JGP Journal of General Physiology

COMMENTARY

Subtype-selective targeting of NMDA receptors—A potent new compound emerges

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Subtype-selective modulation of *N*-methyl-D-aspartate receptors (NMDARs) remains a major goal in neuropharmacology, with the potential to advance basic research and enable targeted therapies for disorders involving dysregulated glutamatergic signalling. In this volume of the *Journal of General Physiology*, Lotti et al. describe UCM-101, a newly optimized GluN2A-selective allosteric inhibitor derived from the weakly active scaffold TCN-213. Introduction of a single ethyl group resulted in a 7.5-fold increase in potency, yielding an inhibitor with an IC_{50} of 110 nM at GluN1/2A receptors and up to 118-fold selectivity over other NMDAR subtypes under physiologically relevant conditions. A 1.7 Å crystal structure of the GluN1–2A ligand-binding domain (LBD) revealed that UCM-101 adopts an extended conformation spanning the inter-subunit allosteric pocket, engaging a previously unexploited "UCM-subsite" distinct from those used by TCN- or MPX-class modulators. Despite its novel orientation, UCM-101 stabilizes the inactive, open-clamshell conformation of the GluN1 LBD, thereby reducing glycine affinity and preventing receptor activation. Mutagenesis identified new selectivity determinants (GluN2A V529, M788, and T797) that are not utilized by TCN-201, demonstrating that different scaffolds exploit distinct microenvironments within the same allosteric site. Functionally, UCM-101 produced robust inhibition of NMDAR-mediated synaptic currents in hippocampal slices (89% at 3 µM) and displayed similar potency at triheteromeric GluN1/2A/2B receptors. Together, these findings validate the mechanistic framework for GluN2A-selective inhibition while broadening the structural landscape for ligand engagement. UCM-101 provides both a potent research tool and a promising scaffold for the development of next-generation subtype-selective NMDAR modulators.

Subtype-selective regulation of the N-methyl-D-aspartate receptor (NMDAR) activity has been considered a crucial goal in the field of neuropharmacology for facilitating basic research and for therapy over several decades (Hanson et al., 2024). NMDARs are key mediators of excitatory neurotransmission, playing a critical role in synaptic plasticity, learning, memory, and neuronal survival (Hansen et al., 2021). Dysregulated NMDAR signaling contributes to a broad spectrum of neurological and psychiatric conditions, including depression, schizophrenia, Parkinson's disease, and Alzheimer's disease. More recently, anti-NMDAR autoimmunity has emerged as the most common cause of autoimmune encephalitis, characterized by psychosis, seizures, cognitive impairment, and autonomic dysfunction (Guasp and Dalmau, 2025). Together, these observations underscore the essential roles of NMDARs in both normal brain physiology and diverse disease states, and highlight the need for precise pharmacological approaches to tune receptor activity. Despite decades of effort, only a limited number of NMDAR-targeting therapeutics have advanced to clinical practice. Memantine, used in Alzheimer's disease and epilepsy, and S-ketamine, approved for treatment-resistant depression, are both open-channel blockers with relatively weak subtype selectivity (Krystal et al., 2024). Consequently, they broadly suppress NMDAR function throughout the brain, restricting therapeutic precision and contributing to undesirable side effects.

NMDARs are tetrameric ligand-gated ion channels comprising two GluN1 subunits and two GluN2 (A–D) and/or GluN3 (A–B) subunits (Hansen et al., 2021). Their biophysical properties, signaling functions, and regional expression patterns vary according to subunit composition and GluN1 splice variants. Classical GluN1/GluN2 receptors require co-agonist binding of glycine (to GluN1) and glutamate (to GluN2), whereas GluN1/GluN3 receptors are activated by glycine alone. Considerable progress has been made in developing subtype-selective allosteric modulators and understanding their mechanisms of binding and actions (Fig. 1). Ifenprodil, a phenylethanolamine derivative, selectively inhibits GluN2B-containing receptors (Gallagher et al., 1996; Reynolds and Miller, 1989) by binding to the GluN1–GluN2B interface within the amino-terminal domain (ATD) (Karakas et al., 2011). A positive allosteric modulator (PAM), PYD-106 (Khatri et al., 2014), binds specifically to the diheteromeric GluN1-2C NMDAR at the ATD-ligand-binding domain (LBD) interface (Chou et al., 2022a; Zhang et al., 2023).

