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SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

A STUDY OF SEIZURE SUSCEPTIBILITY IN BRAIN SPECIFIC PERK KNOCK OUT CRE-RECOMBINASE MICE

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ABSTRACT

PERK is known to play key regulatory roles on endoplasmic reticulum homeostasis and it is essential for the normal function of secretary cells in the pancreas and skeletal system. A tissue specific, PERK knock out mouse model has an ablation of PERK specific to the brain (BrPERK-KO). These mice were initially observed having random seizures, those not previously observed in mice in our laboratory. Seizure susceptibility tests were conducted between Wild type, BrPERK-KO, and Lox-P strain mice and the results were analyzed. WT and Lox-P strain mouse models showed similar levels of PERK expression that were greater than the BrPERK-KO strain. The mice without PERK in the forebrain show a trend towards a shorter latency to seizure development than their counterparts with normal PERK expression in the forebrain. Due to the similarity of pancreatic beta cells and neuronal cells and PERK's known role in regulating calcium dynamics in the pancreas, we hypothesize that PERK may play a role in neuronal calcium dynamics as well.

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I would like to principally thank Dr. Cavener for allowing me to work in his laboratory for the past four years. Through this experience, I have been given many "perks" that have allowed me to study many different pathways and diseases, and I have gained an immeasurable understanding of and appreciation for the research field. I have also gained many friendships through shared excitement at the success, or shared disappointment at the failure, of countless experiments.

I would also like to thank Siying Zhu, a graduate student in our laboratory, with whom I worked closely with. All of my work was done with the assistance and guidance of Siying and none of this would have been possible without her help.

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Introduction

Epilepsy is defined as a state of persistently increased neuronal excitability that manifests as sporadic seizure generation (DeLorezno 2005). Epilepsy, often called a seizure disorder, is diagnosed by reoccurrences of the seizure phenotype. Epilepsy is defined as at least two unprovoked (no identifiable cause) seizures that occur more than 24 hours apart (Rho 2010). Estimates place the incident rate of epilepsy at over 2 percent of the population and less than fifty percent of the cases can be cured through medication and treatment (Duncan 2006).

Epilepsy and epileptic syndromes are classified as either idiopathic or acquired (DeLorenzo 2005). Idiopathic epilepsy is caused by inherited genetic mutations and other brain morphologies whereas acquired epilepsy is the result of an injury or other defect in the brain. Both forms of epilepsy have disruptions in neuronal homeostasis due to disruptions in normal neuronal plasticity and functioning (Rho 2010). The brain's primary function is transmitting electrical impulses. When normal brain function is compromised, brain seizures occur.

Epilepsy and Seizure Activity

A seizure is defined as a temporary disruption in normal brain functioning due to excessive and abnormal discharge of cortical neurons (Rho 2010). Seizures can be considered symptomatic, when they are linked to identifiable diseases or brain abnormalities, or cryptogenic, when no cause for the seizures is determined. The abnormal functioning or activity of neurons in the brain typically shares two common features during the generation of a seizure; hyperexcitability and synchrony (Somjen 2004). Hyperexcitability is the abnormal responsiveness of neurons to excitatory input that leads to excessive neuronal firing and bursts of multiple action potentials (Rho 2010). Synchronization of neuronal responses is critical to brain

functioning and allows for coordinated activity. Hypersynchronous electrical discharges from a group or groups of nerve cells in the cerebral cortex of the brain result in abnormal bursts of action potentials. These action potentials lead to the phenotypic jerkiness and loss of bodily control associated with seizures.

Action potentials are basic chemical and electrical signals that are regulated by ion channels in biological membranes and are fundamental to neurotransmission in the central nervous system (CNS) (Tatum et al. 2009). During an action potential the ion channel gate opens to allow an influx of positively charged ions into the cell, which results in a depolarization and a transmission of current through the cell. Depending on the receptor type, action potentials can be inhibitory or excitatory. Glutamate receptors are responsible for most excitatory action potentials in the nervous system (DeLorenzo 2005). One pathway of seizure development is due to excessive excitation of the receptors to lead to hyperexcitability and abnormal firing in neuronal populations. Overstimulation of these cells leads to an accumulation of intracellular calcium levels, which lead to continual stimulation and ultimately cell death. Calcium is essential in the generation of action potentials in synaptic transmission and is a key secondary messenger to glutamate excitotoxicity. During a seizure, calcium levels outside of the cell decrease as it enters the cell where the endoplasmic reticulum (ER) soaks up calcium like a sponge (Somjen 2004). The ER is a key regulator of calcium levels in neurons and keeps calcium levels in homeostasis; however when this system is disrupted or constantly undergoing stress, it can induce abnormal functions like seizures and cell death.

Role of Protein Kinase PERK

PERK, a transmembrane protein kinase of the PEK family is located in the endoplasmic reticulum (ER) membrane and is linked to insulin processing and the stress response (Liang 2006). Protein kinases play an important role in protein, enzyme regulation, and protein

activation or deactivation. The protein kinase PERK is known to play many roles in protein folding and trafficking in the endoplasmic reticulum during times of stress.

PERK EIF2 is a protein kinase that primarily regulates protein trafficking and folding in the endoplasmic reticulum in response to endoplasmic reticulum (ER) stress. (Liang 2006). PERK is present in many tissues including the brain, pancreas, liver, and thymus. The global knockdown of PERK results in a phenotype similar to that exhibited by humans diagnosed with the recessively linked disorder known Wolcott- Rallison Syndrome (WRS) (Senee 2012). WRS is characterized by the early onset insulin-dependent diabetes, epiphyseal dysplasia, and growth retardation. PERK has interactions in a lot of key developmental regulated pathways, with many downstream effects on the unfolded protein response, calcium regulation, and ER stress (Wang 2013).

The endoplasmic reticulum is the major calcium storage unit in the cell (Teske 2011). It regulates the amount of calcium in the cell and contains many regulations on its sequestering and release in response to stress (Liang 2006). Disturbing the ER calcium levels can lead to a disruption in homeostasis and calls for the integrated stress response. PERK plays a fundamental role in the ER stress response and is required for normal ER function and when calcium levels are low (Wang 2013). ER stress can result in many diseases including diabetes, cancer, and neurodegenerative disorders (Teske 2011). PERK modulates calcium secretion from the ER in order to maintain homeostasis (Wang 2013). Thus, PERK plays a large role in the calcium balance in many cells and the ablation of PERK has many negative downstream affects in calcium regulation, cell functioning, and cell death. Recently, PERK has begun to be the subject of studies of the brain to try to determine or pinpoint its role in brain function and cell signaling.

