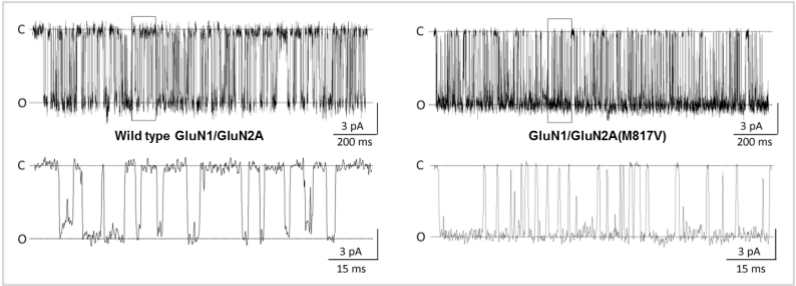
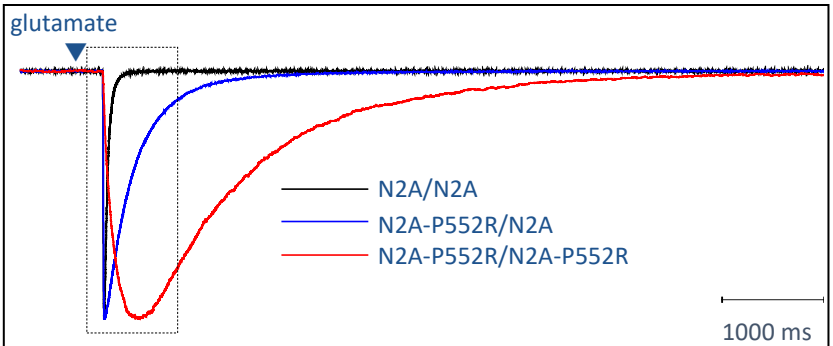
Functional Effects of Glutamate Receptor Mutations in Human Diseases

Many early life disorders, including epilepsy and developmental delay, can involve genetic errors, most often *de novo* mutations that produce missense variants. Genome or exome DNA sequencing can provide pediatric patients who experience these disorders with definitive diagnoses, reducing unnecessary testing, bringing clarity to the family, and focusing basic and clinical research resources. These diagnoses have yielded clinical insight showing that the majority of epilepsies that start prior to one year of life (e.g. infantile epileptic encephalopathies) occur due to *de novo* genetic errors. However, a large gulf still remains between the genetic information describing missense variants in patients and our understanding of how these variants affect the function of the proteins encoded by the affected gene. This lack of functional understanding prevents the translation of genetic information into a better mechanistic understanding and treatment of disease. We are working to directly solve this problem.

Multiple lines of evidence support the idea that genetic variants in genes encoding ion channels can give rise to neurological disease. It is now clear that *de novo* variants in many of the 18 genes encoding glutamate receptor subunits are a major contributor to channelopathies--diseases arising from altered expression or function of an ion channel. We work in partnership with the Center for Functional Evaluation of Rare Variants at Emory and the Simon's Foundation to elucidate the functional consequences of all *de novo* NMDA receptor variants, which exceed 1000 in number. Many of these variants profoundly alter receptor function. We are working to establish the relationship between allelic frequency and functional effects to establish diagnostic criteria for patients with rare NMDA receptor variants. In addition, we are analyzing our functional data for hundreds of variants in the context of clinical data to explore the mechanisms underlying these conditions. All functional data we generate is placed on a public database (the GRIN Portal) hosted by the Broad Institute.



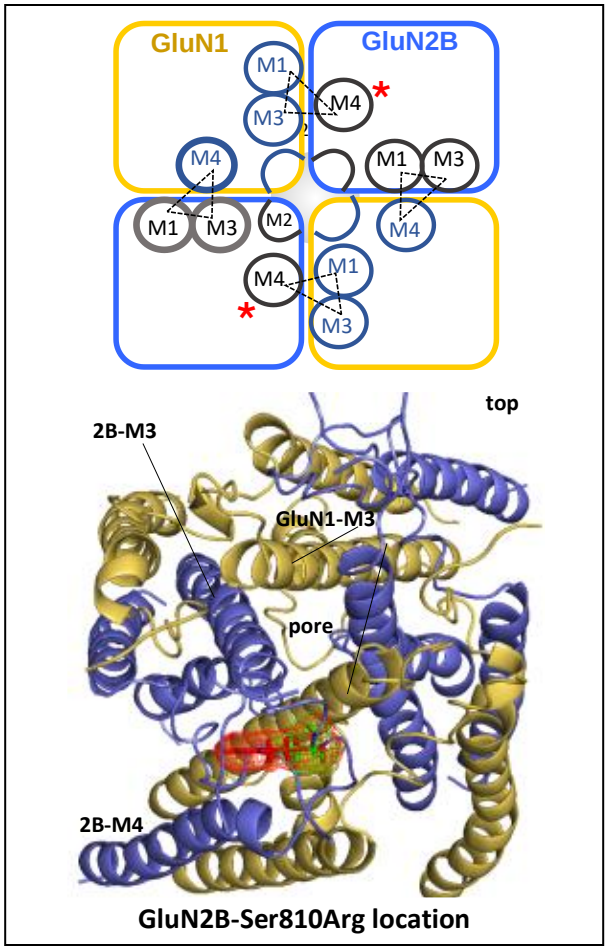
Single channel currents from GluN1/GluN2A and variant GluN1/GluN2A-M817V receptors (O is open, C is closed).

