Identification and characterization of novel NMDA receptor positive allosteric modulators (PAMs)

David H Hackos
Dept. of Neuroscience
Genentech, Inc.

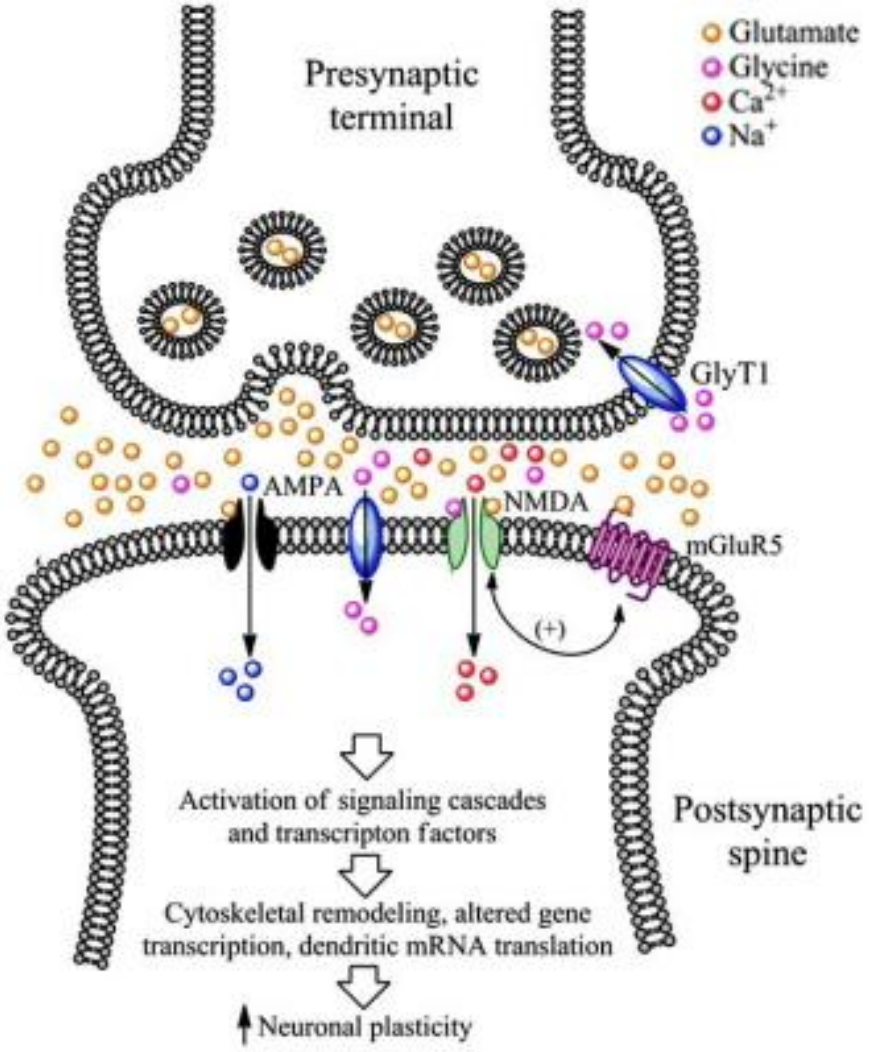
Schizophrenia and NMDA receptors

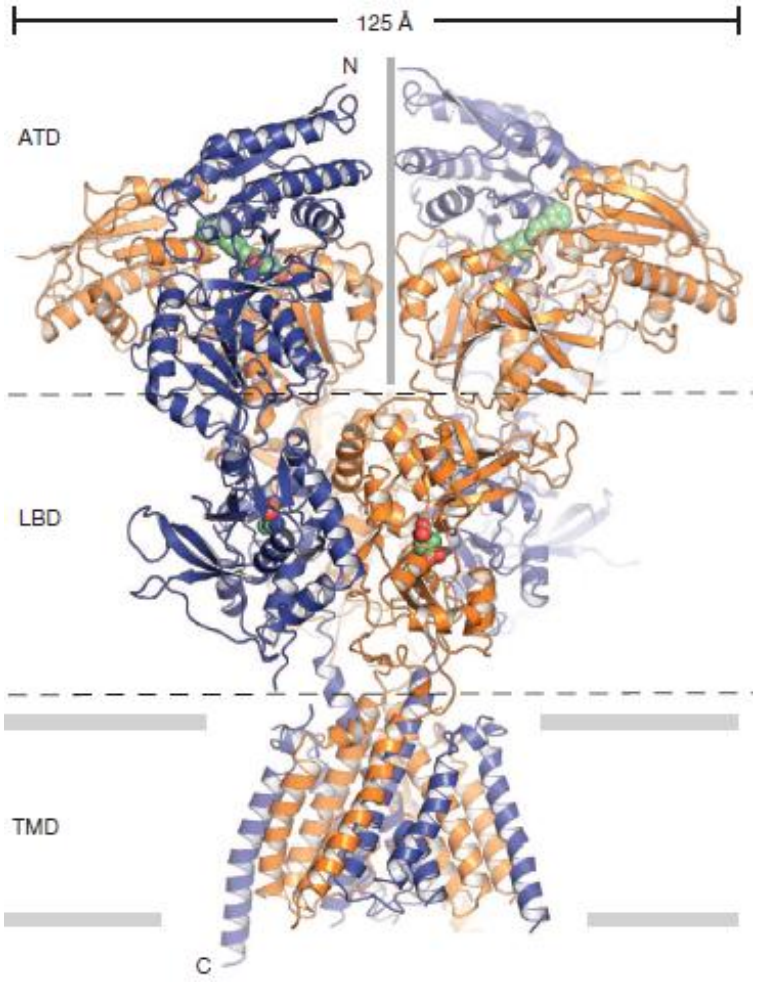
Schizophrenia

- A common disorder - about 1% of general population
- Mechanism and cause is poorly understood
- Antipsychotics treat some symptoms, but show limited efficacy in negative and cognitive symptoms

- NMDA receptor blockers (such as PCP) induce schizophrenia-like symptoms in normal individuals
- Animal models of NMDAR hypofunction exhibit schizophrenia-like phenotypes
- Previous generation NMDAR enhancers, such as GlyT inhibitors, indirectly potentiate NMDARs.

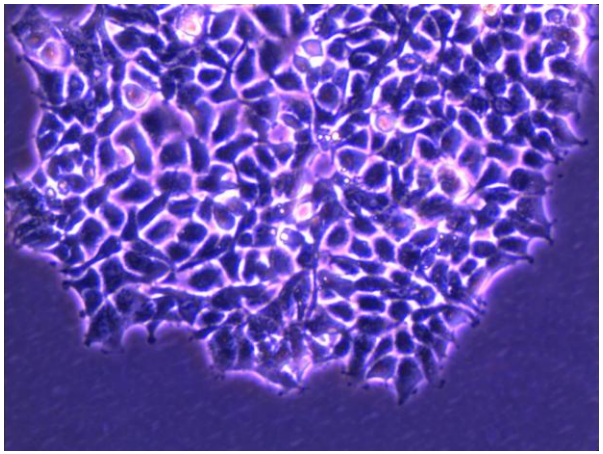
The structure and function of NMDA receptors





Gouaux et al Nature 2014, 511, 191

uHTS screen for NMDA receptor PAMs



GluN1/GluN2A-expressing 293 cells

Induce with Dox

Culture in the presence of 1mM ketamine

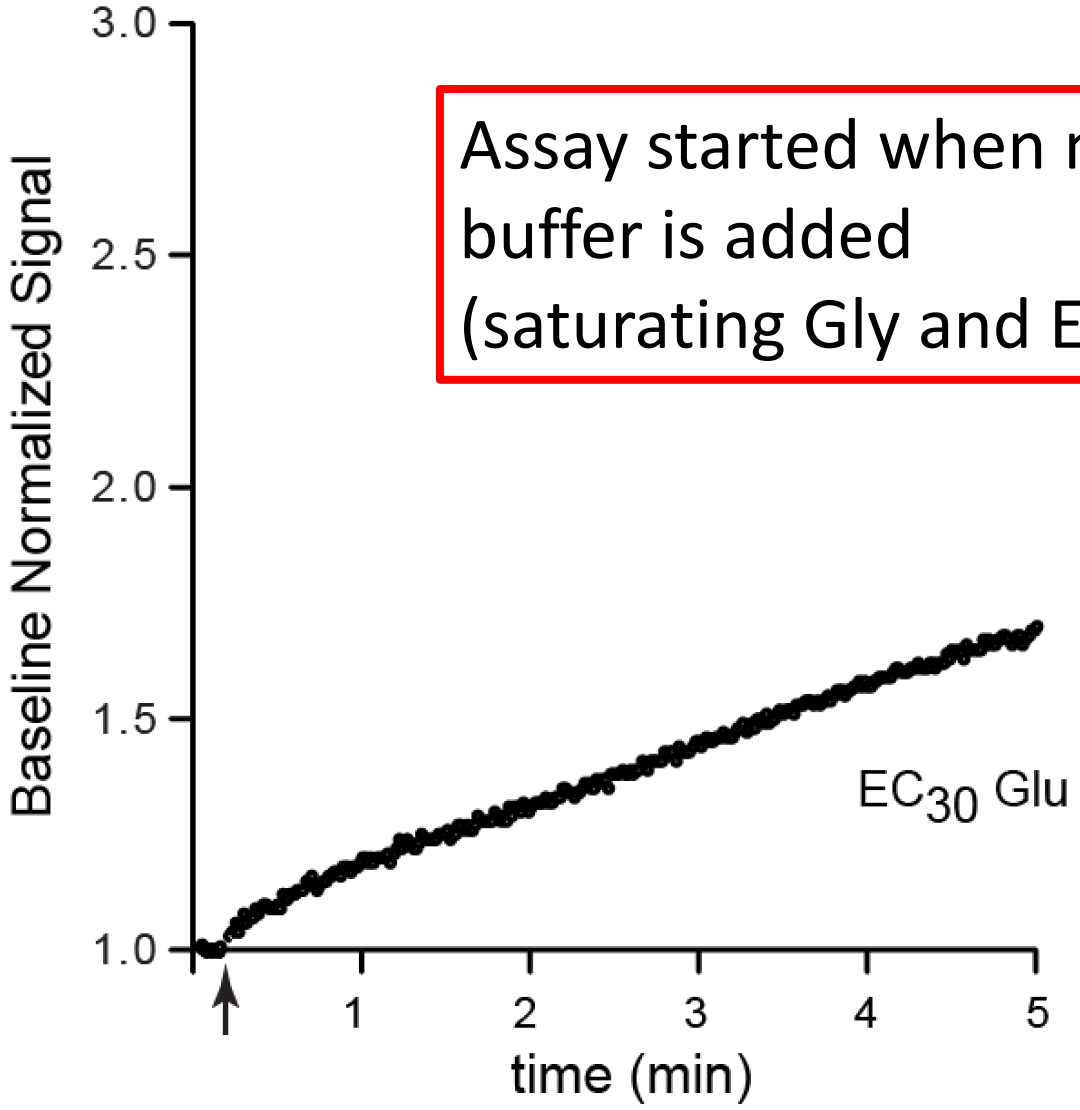
Wash cells to remove ketamine

Plate cells in acidic media (pH 5.5)

