THE ROLE OF THE AMYGDALA IN MEDIATING GENETIC DIFFERENCES IN SENSITIVITY TO ANXIOLYSIS BY BENZODIAZEPINES IN MICE

by

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Abbreviations

5,7-DHT	5,7-Dihydroxytryptamine
5-HT	Serotonin
AA	Anterior amygdala
ABL	Basolateral amygdala
ABM	Basomedial amygdala
Ach	Acetylcholine
ACTH	Adrenocorticotropin hormone
AHA	Anterior hypothalamus
AL	Anterolateral
AME	Medial amygdala
ARH	Arcuate nucleus of the hypothalamus
AT	Anterior thalamus
AV	Anteroventral thalamic nucleus
bCCM	Beta-carboline
BLA	Basolateral amygdala
BST	Bed nucleus of the stria terminalis
BZs	Benzodiazepine
cc	Corpus Callosum
CCK	Cholecystokinin
CDP	Chlordiazepoxide
CEA	Central nucleus of the amygdala
CIN	Cingulate cortex
CLA	Claustrum
CPP	Conditioned place preference
CPU	Caudate/Putamen
CRF	Corticotropin releasing factor
CS	Conditioned stimulus
CSF	Cerebrospinal fluid
DBI	Diazepam binding inhibitor
DHP mice	Diazepam high performing mice
DLP mice	Diazepam low performing mice
DMCM	carboline
DMH	Dorsal medial hypothalamus
dPAG	Dorsal periaqueductal grey
DR mice	Diazepam resistant mice
DRN	Dorsal raphe nucleus
DS mice	Diazepam sensitive mice
EP	Entopeduncular nucleus
fi	Hippocampal fimbria
fx	Fornix
GABA	γ-aminobutyric acid
GLD	Dorsolateral geniculate
GP	Globus pallidum
GVG	γ-vinyl-γ-aminobutyric acid
H	Habenula
HPA	Hypothalamic/pituitary/adrenal
HPC	Hippocampus
HR rats	High responders
ic	Internal capsule

IC	Inferior colliculus
IS	Inescapable shock
LHA	Lateral hypothalamic nucleus
LORR	Loss-of-righting reflex
LR rats	Low responders
MB	Mammillary body
MES	Maximal electric shock
N	Neocortex
NA	Nucleus accumbens
NB	Nucleus basalis
NBM	Nucleus basalis magnocellularis
NO	Nitric oxide
ot	Optic tract
PAG	Periaqueductal grey
PAM	Periamygdalar cortex
PET	Positron emission tomography
PH	Posterior hypothalamus
PIR	Piriform cortex
PT	Paratenialis thalamic nucleus
PTZ	Pentylenetetrazol
PV	Paraventricular thalamic nucleus
QAR	Quantitative autoradiography
RAS	Reticular activating system
REM	Rapid eye movement
RT	Nucleus reticularis
SCN	Suprachiasmatic nucleus
SN	Substantia nigra
SNR	Substantia nigra reticulata
TBPS	thio....
THE	Tonic hindlimb extension
VB	Ventral thalamus, pars basalis
Veh	Vehicle
VL	Lateral ventricle
VM	Ventromedial thalamus
VMH	Ventral medial hypothalamus
vPAG	Ventral periaqueductal grey
VP/SI	Ventral pallidum/substanstia innominata
VTA	Ventral tegmental area

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I avow my gratitude for the assistance provided by a great many people. Of course, a thesis is a dynamic system with multiple connections between people, ideas, and work. The document is just a cross-sectional view of this constantly changing, complex system. How to reflect much of this dynamic system in a relative paucity of words?

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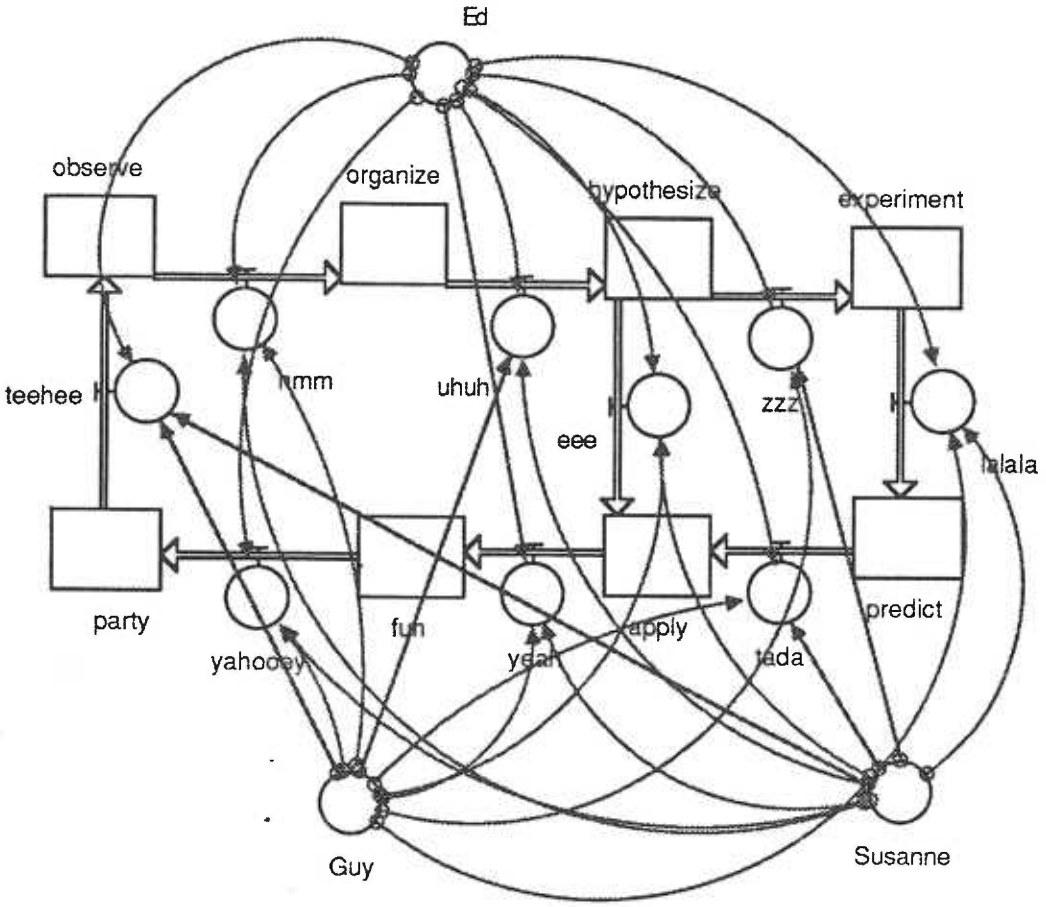

Abstract

Benzodiazepines (BZs) are clinically useful anxiolytics. They also produce sedation, muscle relaxation, ataxia, and protection against seizures. In most clinical situations, more specificity of effects is desired. Therefore, delineating the mechanisms specifically underlying their many effects is a major goal of research. By employing selectively bred lines of animals, mechanisms of drug effects can be studied (Crabbe et al. 1994). Thus, to examine the physiological bases underlying the behavioral effects of BZs, mice were selectively bred for either sensitivity or resistance to the ataxic effect of diazepam (Gallaher et al. 1987). Interestingly, the line sensitive to the ataxic effect (DS mice) is resistant to the anxiolytic effect. Conversely, the line resistant to ataxia (DR mice) is sensitive to anxiolysis. For the seizure protective effects of BZs, the lines are equally sensitive. Therefore, it is possible that separate underlying mechanisms mediate ataxia, anxiolysis, and seizure protection. Understanding each of these mechanisms will be valuable in developing more specific treatments for anxiety. In the studies reported here, experiments were performed to determine a neuroanatomic locus which could specifically contribute to differences in sensitivity to anxiolysis in these mice.

The amygdala has been implicated as a site which can mediate the anxiolytic effect of BZs. It is possible that there are differences in sensitivity to BZs in the amygdala of DS and DR mice. These differences could contribute to the differences in sensitivity to anxiolysis. If the amygdala is capable of mediating differences in sensitivity to anxiolysis, then direct injection of BZs into this structure should result in greater anxiolysis in DR mice than in DS mice.

To test this hypothesis, chlordiazepoxide (CDP), a BZ, was bilaterally micro-injected into the amygdala of the DS and DR mice. Stereotaxic surgery in

anesthetized subjects was used to implant guide cannula, and the micro-injections were subsequently performed in unrestrained, unanaesthetized subjects. The anxiolytic effect of the micro-injections was measured using a plus maze. Immediately following injections, locomotor activity was diminished in both lines of mice. In DR mice, CDP reversed the decrement in locomotor activity. The total number of arm entries after vehicle injection was 4.2 ± 1.2, and the total number of entries after CDP injection was increased to 10.9 ± 1.7 ($p < 0.01$). This reversal of stress-induced freezing is a potential measure of anxiolysis. CDP micro-injections also increased the percent of open arm entries in DR mice when tested 24 min after injection (vehicle = $16.4\% \pm 2.2$; CDP = $41.0\% \pm 3.9$; $p < 0.001$). Increases in this measure are interpreted as indicating anxiolysis. Neither of these anxiolytic effects of CDP micro-injections were found in DS mice. These results suggest that the amygdala can mediate the difference in sensitivity to anxiolysis in these mice.

A variety of other effects produced by BZs were also evaluated following micro-injections. First, the total number of entries into either closed or open arms of the plus maze was used as a measure of total activity; an measure of either locomotor hypoactivity or locomotor activation. Muscle relaxation was examined by measuring forelimb grip strength, ataxia was measured with an accelerating rotarod, and seizure protection was measured using tail-vein infusions of pentylenetetrazol. Micro-injections of CDP into the amygdala did not affect these other measures in either line, indicating that the amygdala does not mediate any of these effects of BZs. In addition, the results indicate that anxiolysis, per se, does not indirectly contribute to BZs' effects on these other measures. That is, these tests measure separate phenomena which can be mediated by separate neuroanatomic systems. For example, locomotor activation is not necessarily the result of anxiolysis, and has a different underlying neuroanatomic substrate.

The anatomic specificity of the micro-injections was tested by injecting CDP into the caudal ventrolateral caudate, a region which neighbors the anterolateral amygdala. These injections did not affect any of the measures in either DS or DR mice. This finding suggests that the ventrolateral caudate does not mediate anxiolysis, ataxia, muscle relaxation, locomotor sedation, or protection against seizures. Additionally, it is unlikely that the results of the amygdala infusions were the consequence of diffusion of CDP into nearby structures.

This thesis confirms the utility of using anatomically specific injections in mice. The DS and DR mice can now be examined for additional differences in other neuroanatomic areas. More directly, the observed differences in anxiolysis provide the impetus for further biochemical, electrophysiologic, and neurochemical examinations of the amygdala.

Introduction

1.1 Purpose of the study

Benzodiazepines (BZs) are used to treat a variety of conditions (Hollister et al. 1993). In addition to their use as anxiolytics and hypnotic sedatives, they are used as muscle relaxants, as treatments for seizures and intestinal disorders, as retrograde amnesics for surgical procedures, and as serenics. These medications can also produce ataxia, tolerance, REM sleep impairment, and deficits in attention (Woods and Winger 1995). Additionally, hyperexcitability and psychoses can be produced in some people (Bixler et al. 1987). Following their introduction in the 1960s (Tobin and Lewis 1960), they became one of the most commonly prescribed classes of medications in the world. A survey conducted in 1981 found that the prevalence of BZ use in the USA was 12.9% of the population (Roth 1989).

In many clinical situations, several of these various responses constitute unwanted side-effects. That is, for a particular treatment, only a subset of the effects is desired. For other effects such as ataxia, there is no known clinical use. Thus, there is a need for more behaviorally selective drugs. The rational design of more specific treatments requires an understanding of the mechanisms underlying each of the effects.

Towards this end, mouse lines that differ in sensitivity to BZs have been created through selective breeding (Gallaher et al. 1987). DS (diazepam-sensitive) mice have increased sensitivity to the ataxic effects of diazepam while DR (diazepam-resistant) mice are relatively resistant to the ataxic effects. Conversely, DR mice are more sensitive to the anxiolytic and locomotor-activating effects of diazepam (Courtney and Gallaher 1991; Courtney et al. in prep.; Phillips and Gallaher 1992). The two lines are equally sensitive to the ability of BZs to protect against the seizures produced by pentylenetetrazol (PTZ) infusions (Gallaher and Gionet 1988). If each of these effects

of BZs were mediated by the same mechanism, then it would be expected that each of the phenotypes would be positively correlated. However, both negative correlations and a lack of correlations have been found for various BZ effects in DS and DR mice, suggesting that different systems may be involved in mediating these distinct effects. Negative correlations do not prove that effects are mediated by distinct mechanisms, however, as a relationship between the phenotypes can exist with negative correlations. For example, sensitivity to locomotor activation may mediate resistance to locomotor sedation or ataxia. Thus, the implications of the phenotypic correlations require further evaluation.

An review of genetic differences for various BZ-affected behaviors is provided in section 2.0 of the introduction. In brief, the results of these studies suggest (but do not prove) that various BZ effects are, in fact, influenced by seperate underlying mechanisms. Conversely, some effects such as ataxia and locomotor sedation may be mediated by similar mechanisms. If the various behavioral effects of BZs are mediated by different systems, the different systems may reflect anatomically different neural circuitry (Figure 1). This possibility can be evaluated.

As mentioned, these mice differ in sensitivity to anxiolysis. Because treating anxiety is an important clinical use of BZs, it is important to understand the mechanisms of anxiolysis as distinct from other effects. It is proposed that the difference in anxiolytic sensitivity in these mice can be utilized to examine the mechanisms that specifically underlie anxiolysis, as opposed to the mechanisms that underlie other BZ effects. A critical step in this process is proposed--to wit, an examination of the differences in DS and DR mice at a neuroanatomic level.

Commonly, selectively bred animals are examined for differences which correlate with the selected trait (Crabbe and Phillips 1993; Crabbe et al. 1990). However, this thesis does not involve a similar examination of the DS and DR mice.

These mice were selectively bred for differences in sensitivity to ataxia, and correlation of ataxia with other phenotypes is ongoing. However, the purpose of this thesis is not to determine correlations between ataxic sensitivity and other differences.

The finding that the sensitivity to anxiolysis in these mice is paradoxically opposite to sensitivity to ataxia allows a novel approach to delineating the mechanisms underlying anxiolysis. That is neuroanatomic basis for their difference in sensitivity to anxiolysis, as opposed to other BZ effects such as ataxia, can be explored.

Because the specific interest of this thesis is the genetic difference in anxiolytic sensitivity, and because numerous studies in the rat implicate the amygdala in mediating anxiolysis (See section 3.1), this study has focused on the role of the amygdala in mediating the difference in anxiolytic sensitivity in these mice. It is hypothesized that other BZ effects are mediated by separate physiologic/neuroanatomic mechanisms. Therefore, the proposal that the amygdala does not mediate other BZ effects was also examined. The specific questions are: (1) Do injections of BZs into the amygdala of mice results in anxiolysis? (2) Do intra-amygdala BZ injections result in more anxiolysis in DR mice than DS mice? (3) Are the intra-amygdala injections behaviorally specific? That is, do intra-amygdala injections fail to produce changes in locomotor activity, rotarod performace, grip strenght, or sensitivity to various seizure endpoints? (4) Are the intra-amygdala injections anatomically specific? That is, will injections into a neighboring area fail to produce any anxiolysis?

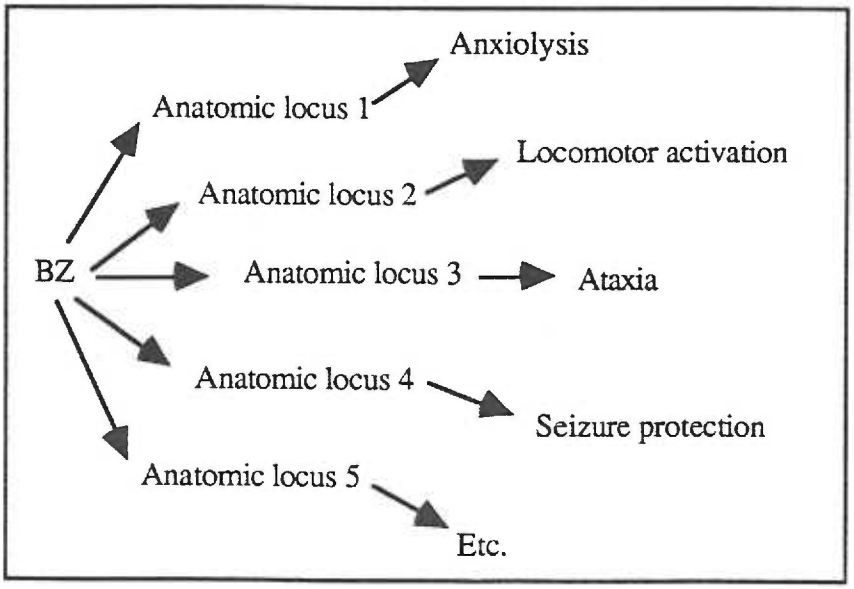

Figure 1. Schematic of neuroanatomic loci mediating differing BZ effects. In this schematic, the various effects of BZs are represented as being mediated by separate neuroanatomic loci. This possibility can be tested by injecting BZs directly into specific anatomic circuits. Another possibility, not seen in this figure, is that several effects may be mediated by common neuroanatomic loci. It is also possible that more than one locus may mediate a particular behavior.

To evaluate the role of the amygdala, methods for intra-cerebral infusions followed by behavioral testing in mice were developed. Then both DS and DR mice were bilaterally cannulated using stereotaxic surgery. After recovery from surgery, chlordiazepoxide (CDP; Librium) was infused into the amygdala. Following infusion, the mice were examined for anxiolysis and several other effects normally produced by systemic BZ injections: muscle relaxation, ataxia, locomotor hypoactivity, locomotor activation, and protection against PTZ-induced seizures. Differences in the anxiolytic effect of intra-amygdala CDP injections in DS and DR mice were examined. It was

expected that the amygdala could mediate differences in sensitivity to anxiolysis. That is, anxiolysis could be more readily produced in one line (DR) than the other (DS). These differences would implicate the amygdala in genetic influences on sensitivity to BZ-induced anxiolysis.

Furthermore, the amygdala was not expected to be involved in mediating other effects of BZs. By implicating the amygdala in a specific behavioral difference, more focused examinations of this region would be justified. For example, electrophysiologic, morphologic, and biochemical differences between DS and DR in the amygdala (or its projection sites) could be examined. Any functional differences between DS and DR mice in this specific anatomic location could therefore be implicated in the differences in anxiolysis--and not be implicated in sensitivity differences for other BZ effects.

For example, functional differences in specific cell types in the amygdala of DS and DR mice would implicate these cellular differences in anxiolytic sensitivity but not ataxic sensitivity. Or receptor subtype differences in the amygdala would be specifically implicate these receptor subtypes in anxiolysis. The relationship of the amygdala with other areas could also be explored. For example, the firing rate of dorsal raphe neurons or the level of serotonin in the hippocampus could be measured following intra-amygdala BZ injections. Differences in these measures in DS and DR mice would specifically implicate these phenomena in anxiolysis.

Although identification of the amygdala as a locus involved in differences in anxiolytic sensitivity was a purpose of this thesis, identification of the anatomical areas that underlie other behavioral effects was beyond the scope of this project. However, similar studies to examine other BZ-influenced behaviors could be executed in future experiments. For example, the neuroanatomic loci that mediate locomotor activation could be identified using micro-injections. Functional differences in these specified

locations could then be implicated in differences in sensitivity to locomotor activation. Similarly, ataxia and the other behavioral effects of BZ could be examined.

To reiterate, a robust difference in sensitivity to anxiolysis has developed in DS and DR mice, though they were not specifically bred for this difference. This thesis focuses on a neuroanatomic evaluation of the mechanisms underlying this specific behavioral difference. Future experiments are necessary to explore the neuroanatomy underlying additional behavioral differences. Ultimately, a more complete picture of the anatomic loci involved in mediating and influencing the many behavioral effects of BZs is desired.

1.2 Methods in functional neuroanatomy

A number of techniques are available for examining the functional neuroanatomy of BZs. One set of techniques relies on imaging. For example, the density of receptors in various brain areas can be examined using either quantitative autoradiography (Benavides et al. 1993; Young and Kuhar 1980) or immunohistochemistry (Benke et al. 1991c; Thompson et al. 1992). In situ hybridization can be used to determine the levels of mRNA in various brain areas (Wisden et al. 1992). Positron emission tomography (PET) can also be used to measure *in vivo* binding of BZs (Halldin et al. 1992). BZ-induced changes in regional glucose uptake can be measured using radiolabeled deoxy-glucose (Laurie and Pratt 1993), and regional changes in blood flow can be measured using PET scans (Friston et al. 1992).

These types of techniques provide some indication about where a drug might act and which brain areas might be affected. However, BZ receptors exist throughout the brain (Young and Kuhar 1980), and they are capable of affecting the function of most neuroanatomical areas (Piercey et al. 1991). Therefore, these techniques do not indicate which areas are responsible for which specific effects.

Another useful technique involves microdialysis. Benzodiazepines are capable of affecting the release of many other neurotransmitters in specific brain areas; for example, serotonin in the hippocampus (Wright et al. 1992a), dopamine in the nucleus accumbens (Horger et al. 1995), and histamine in the caudate (Chikai et al. 1993). This information can be used to determine the circuits involved in the effects of BZs, although it is difficult with this technique to determine which neurochemical effects are associated with which behavioral effect. For example, which of BZ's many behavioral effects is influenced by the effect on hippocampal serotonin?

A more direct method of assessing which neuroanatomical areas may mediate the effects of BZs is to directly inject the compounds into specific brain areas. These micro-injections can be followed by behavioral and physiological evaluation. In this manner, the role of distinct and specific loci in various behaviors can be tested. This method is made feasible through the use of stereotaxic instruments and micro-injection techniques. As early as 1979 this method has been successfully used to examine BZs (Nagy et al. 1979).

Conclusions using this technique are limited by the anatomic specificity of the injections, and this is influenced by the size of the brain area being examined. This has restricted the use of this technique in mice, with their smaller sized brains. The animal generally used for these studies has been the rat.

However a number of genetic manipulations are now feasible in mice; for example, selective breeding, genetic knockouts, and recombinant inbred analysis (Crabbe et al. 1994). The genome of the mouse is also being densely mapped (Takahashi et al. 1994). Functional neuroanatomical experiments will be invaluable in determining the effects on the underlying neurophysiology as influenced by these genetic manipulations.

Towards this end, stereotaxic surgery and micro-injections have been developed for examinations of mice in this thesis. The genetic influences on behavior in DS and DR mice can therefore be examined at the level of functional neuroanatomy, and differences delineated at this level. The approach of combining murine genetics with functional neuroanatomy is proposed to offer a powerful tool for exploring the mechanisms of behavior.

1.3 Selective Breeding for diazepam sensitivity

The DS and DR lines (Gallaher et al. 1987) were created using a mass selection procedure (DeFries 1981; Falconer 1983; Roberts 1981). Each line consisted of ten breeding pairs that were selected for sensitivity to diazepam (the DS line) or resistance to diazepam (the DR line). Sensitivity was defined as the duration of ataxia on a fixed-velocity rotarod after a standard dose of diazepam. The original stock population was obtained from a heterogeneous line of mice (HS/Ibg) created by systematic crossbreeding of eight different inbred mouse lines (McClearn et al. 1970). Selective breeding of the DS and DR lines occurred for 36 generations. An estimated heritability for sensitivity to ataxia in this outbred stock population was about 0.2 (Gallaher et al. 1987). Selection was discontinued after 36 generations. Since then, each line has been randomly bred with ten breeding pairs for each generation.

The use of DS and DR mice in this study has several advantages. The main advantage is that, although selected for differences in sensitivity to ataxia, they also exhibit robust differences in sensitivity to the anxiolytic effect of BZs. And interestingly, the "DS" mice are resistant to anxiolysis while the "DR" mice are sensitive to anxiolysis. This paradoxical difference could be useful in distinguishing the differences in mechanisms influencing anxiety and ataxia.

By implicating particular neuroanatomic regions with specific differences in sensitivity, physiological differences in these neuroanatomic regions can be implicated

in specific behavioral differences (Figure 2). That is, if neuroanatomic region '1' mediates differences in effect 'A' then physiologic differences in region 1 will be implicated in differences in sensitivity to effect 'A.' Similary, differences in any physiologic changes following specific injection into region '1' will be implicated in effect 'A.' Likewise, if neuroanatomic region '2' mediates differences in effect 'B'

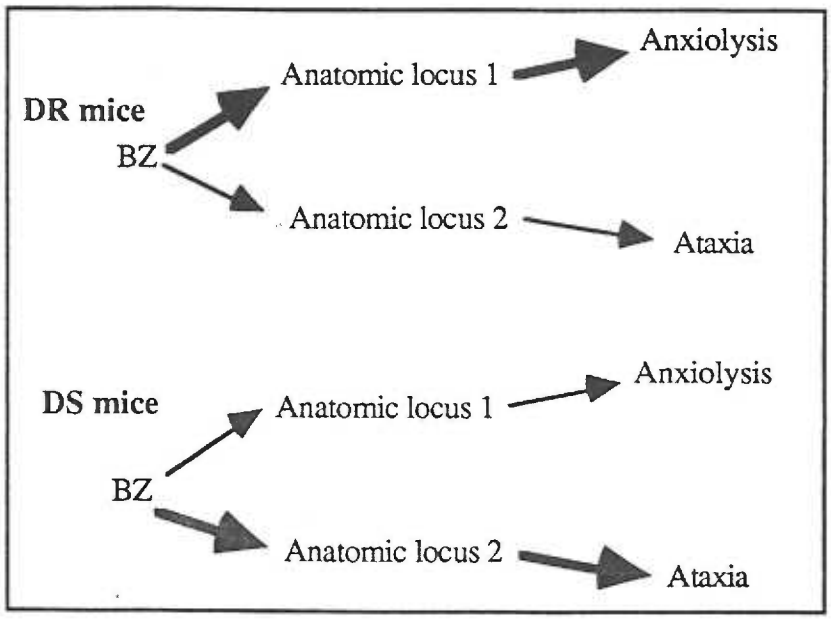

Figure 2. Schematic of differing anatomic loci for anxiolysis and ataxia in DS and DR mice. This diagram illustrates the hypothesis that the anatomic loci mediating anxiolysis and ataxia are different. Furthermore, the ability of BZs to affect the anatomic locus mediating anxiolysis is greater in DR mice than DS mice, as indicated by the thickness of the arrows.. Conversely, the ability of BZs to affect the anatomic locus mediating ataxia is greater in DS mice, as indicated by the thickness of the arrows. Physiological differences in anatomic locus 1 can be implicated in mediating differences in sensitivity to anxiolysis. Physiological differences in anatomic locus 2 can be implicated in mediating differences in sensitivity to ataxia.

then physiologic differences in region 2 will be implicated in differences in sensitivity to effect 'B.' Thus, step one is identification of neuroanatomic regions that can contribute to behavioral differences. Step two is to perform more focused examinations of these regions. In the case of anxiolysis, neuroanatomic loci which may contribute to differences in anxiolysis will be identified. Once identified, these loci can be examined in future studies for physiological differences.

Although a finding of correlated differences between the physiology of neuroanatomic loci and the behavior(s) associated with those loci is not conclusive proof that those differences are causally related, such differences will justify future manipulations to determine physiological relevance. In this manner, known genetic/behavioral correlations can be correlated with differences at biochemical, neurochemical, and neuroanatomic levels. This basic strategy of correlating behavioral differences with other differences is being applied to a number of other selectively bred lines; for example, the neurophysiological bases of emotional reactivity (Castanon and Mormede 1994; Charnay et al. 1995), alcohol withdrawal severity (Crabbe and Phillips 1993), and analgesic sensitivity to opiates (Belknap and O'Toole 1991) .

Because the mice used in this study differ in their sensitivity to a number of effects of BZs, an approach is introduced which begins with an examination of the specific neuroanatomic loci involved in specific behaviors. It is the ultimate goal of this approach to identify causal links among several levels of inquiry including genetics, cellular physiology, neuroanatomical systems, and behavior.

To summarize, genetic differences cause molecular differences which result in differences at the level of the cell. These differences result in neuroanatomic and neurochemical differences which consequently result in differences at the level of functioning circuitry. These differences are then manifested as behavioral differences

(Table 1). By identifying the mechanisms responsible for differences in sensitivity to the various effects of BZs, it is expected that the functional systems involved in mediating these effects will be better understood. The neuroanatomic loci involved in mediating each these effects can be examined to identify these mechanisms. Further examination of these specific loci will implicate the differences in those areas with specific behavioral differences.

The DS and DR lines have behavioral differences in sensitivity to BZs as a result of genetic differences. That is, selective breeding has resulted in a segregation of genes through direct selectional pressure, through genetic drift, or through a combination of these mechanisms. These genetic differences have resulted in differences in sensitivity to some behavioral effects of BZs. (The DS mice are more senstitive to ataxia, the DR mice are more sensitive to anxiolysis, and the lines are equally sensitive to seizure protection.) By finding differences at neuroanatomic levels, and subsequently at the other levels of examination, connections between the different levels of inquiry can be determined.

Several caveats need to be noted. One, these mice were bred solely for differences in response to diazepam on the rotarod. Secondly, replicate lines do not exist for the DS and DR mice. Finally, these lines of mice have been partially inbred during their selection. These caveats influence the interpretation of differences found between these selectively bred lines (Crabbe et al. 1990). The development of the DS and DR mice may have resulted in a segregation of genes and traits genetically unrelated to ataxic sensitivity to BZs. That is, genetic drift resulting from inbreeding may have fixed unrelated genes and traits in these lines. These genes and traits may be unrelated to BZ sensitivity.

Differences	Mechanism
Genetic	genetic differences arise through selective breeding: genes to be identified
Molecular	Unknown: to be identified
Cellular	Unknown: to be identified
Neuroanatomic	Unknown: to be identified
Neurochemical	Unknown: to be identified
System	Unknown: to be identified
Behavior	Behavioral differences are observed

Table 1. Levels of differences between selectively bred mice. Behavioral differences in mice resulting from genetic difference are mediated by several levels, from molecules to functioning anatomic circuits. Understanding the mechanisms of action of BZs requires an understanding at each level.

Therefore, any given cellular or neuroanatomic difference cannot necessarily be causally attributed to any particular behavioral differences. A physiological link between behavioral differences and cellular/neuroanatomic differences will only be a strongly suggested probability, subject to further testing (Henderson 1989). While confirmation of correlated differences will require additional experiments, an advantage of using these lines is that correlations between physiology and behavior provides a rationale for more focused examinations to confirm or refute these correlations.

A second advantage of using the DS and DR mice is that for each of the behaviors to be analyzed, at least one of the lines has been demonstrated to exhibit robust sensitivity to the effect of systemic diazepam. This decreases the interpretative difficulty of finding no behavioral effects after intra-cerebral injections. Negative behavioral findings in some studies can be the result of using a genetically resistant line. This problem can be avoided by using lines known to be both sensitive and resistant to the effect. If a particular behavioral response is not produced in either line,

the conclusion that the neuroanatomical locus is not involved in mediating that behavior is strengthened and more generalizable.

A third advantage of investigating genetic differences in sensitivity in mice is that an understanding of individual differences in human BZ sensitivity can be better attained. It is possible that the differences in the DS and DR mice may be homologous to the differences in humans at the genetic, biochemical, cellular, and/or neuroanatomic level. Since there are genetic differences in sensitivity to various drug effects in humans (Crabbe et al. 1994; Rang and Dale 1991), individualized clinical treatment is aided by understanding and predicting these differences in sensitivity.

In summary, there are several advantages for using DS and DR mice in determining the amygdala's role in BZ anxiolysis. One, if the amygdala can mediate genetic differences in sensitivity to BZ anxiolysis, then subsequent electrophysiologic or neurochemical differences between amygdalas can be correlated specifically with sensitivity to BZ anxiolysis. These correlations can be verified with future experiments. In this fashion, the mechanisms underlying the anxiolytic effect of BZs, from gene to molecule to anatomic circuitry can be determined. In addition, the ground-work is paved such that several other effects of BZs can be similarly examined in the DS and DR mice, such as locomotor activation and sedation. A second advantage of using DS and DR mice is that if a behavior is not affected in either line, this result will be more generalizable to genetically different mouse lines. Third, the mechanisms underlying genetic differences in mice can potentially be used to understand the individual genetic differences in humans.

1.4 Molecular pharmacology of the BZ receptor

The central benzodiazepine receptor is comprised of a number of subunits. At least fifteen subunits have been described to date (Barnard 1995). These subunits assemble to form a chloride ionophore, generally a pentamer, which is gated by the

neurotransmitter γ-amino butyric acid (GABA) (Smith and Olsen 1995). BZs act to enhance the current produced by GABAergic stimulation. By augmenting the GABA-stimulated chloride flux, the effect of BZs is usually to maintain the cell in a hyperpolarized, inhibited state. Various combinations of subunits may combine to form receptors with differing pharmacologies and functions (Doble and Martin 1992; Mohler et al. 1995).

Various ligands at the BZ receptor have different affinities depending on the brain region examined. For example, zolpidem and Cl218872 are compounds that exhibit heterogeneous binding (Luddens et al. 1995). This type of finding was an early clue that there are different types of receptors in the brain in various neuroanatomical loci. Another indication that different BZ receptor subtypes exist is that different ligands have different potencies and efficacies at producing various effects. For example, zolpidem can be more potent at eliciting sedation and seizure protection than at producing myorelaxation. Conversely, quazepam and bretizolam are more potent at producing myorelaxation than sedation and seizure protection (Perrault et al. 1990). Another indication that the different effects may be mediated by different receptor types is the observation that chronic BZ treatment can result in tolerance to some behaviors and not others (Rosenberg et al. 1991).

The realization that the BZ subunits may combine into potentially hundreds of types of central BZ receptors has led to two related lines of research. One, ligands have different potencies and efficacies at producing behavioral effects. These ligands are therefore being examined for different potencies and efficacies in various subunit combinations expressed *in vitro*. In this manner, the *in vitro* effect of a drug on particular subunit combinations can be correlated with its behavior effects (Costa and Guidotti 1996). This information will provide a rational basis for producing compounds with specific behavior effects.

The other strategy is to determine which receptor subunits are colocalized in receptors in various cellular and neuroanatomical locations (Mohler et al. 1995). One way in which this is done is to immunoprecipitate BZ receptors using antibodies to specific subunits and to then examine their association with other subunits (Benke et al. 1991b; Fritschy and Mohler 1995). With this technique, the *in vivo* composition of receptors is determined. Immunoprecipitated receptors can also be analyzed for their ability to bind differently to various ligands.

The information about the subtypes of receptors in various brain areas can be combined with information about the neuroanatomical circuits in which BZs act to produce particular behavioral effects. However, there is a lack of detailed information regarding the functional neuroanatomy of BZs. One purpose of the experiments reported in this study is to determine which specific loci mediate specific behaviors. This information can then be combined with the data regarding the types of receptors in specific loci and the efficacies of behaviorally specific ligands at those receptor types.

2.0 Genetics of BZ sensitivity

Although this study does not directly examine the issue of genetic correlations between BZ's behavioral effects, several other studies are providing this type of data. Genetic influences on different BZ effects have been observed to (1) positively correlate, (2) negatively correlate, and (3) not correlate. A finding in these studies is that several of the effects of BZs do not consistently correlate in one direction. This suggests that these effects can be influenced by separate genes and may therefore be mediated by separate physiological processes. This observation is consistent with the findings of the binding results of other experiments. However, some behavioral effects consistently correlate. This suggests that these effects are influenced by common genes

and may therefore be mediated by common physiological processes. A survey of some of these genetic influences on BZ sensitivity will be presented.

2.1 Genetics of BZ sensitivity in mice

A large degree of variation in sensitivity to the loss-of-righting reflex (LORR) following diazepam injection was observed in Swiss Webster mice (Wong et al. 1986). Because these are outbred mice, it has been hypothesized that some of this variation may have been the result of genetic variation (Wong et al. 1986). This type of variability in sensitivity to BZs has been found in other studies (File 1983b). Both environmental factors as well as genetic factors may have contributed to this phenotypic variation.

Mouse lines have subsequently been selected for enhanced sensitivity to diazepam-induced LORR--demonstrating that genetic differences contributed to the phenotypic differences (Wong and Teo 1990). In addition, the mice that were more sensitive than control mice to the LORR were also more sensitive to the ataxic effects of diazepam on the rotarod (Wong and Teo 1990). This suggests that these two effects may share a common mechanism. Sensitivity to protection against PTZ-induced seizures was not altered by this genetic selection (Wong and Teo 1990). This result suggests that protection against seizures may be influenced by separate genes and may therefore have a different mechanism.

Though the previous study did not find differences in seizure protective effects, there are strain differences in mice in the ability of BZs to protect against PTZ-induced seizures (File 1983a; File et al. 1985; Wilks et al. 1987). This suggests that seizure protection can be genetically influenced, though the genes are not the same as those that influence LORR and ataxia.

Genetic differences in the effects of BZs on exploratory activity in a light/dark paradigm have also been examined in inbred strains of mice (Crawley and Davis 1982;

Mathis et al. 1994). The results indicate that genetics also contribute to sensitivity to anxiolysis. Differences in anxiolytic sensitivity have also been found in mice selectively bred for other traits. For example, LS mice (bred for sensitivity to ethanol on LORR) were more sensitive to the anxiolytic effects of diazepam on the plus maze than SS mice (bred for resistance to ethanol on LORR). LS mice were also more sensitive to LORR induced by flurazepam. In these mice, sensitivity to LORR and anxiolysis positively correlated.

Interestingly, diazepam was more potent at increasing total entries in SS mice than LS mice (Stinchcomb et al. 1989). Thus, anxiolysis and locomotor activation were negatively correlated in these mice, while anxiolysis and ataxia were positively correlated. SS mice were also more sensitive to the protection against convulsions induced by 3-mercaptoproprionic acid, and the two lines were equivalent in the decrease in temperature produced by diazepam (Marley et al. 1988).

A number of other studies have found other types of differences in sensitivity to BZs. Mice which were selectively bred for low aggression were sensitive to decrements in locomotor behavior produced by CDP (Weerts et al. 1992). There are also strain differences in sensitivity to seizures induced by inverse agonists at the BZ receptor (Martin et al. 1994; Nutt and Lister 1988). Also, strain differences in sensitivity to BZ-induced hypothermia have been found (Jackson and Nutt 1992). Therefore, there can be genetic influences on hypothermia as well.

The DS and DR mice were created to utilize genetic differences in further exploring the neurophysiology of BZs (Gallaher et al. 1987). Consistent with the results of the above studies, genetic sensitivity to the ataxic effect of BZs did not correlate with sensitivity to the seizure-protective effects (Gallaher et al. 1991). In addition, sensitivity to both the locomotor activating effects (Phillips and Gallaher 1992) and the anxiolytic effects (Courtney et al. in prep.) were found to correlate with

resistance to the ataxic effect in the DS and DR mice. This is in contrast to the results found with the LS and SS mice. In that experiment, sensitivity to anxiolysis and ataxia positively correlated. The genetic relationship between these two effects is still under study.

Using quantitative autoradiography, no differences in BZ binding have been found between the DS and DR lines (Gallaher et al. 1991). However, DS cortical synaptosomes were more sensitive to BZ enhancement of GABA-stimulated Cl^- flux (Gallaher et al. 1991). These results suggest that there may not be differences in the number or affinity of BZ receptors, though the efficacy of BZs at augmenting Cl^- flux may differ. There may be more subtle differences in efficacy in distinct neuroanatomical areas. This is still to be determined.

DS mice are also more sensitive than DR mice to the ataxic effects of ethanol and phenobarbital, and they are equally sensitive to the ataxic effects of pentobarbital. Consistent with this, the uptake of Cl^- in synaptosomes was enhanced by flunitrazepam in DS but not in DR mice. Both ethanol and Phenobarbital, and not pentobarbital, were also able to augment Cl^- flux more in DS mice than DR mice (Allan et al. 1988). Therefore, sensitivity to these cellular effects correlated with sensitivity to the ataxic and sedative effects. These results suggest that the ability of BZs to enhance GABA uptake in synaptosomes may underlie the ataxic and sedative effects.

The lines also differed in anesthetic sensitivity to halothane and enflurane (McCrae et al. 1993). Additionally, the DS mice were more sensitive to the potentiation of GABA-induced chloride flux produced by halothane (Quinlan et al. 1993). Therefore, sensitivity to anesthesia is another effect which is correlated with the sensitivity of cortical synaptosomes.

However, the lines are equally sensitive to the seizure protective effects of BZs. Also, the DR mice are more sensitive to the anxiolytic and locomotor activating effects.

Thus, the differences in efficacy in whole brain synaptosomes may be not directly underlie these effects. It is expected that other physiologic differences may underlie these differences in behavioral sensitivity.

To confirm many of these correlations and explore others, another set of selectively bred mice are being developed (Gallaher et al. 1992). Two independent but comparable lines are being selectively bred for resistance to the ataxic effect of BZs (diazepam high performing mice, DHP-1 and DHP-2), and two other lines are being independently selected for sensitivity (diazepam low performing mice, DLP-1 and DLP-2). Initial examinations of these lines indicated that the genes influencing sensitivity to anxiolysis do not co-segregate with the genes influencing sensitivity to ataxia (Lotrich and Gallaher, personal comm.). This is consistent with the supposition that these two effects of BZs are mediated by separate systems.

In summary, there is a wide range of effects produced by BZs that are influenced by genetic differences: LORR, ataxia, seizure protection, locomotor sedation, anxiolysis, exploratory activity, and hypothermia. In some experiments, some of these effects have consistently correlated, suggesting that these effects share a common underlying mechanism; for example, ataxia, LORR, and locomotor hypoactivity. Other effects have not consistently correlated. This suggests that the underlying neurophysiology for these effects may be different. The observation that different sets of genes can separately affect sensitivity to a number of measures provides a tool for delineating the mechanisms mediating each of the distinct effects.

2.2 Human genetics of BZ sensitivity

Despite the fact that humans exhibit a large degrees of variation in response to BZs (Malamed and Quinn 1995), there have been few systematic studies examining human sensitivity to BZs. It is possible that some of this variation may be the result of

environmental causes (Branch 1987). However, one study that examined human twins found that there is genetic variation in sensitivity to the anxiolytic and euphoric effects of diazepam (Alda et al. 1987). This difference was not the result of any pharmacokinetic differences. Differences in sensitivity to the euphoric effects may be related to a family history of alcoholism (Ciraulo 1989 and de Wit 1991).

Also, patients with panic disorder had diminished sensitivity to diazepam-induced suppression of sympathetic nervous system (Rimon et al. 1995). Because there is evidence that panic disorder may have a genetic influence, the sensitivity to diazepam may also be influenced by genetics in humans. Similarly, individuals with lower concentrations of cerebrospinal fluid GABA did not benefit from alprazolam therapy. Although the role of genetics in these differences was not investigated in that study, there is a possibility that the effectiveness of alprazolam was influenced by differences in genetics (Rimon et al. 1995).

Differences in sensitivity to diazepam's effects on eye saccades has also been found. For example, novelty seeking was found to correlate with sensitivity to diazepam (Cowley et al. 1993). It is again possible that these diffences may be genetically mediated, however this remains to be determined.

In summary, a few studies found differences in sensitivity to BZs which correlated with biological or psychological differences. Part of this sensitivity may be under genetic control. Some of the paradoxical effects of BZs such as psychoses and hyperexcitability in humans may also be influenced by genetics (Short et al. 1987). Understanding the neurophysiology of these differences and predicting them will be useful in developing more effective treatments for sensitive or resistant individuals.

3.0 Anxiety: How is it defined and studied in animals?

A variety of approaches have been developed for modeling anxiety in animals (Treit 1985; Green and Hodges 1991; Sanger 1994) (Table 2). Implicit in many of these paradigms is the assumption that "anxiety" is the result of novel or exposed situations, the anticipation of punishment or harm, and/or the presence of punishment.

(I)	Tests of unconditioned behaviors		
	(1)	Single, novel environment paradigms	
		(a) Exploratory behavior	
		Open field test	(Fukuda & Iwahara 1974)
		Hole-board test	(File & Wardill 1975)
		Staircase test	(Simiand et al. 1984)
		(b) Socializing behavior	(File & Hyde 1978)
		(c) Eating behavior	(Poschel 1971)
	(2)	Behaviors in "threatening" environments	
		(a) Risk assessment behaviors	
		in presence of a cat	(Blanchard et al. 1990)
		(b) Stretch attend posture	(Pollard & Howard 1988)
	(3)	Two environment paradigms	
		(a) Light-dark crossing	(Crawley 1981)
		(b) Plus maze	(Montgomery 1958)
		(c) Zero maze	(Shepherd et al. 1994)
		(d) Thigmotaxis	(Treit & Fundytus 1989)
(II)	Tests using conditioned behavior		
	(1)	Inhibition of behavior	
		(a) Four plate test	(Boissier et al. 1968)
		(b) Geller-Seifter conflict paradigm	(Geller & Seifter 1960)
		(c) Vogel water-lick conflict	(Vogel et al. 1971)
		(d) Passive avoidance	(Gray 1982)
		(e) Active two-plate avoidance	(Green & Hodges 1991)
		(f) Successive sucrose constrast	(Flaherty & Rowan 1986)
	(2)	Increases in behavior	
		(a) Potentiated startle	(Davis 1986)
		(b) Conditioned defensive burying	(Treit et al. 1981)

Table 2. Anxiety paradigms in rodents. This list includes many of the models commonly used to evaluate anxiety in rodents, but is not comprehensive. A number of variations on each of these models exists (adapted from Green and Hodges 1991; Sanger 1994)..

