

Behavioral and Biochemical Effects of Chronic Quetiapine and Venlafaxine in a Nonhuman Primate Model of Stress Induced Depression

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ABSTRACT

This study examined the effects of venlafaxine and quetiapine in a nonhuman primate model predictive of antidepressant drug effects. Twelve experimentally naïve male cynomolgus monkeys were trained to respond under a food-reinforcement schedule shown to have predictive validity in models of antidepressant activity (a differential-reinforcement-of-low rates; DRL schedule). In addition, monkeys were exposed to social stress by being an intruder in another social pen once per month, immediately before operant test sessions. Monkeys were treated chronically (3 months) with either quetiapine (3.0 mg/kg, p.o., s.i.d.), venlafaxine (1.5 mg/kg, p.o., b.i.d.) or served as controls (n=4/group). At the end of testing, brains were examined for several markers related to depression and antidepressant drug action. Being an intruder disrupted DRL performance with no evidence of improvement over time in the control animals. Chronic quetiapine treatment improved performance in three of four monkeys, while half of the animals treated with venlafaxine showed an antidepressant-like effect. BDNF variant 5 mRNA was significantly increased in the anterior hippocampus of quetiapine-treated monkeys. CREB protein expression was increased in the anterior and posterior hippocampus of venlafaxine-treated monkey, while phosphorylated CREB was decreased in the anterior hippocampus of the quetiapine-treated group. These findings suggest that quetiapine and venlafaxine's antidepressant-like effects are mediated, at least in part, through the hippocampus.

Introduction

Depression continues to be a common, debilitating mental health condition that affects approximately 121 million people worldwide and is currently one of the leading causes of disability.¹ The American Psychiatric Association² guidelines indicate that pharmacological medication is the treatment of choice for moderate to severe depression. While multiple classes of antidepressant medications are available, a meta-analysis reported that venlafaxine, a serotonin and norepinephrine reuptake inhibitor, appeared more efficacious than selective serotonin reuptake inhibitors and at least as effective as tricyclic antidepressants in treating major depression.³ Clinical trials have indicated that more than half of patients show an inadequate response to current antidepressant therapy;^{4,5} therefore, continued research is necessary to find more efficacious medication therapies.

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Author contributions:

R.E.B., M.A.N. and S.E.H. designed the experiments. R.E.B. performed the behavioral studies. S.E.H., S.M. and M.P. performed the molecular biology studies. The manuscript was written by R.E.B., M.A.N., M.P. and S.E.H.

Atypical antipsychotics are also known to be effective agents in treating bipolar depression and when used in combination with antidepressants can effectively reduce depressive symptoms in treatment-resistant individuals.⁶ One possible mechanism by which these compounds exert antidepressant effects is by an active metabolite(s). For example, the primary metabolite of the atypical antipsychotic quetiapine is N-desalkyl quetiapine, also known as norquetiapine. The antidepressant effects of quetiapine are due in part to its affinity at serotonergic and noradrenergic receptors; however, norquetiapine exhibits greater affinity than quetiapine at these receptors and functions as a potent noradrenergic reuptake inhibitor. Quetiapine is indicated for schizophrenia, acute depressive bipolar episodes, as adjunctive therapy for acute manic episodes associated with bipolar I disorder and as adjunctive therapy and monotherapy for the maintenance of bipolar I disorder.⁷ Previous reviews suggested that quetiapine monotherapy exhibits greater efficacy than placebo in treating acute depressive episodes in bipolar I disorder⁸⁻⁹ and significantly improves symptoms¹⁰⁻¹¹ and delays relapse in individuals diagnosed with major depressive disorder.¹² Thus, the parent compound as well as the active metabolite quetiapine mediates the antidepressant effects of quetiapine. The present study was undertaken to directly compare the antidepressant effects of quetiapine with the serotonin noradrenergic reuptake inhibitor venlafaxine in reducing the “depressive-like” effects of social stress in a nonhuman primate model.

There is general consensus in the literature that stress exposure may play a role in the onset of major depression and depressive illnesses.¹³⁻¹⁸ Chronic social stress, the most common type of stressor in humans, leads to a significant increase in the risk for depression.¹⁹ The resident-intruder model is an ethologically relevant model of social stress used to examine the association between stress and psychopathologies, such as depression.²⁰ The paradigm involves placing a subject (intruder) in close proximity to unfamiliar conspecifics (residents) in a manner that allows the intruder to see and hear the residents. The consequent eliciting behavioral responses in the intruder resembles characteristics associated with depression, such as anhedonia, decreased social interactions and subordinate posture.

Previous studies have demonstrated that the resident-intruder paradigm suppresses hippocampal neurogenesis in adult intruder rats,²¹ adult intruder tree shrews,²² and adult intruder marmoset monkeys²³—an effect that is augmented by antidepressant administration.

