An Exploration of Panic Disorder: The Differentiation Between *fear* and *anxiety* using Diazepam and Citalopram.

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TABLE OF CONTENTS

Introduction……1
The Behavioral Approach……2
Behavioral Inhibition System……..4
Neurological Differences……4
Pharmacological Differences……...5
Conditioned Fear Paradigm……6
Selective 5-HT Reuptake Inhibitors & Panic Disorder…….……...7
Recent Studies……7
Present Study……...8
Methods……..10
Animals……..10
Apparatus……...10
Drugs……..10
Procedure……...11
Training……..11
Testing……11
Analysis of Results……12
Results……14
Discussion……..18
Present Investigation……18
Further Limitations……20
Relevance to Humans……21
Future Research……21
References……24

LIST OF TABLES

Table 1. *Summary of ANOVA for Responses Rates*…….14

Tables 2. *Means and Standard Deviations of Response Rates*……15

LIST OF FIGURES

Figure 1. Average Baseline of Responses per minute……...16

Figure 2. Means and Standard Deviations of Responses per minute……....16

Figure 3. Citalopram Doses vs. Responses per minute…….….17

Figure 4. Diazepam Doses vs. Responses per minute……...17

ABSTRACT

Due to high prevalence rates of panic disorder, a better pharmacological treatment needs to be distinguished. Through the use of the principle of defensive direction, which Perkins et al. (2009, pg. 12617) defines by stating "Anxiety is elicited by threats requiring approach and fear by threats that need not be approached, but simply avoided," and a conditioned fear paradigm via the avoidance schedule, eight male Sprague dawley rates were tested using a within subjects design. Citalopram (administered via cumulative dosing) and Diazepam (administered via the Latin square method) were used as the panicolytic and anxiolytic respectively. Results showed one significant main effect of dose, specifically between the baseline doses and high doses, but no differentiation between the two drugs. These data suggest looking into other ways to measure panicolytics and anxiolytics pre-clinically in rodent model.

Introduction

The lifetime prevalence rates of panic disorder have recently been increasing from 4.7% in 2005 (Kessler R.C., Berglund P., Demler O., Jin R., & Merikangas K.R., 2005) to 6.8% in 2007 (Kroenke K, Spitzer R.L., Williams J.B., Monahan P.O., & Lowe B., 2007). This rise suggests a need for clinical improvement and therefore a better distinction of the disorder from others with respect to pharmacological treatment, especially anxiety. Though there are many distinguishing features between "Anxiety" and "Fear," it is not uncommon to find the words used interchangeably. Even the DSM-IV-TR does not clearly define and differentiate between the two constructs. First, the DSM classifies Panic Disorder with and without Agoraphobia as Anxiety Disorders. Panic Disorder is "characterized by recurrent unexpected Panic Attacks about which there is a persistent concern," (DSM-IV-TR, APA, 2000, p. 430). Furthermore, the DSM-IV-TR (2000, p. 429) defines a Panic attack as a "discrete period in which there is the sudden onset of intense apprehension, fearfulness, or terror, often associated with feelings of impending doom." This definition infers that Panic Disorder is associated with *fear* behavior, which as will be further explored, is not the same as *anxiety* behavior. Second, the DSM explains that Anxiety Disorder is distinguished by the onset of 'anxiety' symptoms, such as increased heart rate and tensing of the muscles, due to an inappropriate stimulus. Those symptoms deal with an organisms preparation to flee, which in evolutionary terms is a behavior associated with *fear*. The problem lies in that each disorder uses the other in its definition; this circular logic is common among many schools of thought within Psychology. A distinction needs to be made objectively between the two in order to provide the most effective treatment.

The Behavioral Approach

Until recently, the analysis of human emotion has been a battle between biology and every school of psychology. From the theories of mind-body dualism sprouted many attempts to capture the reasoning behind why humans feel the way they do. Whether it be Freud's concept of angst or Zajonic's Primacy of Affect, all schools of thought have their reasons (Barlow, 2002). What has failed to be recognized, though, is that all of these basic theories have the same underlying approach: humans are animals, too. In the presence of a threat, whether real or not, people *behave.*

The study of emotion as a behavior is not a new concept. In 1872, Charles Darwin published *The Expression of the Emotions in man and animals* suggesting that "emotions are innate patterns of reaction and responding that have evolved in many life forms because of their functional significance," (Barlow, 2002). Throughout his book he set a precedent in the theory of emotional behavior. Darwin pointed out not only that emotion is present at birth, but also that it is present in every human culture and across many different species. His overall approach was that the fundamental expression of emotion was nonverbal; a smile to a passing friend instead of saying "hello."

In keeping with his natural selection point of view, Darwin argued that the expression of emotion has evolutionary advantages. More specifically, he focused on the concepts of flight/fight/freezing and their functions as "biological survival mechanisms," (Barlow, 2002). For example, consider what happens when an animal is faced with a threat from which they must flee. Among many other physiological changes, the cardiovascular system is activated. Blood vessels constrict so more blood reaches the muscles necessary to move and breathing increases to provide more oxygen to that blood. Pupils constrict for better vision and urination is almost

inevitable. These are the same symptoms associated with fear in humans, clinically referred to as panic attacks. From Darwin's point of view, "fear," as well as many other emotions, are "alarms", while the behavior of fleeing or fighting are "alarm reactions" (Barlow, 2002). Darwin suggested that this type of behavior was evolutionarily favored and that organisms without these tendencies were unable to survive.