As a result of the experimental "anxiety," animals substitute one set of behaviors for another. For example, (a) drinking, (b) eating, (c) socializing, and (d) exploratory behaviors are replaced by (a) avoidance of the anxiety-provoking agent/area, (b) increased wariness, freezing, and susceptibility to startle, and (c) species-specific burying of the anxiety-provoking agent.

In the paradigms measuring behavior in one environment, anxiety is presumed to decrease exploratory and social behavior. Anxiolytic agents restore these behaviors. In the paradigms in which subjects can choose between two environments, there is avoidance of the anxiety-provoking area (light area, open arm, or the center of an open field). Anxiolytics increase the percentage of time spent in these areas. In the conflict tests, animals are trained to avoid an aversive stimulus (for example, a shock). This avoidance is decreased by anxiolytics. In the potentiated startle paradigm, exposure to a stimulus which has been paired with an aversive stimulus is used to enhance the startle reflex. This enhancement is reversed by anxiolytics. In the conditioned defensive burying paradigm, an aversive object (an electrified metal probe) is placed in the cage. Rats will normally bury this type of object. This burying behavior can be reversed by anxiolytics.

There are four general types of hypotheses for the effects of BZs in these paradigms. The disinhibition hypothesis states that BZs release behavior which has been suppressed by the punishment, novel stimuli, or non-reinforcement. For example, a behavior inhibition system may become activated in response to novel stimuli, punishment, or non-reward. This system would then inhibit ongoing behavior, increasing arousal and attention. Activity of this system is hypothesized to constitute "anxiety." BZs may inhibit this system (Gray 1982).

Perseveration is a second explanation for the effects of BZ on these paradigms (Dantzer 1977). This is an inability to change behavior patterns in the face of

punishment or non-reinforcement. A third possible explanation is that BZs reduce the tolerance for reward delay (Thiebot et al. 1985), and a fourth hypothesis is that global information processing is altered (Ljungberg 1987). These hypotheses could account for a number of the behavioral effects of BZs in a number of anxiety paradigms. The specific psychological explanation which accounts for these effects of BZs remains under study.

Plus Maze: The plus maze is the paradigm used in the current study. This procedure is a well-validated paradigm for measuring anxiolysis (Green and Hodges 1991). Avoidance of the open arms in favor of the closed arms is used as a measure of anxiety. The basic principle is that the open arms are more anxiety-provoking than the closed arms (Montgomery 1958). Therefore, animals avoid the open arms in favor of the relatively "safe" enclosed arms. It is hypothesized that rodents avoid the open arms because of the open space and not as a result of height or novelty (Treit et al. 1993a). Consistent with the hypothesis that anxiety is involved in the avoidance of the open arms, a variety of drugs known be anxiolytic in humans are effective in increasing open arm activity. Also, hormonal levels have been measured in rats confined to the open arms. The findings of increased corticosterone are consistent with the hypothesis that performance on this task is influenced by systems which are homologous to human anxiety (Pellow and File 1986). Rats confined to the open arms also exhibited increased levels of defecation and freezing behavior (Pellow et al. 1985).

The plus maze was first used for the mouse by Lister (Lister 1987). A factor analysis of the results indicated that one factor (anxiety) was reflected in percent open time, percent open entries, and in part, by the total number of entries. A second factor was related to total entries and total locomotor activity. A third factor was correlated with head dipping behavior. An additional study using unmedicated mice found similar results. The first factor was related to "anxiety," and the second correlated with

measures of "activity." In this study, a third factor correlated with the percent of time spent in the center (Rodgers and Johnson 1995).

One feature of the plus maze is that it simultaneously measures both locomotor activity and anxiety. These studies indicate that the percent of open arm entries (anxiety) can potentially be independent of total arm entries (activity). For example, anxiogenic treatments could either increase or decrease total arm entries though the percentage of open arm entries would be decreased. Likewise, anxiolytic treatments could either increase or decrease total arm entries--while increasing the percentage of open arm entries.

However, these two measurements (percent open entries and total activity) may not always be totally independent (Dawson et al. 1995). It has been suggested that treatments which affect locomotor activity may confound the measure of percent open arms entries (Dawson et al. 1995). The exact relationship between locomotor activation and anxiolysis remains under study. Although the studies by Lister (1987) support the notion that these two measurements reflect different underlying effects, the potential interaction must be regarded with care.

Lengthy training, often necessary in many conflict paradigms, is not needed with the plus maze. Thus, this paradigm is well-suited for measuring anxiolytic effects in surgically cannulated subjects. Additionally, the plus maze is capable of measuring anxiogenic effects (Rodgers and Johnson 1995). Because of these characteristics, the plus maze was chosen for the studies reported in this thesis.

4.0 Neuroanatomy

This section provides a review of the neuroanatomy mediating BZ effects, intended to familiarize the reader with the current state of knowledge regarding the neuroanatomic loci at which BZs act. The main focus is anxiolysis. However other effects of BZs will also be reviewed (Table 3).

Anxiolysis	**Seizure protection**
Amygdala	Substantia nigra reticulata
Septum	Mammillary bodies
Mammillary body	Anterior thalamus
Dorsal raphe nucleus	Inferior colliculus
Dorsal periaqueductal grey	Cerebellum
Hippocampus	Hippocampus
Inferior colliculus	Reticular formation
Hypothalamus	Cortex
Cortex	
	Amnesia
Sedation	Medial septum
Cortex	Amygdala
Ventral pallidum	Hippocampus
Nucleus accumbens	Dorsal raphe nucleus
Amygdala	Nucleus basalis
Hypnosis	**Attention**
Dorsal pedunculopontine tegmentum	Nucleus basalis
Reticular activating system	Cortex
Tuberomammillary nucleus	
Medial preoptic nucleus	**Ataxia**
Cortex	Cerebellum
	Vestibular nuclei
Locomotor activation	Nucleus basalis
Dorsal raphe nucleus	Inferior olive
Medial raphe nucleus	Caudate
Ventral tegmemtal area	
Dentate gyrus	
Medial septum	
Preoptic area	
Mediodorsal thalamus	

Table 3. Selected neuroanatomic areas potentially mediating various BZ effects. This table lists several BZ effects and some of their possible underlying anatomic loci, though not all BZ effects and potential loci are included (see text for references).. The following text reviews the role of these possibile regions in mediating BZ effects.

4.1a Neuroanatomy of anxiety

A variety of neuroanatomical structures have been implicated in anxiety; for example, the amygdala, the septal nuclei, the dorsal and medial raphe nucleus, the dorsal periaqueductal grey, the hippocampus, the mammillary bodies, the ventromedial thalamus, the hypothalamus, the substantia nigra, the inferior colliculus, and various areas of the cortex (Pratt 1992). Lesions, electrical simulation, or localized chemical stimulation of these areas directly address the issue of their involvement in anxiety. The neuroanatomic sites in which BZs act to reduce anxiety has either been inferred based on the results of these studies or directly tested by micro-injections of BZs into these areas.

Amygdala: Surgical lesions of the amygdala reduce anxiety in humans. There is decreased aggression, as well as an increase in "placidity" and "indifference" (Aggleton 1993). The human amygdala is also required to recognize fear expressions in the faces of others. In patients with amygdala lesions, identities of pictures could be recognized but not the emotion expressed in the picture (Adolphs et al. 1995).

A number of lesion experiments in rats also implicate the amygdala. Lesions of the central nucleus of the amygdala (CEA) produce anxiolytic effects in several conflict paradigms (Yamashita et al. 1989b; Yadin et al. 1991; Kopchia et al. 1992). Interestingly, in the shock-probe paradigm CEA lesions increase shock probe contacts but do not affect probe burying behavior or open arm exploration in the plus maze (Treit et al. 1993b). This latter study suggests that BZs may act in the CEA to increase punished behavior, but lesions of this area may not be responsible for increases in open arm exploration in the plus maze or decreases in the duration of burying behavior--two other indexes of anxiolysis.

In the potentiated startle paradigm, both CEA and anterolateral/basolateral amygdala (AL/BLA) lesions block fear conditioning (Campeau and Davis 1995).

Conversely, kindling of the amygdala results in enhanced potentiated startle (Rosen et al. 1996). Lesions here also block both the acquisition and the expression of potentiated startle (Kim and Davis 1993). Furthermore, lesions of the AL/BLA block shock sensitization of startle (Sananes and Davis 1992). Because the amygdala projects to the startle circuitry in the reticular activating system, this connection may be responsible for affecting the startle responses (Davis et al. 1993). In support of this, neurons in the amygdala are capable of acquiring responses to stimuli which have been paired with aversive events. For example, repeated pairing of a tone with foot shocks increases the magnitude of tone-elicited electrophysiological responses in the lateral amygdala, and convert unresponsive cells into tone-responsive cells (Quirk et al. 1995).

Freezing has also been used as a measure of conditioned fear. A stimulus which had been paired with shock is presented to the animal, resulting in analgesia and freezing. Lesions of the BLA block both the conditioned analgesia and freezing (Helmstetter 1992a; Watkins et al. 1993). Lidocaine in the amygdala, producing a temporary lesion, also blocks conditioned stimulus-induced freezing (Helmstetter 1992b). In addition, amygdala lesions eliminate freezing in a shuttle box in rats which have undergone inescapable shock training (Maier et al. 1993b), and freezing following footshock is blocked by amygdala or ventral periaqueductal grey (vPAG) lesions (Kim et al. 1993).

Activation of the hypothalamic-pituitary-adrenal axis may also result from aversive/anxiety-provoking events. Both conditioned stress and immobilization stress result in increases in corticosterone and renin. Lesions of the CEA blocked these effects (Roozendaal et al. 1992; Vandekar et al. 1991).

Consistent with these lesion studies, one early study found that BZ micro-injection into the CEA but not the medial or basolateral amygdaloid nuclei had

anticonflict actions (Shibata et al. 1982). A later study by this same group again reported anticonflict effects of CEA injections of diazepam (Kataoka et al. 1987).

On the other hand, several groups have observed that BZs have their anti-conflict effects when injected into the anterolateral and basolateral (AL/ABL) nuclei of the amygdala (Scheel-Kruger and Petersen 1982; Thomas et al. 1985). One study found that a ligand at the BZ receptor, zopiclone, does not have any anticonflict effects when injected in the CEA (Yamashita et al. 1989a). These findings are supported by a more recent study demonstrating that BZ micro-injection in the basolateral nucleus but not the CEA increases open arm activity (Green and Vale 1992). A later study by Shibata et al. (1989) reported that both CEA and ABL injections of BZs result in anticonflict behavior, and that more anterior areas of the amygdala mediate this effect. This is similar to an early finding that the anterior regions of the amygdala mediate diazepam's anxiolytic effects (Nagy et al. 1979).

A more recent study found that midazolam injections into the amygdala causes an increase in contact with a shock-probe but do not affect burying or open arm activity in the plus maze (Pesold and Treit 1994). Histological examination of the brains from this last study revealed that injections were into the CEA. A subsequent study demonstrated that injections of midazolam into the BLA result in an increase in the percentage of open arm entries into the plus maze, while injections into the CEA do not. Conversely, injections into the CEA result in an increase in shock-probe burying, while injections into the BLA do not (Pesold and Treit 1995). Therefore, prior findings can be explained by the CEA mediating some anti-conflict effects of BZs, with the AL/ABL mediating effects on the plus maze and on shock-probe burying behavior.

Site	Paradigm	Anxiolysis	Reference
CEA	Conflict	Yes	(Shibata et al. 1982)
CEA	Conflict	Yes	(Kataoka et al. 1987)
CEA	Conflict	No	(Yamashita et al. 1989a)
CEA	Conflict	Yes	(Shibata et al. 1989)
CEA	Shock Probe	Yes	(Pesold and Treit 1994)
CEA	Shock Probe	Yes	(Pesold and Treit 1995)
CEA	Plus Maze	No	(Green and Vale 1992)
CEA	Plus Maze	No	(Pesold and Treit 1994)
CEA	Plus Maze	No	(Pesold and Treit 1995)
CEA	Stress ulcers	Yes	(Sullivan et al. 1989)
AL/ABL	Conflict	No	(Shibata et al. 1982)
AL/ABL	Conflict	Yes	(Scheel-Kruger and Petersen 1982)
AL/ABL	Conflict	Yes	(Peterson et al. 1985)
Al/ABL	Conflict	Yes	(Shibata et al. 1989)
AL/ABL	Plus Maze	Yes	(Green and Vale 1992)
AL/ABL	Plus Maze	Yes	(Pesold and Treit 1995)
AL/ABL	Stress-induced Freezing	Yes	(Helmstetter 1993b)
AL/ABL	Shock Probe	No	(Pesold and Treit 1995)
Anterior Amygdala	Conflict	Yes	(Nagy et al. 1979)
Amygdala	Conflict	Yes	(Hodges et al. 1987)
Amygdala	Light/Dark	Yes	(Costall et al. 1989)
Amygdala	Social Interaction	Yes	(Higgins et al. 1991)
Amygdala	Thigmotaxia	Yes	(McNamara and Skelton 1993b)
Amygdala	Conditioned Avoidance	Yes	(Harris and Westbrook 1995a)

Table 4: Anxiolysis following BZ injection in the amygdala.

The results of several studies which have micro-injected BZs into the amygdala are presented. Anxiolysis is indicated by a 'Yes,' while no affect on anxiolysis is indicated by a 'No.'

Micro-injections of BZs into the amygdala also increase social interaction (Higgins et al. 1991), another potential anxiolytic effect. Furthermore, the anxiolytic effect of systemically administered CDP on the social interaction test is blocked by BLA injection of flumazenil, though flumazenil has no effect on its own (Sanders and

Sheckhar 1995). These studies suggest that the effect of systemic CDP in the social interaction test is mediated by the BLA.

The effect of intra-amygdala injections has also been tested in a thigmotaxia paradigm. Chlordiazepoxide was infused into the amygdala, medial septum, hippocampus, cerebellum, frontal cortex, or nucleus basalis/substantia innominata. Only intra-amygdala infusions diminished thigmotaxis. Cue learning and swim speed were not affected (McNamara and Skelton 1993a). This suggests that the amygdala is also involved in mediating this anxiolytic effect of BZs.

Stress-induced hypoalgesia and defensive freezing are also attenuated by diazepam in the BLA. In one study, both hypoalgesia and freezing occurred during an 8-min period following a series of three brief foot-shocks (Helmstetter 1993b). Intra-BLA diazepam attenuated both the hypoalgesia and freezing. In another study, rats were exposed to a heated floor of a hot plate and tested for conditioned avoidance. Both hypoalgesia and conditioned avoidance were reduced by intra-amygdala midazolam. However, when midazolam was injected into the vlPAG, only the hypoalgesic response was attenuated and not the avoidance response. It was hypothesized that fearful stimuli activate the amygdala which activates the PAG to induce freezing and hypoalgesia (Harris and Westbrook 1995b).

It is possible that the amygdala may be involved in a number of other behavioral and physiologic responses to fearful situations. For example, benzodiazepines injected into the CEA also attenuate the production of stress ulcers in rats (Ray et al. 1989; Sullivan et al. 1989). There has also been one prior study examining micro-injections of BZs in mice. Using the light/dark paradigm, injection of BZ into the amygdala reduces aversive responses to a brightly lit area. (Costall et al. 1989).

The anxiolytic effects of intra-amygdala injections of BZs are likely the result of actions at the GABA/BZ receptor. There are GABA immunoreactive interneurons in

the amygdala (Pare and Smith 1993), with the GABAergic receptors usually on the soma or on proximal dendrites (Farb et al. 1992). Using micro-puncture to measure the kD and Bmax in various areas of the brain, the highest binding of BZs in the amygdala is in the lateral nucleus, though binding exists in the other amygdala nuclei as well (Thomas et al. 1985).

Four different antagonists, flumazenil, ZK 93426, FG 7142, CGS 8216, when injected systemically, antagonize the anticonflict actions of AL/ABL midazolam micro-injections supporting an action of BZ at the GABA complex in this brain area (Peterson et al. 1985). Also, while infusion of a BZ into the amygdala increases punished responding, inverse agonists infused here decrease punished responding (Hodges et al. 1987). Each of these findings is consistent with BZs acting at the GABA/BZ receptor.

A number of studies have examined the effectiveness of systemic BZs following lesions of the amygdala. In both amygdala-lesioned and non-lesioned animals, diazepam increases open arm activity. Also, diazepam decreases burying behavior in both lesioned and non-lesioned animals. This indicates that an intact amygdala is not necessary for diazepam's anxiolytic effects in both of these anxiety paradigms (Treit et al. 1993c). Another study, using the conditioned suppression of drinking paradigm, found that chlordiazepoxide increases punished responding after amygdala central nucleus lesions (Kopchia et al. 1992). In fact, the efficacy of diazepam is increased following lesioning. Using another conflict paradigm after both whole amygdala lesions as well as CEA lesions, BZs have anti-punishment effects. Again, it was observed that the lesions may even enhance the anxiolytic effects (Yadin et al. 1991).

These studies suggest that the amygdala may not be solely responsible for mediating anxiolytic effects. One drawback of lesions studies, however, is that

compensatory changes may occur following lesioning. These changes may be reflected by the increase in efficacy observed following lesions.

To more specifically block the action of BZs in the amygdala without lesioning, flumazenil (a BZ receptor antagonist) can be injected into the amygdala to block the anxiolytic effect of systemic BZs. The anxiolytic effect of systemic BZs on the social interaction test is blocked by BLA injection of flumazenil (Sanders and Sheckhar 1995). Also, intra-amygdala flumazenil blocks the effect of BZs on punished responding of rats (Hodges et al. 1987). These studies support the conclusion that at least some effects of systemic CDP in anxiolysis paradigms are mediated by the BLA.

In summary, intra-amygdala injections of benzodiazepines have produced anxiolytic effects in a wide variety of paradigms. There is some indication that "anxiety" in different paradigms might be differentially influenced by AL/ABL or CEA injections. This suggests that these differing paradigms may be measuring different phenomena. Some studies have attenuated the anxiolytic effect of BZs by injecting an antagonist directly into the amygdala. However, other studies have found that lesions of the amygdala do not block the anxiolytic effect BZs. Therefore, although the amygdala is strongly implicated in BZ-induced anxiolysis, additional neuroanatomical areas may also be involved.

Septum: Septal cholinergic neurons are sensitive to diazepam (Kumamoto and Murata 1995), and may mediate anxiolytic effects. Septal lesions have produced anti-conflict effects. However, these effects were less robust than those found after amygdala lesions (Yamashita et al. 1989b). Later studies found that septal lesions can actually increase "anxiety" as measured with conflict responding (Yadin et al. 1991). In the potentiated startle paradigm, septal lesions actually increase baseline startle, though the lesions have no affect on potentiated startle. These lesions do not attenuate diazepam's anxiolytic effects (Melia and Davis 1990). Septal lesions also do not affect

the number of shock probe contacts (Treit et al. 1993b). Furthermore, injections of CDP into the medial septum do not produce any anxiolytic effects in a thigmotaxia paradigm (McNamara and Skelton 1993a). These studies indicate that the septum may not mediate the anxiolytic effects of BZs.

However, using the plus maze to measure anxiolysis, one group has found that septal lesions increase open arm entries in the plus maze and mean duration of probe burying in the shock probe paradigm (Pesold and Treit 1992). Anxiolytic septal lesions were in the posterior septal nuclei, though the number of shock probe contacts were not affected by lesioning. This study suggests that the septum may mediate certain types of anxiolytic effects but not others. Consistent with this lesion study, intra-septal midazolam increases open arm activity in the plus maze and decreased burying behavior but does not affect the number of contacts with the shock-probe (Treit et al. 1993b).

In summary, the septum may mediate some anxiolytic effects and not others. Because the septum is not a homogenous structure, it is possible that some of the conflicting evidence is the result of injections into different structures. The contribution of various septal nuclei to anxiolysis remains under study.

Mammillary body: The mammillary bodies (MB) receive GABAergic innervation from extrinsic sources (Gonzaloruiz et al. 1993), and contains BZ receptors. Being part of the limbic Papez circuit, the MB may be involved in the modulation of emotions such as anxiety. Consistent with this, lesions of the mammillary body increase open arm exploration in the plus maze paradigm (Beracochea and Krazem 1991; Laurie et al. 1990), as well as increase conflict responding (Yamashita et al. 1989a).

Three different BZ's, diazepam, chlordiazepoxide, and midazolam, increase punished responding without changing unpunished responding (Kataoka et al. 1982), when injected into the MB. Zopiclone also has an anti-conflict effect when injected into

the MB (Yamashita et al. 1989a). It has been postulated that the MB connects with the frontal cortex via the anterior thalamus to produce this disinhibition.

Inconsistent with these results, one group has found that neither GABA or muscimol has anti-conflict effects when injected into the MB (Kataoka et al. 1987). Also, while mammillary lesions are anxiolytic on the plus maze, they do not block the anxiolytic effect of diazepam (Laurie et al. 1990).

Thus, there is some evidence that the MB is involved in mediating some aspects of anxiolysis induced by BZs. Both lesions and micro-injections of BZs have resulted in anxiolytic effects. Because the data are inconsistent, however, the role of BZs in the MB remains uncertain.

Dorsal raphe nucleus (DRN): GABAergic dendritic profiles contact both GABAergic interneurons and serotonergic projection neurons in the DRN. Thus, GABAergic stimulation may inhibit or disinhibit serotonergic neurons (Harandi et al. 1987). In vitro experiments show that BZs can facilitate the release of serotonin from midbrain slices (Thiebot et al. 1982a). Because a number of serotonergic drugs have anxiolytic effects, it has been hypothesized that benzodiazepines may produce anxiolysis by modifying the serotonergic system.

Using 5,7-DHT to specifically lesion serotonergic neurons in the DRN, anti-punishment effects are produced (Thiebot et al. 1982b; Thiebot et al. 1984). DRN lesions but not median raphe lesions with 5,7-DHT also have an anxiolytic effect using the social interaction paradigm (File et al. 1979). BZs injected directly into the dorsal raphe have anti-punishment effects. This effect of BZ micro-injections is abolished by prior 5,7-DHT lesions. Therefore, BZs can act on serotonergic neurons to produce anxiolysis.

However, DRN lesions with 5,7-DHT do not block the anti-punishment effects of systemically administered diazepam. And infusion of flumazenil into the DRN does

not block systemic diazepam's anxiolytic effects (Thiebot et al. 1984). Therefore, systemic BZs can also act in other areas of the brain to produce anxiolysis.

An inverse agonist, bCCM, reduces social interaction when injected either systemically or directly into the DRN. Flumazenil injected into the DRN has no effect, but blocks the effects of systemic bCCM. Inverse agonists at the BZ receptor typically attenuate the opening of the chloride channel in reponse to GABA, thus producing opposite effects than BZs. Thus to the extent that changes in social interaction reflect changes in anxiety, bCCM is acting in the DRN (Hindley et al. 1985; Jones et al. 1986). These results suggest that inverse agonists at the BZ receptor are acting in the DRN to increase anxiety, as measured with the social interaction paradigm.

In a different paradigm, mice were placed in a two compartment box (light and dark). Injection of BZ into the dorsal raphe nucleus reduces the aversive response to the brightly illuminated area as do injections into the amygdala (Costall et al. 1989).

One set of studies has measured the ability to escape in a shuttle box paradigm after inescapable shock (IS). Normally, exposure to IS produces deficits in the ability to subsequently perform in the shuttle box. This effect has been termed "learned helplessness." CDP micro-injections into the DRN given immediately prior to IS training block the subsequent learning deficit. If anxiety is mediating "learned helplessness" effects, then BZs may be acting in this structure to remove the anxiety of inescapable shock, and thus remove the "learned helplessness" effect (Maier et al. 1994). Lesions of the DRN also eliminated the escape deficit (Maier et al. 1993a). However, lesions of the amygdala did not affect the escape deficit. This suggest that a different type of "anxiety" may be involved in this paradigm.

Flumazenil or CGS8216, BZ receptor antagonists, have been injected into the DRN. Both compounds block subsequent enhancement of fear conditioning and interference with shuttle box escape when administered before IS, but have no effect

when given before shuttle box testing (Maier et al. 1995). Because these compounds are antagonists, this suggests that endogenous inverse agonists may be released in the DRN during IS training, resulting in the subsequent performance deficits.

In summary, the DRN may play a role in mediating several types of anxiolytic effects. It appears to mediate the anxiogenic effect of inverse agonists in the social interaction paradigm, and the ability of BZs to protect against "learned helplessness." The DRN may also mediate anticonflict effects and BZ-induced attenuation of aversive responses to bright areas.

Dorsal periaqueductal grey (dPAG): The CEA sends projections to about 50% of the cells in the PAG. It has been hypothesized that the dPAG and may mediate the "fear" effects of amygdala stimulation (Da Costa Gomez and Behbehani 1995). Benzodiazepines have been injected into the dPAG, and the effect measured on the plus maze. An increase in percent open arm entries is observed, and this anxiolytic effect is blocked with flumazenil (Russo et al. 1993). However, flumazenil injected into the dPAG does not block the anxiolytic effects of systemically injected diazepam on the plus maze (Russo et al. 1993). This is expected since diazepam can act in other places such as the amygdala to produce anxiolysis. Nevertheless these results suggest that BZs can also act in the dPAG to reduce anxiety.

Other paradigms suggest a role for the dPAG in mediating anxiolysis. For example, diazepam micro-injected in the rostral midbrain central grey decreases thigmotaxis (McCarthy et al. 1995). And measuring escape threshold, BZs micro-injected in the dPAG attenuate escape responses in the shuttle box during to electrical stimulation of the dPAG (Audi and Graeff 1984). Further evaluation of the dPAG in the behavioral effects of BZs remains under study.

Hippocampus: Lesions of the dorsal hippocampus do not affect performance in a Vogel-type conflict paradigm (Yamashita et al. 1989b). However, injection of

diazepam into the dorsal hippocampus has anticonflict effects in a similar paradigm (Kataoka et al. 1991). On the other hand, while zopiclone micro-injected into the MB has anti-conflict effects, it does not when injected into the dorsal hippocampus (Yamashita et al. 1989a). These conflicting results indicate that the exact role which BZs play in this structure needs additional analysis.

Using a learned helplessness paradigm, infusions of diazepam (dissolved in ethanol) into the hippocampus decreases the number of escape failures produced by inescapable shock. Control injections into the anterior neocortex or lateral geniculate body do not produce this effect (Campbell et al. 1980). This suggests that the hippocampus may be involved in mediating this type of "anxiolysis."

Most work on the hippocampus has involved serotonergic drugs. For example, buspirone has anxiolytic effects in the plus-maze and the open field thigmotaxis paradigms when injected directly into the dentate region of the hippocampus (Kostowski et al. 1989). BZ's may influence behavior in these paradigms by altering the release of serotonin in this structure. Local infusion of BZs into the hippocampus can reduce serotonin release in the hippocampus (Nishikawa and Scatton 1986). For further information regarding the role of serotonin, see section 4.1b.

Inferior colliculus (IC): Micro-injections of midazolam into the IC increase the latency to avoid areas associated with shock in the shuttle box task (Melo et al. 1992). It is possible that the IC may play a role in anxiolysis induced by BZs, however this requires further evaluation.

Hypothalamus: Many of the neuroanatomic areas implicated in anxiety have strong projections to the ventromedial hypothalamus (VMH). Stimulation of the VMH results in aversion as measured with a shuttle box. BZ's micro-injected here attenuate that effect (Milani and Graeff 1986). However, lesions of the VMH do not affect

conflict performance (Yamashita et al. 1989b). The role of this structure in mediating this and other anxiolytic effects of BZs has not been fully explored.

Infusion of muscimol into the posterior hypothalamus (PH) has an anticonflict effect. Picrotoxin micro-injections are anxiogenic. There is no effect after infusion of these compounds into the lateral hypothalamus (Shekhar et al. 1990). Infusion of muscimol into the PH also results in decreased avoidance behavior, measured using shock avoidance task. GABAergic antagonists injected into the PH increase avoidance behavior. Again, no affect of these treatments is produced by injection into the lateral hypothalamus (Shekhar et al. 1987). These results suggest that the PH may be capable of mediating anxiolytic effects.

GABA receptor blockade in the dorsal medial hypothalamus (DMH) has pro-conflict effects and is anxiogenic in the plus maze. Muscimol is anxiolytic in the plus maze after DMH injections. These effects are also seen in the social interaction paradigm (Shekhar 1993; Shekhar and Katner 1995). Therefore, it is possible that this area may also be involved in mediating BZ-induced anxiolysis. The relative roles of each of these hypothalamic structures remains unknown. Nevertheless, the initial findings warrant continued investigation into their role in mediating anxiolysis.

Cortical areas: Lesions of the frontal cortex increase punished responding in a conflict paradigm (Yamashita et al. 1989b). However, cortical injections of CDP do not produce thigmotaxis (McNamara and Skelton 1993b), and similar injections of zopiclone do not produce anti-conflict effects (Yamashita et al. 1989a). Injections of diazepam into the anterior neocortex also do not produce anti-conflict effects (Campbell et al. 1980) . The role of the cortex in primates in mediating anxiolysis remains speculative.

Neuroanatomy of anxiety summary: Since it appears that diazepam can have anxiolytic effects despite lesioning various areas, one study combined lesions of the

amygdala, the dorsal raphe, the locus coeruleus, and the mammillary bodies. Despite having lesions in all four areas, systemic CDP still had anticonflict effects (Grishkat et al. 1993). This suggests that numerous areas are capable of mediating the anxiolytic effects of BZs.

Most evidence, however, suggests that the amygdala is involved in mediating anxiolytic effects in many different paradigms. The particular nuclei within the amygdala (CEA or AL/BLA) may be involved in different types of anxiety, as measured with different paradigms. Numerous other areas also may be involved in mediating anxiolysis. Lesions of the amygdala often do not block anxiolytic effects, and injections of BZs into other areas can produce different types of effects. The role of these various areas in the different anxiety paradigms is still an active area of investigation.

4.1b Neurochemistry of anxiety

A number of neurotransmitters interact with BZs in affecting anxiety. Many of these interactions implicate various functional circuits. This may have implications for delineating the neuroanatomic mechanisms involved in anxiolysis.

Serotonin (5-HT): There are a variety of papers examining the effects of BZ's on 5-HT release. One study found that the systemic diazepam causes a reduction in (5-HT) synthesis in the hippocampus but not in the cerebral cortex, striatum, cerebellum, or spinal cord. Local infusion of diazepam into the hippocampus also results in a decrease in 5-HT in the hippocampus. Local infusion of diazepam into the NRD, however, does not affect 5-HT in the hippocampus. One conclusion is that anxiolytic drugs can act in the hippocampus to inhibit serotonergic activity (Nishikawa and Scatton 1986), thus influencing anxiety (Andrews and File 1993b). Other studies support this hypothesis. An increase in extracellular 5-HT is produced in the ventral hippocampus after exposure to the plus maze. GABA tonically inhibits 5-HT release in

this region, and systemic as well as BZs administered through the microdialysis probe further inhibits this release (Pei et al. 1989 and Wright et al. 1992b).

Nevertheless, there is evidence that decreases in 5-HT release may not be involved in anxiolysis. Similar to the hippocampus, exposure to the plus maze increases frontal cortex 5-HT release in guinea pigs, and systemically administered diazepam attenuates this release. However, flumazenil blocks the release of 5-HT into the frontal cortex despite having no behavioral effects on the plus maze. Thus, there is not a simple relationship between frontal cortex 5-HT release and behavior on the plus maze (Rex et al. 1993). In subjects lesioned with 5,7-DHT, there was actually an increase in sensitivity to the anticonflict effects of diazepam (Soderpalm and Engel 1991). Therefore, although diazepam does block 5-HT release in the hippocampus and frontal cortex, these effects may not be involved in anxiolysis.

Norepinephrine: Conflict situations reduce norepinephrine activity in the frontal cortex, CEA, MB, and dorsal hippocampus. Exposure to conflict situations also increases 5-HT activity in the frontal cortex, the CEA, the BLA, and the medial septum. Diazepam has been found to block these changes in the frontal cortex, CEA, MB and dorsal hippocampus. One hypothesis is that BZs affect these monoamine systems, and this is responsible for anxiolysis (Sakurai-Yamashita et al. 1989). Conversely, FG 7152, an inverse agonist, causes activation of norepinephrine in a number of brain areas (Ida et al. 1991). This has been hypothesized to mediate its anxiogenic effect. These hypotheses have not been directly tested.

Benzodiazepines can result in continued lever pressing during extinction, and this disinhibition may be related to anxiolysis. One study examined lever pressing during extinction and found that lesioning the dorsal noradrenergic bundle did not influence BZ effects on nonrewarded lever pressing (Salmon et al. 1989). Therefore, attenuation of norepinephrine release by BZs is not required for this effect.

Dopamine: Stress as well as inverse agonists at the BZ receptor increase dopamine outflow in the medial prefrontal cortex and the shell of the nucleus accumbens (Bassareo et al. 1996). The increase in dopamine release produced by these treatments is attenuated by BZs (Horger et al. 1995). These alterations have been hypothesized to play a role in the behavior effects of anxiety. On the other hand, some studies have found that while diazepam does lower baseline dopamine release in the nucleus accumbens, it does not alter stress-induced changes in dopamine release (Imperato et al. 1990). Therefore, while it is possible that alterations of dopamine outflow may be involved in anxiolysis, the relationship between effects on dopaminergic systems and anxiety is still undetermined.

Nitrous and nitric oxide (NO): Both CDP and nitrous oxide are anxiolytic in the mouse staircase test. Flumazenil blocks the anxiolytic effect of both drugs (Quock and Nguyen 1992). Flumazenil also blocks the anxiolytic effects of nitrous oxide and CDP in the rat social interaction test (Quock et al. 1993). This indicates that nitrous oxide's effects may be mediated by endogenous BZs.

Inhibition of the synthesis of NO with L-N-nitro arginine antagonizes the anxiolytic and locomotor activating effects of CDP (Quock and Nguyen 1992). This is evidence that the presence of NO is necessary for the anxiolytic and locomotor activating effect of CDP. Thus, release of NO may mediate these BZ effects. The interaction between these systems is still under exploration.

Cholecystokinin (CCK): Flumazenil blocks the effects of CCK agonists and antagonists in the plus maze (Chopin and Briley 1993). This suggest that CCK may affect anxiety by a release of endogenous benzodiazepines.

Opiates: Naloxone blocks the anxiolytic effects of BZs in both conflict tests and the plus maze (Agmo et al. 1995). This suggest that the opioid system may be involved in influencing the anxiolytic effect of BZs. Consistent with this, in humans naltrexone

can diminish the euphoric and anxiolytic effects while enhancing the sedative effect (Swift et al 1998). Interestingly, naltrexone pretreated people have a similarity to DS mice.

Corticotrophin releasing factor (CRF): Flumazenil antagonizes the anxiogenic effects of CRF in a conflict paradigm. This suggests that the anxiogenic effect of CRF requires the release of an inverse agonist. A CRF antagonist (α-helical CRF) does not block the anxiogenic effect of DMCM, an inverse agonist (Deboer et al. 1992). This suggests that the anxiogenic effect of inverse agonists is not dependent upon CRF. There may be an interaction between the CRF and BZ systems in controlling the level of anxiety.

Melatonin: Melatonin or diazepam were co-injected at small doses, and each drug alone had no effect on a four-plate crossing paradigm. An increase in punished crossing was observed when co-injected. This indicates that these two compounds act synergistically in effecting anxiety (Guardiolalemaitre et al. 1992). Thus, there may be an interaction with the melatonin system as well.

Endogenous BZ-like molecules: Exposure to new environments or to inhibitory avoidance training results in both an increase in BZ-like molecules in the septum, hippocampus, and amygdala and an increase in anxiety. The degree of anxiety on the plus maze is correlated with the degree of increase in the BZ-like molecules (Dacunha et al. 1992). This suggest that endogenous ligands at the BZ receptor may act in these areas to affect anxiety.

Numerous other neurochemical systems may interact with benzodiazepines to mediate or influence the anxiolytic effect of benzodiazepines. Efforts to delineate these mechanisms is still in progress.

4.2 Sedation and Sleep Induction

Though BZs are "sedative/hypnotics," the meaning 'sedation' is unclear. 'Sedation' has been equated with somnolence, drowsiness, calmness, tranquillity, and lack of anxiety (Wansbrough and White 1993). In the animal literature, it has been equated with decrements in locomotor activity and other performance measures. Benzodiazepines are used clinically to induce sleep. However, the exact relationship between "sedation" and sleep induction is unclear. Because sedation and sleep induction may represent distinct effects, the neuroanatomy of both will be briefly reviewed here.

4.2a Models of sedation

In humans, midazolam impairs a subject's ability to discriminate the duration of tones. Reaction time is also impaired (Polster et al. 1993a; Rammsayer 1992). Both effects are used to measure sedation. Peak saccade velocity is also used to assess the sedation, with BZs decreasing the saccade velocity (Richens et al. 1993). The rate of substituting visual symbols for numerical digits is another measure (Fleishaker et al. 1995).

It is unclear if the different measurements reflect similar phenomena. For example, diazepam affects (1) subjective rates of drowsiness, (2) a digit cancellation task, and (3) rate of rehearsal of words. However, diazepam's effects on these tasks in different people does not correlate (Rich and Brown 1992). This suggests that these tasks measure different effects. A similar study utilized the digit symbol substitution task to measure psychomotor speed. A subjective impression of sedation was assessed with a visual analog scale. These two measures did not correlate (Weingartner et al. 1995). Therefore, there may be different types of "sedation," many of which BZs are capable of eliciting.

In animals, discrimination between two tones can be used to measure "sedation." In these paradigms, two different tones indicate which of two levers would provide food. Discrimination between these two tones is impaired by BZs (Tan et al. 1990). Locomotor activity and performance on various tasks can also be decreased by BZ administration (Lotrich and Gallaher, personal comm; Burke et al. 1994). These decrements in locomotor activity have been considered measures of sedation.

4.2b Anatomy

Cortical somatosensory evoked potentials are increased in latency and decreased in amplitude by diazepam (Todorova 1993), supporting the hypothesis that the slowing of cortical perceptual processing mediates sedation. However, when diazepam was injected into humans at doses in which no sedation was reported, diazepam reduced regional cerebral blood flow (as measured with Xenon 133) in all examined cortical areas. Therefore, decreased metabolic activity in these areas may not be involved in subjective sedation (Mathew et al. 1985).

In animal studies, picrotoxin was injected into the ventral pallidum, a projection site of the nucleus accumbens, increasing locomotor activity. Because picrotoxin inhibits chloride flux at the GABA/BZ receptor, BZs may act here to produce an opposite effect (Mogenson et al. 1983). That is, BZs may act in the pallidum to decrease locomotor activity. Another study has also found that biccuculline or picrotoxin injected into the ventral pallidum/substantia innominata (VP/SI) increased locomotor activity. This effect could be blocked by co-injection of muscimol (Austin and Kalivas 1990). This area projects to the pedunculopontine nucleus and the mediodorsal thalamus (Churchill et al. 1991; Churchill et al. 1996a; Churchill et al. 1996b) and may be involved in regulating activity.

The nucleus accumbens may also mediate BZ locomotor hypoactivity. Local perfusion of flurazepam into the nucleus accumbens decreased the amount of dopamine

release. Because increases in dopamine in this structure are normally associated with locomotor activation, it is possible that this effect could decrease locomotor behavior (Zetterstrom and Fillenz 1990).

Another area which could be involved in hypoactivity is the BLA. The BLA projects to the nucleus accumbens (Burns et al. 1993), and stimulation of this nucleus produces locomotor activation. Biccuculline, which decreases chloride flux through the GABA/BZ receptor, also increased locomotor activity when injected into the BLA (Sanders and Shekhar 1991). Because BZs may produce the opposite effect, they may decrease locomotor activity when injected here.

In summary, a number of areas may be involved in sedation/hypoactivity. Because BZs interfere with a wide range of psychomotor skills, the cortex has been hypothesized to mediate these effects. However, there is limited evidence to support this. Also, a number of nuclei within the limbic/motor areas may mediate different aspects of locomotor hypoactivity. The involvement of different neuroanatomic circuits in "sedation" is fairly unexplored and needs to be further tested.

4.2c Sleep

BZs also have numerous effects on sleep. For example, transient electroencephalogram arousals occur during sleep, occasionally resulting in brief waking episodes. BZs elevate this arousal threshold during sleep (Roehrs et al. 1993). BZs also decrease the latency to sleep onset (Stutzmann et al. 1992). Short wave sleep is increased by BZs at the expense of both REM (rapid eye movement) and delta sleep (Stutzmann et al. 1992).

However, some drugs which act at the BZ receptor have different effects on the sleep architecture. For example, zopiclone increases SWS without depressing REM sleep (Stutzmann et al. 1992), even increasing delta sleep (Stutzmann et al. 1993).

These pharmacologic differences suggest that the effects of BZs on the various stages of sleep may be mediated by different systems. Sleep induction may be a reflect a number of separate physiological phenomena.

4.2d Anatomy

The lateral dorsal pedunculopontine tegmental nucleus contains cholinergic neurons which project to the thalamus and the entire brainstem reticular formation. These neurons fire during waking states and paradoxical (REM) sleep, and because these neurons are innervated by many GABAergic interneurons, they may the locus of action for the sleep-inducing effects of BZs (Jones 1993).

There are also a number of GAD-positive neurons throughout the reticular activating system (RAS) intermingled with monoaminergic and glutaminergic projection neurons. Inhibition of firing by BZs in this area could effect sedation (Ford et al. 1995). Stimulation of the RAS increases rhythmical slow activity in the hippocampus. This type of hippocampal activity is associated with states of arousal. BZs inhibit this activation (Coop et al. 1991). Therefore, BZs may act in the RAS to result in either sedation or sleep-induction.

Sleep promotion has been hypothesized to be in or close to the medial preoptic/anterior hypothalamus (Szymusiak 1995). BZs may selectively inhibit the neurons which normally produce arousal in this area. The anterior hypothalamus has also been directly implicated in sleep-induction. Triazolam injected into the medial preoptic area enhances sleep time, but not with control injections into the nearby lateral preoptic area or the diagonal band of Broca (Mendelson and Martin 1992).

Electrophysiologic monitoring of the nucleus basalis magnocellularis (NBM) indicates that some neurons here fire immediately prior to the transition from waking to sleep. These may be involved in triggering sleep. Electrical simulation here evokes

sleep, and lesions cause insomnia. Other neurons in the NBM may promote arousal. The role or BZs in sleep here remains hypothetical.

Blockade of GABA receptor in the posterior hypothalamus has been observed to result in locomotor activation (Shekhar et al. 1990). GABA injected here was found to decrease wakefulness (Ericson et al. 1991). Histaminergic neurons arise in the posterior hypothalamus from the tuberomammillary nucleus, and the firing rate of these neurons is increased during arousal states. Systemic diazepam decreases the release of histamine in freely moving rats measured with microdialysis (Chikai et al. 1993). This anti-histaminergic property may be responsible for some of the sedative or hypnotic effects of BZs.

In summary, a number of neural systems may be involved in sleep and sedation. Sleep and sedation may not be unitary phenomena. BZs have effects in many of the neuroanatomic areas involved in these systems. Further experimentation is required to delineate the systems that specifically mediate the various effects.

4.3 Ataxia and coordination

Because the cerebellum is involved in coordination of movement, this structure has been hypothesized to mediate the ataxic effects of BZs. Supporting this hypothesis, injections of the GABA agonist piperidine in the fastigial cerebellar nucleus results in ataxia (Miller et al. 1993). Also, an injection of the BZ inverse agonist, Ro15-4513, into the cerebellum protects against ataxia induced by the ethanol (Dar 1995). Consistent with this, a line of rats which have a point mutation in the $\alpha 6$ subunit has increased sensitivity to the ataxic effects of BZs. The $\alpha 6$ subunit is almost exclusively expressed in cerebellar granule cells, and the mutation results in enhanced in vitro sensitivity of the BZ receptor to BZs (Korpi et al. 1993; Korpi and Seeburg 1993).

Other neuroanatomic areas involved in coordination may also be involved. For example, the medial vestibular nucleus has binding sites and is sensitive to GABA. It may mediate some ataxic effects (Hutchinson et al. 1995). The inferior olive, which projects climbing fibers to the purkinje cells of the cerebellum, has BZ receptors (Frostholm et al. 1992). This nucleus may also be involved in mediating ataxic effects.

Areas of the forebrain may also be implicated in motor coordination. The ability of rats to walk across a beam was impaired following infusion of GABA into the nucleus basalis magnocellularis or the frontal cortex (Majchrzak et al. 1992). These areas may also be involved in the attention and sedative effects of BZs. Therefore, behavioral tasks which measure ataxia may also be measuring some other aspects of "sedation."

