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Less NMDA Receptor Binding in Dorsolateral Prefrontal Cortex and Anterior Cingulate Cortex Associated With Reported Early-Life Adversity but Not Suicide

Mark D. Underwood, Mihran J. Bakalian, Virginia L. Johnson, Suham A. Kassir, Steven P. Ellis, J. John Mann, Victoria Arango

Division of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New York, NY (Drs Underwood, Mann, Arango, Ellis, Mr Bakalian, Ms Johnson, and Ms Kassir); Department of Psychiatry, Columbia College of Physicians and Surgeons, New York, NY (Drs Underwood, Mann, Arango, and Ellis).

Correspondence: Mark D. Underwood, PhD, Division of Molecular Imaging and Neuropathology/New York State Psychiatric Institute, Department of Psychiatry/Columbia Psychiatry, 1051 Riverside Drive, Box 42, New York, NY 10032 (mu20@cumc.columbia.edu).

Abstract

Background: Glutamate is an excitatory neurotransmitter binding to 3 classes of receptors, including the N-methyl, D-aspartate (NMDA) receptor. NMDA receptor binding is lower in major depression disorder and suicide. NMDA receptor blocking with ketamine can have antidepressant and anti-suicide effects. Early-life adversity (ELA) may cause glutamate-mediated excitotoxicity and is more common with major depression disorder and in suicide decedents. We sought to determine whether NMDA-receptor binding is altered with suicide and ELA.
Methods: A total 52 postmortem cases were organized as 13 quadruplets of suicide and non-suicide decedents matched for age, sex, and postmortem interval, with or without reported ELA (≤16 years). Tissue blocks containing dorsal prefrontal (BA8), dorsolateral prefrontal (BA9), or anterior cingulate (BA24) cortex were collected at autopsy. Psychiatrically healthy controls and suicide decedents underwent psychological autopsy to determine psychiatric diagnoses and details of childhood adversity. NMDA receptor binding was determined by quantitative autoradiography of [3H]MK-801 binding (displaced by unlabeled MK-801) in 20-µm-thick sections.
Results: [3H]MK-801 binding was not associated with suicide in BA8, BA9, or BA24. However, [3H]MK-801 binding with ELA was less in BA8, BA9, and BA24 independent of suicide ($P < .05$). [3H]MK-801 binding was not associated with age or postmortem interval in any brain region or group.
Conclusions: Less NMDA receptor binding with ELA is consistent with the hypothesis that stress can cause excitotoxicity via excessive glutamate, causing either NMDA receptor downregulation or less receptor binding due to neuron loss consequent to the excitotoxicity.

Keywords: autoradiography, glutamate, ketamine, major depressive disorder, postmortem

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Significance Statement

Glutamate is an excitatory neurotransmitter implicated as playing a role in mood disorders, and the NMDA type of glutamate receptor is altered in major depressive disorder and suicide. Ketamine is an NMDA receptor blocker that has antidepressant and anti-suicide effects, and neurotoxicity is hypothesized to be associated with early-life adversity. Using postmortem brain samples of the prefrontal and anterior cingulate cortex, we examined NMDA receptor binding in suicide decedents with and without early-life adversity and compared them with psychiatrically healthy controls with and without early-life adversity. We found less NMDA receptor binding in the prefrontal cortex with early-life adversity, but not with suicide, as cause of death. Early-life adversity may cause excitotoxicity, leading to less NMDA receptor binding, and it be a risk factor for developing major depression or suicide in adulthood.

Introduction

Glutamatergic neurotransmission abnormalities are reported in major depressive disorder (MDD) (Yuksel and Ongur, 2010; Serafini et al., 2015; Dean et al., 2016) as well as in several neurodegenerative disorders (Meldrum, 2000). Early-life adversity (ELA) during critical periods of neurodevelopment may impair normal negative feedback control of the hypothalamic-pituitary axis and cause hyperexcitatory neurotransmission and possible toxicity involving glutamate (Gunn et al., 2013; Maccari et al., 2014; Acosta, 2018).

Glutamate, as a neurotransmitter, broadly acts via 2 receptor classes: ionotropic glutamate receptors and metabotropic glutamate receptors. Ionotropic glutamate receptors mediate most excitatory neurotransmission in the brain, and 3 classes of these receptors are based in their selectivity for agonists: N-methyl-D-aspartate (NMDA), kainate, and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (Dingledine et al., 1999; Traynelis et al., 2010).