Though Darwin met with much criticism from creationists, his theories shaped the development of B.F. Skinner's (1974) radical behaviorism. Skinner states that behavior is lawful; a particular behavior is selected by the consequence of having done it before, similar to Darwinian theory. As for emotion and human defense behavior, the terms *fear* and *anxiety* are not among the common language of behaviorism. Due to the inclusion of private events and the ongoing controversy of the definitions, it seems the topics have been pushed aside awaiting proper research (Dymond & Roche, 2009). Before a differentiation in human defense behavior can be made in a behavioral analytic point of view, the topic of private events much be addressed. It seems that Skinner's (1974) precedent of not analyzing private events "within the skin" has set the stage for behavior analysts to look beyond emotional variables. It must be remembered that Skinner did acknowledge private events, such as anxiety and fear, but they were thought of as dependent variables in contingencies controlling the behavior of humans. Due to this view, he cautiously warned his fellow students to try and avoid using non-observable events to explain observable behavior, (Skinner, 1974). His view has lead to much confusion, criticism, and limitations in behavioral research in relationship to clinical diagnosis and treatment of emotional disorders. With today's technology, however, we can 'observe' much more in terms of the neurology of anxiety and panic disorders.

Behavioral Inhibition System (BIS)

A better differentiation between fear and anxiety can be explained through the definition of each construct. According to the most recent Behavioral Inhibition System (BIS), there are distinctions between fear and anxiety in relationship to function, behavior, and pharmacology. The theory explains that functionally, the fear construct is responsible for moving an animal away from danger while anxiety moves an animal toward danger. Behaviorally, fear involves the fight/flight/freezing mechanism and anxiety increases risk assessment and defensive quiescence (McNaughton, & Corr, 2004). Risk assessment in animals is defined as "orientation toward a potential threat" and defensive quiescence is a state in which an animal is still but ready to defend (Blanchard, et. al., 1997). Lastly, the pharmacological difference is that fear is insensitive to anxiolytics while anxiety is sensitive.

Neurological Differences

Beyond the BIS Theories, neurological differences between fear and anxiety have also been found. Anxiety has been linked to the septo-hippocampal system (McNaughton & Corr, 2004). This concept comes from the behavioral similarities between anxiolytics and hippocampal lesions as well as anxiolytics and hippocampal-sensitive memories. The most consistent evidence has been the positive correlation between changes in hippocampal electrical activity and anxiolytic drugs (McNaughton & Corr, 2004). Panic disorder, however, has been found to be associated with activity at the Peroaqueductal gray as well as the Amygdala. Panic Disorder without agoraphobia is a fear-like response without an association with a conditioned stimulus and it has been found that activiation of the Peroaqueductal gray produces a similar response (Copland & Lydiard, 1998). Additionally, Panic Disorder with Agoraphobia is a fear-like

response with a conditioned stimulus and is thought to be linked to adaptations in the Amygdala caused by stimuli (Copland & Lydiard, 1998).

Pharmacological Differences

Through the use of the principles of the behavior inhibition system, Blanchard et al. (1997) examined pharmacological differences in rats. Specifically, they looked at the differentiation of anxiolytic and panicolytic drugs using rat and mouse defense test batteries. Due to the similarities in defensive behavior among mammals, they assume "the neurobehavioral systems controlling defensive behaviors of laboratory animals such as rats and mice may be, to a considerable degree, homologous with those of humans," (Blanchard et al., 1997, p. 783). Their findings are consistent with the principles of the behavior inhibition system in that the anxiolytic (benzodiazeoines at clinically effective doses) drugs used reduced risk assessment behaviors. Furthermore, the panicolytic (yohimbine) drug was found to reduce flight behaviors.

Blanchard, Griebel, and Blanchard (2003) later published a review with factor analyses confirming the validity of their mouse defense test battery. The factors included cognitive aspects of defensive behaviors, flight responses, defensive threat/attack reactions, and contextual defensiveness in an attempt to model different aspects of human anxiety. They tested these factors against many anxiolytic agents, but were particularly concerned with benzodiazepines. Because Benzodiazapines mimic the action of GABA at $GABA_A$ receptors, chemically unrelated drugs were developed to selectively bind to $GABA_A$ receptor subtypes in hopes to decrease unwanted side effects such as drug dependence, rebound insomnia, and amnesia (Blanchard et al., 2003). First, the effects of nonselective $GABA_A$ –benzodiazepine receptor ligands on the test battery were found to be less effective on defensive behaviors than full, more selective, agonists. In particular, Flumezenil, a partial agonist, had no affect on risk assessment but greatly increased

flight reactions, suggesting that clinical observations of this drug are true in that it "increases the frequency of panic attacks in panic disorder patients," (Blanchard et al., 2003, p. 104). Second, the effects of subtype-selective $GABA_A$–benzodiazepine receptor ligands, particularly the α_1 subtype, found little to no affects on flight measures. These findings suggest that the α_1 subtype receptor is not involved in panic responses. Overall, Blanchard, Griebel, and Blanchard (2003) suggested that their Mouse Defense Test Battery could be used for preclinical drug trials for defense-related disorders.

Conditioned fear paradigm

Another popular way to study panic disorder in preclinical drug trials is through the conditioned fear paradigm. This stems from the original Pavlovian Respondent conditioning. It is a way of learning such that a neutral stimulus is paired with an aversive stimulus in succession to the point that the neutral stimulus elicits the same response the aversive stimulus would have. We then call the neutral stimulus a conditioned stimulus. Neurologically, the amaygdala receives the sensory input of the stimulus and "decides" what autonomic and behavioral responses will occur. Of the many places the amygdala can send information, the locus ceruleus is one and is responsible for increasing norepinehprine and therefore increasing physiological responses associated with behavioral fear. If there is a deficit in the processing pathways reaching the amygdala, the stimulus can be misinterpreted and lead to inappropriate responses of fear by sending misinformation to the locus ceruleus (Gorman, J., Kent, J., Sullivan, G., & Coplan, J., 2000). That is to say if the stimulus has become a conditioned to an aversive event, the amygdala will send the same response for the conditioned stimulus as it would the aversive stimulus. In addition, there is a strong similarity between the autonomic and behavioral responses of panic attacks and the activity of these brain regions in animals undergoing

conditioned fear paradigms (Gorman et al., 2000). This suggests that a conditioned fear procedure is a good measure of panic disorder in preclinical rodent models.

