Mechanisms Underlying the Comorbidity of Autism Spectrum Disorder and Epilepsy: SCN2A Mutations and Treatments

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Summary

Epilepsy and Autism Spectrum Disorders (ASDs) are distinct disorders that share a few common contributing factors; one such factor is the SCN2A gene. Individuals who experience these diseases often have symptoms that relate to specific types of mutations that occur within this gene, such as intellectual disability in autism and a high frequency of seizures in epilepsy. The SCN2A gene is responsible for the voltage-gated sodium channel (VGSC) pathway which is a pathway integral in early brain development. One of the key pathways in the sodium channel is the NaV1.2 protein pathway. Mutations in this protein are especially associated with ASD and epilepsy. Researchers have studied mutations that occur within the SCN2A gene that can trigger malfunctions; these mutations can cause either increased (gain-of-function) or decreased (loss-of-function) function in the sodium channel. Increased function leads to the sodium channel being inappropriately open while decreased function leads to it being inappropriately closed. Although the impact SCN2A mutations have on epilepsy and ASD is well-studied, there are still some significant areas that need more research. One such gap in knowledge that can assist in understanding the SNC2A gene is the lack of understanding of the exact relationship between sodium permeability of the sodium channel and the location of the mutation in the gene. Another area to study is the relationship that specific mutations, such as R927C, have on sodium selectivity. The last area of focus deals with the variety of treatment options. There are two main potential treatments, non-sodium channel blockers and sodium channel blockers. Right now, the best time in development to apply non-sodium inhibiting drugs is not known, so determining the exact time in development that these drugs have the best effect is important. On the other hand, the exact effect that sodium channel blockers have on the sodium channel is still not known and further research is necessary to establish a comparison to normal functioning channels. By comparing these various aspects of the SNC2A gene, researchers will be able to better understand how to better assist in treatment for individuals that suffer from ASD and Epilepsy.

Background

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that commonly co-occurs with other neurological conditions, such as epilepsy. Since ASD and epilepsy are both heterogeneous diseases, they can present in multiple ways that have varying levels of severity (Lee et al., 2015). However, ASDs have a few distinct features that are used as diagnostic criteria. First, ASD leads to impairments in both social interactions and communication. The second is that there are repetitive, restricted behaviors. The third is that these symptoms must be presented in early childhood (Lauritsen, 2013). Epilepsy is defined in one of three ways. The first is if a person has two unrelated, unprovoked seizures that occur with at least one day between onsets. The second is one seizure, with a greater than 60% risk of more seizures in the next ten years, following two more unprovoked seizures. The last is a diagnosis of epilepsy syndrome (Fisher, 2015). The exact comorbidity of ASD and epilepsy is not clear, with multiple studies projecting different numbers (18-35%); however, it is understood that there is not an insignificant overlap between the two disorders (Brooks-Kayal, 2010; Lee et al., 2015).

Despite the exact connection between ASD and epilepsy remaining unknown, there are a few specific genes associated with the development of both disorders (Brooks-Kayal, 2010; Wolff et al., 2019). One of these genes is SCN2A (Kamiya et al., 2004; Sampaio et al., 2019; Wolff et al., 2019). SCN2A is one of multiple voltage-gated sodium channel (VGSC) genes (Kamiya et al., 2004). Due to its numerous functionalities, SCN2A is a leading target for focus in understanding and treating numerous neurological disorders (Sander et al., 2019). Importantly, SCN2A is the gene that encodes NaV1.2. NaV1.2 is a protein that plays a role in cortical organization, excitability, and synaptogenesis as well as in initiation and conduction of action potentials (Kamiya et al., 2004; Wolff et al., 2019). It is widely expressed in the central nervous system (CNS), but not in the peripheral nervous system (PNS) (Sanders et al., 2019). This channel is the dominant sodium channel expressed in early brain development, which is the period where most ASD mutations have an effect; however, the exact contributions of NaV1.2 dysfunction on ASD endophenotypes is not known (Wang et al., 2021). Early on in development, the 2nd trimester to around 1-2 years old, NaV1.2 is the only sodium channel isoform expressed in the axon initial segment (AIS), which is the site of action potential initiation. After the formation of the cortex, NaV1.2 is mainly replaced by NaV1.6 which has a lower voltage threshold. NaV1.2 is then restricted to areas closest to the soma and functions as a reserve pool, thought to help action potential backpropagation (Sanders et al., 2019).

