Summary

function to see if there is a measurable difference in the sodium permeability of specific mutations. The unaffected NaV1.2 will also be used as a control. As far as research has shown, there has been no direct comparison of solely testing the sodium channel permeability in these mutations. Thus, there is no information showing the relationship between the location of the mutation and the effect on the protein. The difference in potential channel permeability will be measured by first using an inactivation inhibitor, veratridine, to make sure the channels are open, then using a fluorescent Vm indicator dye, read on a fluorescence imaging plate reader (FLIPR), to measure the change in fluorescence.

Aim 2: How does the specific mutant variant R937C of the SCN2A gene impact the selectivity of the sodium channel protein, NaV1.2?

The goal of this aim is to understand and evaluate if variant R937C impacts the selectivity of protein NaV1.2. This mutant variant causes complete loss-of-function in the NaV1.2 protein. Studying specific loss-of-function mutations, such as R937C, and gaining a more complete understanding of the exact effect on the NaV1.2 protein can lead to potential pharmaceutical treatments. This goal will be accomplished by focusing on how R937C cellularly changes the sodium channel activity of NaV1.2. Previous research has indicated that the R937C mutation is located in the transmembrane of the pore loop and potentially causes a complete blockage of the sodium channel (Begemann et al., 2019). This potential for complete blockage will be tested using rat hippocampal cell lines. Fluorescence will theS ‘ P cence δ

gene function. The other two partial loss-of-function mutations would allow for a signifcantly higher level of sodium to pass through. These results would show that the level of sodium that ends up passing through after the mutations are not the ultimate deciding factor on if the mutations cause complete or partial loss-of-function. The results will also show if afecting the sodium permeability will lead to other ions gaining access through the transport channel; it is likely that the levels will difer between the complete and partial loss-of-function mutations.

AIM 2: How does the specifc mutant variant R937C of the SCN2A gene impact the selectivity of the sodium channel protein, NaV1.2?

Rationale: Due to SCN2A mutations being associated with autism spectrum disorder (ASD), and various epilepsy types, elucidation of the R937C mutation's mechanism of dysfunction could provide insight into potential pharmaceutical treatment strategies (Ben-Shalom et al., 2017; Ogiwara et al., 2018). However, little is known about the mechanism underlying how the mutation afects the NaV1.2 sodium channel's function (Begemann et al., 2019). Thus, the goal of this aim is to understand and evaluate how the mutation variant R937C impacts the selectivity of protein NaV1.2. As NaV1.2 protein channels span the cell membrane, allowing for the selective movement of ions, it is suggested that the channel is completely blocked by the R937C mutation, thereby altering neuronal excitability (Begemann et al., 2019). Previous research has also implicated that glutamate residues may potentially assist in the protein's normal function as it complexes to the NaV1.2 pore loop allowing it to potentially modulate channel selectivity. However, it is unknown whether R937C impacts glutamate's interaction with Nav1.2. Through amino acid stabilization of glutamate residues in the pore loop of the protein, the selectivity of the channel is altered (Begemann et al., 2019). The plan is to focus on how the R937C mutation blocks the sodium channel function, thus also impacting glutamate residue stabilization on channel selectivity and function. To determine this, a multi-conditional immunohistochemical assay will be performed on in vitro hippocampal neurons of transgenic mice.

Methods: Rats will be genetically altered to express the mutated version of SCN2A (R937C). Rats will then be euthanized at an age of 12 months and hippocampal neurons will be sampled. Next, an adaptation of calcium channel imaging for the sodium channel will be used to visualize channel è ρ a' hippo ļ M] ƥ ɔ M adapmM 12 ¥

can be more efective than that of non-sodium channel inhibition in affecting neuronal development (Cheah et al., 2019). Although there is an established precedent in using these drugs to treat epilepsy, there is a lack in the literature of the specifc efects these medications show on the sodium channel itself. As such, this research will help fll in that gap using a mice model to show the diference in sodium channel function of the

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