Selective 5-HT reuptake inhibitors & Panic Disorder

Selective 5-HT reuptake inhibitors work by blocking the re-absorption of serotonin at presynaptic cells and causing a buildup of serotonin at the synapse (McKim, 2007). This build up of 5-HT in the locus ceruleus is inhibitory. This means that 5-HT decreases the responses to the locus ceruleus, which produces the physiological fear response suggesting that the serotonergic effect of SSRIs secondarily effect the production of panic disorder symptoms (Gorman et al., 2000). This idea is further supported by a study done by Coplan & Lydiard (1998) showing a decrease in a norepenephrine metabolite after treatment with Fluoexetine, an established SSRI. In addition to affecting the locus ceruleus, 5-HT buildups have been shown to affect the periaqueductal gray region which modulates defense and escape responses. They are also related to a decrease in the corticotrophin-releasing factor which produces cortisol, a neurotransmitter that produces physiological fear responses (Gorman et al., 2000). In addition to the available information on serotonin antagonists, clinical observations have also shown that 5-HT receptor partial agonist mCPP have exacerbated symptoms in patients with panic disorder (Griebel, G., Perrault, G., & Sanger, D., 1997). This further supports the use of SSRIs as a pharmacological treatment for Panic Disorder.

Recent Studies

Recent studies, like the one done by Perkins et al. (2009), use clinically effective drugs to help explain human defense behavior. Specifically, they look at the differences between fear and anxiety through a theoretical principle known as “defensive direction.” Perkins et al. (2009, pg. 12617) defines this principle by stating “Anxiety is elicited by threats requiring approach and

fear by threats that need not be approached, but simply avoided." In 2009, Perkins et al. translated the Mouse Defense Test used by the Blanchards into a computerized version for humans. Using a force-sensing joystick to measure anxiety and an average velocity of the participant's cursor as a measure of fear, the researchers examined the defensive direction principle in humans. Lorazepam (an established anxiolytic benzodiazapem) was hypothesized to affect behavior during approach to threat. Citalopram (an established panicolytic SSRI) was hypothesized to affect behavior during departure from threat. Results showed a significant main effect between drug and risk assessment caused by lorazepam, but no significant main effect was found between drug and flight intensity during departure from threat.

These results provide validation for one part of the defensive direction principle in humans because lorazepam, an anxiolytic, reduced risk assessment relative to the placebo and citalopram did not. However, citalopram did not affect panic behaviors. Perkins et al. (2009) explain that this could be due to the need for several weeks of chronic administration to be effected by SSRIs. More importantly, Citalopram has not been tested using acute administration in rodents. The authors also explain that the absence of citalopram effects may be due to the low level of threat during their study. Due to the differences in brain regions used during low threat and panic reactions, "citalopram, as an established panicolytic, may act more powerfully in the brain systems responsible for mediating intense fear than those that control mild fear," (Perkins et al., 2009). The lack of rodent evidence in regards to citalopram as a panicolytic during high threat situations suggests that more research needs to be done.

Present Study

The goal of the current study is to account for the lack of evidence regarding acute Citalopram effects in rodent models of Panic disorder and establish a way to pre-clinnically

measure panic disorder in rodent models. By training and implementing a conditioned-fear paradigm via the avoidance schedule and making comparisons with the anxiolytic Diazepam and the SSRI/panicolytic Citalopram, a more sophisticated, yes simplified, behavioral approach to the concepts of fear and anxiety will be created. Specifically, the main questions of the study are is there an empirical difference in fear and anxiety? Also, can the administration of acute citalopram in rodent models serve as an appropriate panicolytic drug?

Overall, if the DSM is correct in its definitions, then no differences in avoidant responses in relationships to the two drugs will be found. However, if the defensive direction principle holds true with the neurological information available regarding SSRIs, citalopram (the panicolytic) should decrease the number of avoidant responses while diazepam (the anxiolytic) should have no affect on the responses.

Methods

Animals

Eight female Sprague Dawley rats from the Allegheny College Rat General Colony were used. They were approximately seven months old at the start of treatment and their weights ranged from 276 grams to 318 grams. Their weights were recorded on a daily basis to ensure appropriate administration of saline/drug dose. They were given free access to food and water throughout the entire experiment, except during one continuous reinforcement training session to re-establish lever pressing behavior, in which case they were deprived of water twenty-three hours before the session. Each animal had a history of lever pressing behavior and discrimination training due to prior experience in another lab. The experiment was approved by the Allegheny College Animal Research Committee and abided by all ethical procedures.

Apparatus

A standard Lafayette Instruments Co. operant conditioning chamber (model # 80001) was used. The chamber is equipped with two levers and two stimulus lights. Scheduling of experiments and data collections was done through a Lafayette operant conditioning console (model # 81335).

Drugs

Citalopram hydrobromide (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) was the panicolytic used, obtained from Sigma-Aldrich Co. (St. Louis, MO). The drug was administered through intraperitoneal injections (IP) in a volume of 1 cc/kg. The vehicle used to dissolve the Citalopram hydrobromide was 0.9% saline. The drug was dissolved into concentrations of 1.0 and 2.5 mg/kg on that basis that 10 mg/kg of Citalopram in rats equivalent to 49 mg/day in humans, and that clinical doses are 20-60 mg/day

(Hashimoto et al., 2009). Also, other studies examining 5-HT receptors in combination with discriminative stimuli have used Citalopram doses of 2.5 mg/kg (Millan, M., Girardon, S., & Dekeyne, A., 1998). Saline injections served as the control.

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2(3*H*)-one) was the anxiolytic used, obtained from Sigma-Aldrich Co. (St. Louis, MO). The drug was administered through intraperitoneal injections (IP) in a volume of 1 cc/kg. The vehicle used to dissolve the Lorazepam was 0.9% saline. The three doses used were dissolved into concentrations of 0.025, 0.05 and 2.0 mg/kg (Casarrubea, M., Sorbera, F., Magnusson, M., & Crescimanno, G., 2010). Saline injections served as the control.

