AWARD NUMBER: W81XWH-17-1-0537

TITLE: Development of Pharmacotherapies for the Treatment of Hydrocephalus

PRINCIPAL INVESTIGATOR: Bonnie L. Blazer-Yost, Ph.D.

CONTRACTING ORGANIZATION: Trustees of Indiana University
Indianapolis, IN 46202

REPORT DATE: Sept 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2018	Annual	1 Sep 2017 - 31 Aug 2018

4. TITLE AND SUBTITLE
Development of Pharmacotherapies for the Treatment of Hydrocephalus

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0537

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bonnie L. Blazer-Yost

E-Mail: bblazer@iupui.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Indiana University
Trustees of Indiana University
980 Indiana Ave. RM 2232
Indianapolis, IN 46202

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The proposed studies aim to test the efficacy and mechanism of action of TRPV4 antagonists for the treatment of hydrocephalus. Whether the cause of hydrocephalus is brain hemorrhage as in pre-term infants, idiopathic normal pressure hydrocephalus of the elderly or post-traumatic hydrocephalus of any age, reducing the production of cerebrospinal fluid (CSF) with a pharmaceutical agent is a promising, novel treatment with the potential to revolutionize clinical outcomes. Preliminary data suggest that TRPV4 antagonists represent such a potential drug treatment. The proposed studies will characterize and use unique rodent models of hydrocephalus to study the efficacy of drug treatment. In addition, cultured choroid plexus (CP) cells will be used to study the mechanisms of action of the drug. In the first year we have made progress in all the proposed first year experiments listed in the SOW. Specifically we have further characterized a rat model of the disease and have performed TRPV4 antagonist treatment in pre-weaning animals. We are in the process of performing behavioral studies in an adult rat model of the disease and correlating these with disease severity. Finally the CP cell line has been used to characterize regulation of important transport proteins involved in CSF production. All of these studies will provide a deeper understanding of the function of the CP and will advance the study of potential drug treatment for hydrocephalus.

15. SUBJECT TERMS
Hydrocephalus; choroid plexus cell line; in vivo animals studies; drug development

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The proposed studies aim to test the efficacy and mechanism of action of TRPV4 antagonists for the treatment of hydrocephalus in rodent models. Whether the cause of hydrocephalus is brain hemorrhage as in pre-term infants, idiopathic normal pressure hydrocephalus of the elderly or post-traumatic hydrocephalus of any age, reducing the production of cerebrospinal fluid (CSF) with a pharmaceutical agent is a promising, novel treatment with the potential to revolutionize clinical outcomes. Preliminary data suggest that TRPV4 antagonists represent such a potential drug treatment. The proposed studies will characterize and use unique rodent models of hydrocephalus to study the efficacy of drug treatment. In addition, cultured choroid plexus (CP) cells will be used to study the mechanisms of action of the drug. In the first year we have made progress in all the proposed first year experiments listed in the SOW. Specifically we have further characterized a rat model of the disease and have performed TRPV4 antagonist treatment in pre-weaning animals. We are in the process of performing behavioral studies in an adult rat model of the disease and correlating these with disease severity. Finally the CP cell line has been used to characterize regulation of important transport proteins involved in CSF production. All of these studies will provide a deeper understanding of the function of the CP and will advance the study of potential drug treatment for hydrocephalus.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Hydrocephalus; TRPV4 antagonists; choroid plexus; cerebrospinal fluid production; drug development; behavioral studies

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

STATEMENT OF WORK – 10/19/16

Site: Indiana University
School of Science, 723 West
Michigan Street, Indianapolis
PI: Blazer-Yost

Yellow completed
Pink started
Green – problems with proposed experiments – conducting alternative experiments

Specific Aim 1 (specified in proposal)	Time-line	Investigator
Major Task 1: Start and maintain the breeding colony for the TMEM67(-/-) and TMEM67(+/-) rats	Month	
Subtask 1 Paired matings of TMEM67(+/-) animals in the colony currently approved by local IACUC – to be used for all experiments	3-36	Dr. Blazer-Yost
Local IRB/IACUC Approval for all three animal models	1	Dr. Blazer-Yost
Milestone Achieved: HRPO/ACURO Approval for all three animal models	4	Dr. Blazer-Yost
Milestone(s) Achieved: First animals ready to start MRI analyses as soon as possible after HRPO/ACURO Approval granted	6	Dr. Blazer-Yost
Major Task 2: MRI experiments – TMEM67(-/-) rat pups- days 7 and 15 (48 pups; 6 each wt males and females, 6 each TMEM(-/-) males and females with and without drug treatment)	Month	
Subtask 1 – conduct the MRI studies on rat pups as outlined in S.A. #1	6-14	Dr. Territo
Subtask 2 – calculate ventricular volumes and summarize data	15-16	Dr. Territo
Milestone(s) Achieved: All experiments using TMEM67(-/-) completed and data summarized for publication	17	Drs. Territo/Blazer-Yost
Major Task 3: MRI experiments – TMEM67(+/-) rats - day 240 + 270 (48 adults; 6 each wt males and females, 6 each TMEM67(+/-) males and females with and without drug treatment	Month	
Subtask 1 – conduct the MRI studies on adult rats as outlined in S.A. #1	16 -24	Dr. Territo
Subtask 2 – calculate ventricular volumes and summarize data	24-26	Dr. Territo
Milestone(s) Achieved: All experiments using TMEM67(+/-) adult rats completed and data summarized for publication	27	Drs. Territo/Blazer-Yost
Major Task 4: Start the breeding colony for the $Gas8^{GT}$ mice		
Subtask 1 Paired matings of $Gas8^{GT}$ animals in the colony approved by local IACUC	22-32	Dr. Berbari
Milestone(s) Achieved: First $Gas8^{GT}$ ready to start MRI analyses	24	Dr. Berbari
Major Task 5: : MRI experiments – $Gas8^{GT}$ mice (48 pups; 6 each wt males and females, 6 each $Gas8^{GT}$ males and females with and without drug treatment)	Month	
Subtask 1 – conduct the MRI studies on mice as outlined in S.A. #1	24-30	Dr. Territo
Subtask 2 – calculate ventricular volumes and summarize data	30-32	Dr. Territo
Milestone(s) Achieved: All experiments using Gas8 mice completed and data summarized for publication	34	Drs. Territo/Berbari/ Blazer-Yost