Brain-specific Lox-P strain PERK Connection

The Cre-recombinase/LoxP model is widely used in order to excise certain genes from specific tissues (Schipani 2002). Mice containing Lox-P strain were mated to the brain-specific

Cre-recombinase (CamKII-Cre). Mice with floxed PERK contain two lox P sequences flanking PERK, the gene of interest, which will be targeted by Cre-recombinase when expressed together. When a mouse contains both the Cre-transgene and floxed PERK sites, PERK is excised, leaving lack of PERK expression in the targeted tissue. In this experiment, the transgene specifically targets PERK in the forebrain of mouse models (Trinh 2012).

Mice containing this brain specific knockdown of PERK were studied in a series of behavior experiments to observe its correlation to motor development and its downstream affects on other proteins and pathways (Trinh 2012). When PERK is knocked down, levels of eif2alpha and ATF4 expression decrease. Mice lacking PERK exhibited cognitive defects associated with many neurological disorders. They experienced decreased information processing capabilities, increased hyperactivity and decreased cognitive function. Consequently, research shows that PERK plays a major role in the normal functioning of the brain and was also found to decrease excitatory receptor transmission.

Protein Kinase PERK and Epileptic Triggers

Factors provoking or heightening susceptibility to epileptic seizures are complex and limit their predictability. Seizures may be triggered by various factors that alter normal cellular homeostasis. As noted earlier, the protein kinase PERK is known to play many roles in calcium dynamics and protein folding and trafficking in the endoplasmic reticulum during times of stress (Liang 2006). PERK is known primarily for its role in the pancreas and is highly expressed in beta cells, where it regulates insulin and calcium dynamics (Wang 2013). Beta cells and neurons share similar mechanisms of membrane depolarization as well as specific cell markers (Eberhard 2013). Pancreatic multipotent precursor cells can differentiate into neuroectoderm cells. This concludes common embryonic origin and similar gene expression between the two cell types. Thus, it would not be uncommon for the two cell types to share similar proteins and pathways.

PERK has many downstream targets during its unfolded protein response (UPR), one called calcineurin (Wang 2013). Calcineurin is a calcium dependent phosphatase that both regulates and is regulated by PERK. Calcineurin is primarily regulated by calcium and is highly expressed in the central nervous system (Motohiro 1999). Calcineurin has recently been discovered to play essential roles in the brain, regulating many neuronal processes like synaptic function and neurotransmitter release. Due to PERK's known function in calcium regulation and close interaction with calcineurin, we have hypothesized that PERK plays a role in neuronal calcium dynamics as well.

Initially, BrPERK-KO and Alzheimer carrying mice were frequently observed experiencing random and recurrent unprovoked seizures throughout the course of a day. In an attempt to explain the occurrence of such seizures, we conducted a series of experiments to determine the effects of a BrPERK-KO genotype on seizure susceptibility in mice. We induced seizures in our subjects using Pentylenetetrazol (PTZ), a common drug that leads to the development of seizures. The occurrence and duration of seizures was compared throughout the mouse strains in order to see if PERK plays a role in seizure susceptibility, calcium regulation and brain functioning that may have future implications in epilepsy research

Materials and Methods

BrPERK-KO Mice

Floxed PERK mice were generated as described by Zhang et al. (2002). Mice expressing the Cre recombinase transgene, CAMKII-Cre, were used from the work of Trinh et al. (2012). All mice used were on similar B6 background. Mice with the Cre-transgene were crossed with floxed PERK mice in order to create the experimental mouse models (Trinh, et al. 2012). The F1 generation expressing both the Cre recombinase and the floxed p sites were then backcrossed to mice containing lox-p sites in order to create mice expressing both systems. Mice expressing both systems have an elimination of PERK (BrPERK-KO) and mice not expressing the Cre-transgene do not have an elimination of PERK (Lox-p strain). BrPERK-KO mice contain Lox-P strain that targets the floxed PERK sites and eliminates the expression of PERK from the forebrain. Mice lacking the Lox-P strain do not eliminate PERK expression at the lox-p sites, and therefore, PERK expression levels should not be affected. The presence of the conditional PERK allele and Lox-P strain was determined using PCR-specific primers. The presence of PERK was also determined via PCR previously by Trinh et al. (2012).

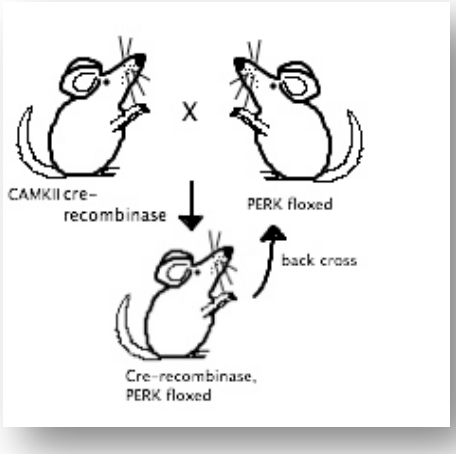

Figure 1. Generation of P2-3/2-3 Cre mice. The specific mating strategy used in order to generate the experimental mouse strain.

Real Time Polymerase Chain Reaction

cDNA was prepared from the hippocampus of WT, BrPERK-KO, and Lox-P strain mice. Supermix was made following basic protocol for Quantum Supermix. The protocol per sample is 12.5 µl of Supermix, 2 µl of primer with a 400nm concentration, and 5.5 µl of water. Each individual well contained 20 µl of the super mix, and 5 µl of each cDNA sample to make a total of 25 µl in each well. Each sample was run with mPERK primer as well as GADPOH as a reference sample. Water was used as the negative control for both sets of primers.

The samples were processed by StepOne Software. SYBR Green detection was used to quantify the amplification. The amplification of GADPH in each cDNA sample was used to normalize the data for the test gene. The CT values were then extracted and quantified for comparison between the genotypes. Mean and standard error were calculated in order to conduct a t-test on the values to determine statistical significance.

Western Blot

Western blots were performed using previous procedures described by Banko et al. (2005). Tissues were taken from the forebrain of similarly aged mice.

