TECHNICAL COMMENTARY

NMDA receptor function

NeuRA Discover. Conquer. Cure.

SCHIZOPHRENIA LIBRARY

Introduction

Converging evidence suggests that N-methyl-d-aspartate (NMDA) receptor hypofunction may contribute to the expression of schizophrenia. The NMDA receptor consists of several subunits; the NR1 subunits that bind coagonists glycine and d-serine, the NR2 subunits that bind the neurotransmitter glutamate, and the NR3 subunits that bind glycine. The NMDA receptor is activated by binding glutamate and a coagonist.

Glutamate is the major excitatory neurotransmitter in the brain and is crucial to normal brain function. In schizophrenia, there may be changes in levels of glutamate and its metabolites, and changes in levels or activity of mechanical components of the NMDA receptor system, such as the receptors that 'receive' glutamate, or the transporters that 'remove' it.

Method

We have included only systematic reviews (systematic literature search, detailed methodology with inclusion/exclusion criteria) published in full text, in English, from the year 2000 that report results separately for people with a diagnosis of schizophrenia, schizoaffective disorder, schizophreniform disorder or first episode schizophrenia. Reviews were identified by searching the databases MEDLINE, EMBASE, CINAHL, Current Contents, PsycINFO and the Cochrane library. Hand searching reference lists of identified reviews was also conducted. When multiple copies of reviews were found, only the most recent version was included. Reviews with pooled data are prioritised for inclusion.

Review reporting assessment was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist which describes a preferred way to present a meta-analysis[1]. Reviews rated as having less than 50% of items checked have been excluded from the library. The PRISMA flow diagram is a suggested way of providing information about studies included and excluded with reasons for exclusion. Where no flow diagram has been presented by individual reviews, but identified studies have been described in the text, reviews have been checked for this item. Note that early reviews may have been guided by less stringent reporting checklists than the PRISMA, and that some reviews may have been limited by journal guidelines.

Evidence was graded using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group approach where high quality evidence such as that gained from randomised controlled trials (RCTs) may be downgraded to moderate or low if review and study quality is limited, if there is inconsistency in results, indirect comparisons, imprecise or sparse data and high probability of reporting bias. It may also be downgraded if risks associated with the intervention or other matter under review are high. Conversely, low quality evidence such as that gained from observational studies may be upgraded if effect sizes are large, there is a dose dependent response or if results are reasonably consistent, precise and direct with low associated risks (see end of table for an explanation of these terms)[2]. The resulting table represents an objective summary of the available evidence, although the conclusions are solely the opinion of staff of NeuRA (Neuroscience Research Australia).

Results

We found six systematic reviews that met our inclusion criteria[3-8].

- Moderate to high quality evidence suggests a medium-sized increase in peripheral glutamate in people with schizophrenia compared to controls. The effect is largest in drug-naïve and non-medicated patients and in studies using high-performance liquid chromatography to measure glutamate.

Margarete Ainsworth Building, Barker Street, Randwick NSW 2031. Phone: 02 9399 1000. Email: info@neura.edu.au

NMDA receptor function

- Moderate quality evidence suggests small to medium-sized reductions in glutamate and increases in glutamine in the frontal cortex of people with schizophrenia when measured using MRS.
- Moderate to high quality evidence finds a medium-sized decrease in glutamate in the thalamus of people at clinical high risk, and a medium-sized increase in glutamate+glutamine in the frontal lobe of people at genetic high risk. There were no other significant differences in other brain regions.
- Moderate quality evidence suggests medium-sized effects of decreased NR1 mRNA expression and protein levels in people with schizophrenia.
- Moderate to high quality evidence suggests increased blood serine levels in people with schizophrenia, particularly in males and in studies measuring serine in plasma.
- Moderate quality evidence suggests a medium-sized, increased odds of NMDAR antibody seropositivity in patients vs. controls using a high-specificity threshold, but not a low-specificity threshold.