Specific Aim 2		
Major Task 1: Neurohistology & neuronal counting of TMEM67(+/-) rat model of slowly progressing hydrocephalus (8 months old; baseline data; 24 rats)	Month	
Subtask 1: Perfusion, brain sectioning	8-9	Dr. Goodlett
Subtask 2: Immunohistochemical processing of brain sections and analysis with confocal & light microscopy	8-11	Dr. Goodlett / Dr. Lamb
Subtask 3: Nissl staining and stereological counting of neocortical neurons	8-12	Dr. Goodlett
Subtask 4: Data summary + statistical analysis of neurohistological data	11-12	Dr. Goodlett
Milestone(s) Achieved: All baseline neurohistological experiments using TMEM67(+/-) rats completed and data summarized for publication	12	Drs. Goodlett / Lamb / Blazer-Yost
Major Task 2: Treatment & Behavioral Testing of TMEM67(+/-) rat model of slowly progressing hydrocephalus; 1st cohort (n=48) given MRI in Aim 1; 2nd cohort without MRI (n=48)	Month	
Subtask 1: 30-day treatment with TRPV4 antagonist or vehicle; ~20 rats approximately every 2 months (treatment spans both cohorts)	16-32	Dr. Blazer-Yost/Dr. Goodlett
Subtask 2a: Behavioral testing of rats of cohort 1 (given MRIs); Subtask 2b: Behavioral testing of rats of cohort 2 (no MRIs)	16-24 24-32	Dr. Goodlett
Subtask 3a: Data summary / analysis of behavioral data of cohort 1 Subtask 3b: Data summary / analysis of behavioral data of cohort 2	24-26 32-34	Dr. Goodlett
Milestone(s) Achieved: Behavioral TMEM67(+/-) adult rat experiments completed, data summarized, integrated with MRI/neurohistology	34	Drs. Goodlett / Territo / Blazer-Yost
Major Task 3: Neurohistology & neuronal counting of TMEM67(+/-) rat model of slowly progressing hydrocephalus (96 rats, 48 from cohort 1 and 48 from cohort 2)	Month	
Subtask 1: Perfusion, brain sectioning (across both cohorts)	16-32	Dr. Goodlett
Subtask 2: Immunohistochemical processing of brain sections and analysis with confocal & light microscopy (across both cohorts)	16-34	Dr. Goodlett / Dr. Lamb
Subtask 3: Nissl staining and stereological counting of neocortical neurons (across both cohorts)	16-34	Dr. Goodlett
Subtask 4: Statistical analysis of neurohistological data	32-34	Dr. Goodlett
Milestone(s) Achieved: Neurohistology TMEM67(+/-) experiments completed, data summarized and integrated with MRI & behavior	34	Drs. Goodlett / Territo / Lamb/ Blazer-Yost
Publication		
Major Task 1: 1-3 publications ready for submission	Month	
Subtask 1: Prepare the data regarding the effect of TRPV4 antagonist treatment on ventricular size, behavior & histology, write publications	20-34	Drs. Blazer-Yost/ Territo/Goodlett/Lamb

		Berbari/Fulkerson
Specific Aim 3		
Major Task 1: TRPV4 immunohistochemical staining of TMEM67(-/-) rat pups for developmental changes in expression 160 animals (2 genders x 5 time points x 4 animals per time point x 2 (wt or hydrocephalic) x 2 (drug or vehicle)	Month	
Subtask 1: Treatment of animals as per protocol; preservation of brain	14-20	Drs. Blazer-Yost/ Berbari
Subtask 2: Cryosectioning, immunostaining, confocal analysis (brain)	16-22	Drs. Blazer-Yost/ Berbari
Milestone(s) Achieved: Determination of developmental changes in TRPV4 expression in severely hydrocephalic rats	23	Drs. Blazer-Yost/ Berbari/Goodlett
Major Task 2: TRPV4 immunohistochemical staining of TMEM67(+/-) adult rats to determine developmental changes in expression of TRPV4 (64 animals: 2 sexes x 2 time points x 4 rats per time point x 2 (wt or hydrocephalic) x 2 (drug or vehicle)	Month	
Subtask 1: Treatment of animals as per protocol; preservation of brain	22-28	Drs. Blazer-Yost/ Berbari
Subtask 2: Cryosectioning, immunostaining, confocal analysis (brain)	24-30	Drs. Blazer-Yost/ Berbari
Milestone(s) Achieved: Determination of developmental changes – TRPV4 in slowly developing, chronically hydrocephalic rats	31	Drs. Blazer-Yost/ Berbari/Goodlett
Major Task 3: Immunohistochemical staining of $Gas8^{GT}$ mice pups to determine developmental changes in expression of TRPV4. 160 animals (2 sexes x 5 time points x 4 mice per time point x 2 (wt or hydrocephalic) x 2 (drug or vehicle)	Month	
Subtask 1: Treatment of animals as per protocol; preservation of brain	28-33	Drs. Blazer-Yost/ Berbari
Subtask 2: Cryosectioning, immunostaining, confocal analysis (brain)	32-34	Drs. Blazer-Yost/ Berbari
Milestone(s) Achieved: Determination of developmental changes – TRPV4 in severely hydrocephalic mice	35	Drs. Blazer-Yost/ Berbari/Goodlett
Specific Aim 4		
Major Task 1: Electrophysiological analyses of ion transporters involved in the response to TRPV4 stimulation in PCP-R cell line	Month	
Subtask 1: Analysis of Ca^{2+}-activated Cl^- channels in the PCP-R (porcine) cell line	1-3	Dr. Blazer-Yost
Subtask 2 Analysis of Ca^{2+}-activated K^+ channels in the PCP-R cell line	3-6	Dr. Blazer-Yost
Major Task 2: Electrophysiological analyses of the ion transporters involved in the response to a TRPV4 agonist in the HIBCPP cell line		

Subtask 1: Characterization of the response to TRPV4 activation in the HIBCPP (human; no identifiable information on source) cell line	6-9	Dr. Blazer-Yost
Subtask 2: Analysis of Ca^{2+}-activated Cl^- channels—HIBCPP cell line	9-12	Dr. Blazer-Yost
Subtask 3: Analysis of Ca^{2+}-activated K^+ channels—HIBCPP cell line	12-15	Dr. Blazer-Yost
Milestone(s) Achieved: Identification of ion channel activated in response to TRPV4-induced changes in intracellular calcium	15	Dr. Blazer-Yost
Specific Aim 5		
Major Task 1: Obtain tissue from all three animal models, section and identify the presence and polarization of identified transport proteins. No new animals – tissue used from Specific Aim 3	Month	
Subtask 1: Obtain tissue from animals – obtained from the same brains as those prepared in Specific Aim 3	14-34	Dr. Blazer-Yost
Major Task 2: Obtain tissue from two tissue culture models, stain and identify the presence and polarization of transport proteins	Month	
Subtask 2: Grow and fix cells from the PCP-R cell line	14-18	Dr. Blazer-Yost
Subtask 2: Grow and fix cells from the HIBCPP cell line	18-22	Dr. Blazer-Yost
Subtask 3: Conduct immunohistochemical localization in cells and animal tissues and visualize by confocal imaging	24-30	Dr. Blazer-Yost
Milestone(s) Achieved: Comparison of expression of transport proteins in *vivo* and *in vitro*	30	Drs. Blazer-Yost/ Berbari/Goodlett
Publication		
Major Task 1: 2-3 publications ready for submission	Month	
Subtask 1: Prepare the data regarding the effects of TRPV4 agonists in cultured cells	16-18	Drs. Blazer-Yost
Subtask 1: Prepare the data for comparison of transporters in native choroid plexus with those found in cultured cell lines	30-36	Drs. Blazer-Yost Berbari/Fulkerson
Milestone: Publish high impact papers and present the data obtained in these studies at national meetings	12-36	Drs. Blazer-Yost/ Territo/Goodlett Berbari/Fulkerson

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

All of the data presented below are correlated with schedule provided in the SOW above.

