A Model for Understanding Epilepsy in *Peromyscus*

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Epilepsy is one of the most common human neurological disorders and can arise from any number of causes, including inherited brain lesions and genetically modified cell function. A mutant in the subspecies *Peromyscus maniculatus bairdii* (BW) displays a proclivity for audiogenic seizures initiated through the inferior colliculus at 105-110 dB and it is known that there is a single gene locus for this spontaneous mutation. Gene expression analysis of BW deer mice using amplified samples of RNA from the inferior colliculus can help to map the genome and identify microsatellite markers associated with this gene locus. Two rounds of RNA amplification using liver tissue and two rounds using tissue of the inferior colliculus yielded these ratios of final to initial mass, respectively, 175x, 95x, 1.4x, and 2.2x. RNA amplification is an effective procedure to increase RNA quantity derived from small masses of tissue and allows for later gene expression analysis. Additionally, manganese-enhanced MRI (MEMRI) may provide tissue contrast between BW and wild type *Peromyscus* to determine morphological difference in brain structure. Manganese is taken up semi-permanently in voltage-gated calcium channels during neuron activation. Therefore, activation of neurons after manganese injection can reveal the neuronal pathway of a seizure. Although initial analysis of scans comparing BW and wild type *Peromyscus* did not reveal any major morphological differences in brain structure, further examination by an expert in the field may yield interesting results.

Introduction

Epilepsy, also known as "seizure disorder", is a neurological condition characterized by recurring seizures and is a significant health problem in the United States and in South Carolina. It is estimated that 2.5 million Americans and between 55,000 to 65,000 South Carolinians have epilepsy. Three percent of the population will develop epilepsy by age 75 and one in every ten individuals will have a seizure in his or her lifetime[1]. Epilepsy can arise from either acquired brain changes or from an inherited susceptibility to cortical hyperexcitability, which may be a result of a single gene mutation or a result of a more complex polygenic and multifactorial pattern. At least 40-50% of epilepsies have a presumed genetic basis. Although few human epilepsy syndromes are inherited in a simple Mendelian manner, single gene animal models offer valuable opportunities to isolate gene mutations, to identify underlying molecular mechanisms and to explore strategies for therapy.

The human epilepsy genes identified to date represent widely diverse functions of cells rather than only those that regulate neuronal membrane excitability [2]. Animal models offer valuable opportunities to isolate gene mutations, to identify underlying molecular mechanisms and to explore strategies for therapy. Both monogenic and polygenic seizure disorders occur in mice[3], however the best studied rodent models of generalized epilepsy involve susceptibility to audiogenic seizures (AGS). After intense sound stimulation animals prone to AGS generally display wild running, clonus (rhythmic muscle spasms) and tonus (rigid muscle stiffness). Audiogenic seizures require activation of brainstem auditory pathways and are initiated through the midbrain inferior colliculus[4]. Unlike other epilepsy models, AGS represent a generalized seizure that is readily initiated by sound stimulation to produce easily quantifiable seizure components from video monitoring. In 1935 a spontaneous AGS mutation appeared among laboratory stocks of *Peromyscus maniculatus artemisiae* at the University of Michigan and has been maintained as a separate stock since, currently housed at the Peromyscus Genetic Stock Center at the University of South Carolina, denoted as 'epl'.

Mice of the genus *Peromyscus* provide a rare opportunity to combine laboratory studies with natural genetic variation found in present-day wild populations. Research on this genus has been widespread across so many disciplines that the genus has aptly been referred to as "The *Drosophila* of North American Mammalogy"[5, 6]. The genus *Peromyscus* contains the two most abundant native North American mammals (the deer mouse, *P. maniculatus*, and the white-footed mouse, *P. leucopus*) as well as one of the most endangered mammals in the United States (*P. polionotus trissyllepsis*)[7]. Members of the genus are found from Alaska to Central America and from the Atlantic to the Pacific. They occur in a wide range of habitats including sea-level wetlands and beaches, forests, prairies, deserts, and mountains of elevation up to 14,000 ft. Variation is seen in morphology, physiology, behavior, growth, coat-color, diet and habitat. Many of these differences exist within the *P. maniculatus* species complex, whose members exhibit sufficient inter-fertility to make genetic analysis possible. Though superficially resembling laboratory mice (*Mus domesticus*) and rats

(*Rattus norvegicus*), deer mice are not closely related to either species. Instead, *Mus* and *Rattus* share a much more recent common ancestor with each other than with *Peromyscus*. In phylogenetic terms *Peromyscus* not only aids researchers in understanding the *Mus/Rattus* lineage by serving as an outgroup, but also provides a species intermediate between the two major rodent genetic models and humans[8].