Since SCN2A initiates action potentials, it is essential for defining neuronal excitability. In this aspect, ASDs are primarily connected to loss-of-function mutations while epilepsy is associated with gain-of-function mutations (Wang et al., 2021; Wolff et al., 2019). SCN2A is located on the positive strand of Chromosome 2. The mRNA transcript has 27 exons, which encodes a 2,005 amino acid protein for NaV1.2. There are few specific areas on the gene known to be associated with disorder variants. These include: the S4v-S5 in voltage sensing domain, the intracellular N- and C- Terminals, and the pore loops around the ion selectivity filter (Sander et al., 2019). The main types of mutations seen that lead to ASD or epilepsy are missense or truncating mutations (Kamiya et al., 2004; Sander et al., 2019; Wolff et al., 2019). Missense mutations can potentially alter the biophysical properties of the sodium channel, especially in terms of sensing voltage or permitting ion flux. It is common for a one point variant to alter multiple aspects of the channel. This can lead to concerted changes, such as loss-of-function or gain-of-function, or a combination of the two depending on which amino acid is changed (Sander et al., 2019).

Interestingly, despite the importance of SCN2A on both ASDs and epilepsy, more people with epilepsy are screened for SCN2A variances, meaning the exact prevalence in ASDs is unknown (Wolff et al., 2019). There are hundreds of disorder-associated mutation variants, of which less than 30 have been electrophysiologically assessed (Sander et al., 2019). A better understanding of the mutations associated with SCN2A can lead to better treatments for the associated disorders. As such, restoring NaV1.2 to a “normal” level of expression may help both gain- and loss-of-function associated diseases. However, there is a need to normalize the level without increasing or decreasing the channel activity too much, which can lead to the opposite issues than those the patient originally suffered (Sander et al., 2019).

Significance

Gaining further understanding on the mutations affecting the SCN2A gene is significant for a number of reasons. The reason this research is important is due to how this gene is connected with numerous neurological diseases; most prominently the SCN2A gene has shown significant overlap when related to ASD and epilepsy (Lee et al., 2015). Further research on the mechanistic effect mutations have on this gene and specifically the protein, NaV1.2, will provide a greater understanding of the diseases themselves. Exploring the specific benefit of different potential drug therapies during different developmental periods will also allow for more refined treatments, by being able to select the most effective drug option for the patient.

Specific Aims:

Aim 1: How is the potential sodium channel permeability affected in complete loss-of-function mutations vs partial loss-of function mutations?

The goal of this aim is to determine how the sodium channel permeability is affected in complete loss-of-function mutations as opposed to partial loss-of-function mutations, specifically in relation to the location of the mutation. This will be accomplished by using NaV1.2 mutations known to cause either partial or complete loss-function such as the complete loss-of-function in R937C and HEK293 mutant proteins and the partial loss-of-function mutation, T1420M, D12N, and D82G mutant proteins. Complete loss-of-function mutations have been shown to block the channel, while partial loss-of-function mutations have been shown to partially block the sodium channel or affect it in other ways such as depolarization (Sanders et al., 2018). Multiple mutations will be used for each variation in loss-of-

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 then be used to measure the NaV1.2 protein level activity. Thus, fluorescence will be able to show if there is a complete blockage or if some products manage to leak through. As well as using fluorescence, glutamate colonization measures will be used to test the level of glutamate residue left over in the mutated gene and the control.

Aim 3: When is the best time during neuronal development to introduce non-sodium channel inhibiting antiepileptic drug treatments (AEDs) to optimally alleviate the phenotypes of both ASD and epilepsy?

