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Preliminary communication

Shank3 as a potential biomarker of antidepressant response to ketamine and its neural correlates in bipolar depression

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ABSTRACT

Background: Shank3, a post-synaptic density protein involved in N-methyl-D-aspartate (NMDA) receptor tethering and dendritic spine rearrangement, is implicated in the pathophysiology of bipolar disorder. We hypothesized that elevated baseline plasma Shank3 levels might predict antidepressant response to the NMDA receptor antagonist ketamine.

Methods: Twenty-nine subjects with bipolar depression received a double-blind, randomized, subanesthetic dose (.5 mg/kg) ketamine infusion. Of the patients for whom Shank3 levels were collected, 15 completed baseline 3-Tesla MRI and 17 completed post-ketamine [^{18}F]-FDG PET.

Results: Higher baseline Shank3 levels predicted antidepressant response at Days 1 ($r=-.39$, $p=.047$), 2 ($r=-.45$, $p=.02$), and 3 ($r=-.42$, $p=.03$) and were associated with larger average ($r=.58$, $p=.02$) and right amygdala volume ($r=.65$, $p=.009$). Greater baseline Shank3 also predicted increased glucose metabolism in the hippocampus ($r=.51$, $p=.04$) and amygdala ($r=.58$, $p=.02$).

Limitations: Limitations include the small sample size, inability to assess the source of peripheral Shank3, and the lack of a placebo group for baseline Shank3 levels and comparative structural/functional neuroimaging.

Conclusions: Shank3 is a potential biomarker of antidepressant response to ketamine that correlates with baseline amygdala volume and increased glucose metabolism in the amygdala and hippocampus.

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1. Introduction

Shank3 is a post-synaptic density scaffolding and tethering protein that plays an important role in glutamatergic neurotransmission. Transfected Shank3 neurons exhibit increased functional N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors at dendritic spines (Roussignol et al., 2005). Chronic treatment of these transfected Shank3 neurons with the glutamate receptor antagonist MK801 decreased spine density and increased spine length, a marker of immature synapses (Roussignol et al., 2005). Shank3 haploinsufficiency decreased protein expression, reduced excitatory postsynaptic currents, and impaired spatial learning and memory (Kouser et al., 2013). Both *in vitro* and *in vivo* studies demonstrated that Shank3 knock-out decreases spine density and leads to autistic-like behaviors (Roussignol et al., 2005). In mice deficient in ubiquitin transfer (Uba6-Use1), altered spine density was observed in the amygdala and hippocampus in conjunction with increased amygdala levels of Shank3 (Lee et al., 2013; Peca et al., 2011); these mice also displayed hyperactive and anxious behaviors.

In addition to autism, Shank3 has been implicated in major depressive disorder (MDD), bipolar disorder (BD), dementia, and schizophrenia. A putative role for Shank3 in BD was recently demonstrated in transgenic Shank3 duplication mice, which display manic-like hyperactivity and seizures (Guilmatre et al., 2014). Cultured hippocampal neurons from these mice had increased spine density and excitatory synapses that were reversed by a specific small interfering RNA. Interestingly, cross-breeding with Shank3 heterozygotes or administration of the mood stabilizer valproate (but not lithium) improved these phenotypes, suggesting a synaptic imbalance underlying manic-like behaviors in rodents (Han et al., 2013).

The NMDA receptor antagonist ketamine has rapid antidepressant effects in both MDD (Zarate et al., 2006), and bipolar depression (Diazgranados et al., 2010; Zarate et al., 2012). NMDA receptor antagonism rapidly increases spine density (Duman, 2014);

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thus, biomarkers of glutamatergic neurotransmission or neuroplasticity that reflect target engagement would be particularly useful in depression research. Towards this end, studies from our and other laboratories have sought biomarkers of rapid antidepressant response (Niciu et al., 2014).

Here, we sought to determine whether elevated peripheral levels of Shank3 would positively correlate with antidepressant response to ketamine in individuals with bipolar depression. A secondary aim of this study was to assess the relationship between Shank3 and other markers that may be related to glutamatergic signaling and dendritic spine rearrangement or neuroplasticity. Specifically, we assessed the relationship between baseline peripheral Shank3 and vGlut1 levels because a prior study had linked increased vesicular glutamate transporter 1 (vGlut1/SLC17A7) brain levels with Shank3 overexpression (Han et al., 2013). Peripheral Shank3 levels were also correlated with structure and function in the hippocampus and amygdala because both regions have been implicated in the etiology, pathogenesis, and/or treatment of BD (Nugent et al., 2014; Sheline et al., 2003; Vassilopoulou et al., 2013; Zhang et al., 2011). Finally, we assessed glucose metabolism via [^{18}F]-fluorodeoxyglucose positron emission tomography ([^{18}F]-FDG-PET) because most glucose in the CNS is ultimately cycled into glutamate (Sheline et al., 2003; Shen et al., 1999).

2. Methods

Twenty-nine subjects with treatment-resistant BD participated in this study. Subjects were a subset from two identical controlled studies for which efficacy and other clinical results were previously reported (Diazgranados et al., 2010; Zarate et al., 2012). The relevant methods are summarized below.