Pentylenetetrazol seizure testing

Pentylenetetrazol, PTZ, (Sigma-Aldrich Corp., St. Louis, MO, USA) solution was made by dissolving PTZ in sterile saline. This was done specifically for each experimental dosage used. Each experimental dosage used was administered to mice of the same age. The mice were weighed and then injected subcutaneously with a calculated, weight-specific PTZ solution and immediately placed in a clean roofless cage under direct observation and overhead video recording for 30 minutes. Trials containing BrPERK-KO and Lox-P mice were conducted using the PTZ dosages of 42.5 mg/kg body weight and 50mg/kg body weight. Trials containing BrPERK-KO mice and WT B6 mice were also conducted using the PTZ dosages of 35mg/kg, 65 mg/kg, and 50mg/kg. The experimenter was blind to genotype at the time of the experiment and during the review of the video for analysis. Latency to onset of the first determinable seizure was

recorded in seconds. Seizure severity was determined for all mice at all five stages. The duration of generalized seizures was also recorded in seconds as the length of time the mouse experiences a stage 4 seizure. Duration extended into stage 5 seizures when necessary, as stage 5 results from stage 4. Seizure severity was determined based on the following scale.

Stage	Seizure type	Visual Description
1	Absence	Inability to move, stuck in an uncomfortable position, absence of much movement
2	Partial	Slight, short twitching of one limb or part of body at a time
3	Myoclonic	Straub tail, slight body jerks, overall shakiness
4	Generalized Clonic	Severe loss of control, total body spasms lasting longer than 5 seconds
5	Clonic-Tonic	Clonus seizure into forelimb and hind limb extension, rigidity. Can result in death

Table 1: Seizure Rating Scale. This was used to determine seizure severity in each mouse strain after PTZ injection (Harai181).

Statistical analysis

Each seizure level reached per mouse in each category was represented as a ratio of the total number of mice in the same experiment with the same genotype. These values were compared for any notable differences. Fischer's exact test was conducted in order to determine significance. 2-tailed t-tests were conducted on all quantitative data, including duration of seizure and latency to seizure onset. Standard mean of duration of seizure and latency to onset was tabulated for each set of mice. Standard error was also calculated for each group.

Results

Cre-mediated deletion of PERK shows diminished PERK expression levels in the forebrain

To evaluate the role of PERK in seizure susceptibility, we generated mice containing CAMKII**α**-Cre transgene specific to the forebrain to eliminate PERK (Trinh 677, Figure 2A). Western blot assays confirmed the efficiency of Cre-mediated deletion of PERK in the forebrain of Cre- Recombinase/Lox-P containing mice, and confirmed the presence of PERK in the forebrain of both WT and Cre lacking mouse strains (Figure 2B). Western blot analysis of tubulin-stained samples served as controls to the amount of protein loaded for each sample (Figure 2C).

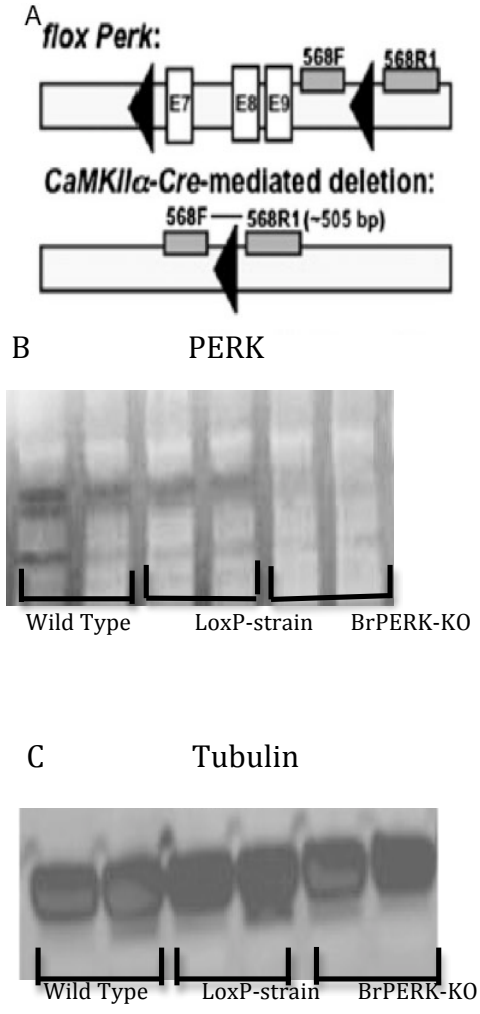

Figure 2. Lox-P Cre-mediated deletion of PERK eliminates PERK expression in the forebrain.

(A) The top region shows LoxP sites flanking exons 7- 9 of PERK and grey boxes represent primers used to detect recombination. The bottom region shows the elimination of PERK in the forebrain upon recombination with CAMKII**α**-Cre as seen in BrPERK-KO mice (Trinh 677).

(B) Western blot showing PERK in the forebrain in WT, LoxP-strain mice the disruption of PERK expression in BrPERK-KO mice.

(C) Western blot showing Tubulin expression levels as a control for each genotype.

In order to determine the efficiency of the Cre-Recombinase/Lox-P system, PCR analysis was performed on tissue from the hippocampus for quantitative analysis of PERK expression levels in each mouse strain. It showed significant differences between Wild Type and BrPERK-KO mice. Lox-P strain mice and BrPERK-KO mice were also compared in order to make sure that the Lox-P sites had no affect of PERK expression level. These two strains also showed significant difference in PERK expression levels (Figure 2). Mouse lacking the Cre-transgene (LoxP strain) showed no significant difference in expression level when compared to WT mice, as expected.

