

The specificity of platelet glutamate receptor
sensitivity as a putative marker for
schizophrenia

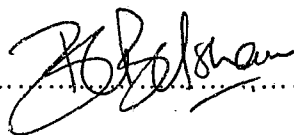
Brendan Clive Belsham

A research report submitted to the Faculty of Health Sciences,
University of the Witwatersrand, in partial fulfilment of the
requirements for the degree of
Master of Medicine in the branch of Psychiatry

Johannesburg, 2000

Declaration

I, Brendan Clive Belsham, declare that this research report is my own work, although I received assistance in the laboratory work and statistical methods. It is being submitted for the degree of Master of Medicine in the branch of Psychiatry. It has not been submitted before for any degree or examination at this or any other University.



.....

..... 26th day of MAY 2000.

Publication arising from this study

Berk M, Plein H, Belsham BC. The Specificity of Platelet Glutamate Receptor Supersensitivity in Psychotic Disorders. *Life Sciences*. In Press.

(See Appendix 1)

Abstract

Hypoglutamatergic function is implicated in the pathogenesis of schizophrenia, and supersensitivity of platelet N-methyl-D-aspartate (NMDA) receptors has been reported in schizophrenia. The aim of this study was to examine the platelet glutamate receptor sensitivity in patients with schizophrenia as well as other psychotic conditions, and matched controls, in order to assess if this is a specific marker of schizophrenia or occurs in other psychotic conditions. Glutamate receptor sensitivity was assessed using the intracellular calcium response to glutamate measured with spectrofluorometry. The percentage responses to glutamate stimulation of the schizophrenic subjects and those with depression with psychotic features were significantly greater than control subjects ($p < 0.005$). The mania with psychotic features group was not significantly different to controls. This data suggests that platelet glutamate receptors may be supersensitive in schizophrenia and depression with psychotic features. The platelet may be a possible peripheral marker of glutamate function in schizophrenia and depression with psychotic features.

Acknowledgements

I wish to thank the following:

My supervisor, Professor Michael Berk, for his guidance in the preparation of this research report.

Dr Helene Plein, of the Department of Pharmacology, for the laboratory work and statistical methods; without her assistance I would not have been able to produce this work.

The medical and nursing staff of Chris Hani Baragwanath Hospital, for their help in recruiting subjects for the study.

The Departments of Psychiatry and Pharmacology, for funding this work.

The patients and controls, for agreeing to participate in the study.

Table of Contents

| | Page |
|---|------|
| DECLARATION | ii |
| PUBLICATION ARISING FROM THE STUDY | iii |
| ABSTRACT | iv |
| ACKNOWLEDGEMENTS | v |
| TABLE OF CONTENTS | vi |
| LIST OF FIGURES AND TABLES | ix |
| 1.0 INTRODUCTION | 10 |
| 1.1 Glutamate as a neurotransmitter | 10 |
| 1.2 Schizophrenia and Glutamate | 11 |
| 1.2.1 Introduction | 11 |
| 1.2.2 Post-mortem studies | 12 |
| 1.2.3 In vivo neurochemical studies | 13 |
| 1.2.4 Pharmacologic studies | 14 |
| 1.2.5 Genetic studies | 15 |
| 1.2.6 Interactions with other neurotransmitters | 15 |
| 1.2.7 Possible mechanisms | 16 |
| 1.2.8 Conclusions | 17 |
| 1.3 Mood disorders and glutamate | 18 |
| 1.4 The platelet as a peripheral marker | 19 |
| 1.5 Aims and hypothesis of the study | 21 |

| | | |
|-------|---|----|
| 2.0 | METHODOLOGY | 22 |
| 2.1 | Sample | 22 |
| 2.1.1 | Patient groups | 22 |
| 2.1.2 | Exclusion criteria | 22 |
| 2.1.3 | Rating scales | 22 |
| 2.1.4 | Controls | 23 |
| 2.2 | Laboratory methods | 23 |
| 2.2.1 | Platelet collection | 23 |
| 2.2.2 | Loading of platelets with fura-2-AM | 23 |
| 2.2.3 | Fluorescence measurements and addition of glutamate | 24 |
| 2.3 | Statistical analysis | 24 |
| 2.4 | Ethics | 24 |
| 2.5 | Consent | 25 |
| 2.6 | Funding | 25 |
| 3.0 | RESULTS | 26 |
| 3.1 | Demographic and clinical data | 26 |
| 3.2 | Biochemical data | 27 |
| 4.0 | DISCUSSION | 31 |
| 4.1 | Limitations of the study | 31 |

| | | |
|------------|---|----|
| 4.2 | Conclusions | 32 |
| APPENDICES | | 35 |
| Appendix 1 | Letter of acceptance from LIFE SCIENCES Journal | 35 |
| Appendix 2 | Chemical reaction | 36 |
| Appendix 3 | Ethics clearance | 37 |
| Appendix 4 | Hamilton Rating Scale for Depression | 38 |
| Appendix 5 | Brief Psychiatric Rating Scale | 39 |
| Appendix 6 | Consent Form | 40 |
| Appendix 7 | Mini-International Neuropsychiatric Interview | 41 |
| REFERENCES | | 51 |

Lists of and Tables and Figures

| Tables | Page |
|---|-------------|
| 3.1 Demographic data and rating scale scores of patient groups and controls | 26 |
| 3.2 Platelet intracellular calcium levels at baseline and at 1 μ M of glutamate in the different study groups | 27 |
| Figures | |
| 3.1 Mean baseline platelet intracellular calcium concentrations in the three patient groups and controls | 28 |
| 3.2 Mean intracellular calcium concentrations in the four study groups before and after stimulation with glutamate at 1 μ M | 29 |
| 3.3 The platelet intracellular calcium response to glutamate stimulation in the four study groups: percentage response | 30 |

1.0 Introduction

1.1 Glutamate as a neurotransmitter

The amino acid glutamate is the most abundantly active neurotransmitter in the mammalian brain, acting at more than 50% of all synapses in the brain. It is also the principal excitatory neurotransmitter in the human cortex. It is synthesised from glucose and glutamine in presynaptic neuron terminals and is stored in synaptic vesicles. Once released into the synaptic cleft, it acts on receptors and its action is terminated by reuptake into the presynaptic neuron. It is catabolized by the enzyme glutamic acid decarboxylase (GAD). Glutamatergic pathways are principally corticofugal, in contrast to monoaminergic pathways, which mainly run towards the cortex from the brainstem and spinal cord. There are also intracortical pathways, especially in the medial temporal lobe structures (1).

There are eight known glutamate receptors. The N-methyl-D-aspartate [NMDA] receptor is the best known. It is an ionotropic receptor, in that it allows the passage of calcium, and to a lesser extent sodium and potassium ions. Its ion channel opens when the receptor is bound by both glutamate and glycine, whilst the membrane potential rises to above -65 mV. Other receptors include the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], kainate and *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) receptors. The latter is a metabotropic receptor, in that it is linked to G proteins which in turn activate intracellular second messengers (2).

Excitatory amino acids, especially glutamate, are known to have a number of physiologic functions. The NMDA receptor is regarded by some to be the essential cellular feature of memory. By a process known as long-term potentiation (LTP), this receptor is thought to trigger a cascade of intracellular events leading to the formation of memory (2). Glutamate is abundant in the pyramidal cells of the cortex, the cerebellum, the striatum and corticostriatal projections, and is thus thought to be important in movement. It is also postulated to be involved in perception (3). Excitatory amino acid systems have the potential to promote or inhibit neuronal development in a wide variety of tissues. Hence the importance of these systems in developmental stages, when neuronal plasticity is critical in the formation of adequate neuronal connections. It is thought that part of the pathophysiology of schizophrenia relates to abnormal neuronal development, as evidenced by abnormal cell layer morphology in temporal lobe structures (4).

1.2 Schizophrenia and glutamate

1.2.1 Introduction

Schizophrenia has been estimated to affect 1% of the population (5). It is a chronic, debilitating illness, which carries a significant economic and human cost to society. It is widely acknowledged that schizophrenia has biological underpinnings and research into its pathogenesis has been dominated by various neurotransmitter theories. Although the dominant neurotransmitter theory is that of dopamine, recent attempts to clarify the neurochemical mechanisms of this disorder have shifted to the amino acids. A major reason for this is the recognition of the role played by the cerebral cortex in schizophrenia, and hence its two primary neurotransmitters, glutamate and gamma-aminobutyric acid (GABA).

Hypofunction of the NMDA receptor was first implicated in schizophrenia when reduced levels of glutamate were found in the cerebrospinal fluid of patients with schizophrenia (6). Although this hypothesis initially failed to attract many adherents, recent years have seen the emergence of numerous lines of evidence to support this theory.

It was observed that the street-drug phencyclidine (“angeldust”), the anaesthetic ketamine, and MK-801, all NMDA receptor antagonists, cause a psychosis resembling both the positive and negative symptoms of schizophrenia (7). This drug model of schizophrenia is thus an improvement on the amphetamine model, in which only positive symptoms are seen, and which is the best drug model of the dopamine hypothesis of schizophrenia. However, its specificity is questionable, since subjects taking these substances also display high scores on dissociative phenomenon rating scales (8).

1.2.2 Post-mortem studies

Radioligand binding studies have shown, in general, decreased glutamate binding to kainate receptors in medial temporal cortices of schizophrenics, and increased glutamate binding to NMDA and AMPA receptors in frontal cortices of schizophrenics (9,10). Although interpretation of this data is difficult, it is at least partially consistent with diminished glutamatergic function.

A study measuring mRNA coding for five different subunits of the NMDA receptor in cortical tissue found no significant differences between schizophrenics and controls,

although a relative increase in one subunit was found in the prefrontal cortex (11).

Decreased mRNA encoding for non-NMDA glutamate receptors has been demonstrated in the hippocampus of subjects with schizophrenia (12).

Certain studies have utilized the co-localization of neuropeptides with glutamatergic neurons to investigate this hypothesis. A study using quantitative receptor autoradiography demonstrated a reduction in the number of neurotensin receptors expressed by a population of glutamatergic neurons in the entorhinal cortex of a sample of schizophrenics (13). In addition, a reduction in mRNA encoding for cholecystokinin in the entorhinal cortex of brains of patients with schizophrenia has been documented (8).