The differential reinforcement of low rate (DRL) schedule of reinforcement has been used widely for screening compounds for antidepressant-like drug activity.²⁴⁻²⁶ Under this schedule, a minimum amount of time must elapse between responses for reinforcement to occur. Administration of clinically effective antidepressants produce a unique profile, they decrease response rates and increase reinforcement frequency, whereas stimulants, opiates, anxiolytics, alcohol and antipsychotics do not.^{25,27-29} The sensitivity and predictive validity of DRL schedules for antidepressants is thought to be due to their ability to improve temporal discriminations, improve “waiting” capacity/decreased impulsivity, or an interaction of these factors with stress related to DRL contingencies. The latter point is parsimonious with the ability of antidepressants to interfere with the effects of stressors on CNS function.²⁴

In terms of CNS mechanisms implicated, the biochemical effects of stress and depression have been well studied in the hippocampus (e.g.³⁰). A reduction in volume of the anterior hippocampus is associated with depression and response to stress in humans³¹⁻³² and nonhuman primates.³³⁻³⁴ Moreover, stress, which predisposes individuals to depression, inhibits hippocampal neurogenesis,²²⁻²³ whereas antidepressants enhance hippocampal neurogenesis.³⁵⁻³⁸

The present study used experimentally naïve cynomolgus monkeys to compare the antidepressant-like effects of quetiapine and venlafaxine following exposure to the resident-intruder model of acute social stress. We hypothesized that exposure to socially derived stress (i.e., being the intruder) would decrease DRL reinforcement frequency, an effect that would be reversed to a similar degree by quetiapine and venlafaxine administration. Moreover, we hypothesized that chronic administration of these compounds would be positively associated with elevated brain derived neurotrophic factor (BDNF) signaling and CREB levels in the hippocampus of these subjects.^{35,39}

Materials and Methods

Animals: Twelve experimentally naïve, individually housed, adult male cynomolgus monkeys (*Macaca fascicularis*) served as subjects. Each monkey was fitted with an aluminum collar (Model B008, Primate Products, Redwood City, CA) and trained to sit calmly in a primate chair (Primate Products). Monkeys were weighed weekly and fed enough food daily (Purina Monkey Chow and fresh fruit and vegetables) to maintain body weights at approximately 95% of free-feeding levels. Water was available ad libitum in the home cage. Animal housing, handling, and all experimental procedures were performed in accordance with the 2011 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research and were approved by the Animal Care and Use Committee of Wake Forest University. Environmental enrichment was provided as outlined in the Wake Forest University Non-Human Primate Environmental Enrichment Plan.

Operant Procedure: Monkeys were trained to respond under a DRL *t*-sec schedule of food reinforcement. Under this schedule, responses must be spaced at least *t* sec apart, the inter-response time (IRT), in order for food reinforcement. Each monkey was first trained to respond under a fixed-ratio 1 schedule, in which each response delivered a banana-flavored food pellet. Next, the IRT value was increased over successive sessions. We individualized the IRT values for each monkey so that they did not receive the maximal number of food reinforcers per 60 min. session. For most animals, this value was 30 sec. When responding was stable ($\pm 20\%$ of the mean for 3 consecutive sessions, with no trends in responding), monkeys were exposed to the intruder model (see below) once a week, for two weeks (baseline), and then once monthly.

Intruder paradigm: For these studies, one monkey (the intruder) was placed in a quadrant of a pen, with visual and olfactory contact, but protected from direct contact with the other four socially housed monkeys in the pen. Behavioral responses of each monkey were monitored over the 15-min. observation period. Prior to placement in the pen and after the 15 min. in another group's pen, the intruder was placed in the primate chair and 3 ml blood sample was obtained

for assessment of plasma cortisol levels. Immediately after completing the blood sample, the monkey was placed in the operant chamber and the experimental session was begun.

Treatment Groups: Following stable DRL performance and two baseline intruder sessions, monkeys were randomly assigned to either the quetiapine, venlafaxine or control group ($n=4/\text{group}$). Monkeys received quetiapine (3.0 mg/kg, p.o., in the evening) or venlafaxine (1.5 mg/kg, p.o., twice daily) in a food treat, or simply a food treat for control monkeys, 7 days per week. Treatment continued for 3 months to determine the time course of effects and to evaluate tolerance to the behavioral effects of each drug. Every 4 weeks, monkeys were placed into the intruder pen prior to DRL sessions.

Data Analysis – Behavior: The primary dependent variables were response rates and total number of food reinforcers earned per session. Separate one-way repeated measures ANOVAs were conducted to determine if each treatment affected reinforcement frequency (with Time as the factor). To compare the drugs, a two-way repeated measures ANOVA with Treatment and Time as factors, was conducted. Dunnett's post-hoc *t*-tests were used to compare drugs at each time point. The first two intruder sessions were considered baseline. Data for the intruder sessions is represented as the percent of the previous weeks performance. After determining the baseline intruder effects, responding under the $\text{IRT} > t$ -sec of reinforcement occurred once per week, without exposure to the intruder paradigm, and the percent of maximum reinforcers and response rate were the primary dependent variables.

