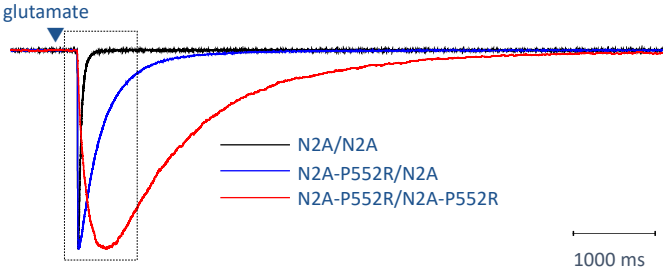
Functional Effects of Glutamate Receptor Mutations in Human Diseases

A large number of early life disorders, including epilepsy and developmental delay, involve genetic errors, most often *de novo* mutations. These devastating conditions can result in refractory seizures, intellectual disability, and lifelong medical problems that create an enormous burden on families. Stunning advances in DNA analysis have increasingly enabled definitive diagnoses to be made, reducing unnecessary testing, bringing clarity to the family, and focusing basic and clinical research resources. These diagnoses have yielded clinical insight, for example 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 and expanding gulf has developed between genetic information describing rare variants and *de novo* mutations in patients and our understanding of how these genetic variants affect the function of the proteins they encode. The lack of functional understanding blunts the promise that genetic analysis holds for improved diagnosis and effective treatment. Furthermore, it prevents translation of genetic information into a better understanding of the basis for disease. This imbalance between genetic and functional information exists because (1) the rate at which sequencing can be accomplished far exceeds the rate at which functional data can be collected, (2) basic functional evaluation of expressed gene variants, proteins, is expensive and requires unique protein-specific assays, and (3) there are few coordinated and systematic efforts to address functional consequences of genetic variation in a manner that is nationally accessible.

Strong genetic, statistical, and biological data support the idea that ion channel variants contribute to and give rise to disease, particularly neurological disease. This has created an opportunity to derive new insight into disease mechanisms from study of human glutamate receptors *de novo* mutations. My lab has worked in partnership with NINDS-funded Center for Functional Evaluation of Rare Variants and CURE to determine the function of all *de novo* NMDA receptor mutations as well as ultra-rare variants. We are also working to establish for the first time the relationship between allelic frequency and functional effects, and use these two data sets to establish diagnostic criteria for patients with rare NMDA receptor variants. This information will be useful for clinicians as they explore ways to attenuate profound gain-of-function mutations. In addition, these mutations reveal in exquisite detail the key gating residues that are relevant for human disease.



Whole cell current recording of recombinant 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. The GluN2A-P552R mutation did not alter receptor trafficking or maximal open probability, but dramatically slowed the deactivation time course, which sets the time course and charge transfer for the synaptic current, leading to a large gain-of-function. This mutation is neurotoxic when transfected into neurons. From Ogden et al., 2017.