Another technique of evaluating glutamate function involves measuring the activity of an intraneuronal enzyme. N-acetyl-alpha-linked acidic dipeptidase (NAALADase) is present in glutamatergic neurons and is responsible for converting N-acetylaspartylglutamate (NAAG), abundant in the limbic system, to N-acetyl aspartate (NAA) and glutamate (see Appendix 2). One group found increased levels of NAAG as well as decreased NAALADase activity and glutamate levels in the hippocampus and prefrontal cortex of patients with schizophrenia (14). NAAG has been shown to block NMDA receptors.

1.2.3 In vivo neurochemical studies

More recently, studies have utilised the unique proton magnetic resonance properties of NAA. This has the advantage of providing in vivo neurochemical information. Thus using magnetic resonance spectroscopy, reduced levels of NAA in the temporal lobes of

schizophrenics have been demonstrated (15). Using a modification of this technique, highly localised decreases in NAA in the rostral hippocampus and dorsolateral prefrontal cortex of people with schizophrenia have been demonstrated (16).

1.2.4 Pharmacologic studies

Glycine, which acts as an obligatory co-agonist at the NMDA receptor, enhances the activation of the NMDA - gated voltage dependent cation channel, by increasing the frequency of channel openings. Both open-label(17) and placebo-controlled crossover trials (18,19) have reported improvements in negative symptoms in treatment refractory schizophrenics treated with glycine.

D-cycloserine, an antituberculous drug, is a selective partial agonist at the glycine recognition site, producing 40-60% of the activity of glycine; it readily crosses the blood-brain barrier. A placebo-controlled dose-finding trial of D-cycloserine added to conventional neuroleptics in schizophrenics showed a significant improvement in negative and cognitive symptoms at 50mg daily (20). A similar study involving clozapine treated patients was negative (21). An independent group found a significant improvement in negative symptoms in unmedicated schizophrenics (22).

Currently used antipsychotics also have effects on the NMDA receptor. Haloperidol upregulates NMDA receptor number and binding, and clozapine increases brain glutamate concentrations in rats (23).

Other pharmacological studies have focused on the AMPA receptor. For example, a study assessing piracetam, which acts as an AMPA agonist, yielded encouraging results in schizophrenia (24).

1.2.5 Genetic studies

Previous research has consistently implicated genetic factors in the pathogenesis of schizophrenia, and it is possible that there may be a genetic basis for aberrant glutamate functioning. In addition to the earlier mentioned postmortem studies measuring cortical mRNA, a linkage study assessed the relationship between allelic variants of the GluR6 glutamate receptor gene and 23 families containing multiple cases of schizophrenia (25). Although the results were negative, this is another burgeoning area of research.

1.2.6 Interactions with other neurotransmitters

Although glutamate may play a role in schizophrenia, there is unequivocal evidence for the involvement of other neurotransmitter systems. Indeed it is known that there are significant interactions between glutamate and other neurotransmitters, especially dopamine, GABA and certain neuropeptides.

According to the dopamine hypothesis, diminished cortical dopamine function accounts for the negative schizophrenic symptoms, whilst an excess of mesolimbic or subcortical dopamine activity accounts for delusions and hallucinations. It is known that dopamine receptors situated in corticostriatal glutamatergic nerve terminals mediate an inhibition of

glutamate release in the striatum. Therefore, excessive subcortical dopamine activity may cause secondary inhibition of NMDA receptor function (26). Conversely, NMDA receptors located on dopaminergic fibres have been shown to inhibit dopamine release (27). Thus at the subcortical level there may be a reciprocal antagonism between dopamine and glutamate.

It has also been shown that dopamine, via activation of the D1 receptor, facilitates neocortical NMDA receptors (28). Thus diminished cortical dopamine activity is consistent with decreased glutamatergic function.

1.2.7 Possible mechanisms

If the NMDA receptor is hypofunctional, it remains unclear how this comes about, and how it manifests in the clinical expression of psychosis. One of the consistent findings of studies assessing the effects of NMDA antagonists, such as phencyclidine, is an associated excessive release of glutamate and consequent overstimulation of postsynaptic neurons. It has been suggested that hypofunctional NMDA receptors may lead to a loss of tonic stimulation of inhibitory GABAergic interneurons, which in turn leads to disinhibition of certain excitatory projections, some of which are glutamatergic. This is thought to result in the bombardment of many other neurons in their projection fields with unmodulated noise, and the clinical expression of psychotic thinking (26,29).

It has further been suggested that this disinhibited NMDA activity leads to aberrant LTP and the formation of fixed abnormal memories, or delusions. This is thought to occur at the subcortical level and to affect cortical processing by way of the mediodorsal thalamus. It has

been hypothesized that the mechanism by which delusions become fixed involves a decrease in excitatory inputs overcoming extensive GABA-ergic influences (28, and Hurlock, personal communications); this is supported by decreased absolute levels of glutamate found in schizophrenic brains.

It has been proposed that the phenomenon of excitotoxicity may explain the long-term clinical deterioration observed in schizophrenics. By this process, excessive NMDA-mediated calcium influx leads to a series of intracellular events culminating in the formation of toxic free radicals and cell death (30). In the long-term this is thought to contribute to a neurodegenerative process. However there are arguments against this hypothesis. In the spectroscopic studies assessing NAA, no difference was found between chronic and first-episode schizophrenics in terms of either the magnitude of the reduction or the localisation of the changes. In addition, studies using computerised tomography to assess ventricular size have failed to show neuronal loss in schizophrenics followed over several years. Also, functional data suggest that memory function, strongly linked to the NMDA receptor, does not decrease over time. These studies suggest that whilst glutamate function may be altered in schizophrenia, it may not contribute to an excitotoxic/neurodegenerative process (8).

1.2.8 Conclusion

Thus there is emerging evidence implicating altered glutamatergic function in schizophrenia. However, due to the complex interplay between the various neurotransmitter systems, it is unlikely that a single neurotransmitter will prove to be the sole explanation for the pathophysiology of schizophrenia.

Similarly, it is likely that neurotransmitter dysfunction per se may represent a small part or epiphenomenon of the actual pathophysiologic mechanism. For example, both epidemiological and neuroanatomical studies support the neurodevelopmental hypothesis, which holds that abnormal brain development is the decisive etiologic factor in schizophrenia, and altered neurotransmitter function is a later consequence (8).

1.3 Mood disorders and glutamate

Research has investigated the role of the excitatory amino acids in depression, but has met with conflicting evidence. One group found elevated plasma and platelet levels of glutamate in depressed patients compared to controls (31). On the other hand, there has been at least one negative study; which found no difference in serum levels of glutamate between patients with depression and age and sex matched controls (32). Also, changes in plasma glutamate have been described in other conditions such as migraine, which may be comorbid with depression (33).

Antidepressant therapy seems to reduce plasma levels of glutamate (32). Tricyclic antidepressant drugs appear to modify the activity of glutamatergic neurons (34). Regionally selective changes in the NMDA receptor complex occur with chronic antidepressant administration, which may be mediated by regionally selective changes in excitatory amino acid concentrations (35).

A competitive NMDA antagonist (2-amino-7-phosphonoheptanoic acid [AP-7]), the non-competitive NMDA antagonist, dizocilpine (MK801), and a partial agonist at strychnine-insensitive glycine receptors (1-aminocyclopropanecarboxylic acid [ACPC]) have all shown efficacy in animal models of depression (36,37).

In a post-mortem study, [3H]MK-801-binding to NMDA receptors did not differ between 22 suicide victims and 20 controls in any of nine brain regions studied (38). However, this study was not specifically of depression, and post-mortem studies are complicated by issues of retrospective diagnosis and post-mortem brain changes.

Lamotrigine is an anticonvulsant which is being used in bipolar disorder for its mood stabilising properties. Its mechanism of action probably includes the inhibition of excessive release of glutamate. The efficacy of lamotrigine in the depressive phase of bipolar disorder has been demonstrated in a placebo controlled design (39). Whilst this suggests the involvement of glutamate, alternate mechanisms of action of the drug may be operative, such as inhibition of sodium and calcium channels.

Also, low levels of the enzyme glutamic acid decarboxylase (GAD) have been demonstrated in patients with major depression and bipolar disorder (40).

1.4 The platelet as a peripheral marker

Psychiatric practise suffers from a lack of external validating criteria; that is, of biological markers which might enhance the reliability and validity of clinical diagnosis. Accessible

peripheral markers remain an elusive goal in psychiatric illness in general, and schizophrenia in particular. Their potential benefits include enriching knowledge of the biological underpinnings of psychiatric illness, the refinement of psychiatric diagnosis, and facilitating the early diagnosis of conditions whose prognoses are known to worsen with the duration of untreated illness. Schizophrenia is one such condition.

Such peripheral markers are divided into state and trait markers. State markers are detectable in a given individual only under certain conditions, for example when a patient has relapsed; trait markers are constantly present in a given individual with a certain illness.

Increases in second messenger free intracellular calcium in response to agonist stimulation have been linked to the NMDA receptor complex in non-neuronal cell lines (41). NMDA receptors have been isolated on platelet membranes, and kinetic properties of glutamate uptake in platelets and brain slices are similar (42,43). While there is no compelling data proving that the platelet data accurately reflects central changes, these studies justify further investigation into the disease using the platelet as a possible peripheral marker. Indeed, platelet intracellular calcium is widely used as an indirect marker of receptor alterations in psychiatric illnesses. It is accepted in the field that the second messenger (calcium) response is the most physiological means of assessing receptor function.

(44,45,46).

It was hypothesised that the NMDA receptors of schizophrenics would be supersensitive as a compensation for chronically attenuated glutamatergic and NMDA receptor functioning. A

previous study showed lowered intracellular baseline calcium concentrations, as well as significant supersensitivity of glutamate receptors in schizophrenics versus controls (47). Thus this prior work appears to demonstrate the sensitivity of this test as a putative peripheral marker of schizophrenia; however its specificity as a test for schizophrenia is yet to be established.