Procedure

training. After the animals were obtained, they were introduced to the apparatus after a twenty-three hour water deprivation. For one session, the lever pressing behavior was examined under a continuous reinforcement schedule. During the following session, the avoidance test was introduced. The S^D (light on) and S^Δ (light off) periods alternated for ten minutes. If the lever was not pressed within 5 seconds in the S^D period, a 0.5 second, 0.4mA shock followed (Gilbert, 1971). Reinforcement was provided by removal of the shock via a lever press. Baseline data was collected until each rat showed steady rates of responding and percent errors no longer showed change from session to session.

testing. Rats were weighed before each session. Testing lasted a total of nine days for Diazepam. The first session was a recorded baseline procedure in the Avoidance schedule. The second session introduced saline injections, a pre-test pause of 0.5 hours and ten minutes in the Avoidance schedule. The third session introduced the first drug dose, a pretest pause of 0.5 hours, and ten minutes in the avoidance schedule. This process was repeated three times for

Diazepam as there were three. Pre-test pauses during Diazepam sessions lasted one half hour as that is the peak concentration time for the drug (Ator, N. & Kautz, M., 2000). To avoid order-effects, the Latin square was used. This means that each combination of drug administration was accounted for. Also, because there were only four Skinner boxes available for testing, each random group alternated going first and second.

Unfortunately, the Citalopram was placed on back order and was not delivered in time to do the original Latin square procedure. Therefore, a less time-consuming method of drug administration was used: cumulative dosing. This allowed for both doses of Citalopram to be given in a single day "with each successive dose being larger than the preceding one," (Thompson, D., Moerschbaecher, J., & Winsauer, P., 1983, pp. 175). When compared to non-cumulative dosing, Thompson et al. (1983) found cumulative dosing to have qualitatively similar drug effects on a four-response chain. This method is now widely used in pre-clinical drug trials.

The procedure for the present study was as follows. A ten minute baseline was recorded in the avoidance schedule. Next, saline injections were given, followed by a pre-test pause of forty-five minutes, and ten minutes in the avoidance schedule. The first dose of Citalopram was 1.0 mg/kg, followed by a pre-test pause of forty-five minutes, and ten minutes in the avoidance schedule. The second dose given was 1.5 mg/kg (to give a cumulative dose of 2.5 mg/kg), followed by a shortened pre-test pause of one half hour (for cumulative peak effects of the drug), and finally ten minutes in the avoidance schedule. Pre-test pauses during Citalopram sessions lasted forty-five minutes as that it the peak concentration time (Hashimoto et al., 2009). Data was recorded as responses per minute for both administration methods.

Analysis of Results

Data was reported as frequency of avoidance responses per minute, which is the dependent measure. A 2x3 two-way ANOVA was used. The first independent variable is drug type (panicolytic and anxiolytic), and the second is dose (each drug uses two doses). Due to the differences in methods used for each drug administration, only baseline, high and low doses were analyzed for each drug.

Results

A two-way ANOVA was conducted to examine the effects of drug, dose, and the interaction between the two factors on response rates in the avoidance schedule (N=8). Table 1 displays the two-way ANOVA results which revealed a significant main effect of Dose, $f(2,42)=5.41$, $p<0.05$, but no significant main effect of Drug or a significant interaction between the two factors. In order to examine the significance between doses, a Tukey's HSD test and a Fischer's Protected t-Test were conducted to compare across doses of both drugs. The main effect only accounted for 18% of the variability among response rates.

Table 1.
Summary of ANOVA for Responses Rates

Sources of Variances	*SS*	*df*	*MS*	*F*	$\acute{\eta}^2$
Factors					
Drug (A)	5.45	1	5.45	2.26	
Dose (B)	15.38	2	7.69	5.41*	0.18
AxB	6.06	2	3.03	1.98	
Subjects					
AxS	16.84	7	2.41		
BxS	19.91	14	1.42		
AxBxS	21.42	14	1.53		
Total	85.06	47	21.53		

* $p<0.05$

Figure 1 presents the combined average baseline data to show a trend in steady response rates prior to treatment. Table 2 and Figure 2 display descriptive statistics for both drugs. As expected, the baseline responses rates (M=2.92) are significantly higher than the high dose responses rates (M=1.53). Though no significant differences were found between the low dose responses rates (M=2.31) and the high dose responses rates or between the baseline and the low dose response rates, general trends suggest a decrease in response rates as doses increase.

In regards to Citalopram (See Figure 3), no significant differences were found between baseline response rates (*M*=2.94, *SD*=2.50), low dose responses rates (*M*=2.09, *SD*=1.81), or high dose responses rates (*M*=0.71, *SD*=0.76).

In regards to Diazepam, no significant differences were found between the baseline response rates (*M=2.89, SD*=2.86), the low dose response rates (*M*=2.53, *SD*=2.28) or the high dose response rates (*M*=2.35, *SD*=1.69), though general trends suggest a marginal decrease (See Figure 4).

