J. Pharmacol. Exp. Ther. 193: 796-803, 1975.

Fuxe, K., Butcher, L.L. and Engel, J.: DL-5-Hydroxytryptophan-induced changes in central monoamine neurons after peripheral decarboxylase inhibition. J. Pharm. Pharmacol. 23: 420-424, 1971.

Fuxe, K., Agnati, L.F., Bolme, P., Hökfelt, T., Lidbrink, P., Ljungdahl, A., Perez de la Mora, M. and Ögren, S.-O.: The possible involvement of GABA mechanisms in the action of benzodiazepines on central catecholamine neurons. In: Mechanism of Action of Benzodiazepines, ed. by E. Costa and P. Greengard, Raven Press, New York, pp. 45-61, 1975.

Fuxe, K., Ögren, S-O., Agnati, L., Gustafsson, J. and Jonsson, G.: On the mechanism of action of the antidepressant drugs amitriptyline and nortriptyline. Evidence for 5-hydroxytryptamine receptor blocking activity. Neurosci. Letters 6: 339-343, 1977.

Gähwiler, B.H.: Diazepam and chlordiazepoxide: Powerful GABA antagonists in explants of rat cerebellum. Brain Res. 107: 176-179, 1976.

Gallager, D.W., Sanders-Bush, E., Aghajanian, G.K. and Sulser, F.: An evaluation of the use of intraventricularly administered [^{3}H]-5-hydroxytryptamine as a marker for endogenous brain 5-hydroxytryptamine. Brain Res. 93: 111-122, 1975.

Gallager, D.W.: Benzodiazepines: Potentiation of a GABA inhibitory response in the dorsal raphe nucleus. Eur. J. Pharmacol. 49: 133-143, 1978.

Garattini, S. and Samanin, R. (Eds.): Central Mechanisms of Anorectic Drugs, Raven Press, New York, 1978.

Gaston, M.E. and Freed, L.: Effects of amygdaloid lesions in a fear conditioning situation not involving instrumental learning. Psychon. Sci. 16: 55-56, 1969.

Geller, I. and Seifter, J.: The effects of meprobamate, barbiturates, d-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1: 482-492, 1960.

Geller, I., Kulak, J.T. and Seifter, J.: The effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. Psychopharmacologia 3: 374-385, 1962.

Geller, I., Bachman, E. and Seifter, J.: Effects of reserpine and morphine on behavior suppressed by punishment. Life Sci. 4: 226-231, 1963.

Geller, I.: Relative potencies of benzodiazepines as measured by their effects on conflict behavior. Arch. Int. Pharmacodyn. Ther. 149: 243-247, 1964.

Geller, I. and Blum, K.: The effects of 5-HTP on para-chlorophenylalanine (p-CPA) attenuation of "conflict" behavior. Eur. J. Pharmacol. 9: 319-324, 1970.

Geller, I., Hartmann, R.J. and Croy, D.J.: Attenuation of conflict behavior with cinanserin, a serotonin antagonist: Reversal of the effect with 5-hydroxytryptophan and α-methyltryptamine. Res. Comm. Chem. Path. Pharmacol. 7: 165-174, 1974.

Gelpi, E., Peralta, E. and Segura, J.: Gas chromatography-mass spectrometry of catecholamines and tryptamines: Determination of gas chromatographic profiles of the amines, their precursors and their metabolites. J. Chromatogr. Sci. 12: 701-709, 1974.

Goldberg, M.E., Manian, A.A. and Efron, D.H.: A comparative study of certain pharmacological responses following acute and chronic administrations of chlordiazepoxide. Life Sci. 6: 481-491, 1967.

Goldberg, M.E. and Ciofalo, V.B.: Effect of diphenylhydantoin sodium and chlordiazepoxide alone and in combination on punishment behavior. Psychopharmacologia 14: 233-239, 1969.

Goridis, C. and Neff, N.H.: Evidence for a specific monoamine oxidase associated with sympathetic nerves. Neuropharmacology 10: 557-564, 1971.

Graeff, F.G. and Schoenfeld, R.I.: Tryptaminergic mechanisms in punished and nonpunished behavior. J. Pharmacol. Exp. Ther. 173: 277-283, 1970.

Graeff, F.G.: Tryptamine antagonists and punished behavior. J. Pharmacol. Exp. Ther. 189: 344-350, 1974.

Green, A.R., Koslow, S.H. and Costa, E.: Identification and quantitation of a new indolealkylamine in rat hypothalamus. Brain Res. 51: 371-374, 1973.

Greenblatt, D.J. and Shader, R.I.: Benzodiazepines in Clinical Practice, Raven Press, New York, pp. 64-79, 1974.

Greenblatt, D.J. and Shader, R.I.: Pharmacotherapy of anxiety with benzodiazepines and β-adrenergic blockers. In: Psychopharmacology: A Generation of Progress, ed. by M.A. Lipton, A. DiMascio and K.F. Killam, Raven Press, New York, pp. 1381-1390, 1978.

