Brain-Derived Neurotrophic Factor and Initial Antidepressant Response to an *N*-Methyl-D-Aspartate Antagonist

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Objective: A model has been proposed to explain the pathophysiology of mood disorders based on decreased neurotrophin levels during mood episodes; treatment with antidepressants and mood stabilizers is associated with clinical improvement. This study investigated whether changes in brain-derived neurotrophic factor (BDNF) levels are associated with the initial antidepressant effects of ketamine, a high-affinity *N*-methyl-d-aspartate (NMDA) antagonist.

Method: Twenty-three subjects aged 18 to 65 years with *DSM-IV* major depressive disorder (treatment resistant) participated in this study, which was conducted between October 2006 and May 2008. The subjects were given an open-label intravenous infusion of ketamine hydrochloride (0.5 mg/kg) and rated using various depression scales at baseline and at 40, 80, 120, and 230 minutes postinfusion. The primary outcome measure was the Montgomery-Asberg Depression Rating Scale score. BDNF levels were obtained at the same time points as depression rating scale scores.

Results: Despite a significant ($P < .001$) improvement in MADRS scores after subjects received ketamine treatment, no changes in BDNF levels were observed in subjects after they received ketamine compared to baseline. Also, no association was found between antidepressant response and BDNF levels.

Conclusions: This study demonstrates that ketamine's rapid initial antidepressant effects are not mediated by BDNF. Further studies are necessary to shed light on the neurobiological basis of these effects.

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Individuals with major depressive disorder (MDD) exhibit diverse brain abnormalities associated with impairment of plasticity cascades, including morphological and functional changes in limbic and cortical areas.[1,2] Similarly, increasing evidence has implicated neurotrophic factors in the pathophysiology of mood disorders.[3] Neurotrophic factors have been shown to play an important role in neuronal plasticity, axonal growth, and connectivity.[3]

Brain-derived neurotrophic factor (BDNF) is the most studied neurotrophin in the pathophysiology of MDD. Postmortem studies have found that depressed subjects have decreased hippocampal and cortical BDNF levels,[4,5] and BDNF has been found to be down-regulated by stress, which has been directly implicated in the pathophysiology of depression.[6] Reinforcing this association, a polymorphism in the BDNF gene has been linked with depression-related traits.[7] Reductions in peripheral BDNF levels have also been described during major depressive episodes, with reports of rescue effects following treatment with various antidepressants.[5,8–11] Diverse classes of antidepressants induce differential regulatory effects on BDNF levels and expression in humans and animals during depressive states,[12–16] with differences observed between acute and chronic treatment. Recently, a meta-analysis showed a significant decrease in BDNF levels in depressed subjects compared to healthy controls.[7] Similarly, the authors found higher BDNF levels after chronic treatment with antidepressants[7]; however, not all studies have found increased BDNF levels after antidepressant treatment.[17]

Neurotrophins are present in blood and cross the blood-brain barrier through a high-capacity, saturable transport system.[18] Specifically, BDNF is expressed in many body tissues at relatively high levels.[19] A strong association has been reported between serum and cortical BDNF levels ($r = 0.81$) in rats,[5] suggesting a relationship between central and peripheral levels. However, although plasma BDNF appears to reflect its brain levels, little is known about which brain regions are more influential in the correlated peripheral changes.

The possibility of therapeutics with rapid antidepressant effects is a new and promising paradigm in the research and

treatment of MDD. Although these effects may involve the activation of plasticity pathways, overall their potential neurobiological basis is still poorly understood.[20] Glutamatergic modulating agents, in particular ketamine (a high-affinity *N*-methyl-d-aspartate [NMDA] antagonist), has been shown to induce rapid antidepressant effects both in humans and in preclinical models.[21,22] In addition, previous studies have shown that ketamine's antidepressant effects in patients with treatment-resistant MDD were not only rapid but also sustained for more than 1 week, well beyond the 2-hour half-life of ketamine.[21,22] Studies are currently underway to determine the biological underpinnings of ketamine's antidepressant effects. One report found that the acute administration of ketamine induced both antidepressant-like effects and increased hippocampal BDNF levels in rats.[23] Another study suggested that the cellular mechanisms underlying ketamine's antidepressant effects might take place through enhanced glutamatergic throughput of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors relative to NMDA, which leads to synaptic potentiation.[24] These findings are notable in the context of the present investigation. It is now clear that in the mature central nervous system, the major function of BDNF is to regulate "here and now" synaptic plasticity.[25] Thus, while one might not expect major neuronal remodeling to occur in the short time frame that ketamine exerts its antidepressant effects, ketamine-induced increases in BDNF levels could have a major effect on synaptic function.