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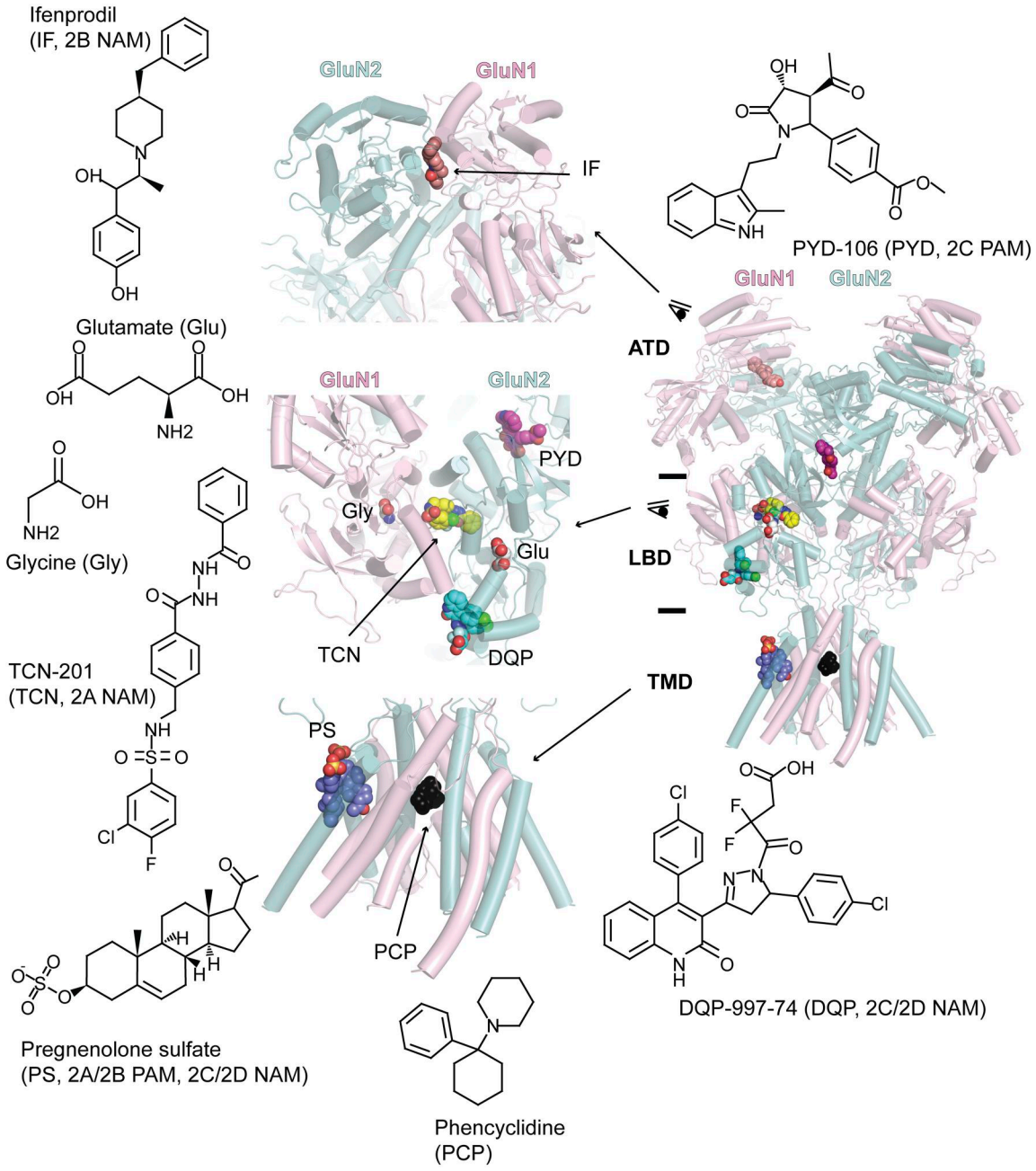