Major depression and suicide risk may be linked to NMDA receptor overactivation because ketamine, an NMDA receptor antagonist (Sucher et al., 1996), is a robust and rapidly acting antidepressant and is an anti-suicidal medication (Malhotra et al., 1997; Niciu et al., 2014; Abdallah et al., 2015; Grunebaum et al., 2018). Major depression and suicide are associated with childhood abuse history (Brown et al., 1999; McHolm et al., 2003; Nelson et al., 2017; Goldberg et al., 2019). In suicide decedents and in mood disorders, neurochemical and structural alterations are reported most consistently in the prefrontal cortex and anterior cingulate cortex (see Pandey, 2013; Lutz et al., 2017 for review). Therefore, we sought to determine whether NMDA receptor binding in the dorsal (BA8), dorsolateral (BA9) prefrontal, and anterior cingulate (BA24) cortices is different in suicide and with reported ELA.

Methods

Subjects

A total of 52 subjects were assigned to 13 quadruplets, consisting of: psychiatrically healthy controls who did not die by suicide with no reported history of ELA, suicide with no reported ELA, psychiatrically healthy controls with ELA, and suicide with ELA. The quadruplets were formed (Table 1) and the cases assigned as close as possible based on age (±5 years), sex, race, and postmortem interval (PMI, ±5 hours). The race distribution for the sample was 43 whites and 6 African Americans; 3 were recorded as Latino without identifying race. Toxicology screening in blood and/or brain was performed in all 52 cases, and the toxicology was clear in 39 cases (Table 2). The male to female ratio was 12:1 because suicides were overwhelmingly in males, and we matched on sex. The brain collection of the Division of Molecular Imaging and Neuropathology at the New York State Psychiatric Institute was the source of the brain samples. The respective Institutional Review Boards for Human Use Considerations of the New York State Psychiatric Institute, the University of Pittsburgh, and the Republic of Macedonia approved the procedures for collection and use of brain tissue. All subjects died suddenly and did not have a protracted agonal state. The coroner or medical examiner diagnosed suicides on the basis of the criteria of evidence of intent and a self-inflicted fatal act. Cases with an undetermined cause of death were excluded.

The next-of-kin agreed to a face-to-face interview for the purpose of a psychological autopsy that included diagnoses based on DSM-IV criteria, and we used the Structured Clinical Interview for DSM-I and -II (Spitzer et al., 1989; Kelly and Mann, 1996). All diagnoses were made at a consensus conference with experienced psychiatrists, psychologists, and other researchers present.

The demographic portion of our psychological autopsy interview (called BDEMO in our psychological autopsy assessment packet) solicits information about the developmental history of the decedent that is used for the determination of reported ELA. The presence of ELA was determined from information including the age of the decedent at which living status of parents changed, separation from parents prior to age 16 years, adoption status, history of physical and/or sexual abuse prior to age 16 years, living status of siblings, and family history of psychiatric illnesses. ELA was treated as a dichotomous variable. No attempt was made to quantify the amount or severity of the ELA. For validation of the ELA determination, we analyzed data collected from 359 live patients who were administered the demographic questions used in the psychological autopsy interview and also the Childhood Trauma Questionnaire (CTQ) (Bernstein and Fink, 1998), a widely used instrument for determining childhood traumatic events and a standard in the field. We found 257 of the 359 people gave corresponding responses; adversity from the demographics form ("adverse.BDEMO") and adversity from the CTQ ("adverse.CTQ") were simultaneously true (adversity present) in 21 cases and simultaneous false (no adversity) in 236 cases. From a logistic regression using "adverse.BDEMO" to predict "adverse.CTQ," $P = .00000686$ and the odds ratio is 8.6. This indicates that the determination of ELA by psychological autopsy has high correspondence with that from the CTQ.