The purpose of this aim is to investigate whether or not there is a critical period within neuronal development that is optimal to administer non-sodium channel inhibiting antiepileptic drug treatments (AEDs) for SCN2A-related disorders. This aim will further expand upon the already known periods of SCN2A-related disorder development and when treatments tend to work best. Non-sodium channel inhibiting AEDs have proven to be the preeminent treatment for individuals that exhibit the phenotypes of both ASD and epilepsy (Sanders et al., 2018). The current study will aim to bridge this gap in knowledge by using three well known types of non-sodium channel inhibiting AEDs including levetiracetam (LEV), benzodiazepines (BZDs), and valproate to assess their effects at different developmental periods within SCN2A mutant transgenic mice. The severity of epilepsy and ASD phenotypes will be assessed within the mice after treatment to assess when is the best time to administer AEDs. The type of AED that presents the most optimal results will be determined from this study to provide better insight on treatment options.

Aim 4: How effective are sodium channel inhibiting drugs on the epileptic effects caused by gain-of-function mutations and do those drugs bring the channel function back to wild type levels?

The goal of this aim is to investigate the effectiveness of sodium blocking drug treatments on gain-of-function mutations in SCN2A. Gain-of-function mutations are primarily associated with epilepsy behaviors, and as such, three epilepsy treatments will be tested. Those drugs are carbamazepine, phenytoin, and lamotrigine. Although these drugs are known as epilepsy treatments, their exact effect on the mutated gene is not known. To test this gap in knowledge, a mouse model showing normal function in SCN2A will be used as a control comparison point, as well as mutated mice who will not be given any drug treatments. These mice will be kept in the same environmental conditions and their behavior will be observed and measured. Blood tests and assays to determine sodium levels will also be collected before and after the administration of the drug treatments. At the end of the study, the mice will be euthanized and their brains dissected. Thus, the benefits of the sodium blocking drug treatments will be measured against each other in multiple avenues.

Design and Methods:

AIM 1: How is the potential sodium channel permeability affected in complete loss-of-function mutations vs partial loss-of-function mutations?

Rationale: Previous studies have shown that not all loss-of-function mutations in the SCN2A gene have the same level of effect. Some mutations, such as those found in R937C, R937H, and C1386R cause complete loss-of-function, while others, such as T1420M, D12N, and D82G only cause a partial loss-of-function (Begemann et al., 2019; Ben-Shalom et al., 2017). These loss-of-function mutations are primarily associated with ASD, but are also present in some patients with seizures (Begemann et al., 2019; Wolff et al., 2019). The overall effects of these known mutations have been studied, but there are no studies directly comparing the permeability of the sodium channel on its own as opposed to the relation back to the action potential. This lack in literature is surprising, considering that NaV1.2, is one of the voltage-gated channels that regulate sodium permeability, meaning that the effect the mutations have directly influences the permeability (Gilchrist et al., 2014). This type of channel works by enabling sodium ions to cross the membranes with the most selectivity (Naylor et al., 2016). Thus, it is possible that these mutations may lead to another ion gaining more selectivity, thus stopping or lessening the important cascade effect leading to excitability (Echevarria-Cooper et al., 2021; Naylor et al., 2016). There is an area known to be important in sodium selectivity. This region is known as the pore region. The pore region consists of four conserved amino acid residues that are necessary for proper sodium channel function, specifically regarding sensitivity of the channel toward sodium (Begemann et al., 2019; Naylor et al., 2016). Of the mutations of interest, R937C, R937H, and T1420M are located in the pore loop, D12N and D82G are located on the N-terminal domain, and C1386R is located in the outer vestibule (Begemann et al., 2019; Ben-Shalom et al., 2017). This suggests that there may be a difference in the sodium channel permeability, specifically relating to sodium, either between the complete loss-of-function mutations compared to the partial loss-of-function, or there may be a difference reliant on whether the mutation is located in the gene. Thus, the purpose of this aim is to measure and compare sodium permeability in relation to degree of function and location.