Margarete Ainsworth Building, Barker Street, Randwick NSW 2031. Phone: 02 9399 1000. Email: info@neura.edu.au

Brouwera A, Luykxa JJ, van Boxmeera L, Bakkera SC, Kahna RS

NMDA-receptor coagonists in serum, plasma, and cerebrospinal fluid of schizophrenia patients: A meta-analysis of case–control studies

Neuroscience and Biobehavioral Reviews 2013; 37: 1587-1596

View review abstract online

Comparison	**Blood or CSF NMDA receptor coagonists in people with schizophrenia vs. controls.**
Summary of evidence	**Moderate to high quality evidence (large sample, inconsistent, precise, direct) suggests increased blood serine levels in people with schizophrenia, particularly in males and in plasma. There were no differences in CSF serine levels or blood or CSF l-erine, d-serine, glycine, alanine, proline or aspartate.**

NMDA receptor coagonists

A significant, small effect of increased blood serine levels in people with schizophrenia compared to controls;

19 studies, N = 1,671, SMD = 0.28, 95%CI 0.02 to 0.54, $p = 0.034$, I^2 82.23%, $p < 0.001$

Subgroup analysis of sex indicated a significantly higher level of blood serine in male patients compared to male controls (SMD = 0.49), with no significant differences between female patients and female controls.

Subgroup analysis of sample indicated a significantly higher level of serine in people with schizophrenia in plasma studies (SMD = 0.403), with no significant differences in serum-based studies.

There were no significant differences in CSF serine, blood or CSF l-serine, d-serine, glycine, alanine, proline, or aspartate.

Authors report no evidence of publication bias.

Consistency in results	Inconsistent
Precision in results	Precise
Directness of results	Direct

Catts VS, Laib YL, Weickert CS, Weickert TW, Catt SV

A quantitative review of the postmortem evidence for decreased cortical N-

methyl-d-aspartate receptor expression levels in schizophrenia: How can we link molecular abnormalities to mismatch negativity deficits? **Biological Psychology 2016; 116: 57-67** View review abstract online	
Comparison	**NMDA receptor subunit mRNA expression and protein levels in the cortex of people with schizophrenia compared to controls.**
Summary of evidence	**Moderate quality evidence (small to medium-sized samples, unable to assess consistency, precise, direct) suggests medium-sized decrease in NR1 mRNA expression and protein levels in people with schizophrenia.**
NR protein and mRNA	
Medium-sized effects of decreased NR1 mRNA expression and protein levels in the frontal cortex of people with schizophrenia; mRNA: 5 studies, N = 176, SMD = -0.64, 95%CI -1.08 to -0.20, *p* = 0.004 Protein: 5 studies, N = 190, SMD = -0.44 95%CI -0.80 to -0.07, *p* = 0.02 There were not enough studies of other cortical regions for meta-analysis. A qualitative review of NR2 (A, B and D) and NR3A subunits suggested no consistent changes in cortical mRNA expression or protein levels.	
Consistency in results	No measure of consistency is reported, data appears inconsistent.
Precision in results	Precise
Directness of results	Direct

Marsman A, van den Heuvel MP, Klomp DWJ, Kahn RS, Luijten PR, Hulshoff Pol HE **Glutamate in schizophrenia: a focused review and meta-analysis of ^{1}H-MRS studies** **Schizophrenia Bulletin 2013; 39(1): 120-9** View review abstract online	
Comparison	**Glutamate, glutamine and N-acetyl aspartate (NAA) levels (measured by ^{1}H-MRS) in the medial frontal cortex, hippocampus**

	and thalamus of schizophrenia patients vs. controls.
Summary of evidence	**Moderate quality evidence (medium to large samples, direct, unable to assess precision or consistency) suggests reduced Glu and increased Gln levels in the frontal cortex of people with schizophrenia, with greater reductions associated with age. Lower quality evidence is unclear about hippocampus and thalamic levels.**
Glu, Gln and NAA	
Medial frontal cortex *A significant, small reduction in glutamate level in people with schizophrenia;* 9 studies, N = 337, *d* = -0.391, *p* = 0.006 Meta-regression showed a progressive decrease with age in patients compared to controls (*p* = 0.008). *A significant, medium-sized increase in glutamine in people with schizophrenia;* 8 studies, N = 275, *d* = 0.403, *p* = 0.045 Meta-regression showed a progressive decrease with age in patients compared to controls (*p* = 0.0005). *No significant difference in total glutamate + glutamine levels between patients and controls;* 8 studies, N = 330, *d* = 0.122, *p* = 0.393 *No significant difference in glutamate/glutamine ratio levels between patients and controls;* 6 studies, N = 228, *d* = 0.308, *p* = 0.062 Meta-regression showed a progressive decrease with age in patients compared to controls (*p* = 0.02). *A significant, small reduction in NAA levels in people with schizophrenia;* 19 studies, N = 779, *d* = -0.320, *p* = 0.019 Meta-regression showed a progressive decrease with age in patients compared to controls (*p* = 0.04). *A significant, small reduction in NAA/glutamate ratio in people with schizophrenia;* 7 studies, N = 247, *d* = -0.357, *p* = 0.038 Meta-regression showed a progressive decrease with age in patients compared to controls (*p* = 0.049). Hippocampus *No significant differencez in total glutamate levels between patients and controls;* 3 studies, N = 107, *d* = 0.031, *p* = 0.92 Thalamus *No significant difference in glutamate levels between patients and controls;* 3 studies, N = 128, *d* = -0.286, *p* = 0.20	