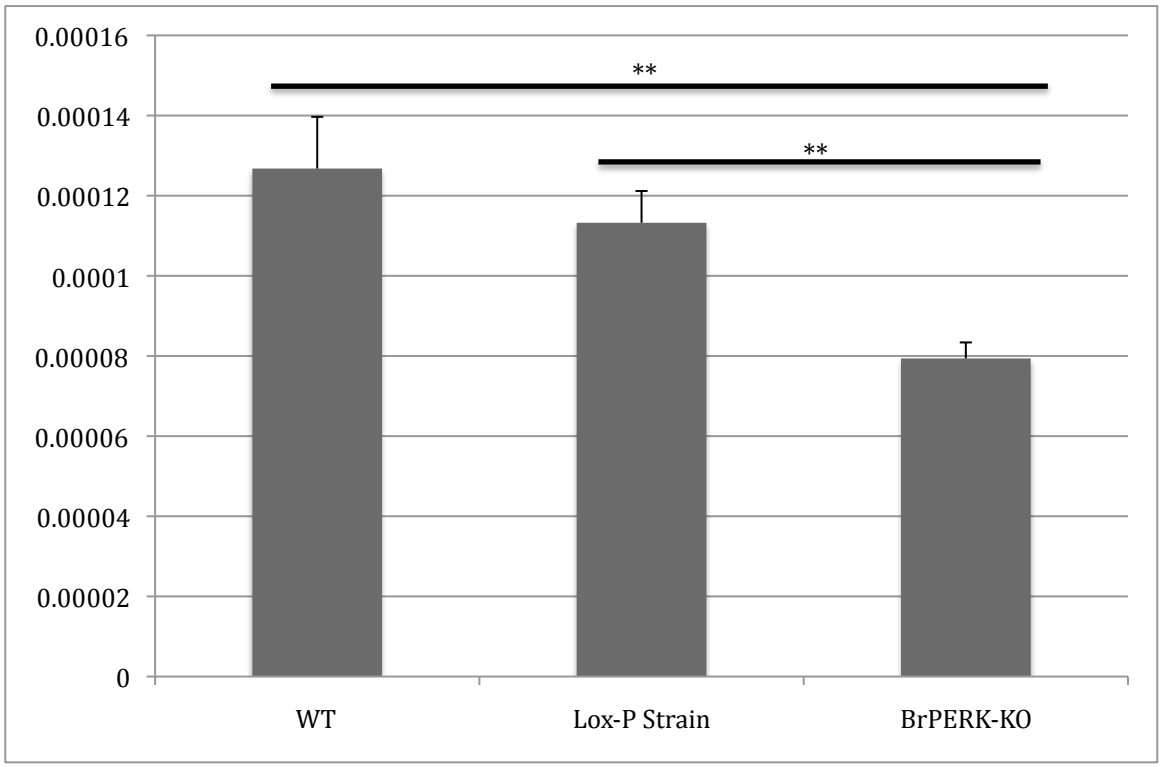

Figure 3. PERK expression levels in the hippocampus are reduced significantly in Cre-expressing mice (BrPERK-KO). Cre-mediated deletion of PERK expression levels in the hippocampus of BrPERK-KO mice is significantly lower than levels in the hippocampus of Lox-P strain and WT mice $p < 0.01$ (**).

Impact of varying PTZ dosages on seizure development

Stage 1 through 3 seizures, are the most difficult to identify. Stage 1 seizures, or absence seizures, are characterized by a paralysis state, or lack of movement. Stage 2 seizures are called partial, and occur when one limb or body part experiences uncontrollable jerks. Stage 3, or myoclonic seizure, can be seen by Straub tail, when the tail sticks straight up, or slight jerks of the entire body. We looked for doses to induce an even spread of Stage 4 into Stage 5 seizures. Stage 4 seizures, or generalized-clonic seizures, are the typical seizure phenotype seen in many epileptic models. Mice experiencing stage 4 seizures show no control over any limbs and have spasms that spread throughout the body. Stage 5 results from the prolonged generalized seizure, to result in a tonic phase, or when the mouse experiences hind limb extension and rigidity. This can sometimes result in death if severe enough.

Doses of 35 and 65 mg/kg of body weight PTZ used in this experiment induced seizures in both WT and BrPERK-KO mice in a dose-dependent manner. Doses of 35 mg/kg did not induce seizures past stage 3, myoclonic (Figure 4). Almost all of the mice exhibited seizure stage 1, absence. Eight of eleven (73%) WT and eight of twelve (75%) BrPERK-KO mice exhibited partial seizures, and eight of twelve (75%) WT and eleven of twelve (92%) BrPERK-KO mice exhibited stage 3. Statistically the two genotypes showed no differences in seizure development at this dosage level. Since this dosage was too low, we decided to try a dosage of 65mg/kg. Mice injected with 65mg/kg PTZ reached all stages of seizure severity (Figure 5). Seven out of seven (100%) BrPERK-KO mice reached stage 5 seizures and six out of seven (86%) died. Eight out of nine (89%) WT mice experienced stage 5 seizures and four out of nine (44%) died. This dosage was too strong in order to produce appropriate control results. BrPERK-KO mice trended towards a higher percentage of death than WT mice. Fischer's exact test ($p=0.2$) for these differences showed no significance. Stage 4 and

5 seizure duration was 20.86 seconds for BrPERK-KO mice, and 18.556 seconds for WT mice (Figure 6). Two-tailed t-test yielded a non-significant p-value of 0.52. Latency to onset of seizure showed a non-significant trend (p-value= 0.67) towards BrPERK-KO mice (108.6 seconds) being shorter than WT (127.6 seconds). (Figure 7).

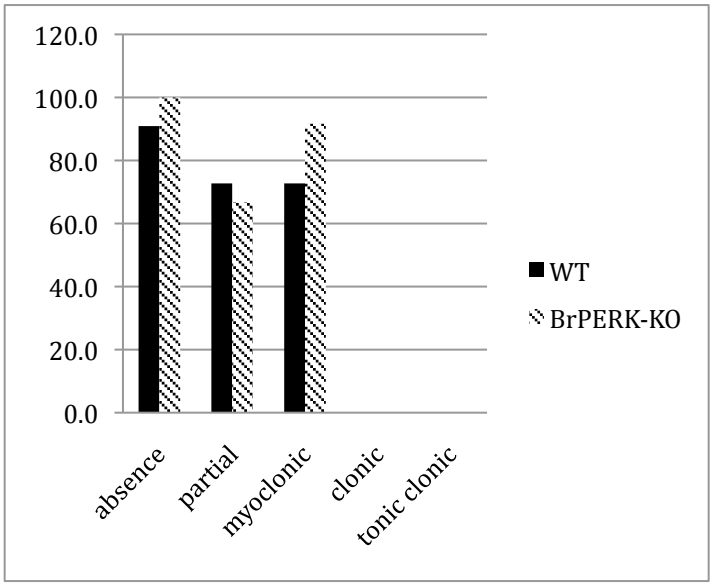

Figure 4. 35 mg/kg PTZ seizure distribution in WT and BrPERK-KO mice. This figure shows the percentage of mice exhibiting each seizure phenotype.