Necropsy and Dissection: Following completion of behavioral studies, monkeys were anesthetized with pentobarbital and transcardially perfused with ice-cold saline. The hippocampus was dissected according to anatomical landmarks and stereotaxic atlas. The remainder of the brain was blocked in 4 mm slabs and stored at -80°C for subsequent studies. The hippocampus from one hemisphere was used for regional protein and mRNA analysis. The anterior hippocampus was delineated from the posterior hippocampus by the presence of the uncus.³³

mRNA isolation and qPCR: Dissected tissue samples were pulverized under liquid nitrogen with an aliquot of pulverized tissue used for RNA isolation. RNA was isolated and reverse transcribed as described previously.⁴⁰⁻⁴¹ The integrity of total RNA for each sample was assessed using an Agilent 2100 Bioanalyzer and RNA 6000 Nano Lab Chip. From all monkey samples, 2.0 µg of total RNA was reverse transcribed using random hexamers and superscript III. The resulting cDNA products was diluted 1:100 with RNase-free water for samples and cDNA from the pooled samples was serially diluted in 2 fold dilutions from 1:20-1:1280 for use as standards.

qPCR methodology and analysis were performed as previously described.⁴⁰⁻⁴¹ Using a 384 well format with the ABI Prism 7900HTS real-time detector, 0.5 µl aliquots of Taqman Expression Assay (20X), 5.5 µl 2X Absolute QPCR ROX PCR Mastermix (Abgene), and 4.5µl diluted cDNA (either sample or pooled standard) were mixed together and pipetted into single wells of the PCR plate. For no template controls (NTC) for each gene tested, water was added in lieu of cDNA. Each sample, including NTC, was run in triplicate. Thermocycling conditions: 1) one cycle 2 min at 50°C; 2) one cycle 15 min at 95°C; and 3) 40 cycles 15 sec at 95°C and 1 min at 60°C. Fluorescence was measured during the 60°C step for each cycle. Reactions were quantified by the standard curve method using SDS2.1 software generating a mean quantity value (Qty mean) for each sample from the triplicates of that sample for each gene of interest. Endogenous controls were selected for each experiment from a set of eleven candidate reference transcripts (Table 1) using geNorm software which allows the determination of the most stable reference genes from a given test panel of genes. By computing the average pairwise variation (V)

for each control gene paired with all other tested control genes, geNorm calculates the gene expression stability measure M42, minimizing any bias in the data as a result of normalization. The gene expression normalization factor is then calculated based on the geometric mean of a user-defined number of reference genes.⁴² Thus, data for each gene of interest was expressed as Qty mean for the gene of interest/geometric mean of Qty mean values for the selected endogenous control genes. Normalized values were then expressed as percent control. All primer sets were tested for optimal primer and input cDNA concentrations to ensure linear amplification.

Immunoblotting: Tissue samples were homogenized in RIPA lysis buffer (Upstate Biotechnology) containing protein phosphatase and protease inhibitor cocktail (#78440, Thermo Scientific), centrifuged, and supernatant (crude total protein) was removed. Protein was quantified using the bicinchoninic acid protein assay kit (Pierce Biotechnology) and equivalent final protein concentrations of each sample were diluted in Laemmli sample buffer, boiled at 95°C for 5 min, and subjected to electrophoresis⁴³⁻⁴⁵ (see 46 for complete details). Proteins were separated on Tris-HCl SDS-PAGE gels (Biorad) and transferred to nitrocellulose membranes. Blots were incubated with Odyssey Blocking Buffer (LI-COR) at room temperature for 2 h, followed by incubation in primary antibodies of interest overnight at 4°C. Blots were thoroughly washed and subsequently incubated with the appropriate host secondary antibody IRDye680 or IRDye800 (Rockland Immunochemicals, Gilbertsville, PA and Molecular Probes, Eugene, OR) for 1 h. To assure equal loading of the samples, membranes were incubated with a monoclonal mouse anti-GAPDH antibody for 1 h (Santa Cruz; 1:500), detected with an IRDye680 conjugated rabbit anti-mouse antibody (LI-

Table 1. Reference Transcripts

β-2 microglobulin (B2M)	transferrin receptor (TFRC)	β -glucuronidase (GUSB)	β-actin (ACTB)
ribosomal protein 18S (18S)	TATA box binding protein (TBP)	phosphoglycerate kinase 1 (PGK1)	ribosomal protein; large (RPLPO)
peptidylprolyl isomerase A (cyclophilin A) (PPIA)	hypoxanthine phosphoribosyltransferase 1 (HPRT1)	glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	

COR, 1:10,000). The Odyssey Infrared Imaging System (LICOR) was used for visualizations. Primary antibodies were as follows: CREB (#9104S) and phospho-CREB S133 (#9191S) from Cell Signaling; TrkB (#610101; BD Biosciences); and proBDNF (#AB1779SP; Millipore).