There are also a number of different types of GABAergic interneurons and projections neurons in the striatum (Kawaguchi et al. 1995). The inverse agonist Ro15-4513 has been micro-injected into the striatum, attenuating the ataxic effects of systemically administered ethanol (Meng and Dar 1994). Therefore, it is possible that the striatum is involved in mediating sedation or ataxia.

4.4 Locomotor activation

At lower doses, systemically administered BZs result in locomotor activation rather than sedation. Various BZs micro-injected into the nucleus raphe, result in increased locomotor activity (Sainati and Lorens 1982a). This effect depended upon intact ascending serotonin projections (Sainati and Lorens 1982b). This suggests that serotonergic neurons in the nucleus raphe may mediate the locomotor activating effect of BZs. Diazepam injected into the dorsal raphe nucleus also increases wakefulness (Mendelson 1990), and muscimol, when injected into the median raphe, produces hyperactivity (Wirtshafter et al. 1988). This supports the role of this structure in mediating arousal and locomotor activation by BZs. Systemic injection of high doses

of haloperidol do not attenuate this hyperactivity. Therefore, this effect may not be mediated by dopamine systems.

These results suggest that changes in the serotonergic system may be responsible for locomotor activation. Supporting this, injections of muscimol into the nucleus raphe produce a hippocampal theta rhythm. Because the theta rhythm has been associated with arousal, this is consistent with involvement of the DRN in locomotor activation (Kinney et al. 1995). However, in microdialysis studies, the degree of 5-HT release does not correlate with total entries on the plus maze (Wright et al. 1992a). Therefore, other systems may also be involved.

For example, injection of muscimol into the preoptic area of the rat increases locomotor activity. This effect is blocked by systemic haloperidol. This area, a putative sleep center, may also be involved in both sedation and arousal (Osborne et al. 1993). The mediodorsal nucleus of the thalamus is innervated by GABAergic neurons arising from a number of areas including the ventral pallidum (Churchill et al. 1996b). Muscimol injected into this thalamic area results in increased locomotor activity (Churchill et al. 1996a). It is feasible that this and other thalamic areas may mediate the locomotor activating effects of BZs.

Numerous other areas can be postulated to mediate BZ-induced locomotor activation. A basal forebrain lesion produces increased locomotor hyperactivity, and this is reversed with a BZ antagonist, ZK 93426 (Sarter and Steckler 1989). Injection of muscimol into the medial septum also increases locomotor activity (Osborne et al. 1993). These studies implicate both areas as possible mediators of locomotor activation.

Lidocaine in the dentate gyrus also increases locomotor activity (Flicker and Geyer 1982a). This temporary functional lesion may simulate the effect of

hyperpolarization produced by BZs. It is possible, therefore, that BZs can act in the hippocampus to increase locomotor activity.

The ventral tegmental area (VTA) has also been hypothesized to mediate locomotor activation. Low doses of muscimol injected into the VTA increased locomotor behavior (Willick and Kokkinidis 1995). Furthermore, in the VTA there is a slight excitation of dopamine neurons by BZs, through inhibition of inhibitory interneurons (O'Brien and White 1987). Because dopamine release by these neurons is implicated in locomotor activation, this area may mediate this effect of BZs.

4.5 Seizure protection

There are a number of animal models of seizures and epilepsy (reviewed by (Fisher 1989)). Topical convulsants, injury, or electrical stimulation of an area have been used. For generalized tonic-clonic seizures, genetically prone strains of mice, rats, gerbils, fruitflies, and baboons, are available. Maximal electrical shock (MES), systemic chemical convulsants, and metabolic derangements also produce seizures. Seizures can also be elicited by some types of sensory stimulation. For example photosensitive baboons react with seizures to light and some strains of mice react to tail-spins.

The anatomical circuits involved in these many types of seizures are likely to be different. In a review of the anatomy of seizures, it is suggested that the prepiriform, piriform, and entorhinal cortices play a predominant role in limbic motor seizures in conjunction with the hippocampus, amygdala, substantia innominata, and mediodorsal thalamus (Gale 1992a). For example, metabolic activity mapping of seizures triggered by electrode stimulation in the amygdala demonstrated that the basolateral amygdala was first to be activated, followed by the hippocampus and other limbic areas. Some thalamic areas also were involved (Handforth and Ackermann 1995). On the other hand, seizures involving running-and-bouncing or tonic hindlimb extension are

proposed to depend on inferior colliculus activity. And tonic hindlimb extension (THE) appears to depend upon the nucleus reticularis pontis oralis (Gale 1992b).

Infusions of bicuculline into the tectum has anticonvulsant actions against maximal electric shock convulsions. However injection of bicuculline into this area is proconvulsant for other types of seizures (Weng and Rosenberg 1992). This is further evidence that particular brain areas may be differentially involved in different types of seizures.

One important pathway implicated in PTZ-induced seizures is the projection from the mammillary bodies (MB) to the anterior thalamus (AT). Muscimol injections in the AT have protected against repetitive high-voltage seizure discharges produced by systemic PTZ. Injections into other thalamic nuclei, the striatum, MB, or cortex have no effect (Mirski and Ferrendielli 1986). Lesions of the tract from the MB to the AT also protect against tonic seizures produced by systemic PTZ (Mirski and Ferrendelli 1984; Mirski and Ferrendelli 1987). It is hypothesized that the mammillothalamic tracts are the major route of activity from the brainstem to the thalamus, and activity in the thalamus is the gateway to generalized seizure activity (Mirski and Ferrendelli 1987).

Another important area is the substantia nigra (SN). The SN has excitatory projections to the superior colliculus which can influence the limbic system, the thalamo-cortical system, and brainstem circuits. Inhibition at the SN influences many different types of seizures (Gale 1992a).

The substantia nigra reticulata (SNR) has been directly examined as an important locus where GABAergic agents provide anti-convulsant activity. Electrical stimulation of the amygdala, olfactory structures, and lateral entorhinal cortex produces seizures while injection of muscimol into the SNR raises the seizure threshold for all these seizures.(McNamara et al. 1984). In photosensitive baboons, GABA is partially

anticonvulsant when injected into the SNR (Silva-Barrat et al. 1988), and muscimol injections into the SNR have protect against both MES-induced THE and PTZ-induced tonic-clonic seizures (Iadarola and Gale 1982).

Clonazepam micro-injections into the SNR also protect against generalized seizures elicited by amygdala stimulation (King et al. 1987). In addition, midazolam and flurazepam block PTZ-induced seizures when injected into the SNR (Zhang et al. 1989). Micro-injection of BZs into the SNR also protects against bicuculline-induced and MES seizures (Zhang et al. 1991). Conversely, injection of the inverse agonist DBI into the SNR enhanced kindled seizures and partially reversed the protective effects of diazepam (Shandra et al. 1990; Shandra et al. 1991).

This set of studies strongly suggests that the SNR is a crucial locus for mediating the seizure-protective effects of BZs. In support of this, the SNR has glutamate decarboxylase-containing terminals which contact both dopamine and other GABAergic neurons (Mendez et al. 1993; Santiago and Westerink 1992). Diazepam inhibits firing of cells in the SNR by 50% (Mereu et al. 1983). Furthermore, tolerance to diazepam is seen in the cells of the SNR after 2 days of flurazepam treatment, and this may be the cause of tolerance to the seizure-protective effects (Rosenberg et al. 1990).

Another area which may be involved in mediating the protective effects of seizures is the inferior colliculus (IC). In the genetically epilepsy-prone rat, seizures can be induced acoustically. In these seizure prone rats, neurons in the IC are electrophysiologically less sensitive to flurazepam. The IC has projections to the reticular formation, and these may mediate the spread of convulsive activity to other areas of the brain (Faingold 1980; Faingold et al. 1986). Bilateral injection of muscimol and CDP into the IC suppresses all sound-induced seizures (wild running,

clonus, and tonus) in ethanol-withdrawn rats. There was little or no effect when CDP was injected into the medial septum (Frye et al. 1983).

The cerebellum may be another locus in which BZs exert anti-convulsant effects. GABA agonists injected into the fastigial nucleus of the cerebellum result in protection against bicuculline-induced myoclonic, clonic, and tonic seizures. Slightly dorsal, anterior, or dentate injections do not have this protective effect (Miller et al. 1993).

The hippocampus is another structure hypothesized to mediate the seizure protective effects of BZs. Polysynaptic responses appear in the dentate gyrus after PTZ infusion, consistent with a role in seizure activity (Stringer 1995). There are several cells in the dentate gyrus, each containing glutamic acid decarboxylase, which project to the dendrites and somata of granule cells (Ribak 1992). The inhibition produced by these cells may mediate protection against seizures. Consistent with this, the strength of inhibition of CA1 hippocampal cells is decreased by chronic BZ treatment. The time-course for this corresponds to the time-course for tolerance to anti-seizure effects (Zeng et al. 1994). Also consistent with a role for the hippocampus, diazepam is less effective in protecting against seizures in hippocampally lesioned animals (Czuczwar et al. 1982).

A number of other areas are implicated in studies using γ-vinyl-γ-aminobutyric acid (GVG), an irreversible inhibiter of GABA transaminase. For example, pentylenetetrazol (PTZ) tonic clonic seizures are prevented by GVG injections into the anterior thalamus, the caudal hypothalamus, the superior colliculus, cerebellar nuclei, medial medullary, pontine and mesencephalic tegmentum including the vestibular nuclei, the reticular formation, and portions of the central gray. Vestibular, cerebellar, and reticular injections of GVG also prevented MES seizures (Miller et al. 1987).

4.6 Amnesia

BZs are reported to affect explicit but not implicit memory (Ghoneim and Mewaldt 1990; Polster et al. 1993b). Implicit memory tasks include recognizing degraded pictures and words, while an example of explicit memory is the ability to remember a list of words (Fleishaker et al. 1995). Spatial memory is also impaired by BZs. For example, diazepam specifically affects spatially learning in a Morris water maze, but does not affect cue learning in this task (Brioni and Arolfo 1992). This indicates that BZs may affect some types of memories more than others.

The effect of BZs on memory does not seem to be the result of sedation (Curran 1991; Curran and Birch 1991). For example, there is a difference in the dose response curves for performance on explicit memory tests and subjective rankings of sedation following triazolam injection (Weingartner et al. 1995). This has led to the suggestion that the amnesic effects are mediated by a different mechanism (Hommer et al. 1993). In animals, after 14 days of CDP treatment, there is tolerance to ataxia, locomotor sedation, and acquisition of inhibitory avoidance. However there is no tolerance to the effects on acquisition of performance on a radial arm maze (Shumsky and Lucki 1994). This again suggests that effect on acquisition of memory involves a system different from sedation.

The anatomic locus in which BZs affect spatial memory has been tested by injecting chlordiazepoxide into the amygdala, medial septum, hippocampus, cerebellum, frontal cortex, and the nucleus basalis magnocellulari/substantia innominata. Only infusions into the medial septum prevented spatial learning in the Morris water maze. Cue learning and swim speed were not affected (McNamara and Skelton 1993b). This strongly implicates the medial septum as the locus which mediates this effect of BZs. It was hypothesized that CDP impairs spatial learning by

inhibiting septo-hippocampal GABAergic projection neurons (McNamara and Skelton 1993c). GABAergic interneurons may also be involved (Wood 1986).

CDP infused into the medial septum produce a working memory deficit in another spatial memory task, the radial arm maze. Injections into the lateral septum, the anterior cingulate, and the NBM are not effective (Stackman and Walsh 1992; Stackman and Walsh 1995). In addition, medial septal lesions affect spatial memory in a Y-maze. However, the lesions do not affect the ability to choose a goal box which was visually cued by an object (Kelsey and Vargas 1993). These studies suggest that the spatial memory deficit produced by intra-septal BZs may generalize to a number of spatial memory tasks.

One mechanism proposed for the effect of BZs in the medial septum is decreased acetylcholine (Ach) release in the hippocampus. Diazepam micro-injections into the medial septum decrease Ach in the hippocampus by 50%. Interestingly, micro-injections of flumazenil increase Ach release by 95% (Imperato et al. 1994).

The effect of systemic CDP has been evaluated in a group of animals with medial septal lesions. Although the lesions impaired performance in the Morris water maze, CDP attenuated this impairment (Farber 1993). This later finding suggests that BZs may be acting in other areas to enhance memory. However, intraseptal infusions of flumazenil failed to attenuate the spatial learning deficit produced by systemically administered CDP (Wood 1996). Thus, it is possible that BZs are also acting in other areas to diminish spatial memory.

Another memory task is inhibitory avoidance in which subjects are exposed to a location previously paired with an aversive shock stimulus. Avoidance of this location is a test of memory. Lesions of the amygdala block the amnestic effects of diazepam in this inhibitory avoidance task (Tomaz et al. 1991). More specific lesions of basolateral amygdala also block the effects of diazepam on retention in this paradigm (Tomaz et al.

1992). This suggests that the basolateral amygdala mediates this effect of BZs. In support of this conclusion, amnesia results from diazepam injections into the AL/BLA but into the CEA (Tomaz et al. 1993). This finding has been replicated several times (de Souza Silva et al. 1993; de Souza Silva and Tomaz 1995; Dickinson-Anson and McGaugh 1993).

Using a multiple trial inhibitory avoidance task, bicuculline injections in the amygdala block BZ-induced amnesia. This again supports the proposal that amnesic effects of BZs may be mediated by GABAergic systems in the amygdala (Dickinson-anson et al. 1993). Bicuculline injections into the medial septum did not block the amnestic effect a systemic BZs on this task. This suggest that inhibitory avoidance is different from the spatial memory amnestic effect (Dickinson-Anson and McGaugh 1994).

There is also some evidence that the right amygdala may play a larger role in the amnestic effect of BZs than the left amygdala. When lidocaine is injected into the right or left amygdala immediately prior to inhibitory avoidance training, memory of the aversive task is more affected by the right-sided injection (Coleman and McGaugh 1993; Coleman-Mesches and McGaugh 1995).

In another learning paradigm, the DRN is implicated in possible amnestic effects. CDP injected into the dorsal raphe nucleus blocks the learning deficits induced by inescapable shock. This effect on "learned helplessness" may therefore be mediated by the DRN (Grahn et al. 1993).

Although McNamara and Skelton (1993b) did not find that CDP injections into the nucleus basalis (NB) affected spatial memory, another group has found that working memory in a double Y maze is affected by NB lesions. This suggests that the NB may be involved in mediating some amnestic effects of BZs (Mallet et al. 1995).

Consistent with this, the inverse BZ agonist b-CCM enhanced recognition performance in a two trial recognition task, when injected into the NB (Mayo et al. 1992).

The hippocampus is potentially an area in which BZs may influence memory. Rats were tested in a maze in which there were four choice points, with three gates at each choice point. CDP injected into the hippocampus increased the number of errors needed to complete the task (Ohno et al. 1992). Therefore, this structure may also be involved in mediating amnesic effects in this type of paradigm.

Flumazenil has also been found to enhance retention in an avoidance task after injection into the hippocampus. This suggests that the hippocampus may be involved in avoidance learning. Flumazenil injected into the amygdala and septum also enhanced retention of the avoidance task. It is proposed that memory is influenced by the release of some endogenous BZ in all three areas (Cunha et al. 1990).

4.7 Attention

When human subjects are required to respond to signals cued with valid or invalid cues, triazolam decreases the ability to disengage attention and respond correctly (Johnson et al. 1995). This impairment of attentional mechanisms by BZs has been studied in a number of animal models. For example, in one paradigm, animals are required to detect a brief 50 ms light, signalling the availability of food. Diazepam decreases performance in this task (Dudchenko et al. 1992). This has been interpreted as an interruption in attention. In detecting visual stimuli of 25-500 ms in length, CDP attenuates the number of correct responses to these cues, and nucleus basalis (NB) lesions reduce CDP's potency in this effect (Dudchenko et al. 1993). In a similar task, CDP injections directly into the NB impair performance. Also, micro-injection of an inverse agonist, b-CCM, facilitate performance (Travers et al. 1993). These results suggest that decrements in attention can be mediated by the NB.

Discrimination paradigms to measure attention have been utilized in other studies. For example, when different behaviors are required in response to a slowly or a quickly pulsing light, muscimol injections into the NB impair performance (Muir et al. 1992). In a similar study, subjects required to discriminate between a flickering and a constant light have impaired performance following muscimol injections into either the NBM or the substantia innominata. Interestingly, CDP micro-injections here have no effect (Dudchenko and Sarter 1992). These results suggest that the GABAergic inhibition of activity in the NB can interfere with the ability to perform in discrimination paradigms. However, BZs may not be effective. Therefore, although enhanced GABAergic activity in the NB can affect discrimination performance, the effect of systemic BZs on this paradigm may not be mediated by this locus.

Infusions of CDP or b-CCM into the basal forebrain was tested on a similar task of behavioral vigilance. Animals were trained to discriminate between visual signals of various lengths and non-signal events. CDP injections into the NB decrease the number of correct responses to visual signals, without affecting the relative number of correct rejections. Conversely, b-CCM decrease the number of correct rejections, without affecting the number of correct acceptances (Holley et al. 1995). This is evidence that BZs in the NB impair performance on some tasks which require attention.

It has been proposed that decreased cholinergic output is the mechanism for these attention deficits. Supporting this, direct injection of muscimol into the NB decreases Ach in the cortex (Casamenti et al. 1986). Electrophysiologically monitering single cells, GABA injected into the NB decreases the likelihood of conditioned stimulus elicited changes in frontal cortex firing rates (Rigdon and Pirch 1984), an effect potentially mediated by Ach. However, there is also evidence that the attentional effects are not mediated by changes in Ach release. Imidazenil, abecarnil, diazepam, and midazolam all inhibit basal hippocampal Ach release. However, these different

compounds have different effects on cognition and attention (Dazzi et al. 1995). Therefore, alterations in Ach release may not be responsible for effects on attention.

4.8 Other effects

Muscle relaxation: Benzodiazepine receptors exists in the spinal cord and may mediate the muscle relaxant properties of BZs (Bohlhalter et al. 1996). However, there is no direct evidence that receptors here mediate this effect. Other areas of the brain involved in muscle tone and movement may be directly affected by BZs to produce muscle relaxation. For example, opioid manipulations of the striatum affected muscular rigidity in the gastrocnemius-soleus (Melzacka et al. 1985). It is possible that BZs may similarly act in the striatum and other areas of the basal ganglia to affect muscle tension.

Aggression: Depending upon the type of aggression which is measured, BZs can either decrease or increase aggression (Bond et al. 1995). Lesions of the VMH and lateral septum can increase aggressiveness (Brayley and Albert 1977, Brady and Nauta 1953). Interestingly, stimulation of the lateral septum can attenuate the increase in aggressiveness caused by VMH lesions (Brayley and Albert 1977). It is possible that BZs could act in either of these areas to affect aggression. However, although BZ receptors exist in these areas, the role of these structures in mediating BZ's effects on aggression is not known.

One study has examined the effect of a micro-injection of an inverse agonist, DBI, on the aggressive effects of diazepam. It was found that injections into the substantia nigra interfered with seizure protection but did not affect diazepam's attenuation of aggression (Shandra et al. 1990).

Hypothermia: BZs decrease temperature in mice. Drugs effects were antagonized by flumazenil (Taylor et al. 1985). However the locus of action is not known. Temperature regulation centers of the hypothalamus may be involved.

Heart rate: BZs can increase heart rate, possibly by decreasing the vagal tone (DiMicco 1987). Pre-ganglionic neurons in the vagal motor nuclei are inhibited by GABA. The hypothalamus may also be involved in mediating other effects of BZs on heart rate (Soltis and DiMicco 1991). Changes in heart rate may also be secondary to anxiolysis.

Corticosterone effects: Restraint stress results in increased CRF mRNA in the paraventricular nucleus of the hypothalamus. This is blocked by CDP (Imaki et al. 1995). Diazepam also inhibits the other aspects of the hypothalamic-pituitary axis, causing decreases in adrenocorticotropin hormone and corticosterone levels (Pivac and Pericic 1993). These effects may be the result of decreases in anxiety or direct effects on the hypothalamus and/or pituitary.

Feeding effects: BZs can induce eating (Sanger 1984). Diazepam also enhances feeding behavior induced by electrical stimulation of the lateral hypothalamus (Bielajew and Bushnik 1994). Because GABA in the medial hypothalamus and amygdala can stimulate feeding (Minano et al. 1992), it is possible that these areas mediate this effect of BZs. However, this has not been directly investigated.

Circadian rhythm effects: BZs induce phase advances in circadian rhythms as measured by wheel running activity, and there is electrophysiological evidence for a role of BZs in the suprachiasmatic nucleus (SCN). CDP or flurazepam can decrease cell firing of SCN cells in vitro (Mason et al. 1991). Also, because the ability of BZs to produce phase advances is blocked by geniculo-hypothalamic tract lesions, it is possible that BZs may act in places other than the SCN (Biello et al. 1991). Additionally, it is possible that the phase advances are the result of other effects of BZs such as sedation (Biello et al. 1991).

Reward: BZs can produce conditioned place preference (CPP) (File 1986). Diazepam's ability to produce CPP can be blocked by ritanserin, a 5-HT_2 antagonist.

However, ritanserin does not block open arm exploration of rats on the elevated plus maze. This indicates that the anxiolytic effect is not causing the rewarding effect (Nomikos and Spyraki 1988). Opioid systems may be involved, as CPP induced by diazepam is blocked by naloxone (Spyraki et al. 1985).

Both haloperidol and nucleus accumbens lesions block diazepam-induced CPP (Spyraki and Fibiger 1988). Also muscimol injected into the ventral tegmental area is capable of enhancing intra-cranial self-stimulation (Willick and Kokkinidis 1995). Therefore it is possible that the rewarding effect of BZs is mediated by the mesolimbic dopamine system (Koob 1992). However, systemic BZs have only been observed to decrease dopamine release in the nucleus accumbens (Finlay et al. 1992). Thus, the role of the mesolimbic dopamine system in mediating rewarding effects of BZ remains unresolved.

4.9 Summary of Anatomy

BZs are capable of producing a number of behavioral and physiological changes: anxiolysis, sedation, sleep-induction, ataxia, locomotor activation, seizure protection, amnesia, attention deficits, muscle relaxation, aggression, hypothermia, corticosterone release, hyperphagia, circadian phase shifts, and possible reward effects. Various neuroanatomic systems may mediate these different effects. Although there is some suggestive evidence for the involvement of numerous neuroanatomic areas in mediating each of these effects, further studies are clearly necessary to determine those areas which do, in fact, mediate each effect. That is, a major task for understanding the physiology of BZs will be to empirically clarify the neuroanatomy and neurochemistry involved in each individual effect.

5.0 Specific Rationale and Hypotheses

- Mouse lines have been developed by selective breeding which exhibit behavioral differences following acute BZ administration. In particular, DR mice exhibit a dose-dependent anxiolytic response on the plus maze following systemic diazepam, whereas DS mice do not. This is paradoxically distint from the originally targeted behavioral difference--DS mice being selectively bred to be more sensitive than DR mice to the ataxic effect of BZs.
- Detailed study of the mechanisms underlying these behavioral differences requires a knowledge of the anatomic structures which mediate each of the specific behaviors being examined. This will permit a more focused examination of specific neural areas.
- A review of the literature suggests that the amygdala is involved in mediating several types of anxiolytic effects. Therefore, an analysis of the amygdala's role in mediating anxiolysis is an appropriate starting point.
- Positive identification of the amygdala as a locus which contributes to observed differences in anxiolytic sensitivity in DS and DR mice provides a critical foundation for further examination of neurophysiolygic mechanisms in this region of the brain.
- **Hypothesis 1: The amygdala mediates BZ-induced anxiolysis, as well as genetic differences in sensitivity to anxiolysis.**
    - **Prediction 1a: Injection of CDP into the amygdala will result in an anxiolytic effect as measured with the plus maze.**
    - **Prediction 1b: CDP injected into the amygdala will produce anxiolysis in the DR mice and not the DS mice.**

- This hypothesis will be tested by direct injection of CDP into the amygdala. Following injection, the mice will be tested on the plus maze. This experiment will determine (1) if anxiolytic effects can be measured on the plus maze in mice after intra-amygdala injection procedures, (2) if CDP produces an anxiolytic response following injection into the amygdala, and (3) whether the DS and DR mice differ in sensitivity to any anxiolytic effects of intra-amygdala CDP. Evidence from prior experiments indicates that BZ micro-injections *will* be anxiolytic. It is expected that this effect will be seen in DR and not DS mice.
- **Hypothesis 2: The amygdala does not mediate locomotor activation, muscle relaxation, ataxia, locomotor hypoactivity, or seizure protection.**
    - **Prediction 2: Injection of CDP into the amygdala is specific for anxiolysis. That is, the micro-injections will not affect muscle relaxation, ataxia, locomotor sedation, or activation. The injections also will not protect against PTZ-induced seizures.**
- Many behavioral methods relevant to benzodiazepines are currently available in this laboratory. Therefore, several other behaviors will be sequentially evaluated: locomotor activity on the plus maze, muscle relaxation, ataxia, and protection against PTZ-induced seizures.
- If the intra-amygdala micro-injections specifically produce anxiolysis and not the other effects, this will indicate that this structure is involved specifically in anxiolysis. Therefore, other neuroanatomic areas must mediate the other effects.
- If the amygdala specifically mediates differences in anxiolytic effects, then any physiological differences of the amygdala revealed in future experiments using

DS and DR mice will be implicated in the anxiolytic differences, and not differences in locomotor activation, ataxia, or seizure protection.

- **Hypothesis 3a: Injections into the ventrolateral caudate will not diffuse into neighboring regions to affect anxiety.**
- **Hypothesis 3b: The ventrolateral caudate does not mediate anxiolysis**
  - **Prediction 3: Injection of CDP into the ventrolateral caudate will not result in an anxiolytic effect as measured with the plus maze.**
- Microinjections should provide adequate spatial resolution to localize the effect to the amygdala. It is expected that the injection of BZs into the amygdala is not resulting in diffusion into nearby structures to produce anxiolysis. This anatomical specificity will be tested by direct injection into a nearby structure, the ventrolateral caudate.
- It is expected that CDP will remain localized to the injection site for at least as long as behavioral testing occurs in these experiments. Thus, it is unlikely that micro-injections into the amygdala are diffusing into nearby structures. Similarly, if injections remain localized, then diffusion into the amygdala from the caudate should not occur.
- There is no evidence that the ventrolateral caudate participates in anxiety or anxiolysis. Therefore, it is expected that the injection of BZs into this locus will not result in anxiolysis.
- **Hypothesis 4: The caudate is capable of mediating muscle relaxation, ataxia, locomotor activation, protection against PTZ induced seizures, and/or locomotor sedation.**

- **Prediction 4: Injection of CDP, a benzodiazepine, into the ventrolateral caudate will produce muscle relaxation, ataxia, locomotor activation, protection against PTZ induced seizures, and/or locomotor sedation.**

- The role of the caudate in possibly mediating a number of other effects which are produced by BZs is unknown. It is unlikely that the amygdala mediates ataxia or muscle relaxation, however there is some evidence for a potential role for the caudate in muscle tone and overall behavioral activity. These effects will be evaluated following direct injection of CDP into the caudate.
- The DS mice are particularly sensitive to ataxia, and locomotor hypoactivity. If the caudate mediates either of these effects of systemic BZs, then micro-injection into this structure will result in DS mice being affected. Because the DS mice are sensitive to these effects and the DR mice are relatively resistant, the performance of the DS mice should be more influenced than the DR mice.

METHODS

1.0 Animals

Diazepam sensitive (DS) mice and diazepam resistant (DR) mice have been selectively bred for performance on a fixed-speed rotarod after injection with diazepam (Gallaher et al. 1987). Selective breeding occurred for 36 generations, followed by random mating within lines. Naive male mice from generations 40 to 43 were used in these studies (ages 80-190 days).

Mice were maintained on a 12/12 light/dark cycle. Food and water were provided ad libitum. Prior to surgery, mice were housed in shoe box sized cages. Following stereotaxic surgery, mice were housed in larger 12"x12"x12" rat cages.

2.0 Chemicals and drugs

Glutaraldehdye was obtained from Electron Microscopy Services (Fort Washington, PA), pentylenetetrazol from Aldrich (Milwaukee, WI), and Durelon carboxylate cement from ESPE (Norristown, PA). Chlordiazepoxide and all other chemicals were from Sigma (St. Louis, MO).

3.0 Surgical procedure

Overview Anesthetized mice were positioned into a Kopf (Model 900; Tujunga, CA) stereotaxic apparatus. The surface of the skull was cleaned of periosteal tissue, and a 1/8th inch screw was secured to a caudal area of the parietal bone. To establish the coordinates for placement of the holes and guide cannula, bregma was used as a landmark. Holes (0.03 inch diameter) were drilled bilaterally through the skull without entering brain tissue, and the guide cannulae were lowered through the

holes to their correct vertical placement. To ensure secure fixation of the guide cannula, dental cement was applied to skull and guide cannula. After the cement had hardened, the mice were removed from the apparatus and recovered in a heated cage.

- Guide cannula placement into the electrode holders of the stereotaxic apparatus: Prior to the placement of a mouse into the stereotaxic apparatus, two guide cannulae were tightly secured to the electrode holders of the stereotaxic apparatus using the electrode clamps. The cannulae were positioned in the clamps so that enough room would be available for cementing.

Also, the stylets were inserted into the guide cannula prior to surgery. These were lowered until the stylet tips were flush with the bottom of the cannula. This permitted no blood or cement to enter into the lumens of the cannulae during surgery. After surgery, the stylets were lowered completely to project 0.5 to 1.0 mm beyond the end of the guide cannulae.

- Proper alignment of the stereotaxic apparatus: Prior to surgery, it was important that the arms of the stereotaxic apparatus were exactly perpendicular to the frame. This was tested by maneuvering the arms laterally and medially. When the arms were perpendicular to the frame, the position of the arms relative to the planes of ear bars was a constant.

- Anesthesia: Mice were anesthetized with anesthetic cocktail as currently approved in the Portland Veterans Administration animal research facility [Xylazine (2.5 ml of 20 mg/ml stock solution), Acepromazine (1 ml of 10 mg/ml stock solution), Ketamine (5 ml of a 100 mg/ml solution), and saline (1.5 ml)]. This mixture was available from the VA ARF. The mixture was diluted in saline (1:3), and was administered (s.c.) in a volume of 10 mL/kg minus 0.1 mL.

After 20 minutes, the mice were tested for adequate anesthesia. Depth of anesthesia was measured by dabbing the eyes with a cotton swab which had been

soaked in 0.9% saline. The hind foot was also gently pinched with a pair of forceps. Anesthesia was indicated by an absence of a blink reflex and by an absence of a foot withdrawal reflex. If more anesthetic was required, a supplement (0.005 to 0.015 mL) was provided. Throughout the stereotaxic procedure, the eyes were kept moist by dabbing them with the 0.9% saline-soaked swab.

- Initial incision: Following adequate anesthesia, a pair of scissors was used to create a small midline incision over the dorsal scalp to allow access to the cranial surface. The length of the incision proceeded a few millimeters anterior to bregma to a few millimeters posterior to lambda.
- Mouse positioning into stereotaxic apparatus: A cardboard platform has been constructed for the mouse to lay on during surgery. This platform maintains a comfortable position for the mouse, and places the body at the correct height for securing the head in the stereotaxic apparatus. A mouse bite bar has been constructed (Dr. Dan Feller, OHSU/VAMC) for attachment to the Kopf stereotaxic instrument. The bite bar was specifically designed for securing a mouse in the apparatus.

To position the head, the incisors were lowered through the bite hole of the bite bar, and the nose clamp was slightly tightened. The ears bars were softly maneuvered into the ears. The insertion of the ear bars was slightly posterior to the ear canal and below a ledge of the skull which then sat on the tips of the ear bars. The ear bars were gradually inserted further into the ear until the head was stable. The nose clamp was then further tightened. By positioning the ear bars posterior to the ear canal, damage to the tympanic membrane was avoided. In addition, the head could be more securely positioned than if the bars were directly inserted into the ear canals.

- Cranial surface cleaning: The cranial surface was wiped clean with 100% ethanol using a cotton swab. The surface was cleaned for three reasons. One, the ethanol sterilized the exposed tissue. Two, the dental cement required exposed skull

bone for secure attachment. Three, a clean skull was necessary for optimal visualization of bregma, lambda, and the sagittal suture. Therefore, all of the periosteal membranes were wiped from the surface.

- Precise positioning of the head: Precise positioning of the head in the stereotaxic holder was crucial for correct cannula placement. Positioning was accomplished by manipulating the placement of the ear bars and by loosening the nose clamp. The head was then gently moved and resecured. The sagittal suture was made exactly parallel to the anterior/posterior plane of the stereotaxic apparatus, and the head was positioned so that lambda and bregma were at the same height. Once precise positioning was established, bregma was marked with an ultra-fine point pen.
- Screw fastened to cranium: Once the mouse was securely positioned in the stereotaxic apparatus, a small 1/8" screw was fastened to the left, posterior skull. For this, a small hole was first created using a 25g needle. A 0.031" drill was then used to create a hole in the skull. This hole was enlarged with a larger bore drill. If bleeding occurred, a cotton swab was held to the area until the hemorrhage stopped. While a screw was held with a pair of forceps, it was drilled half-way into the hole with a small screwdriver. The hole was slightly smaller than the screw allowing firm attachment.
- Marking of cannula placement: Using the dials to maneuver the arms of the stereotaxic apparatus, the right cannula was positioned over bregma. The cannula was lowered until it was touching bregma, and the three coordinates were recorded: Anterior/Posterior, Medial/Lateral, and Dorsal/Ventral. The guide cannula was then moved laterally and posteriorly to a position relative to bregma. For injections into the basolateral amygdala the coordinates used for the guide cannulae were: 1.6 mm posterior, 3.4 mm lateral, and 2.3 mm ventral to bregma. For the ventrolateral caudate, the coordinates used were: 0.5 mm posterior, 3.2 mm lateral, and 1.2 mm ventral to bregma (Slotnik and Leonard 1975). The cannula was lowered until the skull

was touched, and the exact spot was marked with a pen. The cannula was then dialed away from the area.

A small hole was drilled through the skull using a 25g needle, and then enlarged using a 0.031" drill bit. The drilling penetrated the skull without entering the dura mater. If the arachnoid space was entered and bleeding occurred, a cotton swab was held to the area until the hemorrhage stopped.

The opposite side was treated in a similar manner to create a hole for the left cannula. Once holes were drilled bilaterally, both guide cannula were returned to the pre-calculated coordinates for lowering into the holes. The guide cannulae were then lowered through the drilled holes to the calculated depth. For both injection areas, the depth to which the cannula were lowered resulted in some cortical brain tissue penetration. The location of the guide cannula tips, 2.5 mm above the intended injection site, was in cerbral cortex, resulting in expected damage to this area.

- Cementing: Cannulae were secured to the cranial surface using carboxylate dental cement. The cement mixture was applied to the cranium, covering as much skull surface as possible. Some cement was pushed between the skin and the skull surface. This assisted in maintaining the cement on the skull surface, and also helped with wound closure. Once the cannulae were satisfactorily cemented to surface, the cement was allowed to dry for 10 minutes.

- Removal of mouse from the stereotaxic apparatus: Once the cement was hard, the electrode clamps were loosened, the guide cannulae were released from the electrode holders, and the stereotaxic arms were moved away. The mouse was then removed from the stereotaxic apparatus. The stylets were lowered completely through the guide cannulae.

- Guide cannula positions: Implanted guide cannula were situated so that the injection cannnula would project 2.5 mm beyond the tip following insertion. For

injections into the amygdala, the tips of the guide cannula were situated in the dorsolateral 'parietal' cortex. Ultimately this produced small, core-shaped ablations of this region of the cortex. See Figure xx for an example of typical damage.

- Recovery: After surgery, the mouse was placed in a large rat cage. One half of the bedded floor of the rat cage was slightly heated with a heating pad, while the other half was room temperature. Recovering mice could change floor positions to regulate their heat exposure. The mouse began to awaken one hour after surgery, and after several hours, the mouse was fully awake. Mice taken from the same cages before surgery were allowed to recover in the same cages after surgery.
- Additional handling after surgery: The mice continued to be housed in rat cages. They were handled at least three times per week between surgery and behavioral testing for two reasons. One, the stylets in the cannulae lumens were regularly checked so that cannula patency was maintained. Second, repeated handling of the mice in this manner was necessary to habituate them to the stress of handling.

4.0 Construction of guide cannulae, stylets, and the intra-cerebral injection assembly

- Overview: Guide cannulae were secured into the cranium during stereotaxic surgery. They served as guides for the injection cannulae. They were 1.5 cm in length and were constructed with 25 gauge stainless steel tubing (OD 0.020" ID 0.010"). Obturator stylets (used for guide cannula patency) were 1.55 cm in length and constructed with stainless steel wire (OD 0.0095"). These were designed to tightly fit into the lumen of the guide cannula. Injection cannulae extended 1.75 cm in length and were constructed with 32 gauge stainless steel tubing (OD 0.009" ID =0.004"). These were inserted through the guide cannula for infusion of substances.

The injection assembly consisted of an injection cannula firmly attached to a segment of 25 gauge stainless steel tubing. At the proximal end of the injection cannula, a 7 mm length of borosilicate glass tubing (ID slightly greater than 0.02") was fixed to the 25 gauge stainless steel tubing. The glass tubing was from a section of a 20 µL glass micropipet (Fisher).

When the injection cannula was fully inserted through the guide cannula, the glass tubing of the injection assembly fit on the outside of the guide cannula. This securely immobilized the injection cannula during infusions. Also, when the injection cannula was completely inserted through the guide cannula, the distal end projected 2.5 mm beyond the distal end of the guide cannula. The design allowed quick insertion of the injection cannula to the correct depth as well as quick removal of the injection cannulae following infusion. The animal was allowed to freely move about the cage without restraint during injection. PE-20 tubing (ID 0.015" OD 0.043"; Intramedic) connected the injection assembly to a glass injection syringe (10 µL, Hamilton). Injections were controlled using a dual syringe pump (Stoelting model 101).

- Guide cannula construction: Guide cannulae were made from 25g steel tubing (ID 0.01" OD 0.02"; Small Parts Inc.). They were cut to exactly 15 mm in length. One end of the cannula was slightly tapered. This was the end into which the injection cannula was inserted. The tapering allowed the guide cannula to more easily slip into the glass section of the injection cannula assembly. The guide cannula was bored before surgery to ensure that the lumen was free of any obstruction. This allowed both the stylet and the injection cannula to be easily inserted.
- Stylet construction: Stylets were made from 0.0095" wire (Small Parts Inc.) They were cut to approximately 22 mm in length. One end of the wire was tapered to facilitate insertion into the guide cannula. The wire was inserted into a 15 mm guide cannula until 0.5 mm of wire projected from the end of the guide cannula. The other

end of the wire was then bent so that no further progression was permitted. At this stage, the stylet could be fully inserted into a guide cannula until 0.5 mm projected from one end. The stylet was now slightly bent to ensure that the stylet could not easily fall out of the guide cannula. That is, the stylet could fit tightly into the guide cannula but was readily removable by hand.

- Injection cannula assembly

(a) A 2.5 cm to 3 cm length of 25g steel tubing (ID 0.01" OD 0.02"; Small Parts Inc.) was cut. The ends were evenly ground and the lumen was bored so that a wire (OD 0.0095"; Small Parts Inc.) could easily pass through.

(b) A precut 3 cm length of 32g steel tubing (OD 0.009" ID 0.004"; Small Parts Inc.) was carefully bored so that a wire (0.003"; Hamilton) could easily pass through. It was crucial that this tubing have no blockages.

(c) The 32g tubing was inserted into the 25g tubing so that about 18 mm of 35 g tubing protruded from the end. The 32g tubing was glued to the 25g tubing. This was done so that (i) the two pieces were firmly fastened to one another and (ii) no water or air could pass at their junction. Both of the lumens had to remain patent. A small droplet of acrylate glue at the junction was sufficient to form this seal. After gluing, the 32g tubing now projected 1.75 mm from the end of the 25g tubing. This formed the steel portion of the injection assembly (Figure 3).

(d) A 0.5 cm length of glass tubing was made by manually snapping off a small section from a microliter pipet (20 µL, Fisher). This section could be inserted over a small length of 25g steel tubing. The ends of the glass tubing were then ground until they become smooth. The 25g steel tubing easily slid into and through the glass tubing. Tygon tubing (040x070; Tygon) was now slipped over 75% of the glass tubing, and a 1.0 cm length of PE-200 tubing (Intramedic) was slipped over 75% of the

Tygon tubing. All of these junctions were extremely tight and no gluing was necessary.

(e) The steel tubing portion of the injection assembly was now connected to the glass and plastic portion. The 32g steel tubing was inserted through the lumen of the glass and plastic portion until the junction of the 32g and 25g tubing reached the glass tubing. The steel portion could now be glued to the plastic portion. With the two sections held tightly together, some acrylate glue was placed into the space between the 25g steel tubing and the PE-200 tubing. These were held together until the glue had dried.

(f) One end of a 0.5 meter length of PE-20 tubing (Intramedic) now slid over the 25g end of the injection assembly. The other end of the PE-20 tubing was slid over a needle connected to a 10 μL glass (Hamilton) syringe. These connections were water-tight, and could be undone. They did not need gluing.

(g) The completed injection assembly was water-tight. Water easily passed through the PE-20 tubing and out of the 32g end of the injection assembly. When completely inserted through a 15 mm length of 25g steel tubing, the glass portion of the assembly could slip over the end of the 25g tubing, and 2.5mm of 32g tubing projected from the other end.

(h) Maintenance of the injection assembly was required for proper functioning. Insertion of the 0.003" wires through the lumen was used the to clean the cannulae and to unclog any blockages.

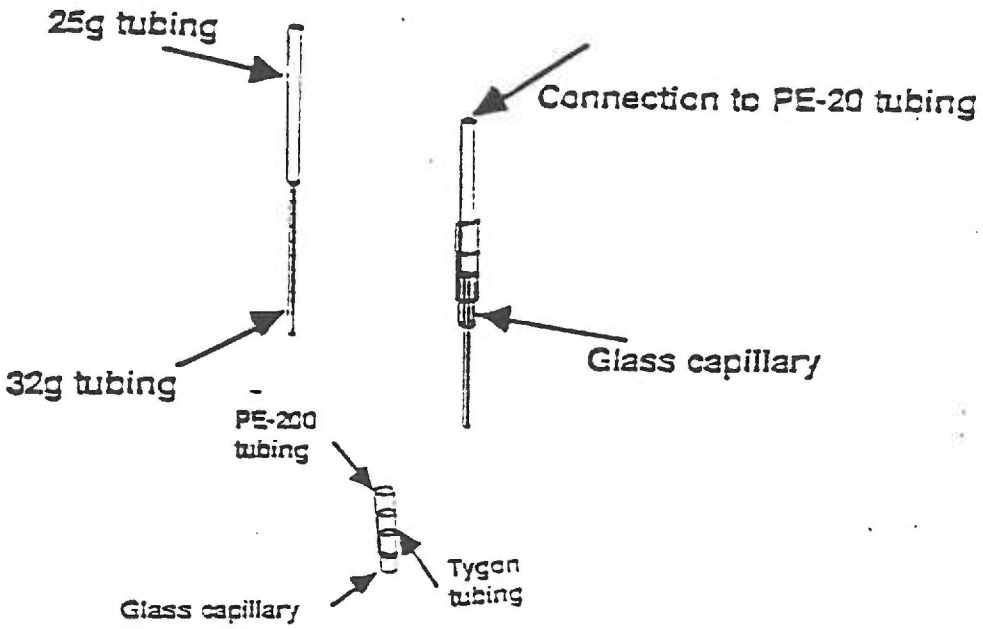

Figure 3. Injection cannula construction The injection cannula is constructed from (a) 25g and 32g steel tubing and (b) glass capillary, PE-200 tubing, and tygon tubing.

5.0 Intra-cerebral injections

- Overview: The mice were firmly held by the dorsal neck scruffs to immobilize the heads, and the stylets were removed. The 32g cannulae from the injection assemblies were fully inserted through the lumens of the guide cannulae until the glass portions of the injection assembly had slipped over the ends of the guide cannulae. When fully inserted, the injection cannulae projected 2.5 mm beyond the tip of the guide cannulae.

The mice were then released into a "shoe-box" size cage. Infusion of 0.5 µL over 80 seconds was begun. The mice were injected with either an artificial CSF vehicle or a 200 mM CDP solution. This dose was selected for three reasons. One, this dose was found to produce a variety of behavioral effects (Audi and Graeff 1984;

Hodges et al. 1987; Kataoka et al. 1982; Maier et al. 1994; Sullivan et al. 1989).. Two, this was the maximal dose achievable in solution using artificial CSF with Tween 80. Three, by maximizing the amount of CDP infused, the probability of eliciting a response could be maximized. Three minutes after the initiation of infusion, the mice were again immobilized, and the injection cannulae were removed. The mice then remained in the "shoe-box" cage until behavioral testing.

- Injection Solutions: CDP for injections was suspended (200 nmol/μL) in artificial CSF with 1 drop of Tween 80 per mL. This concentration of CDP was not completely soluble in the vehicle, and some remained suspended. This concentration could be consistently infused through the narrow lumen of the injection cannulae. No solid pieces larger than the lumen could be injected into the brain. During infusions and behavioral testing, the solution was constantly agitated using a magnetic stir bar to maintain a consistently homogenous solution.