1.5 Aims and hypothesis of the study

The primary aim of this study thus is to determine whether supersensitivity of NMDA receptors is specific for schizophrenia, or whether it is a non-specific marker of psychosis. The other psychotic conditions chosen were mania with psychotic features and psychotic depression. These two mood disorders were chosen to assess if the supersensitivity of the NMDA receptors would occur in these disorders.

A related objective is to replicate earlier work, which found lowered intracellular calcium levels in schizophrenics versus controls, and supersensitive NMDA receptors.

There are also broader aims, namely adding to the existing knowledge of the glutamate hypothesis of schizophrenia, and further investigating the possibility of peripheral markers for psychiatric illnesses (specifically schizophrenia).

2.0 Methodology

2.1 Sample

2.1.1 Patient groups

The sample comprised male and female patients admitted to Chris Hani Baragwanath Hospital, between the ages of 18 and 70 years, diagnosed on a structured clinical interview (Mini International Neuropsychiatric Interview, see appendix 7) as suffering from schizophrenia, or mania with psychotic features, or psychotic depression. The sample was recruited between February and July of 1998. To facilitate accurate diagnosis, collateral information from family and hospital records was sought. If the diagnosis was in any way unclear, the patient was not included in the study.

2.1.2 Exclusion criteria

All patients had not taken psychotropic medication for a period of two weeks (four weeks for depot preparations, five weeks for fluoxetine). Exposure to benzodiazepines for acute behavioural control was not an exclusion criterion. Patients with diagnoses of psychoactive substance use disorders or a positive urine cannabis assay were excluded from the study, although social use of alcohol and cigarette use were not exclusion criteria. Patients with significant medical illness (e.g. diabetes, hypertension) were excluded.

2.1.3 Rating scales

The schizophrenic group and the mania group were rated on the Brief Psychiatric Rating Scale (BPRS, see appendix 5), and the depression group was rated on the Hamilton Depression Rating Scale (HAMD, see appendix 4).

2.1.4 Controls

Sex, age and race matched controls, with no psychiatric history, were recruited; they were mainly staff at the hospital.

2.2 Laboratory methods

2.2.1 Platelet Collection

20ml blood was freshly drawn by venipuncture from an antecubital vein and placed in an acid-citrate-dextrose buffer containing 100 μ M aspirin. Platelet-rich-plasma was obtained by centrifugation for 15 minutes at 150g. This suspension was then recentrifuged for five minutes at 850g in order to obtain a platelet pellet. The platelets were then resuspended in an assay buffer (containing 137 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 5mM dextrose, 5 mM HEPES, pH 7,4).

2.2.2 Loading of platelets with fura-2-AM

The platelets were incubated at 37°C for 45 minutes with 4 μ M final concentration of fura-2-AM. Fura-2-AM is a calcium indicator which is used in studies assessing intracellular calcium. After the loading period, the platelets were kept at room temperature until fluorescence measurements were performed. Before fluorescence measurement, the platelets were spun down at 350g for five minutes. The supernatant was discarded and the pellet resuspended in a HEPES buffer (containing 145 mM NaCl, 1 mM MgCl₂, 10 mM HEPES, 5 mM glucose, 0,5 mM Na₂HPO₄, 1 mM CaCl₂, pH 7,55). The cell count was adjusted to 50 x10⁶ platelets/ml.

2.2.3 Fluorescence measurements and addition of glutamate

Fluorescence was measured in a Perkin-Elmer LS50 spectrophotometer with continuous gentle stirring. The excitation wavelengths were 340 nm and 380 nm with an emission wavelength of 510 nm. Glutamate concentrations of 0-100 μ M were added sequentially. The cytoplasmic free calcium concentration was measured by lysing the cells with Triton-X-100 in order to obtain maximum fluorescence, and then quenching the dye with 2 mM EGTA, which is an intracellular calcium chelator. This is in accordance with the method of Grynkiewicz, Poenie & Tsien (48).

2.3 Statistical analysis

Wilcoxon signed rank tests were used for these analyses. Non-parametric statistical methods (or distribution-free tests) were used because the sample sizes were small (less than 30), and it was thus not possible to determine whether the data were normally distributed. Percentage increases from baseline intracellular calcium were calculated in order to cancel the effect different baseline values would have had on the results.

2.4 Ethics

The protocol was passed by the Committee for Research on Human Subjects of the University of the Witwatersrand (see appendix 3).

2.5 Consent

Written informed consent was required for all subjects (see appendix 6). If patients could not give informed consent they were excluded from the study.

2.6 Funding

This was provided by the departments of Psychiatry and Pharmacology, University of the Witwatersrand.

3.0 Results

3.1 Demographic and clinical data

Table 3.1 illustrates the demographics and rating scale scores of the patient groups and controls included in this study. Except for the schizophrenia group, which contained a disproportionate number of males, the gender ratios were not statistically different. There were no statistically significant differences in the average ages across the four groups. The mean BPRS scores were similar in the mania and schizophrenia groups.

Table 3.1 Demographic data and rating scale scores of patient groups and controls

| Group | Number | Female | Male | Mean age (years) | BPRS score | Mean HAM-D score |
|-------------------------------|--------|--------|------|------------------|------------|------------------|
| Psychotic depression | 10 | 7 | 3 | 29,3 | - | 34,4 |
| Schizophrenia | 12 | 1 | 11 | 27,9 | 31,6 | - |
| Mania with psychotic features | 10 | 5 | 5 | 30,1 | 28,5 | - |
| Controls | 12 | 6 | 6 | 28 | 0 | 0 |

3.2 Biochemical data

Baseline platelet intracellular calcium levels were significantly lower than age, race, and sex matched control subjects in all the psychiatric groups studied ($p < 0.01$). All patient groups showed similarities in terms of baseline platelet intracellular calcium levels ($p > 0.05$). (See Table 3.2 and Figure 3.1)

Figure 3.2 depicts the change in intracellular calcium concentrations before and after stimulation with glutamate. However, in order to control for the differing baseline levels of intracellular calcium, the percentage change in intracellular calcium with glutamate stimulation was calculated (see Figure 3.3). Both the schizophrenia ($p = 0.004$, Wilcoxon signed rank test) and depression with psychotic features ($p = 0.007$) groups showed significant supersensitivity compared to the control group. The mania with psychotic features group did not differ significantly from controls ($p = 0.075$).

Table 3.2 Platelet intracellular calcium levels at baseline and at $1\mu\text{M}$ glutamate in the different study groups.

| <u>Psychiatric Group</u> | N | Mean baseline $[\text{Ca}^{2+}]_i$ | Mean $[\text{Ca}^{2+}]_i$ at $1\mu\text{M}$ glutamate |
|--------------------------|----|------------------------------------|---|
| Psychotic depression | 10 | 76,55; SD= 48,79 | 120,51; SD= 78,24 |
| Schizophrenia | 12 | 76,75; SD= 35,75 | 108,77; SD= 51,91 |
| Mania | 10 | 75,72; SD= 38,70 | 89,66; SD= 43,47 |
| Controls | 12 | 136,43; SD= 55,75 | 156,91; SD= 62,06 |

SD = Standard deviation

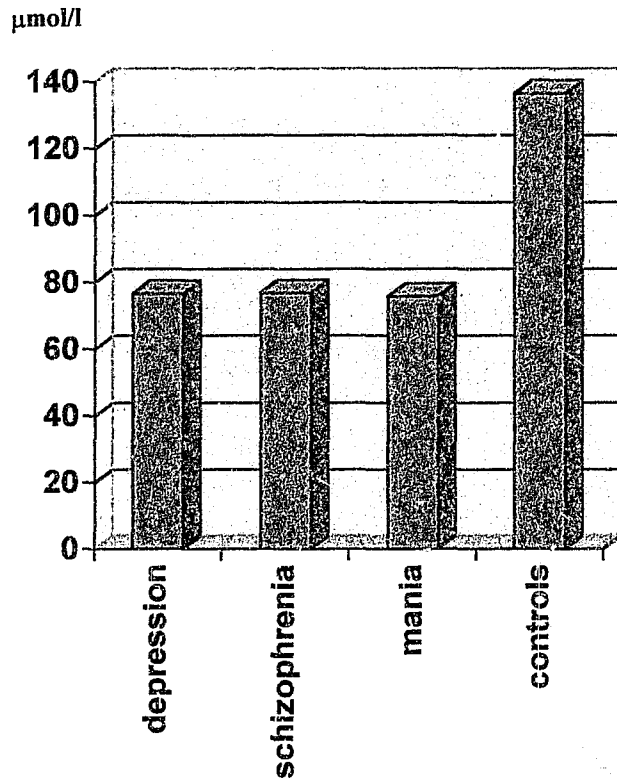


Figure 3.1 Mean baseline platelet intracellular calcium concentrations in the three patient groups and controls