Guerrero-Figueroa, R., Gallant, D.M., Guerrero-Figueroa, C. and Gallant, J.: Electrophysiological analysis of the action of four benzodiazepine derivatives on the central nervous system. In: The Benzodiazepines, ed. by S. Garattini, E. Mussini and L.O. Randall, Raven Press, New York, pp. 489-511, 1973.

Guidotti, A.: Synaptic mechanisms in the action of benzodiazepines. In: Psychopharmacology: A Generation of Progress, ed. by M.A. Lipton, A. DiMascio and K.F. Killam, Raven Press, New York, pp. 1349-1374, 1978.

Gyermek, L.: 5-Hydroxytryptamine antagonists. Pharmacol. Rev. 13: 399-439, 1961.

Haefely, W., Kulcsar, A., Mohler, H., Pieri, L., Polc, P. and Schaffner, R.: Possible involvement of GABA in the central actions of benzodiazepines. In: Mechanism of Action of Benzodiazepines, ed. by E. Costa and P. Greengard, Raven Press, New York, pp. 131-151, 1975.

Haefely, W.: Synaptic pharmacology of barbiturates and benzodiazepines. Agents and Actions 7: 353-359, 1977.

Haefely, W.E.: Behavioral and neuropharmacological aspects of drugs used in anxiety and related states. In: Psychopharmacology: A Generation of Progress, ed. by M.A. Lipton, A. DiMascio and K.F. Killam, Raven Press, New York, pp. 1359-1374, 1978.

Haigler, H.J. and Aghajanian, G.K.: Peripheral serotonin antagonists: Lack of antagonism to serotonin at identified serotonergic synapses in rat brain. J. Neural Transmission 35: 257-273, 1974.

Hamon, M., Bourgoin, S., Jagger, J. and Glowinski, J.: Effects of LSD on synthesis and release of 5-HT in rat brain slices. Brain Res. 69: 265-280, 1974.

Hamon, M., Bourgoin, S., Enjalbert, A., Bockaert, J., Hery, F., Ternaux, J.P. and Glowinski, J.: The effects of quipazine on 5-HT metabolism in the rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 294: 99-108, 1976.

Hartmann, R.J. and Geller, I.: p-Chlorophenylalanine effects on a conditioned emotional response in rats. Life Sci. 10: 927-933, 1971.

Heise, G.A. and Boff, E.: Continuous avoidance as a baseline for measuring behavioral effects of drugs. Psychopharmacologia 3: 264-282, 1962.

Heise, G.A., Laughlin, N. and Keller, C.: A behavioral and pharmacological analysis of reinforcement withdrawal. Psychopharmacologia 16: 345-368, 1970.

Hess, S.M., Chasin, M., Free, C.A. and Harris, D.N.: Modulators of cyclic AMP systems. In: Mechanism of Action of Benzodiazepines, ed. by E. Costa and P. Greengard, Raven Press, New York, pp. 153-167, 1975.

Hillestad, L., Hansen, T. and Melsom, H.: Diazepam metabolism in normal man. II. Serum concentration and clinical effect after oral administration and cumulation. Clin. Pharmacol. Ther. 16: 485-489, 1974.

Hirano, M., Kim, J.S., Saito, M., Uchimura, H. and Ito, M.: Monoamine oxidase activities for serotonin and tyramine in individual limbic and lower brain stem nuclei of the rat. J. Neurochem. 30: 263-267, 1978.

Hobbs, S.N.: Response suppression on two operant schedules by rats with septal lesions. Neurosci. Abstracts 2: 388, 1976.

Hökfelt, T., Ljungdahl, A., Fuxe, K. and Johansson, O.: Dopamine nerve terminals in the rat limbic cortex: Aspects of the dopamine hypothesis of schizophrenia. Science 184: 177-179, 1974.

Hunt, P. and Raynaud, J.: Benzodiazepine activity: Is interaction with the glycine receptor, as evidenced by displacement of strychnine binding, a useful criterion? J. Pharm. Pharmacol. 29: 442-444, 1977.

Huppert, F.A. and Iversen, S.D.: Response suppression in rats: A comparison of response-contingent and noncontingent punishment and the effect of the minor tranquilizer, chlordiazepoxide. Psychopharmacologia 44: 67-75, 1975.

Isaacson, R.L., Douglas, R.J. and Moore, R.Y.: The effect of radical hippocampal ablation on acquisition of avoidance response. J. Comp. Physiol. Psychol. 54: 625-628, 1961.

Isaacson, R.L.: The structure of the limbic system. In: The Limbic System. Plenum Press, New York, pp. 1-57, 1974.

Jacobowitz, D.M. and Palkovits, M.: Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in rat brain. 1: Forebrain. J. Comp. Neurol. 157: 13-28, 1974.

Jacobs, B.L.: An animal behavior model for studying central serotonergic synapses. Life Sci. 19: 777-786, 1976.