Given the potential role of BDNF in synaptic plasticity, the present study sought to investigate the role of BDNF in ketamine's initial antidepressant effects.

METHOD

Twenty-three patients aged 18 to 65 years old participated in this study between October 2006 and May 2008. Participants fulfilled *DSM-IV* criteria for MDD (treatment resistant) and had no diagnosis of alcohol or substance abuse or dependence in the past 90 days, as determined by the Structured Clinical Interview for *DSM-IV*.[26] Patients with a *DSM-IV* diagnosis of bipolar disorder or those who had a history of antidepressant- or substance-induced hypomania or mania were excluded. Inclusion criteria were a Montgomery-Asberg Depression Rating Scale (MADRS)[27] score of at least 22, a current or past history of lack of response to 2 adequate antidepressant trials, and a current major depressive episode of at least 4 weeks' duration. All patients were drug free, were in good health, and were unmedicated for at least 2 weeks prior to the ketamine infusion, as determined by medical history, physical examination, routine blood laboratories, electrocardiogram, urinalysis, and urine toxicology. Patients received a complete description of the study, and written informed consent was subsequently obtained. The study was approved by the Combined Neuroscience Institutional Review Board of the National Institutes of Health.

Patients underwent a single, open-label infusion of ketamine hydrochloride (0.5 mg/kg), infused over 40 minutes, followed by a double-blind randomization to riluzole or placebo at 6 hours postinfusion. Here we report only the results of the open-label ketamine phase (up to 230 minutes postinfusion). A previous study found that 88% of all responders to ketamine reached response criteria by 230 minutes.[22] Ratings included the MADRS, the 17-item Hamilton Depression Rating Scale (HDRS),[28] the Beck Depression Inventory (BDI),[29] the Brief Psychiatric Rating Scale (BPRS),[30,31] and the Young Mania Rating Scale (YMRS).[32] Ratings were obtained at baseline (60 minutes prior to the infusion) and at 40, 80, 120, and 230 minutes postinfusion. Blood samples were collected using the vacutainer system at 60 minutes before and at 40, 80, 120, and 230 minutes after a single intravenous infusion of ketamine—the same time points as those used with the depression ratings. Response was defined as a 50% or greater decrease from baseline in MADRS scores.

Blood samples were centrifuged at 1,000 rpm at 4°C for 5 minutes and stored at −80°C until assay. Brain-derived neurotrophic factor was measured using an anti-BDNF sandwich ELISA kit (Chemicon International, Temecula, California) according to the manufacturer's instructions. Plasma was diluted 1:2 with sample buffer and carried out in duplicate blind to clinical information. Brain-derived neurotrophic factor standard solution was diluted to concentrations from 7.8 to 500 pg/mL of BDNF in a microplate reader in order to create the standard curve for BDNF levels. After the addition of streptavidin enzyme, substrate, and stop solution, BDNF levels were determined by absorbance in 450 nm using optical density values based on the standard curve values.

Statistics

A linear mixed model was used to examine changes in BDNF levels and depression rating scale scores over time. Schwarz's Bayesian information criterion was used to determine the best-fitting covariance structure that was first-order autoregressive. Initially, time was the only factor. A fixed intercept was included along with a random effect for subject. An additional analysis of BDNF levels added a fixed effect for antidepressant response versus nonresponse and its interaction with time. Montgomery-Asberg Depression Rating Scale score was the primary measure per protocol, but scores obtained from other psychiatric rating scales were also tested for confirmation. Bonferroni post hoc tests examined the change from baseline at each time point as well as group differences at each time point. Further analysis examined the influence of sex, age, and body mass index (BMI) separately to avoid potential confounds by adding these variables as covariates.