Artificial CSF had the following composition:

NaCl	277 mOsm/L
KCl	6.7 mOsm/L
$CaCl_2$	4.5 mOsm/L
$MgCl_2$	3.0 mOsm/L
NaH_2PO_4	2.9 mOsm/L
Na_2HPO_4	14.6 mOsm/L
Glucose	5.4 mOsm/L
pH	7.4

- Preparation for injections: The injection assembly, the connecting polyethylene tubing (PE-20), and 10 μL injection syringes were filled with the solution to be injected. No air bubbles were permitted. The dual syringe pump (Stoelting model 101) was programmed to infused 0.5 μL (bilaterally) over 80 seconds. Before each injection, the patency of the entire infusion system was checked.

- Injections: The subjects were slightly restrained by tightly holding the scruffs of their necks to minimize head movement. The obdurator stylets were removed from the guide cannulae, the injection cannulae were fully inserted into the lumens of the

guide cannulae, and the attachments were checked for secure insertion. This procedure required approximately 30-45 seconds. The injection cannulae remained secure during free movement of the animals. The mice were placed into shoe-box size cages, and infusion was initiated.

Three minutes after the initiation of infusion, the mice were again immobilized, and the injection cannulae were removed. Removal of the injection cannulae required approximately 15 seconds. The mice then remained in the "shoe-box" cages until behavioral testing. Recommendations for maximizing specificity of injections were followed: (1) Use of fine gauge injection needles to minimize tissue damage, (2) injection beyond the tip of the guide cannula (2.5 mm) to minimize backflow of injectate through the large tract created by the guide cannula, (3) use of a slow injection to allow fluid to be absorbed by the tissue, minimizing tissue damage, and (4) habituation of the animals to the injection procedure, minimizing influences of stress (Jacquet and Lajtha 1973).

6.0 Behavioral testing

- Plus maze Apparatus: The plus maze consisted of four elevated runways arranged in the shape of a cross, and was made of black acrylic plastic. Each runway was raised 20 inches above the base and was 11.5 inches long and 2 inches wide. Two opposing runways were enclosed with clear acrylic plastic walls (6 inches high) while the other two runways remained open. To minimize falling from open runways, these were surrounded with plastic lips (1/32 inches high) to provide a tactile indication of the edge. Sawdust was placed at the base of the plus-maze to prevent injuries from falling.

Performance on the plus maze was monitored using a program written by Edward J. Gallaher for this purpose (Microsoft QuickBasic on a Macintosh Classic computer). As a mouse entered into either open or enclosed arms, the appropriate key

was pressed. The program then summated the total number of entries into either open or closed arms, the total number of arm entries, and the duration spent in either open or closed arms.

- Plus maze Procedure: A subject was released in the center of the plus maze where the four runways meet. For a period of five minutes, the number of entries into either open or enclosed arms as well as the time spent in each was monitored and recorded. An entry into an arm was counted when all four paws of the subject entered that arm. After five minutes, the subject was returned to its home cage, and the plus maze was wiped clean with a bleach solution. If the mouse fell from the plus maze during testing, it was immediately placed back onto the central position. Ataxia can increase the probability of falling, though this rarely occurred in these experiments.

The total number of entries a subject made was used as an index of total locomotor activity. The percent of total entries made into the open arms of the plus-maze was used as an index of anxiety (a baseline is generally about 10-25% open arm entries). A larger percentage of open entries indicated an anxiolytic effect. Animals with zero arm entries (those which remained in the central square for the 5 min duration), were excluded from analyses using percent open entries.

- Muscle relaxation Apparatus: A digital strain gauge (Accuforce Cadet; Ametek) was securely mounted onto 1/2 inch thick plastic. A wire triangular ring (2.5 inches x 2.5 inches x 4 inches) made from 3/32" thick rod was attached to the extension assembly of the strain gauge. The 4 inch length of this triangular ring served as the forelimb grip-bar and was aligned parallel to the plastic surface.
- Muscle relaxation Procedure: The procedure was adapted from Nevins et al. (1993). Each subject was held by the tail about 3/4 of the distance from the tail base and was lowered toward the triangular ring until its forepaws grasped the grip bar. The subjects were then steadily pulled (about 1 inch/second) away from the ring until the

grip was broken. The greatest extending force exerted on the strain gauge was recorded. Three successive trials were given per animal; each trial separated by about 5 seconds. Performance was reported as the average of the three trials. The strain gauge was re-zeroed between each trial.

The subjects were tested for muscle tension before treatment and then retested after treatment. The effect of a treatment was then expressed as a percentage of pre-treatment performance.

- Rotarod Apparatus: The rotarod apparatus consisted of a rotating horizontal cylinder, 5 cm in diameter, covered with 320 wet-dry sandpaper to provide a uniform surface. The cylinder was divided into sections 10 cm wide by means of plastic disks, 6 cm thick and 25 cm in diameter. To avoid spontaneous jumping by the mice, the rotarod was suspended 60 cm above a bed of sawdust. Rotation was powered by a Bodine 115V (1/50 HP) electric motor (model NSH-12). Voltage was controlled by a Bodine DC speed controller with extended range. To this controller, an auxiliary circuit was added to produce a smooth linear acceleration of 20 RPM per minute.
- Rotarod Procedure: Mice were placed on a stationary rotarod. Acceleration began after 3 to 5 seconds. A multi-channel stopwatch program (Microsoft QuickBasic on a Macintosh Classic computer) was used to monitor the time from the beginning of acceleration until the subject fell. As each mouse fell, the appropriate key was pressed on the computer to indicate the duration of performance. This was automatically converted to the RPM at which the individual animal fell.

Mice were tested three times, separated by about 30 seconds between trials. The mean of the second and third trials was used to indicate the individual's performance. The subjects were tested for rotarod performance before treatment and then retested after treatment. The effect of a treatment was then expressed as a percentage of pre-treatment performance.

- Seizure Threshold Procedure: The subject was placed in a restraining tube with its tail extended out one end of the tube. To dilate the tail veins, the tail was heated by submerging it for 1 min in a beaker of water maintained at 44 - 47 degrees C. The restraining tube was then clamped to a ring stand for stability. A 27 gauge x 3/8 inch butterfly (Abbot Laboratories) was quickly inserted into a lateral tail vein. Infusion was initiated using a foot switch which simultaneously turned on a timer (GraLab Model 605) and an infusion pump (Sage Model 355). PTZ (0.5 mg/ml) was infused at a rate of 5.0 ml/min--giving an infusion rate of 0.25 mg/min. Latencies to seizure thresholds were recorded and later converted to mg/kg PTZ administered.

Four seizure endpoints were recorded and identified as follows. (1) Myoclonic jerks were jerks of the head back toward the shoulders of about 1 millimeter. (2) Face and forelimb seizures were clonic seizures wherein the head bent downward and/or rolled left or right, the front limbs were drawn in, and paws curled into fists and moved in slow boxing motions. (3) Running and bouncing seizures were full body clonic seizures that simulated wild involuntary running. (4) Tonic hindlimb extensions were sustained full body tensing with slow extension of the front and rear paws.

7.0 Histology

The placement of the injections was histologically verified. Following behavioral testing, the brains were removed from animals and placed into a glutaraldehyde fixative [NaCl 9 g/L; 0.1M phosphate buffer (pH 7.3) 50mL/L; glutaraldehyde (50%) 50mL/L]. They remain refrigerated in this fixative for at least three days. Perfusion of the subject with fixative prior to removal of the brain was not necessary for the gross histological verifications used in this study. Fixed brains were sliced using a Vibratome (Series 1000, Pelco) to a thickness of 150 μm. Slices were mounted on slides and allowed to dry. The tracts made through the tissue by the

cannulae were inspected and the injection site noted. Grey matter could be differentiated from white matter in these slices without staining. Using these unstained landmarks, the location of the injection site could be verified. The brains were coded so that the verification of injection site was performed blind to the drug treatment and performance of the animal.

For amygdala injections, landmarks used were (1) the dorsal hippocampus, (2) the corpus collosum extending lateral to the amygdala, and (3) the optic tracts radiating just medial to the amygdala. A distinct branching of the white matter tracts distinguished the AL/ABL from the CEA (Figure 4). The intended injection area was the AL or ABL, 1.6 mm posterior to bregma. Misses were noted when the tip of cannula was (1) lateral to the tip of the corpus callosum and into the cortex (2) medial to the white tract and into the CEA or optic tracts, (3) dorsal to the amygdala and into the caudate or globus pallidum, or (4) extremely caudal and into the lateral ventrical or ventral hippocampus.

Injections into the ventrolateral caudate were counted as "missses" if they were (1) lateral or dorsal to the corpus callosum and into the cortex (most common), (2) in the caudate but anterior to the anterior commissure, (3) into the caudate or globus pallidus but more medial than the underlying tip of the corpus callosum (Figure 5).

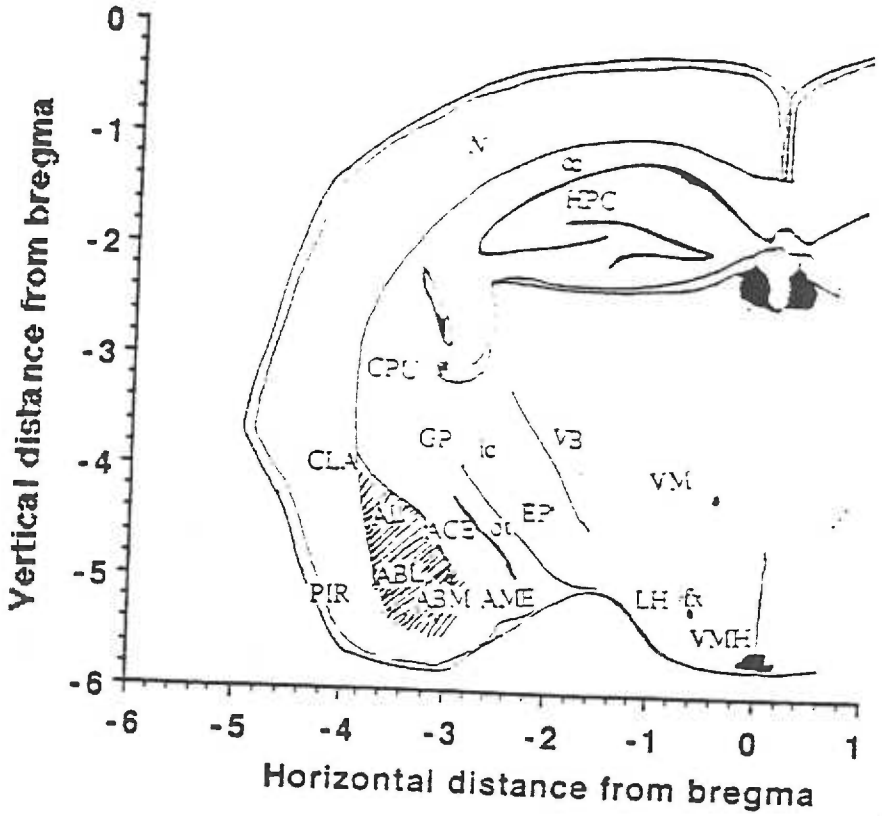

Figure 4. Location of injections in the amygdala. This schematic of a cross-sectional coronal brain slice, 1.6 mm posterior to bregma, indicates the intended injection site for amygdala injections. Injections into the shaded region were considered to be "hits." Injections outside of this region were considered "misses."

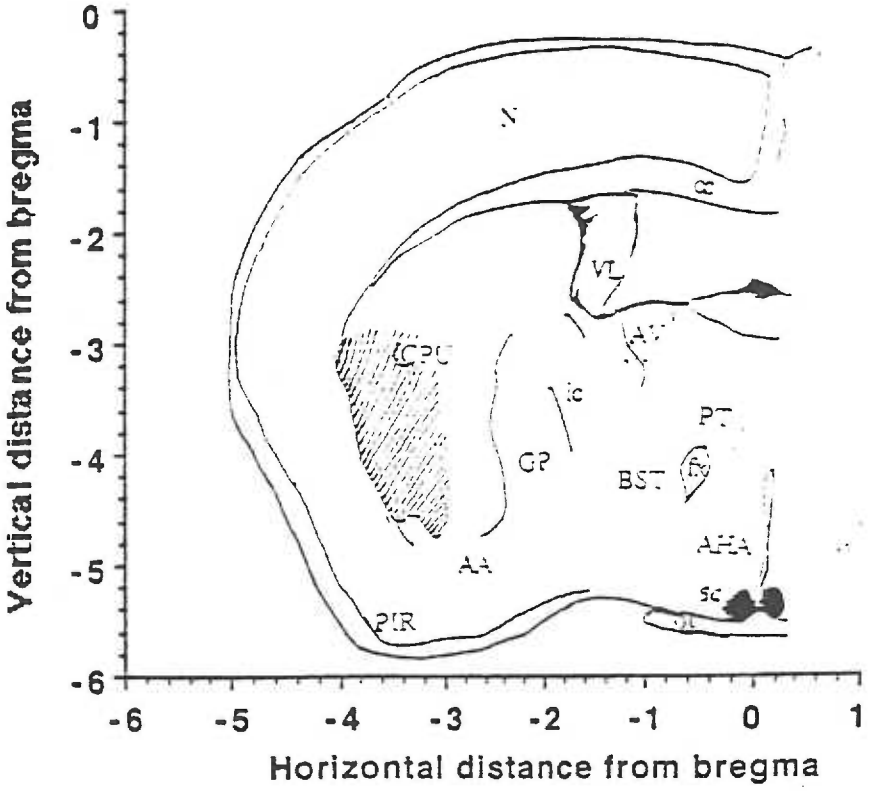

Figure 5. Location of injections in the ventrolateral caudate. This schematic of a coronal brain slice from 0.5 mm posterior to bregma indicates the intended injection site for caudate injections. Injections into the shaded region were considered "hits." Injections outside this region were considered "misses."

8.0 Statistics

The results of each experiment were analyzed using two-way ANOVAs. The comparisons were DS mice vs DR mice, CDP injection vs vehicle injection, and the interaction. Post-hoc comparison were made using Scheffe's S test. Significant differences are defined by p values less than 0.05. Both StatView 4.01 (MacIntosh) and SuperANOVA (MacIntosh) were used.

Exlusion criteria were used. Only subjects with histologically verified bilateral injections were used in these analyses. Subjects with zero arm entries on the plus maze were excluded from analyses of percent open arm entries. Also, during evaluation of seizure protection, the intravenous infusion site was lost during the procedure, resulting in loss of later seizure endpoints in some subjects.

9.0 Experimental Protocols

- <u>Experiment (1) Behavioral battery following amygdala infusion</u>

Seven to fourteen days following stereotaxic surgery, mice were bilaterally infused with either CDP (200 mM) or artificial CSF (0.5 μL) and sequentially tested on a variety of tasks. Only data for bilateral injections into the basolateral or anterolateral amygdala is included in the analyses. Mice were injected and behaviorally tested only once. 46 DS (23 CDP, 23 vehicle) and 46 DR (20 CDP, 26 vehicle) mice from generation 40 were used.

This first experiment was to evaluate whether injection of CDP into DR and DS mice would affect anxiety or a number of other measures. For this, the mice were first tested for baseline muscle relaxation (1-2 minutes), were given a brief rest (about 30 seconds), and then tested for baseline rotarod performance (2-4 minutes). Intra-cranial infusions (3 minutes) then occurred. Five minutes following the infusion, the mice were tested on the plus-maze. Following this, the subjects were tested for muscle

relaxation and rotarod performance. The final test was for protection against PTZ-induced seizures (Figure 6). The mice were sacrificed, and the brains were removed and placed in vials containing glutaraldehyde fixative.

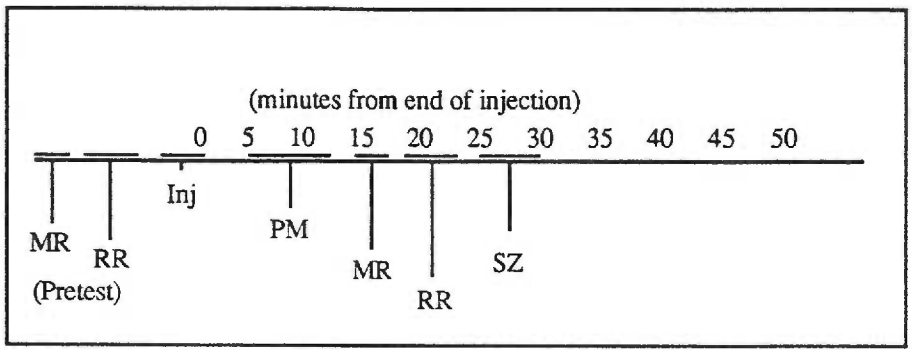

Figure 6: Time line for behavioral testing in Experiment 1. Pretesting of grip strength (MR) and rotarod performance (RR) was followed by injection (Inj). After five minutes, plus maze testing (PM) occurred. Then, MR, RR, and protection against seizures (SZ) were evaluated.

- **Experiment (2) Plus maze testing following amygdala infusion**

In order to minimize freezing on the plus maze following intra-amygdala injections, the mice were tested on the plus maze 24 (± 2) minutes following injection. The results of Experiment 1 demonstrated that total locomotor activity was markedly diminished 5 minutes following the infusion procedure. This interfered with the measurement of percent open arm entries. Initial pilot data (not shown) suggested that locomotor activity on the plus maze could return to baseline following a period of 24 minutes. Therefore, the mice were tested in Experiment 2 at this later time period.

Additionally, the tests for seizure protection in Experiment 1 occurred 24 min following injection. One concern was whether concentrations of behaviorally active CDP remained in the amygdala at 24 to 30 min. The lack of any seizure protection could theoretically be the result of diffusion of CDP from the infusion site over this time course. Thus, Experiment 2 was also designed to test whether behaviorally active CDP remained in the AL/ABL after 24 to 30 min.

Seven to fourteen days following stereotaxic surgery, mice were bilaterally infused with either CDP (200 mM) or artificial CSF and then, after a 24 min period, tested on the plus maze (Figure 7). Following testing, the mice were sacrificed, and the brains were removed and placed in vials containing glutaraldehyde fixative. Only data for bilateral injections into the basolateral or anterolateral amygdala is included in the analyses. 32 DS (16 CDP, 16 Vehicle) and 30 DR (16 CDP, 14 Vehicle) mice from generations 40 and 41 were given CDP or vehicle injections.

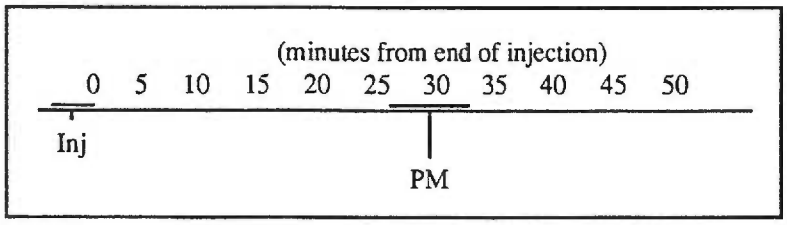

Figure 7: Time line for behavioral testing in Experiment 2. Twenty-four minutes following injections, plus maze testing (PM) occurred.

- **Experiment (3) Seizure threshold following amygdala infusion**

Plus maze testing began 5 min after injection in experiment 1 and lasted for 5 min. However, seizures were not evaluated until about 24 min following injection. One concern was whether behaviorally active CDP remained in the amygdala at this later time. The lack of any seizure protection at 24 minutes could theoretically be the result of diffusion of CDP from the infusion site. Experiment 3 was designed to measure seizure protection 7 min following injection. It is expected that the amount of CDP in the AL/ABL at 7 minutes will be equivalent to the amount present during plus maze testing in Experiment 1.

Seven to fourteen days following stereotaxic surgery, mice were bilaterally infused with either CDP (200 mM) or artificial CSF and then tested for protection against PTZ-induced seizures. Testing for seizure protection was begun approximately 7 minutes after the infusion (Figure 8). The mice were sacrificed, and the brains were removed and placed in vials containing glutaraldehyde fixative. Only data for bilateral injections into the basolateral or anterolateral amygdala is included in the analyses. 50 DS (26 CDP and 24 vehicle) and 44 DR (24 CDP and 20 vehicle) mice from generation 42 were used.

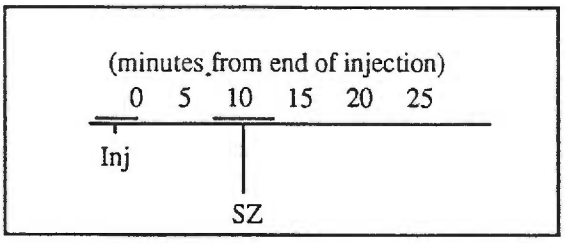

Figure 8: Time line for behavioral testing in Experiment 1. Seven minutes following injection, protection against seizures (SZ) was evaluated.

- **Experiment (4) Plus maze performance following caudate infusion**

Behavioral evaluation on the plus maze occurred 24 minutes following injection in experiment 2. In order to evaluate the anatomic localization of BZ micro-injections into this structure, injections into the nearby ventrolateral caudate (about 1.6 mm distant) were performed. In this experiment, plus maze testing also occurred at 24 minutes following injection in order to directly compare the results with those of experiment 2.

Seven to fourteen days following stereotaxic surgery, mice were bilaterally infused with either CDP (200 mM) or artificial CSF and then tested on the plus maze. As in Experiment 2, plus maze testing occurred 24 minutes (±2 minutes) after the infusion (Figure 7). The mice were sacrificed, and the brains were removed and placed in vials containing glutaraldehyde fixative. Only data for bilateral injections into the ventrolateral portion of the caudate (caudal to the anterior commissure) was included in the analyses. 39 DS (19 CDP and 20 vehicle) and 47 DR (24 CDP and 23 vehicle) mice from generation 42 were used.

- **Experiment (5) Behavioral battery following caudate infusion**

The caudate may possibly be involved in several behavioral effects of BZs. Seven to fourteen days following stereotaxic surgery, mice were bilaterally infused with either CDP (200 mM) or artificial CSF and sequentially tested on a variety of tasks. The first post-infusion behavioral test was the plus maze. Testing on this apparatus occurred 24 minutes following infusion, as freezing no longer occurred during this period.

The mice were first tested for baseline muscle relaxation (1-2 minutes), were given a brief rest (about 30 seconds), and then tested for baseline rotarod performance

(2-4 minutes). Intra-cranial infusions (3 minutes) then occurred. Twenty-four minutes following the infusion, the mice were tested on the plus-maze. Following this, the subjects were tested for muscle relaxation and rotarod performance. The final test was for protection against PTZ-induced seizures (Figure 9). The mice were sacrificed, and the brains were removed and placed in vials containing glutaraldehyde fixative. Only data for bilateral injections into the ventrolateral caudate is included in the analyses. 23 DS (11 CDP and 12 vehicle) and 22 DR (12 CDP and 10 vehicle) mice from generations 42 and 43 were used.

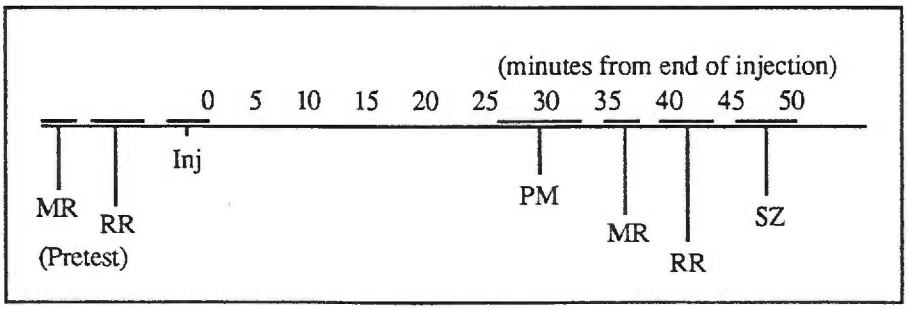

Figure 9: Time line for behavioral testing in Experiment 5. Pretesting of grip strength (MR) and rotarod performance (RR) was followed by injection (Inj). After 24 minutes, plus maze testing (PM) occurred. Then, MR, RR, and protection against seizures (SZ) were evaluated.

Results

1.0 Experiment 1: Behavioral testing 5 minutes after infusion

The percentage of animals in this initial experiment with bilateral injections into the amygdala was 39%. There are a number of reasons why the cannulae may have missed the injection site. One, there was variation in the size and shape of the mouse craniums. Thus the location of the amygdala relative to bregma may exhibit phenotypic variation. The presence of phenotypic variation has previously been described, with genetic differences in the location of various structures relative to bregma (Wahlsten 1975). Also, there may have been movement and tilting of the guide cannulae during the week before behavioral testing. The mice were observed to lie on the cannulae, as well as rub the cannulae against the sides of the cages. This may have shifted the placement of the guide cannulae. Only the behavior of animals with bilateral injections into the amygdala was analyzed.

The typical amygdala injection site was into the anterolateral nucleus of the amygdala, with "misses" most commonly into the neighboring cortex or dorsally into the lateral caudate. Figure 10 is a plot of injection sites demonstrating the common areas which were included as "hits" and those areas counted as "misses." This schematic summarizes a representative sample of the injection cannula tip locations, though, for clarity, not all are included.

The typical extent of damage by both the guide cannula as well as the injection cannula included a core of parenchymal ablation by the guide cannula and a hemmorhagic tract created by the injection cannula (Figure 11). The ventral extent travelled by the flat-tipped end of the injection cannula was noted in consecutive coronal slices and recorded.

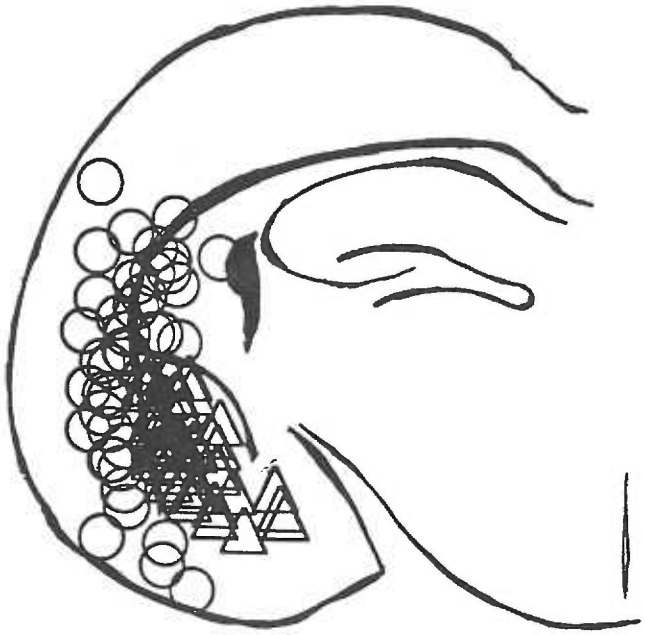

Figure 10. Location of injection sites. Triangles indicate injection sites counted as "hits," and circles indicate those counted as "misses." Note that the majority of misses were lateral or dorsal to the anterolateral amygdala. The majority of hits were in the anterolateral nucleus, though some injections extended into the basolateral nucleus.

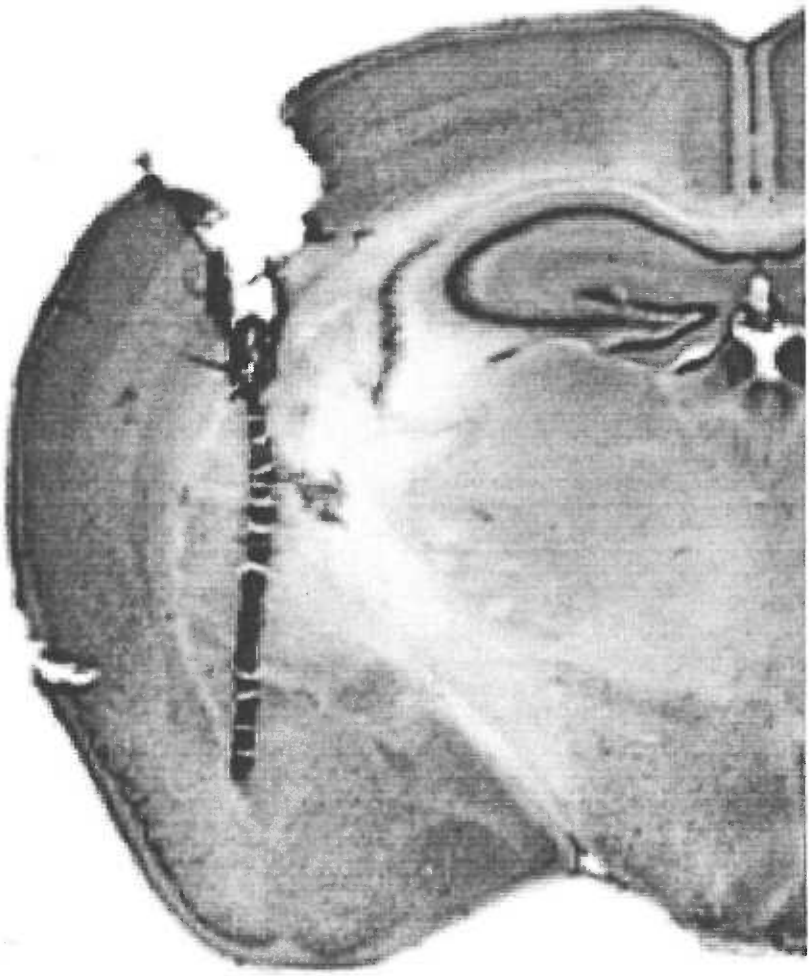

Figure 11: Histology of cannula placement in the amygdala. A typical slice with an injection tract was stained and scanned into the computer to demonstrate the location of injections and anatomy. Note that the tip of the injection cannula extends to the lateral portion of the anterolateral amygdala (compare with Figure 4). The injection site was recorded as the most ventral extent of the tract observed in consecutive slices. This was the most common site of injection in these experiments. The parenchymal damage seen in this picture is typical of that produced in these studies. Because of the angle of slicing, the contralateral injection tract was rarely seen in the same 150 μm slice.

On the plus maze, the total (closed plus open) number of entries was low for both DS and DR mice when tested five minutes after infusion (Figure 12). This may be due to freezing behavior resulting from handling and injection trauma during intra-cranial infusions. A two-way ANOVA indicated that (i) the DR mice had more total entries than DS mice [$F(1,32) = 17.66$; $P = 0.0002$] (Figure 12); (ii) there was also a significant effect of the CDP injection [$F(1,32) = 10.49$; $P = 0.0028$], and (iii) there was a significant treatment interaction [$F(1,32) = 6.31$; $P = 0.017$]. This significant interaction indicates that CDP treatment had a different effect in DR mice than in DS mice. Post-hoc tests revealed that total entries following CDP injection in DR mice were significantly increased relative to vehicle injections, although there was no significant effect of CDP treatment in DS mice. Baseline performance after vehicle injection in DS and DR mice did not differ. Taken together, these results indicate that CDP treatment increased total entries in DR mice, but not in DS mice.

CDP injection did not result in significant effects on percent open entries [$F(1,29) = 0.015$; ns] (Table 5). There were also no differences on this measure between DS and DR mice [$F(1,29) = 1.64$; ns], nor was there any significant interaction [$F(1,29) = 1.06$; ns]. Note that because three subjects had zero open arm entries, they were excluded from this analysis.

Injection of CDP was expected to result in an increase in the percent of open entries. There was a statistically insignificant trend for CDP to increase the percent of open entries of DR mice ($p = 0.47$ using Scheffe's S test).. However, a large degree of variation in performance was observed, which may have resulted in a decreased power to detect any effects of CDP injections. Much of this variation was likely the result of diminished activity in the mice.

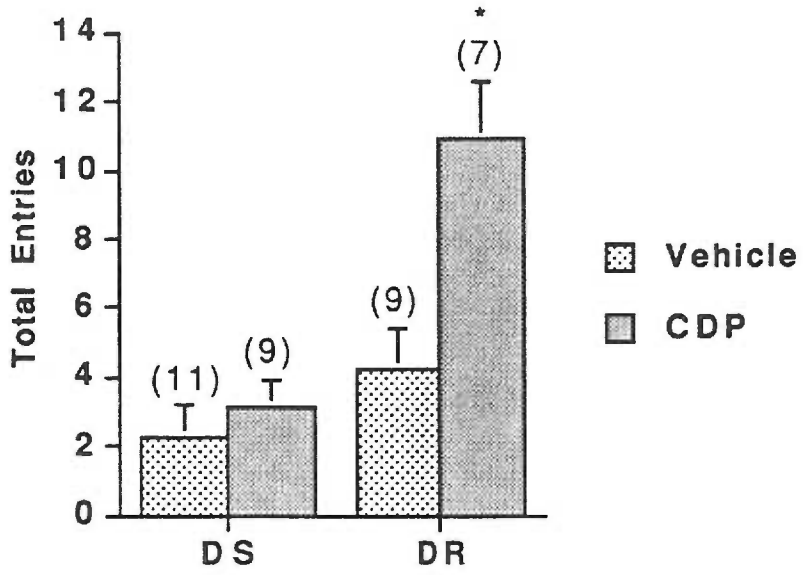

Figure 12. Total (closed and open) entries on the plus maze 5 minutes after injection into the amygdala. In Experiment 1, vehicle or CDP was injected into the amgydala of DS and DR mice. Five minutes following injection, they were tested on the plus maze. Results are mean ± SEM. The number of mice tested is in parentheses. There was a significant effect of line ($p < 0.001$) and treatment ($p < 0.005$), as well as a significant interaction ($p < 0.05$). Post-hoc comparisons demonstrated that CDP injections increased total entries in DR mice compared to the other three treatment groups, as indicated by an asterisk.

Anxiety (percentage open arm entries on the plus maze)			
DS_{Veh}	22.2 ± 12.9 (8)	DS_{CDP}	25.0 ± 13.8 (9)
DR_{Veh}	12.0 ± 8.2 (9)	DR_{CDP}	37.9 ± 8.4 (7)

Table 5. Performance of DS and DR mice on the plus maze. Testing began 5 minutes after injection. In Experiment 1, vehicle or CDP was bilaterally injected into the amygdala of DS and DR mice. Results are mean ± SEM. The number of mice tested is in parentheses.

There was no significant effect of drug treatment on forelimb grip strength (Table 6) in DR and DS mice [$F(1,32) = 0.02$; ns]. There was also no difference between the lines [$F(1,32) = 0.78$; ns], nor was there any significant interaction [$F(1,32) = 0.19$; ns].

Additionally, there was no significant effect of drug treatment on performance on the rotarod (Table 6) in DS and DR mice [$F(1,32) = 0.0008$; ns], nor was there a significant interaction [$F(1,32) = 0.26$; ns]. However, the DR mice performed better on the rotarod than DS mice, regardless of the injection [$F(1,32) = 9.503$; $p = 0.004$].

There were no significant differences between the lines in sensitivity to any of the four PTZ-induced seizures (Table 7): myoclonus [$F(1,21) = 0.19$; ns], face/forelimb clonus [$F(1,18) = 0.32$; ns], tonic/clonic running and bouncing [$F(1,15) = 0.07$; ns], and tonic hindlimb extension [$F(1,14) = 0.27$; ns]. There was also no effect of CDP infusion in the mice: myoclonus [$F(1,21) = 0.54$; ns], face/forelimb clonus [$F(1,18) = 0.20$; ns], tonic/clonic running and bouncing [$F(1,15) = 0.05$; ns], and tonic hindlimb extension [$F(1,14) = 0.54$; ns]. There also were no significant interactions: myoclonus [$F(1,21) = 0.91$; ns], face/forelimb clonus [$F(1,18) = 0.12$; ns], tonic/clonic running and bouncing [$F(1,15) = 0.29$; ns], and tonic hindlimb extension [$F(1,14) = 0.33$; ns]. Note that their was attrition of subjects during seizure testing. This was the result of loss of intravenous access during the seizure latency protocol. As this access was lost, later seizure endpoints could not be evaluated.

This initial experiment indicated that the infusion of CDP reversed the handling-induced freezing in the DR mice, as seen by an increase in total plus maze entries. This was not seen in the DS mice, indicating a genetic difference between the lines in sensitivity to this effect. The more typical anxiolytic measure, an increase in open arm entries, was not produced in either line, although there was a trend for this in the DR

mice. Neither line exhibited muscle relaxation, ataxia, or protection from four different PTZ-induced seizures.

The limited activity exhibited by the mice only five minutes following injection decreased the power to detect changes in open arm activity, anxiolysis. Thus, experiment two was designed, measuring of plus maze activity 24 minutes following infusion, when baseline activity levels had returned to normal.

Muscle Relaxation (percentage on forelimb grip strength)

DS_{Veh}	94.2 ± 2.3 (11)	DS_{CDP}	93.7 ± 3.5 (9)
DR_{Veh}	92.5 ± 2.0 (9)	DR_{CDP}	94.6 ± 4.0 (7)

Ataxia (percentage of baseline performance on accelerating rotarod)δ

DS_{Veh}	99.2 ± 7.8 (11)	DS_{CDP}	104.9 ± 15.6 (9)
DR_{Veh}	140.4 ± 8.9 (9)	DR_{CDP}	134.5 ± 13.0 (7)

Table 6: Muscle relaxation and ataxia. In Experiment 1, vehicle or CDP was bilaterally injected into the amygdala of DS and DR mice. Results are mean ± SEM. The number of mice tested is in parentheses. A significant difference between DS performance and DR performance is indicated by a δ. Regardless of injection, the DR mice performed better on the rotarod during their second set of trials.

Myoclonus (PTZ mg/kg)			
DS_{Veh}	47.3 ± 7.7 (9)	DS_{CDP}	48.7 ± 3.9 (6)
DR_{Veh}	50.6 ± 4.0 (6)	DR_{CDP}	40.8± 1.6 (5)
Face and Forelimb (PTZ mg/kg)			
DS_{Veh}	53.3 ± 9.2 (8)	DS_{CDP}	59.4 ± 4.5 (5)
DR_{Veh}	54.6 ± 3.8 (5)	DR_{CDP}	55.7 ± 1.7 (5)
Running/Bouncing (PTZ mg/kg)			
DS_{Veh}	58.4 ± 13.1 (6)	DS_{CDP}	65.7 ± 4.9 (5)
DR_{Veh}	61.1 ± 4.9 (5)	DR_{CDP}	58.0 ± 3.8 (3)
Tonic Hindlimb Extension (PTZ mg/kg)			
DS_{Veh}	63.9 ± 13.8 (6)	DS_{CDP}	68.3 ± 5.2 (5)
DR_{Veh}	81.5 ± 10.7 (4)	DR_{CDP}	62.7 ± 4.6 (3)

Table 7. Results of injections into the amygdala on protection against PTZ-induced seizures. In Experiment 1, vehicle or CDP was bilaterally injected into the amygdala of DS and DR mice. Results are mean ± SEM. The number of mice tested is in parentheses. Testing began an averag of 24 minutes following injection.

2.0 Experiment 2: Behavioral testing at 24 minutes after infusion

This experiment evaluated performance on the plus maze 24 minutes after injection for two reasons. First, when mice were tested on the plus maze 5 minutes following injection, they were observed to exhibit freezing. The low level of locomotor activity resulted in a large degree of variability in the percent of open arms which were entered. This variability in performance may have interfered with the ability to detect anxiolysis, as defined by an increase in the percent of open arm entries. It was expected that this freezing behavior might be attenuated if the mice were allowed to

recover in their home cage from the handling stress before behavioral testing. Also, the subjects in Experiment 1 were tested for seizure protection about 24 minutes after injection, and no protective effects were found. Therefore Experiment 2 was designed to evaluate whether behaviorally active CDP remained in the amygdala after this time period.

The percentage of animals with bilateral injections into the amygdala was 74%, resulting from improving surgical technique. Again, only animals with bilateral amygdala injections were analyzed. When tested on the plus maze 24 minutes after infusion, freezing behavior was no longer apparent. This is reflected in a higher number of total entries on the plus maze than in experiment one (Figure 13). DR mice had more total arm entries on the plus maze than did DS mice [$F(1,42) = 12.06$; $p = 0.001$], however there was no effect of drug treatment [$F(1,42) = 0.34$; ns]. Also, there was no significant interaction effect [$F(1,42) = 0.62$; ns]. These analyses indicate that although baseline locomotor activity differs between DS and DR mice, injection of CDP does not affect either line on this measure.

CDP treatment produced a significant increase in the percentage of open arm entries compared to vehicle treatment [$F(1,42) = 6.67$; $p = 0.013$]. There was also a significant difference between the lines [$F(1,42) = 15.53$; $p = 0.0003$] on this measure of anxiolysis, although there was no difference between baseline performance in these two lines. Additionally, the interaction was significant [$F(1,42) = 8.97$; $p = 0.005$]. Inspection of the data indicates that CDP treatment had an anxiolytic effect in DR mice and not in DS mice. Post-hoc analyses indicate that CDP did significantly increase the percent of open entries in DR mice ($p = 0.003$), though CDP did not have this effect in DS mice ($p = 0.94$).

These results indicate that the amygdala is capable of mediating the anxiolytic effect of CDP in this paradigm. Furthermore, there is a genetic difference in sensitivity

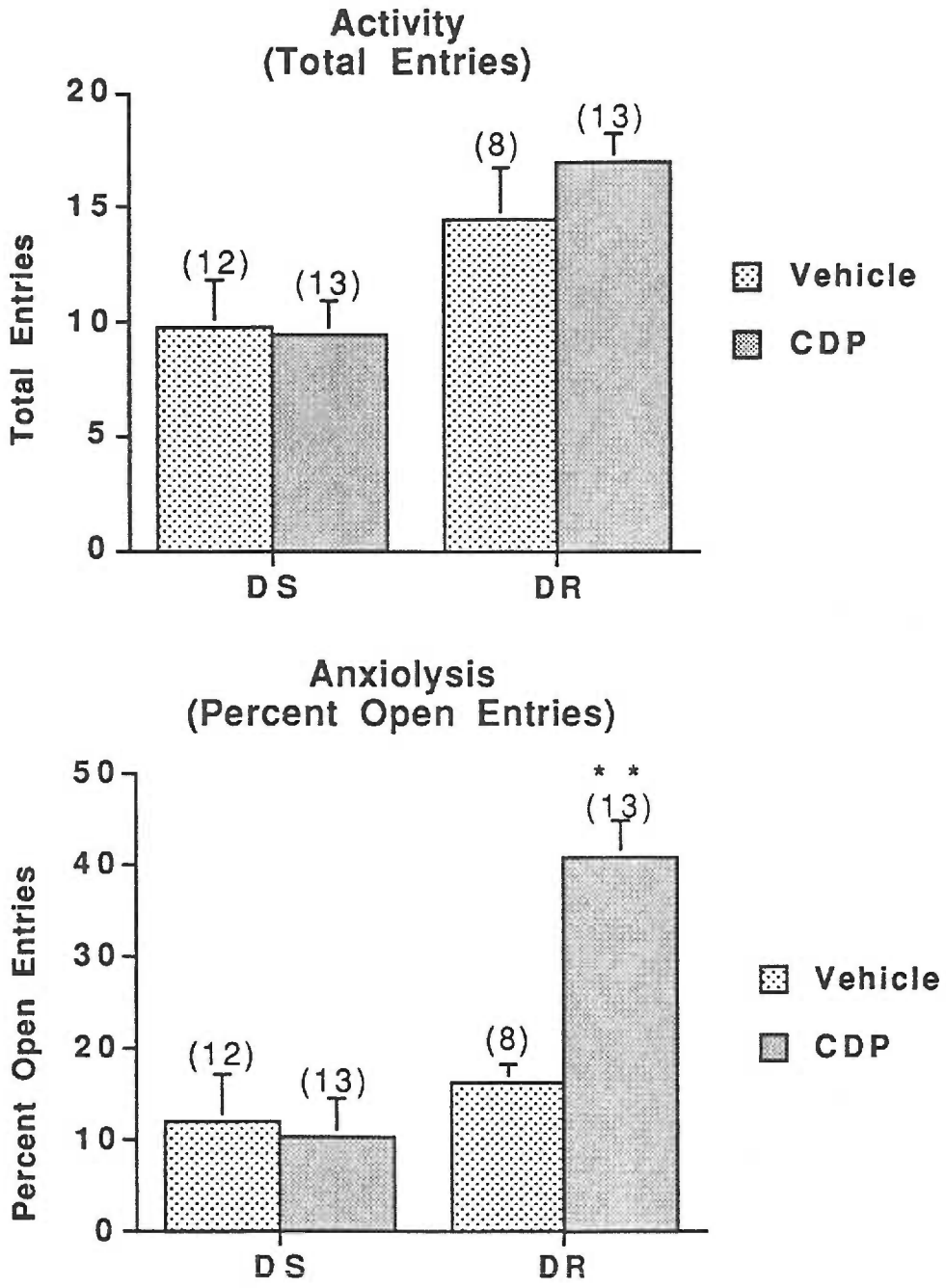

Figure 13: Plus maze performance of DS and DR mice 24 minutes after CDP or vehicle injections into the amygdala. Experiment 2. The number of mice tested is in parentheses. A significant difference between vehicle and CDP injections is indicated by a ** ($p < 0.001$).

to this effect, as no anxiolysis was seen following infusion into DS mice. Additionally, this experiment indicates that behavioral effects can be measured 24 minutes following localized infusion.