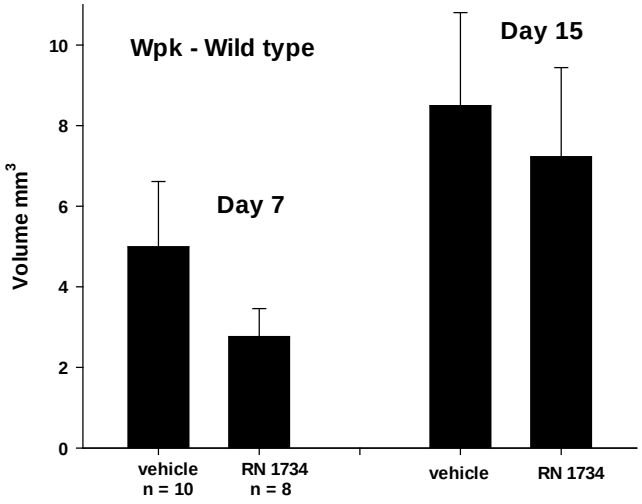
Specific Aim #1

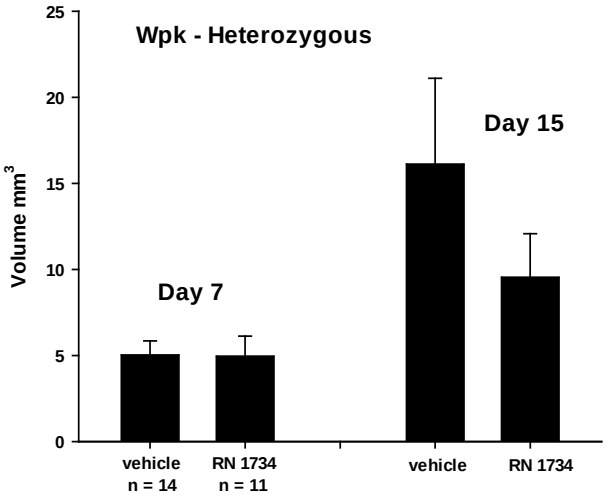
Major Task #1: Start and maintain the breeding colony for the TMEM(-/-) and TMEM (+/-) rats.

All milestones of obtaining approvals from the local IACUC as well as ACURO have been met. As the experiments progress, it may be necessary to obtain protocol amendments – indeed some of these have already been necessary in the on-going experiments. The protocol amendments are first approved by the local IACUC and then submitted to ACURO. No studies funded by the grant are initiated until ACURO approval is granted.
The paired matings are on-going and will continue for all three years of funding.

Major Task 2: MRI experiments – TMEM67(-/-) rat pups- days 7 and 15 (48 pups; 6 each wt males and females, 6 each TMEM(-/-) males and females with and without drug treatment)

Subtask 1 was been initiated on time and Subtask 2 has been initiated early. The results of these combined 2 subtasks are provided below Figure 1. We expected to achieve the milestone of publication in Major Task 2 on time (17 months).





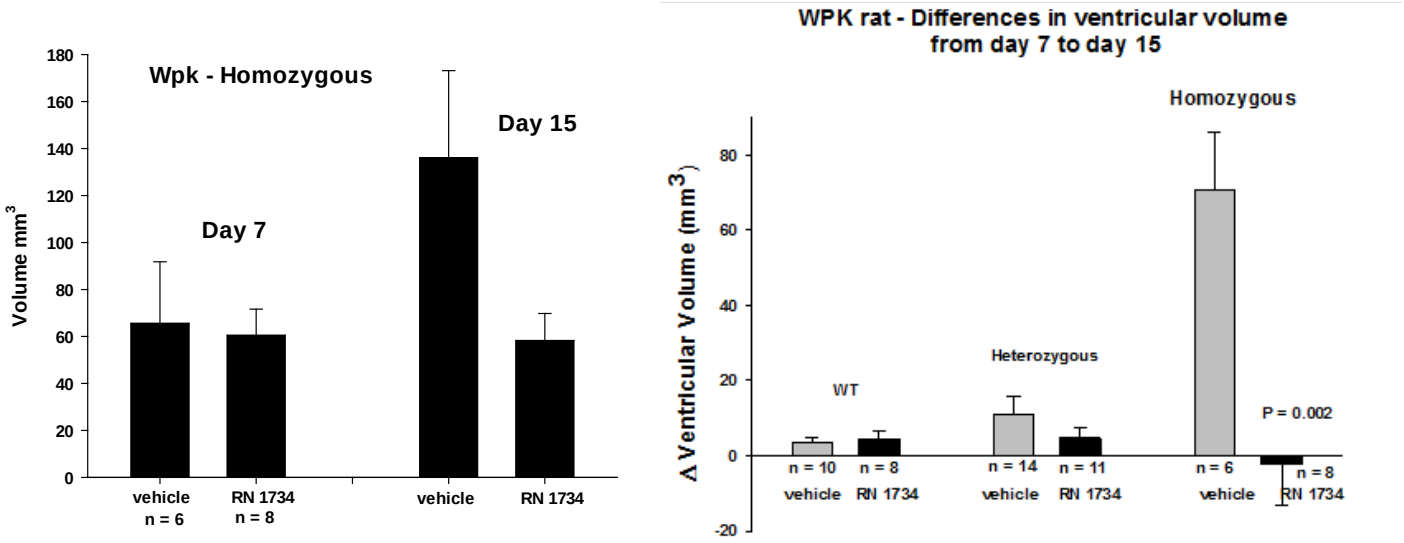


Figure 1: MRI quantitation of lateral ventricle volumes of wild type, heterozygous and homozygous TMEM67 rats at post natal days 7 and 15. The first three bar graphs represent the actual ventricular volumes of each of the genotypes at the indicated post-natal days. RN1734 is a TRPV4 antagonist and was injected i.p. daily at a concentration of 4 mg/kg body weight from post-natal day 7 to 14. The number of animals used in each treatment group is indicated by the n number at the base of the first set of columns. The fourth graph shows the difference in ventricular volume between day 7 and day 15 for each of the genotypes. In the homozygous animals, treatment with the TRPV4 antagonist significantly reduced the increase in ventricular volume as measured a Students T test.