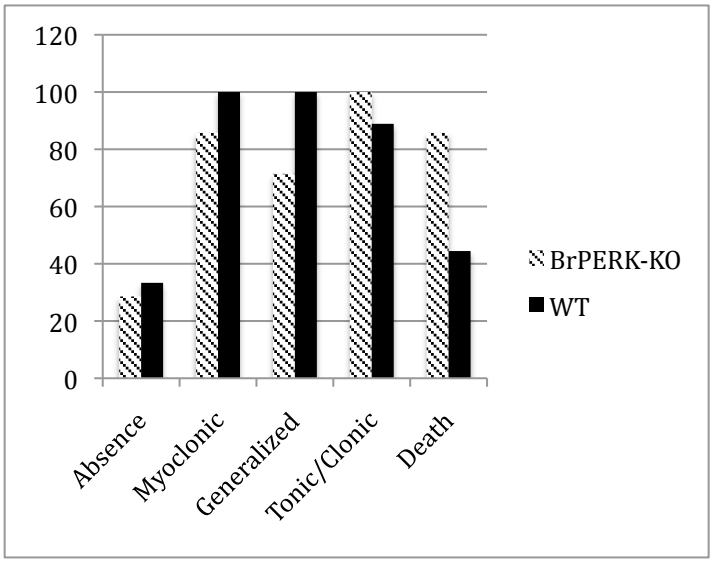

Figure 5. 65 mg/kg PTZ seizure distribution in WT and BrPERK-KO mice. This figure shows the percentage of mice exhibiting each seizure phenotype.

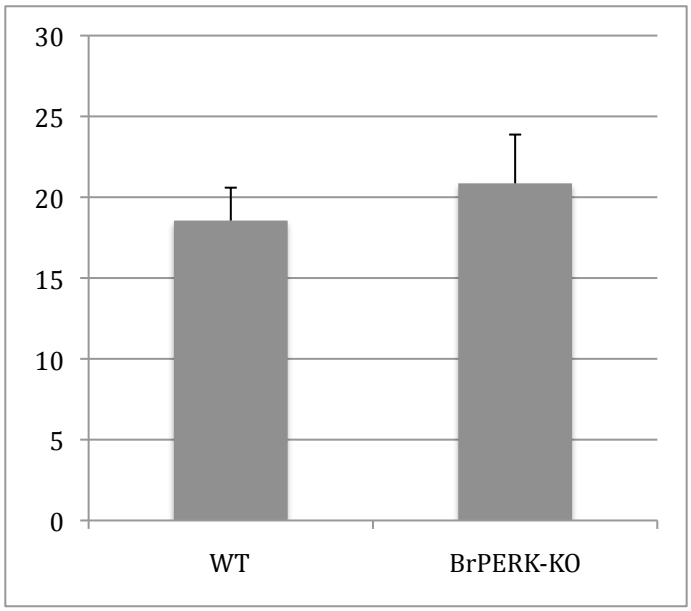

Figure 6. Average duration of stage 4-5 seizures following 65 mg/kg PTZ injection. BrPERK-KO mice show a trend towards longer average seizure duration than WT. No statistical significance.

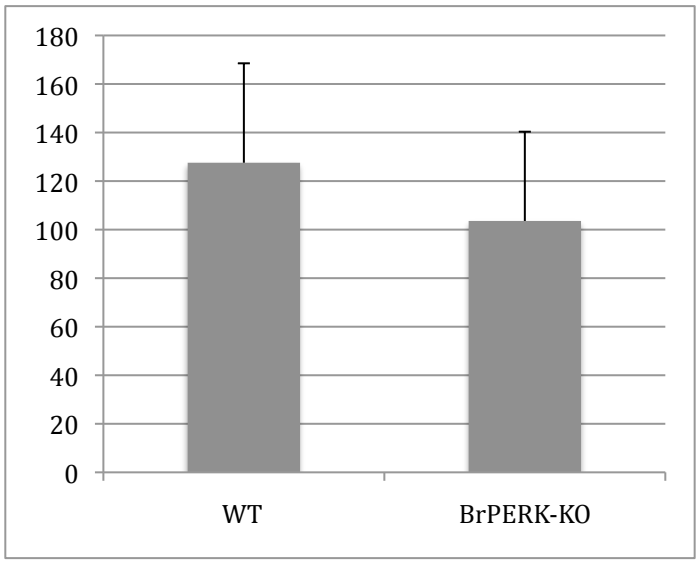

Figure 7. Average latency to stage 4 seizure following 65mg/kg PTZ injection. BrPERK-KO mice show a trend towards shorter latency to onset than WT counterparts. No statistical significance.

Optimal PTZ dosages and notable trends

Due to the possibility of the Lox-p sites playing a role in seizure susceptibility, the Lox-P mouse strain was added as a control to compare to the BrPERK-KO mice. Doses of 42.5mg/kg

PTZ injections were administered to Lox-P strain and BrPERK-KO mice, and 50mg/kg PTZ injections were administered to Lox-P strain, BrPERK-KO, and WT mice. These two dosages of PTZ showed a more comparable range of seizure phenotypes in all three strains of mice. Fifty mg/kg injection showed no significant difference in seizure severity among the three groups of mice (Figure 8). Three out of five (60%) WT mice developed stage 4 seizures, where as eleven out of twelve (92%) BrPERK-KO and eight out of nine (89%) Lox-P strain mice developed stage 4 seizures. Due to population size this difference is not significant. Doses of 42.5 mg/kg showed similar results between Lox-P strain and BrPERK-KO mice (Figure 9). Only two out of nine (22%) BrPERK-KO mice exhibited stage 4 and 5 seizures and only one out of nine (11%) Lox-P strain mice exhibited stage 4 and 5 seizures. Neither difference was significant. Due to the non-significant difference in seizure susceptibility between Lox-P strain mice and WT, Lox-P sites most likely do not play a role in seizure susceptibility.