Jacoby, J.H., Poulakos, J.J. and Bryce, G.F.: On the central antiserotonergic actions of cyproheptadine and methysergide. Neuropharmacology 17: 299-306, 1978.

Jenner, P., Chadwick, D., Reynolds, E.H. and Marsden, C.D.: Altered 5-HT metabolism with clonazepam, diazepam and diphenylhydantion. J. Pharm. Pharmacol. 27: 707-710, 1975.

Jequier, E., Lovenberg, W. and Sjoerdsma, A.: Tryptophan hydroxylase inhibition: The mechanism by which p-chlorophenylalanine depletes rat brain serotonin. Mol. Pharmacol. 3: 274-278, 1967.

Karoum, F., Garrison, C.K., Neff, N. and Wyatt, R.J.: Trans-synaptic modulation of dopamine metabolism in the rat superior cervical ganglion. J. Pharmacol. Exp. Ther. 201: 654-661, 1977.

Kehr, W.: Effect of lisuride and other ergot derivatives on monoaminergic mechanisms in rat brain. Eur. J. Pharmacol. 41: 261-273, 1977.

Keller, H.H., Schaffner, R. and Haefely, W.: Interaction of benzodiazepines with neuroleptics at central dopamine neurons. Naunyn-Schmiedeberg's Arch. Pharmacol. 294: 1-7, 1976.

Kellicutt, M.H. and Schwartzbaum, J.S.: Formation of a conditioned emotional response (CER) following lesions of the amygdaloid complex in rats. Psychol. Rep. 12: 351-358, 1963.

Kemble, E.D. and Tapp, J.T.: Passive and active avoidance performance following small amygdaloid lesions in rats. Physiol. Behav. 3: 713-718, 1968.

Kilts, C.D., Vrbanac, J.J., Rickert, D.E. and Rech, R.H.: Mass fragmentographic determination of 3,4-dihydroxyphenylethylamine and 4-hydroxy-3-methoxyphenylethylamine in the caudate nucleus. J. Neurochem. 28: 465-467, 1977.

Kizer, J.S., Kopin, I.J. and Zivin, J.A.: Estimates of catecholamine turnover rates in individual hypothalamic nuclei of the rat by use of alpha-methyl-para-tyrosine. Brit. J. Pharmacol. 243P, 1975.

Kizer, J.S., Palkovits, M. and Brownstein, M.J.: The projections of the A8, A9 and A10 dopaminergic cell bodies: Evidence for a nigral-hypothalamic-median eminence dopaminergic pathway. Brain Res. 108: 363-370, 1976.

Knowler, W.C. and Ukena, T.E.: The effects of chlorpromazine, pentobarbital, chlordiazepoxide and d-amphetamine on rates of licking in the rat. J. Pharmacol. Exp. Ther. 184: 385-397, 1973.

Ko, H., Lahti, R.A., Duchamp, D.J. and Royer, M.E.: A GC-MS procedure for the measurement of dopamine in mouse striatal tissue. Analyt. Letters 7: 243-255, 1974.

Koe, B.K. and Weissman, A.: p-Chlorophenylalanine: A specific depletor of brain serotonin. J. Pharmacol. Exp. Ther. 154: 499-516, 1966.

Konig, J.F.R. and Klippel, R.A.: The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem, Williams and Wilkins, Baltimore, 1963.

Korf, J., Roth, R.H. and Aghajanian, G.K.: Alterations in turnover and endogenous levels of norepinephrine in the cerebral cortex following electrical stimulation and acute axotomy of cerebral noradrenergic pathways. Eur. J. Pharmacol. 23: 276-282, 1973.

Koslow, S.H., Cattabeni, F. and Costa, E.: Norepinephrine and dopamine: Assay by mass fragmentography in the picomole range. Science 176: 177-180, 1972.

Koslow, S.H., Racagni, G. and Costa, E.: Mass fragmentographic measurement of norepinephrine, dopamine, serotonin and acetylcholine in seven discrete nuclei of the rat tel-diencephalon. Neuropharmacology 13: 1123-1130, 1974.

Krieckhaus, E.E., Simmons, H.J., Thomas, G.J. and Kenyon, J.: Septal lesions enhance shock avoidance behavior in the rat. Exp. Neurol. 9: 107-113, 1964.

Krogsgaard-Larsen, P., Johnston, G.A.R., Curtis, D.R., Game, C.J.A. and McCulloch, R.M.: Structure and biological activity of a series of conformationally restricted analogues of GABA. J. Neurochem. 25: 803-808, 1975.

Kuribara, H., Ohashi, K. and Tadokoro, S.: Rat strain differences in the acquisition of conditioned avoidance responses and in the effects of diazepam. Jap. J. Pharmacol. 26: 725-735, 1976.

Ladinsky, H., Consolo, S., Peri, G. and Garattini, S.: Increase in mouse and rat brain acetylcholine levels by diazepam. In: The Benzodiazepines, ed. by S. Garattini, E. Mussini and L.O. Randall, Raven Press, New York, pp. 241-242, 1973.