Significance: These results further underscore and extend the preliminary data that was presented in the initial grant application and represent the most exciting findings to date. The MRI provides a quantitative measurement of changes in ventricular volume. The results show that in this model of hydrocephalus, the TRV4 antagonist mitigates the hydrocephalic development.

Major Task 3: MRI experiments – TMEM67(+/-) rats - day 240 + 270 (48 adults; 6 each wt males and females, 6 each TMEM67(+/-) males and females with and without drug treatment.

Major task 3 has been initiated early and the preliminary results indicate a change in the timing of drug treatment in the experimental protocol of the adult animals. In figure 2 the volumes of the lateral ventricles of wild-type and heterozygous animals are shown as measured at day 240 (+/- 3 days). While the heterozygous animals show ventricular volumes that are statistically greater than the wild-type, the variability of the volumes in the heterozygous animals may make drug treatment data difficult to interpret if the treatment results in a partial decrease in the development of the hydrocephalus. For this reason it has been decided to shift the time of initiation of drug treatment to start at post-natal day 300 and continue to post-natal day 330. This will provide the same number of days of drug treatment but will take place in older animals.

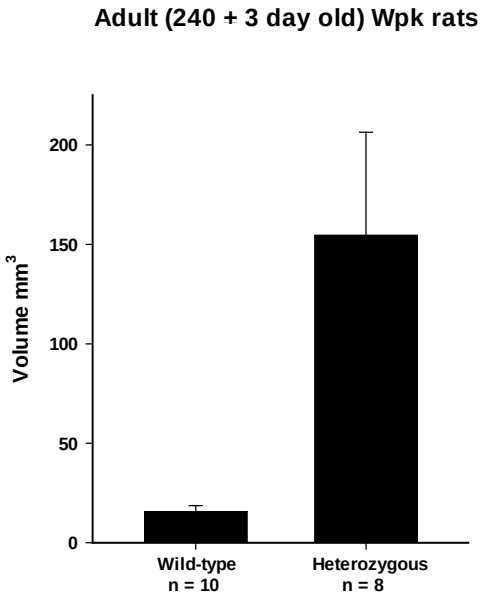


Figure 2: MRI quantitation of lateral ventricle volumes of wild type and heterozygous and TMEM67 rats at post natal day 240 (+/- 3 days). The number of animals used in each genotype is indicated by the n number at the base of the columns. The values are averages +/- SEM.

Significance: These preliminary data indicate that there is a large variability in the ventricular volumes of the heterozygous rats at postnatal day 240. Therefore, for the drug treatment it has been decided to use animals starting at post natal day 300.

The initial drug treatment experiments have been initiated but there are no data available at this time. We anticipate that the subtasks under this major task will be completed at or before the scheduled time on the SOW.

Major Task 4: Start the breeding colony for the Gas8^{GT} mice

This major task was scheduled to begin at the end of the second year of funding. However, we were forced to move the scheduled time ahead because the laboratory that agreed to provide this mouse model (Dr. Brad Yoder, University of Alabama, Birmingham) decided to phase out the colony at their institution. The decision was made that it was safer to start the experiments early rather than risk the time and expense of deriving the colony from frozen gametes.

The mice were obtained and the colony was established in our facility. However, we discovered that this model had been crossed onto a black 6 (B6) background. The animals were found to have very severe and rapidly progressing hydrocephalus. Due to the severity of the disease, many of the pups had to be sacrificed by post-natal day 6. This model would, therefore, not be compatible with drug testing (major Task #5).

Given the predisposition of B6 mice to develop hydrocephalus, and information regarding greater disease penetrance on this background (Danielian et al 2016, Lewis et al 2016), we are in the process of crossing the current GAS8 knockout mouse onto a 129S1/SvlmJ (Stock #002448) background (their original background) and the other two backgrounds (BALB/cJ (Stock #000651) and FVB/NJ (Stock #001800)).

We will utilize heterozygous females from the current background, and breed them with pure inbred males of the new background. Daughters of these litters will be bred back to their original males and so on in order to continue to obtain animals with purer and purer genetic backgrounds associated with each strain. After approximately 10-12 generations, the mice should be sufficiently on each background for us to test for changes in disease penetrance and severity by crossing two heterozygous siblings and assessing the offspring. Through this process we predict that both the

Gas8 models will reduce the severity of the hydrocephalus phenotype once moved to the different genetic backgrounds. This will allow for potential drug efficacy trials.

Because these studies have been initiated early, we anticipate that we have sufficient time perform the back-crossings and still stay on schedule for the planned experiments.

Major Task 5: : MRI experiments – Gas8^{GT} mice (48 pups; 6 each wt males and females, 6 each Gas8^{GT} males and females with and without drug treatment)

This major task is scheduled to start at the end of year 2 of funding. We anticipate that it will be initiated at or before the scheduled time period and will be completed within the proposed time frame as listed in the SOW.

Specific Aim #2

Major Task 1: Neurohistology & neuronal counting of TMEM67(+/-) rat model of slowly progressing hydrocephalus (8 months old; baseline data; 24 rats).

There has been a change in the scope and timing of this major task. As per Specific Aim 1, Major Task 3 above we have decided to look at the adult animals starting at 10 months rather than 8 months. For consistency, all the adult studies will follow the 10 month starting point. We have aged the animals, prepared perfused brains and initiated the immunohistochemical processing. In addition, the added costs of maintenance and production of the animals now limits the number of rats available to complete separate groups originally planned for the baseline neurohistological characterization, so the revised plan will omit those studies to assure that sufficient numbers will be available for the complete analysis of the pharmacological treatment effects. Given the change in scope and timing, we do not have sufficient results to summarize at this point. However, since over half of the brains have been collected, we anticipate that the timeline as given in the SOW will not be delayed more than 3 months. This is off-set by other Major Tasks that are progressing ahead of schedule.

Major Task 2: Treatment & Behavioral Testing of TMEM67(+/-) rat model of slowly progressing hydrocephalus; 1st cohort (n=48) given MRI in Aim 1; 2nd cohort without MRI (n=48)

This major task has been initiated ahead of schedule. As above, the drug treatments on the animals will begin at postnatal day 300 rather than the originally proposed postnatal day 240. We have 23 animals that have completed the MRIs and behavioral testing in the first cohort. All of the behavioral tests listed in the text of the proposal are being conducted. In some cases the scoring is not yet completed and in other it will only be completed when all the animals have been tested. For one component of the multivariate concentric square field (MCSF) test, the data have been analyzed by two independent observers and is summarized in Figure 3 below.

