Glorious Glutamate:
An Update for Today’s Practicing Clinician

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Faculty Disclosure

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Disclosure

• The faculty have been informed of their responsibility to disclose to the audience if they will be discussing off-label or investigational use(s) of drugs, products, and/or devices (any use not approved by the US Food and Drug Administration).
  – The off-label or investigational use of ketamine, esketamine, rapastinel, D-cycloserine, riluzole, CP-101,606, CERC 301, basimglurant, JNJ-4011813, ceftriaxone, nitrous oxide, dextromethorphan-quinidine, AVP 786, and ayahuasca for the treatment of depression will be discussed.

• This activity has been independently reviewed for balance.

Learning Objectives

• List tasks that glutamate performs and how in various psychiatric disorders this negatively impacts outcomes

• Apply this emerging understanding to various currently, and soon to be released pharmacologic interventions

• Assess how this emerging knowledge base regarding glutamate can be used in daily clinical practice to improve outcomes
Why Bother Learning about Glutamate?

Here’s Why …

The Magnificent 7 Reasons Why →

It is the single most potent neurotransmitter in the entire brain.
It is the single most commonly found neurotransmitter in the brain.
It plays an exceptionally large role in learning, cognition, and mood.
It is the major contributor to increased synaptogenesis and neuronal plasticity.
It is the major source of creation of its single greatest antagonist – GABA.
It can be quite neurotoxic in excess.
It isn’t as well understood as it should be.

It was only about 25 years ago that Glutamate’s role as a neurotransmitter was discovered.
Well …

Does Glutamate **Now** Have
Our Attention???

**Glutamate Pathology in Psychiatry
and Neuropsychiatry**

Disorders where Astrocyte / Glutamate Pathology Plays a Role:

1. Neurological Disorders
   - Autism
   - Down Syndrome
   - Fragile X Syndrome
   - Brain Trauma (CTE)

2. Neurodegenerative Disorders
   - Wernicke and Korsakoff
   - Stroke

3. Psychiatric Disorders
   - Schizophrenia
   - Addictive Disorders
   - Major Depression

CTE = chronic traumatic encephalopathy.
Why Understanding Glutamate is So Critical – It Plays A Central Role in Psychiatry


But… Some Things are Just Complicated – Glutamate’s Life Cycle is Complex. There’s No Getting Around This Fact of Life...
Glutamate’s Life Story: The Simplistic View

CNS = central nervous system; TCA = tricarboxylic acid.

The glutamatergic synapse. Glutamate is an amino acid, a building block for proteins, therefore, it is abundant in all cells of the body. It is also the most important excitatory neurotransmitter in the CNS. Glutamate is synthesized in axon terminals of glutamatergic neurons. It can be produced from α-ketoglutarate—a TCA-cycle intermediate—or from glutamine. For glutamate synthesis from glutamine, the enzyme glutamine synthase is transported to the axon terminal. In the cytosol, it converts glutamine into glutamate. Transporters then concentrate glutamate in vesicles. Release of glutamate is triggered by influx of calcium (Ca^{2+}) into the presynaptic neuron. The synaptic vesicles fuse with the cell membrane and release glutamate into the synaptic cleft. Glutamate is taken up by the postsynaptic neuron, by glia, or it is recycled in the presynaptic neuron.

The Complex, Drama Filled Life of Glutamate


Neurotransmitter glutamate (GLU) is subsequently taken up mainly by astrocytes. GLU is amidated to glutamine (GLN) via GLN synthetase (GS) and GLN is subsequently released from the astrocyte via specific transporters followed by uptake into the neuron. In the neuron, GLN is deaminated by phosphate activated glutaminase (PAG) to GLU, which completes the GLU-GLN cycle. A net transfer of nitrogen from the astrocyte to the neuron occurs as part of the GLU-GLN cycle. This nitrogen transfer may be counteracted by transport of a neuro-inactive amino acid (AA) likely alanine or one of the branched chain amino acids. The GLU-GLN cycle is coupled to an amino acid-keto acid (KA) cycle via the action of glutamate dehydrogenase (GDH) and the relevant aminotransferase (AT).

Neurotransmitter GLU is mainly taken up by surrounding astrocytes subsequent to interaction with receptors in the synapse. In the astrocyte, GLU is either converted to GLN catalyzed by GS as part of the GLU-GLN cycle or metabolized in the TCA cycle. GLN is transferred to the glutamatergic neuron to be used for synthesis of glutamate catalyzed by PAG. GLU enters the TCA cycle by the action of GDH or an AT, and the carbon skeleton may either be completely oxidatively metabolized via pyruvate recycling including malaic enzyme (ME) activity or phosphoenolpyruvate carboxykinase and pyruvate kinase. Alternatively, the carbon skeleton supports the pool of TCA cycle intermediates and in that way potentially increases the oxidation of acetyl CoA in the TCA cycle. De novo synthesis of GLU and GLN from glucose occurs via the concerted action of pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) making a net synthesis of TCA cycle intermediates. CIT = citrate; OAA = oxaloacetate; PYR = pyruvate.
Vesicular Glutamate Transporters

- Cytosolic glutamate crosses the vesicular membrane via the activity of VGLUTs.
- VGLUT1 and 2 are primarily expressed in glutamatergic neurons; whereas, VGLUT3 is somewhat unique in that it has been detected in GABAergic, cholinergic, and monoaminergic neurons.