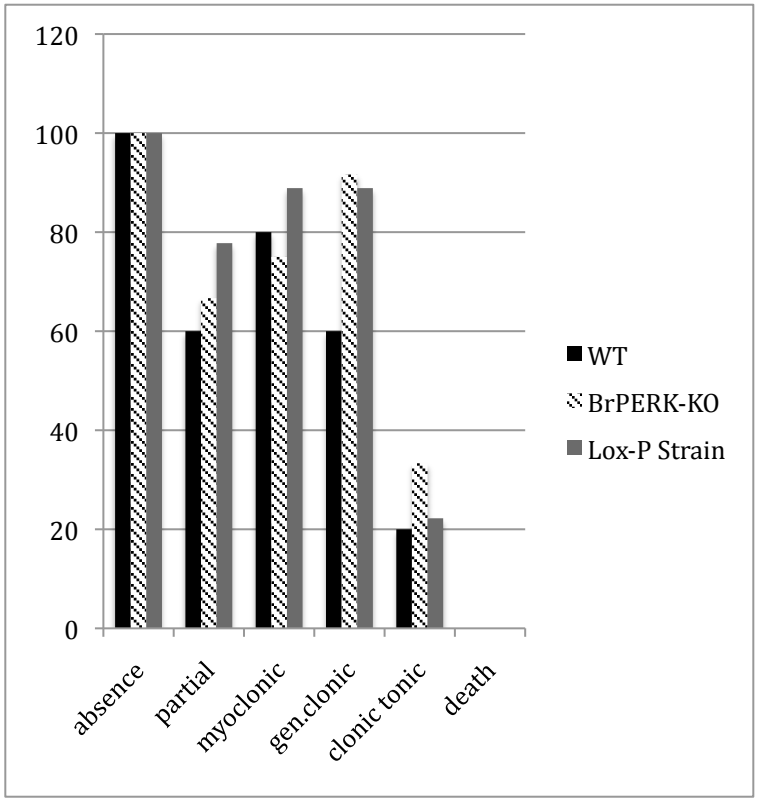

Figure 8. 50mg/kg PTZ injection seizure distribution. This figure shows the percentage of mice exhibiting each seizure phenotype.

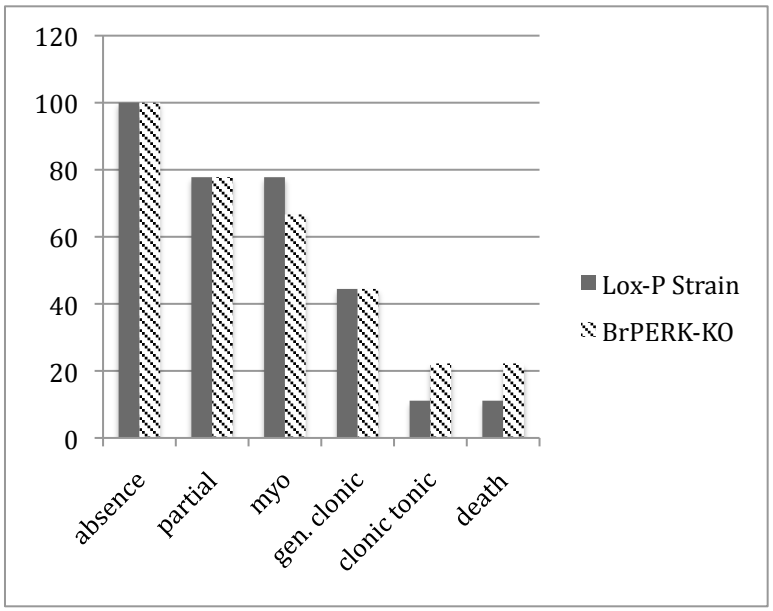

Figure 9. 42.5 mg/kg PTZ injection seizure distribution.

Average latency to seizure onset and duration of seizure were calculated for both Lox-P strain and BrPERK-KO mice in each group. Due to the small sample size of the WT littermates and small percentage that reached stage 4, Lox-P strain and BrPERK-KO were the only 2 strains analyzed for duration and latency in the 50mg/kg injection sample of mice. Seizure duration did not differ between the two groups under either PTZ treatment (Figure 10). Average latency to seizure showed significant trends in each experimental group. In the 42.5 mg/kg group, BrPERK-KO mice had an average latency of 212.2 seconds and Lox-P strain mice had a latency of 278.26 seconds (Figure 11). However despite the trend, these differences were not statistically significant (P-value=0.205). In the 50 mg/kg group, BrPERK-KO mice had an average latency of 137.4 seconds while Lox-P strain mice had a latency of 201.125 (Figure 12). These results did not exhibit statistical significance (P-value=0.09). However, the notable trend may be due to the elimination of PERK in the forebrain of BrPERK-KO mice or due to the Cre-recombinase

system. Due to the differences in results, it most likely is not a result of the Lox-P site, since both strains have it. Finally, an inverse relationship between latency to onset was confirmed when latency to onset of type 4 seizures consistently decreased with increases in PTZ dosage in all mouse models. It is most easily seen in reviewing the large population of BrPERK-KO mice (Figure 13). This adds credibility to the measurement of latency as a parameter of comparison.

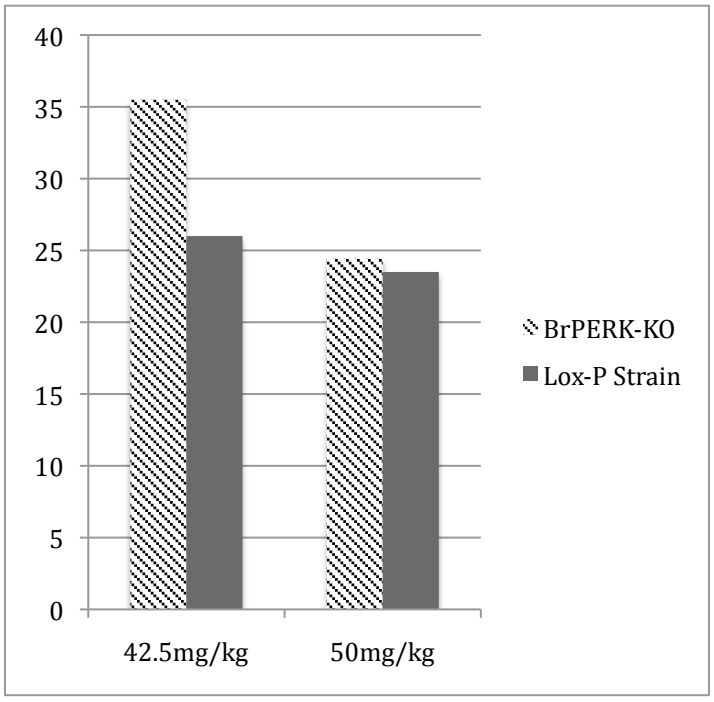

Figure 10. Average seizure duration for Lox-P strain and BrPERK-KO at different dosages of PTZ injection. This figure compares the duration of stage 4 seizures in each mouse strain at differing PTZ dosages.