Method: Data will be collected from induced pluripotent cells (iPCs) designed to mimic the R937C, R937H, T1420M, D12N, and D82G mutations in the SCN2A as well as a normal functioning SCN2A cell culture, or the mutated cells will be collected from an institute with the necessary mutations already induced. Before any measurement, the Goldman–Hodgkin–Katz voltage equation will be used to calculate the reversal potential of the sodium channel to determine the selectivity of the control cell. This provides a theoretical basis on which to compare the actual results. The iPC control and mutated sodium channel cells will then be placed and cultured on petri dishes and the sodium channel permeability will be measured in the method described by Benjamin et al. (2006). After the cultures are grown, cells from each mutation will be transferred into poly-D-lysine pre-coated well plates in media a day prior to the assay. Before the assay, all cells will be washed twice with an assay buffer and then the FLIPR Vm dye will be added to each well and integrated into the culture by being placed at 37 °C for one hour. This fluorescent dye attaches to sodium ions, so only the permeability of sodium ions will be measured, and not any other ions present in the cell. After the hour, the wells will be placed on a FLIPR96 Penta High-Throughput Cellular Screening System and will be monitored. During the monitoring, veratridine will be added to stop the inactivation inhibitor for the sodium channels and the cells will be hit with a consistent electrical pulse. This will force the cells to be pushing as much sodium as they are able through the voltage-gated sodium channel (Benjamin et al. 2006). The concentration of sodium passing through the channels will be measured based on the fluorescence read using the FLIPR software. The same process will then be replicated using a fluorescence dye that attaches to other potential transport ions. Statistical analysis will be performed using ANOVA and unpaired t-tests. Significance will be defined as a value less than 0.05.

Predictions: Since two different questions are being asked related to the sodium permeability, there are two likely results. The first, most likely, result is that the complete loss-of-function mutations cause a complete halt to sodium permeability, preventing any sodium from passing through the channels and completely arresting any possible action potential. However, there could still be some end result sodium due to other sodium ion channels. Thus, the partial loss-of-function mutations will necessarily allow some sodium to pass through the voltage channel in order to initiate the action potential. However, there is another result that could co-occur. In this case, the one partial loss-of-function mutation connected to the pore loop will have lower levels of sodium passing through the channel than the other partial loss-of-function mutations due to the importance of the pore loop in

gene function. The other two partial loss-of-function mutations would allow for a significantly 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 affecting the sodium permeability will lead to other ions gaining access through the transport channel; it is likely that the levels will differ between the complete and partial loss-of-function mutations.

AIM 2: How does the specific 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 affects 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 activity between R937C neurons and control neurons. This would be performed in healthy controls, both with and without glutamate residues present, as well as with samples exhibiting the R937C mutation. First a base-line sodium flux should be determined for the NaV1.2 channel without the mutation, and with and without the glutamate residues present. Since glutamate can be fluorescently tagged with green fluorescent protein (GFP), a comparative analysis of relative fluorescence can then be performed to determine sodium influx changes in the hippocampal neurons between the four conditions. Additionally, cryo-electron microscopy of the protein's structure will be performed for each condition to better visualize and understand protein shape. Utilizing cryo-electron microscopy will confirm that any alterations appearing are based on residue binding and mutation presence. These scans could also be compared to protein structure as well as exhibit how R937C is altering protein structure and thus altering glutamate presence to create a total loss of function in NaV1.2. Statistical analysis will be performed using ANOVA and unpaired t-tests. Significance will be defined as a value less than 0.05.

Predictions: It is expected that glutamate residue stabilization in the pore loop of the NaV1.2 channel will modulate its selectivity. The prediction is that there will be a higher relative fluorescence of sodium ions in the channel mutants compared to the normal controls. As the mutation alters the channel function, it should not be permeable to the ions, and thus a high concentration of the ions should be visible in the extracellular environment of the channel. Lastly, a colocalization of glutamate residue in the pore loop of NaV1.2 is expected and more residue evident in mutated neurons where the selectivity of NaV1.2 is at its highest.