ELISA: Tissue levels of mature BDNF were measured using the BDNF Emax ImmunoAssay System (Promega Corporation, Madison, WI). To minimize assay variance, levels of mature BDNF in both the anterior and posterior hippocampus for all subjects were analyzed in the same assay. All samples were analyzed in triplicate. Final absorbance was read at 450 nm with a SpectraMax microplate reader/spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

Data Analysis – Biochemistry: Levels of gene expression were calculated using SDS 2.1 software (Applied Biosystems, Foster City, CA) to interpolate the Ct values for each well onto a standard curve generated from the Ct values of a dilution series of standards. These values were then averaged across triplicates after removal of outliers and expressed relative to the quantity value for the mean of the endogenous control genes measured in the same sample on the same plate. This relative value was used for subsequent statistical analysis. Experiments determining relative gene expression for each candidate gene were run independently. ANOVA was used to compare group means between quetiapine, venlafaxine and controls.

For immunoblotting, background-subtracted intensity values for each sample was calculated using Odyssey version 1.2 software. Data from each region was analyzed using one-way ANOVA with treatment as the factor and hybridization intensity relative to GAPDH as the dependent variable. Post-hoc analysis will be performed using Tukey’s test. In all cases, significance was defined as $p < 0.05$.

Results

Baseline DRL >t-sec behavior: Prior to beginning drug treatments, the groups did not differ in baseline response rates or reinforcement frequency under the DRL contingencies (Table 2). On average, response rates varied between 0.05-0.08 response/sec and monkeys received between 25-50 reinforcers

Table 2. Baseline Response Rates (resp/sec) and Reinforcement Frequency (SR/session) under the DRL Schedule Prior to Drug Treatment

Response Rates (resp/sec)					
Control		Quetiapine		Venlafaxine	
C-7477	0.07	C-7896	0.04	C-7705	0.05
C-7895	0.16	C-7669	0.04	C-7667	0.04
C-7828	0.02	C-8127	0.07	C-7704	0.07
C-8125	0.06	C-8126	0.06	C-7897	0.01
0.08 (.03)		0.05 (.01)		0.05 (.01)	
Reinforcement Frequency (SR/session)					
Control		Quetiapine		Venlafaxine	
C-7477	27.75	C-7896	25.00	C-7705	33.67
C-7895	56.00	C-7669	30.00	C-7667	64.33
C-7828	53.00	C-8127	25.00	C-7704	49.33
C-8125	39.33	C-8126	16.33	C-7897	35.67
45.58 (6.5)		26.67 (2.8)		49.11 (7.1)	

per session. For control monkeys (Fig. 1, left panels), after the first two weeks, response rates and reinforcement frequency under non-intruder DRL schedule were relatively stable for the remainder of the 12-week study. Chronic quetiapine (Fig. 1, middle panels) and venlafaxine (Fig. 1, right panels) treatment also did not affect weekly DRL responding under non-intruder conditions.

Repeated intruder challenge.

Cortisol assessments: For the 12 monkeys studied twice in the intruder (24 total test sessions), the mean increase in cortisol was approximately 20%, but with the large amount of variability, this failed to reach statistical significance. Nonetheless, it is important to note that cortisol increased from baseline in all but 3 intruder trials (i.e., 21 of 24 intruder sessions; one monkey from each group failed to show cortisol elevations; data not shown).

Behavioral assessments: Prior to the start of drug treatments, each monkey was tested twice in the intruder paradigm. Being an intruder resulted in decreases in response rates