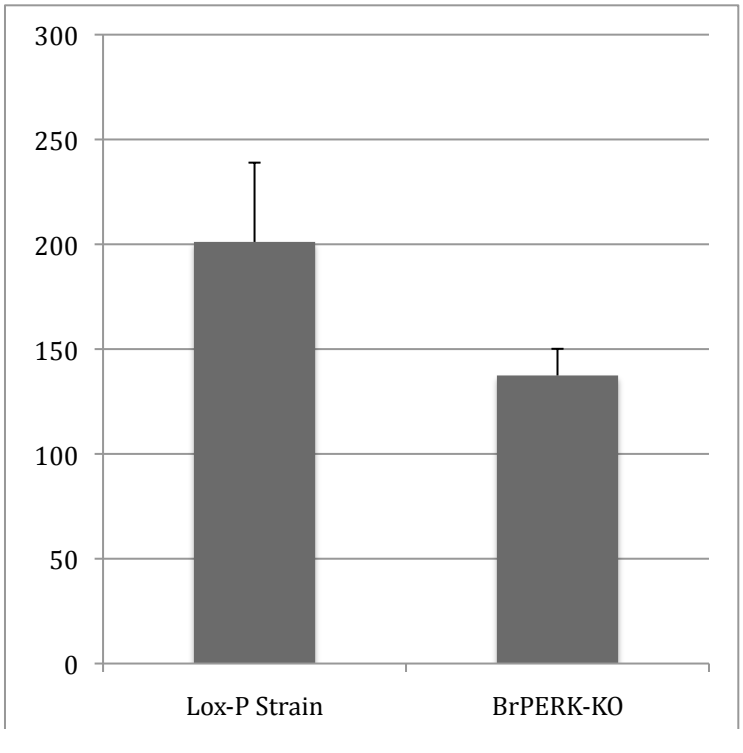

Figure 11. Latency to seizure development (seconds) after 42.5 mg/kg PTZ injection. This graph shows differences between latency to stage 4-seizure development between both Lox-P strain and

BrPERK-KO mouse models (p-value = 0.205).

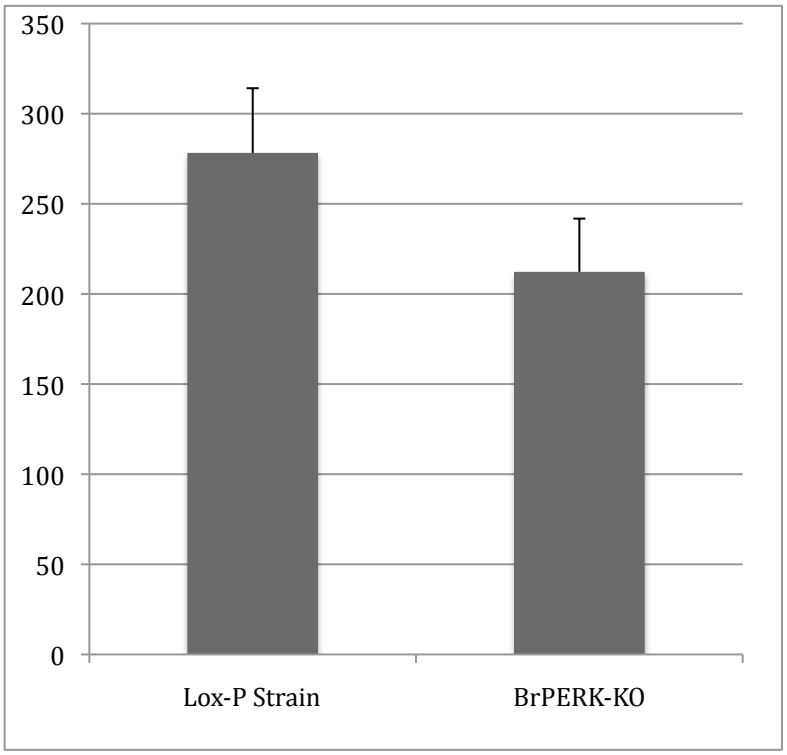

Figure 12. Latency to seizure development (seconds) after 50mg/kg PTZ injection. This graph shows the differences between latency to stage 4-seizure development between both Lox-P strain and BrPERK-KO mouse models (p-value = 0.09).

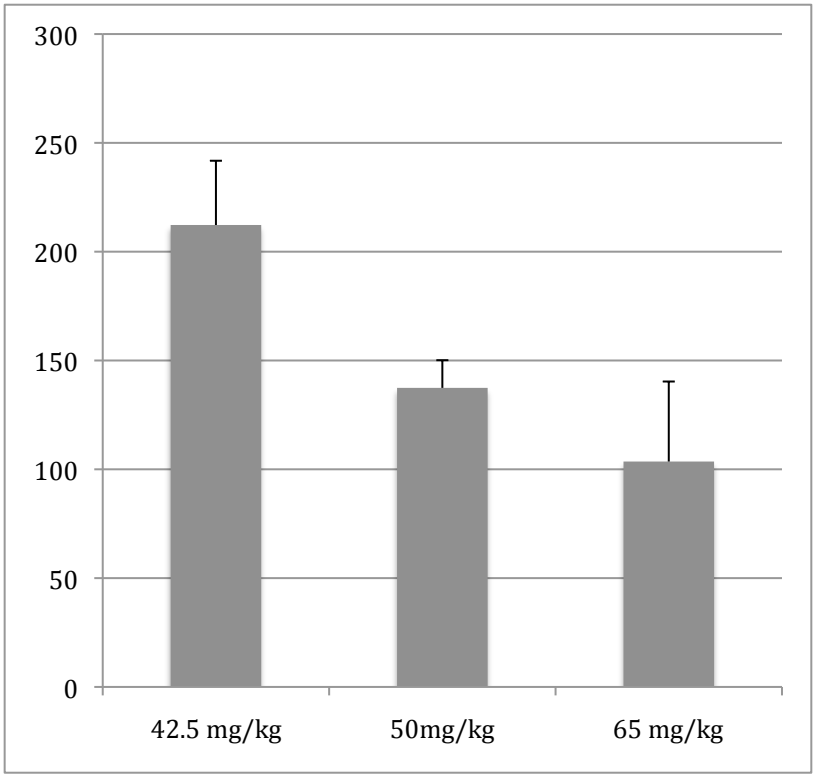

Figure 13. Latency to stage 4 –seizure with increasing PTZ dosage in BrPERK-KO mice. This graph shows a decrease in latency as the PTZ dosage increases in BrPERK-KO mice.