Lauener, H.: Conditioned suppression in rats and the effect of pharmacological agents thereon. Psychopharmacologia 4: 311-325, 1963.

Lidbrink, P., Corrodi, H., Fuxe, K. and Olson, L.: The effects of benzodiazepines, meprobamate and barbiturates on central monoamine neurons. In: The Benzodiazepines, ed. by S. Garattini, E. Mussini and L.O. Randall, Raven Press, New York, pp. 203-223, 1973.

Lidbrink, P., Corrodi, H. and Fuxe, K.: Benzodiazepines and barbiturates: Turnover changes in central 5-hydroxytryptamine pathways. Eur. J. Pharmacol. 26: 35-40, 1974.

Lindström, B., Sjöquist, B. and Anggard, E.: Preparation of deuterium labelled catecholamines, catecholamine precursors and metabolites for use as internal standards in mass fragmentographic determination and for turnover studies. J. Labelled Compounds 10: 187-194, 1974.

Lippa, A.S., Greenblatt, E.N. and Pelham, R.W.: The use of animal models for delineating the mechanisms of action of anxiolytic agents. In: Animal Models in Psychiatry and Neurology, ed. by I. Hanin and E. Usdin, Raven Press, New York, pp. 279-292, 1977a.

Lippa, A.S., Nash, P.A. and Greenblatt, E.N.: Preclinical neuropsychopharmacological testing procedures for anxiolytic drugs. In: The Anxiolytics. Vol. 3, ed. by S. Fielding and H. Lal, Futura Publishing Co., 1977b.

Lippa, A.S. and Regan, B.: Additional studies on the importance of glycine and GABA in mediating the actions of benzodiazepines. Life Sci. 21: 1779-1784, 1978.

Lippmann, W. and Pugsley, T.A.: Effects of benzoctamine and chlordiazepoxide on turnover and uptake of 5-hydroxytryptamine in the brain. Brit. J. Pharmacol. 51: 571-575, 1974.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275, 1951.

MacDonald, R. and Barker, J.L.: Benzodiazepines specifically modulate GABA-mediated postsynaptic inhibition in cultured mammalian neurones. Nature 271: 563-564, 1978.

Mandell, A.J.: Redundant mechanisms regulating brain tyrosine and tryptophan hydroxylases. Ann. Rev. Pharmacol. Toxicol. 18: 461-493, 1978.

Mao, C.C., Guidotti, A. and Costa, E.: Evidence for an involvement of GABA in the mediation of the cerebellar cGMP decrease and the anticonvulsant action of diazepam. Naunyn-Schmiedeberg's Arch. Pharmacol. 289: 369-378, 1975.

Mao, C.C., Marco, E., Revuelta, A., Bertilsson, L. and Costa, E.: The turnover rate of γ-aminobutyric acid in the nuclei of telencephalon: Implications in the pharmacology of antipsychotics and of a minor tranquilizer. Biol. Psychiat. 12: 359-371, 1977.

Margules, D.L. and Stein, L.: Increase of "antianxiety" activity and tolerance of behavioral depression during chronic administration of oxazepam. Psychopharmacologia 13: 74-80, 1968.

McCrea, D.A., Jordan, L.M. and Lake, N.: Microiontophoresis in the amygdala of rat. Canada Physiol. 4: 189, 1973.

McMillan, D.E.: Drugs and punished responding. III. Punishment intensity as a determinant of drug effect. Psychopharmacologia 30: 61-74, 1973.

Miczek, K.A.: Effects of scopolamine, amphetamine and chlordiazepoxide on punishment. Psychopharmacologia 28: 373-389, 1973a.

Miczek, K.A.: Effects of scopolamine, amphetamine and benzodiazepines on conditioned suppression. Pharmacol. Biochem. Behav. 1: 401-411, 1973b.

Miczek, K.A. and Luttinger, D.: Differential attenuation of two kinds of conditioned suppression by d-amphetamine and pentobarbital. J. Pharmacol. Exp. Ther. 205: 282-290, 1978.

Millenson, J.R. and Leslie, J.: The conditioned emotional response (CER) as a baseline for the study of anti-anxiety drugs. Neuropharmacology 13: 1-9, 1974.

Möhler, H. and Okada, T.: Benzodiazepine receptor: Demonstration in the central nervous system. Science 198: 849-851, 1977.

Möhler, H. and Okada, T.: Biochemical identification of the site of action of benzodiazepines in human brain by ^{3}H-diazepam binding. Life Sci. 22: 985-996, 1978.

Moir, A.T.B. and Eccleston, D.: The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. J. Neurochem. 15: 1093-1108, 1968.

Molinengo, L. and Gamalero, S.R.: Behavioral action and tranquilizing effect of reserpine, diazepam and hydroxyzine. Arch. Int. Pharmacodyn. Ther. 180: 217-231, 1969.