Consistency in results	Unable to assess; no measure of consistency is reported.
Precision in results	Unable to assess; no measure of precision is reported.
Directness of results	Direct

Pearlman DM, Najjar S

Meta-analysis of the association between N-methyl-D-aspartate receptor antibodies and schizophrenia, schizoaffective disorder, bipolar disorder, and major depressive disorder

Schizophrenia Research 2014; 157: 249-258

View review abstract online

Comparison	**Comparison of N-methyl-D-aspartate receptor antibodies in patients with schizophrenia vs. controls.** **Note: samples were predominately people with schizophrenia, but also included people with bipolar and major depressive disorders.**
Summary of evidence	**Moderate quality evidence (large sample size, imprecise, inconsistent, direct) suggests a medium-sized, increased odds of NMDAR antibody seropositivity in patients vs. controls using a high-specificity threshold, but not a low-specificity threshold.**

Blood N-methyl-D-aspartate receptor (NMDAR) antibodies

Significant, medium sized increased odds of NMDAR antibody seropositivity among patients vs. controls using a high-specificity threshold, but not a low-specificity threshold;

High-specificity threshold: 5 studies, N = 3,387, OR = 3.10, 95%CI 1.04 to 9.27, $p = 0.043$, $I^2 = 68\%$, $p = .025$

Low-specificity threshold: 4 studies, N = 3,194, OR = 2.31, 95%CI 0.55 to 9.73, $p = 0.25$, $I^2 = 90\%$, $p < 0.001$

All study participants were 4.5 times more likely to test seropositive for NMDAR antibodies based on a high-specificity threshold (1.320 dilution) than on a low-specificity threshold (1.10 dilution).

No significant differences were reported between people with first episode vs. chronic schizophrenia or schizoaffective disorder.

Consistency	Inconsistent
Precision	Imprecise

Directness	Direct

Song J, Viggiano A, Monda M, De Luca V

Peripheral Glutamate Levels in Schizophrenia: Evidence from a Meta-Analysis

Neuropsychobiology 2014; 70: 133-141

View review abstract online

Comparison	**Peripheral (serum or plasma) glutamate levels in people with schizophrenia vs. controls.**
Summary of evidence	**Moderate to high quality evidence (large sample, inconsistent, precise, direct) finds a medium-sized increase in peripheral glutamate in people with schizophrenia. The effect is largest in drug-naïve and non-medicated patients and in studies using high-performance liquid chromatography.**

Peripheral glutamate

A significant, medium-sized effect of increased levels of glutamate in people with schizophrenia than in controls;

10 studies, N = 614, SMD = 0.64, 95%CI 0.21 to 1.06, I^2 = 80.0%, p = 0.000

Larger effect sizes were found in the subgroup analyses of studies of drug-naïve (SMD = 0.90) or non-medicated patients (SMD = 0.82).

Larger effect sizes were found in studies using high-performance liquid chromatography (SMD = 1.075) than in studies using ion exchange column chromatography or fluorometric assay.

There were no differences in effect size according to age, sex, ethnicity, medication, sample (serum or plasma), or whether patients were fasting.

There was no evidence of publication bias.

Consistency in results	Inconsistent
Precision in results	Precise
Directness of results	Direct

Wenneberg C, Glenthoj BY, Hjorthoj C, Buchardt Zingenberg FJ, Glenthoj LB, Rostrup E, Broberg BV, Nordentoft M

Cerebral glutamate and GABA levels in high-risk of psychosis states: A focused review and meta-analysis of ^{1}H-MRS studies