Brain collection

After removal of the entire brain from the cranium, the dura mater was stripped and the brainstem separated from the rest of the brain by a transverse cut at the anterior margin of the superior colliculus. The cerebellum was removed at the peduncles. The forebrain was then bisected and the right

Table 1. Demographics of Cases

	Age (years) Mean ± SEM	Sex (M:F)	PMI (h) Mean ± SEM	Brain pH Mean ± SEM
Nonsuicide, no ELA	35 ± 5	12:1	14 ± 1	6.51 ± 0.11
Suicide, no ELA	36 ± 5	12:1	15 ± 1	6.46 ± 0.09
Nonsuicide, ELA	36 ± 5	12:1	13 ± 1	6.54 ± 0.07
Suicide, ELA	34 ± 5	12:1	20 ± 2	6.58 ± 0.07

Abbreviations: ELA, early life adversity; F, female; M, male; PMI, postmortem interval.
There were no statistical differences between any of the groups ($P > .05$, all comparisons).

Table 2. Manner of Death, Toxicology, and Psychological Autopsy Diagnoses for the Cases Studied

	Manner of death (n)	Toxicology (n)	Axis I diagnosis (n)
Nonsuicide, no ELA	CVA (3) GSW (1) Stabbing (4) MVA (2) Fall from height (1) Accident (2)	Clear (11) Benzodiazepine, opiate (1) Cannabis (1)	None (13)
Suicide, no ELA	Fall from height (2) Hanging (7) GSW (1) Subway (1) Poison (2)	Clear (8) Benzodiazepine (2) Opiate (1) Benzodiazepine, opiate (1) Benzodiazepine, opiate, cannabis (1)	MDD (11) None (1) Other (1)
Nonsuicide, ELA	CVA (9) Stabbing (1) MVA (1) Electrocution (1) Drowning (1)	Clear (12) Benzodiazepine (1)	Gambling Disorder (1) None (12)
Suicide, ELA	Hanging (6) GSW (2) Overdose (2) Asphyxiation (1) Poisoning (2)	Clear (8) Benzodiazepine (4) Benzodiazepine, barbiturate (1)	None (1) MDD (11) MDD with Gambling Disorder and OCD (1)

Abbreviations: CVA, cardiovascular accident; ELA, early-life adversity; GSW, gunshot wound; MVA, motor vehicle accident; MDD, major depressive disorder; OCD, obsessive compulsive disorder.

hemicerebrum cut into 2-cm-thick slabs in the coronal plane and the slab placed on a glass plate, frozen in liquid Freon-12 (DuPont), and then placed in prelabeled plastic bags and transferred to a –80°C freezer. Cerebellar tissue was used for brain toxicology. The remaining tissue was placed in formalin for neuropathological examination consisting of gross and microscopic examination.

Quantitative Receptor Autoradiography of the NMDA Receptor Using [3H]MK-801

Quantitative in vitro receptor autoradiography in postmortem human brain was conducted as previously described (Arango et al., 1990, 1995, 2001; Underwood et al., 2012). In brief, a coronal slab of brain containing BA8, BA9, and BA24 was dissected frozen, and those blocks were cut into 20-µm-thick sections placed onto glass slides. Sequential cortical sections were used for total binding, nonspecific binding, and Nissl staining. Sections were collected so that 6 sections were used for each autoradiography assay: 3 sections for total binding and 3 near-adjacent sections for nonspecific binding.

For the labeling of NMDA receptors, we followed the protocol of Kravitz (Kravitz et al., 2013) with minor modifications and which is briefly summarized. Sections were preincubated for 30 minutes at room temperature in 50 mM Tris acetate buffer containing glutamate and glycine to remove endogenous ligands and any possible exogenously administered drugs from the tissue. NMDA receptors are unique among synaptic receptors in their requirement for the binding of the agonists, glutamate and glycine or d-serine (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Benveniste and Mayer, 1991; Clements and Westbrook, 1991, 1994). Following preincubation, sections were incubated with 5 nM of [3H]MK-801 (dizocilpine maleate, specific activity 20 Ci/mmol, Perkin Elmer life Science, Waltham, MA). Nonspecific binding was determined by incubation of adjacent sections with 10 µM of nonradiolabeled MK-801 as displacer (Kravitz et al., 2013). Sections were then washed in incubation buffer at 4°C to remove unbound radioligand, briefly dipped in water to remove buffer salts, then washed for 90 minutes in ice-cold buffer, dipped once in ice-cold d-H_2O, and dried under a stream of cold air from a handheld blower to prevent diffusion of the ligand and placed in a desiccator overnight to dry. Dried slides were arranged in X-ray film cassettes along with slide-mounted tritium standards (American Radiolabeled Chemicals, Inc.) and exposed to tritium-sensitive film (Biomax MR film from Kodak). Sections were exposed for approximately 12 weeks, and the films were developed (Kodak D-19) following the manufacturer's directions. Adjacent tissue sections were

fixed in 10% buffered formalin and stained for Nissl substance with thionin or cresyl violet.