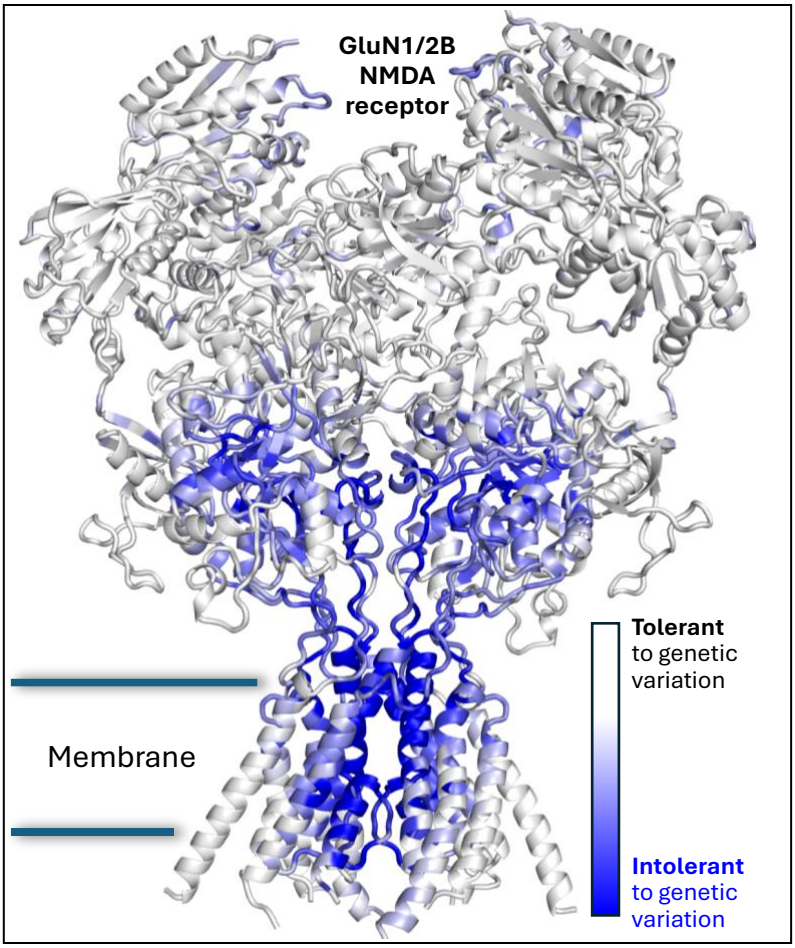


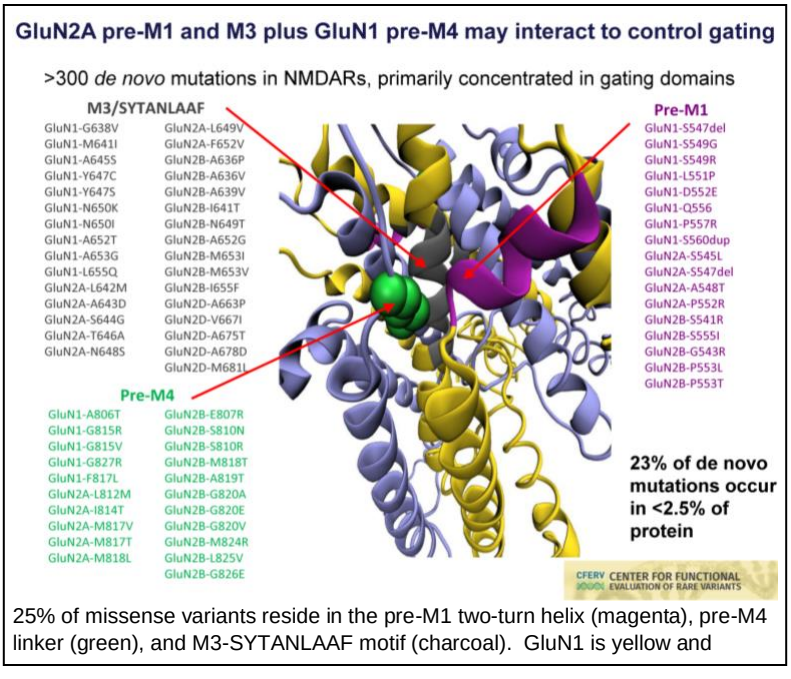
Whole cell current from GluN1/GluN2A receptors with 0, 1, or 2 copies of mutant GluN2A-P552R subunit. The receptors were activated by brief (5 ms) application of a maximally effective concentration of glutamate and glycine.

In addition, the functional information we obtain is being used by clinicians to stratify patients for ongoing and future clinical trials, which helps to diminish patient heterogeneity and increase the power of these smaller trials for rare diseases. This is essential given the low number of available patients to enroll. This information also can serve as a training set for machine learning algorithms optimized to predict the functional consequences of future variants, and further expand information that can be ascertained from future sequencing.

Lastly, functional data has revealed in exquisite detail the structural basis of ion channel function since many variants have been identified in regions previously not known to be involved in gating. This work has further validated the predictive power of the analysis of intolerant regions within the gene, which show a reduced number of variants in the healthy population. These intolerant regions are identified through algorithms that we helped to develop. Our functional data show that the regions that are predicted to be intolerant harbor a large number of variants.







25% of missense variants reside in the pre-M1 two-turn helix (magenta), pre-M4 linker (green), and M3-SYTANLAAF motif (charcoal). GluN1 is yellow and