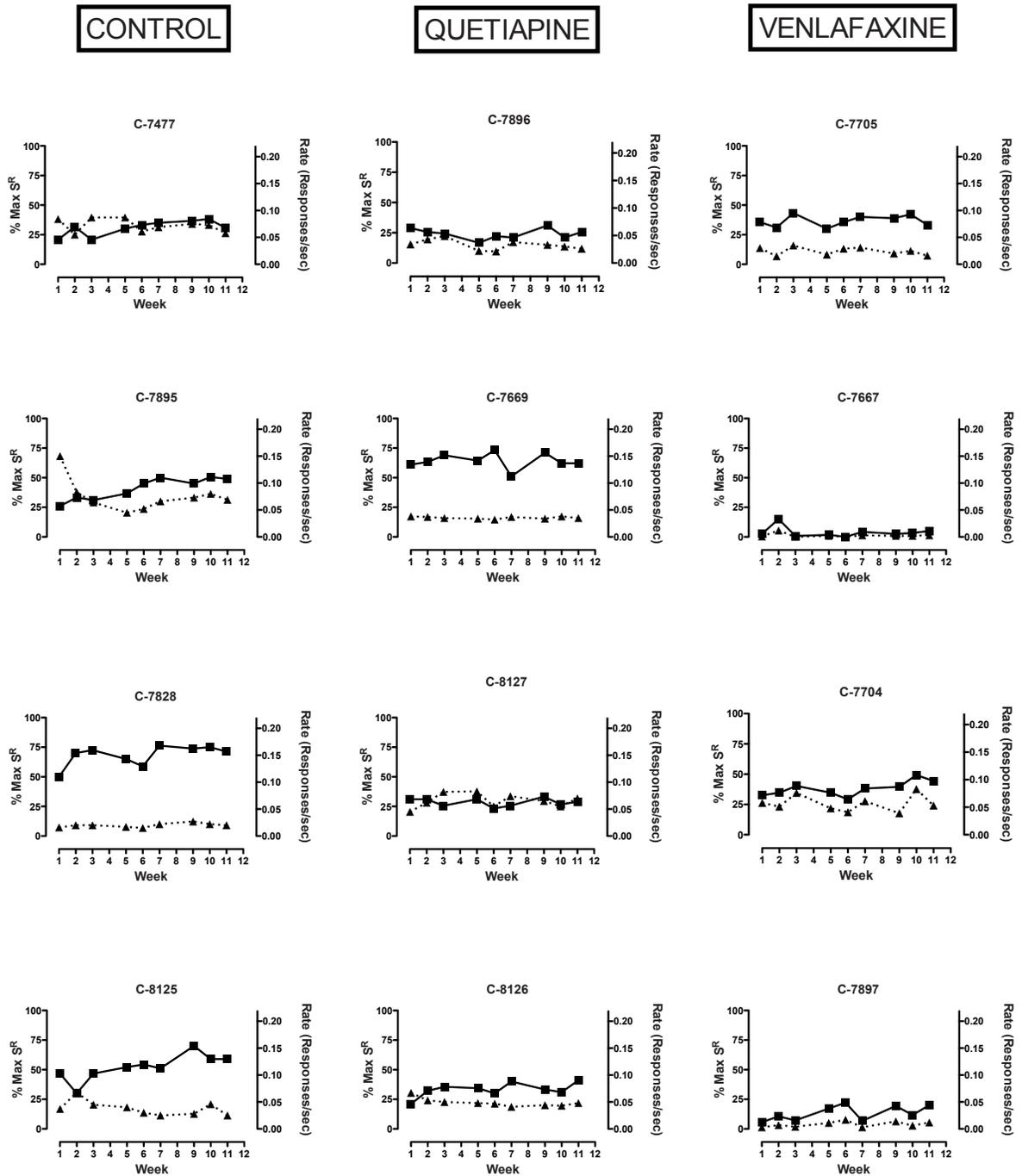


Figure 1. Effect of treatment on reinforcement frequency (square with solid line) and response rates (triangle with dashed-line) on weekly IRT>t performance in controls (left panels), quetiapine- (middle panels) and venlafaxine- (right panels) treated monkeys for each subject.

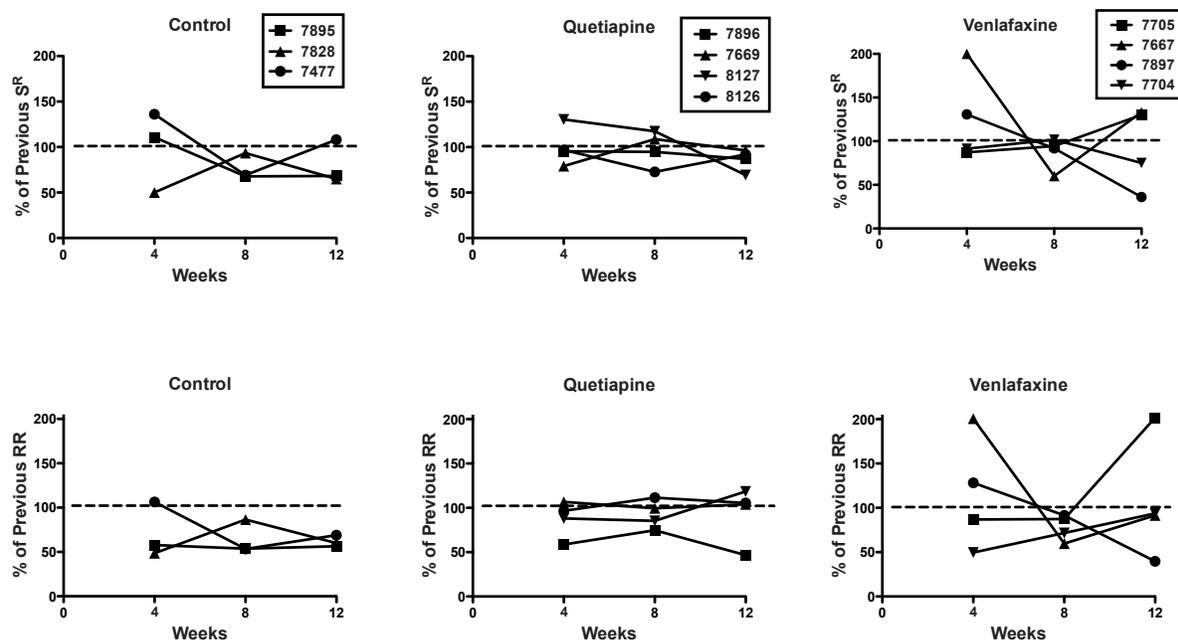


Figure 2. Effects on reinforcement frequency (top panels) and response rates (bottom panel) of intruder sessions across 12 weeks of treatment in controls (left panels), quetiapine- (middle panels) and venlafaxine- (right panels) treated monkeys for each subject.