Quantitation of autoradiograms (Figure 1) was accomplished using a computer-based image analysis system (Imaging Research Inc., St. Catharines, Ontario, Canada). First, shading correction was established by acquiring a blank field at medium luminance to correct each of the image pixels (proportional shade correction). Images of the radioactivity standards were calibrated to femtomoles of radioligand per milligram of tissue, providing density values in units of radioligand concentration, corrected for nonlinearities. Calibrated images of total binding and nonspecific binding were aligned on separate channels and linked. Samples of receptor binding in a given brain region were averaged to produce one specific binding density measure for that individual.

Statistical Analyses

Statistical tests were conducted using IBM SPSS Statistics for Windows (Version 25, IBM Corp., Armonk, NY). Binding was the outcome variable, with "suicide" and "ELA" predictors and included interaction terms, "brain region" as a fixed effect, and "quadruplet" as a random effect using a General Linear Mixed Model (SPSS procedure UNIANOVA). Significance was assumed at $\alpha = .05$ (2-tailed). Post hoc analyses were performed when "brain region" was significant to identify which region(s) and which binding layer (inner, outer) had significant association with suicide and/or ELA. Correction for multiple comparisons were then made using Bonferroni correction. Correlations were tested using bivariate Pearson's correlation coefficient (SPSS Procedure CORRELATIONS). Data are presented as mean ± SEM.

Results

There were no Axis I diagnoses in any of the psychiatrically healthy controls without ELA or in 12 of the 13 psychiatrically healthy controls with ELA; 1 case had a diagnosis of gambling disorder (Table 2). In the suicide decedents, 11 of 13 without ELA and 12 of 13 with ELA had a diagnosis of MDD; 1 suicide decedent without ELA and 2 suicide decedents with ELA had no Axis I diagnosis detected. One suicide decedent without ELA had psychosis, and 1 suicide decedent with ELA had MDD with gambling disorder and OCD (Table 2).

In psychiatrically healthy controls (n = 24), [3H]MK-801 binding did not differ across the 3 brain regions assayed ($F_{2,51} = 2.284$, $P = .102$): anterior cingulate cortex (BA24: 36 ± 3 nCi/mg), dorsal prefrontal cortex (BA8: 34 ± 3 nCi/mg), and dorsolateral prefrontal cortex (BA9: 37 ± 3 nCi/mg). The [3H]MK-801 binding had a bilaminar appearance with more binding in the outer isodensity band which corresponds to cellular layers I-III, than the inner binding layer which corresponds to layers IV-VI (Figure 1). The inner isodensity band has 31–33% less binding