Load cells with calcium indicator dye

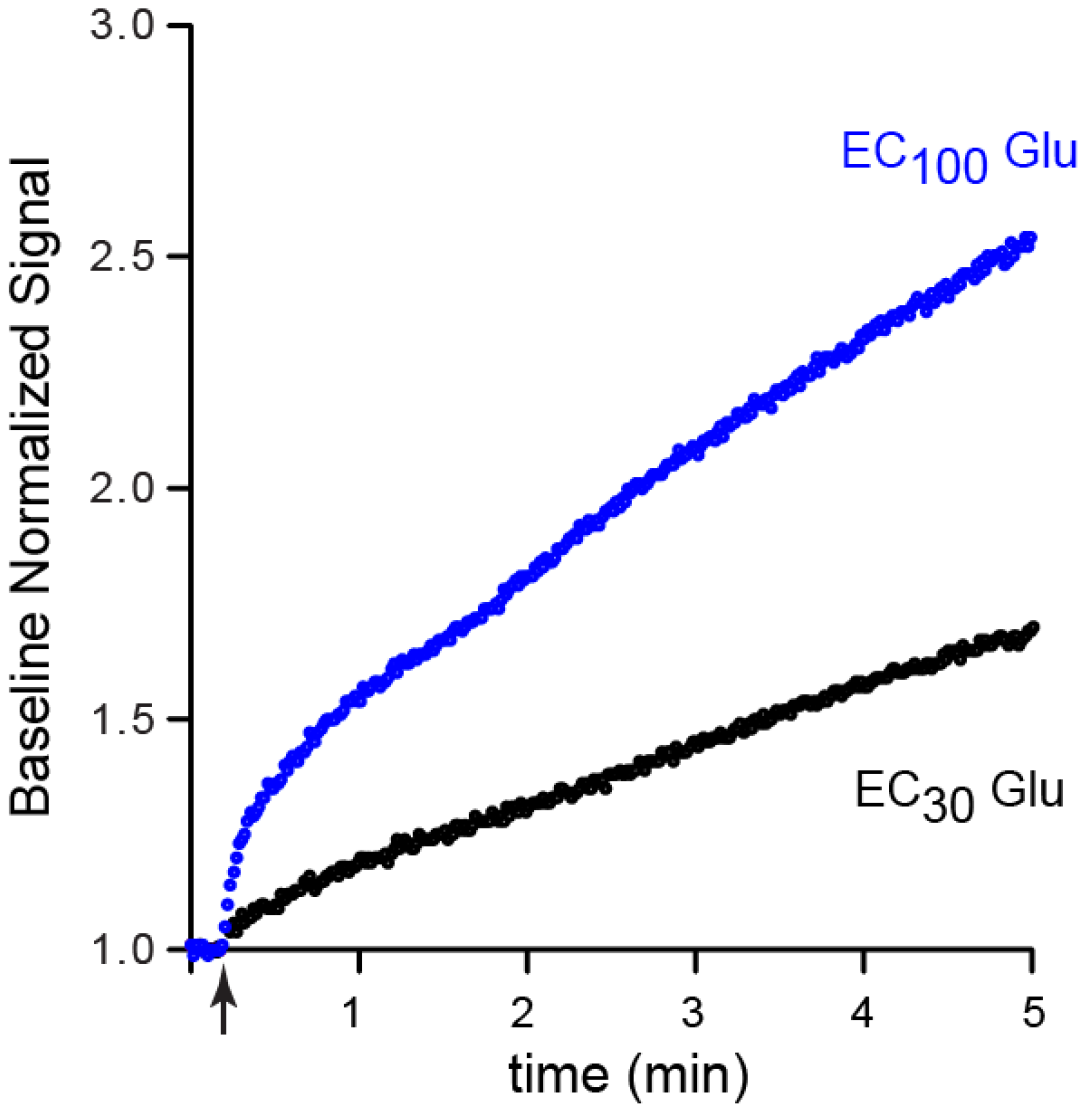
Hamamatzu FDSS

uHTS screen for NMDA receptor PAMs

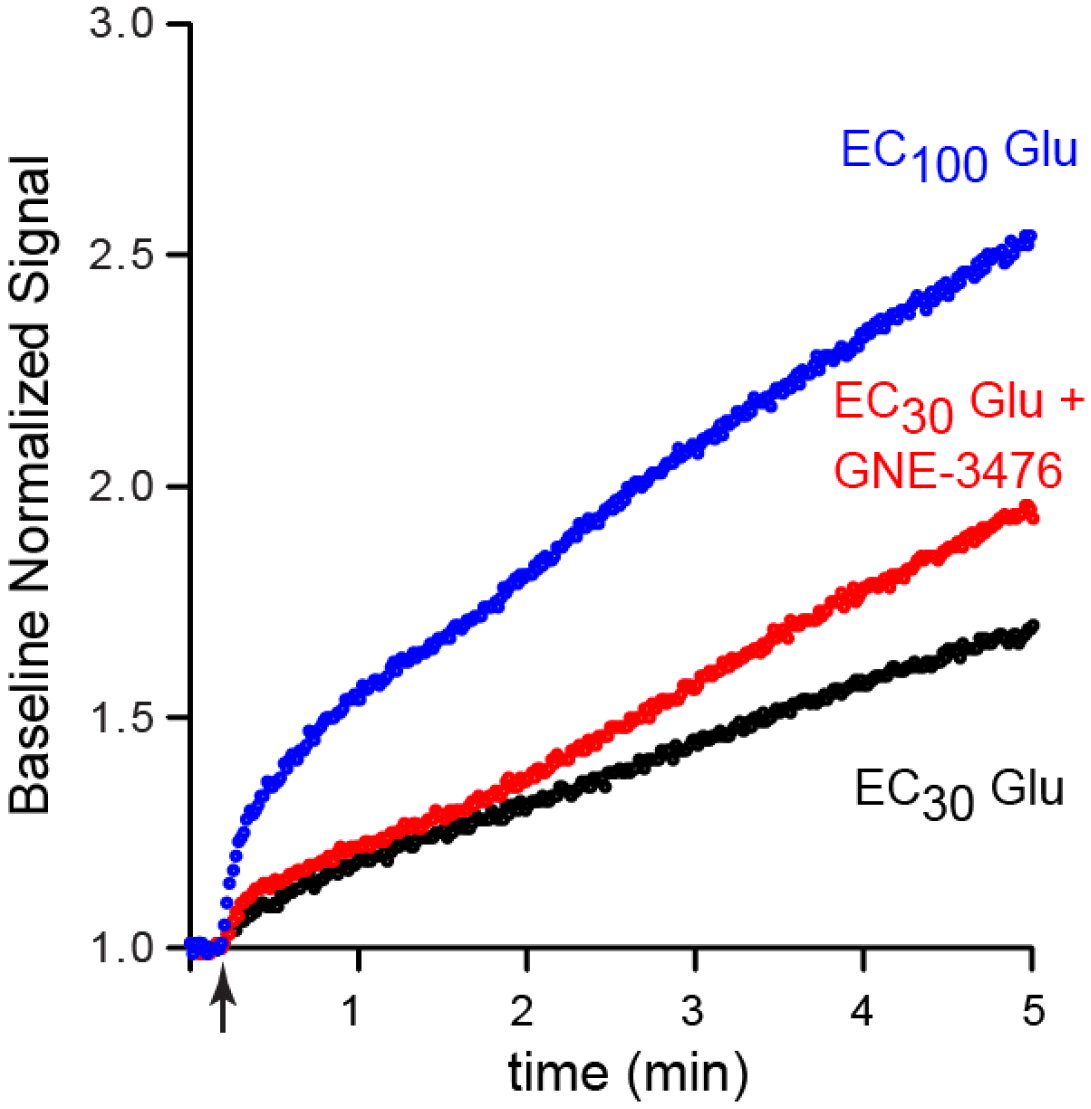


Assay started when neutralization buffer is added
(saturating Gly and EC30 Glu)