Morillo, A.: Effects of benzodiazepines upon amygdala and hippocampus of the rat. Int. J. Neuropharmacol. 1: 353-359, 1962.

Morot-Gaudry, Y., Hamon, M., Bourgoin, S., Ley, J.P. and Glowinski, J.: Estimation of the rate of 5-HT synthesis in the mouse brain by various methods. Naunyn-Schmiedeberg's Arch. Pharmacol. 282: 223-238, 1974.

Nadel, L.: Dorsal and ventral hippocampal lesions and behavior. Physiol. Behav. 3: 891-900, 1968.

Nagel, J.A. and Kemble, E.D.: Effects of amygdaloid lesions on the performance of rats in four passive avoidance tasks. Physiol. Behav. 17: 245-250, 1976.

Nagy, J. and Decsi, L.: Location of the site of the tranquilizing action of diazepam by intralimbic application. Neuropharmacology 12: 757-768, 1973.

Nakamura, M. and Fukushima, H.: Effects of benzodiazepines on central serotonergic neuron systems. Psychopharmacology 53: 121-126, 1977.

Nakamura, M. and Fukushima, H.: Effects of reserpine, para-chlorophenylalanine, 5,6-dihydroxytryptamine and fludiazepam on the head twitches induced by 5-hydroxytryptamine or 5-methoxytryptamine in mice. J. Pharm. Pharmacol. 30:254-256, 1978.

Neckers, L.M. and Meek, J.L.: Measurement of 5-HT turnover rate in discrete nuclei of rat brain. Life Sci. 19: 1579-1584, 1976.

Neff, N.H., Tozer, T.N. and Brodie, B.B.: Application of steady state kinetics to studies of the transfer of 5-hydroxyindoleacetic acid from brain to plasma. J. Pharmacol. Exp. Ther. 158: 214-218, 1967.

Oishi, H., Iwahara, S., Yang, K.M. and Yogi, A.: Effects of chlordiazepoxide on passive avoidance responses in rats. Psychopharmacologia 23: 373-385, 1972.

Olds, M.E. and Olds, J.: Effects of anxiety-relieving drugs on unit discharges in hippocampus, reticular midbrain and pre-optic area in the freely moving rat. Int. J. Neuropharmacol. 8: 87-103, 1969.

Oppizzi, G., Verde, G., De Stefano, L., Cozzi, R., Botalla, L., Liuzzi, A. and Chiodini, P.G.: Evidence for a dopaminergic activity of methysergide in humans. Clin. Endocrin. 7: 267-272, 1977.

Padjen, A. and Bloom, F.: Problems in the electrophysiological analysis of the site of action of benzodiazepines. In: Mechanism of Action of Benzodiazepines, ed. by E. Costa and P. Greengard, Raven Press, New York, pp. 93-102, 1975.

Palkovits, M.: Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res. 59: 449-450, 1973.

Palkovits, M., Saavedra, J.M., Kobayashi, R.M. and Brownstein, M.: Choline acetyltransferase content of limbic nuclei of the rat. Brain Res. 79: 443-450, 1974.

Pellegrino, L.: Amygdaloid lesions and behavioral inhibition in the rat. J. Comp. Physiol. Psychol. 65: 483-491, 1968.

Pellegrino, L.J. and Cushman, A.J.: A Stereotaxic Atlas of the Rat Brain. Appleton-Century-Crofts, New York, 1967.

Pericic, D., Walters, J.R. and Chase, T.N.: Effect of diazepam and pentobarbital on aminooxyacetic acid-induced accumulation of GABA. J. Neurochem. 29: 839-846, 1977.

Polc, P., Möhler, H. and Haefely, W.: The effect of diazepam on spinal cord activities: Possible sites and mechanisms of action. Naunyn-Schmiedeberg's Arch. Pharmacol. 284: 319-337, 1974.

Polc, P. and Haefely, W.: Effects of two benzodiazepines, phenobarbitone, and baclofen on synaptic transmission in the cat cuneate nucleus. Naunyn-Schmiedeberg's Arch. Pharmacol. 294: 121-131, 1976.

Poling, A., Urbain, C. and Thompson, T.: Effects of d-amphetamine and chlordiazepoxide on positive conditioned suppression. Pharmacol. Biochem. Behav. 7: 233-237, 1977.

Randall, L.O.: Pharmacology of methaminodiazepoxide. Dis. Nerv. Syst. 21: (March suppl) 7-10, 1960.

Randall, L.O., Schallek, W., Heise, G.A., Keith, E.F. and Bagdorn, R.E.: The psychosedative properties of methaminodiazepoxide. J. Pharmacol. Exp. Ther. 129: 163-171, 1960.

Rastogi, R.B., Agarwal, R.A., Lapierre, Y.D. and Singhal, R.L.: Effects of acute diazepam and clobazam on spontaneous locomotor activity and central amine metabolism in rats. Eur. J. Pharmacol. 43: 91-98, 1977.