VGLUT = vesicular glutamate transporter; GABA = γ-aminobutyric acid.

Getting to Know EAATs:
An Important Player in Glutamate’s Life Cycle

To terminate the action of glutamate and maintain its extracellular concentration below excitotoxic levels, Na⁺-dependent high affinity glutamate transporters (EAATs) located on the plasma membrane of neurons and glial cells rapidly remove glutamate from the extracellular space.

EAATs = excitatory amino acid transporters.
Glutamate is packaged into presynaptic vesicles by VGLUT proteins and synaptically released in a voltage-dependent manner through vesicular interactions with SNARE proteins.

Synaptically-released Glutamate is recycled from the extracellular space by EAATs expressed predominantly on astroglia.

In astrocytes, Glutamate is converted to Glutamine by Glutamine synthetase and exported extracellularly to be taken up again by neurons.

Glutamate receptors are present on presynaptic and postsynaptic neurons as well as on glial cells.

These include both ionotropic receptors (NMDA, AMPA/KA) and metabotropic receptors (mGluRs). The effect of Glutamate is determined by the receptor subtype, localization (synaptic, perisynaptic, and extrasynaptic), and interactions with various scaffolding and signaling proteins (not shown) in the postsynaptic density. Glutamate receptor stimulation results not only in rapid ionotropic effects but also in synaptic plasticity, eg, long-term potentiation and long-term depression, via cognate signal transduction cascades.

Astrocytes and neurotransmitter homeostasis. Astrocytes take up glutamate, GABA, adenosine, and monoamines.

Glutamate is converted to glutamine (by glutamine synthetase (GS), which is shuttled to neurons for subsequent conversion into glutamate and GABA.

GABA accumulated by astrocytes is mainly consumed by tricarboxylic acid cycle (TCA); adenosine is converted to adenosine monophosphate (AMP) by adenosine kinase (ADK).

Monoamines are degraded by astroglia monoamine oxidase B (MAO-B).

Glutamate is a Firm Believer in the System of Checks and Balances: 

*It Gives Birth to Its Greatest Opposer – GABA*

- THE greatest excitatory neurotransmitter in the brain is – GLUTAMATE
- It produces, THE greatest inhibitory neurotransmitter in the brain – GABA
- A comedy of errors, or is this the ultimate system of checks and balances in the human brain?

Glutamate is one of the 20 amino acids in human beings. It's non-essential and the body can create its own glutamate.

Glutamate may be synthesized in 2 separate ways:

1. Through the Krebs cycle: Firstly, it may be synthesized from α-ketoglutarate, by either glutamate dehydrogenase or by a variety of amino-transferases
2. Secondly, glutamate may be synthesized from other amino acids; the “glutamate family” of amino acids comprise glutamine, arginine, proline, and histidine
3. Taste buds: Umami tastants, particularly glutamate, are thought to mediate appetitive responses to protein-rich foods. As such, they play a fundamental role in evaluating the nutritional value of foods

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**Tripartite Synapse and the Synaptic Cradle**

- The tripartite synapse model principally focuses on a bi-directional rapid neuronal-glial communications; neurotransmitter-induced glial Ca²⁺ signaling with subsequent exocytotic release of neurotransmitters from the astroglia
- Astrocytes control emergence and shaping of synaptic networks, they regulate ionic homeostasis of the synaptic cleft, control neurotransmitters dynamics, prevent or allow neurotransmitter spillover, and contribute to synaptic extinction

ECM = extracellular matrix.
**A Closeup View of the Tripartite Neuron**

How Astrocyte and Glutamate Functioning Impacts Monoamine Functioning

EAAT = excitatory amino acid transporters 1 (SLC1A3) and 2 (SLC1A2); NKA = Na+/K+ ATPase or ATP-dependent Na+/K+ pump, the α2 subtype (ATP1A2) is predominantly expressed in astrocytes; NKCC1 = Na–K–Cl co-transporter (SLC12A1); NCX = sodium–calcium exchanger expressed in 3 isoforms (SLC8A1, SLC8A2 and SLC8A3); NAAT = Na+-dependent ascorbic acid transporter (SLC23); NBC = sodium-bicarbonate co-transporter (SLC4A1); CNT2 = high-affinity Na+-dependent concentrative adenosine transporter (CNT2); ASCT2 = alanine–serine–cysteine transporter 2; MCT-1 = mono-carboxylate transporter 1 (SLC16A1); Kir4.1 = inward rectifier Kir4.1 channel; NHE = sodium-proton exchanger 1 (SLC9A1); GAT = GABA transporters 1 (SLC6A1) and 3 (SLC6A11); SN1,2 = Na+/H+-dependent sodium-coupled neutral amino acid transporters 1 (SLC38A3) and 2 (SLC38A5); GlyT1 = glycine transporter 1 (SLC6A9).