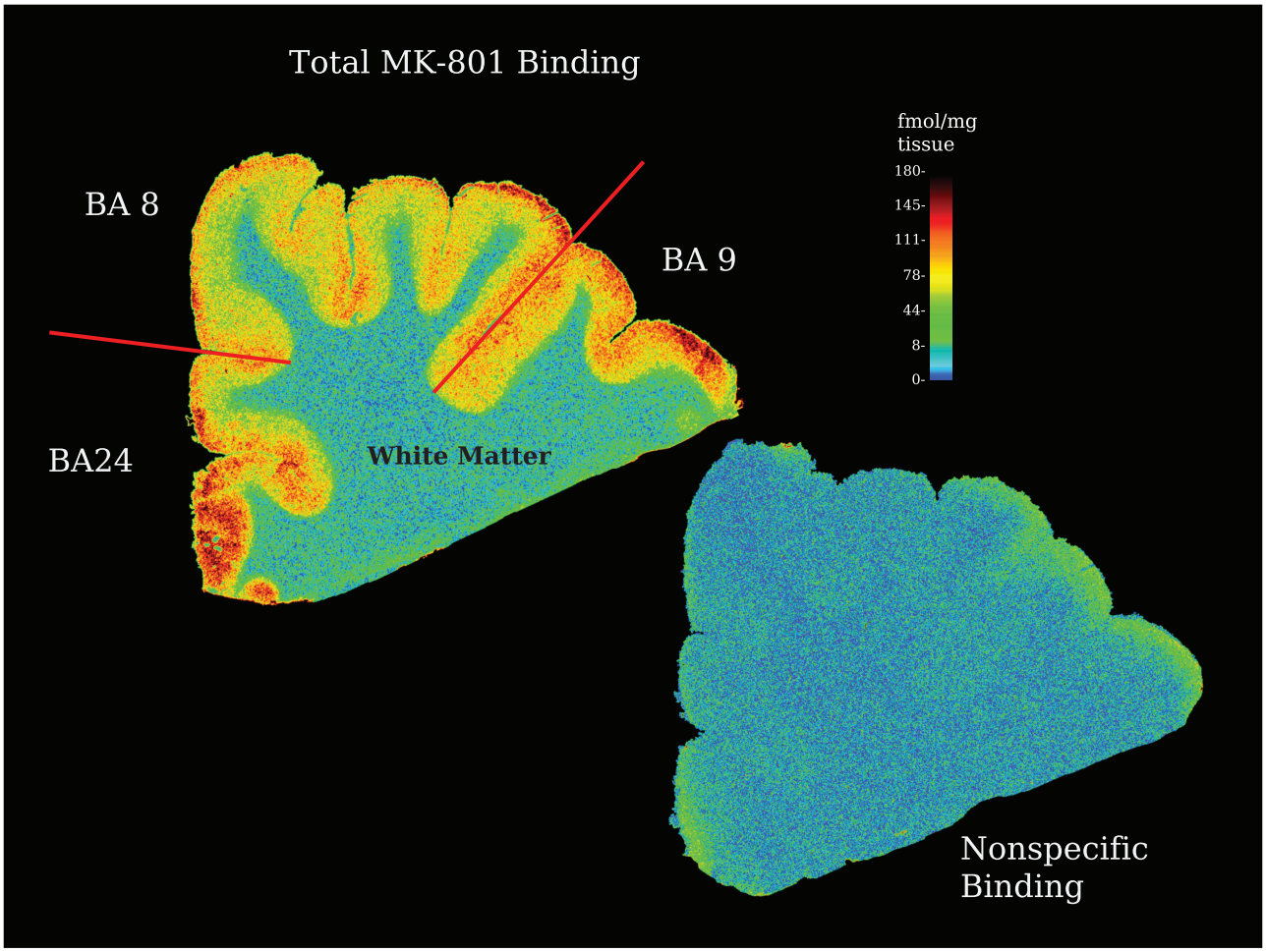

Figure 1. Autoradiogram of [3H]MK-801 binding in dorsal prefrontal cortex (BA8), dorsolateral prefrontal cortex (BA9) and anterior cingulate cortex (BA24) in a representative case. Non-specific binding was determined with incubation with non-radiolabeled MK-801.

compared with the outer band of binding ($F_{1,1}$=991.52, P=.019). The amount of binding in the inner and outer layers was statistically different in all 3 regions: BA8 (inner: 27 ± 5 nCi/mg; outer: 40 ± 8 nCi/mg, $F_{1,30}$=151, P<.001), BA9 (inner: 29 ± 6 nCi/mg; outer: 43 ± 9 nCi/mg, $F_{1,29}$=120, P<.001), and BA24 (inner: 28 ± 6 nCi/mg; outer: 42 ± 9 nCi/mg, $F_{1,23}$=86, P<.001).