Table 2.
Means and Standard Deviations of Response Rates (N=8)

	Diazepam		Citalopram		Marginal Means
Dose	M	SD	M	SD	M
Baseline	2.94	2.5	2.89	2.86	2.92
Low	2.09	1.81	2.53	2.28	2.31
High	0.71	0.76	2.35	1.69	1.53
Marginal Means	1.91		2.59		

Note: Higher numbers indicate higher response rates

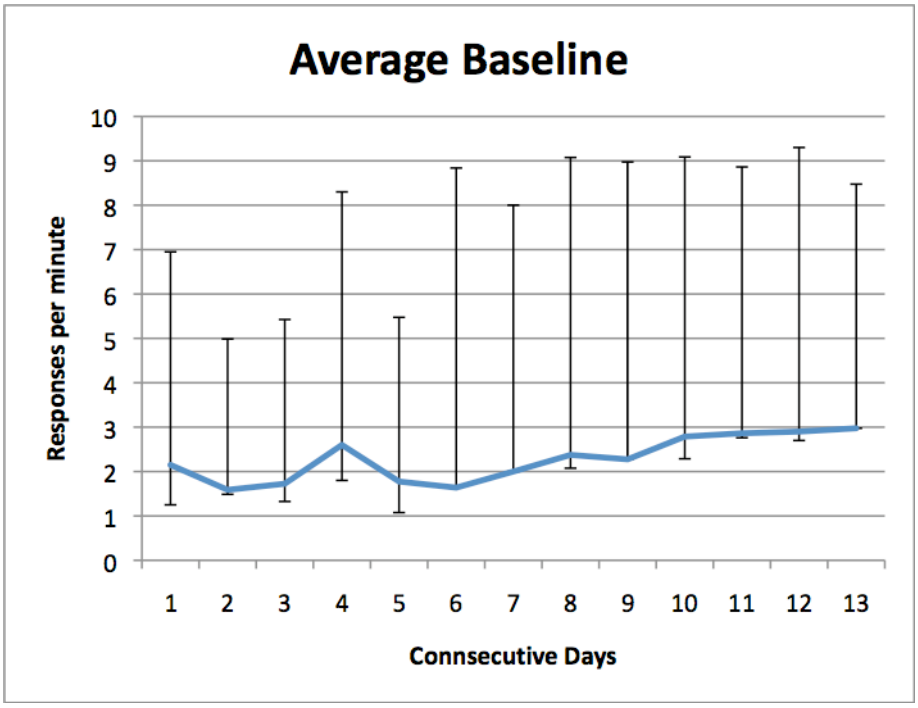
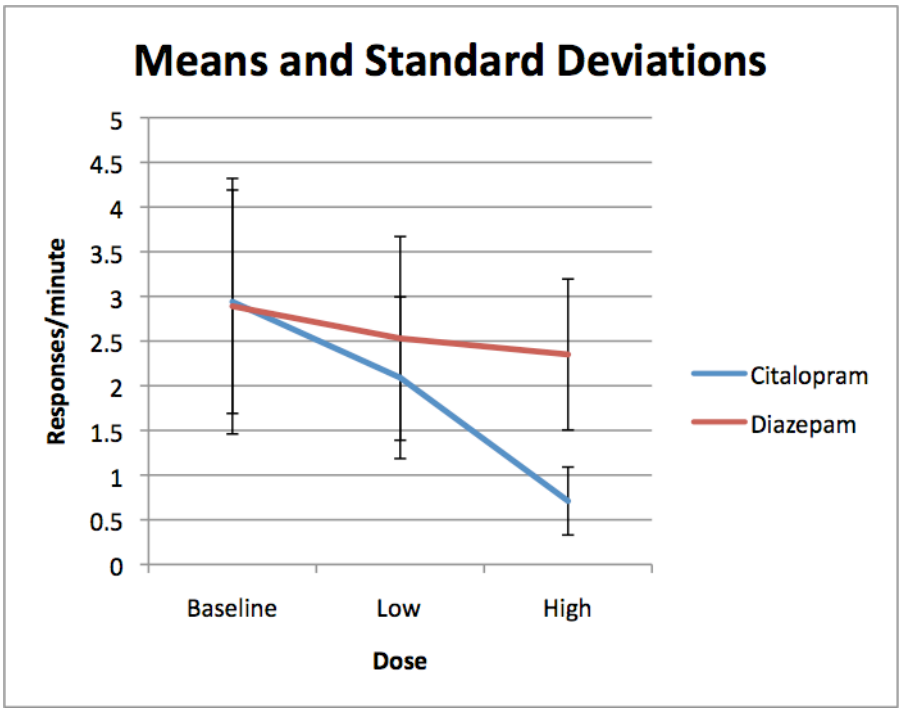


Figure 1. Baseline results of all 8 rats averaged per day. Note: error bars represent a range from highest to lowest individual responses per minute



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Figure 2. Means and Standard Deviations of Response Rates. Note: error bars represent standard deviations.

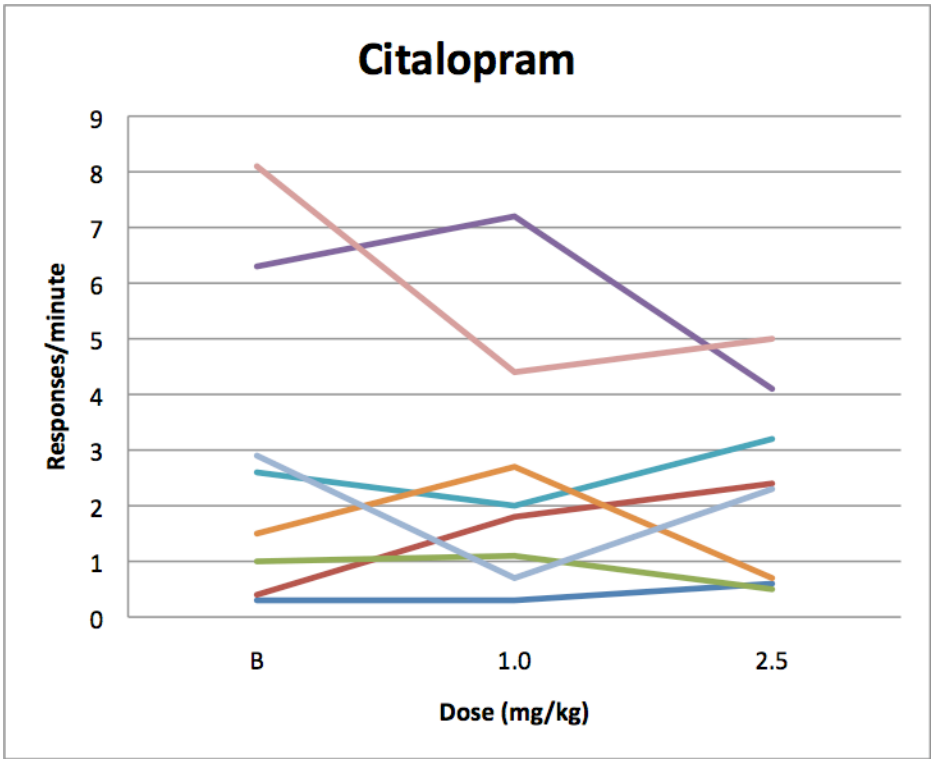


Figure 3. Citalopram response rates. Note: Higher numbers indicate higher response rates.