Pearson correlations were used to understand the association between BDNF levels and depression rating scale scores. Correlations were performed with the raw values,

Table 1. Demographic and Clinical Characteristics of Subjects With Major Depressive Disorder (treatment resistant) Receiving Ketamine (N = 23)

Characteristic	Mean	SD
Age, y	43.9	13.9
Age at onset, y	19.2	8.5
Body mass index	30.9	7.6
Illness duration, y	24.8	12.4
Episode duration, mo	80.7	108.5
No. of episodes	31.7	45.3
MADRS score	33.5	4.3
HDRS-17 score	20.8	4.4
BDI score	26.0	7.3
BPRS score	36.9	5.7
YMRS score	5.0	3.1
	N	%
Sex, male	14	61
Education, college	12	63[a]

[a]Information was unavailable for a few subjects.
Abbreviations: BDI = Beck Depression Inventory, BPRS = Brief Psychiatric Rating Scale, HDRS-17 = 17-item Hamilton Depression Rating Scale, MADRS = Montgomery-Asberg Depression Rating Scale, YMRS = Young Mania Rating Scale.

changes from baseline, baseline BDNF levels with raw depression rating scale scores, and baseline BDNF levels with changes in depression rating scale scores, all at each time point. Significance was evaluated at $P < .05$, 2-tailed.

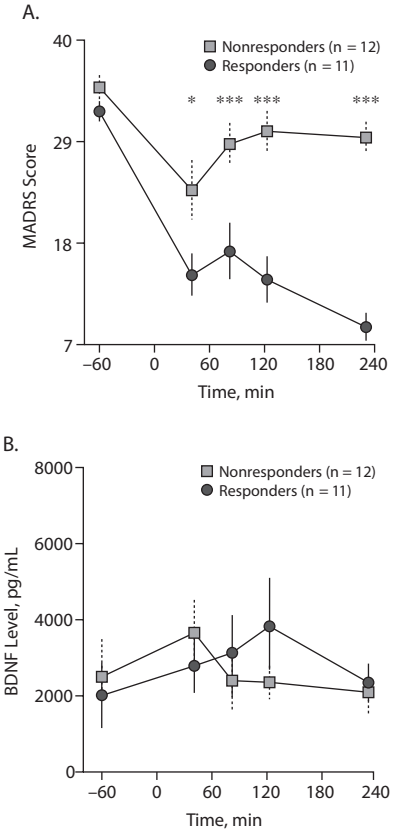
RESULTS

Table 1 summarizes the demographic and clinical characteristics of subjects participating in the study. The initial examination of BDNF levels over time showed no significant changes ($F_{4,73} = 1.15$, $P = .34$). This suggests that ketamine infusion did not substantially alter BDNF levels. However, MADRS scores changed significantly ($F_{4,81} = 24.70$, $P < .001$). Post hoc tests showed significant improvement beginning at the first postinfusion time point and lasting through the final one.

Given the change in depression rating scale scores but lack of change in BDNF levels, a variety of approaches were taken to understand any potential relationship between these 2 measures. First, a linear mixed model compared BDNF levels over time in ketamine responders versus nonresponders. This model showed no significant effect of time ($F_{4,69} = 1.15$, $P = .34$), response ($F_{4,26} = 0.23$, $P = .64$), or their interaction ($F_{4,69} = 0.50$, $P = .74$) (Figure 1). Further models covarying age, sex, and BMI separately showed no statistically significant results.

Next, correlations between BDNF levels and MADRS scores were performed for the raw values at each time point. The only significant correlation was at the 80-minute time point ($r = 0.43$, $P = .04$), but this did not remain significant after we corrected for multiple comparisons. Furthermore, similar correlations with the HDRS were not statistically significant at any time. Additional correlations between changes in BDNF levels and changes in depression rating scale scores, and baseline BDNF levels and either depression rating scale scores or changes in depression rating scale scores, did not yield any significant relationships (r's < 0.29, P's > .19).