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**Glutamate / Astrocyte Interactions and Its Importance in Health and Psychiatric Disorders**

Glutamate Interventions are So Much More than Just Glutamate Interventions – *The Quartet of Other Effects and Benefits*

**Upstream**

- Opioid Receptors
- Norepinephrine Transporters
- Serotonin Transporter
- Adrenergic Receptors

**Downstream**


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Glutamate as a Great “Concert Master”: *The INTRAcellular Players That Listen to Glutamate’s Commands*

- **Glutamate**
- **BDNF** = brain-derived neurotrophic factor
- **PSD-95** = postsynaptic density protein – a major scaffolding protein for both neurons and dendrites
- **mTOR** = modulation (mammalian target of rapamycin)

“The Party is in Here”: The Real Action from Glutamate-Based Interventions is Intracellular

NMDA = N-methyl-D-aspartate receptor
GABA = γ-aminobutyric acid receptor
AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid receptor
VDCC = voltage dependent calcium channel
BDNF = brain-derived neurotrophic factor
PSD-95 = (postsynaptic density protein) – a major scaffolding protein for both neurons and dendrites
mTOR = modulation (mammalian target of rapamycin)


Hold Up!
Before We Take Too Brain-Centric a View: Glutamate in the Body
Glutamate in the Periphery
Multiple Roles, Multiple Tasks

Peripheral glutamate mechanisms. Nociceptive free nerve endings store glutamate in neurotransmitter vesicles (white circles) and release glutamate into peripheral tissue following noxious stimulation (leftward going red arrows). Glutamate released from the same or a nearby terminal can interact with excitatory amino acid receptors (EAAR; chevrons) to activate or sensitize the terminal.


And Let’s Now Focus on Glutamate’s Targets:
Its Army of Receptors
Glutamate Receptor Classification

NMDA receptors are among the most tightly regulated in the mammalian brain and unique in requiring co-agonists for activation.

At least 6 binding sites have been identified that regulate the probability of ion channel opening, viz., sites for 2 obligatory co-ligands (glutamate and glycine), polyamines, and cations (Mg$^{2+}$, Zn$^{2+}$, and H$^+$). NMDA receptor ligands are short-chain dicarboxylic amino acids (NMDA, glutamate, aspartate, etc.).

Glutamate, the most potent neurochemical agonist identified in the CNS.

Ionotropic and Metabotropic Glutamate Receptors

Role of Neurons and Astrocytes

2 Types of Glutamate Release – “Burst” Release vs “Drip-Drip” Release

Extracellular glutamate in the nervous system may thus be divided into 2 functionally and spatially distinct pools:

1) “Synaptic glutamate,” a transient (few milliseconds) burst of high (0.5–5 mM) glutamate that appears in the synaptic cleft when glutamate-filled synaptic vesicles in neurons fuse with the presynaptic membrane.

2) “Ambient extracellular glutamate,” a relatively steady background of glutamate, mostly from glia, that fills most of the extracellular space at lower concentration (1–5 uM).

Oh, Magnificent NMDA Receptor

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Glutamate / NMDA Receptor

- NMDARs contain 4 subunits which are a combination of: NR1, NR2, and NR3
- There is consensus that NMDARs are tetramers composed of 2 NR1 subunits and 2 NR2 subunits, less commonly including NR3 subunits
- The combination of NR1 with different NR2 subunits results in diverse electrophysiological and pharmacologic responses
- There is a binding place in the channel pore for Mg\(^{2+}\), and at resting membrane potential, Mg\(^{2+}\) is attached to this binding site, blocking ion flow through the channel
- They are mostly located on dendrites


AMPA Receptors

- AMPARs are activated in the presence of glutamate, thus inducing a fast excitatory synaptic signal involved in early glutamatergic effects in the synapse
- These effects play a crucial role in calcium metabolism, synaptic strength, and oxidative stress. Indeed, AMPAR activation opens the pore permitting the inward flow of sodium, which results in the depolarization of the neuronal membrane
- At mature synapses, AMPARs can be co-expressed with NMDARs, thereby contributing to synaptic plasticity and neuroprotection
- It is important to note that AMPARs have a lower affinity for glutamate than NMDARs, which allows for a more rapid dissociation of glutamate and a fast deactivation of the AMPAR

Enhanced AMPA to NMDA throughput as a key mechanism for the rapid and sustained antidepressant efficacy of ketamine and its metabolite (2R,6R)-HNK. In this model, AMPA receptor activation (but not NMDA antagonism) is the key mechanism that induces antidepressant effects via activation and blockade of different downstream targets.

**CREB = cyclic adenosine monophosphate response element binding protein; eEF2 = eukaryotic elongation factor; HNK = hydroxynorketamine.

**Ketamine-induced BDNF release is dependent on activation of glutamate-AMPA receptors and L-type VDCCs. (A) Cortical neurons were incubated with NBQX (50 µM) 20 min prior to ketamine, and medium was collected 15 min later (after ketamine). Incubation with the AMPA receptor antagonist completely blocked ketamine-induced BDNF release (n=6; drug x drug interaction, F1,20 = 13.209, *P<.01)


Incubation of primary neuronal cultures with ketamine rapidly increases BDNF release.