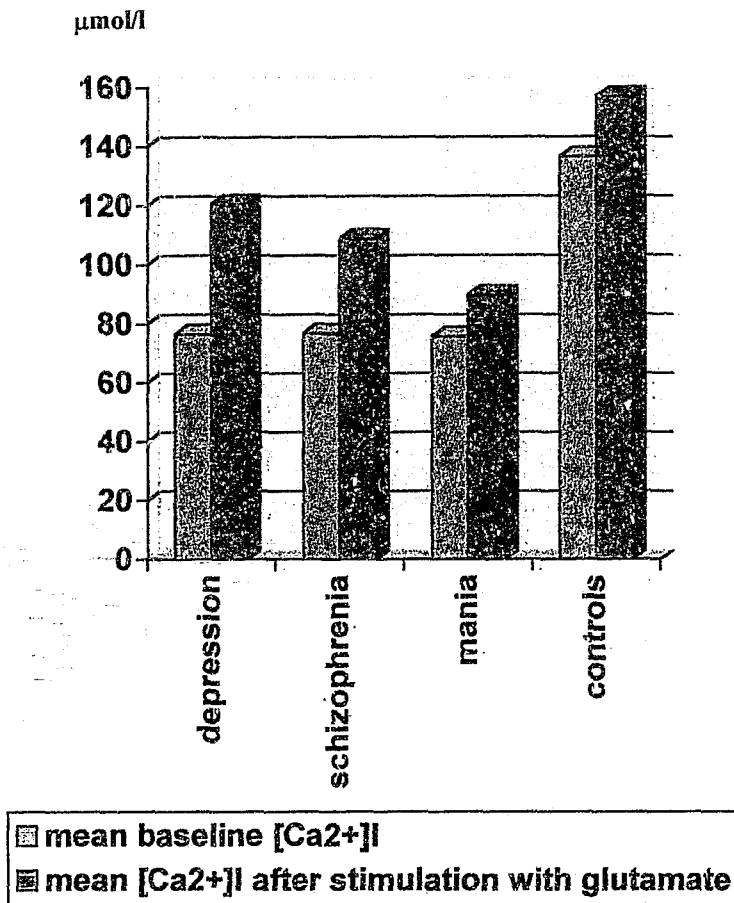
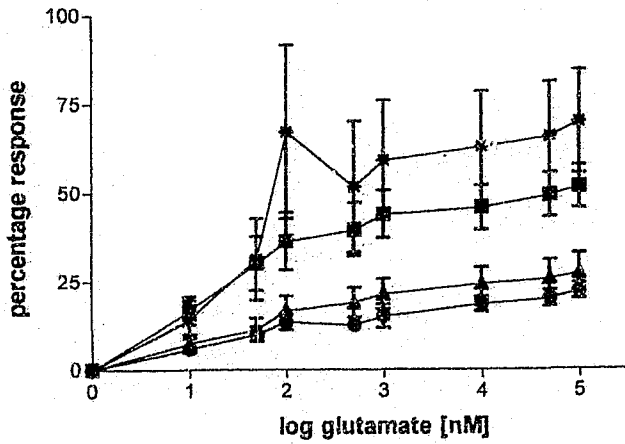


Figure 3.2 Mean intracellular calcium concentrations in the four study groups before and after stimulation with glutamate at concentration of $1 \mu\text{M}$



- Schizophrenia
- ▲ Mania
- * Depression
- Controls

Figure 3.3 The platelet intracellular calcium response in the four study groups:

percentage response

4.0 Discussion

4.1 Limitations of the study

Although every attempt was made to enhance the reliability and validity of this research, there were nonetheless certain difficulties. The cross-sectional diagnoses of the various psychotic disorders is a possible pitfall, as it is known that patients presenting with, for example, a maniform psychosis may subsequently receive the diagnosis of schizophrenia or bipolar disorder. Also, there was only one interviewer and thus inter-rater reliability could not be assessed. To facilitate accurate diagnosis, collateral information from family members and hospital records was sought. If the diagnosis was in any way unclear, the patient was not included in the study.

Unfortunately it was not possible to stratify the schizophrenic group into first-episode versus longstanding cases. This would have provided interesting information, since there may have been differences in this marker between the two groups, given the fact that schizophrenia is thought to be associated with neurodegeneration.

Also, it was not feasible to divide the psychotic depression group into bipolar versus unipolar depression, as again there may have been interesting differences. For example, since the psychotic depression group probably contained bipolar patients, and the mania group by definition contained bipolar patients, it might be argued that there should rather have been a single bipolar group.

The schizophrenic group contained a predominance of males. This may have affected the results, since there are gender differences amongst certain neurotransmitters, for example the serotonergic system. However the other groups, including the controls, did not show any gender differences.

Since a number of glutamate receptors are known to have calcium as their second messenger, it might be argued that the intracellular calcium response is not specific for the NMDA receptor. However, the addition of MK801, a specific NMDA antagonist, to the solution clearly blocked the response, suggesting that this is a valid test for the NMDA receptor.

It has been claimed that informed consent is not possible from psychotic individuals. However, not all reasoning faculties are necessarily affected when a person is psychotic, and internationally it is accepted practice to obtain informed consent from psychotic subjects.

8.2 Conclusions

Despite these difficulties, this study replicates earlier work which found that baseline levels of platelet intracellular calcium in schizophrenic patients are lower than age and sex matched controls. It also replicates the finding of a supersensitive platelet intracellular calcium response to glutamate stimulation in schizophrenics (47).

Within the schizophrenic group, no correlation between symptom severity and glutamate receptor sensitivity was found, although this may be an artefact of the sample size. Also, this group was heterogeneous in the duration of illness represented, which may also have affected the results, since schizophrenia is known to be a chronic progressive disorder.

No post treatment measurements were made to elucidate the state or trait status of the marker.

The results showed a supersensitive platelet intracellular calcium response to glutamate stimulation in the patients with major depression with psychotic features, but not in manic patients with psychotic features. These differences suggest that whilst supersensitive glutamate receptors are not unique to schizophrenia, neither are they a non-specific marker of psychosis.

Although it was not determined which of the patients with psychotic depression had underlying bipolar illnesses, these results suggest that this marker may be a feature of the depressive rather than the manic phase of bipolar disorder. Further, data on the behaviour of this marker in patients with non-psychotic depression would be helpful to elucidate if this is a marker of depressive disorders or only occurs in the psychotic forms of the illness. A recent study addressed this question, and the results suggested that glutamate receptor sensitivity is in fact increased in non-psychotic depression compared to controls (41).

Increased sensitivity to glutamate in depressed subjects, as seen in this study, may be due to NMDA receptor upregulation as a consequence of decreased glutamate concentrations. This

is however not in accordance with the available literature, which if anything suggests increased glutamate levels in platelets and serum of depressed subjects (32).

Seen within the broader context of the glutamate hypothesis of schizophrenia, this study has strengthened the available evidence for glutamate receptor dysfunction in this illness; specifically, it has contributed to the theory of attenuated activity of the NMDA receptor in schizophrenia.

The findings of this study further support the use of the platelet as a possible peripheral marker in schizophrenia, and also in psychotic depression. In so doing, it is hoped that it has contributed to the quest for suitable biological markers for psychiatric illnesses. Indeed, as evidence-based medicine and managed care become integral influences in psychiatric practice, such objective measures will be essential tools for the clinical psychiatrist.

APPENDICES

Appendix 1: Letter of acceptance from LIFE SCIENCES journal



LIFE SCIENCES

including Pharmacology Letters

Published by Elsevier Science Inc.
655 N. Alvernon Way, Suite 119
Tucson, Arizona 85711
Phone: (520) 321-7778
Fax: (520) 321-7781

Tuesday, April 18, 2000

Dr. Michael Berk
Dept. of Psychiatry
University of Witwatersrand
7 York Road
Parktown
Johannesburg, 2193
South Africa

RE: Manuscript #EE-0630-99

Dear Dr. Berk,

We are pleased to provide you with the publication information for your manuscript

THE SPECIFICITY OF PLATELET GLUTAMATE RECEPTOR SUPERSENSITIVITY IN PSYCHOTIC DISORDERS.

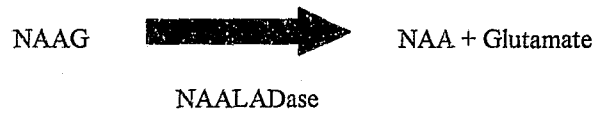
Your manuscript will appear in LIFE SCIENCES in Vol. 66, No. 25, on pages 2427-2432. This issue will have a publication date of 01/18/2000.

Thank you for using LIFE SCIENCES as a means of communicating your work.

Sincerely yours,

Christine K. Wade
Managing Editor

Appendix 2: Chemical reaction




NAAG - N-acetylaspartylglutamate – (abundant in limbic system)

NAALADase - N- acetyl-alpha- linked acidic dipeptidase

NAA - N-acetyl aspartate

Appendix 3: Ethics Clearance

Department of Psychiatry

 **UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**
7, Jeffery Road, Rosebank, 2195 South Africa • Telegrams: LITHALS • Telephone: 911 1947-2025 • Fax: 911 547-2125

Department of Psychiatry
Email: G39berk@chiron.wits.ac.za

26 January 1998


Prof PE Cleason-Jones
CRHS
Research Office
Senate House

Dear Prof Cleason-Jones,

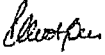
I wish to apply for an extension to protocol 29/2/92. In that protocol, we examined platelet intracellular calcium and the intracellular calcium response to various agonists in Bipolar Disorder. We again wish to examine the same parameters, using glutamate as one of the laboratory agonists, in Bipolar Disorder, Schizophrenia and Depression (extension previously approved).

The protocol is essentially unaltered, the only change is a lab one, in that we will use glutamate rather than serotonin as an agonist.


Yours Sincerely



Michael Berk
Associate Professor
Department of Psychiatry

Approved. 27/1/98


Deputy Registrar - Research

 **UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**
Private Bag 3, WITS 2050, South Africa • Telegrams: LITHALS • Telex: 4-271150A • Telephone: (011) 716-1111

20 February 1998


Professor Michael Berk
Department of Psychiatry
Wits Medical School

Dear Professor Berk

RE: EXTENSION ON PROTOCOL 29/2/92

This letter serves to confirm that an extension on protocol 29/2/92 has been granted by the Chairman of the Committee for Research on Human Subjects (Medical). Copy attached.