uHTS screen for NMDA receptor PAMs

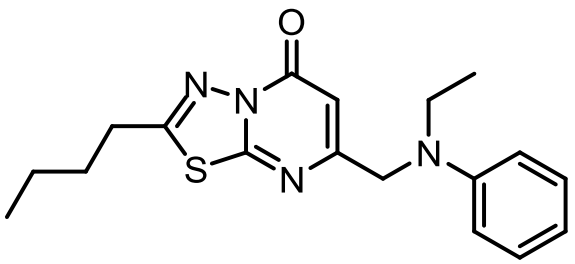


uHTS screen for NMDA receptor PAMs



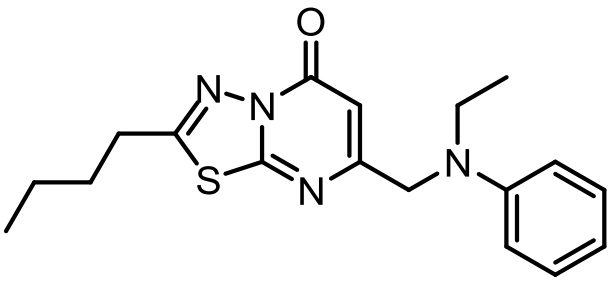
2.5 million compounds screened

7 verified hits

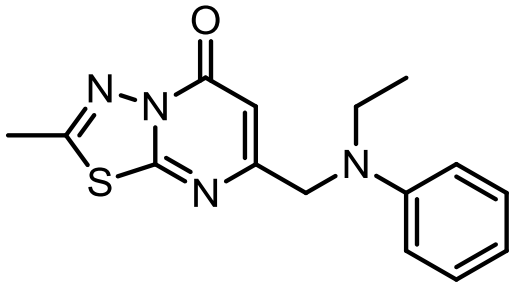


GNE-3476

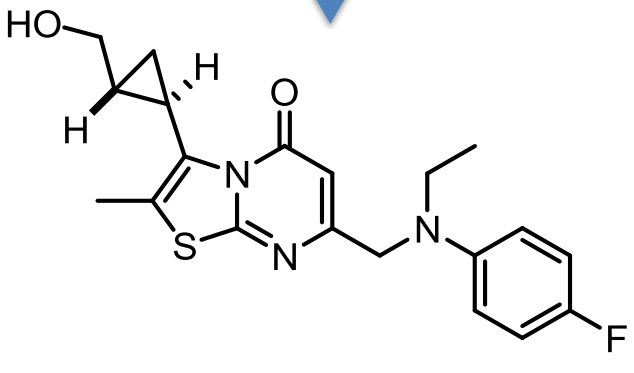
Medicinal chemistry efforts led to drug-like compounds