AIM 3: When is the best time during neuronal development to introduce non-sodium channel inhibiting antiepileptic drug treatments (AEDs) to optimally alleviate the phenotypes of both ASD and epilepsy?

Rationale: The goal of this aim is to understand if there is a critical period of neuronal development in which non-sodium channel inhibiting antiepileptic drug treatments (AEDs) should be administered. This is a gap that should be addressed to understand how AEDs can work to optimally reduce the phenotypes of both ASD and epilepsy. The co-occurrence of both SCN2A-related disorders and their phenotypes have been studied in previous research. Phenotypes of both disorders as they occur together are understood as seizures due to gain-of-function (GoF) and intellectual disabilities, motor delay and verbal delay caused by loss-of-function (LoF) (Sanders et al., 2018). AEDs have proven to be the most effective course of treatment for individuals that experience both ASD/ID and childhood onset epilepsy (Sanders et al., 2018). Previous research on treatments for SCN2A-related disorders demonstrates that non-sodium channel inhibiting AEDs such as levetiracetam (LEV), benzodiazepines (BZDs), and valproate produced the most promising results in children aged one or older to induce seizure reduction (Sanders et al., 2018). Age is a risk factor for the development of epilepsy in children with ASD (Stafstrom et al., 2015). Previous studies have assessed the effects of these three AEDs in infants less than 3 months of age and greater than 3 months of age (Wolff et al., 2017). Previous studies have mentioned that children with SCN2A-related epilepsy are known to present seizures as early as in utero, during the neonatal period, and individuals experience onset around 3 months of age (Sanders et al., 2018). The same study states that children with SCN2A-related ASD/ID do not typically show symptoms until about 6 months of age. Therefore, providing early intervention with AEDs and determining a critical developmental period may prove to be the most beneficial for treating the disorders. The plan is to study the effects of each of the three AED types at four different stages in SCN2A mutant transgenic mice. More research needs to be conducted on the treatment response in SCN2A-related disorders and the seizures that begin from birth till about 12 months. This study would provide more insight to the lack of research surrounding treatment efficacy and outcomes.

Methods: To understand the effects of levetiracetam (LEV), benzodiazepines (BZDs), and valproate on the co-occurrence of ASD and epilepsy, transgenic mice with the SCN2A mutation will be used to assess the efficacy of each AED at four different periods of development. The treated transgenic mice will be compared to matched untreated transgenic mice to provide insight on the phenotypes that are alleviated post-treatment. The transgenic mice population will be split in half where one half will express greater NaV1.2 activity (GoF), while the other group will express a decrease in NaV1.2 activity (LoF) to represent the two main different variants. The four main developmental periods would be 3 months of age, 6 months, 9 months, and 12 months after the transgenic mice express the SNC2A mutation. At each of these developmental periods, the transgenic mice will be administered either levetiracetam, benzodiazepines, or valproate. After drug administration to each of the groups, the mice would be in observation for the next 3 months to see how the drugs affect each group as they continue to develop. The treated mice would be observed alongside the untreated mice to observe the alleviation or advancement of phenotypes. Expression of phenotypes would be rated on a standardized rating report by experimenters. Statistical analysis will be performed using ANOVA and unpaired t-tests. Significance will be defined as a value less than 0.05.

Predictions: It is expected that the GoF SCN2A mutant transgenic mice group would respond best to BZDs, as they are the first choice of treatment for epilepsy (Rajadhyaksha et al., 2021). The LoF SCN2A mutant transgenic mice group should respond best to levetiracetam based on previous research conducted by Wolff et al., in 2019. Of all developmental periods, the GoF mice would be predicted to do well in the 3-6 month of age category, while the LoF mice would do better in the 9-12 month of age category. There would be an overall improvement in both groups when compared to the non-treatment group.