Yours sincerely,



ANISA KESHAV
SECRETARY
COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)

Appendix 4: The Hamilton Rating Scale for Depression

| Item | Grading |
|---|----------------|
| 1. depressed mood | 0-4 |
| 2. feelings of guilt | 0-4 |
| 3. suicidal impulses | 0-4 |
| 4. insomnia early | 0-2 |
| 5. insomnia middle | 0-2 |
| 6. insomnia late | 0-2 |
| 7. work and activities | 0-4 |
| 8. retardation | 0-4 |
| 9. agitation | 0-4 |
| 10. anxiety psychic | 0-4 |
| 11. anxiety somatic | 0-4 |
| 12. somatic symptoms (gastrointestinal) | 0-2 |
| 13. somatic symptoms (general) | 0-2 |
| 14. genital symptoms | 0-2 |
| 15. hypochondriasis | 0-4 |
| 16. loss of weight | 0-2 |
| 17. insight | 0-2 |
| 18. diurnal variation | 0-2 |
| 19. depersonalisation and derealisation | 0-4 |
| 20. paranoid symptoms | 0-4 |
| 21. obsessional and compulsive symptoms | 0-2 |

Appendix 5: The Brief Psychiatric Rating Scale

| Item | Grade |
|--|--------------|
| 1. somatic concerns | 0-4 |
| 2. anxiety (psychic) | 0-4 |
| 3. emotional withdrawal | 0-4 |
| 4. conceptual disorganisation | 0-4 |
| 5. self-deprecation and guilt feelings | 0-4 |
| 6. anxiety (somatic) | 0-4 |
| 7. specific motor disturbances | 0-4 |
| 8. exaggerated self-esteem | 0-4 |
| 9. lowered mood | 0-4 |
| 10. hostility | 0-4 |
| 11. suspiciousness | 0-4 |
| 12. hallucinatory behaviour | 0-4 |
| 13. decreased psychomotor activity | 0-4 |
| 14. uncooperativeness | 0-4 |
| 15. unusual thought content | 0-4 |
| 16. blunted or inappropriate affect | 0-4 |
| 17. increased psychomotor activity | 0-4 |
| 18. disorientation and confusion | 0-4 |

Appendix 6: Information and consent form

We are doing a research project on people who suffer from mental illnesses, including depression, schizophrenia and mania. We wish to measure certain chemicals, calcium and glutamate, in blood cells called platelets in people who have these illnesses. The aim of the study is to develop blood tests for people with mental illnesses. Participation in the study is voluntary and you are free to refuse to participate or withdraw your consent at any time. If you choose not to participate, you will not be disadvantaged in any way. The study simply involves taking a blood test and answering a few questions. The results of the study will be strictly confidential and your name will not be used. There will be no costs to you from the study. Your help with the project is much appreciated.

Signed

Date

Witnessed

Date

Appendix 7: Mini-International Neuropsychiatric Interview

M.I.N.I.

Mini-International Neuropsychiatric Interview, Clinician Rated (version 4.4)

© 1992, 1994 Sheehan DV & Lecrubier Y.

D. Sheehan, J. Janavs, E. Knapp, M. Sheehan, R. Baker, K.H. Sheehan, University of South Florida, Tampa, U.S.A.
Y. Lecrubier, E. Weiller, L.I. Bonora, P. Amarim, J.P. Lepine, INSERM-Hôpital de la Salpêtrière, Paris, France.

PATIENT NAME: _____ PROTOCOL NUMBER: _____
DATE OF INTERVIEW: _____ Time Interview Began: _____
DATE OF BIRTH: _____ Time Interview Ended: _____
INTERVIEWER'S NAME: _____ Total Time: _____

 means: Go to end of disorder, circle NO and move to next disorder.

A. MAJOR DEPRESSIVE EPISODE

- A 1 Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks? NO YES 1
- A 2 In the past two weeks, have you been less interested in most things or less able to enjoy the things you used to enjoy most of the time? NO YES 2

If Both A1 & A2 = NO, Circle NO in A4b and skip to B1

- A 3 In the past two weeks, when you felt depressed or uninterested, most of the time:
- a Did your appetite change significantly or did your weight increase or decrease \pm 8 lbs. (i.e., $\pm 5\%$ of body weight) without trying intentionally? NO YES 3
 - b Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning waking or sleeping excessively)? NO YES 4
 - c Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still? NO YES 5
 - d Did you feel tired or without energy most of the time? NO YES 6
 - e Did you feel worthless or guilty (most of the time)? NO YES 7
 - f Did you have difficulty concentrating or making decisions? NO YES 8
 - g Did you consider hurting yourself, feel suicidal, or wish that you were dead? NO YES 9
- A 4 a ARE 3 OR MORE ITEMS FROM A3 CODED YES - OR 4 ITEMS FROM A3 IF A1 OR A2 ARE CODED NO? NO YES
- b CODES POSITIVE FOR CURRENT MDE (A1 & A4a = YES) and/or (A2 & A4a = YES)?

| | |
|--------------------------|-----|
| NO | YES |
| Major Depressive Episode | |
| CURRENT | |

IF PATIENT CODES POSITIVE FOR MAJOR DEPRESSION (A4b = YES), SKIP TO BIPOLAR DISORDERS



B. DYSTHYMIA

If patient currently meets criteria for major depressive episode, do not explore this section.

| | | | | |
|-----|--|----|-----|----|
| B 1 | Have you felt sad, low or depressed most of the time for the last two years? | NO | YES | 10 |
| B 2 | Was this period interrupted by your feeling OK for two months or more? | NO | YES | 11 |
| B 3 | During this period of feeling depressed most of the time: | | | |
| a | Did your appetite change significantly? | NO | YES | 12 |
| b | Did you have trouble sleeping or sleep excessively? | NO | YES | 13 |
| c | Did you feel tired or without energy? | NO | YES | 14 |
| d | Did you lose your self-confidence? | NO | YES | 15 |
| e | Did you have trouble concentrating or making decisions? | NO | YES | 16 |
| f | Did you feel hopeless? | NO | YES | 17 |
| B 4 | Did the symptoms of depression cause you significant distress or impair your ability to function at work, socially, or in your other daily activities? | NO | YES | 18 |

ARE 2 OR MORE ITEMS FROM B3 & B4 CODED YES?

| | |
|-------------------------|-----|
| NO | YES |
| Dysthymia & Current? | |

NOTE: The diagnosis of double depression and major depression in partial remission are not explored in the MINI but can be explored in detail with additional questions in the MINI Plus.

C. BIPOLAR DISORDERS

| | | | | |
|-------|---|----|-----|---|
| C 1 a | Have you ever had a period of time when you were feeling 'up' or 'high' or so full of energy or full of yourself that you got into trouble, or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.) | NO | YES | 1 |
| | If patient is puzzled or unclear about what you mean by 'up' or 'high', clarify as follows: By 'up' or 'high' I mean: •having elated mood, •increased energy, •needing less sleep, •having rapid thoughts, •being full of ideas, •having an increase in productivity, creativity, motivation or impulsive behavior? | | | |
| b | Have you ever been persistently irritable, so that you shouted or started fights or arguments with people outside your family? | NO | YES | 2 |

If NO to all of C1a-b, Circle NO in C5 and skip to D1

| | | | | |
|-----|---|----|-----|---|
| C 2 | Have you been feeling 'up' or 'high', full of energy or irritable in the past month (manic symptoms)? | NO | YES | 3 |
|-----|---|----|-----|---|

NOTE: If currently manic (C2 = YES), explore only current episode. If no current mania, explore most symptomatic past episode.

| | |
|-----|--|
| C 3 | During the times when you felt high, full of energy, or irritable did you: |
|-----|--|

| | | | | | |
|-------|---|--|-----------------------------|---------------|----|
| | a | Feel that you could do things others couldn't do, or that you were an especially important person? | NO | YES | 4 |
| | b | Need less sleep (e.g., feel rested after only a few hours sleep)? | NO | YES | 5 |
| | c | Talk too much without stopping, or so fast that people had difficulty understanding? | NO | YES | 6 |
| | d | Have thoughts racing through your head so fast that you had difficulty keeping track of them? | NO | YES | 7 |
| | e | Become easily distracted so that any little interruption could distract you? | NO | YES | 8 |
| | f | Become so active or physically restless that others were worried about you? | NO | YES | 9 |
| | g | Want so much to engage in pleasurable activities that you ignored the risks or consequences? (e.g., spending sprees, reckless driving, or sexual indiscretions)? | NO | YES | 10 |
| | | Summary of C3: Are 3 of the C3 answers coded YES (or 4 if C1a is NO)? | NO | YES | |
| | | RULE: Elation/Expansiveness requires only three C3 symptoms while the other criteria require four of the C3 symptoms. | | | |
| C 4 | | Did these symptoms last at least a week and cause problems beyond your control at home, work, school, or were you hospitalized for these problems? | NO | YES | 11 |
| | | | HYPOMANIC EPISODE | MANIC EPISODE | |
| C 5 a | | CODES POSITIVE FOR CURRENT MANIC EPISODE? HYPOMANIC EPISODE (C2 = YES and C3(summary)=YES) | NO | YES | |
| | | | NO Current Bipolar Disorder | YES | |
| | b | Prior to the last month, has the patient ever had a manic (hypomanic) episode? (C3 = YES and C1a-b=YES) | NO | YES | |
| | | | NO Past Bipolar Disorder | YES | |
| | | NOTE: The diagnosis of past major depression is not explored in the MINI but can be explored in detail with additional questions in the MINI Plus. | | | |

D. PANIC DISORDER

| | | | | | |
|-----|---|--|----|-----|---|
| D 1 | | PANIC DISORDER | | | |
| | a | Have you, on more than one occasion, had spells or attacks when you suddenly felt anxious, frightened, uncomfortable or uneasy in a situation where most people would not feel that way? | NO | YES | 1 |
| | b | At any time in the past, did any of those spells or attacks come on unexpectedly or occur in an unpredictable or unprovoked manner? | NO | YES | 2 |
| | c | Have you ever had one such attack followed by a month or more of persistent fear of having another attack, or worries about the consequences of the attack? | NO | YES | 3 |

d During the worst spell that you can remember.