Suicide and ELA

[3H]MK-801 binding was not associated with suicide ($F_{1,11}$=.548, P=.475) in any of the 3 Broadman areas (suicide × BA interaction: $F_{2,22}$=0.542, P=.589) (Figure 2). However, [3H]MK-801 binding was associated with ELA ($F_{1,11}$=5.935, P=.033) with no significant interactions between ELA, suicide, and/or region (Figure 2). The absence of a significant interaction between brain region and ELA ($F_{2,22}$=.389, P=.682) suggested there was less [3H]MK-801 binding associated with reported ELA in all 3 of the regions (BA8, BA9, and BA24). In post hoc univariate ANOVA analyses, the difference in BA8 was not statistically significant ($F_{1,11}$=4.126, P=.067) but was in BA9 ($F_{1,11}$=5.650, P=.037) and BA24 ($F_{1,11}$=5.576, P=.037). To determine whether the difference was confined to the inner or outer binding layer, post hoc analysis of layers was performed in the regions considered separately, including BA8 given that the P value approached statistical significance: [3H]MK-801 binding was 21% lower with ELA in the inner portion of BA8 ($F_{1,11}$=4.821, P=.05), in the outer and inner portions of BA9 (outer: 16% lower, $F_{1,11}$=5.505, P=.039; inner: 18% lower, $F_{1,11}$=5.861, P=.034) and the anterior cingulate cortex (outer: 15% lower, $F_{1,11}$=6.347, P=.028; inner: 20% lower, $F_{1,11}$=7.647, P=.018). There was no interaction between reported ELA and the suicide status ($F_{1,9.3}$=.327, P=.581), indicating there is less [3H]MK-801 binding regardless of suicide as cause of death.

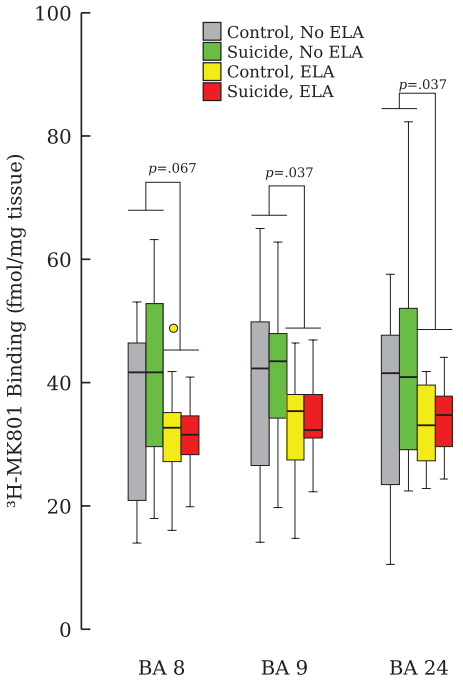

Figure 2. Boxplot of [3H]MK-801 binding in in dorsal prefrontal cortex (BA8), dorsolateral prefrontal cortex (BA9) and anterior cingulate cortex (BA24). Group data are presented for the quadruplets of cases with and without suicide and reported Early Life Adversity (ELA). Note there is less binding with reported ELA in BA9 and BA24. Box plot present the median and the 25th and 75th percentiles ± 1.5 times the interquartile range. Note there was one psychiatrically healthy control with ELA that was more than 1.5 times the interquartile range. Exclusion of the case did not affect the outcome.

The groups did not differ in age or brain tissue pH (Table 1), and the amount of [3H]MK-801 binding was not correlated with age (r=0.177, P=.235; r=0.231, P=.119; r=0.232, P=.117 for areas BA8, BA9, and BA24, respectively) or brain tissue pH (r=0.08, P=.602; r=0.005, P=.973; r=0.029, P=.848) when examined in all cases together, suggesting that neither age nor brain tissue pH explains the lower binding with reported ELA. The PMI in suicide decedents with ELA was greater than the other 3 groups (F=3.672, P=0.019); the other 3 groups did not differ from each other (P=0.505). However, PMI did not correlate with the amount of [3H]MK-801 binding in BA8, BA9, or BA24 (r=0.092, P=.538; r=0.059, P=.695; r=0.084, P=.574, respectively) and ELA remained significant when PMI was included in the model ($F_{1,11}$=5.961, P=.034), suggesting that the difference in PMI does not explain the difference in binding observed with ELA. There were no correlations between these variables and the amount of [3H]MK-801 binding when considered in suicide decedents. When looking at suicide as cause of death, or reported ELA, or in the groups separately, we found a negative correlation between brain pH and [3H]MK-801 binding only in BA8 only in psychiatrically healthy controls with reported ELA (r=–0.704, P=0.011; supplementary Figure 1). When brain pH was included in the model, ELA remained a significant main effect ($F_{1,15}$=6.152, P=.025), suggesting the ELA effect was not explained by pH. To determine whether the cases with positive toxicology were outlier points, boxplots of binding across gray matter were examined. One outlier was identified: a nonsuicide with ELA had more binding (between 1.5 and 3 interquartile ranges above the 75th percentile) in BA8; the case was positive for benzodiazepines. Reanalysis excluding the outlier did not significantly alter the outcome, and ELA remained a significant main effect for [3H]MK-801 binding ($F_{1,11}$=5.972, P=.033).