AIM 4: How effective are sodium channel inhibiting drugs on the epileptic effects caused by gain-of-function mutations and do those drugs bring the channel function back to wild type levels?

Rationale: The purpose of this aim is to see how sodium channel blocking drugs affect the gain-of-function mutation in the SCN2A gene and see if those blockers cause the mutated genes to function at a similar level to control mice. Since autistic behavioral symptoms are harder to measure in non-human models and are also associated with loss-of-function mutations, the primary behavioral effect of interest is that of epilepsy. However, using non-human models also allows researchers to look at the physical and chemical changes that occur in the brain in a way not allowed in human models. Since the focus of this aim is on sodium inhibiting drugs, it is important to note some commonly used sodium channel blockers. Such blockers include the drugs carbamazepine, phenytoin, and lamotrigine (Mason and Cummins, 2020). There are instances where sodium channel inhibitor

can be more effective 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 specific effects these medications show on the sodium channel itself. As such, this research will help fill in that gap using a mice model to show the difference in sodium channel function of the mutated mice before and after the application of the three commonly used sodium channel blockers. There are two specific gain-of-function mutations commonly seen in the NaV1.2 protein. These mutations are E1211K and I1473M which are both located in the pore loop (Ben-Shalom et al., 2017). A benefit of mice models is that they have a higher homology percentage to humans than other common animal models (Sanders et al., 2019). There is previous mouse model research on the NaV1.1 protein. The specific species of mice that were used were mice of the Flox heterozygous mouse model. In this research, they used immunochemistry tests to track the NaV1.1 protein (Cheah et al., 2019). Other previous research studying the performance of sodium channel blockers were performed by Kim et al. (2020).

Methods: To display how sodium channel blocking drugs affect the mutated SNC2A gene in mouse models, mice showing the gain-of-function mutations E1211K and I1473M will first be bred or acquired from other researchers. Variations will be made where there will be both a wild type copy of the gene as well as the two gain-of-function mutations. To make sure that the sample size is large enough to show significance in potential differences, there will be a sample size consisting of 30 mice for each experimental category. These categories will consist of the control mice, and the gain-of-function mice broken down into four categories. There will be one category for each sodium blocking drug (carbamazepine, phenytoin, and lamotrigine) and one category of mutated mice that will not be treated. To compare the sequenced genome data between the mutated and non-mutated mice, a MiSeq Sequence will be used. To ensure that environmental differences are not significant factors, the mice will be kept under the same temperatures, and food and light cycles will remain constant throughout the entire experiment. All mice will be around the same age to mitigate age differences affecting results. To ensure this, all mice will be bred in the same duration. In previous studies, all mice were left in rooms for a 20 min period before any behavioral tests were done so that the mice would be able to get assimilated in the testing room (Mantegazza, 2019). This will be similarly followed to allow for the behavioral effects not to be influenced due to the mice adjusting to the testing area through consideration of controls. The following behavior tests will be conducted: a memory test, an anxiety test, and a measurement of any epileptic episodes that occur during the testing period. The behavioral tests will be done daily over a period of three months before the drug treatment will be added. Blood levels will be collected before the drug treatment, as well as after. There will be another three months of behavioral study during which the drug treatments are applied, then all the mice will be euthanized and the brain dissected to look for any damage present in the non-treated mutant mice. Statistical analysis will be performed using ANOVA and unpaired t-tests. Significance will be defined as a value less than 0.05.

Predictions: It is predicted that the mice will show a negative correlation in epileptic and ASD behaviors with the addition of a sodium blocker at the NaV1.2 pathway. Efficacy of the drug would potentially have an impact on not just the structure of the SNC2A gene but the four homologous domains as well which could assist in identifying exactly how the mutations react to the sodium channel blocker. The mice would show signs of improvement through the various behavioral tests that will be conducted and have less seizures. It is unknown which drug treatment will have the best effect, but that will also be measured. It is also predicted that the brains of the mice that were subjected to a gain-of-function mutation but never treated would have more damage to the brain.

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