- | | | | | |
|----|--|----|-----|----|
| 1 | Did you have skipping, racing or pounding of your heart? | NO | YES | 4 |
| 2 | Did you have sweating or clammy hands? | NO | YES | 5 |
| 3 | Were you trembling or shaking? | NO | YES | 6 |
| 4 | Did you have shortness of breath or difficulty breathing? | NO | YES | 7 |
| 5 | Did you have a choking sensation or a lump in your throat? | NO | YES | 8 |
| 6 | Did you have chest pain, pressure or discomfort? | NO | YES | 9 |
| 7 | Did you have nausea, stomach problems or sudden diarrhea? | NO | YES | 10 |
| 8 | Did you feel dizzy, unsteady, lightheaded or faint? | NO | YES | |
| 9 | Did you feel detached from things around you or detached from part of your body? | NO | YES | 12 |
| 10 | Did you fear that you were losing control or going crazy? | NO | YES | 13 |
| 11 | Did you fear that you were dying? | NO | YES | 14 |
| 12 | Did you have tingling or numbness in parts of your body? | NO | YES | 15 |
| 13 | Did you have hot flushes or chills? | NO | YES | 16 |

Summary D1d: Are at least 4 of the above D1d symptoms coded YES? NO YES

e CODES POSITIVE FOR LIFETIME PANIC DISORDER?
D1a and D1b and D1c and Summary of D1d = YES
IF a IS CODED NO, SKIP TO D1g.

| | |
|----------------------------|-----|
| NO | YES |
| Panic Disorder Lifetime | |

f In the past month, did you have such attacks repeatedly, or did you have one attack followed by persistent fear of having another attack? (It is denied by the patient - challenge by reviewing the symptoms entered in D1d)

| | | |
|---------------------------|-----|----|
| NO | YES | 17 |
| Panic Disorder Current | | |

IF D1f IS CODED YES SKIP TO E1.

g Apart from the panic attacks with 4 or more symptoms that we just discussed, in the past month, did you have sudden attacks of only 3, 2 or 1 of the above symptoms.

| | | |
|---------------------------------------|-----|----|
| NO | YES | 18 |
| Current Limited Symptom Attacks | | |

CODES POSITIVE FOR CURRENT LIMITED SYMPTOM ATTACKS.

E. AGORAPHOBIA

E 1 a Do you feel particularly uneasy in places or situations from which escape might be difficult or embarrassing, or help might not be available: like being in a crowd, standing in a line, being alone away from home, crossing a bridge, or traveling in a bus, train or car?

| | | |
|----|-----|----|
| NO | YES | 19 |
|----|-----|----|

b Do you fear these situations so much that you avoid them, suffer through them, or need a companion to face them?

| | | |
|------------------------|-----|----|
| NO | YES | 20 |
| Agoraphobia Current | | |

c Patient codes positive for Panic Disorder, Current. Patient does not code positive for Agoraphobia, Current

| | |
|--|-----|
| NO | YES |
| PANIC DISORDER Current without Agoraphobia | |

☑ means: Go to end of disorder, circle NO and move to next disorder

d Patient codes positive for Panic Disorder (current) with Agoraphobia, Current.

| | | |
|---------------------------------|---------|-----|
| NO | Current | YES |
| PANIC DISORDER with Agoraphobia | | |

e Patient codes positive for Current Agoraphobia without history of Panic Disorder, Lifetime.

| | | |
|---|---------|-----|
| NO | Current | YES |
| AGORAPHOBIA without history of Panic Disorder | | |

F. SOCIAL PHOBIA

F 1 a In the past month, were you fearful or embarrassed being the focus of attention or fearful of being humiliated? This includes things like speaking in public, using public toilets, writing while someone watches, or being in social situations.

| | | | |
|--------------------------|----|-----|---|
| <input type="checkbox"/> | NO | YES | 1 |
|--------------------------|----|-----|---|

b Is this fear excessive or unreasonable?

| | | | |
|--------------------------|----|-----|---|
| <input type="checkbox"/> | NO | YES | 2 |
|--------------------------|----|-----|---|

c Do you fear these situations so much that you avoid them or suffer through them?

| | | | |
|--------------------------|----|-----|---|
| <input type="checkbox"/> | NO | YES | 3 |
|--------------------------|----|-----|---|

d Does this fear disrupt normal work or social functioning or cause marked distress?

| | | |
|-----------------------|---------|-----|
| NO | Current | YES |
| Social Phobia Current | | |

G. SPECIFIC PHOBIA

G 1 a In the past month, have you been excessively afraid of things like: flying, driving, heights, storms, animals, insects, or seeing blood or needles?

| | | | |
|--------------------------|----|-----|---|
| <input type="checkbox"/> | NO | YES | 1 |
|--------------------------|----|-----|---|

b Is this fear excessive or unreasonable?

| | | | |
|--------------------------|----|-----|---|
| <input type="checkbox"/> | NO | YES | 2 |
|--------------------------|----|-----|---|

c Do you fear these situations so much that you avoid them or suffer through them?

| | | | |
|--------------------------|----|-----|---|
| <input type="checkbox"/> | NO | YES | 3 |
|--------------------------|----|-----|---|

d Does this disrupt normal work or social functioning or cause marked stress?

| | | |
|-------------------------|---------|-----|
| NO | Current | YES |
| Specific Phobia Current | | |

H. OBSESSIVE COMPULSIVE DISORDER

*Skip to Question H4

H 1 In the past month, have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing? (e.g., the idea that you were dirty or had germs, or of hurting someone even though you didn't want to) (DO NOT INCLUDE SIMPLY EXCESSIVE WORRIES ABOUT REAL LIFE PROBLEMS, DO NOT INCLUDE OBSESSIONS DIRECTLY RELATED TO EATING DISORDERS, SEXUAL BEHAVIOR, PATHOLOGICAL GAMBLING, OR ALCOHOL OR DRUG ABUSE BECAUSE THE PATIENT MAY DERIVE PLEASURE FROM THE ACTIVITY AND MAY WANT TO RESIST IT ONLY BECAUSE OF ITS NEGATIVE CONSEQUENCES.)

| | | | |
|--------------------------|-----|-----|---|
| <input type="checkbox"/> | NO* | YES | 1 |
|--------------------------|-----|-----|---|

H 2 Did they keep coming back into your mind even when you tried to ignore or get rid of them?

| | | | |
|--------------------------|-----|-----|---|
| <input type="checkbox"/> | NO* | YES | 2 |
|--------------------------|-----|-----|---|

| | | | | |
|-----|---|------|-----|---|
| H 3 | Do you think that these obsessions are the product of your own mind and that they are not imposed from the outside? | NO | YES | 3 |
| H 4 | In the past month, did you do something repeatedly without being able to resist doing it, like washing excessively, counting or checking things over and over? | NO** | YES | 4 |
| H 5 | Did you recognize that either these obsessional thoughts or compulsive behaviors were excessive or unreasonable? | NO | YES | 5 |
| H 6 | Did these obsessions or compulsions significantly interfere with your normal routine, occupational functioning, usual social activities, or relationships, or did they take more than one hour a day? | NO | YES | 6 |

**IF NO TO H4 AND TO H1 OR H2: GO TO END OF DISORDER, CIRCLE NO AND MOVE TO NEXT DISORDER.

CODES POSITIVE FOR CURRENT OCD IF EITHER (H1 & H2 & H3 & H5 & H6) OR (H4 & H5 & H6) IS YES.

| | |
|-------------------|-----|
| NO | YES |
| CURRENT O.C.D. | |

I. GENERALIZED ANXIETY DISORDER

Skip this disorder if the patient's anxiety is restricted exclusively to or better explained by any disorder prior to this point.

| | | | | |
|-------|---|----|-----|---|
| I a 1 | Have you worried excessively or been anxious about 2 or more things (e.g., finances, children's health, misfortune) over the past 6 months? More than most others would? Are these worries present most days? Have several people told you that you worry too much? | NO | YES | 1 |
| 2 | Do you find it difficult to control the worries or do they interfere with your ability to focus on what you are doing? | NO | YES | 2 |
| I b | During these worried periods when you are anxious, do you: (DO NOT CODE SYMPTOMS OCCURRING ONLY DURING PANIC ATTACKS) | | | |
| 1 | Feel restless, keyed up or on edge? | NO | YES | 3 |
| 2 | Feel tense? | NO | YES | 4 |
| 3 | Feel tired, weak or exhausted easily? | NO | YES | 5 |
| 4 | Have difficulty concentrating or find your mind going blank? | NO | YES | 6 |
| 5 | Feel irritable? | NO | YES | 7 |
| 6 | Have difficulty sleeping? | NO | YES | 8 |
| | Summary of 1b: Are at least 3 of 1b answers YES? | NO | YES | |

CODES POSITIVE FOR CURRENT GAD (1b (summary) = YES)?

| | |
|----------------|-----|
| NO | YES |
| CURRENT GAD | |

J. ALCOHOL ABUSE AND DEPENDENCE

| | | | | | | | | |
|-------------------------------|--|--|-----|----|----|-----|-------------------------------|--|
| J 1 | IN THE PAST 12 MONTHS, have you had 3 or more alcoholic drinks within a 3 hour period on 3 or more occasions? | NO | YES | 1 | | | | |
| J 2 | IN THE PAST 12 MONTHS: | | | | | | | |
| a | Did you need to drink more in order to get the same effect that you did when you first started drinking? | NO | YES | 2 | | | | |
| b | When you cut down on drinking did your hands shake, did you sweat or feel agitated? Did you drink to avoid these symptoms or to avoid being hungover, e.g., 'the shakes', sweating or agitation? | NO | YES | 3 | | | | |
| c | During the times when you drank alcohol, did you end up drinking more than you planned when you started? | NO | YES | 4 | | | | |
| d | Have you tried to reduce or stop drinking alcohol? | NO | YES | 5 | | | | |
| e | On the days that you drank, did you spend more than two hours per day in obtaining alcohol, drinking, and in recovering from the effects of alcohol? | NO | YES | 6 | | | | |
| f | Did you spend less time working, enjoying hobbies, or being with others because of your drinking? | NO | YES | 7 | | | | |
| g | Have you continued to drink even though you knew that the drinking caused health or mental problems? | NO | YES | 8 | | | | |
| h | CODES POSITIVE FOR CURRENT ALCOHOL DEPENDENCE? (At least 3 of J2 are coded YES) | <table border="1" style="width: 100px; height: 30px;"> <tr> <td style="width: 50%; text-align: center;">NO</td> <td style="width: 50%; text-align: center;">YES</td> </tr> <tr> <td colspan="2" style="text-align: center;">Alcohol Dependence Current</td> </tr> </table> | | | NO | YES | Alcohol Dependence Current | |
| NO | YES | | | | | | | |
| Alcohol Dependence Current | | | | | | | | |
| J 3 a | In the PAST 12 MONTHS, have you been intoxicated, high, or hungover more than once when you had other responsibilities at school, at work, or at home? Did this cause any problems? (Code YES only if this caused problems.) | NO | YES | 9 | | | | |
| b | In the PAST 12 MONTHS, were you intoxicated in any situation where you were physically at risk, e.g., driving a car, boating, using machinery, etc.? | NO | YES | 10 | | | | |
| c | In the PAST 12 MONTHS, have you had any legal problems because of your drinking, e.g., perjury, or disorderly conduct? | NO | YES | 11 | | | | |
| d | In the PAST 12 MONTHS, have you continued to drink even though your drinking caused problems with family or other people? | NO | YES | 12 | | | | |
| e | CODES POSITIVE FOR CURRENT ALCOHOL ABUSE? (J3a or b or c or d = YES) | <table border="1" style="width: 100px; height: 30px;"> <tr> <td style="width: 50%; text-align: center;">NO</td> <td style="width: 50%; text-align: center;">YES</td> </tr> <tr> <td colspan="2" style="text-align: center;">Alcohol Abuse Current</td> </tr> </table> | | | NO | YES | Alcohol Abuse Current | |
| NO | YES | | | | | | | |
| Alcohol Abuse Current | | | | | | | | |