3.0 Experiment 3: Seizure protection 7 minutes following infusion

In Experiment 1, CDP had a behavioral effect in DR mice--increasing total arm entries in DR mice 5 minutes following infusion. However, CDP injection did not protect against seizures, tested at 24 minutes after infusion. It is possible that the local concentration of CDP may have declined after 24 minutes, leading to a false negative conclusion regarding seizure protection. Therefore, Experiment 3 was designed to evaluate seizure protective effects 7 minutes following injection.

The percentage of animals with bilateral injections into the amygdala was 61%, similar to Experiment 2. Four types of seizures produced by PTZ were analyzed (Figure 14). When tested for seizure susceptibility seven minutes after infusion, no differences between DS and DR mice were found; myoclonus [$F(1,48) = 2.28$; ns], face/forelimb clonus [$F(1,42) = 1.24$; ns], tonic/clonic running and bouncing [$F(1,39) = 0.99$; ns], and tonic hindlimb extension [$F(1,39) = 0.96$; ns]. Similar to Experiment 1, there was attrition of some subjects during testing as a result of loss of venous access. Therefore, later seizure endpoints were not evaluated in these subjects.

Similar to Experiment 1, there was also no effect of CDP infusion; myoclonus [$F(1,48) = 0.24$; ns], face/forelimb clonus [$F(1,42) = 0.02$; ns], tonic/clonic running and bouncing [$F(1,39) = 0.04$; ns], and tonic hindlimb extension [$F(1,39) = 0.03$; ns]. There were also no significant interactions between drug treatment and line: myoclonus [$F(1,48) = 1.63$; ns], face/forelimb clonus [$F(1,42) = 2.14$; ns], tonic/clonic running and bouncing [$F(1,39) = 1.46$; ns], and tonic hindlimb extension [$F(1,39) = 1.78$; ns].

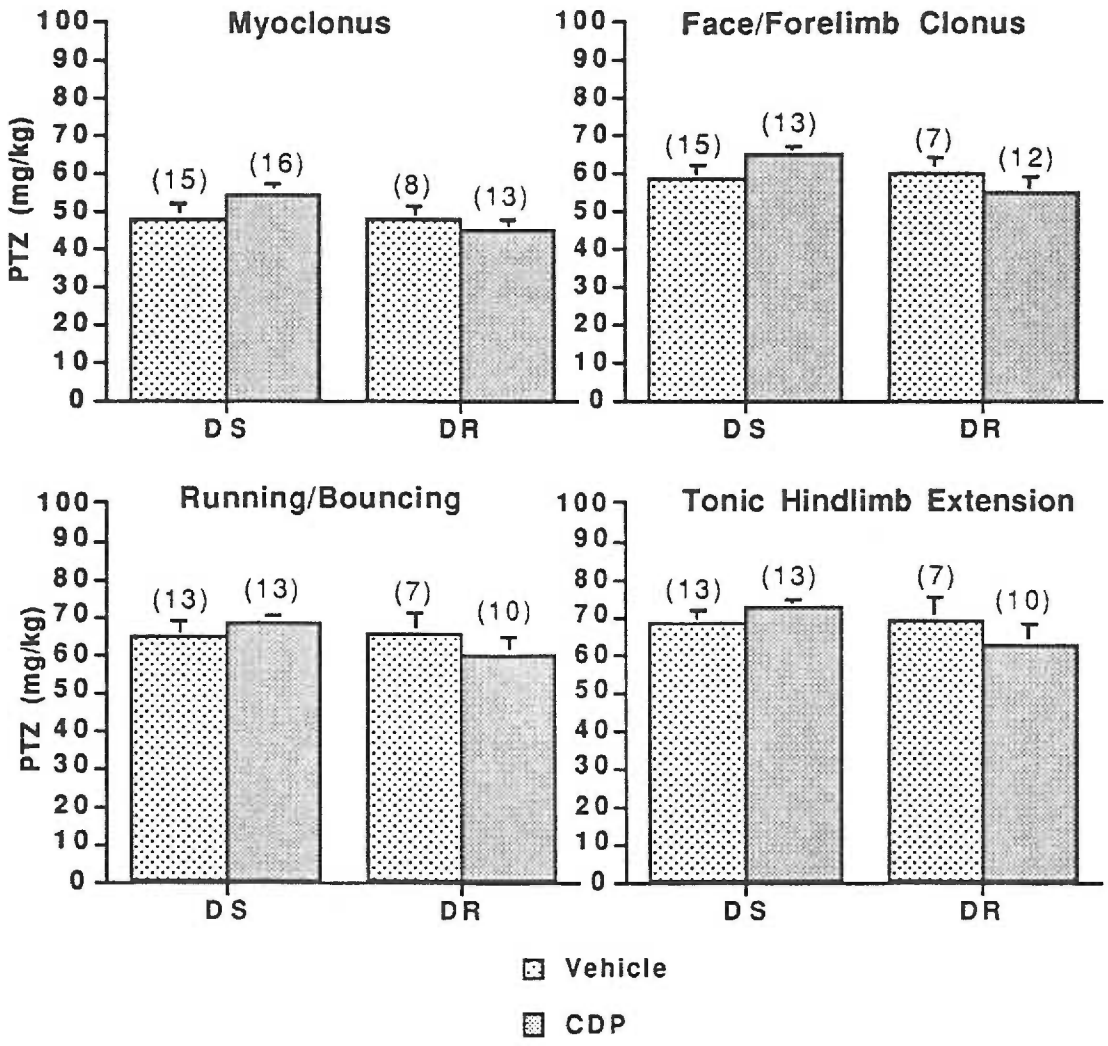

Figure 14: PTZ-induced seizures in DS and DR mice 7 minutes after CDP or vehicle injections into the amygdala. In Experiment 3, vehicle or CDP was injected into the amygdala of DS and DR mice. The number of mice tested is in parentheses. PTZ was infused until each seizure end-point was reached. The amount of PTZ required to induce each type of seizure is reported. There were no significant differences between DS and DR mice, and there was no effect of CDP infusion.

Consistent with the result of Experiment 1, the amygdala does not mediate the PTZ-induced seizure protective effects of CDP. Thus, the results of Experiment 1 cannot simply be explained by diffusion of CDP from the amygdala. This conclusion is also consistent with Experiment 2, in which behavioral effects of CDP infusion could be measured 24 minutes following infusion.

4.0 Experiment 4: Control for diffusion to adjacent brain areas

In Experiment 2, anxiolytic effects were produced in the DR mice by CDP injection into the amygdala. Testing for anxiolysis occurred 24 minutes following injection, and lasted for 5 minutes. It is possible that CDP may have diffused to a nearby structure, and this may have mediated the anxiolytic effect. Therefore, Experiment 4 was designed to evaluate anxiolysis after injection into the nearby ventrolateral caudate. As in Experiment 2, the mice were tested on the plus maze 24 minutes after infusion.

The percentage of animals with bilateral injections into the ventrolateral caudate was 54%. This is a lower percentage than in the prior two experiments. The majority of injections which missed this site were lateral to the caudate, and it is possible that the geometric relationship between this locus and bregma is different in the albino CF-1 mice used to construct the atlas (Slotnik and Leonard 1975). This indicates that in murine micro-injection studies, it is important to be cognizant of genetic differences in gross anatomy. As in the prior experiments, only behavior following bilateral injections was analyzed.

As in Experiments 1 and 2, DR mice had more total arm entries than did the DS mice [$F(1,43) = 4.59$; $p = 0.04$]. This was regardless of CDP or vehicle treatment. In each of these three experiments, the mice have increased baseline activity. No effect of

drug treatment was observed on this measure of activity (Figure 15) in either line [$F(1,43) = 0.26$; ns]. There was also no significant interaction [$F(1,43) = 0.41$; ns]. Thus, this experiment replicates the findings of Experiment 2 that there are differences in baseline exploratory activity. However, this area of the caudate does not mediate any effects of CDP on locomotor activity.

There was no effect of drug treatment on the percentage of open arm entries [$F(1,43) = 1.04$; ns], there was no difference between DR and DS mice in the percentage of open arm entries [$F(1,43) = 0.37$; ns], and there was no significant interaction [$F(1,43) = 0.90$; ns]. These analyses indicate that CDP did not affect plus maze performance in either line of mice when injected into the ventrolateral caudate. Additionally, they replicate the finding of Experiment 2 that baseline anxiety is similar in the DS and DR mice.

These caduate injections were dorsal and slightly anterior to the amygdala injections. In fact, the cannula tract to the amygdala traverses the ventrocaudal caudate. Most non-spherical diffusion occurs through backflow along the tract produced by the injection cannula (Jacquet and Lajtha 1973). By demonstrating that infusions into a nearby structure, the caudate, did not result in similar behavioral results, this experiment provides further evidence that Experiments 1, 2, and 3 were the result of CDP localized to the amygdala injection site.

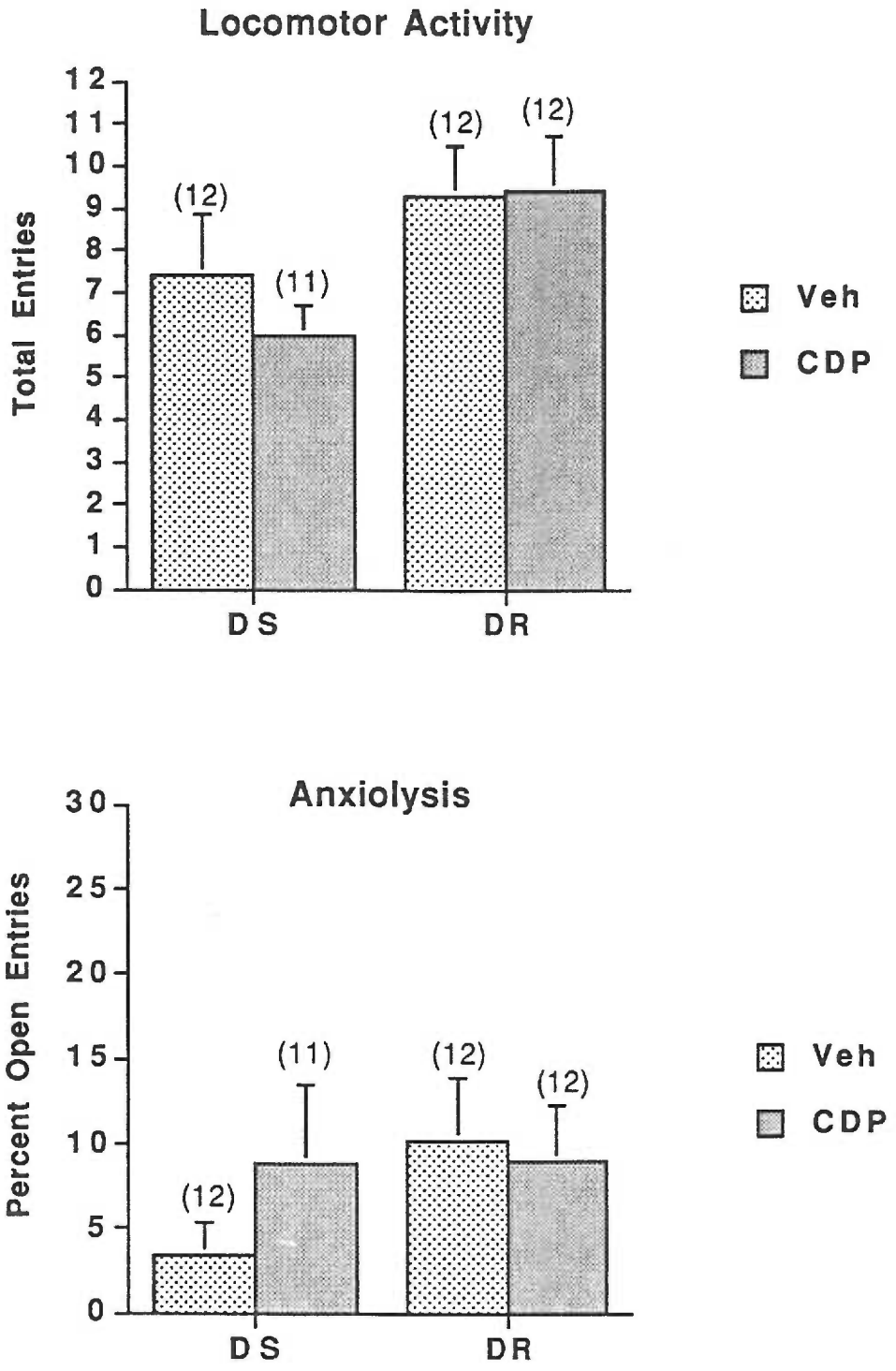

Figure 15. Plus maze performance of DS and DR mice 24 minutes after injections into the ventrolateral caudate. Experiment 4. No significant differences between CDP and vehicle injections were observed. Number of mice is in parentheses.

5.0 Experiment 5: Behavioral testing after caudate infusions

Although, the caudate does not mediate the anxiolytic effect of CDP, it is possible that it may mediate other effects. Mice were tested on a series of behavioral tasks beginning 24 minutes following injection. Because many of the misses in Experiment 4 were lateral to the caudate, guide cannula were placed 0.1 mm more medial than in Experiment 4. Care was also taken to minimize lateral tilting of the cannulae. The percentage of animals with bilateral injections into the ventrolateral caudate was 91% in this experiment. This increase in the number bilateral hits results from awareness of the difference in anatomy between the mice used in this study and the mice used for construction of the atlas. In addition, improving surgical technique, and the larger target area also were important factors.

There was no significant effect of drug treatment on locomotor activity (Table 8) in both lines of mice [$F(1,37) = 0.02$; ns]. Also, there was no effect of drug on the percentage of open arm entries [$F(1,37) = 0.65$; ns]. These findings replicate those of Experiment 4. Consistent with Experiments 2 and 4, DR mice had more total arm entries than did DS mice [$F(1,37) = 7.80$; $p = 0.008$], but they did not differ in their percentage of open arm entries [$F(1,37) = 2.07$; ns]. There was no significant interaction for either of these measures: total entries [$F(1,37) = 0.07$; ns] or percent open entries [$F(1,37) = 0.09$; ns].

There was no significant effect of CDP injection on the measure of forelimb grip strength [$F(1,37) = 1.03$; ns], and there was no significant effect of drug treatment on performance on the rotarod [$F(1,37) = 0.0003$; ns] (Table 9). As in Experiment 1, DR performance on the rotarod was significantly better than the performance of DS mice [$F(1,37) = 21.68$; $p < 0.0001$], though there were no difference between the lines in grip strength [$F(1,37) = 0.93$; ns]. There were also no significant interactions for muscle relaxation [$F(1,37) = 0.38$; ns] or rotarod performance [$F(1,37) = 0.42$; ns].

Locomotor activity (total entries on the plus maze) **			
DS_{Veh}	6.5 ± 1.0 (12)	DS_{CDP}	5.8 ± 1.2 (10)
DR_{Veh}	10.8 ± 2.3 (9)	DR_{CDP}	11.0 ± 2.2 (10)
Anxiety (percentage open arm entries on the plus maze)			
DS_{Veh}	15.5 ± 8.6 (12)	DS_{CDP}	7.4 ± 4.5 (10)
DR_{Veh}	24.0 ± 7.3 (9)	DR_{CDP}	20.2 ± 7.6 (10)

Table 8. Plus maze performance 24 minutes after injections into the ventrolateral caudate. In Experiment 5, vehicle or CDP was bilaterally injected into the ventrolateral caudate of DS and DR mice. Results are mean ± SEM. The number of mice tested is in parentheses. A significant difference ($p < 0.01$) between DS performance and DR performance is indicated by a **.

Muscle Relaxation (percentage on forelimb grip strength)			
DS_{Veh}	100.2 ± 4.1 (12)	DS_{CDP}	106.1 ± 3.7 (10)
DR_{Veh}	99.0 ± 2.0 (9)	DR_{CDP}	100.4 ± 3.6 (10)
Ataxia (percentage on accelerating rotarod performance) ***			
DS_{Veh}	100.4 ± 7.9 (12)	DS_{CDP}	107.0 ± 7.7 (10)
DR_{Veh}	155.8 ± 10.2 (9)	DR_{CDP}	148.8 ± 14.8 (10)

Table 9. Results of injections into the ventrolateral caudate on muscle relaxation and ataxia. In Experiment 5, vehicle or CDP was bilaterally injected into the ventrolateral caudate of DS and DR mice. Results are mean ± SEM. The number of mice tested is in parentheses. A significant difference ($p < 0.001$) between DS performance and DR performance is indicated by a ***.

There were no differences between the lines in sensitivity to any of the four PTZ-induced seizures (Table 10): myoclonus [$F(1,29) = 0.48$; ns], face/forelimb clonus [$F(1,29) = 0.002$; ns], tonic/clonic running and bouncing [$F(1,28) = 1.00$; ns], and tonic hindlimb extension [$F(1,24) = 0.11$; ns]. There was also no effect of drug

infusion in either line. myoclonus [F(1,29) = 0.03; ns], face/forelimb clonus [F(1,29) = 0.42; ns], tonic/clonic running and bouncing [F(1,28) = 1.76; ns], and tonic hindlimb extension [F(1,24) = 0.16; ns]. No significant interactions were found: myoclonus [F(1,29) = 0.03; ns], face/forelimb clonus [F(1,29) = 1.35; ns], tonic/clonic running and bouncing [F(1,28) = 0.02; ns], and tonic hindlimb extension [F(1,24) = 2.39; ns].

Myoclonus (PTZ mg/kg)			
DS_{Veh}	43.3 ± 1.3 (9)	DS_{CDP}	42.7 ± 1.9 (9)
DR_{Veh}	42.0 ± 1.4 (7)	DR_{CDP}	41.9 ± 1.3 (8)
Face and Forelimb (PTZ mg/kg)			
DS_{Veh}	49.7 ± 1.3 (9)	DS_{CDP}	50.9 ± 2.2 (9)
DR_{Veh}	52.4 ± 3.4 (7)	DR_{CDP}	48.0 ± 2.8 (8)
Running/Bouncing (PTZ mg/kg)			
DS_{Veh}	61.2 ± 5.6 (9)	DS_{CDP}	55.9 ± 2.3 (8)
DR_{Veh}	57.3 ± 5.5 (7)	DR_{CDP}	50.9 ± 3.0 (8)
Tonic Hindlimb Extension (PTZ mg/kg)			
DS_{Veh}	52.5 ± 9.2 (7)	DS_{CDP}	59.8 ± 1.9 (8)
DR_{Veh}	64.4 ± 7.8 (7)	DR_{CDP}	52.1 ± 4.0 (6)

Table 10. Results of injections into the ventrolateral caudate on protection against PTZ-induced seizures. In Experiment 5, vehicle or CDP was bilaterally injected into the ventrolateral caudate of DS and DR mice. Results are mean ± SEM. The number of mice tested is in parentheses.

Discussion

1.0 Overview of experimental results

1.1 Experiment 1

The average number of total entries on the plus maze was low in both DS and DR mice five minutes after receiving vehicle injections into the amygdala (Figure 12). The normal level of baseline activity (Courtney and Gallaher 1991; Courtney et al. in prep.; Phillips and Gallaher 1992) is seen in Experiments 2, 4, and 5 (Figures 13, 15 and Table 8). It is possible that both lines of mice were exhibiting freezing behavior in Experiment 1 as a result of the injections and handling. Analysis of the data indicated that this low level of locomotor activity was increased by CDP micro-injections in the DR line, but not the DS line.

Other studies have found that freezing results from the presentation of a stressor (Fanselow and Helmstetter 1988). Defeat stress has also been observed to decrease plus maze exploration (Skutella et al. 1994). Additionally, freezing has been observed following brief foot shocks. Systemic diazepam or direct injection into the BLA attenuated this type of freezing behavior (Helmstetter 1993b). In a different study, rats were presented a heated floor to which they previously had been exposed. Following vehicle injections into the amygdala, the rats froze and avoided the floor. Midazolam injected into the amygdala resulted in this avoidance response being attenuated (Harris and Westbrook 1995a).

These studies are consistent with the explanation that the low locomotor activity seen in Experiment 1 resulted from handling during the injection--a procedure which was likely stressful to the subjects. Because of the potential for this problem, the mice in this experiment were repeatedly exposed to the handling procedure prior to the

injections and behavioral testing. Nevertheless, they did not become completely habituated to this type of restraint.

Because CDP injections in the amygdala increased total entries in DR mice, the most plausible explanation is that intra-amygdala CDP attenuated stress-induced freezing. The increase in total entries seen after CDP injections into the amygdala of the DR mice (Figure 12) is consistent with the results of the Helmstetter (1993a) who also found an attenuation of freezing following a similar treatment in rats. The number of total entries exhibited by the DR mice following intra-amygdala injections of CDP was similar to the number normally seen after systemic vehicle treatments (Courtney et al. in prep.). This indicates that the CDP treatment was reversing the stress-induced decrease in activity, as opposed to directly increasing locomotor activity. The ability to reverse stress-induced freezing was not seen in the DS mice, suggesting one important difference between the two strains. That is, the amygdala mediates an attenuation of stress-induced freezing in DR and not DS mice.

Increases in percent open entries is a standard measure of anxiolysis in this paradigm (Lister 1987). Prior reports with rats suggested that the amygdala could mediate this effect of BZs (Green and Vale 1992; Pesold and Treit 1995). However, CDP injections into the amygdala did not significantly increase percent open entries in either line, though there was a trend towards an increase in the DR mice.

Because the total number of arm entries was low, the variability in percent open arm entries was high (Table 5). Because of the high variability, the power to detect differences in the mice between vehicle and CDP treatment was very low. That is, the ability to measure anxiolysis was minimal, and an anxiolytic effect may have been missed. Therefore, the ability of the amygdala to mediate anxiolysis in DS and DR mice was re-examined as described later in Experiment 2.

Experiment 1 did not identify any other effects of intra-amygdala injections of CDP. This indicates that the amygdala does not mediate (a) any muscle relaxant effects, (b) any ataxic effects, (c) any sedative effects, or (d) any protection against PTZ-induced seizures. The DS mice are very sensitive to the muscle relaxant effects and ataxic effects of systemic BZs (Gallaher et al., personal comm.). Because of this sensitivity, if the amygdala mediated these effects, they would likely be seen in this line. Therefore it is unlikely that the amygdala mediates these effects in this or other less sensitive lines of mice.

In addition, the DS and DR mice are sensitive to the seizure protective effect of BZs. This robust effect of systemic BZs is not seen following localized injection. Therefore, it is likely that the amygdala does not mediate seizure protective effects in other lines as well.

There are three caveats to the first experiment. One, the variability in plus maze performance was high because of the stress-induced freezing. Two, only the results of bilateral injections were examined. Because of the low percentage of bilateral injections, the number of subjects examined was low, thus decreasing the power to detect any possible differences. Three, behavioral testing for seizure sensitivity occurred 20 to 25 minutes following the micro-injection. Thus, it is possible that the CDP diffused from the amygdala during this time, explaining the lack of any seizure protective effects. Therefore, Experiments 2 and 3 were performed.

1.2 Experiment 2

In this experiment, plus maze testing began 24 minutes after CDP micro-injection to avoid the effect of handling-induced freezing. Consistent with the hypothesis that the amygdala does not mediate the locomotor activating effects of BZs, there was no increase in total entries produced by BZ injection (Figure 13). This supports the hypothesis that the increase in total entries seen in Experiment 1 was the

result of an attenuation of freezing. The DR mice are very sensitive to the locomotor activating effects of systemic BZs. If the amygdala was involved in mediating this effect, it would have been produced in the DR mice in this experiment.

Intra-amygdala CDP resulted in an increase in the percentage of open arm entries in DR mice. This anxiolytic effect was not observed in DS mice. Because there was no difference between vehicle and CDP treatment in DS mice, this strengthens the conclusion of Experiment 1; i.e. the amygdala is capable of mediating the anxiolytic effects of BZs in DR mice but not in DS mice. Since the number of mice was higher in Experiment 2, it is unlikely that the failure to find an effect of intra-amygdala CDP infusion in DS mice is the result of a lack of statistical power.

The type of anxiolytic effect observed in Experiment 2 differs from Experiment 1. In Experiment 1, CDP appears to have reversed stress-induced freezing as opposed to having increase the percent of open arm entries. It appears that the amygdala can mediate two types of "anxiolytic" effects in DR mice. Alternatively, each of these two measurements may reflect different behaviors representing the same underlying phenomenon. Nevertheless, both experiments suggest that the amygdala can mediate a difference in anxiolytic sensitivity between DS and DR mice.

Furthermore, the finding that a behavioral effect was observed 24 minutes after micro-injection indicates that CDP remained in the injection area at least this length of time. Therefore, it is unlikely that the negative seizure effects were the result of diffusion of the drug away from the injection site. To more carefully explore this issue, seizure protective effects were reexamined in Experiment 3.

1.3 Experiment 3

The amygdala has a role in a number of types of seizure (During et al. 1992; Gale 1992a; Gale 1992b; Handforth and Ackermann 1995; McNamara et al. 1984;

Suzuki et al. 1994; Tietz et al. 1985), in particular "limbic seizures." In Experiment 1, four different seizure endpoints were evaluated: myoclonus, face-and-forelimb clonus, tonic-clonic running and bouncing, and tonic hindlimb extension. There is evidence that each of the seizure types are mediated by different anatomical areas (Gale 1992b), and some of these seizures may involve the amygdala. However, the results of Experiment 1 in this study suggested that the amygdala does not mediate protection against PTZ-induced seizures (Figure 14). This conclusion included two seizure endpoints which may involve the amygdala, myoclonus and face-and-forelimb seizures (Gale 1992a; Gale 1992b).

However, in Experiment 1 seizure sensitivity was measured 20 to 25 minutes after injection, whereas the behavioral effects on the plus maze occurred between 5 and 10 minutes following injection. It is feasible that the local concentration of CDP in the amygdala may have decreased after 20 min, resulting in a loss of behavioral activity. Because of this possibility, seizure sensitivity was tested in a separate group of animals 7 min after injection. Also, a larger number of subjects were tested to increase the statistical power to detect any possible effects of CDP infusion.

Consistent with the results of Experiment 1, no protection against any of these seizure types was again observed. Thus, although there are loci in the brain which are capable of mediating the protective effects (King et al. 1987) (Zhang et al. 1989), the results of these experiments strongly suggest that enhancement of GABAergic activity in the amygdala does not protect against the seizures produced by systemic PTZ.

1.4 Experiment 4

The most robust demonstration of anxiolysis after injection into the amygdala was found when testing was performed 24 min after infusion. It is possible that the drug could have diffused to another area. This locus, and not the amygdala, may be responsible for the anxiolytic effects. The anatomical specificity of the injection was

therefore examined. This test was performed by injecting CDP into the ventrolateral caudate, at a site about 1.7 mm dorsal and anterior to the amygdala.

No anxiolytic effects were found after injection into this site (Figure 15). This outcome suggests that the injections remained localized to the injection site. The results also suggest that this area of the caudate is not involved in mediating the anxiolytic effect of BZs, consistent with other studies of the caudate (Costall et al. 1989).

The caudate has a role in motor behavior. Therefore, it was possible that it may have mediated either the locomotor activating effect (to which the DR are sensitive) or the locomotor sedating effect (to which the DS mice are sensitive). Neither result was observed in this study. Therefore, the caudate does not appear to mediate these other effects of BZs.

1.5 Experiment 5

The ventrolateral caudate may mediate other effects of BZs. In particular, it has been found that injections of RO 15-4513, an inverse agonist, into the caudate attenuates the ataxic effects of systemic ethanol (Meng and Dar 1994). This compound attenuates the opening of the chloride ionophore in response to GABA. It has also been observed to diminish the effect of alcohol on GABAA receptors. This raises the possibility that the caudate may mediate either sedative or ataxic effects of BZs. Therefore, the role of the caudate in mediating a number of behavioral effects of BZs was tested. The current results suggest that this area does not mediate any of the behavior effects of BZs: muscle relaxation, ataxia, locomotor sedation, or protection against seizures (Tables 8, 9, and 10). In addition, the finding of Experiment 4 that injections of CDP into this locus do not produce anxiolytic or locomotor activating effects was confirmed.

It is possible, however, that other areas of the caudate may mediate the ataxic effects. The injections performed by Dar (Meng and Dar 1994) were in the dorsal

caudate in an area more anterior than that used in this study. More ventral and anterior regions of the caudate, near the nucleus accumbens, may be involved in effects on locomotor activity (Koob 1992). The injections used in this study were posterior to the anterior commissure, and closer to the amygdala. The main purpose of Experiments 4 and 5 was to demonstrate the anatomical specificity of the amygdala injections. Therefore caudal, ventrolateral sections of the caudate were evaluated. Examinations of the dorsal caudate and other regions will occur in future studies. For further discussion of the spatial and temporal resolution of micro-injections, see section 3.2 of the discussion.

2.0 General Conclusions

2.1 Anxiolysis

Bilateral injections into the amygdala of DR mice resulted in both an attenuation of stress-induced freezing 5 minutes after injection (Figure 12) as well as an increase in the percentage of entries into the open arms 24 minutes after injection (Figure13). Neither of these effects were seen in DS mice. These experiments demonstrate that the amygdala is capable of mediating anxiolytic effects in DR mice but not DS mice.

However, the current study does not address the influence of other neuroanatomical areas which have been implicated in mediating anxiolysis (see introduction, section 4.1). Though a role for the amygdala in potentially mediating this difference has been established in this study, the possibility DR and DS mice may also differ in other loci has not been excluded. Nevertheless, the stereotaxic surgery and micro-injection techniques have been refined so that additional neuroanatomic areas can similarly be examined.

2.2 Ataxia

Injections of CDP into the amygdala did not produce ataxia in either line (Table 6). It has been proposed that the anxiolytic response may contribute to the relative improvement observed in DR mice when tested for ataxia on the rotarod (Courtney et al. personal comm.). That is, decreased anxiety could result in enhanced rotarod performance. Because there was no alteration of rotarod performance in the DR mice, however, the current results suggest otherwise. Anxiolysis produced by intra-amygdala injections did not improve rotarod performance. The results of these experiments suggest that the amygdala does not directly mediate the ataxic effects of BZs, nor does it indirectly improve rotarod performance through effects on anxiety. Additionally, the ventrolateral caudate does not appear to mediate the ataxic effect of CDP.

2.3 Muscle Relaxation

It is possible that some muscle relaxation produced by systemic BZs is secondary to decreased anxiety. The assay used to detect muscle relaxation was capable of detecting small changes in grip strength. Such changes were not observed after intra-amygdala injections of BZs, however--even in the presence of anxiolysis, indicating that muscle relaxation is not the result of anxiolysis (Table 6). The results also indicate that other areas of the mouse central nervous system must mediate the muscle relaxant effect of BZs. As injections into the ventrolateral caudate did not affect grip strength, this locus is also not an area which mediates BZ muscle relaxant effects.

2.4 Locomotor activation

Surprisingly, injections of CDP into the amygdala also did not have locomotor activating effects in either line (Figure 13). Although stress-induced freezing was reversed in the DR line, the total number of open entries was only increased to previous

baseline levels (Figure 12). When plus maze testing was performed 24 minutes after injection, there was no evidence of CDP-induced activation.

It has been hypothesized that the anxiolytic effects of CDP after systemic injections may be confounded by its locomotor activating effects. That is, increased exploratory activity may result in an increase in the percentage of open arm entries, independent of effects on anxiety (Dawson et al. 1995). However, because anxiolysis was observed in the absence of locomotor activation, these two behaviors can reflect separate behavioral and physiological effects of BZs. Although the DR mice are more sensitive than DS mice to both effects after systemic injections, the amygdala only mediates the anxiolytic effect and not the activating effect.

The results also suggest that if activity has been decreased by stress, BZs may increase activity by attenuating this effect. In these instances, the amygdala may mediate a type of locomotor activation. However the amygdala does not mediate the high level of locomotor activation seen in DR mice following systemic BZ injections (Courtney et al. personal comm.; Phillips and Gallaher 1992). Additionally, although the caudate is involved in locomotor activity, the ventrolateral caudate does not mediate either sediation or locomotor activation by CDP.

2.5 Seizure protection

The results of Experiments 1 and 3 demonstrate that the amygdala does not mediate the ability of BZs to protect against PTZ-induced seizures (Table 7, Figure 14). It is possible that higher doses of CDP in this area may have been necessary. However, the concentration used was the highest possible, given the limited solubility of CDP. This dose was capable of producing anxiolysis, and thus was having a physiological effect in the amygdala. Because similar doses of systemic BZs produce both anxiolysis and seizure protection, it is unlikely that a difference in the dose-response curve in the amygdala is an explanation. Given the findings that BZs have

antiseizure effects when micro-injected into other structures (King et al. 1987), it is likely that BZs act in these locations and not the amygdala. This will need to be tested in the DS and DR mice.

3.0 Methodological implications

3.1 Handling

In all experiments, the subjects were routinely handled following surgery and before behavioral testing. This served two purposes: to maintain the patency of the guide cannula, and to habituate the subjects to the handling procedure. Rats have been similarly tested on the plus maze following intra-amygdala injections (Green and Vale 1992; Pesold and Treit 1994; Pesold and Treit 1995). In these procedures, the subject was habituated to the handling procedure and was gently held during the intra-cranial injection. Rats were observed to exhibit minimal restraint stress during the injection procedure (C. Pesold, personal comm.), and freezing was not reported to occur in these experiments. In neuroanatomical studies of anxiolysis, this difference between rats and mice is a significant variable.

Handling history has implications for anxiolysis testing. For example, after 28 days of handling of rats, no anxiolytic effect of BZs on the plus maze was observed (Brett and Pratt 1990). Other groups have also found that handling history can modify the anxiolytic effect of BZs on the plus maze (Andrews and File 1993a). Handling habituation has also attenuated the anxiolytic effect of BZ in other paradigms (Boix et al. 1989). Furthermore, while diazepam affected 5-HT levels in the hippocampus, cortex, and hypothalamus, this effect was not found in handling habituated subjects (Boix et al. 1990). Handling habituation resulted in a 40% increase in GABA binding (Biggio et al. 1984). These results indicate that handling can alter sensitivity to BZs.

In addition, there may also be genetic differences in sensitivity to the effects of handling (Brush 1991).

The mice used in these experiments were routinely handled during cage changes, and this may have been involved in the development of differences in sensitivity. Alternatively, the habituation to brief head immobilization may also have played a role. That is, if the DS and DR mice are differentially sensitive to handling, both types of handling could have been involved in the development of differences in sensitivity to anxiolysis.

The mice may also differ in their sensitivity to the acute stress of the injection procedure. Sham injections, and intraperitoneal injections are capable of affecting plus maze performance (Lapin 1995). Inescapable shock and escapable shock also both affect plus maze performance (Grahn et al. 1995), and acute stress can decrease locomotor activity (Acosta and Rubio 1994). Aversive conditions and stress effects the levels of endogenous BZs, and this effect may be influenced by genetics (Teruel et al. 1991). The brief immobilization of the head and insertion of the injection cannulae in this study could have produced similar effects.

When tested 5 min after vehicle injection, both DS and DR mice exhibited similar behaviors. There were no baseline differences in total entries or percent open entries. That is, the injection procedure did not affect baseline behavior differently in the two lines. However, the stress of injection may have affected sensitivity to BZs. In particular, this may have rendered the DS mice insensitive to the anxiolytic effect of CDP. This possibility has not been excluded. Nevertheless, regardless of the etiology of the differences, the results suggest that they can be mediated by the amygdala.

3.2 Diffusion

The anatomic specificity of the intra-amygdala injections were evaluated by bilaterally injecting a similar amount of CDP into a nearby structure, the ventrolateral

caudate. This area of the caudate, posterior to the anterior commissure, was chosen for two reasons. It was slightly dorsal and near (1.7 mm) to the amygdala injection site. Also, it was not expected to mediate the anxiolytic effect of CDP. Because there were no behavioral effects of injections into this structure, it was concluded that the results of the intra-amygdala injection were not the consequence of CDP diffusing into other locations near the amygdala. In other micro-injection experiments, this type of anatomic control has been used to verify anatomic specificity. For example, mice were able to discriminate 0.1 μL morphine injections from vehicle injections into the lateral hypothalamus. Injections 1.1 mm distant were not effective (Cazala and David 1995).

Because behavioral testing on the plus maze was most successful when testing occurred 24 minutes after injection, a more quantitative determination of the extent of diffusional spread during this time is required. Additionally, for experiment five, testing began 24 minutes after injection and was not completed until 40 to 45 minutes after injection. Interpretation of the results of this experiment requires an evaluation of the concentration of CDP remaining in the caudate over this time.

A few experiments have been performed which indicate that micro-injections of BZs remain within localized neuroanatomic areas over these time courses. For example, 0.5 μL of [^{3}H]-methylclonazepam was injected into the substantia nigra. After 30 minutes, the brains were removed and sliced. The slices were evaluated using quantitative autoradiography (QAR). The tritiated BZ remained within a 0.4 mm radius over this time course (King et al. 1987). Therefore, BZs can remain in injection areas for up to 30 minutes without extensive diffusion. In this case, the extent of diffusion was smaller than the size of the rat substantia nigra. It is expected that the 0.5 μL injections used in the five experiments of this thesis also remained similarly localized.

A study was performed in the 1960s using [^{14}C]-methylmorphine sulphate (Lomax 1966). Brains were removed and sliced various times after injection of 1.0 μL

into the hypothalamus (twice the volume used in the experiments reported in this thesis). The slices were examined for radioactivity using liquid scintillation. It was found that most of the methylmorphine sulphate remained within a radius of 0.6 mm, even after one hour. While the concentration gradient of methyl-morphine flattened over the course of one hour, the total radius of spread did not drastically increase. This study is an early piece of evidence that micro-injected compounds remain within localized areas.

Volume and Substance	Time evaluated	Radius of diffusion	Reference
0.5 µL Clonazepam	30 min	0.4 mm	(King et al. 1987)
1.0 µL m-Morphine	60 min	0.6 mm	(Lomax 1966)
0.33 µL SCH23390	60 min	0.8 mm	(Caine et al. 1995)
0.3 µL DAMGO	40 min	0.7 mm	(Bals-Kubik et al. 1993)
0.3 µL U-69593	40 min	0.5 mm	(Bals-Kubik et al. 1993)
1.0 µL U-69593	40 min	0.7 mm	(Bals-Kubik et al. 1993)

Table 11. Diffusion following injection of radiolabeled ligands (0.3 to 1.0 µL). Radiolabeled compounds have been micro-injected into localized neuroanatomic areas in a few studies. The approximate extent of diffusion of these substances is summarized here.

Booth et al (Booth 1968) injected 1 µL of [^{3}H]-norepinephrine into the lateral hypothalamus. Using dissection, brain regions were removed and specific regions were analyzed using liquid scintillation. These authors found that norepinephrine diffused further than the methyl-morphine sulphate, though most of the norepinephrine

remained within 2 mm of the injection site. In this study, the concentration curve flattened between 10 and 20 minutes.

In another study, [^{3}H]- acetylcholine, norepinephrine, and serotonin were injected into the hypothalamus (Myers et al. 1971). The brains were dissected after 35 to 40 minutes. Consistent with the other studies, the injected compounds remained within the hypothalamus. A similar experiment was performed using either [^{3}H]-Ro 15-4513 or [^{3}H]-cyclohexyladenosine. Following brain dissection, it was observed that injections into the striatum remained in the striatum after 30 minutes (Meng and Dar 1994).

A time course of diffusion has been evaluated for injections of various volumes of [^{3}H]-bicuculline (Yoshida et al. 1991). Five, twenty, and thirty minutes after injection, brain slices were taken for QAR analysis . Three injection volumes were used: 1.2 μL, 1.5 μL, and 2.0 μL. The extent of diffusion gradually increased over time. After 30 minutes, the 1.5 μL injection acquired a radius of about 1.5 mm. The size of the injection also influenced the extent of diffusion. In all cases, the injectate remained within a discreet area, the caudate.

The time course of diffusion was also evaluated for [^{3}H]-SCH 23390 after injection of 0.33 μL into the amygdala (Caine et al. 1995). Immediately after injection, the radius was about 0.5 mm. After twenty minutes, this radius had increased to approximately 0.7 mm, and after 60 minutes the radius was about 0.8 mm. Thus, over the course of one hour, the extent of diffusion only gradually increased. The concentration of the radio-ligand decreased over this time period. After one hour, the concentration dropped to one fifth of the original concentration.

Other studies found similar results. Injections of [3H]-DAMGO (0.3 μL) were confined to within 0.7 mm of the site of injection after forty minutes, while similar volumes of [3H]-U-69593 remained within 0.5 mm of the injection site (Bals-Kubik et

al. 1993). When a volume of 1.0 μL of [3H]-U-69593 was injected, it remained within 0.7 mm of the injection site after 40 minutes. Similarly, Johnson et al (Johnson et al. 1996) injected 0.3 μL of [^{3}H]-dopamine into the core of the nucleus accumbens. After twenty minutes, the dopamine remained within the nucleus accumbens. The diffusion of these substances is again consistent with the probability that the CDP injections in this experiment remained localized to the injection site over 45 minutes.

A number of early studies have examined larger injection volumes. The extent of diffusion at various times after injection of 2.5 μL [3H]-morphine into the thalamus or the hypothalamus was analyzed. After 60 minutes, the morphine remained within 2 mm from the injection site (Schubert et al. 1970). Injections of a 5 μl volume of tritiated dopamine remained within the striatum 25 minutes after injection (Fog and Pakkenberg 1971). Three microliters of [3H]-thyroxine was injected into the brains of rabbits, and the extent of diffusion was examined using QAR. The radius of spread was between 2 and 2.5 mm (Harrison 1961). Thus, even with these large volumes, the injected substances remain fairly localized.

Dye injections have also been used to examine diffusion. Myers (Myers 1971) reported that after injection of several types of dyes (0.5 μL), the extent of diffusion after 30-35 minutes was only 0.3 to 0.8 mm from the injection site. The extent of spread depended upon the dye used. Injections of 1.0 μL resulted in diffusion of up to 1.1 mm from the injection site. Five different volumes were examined in this study (0.5 to 4.0 μL). It was noted that the extent of diffusion most correlated with the size of the injection. An analysis of fast green dye by Yoshida et al (Yoshida et al. 1991) found that diffusion of this substance was approximately similar to diffusion of [^{3}H]-bicuculline. These studies are also consistent with the conclusion that the 0.5 μL injections of CDP remained within the injected structures for at least 45 minutes.

A number of other techniques have also been used to examine diffusion. Two studies have examined the spread of lidocaine (Sandkuhler et al. 1987) (Fink and Cairns 1984). Because lidocaine is able to block electrical stimulation of nerves, the blockade can be used as a measure of the concentration of lidocaine. In one study, stimulation of dorsal column fibers in the spinal cord was used to detect lidocaine. One microliter was injected 1 mm from the stimulation site. Neural blockade was observed within 2 minutes of injection, a maximal effect was seen at 23 minutes, and the effect was gone by 90 minutes.

Because catecholamines fluoresce after glutaraldehyde fixation, several studies have examined diffusion using this technique (Bondareff et al. 1970; Montgomery and Singer 1969; Routtenberg et al. 1968; Ungerstedt et al. 1969). Flicker et al (Flicker and Geyer 1982b) infused norepinephrine at a constant rate of 0.025 μL per minute. Infusion occurred for 10, 20, or 40 minutes. After one microliter had been infused over 40 minutes, norepinephrine fluorescence was mainly found in a area limited to 0.1 mm from the infusion site. No infused norepinephrine was found 0.5 mm from the infusion site. In this study, Evan's blue dye was also infused. In this case, a the dye diffused 0.75 mm from the injection site after a 40 minute infusion.

HPLC measurements have also been used. Microdissections of the striatum were used to measure the levels of micro-injected morphine. The concentration of morphine in the striatum paralleled the time-course of its effects on EMG activity. Both declined with a half-life of about 10 minutes (Melzacka et al. 1985).

One concern in diffusion experiments which has been raised is the difference between lipophilic (eg. naloxone) and lipophobic drugs (eg. morphine). It has been hypothesized that lipophilic drugs may leave the injection site more quickly (Schroeder et al. 1991). Because lipophilic compounds can diffuse through the blood-brain barrier, it is hypothesized that diffusion into the capillaries may account for a more

rapid removal. There is some evidence for this. The hydrophilic compound methyl-naloxonium remained in the hindbrain for a longer period of time than the more hydrophobic naloxone (Schroeder et al. 1991). However, these levels were measured by dissecting out brain areas rather than by using QAR. Therefore, the extent of spread of the two compounds was not examined.

Computer models have been developed to evaluate diffusion (Lotrich and Gallaher 1996). These indicate that highly lipophilic molecules will distribute into the lipid membranes at the injection site, thus limiting the extent of diffusion. It is hypothesized that these compounds will only gradually be removed as the molecules leave the membranes, cross through the extracellular space, and then exit through the blood-brain barrier (Lotrich and Gallaher 1996). Thus lipophilic compounds will remain in localized areas for relatively long periods of time. This conclusion is consistent with the results of the studies reviewed above. Although it is difficult to make comparisons between studies, the prior experiments suggest that more lipophilic compounds remained more localized than the more hydrophilic compounds.