by approximately 30% following the first intruder and approximately 20% following the second intruder (data not shown). Although separate one-way ANOVAs were not significant, individual-subject data revealed group differences following once per month intruder challenges. Three of four control monkeys showed decreases in reinforcement frequency and response rates that remained impaired when tested at Weeks 4, 8, and 12, with one monkey (C-8125) showing increases during Weeks 8 and 12 (Fig. 2, left panels). In contrast to what was observed in control monkeys following repeated intruder challenges, three of four monkeys treated with quetiapine no longer had reductions in reinforcement frequency or response rates (Fig. 2, middle panels). For monkeys treated chronically with venlafaxine, individual-subject data indicated that two monkeys no longer had reductions in reinforcement frequency while two monkeys (C-7897 and C-7667) did not improve over the 12 weeks of treatment; in the latter two monkeys, being an intruder continued to reduce response rates and reinforcement frequency (Fig. 2, right panels).

Expression of BDNF variants and TrkB receptor isoforms in the hippocampus. In the anterior hippocampus, BDNF Variant 5 mRNA was significantly different between the groups [$F(2,10)=8.83$, $P=0.009$]. Post-hoc analysis revealed BDNF Variant 5 levels were significantly greater for quetiapine-treated subjects compared to controls and venlafaxine-treated monkeys ($P<0.05$). There were no significant differences between the groups for any of the remaining variants: Var 1, Var 2, Var 3 and Var 4. Furthermore, there was no significant effect of treatment by group for any of the TrkB isoforms in the anterior hippocampus: TrkB pan, TrkB TK+ and TrkB T1 (Fig. 3, top panel).

In the posterior hippocampus, BDNF Variant 2 mRNA levels were significantly different between the groups [$F(2,10)=8.29$, $P=0.011$]. Post-hoc analysis revealed BDNF Variant 2 levels were significantly greater for quetiapine-treated monkeys than controls ($P=0.013$) or venlafaxine-treated monkeys ($P<0.05$). There were no significant differences between the

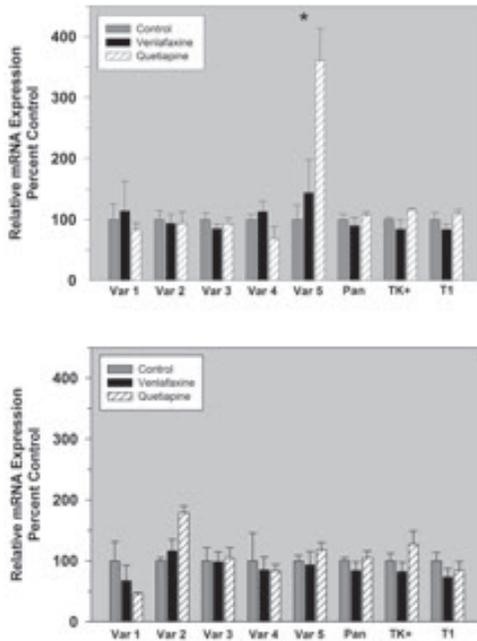


Figure 3. Effects on quetiapine and venlafaxine administration on mRNA expression of BDNF variants and TrkB isoforms in anterior (top) and posterior (bottom) hippocampus.

groups for any of the remaining variants: Var. 1, Var. 3, Var. 4 and Var. 5. Furthermore, there was no significant effect of treatment by group for any of the TrkB isoforms in the anterior hippocampus: TrkB pan, TrkB TK+ and TrkB T1 (Fig. 3, bottom panel).

Protein levels in anterior and posterior hippocampus. In the anterior hippocampus, neither mature BDNF nor proBDNF were significantly different between the groups (data not shown). The protein levels of the TrkB receptor, primary receptor of BDNF, were not significantly different between the groups in the anterior hippocampus (data not shown). Analysis of CREB

and phosphorylated CREB (serine 133) revealed significant differences in CREB [$F(2,10)=9.23$, $P=0.008$] and pCREB relative to CREB levels in this region [$F(2,10)=6.59$, $P=0.02$]. Post-hoc analysis revealed CREB protein levels were significantly greater in the venlafaxine group compared to controls ($p=0.008$) and pCREB/CREB levels were significantly lower in the quetiapine group compared to controls ($P<0.05$) (Fig. 4).