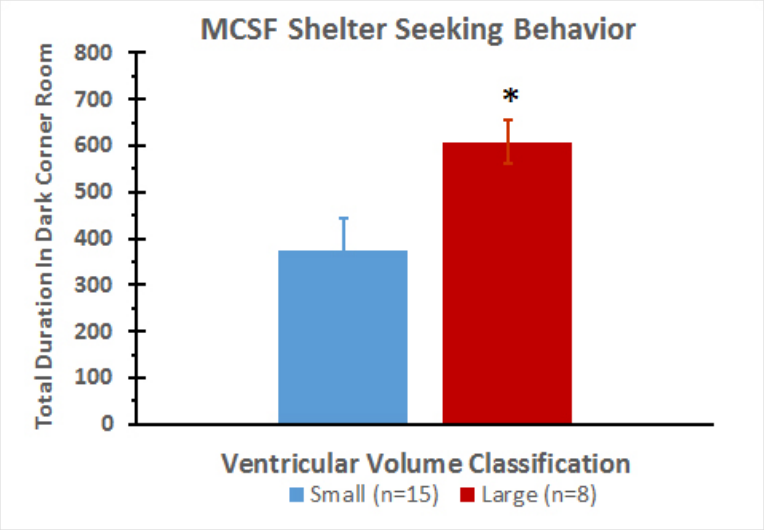


Figure 3. Enlarged ventricular volume was associated with increased shelter seeking in the Multivariate Concentric Square Field (MCSF) behavioral test (*p<0.05). A total of 23 rats have completed the MCSF testing, all of which had quantitative MRI imaging 15 days before behavioral testing to determine ventricular volumes. Regardless of genotype, sex, and drug treatment, rats that were classified with large ventricles (mean = 175.8 µl; range: 99.7-296.4 µl, n=8) spent significantly more time in the Dark Corner Room (a measure of shelter seeking) than did rats classified with small ventricles (mean =24.6 µl, range 5.7-72.8 µl; n=15). The increased time in this small, dark enclosed room during exploration of this complex environment was inversely correlated with total activity counts in the apparatus (r=-0.67), consistent with reduced exploration and increased shelter seeking in the first encounter in the novel environment.

Significance: These findings suggest that brain pathology associated with enlarged ventricles in the adult rats changes the typical behaviors elicited in novel settings, increasing anxiety-like responses while diminishing the motivation to explore. Ongoing analyses of the MCSF test, Novel Objection Recognition memory, and spatial learning and memory in the Morris Water Maze will determine the reliability and generality of behavioral changes associated with ventricular enlargement.

Major Task 3: Neurohistology & neuronal counting of TMEM67(+/-) rat model of slowly progressing hydrocephalus (96 rats, 48 from cohort 1 and 48 from cohort 2).

The Major Task is scheduled for year 2. However, it is worth noting that 23 brains have been perfused and that the sectioning on these has been initiated. We anticipate this task will be completed on or before the schedule in the SOW.

Specific Aim #3

Major Task 1: TRPV4 immunohistochemical staining of TMEM67(-/-) rat pups for developmental changes in expression
160 animals (2 genders x 5 time points x 4 animals per time point x 2 (wt or hydrocephalic) x 2 (drug or vehicle)

This Major Task is scheduled for the second year of funding. The antibody to be used in the rat tissue has been identified and the staining optimized (Figure 4). Two of the graduate students have

become expert in confocal imaging. All techniques and treatment protocols are in place and it is anticipated that the proposed experiments will progress as scheduled in the SOW.

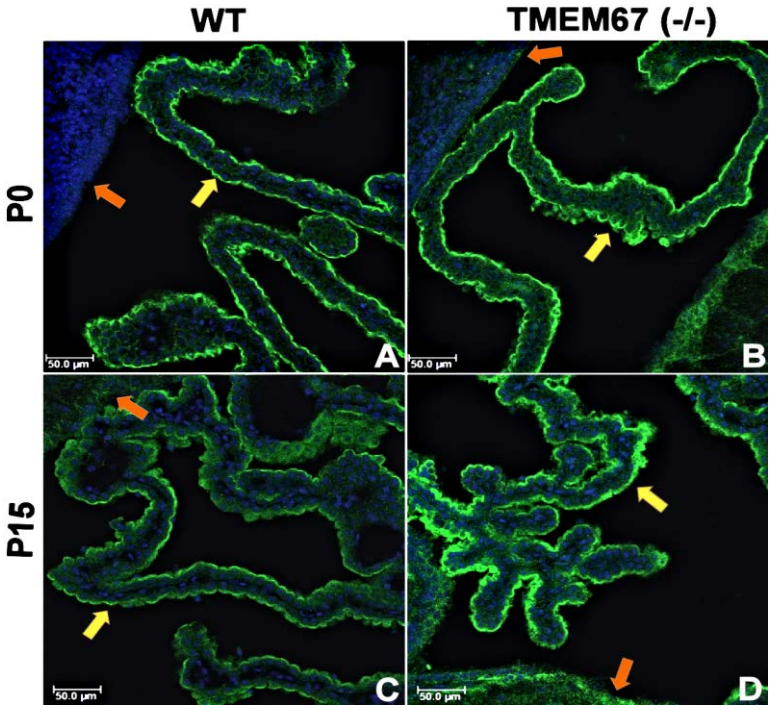


Figure 4: Immunofluorescence of TRPV4 in the *Wpk* rat Choroid Plexus. WT (A & C) and hydrocephalic (B & D) choroid plexus (yellow arrows) at birth (P0, A-B) and at the time of sacrifice (P15, C-D). At birth, TRPV4 in hydrocephalic rats (B) is similar to the WT (A). At P15, this expression increases in the hydrocephalic rat (D) but not in the WT (C). In WT animals there is no detectable TRPV4 expression in the ependymal cells lining the ventricle (orange arrows). In contrast, in the hydrocephalic rats, the ependymal cells show detectable expression of TRPV4. In both phenotypes, TRPV4 localized to the apical membrane of the choroid plexus.

Significance: As shown in Figure 4, there appears to be an up-regulation of TRPV in the choroid plexus of the hydrocephalic animals compared to a wild-type littermate at post-natal day 15. In addition the hydrocephalic animals show TRPV4 staining of the ependymal cells that is not present at birth or in the wild-type animals at day 15. This indicated an increased expression of the transporter that is the target of the drug treatment in these studies.