Discussion

In the present study, we found specific [3H]MK-801 binding in the dorsal, dorsolateral and anterior cingulate in the prefrontal cortex, and there was less binding associated with reported ELA. We did not find altered binding in suicide decedents, the vast majority of whom were depressed, compared with age- and PMI-matched psychiatrically healthy controls with and without ELA who did not die by suicide.

The receptor binding in prefrontal cortex was comparable in anatomical distribution and level with that reported in the literature (Dean et al., 2016). In cortex, there were 2 isodensity layers of binding: the outer layer corresponding to layers I–III and a portion of IV, and the inner layer corresponding to the remainder of IV plus V and VI. Lower binding associated with reported ELA was observed in the inner band in BA8 and in both the inner and outer bands in BA9 and BA24, indicating that the difference was not restricted to a single Brodmann area, isodensity band, or cortical layer. NMDA receptors are located predominantly on postsynaptic densities on dendrites with synthesis occurring in the cell body (Conti, 1997; Conti et al., 1999) and on both astrocytes and neurons (Dzamba et al., 2013). We examined the effects on binding of reported ELA defined as prior to age 16 years, but we do not have a more granular time frame to determine whether the origins of the binding difference occurred during a more specific or limited critical window of neurodevelopment.

Maternal separation is an animal model of maternal neglect resulting in behavioral and neurochemical alterations lasting

into adulthood (Lehmann and Feldon, 2000; Carlyle et al., 2012; Vetulani, 2013). The NMDA receptor is formed from 3 families of subunits, GRIN1, GRIN2, and GRIN3 (Paoletti and Neyton, 2007), with the different subunits regulated differently. Early-life stress in the Flinders Sensitive Line of rats was associated with lower expression of the NMDA NR1 subunit in hippocampus synaptosomes (Ryan et al., 2009) and was reversed by administration of the antidepressant escitalopram. Conversely, maternal separation in rats increased expression of the NMDA NR2 receptor subunit (Wieck et al., 2013). In addition, ELA can have both acute and long-lasting effects such as on DNA methylation of the NMDA receptor 2b subunit (*Grin2b*), resulting in decreased gene expression, with some changes being sex-specific and brain region dependent (Kundakovic et al., 2015). We found less NMDA binding associated with ELA, raising the possibility that the ELA exposure affected DNA methylation, resulting in reduced NMDA receptor gene expression and lower levels of NMDA receptors. More research is needed to clarify the regulation of NMDA receptor expression and how gene and environment impacts expression.

We did not detect a difference in [3H]MK-801 binding associated with suicide as cause of death. Other studies are in agreement with our findings such as a report of no difference in [3H]MK-801 binding in frontal and parietal cortex in controls and suicide decedents (Palmer et al., 1994). Likewise, no difference in [3H]MK-801 binding between controls and suicides was reported in BA19, BA46, BA40, or BA24 (Dean et al., 2016). [3H]MK-801 binding was also reported to not differ between controls and suicides in motor cortex, frontal cortex, occipital cortex, temporal cortex, hippocampus, thalamus, putamen, caudate, and cerebellum (Holemans et al., 1993). No difference in [3H]MK-801 binding in the striatum in suicides is reported elsewhere (Weissman et al., 1991; Noga et al., 1997). Taken together, the cumulative evidence indicates there is no difference in NMDA receptor binding in suicide.