15 min (n=12; t[22] = 3.10, **P<.01), 60 min (n=6; t[10] = 3.33, **P<.01), and 6 hour (n=3; t[4] = 3.14, *P<.05) incubations.
**NMDA Receptor and Glycine**

**Another Important Player in the Glutamate Homeostasis**

NMDA Receptor and Its Various Ligands

Schematic diagram of NMDA receptor complex. The NMDA receptor is an ionotropic glutamate receptor for controlling synaptic plasticity and memory function. Glutamate (and NMDA) binds to the agonist site on the NMDA receptors. PCP, ketamine, and dizocilpine bind to the PCP receptor in the inside of the NMDA receptors. Glycine and D-serine bind to a glycine modulatory site on the NMDA receptors. The NMDA receptor is blocked by Mg²⁺ in a voltage sensitive manner. Activation of NMDA receptor by binding of both glutamate and glycine results in the opening of the channel. This allows voltage-dependent flow of Na⁺ and small amounts of Ca²⁺ ions into the cell and K⁺ out of the cell. The symbol (−) denotes inhibitory effect.

PCP = phencyclidine; 7-CK = 7-chlorokynurenic acid; L689,560 = trans-2-carboxy-5,7-dichloro-4-phenylaminocarbonyl; AP5 = 2-amino-5-phosphonovaleric acid; CGS-19775 = cis-4-phosphonomethyl-2-piperidinecarboxylic acid.


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**Glycine – (an NMDA Agonist)**

**Another Way to Modulate the Glutamate System**

Glycine, an important amino acid released by both glial cells and glutamatergic neurons, elicits persistent bidirectional modifications in NMDAR-mediated synaptic responses

Activation of GlyR by glycine has additional contributions to the glycine-induced NMDAR endocytosis as well as suppression of NMDAR function

Net effect mediated by glycine is a depression of the NMDA response

Focus on Metabotropic Glutamate Receptors

- Unlike ionotropic glutamate receptors that depend on cation flux, metabotropic glutamate receptors exert their effects via the recruitment and activation of intracellular trimeric G-proteins and downstream signal transduction pathways.
- Like all G-protein coupled receptors, metabotropic glutamate receptors are 7 transmembrane domain-spanning receptors.
- 8 metabotropic glutamate receptors have been identified. Classified into 3 groups:
  - **Group I:** mGluR1 and mGluR5
  - **Group II:** mGluR2 and mGluR3
  - **Group III:** mGluR4–8

There is presently intense effort to develop both positive and negative modulators of presynaptic group II and III metabotropic glutamate receptors in an effort to treat a plethora of neuropsychiatric illnesses.


Glutamate Modulation (*in this case NMDA Antagonism via ketamine*) Enhances Synaptogenesis

What Happens after Both Ionotropic and Metabotropic Glutamate Receptors are Modulated? **Intracellular Action**

- Major intracellular players in the “after the receptors are modulated” story are the following:
  - PSD-95 – a major scaffolding protein for both neurons and dendrites
  - Multiple Kinase enzyme systems (eg CAMKII, GTPases)
  - mTOR modulation
  - GSK-3 Modulation

CAMKII = Ca2+/calmodulin-dependent protein kinase II; GTP = guanosine triphosphate.

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**Glutamate / NMDA Functioning:**

* A Tale of Two Extremes

- Optimum
- Suboptimum

# Shifting Our Focus to Glutamate and Its Role in Psychopathology

**What Goes Wrong with Glutamate in Psychiatric Disorders?** *Answer: Multiple Things*

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Astrocyte Dysfunction</th>
<th>Impact on Glutamate Homeostasis</th>
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<tbody>
<tr>
<td>Schizophrenia</td>
<td>Functional asthenia, morphological atrophy, and pathological remodeling</td>
<td>Decrease in number of astrocytes and GFAP-positive profiles associated with impaired glutamate uptake and glutamine synthesis. Abnormal glutamate homeostasis together with an increased astrogial production and release of kynurenic acid (exogenous inhibitor of NMDA and Ach receptors) and altered synthesis of α-serine (positive modulator of NMDA receptors) contributes to pathological glutamatergic transmission implicated in pathogenesis of the disease.</td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>Functional asthenia and morphological atrophy</td>
<td>Decrease in number of astrocytes and GFAP expression, associated with decreased glutamate uptake and secretion of growth factors and cytokines as well as impaired glutamine synthesis, altered gap junctional connectivity in glial syncytia, which all may contribute to abnormal connectivity in neural networks and neurotransmission disbalance</td>
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GFAP = glial fibrillary acidic protein; Ach = acetylcholine.
Evidence for Glutamate Pathology in Psychiatry: Interesting Facts

- Proton magnetic resonance spectroscopy studies have noted glutamate reductions in the dorsolateral PFC of individuals with MDD as well as in other PFC areas such as the dorsomedial and dorsal anterolateral PFC and ACC.
- Glutamatergic genes found to be particularly relevant for mood disorders include:
  - GRIA3, which codes a protein of the AMPA receptor
  - GRIK4 and GRIK2, which code proteins of the kainate receptor
  - GRM7, a gene that encodes a protein of the metabotropic receptor

PFC = prefrontal cortex; ACC = anterior cingulate cortex.