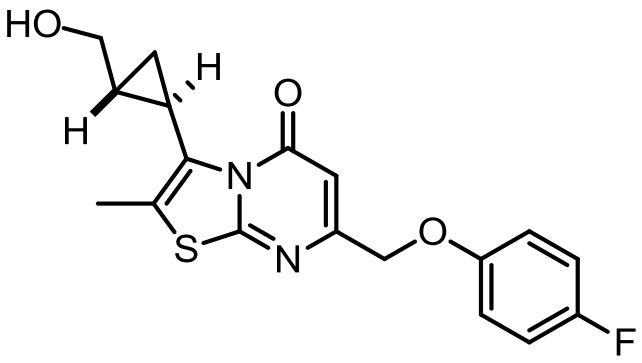
GNE-3476



GNE-3419

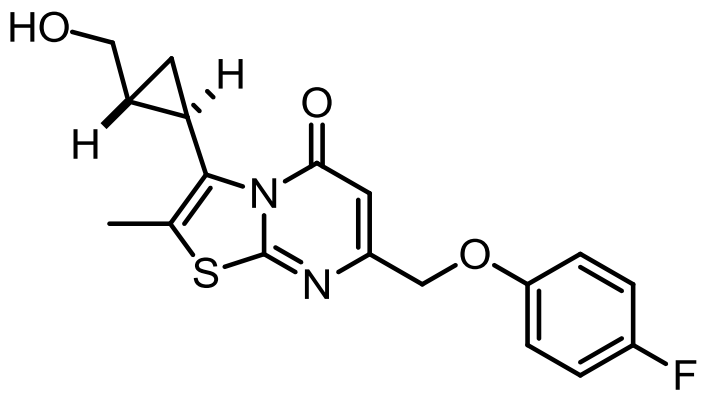


GNE-7728

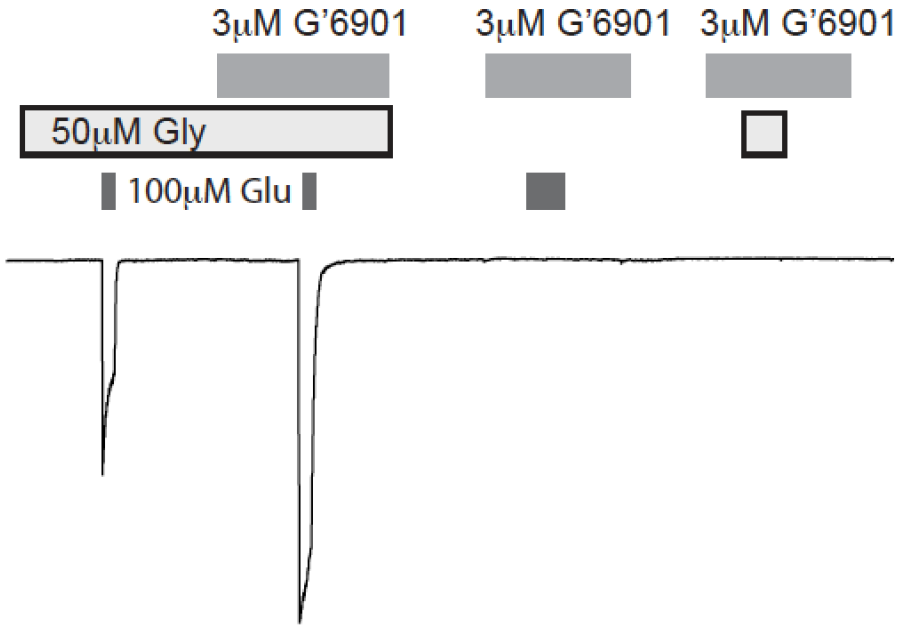


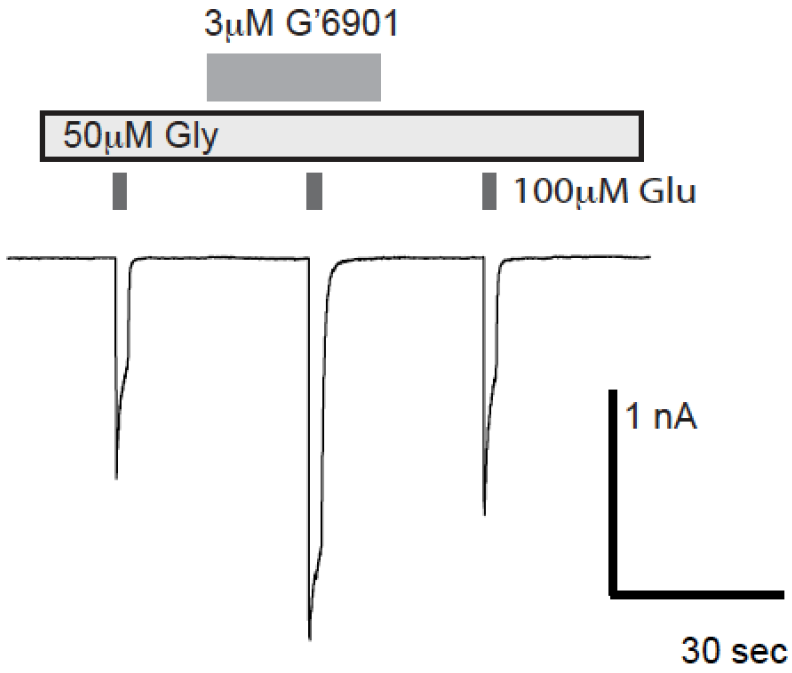
GNE-6901

Example PAM discovered by the Genentech NR2A PAM project team

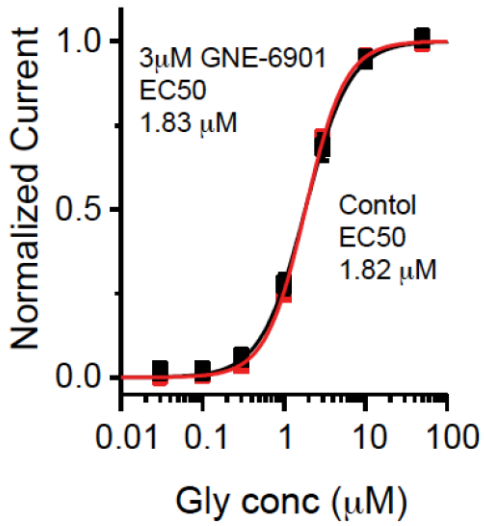


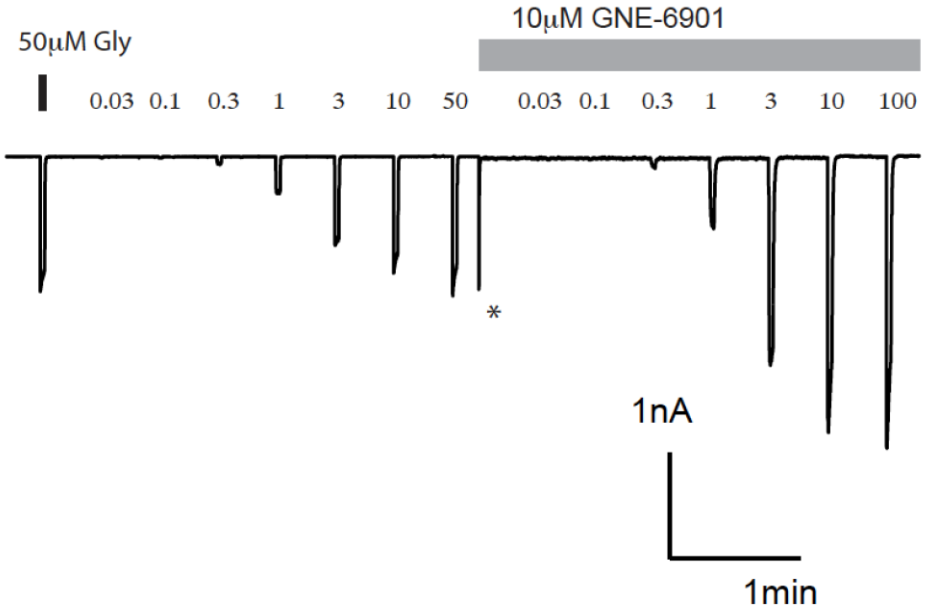
GNE-6901

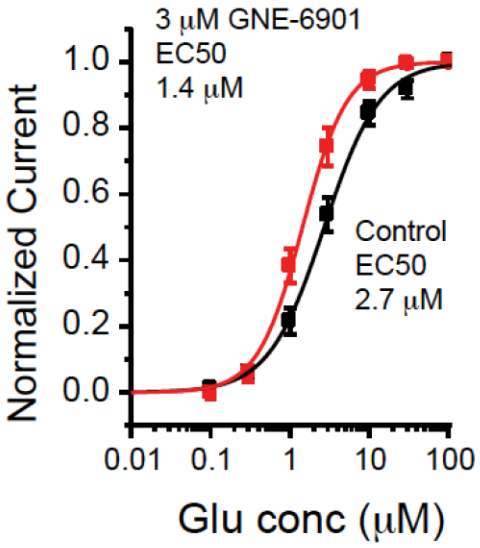


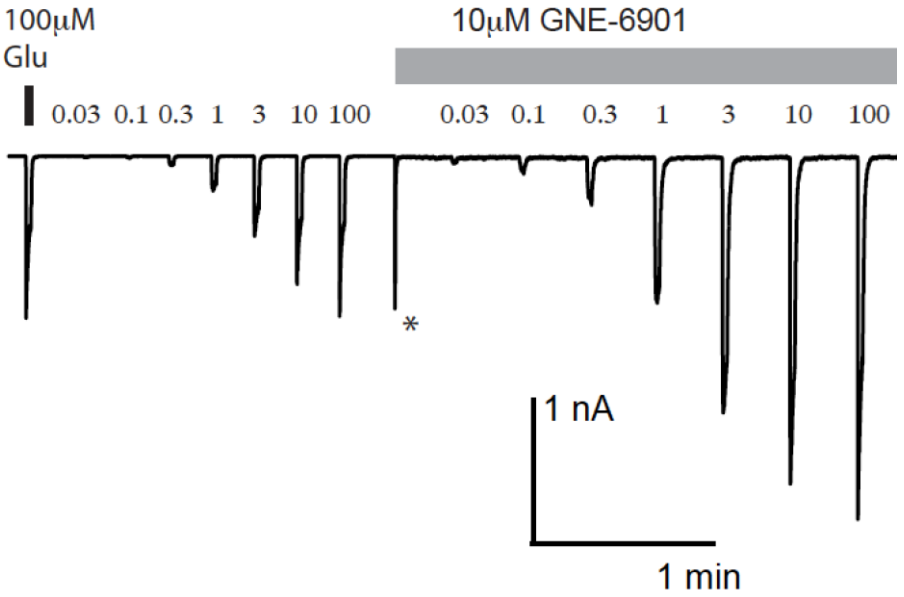


GNE-6901 slightly shifts Glu potency with no effect on Gly potency

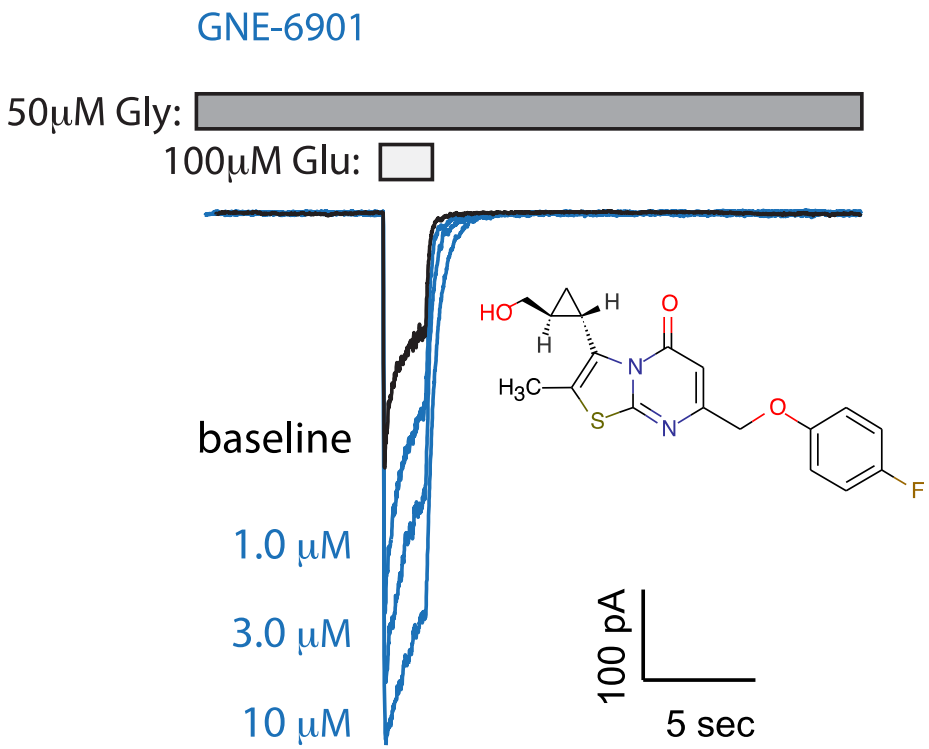


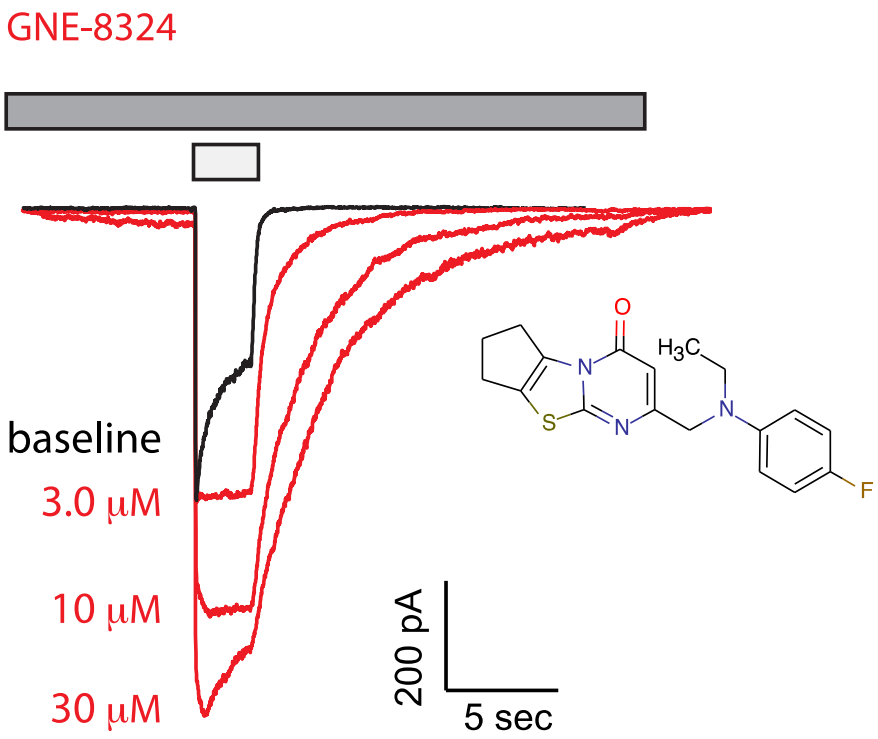




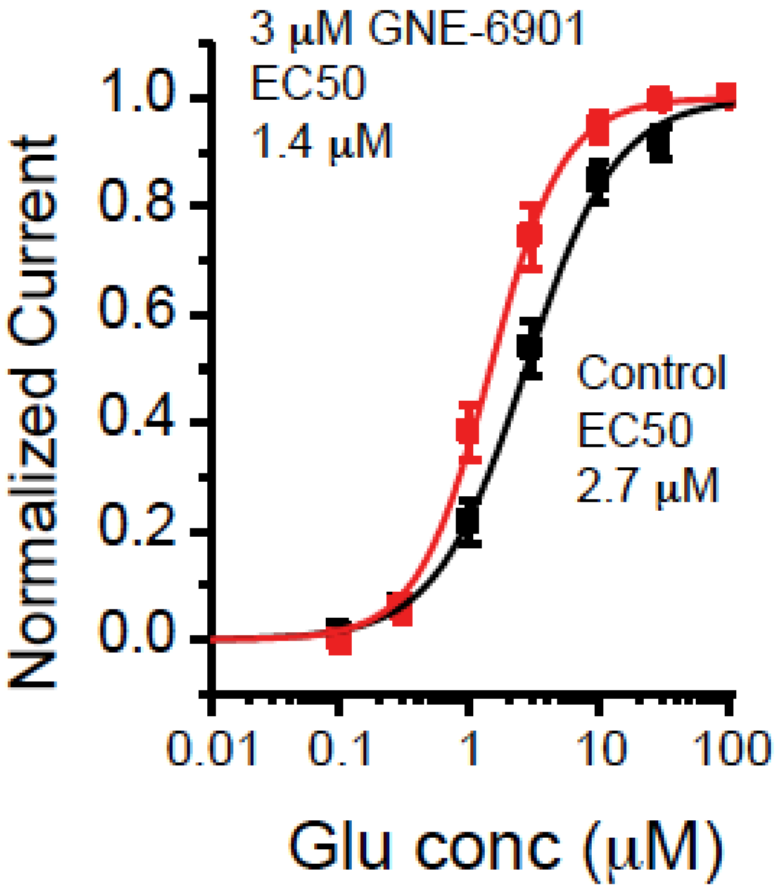