Major Task 2: TRPV4 immunohistochemical staining of TMEM67(+/-) adult rats to determine developmental changes in expression of TRPV4 (64 animals: 2 sexes x 2 time points x 4 rats per time point x 2 (wt or hydrocephalic) x 2 (drug or vehicle).

This Major Task is scheduled predominately for the third year. As noted above, all techniques are in place and it is anticipated that these experiments will be completed according to the schedule in the SOW.

Major Task 3: Immunohistochemical staining of Gas8^{GT} mice pups to determine developmental changes in expression of TRPV4. 160 animals (2 sexes x 5 time points x 4 mice per time point x 2 (wt or hydrocephalic) x 2 (drug or vehicle).

This is scheduled for the third year of funding and the studies have not been initiated at this point.

Specific Aim 4

Major Task 1: Electrophysiological analyses of ion transporters involved in the response to TRPV4 stimulation in PCP-R cell line

Explanation of electrophysiological methods used for subtasks 1 and 2:
For electrophysiological analyses, PCP-R cells were cultured on 6-well, Transwell filters for 9-12 days. Ussing-style electrophysiological techniques were used to monitor transepithelial resistance (TER) or conductance as well as changes in electrogenic transepithelial ion flux. Filters were excised, mounted in Ussing chambers, and connected to a DVC-1000 Voltage/Current Clamp (World Precision Instruments) with voltage and current electrodes on either side of the membrane. Each half of the chamber contains a tapered fluid compartment with fittings for voltage electrodes (close to the epithelial membrane) and current electrodes (at the opposite end of the chamber). Each fluid chamber was water jacketed to maintain a constant temperature (37^{o}C). The cells were bathed in serum-free media. Media were circulated in the chambers and oxygenated by means of a 5% CO_2/O_2 gas lift. The spontaneous transepithelial potential difference was measured and clamped to zero, and the resultant short-circuit current (SCC) was monitored continuously as a measurement of net transepithelial ion flux. As per convention, a positive deflection in the SCC is either anion secretion (from blood to CSF) or cation absorption (CSF to blood) and a negative deflection indicates the opposite. TER is recorded every 200 seconds throughout each experiment by applying a 2 mV pulse and using the resulting deflection in the SCC to calculate the TER and conductance by Ohm's law. Cultures that showed basal TERs of less than 500 $\Omega.cm^2$ were not used. Conductances, a measurement of transepithelial permeability, were also calculated from the change in SCC during the voltages pulses as $\Delta I/\Delta V$. In all cases, the graphs shown in each panel represent a series of control and experimental cultures that were grown and analyzed in parallel.

Subtask 1: Analysis of Ca^{2+}-activated Cl^- channels in the PCP-R (porcine) cell line

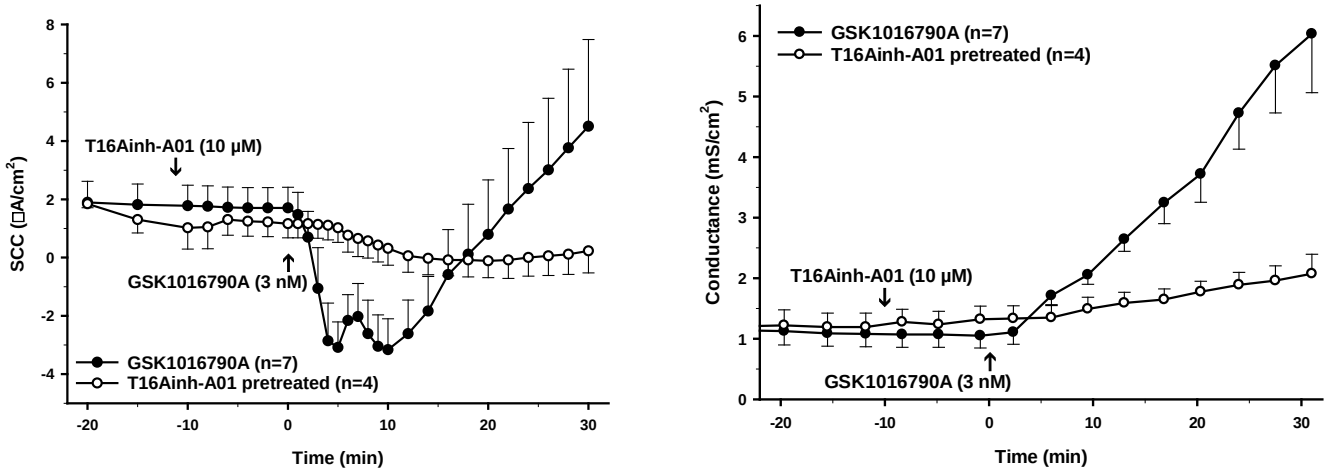


Figure 5: Effect of T16Ainh-A01 pretreatment in the PCP-R Cell line. T16Ainh-A01 is an inhibitor of the TMEM16A chloride channel. Pretreatment of the PCP-R cells 10 minutes prior to addition of the agonist shows significant inhibition of the TRPV4-mediated current change. The increase in conductance, a measure of cell permeability, seen with GSK treatment is also shown to be inhibited

upon pretreatment with T16Ainh-A01. This demonstrates that TMEM16A is an integral component of TRPV4-mediated ion flux.