Glutamatergic genes found to be particularly relevant for mood disorders include:

1. Presynaptic release of glutamate
2. Postsynaptic ionotropic receptors for glutamate (NMDA and AMPA receptors)
3. Reuptake of glutamate by glial glutamate membrane transporters
4. Glutamate metabolism and recycling by the glutamate/glutamine cycle

Evidence of Abnormal Glutamate Trafficking in Depression: Meta-Analysis of Peripheral Blood Glutamate Levels and Major Depression

Systematic review and meta-analysis of 12 association studies between blood glutamate levels and MDD in a total of 529 MDD patients and 590 controls

Results:
Blood glutamate levels were significantly higher in MDD patients than in controls (standardized mean difference = 0.54, 95% CI = 0.27–0.82, \( P = 8.5 \times 10^{-5} \))

Authors’ Conclusion:
Findings suggest that altered glutamate levels may be implicated in MDD, which provides further evidence of glutamatergic dysfunction in MDD

Glutamate and Schizophrenia

- Primary glutamate-based hypothesis in schizophrenia is: NMDA receptor hypofunction hypothesis (It’s based on a few lines of thinking)
- Potent NMDA receptor antagonists like PCP precipitate states virtually indistinguishable from schizophrenia (with positive, negative, and cognitive symptoms)
- Genetic studies show polymorphisms in both inotropic and metabotropic glutamate captor coding genes
- Clozapine, thought to be greater efficacy than other antipsychotics, is an NMDA receptor antagonist
- Some co-agonists at NMDA site (such as D-cycloserine, D-serine, glycine), when administered with traditional antipsychotics show greater efficacy on positive, negative, and cognitive symptoms of schizophrenia

Glutamate and Anxiety

Examining a Deeply Interwoven Link

- A growing body of evidence suggests that glutamatergic neurotransmission may be involved in the biological mechanisms underlying stress response and anxiety-related disorders
- The glutamatergic system mediates BOTH the acquisition and extinction of fear-conditioning
- Amygdalar and hippocampal NMDA and metabotropic glutamate receptors are involved in the mechanisms related to fear-conditioning and inhibitory-avoidance memory formation
- Glutamate receptors seem to play an important role in fear-mediated learning, affecting both hippocampal-dependent associative learning and amygdala-dependent emotional processing during and after a stressful event
- 85% to 95% of the neurons in BLA are glutamatergic and project to ventral striatum and PFC
- Glutamate not only exerts its effects through direct activation of glutamate neurotransmission but it also modulates the release of other neurotransmitters involved in stress-response, such as serotonin dopamine monoamines and GABA

BLA = basolateral amygdala.

Pain and Glutamate – How NMDA Antagonism Can Impact Pain – Acute and Chronic

- NMDA antagonism reduces postsynaptic neuron excitability – thereby reducing pain fiber firing rate
- NMDA antagonism via ketamine is an agonist of opioid receptors
- NMDA antagonism activates the monoaminergic descending inhibitory system that modulates nociception in the dorsal horn
- NMDA antagonism via ketamine suppresses the production of pro-inflammatory cytokines (TNF-α, IL-6)
- NMDA antagonism via ketamine exerts a direct anti-inflammatory effect on macrophages

TNF = tumor necrosis factor; IL = interleukin.
Glutamate and Inflammation: A “Brain–Body” Link

Glutamate disruptions adversely affect production of IL-6, TNF-α etc. from microglia and macrophages

Disorders Where We Clinicians Should Consider the Role of Glutamate Pathology (and Interventions)

- Schizophrenia
- Bipolar Depression
- Traumatic Brain Injury
- Neurodegenerative Disorders
- Chronic Pain
Finally, We Shift Our Attention to Glutamate-Centric Interventions

Glutamate Targets in Psychiatry – Where are They?  
**Answer:** Literally, Everywhere!

1) NMDA receptor antagonists (ketamine, NR2B subunit antagonists, memantine, magnesium, and zinc)  
2) Positive modulators of AMPA  
3) Group I mGluR antagonists, group II mGluR antagonists and agonists, and group III mGluR agonists  
4) EAAT2 enhancer (ceftriaxone)  
5) Possible indirect NMDA receptor modulator (minocycline)  
6) Inhibitor of glutamate release, antagonist of NMDA, AMPA, and kainate receptors, and potentiatior of glutamate uptake (riluzole)
The are Many, Many Correct Roads to Rome!

Similarly, There are Many, Many Paths to Glutamate Modulation

Which Road Should I Take to Get to Rome? Answer: There are Multiple Paths

Do Our “Classic” Antidepressants and Mood Stabilizers (SSRIs, SNRIs, etc.) Have an Impact on Glutamate/Astrocytes?

- Amitriptyline, fluoxetine, clomipramine, and paroxetine increase synthesis and release of glial cell-derived neurotrophic factor in C6 astrocytoma cell line
- SSRIs and SNRIs were found to downregulate NMDA and activate AMPA
- Lithium and valproate decreased hippocampal GluR1 expression in the hippocampus
- Lamotrigine limited glutamate release by blocking voltage-operated sodium channels and potentiates AMPAR activity

SSRI = selective serotonin reuptake inhibitor; SNRI = serotonin norepinephrine reuptake inhibitor.

Let’s Meet Target #1 in Glutamate-Based Therapies

NMDA Receptor

- Ketamine subunit selectivity for GluN1/GluN2A and GluN2B has been discovered
- The psychotomimetic effects of ketamine are produced by GluN1/GluN2A subunits rather than GluN1/GluN2B

A Tale of Two Cities – Agonism AND Antagonism of Glutamate NMDA are Both Clinical Approaches to Treating Major Depression

Glutamate Activity

NMDA Receptor Partial Agonism
eg – Rapastinel D-cycloserine

NMDA Receptor Antagonism
eg – Ketamine Esketamine


How a Glutamate NMDA Antagonist Work

AMPARs are required for ketamine’s effects, although the exact role they play has yet to be defined. Pretreatment with the AMPAR antagonist NBQX prevents the antidepressant-like actions of ketamine


A simplified cortical microcircuit is shown to demonstrate the 2 potential sites of action for ketamine, which leads to increased protein synthesis and excitatory synapses. Local interneurons (iIN), including fast spiking parvalbumin-expressing neurons, provide inhibition of principal pyramidal neurons (PN) that have low basal firing rates.