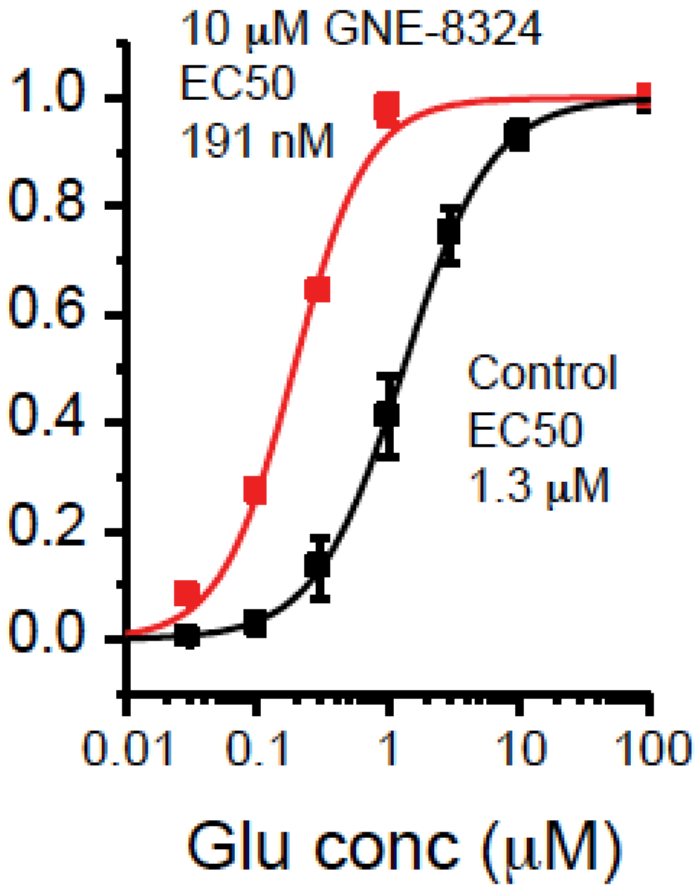
Different impacts of related NR2A PAMs



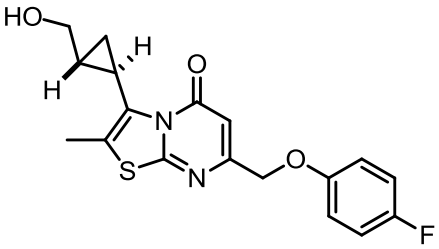


GNE-8324 slows deactivation by increasing glutamate potency

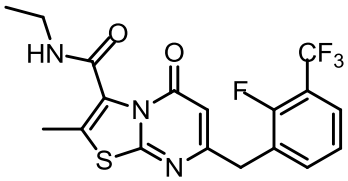




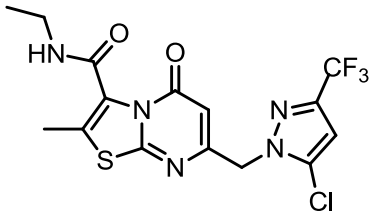
Deactivation increases with substitution on the right-hand side



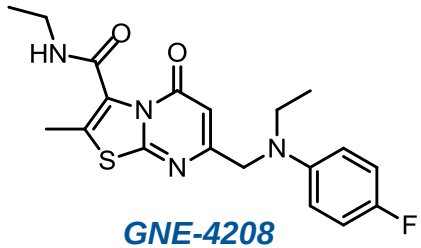
GNE-6901
GluN2A **0.38 µM**



GNE-8016
GluN2A **1.2 uM**

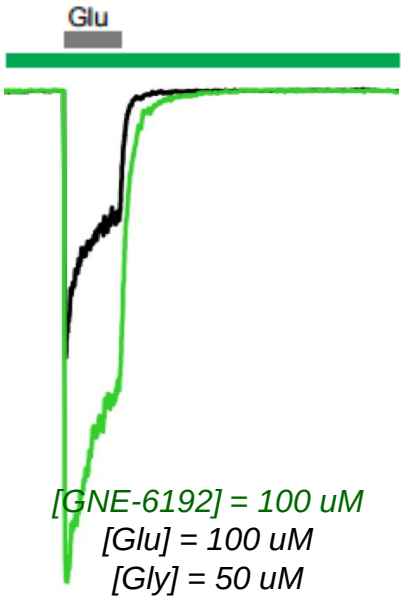


GNE-9178
GluN2A **0.44 uM**



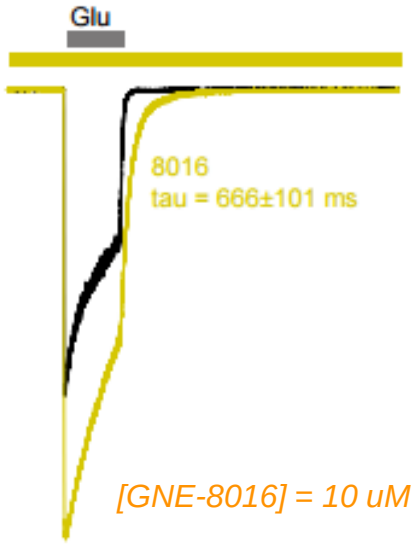
GNE-4208
GluN2A **0.09 uM**

Ether



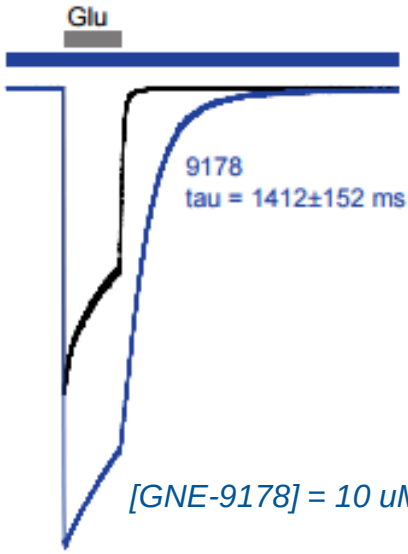
[GNE-6192] = 100 uM
[Glu] = 100 uM
[Gly] = 50 uM