The mutation denoted 'epl' that occurred naturally in *Peromyscus maniculatus artemisiae* in 1935 has been maintained in the outbred *Peromyscus maniculatus bairdii* (BW) background by the Peromyscus Genetic Stock Center. This mutation exists as a homozygous recessive trait and only those animals receiving both 'epl' alleles are prone to AGS. The animals were tested for audiogenic seizures - as characterized by wild running followed by clonus and tonus - by jiggling keys as published[9]. In collaboration with Dr. Jay Coleman (Dept. of Psychology, University of South Carolina), an expert in audiogenic seizures (AGS), we have characterized the susceptibility of the 'epl' animals in a controlled manner using white noise at different intensities. Eight 'epl' animals and eight controls were placed in a sound proof chamber individually and were subjected to white noise starting at 60 dB. If the animals did not show signs of AGS within two minutes, the sound intensity was raised by 5 dB. All 'epl' animals had seizures between 105 and 110 dB, while the controls did not seize even at 120 dB. The low intensity of the required sound stimulus allowed to design a portable test system that can be used at the PGSC consisting of a CD player, a 200W 4 channel amplifier and 4 speakers each with a 100W output. The unit is calibrated to dB intensities. We speculated, that in contrast to other rodent AGS models, where the intensity of the initial sound stimulus is 120dB and higher and therefore the cochlea is severely damaged, the hearing of the *Peromyscus* 'epl' animals will not be impaired using 105 dB sound and the AGS stimulation can be repeated. We have induced AGS in six 'epl' animals six times in row with 48 hr intervals.

Gene expression analysis of *Peromyscus* involves the analysis of microsatellite markers associated with the 'epl' gene locus. It is known that there is a single gene locus for the expression of epilepsy in BW deer mice and the recently assembled genetic map of *Peromyscus* will allow us to map the epl locus. At the dawn of the human genetic map, Lander and Botstein proposed a mapping strategy of recessive traits, namely homozygosity mapping[10]. Such strategy has been successfully used to map a recessive trait in the American Quarter Horse[11]. Initially a large number of microsatellites are amplified from a few affected individuals and those markers that do not show significant difference from the expected heterozygousity values are excluded from subsequent studies. Next, the remaining candidate microsatellite markers are amplified from a larger number of affected animals as well as their healthy relatives. Markers that remain homozygous in all affected animals but heterozygous in the healthy population are most likely linked to the epl locus. Once these are identified, the homologous DNA sequence of rat, mouse and *Peromyscus* should reveal a short list of actual candidate genes responsible for AGS susceptibility. Direct sequencing of these from wild type and 'epl' animals will reveal the mutation responsible for the epileptic trait. Additionally, a mapping effort in collaboration with Rachel O'Neill (University of Connecticut) revealed differential expression of proteins in the inferior colliculus between wild type and 'epl.' Microarray analysis of isolated RNA was performed with 4 wild type and 4 epl / epl mice using a cross-species platform[12]. Some of these proteins are known to be associated with neuronal activity and real-time quantitative PCR will confirm their differential expression. Homogenization and purification of *Peromyscus* RNA, especially in the inferior colliculus, indicates that the initial quantity of tissue is of too low a mass to yield useful and plentiful samples of RNA. cDNA libraries of brain samples have been hindered by the relatively small sample size collected. However, reverse transcriptase synthesis of cDNA and subsequent transcription can provide RNA yields of up to 5,000 times the original sample and creates a model for the study of gene expression[13].