Ketamine selectively antagonizes the PN to iIN excitatory synapses leading to loss of tonic inhibition and increased firing followed by LTP-like synaptic plasticity and an increase in excitatory synapses.
How Ketamine Works

1) Proposed mechanism of ketamine’s antidepressant action, whereby ketamine, through a blockade of tonic GABAergic inhibition …

2) causes a surge in glutamate release and cycling.

3) The resulting increased glutamatergic transmission through AMPARs (whose surface expression may be independently upregulated by the suppression of spontaneous NMDAR-mediated neurotransmission) …

4) leads to increased BDNF-dependent levels of synaptogenesis …

5) that ultimately contribute to the rapid and sustained antidepressant effects.


How NMDA Antagonism Works: A Receptor and Intracellular Cascade Story

- NMDA receptor blockade …
- Lead to increased glutamate release from pyramidal neurons
- Activation of postsynaptic AMPARs by increased glutamate transmission …
- release of BDNF. This neurotrophic factor binds to related kinase B (TrkB) receptors, and mTOR is activated …
- Leading to transphosphorylation and downstream activation of the extracellular signal-related kinase (ERK) and suppression of glycogen synthase kinase 3 (GSK-3)

This is to illustrate that the "real action" of the pharmacologic manipulation of glutamate is intracellular. It shows the importance of the mTOR system, GSK-3, BDNF, scaffolding proteins, and synaptogenesis.

Why Ketamine Matters to Us Clinicians?  
NIMH Researchers’ Thoughts

“It is important to note that, within four hours to one day, a single infusion of ketamine in TRD patients achieved response rates comparable to that seen following eight weeks of treatment with monoaminergic-based antidepressants in non-TRD patients.

The fact that ketamine is capable of inducing remission in approximately one-third of TRD patients within a single day is in stark contrast to the effectiveness of monoaminergic-based approaches, which usually require 10–14 weeks of chronic use to produce similar remission rates.”

TRD = treatment-resistant depression.

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Some Interesting Things about Ketamine

- Non-competitive NMDA receptor antagonists like ketamine produce a block only when the channel is in its open state after activation; it is use-dependent. This means that the drug will act selectively at the site of excess NMDA activation

- Ketamine induces monoaminergic activation and inhibits reuptake

- It targets μ and δ receptors from the opioid family receptors

- Adipokines – ketamine affects levels of leptin, resistin, and adiponection. Ketamine can putatively reverse metabolic dysfunction in depression

- AMPA may—through 4 mechanisms—positively affect brain functions
  - mTOR activation
  - eEF2 activation (eukaryotic elongation factor)
  - GSK3 inhibition
  - BDNF level increase through exocytosis of BDNF containing vesicles

Remarkable, Iconoclastic Things with Ketamine

• Rapidity of antidepressant activity onset
• Many studies demonstrate 4 hours to 1 day onset of action
• Ability to induce remission in one-third of patents with TRD
• No significant challenges for use in bipolar depression (low switching rates)
• Improvement in 3 domains that were particularly vexing challenges with SSRIs
  – Anhedonia
  – Fatigue
  – Suicidal ideations (ketamine’s ability to reduce suicidal-ideation measures may occur independently of its antidepressant effects)

DOWNSIDE: Ketamine while rapid, had transient antidepressant effects and caused brief dissociative and psychotomimetic effects


Modulating Glutamate May Impact Both “Poles”: Impact on Depression and Wellness

Note:
Early antidepressant action from Glutamate NMDA antagonism and AMPA agonism

AND
Later, but substantial increase in Wellness traits such as Happiness, Energy, Calmness, Self-esteem

14 participants completed 6 IV infusions of 0.5 mg/kg ketamine over 40 minutes on a Monday–Wednesday–Friday schedule.

Glutamate Interventions Can Be Rapidly Anti-Suicidal Even with One Dose: *Results from a Meta-Analysis*

This meta-analysis investigated studies of single-dose IV ketamine for the treatment of any psychiatric disorder; only comparison intervention trials (using saline placebo or midazolam as a control). 10 trials were included.

**Clinician-Rated Change**

![Graph of Clinician-Rated Change](image)

**Patient-Rated Change**

![Graph of Patient-Rated Change](image)

*P<.05, ***P<.001.

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**Intranasal Esketamine**

*A Promising Glutamate-Based Therapy*

28 placebo-treated participants with moderate-to-severe symptoms were rerandomized (1:1:1:1) to 1 of the 4 treatment arms; those with mild symptoms continued receiving placebo. Participants continued their existing antidepressant treatment during the study. During the open-label phase, dosing frequency was reduced from twice weekly to weekly, and then to every 2 weeks.