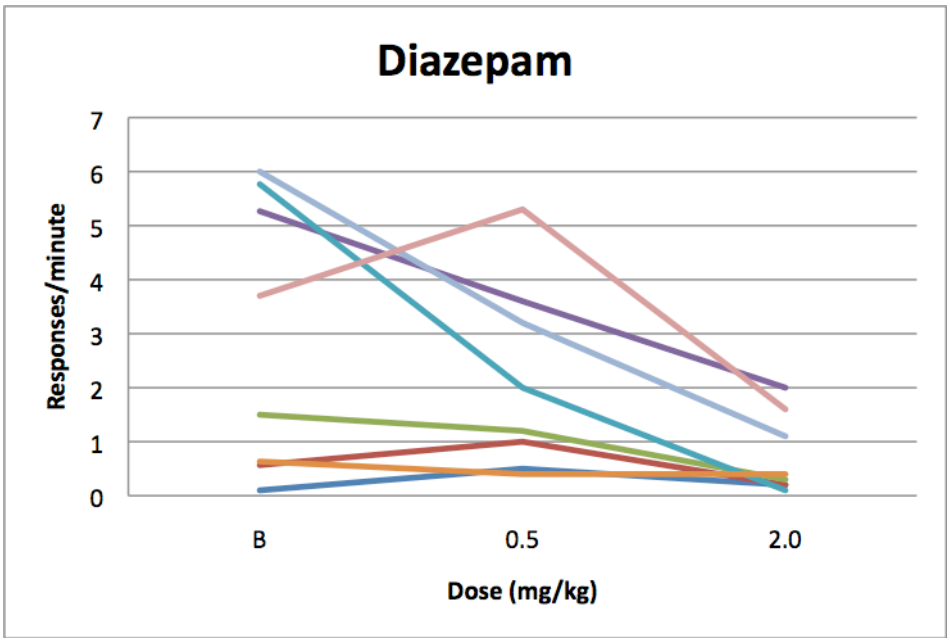


Figure 4. Diazepam response rates. Note: Higher numbers indicate higher response rates.

Discussion

Present Investigation

Overall, one significant main effect of dose was found. Among doses, significant differences were found between baseline and high dose groups, but not between baseline and low dose or low dose and high dose of the Citalopram and Diazepam.

However, the results for Citalopram suggest a small general decrease in responding as dose level increases. Millan et al. (1999) found similar dose-dependent generalization, but their task did not include an aversive stimulus. Results such as these have been seen in other studies using the Avoidance schedule as a paradigm to measure anti-depressant effects of 5-HT reuptake inhibitors. Martin, P., Soubri, P. & Puech, A. (1990) found significant differences in escape responses after 1mg/kg doses in I.P. administered Citalopram ($p<0.05$). However, this experiment used a more chronic administration (three doses per day of testing) suggesting further research should be done with acute Citalopram doses in rats. Research done by Hashimo, et al. (2009) showed a significant decrease ($p<0.05$) in freezing behaviors of rats undergoing a conditioned fear paradigm after chronic administration of 10mg/kg twice daily.

One noticeable objective observation found during Citalopram administration was increased aggressive behaviors of the rats such as chasing and biting at one another. Though they used apomorphin-induced aggression, Matto, V., Allikmets, L., & Skrebuhhova, T., (1998) found similar aggressive behaviors. After repeated studies, they showed that Citlaopram, and other 5-HT reuptake inhibitors of certain subtypes, have mediating effects on induced aggressive behaviors. Further research needs to be done in this area.

In regards to Diazepam, no significant differences were found between doses, but a much larger decrease in responding as dose increased was seen compared to Citalopram. The results

infer that the conditioned-fear paradigm within the avoidance schedule may not be a good measure of distinction between fear and anxiety behaviors, as they are two separate things by evolutionary standards and the principle of defensive direction. Blanchard et al. (1997) found results regarding Diazepam in their Mouse Defense Test Battery such that both acute and chronic administration of the drug had significant anxiolytic effects on risk assessment. By the definition of defensive direction principles, risk assessment is a behavior associated with anxiety and not fear. Very weak effects were found on flight behaviors further suggesting that anxiolytics do not effectively alter fear behaviors.

However, the marginal effect on behavior seen with Diazepam can also be attributed to the sedative and amnesic effects of the drug. Brian, Kusumorini, & Benton, D., (1991) studied anxiety in laboratory rodents and suggested that decreases in responding may simply reflect the sedative and muscle relaxation properties of Benzodiazepines in general. Furthermore, rats undergoing object recognition tasks have shown significant impairment of memory after 0.2 mg/kg I.P. suggesting the possibility of the rats "forgetting" the learned task (Bertaina-Anglade, V., Enjuanes, E., Morillon, D., & Rochelle, C., 2006).

In addition to the marginal effects seen, the difference in administration between the two drugs should also be taken into account. Diazepam experimentation was conducted directly after baseline data collection. Citalopram data was collected after a large time gap due to the delivery date of the drug. Therefore, it is possible that the learning of the task had a significant effect on data collection for both drugs such that when the rats were undergoing Citalopram treatment, they were re-learning the task. This could account for the smaller decrease in responding.