Phenyl



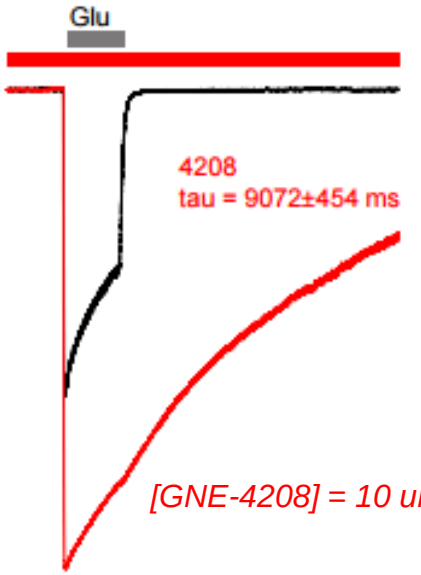
[GNE-8016] = 10 uM

Pyrazole



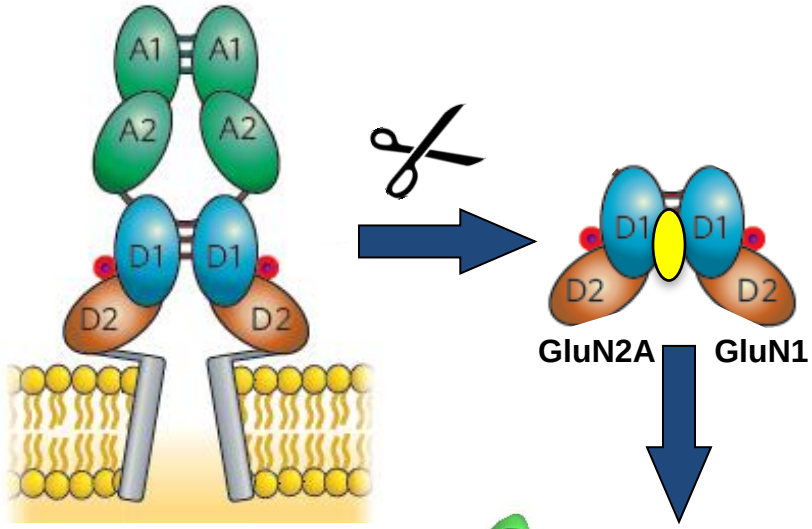
[GNE-9178] = 10 uM

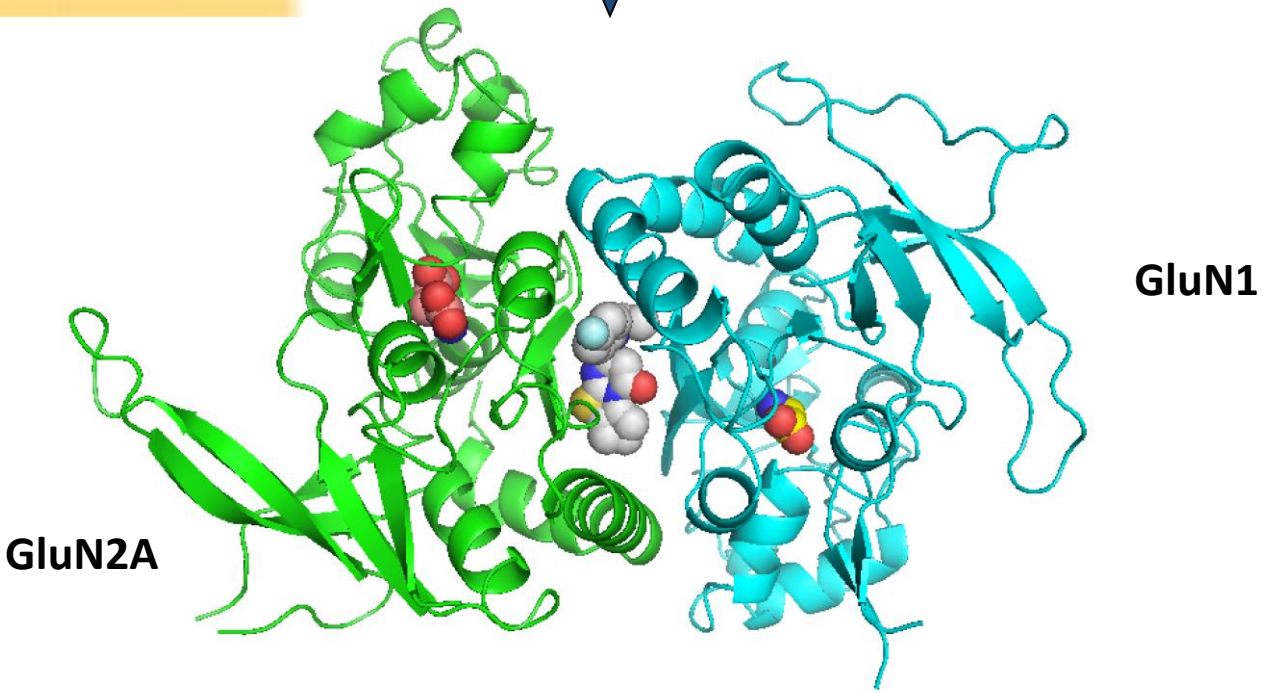
Aniline



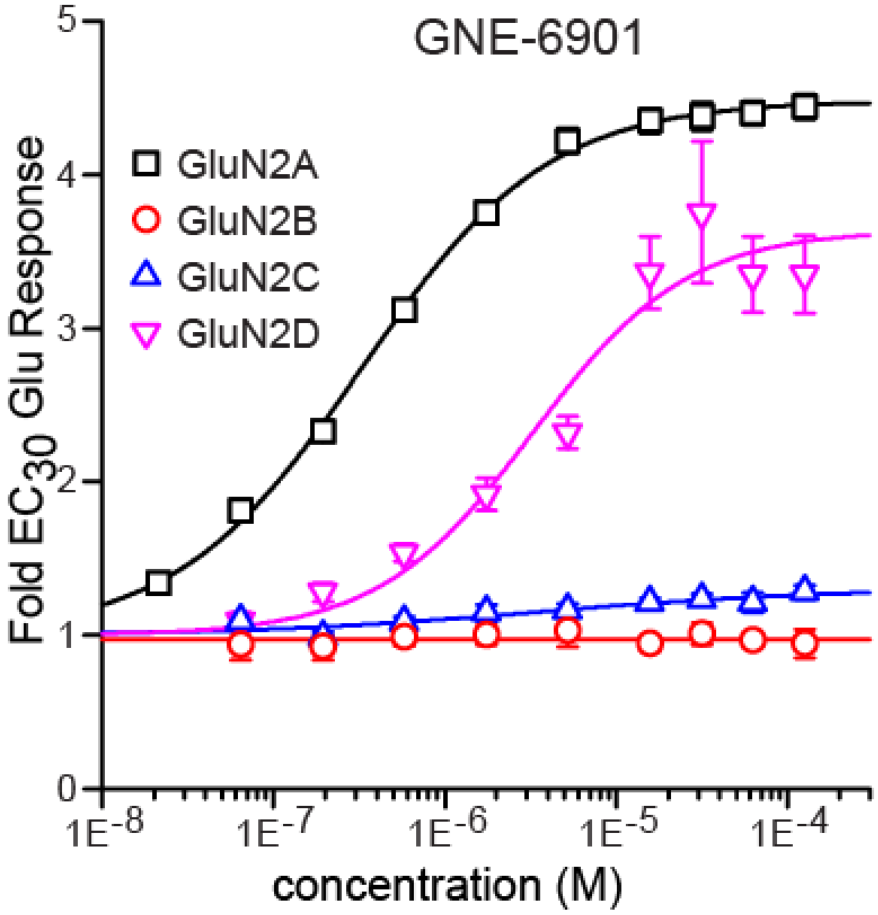
[GNE-4208] = 10 uM

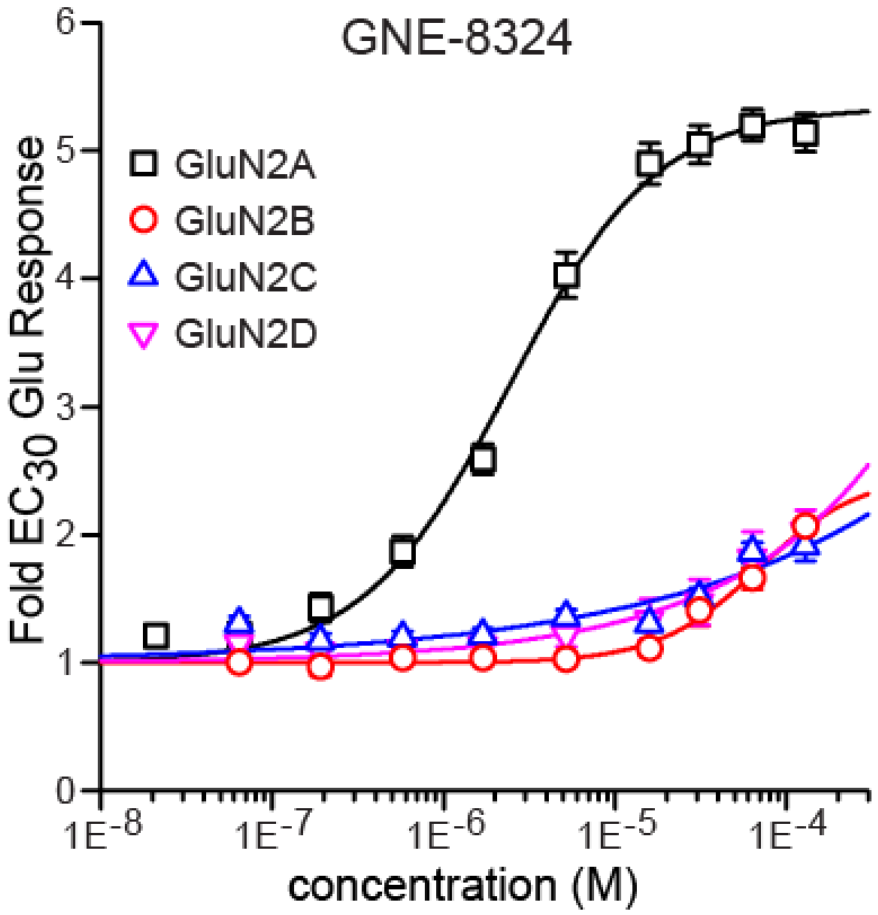
Structure of the binding site within the ligand-binding domain (LBD)



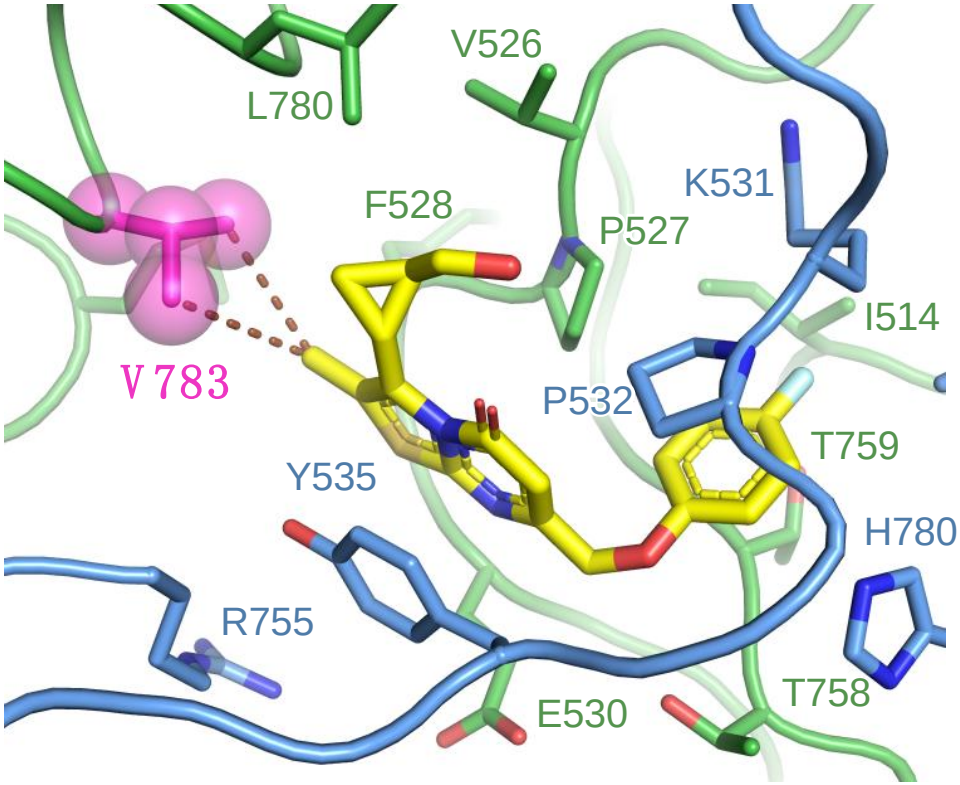


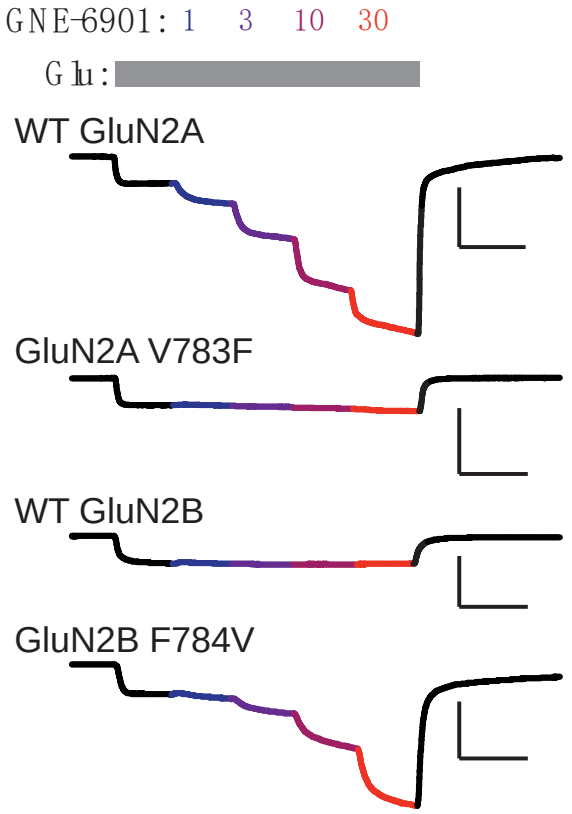
Compounds of this series are highly selective for GluN2A