As predicted by Schroeder et al (Schroeder et al. 1991), highly lipophobic compounds will remain in the brain because of inability to exit through the blood-brain barrier. However, the computer model predicts that these compounds will diffuse to a much greater extent than lipophilic compounds. Compounds of intermediate lipophilicity are predicted to diffuse to a less extent than the lipophobic compounds. However, they are also predicted to be removed from the brain more rapidly. Thus, the concentration gradient of lipophobic substances is expected to flatten over time. And substances of intermediate lipophilicity are expected to be removed through the blood-brain barrier more quickly.

In conclusion, the results of these computer models as well as the results of empirical studies suggest that the 0.5 μL injections of CDP used in this study are

expected to remain for at least 45 minutes in a confined locus--less than one millimeter from the injection site. The most similar study empirically measuring diffusion found that a 0.5 μL injection of clonazepam remained within a 0.4 mm radius. This is supported by the finding that injections into the nearby ventrolateral caudate did not have a behavioral effect.

The exact extent of diffusion and loss of compound over time remains undetermined however. The estimates of diffusion, preclude any claim that injections were limited to any subnuclei within the amygdala. That is, although successful infusions were made into the AL/ABL, CDP could have diffused into neighboring amygdala nuclei as well (Figure 16). Therefore, the conclusions of this thesis are limited to more general statements about the amygdala as a whole, rather than about specific nuclei within the amygdala.

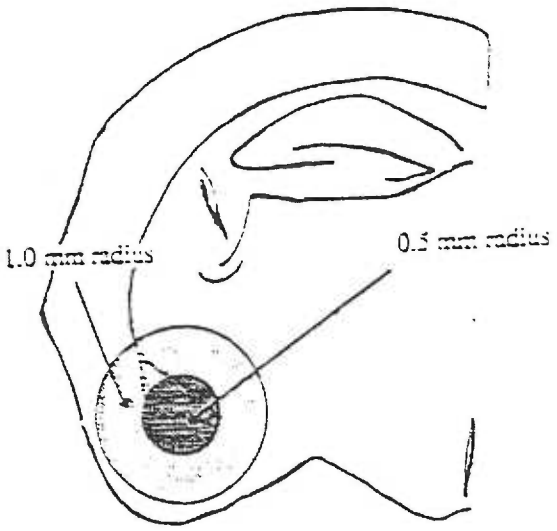

Figure 16. Relative size of the mouse brain to potential diffusion diameters. A schematic of a coronal slice from the mouse brain at the level of the amygdala is presented with two circles indicating the distance of diffusion assuming either spherical diffusion of 0.5 mm or 1.0 mm radius. Note that diffusion of even 0.5 mm can involve multiple subnuclei within the amygdala (see also Figure 10).

3.3 Concentration of CDP

Each micro-injection consisted of 0.5 μL of 200 mM CDP. Therefore, 100 nmol of CDP were bilaterally infused. This or lesser amounts of micro-injected CDP has resulted in behavioral effects in a number of experiments (Audi and Graeff 1984; Hodges et al. 1987; Kataoka et al. 1982; Maier et al. 1994; Sullivan et al. 1989). Because this and smaller amounts of micro-injected CDP were able to produce behavioral effects in these experiments, this concentration was chosen for this thesis. The concentration was also chosen because it is the amount which could be maximally injected through the narrow injection cannulae.

Chlordiazepoxide (molecular weight 299.75 g/mole) is less lipid soluble than many other BZs. Octanol/buffer partition coefficients for CDP and diazepam are 28 and 309 respectively (Greenblatt et al. 1983). In a more natural system, synaptosome/buffer partition coefficients for CDP, clonazepam, and diazepam are 16, 30, and 79 respectively (Perillo et al. 1995). Because it is fairly lipid soluble, systemically injected chloridazepoxide is rapidly concentrated by the brain--though not as rapidly as diazepam (Van Der Kleijn 1969). The maximal brain concentration following systemic injection of 25 g/kg and 50 g/kg CDP is 22 μg/g and 26.3 μg/g respectively (Maleque and Ahmad 1977). These are typical maximal doses used in behavioral experiments. The amount of CDP micro-infused in these experiments (100 nmol) was 30 μg. Assuming that the CDP remains within a 0.5 to 1mm radius (0.5 to 4 μL of brain tissue), the concentration is well in excess of that achieved following systemic injections.

CDP injection into the amygdala or into the ventrolateral caudate did not affect a number of behaviors in several experiments in this thesis. It is possible that higher concentrations would have affected these behaviors. However, this possibility is unlikely. This injection concentration was chosen to be high (about a thousand fold

higher than than achieved following systemic injections), increasing the likelihood of producing an effect. Nevertheless, the possibility that higher concentrations could have produced different effects has not been completely excluded. This could only be accomplished with more hydrophilic or more potent ligands, to allow greater concentrations in the infusion solution. CDP is not as potent as many ligands at the BZ receptor. The IC50 for inhibition of diazepam binding is 352 nM, as compared to diazepam and midazolam which are 8.1 nM and 4.8 nM, respectively (Haefly et al. 1985). Nevertheless, saturation of BZ receptors is expected at the concentration used in these experiments.

3.4 Unilateral infusions.

Only successful bilateral infusions were included for analysis in this study. A number of subjects received an infusion into one amygdala and the other infusion into a nearby area (the cortex, the caudate or globus pallidus, etc.) Although these subjects could be grouped into a "unilateral injection" group for analysis, in reality each subject received a different treatment, making interpretation difficult. There is variation between subjects in performance on the plus maze. Therefore, analyses of the behavioral effects of infusions require pooling of similarly treated subjects and subsequent comparison between treatment groups. For this reason, the results of unilateral injections could not be evaluated.

The unintentionally "missing" infusions were into several different structures of unknown relevance to anxiolysis or the other behaviors. Additionally, the "misses" were of varying distance from the intended injection site. Some "misses" may have potentially diffused back into the amygdala. Both the injections into differing nuclei, as well as injections at differing distances from the AL/ABL add layers of interpretive difficulty. For example, does an amygdala infusion combined with a contralateral

temporal cortex infusion result in anxiolysis? Or is the unilateral infusion sufficient for anxiolysis? These possbilities could not distinguished in these experiments.

3.5 Statistics

The results of these experiments were analyzed using two-way ANOVAs. Post-hoc, paired comparisons were then made using Scheffe's S test. The results could also have been analyzed using available *a-priori* multiple comparison procedures. For example, comparisons between treatment groups could have been made using Holm's , Dunn's , Dunn-Sidak's test. Of these procedures, Holm's test is the most powerful. That is, it is less likely to make type II errors. Each of these tests holds the familywise type I error rate at less than α. This is done by modifying the α used for each planned comparison (Kirk 1995).

By using the two-way ANOVA, however, the significance of the interaction between line and drug could be more easily determined. This statistic indicates whether CDP had a greater effect in one line than the other. This evaluation was of interest in this study, but could not have been performed using standard *a-priori* planned comparisons. Thus in order to evaluate the interaction, some loss of power was justified. Additional comparisons were then be made using Scheffe's S test, which is robust to unequal n's.

4.0 Possible mechanisms of genetic difference in sensitivity to anxiolysis

An anxiolytic effect was found in DR mice and not in DS mice after injection of CDP into the amygdala. There are many potential reasons for this.

4.1 Differences at the BZ receptor

It is possible that the DR mice have a greater number of BZ receptors in the amygdala than the DS mice. Alternatively, the receptors in this locus may have a higher affinity for BZs. However, using whole brain assays, no difference in affinity (kD) or density (Bmax) of receptors have been found in DS and DR mice (Allan et al. 1988). Differences in flunitrazepam binding were also examined using quantitative autoradiography. Again, no differences between DS and DR mice were found (Gallaher et al. 1991). These results suggest that the number and affinity of receptors may not be an explanation for the differences between DS and DR mice.

Although no differences in the density of BZ receptors have been found between DS and DR mice, genetic differences in BZ binding densities have been observed in other studies. For example, BALB mice have fewer cortical BZ binding sites than C57/BL, AKR/J, or ICR mice, and diazepam potentiation of Cl^- flux was decreased in BALB mice. BALB mice also exhibited more "emotionality" in the open field than the other strains. That is, they defecated and froze more often (Mihic et al. 1992). These results suggests that cortical BZ binding may be associated with the level of baseline "emotionality."

Consistent with this, outbred mice have been divided into anxious and nonanxious groups depending upon their baseline performance on the plus maze. Anxious mice ventured into the open arms less frequently. Cortical BZ binding was examined in these mice, and it was found that anxious animals had lower BZ binding than nonanxious animals (Rago et al. 1988).

Both genetic and environmental factors can influence the density of receptors, and these binding differences may have been the result of either genetics and/or environment. For example, postnatal handling decreased baseline anxiety, and this was correlated with changes in the density of BZ receptors (Teruel et al. 1991). However,

the results of the studies by Rago et al. and Mihiic et al. are consistent with the suggestion that cortical BZ binding is associated with baseline differences in anxiety-related behaviors.

Rats have also been grouped into anxious or nonanxious animals based on baseline plus maze performance. Again, anxious rats were found to have a decreased number of BZ receptors in the cortex. Decreased binding was also found in the hippocampus (Harro et al. 1990). Genetic susceptibility to hypertension has also been associated with lower GABA binding. In this case, the lower binding was observed in the hypothalamus and the amygdala (Kunkler and Hwang 1995). These studies collectively suggest that differences in the BZ binding are associated with differences in the baseline level of anxiety, and perhaps spontaneous hypertension.

In summary, although BZ receptor density may be associated with baseline measures of anxiety, the prior studies with DS and DR mice suggest that differences in BZ binding do not correlate with differences in anxiolytic sensitivity to BZs. In support of this, LS mice are more sensitive to the anxiolytic effects of diazepam on the plus maze than SS mice, and no differences in cortical BZ binding have been found in these lines (Stinchcomb et al. 1989). Thus, differences in sensitivity to BZs may not be a function of variability in BZ binding.

Another possible explanation for the difference in anxiolytic sensitivity between DS and DR mice is that BZs are more efficacious at the amygdala receptors in DR mice. Differences in efficacy have been found to be independent of differences in affinity or density. For example, chronic treatment with BZs can affect the ability of BZs to enhance GABA-stimulated CL- flux without affecting binding (Hu and Ticku 1994). Consistent with this hypothesis, BZ's were found to have more efficacy in DS mice than DR mice in enhancing chloride flux in cortical synaptosomes (Allan et al. 1988). However, this explanation is more consistent with higher sensitivity of DS mice to the

ataxic and sedative effects of BZs. It may be that the receptor types in the amygdala differ such that BZs are more efficacious at enhancing amygdala chloride flux in DR mice. This remains to be determined.

There are a number of mechanisms which could produce differences in receptor efficacy. (A) A point mutation may result in differences in efficacy. For example, a naturally occurring point mutation has been discovered in the α6 subunit of the BZ receptor in rats. This subunit which is expressed in the granule cells of the cerebellum is associated with increased sensitivity to the ataxic effects of ethanol (Korpi et al. 1993; Korpi and Seeburg 1993). Point mutations in other α subunits have been observed, in vitro, to affect affinity for BZs (Sigel et al. 1992). Additionally, point mutations have been discovered in the γ2 subunit which affect the efficacy of BZs (Mihic et al. 1994).

(B) Splice variations in the subunits can also affect efficacy. Differential splicing of the γ2 subunit into long or short forms is capable of altering the efficacy of ethanol at the receptor (Wafford et al. 1991). In different neuroanatomical regions, the proportion of BZ receptors with splice variants of this subunit varies (Wang and Burt 1991). Alternate splicing of the α6 subunit has also been observed to affect receptor function (Korpi et al. 1994). The β3 subunit is also differentially spliced in various brain nuclei. The functional consequences of this are not known (Kirkness and Fraser 1993). The DS and DR mice may differ in the splicing of some subunits which then results in differences in efficacy of BZs in different nuclei.

(C) A BZ receptor/chloride ionophore is comprised of a number of subunits. There are an estimated 800 different types of functional combinations of subunits which are possible in the brain (Yeh and Grigorenko 1995). Various compositions of these subunits results in receptors with different functional attributes (Costa and Guidotti 1996; Doble and Martin 1992). One difference between DS and DR mice could be in

the proportions of different subunits in different receptors in various neuroanatomical areas. This could be evaluated with detailed *in situ* hybridization studies or subunit-specific antibody binding studies.

(D) Phosphorylation affects the function of BZ receptors. PKCγ can phosphorylate BZ receptors to produce functional consequences (Valenzuela et al. 1995). PKA can also phosphorylate various subunits in receptors producing changes in function (Moss et al. 1992; Tehrani and Barnes 1994). There is evidence that differences in phosphorylation of the BZ receptor may affect seizure sensitivity (Tehrani and Barnes 1995). Differences in the levels of phosphorylation could produce differences in BZ receptors in DS and DR mice.

(E) BZ receptors are also differentially glycosylated. For example, the α5 subunit was found to have different degrees of O-glycosylation (Sieghart et al. 1993). It is possible that glycosylation may have functional consequences, and the DS and DR receptors may have different types of glycosylation.

(F) The lipid micro-environment may also influence the function of BZ receptors (Koenig and Martin 1992). If the composition of lipids in the membranes in DS and DR mice differ, this may contribute to differences in sensitivity.

(G) Other ligands at the ionophore may affect the binding and efficacy of BZs. For example, S-adenosylhomocysteine was found to inhibit the binding of flunitrazepam. It is possible that this or a related compound may bind to the BZ receptor site (Tsvetnitsky et al. 1995). Conversely, the amino acid l-lysine is capable of enhancing the binding of diazepam at the BZ receptor (Chang and Gao 1995). If the DS and DR mice differ in the amount of these and related compounds at the synapse, then this may be a factor in the sensitivity differences which exist.

Neurosteroids can also affect the function of the BZ receptor. It is possible that various levels of these can affect the sensitivity to BZs. It is also possible that there is a

genetic influence on the sensitivity of various receptors to neurosteroids (Finn et al 1997). Differential activity of the steroids at these receptors could influence sensitivity to exogenously administered BZs.

(H) A peptide has been discovered, diazepam binding inhibitor (DBI), which may endogenously act as an inverse agonist at the BZ receptor (Barbaccia et al. 1990; Ferrarese et al. 1989). Using in situ hybridization, mRNA for this compound has been observed in non-neuronal cells (Alho et al. 1990; Alho et al. 1991; Slobodyansky et al. 1992; Tong et al. 1991), though the peptide has been observed to colocalize with GABA in axon terminals (Costa 1991; Costa and Buidotti 1991). A number of treatments, including stress and chronic BZ treatment, can alter the level of this compound (Ferrarese et al. 1993; Ferrarese et al. 1991; Gavish et al. 1992). It is possible that endogenous levels of DBI may compete with exogenously administered BZs. DBI is most concentrated in the hypothalamus, the amygdala, the cerebellum, discreet areas of the thalamus, hippocampus, and cortex. Different levels of this compound may be present in different areas in DS and DR mice. This would influence sensitivity to BZs.

(I) Differences in the level of GABA in the synaptic cleft may also influence sensitivity to BZs. BZs only augment the chloride flux elicited by GABA. They do not result in flux in the absence of GABA. Therefore, the effect of BZs at the receptor may depend on the presence or absence of GABA. If there is no GABA, then BZs will have no effect. Alternatively, if GABA is being maximally effective, then BZs will again have no effect. A number of possible factors could influence levels of GABA (Remiszewska et al. 1992).

(J) The type of cells in which of BZ receptors are located may also influence their function. There is evidence that GABAA receptors with flunitrazepam-specific binding exist on glial cells (Bureau et al. 1995; Fraser et al. 1994). The function of

these receptors is expected to be different from receptors on neuronal cells. The DS and DR mice may differ in the distribution of receptors between glial and neuronal cells. These could have an influence on sensitivity.

(K) The location of receptors on cells may also influence their function. Using immunohistochemistry, it has been found that receptors with different subunits exist in different sections of the same neuron (Baude et al. 1992; Nusser et al. 1995). It is possible that the routing of particular subunits to various neuronal areas may be different in DS and DR mice. This could influence the functional sensitivity to BZs.

4.2 Differences in neural circuitry.

The amygdala is probably involved with other neuroanatomic areas in influencing anxiety-related behaviors. It is possible that downstream sites to which the amygdala projects may differ in the DS and DR mice. Therefore, inhibition of the amygdala by BZs may have similar electrophysiological effects in the amygdala, but downstream effects may differ.

For example, the electrophysiological activity of neurons in the bed nucleus of the stria terminalis (BNST) in response to medial amygdala stimulation can be altered by adrenalectomy (Sanchez et al. 1995). Adrenalectomy may have altered the BNST neurons without altering the amygdala. Similarly, genetic differences in the DS and DR mice might have resulted in differences in areas to which the amygdala projects, rather than the amygdala itself.

One possibility is suggested by the finding that the level of nitric oxide may influence sensitivity to anxiolysis (Quock and Nguyen 1992). Thus, differences in the synthesis of this molecule may influence sensitivity. Additionally, naloxone can block the anxiolytic effect of BZs (Agmo et al. 1995). Therefore, downstream opioid sites may be involved in the differences seen in the DS and DR mice. Different levels of various neurotransmitters or their receptors--such as nitric oxide or endorphin--may be

involved in the differences in sensitivity to BZs. These differences may be downstream from the effect of BZs at their own receptors.

4.3 Miscellaneous contributions to genetic differences

Some of the genetic differences in the DS and DR mice may not be directly related to the BZ receptor. For example, protein malnutrition in early life results in changes in plus maze exploration and decreased sensitivity to the anxiolytic effect of diazepam on the plus maze (Almeida et al. 1988). The decreased sensitivity of DS mice to the anxiolytic effects of BZs may be mediated through a parallel mechanism.

Differences in hormone levels or sensitivity to various hormones in DS and DR mice may also indirectly influence receptor function. For example, exposure to various hormones resulted in alterations in the expression of specific BZ subunits in specific areas of the hippocampus. The precise subunits which were affected depended on the region examined and the hormone given (Weiland and Orchinik 1995).

These dietary or hormonal effects may be involved in causing changes in the BZ receptors. Alternatively, the effects may be involved in several possible downstream effects. Variability in these effects would be the result of allelic variation in number of genes not directly related to BZ receptors.

4.4 Other genetic differences in the amygdala

Other experiments have found genetic variability in the effect of intra-amygdala injections. RHA rats were bred for high avoidance performance in an active two-way avoidance task. RLA rats were bred for low avoidance in this task. Injection of vasopressin into the central amygdala nucleus (CEA) of the RHA rats did not affect their response to a conditioned emotional stressor. The stressor was a probe which had been previously paired with a shock. However, injection of vasopressin into the CEA of the RLA rats enhanced bradycardia and immobility in response to the stressor

(Roozendaal et al. 1992). Thus the amygdala appears to mediate vasopressin-enhancement of freezing in RLA rats and not RHA rats.

Similar results were found with norepinephrine infusions. Intra-amygdala injections of norepinephrine enhanced freezing the RLA rats, but enhanced burying behavior in RHA rats (Roozendaal et al. 1993). Therefore, there was an anxiogenic effect of norepinephrine infusion in both lines. However, the behavioral expression of "anxiety" was different between the lines.

High responders (HR) and low responders (LR) were chosen from outbred Wistar rats. These mice exhibited large increases (HR) or no increases (LR) in locomotor activation in response to a novel environment. Both lines exhibited high levels of neophobia--based on their avoidance of novel rat chow in novel environments. Propranolol, a beta-adrenergic antagonist, reduced neophobia in HR but not in LR rats when injected into the AL/BLA. This suggests that the amygdala is more sensitive to the anxiolytic effect of adrenergic antagonism in HR rats. Conversely, a beta-adrenergic agonist decreased neophobia in LR rats but not HR rats (Roozendaal and Cools 1994). This suggests that the amygdala of LR rats is more sensitive to anxiolysis induced by this class of drugs. Thus, there is evidence in other genetically different lines or rodents that the amygdala can be involved in mediating differences in sensitivity to varying classes of drugs.

The DR mice had a higher baseline level of locomotor activity than DS mice. They were also more sensitive to the anxiolytic effects. This is homologous to the findings in the HR and LR rats., suggesting that there may be a relationship between activity levels and susceptibility to anxiolysis for a variety of anxiolytic treatments. This hypothesis requires experimental testing for verification.

Nevertheless, these studies suggest that a number of related functions of the amygdala are influenced by genetic differences. In these other experiments, the ability

of the amygdala to mediate the effects of a number of drugs has been correlated with genetic and behavioral differences. Further exploration of these differences will be valuable in understanding its role in anxiety.

5.0 Amygdala projections

The involvement of the amygdala in the potentiated startle paradigm has been extensively studied (Davis 1992). It has been demonstrated that projections from the amygdala to the nucleus reticularis pontis caudalis are involved in the augmentation of the startle reflex, a model of "fear." Additionally, it has been hypothesized that projections to the lateral hypothalamus are involved in sympathetic activation, projections to the dorsal motor nucleus of the vagus are involved in parasympathetic activation, projections to the ventral tegmental area are involved in activation of the dopaminergic system, projections to the locus coeruleus activate the adrenergic system, projections to the dorsal lateral tegmental nucleus activate the cholinergic system, and projections to the central grey mediate freezing responses (Davis 1992). It is possible that the projection to the central grey from the amygdala may be more sensitive to BZs in DR mice than in DS mice. This could explain the finding that intra-amygdala injection attenuated the freezing response in the DR mice but not the DS mice. However, it is possible that projections to others areas such as the locus coeruleus or the nucleus accumbens may also be involved in regulating motor activity in response to aversive situations. These multiple possibilities require testing.

For example, are their differences in firing rates of dPAG, dorsal raphe, or ventral tengmental neurons following intra-amygdala infusions in DR and DS mice? If differences were observed, they would be strongly implicated in the anxiolytic differences between these lines, and consequently in the circuitry of anxiety. Alternatively, are their differences in turnover of serotonin or dopamine between DS

and DR mice in various brain areas such as the hippocampus or frontal cortex following intra-amygdala BZ injections?

In addition to direct influences on adrenergic, cholinergic, and dopaminergic systems, the amygdala also has projections to the central grey, various hypothalamic areas, and the entorhinal cortex (Pratt 1992). Lesions of these latter areas have implicated them in the circuitry of anxiety. It is possible that projections to one or all of these areas may influence avoidance of open arms. For now, the projections from the amygdala which may mediate increases in the percent of open arm entries is not known. The difference observed between the DS and DR mice provides an excellent model for testing these possbilities.

A hypothesized circuitry (Figure 17) based on a modification and extension of the Papez circuit has been proposed by Pratt (1992). Anxiety is hypothesized to arise as a result of the operation of this interconnected network of nuclei. The exact relationship between each of the neuroanatomic areas is left undefined in this schematic. It is likely that the amygdala directly interacts with several other areas than those identified in Figure 17. Nevertheless, it is likely that the behavioral observations in anxiety paradigms such as the plus maze are the result of the interaction of numerous areas such as those depicted.

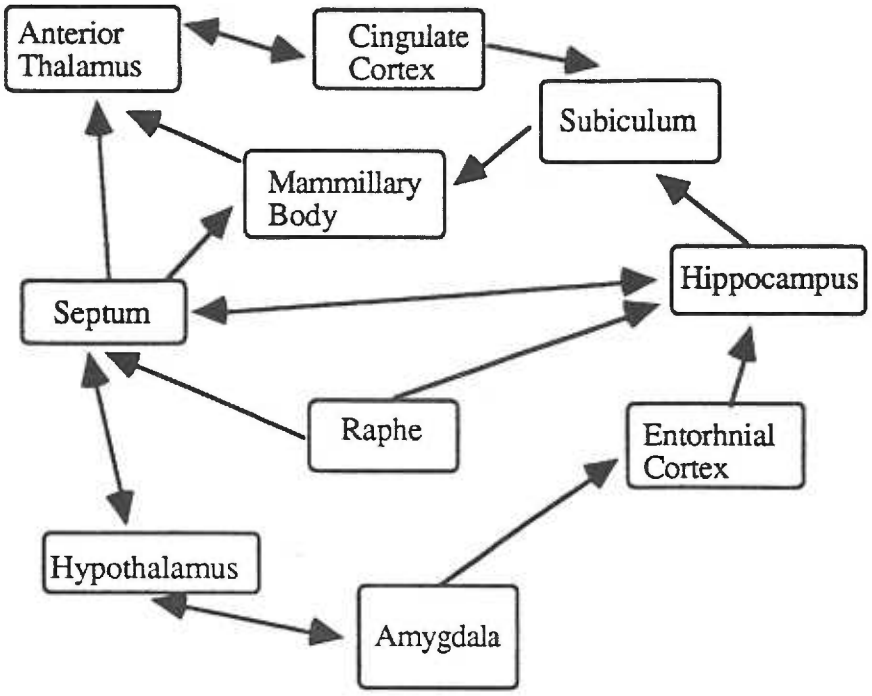

Figure 17. Possible circuitry involved in anxiety. The amygdala has connections with a limbic network. Both lesioning and micro-injections have implicated these structures in anxiety. This figure has been adopted from Pratt (1992)..

An alternative model has been proposed by Graeff et al (1993). This model concentrates more heavily on the amygdala projections to the dPAG and dorsal raphe nucleus, implicating them in anxiety. This proposed circuitry also involves projections from the dorsal raphe nucleus to the dPAG and back to amygdala. In this model, the amygdala evaluates dangerous contexts in conjunction with the hippocampus. Then when threat is determined, the dPAG is activated by the amygdala, and the dPAG then coordinates the appropriate responses; for example, freezing and avoidance. Activation of the dorsal raphe nucleus is hypothesized to inhibit the dPAG, while further activating the amygdala. That is, subjects engage in "anxiety" behaviors other than freezing.

This may modulate the behavioral pattern which is produced by anxiety-provoking situations; for example, freezing or open arm avoidance.

A model proposed by Gray (1982) involves a behavioral inhibition system which is primarily controlled by interactions of the hippocampus and septum. In the face of punishment or decreased reward, associated behavior is attenuated. It is possible that the amygdala may modify this system. Anxiety would reflect a change in the state of this system when danger activates the amygdala.

Of course, the exact circuitry is unkown. However, it is expected that sensitivity to the effect of BZs on the circuitry involved in open-arm avoidance will be greater in DR mice than in DS mice. This expectation can therefore be utilized to examine the physiology of other brain areas following intra-amygdala BZ injections in DS and DR mice. Examination of the effect of BZs on different circuits in these two lines may offer a clue as to which connections of the amygdala are, in fact, involved in plus maze behavior. Continued evaluation of the amygdala differences in the DR and DS mice may lead to answer to this question.

6.0 BZ receptors

Evidence obtained in this study indicates that different neuroanatomic areas mediate different effects of BZs. One purpose of this project has been to initiate a larger project of delineating which areas are involved in which behaviors. Concurrently in other labs, a number of other techniques are being used to identify which receptor subtypes are in particular areas. This information will be of use in understanding the biology underlying specific effects and in designing more specific therapies. The localization of particular subunits in BZ receptors to specific loci is also being combined with information regarding regional differences in binding and efficacy of various BZ ligands. Furthermore, differences in potency and efficacy of various ligands on

behavior is being combined with knowledge about differences in potency and efficacy at specific receptor subtypes. This information will be valuable in evaluating the role of particular neuroanatomic loci in particular behavior effects. A brief review of these studies is presented to provide a perspective on how the functional studies of neuroanatomy will interact with studies of receptors subtypes and receptor localization.

6.1 Receptor localization and receptor types

Using *in situ* hybridization, a number of brain regions have been examined for the expression of 13 different subunits (Araki et al. 1992; Wisden et al. 1992). Immunohistochemistry has also been used to directly assess the level of differing subunits in various areas (Benke et al. 1991c; Hartig et al. 1995; Thompson et al. 1992; Zimprich et al. 1991). Using both techniques, various neuroanatomic areas were demonstrated to express different levels of these subunits. Immunohistochemistry is also capable of determining the location of specific subunits on certain cell types in specific areas. For example, in the dorsal raphe nucleus, serotonergic neurons predominantly express receptors with the α3 subunit while GABAergic neurons express both the α3 and α1 (Gao et al. 1993).

Although the imaging studies determined the levels of the subunits in various neuroanatomical areas, the composition of the actual receptors in the areas has not been resolved. Immunoprecipitation can be used to isolate receptors from various brain areas, and this technique is currently being used for this purpose. This technique requires antibodies which have been created for specific subunits. These are used to isolate receptors containing specific subunits. One early study using this technique found that 63% of benzodiazepine receptors contain the α1 subunit. Receptors containing the α3 subunit came from a separate population of receptors (Benke et al. 1991a). In another study, deglycosylated receptors containing the α3 subunit came in three separate molecular sizes indicating that this subunit occurs in at least three

different receptor types (Buchstaller et al. 1991). A number of different types of α subunits can co-occur in the same receptors, though most receptors contain the α1 subunit (McKernan et al. 1991).

One study has precipitated receptors containing α1, α2, α3, α5, β2,3, γ2, or δ subunits. At least 12 different types of receptors, with different combinations of subunits, were verified to exist in the brain. Again, the most common receptor contained the α1 subunit. Other α subunits were found in discreet cell populations. Five combinations apparently lacked beta subunits and were minor populations (Fritschy and Mohler 1995). One interesting finding of these studies was that splice variants can co-occur in the same receptors. For example the long and short form of the γ2 subunit can co-exist in the same receptor. However, this depended upon the brain region examined (Khan et al. 1994). As these studies proceed, the composition of receptors found in various brain regions will be determined. When the function of BZs in these brain regions is resolved, the behavioral effect of ligands specific for certain receptor types will be predictable. Thus, intrepreting the functional importance of the difference molecular subtypes will be assisted by an understanding of the role of the neuroanatomic nuclei in which the subtypes are found.

6.2 Regional differences in BZ receptors

Consistent with the findings that various loci in the brain contain different types of receptors, a number of studies have found that some ligands have different binding affinities in different brain regions (Luddens et al. 1995; Sieghart and Schlerka 1991). For example, zolpidem inhibition of flunitrazepam has been examined using QAR. Scatchard analyses of this data resolved three different binding sites for this ligand. The percentage of each of these sites varied between neuroanatomical regions (Benavides et al. 1993). High affinity zolpidem binding was found in areas of the brain which are rich in α1, β2, and γ2 subunits (Criswell et al. 1993). Clonazepam

only bound to 60% of the receptors in the striatum to which diazepam bound, and less than 20% of such receptors in the spinal cord (Massotti et al. 1991). These binding differences may be important factors in the different behavioral effects of these compounds (Guidotti et al. 1990).

A number of other regional differences exist in the GABA ionophores. These differences may reflect regional variation in the existence of subtypes of the receptors. For example, there are differences in various brain regions in the ability of bicuculline to affect TBPS binding (Peris et al. 1991). Also, steroids have been observed to increase TBPS binding in some regions of the brain, decrease it in some, and have biphasic effects in others (Sapp et al. 1992). Regional variation has also been found in enhancement of BZ binding by pentobarbital (Carlson et al. 1992) and enhancement of BZ binding by steroids (Friedman et al. 1993). The ability of zolpidem to enhance GABA binding to its receptor also varied, depending on the region examined (Ruano et al. 1993). The ability of ligands to affect GABA or TBPS binding may relate to their functional ability to enhance chloride flux. Therefore, these findings indicate that the various brain regions have functionally distinct receptors.

6.3a Ligands with specific behavioral effects

A number of ligands at the BZ receptor have relatively specific behavior effects (Chen et al. 1996; Gardner 1992). For example, RP59037 and RP60503 are two new compounds which act at the BZ receptor site. Both are anxiolytic in conflict tasks and the plus maze. And both are also capable of protecting against PTZ-induced seizures. However each produces little sedation or myorelaxation (Doble et al. 1993). Other studies have found that while diazepam, bretazenil, and Ro42-8773 have anxiolytic and seizure protective effects, only diazepam results in anterograde amnesia in a passive avoidance task (Martin et al. 1993).

F2692 is another ligand which is reported to have only anxiolytic effects, with little anticonvulsant, sedative, myorelaxant, or amnesic effects (Assie et al. 1993). Apigenin, a naturally occurring substance, acts at the central BZ receptor and has anxiolytic effects but no anticonvulsant effects (Viola et al. 1995). Even when two drugs such as diazepam and temazepam affect a number of behaviors, the potencies are different. Tetrazepam has 1/6th the potency of diazepam for anxiolytic effects and 1/70th the potency for causing LORR (Keane et al. 1988). This suggests that they may have different potencies at different receptor types.

Different types of anxiolytic tasks are also differentially sensitive to various ligands. For example, bretazenil and ZK95962 both increased punished exploratory activity in a four-plate paradigm in mice. However both had weak anxiolytic activity on the plus maze (Jones et al. 1994). Diazepam, midazolam, zolpidem, alpidem, clonazepam, alprazolam, and bretazenil all had anti-conflict effect in a Vogel paradigm. However if conflict was enhanced by co-administration of PTZ, only clonazepam, alprazolam and bretazenil suppressed this enhanced conflict effect. Interestingly, clonazepam and alprazolam are also uniquely capable of treating panic disorders, unlike the other BZs (Giusti et al. 1991).

Abecarnil is a ligand which was found to be relatively specific for reducing anxiety in animal models. This compound is currently being examined in humans as a treatment for generalized anxiety disorder (Cooper and Green 1993; Cooper and Greenwood 1992; Duka et al. 1993; Knoflach et al. 1993; Sanger et al. 1994; Spencer and Benfield 1995; Stephens et al.). The ability of abecarnil to produce anxiolysis while antagonizing the LORR effect of BZs suggests that abecarnil is an agonist at some receptor types, while it is a partial agonist or antagonist at others (Stephens et al. 1990).

The differential effects of other compounds may be species dependent however. For example, DN-2327 is sedative in humans though it is not sedative in animal models (Suzuki et al. 1995). This may indicate that there are important species differences in the effects of different ligands at particular receptor subtypes. This will be an important consideration as new compounds are developed and considered for use as specific treatments. The genetic differences between the DS and DR mice also indicate that within species variation may also influence the "specificity" of BZ ligands.

6.3b Ligand efficacy at different receptor types

There are functional differences for a number of ligands at the BZ receptor subtypes, and these can be associated with their variation in behavioral potencies (Costa and Guidotti 1996; Doble and Martin 1992). For example, bretazenil is less efficacious than diazepam in enhancing chloride flux in receptor combinations having a $\gamma 2$ subunit, regardless of the alpha or beta subunit. However bretazenil is equivalent to diazepam in receptors with $\gamma 1$ or $\gamma 3$ (Puia et al. 1992). These different efficacies at different receptor types may be associated with its different behavioral profile. Zolpidem is more potent and efficacious than diazepam in inhibiting the firing of substantia nigra reticulata cells (Mereu et al. 1990). However diazepam is more efficacious than zolpidem in enhancing cortical chloride flux (Biggio 1989-TBE).

A number of studies have examined the different efficacies of ligands in receptor subtypes *in vitro* (Facklam et al. 1992; Herb et al. 1992; Im et al. 1993; Puia et al. 1992; Sigel et al. 1992; Wafford et al. 1993) (Puia et al. 1991; White and Gurley 1995). In each of these studies, the subunit composition of the BZ receptor greatly affected the function of the ionophore. As the contribution of different subunits to sensitivity to various BZ receptor ligands is resolved, the variation in behavioral effect of different ligands will be determined. This knowledge can then be combined with

what is discovered about the neuroanatomic locations of receptors types and the neuroanatomic locations in which BZs produce various behavior effects.

In summery, a number of projects are proceeding in parallel to understand the complexity of BZ effects. (a) The subunit composition of receptors in various loci is being determined, (b) the efficacy and potency of differing ligands at different types of receptors is being determined, (c) the the efficacy and potency of differing ligands in affecting various behaviors is being determined, and (d) the role of differing anatomic loci in various behaviors is being determined.

7.0 Genetic implications of DS and DR differences

An analysis has been performed with a set of recombinant inbred mice. These mice consist of several inbred strains which were created from crosses between C57/Bl and A/J mice. The strains created from this cross were tested for (a) light/dark exploration, (b) the anxiolytic effect of diazepam in a light/dark model, and (c) sensitivity to convulsant effect of b-CCM, an inverse agonist. Two genetic markers were highly associated with sensitivity to the anxiolytic effects of diazepam: Xmv-41, and D10Mit2. This suggest that genes in these regions influence sensitivity to diazepam-induced anxiolysis. Different chromosomal areas were associated with baseline performance and seizure susceptibility (Mathis et al. 1995).

The DS and DR mice exhibit genetic differences in sensitivity to anxiolysis. It is possible that similar genes may be involved; for example, genes in the regions of Xmv-41 and D10Mit2. A number of strategies are available for determining the genes responsible for differences in the amygdala's ability to mediate anxiolysis (Crabbe et al. 1994). For example, congenic strains can be created (Dudek and Underwood 1993) by using successive generations of backcrosses of DS and DR mice. Using this technique, specific chromosomal segments can be transferred onto a constant genetic

background. The contribution of a particular genetic chromosomal region (such as the area around Xmv-41) to a behavior can, therefore, be examined.

Recombinant inbred strains have also been created between LS and SS mice (Erwin and Jones 1993). In this manner, the segregation of particular genetic areas with specific behavioral phenotypes can be evaluated. Crosses between DS and DR mice could similarly be performed. This would directly test the correlation between particular phenotypes and/or genetic loci. The relationship between sensitivity to intra-amygdala injections and systemic injections could be confirmed.

A number of techniques are also now available in mice for direct alteration of particular genes (Takahashi et al. 1994). "Knockout" mice have been created which do not express functional $\gamma 2$ subunits. These mice are insensitive to a number of effects of BZs (Gunther et al. 1995). Genetic techniques can be used to make more subtle alterations in various subunits. These powerful genetic manipulations can, therefore, be used to further explore the neuroanatomic mechanisms underlying BZ effects.

This project has demonstrated that the amygdala can mediate the differences in sensitivity to anxiolysis in DS and DR mice. The genes responsible for this can be mapped and/or tested using the techniques just mentioned: recombinant inbreds, back crosses, genetic knockouts, directed mutations, etc. Because these techniques often require mice, the relationships between molecular genetics and neuroanatomy requires mice as subjects. With stereotaxic surgery available as a tool for working with mice, these and similar types of studies can proceed.

8.0 Summary

Intra-amygdala injections of CDP produced anxiolysis in DR mice but not DS mice. This was observed as both a reversal of stress-induced freezing, as well as an increase in the percentage entries into the open arm of the plus maze. This result

suggests that the amygdala can mediate the difference in genetic sensitivity between these two lines. The injections appear to be anatomically specific because injections into the ventrolateral caudate did not have this effect. The injections were also behaviorally specific as they did not result in locomotor activation, locomotor sedation, muscle relaxation, ataxia, or protection against seizures produced by PTZ. The ventrolateral caudate also does not mediate these other effects. These results are consistent with the hypothesis that the various effects of BZs are mediated by different anatomic and physiological systems.

Hypothesis	Experiment	Result
H1: The amygdala can mediate anxiolysis	1	yes
	2	yes
Anxiolysis in DR but not DS mice	1	yes
	2	yes
H2: The amygdala does not mediate the other behaviors	1	yes
	2	yes
	3	yes
H3: The caudate mediates anxiolysis	4	no
	5	no
Diffusion is localized	4	yes
	5	yes
H4: The caudate mediates other behaviors	4	no
	5	no

Figure 18. Hypotheses and results. Hypotheses were tested in five experiments. The results of these experiments are summarized here. In experiments 1 and 2, intra-amygdala injections of CDP produced anxiolysis in DR mice but not in DS mice. Other behaviors were not affected, and intra-caudate injections of CDP did not produce effects on any of the behaviors.

These results provide the framework for the initiation of a larger project. Further verification of the involvement of the amygdala in mediating differences in sensitivity to anxiolysis can be accomplished using a number of other genetic techniques available in mice; for example, back-crosses and intercrosses between DS and DR mice. Additionally, the neuroanatomic difference in the amygdala of DR and DS mice can be examined at additional levels; thereby determining correlations between anxiolysis and the cellular physiology, neurochemistry, and circuitry of the amygdala. Furthermore, the technique of stereotaxic surgery followed by behavioral testing has been demonstrated in these mice, and further studies similarly examining other behaviors such as ataxia and locomotor activation can now occur.