A further reason for caution when interpreting the results comes from the level of threat in the avoidance schedule used. Though not life-threatening, electric shock is a moderate to high

level of threat. Fanselow (1994) compares electric shock in rats to a brief exposure to a cat. This type of fear activates the Peroaqueductal gray region, which as previously discussed is not involved in anxiety behaviors. Therefore, Diazepam may have affected other areas of fear behaviors that were not measured by this paradigm.

Further Limitations

As explained earlier, the difference in drug administration was a large limitation in this study. It is hard to accurately compare the results of the two different methodologies. Furthermore, the avoidance schedule used to mimic a conditioned-fear paradigm may not be the best choice in distinguishing between fear and anxiety behaviors. Griebel et al., (1997, pg. 793) claim that inconsistencies in drug effects may be effected by measured behaviors such that "spontaneous behaviors may be more reliable than findings from tests based on conditioned responses."

Beyond faults with both drug and paradigm, other observational limitations were observed. Throughout both treatments, the rats showed an increase in learning of equipment malfunctions. If, after one shock, the rat held the lever down for the remainder of the scheduled time, the interval would not restart. Therefore, they could avoid ten minutes worth of shocks by taking one and sitting on the lever for the remainder of the time.

Also, prior to the shock, a clicking sound is made. Observations suggested that rats responded more to this sound than the conditioned light itself. This suggests that the sound functioned as another neutral stimulus turned conditioned and may have altered responding. The shock occurred within less than a second after the sound was emitted. Therefore, if the rats were more conditioned to the sound than the light, they had less time to respond despite any drug effects.

Relevance to Humans

Due to the prevalence rates reported at the beginning of this study, research in Panic Disorder treatment is still needed. Kasper & Resinger (2001) did a follow up study of panic disorder patients seven years after diagnosis and reported that 25% "rated themselves as slightly improved, unchanged or worse," (pp.307). They also report a high co-morbidity with depression which decreases treatment outcomes and usefulness of therapy. Furthermore, they report trials comparing the efficacy of Citalopram against placebos in 475 patients with panic disorder. After eight weeks, which is considered a short term study, 60% of patients were symptom free. A significantly higher proportion reported they were free of panic attacks after one year (Kasper & Resinger, 2001).

Kasper & Resinger (2001) further report a study done with thirty six panic disorder patients and 35 generalized anxiety disorder patients treated with Alprazolam, a benzodiazepine similar to Diazepam. Eighty-eight percent of the panic disorder patients withdrew from the study due to physiological withdrawal symptoms triggering re-emergence of their symptoms. There trials suggest that further research needs to be conducted improving Citalopram dosing as well as considering Benzodiazepines as treatment for panic disorder patients.

Future Research

I suggest this experiment be repeated using the both methods of drug administration. Both drugs should be administered via the Latin square method, as that is the most statistically reliable. Both drugs could also be administered using the cumulative dosing methods, but there is little quantitative research to uphold the findings in comparison to other statistically valid techniques. Furthermore, the study could be repeated with each dose being multiplied per rat to give a better comparison of doses versus response rates.

I also suggest more research be done on the administration of each drug. Specifically, the pre-test pauses of each drug as there was much disagreement in the literature regarding maximum effect times. Also, methods of administration (i.e. I.P., subcutaneous, etc.) should be looked into as many discrepancies were also found in the literature as to the best way for rats to receive doses.

If the same paradigm were to be used for distinguishing fear behaviors, I suggest a 5-HT reuptake inhibitor agonist be used. In addition to saline, this would be a better way to measure if the response rates were affected by 5-HT reuptake inhibitors using this paradigm and then further comparisons with other drugs can then be made.

In addition to further research regarding panicolytic-altering behavior evidence, there seems to be a large body of evidence missing in regards to sex differences among the relationship between human defensive behavior and affective disorders regarding fear and anxiety. I believe this lack of evidence may be due to Darwin's struggle to fit the concepts of sexual differences into his natural selection theory. He developed a second evolutionary theory call Sexual Selection (Anderson, 1994). In essence, this theory still supports the concept of natural selection but fails to account for a survival advantage in direct relationship with sexual differences. I think there is one major component missing: the perception of threat.

According to McLean and Anderson (2009), "compared to men, women are more likely to overestimate the probability of danger." This concept may be evolutionarily adaptive in that women would be more likely to protect their offspring. Females do not follow the same patterns of fight or flight, rather they "tend and befriend" to be more safe, less distressed, and to create social networks (McLean & Anderson, 2009). Despite being adaptive, the overestimation of threat poses complications. If women overestimate a threat, they are more likely to engage in

escape and avoidance. This creates a history of fearful situations and could be the explanation of why “a number of self-report studies indicate that women endorse a greater number and severity of fears,” (McLean & Anderson, 2009).

However, there may be an alternative explanation for these indications. If the male duty is to protect both the female and the offspring, they are more likely to approach the threat. Mclean & Anderson (2009) explain that if boys are more regularly encouraged to confront threats and not engage in avoidance behavior, they are probably being reinforced for their “emotional processing of fears.” This concept also explains the theory of gender role socialization such that the expression of fear is less acceptable for males than females; moreover, females are encouraged to express their emotion and are reinforced because of that. Unfortunately, the empirical evidence for these concepts is.

However, recent studies on the relationship between defensiveness and metabolic syndrome may give some explanation to female defensiveness. Levesque et al. (2009) conducted research regarding the impact of sex and age on defensiveness and metabolic syndrome. Distinguished by avoidance of perceived threats, defensiveness is defined as a personality trait and measured using a social desirability scale. Metabolic syndrome (MS) is defined by multiple dysfunctions in blood pressure, weight, and other areas of the body, and is a risk factor for cardiovascular disease and premature death (Levesque et al., 2009). Overall, their results showed that men and young women who received low scores of defensiveness were more likely to have an increased risk of MS.