2.1. Patient selection

Potential participants were recruited from local inpatient psychiatric units, the internet, and local and national physician referrals. Subjects between 18 and 65 years old with treatment-resistant BD (Type I or Type II) who were currently experiencing a major depressive episode were admitted to the National Institute of Mental Health's Inpatient Mood and Anxiety Disorder Research Unit in Bethesda, Maryland, U.S.A. between October 2006 and November 2012. Subjects were diagnosed using a Structured Clinical Interview for Axis I DSM-IV Disorders (SCID). Additional inclusion criteria included a pre-treatment Montgomery-Åsberg Depression Rating Scale (MADRS) score of $\geq$20, a history of prior non-response to at least one antidepressant trial, and a prospective mood stabilizer trial while at the NIMH (either lithium or valproate for at least four weeks at therapeutic levels (serum lithium: .6–1.2 mEq/L, valproic acid: 50–125 μg/mL)). Exclusion criteria included substance abuse or dependence in the prior three months, a history of uncontrolled medical illness, head trauma, concomitant use of other psychotropic medications for at least two weeks prior to infusion (five weeks for fluoxetine), or clinically significant medical abnormalities discovered during screening. All studies—both the original studies from which these data were drawn (Diazgranados et al., 2010; Zarate et al., 2012) and the present study—were approved by the Combined Neuroscience institutional review board and the Radiation Safety Committee of the National Institutes of Health. All subjects provided written informed consent before study enrollment and were assigned a clinical research advocate.

2.2. Study design and outcome measures

The design of the original phase Ib studies from which these data were drawn (Diazgranados et al., 2010; Zarate et al., 2012) was identical. Both were double-blind, randomized, placebo (saline)-controlled, cross-over trials designed to assess the antidepressant efficacy of ketamine in refractory bipolar depression. All subjects received a single subanesthetic dose (.5 mg/kg) of intravenous ketamine hydrochloride over 40 min. MADRS scores were assessed and peripheral blood was collected at baseline (60 min before infusion), at 230 min, and at Days 1, 2, 3, 7, and 10 post-infusion. Overall response to ketamine was determined by percent change in MADRS score compared to baseline; negative values reflected a reduction in depressive symptoms.

2.3. Peripheral protein measurements

Whole blood samples were centrifuged at 3000 rpm for 10 min and plasma was extracted and frozen at −80 °C. Shank3 and vGlut1 (SLC17A7) levels were measured by enzyme-linked immunosorbent assay (ELISA) (MyBioSource, San Diego, CA). Briefly, biotin and horseradish peroxidase (HRP)-avidin antibodies were diluted 100-fold from 100× concentrate to create 1× dilutions. A stock solution for standard protein concentrations was prepared by centrifugation at 8000 rpm for 30s, followed by reconstitution in 1 mL sample diluent and dilution to create standards of the following concentrations: 1000, 500, 250, 125, 62.5, 31.25, 15.6, and 0 pg/mL. The assay procedure in the MyBioSource kit protocol was then followed. The samples were plated in duplicate with 100 ml of standard or non-diluted sample per well. A microplate reader determined the optical density of each well at 450 nm.

2.4. Neuroimaging

The neuroimaging methods have been described elsewhere (Nugent et al., 2014). Of the bipolar depression patients for whom Shank3 levels were collected, 15 underwent baseline structural 3-Tesla magnetic resonance brain imaging (MRI) before ketamine infusion, and 17 underwent [^{18}F]-FDG PET imaging approximately 200 min post-infusion. Regions of interest (ROI) used to calculate mean PET regional metabolic rate of glucose (rMRGlu) were defined as previously reported (Nugent et al., 2014). Standardized ROIs were drawn on a template image. Individual MRIs were transformed to the space of the template, and the regions were adjusted to account for anatomical variation. The regions were then transformed back to the subject's native space, where they were masked by a binary image of gray matter derived from the MRI image. The resulting gray matter ROI masks were then used to calculate mean rMRGlu. In keeping with our initial hypotheses, the ROIs assessed here were the hippocampus and amygdala.

Baseline volumetric measurements were obtained as described previously (Nugent et al., 2013a). Briefly, the Functional MRI of the Brain (FMRIB) Software Library (FSL) tool FIRST (FMRIB's Integrated Registration and Segmentation Tool) was used to segment the basal ganglia in an automated fashion after registration to standard space using the FSL tool FLIRT (FMRIB's Linear Image Registration Tool) (Nugent et al., 2013a). Left and right lateralized ROIs were averaged prior to analysis. Notably, due to the higher reliability of the right amygdala ROI data (Nugent et al., 2013b), both average and right amygdala ROIs are reported.

2.5. Statistical analyses

All statistical analyses were completed using IBM SPSS Version 21. Improvements in depressive symptoms after ketamine infusion were measured via percent change in MADRS score from baseline to Days 1, 2, and 3. Because Day 3 was the latest time point for which significant differences in antidepressant effects were observed between ketamine and placebo infusions, no further time points were assessed (Diazgranados et al., 2010; Zarate et al.,