Figure 1. **Regulatory landscape of GluN1–GluN2 NMDA receptors.** Canonical NMDARs assemble as heterotetrameric ion channels comprising two GluN1 (light pink) and two GluN2 (light teal) subunits, which bind glycine and glutamate, respectively. Representative PAMs/NAMs, along with open-channel blockers (shown as spheres or chemical structures), are positioned at their corresponding regulatory sites. Structural models for GluN1 and GluN2 were adapted from PDB ID 7SAA. Ifenprodil, PYD-106, DQP-997-74, and TCN-201 occupy distinct sites within the extracellular domain, while PS engages a juxtamembrane lipid-facing pocket. In contrast, classic open-channel blockers, such as phencyclidine (PCP), lodge within the transmembrane ion-conducting pathway. TMD, transmembrane domain.

A negative allosteric modulator (NAM), DQP-997-74 (D'Erasmo et al., 2023), binds selectively to GluN2C/D-containing receptors at the GluN1–2 LBD dimer interface close to the ion channel region at the transmembrane domain (Kang et al., 2025a). Although not subtype-selective, the open channel blockers, such as (S)-ketamine, memantine, and phencyclidine, bind above the selectivity filter and a nearby region clustered by hydrophobic residues, physically blocking the ion-permeating pathway (Chou et al., 2022b; Kang et al., 2025a; Zhang et al., 2021). Furthermore, the natural neurosteroids, which can potentiate NMDAR functions, such as pregnenolone sulfate (PS) and 24S-hydroxycholesterol, bind to a juxtamembrane pocket (Kang et al., 2025b). PS has been shown as PAM in GluN2A/GluN2B and NAM in GluN2C/GluN2D (Malayev et al., 2002). Interestingly, synthetic compounds such as GNE-4123 (Abbott et al., 2025) and EU1622-240 (Chou et al., 2024; Kang et al., 2025b)

also bind to the juxtamembrane sites and modulate the channel activity. Finally, the GluN2A-selective modulators TCN-201 were identified (Bettini et al., 2010), acting at the GluN1–GluN2A interface in the LBD layer (Yi et al., 2016), and this allosteric site is the focus of the study by Lotti et al. (2025).

The work by Lotti et al. (2025) was the product of a powerful collaboration between molecular neuroscientists, structural biologists, and chemists. They introduce UCM-101, a strategically optimized analog of the weakly active compound TCN-213. Adding a single ethyl group resulted in a remarkable 7.5-fold improvement in potency, with UCM-101 now matching established compounds like TCN-201 at a potency of 110 nM. The compound exhibits an impressive binding affinity with a dissociation constant of just 10 nM at GluN1/2A receptors, as well as 59-fold selectivity over a similar subtype, GluN1/2B. Under physiologically relevant conditions (1 μM glycine), UCM-101 shows 17- to 118-fold selectivity for GluN2A-containing receptors over other subtypes. While somewhat less selective than TCN-201 and MPX-004, it substantially improves upon TCN-213.