MRI imaging of wild type and epileptic *Peromyscus* deer mice allows for another avenue of exploration. It is known that the inferior colliculus is responsible for initiating audiogenic seizures, however it is not known whether any major morphological differences exist in brain structure between wild type and 'epl' mice. Therefore, MRI imaging provides a pathway to examine brain structures including but not limited to the inferior colliculus. As imaging *Peromyscus* is a relatively new endeavor and the MRI system used is normally designed for typical laboratory mice (*Musc domesticus*) and rats (*Rattus norvegicus*), MRI optimization has been a concern from the start. *Peromyscus* deer mice are of a much smaller size than typical lab rodents and initial imaging provided images with little resolution and poor contrast. Several revisions of protocol have been adopted, including the introduction of a small head coil to amplify the field of view and the use of a manganese contrast agent. Manganese-enhanced MRI (MEMRI) exploits the paramagnetic nature of the manganese ion (Mn^{2+}) as a contrast agent. Injection of manganese prior to scanning can increase contrast in certain brain structures up to forty-eight hours after treatment[14, 15]. Additionally, manganese readily passes through the blood brain barrier and uptake in the inferior colliculus is maximized at twenty-four hours. Mn^{2+} enters cells through voltage-gated calcium channels and is then retained in the intracellular compartment. Areas affected by manganese enhancement appear as a bright white color in contrast to the surrounding tissue in MRI

images. Since Mn^{2+} is taken up in calcium channels during neuron activation, it is thought that neurons activated after injection of manganese can be targeted as areas of interest.

Methods

For molecular and genetic characterization of the *Peromyscus* genome, samples of the liver and inferior colliculus of both ‘epl’ and wild type deer mice were analyzed. Initially, samples were obtained and homogenized. RNA was isolated using Ambion, Inc. RNAqueous Kit (Category # AM1912) and amplified using Ambion, Inc. MessageAmp II aRNA Amplification Kit (Category # AM1751). The kit utilizes a method of first creating cDNA using reverse transcriptase on collected RNA samples. Next, second strand synthesis on the cDNA creates a double-stranded DNA sample. Transcription using T7 RNA Polymerase creates the final product of amplified RNA (aRNA) with a yield of up to 5,000 fold from the original RNA samples. Two rounds of amplification were carried out using BW liver samples to judge the degree of amplification possible. Afterwards, a round of amplification was performed on an ‘epl’ sample of inferior colliculus as well as a wild type sample of inferior colliculus. Mass and concentration of final and initial samples were measured by ultraviolet-visible spectrophotometry. The integrity of amplified cDNA was confirmed by comparing RNA expression using a collagen protein primer between initial and amplified samples.

For MRI imaging, both wild type and ‘epl’ *Peromyscus* were injected with 100 µL of a manganese contrast agent (0.4% $MnCl2$ within a 4.2% glucose solution) subcutaneously into axillary adipose tissue. Over the course of several weeks, three deer mice homozygous for ‘epl’ and one wild type deer mice were treated. At both 8 and 22 hours after injection, mice received two minutes of sound stimulation at a calibrated 105 dB to activate neurons of the auditory pathway and also to induce the neuronal pathway of a seizure in ‘epl’ mice. At 24 hours after injection, the animals were anesthetized using a dilution of sevoflurane in oxygen and placed on their back in a Bruker Biospec 70/30 USR 7 Tesla Preclinical MRI System using a small head coil to maximize the field of view. T1-weighted, T2-weighted, and inversion recovery scans were run on each animal with differing values for echo and repetition times to maximize tissue contrast. Obtained images were examined for basic morphological differences of brain structure between ‘epl’ and wild type *Peromyscus*.

All methods and protocol are previously approved by IACUC.

Results

Tables 1-4 show the values for initial and final concentration and mass of amplified RNA. Final volume of all samples was 100 µL and the starting concentration for each was ~40 ng / µL. While the final mass for each increased significantly, the final concentration was only significantly increased in the liver samples (Tables 1 and 2).

Figure 1 shows the ratio of final to initial masses recorded through four rounds of amplification, two with liver and two with inferior colliculus. In general, the liver samples show a much higher ratio of amplification in mass. The liver samples were used to judge the efficacy of amplification and since both rounds were successful, amplification of both wild type and ‘epl’ inferior colliculus RNA was performed.