Bibliography

Acosta GB, Rubio MC (1994) GABA(A) receptors mediate the changes produced by stress on GABA function and locomotor activity. Neuroscience Letters 176: 29-31

Adolphs R, Tranel D, Damasio H, Damasio AR (1995) Fear and the human amygdala. Journal of Neuroscience 15: 5879-5891

Aggleton JP (1993) The contribution of the amygdala to normal and abnormal emotional states. Trends in Neurosciences 16: 328-333

Agmo A, Galvan A, Heredia A, Morales M (1995) Naloxone blocks the antianxiety but not the motor effects of benzodiazepines and pentobarbital: experimental studies and literature review. Psychopharmacology 120: 186-194

Alda M, Dvorakova M, Posmurova M, Balikova M, Zvolsky P, Filip V (1987) Pharmacogenetic study with diazepam in twins. Neuropsychobiology 17: 4-8

Alho H, Bovolin P, Slobodyansky E (1990) Diazepam binding inhibitor (DBI) processing: immunohistochemical studies in the rat brain. Neurochemical Research 15: 209-16

Alho H, Harjuntausta T, Schultz R, Peltohuikko M, Bovolin P (1991) Immunohistochemistry of Diazepam Binding Inhibitor (DBI) in the Central Nervous System and Peripheral Organs - Its Possible Role as an Endogenous Regulator of Different Types of Benzodiazepine Receptors. Neuropharmacology 30: 1381

Allan AM, Gallaher EJ, Gionet Se, Harris RA (1988) Genetic selection for benzodiazepine ataxia produces functional changes in the gamma-aminobutyric acid receptor chloride channel complex. Brain Research 452: 118-126

Almeida SS, de-Oliveira LM, Bichuette MZ, Graeff FG (1988) Early malnutrition alters the effect of chlordiazepoxide on inhibitory avoidance. Brazilian Journal of Medical & Biological Research 21: 1033-1036

Andrews N, File SE (1993a) Handling History of Rats Modifies Behavioural Effects of Drugs in the Elevated Plus-Maze Test of Anxiety. European Journal of Pharmacology 235: 109-112

Andrews N, File SE (1993b) Increased 5-HT Release Mediates the Anxiogenic Response During Benzodiazepine Withdrawal - A Review of Supporting Neurochemical and Behavioural Evidence. Psychopharmacology 112: 21-25

Araki T, Sato M, Kiyama H, Manabe Y, Tohyama M (1992) Localization of GABAA-Receptor gamma2-Subunit Messenger RNA-Containing Neurons in the Rat Central Nervous System. Neuroscience 47: 45-61

Assie MB, Chopin P, Stenger A, Palmier C, Briley M (1993) Neuropharmacology of a New Potential Anxiolytic Compound, F2692, 1-(3'-Trifluoromethyl Phenyl) 1,

4-Dihydro 3-Amino 4-Oxo 6-Methyl Pyridazine .1. Acute and Invitro Effects. Psychopharmacology 110: 13-18

Audi EA, Graeff FG (1984) Benzodiazepine receptors in the periaqueductal gray mediate anti-aversive drug action. European Journal of Pharmacolology 103: 279-286

Austin MC, Kalivas PW (1990) Enkephalinergic and GABAergic modulation of motor activity in the ventral pallidum. Journal of Pharmacology and Experimental Therapeutics 252: 1370-1377

Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS (1993) Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. Journal of Pharmacology and Experimental Therapeutics 264: 489-495

Barbaccia ML, Berkovich A, Guarneri P, Slobodyansky E (1990) DBI (diazepam binding inhibitor): the precursor of a family of endogenous modulators of GABAA receptor function. History, perspectives, and clinical implications. Neurochemical Research 15: 161-8

Barnard EA (1995) The molecular biology of GABAA receptors and their structural determinants. In: GABAA Receptors and Anxiety: From Neurobiology to Treatment. Biggio G, Serra M, Sanna E, Costa E, Raven Press, New York, pp 1

Bassareo V, Tanda G, Petromilli P, Giua C, Di Chiara G (1996) Non-psychostimulant drugs of abuse and anxiogenic drugs activate with differential selectivity dopamine transmission in the nucleus accumbens and in the medial prefrontal cortex of the rat. Psychopharmacology 124: 293-299

Baude A, Sequier J-M, McKernan RM, Olivier KR, Somogyi P (1992) Differential subcellular distribution of the a6 subunit versus the a1 and b2/3 subunits of the GABAa/benzodiazepine receptor complex in granule cells of the cerebellar cortex. Neuroscience 51: 739-748

Belknap JK, O'Toole LA (1991) Studies of Genetic Differences in Response to Opioid Drugs. Plenum Publishing Corp, Plenum Publishing Corp, New York

Benavides J, Peny B, Ruano D, Vitorica J, Scatton B (1993) Comparative Autoradiographic Distribution of Central omega (Benzodiazepine) Modulatory Site Subtypes with High, Intermediate and Low Affinity for Zolpidem and Alpidem. Brain Research 604: 240-250

Benke D, Cicinsain A, Mertens S, Mohler H (1991a) Immunochemical identification of the alpha1-Subunit and alpha3-Subunit of the GABAA-Receptor in rat brain. Journal of Receptor Research 11: 407-424

Benke D, Mertens S, Trzeciak A, Gillessen D, Mohler H (1991b) GABAA receptors display association of gamma 2-subunit with alpha 1- and beta 2/3-subunits. Journal of Biological Chemistry 266: 4478-83

Benke D, Mertens S, Trzeciak A, Gillessen D, Mohler H (1991c) Identification and immunohistochemical mapping of GABAA receptor subtypes containing the delta-Subunit in rat brain. FEBS Letters 283: 145-149

Beracochea DJ, Krazem A (1991) Effects of mammillary body and mediodorsal thalamic lesions on elevated plus maze exploration. NeuroReport 2: 793-796

Bielajew C, Bushnik T (1994) Diazepam facilitates stimulation-induced feeding in rats. Pharmacology Biochemistry and Behavior 48: 557-561

Biello SM, Harrington ME, Mason R (1991) Geniculo-hypothalamic tract lesions block chlordiazepoxide-induced phase advances in Syrian hamsters. Brain Research 552: 47-52

Biggio G, Concas A, Serra M, Salis M, Corda MG, Nurchi V, Crisponi C, Gessa GL (1984) Stress and b-carbolines decrease the density of low affinity GABA binding sites; An effect reversed by diazepam. Brain Research 305: 13-18

Bixler EO, Kales A, Brubaker BH, Kales JD (1987) Adverse reactions to benzodiazepine hypnotics: Spontaneous reporting system. Pharmacology 35: 286-300

Blanchard DC, Blanchard RJ, Tom P, Rodgers RJ (1990) Diazepam changes risk assessment in an anxiety/defense test battery. Psychopharmacolgoy 101:511-518

Bohlhalter S, Weinmann O, Mohler H, Fritschy J-M (1996) Laminar compartmentalization of GABAA-receptor subtypes in the spinal cord: An immunohistochemical study. Journal of Neuroscience 16: 283-297

Boissier JR, Simon P, Aron C (1968) A new method for rapid screening of minor tranquilizerrs in mice. European Journal of Pharmacology 4:145-151

Boix F, Teruel AF, Escorihuela RM, Tobena A (1990) Handling-habituation prevents the effects of diazepam and alprazolam on brain serotonin levels in rats. Behavioral Brain Research 36: 209-215

Boix F, Teruel AF, Tobena A (1989) The anxiolytic action of benzodiazepines is not present in handling-habituated rats. Pharmacology Biochemistry & Behavior 31: 541-546

Bond AJ, Curran HV, Bruce MS, O'Sullivan G, Shine P (1995) Behavioral aggression in panic disorder after 8 weeks' treatment with alprazolam. Journal of Affective Disorders 35: 117-123

Bondareff W, Routtenberg A, Narotzky R, McLone DG (1970) Intrastriatal spreading of biogenic amines. Experimental Neurology 28: 213-229

Booth DA (1968) Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. Journal of Pharmacology and Experimental Therapeutics 160: 336-348

Brady JV, Nauta WJH (1953) Subcortical mechanisms in emotional behavior: Affective changes following septal forebrain lesions in the albino rat. Journal of Comparative and Physiological Psychology 46: 339-346

Branch RA (1987) Is there increased cerebral sensitivity to benzodiazepines in chronic liver disease? Hepatology 7: 773-776

Brayley KN, Albert DJ (1977) Suppression of VMH-lesion-induced reactivity and aggressiveness in the rat by stimulation of lateral septum, but not medial septum or cingulate cortex. Journal of Comparative and Physiological Psychology 91: 290-299

Brett RR, Pratt JA (1990) Chronic handling modifies the anxiolytic effects of diazepam in the elevated plus-maze. European Journal of Pharmacology 178: 135-138

Brioni JD, Arolfo MP (1992) Diazepam Impairs Retention of Spatial Information Without Affecting Retrieval or Cue Learning. Pharmacology Biochemistry & Behavior 41: 1-5

Brush FR (1991) Genetic determinants of individual differences in avoidance learning: Behavioral and endocrine characteristics. Experientia 47: 1039-1050

Buchstaller A, Fuchs K, Sieghart W (1991) Identification of alpha1-Subunit, alpha-2-Subunit and alpha-3-Subunit isoforms of the GABAA-Benzodiazepine receptor in the rat brain. Neuroscience Letters 129: 237-241

Bureau M, Laschet J, Bureau-Heeren M, Hennuy B, Minet A, Wins P, Grisar T (1995) Astroglial cells express large amounts of GABAa receptor proteins in mature brain. Journal of Neurochemistry 65: 2006-2015

Burke TF, Miller LG, Moerschbaecher JM (1994) Acute effects of benzodiazepines on operant behavior and in vivo receptor binding in mice. Pharmacology Biochemistry and Behavior 48(1):69-76

Burns LH, Robbins TW, Everitt BJ (1993) Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. Behavioural Brain Research 55: 167-183

Caine SB, Heinrichs SC, Coffin VL, Koob GF (1995) Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. Brain Research 692: 47-56

Campbell JL, Sherman AD, Petty F (1980) Diazepam anxiolytic activity in hippocampus. Communications in Psychopharmacology 4: 387-392

Campeau S, Davis M (1995) Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. Journal of Neuroscience 15: 2301-2311

Carlson BX, Mans AM, Hawkins RA, Baghdoyan HA (1992) Pentobarbital-Enhanced <H-3>Flunitrazepam binding throughout the rat brain - an autoradiographic study. Journal of Pharmacology and Experimental Therapeutics 263: 1401-1414

Casamenti F, Deffenu G, Abbamondi AG, Pepeu G (1986) Changes in cortical acetylcholine output induced by modulation of the nucleus basalis. Brain Research Bulletin 16: 689-695

Castanon N, Mormede P (1994) Psychobiogenetics - Adapted Tools for the Study of the Coupling Between Behavioral and Neuroendocrine Traits of Emotional Reactivity. Psychoneuroendocrinology 19: 257-282

Cazala P, David V (1995) Dose-discrimination performance of mice for self-administration of morphine into the lateral hypothalamus. Pharmacology Biochemistry & Behavior 51: 49-55

Chang Y-F, Gao X-M (1995) L-lysine is a barbiturate-like anticonvulsant and modulator of the benzodiazepine receptor. Neurochemical Research 20: 931-937

Charnay Y, Steimer T, Huguenin C, Driscoll P (1995) [H-3] paroxetine binding sites: Brain regional differences between two psychogenetically selected lines of rats. Neuroscience Research Communications 16: 29-35

Chen S, Chen HA, Davies MF, Loew GH (1996) Putative benzodiazepine partial agonists demonstrate receptor heterogeneity. Pharmacology, Biochemistry, & Behavior 53: 87-97

Chikai T, Oishi R, Saeki K (1993) Microdialysis study of the effects of sedative drugs on extracellular histamine in the striatum of freely moving rats. Journal of Pharmacology & Experimental Therapeutics 266: 1277-81

Chopin P, Briley M (1993) The Benzodiazepine Antagonist Flumazenil Blocks the Effects of CCK Receptor Agonists and Antagonists in the Elevated Plus-Maze. Psychopharmacology 110: 409-414

Churchill L, Bourdelais A, Austin MC, Lolait SJ, Mahan LC, O'Carroll A-M, Kalivas PW (1991) GABAa receptors containing a1 and b2 subunits are mainly localized on neurons in the ventral pallidum. Synapse 8: 75-85

Churchill L, Zahm DS, Duffy P, Kalivas PW (1996a) The mediodorsal nucleus of the thalamus in rats--II. Behavioral and neurochemical effects of GABA agonists. Neuroscience 70: 103-112

Churchill L, Zahm DS, Kalivas PW (1996b) The mediodorsal nucleus of the thalamus in rats--I. Forebrain gabaergic innervation. Neuroscience 70: 93-102

Ciraulo DA, Barnhill JG, Ciraulo AM, Greenblatt DJ, Shader RI (1989) Parental alcoholism as a risk factor in benzodiazepine abuse: a pilot study. American Journal of Psychiatry 146:1333-1335.

Coleman K, McGaugh JL (1993) The right and left amygdalae are differentially involved in the expression of memory for an aversively motivated task. Soc. Neuroscience 2: 504.4

Colemanmesches K, Mcgaugh JL (1995) Differential involvement of the right and left amygdalae in expression of memory for aversively motivated training. Brain Research 670: 75-81

Coop CF, McNaughton N, Lambie I (1991) Effects of GABAa and GABAb receptor agonists on reticular-elicited hippocampal rhythmical slow activity. European Journal of Pharmacology 192: 103-108

Cooper SJ, Green AE (1993) The Benzodiazepine Receptor Partial Agonists, Bretazenil (Ro-16-6028) and Ro-17-1812, Affect Saccharin Preference and Quinine Aversion in the Rat. Behavioural Pharmacology 4: 81-85

Cooper SJ, Greenwood SE (1992) The beta-Carboline Abecarnil, a Novel Agonist at Central Benzodiazepine Receptors, Influences Saccharin and Salt Taste Preferences in the Rat. Brain Research 599: 144-147

Costa E (1991) Prolegomena to the Biology of the Diazepam Binding Inhibitor (DBI). Neuropharmacology 30: 1357-1364

Costa E, Buidotti A (1991) Diazepam binding inhibitor (DBI): a peptide with multiple biological actions. Life Sciences 49: 325-44

Costa E, Guidotti A (1996) Benzodiazepines on trial: a research strategy for their rehabilitation. Trends in Pharmacological Sciences 17: 192-200

Costall B, Kelly ME, Naylor RJ, Onaivi ES, Tyers MB (1989) Neuroanatomical sites of action of 5-HT3 receptor agonist and antagonists for alteration of aversive behaviour in the mouse. British Journal of Pharmacology 96: 325-332

Courtney ND, Gallaher EJ (1991) Anxiolytic and activating effects of diazepam in DS and DR mice tested on the plusmaze. Abstract, Society for Neuroscience 17:512

Courtney ND, Jones GE, Gallaher EJ (personal comm.) Inverse segregation of anxiolytic action of diazepam in genetically selected diazepam-sensitive and -resistant mice.

Cowley DS, Roybyrne PP, Greenblatt DJ, Hommer DW (1993) Personality and Benzodiazepine Sensitivity in Anxious Patients and Control Subjects. Psychiatry Research 47: 151-162

Crabbe JC, Belknap JK, Buck KJ (1994) Genetic animal models of alcohol and drug abuse. Science 254: 1715-1723

Crabbe JC, Phillips TJ (1993) Selective Breeding for Alcohol Withdrawal Severity. Behavior Genetics 23: 171-177

Crabbe JC, Phillips TJ, Kosobud A, Belknap JK (1990) Estimation of genetic correlation: Interpretation of experiments using selectively bred and inbred animals. Alcoholism: Clinical and Experimental Research 14: 141-151

Crawley JN (1981) Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. Pharmacology Biochemistry and Behavior 15:695-699

Crawley JN, Davis LG (1982) Baseline exploratory activity predicts anxiolytic responsiveness to diazepam in five mouse strains. Brain Research Bulletin 8: 609-612

Criswell HE, Simson PE, Duncan GE, McCown TJ, Herbert JS, Morrow AL, Breese GR (1993) Molecular basis for regionally specific action of ethanol on gamma-aminobutyric acidA receptors: generalization to other ligand-gated ion channels. Journal of Pharmacology & Experimental Therapeutics 267: 522-537

Cunha DD, Wolfman C, Huang CH, Walz R, Koya R, Bianchin M, Medina JH, Izquierdo I (1990) Effect of post-training injections of flumazenil into the amygdala, hippocampus and septum on retention of habituation and of inhibitory avoidance in rats. Brazilian Journal of Biology Research 23: 301-306

Curran HV (1991) Benzodiazepines, memory and mood: a review. Psychopharmacology 105: 1-8

Curran HV, Birch B (1991) Differentiating the seative, psychomotor and amnesic effects of benzodiazepines: a study with midazolam and the benzodiazepine antagonist, flumazenil. Psychopharmacology 103: 519-523

Czuczwar SJ, Turski L, Kleinrok Z (1982) Anticonvulsant action of phenobarbital, diazepam, carbamazepine, and diphenylhydantoin in the electroshock test in mice after lesion of hippocampal pyramidal cells with intracerebroventricular kaininc acid. Epilepsia 32: 377-382

Da Costa Gomez TM, Behbehani MM (1995) An electrophysiological characterization of the projection from the central nucleus of the amygdala to the periaqueductal gray of the rat: the role of opioid receptors. Brain Research 689: 21-31

Dacunha C, Destein ML, Wolfman C, Koya R, Izquierdo I, Medina JH (1992) Effect of various training procedures on performance in an elevated Plus-Maze - possible relation with brain regional levels of Benzodiazepine-Like molecules. Pharmacology Biochemistry & Behavior 43: 677-681

Dantzer R (1977) Behavioural effects of benzodiazepines: a review. Biobehavioural Reviews 1: 71-86

Dar MS (1995) Antagonism by intracerebellar Ro15-4513 of acute ethanol-induced motor incoordination in mice. Pharmacology Biochemistry & Behavior 52: 217-223

Davidson RJ, Kalin NH, Shelton SE (1993) Lateralized Response to Diazepam Predicts Temperamental Style in Rhesus Monkeys. Behavioral Neuroscience 107: 1106-1110

Davis M (1979) Diazepam and flurazepam: effects on conditioned fear as measured with the potentiated startle paradigm. Psychopharmacolgy 62:1-7

Davis M (1992) The Role of the Amygdala in Fear and Anxiety. Annual Review of Neuroscience 15: 353-375

Davis M, Falls WA, Campeau S, Kim M (1993) Fear-Potentiated Startle - A Neural and Pharmacological Analysis. Behavioural Brain Research 58: 175-198

Dawson GR, Crawford SP, Collinson N, Iverson SD, Tricklebank MD (1995) Evidence that the anxiolytic-like effects of CDP on the elevated plus maze are confounded by increases in locomotor activity. Psychopharmacology 118: 316-323

Dazzi L, Motzo C, Imperato A, Serra M, Gessa GL, Biggio G (1995) Modulation of basal and stress-induced release of acetylcholine and dopamine in rat brain by abecarnil and imidazenil, two anxioselective gamma-aminobutyric acidA receptor modulators. Journal of Pharmacology and Experimental Therapeutics 273: 241-247

de Souza Silva MA, Guimaraes FS, Graeff FG, Tomaz C (1993) Absence of amnestic effect of an anxiolytic 5-HT3 antagonist (BRL 46470A) injected into basolateral amygdala, as opposed to diazepam. Behavioural Brain Research 59: 141-145

de Souza Silva MA, Tomaz C (1995) Amnesia after diazepam infusion into basolateral but not central amygdala of Rattus norvegicus. Neuropsychogiology 32: 31-36

de Wit H (1991) Diazepam preference in males with and without an alcoholic first-degree relative. Alcohol and Clinical Experimental Research 20:593-600

Deboer SF, Katz JL, Valentino RJ (1992) Common Mechanisms Underlying the Proconflict Effects of Corticotropin-Releasing Factor, a Benzodiazepine Inverse Agonist and Electric Foot-Shock. Journal of Pharmacology and Experimental Therapeutics 262: 335-342

DeFries JC (1981) Current perspectives on selective breeding: example and theory. In: NIAAA Research Monograph 6. McClearn GE, Deitrich RA, Erwin VG, Superintendant of Documents, Washington, DC, pp 11-35

Dickinson-Anson H, Mcgaugh JL (1993) Midazolam administered into the amygdala impairs retention of an inhibitory avoidance task. Behavioral and Neural Biology 60: 84-87

Dickinson-Anson H, Mcgaugh JL (1994) Infusion of the GABAergic antagonist bicuculline into the medial septal area does not block the impairing effects of systemically administered midazolam on inhibitory avoidance retention. Behavioral and Neural Biology 62: 253-258

Dickinson-Anson H, Mesches MH, Coleman K, Mcgaugh JL (1993) Bicuculline administered into the amygdala blocks Benzodiazepine-Induced amnesia. Behavioral and Neural Biology 60: 1-4

DiMicco JA (1987) Evidence for control of cardiac vagal tone by benzodiazepine receptors. Neuropharmacology 26: 553-559

Doble A, Canton T, Dreisler S, Piot O, Boireau A, Stutzmann JM, Bardone MC, Rataud J, Roux M, Roussel G, et al (1993) RP 59037 and RP 60503: anxiolytic cyclopyrrolone derivatives with low sedative potential. interaction with the gamma-aminobutyric acidA/benzodiazepine receptor complex and behavioral effects in the rodent. Journal of Pharmacology and Experimental Therapeutics 266: 1213-26

Doble A, Martin IL (1992) Multiple benzodiazepine receptors: no reason for anxiety. Trends in Pharmacological Sciences 13: 76-81

Dudchenko P, Apple C, Conti T, Sarter M (1993) Basal forebrain-lesion induced blockade of the effects of benzodiazepine receptor ligands on vigilance. Soc. Neuroscience 2: 412.10

Dudchenko P, Paul B, Sarter M (1992) Dissociation Between the Effects of Benzodiazepine Receptor Agonists on Behavioral Vigilance and Responsivity. Psychopharmacology 109: 203-211

Dudchenko P, Sarter M (1992) Failure of a Chlordiazepoxide to Reproduce the Behavioral Effects of Muscimol Administered into the Basal Forebrain. Behavioural Brain Research 47: 202-205

Dudek BC, Underwood KA (1993) Selective Breeding, Congenic Strains, and Other Classical Genetic Approaches to the Analysis of Alcohol-Related Polygenic Pleiotropisms. Behavior Genetics 23: 179-189

Duka T, Schutt B, Krause W, Dorow R, Mcdonald S, Fichte K (1993) Human Studies on Abecarnil a New beta-Carboline Anxiolytic - Safety, Tolerability and Preliminary Pharmacological Profile. British Journal of Clinical Pharmacology 35: 386-394

During MJ, Craig JS, Hernandez TD, Anderson GM, Gallager DW (1992) Effect of amygdala kindling on the invivo release of GABA and 5-HT in the dorsal raphe nucleus in freely moving rats. Brain Research 584: 36-44

Ericson H, Kohler C, Blomqvist A (1991) GABA-Like immunoreactivity in the tuberomammillary nucleus - an electron microscopic study in the rat. Journal of Comparative Neurology 305: 462-469

Erwin VG, Jones BC (1993) Genetic Correlations Among Ethanol-Related Behaviors and Neurotensin Receptors in Long Sleep (LS) X Short Sleep (SS) Recombinant Inbred Strains of Mice. Behavior Genetics 23: 191-196

Facklam M, Schoch P, Haefely WE (1992) Relationship Between Benzodiazepine Receptor Occupancy and Potentiation of gamma-Aminobutyric Acid-Stimulated

Chloride Flux Invitro of 4 Ligands of Differing Intrinsic Efficacies. Journal of Pharmacology and Experimental Therapeutics 261: 1106-1112

Faingold CL (1980) Enhancement of mesencephalic reticular neuronal responses to sensory stimuli with pentylenetetrazol. Neuropharmacology 19: 53-62

Faingold CL, Gehlbach G, Travis MA, Caspary DM (1986) Inferior colliculus neuronal response abnormalities in genetically epilepsy-prone rats: evidence for a deficit of inhibition. Life Sciences 39: 869-878

Falconer DS (1983) Introduction to quantitative genetics, 2nd edition edn. Longman, Longman, London

Fanselow MS, Helmstetter FJ (1988) Conditional analgesia, defensive freezing and benzodiazepines. Behavioral Neuroscience 102: 233-243

Farb C, Aoki C, Milner T, Kaneko T, Ledoux J (1992) Glutamate Immunoreactive Terminals in the Lateral Amygdaloid Nucleus - A Possible Substrate for Emotional Memory. Brain Research 593: 145-158

Ferrarese C, Appollonio I, Bianchi G, Frigo M, Marzorati C, Pecora N, Perego M, Pierpaoli C, Frattola L (1993) Benzodiazepine Receptors and Diazepam Binding Inhibitor - A Possible Link Between Stress, Anxiety and the Immune System. Psychoneuroendocrinology 18: 3-22

Ferrarese C, Appollonio I, Frigo M, Piolti R, Tamma F, Frattola L (1989) Distribution of a putative endogenous modulator of the GABAergic system in human brain. Neurology 39: 443-5

Ferrarese C, Mennini T, Pecora N, Pierpaoli C, Frigo M, Marzorati C, Gobbi M, Bizzi A, Codegoni A, Garattini S, Frattola L (1991) Diazepam Binding Inhibitor (DBI) Increases After Acute Stress in Rat. Neuropharmacology 30: 1445-1452

File SE (1983a) Strain differences in mice in the development of tolerance to the anti-pentylenetetrazole effects of diazepam. Neuroscience Letters 42: 95-98

File SE (1983b) Variability in behavioral responses to benzodiazepines in the rat. Pharmacology Biochemistry & Behavior 18: 303-306

File SE (1986) Aversive and appetitive properties of anxiogenic and anxiolytic agents. Behavioral Brain Research 21: 189-194

File SE, Greenblatt DJ, Martin IL, Brown C (1985) Long-lasting anticonvulsant effects of diazepam in diffrent mouse strains: Correlations with brain concentrations and receptor occupancy. Psychopharmacology 86: 137-141

File SE, Hyde JRG (1978) Can social interaction be used to measure anxiety? British Journal of Pharmacology 62:19-24

File SE, Hyde JRG, MacLeod NK (1979) 5,7-Dihydroxytryptamine lesions of dorsal and median raphe nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. Journal of Affective Disorders. 1: 115-122

File SE, Wardill AG (1975) Validity of head-dipping as a measure of exploration in a modified holeboard. Psychopharmacologia 44:53-59

Fink BR, Cairns AM (1984) Diffusional delay in local anesthetic block in vitro. Anesthesiology 61: 555-557

Finlay JM, Damsma G, Fibiger HC (1992) Benzodiazepine-Induced Decreases in Extracellular Concentrations of Dopamine in the Nucleus Accumbens After Acute and Repeated Administration. Psychopharmacology 106: 202-208

Finn DA, Crabbe JC, Merill C, Young E, Lotrich FE, Jones GE, Gallaher EJ (1996) Genetic differences in sensitivity to a neuroactive steroid. Journal of Pharmacology and Experimental Therapeutics iss:page

Fisher RS (1989) Animal models of the epilepsies. Brain Research Reviews 14: 245-278

Flahery CF, Rowan GA (1986) Successive simultaneous and anticipatroy contrast in the consumption of saccharin solutions. Journal of Experimental Psychology 12:381-393

Fleishaker JC, Garzone PD, Chambers JH, Sirocco K, Weingartner H (1995) Comparison of the spectrum of cognitive effects of alprazolam and adinazolam after single doses in healthy subjects. Psychopharmacology 120: 169-176

Flicker C, Geyer MA (1982a) Behavior during hippocampal microinfusions III. Lidocaine versus picrotoxin. Brain Research Reviews 4: 129-136

Flicker C, Geyer MA (1982b) Behavior during hippocampal microinfusions. I. Norepinephrine and diversive exploration. Brain Research Reviews 4: 79-103

Fog R, Pakkenberg H (1971) Behavioral effects of dopamine and p-hydroxyamphetamine injected into corpus striatum of rats. Experimental Neurology 31: 75-86

Ford B, Holmes CJ, Mainville L, Jones BE (1995) GABAergic neurons in the rat pontomesencephalic tegmentum: Codistribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. Journal of Comparative Neurology 363: 177-196

Fraser DD, Madrick-Donnon La, MacVicar BA (1994) Astrocytic GABA receptors. GLIA 11: 83-93

Friedman L, Gibbs TT, Farb DH (1993) g-Aminobutyric acidA receptor regulation: Chronic treatment with pregnanolone uncouples allosteric interactions between steroid and benzodiazepine recognition sites. Molecular Pharmacology 44: 191-197

Friston KJ, Grasby PM, Bench CJ, Frith CD, Cowen PJ, Liddle PF, Frackowiak RSJ, Dolan R (1992) Measuring the Neuromodulatory Effects of Drugs in Man with Positron Emission Tomography. Neuroscience Letters 141: 106-110

Fritschy JM, Mohler H (1995) GABAa-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. Journal of Comparative Neurology 359: 154-194

Frostholm A, Zdilar D, Luntzleybman V, Janapati V, Rotter A (1992) Ontogeny of GABAA/Benzodiazepine receptor subunit mRNAs in the murine inferior olive - transient appearance of beta3 subunit mRNA and <H-3>muscimol binding sites. Molecular Brain Research 16: 246-254

Frye GD, McCown TJ, Breese GR (1983) Characterization of susceptibility to audiogenic seizures in ethanol-dependent rats after microinjection of g-aminobutyric acid (GABA) agonists into the inferior colliculus, substantia nigra, or medial septum. The Journal of Pharmacology and Experimental Therapeutics 227: 663-670

Fukuda S, Iwahara S (1974) Dose effects of chlordiazepoxide upon habituation of open-field behavior in white rats. Psychologia 17:82-90

Gale K (1992a) GABA and epilepsy: Basic concepts from preclinical research. Epilepsia 33: S3-S12

Gale K (1992b) Subcortical structures and pathways involved in convulsive seizure generation. Journal of Clinical Neurophysiology 9: 264-277

Gallaher EJ, Gionet SE (1988) Seizure thresholds in diazepam-sensitive and -resistant mice. Abstract. Society for Neuroscience. 14: 350

Gallaher EJ, Gionet SE, Feller DJ (1991) Behavioral and neurochemical studies in diazepam-sensitive and -resistant mice. [Review]. Journal of Addictive Diseases 10: 45-60

Gallaher EJ, Helms ML, Gionet SE (1992) Genetic selection for replicate lines of diazepam-sensitive and -resistant mice. Neuroscience Abstracts 18: 360

Gallaher EJ, Hollister LE, Gionet SE, Crabbe JC (1987) Mouse lines selected for genetic differences in diazepam sensitivity. Psychopharmacology 93: 25-30

Gao B, Fritschy JM, Benke D, Mohler H (1993) Neuron-Specific Expression of GABA(A)-Receptor Subtypes - Differential Association of the alpha(1)-Subunit and alpha(3)-Subunit with Serotonergic and GABAergic Neurons. Neuroscience 54: 881-892

Gardner CR (1992) A Review of Recently-Developed Ligands for Neuronal Benzodiazepine Receptors and Their Pharmacological Activities. Progress in Neuro-Psychopharmacology & Biological Psychiatry 16: 755-781

Gavish M, Katz Y, Barami S, Weizman R (1992) Biochemical, Physiological, and Pathological Aspects of the Peripheral Benzodiazepine Receptor. Journal of Neurochemistry 58: 1589-1601

Geller I, Seifter J (1960) The effects of meprobamate, barbiturates, d-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 9:482-492

Ghoneim MM, Mewaldt SP (1990) Benzodiazepines and humans memory: A review. Anesthesiology 72: 926-938

Giusti P, Guidetti G, Costa E, Guidotti A (1991) The preferential antagonism of pentylenetetrazole proconflict responses differentiates a class of anxiolytic benzodiazepines with potential antipanic action. Journal of Pharmacology & Experimental Therapeutics 257: 1062-8

Gonzaloruiz A, Sanzanquela JM, Spencer RF (1993) Immunohistochemical Localization of GABA in the Mammillary Complex of the Rat. Neuroscience 54: 143-156

Graeff FG, Silveira MCL, Nogueira RL, Audi EA, Oliveira RMW (1993) Role of the amygdala and periaqudeuctal gray in anxiety and panic. Behavioural Brain Research 58: 123-131

Grahn RE, Kalman BA, Brennan FX, Watkins LR, Maier SF (1995) The elevated plus-maze is not sensitive to the effect of stressor controllability in rats. Pharmacology Biochemistry & Behavior 52: 565-570

Grahn RE, Kalman BA, Watkins LR, Maier SF (1993) Chlordiazepoxide in the dorsal raphe nucleus eliminates the effects of inescapable shock on shuttlebox escape learning and fear conditioning. Abstract.Society for Neuroscience 2: 664.7

Gray JA (1982) Precis of The neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system. The Behavioral and Brain Sciences 5: 469-534

Green S, Hodges H (1991) Animal models of anxiety. In: Behavioral models in psychopharmacology: Theoretical, industrial, and clinical perspectives. Wilner P, Cambridge University Press, New York, pp 21-49

Green S, Vale AL (1992) Role of Amygdaloid Nuclei in the Anxiolytic Effects of Benzodiazepines in Rats. Behavioural Pharmacology 3: 261-264

Greenblatt DJ, Arendt RM, Abernathy DR, Giles HG, Sellers EM, Shader RI (1983) In vitro quantitation of benzodiazepine lipophilicity: relation to in vivo distribution. British Journal of Anaesthesia 55:985

Grishkat HL, Thomas E, Yadin E (1993) Anticonflict Action of Chlordiazepoxide in Rats with Combined Lesions. Physiology & Behavior 54: 1163-1167

Guardiola-lemaitre B, Lenegre A, Porsolt RD (1992) Combined Effects of Diazepam and Melatonin in Two Tests for Anxiolytic Activity in the Mouse. Pharmacology Biochemistry & Behavior 41: 405-408

Guidotti A, Antonacci MD, Giusti P, Massotti M, Memo M, Schlichting JL (1990) The differences in the pharmacological profiles of various benzodiazepine recognition

site ligands may be associated with GABAA receptor structural diversity. In: GABA and Benzodiazepine Receptor Subtypes. Biggio G, Costa E, Raven Press, New York, pp 73-87

Gunther U, Benson J, Benke D, Fritschy JM, Reyes G, Knoflach F, Crestani F, Aguzzi A, Arigoni M, Lang Y (1995) Benzodiazepine-insensitive mice generated by targeted disruption of the gamma 2 subunit gene of gamma-aminobutyric acid type A receptors. Proceedings of the National Academy of Sciences of the United States of America. 92: 7749-7753

Haefly W, Kyburg E, Gerecke M, Mohler H (1985) Recent advances in the molecular pharmacology of benzodiazepine receptors and in structure activity relationships of their agonists and antagonists. Advances in Drug Research 14:165

Halldin C, Farde L, Litton J-E, Hall H, Sedvall G (1992) [11C]Ro 15-4513, a ligand for visualization of benzodiazepine receptor binding. Psychopharmacology 108: 16-22

Handforth A, Ackermann RF (1995) Mapping of limbic seizure progressions utilizing the electrogenic status epilepticus model and the C-14-2-deoxyglucose method. Brain Research Reviews 20: 1-23

Harandi M, Aguera M, Gamrani H, Didier M, Maitre M, Calas A, Beline MF (1987) g-Aminobutyric acid and 5-hydroxytryptamine interrelationship in the rat nucleus raphe dorsalis: Combination of radioautographic and immunocytochemical techniques at light and electron microscopy levels. Neuroscience 21: 237-251

Harris JA, Westbrook RF (1995a) Effects of benzodiazepine microinjection into the amygdala or periaqueductal gray on the expression of conditioned fear and hypoalgesia in rats. Behavioral Neuroscience 109: 295-304

Harris JA, Westbrook RF (1995b) Effects of benzodiazepine microinjection into the amygdala or periaqueductal gray on the expression of conditioned fear and hypoalgesia in rats. Behavioral Neuroscience 109: 295-304

Harrison TS (1961) Some factors influencing thyrotropin release in the rabbit. Endocrinology 68: 466-477

Harro J, Kiivet R-A, Lang A, Vasar E (1990) Rats with anxious or non-anxious type of exploratory behaviour differ in their brain CCK-8 and benzodiazepine receptor characteristics. Behavioural Brain Research 39: 63-71

Hartig W, Brauer K, Fritschy J-M, Brickner G, Bigl V (1995) Regional and cellular expression sites of the a1 subunit of GABAa receptors in the rat basal forebrain: a cytochemical study with glutamic acid decarboxylase, choline acetyltransferase, calcium-binding proteins and nitric oxide synthase as second markers. Brain Research 692: 215-226

Helmstetter FJ (1992a) The Amygdala Is Essential for the Expression of Conditional Hypoalgesia. Behavioral Neuroscience 106: 518-528

Helmstetter FJ (1992b) Contribution of the Amygdala to Learning and Performance of Conditional Fear. Physiology & Behavior 51: 1271-1276

Helmstetter FJ (1993a) Stress-induced hypoalgesia and defensive freezing are attenuated by application of diazepam to the amygdala. Pharmacology Biochemistry & Behavior 44: 433-438

Helmstetter FJ (1993b) Stress-Induced hypoalgesia and defensive freezing are attenuated by application of diazepam to the amygdala. Pharmacology Biochemistry & Behavior 44: 433-438

Henderson ND (1989) Interpreting studies that compare high- and low-selected lines on new characters. Behavior Genetics 19: 473-502

Herb A, Wisden W, Luddens H, Puia G, Vicini S, Seeburg PH (1992) The Third gamma-Subunit of the gamma-Aminobutyric Acid Type-A Receptor Family. Proceedings of the National Academy of Sciences of the United States of America 89: 1433-1437

Higgins GA, Jones BJ, Oakley NR, Tyers MB (1991) Evidence that the amygdala is involved in the disinhibitory effects of 5-HT3-Receptor antagonists. Psychopharmacology 104: 545-551

Hindley SW, Hobbs A, Paterson IA, Roberts MHT (1985) The effects of methyl b-carboline-3-carboxylate on social interaction and locomotor activity when microinjected into the nucleus raphe dorsalis of the rat. British Journal of Pharmacology 86: 753-761

Hodges H, Green S, Glenn B (1987) Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not on discrimination. Psychopharmacology 92: 491-504.

Holley LA, Turchi J, Apple C, Sarter M (1995) Dissociation between the attentional effects of infusions of a benzodiazepine receptor agonist and an inverse agonist into the basal forebrain. Psychopharmacology 120: 99-108

Hollister LE, Mulleroerlinghausen B, Rickels K, Shader RI (1993) Clinical Uses of Benzodiazepines. Journal of Clinical Psychopharmacology 13: S1-S169

Hommer D, Weingartner H, Breier A (1993) Dissociation of Benzodiazepine-Induced Amnesia from Sedation by Flumazenil Pretreatment. Psychopharmacology 112: 455-460

Horger BA, Elsworth JD, Roth RH (1995) Selective increase in dopamine utlization in the shell subdivision of the nucleus accumbens by the benzodiazepine inverse agonist FG 7142. Journal of Neurochemistry 65: 770-774

Hu XJ, Ticku MK (1994) Chronic Benzodiazepine Agonist Treatment Produces Functional Uncoupling of the gamma-Aminobutyric Acid Benzodiazepine Receptor Ionophore Complex in Cortical Neurons. Molecular Pharmacology 45: 618-625

Hutchinson M, Smith PF, Carlington CL (1995) Further evidence on the contribution of GABAa receptors to the GABA-mediated inhibition of medial vestibular nucleus neurones in vitro. NeuroReport 6: 1649-1652

Iadarola MJ, Gale K (1982) Substantia nigra: Site of anticonvulsant activity mediated by gamma-aminobutyric acid. Science 218: 1237-1240

Ida Y, Elsworth JD, Roth RH (1991) Anxiogenic beta-Carboline FG-7142 produces activation of noradrenergic neurons in specific brain regions of rats. Pharmacology Biochemistry & Behavior 39: 791-793

Im HK, Im WB, Hamilton BJ, Carter DB, Vonvoigtlander PF (1993) Potentiation of gamma-Aminobutyric Acid-Induced Chloride Currents by Various Benzodiazepine Site Agonists with the alpha 1 gamma 2,beta 2 gamma 2 and alpha 1 beta 2 gamma 2 Subtypes of Cloned gamma-Aminobutyric Acid Type-A Receptors. Molecular Pharmacology 44: 866-870

Imaki T, Xiao-Quan W, Shibasaki T, Harada S, Chikada N, Takahashi C, Naruse M, Demura H (1995) Chlordiazepoxide attenuates stress-induced activation of neurons, corticotropin-releasing factor (CRF) gene transcription and CRF biosynthesis in the paraventricular nucleus (PVN). Molecular Brain Research 32: 261-270

Imperato A, Dazzi L, Obinu MC, Gessa GL, Biggio G (1994) The benzodiazepine receptor antagonist flumazenil increases acetylcholine release in rat hippocampus. Brain Research 647: 167-171

Imperato A, Puglisi-Allegra S, Zocchi A, Scrocco MG, Casolini P, Angelucci L (1990) Stress activation of limbic and cortical dopamine release is prevented by ICS 205-930 but not by diazepam. European Journal of Pharmacology 75: 211-214

Jackson HC, Nutt DJ (1992) Strain Differences in Sensitivity to the Hypothermic Effects of Benzodiazepine Receptor Ligands in Mice. Psychopharmacology 109: 365-368

Jacquet YF, Lajtha A (1973) Morphine action at central nervous system sites in rat: Analgesia or hyperalgesia depending on site and dose. Science 182: 490-492

Johnson DN, Weingartner HJ, Andreason P, George DT (1995) An effect of triazolam on visual attention and information processing. Psychopharmacology 121: 145-149

Johnson K, Churchill L, Klitenick MA, Hooks MS, Kalivas PW (1996) Involvement of the ventral tegmental area in locomotion elicited from the nucleus accumbens or ventral pallidum. Journal of Pharmacology and Experimental Therapeutics 277: 1122-1131

Jones BE (1993) The organization of central cholinergic systems and their functional importance in sleep-waking states. In: Progress in Brain Research. Cuello AC, Elsevier, pp 61-71

Jones BJ, Paterson IA, Roberts MHT (1986) Microinjections of methyl-b-carboline-3-carboxylate into the dorsal raphe nucleus: Behavioral consequences. Pharmacology Biochemistry & Behavior 24: 1487-1489

Jones DR, Hall SD, Jackson EK, Branch RA, Wilkinson GR (1988) Brain uptake of benzodiazepines: Effect of lipophilicity and plasma protein binding. Journal of Pharmacology and Experimental Therapeutics 245(3):816-822

Jones GH, Schneider C, Schneider HH, Seidler J, Cole BJ, Stephens DN (1994) Comparison of several benzodiazepine receptor ligands in two models of anxiolytic activity in the mouse: an analysis based on fractional receptor occupancies. Psychopharmacology 114: 191-199

Kataoka Y, Shibata K, Gomita Y, Ueki S (1982) The mammillary body is a potential site of antianxiety action of benzodiazepines. Brain Research 241: 374-377

Kataoka Y, Shibata K, Miyazaki A, Inoue Y, Tominaga K, Koizumi k, Ueki S, Niwa M (1991) Involvement of the dorsal hippocampus in mediation of the antianxiety action of tandospirone, a 5-hydroxytryptamine1A agonistic anxiolytic. Neuropharmacology 30: 475-480

Kataoka Y, Shibata K, Yamashita K, Ueki S (1987) Differential mechanisms involved in the anticonflict action of benzodiazepines injected into the central amygdala and mammillary body. Brain Research. 416

Kawaguchi Y, Wilson CJ, Augoog sJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends in Neurosciences 18: 527-535

Keane PE, Bachy A, Morre M, Biziere K (1988) Tetrazepam: A benzodiazepine which dissociates sedation from other benzodiazepine activities. II. In vitro and in vivo interactions with benzodiazepine binding sites. Journal of Pharmacology and Experimental Therapeutics 245: 699-705

Kelsey JE, Vargas H (1993) Medial Septal Lesions Disrupt Spatial, But Not Nonspatial, Working Memory in Rats. Behavioral Neuroscience 107: 565-574

Khan ZU, Gutierrez A, Deblas AL (1994) Short and long form gamma(2) subunits of the GABA(A)/benzodiazepine receptors. Journal of Neurochemistry 63: 1466-1476

Kim JJ, Rison RA, Fanselow MS (1993) Effects of Amygdala, Hippocampus, and Periaqueductal Gray Lesions on Short-Term and Long-Term Contextual Fear. Behavioral Neuroscience 107: 1093-1098

Kim M, Davis M (1993) Electrolytic Lesions of the Amygdala Block Acquisition and Expression of Fear-Potentiated Startle Even with Extensive Training But Do Not Prevent Reacquisition. Behavioral Neuroscience 107: 580-595

King PH, Shin C, Mansbach HH, Chen LS, McNamara JO (1987) Microinjection of a benzodiazepine into substantia nigra elevates kindled seizure threshold. Brain Research 423: 261-268

Kinney GG, Kocsis B, Vertes RP (1995) Injections of muscimol into the median raphe nucleus produce hippocampal theta rhythm in the urethane anesthetized rat. Psychopharmacology 120: 244-248

Kirk RE (1995) Experimental Design: Procedures for the Behavioral Sciences, Third edn. Brooks/Cole Publishing Company, San Fransisco

Kirkness EF, Fraser CM (1993) A strong promotor is located between alternate exons of a gene encoding the human gamma-aminobutyric acid receptor beta 3 subunit (GABAB3). Journal of Biological Chemistry 268: 4420-4428

Knoflach F, Drescher U, Scheurer L, Malherbe P, Mohler H (1993) Full and partial agonism displayed by benzodiazepine receptor ligands at recombinant gamma-aminobutyric acidA receptor subtypes. Journal of Pharmacology & Experimental Therapeutics 266: 385-91

Koenig JA, Martin IL (1992) Effect of Free Fatty Acids on GABAA Receptor Ligand Binding. Biochemical Pharmacology 44: 11-15

Koob GF (1992) Drugs of Abuse - Anatomy, Pharmacology and Function of Reward Pathways. Trends in Pharmacological Sciences 13: 177-184

Kopchia KL, Altman HJ, Commissaris RL (1992) Effects of Lesions of the Central Nucleus of the Amygdala on Anxiety-Like Behaviors in the Rat. Pharmacology Biochemistry & Behavior 43: 453-461

Korpi ER, Kleingoor C, Kettenmann H, Seeburg PH (1993) Benzodiazepine-Induced Motor Impairment Linked to Point Mutation in Cerebellar GABA(A) Receptor. Nature 361: 356-359

Korpi ER, Kuner T, Kristo P, Kohler M, Herb A, Luddens H, Seeburg PH (1994) Small N-terminal deletion by splicing in cerebellar alpha 6 subunit abolishes GABA(A) receptor function. Journal of Neurochemistry 63: 1167-1170

Korpi ER, Seeburg PH (1993) Natural mutation of GABAA receptor alpha 6 subunit alters benzodiazepine affinity but not allosteric GABA effects. European Journal of Pharmacology 247: 23-7

Kostowski W, Plaznik a, Stefanski R (1989) Intra-hippocampal buspirone in animal models of anxiety. European Journal of Pharmacology 168: 393-396

Kumamoto E, Murata Y (1995) Characterization of GABA current in rat septal cholinergic neurons in culture and its modulation by metal cations. Journal of Neurophysiology 71: 2012-2027

Kunkler PE, Hwang BH (1995) Lower GABA(A) receptor binding in the amygdala and hypothalamus of spontaneously hypertensive rats. Brain Research Bulletin 36: 57-61

Lapin IP (1995) Only controls: Effect of handling, sham injection, and ip injection on behavior of mice in an elevated plus-maze. Journal of Pharmacology & Toxicology Methods 34: 73-77

Laurie DJ, Gray AM, Pratt JA (1990) Influence of mamillary body lesions on the effects of diazepam and FG 7142 in the elevated plus-maze test for anxiety. Neuroscience Letters. Suppl. 38: S105

Laurie DJ, Pratt JA (1993) Flumazenil Induces Localised Increases in Glucose Utilization During Diazepam Withdrawal in Rats. Brain Research 631: 277-286

Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92: 180-185

Lomax P (1966) The distribution of morphine following intracerebral microinjection. Experientia 15: 249-250

Lotrich FE, Gallaher EJ (1996) Micropharmacokinetics: A systems dynamics model of drug diffusion and uptake following localized injection into the brain. In: Simulation in the Medical Sciences: Proceedings of the 1996 Wetern Multiconference. Anderson JG, Katzper M, Society for computer simulation, pp 165

Lotrich FE, Gallaher EJ (personal comm.) Comparison of mouse lines bred for differential sensitivity to diazepam: Use of a conditioned suppression of drinking paradigm.

Luddens H, Korpi ER, Seeburg PH (1995) GABAa/benzodiazepine receptor heterogeneity: neurophysiological implications. Neuropharmacology 34: 245-254

Maier SF, Grahn RE, Kalman BA, Sutton LC, Wiertelak EP, Watkins LR (1993) The Role of the Amygdala and Dorsal Raphe Nucleus in Mediating the Behavioral Consequences of Inescapable Shock. Behavioral Neuroscience 107: 377-388

Maier SF, Grahn RE, Maswood S, Watkins LR (1995) The benzodiazepine receptor antagonists flumazenil and CGS8216 block the enhancement of fear conditioning and interference with escape behavior produced by inescapable shock. Psychopharmacology 121: 250-258

Maier SF, Kalman BA, Grahn RE (1994) Chlordiazepoxide microinjected into the region of the dorsal raphe nucleus eliminates the interference with escape responding produced by inescapable shock whether administered before inescapable shock or escape testing. Behavioral Neuroscience 108: 121-130

Majchrzak M, Brailowsky S, Will B (1992) Chronic Infusion of GABA into the Nucleus Basalis Magnocellularis or Frontal Cortex of Rats - A Behavioral and Histological Study. Experimental Brain Research 88: 531-540

Malamed SF, Quinn CL (1995) Sedation: A guide to Patient Management, 3rd edn. Mosby, Chicago

Maleque A, Ahmad K (1977) Brain levels of chlordiazepoxide in rats. Indian Journal of Physiology and Pharmacology 21:94.