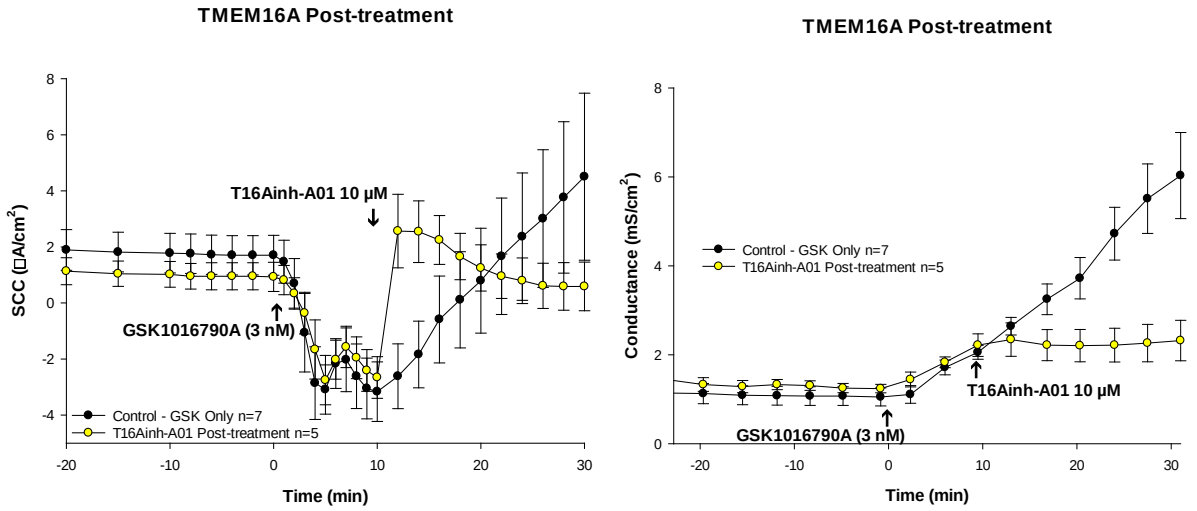


Figure 6: Effect of T16Ainh-A01 post-treatment in the PCP-R Cell line. In addition to being able to inhibit the short circuit current and conductance changes associated with activation of TRPV4 utilizing pretreatment with TMEM16A, we have also demonstrated that the decrease in short circuit current is immediately reversible upon addition of the TMEM16A inhibitor 10 minutes after the addition of the GSK compound. This shows that TMEM16A is capable of specifically regulating TRPV4 activity via chloride ion mobilization. In addition, the conductance increase associated with the GSK compound is stagnated when the TMEM16A inhibitor is utilized. This demonstrates that TMEM16A is capable of preventing the cellular permeability changes associated with TRPV4 activation.

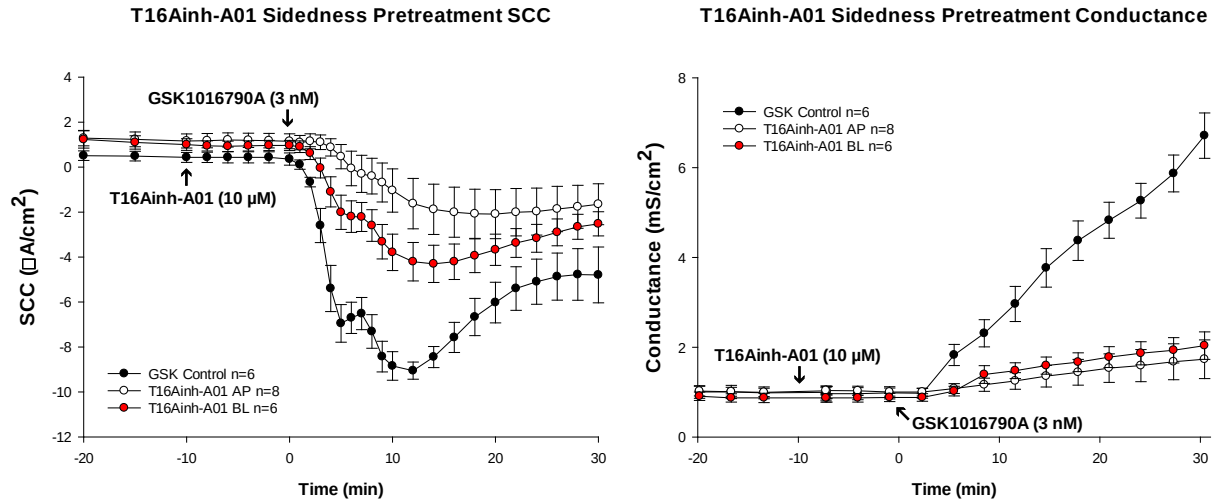


Figure 7: Effect of T16Ainh-A01 on individual membranes of the PCP-R cells. Utilization of the TMEM16A inhibitor on either the apical, or basolateral side individually shows that the chloride channel activity is limited to one particular cellular membrane. These data show that the inhibitor imparts its cellular activity more strongly on the apical membrane than the basolateral membrane, indicating that TMEM16A is likely localized to the apical membrane of the choroid plexus monolayer. The inhibition of conductance change is observed both apically and basolaterally in cells treated with the TMEM16A inhibitor.

Significance: These data suggest that Ca^{2+}-activated Cl^- channels are involved in TRPV4–stimulated electrolyte flux across the choroid plexus epithelium. In addition these channels are critical for the change in transepithelial permeability that is stimulated in response to the TRPV4 agonist.

Subtask 2 Analysis of Ca^{2+}-activated K^+ channels in the PCP-R cell line.

Between the time of submission of the DoD grant and the beginning of the funding, we examined Ca^{2+}-activated K^+ channels in the PCP-R cell line and these findings have been recently published: **https://www.physiology.org/doi/abs/10.1152/ajpcell.00312.2017**

In studies not presented in the publication above we have also examined the effect of an inhibitor of the large conductance channel (BK). The BK channel has been correlated with TRPV4 activity in vascular tissue.

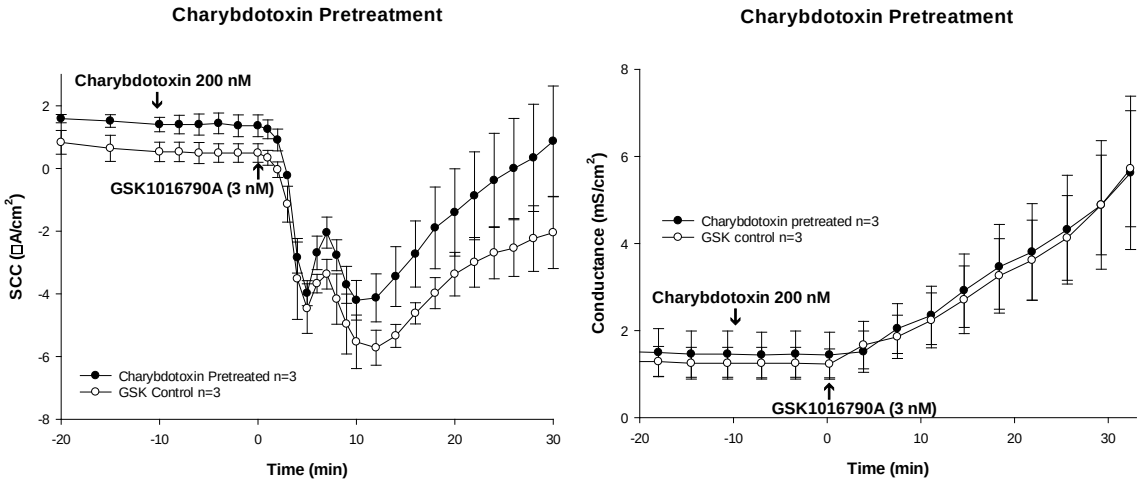


Figure 8: Effect of Charybdotoxin in the PCP-R Cell line. Charybdotoxin is a potent inhibitor of the large conductance potassium channel (BK). We have shown both using the inhibitor and RT-PCR that the BK channel is neither present in the choroid plexus cell line, nor involved in the TRPV4-mediated ion flux pathway. Here, charybdotoxin is used at a concentration which is non-toxic to the cells, and is capable of completely inhibiting the BK channel in tissue. No changes to the short circuit current, or conductance are observed with addition of charybdotoxin to the PCP-R cells. Charybdotoxin is added to the cells at time = -10 minutes, the TRPV4 agonist GSK1016790A is added at time = 0.