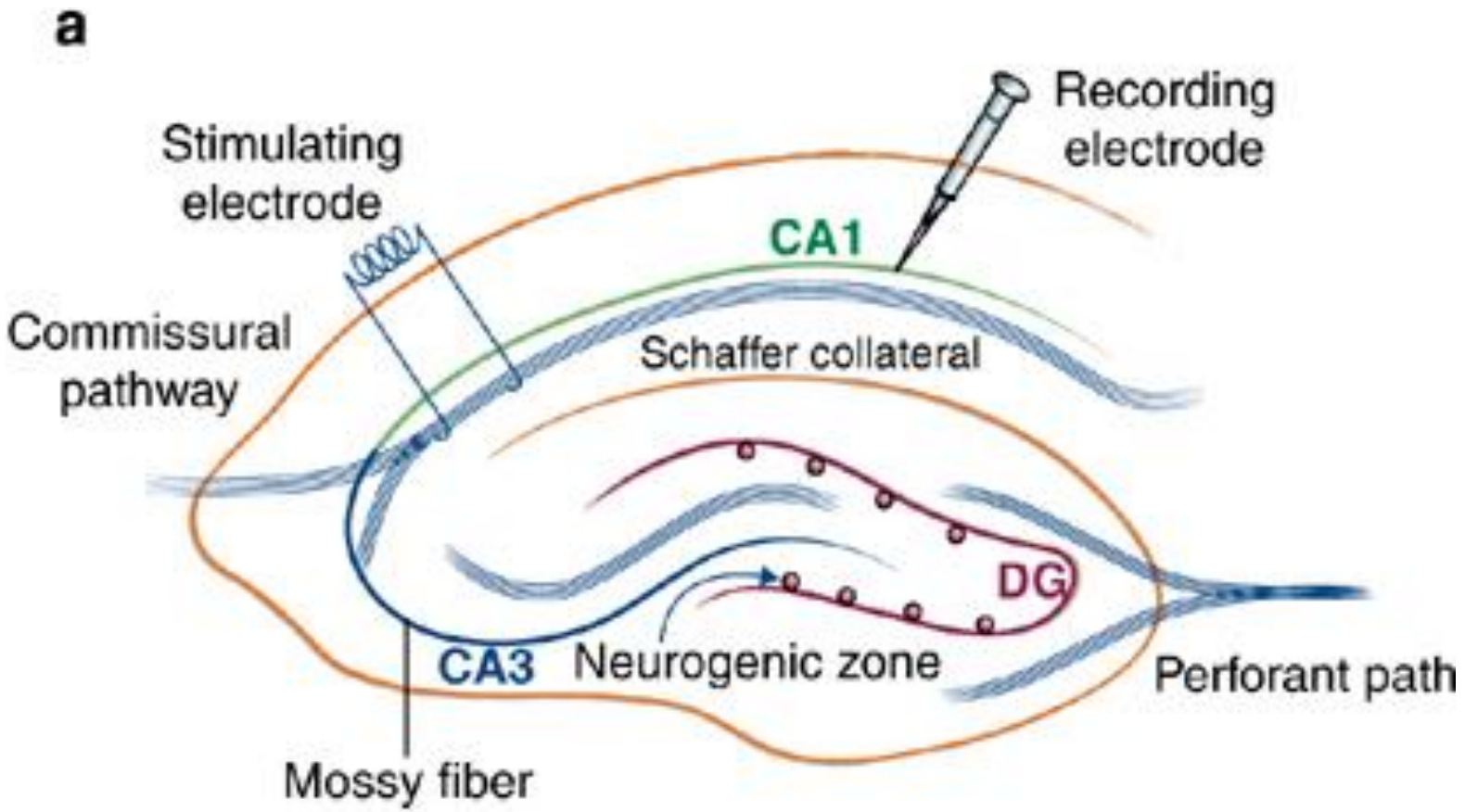
A single residue accounts for the NR2A selectivity of GNE-6901



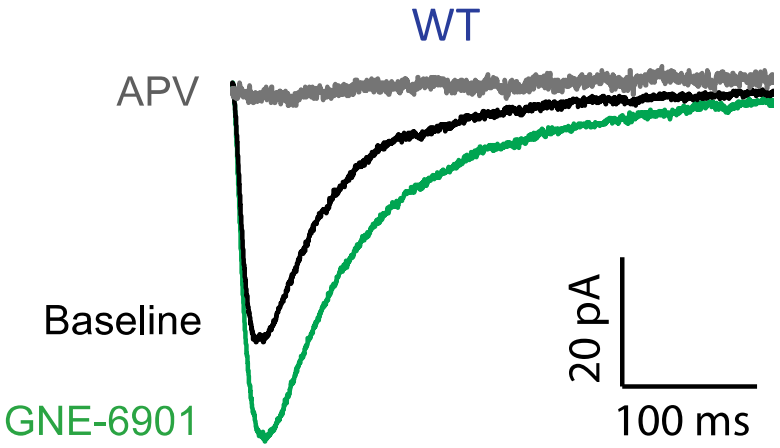


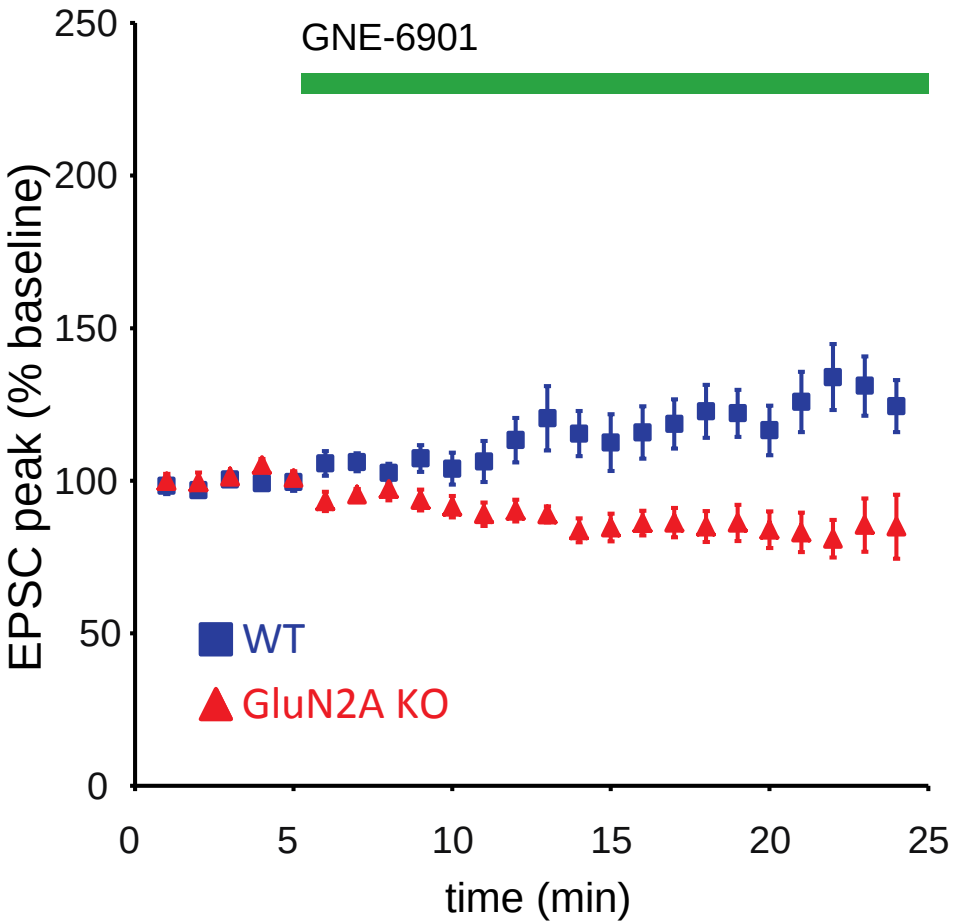
GluN2A V783 = GluN2B F784 (larger side chain)

Do these GluN2A selective PAMs potentiate native NMDA receptors?