Discussion

A Br-PERK knockout mouse is generated by crossing a Floxed PERK mouse with a Cre-recombinase mouse. Lox-P sites flank PERK and when in combination with Cre-recombinase eliminate PERK from the forebrain. Both the Cre-transgene and Lox-P sites have to be studied separately in order to make sure that neither one of these separate elements is effecting seizure susceptibility. In order to make sure that PERK was effectively reduced in the BrPERK-KO strain in comparison to the Lox-P strain, PERK expression levels were compared. The similar expression of PERK in the hippocampus of both WT and Lox-P strain P2-3/2-3 mice confirms the presence of PERK in the Lox-P strain recombinase strain. Thus, Lox-P strain can serve as a good control for PERK expression against BrPERK-KO mice. However, neither WT or Lox-P strain mice serve as a control against the Cre-recombinase, thus future studies must look into the seizure susceptibility of mice expressing the Cre-transgene alone. Western blot analysis also shows a similar PERK expression level in both Lox-P strain and WT mouse lines with a significant reduction in the BrPERK-KO knock out mice, as expected.

It was necessary to work out the dosage of the PTZ in order to see a clear reproducible affect without being too adverse. Initial PTZ seizure testing showed little seizure activity and no difference between the treatment and control groups when a 35mg/kg dosage was used. This dosage level was deemed too low to produce visible results. Conversely, a dosage level of 65 mg/kg proved too high, producing a large percentage of both WT and BrPERK-KO mice reaching the highest seizure stage and ultimately death. The results from these trials provided guidelines for establishing a more suitable dosage of PTZ to induce a measureable nonlethal seizure severity in each mouse strain. Due to the mid range development of stage 4 seizures induced in WT and Lox-P strain mice during 50mg/kg dosage as well as 42.5mg/kg dosage, these are both closer to a recommended dosage for accurate PTZ testing. Stage 4 seizures are the most

easily identified form of seizure, due to explicit descriptions associated with its development. The development of stage 4 seizures is essential when evaluating seizure susceptibility in mice.

Mice expressing Cre-recombinase systems of genetically knocking out genes similar to those used in our experiment have showed some increase in seizure susceptibility compared to WT in other published research (Kim 2012). In our experiment, BrPERK-KO trended towards a slightly greater seizure susceptibility, longer seizure duration, and shorter latency to seizure development than their WT counterparts. Also, during this experiment, WT mice had half as many mice die as their BrPERK-KO counterparts. These findings support the findings by Kim, et al. (2012), who found that two other Cre strains of mice, Emx-1 Cre and Slx5/6-Cre have been proven to have a shorter latency to seizure development than WT littermates (Kim 2012). However, in our case seizure susceptibility could also be due to the lack of PERK expression in the forebrain. Due to the possible implications these findings could have on research involving animal strains genetically altered using the Cre-Recombinase/LoxP system, caution needs to be taken when using these strains for research in the neuroscience and brain function. Lox-P sites may also result in a toxicity that may be playing a role in the seizure severity of BrPERK-KO mice.

Looking at the different genetic components that allowed us to generate the PERK knockout will allow us to see if they independently affect seizure susceptibility. In order to account for possible interactions of the Lox-P site that effect seizure susceptibility, Br-PERK KO mice were compared to control counterparts lacking the Cre-transgene, but expressing LoxP sites. BrPERK-KO mice did not show a statistically significant difference in seizure susceptibility when compared with their Lox-P strain or WT counterparts after a PTZ injection of 50mg/kg. However, the BrPERK-KO mice consistently show a shorter latency to seizure development than their Lox-P strain littermates. Latency was also shown to progressively increase as PTZ dosage

decreased in all strains of mice, making it a reliable parameter. This difference is not a result of unsuccessful recombination or elimination of PERK because Lox-P strain mice show PERK expression levels similar to their WT counterparts. PERK expression levels are significantly reduced in BrPERK-KO mice and lack of PERK could be playing a key role in the shortened latency to seizure. There was also no significance between mice containing the loxP site (Lox-P strain and BrPERK-KO mouse strains) versus the WT counterparts, thus lox p must not be playing a role in seizure susceptibility. Therefore, PERK is another possible factor accounting for these differences. One parameter left unstudied in our experiment was the presence of the Cre-transgene by itself. Thus, in order to eliminate the possible interactions of Cre in seizure susceptibility, a strain expressing the Cre-transgene needs to be tested for seizure susceptibility against a WT control.

Preliminary results of Siying Zhu, a graduate student in our laboratory, suggest a role of PERK in the calcium dynamics of neuronal cell lines. Calcium plays a large role in the development of seizures through ionic currents and synaptic transmission (Eberhard 584). Thus, it is likely that impairment in the regulation of calcium could lead to a shorted latency to seizure development. PERK shares similarities to other protein kinases that are abundant in the brain and play role in synaptic plasticity and calcium regulation (Sierra-Paredes 96). One important modulator of inhibition in the brain is the protein receptor, GABA, which is regulated by calcineurin (97). Due to PERK's known interaction with calcineurin, PERK may be playing a role in regulating calcium in the brain and could be affecting the efficiency and conductance of receptors necessary for normal synaptic transmission.

Our results indicate the possibility that PERK may impact seizure susceptibility. However to confirm the preliminary trends noted in this experiment, further research with larger population strains and more controls need to be conducted. Due to lack of time necessary to

prepare more experimental groups and a limited access to a sufficient number of individual mice, more research should be conducted. The resulting sample size of the mice was relatively small. However due to other published studies and current work being done in our research lab, it is important to consider underlying issues in the calcium dynamics of mice lacking PERK in the brain. These findings could ultimately play a role in seizure development and epilepsy. Since calcium plays an extremely important role in synaptic transmission and signaling in the brain, further understanding of its regulation is essential for studying and possibly preventing diseases like epilepsy and epileptic syndromes. Further research needs to be conducted in this field in order to understand the role of PERK in the brain, how it relates to the development of seizures, and any possible implications these findings could have on new treatments for epilepsy.

Conclusion

In order to accurately study seizure susceptibility in mouse models, an optimal PTZ dosage was first determined. Due to trending differences in seizure susceptibility between WT mice and BrPERK-KO mice and current findings on the Cre-transgene, research on seizures and epilepsy should be held with caution when using a Lox-P/Cre-recombinase system until further research is conducted to identify the specific faults associated with this system. The only way to rule out the possible interactions of the Cre-transgene effecting seizure susceptibility, mice expressing only the Cre-transgene need to be tested for seizure susceptibility in order to provide an accurate control against WT mice. Due to the difference in latency to seizure between Lox-P strain and BrPERK-KO mice, further studies should be conducted on PERK in the brain in order to identify the role it may play in calcium dynamics, seizure generation, and epilepsy.

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