In the posterior hippocampus, neither mature BDNF nor proBDNF were significantly different between the groups. The protein levels of the TrkB receptor, primary receptor of BDNF, were not significantly different between the groups in the posterior hippocampus. Analysis of CREB and phosphorylated CREB (serine 133) revealed significant differences in CREB [$F(2,11)=8.64$, $P=0.008$]; however, pCREB relative to CREB levels in this region were not significantly different between the groups. Post-hoc analysis revealed CREB protein levels were significantly greater in the venlafaxine group compared to quetiapine ($P<0.05$) (Fig. 4).

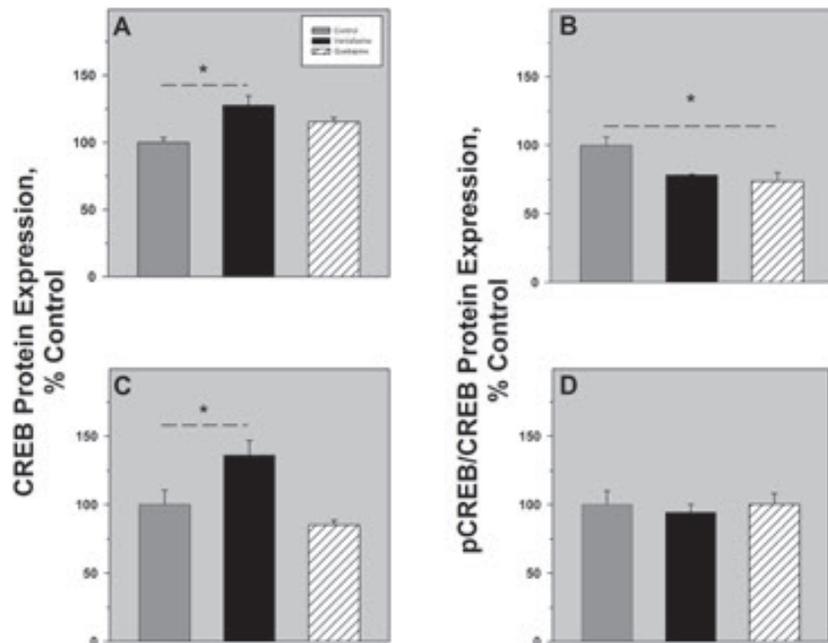


Figure 4. CREB and pCREB expression in the anterior (A,B) and posterior (C,D) hippocampus following venlafaxine and quetiapine administration.

Discussion

The present study utilized a unique animal model to compare the antidepressant-like efficacy of quetiapine with venlafaxine in adult male cynomolgus monkeys. Operant responding was maintained under a schedule of reinforcement that has been shown to be sensitive to clinically effective antidepressant drugs — a DRL schedule. In this study, we examined DRL performance following acute socially derived stress using an intruder model and examined the effects of potential antidepressant compounds. For control monkeys, being an intruder resulted in decreases in response rates and reinforcement frequency — this effect did not dissipate when tested every 4 weeks for 12 weeks. Quetiapine effectively attenuated the intruder-induced reductions in DRL responding in 3 of 4 monkeys, while venlafaxine was effective in half the subjects. Biochemical analyses noted significant changes in hippocampus, some attributed to chronic quetiapine treatment, while other changes were due to chronic venlafaxine administration. These findings highlight the individual differences in response to antidepressants and the distinct mechanisms of action for two drugs with clinical efficacy.

A unique aspect of the present behavioral studies was the combination of DRL responding and intruder-induced socially derived stress, with chronic drug treatment. DRL responding has been shown to be an excellent predictive model of clinical efficacy of antidepressants.²⁴ Surprisingly, there are no studies with quetiapine and only one published study examining the behavioral effects of venlafaxine on DRL responding⁴⁷ and these investigators reported no significant effects on response rates or reinforcement frequency. The present study extended the work with venlafaxine from a rodent model to nonhuman primates and from acute (no effect) administration to chronic drug treatment. Importantly, the present report is the first to extend the behavioral profile of quetiapine, a drug that has been characterized as an antipsychotic, anxiolytic and antidepressant (e.g.⁴⁸⁻⁵¹) to DRL responding in monkeys.

Preclinical models, such as chronic restraint stress and chronic mild stress, have been developed which closely

resemble multiple aspects of depression in humans, such as decreased interest in rewarding stimuli. In the present study, we used an intruder model, which is ethologically relevant and behavioral deficits induced by the model are reversed by antidepressants in rodents (cf.20). To the best of our knowledge, no investigators have combined socially derived stress, using the intruder paradigm, with DRL performance to assess antidepressant-like drug activity. Although there was evidence of individual-subject variability, the present study found disruptions in DRL behavior in control monkeys following exposure to another social group as an intruder. In contrast, 75% of the monkeys treated with quetiapine and 50% of the venlafaxine-treated monkeys showed improved performance, suggesting positive clinical outcomes. Importantly, these studies also included chronic drug administration and there was no evidence of tolerance developing to these positive effects.