K. NON-ALCOHOL PSYCHOACTIVE SUBSTANCE USE DISORDERS

| | | | |
|-------|--|----|-----|
| K 1 a | Now I am going to read to you a list of street drugs or medicines. Stop me if, IN THE PAST 12 MONTHS, you have taken more than once, any of them to get high, to feel better, or to change your mood. CIRCLE EACH DRUG TAKEN: Quaalude, Seconal ("reds"), Valium, Xanax, Librium, Ativan, Dalmane, Halcion, barbiturates, Miltown or tranquilizers. Marijuana; hashish ("hash"), THC, "pot", "grass", "weed", "reeler". Amphetamine: | NO | YES |
|-------|--|----|-----|

"speed", crystal meth, "rush", dexadrine, Ritalin, diet pills. Cocaine: snorting, IV, freebase, crack, "speedball". Narcotics: heroin, morphine, diacodid, opium, demerol, mebadone, codeine, percocan, darvon. LSD ("acid"), mescaline, peyote, PCP ("angel dust", "peace pill"), psilocybin, STP, or "mushrooms". Steroids, "glue", ethyl chloride, nitrous oxide, ("laughing gas"), amyl or butyl nitrate ("poppers"), Ecstasy, MDA, MDMA, nonprescription sleep or diet pills. Any others?
Specify MOST USED Drug(s): _____

b SPECIFY WHICH WILL BE EXPLORED IN CRITERIA BELOW:

If concurrent or sequential polysubstance use:

Each drug class used individually.

Most used drug class only.

If one drug used:

Single drug class only.

K 2 Considering the drug class selected, IN THE PAST 12 MONTHS:

- | | | | | | | | | |
|-------------------------|--|--|-----|---|----|-----|-------------------------|--|
| a | Have you found that you needed to use more of the drug to get the same effect that you did when you first started taking it? | NO | YES | | | | | |
| b | When you reduced or stopped using drugs did you have withdrawal symptoms? (Aches, shaking, fever, weakness, diarrhea, nausea, sweating, heart pounding, difficulties sleeping, or feeling agitated, anxious, irritable, or depressed) Did you use any drug(s) to keep yourself from getting sick (WITHDRAWAL SYMPTOMS) or so that you would feel better? | NO | YES | 2 | | | | |
| c | Have you often found that when you used drug(s), you ended up taking more than you thought you would? | NO | YES | 3 | | | | |
| d | Have you tried to reduce or stop taking these drug(s)? | NO | YES | 4 | | | | |
| e | On the days that you used drug(s), did you spend more than 2 hours per day obtaining, using and recovering from drug(s), or thinking about drug(s)? | NO | YES | 5 | | | | |
| f | Did you spend less time working, enjoying hobbies, or being with family or friends because of your drug use? | NO | YES | 6 | | | | |
| g | Have you continued to use drug(s) even though it caused health or mental problems? | NO | YES | 7 | | | | |
| h | CODES POSITIVE FOR CURRENT PSYCHOACTIVE SUBSTANCE DEPENDENCE (At least three K 2's are coded YES)? specify drug(s): _____ | <table border="1" style="border-collapse: collapse; width: 100px; height: 30px;"> <tr> <td style="text-align: center;">NO</td> <td style="text-align: center;">YES</td> </tr> <tr> <td colspan="2" style="text-align: center;">Current Drug Dependence</td> </tr> </table> | | | NO | YES | Current Drug Dependence | |
| NO | YES | | | | | | | |
| Current Drug Dependence | | | | | | | | |

Considering the drug class selected:

- | | | | | |
|--------------|--|----|-----|---|
| K 3 a | In the PAST 12 MONTHS, have you been intoxicated, high, or hungover from drug(s), more than once, when you had other responsibilities at school, at work, or at home? Did this cause any problem? (Code YES only if this caused problems.) | NO | YES | 8 |
| b | In the PAST 12 MONTHS, have you been high or intoxicated from drugs in any situation where you were physically at risk (e.g., driving a car, boating, using machinery, etc.)? | NO | YES | 9 |

| | | | | | | | | | | |
|------------|---|---|-----|----|----|-----|------------|--|---------|--|
| c | In the PAST 12 MONTHS, have you had any legal problems because of your drug use, e.g., an arrest or disorderly conduct? | NO | YES | 10 | | | | | | |
| d | In the PAST 12 MONTHS, have you continued to use drug(s) even though it caused problems with your family or other people? | NO | YES | 11 | | | | | | |
| e | CODES POSITIVE FOR CURRENT PSYCHOACTIVE SUBSTANCE ABUSE (K3a or b or c or d = YES)? | <table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td>NO</td> <td>YES</td> </tr> <tr> <td colspan="2" style="text-align: center;">Drug Abuse</td> </tr> <tr> <td colspan="2" style="text-align: center;">Current</td> </tr> </table> | | | NO | YES | Drug Abuse | | Current | |
| NO | YES | | | | | | | | | |
| Drug Abuse | | | | | | | | | | |
| Current | | | | | | | | | | |

L. PSYCHOTIC SYNDROMES

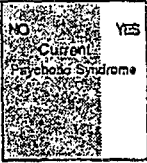


Ask for an example of each question answered positively.
Code Yes only if the examples clearly show a distortion of thought or of perception.
 Before coding, investigate whether delusions qualify as "bizarre".

Delusions are "bizarre" if: clearly implausible, absurd, not understandable, and cannot derive from ordinary life experience.

Incinations are scored "bizarre" if: a voice comments on the person's thoughts or behavior, or when two or more voices are conversing with each other.

Now I am going to ask you about usual experiences that some individuals may experience.

| | | Bizarre | | |
|-----|--|---------|-------------|-----|
| | | NO | YES | YES |
| L 1 | a Have your relatives or friends ever considered any of your beliefs strange or unusual? Please give me an example. Interviewer: Only Code Yes if the examples are CLEARLY delusional ideas of GRANDIOSITY, HYPOCHONDRIASIS, RUIN, GUILT, etc.) | NO | YES | 1 |
| | b IF YES: do they currently consider your beliefs strange? | NO | YES | 2 |
| | | | *skip to L2 | |
| L 2 | a Have you ever believed that people were spying on you, or that someone was plotting against you, or trying to hurt you? | NO | YES | 3 |
| | b IF YES: do you currently believe these things? NOTE: Ask for examples, to rule out actual stalking. | NO | YES | 4 |
| | | | *skip to L2 | |
| L 3 | a Have you ever believed that someone was reading your mind or could hear your thoughts or that you could actually read or hear what another person was thinking? | NO | YES | 5 |
| | b IF YES: do you currently believe these things? | NO | YES | 6 |
| | | | *skip to L2 | |
| L 4 | a Have you ever believed that someone or some force outside of yourself put thoughts in your mind that were not your own, or made you act in a way that was not your usual self? CLINICIAN: Ask for examples and discount any that are not psychotic. | NO | YES | 7 |
| | b IF YES: do you currently believe these things? | NO | YES | 8 |
| | | | *skip to L2 | |
| L 5 | a Have you ever believed that you were being sent special messages through the TV, radio, or newspaper, or that a person you did not personally know was particularly interested in you? | NO | YES | 9 |

| | | | | | |
|-----------------------------|---|---|--|-----|-----------------------|
| | b | IF YES: do you currently believe these things? | NO | YES | YES* 1 *skip to L6 |
| L 6 | a | Have you ever heard things other people couldn't hear, such as voices? Hallucinations are scored "bizarre" only if patient answers YES to the following: Did you hear a voice commenting on your thoughts or behavior or did you hear two or more voices talking to each other? | NO | YES | YES* 1 *skip to L6 |
| | b | If YES, have you heard these things in the past month? Score as "YES Bizarre" if patient heard a voice commenting on their thoughts or behavior or heard two or more voices talking to each other. | NO | YES | YES* 1 *skip to L6 |
| L 7 | a | Have you ever had visions or have you ever seen things other people couldn't see? | NO | YES | 1: |
| | b | IF YES, have you seen these things in the past month? | NO | YES | 1: |
| CLINICIAN'S JUDGMENT | | | | | |
| | 3 | Is the patient currently exhibiting incoherence, disorganized speech, or marked loosening of associations? | NO | YES | 1: |
| L 9 | | Is the patient currently exhibiting disorganized or catatonic behavior? | NO | YES | 1: |
| L 10 | | ARE 1 OR MORE ITEMS FROM L1b, L2b, L3b, L4b, L5b, L6b, CODED YES BIZARRE? CR ARE 2 OR MORE ITEMS FROM L1b, L2b, L5b, L6b, L7b, L8, L9 CODED YES (RATHER THAN YES BIZARRE)? |  | | |
| L 11 | | ARE 1 OR MORE ITEMS FROM L1a, L2a, L3a, L4a, L5a, L6a, CODED YES BIZARRE? CR ARE 2 OR MORE ITEMS FROM L1a, L2a, L5a, L6a, L7a, L8, L9 CODED YES (RATHER THAN YES BIZARRE)? |  | | |
| | 2 | IF L11 CODED YES: DOES THE PATIENT CODE POSITIVE FOR CURRENT MAJOR DEPRESSION OR CURRENT OR PAST BIPOLAR DISORDER? | NO | YES | |
| L 13 | | IF L11 CODED YES: Were any beliefs and experiences you just described (give examples to patient) restricted exclusively to times when you were feeling depressed/high/very irritable? |  | | |

REFERENCE LIST

1. Cooper JR, Bloom FE, Roth RH. *The Biochemical Basis of Neuropharmacology*, ed 7. Oxford University Press: New York, 1996.
2. Kornhuber J, Weller M. Psychotogenicity and N-methyl-D-aspartate Receptor Antagonism: Implications for Neuroprotective Pharmacotherapy. *Biol Psychiatry* 1997;41:135-44.
3. Gasic GP, Holman M. Molecular neurobiology of glutamate receptors. *Annu Rev Physiol* 1992;54:507-36.
4. Arnold SE, Hyman BT, van Hoesen GW, et al. Some cytoarchitectural abnormalities in the entorhinal cortex in schizophrenia. *Arch Gen Psychiatry* 1996;48: 625-33.
5. Kaplan HI, Sadock BJ, editors. *Synopsis of Psychiatry*. Baltimore: Williams & Wilkins, 1998.
6. Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmuller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. *Neurosci Lett* 1980; 20:379-82.
7. Javitt DC, Zukin SR. Recent Advances in the Phencyclidine Model of Schizophrenia. *Am J Psychiatry* 1991;148(10):1301-8.