Reinhard, J.F. and Wurtman, R.J.: Relation between brain 5-HIAA levels and the release of serotonin into brain synapses. Life Sci. 21: 1741-1746, 1977.

Robichaud, R.C. and Sledge, K.L.: The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. Life Sci. 8: 965-969, 1969.

Robichaud, R.C., Sledge, K.L., Hefner, M.A. and Goldberg, M.E.: Propranolol and chlordiazepoxide on experimentally induced conflict and shuttle box performance in rodents. Psychopharmacologia 32: 157-160, 1973.

Robinson, E.: Effect of amygdalectomy on fear-motivated behavior in rats. J. Comp. Physiol. Psychol. 56: 814-820, 1963.

Rubin, B., Piala, J.J., Burke, J.C. and Craver, B.N.: A new potent and specific serotonin inhibitor (SQ 10,643) 2'-(3-dimethylaminopropylthio)cinnamanilide hydrochloride: Antiserotonin activity on uterus and on gastrointestinal, vascular, and respiratory systems of animals. Arch. Int. Pharmacodyn. Ther. 152: 132-143, 1964.

Ruch-Monachon, M.A., Jalfre, M. and Haefely, W.: Drugs and PGO waves in the lateral geniculate body of the curarized cat. IV. The effects of acetylcholine, GABA and benzodiazepines on PGO wave activity. Arch. Int. Pharmacodyn. Ther. 219: 308-325, 1976.

Russo, N.J., Kapp, B.S., Holmquist, B.K. and Musty, R.E.: Passive avoidance and amygdala lesions: Relationship with pituitary-adrenal system. Physiol. Behav. 16: 191-199, 1976.

Saad, S.: Effect of diazepam on gamma-aminobutyric acid content of mouse brain. J. Pharm. Pharmacol. 24: 839-840, 1972.

Saavedra, J.M., Brownstein, M. and Palkovits, M.: Serotonin distribution in the limbic system of the rat. Brain Res. 79: 437-441, 1974.

Saavedra, J.M. and Zivin, J.: Tyrosine hydroxylase and dopamine-β-hydroxylase: Distribution in discrete areas of the rat limbic system. Brain Res. 105: 517-524, 1976.

Saavedra, J.M.: Distribution of serotonin and synthesizing enzymes in discrete areas of the brain. Fed. Proc. 36: 2134-2141, 1977.

Samanin, R., Bendotti, C., Candelaresi, G. and Garattini, S.: Specificity of serotonergic involvement in the decrease of food intake induced by quipazine in the rat. Life Sci. 21: 1259-1266, 1977.

Sanders-Bush, E. and Massari, V.J.: Action of drugs that deplete serotonin. Fed. Proc. 36: 2149-2153, 1977.

Schallek, W., Kuehn, A. and Nailan, J.: Effects of chlordiazepoxide (Librium) and other psychotropic drugs on the limbic system of the brain. Ann. N.Y. Acad. Sci. 96: 303-312, 1962.

Schallek, W., Zabransky, F. and Kuehn, A.: Effects of benzodiazepines on central nervous system of cat. Arch. Int. Pharmacodyn. Ther. 149: 467-483, 1964.

Schallek, W., Schlosser, W. and Randall, L.O.: Recent developments in the pharmacology of the benzodiazepines. Adv. Pharmacol. Chemother. 10: 119-183, 1972.

Schoenfeld, R.I.: Lysergic acid diethylamide- and mescaline-induced attenuation of the effect of punishment in the rat. Science 192: 801-803, 1976.

Schwartzbaum, J.S., Green, R.H., Beatty, W.W. and Thompson, J.B.: Acquisition of avoidance behavior following septal lesions in the rat. J. Comp. Physiol. Psychol. 63: 95-104, 1967.

Sepinwall, J., Grodsky, F.S., Sullivan, J.W. and Cook, L.: Effects of propranolol and chlordiazepoxide on conflict behavior in rats. Psychopharmacologia 31: 375-382, 1973.

Sepinwall, J., Grodsky, F.S. and Cook, L.: Conflict behavior in the squirrel monkey: Effects of chlordiazepoxide, diazepam and N-desmethyldiazepam. J. Pharmacol. Exp. Ther. 204: 88-102, 1978.

Sharma, J.N.: Microiontophoretic application of some monoamines and their antagonists to cortical neurons of the rat. Neuropharmacology 16: 83-88, 1977.

Skinner, B.F.: The steep and thorny way to a science of behavior. Am. Psychol. 30: 42-49, 1975.

Slotnick, B.M.: Fear behavior and passive avoidance deficits in mice with amygdala lesions. Physiol. Behav. 11: 717-720, 1973.

Sloviter, R.S., Drust, E.G. and Connor, J.D.: Serotonin agonist actions of p-chlorophenylalanine. Neuropharmacology 17: 1029-1033, 1978.

Smith, J.E., Lane, J.D., Shea, P.A. and McBride, W.J.: Neurochemical changes following the administration of precursors of biogenic amines. Life Sci. 21: 301-306, 1977.