Mallet PE, Beninger RJ, Flesher SN, Jhamandas K, Boegman RJ (1995) Nucleus basalis lesions: implication of basoamygdaloid cholinergic pathways in memoryq. Brain Research Bulletin 36: 51-56

Marley RJ, Freund RK, Wehner JM (1988) Differential response to flurazepam in long-sleep and short-sleep mice. Pharmacology Biochemistry & Behavior 31: 453-458

Martin B, Marchaland C, Chapouthier G, Motta R (1994) Evidence for a multigenic system controlling methyl-beta-carboline-3-carboxylate (beta-CCM)-induced seizures. Behavior Genetics 24: 285-297

Martin JR, Schoch P, Jenck F, Moreau JL, Haefely WE (1993) Pharmacological characterization of benzodiazepine receptor ligands with intrinsic efficacies ranging from high to zero. Psychopharmacology 111: 415-422

Mason R, Biello SM, M.E. H (1991) The effects of GABA and benzodiazepines on neurones in the suprachiasmatic nucleus (SCN) of Syrian hamsters. Brain Research 552: 52-57

Massotti M, Schlichting JL, Antonacci MD, Giusti P, Memo M, Costa E, Guidotti A (1991) gamma-Aminobutyric acidA receptor heterogeneity in rat central nervous system: studies with clonazepam and other benzodiazepine ligands. Journal of Pharmacology & Experimental Therapeutics 256: 1154-60

Mathew RJ, Wilson WH, Daniel DG (1985) The effect of nonsedating doses of diazepam on regional cerebral blood flow. Biological Psychiatry 20: 1109-1116

Mathis C, Neumann PE, Gershenfeld H, Paul SM, Crawley JN (1995) Genetic analysis of anxiety-related behaviors and responses to benzodiazepine-related drugs in AXB and BXA recombinant inbred mouse strains. Behavior Genetics 25: 557-568

Mathis C, Paul SM, Crawley JN (1994) Characterization of benzodiazepine-sensitive behaviors in the A/J and C57BL/6J inbred strains of mice. Behavior Genetics 24: 171-80

Mayo W, Dellu F, Cherkaoui J, Chapouthier G, Dodd RH, Le Moal M, Simon H (1992) Cognitive enhancing properties of B-CCM infused into the nucleus basalis magnocellularis of the rat. Brain Research 589: 109-114

McCarthy MM, Felzenberg E, Robbins A, Pfaff DW, Schwartz-Giblin S (1995) Infusions of diazepam and allopregnanolone into the midbrain central gray facilitate open-field behavior and sexual receptivity in female rats. Hormones and Behavior 29: 279-295

McClearn GE, Wilson JR, Meredith W (1970) The use of isogenic and heterogenic mouse stocks in behavioral research. In: Contributions to behavior - genetic analysis - the mouse as a prototype, Appleton-Century-Crofts, New York, pp 3-22

McCrae AF, Gallaher EJ, Winter PM, Firestone LL (1993) Volatile anesthetic requirements differ in mice selectively bred for sensitivity or resistance to diazepam: Implications for the site of anesthesia. Anesthesiology & Analgesia 76: 1313-1317

McKernan RM, Quirk K, Prince R, Cox PA, Gillard NP, Ragan CI, Whiting P (1991) GABAa receptor subtypes immunopurified from rat brain with a subunit-specific antibodies have unique pharmacological properties.

McNamara JO, Galloway MT, Rigsbee LC, Shin C (1984) Evidence implicating substantia nigra in regulation of kindled seizure threshold. Journal of Neuroscience 4: 2410-2417

McNamara RK, Skelton RW (1993a) Effects of intracranial infusions of chlordiazepoxide on spatial learning in the Morris water maze. I. Neuroanatomical specificity. Behavioural Brain Research 59: 175-191

McNamara RK, Skelton RW (1993b) Effects of intracranial infusions of chlordiazepoxide on spatial learning in the Morris water maze. I. Neuroanatomical specificity. Behavioural Brain Research 59: 175-191

McNamara RK, Skelton RW (1993c) Effects of intracranial infusions of chlordiazepoxide on spatial learning in the Morris water maze. II. Neuropharmacological specificity. Behavioural Brain Research 59: 193-204

Melia KR, Davis M (1990) Effects of septal lesions on fear-potentiated startle, and on the anxiolytic effects of buspirone and diazepam. Physiology and Behavior 49: 603-611

Melo LL, Cardoso SH, Brandao ML (1992) Antiaversive action of benzodiazepines on escape behavior induced by electrical stimulation of the inferior colliculus. Physiology and Behavior 51: 557-562

Melzacka M, Nesselhut T, Havemann U, Vetulani J, Kuschinsky K (1985) Pharmacokinetics of morphine in striatum and nucleus accumbens: relationship to pharmacological actions. Pharmacology Biochemistry & Behavior 23: 295-301

Mendelson WB (1990) The search for the hypnogenic center. [Review]. Progress in Neuro Psychopharmacology & Biological Psychiatry 14: 1-12

Mendelson WB, Martin JV (1992) Characterization of the hypnotic effects of triazolam microinjections into the medial preoptic area. Life Sciences 50: 1117-1128

Mendez I, Elisevich K, Flumerfelt BA (1993) GABAergic synaptic interactions in the Substantia-Nigra. Brain Research 617: 274-284

Meng Z-H, Dar MS (1994) Intrastriatal Ro14-4513 functionally antagonizes ethanol-induced motor incoordination and striatal adenosinergic modulation of ethanol-induced motor incoordination in rats. Journal of Pharmacology and Experimental Therapeutics 27: 524-534

Mereu G, Carcangiu G, Concas A, Passino N, Biggio G (1990) Reduction of reticulata neuronal activity by zolpidem and alpidem, two imidazopyridines with high affinity for type I benzodiazepine receptors. European Journal of Pharmacology 179: 339-45

Mereu G, Fanni B, Serra M, Concas A, Biggio G (1983) beta-Carbolines activate neurons in the substantia nigra pars reticulata: an effect reversed by diazepam and Ro15-1788. European Journal of Pharmacology 96: 129-132

Mihic SJ, Vanberckel BNM, Odowd BF, Nguyen T, Wu PH (1992) Effects of Sedatives on GABA-Mediated Chloride Flux into Cerebral Cortical Microsacs Prepared from Emotional and Non-Emotional Mice. European Journal of Pharmacology 218: 283-286

Mihic SJ, Whiting PJ, Klein RL, Wafford KA, Harris RA (1994) A single amino acid of the human gamma-aminobutyric acid type A receptor gamma2 subunit determines benzodiazepine efficacy. Journal of Biological Chemistry 269: 32768-32773

Milani H, Graeff FG (1986) GABA-benzodiazepine modulation of aversion in the medial hypothalamus of the rat. Phramacology Biochemistry & Behavior 28: 21-27

Miller JW, Gray BC, Turner GM (1993) Role of the fastigial nucleus in generalized seizures as demonstrated by GABA agonist microinjections. Epilepsia 34: 973-978

Miller JW, McKeon AC, Ferrendelli JA (1987) Functional anatomy of pentylenetetrazol and electroshock seizures in the rat brainstem. Annals of Neurology 22: 615-621

Minano FJ, Sancho MSM, Sancibrian M, Salinas P, Myers RD (1992) GABA(A) receptors in the amygdala - role in feeding in fasted and satiated rats. Brain Research 586: 104-110

Mirski MA, Ferrendelli JA (1984) Interruption of the mammillothalamic tract prevents seizures in guinea pigs. Science 226: 72-74

Mirski MA, Ferrendelli JA (1987) Interruption of the connections of the mammillary bodies protects against generalized pentylenetetrazol seizures in guinea pigs. The Journal of Neuroscience 7: 662-670

Mirski MA, Ferrendielli JA (1986) Anterior thalamic mediation of generalized pentylenetetrazol seizures. Brain Research 399: 212-223

Modica PA, Tempelhoff R, White PF (1990) Pro- and anticonvulsant effects of anesthetics (Part II) [see comments]. [Review]. Anesthesia & Analgesia 70: 433-44

Mogenson GJ, Swanson LW, Wu M (1983) Neuronal projections from nucleus accumbens to globus pallidus, substantia innominata and lateral preoptic-lateral hypothalamic area: an anatomical and electrophysiological investigation in the rat. Journal of Neuroscience 3: 189-202

Mohler H, Benke D, Benson J, Juscher B, Fritschy JM (1995) GABAA-receptor subtypes in vivo: Cellular localization, pharmacology and regulation. In: GABAA Receptors and Anxiety: From Neurobiology to Treatment. Biggio G, Serra M, Sanna E, Costa E, Raven Press, New York, pp 41

Montgomery KC (1958) The relation between fear induced by novel stimulation and exploratory behaviour. Journal of Comparative and Physiological Psychology 48: 254-260

Montgomery RB, Singer G (1969) Histochemical fluorescence as an index of spread of centrally applied neurochemicals. Science 165: 1031-1032

Moss SJ, Smart TG, Blackstone CD, Huganir RL (1992) Functional Modulation of GABA(A) Receptors by cAMP-Dependent Protein Phosphorylation. Science 257: 661-665

Muir JL, Robbins TW, Everitt BJ (1992) Disruptive Effects of Muscimol Infused into the Basal Forebrain on Conditional Discrimination and Visual Attention - Differential Interactions with Cholinergic Mechanisms. Psychopharmacology 107: 541-550

Myers RD, Tytell M, Kawa A, Rudy T (1971) Micro-injection of 3H-acetylcholine, 14C-serotonin and 3H-norepinephrine into the hypothalamus of the rat: Diffusion into tissue and ventricles. Physiology and Behavior 7: 743-751

Nagy J, Zambo K, Desci L (1979) Anti-anxiety action of diazepam after intraamygdaloid application in the rat. Neuropharmacology 18: 573-576

Nevins ME, Nash SA, Beardsley PM (1993) Quantitative grip strength assessment as a means of evaluating muscle relaxation in mice. Psychopharmacology 110: 92-96

Nishikawa T, Scatton B (1986) Neuroanatomical site of the inhibitory influence of anxiolytic drugs on central serotonergic transmission. Brain Research 371: 123-132

Nomikos GG, Spyraki C (1988) Effects of ritanserin on the rewarding properties of d-amphetamine, morphine and diazepam revealed by conditioned place preference in rats. Pharmacology Biochemistry & Behavior 30: 853-858

Nusser Z, Roberts JDB, Baude A, Richards JG, Sieghart W, Somogyi P (1995) Immunocytochemical localization of the a1 and b2/3 subunits of the GABAa receptor in relation to specific GABAergic synapses in the dentate gyrus. European Journal of Neuroscience 7: 630-646

Nutt DJ, Lister RG (1988) Strain differences in response to a benzodiazepine receptor inverse agonist (FG 7142) in mice. Psychopharmacology 94: 435-436

O'Brien DP, White FJ (1987) Inhibition of non-dopamine cells in the ventral tegmental area by benzodiazepines: relationship to A10 dopamine cell activity. European Journal of Pharmacology 142: 343-354

Ohno M, Yamamoto T, Watanabe S (1992) Intrahippocampal Injections of Benzodiazepine and Muscimol Impair Working Memory But Not Reference Memory of Rats in the 3-Panel Runway Task. European Journal of Pharmacology 219: 245-251

Osborne PG, Mataga N, Onoe H, Watanabe Y (1993) Behavioral Activation by Stimulation of a GABAergic Mechanism in the Preoptic Area of Rat. Neuroscience Letters 158: 201-204

Pare D, Smith Y (1993) Distribution of GABA Immunoreactivity in the Amygdaloid Complex of the Cat. Neuroscience 57: 1061-1076

Pei Q, Zetterstrom T, Fillenz M (1989) Both systemic and local administration of benzodiazepine agonists inhibit the in vivo release of 5-HT from ventral hippocampus. Neuropharmacology 28: 1061-1066

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods 14: 149-167

Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus maze: a novel test of anxiety in the rat. Pharmacology Biochemistry & Behavior 24: 524-529

Perillo MA, Garci DA, Arce A (1995) Partitioning of 1,4-benzodiazepines into natural membranes. Molecular Membrane Biology 12(2):217

Peris J, Shawley A, Dawson R, Abendschein KH (1991) Regulation of S-35-TBPS binding by bicuculline is region specific in rat brain. Life Sciences 49: PL49-PL54

Perrault G, Morel E, Sanger DJ, Zivkovic B (1990) Differences in pharmacological profiles of a new generation of benzodiazepine and non-benzodiazepine hypnotics. European Journal of Pharmacology 187: 487-94

Pesold C, Treit D (1992) Excitotoxic Lesions of the Septum Produce Anxiolytic Effects in the Elevated Plus-Maze and the Shock-Probe Burying Tests. Physiology & Behavior 52: 37-47

Pesold C, Treit D (1994) The septum and the amygdala differentially mediate the anxiolytic effects of benzodiazepines. Brain Research 638: 295-301

Pesold C, Treit D (1995) The central and basolateral amygdala differentially mediate the anxiolytic effects of benzodiazepines. Brain Research 671: 213-221

Peterson EN, Braestrup C, Scheel-Kruger J (1985) Evidence that the anticonflict effect of midazolam in amygdala is mediated by the specfic benzodiqazepine receptors. Neurosci. Lett 53: 285-288

Phillips TJ, Gallaher EJ (1992) Locomotor Responses to Benzodiazepines, Barbiturates and Ethanol in Diazepam-Sensitive (DS) and Diazepam-Resistant (DR) Mice. Psychopharmacology 107: 125-131

Piercey MF, Hoffmann WE, Cooper M (1991) The hypnotics triazolam and zolpidem have identical metabolic effects throughout the brain - implications for benzodiazepine receptor subtypes. Brain Research 554: 244-252

Pivac N, Pericic D (1993) Inhibitory effect of diazepam on the activity of the Hypothalamic-Pituitary-Adrenal axis in female rats. Journal of Neural Transmission - General Section 92: 173-186

Pollard GT, Howard JL (1988) Effects of chlordiazepoxide, pentobarbital, buspirone, chlorpromazine, and morphine in the stretched attend posture (SAP) test. Psychopharmacology 94:433-434

Polster MR, Gray PA, O'Sullivan G, McCarthy RA, Park GR (1993a) Comparison of the sedative and amnesic effects of midazolam and propofol. British Journal of Anaesthesia 70: 612-6

Polster MR, Mccarthy RA, Osullivan G, Gray PA, Park GR (1993b) Midazolam-Induced amnesia - implications for the Implicit/Explicit memory distinction. Brain and Cognition 22: 244-265

Poschel BPH (1971) A simple and specific screen for benzodiazepine-like drugs. Psychopharmacology 19:193

Pratt JA (1992) The neuroanatomical basis of anxiety. Pharmacology & Therapeutics 55: 149-181

Puia G, Ducic I, Vicini S, Costa E (1992) Molecular Mechanisms of the Partial Allosteric Modulatory Effects of Bretazenil at gamma-Aminobutyric Acid Type-A Receptor. Proceedings of the National Academy of Sciences of the United States of America 89: 3620-3624

Puia G, Vicini S, Seeburg PH, Costa E (1991) Influence of recombinant g-aminobutyric Acid-A receptor subunit composition on the action of allosteric modulators of g-aminobutyric acid-gated Cl- currents. Molecular Pharmacology 39: 691-696

Quinlan JJ, Gallaher EJ, Firestone LL (1993) Halothane's Effects on GABA-Gated Chloride Flux in Mice Selectively Bred for Sensitivity or Resistance to Diazepam. Brain Research 610: 224-228

Quirk GJ, Repa C, LeDoux JE (1995) Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: Parallel recordings in the freely behaving rat. Neuron 15: 1029-1039

Quock RM, Nguyen E (1992) Possible Involvement of Nitric Oxide in Chlordiazepoxide Induced Anxiolysis in Mice. Life Sciences 51: PL255-PL260

Quock RM, Wetzel PJ, Maillefer RH, Hodges BL, Curtis BA, Czech DA (1993) Benzodiazepine Receptor-Mediated Behavioral Effects of Nitrous Oxide in the Rat Social Interaction Test. Pharmacology Biochemistry and Behavior 46: 161-165

Rago L, Kiivet R-A, Harro J, Pold M (1988) Behavioral differences in an elevated plus-maze: correlation between anxiety and decreased number of GABA and benzodiazepine receptors in mouse cerebral cortex. Naunyn-Schmiedeberg's Archives of Pharmacology 337: 675-678

Rammsayer T (1992) Effects of Benzodiazepine-Induced Sedation on Temporal Processing. Human Psychopharmacology - Clinical and Experimental 7: 311-318

Rang HP, Dale MM (1991) Pharmacology, 2nd edn. Churchill Livingstone, Churchill Livingstone, New York

Ray A, Henke PG, Sullivan RM (1989) Effects of intra-amygdalar thyrotropin releasing hormone (TRH) and its antagonism by atropine and benzodiazepines during stress ulcer formation in rats. Pharmacology Biochemistry & Behavior 36: 597-601

Remiszewska M, Jastrzebski Z, Czyzewskaszafran H, Wutkiewicz M, Czarnecki A (1992) Increased Activity of the GABAergic System in Selected Brain Areas After Chronic Propranolol Treatment in Spontaneously Hypertensive Rats. Biochemical Pharmacology 44: 465-470

Rex A, Marsden CA, Fink H (1993) Effect of Diazepam on Cortical 5-HT Release and Behaviour in the Guinea-Pig on Exposure to the Elevated Plus Maze. Psychopharmacology 110: 490-496

Ribak CE (1992) Local Circuitry of GABAergic Basket Cells in the Dentate Gyrus. Epilepsy Research : 29-47

Rich JB, Brown GG (1992) Selective dissociations of sedation and amnesia following ingestion of diazepam. Psychopharmacology 106: 346-50

Richens A, Mercer AJ, Jones DM, Griffiths A, Marshall RW (1993) Effects of zolpidem on saccadic eye movements and psychomotor performance: a double-blind, placebo controlled study in healthy volunteers. British Journal of Clinical Pharmacology 36: 61-5

Rigdon GC, Pirch JH (1984) Microinjection of procaine or GABA into the nucleus basalis magnocellularis affects cue-elicited unit responses in the rat frontal cortex. Experimental Neurology 85: 283-296

Rimon R, Lepola U, Jolkkonen J, Halonen T, Riekkinen P (1995) Cerebrospinal fluid gamma-aminobutyric acid in patients with panic disorder. Biological Psychiatry 38: 737-741

Roberts RC (1981) Current perspectives on selective breeding: theoretical aspects. In: NIAAA Research Monograph 6. McClearn GE, Deitrich RA, Erwin VG, Superintendant of Documents, Washington, DC, pp 37-58

Rodgers RJ, Johnson NJT (1995) Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacology Biochemistry & Behavior 52: 297-303

Roehrs T, Merlotti L, Rosenthal L, Roth T (1993) Benzodiazepine Associated Reversal of the Effects of Experimental Sleep Fragmentation. Human Psychopharmacology - Clinical and Experimental 8: 351-356

Roozendaal B, Cools AR (1994) Influence of the noradrenergic state of the nucleus accumbens in basolateral amygdala mediated changes in neophobia of rats. Behavioral Neuroscience 108: 1107-1118

Roozendaal B, Koolhaas JM, Bohus B (1992) Central Amygdaloid Involvement in Neuroendocrine Correlates of Conditioned Stress Responses. Journal of Neuroendocrinology 4: 483-489

Roozendaal B, Koolhaas JM, Bohus B (1993) Posttraining Norepinephrine Infusion into the Central Amygdala Differentially Enhances Later Retention in Roman High-Avoidance and Low-Avoidance Rats. Behavioral Neuroscience 107: 575-579

Rosen JB, Hamerman E, Sitcoske M, Schulkin J, Glowa JR (1996) Hyperexcitability: Exaggerated fear-potentiated startle produced by partial amygdala kindling. Behavioral Neuroscience 110: 34-50

Rosenberg HC, Tietz EI, Chiu TH (1991) Differential tolerance to the antipentylenetetrazol activity of benzodiazepines in flurazepam-treated rats. Pharmacology Biochemistry & Behavior 39: 711-716

Rosenberg HC, Tietz EI, Zhang H, Chiu TH (1990) Tolerance to diazepam and methyl-beta-carboline-3-carboxylate measured in substantia nigra of benzodiazepine tolerant rats. Life Sciences 46: 519-25

Roth M (1989) Anxiety disorders and the use and abuse of drugs. Journal of Clinical Psychiatry 50: 30-35

Routtenberg A, Sladek J, Bondareff W (1968) Histochemical fluorescence after application of neurochemicals to caudate nucleus and septal area in vivo. Science 161: 272-274

Ruano D, Benavides J, Machado A, Vitorica J (1993) Regional Differences in the Enhancement by GABA of <H-3>zolpidem Binding to omega-1 Sites in Rat Brain Membranes and Sections. Brain Research 600: 134-140

kusso AS, Guimaraes FS, Deaguiar JC, Graeff FG (1993) Role of benzodiazepine receptors located in the dorsal periaqueductal grey of rats in anxiety. Psychopharmacology 110: 198-202

Sainati SM, Lorens SA (1982a) Intra-raphe benzodiazepines enhance rat locomotor activity: Interactions with GABA. Pharmacology Biochemistry & Behavior 18: 407-414

Sainati SM, Lorens SA (1982b) Intra-raphe muscimol induced hyperactivity depends on ascending serotonin projections. Pharmacology Biochemistry & Behavior 17: 973-986

Sakurai-Yamashita Y, Kataoka Y, Yamashita K, Miyazaki A, Ushio M, Mine K, Niwa M, Ueki S (1989) Conflict behavior and dynamics of monoamines of various brain nuclei in rats. Neuropharmacology 28: 1067-1073

Salmon P, Tsaltas E, Gray JA (1989) Disinhibition by propranolol and chlordiazepoxide of nonrewarded lever-pressing in the rat is unaffected by dorsal noradrenergic bundle lesion. Neuropharmacology 28: 207-10

Sananes CB, Davis M (1992) N-Methyl-D-Aspartate Lesions of the Lateral and Basolateral Nuclei of the Amygdala Block Fear-Potentiated Startle and Shock Sensitization of Startle. Behavioral Neuroscience 106: 72-80

Sanchez MM, Aguado F, Sancheztoscano F, Saphier D (1995) Adrenalectomy alters the response of neurons in the bed nucleus of the stria terminalis to electrical stimulation of the medial amygdala. Brain Research Bulletin 36: 63-69

Sanders SK, Sheckhar A (1995) Anxiolytic effects of chlordiazepoxide blocked by injection of GABAA and benzodiazepine receptor antagonists in the region of the anterior basolateral amygdala of rats. Biological Psychiatry 37: 473-476

Sanders SK, Shekhar A (1991) Blockade of GABAA Receptors in the Region of the Anterior Basolateral Amygdala of Rats Elicits Increases in Heart Rate and Blood Pressure. Brain Research 567: 101-110

Sandkuhler J, Maisch B, Zimmermann M (1987) The use of local anaesthetic microinjections to identify central pathways: a quantitative evaluation of the time course and extent of the neuronal block. Experimental Brain Research 68: 168-178

Sanger DJ (1984) Chlordiazepoxide-induced hyperphagia in rats: lack of effect of GABA agonists and antagonists. Psychopharmacology 84: 388-392

Sanger DJ (1994) Animal Models of Anxiety and the Screening and Development of Novel Anxiolytic Drugs. Humana Press Inc, Crescent Manor/PO Box 2148/Clifton/NJ 07015

Sanger DJ, Benavides J, Perrault G, Morel E, Cohen C, Joly D, Zivkovic B (1994) Recent developments in the behavioral pharmacology of benzodiazepine (omega) receptors: Evidence for the functional significance of receptor subtypes. Neuroscience and Biobehavioral Reviews 18: 355-372

Santiago M, Westerink BHC (1992) The role of GABA receptors in the control of nigrostriatal dopaminergic neurons: dual-probe microdialysis study in awake rats. European Journal of Pharmacology 219: 175-181

Sapp DW, Witte U, Turner DM, Longoni B, Kokka N, Olsen RW (1992) Regional variation in steroid anesthetic modulation of <S-35>TBPS binding to gamma-Aminobutyric Acid(A) receptors in rat brain. J Pharmacol Exp Ther 262: 801-808

Sarter M, Steckler T (1989) Spontaneous exploration of a 6-arm radial tunnel maze by basal forebrain lesioned rats: effects of the benzodiazepine receptor antagonist b-carboline ZK 93426. Psychopharmacology 98: 193-202

Scheel-Kruger J, Petersen EN (1982) Anticonflict effect of the benzodiazepines mediated by a gabaergic mechanism in the amygdala. European journal of pharmacology 82: 115-116

Schroeder RL, Weinger MB, Vakassian L, Koob GF (1991) Methylnaloxonium diffuses out of the rat brain more slowly than naloxone after direct intracerebral injection. Neuroscience Letters 121: 173-177

Schubert P, Teschemacher H, Kreutzberg GW, Herz A (1970) Intracerebral distribution pattern of radioactive morphine and morphine-like drugs after intraventricular and intrathecal injection. Histochemie 22: 277-288

Shandra AA, Godlevskii LS, Mazarati AM, Makul'kin RF (1991) Role of the substantia nigra in antiaggressive and anticonvulsant effects of diazepam during pharmacological kindling. Neirofiziologiya 22: 482-485

Shandra AA, Godlevskii LS, Mazarati AM, Makulkin RF (1990) Role of the substantia nigra in antiaggressive and anticonvulsant effects of diazepam during pharmacological kindling. Neurophysiology-Engl Tr 22: 356-359

Shekhar A (1993) GABA Receptors in the Region of the Dorsomedial Hypothalamus of Rats Regulate Anxiety in the Elevated Plus-Maze Test .1. Behavioral Measures. Brain Research 627: 9-16

Shekhar A, Hingtgen JN, DiMicco JA (1987) Selective enhancement of shock avoidance responding elicited by GABA blockade in the posterior hypothalamus of rats. Brain Research 420: 118-128

Shekhar A, Hingtgen JN, DiMicco JA (1990) GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. Brain Research 512: 81-88

Shekhar A, Katner JS (1995) Dorsomedial hypothalamic GABA regulates anxiety in the social interaction test. Pharmacology Biochemistry & Behavior 50: 253-258

Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT (1994) Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. Psychopharmacology 116:56-64

Shibata K, Kataoka Y, Gomita Y, Ueki S (1982) Localization of the site of the anticonflict action of benzodiazepines in the amygdaloid nucleus of rats. Brain Research 234: 442-446

Shibata S, Yamashita K, Yamamoto E, Ozaki T, Ueki S (1989) Effects of benzodiazepine and GABA antagonists on anticonflict effects of antianxiety drugs injected into the rat amygdala in a water-lick suppression test. Psychopharmacology 98: 38-44

Short TG, Forest P, Galletty DC (1987) Paradoxical reactions to benzodiazepines--a genetically determined phenomenon? Aneasthesia and Intensive Care 15: 330-331

Shumsky JS, Lucki I (1994) Differential regulation of the behavioral effects of chlordiazepoxide. Psychopharmacology 115: 37-45

Sieghart W, Item C, Buchstaller A, Fuchs K, Hoger H, Adamiker D (1993) Evidence for the Existence of Differential O-Glycosylated alpha5-Subunits of the gamma-Aminobutyric Acid(A) Receptor in the Rat Brain. Journal of Neurochemistry 60: 93-98

Sieghart W, Schlerka W (1991) Potency of several type I-benzodiazepine receptor ligands for inhibition of [3H] flunitrazepam binding in different rat brain tissues. European Journal of Pharmacology 197: 103-107

Sigel E, Baur R, Kellenberger S, Malherbe P (1992) Point mutations affecting antagonist affinity and agonist dependent gating of GABAA receptor channels. Embo Journal 11: 2017-23

Silva-Barrat C, Brailowsky S, riche D, Menini C (1988) Anticonvulsant effects of localized chronic infusions of GABA in cortical and reticular structures of baboons. Experimental Neurology 101: 418-427

Simiand J, Keane PE, Morre M (1984) The staircase test in mice: a simple and efficient procedure for primary screening of anxiolytic agents. Psychopharmacology 84:48-53

Skutella T, Montkowski A, Stohr T, Probst JC, Landgraf R, Holsboer F, Jirikowski GF (1994) Corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide treatment attenuates social defeat-induced anxiety in rats. Cellular and Molecular Neurobiology 14: 579-588

Slobodyansky E, Kurriger G, Kultasilinsky K (1992) Diazepam Binding Inhibitor Processing in the Rhesus Monkey Brain - An Immunocytochemical Study. Journal of Chemical Neuroanatomy 5: 169-180

Slotnik BM, Leonard CM (1975) A Stereotactic Atlas of the Albino Mouse Forebrain. US Department of Health, Education, and Welfare, DHEW publication no. (ADM) 75-100., Rockville, MD

Smith GB, Olsen RW (1995) Functional domains of GABAA receptors. Trends in Pharmacological Sciences 16: 162-168

Soderpalm B, Engel JA (1991) Involvement of the GABAa/benzodiazepine chloride ionophore receptor complex in the 5,7-DHT induced anticonflict effect. Life Sciences 49: 139-153

Soltis RP, DiMicco JA (1991) Interaction of hypothalamic and excitatory amino acid receptors controlling heart rate in rats. American Journal of Physiology 261: R427-R433

Spencer CM, Benfield P (1995) Abecarnil in generalised anxiety disorder: An initial appraisal of its clinical potential. CNS Drugs 3: 69-82

Spyraki C, Fibiger HC (1988) A role for the mesolimbic dopamine system in the reinforcing properties of diazepam. Psychopharmacology 94: 133-137

Spyraki C, Kazandjian A, Varonos D (1985) Diazepam-induced place preference conditioning: appetitive and antiaversive properties. Psychopharmacology 87: 225-232

Stackman RW, Walsh TJ (1992) Chlordiazepoxide-Induced Working Memory Impairments - Site Specificity and Reversal by Flumazenil (RO15-1788). Behavioral and Neural Biology 57: 233-243

Stackman RW, Walsh TJ (1995) Anatomical specificity and time-dependence of chlordiazepoxide-induced spatial memory impairments. Behavioral Neuroscience 109: 436-445

Stephens DN, Schneider HH, Kehr W, Andrew JS, Rettig K-J, Turski L, Schmiechen r, Turner JD, Jensen LH, Petersen EN, Honore T, Hansen JB (1990) Abecarnil, a metabolically stable, anxioselective b-carboline acting at benzodiazepine receptors. Journal of Pharmacology and Experimental Therapeutics 253: 334-343

Stephens DN, Turski L, Hillman M, Turner JD, Schneider HH, Yamaguchi M What Are the Differences Between Abecarnil and Conventional Benzodiazepine Anxiolytics. Raven Press, 1185 Ave of the Americas/New York/NY 10036

Stinchcomb A, Bowers BJ, Wehner JM (1989) The effects of ethanol and Ro 15-4513 on elevated plus-maze and rotarod performance in long-sleep and short-sleep mice. Alcohol 6: 369-376

Stringer L (1995) Pentylenetetrazol causes polysynaptic potentials to appear in the dentate gyrus. Neuroscience 68: 407-413

Stutzmann JM, Delahaye C, Allain H (1993) Zopiclone. Data of experimental pharmacology and clinical use. [Review] [French]. Therapie 48: 33-42

Stutzmann JM, Piot O, Reibaud M, Doble A, Blanchard JC (1992) Pharmacological properties and mechanism of action of the cyclopyrrolones. Encephale 18: 393-400

Sullivan RM, Henke PG, Ray A, Hebert MA, Trimper JM (1989) The GABA/behzodiazepine receptor complex in the central amygdalar nucleus and stress ulcers in rats. Behavioral and Neural Biology 51: 262-269

Suzuki M, Uchiumi M, Murasaki M (1995) A comparative study of the psychological effects of DN-2327, a partial benzodiazepine agonist, and alprazolam. Psychopharmacology 121: 442-450

Suzuki Y, Wang L, Edge J, Mimaki T, Walson PD (1994) Anticonvulsant tolerance to clonazepam in amygdala kindled rats: Clonazepam concentrations and benzodiazepine receptor binding. Neuropharmacology 33: 869-874

Swift R, Davidson D, Rosen S, Fitz E, Camara, P (1998) Natrexone effects on diazepam intoxication and pharmacokinetics in humans. Psychopharmacology 135:256-262

Szymusiak R (1995) Magnocellular nuclei of the basal forebrain: substrates of sleep and arousal regulation. Sleep 18: 478-500

Takahashi JS, Pinto LH, Vitaterna MH (1994) Forward and reverse genetic approaches to behavior in the mouse. Science 264: 1724-1733

Tan S, Kirk RC, Abraham WC, McNaughton N (1990) Chlordiazepoxide reduces discriminability but not rate of forgetting in delayed conditional discrimination. Psychopharmacology 101: 550-554

Taylor SC, Little HJ, Nutt DJ, Sellars N (1985) A benzodiazepine agonist and contragonist have hypothermic effects in rodents. Neuropharmacology 24: 69-73

Tehrani MH, Barnes EM (1995) Reduced function of GABAA receptors in tottering mouse brain: role of cAMP-dependent protein kinase. Epilepsy Research 22: 13-21

Tehrani MHJ, Barnes EM (1994) GABAA receptors in mouse cortical homogenates are phosphorylated by endogenous protein kinase A. Molecular Brain Research 24: 55-64

Teruel AF, Escorihuela RM, Tobena A, Driscoll P (1991) Stress and putative endogenous ligands for benzodiazepine receptors: The importance of characteristics of the aversive situation and of differential emotionality in experimental animals. Experientia 47: 1051-1056

Thiebot M-H, Hamon M, Soubrie P (1982a) Attenuation of induced-anxiety in rats by chlordiazepoxide: role of raphe dorsalis benzodiazepine binding sites and serotoninergic neurons. Neuroscience 7: 2287-2294

Thiebot M-H, Hamon M, Soubrie P (1982b) The involvement of nigral serotonin innervation in the control of punishment-induced behavioral inhibition in rats. Pharmacology Biochemistry & Behavior 19: 225-229.

Thiebot M-H, Soubrie P, Hamon M, Simon P (1984) Evidence against the involvement of serotonergic neurons in the anti-punishment activity of diazepam in the rat. Psychopharmacology 82: 355-359

Thiebot M-H, Soubrie P, Simon P (1985) Is delay of reward mediated by shock-avoidance behavior a critical target for anti-punishment effects of diazepam in rats? Psychopharmacology 87: 473-479

Thomas SR, Lewis ME, Iverson SD (1985) Correlation of [3H]diazepam binding density with anxiolytic locus in the amygdaloid complex of the rat. Brain Research. 342: 85-90

Thompson CL, Bodewitz G, Stephenson FA, Turner JD (1992) Mapping of GABAa receptor a5 and a6 subunit-like immunoreactivity in rat brain. Neuroscience Letters 144: 53-56

Tietz EI, Gomez F, Berman RF (1985) Amygdala kindled seizure stage is related to altered benzodiazepine binding site density. Life Sciences. 36: 183-190

Tobin JM, Lewis ND (1960) New psychotherapeutic agent, chlordiazepoxide. Use in treatment of anxiety states and related syndromes. Journal of the American Medical Association 174: 1242-1249

Todorova A (1993) Effects of Diazepam and the Specific Benzodiazepine Antagonist Flumazenil on Somatosensory Evoked Potentials in Rats. Archives Internationales de Pharmacodynamie et de Therapie 321: 14-29

Tomaz C, Dickinsonanson H, Mcgaugh JL (1991) Amygdala Lesions Block the Amnestic Effects of Diazepam. Brain Research 568: 85-91

Tomaz C, Dickinsonanson H, Mcgaugh JL (1992) Basolateral Amygdala Lesions Block Diazepam-Induced Anterograde Amnesia in an Inhibitory Avoidance Task. Proceedings of the National Academy of Sciences of the United States of America 89: 3615-3619

Tomaz C, Dickinsonanson H, Mcgaugh JL, Souzasilva MA, Viana MB, Graeff FG (1993) Localization in the Amygdala of the Amnestic Action of Diazepam on Emotional Memory. Behavioural Brain Research 58: 99-105

Tong Y, Toranzo D, Pelletier G (1991) Localization of diazepam-binding inhibitor (DBI) mRNA in the rat brain by high resolution in situ hybridization. Neuropeptides 20: 33-40

Travers J, McGaughy J, Sarter M (1993) Attentional effects of infusions of benzodiazepine receptor ligands into the substantia innominata of the basal forebrain. Abstract. Society for Neuroscience 2: 412.9

Treit D (1985) Animal models for the study of anti-anxiety agents: a review. Neuroscience and Biobehavioral Reviews 9:203-222

Treit D, Fundytus M (1989) Thigmotaxis as a test for anxiolytic activity in rats. Pharmacology Biochemistry & Behavior 31:959-962

Treit D, Menard J, Royan C (1993a) Anxiogenic Stimuli in the Elevated Plus-Maze. Pharmacology Biochemistry & Behavior 44: 463-469

Treit D, Pesold C, Rotzinger S (1993b) Dissociating the Anti-Fear Effects of Septal and Amygdaloid Lesions Using Two Pharmacologically Validated Models of Rat Anxiety. Behavioral Neuroscience 107: 770-785

Treit D, Pesold C, Rotzinger S (1993c) Noninteractive Effects of Diazepam and Amygdaloid Lesions in Two Animal Models of Anxiety. Behavioral Neuroscience 107: 1099-1105

Treit D, Pinel JPJ, Fibiger HC (1981) Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacology Biochemistry and Behavior 15:619-626

Tsvetnitsky V, Campbell IC, Gibbons WA (1995) S-Adenosyl-l-homocysteine and 5'-methylthioadenosine inhibit binding of [3H]flunitrazepam to rat brain membranes. European Journal of Pharmacology 282: 255-258

Ungerstedt U, Butcher LL, Butcher SG, Anden N-E, Fuxe K (1969) Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. Brain Research 14: 461-471

Valenzuela CF, Kazlauskas A, Brozowski SJ, Weiner JL, Demali KA, McDonald BJ, Moss SJ, Dunwiddie TV, Harris RA (1995) Platelet-derived growth factor receptor is a novel modulator of type A g-aminobutyric acid-gated ion channels. Molecular Pharmacology 48: 1099-1107

Van Der Kleijn, K (1969) Kinetics of distribution and metabolism of diazepam and chlordiazepoxide in mice. Arch. Int. Pharmacodyn. 178(1):193

Vandekar LD, Piechowski RA, Rittenhouse PA, Gray TS (1991) Amygdaloid lesions - differential effect on conditioned stress and Immobilization-Induced increases in corticosterone and renin secretion. Neuroendocrinology 54: 89-95

Viola H, Wasowski C, Levi de Stein M, Wolfman C, Silveira R, Dajas F, Medina JH, Paladini AC (1995) Apigenin, a component of Matricaria recutita flowers, is a central benzodiazepine receptor ligand with anxiolytic effects. Planta Medica 61: 213-216

Vogel JR, Beer B, Clody DE (1971) A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacology 21:1-7

Wafford KA, Bain CJ, Whiting PJ, Kemp JA (1993) Functional Comparison of the Role of gamma-Subunits in Recombinant Human gamma-Aminobutyric Acid(A)/Benzodiazepine Receptors. Molecular Pharmacology 44: 437-442

Wafford KA, Burnett DM, Leidenheimer NJ, Burt DR, Wang JB, Kofuji P, Dunwiddie TV, Harris RA, Sikela JM (1991) Ethanol sensitivity of the GABAA receptor expressed in Xenopus oocytes requires 8 amino acids contained in the gamma 2L subunit. Neuron 7: 27-33

Wahlsten D et al (1975) Implications of genetic variation in mouse brain structure for electrode placement by stereotaxic surgery. Journal of Comparitive Neurology 162:519

Wang JB, Burt DR (1991) Differential expression of two forms of GABAa receptor g2-subunit in mice. Brain Research Bulletin 27: 731-735

Wansbrough SR, White PF (1993) Sedation scales: measures of calmness or somnolence? [editorial]. Anesthesia & Analgesia 76: 219-21

Watkins LR, Wiertelak EP, Maier SF (1993) The Amygdala Is Necessary for the Expression of Conditioned But Not Unconditioned Analgesia. Behavioral Neuroscience 107: 402-405

Weerts EM, Miller LG, Hood KE, Miczek KA (1992) Increased GABA(A)-Dependent Chloride Uptake in Mice Selectively Bred for Low Aggressive Behavior. Psychopharmacology 108: 196-204

Weiland NG, Orchinik M (1995) Specific subunit mRNAs of the GABAa receptor are regulated by progesterone in subfields of the hippocampus. Molecular Brain Research 32: 271-278

Weingartner HJ, Sirocco K, Rawlings R, Joyce E, Hommer D (1995) Dissociations in the expression of the sedative effects of triazolam. Psychopharmacology 119: 27-33

Weng XJ, Rosenberg HC (1992) Infusion of bicuculline methiodide into the tectum - model specificity of proconvulsant and anticonvulsant actions. Epilepsy Res 12: 1-8

White G, Gurley D (1995) Benzodiazepine site inverse agonists can selectively inhibit subtypes of the GABAA receptor. NeuroReport 6: 1313-1316

Wilks L, File SE, Martin IL (1987) Evidence of strain differences in GABA-benzodiazepine coupling. Psychopharmacology 93: 127-132

Willick ML, Kokkinidis L (1995) The effects of ventral tegmental administration of GABAA, GABAB and NMDA receptor agonists on medial forebrain bundle self-stimulation. Behavioural Brain Research 70: 31-36

Wirtshafter D, Klitenick MA, Asin KE (1988) Is dopamine involved in the hyperactivity produced by injections of muscimol into the median raphe nucleus? Pharmacology Biochemistry & Behavior 30: 577-583

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The Distribution of 13-GABAA Receptor Subunit Messenger RNAs in the Rat Brain .1. Telencephalon, Diencephalon, Mesencephalon. Journal of Neuroscience 12: 1040-1062

Wong PT, Teo WL (1990) Diazepam sensitive mice: differential sensitivity to the depressant and anticonvulsant effects of diazepam. Life Sciences 47: 1519-25

Wong PT-H, Yoong YL, Gwee MCE (1986) Marked variation in diazepam sensitivity in swiss albino mice. Life Sciences 39: 945-952

Wood PL (1986) Pharmacological evaluation of GABAergic and glutamatergic inputs to the nucleus basalis - cortical and the septal-hippocampal cholinergic projections. Canadian Journal of Physiology and Pharmacology 64: 325-328

Woods JH, Winger G (1995) Current benzodiazepine issues. Psychopharmacology 118: 107-115

Wright IK, Upton N, Marsden CA (1992a) Effect of established and putative anxiolytics on extracellular 5-HT and 5-HIAA in the ventral hippocampus of rats during behaviour on the elevated X-Maze. Psychopharmacology 109: 338-346

Wright IK, Upton N, Marsden CA (1992b) Effect of established and putative anxiolytics on extracellular 5-HT and 5-HIAA in the ventral hippocampus of rats during behaviour on the elevated X-Maze. Psychopharmacology 109: 338-346

Yadin E, Thomas E, Strickland CE, Grishkat HL (1991) Anxiolytic effects of benzodiazepines in amygdala-Lesioned rats. Psychopharmacology 103: 473-479

Yamashita K, Kataoka Y, Miyazaki A, Shibata K, Tominaga K, Ueki S (1989a) A key role of the mammillary body in mediation of the antianxiety action of zopiclone, a cyclopyrrolone derivative. Japanese Journal of Pharmacology 51: 438-442

Yamashita K, Kataoka Y, Shibata K, Ozaki T, Miyazaki A, Kagoshima M, Ueki S (1989b) Neuroanatomical substrates regulating rat conflict behavior evidenced by brain lesioning. Neuroscience Letters 104: 195-200

Yeh HH, Grigorenko EV (1995) Deciphering the native GABAa receptor: is there hope? Journal of Neuroscience Research 41: 567-571

Yoshida M, Nagatsuka Y, Muramatsu S, Niijima K (1991) Differential roles of the caudate nucleus and putamen in motor behavior of the cat as investigated by local injection of GABA antagonists. Neuroscience Research 10: 34-51

Young WS, Kuhar MJ (1980) Radiohistochemical localization of benzodiazepine receptors in rat brain. Journal of Pharmacology and Experimental Therapeutics 212: 337-346

Zeng X, Xie XH, Tietz EI (1994) Impairment of feedforward inhibition in CA1 region of hippocampus after chronic benzodiazepine treatment. Neuroscience Letters 173: 40-44

Zetterstrom T, Fillenz M (1990) Local administration of flurazepam has different effects on dopamine release in striatum and nucleus accumbens: a microdialysis study. Neuropharmacology 29: 129-134

Zhang H, Rosenberg HC, Tietz EI (1989) Injections of benzodiazepines but not GABA or muscimol into the pars reticulata substantia nigra suppresses pentylenetetrazol seizures. Brain Research 488: 73-79

Zhang H, Rosenberg HC, Tietz EI (1991) Anticonvulsant actions and interaction of GABA agonists and a benzodiazepine in pars reticulata of substantia nigra. Epilepsy Res 8: 11-20

Zimprich F, Zezula J, Sieghart W, Lassmann H (1991) Immunohistochemical localization of the alpha-1, alpha-2 and alpha-3 subunit of the GABAA receptor in the rat brain. Neurosci Lett 127: 125-128