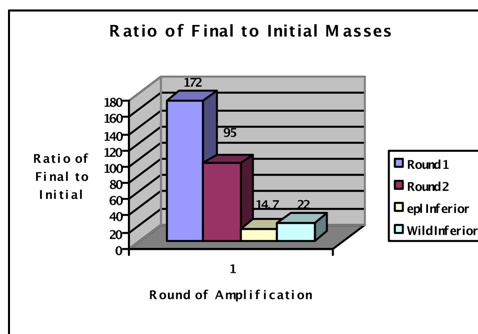

Figure 1. Ratio of the final to initial masses recorded in each amplification

	Volume (µL)	Concentration (ng / µL)	Mass (ug)
Initial	2	44	0.066
Final	100	152	15.2

Table 1. Amplification of ‘epl’ *Peromyscus* liver RNA samples (First Round)

	Volume (µL)	Concentration (ng / µL)	Mass (ug)
Initial	10	44	0.44
Final	100	420	42

Table 2. Amplification of ‘epl’ *Peromyscus* liver RNA samples (Second Round)

	Volume (µL)	Concentration (ng / µL)	Mass (ug)
Initial	10	48	0.48
Final	100	68	6.8

Table 3. Amplification of ‘epl’ *Peromyscus* inferior colliculus RNA samples

	Volume (µL)	Concentration (ng / µL)	Mass (ug)
Initial	10	40	0.4
Final	100	88	8.8

Table 4. Amplification of wild type *Peromyscus* inferior colliculus samples

Inferior colliculus numbers show much less amplification but are consistent with each other. The disparity between the first round liver ratio of mass is due to the fact that only 2 µL was used as initial volume in comparison with 10 µL for every other round. A relatively low concentration increase (3.4x) still accounted for a large mass increase (172x) due to a volume increase from 2 µL to 100 µL. MRI imaging of *Peromyscus* provides a unique opportunity to view the brain structure of *Peromyscus* mice. Only in the past few years has imaging with these animals taken place. Since good image quality is necessary for MRI studies, optimization of *Peromyscus* scans took several approaches. Compared to original scans, three major protocol changes were introduced. First, animals were placed on their backs instead of stomachs to reduce motion artifact from respiratory breathing. Second, a small head coil placed directly on the area of interest narrowed the field of view, but maximized resolution. Third, a manganese contrast agent was used to highlight brain structures of interest. Figure 2 taken in September of 2007, shows a *Peromyscus* scan with the animal anesthetized on its stomach and without manganese injection or the addition of the small head coil. Instead, the image is done using the bore provided with the magnet, ideal for larger laboratory mice and rats, but insufficient for *Peromyscus*. Figure 3, taken in February of 2010, introduces placing the animal on its back, a small head coil placed directly on the animal in the bore itself, and the manganese contrast agent. Both Figures 2 and 3 are of wild type *Peromyscus*. The inferior colliculus is pointed out by a red arrow. As can be seen, Figure 3 is of a much higher image quality and only the area of interest, namely the inferior colliculus, is highlighted, instead of a whole brain scan as in Figure 2,

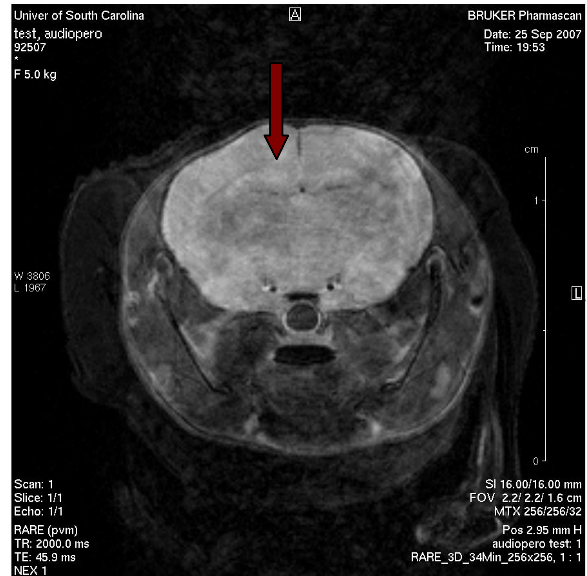

Figure 2. MRI of a non-treated wild type *Peromyscus*

which sacrifices resolution for a scan of the entire brain. There is no motion artifact due to breathing and the inferior colliculus can be seen in its entirety in Figure 3. Additionally, the manganese contrast agent appears to highlight certain areas, but it is indiscernible as to the exact extent of intensification.