Sokal, R.R. and Rohlf, F.J.: Biometry, W.H. Freeman and Company. pp. 299-340, 1969.

Soubrie, P., Simon, P. and Boissier, J.R.: Antagonism of diazepam against central anticholinergic drug-induced hyperactivity in mice: Involvement of a GABA mechanism. Neuropharmacology 15: 773-776, 1976.

Soubrie, P. and Simon, P.: Comparative study of the antagonism of bemegride and picrotoxin on behavioral depressant effects of diazepam in rats and mice. Neuropharmacology 17: 121-125, 1978.

Speth, R.C., Wastek, G.J., Johnson, P.C. and Yamamura, H.I.: Benzodiazepine binding in human brain: Characterization using [^{3}H]-flunitrazepam. Life Sci. 22: 859-866, 1978.

Spevack, A.A., Campbell, C.T. and Drake, L.: Effect of amygdalectomy on habituation and CER in rats. Physiol. Behav. 15: 199-207, 1975.

Squires, R.F. and Braestrup, C.: Benzodiazepine receptors in rat brain. Nature 266: 732-734, 1977.

Stein, L., Wise, C.D. and Berger, B.D.: Antianxiety action of benzodiazepines: Decrease in activity of serotonin neurons in the punishment system. In: The Benzodiazepines, ed. by S. Garattini, E. Mussini and L.O. Randall, Raven Press, New York, pp. 299-326, 1973.

Stein, L., Wise, C.D. and Belluzzi, J.D.: Effects of benzodiazepines on central serotonergic mechanisms. In: Mechanism of Action of Benzodiazepines, ed. by E. Costa and P. Greengard, Raven Press, New York, pp. 29-44, 1975.

Stein, L., Belluzzi, J.D. and Wise, C.D.: Benzodiazepines: Behavioral and neurochemical mechanisms. Am. J. Psychiatry 134: 665-669, 1977.

Stein, L.: Reward transmitters: Catecholamines and opioid peptides. In: Psychopharmacology: A Generation of Progress, ed. by M.A. Lipton, A. Di Mascio and K.F. Killam, Raven Press, New York, pp. 569-581, 1978.

Steiner, F.A. and Felix, D.: Antagonistic effects of GABA and benzodiazepines on vestibular and cerebellar neurones. Nature 260: 346-347, 1976.

Stone, C.A., Wenger, H.C., Ludden, C.T., Stavorski, J.M. and Ross, C.A.: Antiserotonin-antihistaminic properties of cyproheptadine. J. Pharmacol. Exp. Ther. 131: 73-84, 1961.

Straughan, D.W. and Legge, K.F.: The pharmacology of amygdaloid neurones. J. Pharm. Pharmacol. 17: 675-677, 1965.

Suboski, M.D., Marquis, H.A., Black, M. and Platenius, P.: Adrenal and amygdala function in the incubation of aversively conditioned responses. Physiol. Behav. 5: 283-289, 1970.

Sullivan, J.W., Sepinwall, J. and Cook, L.: Anticonflict evaluation of muscimol, a GABA receptor agonist, alone and in combination with diazepam. Fed. Proc. 37: 619, 1978.

Svensson, T.: Attenuated feed-back inhibition of brain serotonin synthesis following chronic administration of imipramine. Naunyn-Schmiedeberg's Arch. Pharmacol. 302: 115-118, 1978.

Swahn, C-G., Sandgarde, B., Wiesel, F-A. and Sedvall, G.: Simultaneous determination of the three major monoamine metabolites in brain tissue and body fluids by a mass fragmentographic method. Psychopharmacology 48: 147-152, 1976.

Tappaz, M.L., Brownstein, M.J. and Palkovits, M.: Distribution of glutamate decarboxylase in discrete brain nuclei. Brain Res. 108: 371-379, 1976.

Taylor, K.M. and Laverty, R.: The interaction of chlordiazepoxide, diazepam and nitrazepam with catecholamines and histamine in regions of the rat brain. In: The Benzodiazepines, ed. by S. Garattini, E. Mussini and L.O. Randall, Raven Press, New York, pp. 191-202, 1973.

Tedeschi, D.H.: Criteria for the selection of pharmacological test procedures useful in the evaluation of neuroleptic drugs. In: The Present Status of Psychotropic Drugs. Pharmacological and Clinical Aspects, ed. by A. Cerletti and F.J. Bove, Excerpta Medica Foundation, Amsterdam, pp. 145-153, 1969.

Tozer, T.N., Neff, N.H. and Brodie, B.B.: Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats. J. Pharmacol. Exp. Ther. 153: 177-182, 1966.

Trulson, M.E., Eubanks, E.E. and Jacobs, B.L.: Behavioral evidence for supersensitivity following destruction of central serotonergic nerve terminals by 5,7-dihydroxytryptamine. J. Pharmacol. Exp. Ther. 198: 23-32, 1976.