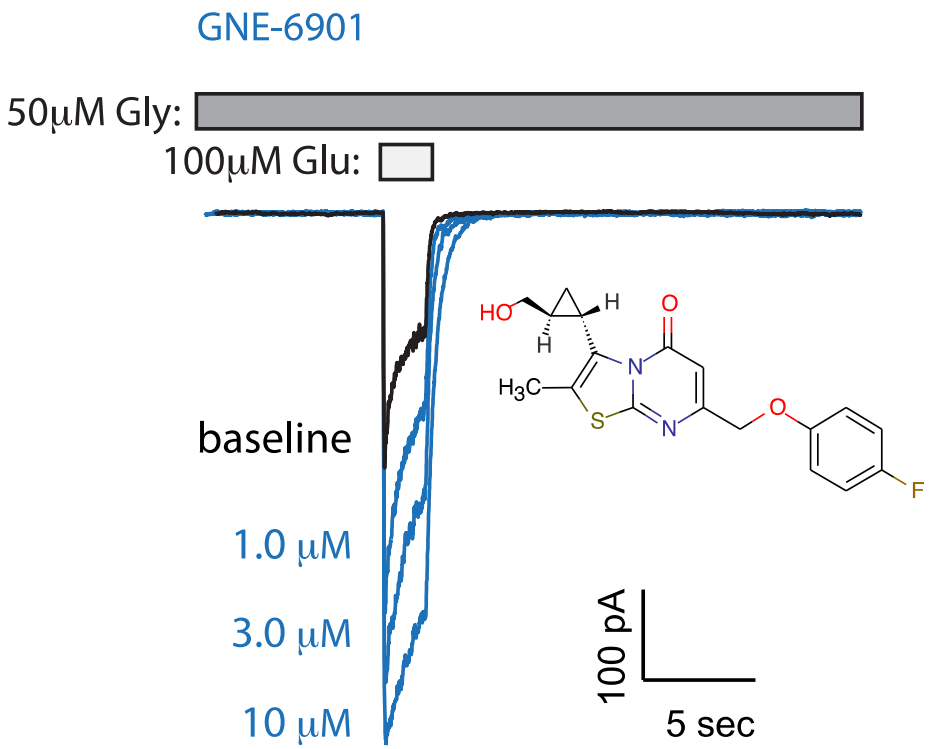


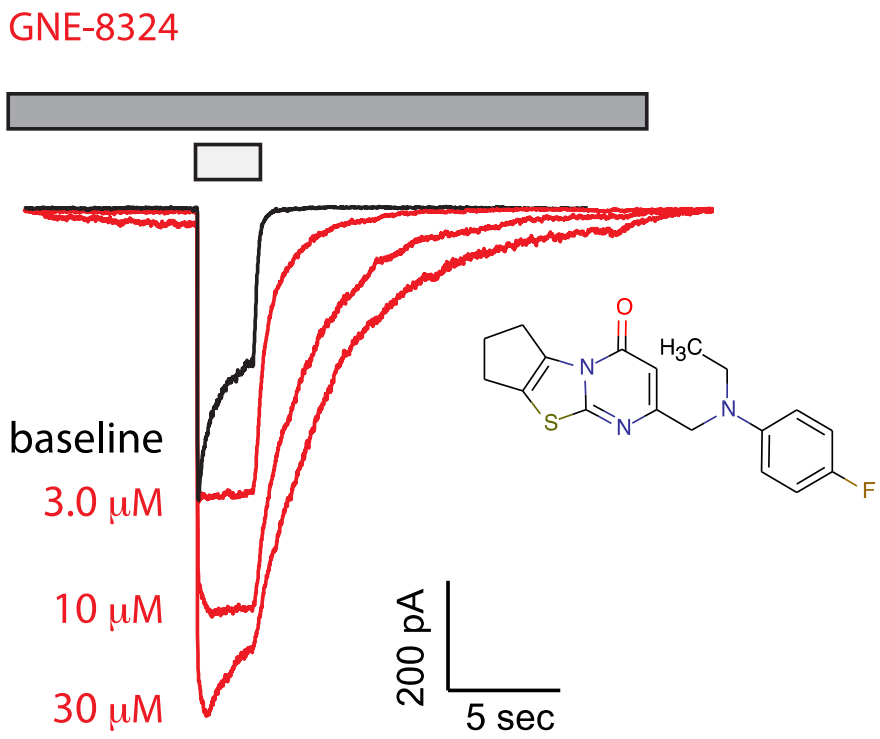
GNE-6901 potentiates NR2A-containing NMDA receptors in hippocampal slices



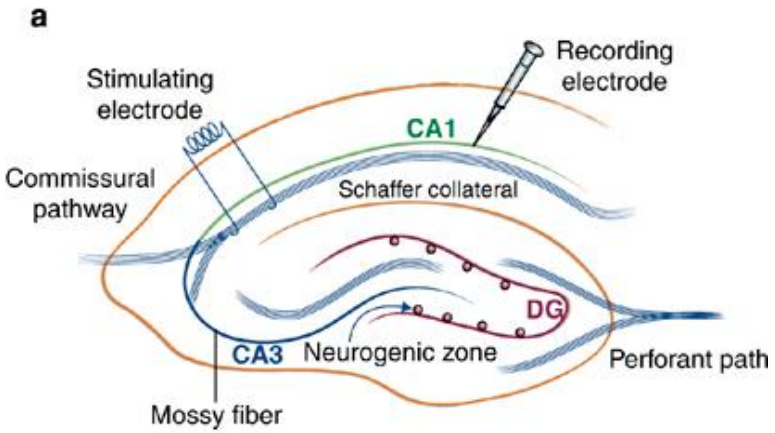


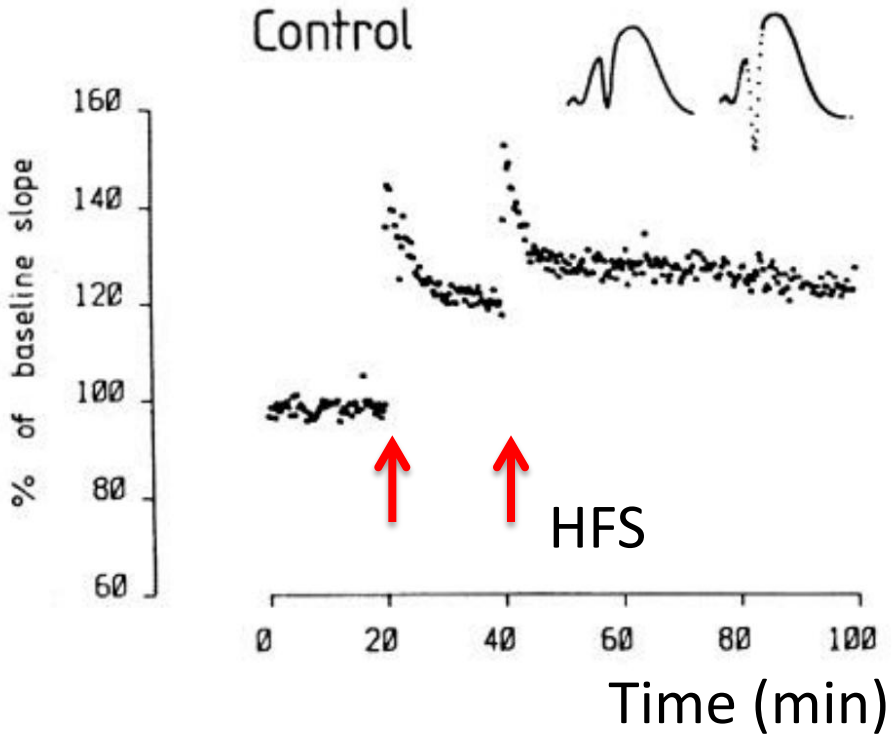
Do PAMs with differential effects on NR2A have differential effects on physiology?



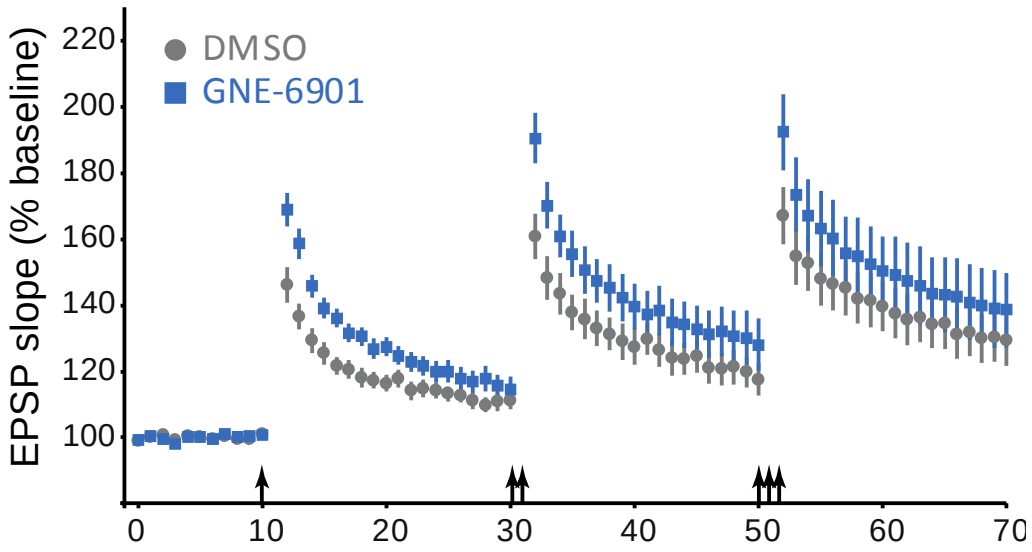


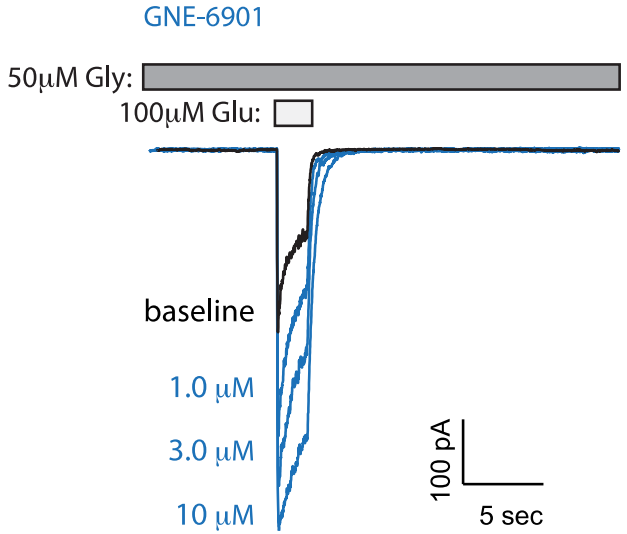
NMDA receptor dependent long term potentiation





GNE-6901 enhances LTP whereas GNE-8324 inhibits LTP





Jesse Hanson, David Hackos

Acknowledgements

Neurobiology
Jesse Hanson
Yelin Chen
Qiang Zhou
Kimberly Scearce-Levie
Morgan Sheng

Biochemical and Cellular Pharmacology
James Herrington
Paul Reynen
Saundra Clausen
Amy Gustafson
Peter Thana
Yichin Liu

Chemistry
Matt Volgraf
Cuong Ly
Elisia Villemure
Richard Pastor
Allen Jiang (PH)
Po-wai Yuen (PH)
Mingcui Liu (PH)
Xifeng Luo (PH)

Compchem
Ben Sellers
Guosheng Wu (PH)
Aijun Lu (PH)

Structural Biology
Patrick Lupardus
Heidi Ackerly Wallweber
Christopher Koth

Protein Production
Baculovirus Expression Group

Legal
Shannon Chi
Robert Hall

IBENS, France
Teddy Grand
Pierre Paoletti


Genentech
A Member of the Roche Group