In conclusion, further research should be done in regards to how to properly distinguish between anxiety and fear behaviors, how to study them pharmacologically, and the treatment of panic disorder versus anxiety disorder in relationship to sex differences.

References

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text revision). Washington, DC.

Anderson, M. B. (1994). *Sexual Selection.* Princeton, N.J.: Princeton University Press.

Ator, N. A., and Kautz, M. A. (2000). Differentiation benzodiazepine and barbiturate like discriminative stimulus effects of lorazepam, diazepam, pentobarbital, imidazenil, and zalopen in two-versurs-three lever procedures. *Behavioral Pharmacology 11*: 1-14

Barlow, D. H. (2002) *Anxiety and its disorders: the nature of anxiety and panic.* Retrieved from http://books.google.com

Bertaina-Anglade, V., Enjuanes, E., Morillon, D., & Rochelle, C. (2006). The object recognition task in rats and mice: a simple and rapid model in safety pharmacology to detect amnesic properties of a new chemical entity. *Journal of Pharmacological and Toxicological Methods, 54*, 99-105.

Blanchard, D. C., Griebel, G., % Blanchard, R. J. (2003). The mouse defense test battery: pharmacological and behavioral assays for anxiety and panic. *European Journal of Pharmacology, 463*, 97-116. doi:10.1016/S0014-2999(03)01276-7.

Blanchard, R. J., Griebek, G., Henrie, J. A., & Blanchard, D. C. (1997). Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. *Neuroschience and Biobehavioral Revieews, 21*(6), 783-798.

Brian, P., Kusumorini, N., & Benton, D. (1991). Anxiety in laboratory rodent: a breif review of some recent behavioural developments. *Behavioural Processes, 25*, 71-80.

Casarrubea, M., Sorbera, F., Magnusson, M., and Crescimanno, G. (2010). T-pattern analysis of diazepam-induced modifications on the temporal organization of rat behavioral response to anxiety in hole board. *Psycholpharmacology.* doi: 10.1007/s00213-010-2123-1.

Copland, J. D. & Lydiard, R. D. (1998) Brain circuits in panic disorder. *Biological Psychiatry, 44*(12), 1264-1276. doi: 10.1016/S0006-3223(98)00300-X.

Dymond, S. & Roche, B. (2009). A contemporary behavior analysis of anxiety and avoidance. *The Behavior Analyst, 32*, 7-27.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review*, *1*(4), 429-438.

Gilbert, R. M. (1971). Signal functions in discriminated avoidance behavior. *Journal of the Experimental Analysis of Behavior, 15*(1), 97-108.

Gorman, J., Kent, J., Sullivan, G., & Coplan, J. (2000) Neuroanatomical hypothesis of panic disorder, revised. *American Journal of Psychiatry 157*:493–505

Griebel, G., Perrault, G. and Sanger, D. (1997). A comparative study of the effect os selective and non-selective 5-HT_2 receptor subtype antagonists in rat and mouse models of anxiety. *Neuropharmacology, 36*(6), 793-802.

Hashimoto, S., Inoue, T., Muraki, I., and Koyama, T. (2009). Effects of acute citalopram on the expression of conditioned freezing in naïve versus chronic citalopram treated rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 33*(1), 113-117. doi: 10.1016/j.pnpbp.2008.10.015

Kasper, S. & Resinger, R. (2001) Panic disorder: the place of benzodiazepines and selective serotonin reuptake inhibitors. *Eurpean Neuropsychopharmacology 11,* 301-321.

Kessler R.C., Berglund P., Demler O., Jin R., & Merikangas K.R. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry 62*, 593–602.

Kroenke K, Spitzer R.L., Williams J.B., Monahan P.O., & Lowe B., (2007). Anxiety disorders in primary care: prevalence, impairment, comorbidity, and detection. *Annals of Internal Medicine 146*, 317–325.

Levesque, K., Bureau, S., Moskowitz, D., Tardif, J., Lavoie, J., Dupuis, G. & D'Antono, B. (2009) Defensiveness and metabolic syndrome: impact of sex and age. *Biological Psychology, 80,* 354-360.

Martin, P., Soubri, P. & Puech, A. (1990). Reversal of helpless behavior by serotonin uptake blockers in rats. *Psychopharmacology, 101,* 403-407.

Matto, V., Allikmets, L., & Skrebuhhova, T. (1998). Apomorphine-induced aggressiveness and [^{3}H]citalopram binding after antidepressant treatment in rats. *Pharmacology Biochemistry and Behavior 59*(3), 747-752.

McLean, M. P. & Anderson, E. R. (2009). Brave men timid women? A review of the gender differences in fear and anxiety. *Clinical Psychology Review, 29,* 496-505.

McKim, W. A. (2007). *Drugs and behavior.* Uppersaddle, NJ: Pearson Education, Inc.

McNaughton, N. & Corr, P. J. (2004). A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. *Neuroscience and Biobehavioral Reviews, 28,* 285-305. doi:10.1016/j.neubiorev.2004.03.005.

Millan, M., Girardon, S., & Dekeyne, A. (1998). 5-HT receptors are involved in the discriminative stimulus effects of citalopram in rats. *Psychopharmacology, 142,* 432-434.

Perkins, A. M., Ettinger, U., Davis, R., Foster, R., Williams, S. C. R., & Corr, P. J. (2009). Effects of lorazepam and citalopram on human defensive reactions: Ethopharmacological differentiation of fear and anxiety. *The Journal of Neuroscience, 29*(40), 12617-12624. doi:10.1523/JNEUROSCI.2696-09.2009

Skinner, B. F. (1974). *About Behaviorism.* New York, New York: Random House Inc.

Thompson, D., Moerschbaecher, J., & Winsauer, P., (1983). Drug effects on repeated acquisition: comparison of cumulative and non-cumulative dosing.*The Journal of the Experimental Analysis of Behavior, 39*, 175-184. doi: 10.1901/jeab.1983.39-175