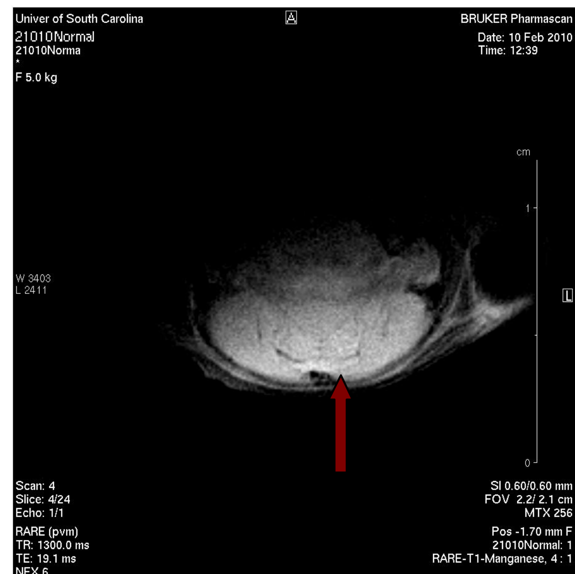

Figure 3. MRI of a treated wild type *Peromyscus*

Figure 4 is a scan also taken in February of 2010 of a treated ‘epl’ *Peromyscus*. The comparison of Figures 3 and 4 should reveal either morphological differences between brain structure and specifically the inferior colliculus, or at least the pathway of neuronal activation in ‘epl’ during a seizure.

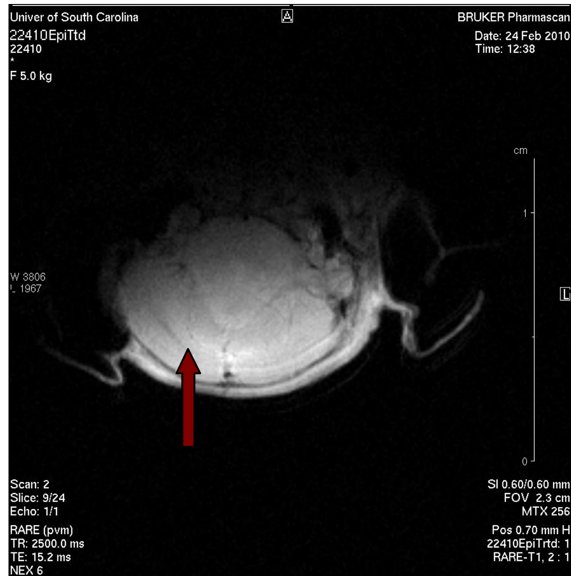

Figure 4. MRI image of a treated 'epl' *Peromyscus*

Comparison of Figures 3 and 4 does not show much in terms of morphological difference between 'epl' and wild type inferior colliculus structure. It can be noted that both images, however, show a bright white contrast at the junction of the right and left inferior colliculus that could possibly indicate manganese staining of the auditory pathway. Additionally, the contrast is much more evident and pronounced in Figure 4 of an 'epl.' Since optimization of images is crucial to MRI, the protocols established for *Peromyscus* are now in place to allow for acquiring images consistently of a good quality.

Repeated scans of both treated wild type and 'epl' and statistical analysis by an expert in the field will yield further results.

Discussion

Genetic characterization of the *Peromyscus* genome requires that sample sizes derived from tissue provide quantities of RNA large enough to use for gene expression analysis. Many times, and especially with tissue derived from the inferior colliculus, homogenized and purified RNA samples are of inadequate quantity. Therefore, in order to create useful samples, an amplification procedure must be adopted. The amplification process used can yield up to 5,000 times the initial input of RNA total mass and would be ideal for amplification use before gene analysis. However, the amplifications performed in lab display yields much less than that. For the 'epl' liver samples, the final yield was 172x for the first round and 95x for the second round. These numbers, although not the maximum yield, provide for a large quantity of RNA from small samples, from 88 ng to 15.2 ug for the first round and from 440 ng to 42 ug for the second round. With samples of inferior colliculus, the final yield was much smaller. 'epl' inferior colliculus showed an amplification of 14.2x, while the wild type samples showed an amplification of 22x. For the purposes of creating a large library of RNA samples for gene analysis, then, the samples of inferior colliculus amplified proved to be of little use. The initial tissue size of the inferior colliculus could be a major factor in this difference. Undiluted and purified liver samples of RNA originally displayed a concentration of 4.4 ug / µL. The same purification process used for inferior colliculus samples displayed concentrations of 48 ng / µL for 'epl' and 40 ng / µL for wild type mammals. These original concentrations of RNA yield from tissue sample represent close to a 100x higher yield in liver tissue. While examination of RNA samples from the inferior colliculus is necessary in continuing the characterization of epilepsy in *Peromyscus* deer mice, multiple amplifications or a more effective method of amplification may be required to produce quantities of inferior colliculus RNA for gene expression analysis.