Figure 1. BDNF Values and MADRS Scores Over 230 Minutes in Subjects With Major Depressive Disorder (treatment resistant) Who Did and Did Not Respond to Ketamine (N = 23)



*$P < .05$, ***$P < .001$ after Bonferroni correction.
Abbreviations: BDNF = brain-derived neurotrophic factor, MADRS = Montgomery-Asberg Depression Rating Scale.

DISCUSSION

This study sought to determine whether BDNF is involved in ketamine's rapid antidepressant effects. The study found that (1) ketamine induced a significant and rapid antidepressant response, consistent with previous studies[21,22]; (2) BDNF levels showed no changes from baseline after ketamine infusion and at any time up to 230 minutes postinfusion (a time point when antidepressant effects and response were manifest); and (3) BDNF levels showed no association with the observed antidepressant response.

Studies have shown that antidepressants targeting different systems result in regulatory effects on BDNF.[12–16] These differential effects may account for the heterogeneous data regarding antidepressant regulation in MDD. Specifically,

in contrast to a previous animal study showing increased BDNF levels after treatment with ketamine (15 mg/kg),[23] our data failed to demonstrate that BDNF is involved in ketamine's rapid antidepressant response in subjects with MDD.

Several studies have shown reduced peripheral BDNF levels in subjects with MDD compared to healthy control subjects, supporting the role of BDNF as a state-dependent biomarker (reviewed by Sen and colleagues[7]). Notably, it has been shown that dissimilar classes of antidepressants can differentially regulate BDNF levels.[15] Given the strong body of evidence showing decreased BDNF levels in subjects with MDD versus healthy controls, here we aimed to include only patients presenting with a major depressive episode in a drug-free state, focusing on the potential association between BDNF levels and ketamine's initial antidepressant effects and the potential role of BDNF as a surrogate outcome.

It is important to mention the complex mechanisms underlying BDNF storage/release from blood cells.[11] Changes in plasma BDNF occur rapidly. A complete plasma/brain BDNF turnover takes approximately 6 minutes; it appears to be bidirectional and seems to be a good marker of recent brain BDNF levels.[18,33,34] Brain BDNF is stable in circulating blood for 60 minutes and crosses the blood-brain barrier intact.[18]

Overall, total plasma BDNF levels do not appear to play a major role in ketamine's rapid antidepressant effects. However, ketamine might have particular effects on exon-specific BDNF transcript levels known to share functions with immediate early genes (exons III and IV), thus potentially bringing about faster modifications than those depending on recurrent protein synthesis for their transcription (exons I and II). In other words, different BDNF transcript regulation may account for the rapid (hours) and sustained (days/weeks) antidepressant actions of ketamine. In this study, we evaluated *only* the rapid initial effects, possibly through fast activation of exons associated with the induction of immediate early genes. It does thus remain a possibility that BDNF levels could change at later time points and might be more immediately involved in ketamine's sustained, rather than acute, antidepressant effects.

Our knowledge of the mechanisms involved in the therapeutic actions of ketamine as an antidepressant is incomplete, and further studies in this area are urgently needed. The present results suggest that increased serum BDNF levels might be neither critical nor enough for a rapid initial improvement in major depressive episodes. Indeed, a recent article highlights the importance of considering new models to understand the complex interplay of neurotrophic factors and other systems to better advance our understanding of depressive disorders.[17] Specifically, activation of AMPA receptor plasticity pathways has recently been proposed to play a critical role in ketamine's rapid antidepressant effects.[24,35] These rapid effects on depressive symptoms (and possibly potentiation of synaptic plasticity) seem to take place through a direct trafficking of AMPA receptors (fast-acting ionic glutamatergic receptors) into the postsynaptic membrane, changing membrane potential without direct regulation by BDNF. We conclude that further studies evaluating the role of BDNF in ketamine's sustained antidepressant effects (days/few weeks) are necessary to provide further insight into this topic.

Drug names: ketamine (Ketalar and others), riluzole (Rilutek and others).

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Financial disclosure: **Drs Zarate** and **Manji** have a patent pending on the use of ketamine in depression and have assigned their patent rights on ketamine to the US government. **Drs Machado-Vieira**, **Yuan**, and **DiazGranados** and **Ms Brutsche** and **Mr Luckenbaugh** have no conflict of interest, financial or otherwise, to disclose.

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