The 1.7-Å crystal structure of the isolated GluN1-2A LBDs reveals UCM-101 occupying the allosteric interface between GluN1 and GluN2A subunits in a fundamentally different orientation than any previously characterized modulator (Yi et al., 2016). While compounds like MPX-007 adopt a compact U-shape, UCM-101 stretches across the interface in an extended conformation, creating a "UCM-subsite" that remains untouched by TCN-201 and MPX compounds. Three hydrogen bonds anchor UCM-101 to GluN2A backbone residues, supplemented by extensive hydrophobic contacts and water-mediated interactions with GluN1 R755. The ethyl group that boosts potency does not form new direct contacts; instead, it rigidifies the molecule, reducing conformational flexibility and enabling more stable formation of conserved interactions. Despite occupying the allosteric site differently, UCM-101 works through the same proposed mechanism as structurally distinct modulators. Using engineered disulfide bonds, the authors demonstrate that UCM-101 stabilizes the inactive state of the GluN1 agonist-binding domain, represented by the open clamshell conformation of the LBD, preventing glycine binding and subsequent channel activation. This mechanistic convergence, despite structural divergence, suggests that the allosteric site functions as a conformational "switch" that can be manipulated by multiple structural solutions. For TCN-201/MPX compounds, a single residue, GluN2A V783, acts as the primary selectivity gatekeeper. The small valine in GluN2A permits binding, while bulkier residues in other subunits create steric clashes. UCM-101's reduced selectivity suggested different interactions, confirmed by mutagenesis: V783 mutations had modest 1.9- to 2.5-fold effects on UCM-101 versus 1.9- to 8.1-fold effects on TCN-201. The authors identified additional UCM-101–specific selectivity determinants: GluN2A V529, M788, and T797. Mutations at these positions reduced UCM-101 potency by two- to fivefold yet had minimal effect on TCN-201, providing direct evidence that different scaffolds exploit different selectivity determinants within the same allosteric site.

In hippocampal brain slices from juvenile mice, UCM-101 (3 μM) reduced NMDAR-mediated synaptic currents by 89%, dramatically outperforming TCN-213 (16%) and substantially exceeding TCN-201 and MPX-004 (both ∼35%). This robust inhibition reflects UCM-101's high potency at GluN2A-containing receptors combined with significant GluN2B activity, validating that the novel binding mode produces strong functional effects in native neurons. At triheteromeric GluN1/2A/2B receptors, increasingly recognized as abundant in mature cortex and hippocampus (Zhang et al., 2025), UCM-101 showed similar potency (IC50 = 240 nM) to its activity at diheteromeric GluN1/2A receptors, suggesting the GluN2A subunit exerts dominant allosteric control in mixed assemblies.

Modified Schild analysis revealed that UCM-101's improvement over TCN-213 stems from dual enhancement: binding affinity increased 78-fold (KB from 780 to 10 nM), and allosteric coupling strength improved 7.5-fold (allosteric binding interaction constant [α] from 0.043 to 0.0057). UCM-101's α value indicates that the glycine affinity decreases 175-fold when the modulator is bound, matching TCN-201's performance despite different structural approaches. The consistency of α values across scaffolds suggests that this parameter is constrained by receptor conformational states, while KB values reflect specific ligand-protein contacts that are amenable to medicinal chemistry optimization.

Lotti and colleagues achieve something rare: simultaneously validating an existing mechanistic framework while expanding the structural space for engaging that mechanism. The allosteric site proves more accommodating to chemical diversity than expected, suggesting that additional novel scaffolds await discovery, potentially with improved selectivity, pharmacokinetics, or safety profiles. The identification of distinct selectivity determinants provides new handles for structure-based design. Rather than optimizing only V783 interactions, medicinal chemists can now explore contacts with V529, M788, or T797, diversifying selectivity strategies. The work also raises questions about whether subtype-specific conformational movements exist outside the LBD. Therapeutically, selective GluN2A modulation remains attractive for depression, Rett syndrome, L-DOPA–induced dyskinesias, and GluN2A-mutation disorders. However, realizing this promise requires modulators with suitable pharmacokinetics, particularly those that exhibit suitable brain penetration. UCM-101 may not be the final answer, but it illuminates new paths forward. Whether UCM-101 transitions toward clinical development or serves as a springboard for next-generation compounds remains to be seen. What is clear is that researchers now have more tools, structural information, and mechanistic insights to guide the challenging quest for subunit-selective NMDAR modulators in the treatment of brain disorders.

Acknowledgments

Christopher J. Lingle served as editor.

The author is funded by the National Institutes of Health (grants NS111745, NS142231, MH085926, and CA300691), Robertson funds at Cold Spring Harbor Laboratory, and the Doug Fox Alzheimer's fund.

Author contributions: Hiro Furukawa: conceptualization, funding acquisition, visualization, and writing—original draft, review, and editing.

Disclosures: The author declares no competing interests exist.

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