Manganese-enhanced MRI has proven effective in analyzing brain structures as manganese may cross the blood brain barrier and can be noted in MRI scans up to 48 hours after injection. Additionally, the manganese ion (Mn^{2+}) has an ionic radius similar to that of the calcium ion and can readily enter cells through voltage-gated calcium channels and remain confined to the intracellular compartment. Activation of neurons after injection of a manganese contrast agent should stain these neurons for analysis under an MRI system. Both 'epl' and wild type animals were injected in the same manner and received the same sound treatment. Sound stimulation was given at intervals before scanning to stain the general auditory pathways in wild type mammals and use them as a comparison to the pathways activated during a seizure in 'epl' deer mice. Although several scans were performed on each animal and the quality of the images proved adequate, no discernible difference existed between brain structure or neuronal activation. It is possible that the manganese agent highlighted certain brain structures, however it would be necessary to consult an expert in the field to determine the statistical differences between images. Since MRI scanning of *Peromyscus* is relatively new, optimization techniques are finally in place to allow for repeated scans of high image quality and resolution.

Limitations in the use of manganese exist in its lethal capabilities and that it may be used as a substitute to calcium in voltage-gated calcium channels. While manganese may be administered safely at relatively low amounts (20 mg / kg), higher quantities will lead to successively more intense adverse effects in the treated animal and eventually in death. Additionally, it is believed

that the manganese ion may in certain circumstances block voltage-gated calcium channels and prevent their firing. Table 5 below displays whether or not a treated 'epl' mouse seized when given sound stimulation.

	8 hours	22 hours
Animal 1	no	yes
Animal 2	no	no
Animal 3	no	yes

Table 5. Occurrence of seizure in 'epl' mice after Mn^{2+} injection

For the three 'epl' deer mice treated in this experiment, not one seized when provided with sound stimulation of 105 dB eight hours after injection. Additionally, when the same procedures were initiated at twenty-two hours after injection, one of the three mice once again did not seize. The mutant subspecies in *Peromyscus maniculatus bairdii* (BW) has been maintained as a separate colony ever since it first displayed AGS at the University of Michigan in 1935. Therefore, all 'epl' treated contain the homozygotic recessive trait of epl / epl which makes them susceptible to AGS. At two minutes of 105 dB sound stimulation, 'epl' animals will seize. The manganese contrast, then, must be acting on the animal in a way to prevent the onset of a seizure. One possibility is that the manganese ion blocks or semi-permanently attaches to voltage-gated calcium channels, preventing the firing of excitable cells. While it is known that the inferior colliculus retains the manganese contrast agent at an optimal time of 24 hours, scanning before this time period may be helpful to map the actual pathway of the seizure. Since not one 'epl' seized 8 hours after injection, it can be assumed that manganese at that time is taken up in calcium channels and blocking neuronal activation. The existence of a seizure in two out of the three mice at 22 hours after injection indicates that manganese may no longer be present and blocking calcium channel activation. Therefore, further scanning to view the pathway of seizure should possibly occur from 8-10 hours after injection, as this time seems to be prime in terms of manganese affecting actual neuronal activation. For inferior colliculus and other brain structure analysis, however, 24 hours after injection is still ideal.

Due to the small size of the *Peromyscus* rodent, MRI scanning of the brain first yielded images of a quality and distinction not useful for research purposes. In order to increase resolution a small head coil was created which could fit around the animal during scans inside of the bore itself[16]. A small implantable coil made of chip capacitors is placed directly on the area of interest, while a volume coil placed on top of this one acts with the implantable coil to increase radiofrequency specificity. While the MRI system contains a coil built-in that was adequate for rat or mouse analysis, the small head coil created was necessary to amplify and minimize the field of view for the smaller *Peromyscus*. A tuning and matching instrument was used to adjust the frequency to the correct value. One benefit of this system is that once implanted, tuned, and matched, it is not necessary to manipulate the small head coil any further and scans can be run consistently. Additionally, placing the anesthetized animal on its back, instead of stomach, increased image consistency and reduced motion artifact from respiratory breathing. All of these changes to protocol have established a model for MRI imaging of *Peromyscus* to continue with reliable and steady results.