![Graph of Intranasal Esketamine](image)

Glutamate NMDA Receptor Antagonist for Depression and Suicidality – Intranasal Esketamine Data

In a double-blind, multicenter, proof-of-concept study, 68 participants were randomly assigned to receive esketamine (84 mg) or placebo twice weekly for 4 weeks, in addition to comprehensive standard-of-care treatment.

- Significantly greater improvement was also observed in the esketamine group on the MADRS suicidal thoughts item score at 4 hours (effect size = 0.67), but not at 24 hours (effect size = 0.35) or at day 25 (effect size = 0.29).

A significantly greater improvement in MADRS score was observed in the esketamine group compared with the placebo group at 4 hours (least-square mean difference = -5.3, SE = 2.10; effect size = 0.61) and at ~24 hours (least-square mean difference = -7.2, SE = 2.85; effect size = 0.65), but not at day 25 (least-square mean difference = -4.5, SE = 3.14; effect size = 0.35).

Therapeutic Effects of Glutamate Intervention Through Ketamine

- PTSD = posttraumatic stress disorder; OCD = obsessive-compulsive disorder.
Ketamine: A Glutamate NMDA Receptor Antagonist, in Refractory Anxiety Disorders

- Open-label, 10 women (50%) and 10 men (50%); 15 patients (75%) met criteria for GAD and 18 (90%) for SAD
- Patients received 1 or 2 weekly ketamine doses of 1 mg/kg injected subcutaneously for 3 months

**Results:**
- Fear Questionnaire ratings decreased by ~50%, as did Hamilton Anxiety ratings
- The most common adverse events were nausea, dizziness, and blurred vision. Of the 20 patients, 18 reported improved social functioning and/or work functioning during maintenance treatment
- Maintenance ketamine may be a therapeutic alternative for patients with treatment refractory GAD/SAD

GAD = generalized anxiety disorder; SAD = social anxiety disorder.

Rapastinel: An NMDA Receptor Partial Agonist (Through Glycine Receptor Partial Agonism)

A Glycine Receptor Partial Agonist, Working on NMDA Receptor, Quickly Impacts Depression

In this double-blind, randomized, placebo-controlled study, a single IV dose of rapastinel (1, 5, 10, or 30 mg/kg) or placebo was administered to 116 participants with MDD who had not benefitted from a trial of at least one biogenic amine antidepressant during the current episode.

Results. Rapastinel, 5 or 10 mg/kg IV, reduced depressive symptoms as assessed by the HAM-D-17 at days 1 through 7. Onset of action as assessed using the Bech-6 occurred within 2 hours. Rapastinel did not elicit psychotomimetic or other significant side effects.


Which Then is the Better Approach to Treating MDD?

Good news for us clinicians, both approaches are helpful. There are enough similarities and dissimilarities to potentially make both approaches viable –

There are neurobiological similarities and dissimilarities: “The results demonstrate similarities as well as differences in the synaptic and behavioral actions of [rapastinel] compared with ketamine.”

Metabotropic Glutamate-Related Compounds in Psychiatry

- **Group I**: mGluR1 antagonists and mGluR5 antagonists
  (example: mGluR1 antagonist, [3-ethyl-2-methyl-quinolin-6-yl]-[4-methoxy-cyclohexyl]-methanone methanesulfonate [EMQMCM])

- **Group II**: mGluR2/3 antagonists and agonists
  (example: LY341495, a group II mGluR antagonist)

- **Group III**: mGluR4, mGluR6, mGluR7, and mGluR8 agonists
  (example: group III mGlu receptor agonist, [1S,3R,4S]-1-aminocyclopentane-1,3,4-tricarboxylic acid [ACPT-I])

More Glutamate-Based Therapies on the Horizon (maybe!)

- D-cycloserine (FDA approved for tuberculosis) – works on glycine site on NMDA receptor

- Riluzole (FDA approved for amyotrophic lateral sclerosis [ALS])

- CP-101,606 and CERC 301 – both work only on NR2B subunit of NMDA receptor as an antagonist

- Basimglurant and JNJ-4011813 – mGluR5 negative and positive modulators
Positive Modulators of AMPA Receptors

- AMPA receptors are involved in the antidepressant actions of NMDA receptor antagonists

- NMDA receptor signaling seems to interact with AMPA receptor signaling directly or indirectly during the antidepressant activities of some types of NMDA antagonists

- There are several classes of AMPA receptor potentiators, including nootropic agents and ampakines (piracetam, aniracetam, and ampakines)


EAAT2 Modulator

- Glutamate transporter EAAT2: the enhancer ceftriaxone

- EAATs located on the plasma membrane of neurons and glial cells rapidly terminate the actions of glutamate and maintain its extracellular concentration below excitotoxic levels

- Ceftriaxone, a β-lactam antibiotic, increased both the brain expression of EAAT2 and its biochemical and functional activities

- Ceftriaxone treatment reduced the immobility time in the forced swim test and the tail suspension test in mice. Removal of excess glutamate by enhanced glutamate uptake may improve depression

Targeted Attack on NMDA Receptor: Focus on NR2B Subunit

- NR2B is a subunit of the NMDA receptor. Investigations into the therapeutic potential of NMDA receptor subunit antagonists stem largely from the hypothesis that—because these agents non-selectively block the NMDA receptor—they may be more specific and have fewer undesirable adverse effects than ketamine.