Ts'o, T.O.T. and Chenoweth, M.B.: Comparison between chronic chlordiazepoxide treatment and shock removal in a conflict situation in rats. Neuropharmacology 15: 99-101, 1976.

Tsuchiya, T. and Kitagawa, S.: Effects of benzodiazepines and pentobarbital on the evoked potentials in the cat brain. Jap. J. Pharmacol. 26: 411-418, 1976.

Tye, N.C., Everitt, B.J. and Iversen, S.D.: 5-Hydroxytryptamine and punishment. Nature 268: 741-743, 1977.

Umemoto, M. and Olds, M.E.: Effects of chlordiazepoxide, diazepam and chlorpromazine on conditioned emotional behavior and conditioned neuronal activity in limbic, hypothalamic and geniculate regions. Neuropharmacology 14: 413-425, 1975.

Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. 82(Suppl. 367): 1-48, 1971.

van Riezen, H.: Different central effects of the 5-HT antagonists mianserine and cyproheptadine. Arch. Int. Pharmacodyn. Ther. 198: 256-269, 1972.

Veening, J.G.; Subcortical afferents of the amygdaloid complex in the rat: An HRP study. Neuroscience Letters 8: 197-202, 1978a.

Veening, J.G.: Cortical afferents of the amygdaloid complex in the rat: An HRP study. Neuroscience Letters 8: 191-196, 1978b.

Vogel, J.R., Beer, B. and Clody, D.E.: A simple and reliable conflict procedure for testing antianxiety agents. Psychopharmacologia 21: 1-7, 1971.

Von Voigtlander, P.F. and Losey, E.G.: On the use of selective neurotoxic amine analogs to measure the blockade of norepinephrine and 5-hydroxytryptamine uptake systems by antidepressants. Res. Comm. Chem. Path. Pharmacol. 13: 389-400, 1976.

Waddington, J.L. and Longden, A.: Rotational behavior and cGMP responses following manipulation of nigral mechanisms with chlordiazepoxide. Naunyn-Schmiedeberg's Arch. Pharmacol. 300: 233-237, 1977.

Waddington, J.L. and Owen, F.: Stereospecific benzodiazepine receptor binding by the enantiomers of oxazepam sodium hemisuccinate. Neuropharmacology 17: 215-216, 1978.

Wang, R.Y. and Aghajanian, G.K.: Inhibition of neurons in the amygdala by dorsal raphe stimulation. Mediation through a direct serotonergic pathway. Brain Res. 120: 85-102, 1977.

Warner, R.S.: Management of the office patient with anxiety and depression. Psychosomatics 6: 347-351, 1965.

Watson, E., Wilk, S. and Roboz, J.: Derivatization and gas chromatographic determination of some biologically important acids in cerebrospinal fluid. Analyt. Biochem. 59: 441-449, 1974.

Weiner, N.: A critical assessment of methods for the determiation of monoamine synthesis turnover rates in vivo. In: Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes, ed. by E. Usdin, Raven Press, New York, pp. 143-159, 1974.

Wiesel, F.A., Fri, C.G. and Sedvall, G.: Determination of homovanillic acid turnover in rat striatum using a monoamine oxidase inhibitor. Eur. J. Pharmacol. 23: 104-106, 1973.

Wilk, S., Watson, E. and Travis, B.: Evaluation of dopamine metabolism in rat striatum by a gas chromatographic technique. Eur. J. Pharmacol. 30: 238-243, 1975.

Wilson, M.C.: The effect of cocaine and d-amphetamine on punished responding. Arch. Int. Pharmacodyn. Ther. 227: 98-105, 1977.

Wise, C.D., Berger, B.D. and Stein, L.: Benzodiazepines: Anxiety-reducing activity by reduction of serotonin turnover in the brain. Science 177: 180-183, 1972.

Wuttke, W. and Kelleher, R.T.: Effects of some benzodiazepines on punished and unpunished behavior in the pigeon. J. Pharmacol. Exp. Ther. 172: 397-405, 1970.

Young, A.B. and Snyder, S.H.: Strychnine binding associated with glycine receptors of the central nervous system. Proc. Nat. Acad. Sci. 70: 2832-2836, 1973.

Young, A.B., Zukin, S.R. and Snyder, S.H.: Interaction of benzodiazepines with central nervous glycine receptors: Possible mechanism of action. Proc. Nat. Acad. Sci. 71: 2246-2250, 1974.

Zakusov, V.V., Ostrovskaya, R.V., Markovitch, V.V., Molodavkin, G.M., Bulayev, V.M.: Electrophysiological evidence for an inhibitory action of diazepam upon cat brain cortex. Arch. Int. Pharmacodyn. Ther. 214: 188-205, 1975.

Zsilla, G., Cheney, D.L. and Costa, E.: Regional changes in the rate of turnover of acetylcholine in rat brain following diazepam or muscimol. Naunyn-Schmiedeberg's Arch. Pharmacol. 294: 251-255, 1976.