The characterization of the *Peromyscus* epilepsy model is an ongoing process overlapping several fields of research. Genetic analysis using associated microsatellite markers to identify gene locus for 'epl' constantly reveals new information about the *Peromyscus* genome. Although amplification of inferior colliculus RNA proved difficult, further research and innovation may expose another novel way to create vast quantities of RNA from limited tissue samples. Past studies show that a manganese contrast agent can be used to identify brain structures or even pathways of neuronal activation under an MRI system. Additionally, the creation of a small head coil specifically for *Peromyscus* scanning has improved image quality and resolution. While the protocols for stimulation after injection display that manganese has been uptaken (i.e. lack of seizure in 'epl' mammals), first examination of acquired images does not show any major morphological differences between 'epl' and wild type *Peromyscus*. Future scanning will provide further evidence for statistical analysis by a trained expert in the field. The protocols set forth promise that discoveries will be made about the origin of epilepsy in *Peromyscus maniculatus bairdii*, although more work must be done to ensure consistency in both the amplification of inferior colliculus tissue as well as in the replication of MRI imaging techniques.

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References

1. Epilepsy Foundation of America, 1999
2. Prasad AN, Prasad C, Stafstrom CE. Recent advances in the genetics of epilepsy:insights from human and animal studies. *Epilepsia*. 1999. 40:1329-1352.
3. Noebels JL. Single-gene models of epilepsy. *Advnaced Neurology*. 1999. 79: 227-38.
4. Ross KC, Coleman JR. Developmental and genetic audiogenic seizure models: behavior and biological substrates. *Neurosci Biobehav Rev.* 2000. 24:639-653.
5. Dewey MJ, Dawson WD. Deer Mice: "The Drosophilia of North American Mammalogy." *Genesis*. 2001. 29: 105-109.
6. Musser GG, Carleton MD. Peromyscus. In: Wilson DE, Reeder DM, editors. Mammal species of the world, 2d ed. Washington and London: Smithsonian Institution Press. 1993. p. 728.
7. Holler NR, Mason DW, Dawson RM, Simons T, Wooten MC. Reestablishment of the Perdido Key Beach Mouse (Peromyscus polionotus trissyllepsis) on Gulf Islands National Seashore. *Conservation Biology*. 1989. 3:397-404.
8. Steppan SJ, Adkins RM, Anderson J. Phylogeny and divergence-date estimates of rapid radiations in muroid rodents based on multiple nuclear genes. *Systematic Biologists*. 2004. 53: 533-553.
9. Dice L. Inheritance of waltzing and of epilepsy in mice of the genus Peromyscus. *Journal of Mammalogy.* 1935. 16:25-35.
10. Lander ES, Botstein D. Homozygosity Mapping – A Way to Map Human Recessive Traits with the DNA of Inbred Children. *Science*. 1987. 236: 1567-1570.
11. Tryon RC, White SC, Bannasch DL. Homozygosity mapping approach identifies a missense mutation in equine cyclophilin B (PPIB) associated with HERDA in the American Quarter Horse. *Genomics*. 90: 93-102.
12. Obergfell DJ, J.D. B, Dewey MJ, Vrana PB, O'Neill RJ. Utility of Affymetrix GeneChip Technology as a Platform for Transcriptome Analyses Within Peromyscines. *Peromyscus Newsletter*. 2006. 22: 100888-10093.
13. Van Gelder RN, von Zastrow ME, Yool A, Dement WC, Barchas JD, Eberwine, JH Amplified RNA Synthesized from Limited Quantities of Heterogenous cDNA *Proceedings of the National Academy of Sciences to the United States of America*. 1990. 87(5):1663-1667.
14. Bilgen M, Peng W, Al-Hafez B, Dancause N, He Y, Cheney PD. Electrical stimulation of cortex improves corticospinal tract tracing in rat spinal cord using manganese-enhanced MRI. *Journal of Neuroscience Methods*. 2006. 156: 17-22.
15. Watanabe T, Frahm J, Michaelis T. Functional mapping of neural pathways in rodent brain in vivo using manganese-enhanced three dimensional magnetic resonance imaging. *NMR Biomedicine*. 17: 554-68.
16. Bilgen M. Magnetic resonance microscopy of spinal cord injury in mouse using a miniaturized implantable RF coil. *Journal of Neuroscience Methods*. 2007. 159: 93-97.