8. Weinberger DR. The Biological Basis of Schizophrenia: New Directions. *J Clin Psychiatry* 1997;58 Suppl 10:22-7.
9. Kerwin R, Patel S, Meldrum B. Quantitative autoradiographic analysis of glutamate binding sites in the hippocampal formation in normal and schizophrenic brain post mortem. *Neuroscience* 1990;39:25-32.
10. Toru M, Kurumaji A, Ishimaru M. Excitatory amino acids: implications for psychiatric disorders research. *Life Sci* 1994;55:1683-99
11. Akbarian S, Sucher NJ, Bradley D, Tafazzoli A, Trinh D, Hetrick WP, et al. Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *J Neurosci* 1996;16(1):19-30
12. Harrison PJ, McLaughlan D, Kerwin RW. Decreased hippocampal expression of a glutamate receptor gene in schizophrenia. *Lancet* 1991;337:450-2.
13. Wolf SS, Hyde TM, Saunders RC, Herman MM, Weinberger DR, Kleinman JE, et al. Autoradiographic characterization of neurotensin receptors in the entorhinal cortex of schizophrenic patients and control subjects. *J Neural Transm Gen Sect* 1995;102(1):55-65.

14. Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE, et al. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Arch Gen Psychiatry* 1995;52:829-36.
15. Yurgelun-Todd DA, Renshaw PF, Gruber SA, Ed M, Wateraux C, Cohen BM, et al. Proton magnetic resonance spectroscopy of the temporal lobes in schizophrenics and normal controls. *Schizophr Res* 1996;19(1):55-9.
16. Bertolino A, Nawroz S, Mattay V, Barnett AS, Duyn JH, Moonen CT et al. A regionally specific pattern of neurochemical pathology in schizophrenia as assessed by multislice proton magnetic resonance spectroscopic imaging. *Am J Psychiatry* 1996;153(12):1554-63.
17. Waziri R. Glycine Therapy of Schizophrenia. *Biol Psychiatry* 1988;23:210-1.
18. Heresco-levy U, Javitt DC, Ermilov M, Mordel C, Horowitz A, Kelly D. Double-blind, Placebo-controlled, Crossover Trial of Glycine Adjuvant Therapy for Treatment-Resistant Schizophrenia. *Br J Psychiatry* 1996;169(5):610-7.
19. Heresco-levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein MA. Efficacy of High-Dose Glycine in the Treatment of Enduring Negative Symptoms of Schizophrenia. *Arch Gen Psychiatry* 1999;56:29-36.

20. Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, et al. A Placebo-Controlled Trial of D-Cycloserine Added to Conventional Neuroleptics in Patients with Schizophrenia. *Arch Gen Psychiatry* 1999;56:21-27.
21. Goff DC, Tsai G, Manoach DS, Flood J, Darby DG, Coyle JT. D-Cycloserine added to clozapine for patients with schizophrenia. *Am J Psychiatry* 1996;153:1628-1630.
22. Van Berckel BN, Hijman R, van der Linden JA, Westernberg HG, van Ree JM, Kahn RS. Efficacy and tolerance of D-cycloserine in drug-free schizophrenic patients. *Biol Psychiatry* 1996;40:1298-1300.
23. Ulas J, Cotman CW. Excitatory amino acid receptors in Schizophrenia. *Schizophr Bull* 1993;19(1):105-13.
24. Noorbala AA, Akhondzadeh S, Davari-Ashtiani R, Amini-Nooshabadi H. Piracetam in the treatment of schizophrenia: implications for the glutamate hypothesis of schizophrenia. *J Clin Pharm Ther* 1999;24(5):369-74.
25. Chen AC, Kalsi G, Brynjolfsson J, Sigmundsson T, Curtis D, Butler R, et al. Lack of evidence for close linkage of the glutamate GluR6 receptor gene with schizophrenia. *Am J Psychiatry* 1996;153(12):1634-36.

26. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* 1995;52:998-1007.
27. Deutsch, SJ, Mastropalo J, Schwartz BL, Rosse RB, Morishisa JM. A "glutarnatergic hypothesis" of schizophrenia: Rationale for pharmacotherapy with glycine. *Clinical Neuropharmacology* 1989;12:1-13.
28. Cepeda C, Levine MS. Dopamine and N-methyl-D-aspartate receptor interactions in the neostriatum. *Dev Neurosci* 1998;20(1):1-18.
29. Farber NB, Newcomer JW, Olney JW. Glycine agonists: What can they teach us about schizophrenia [comment]? *Arch Gen Psychiatry* 1999;56:13-7.
30. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. *The New England Journal of Medicine* 1994;330(9):613-22.
31. Mauri MC, Ferrara A, Boscatti L, Bravin S, Zamberlan F, Alecci M, et al. Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. *Neuropsychobiology* 1998;37(3):124-9.

32. Maes M, Verkerk R, Vandoolaeghe E, Lin A, Scharpe S. Serum levels of excitatory amino acids, serine, glycine, histidine, threonine, taurine, alanine and arginine in treatment-resistant depression: modulation by treatment with antidepressants and prediction of clinical responsiveness. *Acta Psychiatr Scand* 1998;97(4):302-308.
33. Ferrari MD, Odink J, Bos KD, Malessy MJ, Bruyn GW. Neuroexcitatory plasma amino acids are elevated in migraine. *Neurology* 1990;40(10):1582-6.
34. Bouron A, Chatton JY. Acute application of the tricyclic antidepressant desipramine presynaptically stimulates the exocytosis of glutamate in the hippocampus. *Neuroscience* 1999;90(3):729-36.
35. Nowak G, Li Y, Paul IA. Adaptation of cortical but not hippocampal NMDA receptors after chronic citalopram treatment. *Eur J Pharmacol* 1996;295(1):75-85.
36. Kelly JP, Wrynn AS, Leonard BE. The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther* 1997;74(3):299-316.
37. Trullas R, Skolnick P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur J Pharmacol* 1990;185(1):1-10.

38. Holemans S, De Paermentier F, Horton RW, Crompton MR, Katona CL, Maloteaux JM. NMDA glutamatergic receptors, labelled with [3H]MK-801, in brain samples from drug-free depressed suicides. *Brain Res* 1993;616(1-2):138-43.
39. Calabrese JR, Bowden CL, Sachs GS, Ascher JA, Monaghan E, Rudd GD. A Double-Blind Placebo-Controlled Study of Lamotrigine Monotherapy in Outpatients With Bipolar I Depression. *J Clin Psychiatry* 1999;60(2):79-88.
40. Kaiya H, Namba M, Yosida H, Nakamura S. Plasma glutamate decarboxylase activity in neuropsychiatry. *Psychiatry Res* 1982;6(3):335-43.
41. Grant ER, Bacskai BJ, Pleasure DE, Pritchett DB, Gallagher MJ, Kendrick SJ, et al. N-methyl-D-aspartate receptors expressed in a nonneuronal cell line mediate subunit-specific increases in free intracellular calcium. *J Biol Chem* 1997;272(1):647-56.
42. Almazov VA, Popov YG, Gorodinskii AI, Mikhailova OA, Dambinova SA, Gurevich VS. Sites of high affinity binding of L-[3H]-glutamic acid in human platelets. New type of platelet receptors? *Biokhimiya* 1988;53:848-52.
43. Mangano RM, Schwarcz R. The human platelet as a model for the glutamatergic neuron: platelet uptake of L-glutamate. *J Neurochem* 1981;36:1067-76.

44. Berk M, Bodemer W, van Oudenhove T, Butkow N. Dopamine increases platelet intracellular calcium in bipolar affective disorder and controls. *Int Clin Psychopharmacol* 1994;9(4):291-3.
45. Dubovsky SL, Christiano J, Daniell LC, Franks RD, Murphy J, Adler L, et al. Increased platelet intracellular calcium concentration in patients with bipolar affective disorders. *Arch Gen Psychiatry* 1989;46(7):632-8.
46. Mikuni M, Kagaya A, Takahashi K, Meltzer HY. Serotonin but not norepinephrine induced calcium mobilization of platelets is enhanced in affective disorders. *Psychopharmacology* 1992;106:311-4.
47. Berk M, Plein H, Csizmadia T. Supersensitive platelet glutamate receptors as a peripheral marker in schizophrenia. *Int Clin Psychopharmacol* 1999;14(2):119-22.
48. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J Biol Chem* 1985;260(6) 3440-50.
49. Berk M, Plein P, Ferreira D. Platelet glutamate receptor supersensitivity in major depressive disorder. *Life Sci*. In Press.

Author Belsham B C

Name of thesis The Specificity Of Platelet Glutamate Receptor Sensitivity As A Putative Marker For Schizophrenia Belsham B C 2000

PUBLISHER:

University of the Witwatersrand, Johannesburg

©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.