- 2 such agents have been investigated:
  - CP-101,606 (traxoprodil)
  - CERC-301


NMDA Receptor Modulation is No Laughing Matter
N2O as Treatment of Depression

N2O, a non-competitive NMDA receptor inhibitor

In a placebo-controlled, double-blind, crossover study examining 20 patients with TRD who received an inhalation of 50% N2O or 50% nitrogen (placebo), both over 1 hour, those who received N2O experienced a reduction in depressive symptoms (as measured by the HAM-D) at both 2 hours and 24 hours post-inhalation compared to placebo. Adverse effects included anxiety, headache, and nausea/vomiting, but there were no psychotomimetic effects associated with N2O inhalation.

Depressive symptoms improved significantly at 2 hours and 24 hours after receiving N2O compared with placebo (mean HAM-D-21 difference at 2 hours, -4.8 points, 95% CI, -1.8 to -7.8 points, P=002; at 24 hours, -5.5 points, 95% CI, -2.5 to -8.5 points, P<0.001; comparison between N2O and placebo, P<0.001).

4 patients (20%) had treatment response (reduction ≥ 50% on HAM-D-21) and 3 patients (15%) had a full remission (HAM-D-21 ≤ 7 points) after N2O compared with 1 patient (5%) and none after placebo.

“Don’t You Sneeze at Glutamate’s Role in Depression!” – Dextromethorphan in Depression

Dextromethorphan acts on opioid receptors, and at higher doses dextromethorphan acts as a σ-1 receptor agonist and inhibitor of the serotonin and norepinephrine transporters, as well as a non-competitive NMDA receptor antagonist.

Dextromethorphan-Quinidine combination, which is FDA-approved for the treatment of pseudobulbar affect—is currently under investigation as a potential antidepressant agent in patients with MDD.

In addition, AVP 786, a combination of deuterated (d6)-dextromethorphan and an ultra-low dose of quinidine, received fast-track designation for agitation in Alzheimer’s disease; it is currently under investigation for use in depression.


Our Future with Glutamate-Based Therapies

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Can Glutamate Interventions “Change” The Brain? Multiple Lines of Evidence Say … **Yes**

Ketamine as a Probe of Glutamate-Based Interventions

- Ketamine dampens brain regions involved in rumination (the repetitive focusing of attention on negative feelings and thoughts in response to negative mood) by reducing the functional connectivity between the pregenual ACC and the dorsal PCC.
- Ketamine has been shown to interfere with the default mode network by disrupting the “hyper-connectivity”—commonly associated with rumination—between it and the medial PFC in patients with MDD.
- Ketamine increases neural activation in the bilateral MCC, ACC, and insula, as well as the right thalamus. Activation of these areas is consistent with activation of reward-processing areas, suggesting that ketamine may play a role in activating reward neurocircuitry.
- Ketamine appears to reduce brain activation in regions associated with self-monitoring, to increase neural regions associated with emotional blunting, and to increase neural activity in reward processing.

PCC = posterior cingulate cortex; MCC = middle cingulate cortex.


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Ketamine OR SSRI – Tomato or Tomatee? Perhaps Both?

Ketamine has also been used in conjunction with SSRIs. In a double-blind study, 30 MDD patients were randomized to receive either a single administration of ketamine or placebo concomitant to daily treatment with escitalopram (10 mg). Improvement in depressive symptoms was greater, and remission rates were higher, in those that received escitalopram plus ketamine vs those that received escitalopram plus placebo for up to 2 weeks (92.3% vs 57.1%, \( P = .04 \) and 76.9% vs 14.3%, \( P = .001 \), respectively), with a significantly shorter time to response (HR 0.04, 95% CI 0.01–0.22, \( P < .001 \)) and remission (HR 0.11, 95% CI 0.02–0.63, \( P = .01 \)). This suggests that ketamine administration may be used to offer more immediate antidepressant relief during the lag time to monoaminergic antidepressant response.

Physical Exercise and Glutamate

Running also increases the gene expression levels of the NR2B subunit of the NMDA receptor in the dentate gyrus.

Meditation Has a Strong Impact on Brain Glutamate Levels

Magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), diffusion-weighted imaging (DWI), and diffusion tensor imaging (DTI) are correlated with years of meditation and psychological variables in 10 long-term Zen meditators compared to 10 healthy non-meditator controls.

Psychedelics and Glutamate

Single Dose of Ayahuasca: Sustained Alteration in Glutamate Transmission – Even at 2 Months

16 participants ingesting single dose of DMT dose of 0.64 mg/kg

In Conclusion
Why This In Depth Examination and Understanding of Glutamate Matters …

Consequences of a Narrow, Monoamine-Centric View in Psychiatry is …

“None of the antidepressants have been designed with astrocytes (or glutamate) as target in mind.”

And this may be because of …

“(delayed) new drug development, but this is often a slow process due to adherence to existing dogma by research community and drug companies.”

Why We Should Know and Understand Glutamate In Depth

“Glutamatergic transmission is generally believed to be significantly impaired in the contexts of all major neuropsychiatric diseases.”

“…Glutamate homeostasis are affected in all forms of major psychiatric disorders and represent a common mechanism underlying neurotransmission disbalance, aberrant connectome and overall failure on information processing by neuronal networks, which underlie pathogenesis of neuropsychiatric diseases.”


Thank you!