Schizophrenia Research Jan: doi: 10.1016/j.schres.2019.10.050

View review abstract online

Comparison	**Cerebral glutamate levels measured by ^{1}H-MRS in people at high risk of psychosis vs. controls.**
Summary of evidence	**Moderate to high quality evidence (small to medium-sized samples, consistent, precise, direct) finds a medium-sized decrease in glutamate in the thalamus of people at clinical high risk, and a medium-sized increase in glutamate+glutamine in the frontal lobe of people at genetic high risk. There were no other significant differences in other brain regions (occipital, temporal, hippocampal, striatum, cerebellum, or white matter).**
Glu, Glx	
Thalamus *A medium-sized effect showed significantly lower glutamate levels in the thalamus of people at clinical high risk;* 3 studies, N = 218, SMD = 0.50, 95%CI 0.23 to 0.78, p = 0.0003, I^2 = 0% There were no significant differences in other brain regions (frontal, occipital, temporal, hippocampal, striatum, cerebellum, white matter). Frontal *A medium-sized, significant effect showed higher glutamate + glutamine levels in the frontal lobe of people at genetic high risk;* 4 studies, N = 140, SMD = -0.55, 95%CI -0.89 to -0.21, p = 0.001, I^2 = 0% There were no significant differences in other brain regions (thalamus, occipital, temporal, hippocampal, striatum, cerebellum), and in the analysis of the frontal lobe that combined clinical and genetic high-risk individuals.	
Consistency in results	Consistent
Precision in results	Precise

Directness of results	Direct

Explanation of acronyms

CI = confidence interval, Gln = glutamine (glutamate synaptic metabolic), Glu = glutamate neurotransmitter, Glx = glutamine+glutamate, ^{1}H-MRS = Proton Magnetic Resonance Spectroscopy, I^2 = measure of heterogeneity in results, N = number of participants, NAA = N-acetylaspartate, *p* = statistical probability of obtaining that result ($p < 0.05$ generally regarded as significant), SMD = standardised mean difference, vs. = versus

Margarete Ainsworth Building, Barker Street, Randwick NSW 2031. Phone: 02 9399 1000. Email: info@neura.edu.au

Explanation of technical terms

* Bias has the potential to affect reviews of both RCT and observational studies. Forms of bias include; reporting bias which involves the selective reporting of results; publication bias - trials which are not formally published tend to show less effect than published trials, further if there are statistically significant differences between groups in a trial, these trial results tend to get published before those of trials without significant differences; language bias – only including English language reports; funding bias - source of funding for the primary research with selective reporting of results within primary studies; outcome variable selection bias; database bias - including reports from some databases and not others; citation bias - preferential citation of authors. Trials can also be subject to bias when evaluators are not blind to treatment condition and selection bias of participants if trial samples are small.

---

† Different effect measures are reported by different reviews.

Weighted mean difference scores refer to mean differences between treatment and comparison groups after treatment (or occasionally pre to post treatment) and in a randomised trial there is an assumption that both groups are comparable on this measure prior to treatment. Standardised mean differences are divided by the pooled standard deviation (or the standard deviation of one group when groups are homogenous) which allows results from different scales to be combined and compared. Each study's mean difference is then given a weighting depending on the size of the sample and the variability in the data. 0.2 represents a small effect, 0.5 a medium effect, and 0.8 and over represents a large effect [9].

Reliability and validity refers to how accurate the instrument is. Sensitivity is the proportion of actual positives that are correctly identified (100% sensitivity = correct identification of all actual positives) and specificity is the proportion of negatives that are correctly identified (100% specificity = not identifying anyone as positive if they are truly not).

Odds ratio (OR) or relative risk (RR) refers to the probability of a reduction (< 1) or an increase (> 1) in a particular outcome in a treatment group, or a group exposed to a risk factor, relative to the comparison group. For example, a RR of 0.75 translates to a reduction in risk of an outcome of 25% relative to those not receiving the treatment or not exposed to the risk factor. Conversely, a RR of 1.25 translates to an increased risk of 25% relative to those not receiving treatment or not having been exposed to a risk factor. A RR or OR of 1.00 means there is no difference between groups. A medium effect is considered if RR > 2 or < 0.5 and a large effect if RR > 5 or < 0.2[10]. lnOR stands for logarithmic OR where a lnOR of 0 shows no difference between groups. Hazard ratios measure the effect of an explanatory variable on the hazard or risk of an event.

Correlation coefficients (eg, r) indicate the strength of association or relationship between variables. They are an indication of prediction, but do not confirm causality due to possible and often unforseen confounding variables. An r of 0.10 represents a weak association, 0.25 a medium association and 0.40 and over represents a strong association. Unstandardised (*b*) regression coefficients indicate the average change in the dependent variable associated with a 1 unit change in the independent variable, statistically controlling for the other

Margarete Ainsworth Building, Barker Street, Randwick NSW 2031. Phone: 02 9399 1000. Email: info@neura.edu.au

independent variables. Standardised regression coefficients represent the change being in units of standard deviations to allow comparison across different scales.