Low [3H]MK-801 binding in association with ELA was not explained by other factors. [3H]MK-801 binding is reported not to differ by sex, age, PMI, or tissue pH (Dean et al., 2016). A negative correlation between [3H]MK-801 binding and age was reported by Holemans and colleagues, but only in frontal cortex of suicides, and the correlation was not seen in controls or in any other brain region, nor did they find a correlation with PMI or storage time (Holemans et al., 1993). We did not observe any significant relationship between [3H]MK-801 binding and age, brain tissue pH, or PMI. We had too few females to detect any sex effects. Dean and colleagues (Dean et al., 2016) did detect differences in [3H]MK-801 binding in schizophrenia, bipolar disorder, and MDD, but these diagnoses cannot explain our findings since our cases were diagnosed by psychological autopsy and did not have schizophrenia, bipolar disorder, or other Axis I diagnoses or alcohol use disorder. Twenty-three of our 26 suicide decedents had MDD, and since suicide was not a significant variable in our findings and MDD was present in the vast majority of the suicides, MDD is not a likely explanation. Likewise, 13 of the 52 cases had toxicology positive for benzodiazepines and other drugs, raising the possibility that they could have influenced receptor binding. However, 3 of the 6 outlier cases identified had clear toxicology. Of the other 3 positive toxicology cases, 2 had more binding and one had less binding, leaving no clear or consistent indication that the presence of drugs explains our findings.

NMDA receptors are increasingly being targeted for novel treatments of MDD and suicidality due to the rapid antidepressant and anti-suicide effects of low doses of the NMDA receptor antagonist ketamine (Berman et al., 2000; Zarate et al., 2006). The glutamate hypothesis of depression implicates NMDA receptor overactivation in the pathophysiology of MDD (Pittenger et al., 2007; Skolnick et al., 2009). We did not observe any difference in the amount of NMDA receptor binding with suicide and by extension MDD, suggesting that any overactivity at the receptor is not due to an increased number of receptors. Experimentally, NMDA receptor-antagonists produce rapid antidepressant-like responses and reverse some of the behavioral and physiological consequences of chronic stress in rodent models of depression (Autry et al., 2011; Li et al., 2011). Clinically, ketamine can reduce suicide ideation and depressive symptoms, even after a single acute treatment (Berman et al., 2000; Price et al., 2009, 2014; Rao and Andrade, 2010; Ibrahim et al., 2011; Zarate et al., 2012; Ballard et al., 2014; De Gioannis and De Leo, 2014; Murrough et al., 2015; Grunebaum et al., 2018). We did not find a significant difference in the amount of NMDA binding associated with suicide, suggesting the clinical effects of ketamine are not related to a difference in the underlying amount of NMDA receptor concentration in suicide ideators. Conversely, we did observe lower NMDA receptor binding with ELA independent of the suicide status of the decedent. Less NMDA receptor binding we observed may reflect a downregulation of the receptor and possible homeostatic response to the ELA exposure. Moreover, since ketamine is an NMDA receptor antagonist, it suggests the reduced amount of NMDA receptor binding associated with ELA, if it is homeostatic downregulation, is an insufficient response and additional suppression of receptor activity by ketamine contributes to the clinical antidepressant and anti-suicidal action of the drug. Further studies are needed to replicate this finding and to determine whether there is less NMDA receptor binding in other animal models or higher orders of mammals in response to severe stress exposure.

ELA is hypothesized to be a severe stress that can lead to neuroinflammation and elevation of inflammatory cytokines, which can lead to activated microglia derived quinolinic acid and glutamate elevations in the central nervous system, even to the point of excitotoxicity (Piani et al., 1992; Savitz et al., 2013; Dantzer and Walker, 2014; Mechawar and Savitz, 2016; Rizavi et al., 2016). Our finding of less NMDA receptor binding in the prefrontal cortex could be the result of downregulation of the NMDA receptor in response to elevated levels of glutamate. Further studies are needed to examine whether glutamate is elevated in the prefrontal cortex in association with ELA.

Future studies also need to identify the multiple cells, sites, neurotransmitters, and pathways where ketamine acts to bring about both the rapid and long-lasting effects on executive function to reduce depression symptoms and suicide ideation.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Statement of Interest

Dr Mann receives royalties for the commercial use of the C-SSRS from the Research Foundation for Mental Hygiene. Drs Arango, Underwood, Ellis, Mr Bakalian, Ms Johnson, and Mrs Kassir declare no conflicts of interest.

This article was prepared while Dr Arango was employed at Columbia University and the New York State Psychiatric Institute. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

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