Following completion of the behavioral experiments, biochemical analysis was conducted on the anterior and posterior hippocampi from all monkeys involved in the study in order to determine the degree to which venlafaxine and quetiapine affected cell-signaling molecules attributed to the pathophysiology and treatment of depression. Previous studies have shown that the beneficial effects of antidepressant medications are exerted in part through an up-regulation of BDNF expression in the hippocampus.⁵²⁻⁵³ BDNF supports the survival and differentiation of nascent dentate gyrus cells⁵⁴⁻⁵⁵ as well as synaptic integrity and dendritic arborization in CA3 pyramidal neurons⁵⁶. The multiple functions of BDNF are mediated by various regulatory processes including alternative splicing, post-translational proteolysis, receptor subtypes and downstream signaling pathways. Recent studies demonstrate that BDNF splice variant mRNAs are selectively targeted to the cell body or dendritic domains resulting in localized changes in BDNF protein expression.⁵⁶⁻⁵⁷ Previous studies have found that antidepressant administration modulates synaptic structure and function through the regulation of Bdnf splice variant expression.^{56,58} The present results demonstrate that quetiapine and venlafaxine differentially regulated the expression of specific BDNF variants in the anterior

and posterior hippocampus of primate brain. Quetiapine administration increased the expression of BDNF variant 5 in the anterior hippocampus and variant 2 in the posterior hippocampus. In primates, variant 5 is composed of exons 4, 11e and 11j. Exon 4 expression is primarily confined to the cell soma and proximal dendrites and is highly regulated by neuronal activation.⁵⁷ The initiation of transcription for BDNF exon 4 is controlled primarily by the cAMP-response element, CRE—the binding site for phosphorylated CREB. Thus, reduced phosphorylated CREB in the anterior hippocampus in monkeys administered quetiapine appears incompatible with elevated BDNF variant 5 levels. However, increased expression of exon 4 is dependent on binding of bHLH-PAS transcription factors to PasRE in the promoter region. Variant 2C is composed of exons 2c, 11e, and 11j. Exon 2c is localized in neurons, with constitutive expression in distal dendrites, and its expression is dependent on neuronal activation, suggesting a role in neuronal plasticity.⁵⁷ The means by which quetiapine regulates expression of specific BDNF exons between the anterior and posterior hippocampus requires further investigation. Interestingly, neither mature nor proBDNF protein levels were elevated in either region of the hippocampus. The lack of change in protein levels may be due in part to the cellular localization of such changes such that the analysis of the entire anterior or posterior hippocampus masks subtle changes occurring in discrete neuronal populations of hippocampal circuitry.

BDNF transcription is mediated in part by the transcription factor cAMP response element-binding protein (CREB). Antidepressant administration leads to activation of the cAMP dependent protein kinase (PKA) and calcium-dependent kinases that activate CREB, which in turn binds to a calcium response element within the BDNF gene thereby contributing to transcriptional activation. There is general consensus that CREB is involved in antidepressant action of multiple compounds. Previous studies have shown that CREB and phosphorylated CREB protein levels are altered by several antidepressants in various brain regions; however, few studies have examined the effects of venlafaxine or quetiapine on CREB or phosphorylated CREB in brain. Chronic administration of venlafaxine reduced pCREB levels

in cortex⁵⁹ but not in hippocampus of rats.⁶⁰ No studies to date have reported the effects on quetiapine on CREB expression in brain. In the present study, we found that venlafaxine-elevated CREB in the anterior and posterior hippocampal regions, whereas quetiapine decreased CREB phosphorylation in the anterior hippocampus only. The mechanism of these regional effects on CREB expression remains to be determined, but the functional and cellular connectivity differences between these hippocampal regions likely play an important role.⁶¹⁻⁶²

There are some limitations to be noted. Because of the biochemical measures requiring all monkeys in each group to be treated with the same dose, the possibility exists that individualized dosing may have resulted in a more uniform response treated monkeys showing improvements. Nonetheless, the findings are promising for quetiapine considering the improvements noted in three of the monkeys. It would also have been interesting and important to test the “non-responders” with the other antidepressant drug. That would, of course, have negated the biochemical analysis, so future studies should focus on identifying behavioral phenotypes that may predict treatment efficacy, with the goal of trying different treatment drugs when one fails to show a positive outcome.

In summary, we utilized a unique animal model to compare the antidepressant-like efficacy of quetiapine with venlafaxine in adult male cynomolgus monkeys. Antidepressant-like effects on stress-induced disruptions in DRL responding were exhibited in two of the four monkeys treated with venlafaxine and three of four monkeys treated with quetiapine. Importantly, the study utilized chronic drug treatments and tolerance did not develop to these effects on DRL responding. The possibility exists that individualized dosing based on plasma concentrations of the drug may have resulted in a more uniform response within each group; however, equivalent dosing within each group was necessary in order to minimize the influence of this variable for biochemical assessments. In addition, the absence of an experimentally naïve group precluded an assessment of the effects of behavioral manipulations on biochemical measures.

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