Prevalence refers to how many existing cases there are at a particular point in time. Incidence refers to how many new cases there are per population in a specified time period. Incidence is usually reported as the number of new cases per 100,000 people per year. Alternatively some studies present the number of new cases that have accumulated over several years against a person-years denominator. This denominator is the sum of individual units of time that the persons in the population are at risk of becoming a case. It takes into account the size of the underlying population sample and its age structure over the duration of observation.

‡ Inconsistency refers to differing estimates of treatment effect across studies (i.e. heterogeneity or variability in results) that is not explained by subgroup analyses and therefore reduces confidence in the effect estimate. I^2 is the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance) - 0% to 40%: heterogeneity might not be important, 30% to 60%: may represent moderate heterogeneity, 50% to 90%: may represent considerable heterogeneity and over this is considerable heterogeneity. I^2 can be calculated from Q (chi-square) for the test of heterogeneity with the following formula;

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

§ Imprecision refers to wide confidence intervals indicating a lack of confidence in the effect estimate. Based on GRADE recommendations, a result for continuous data (standardised mean differences, not weighted mean differences) is considered imprecise if the upper or lower confidence limit crosses an effect size of 0.5 in either direction, and for binary and correlation data, an effect size of 0.25. GRADE also recommends downgrading the evidence when sample size is smaller than 300 (for binary data) and 400 (for continuous data), although for some topics, this criteria should be relaxed[11].

‖ Indirectness of comparison occurs when a comparison of intervention A versus B is not available but A was compared with C and B was compared with C which allows indirect comparisons of the magnitude of effect of A versus B. Indirectness of population, comparator and or outcome can also occur when the available evidence regarding a particular population, intervention, comparator, or outcome is not available so is inferred from available evidence. These inferred treatment effect sizes are of lower quality than those gained from head-to-head comparisons of A and B.

References

1. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMAGroup (2009): Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *British Medical Journal* 151: 264-9.
2. GRADEWorkingGroup (2004): Grading quality of evidence and strength of recommendations. *British Medical Journal* 328: 1490.
3. Marsman A, Van Den Heuvel MP, Klomp DWJ, Kahn RS, Luijten PR, Hulshoff Pol HE (2013): Glutamate in schizophrenia: A focused review and meta-analysis of 1H-MRS studies. *Schizophrenia Bulletin* 39: 120-9.
4. Song J, Viggiano A, Monda M, De Luca V (2014): Peripheral glutamate levels in schizophrenia: Evidence from a meta-analysis. *Neuropsychobiology* 70: 133-41.
5. Brouwer A, Luykx JJ, van Boxmeer L, Bakker SC, Kahn RS (2013): NMDA-receptor coagonists in serum, plasma, and cerebrospinal fluid of schizophrenia patients: a meta-analysis of case-control studies. *Neuroscience & Biobehavioral Reviews* 37: 1587-96.
6. Catts VS, Lai YL, Weickert CS, Weickert TW, Catts SV (2016): A quantitative review of the postmortem evidence for decreased cortical N-methyl-D-aspartate receptor expression levels in schizophrenia: How can we link molecular abnormalities to mismatch negativity deficits? *Biological Psychology* 116: 57-67.
7. Pearlman DM, Najjar S (2014): Meta-analysis of the association between N-methyl-d-aspartate receptor antibodies and schizophrenia, schizoaffective disorder, bipolar disorder, and major depressive disorder. *Schizophrenia Research* 157: 249-58.
8. Wenneberg C, Glenthoj BY, Hjorthoj C, Buchardt Zingenberg FJ, Glenthoj LB, Rostrup E*, et al.* (2020): Cerebral glutamate and GABA levels in high-risk of psychosis states: A focused review and meta-analysis of 1H-MRS studies. *Schizophrenia Research* Jan: doi: 10.1016/j.schres.2019.10.050.
9. CochraneCollaboration (2008): Cochrane Handbook for Systematic Reviews of Interventions. Accessed 24/06/2011.
10. Rosenthal JA (1996): Qualitative Descriptors of Strength of Association and Effect Size. *Journal of Social Service Research* 21: 37-59.
11. GRADEpro (2008): [Computer program]. Jan Brozek, Andrew Oxman, Holger Schünemann. *Version 32 for Windows*