Significance: Unlike vascular tissue, the Ca^{2+}-sensitive BK channel is not correlated with TRPV4 activity in the choroid plexus epithelial cells.

Major Task 2: Electrophysiological analyses of the ion transporters involved in the response to a TRPV4 agonist in the HIBCPP cell line

We obtained the human choroid plexus cell line (HIBCPP) cell line and established it in culture in our laboratory. Unfortunately as we characterized the cell line, we found that it was not suitable for electrophysiological studies. In summary, the main problem is that the cells never develop a high transepithelial electrical resistance that is characteristic of the choroid plexus epithelial cells in vivo and is necessary for electrophysiological studies. Our laboratory, in general, and Dr. Blazer-Yost specifically, have substantial expertise in epithelial cell culture. We have altered the growing conditions with regard to time, seeding density, filter support type, fetal bovine serum concentration and media change frequency. Despite considerable effort, we were unable to obtain cultures that were useable for electrophysiology. The cells have the characteristics of a cancerous cell line in that they overgrow and never form a single monolayer of cells when they become confluent. Therefore we have to conclude that these cells are not suitable for the current studies. We have also consulted several colleagues who have attempted to use this cell line to study transepithelial transport and they have had similar experiences.

In summary the Major Task #2 under this Specific Aim will not be possible. However, in order to complete the goal of conducting studies regarding ion channel activation in response to TRPV4 stimulation we are exploring the role of inflammatory cytokines on TRPV4-mediated ion flux (Figures 9, 10, 11). Our preliminary data are provided below and we anticipate that these and on-going studies will lead to a publication within the next 6 months.

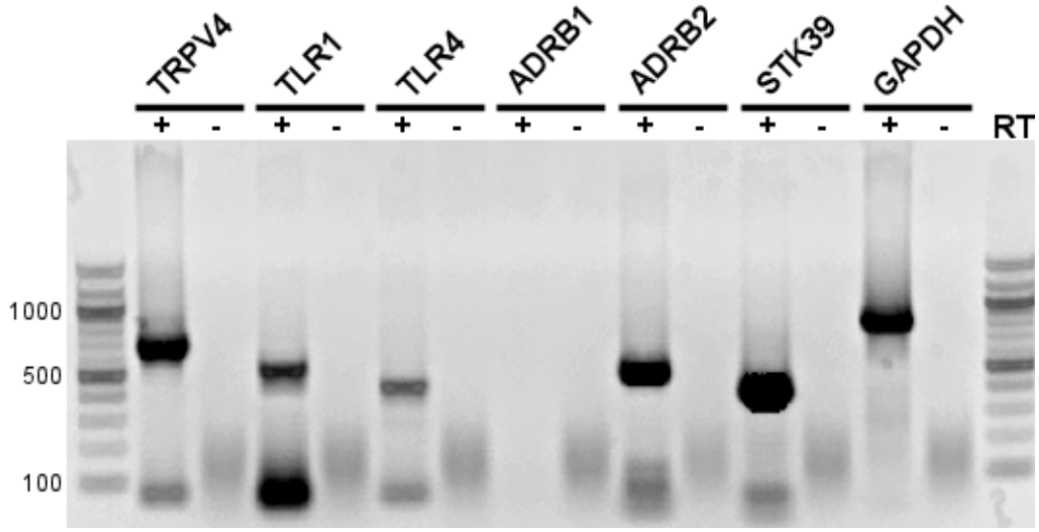


Figure 9: RT-PCR of channels and receptors in PCP-R cells. TRPV4 and receptors for various cytokines and inflammatory mediators. Both Toll-like receptors (TLR1 and TLR4), which are known to respond to pro-inflammatory cytokines, are present. Of the two beta-adrenergic receptors (ADRB1 and ADRB2), which are the receptors for epinephrine, only ADRB2 is present. STK39, the gene which encodes the protein kinase, SPAK, is present. GAPDH is used as a positive control.

Significance: In order to understand whether or not our cell line is able to respond to and/or produce various inflammatory mediators and cytokines, we need to characterize the cells for the necessary receptors.

Pro-inflammatory cytokines in PCP-R cells. IL-1β and TNF-α are prominent pro-inflammatory cytokines seen in neurodegenerative diseases. IL-10 and TGF-β1 are common anti-inflammatory cytokines upregulated in these diseases. In the PCP-R cells, none of these cytokines had immediate significant effects on TRPV4-mediated ion flux, measured as short-circuit current (SCC), nor permeability, measured as conductance. Cytokines were added to apical and basolateral membranes 10 minutes prior to adding TRPV4 agonist, GSK1016790A (data not shown). IL-1β n=3; TNF-α n=6; IL-10 n=4; TGF-β1 n=3.

Significance: Cytokines are upregulated in neurodegenerative diseases. TRPV4 has been reported to activate in response to increases in temperature, such as that seen during inflammation, and to chemical activators, which could include cytokines and inflammatory mediators. These experiments show that stimulation of TRPV4 is not affected immediately after addition of various pro-inflammatory and anti-inflammatory cytokines.

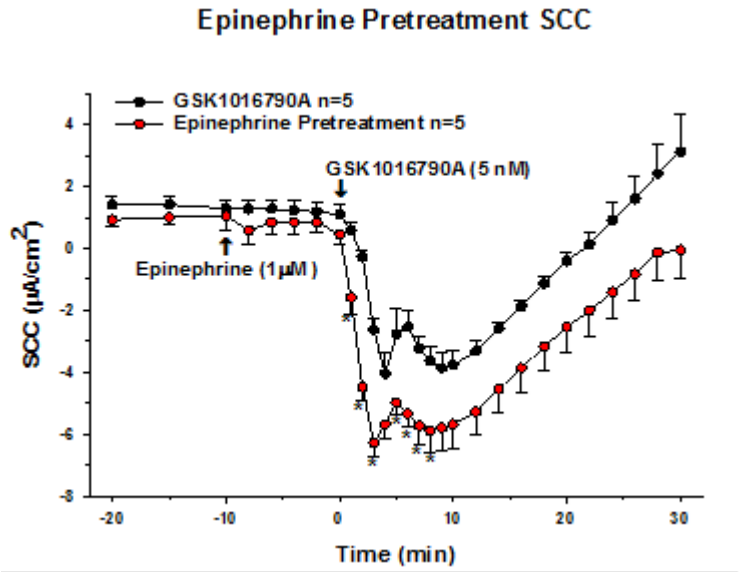


Figure 10: Effect of epinephrine in PCP-R cells. In PCP-R cells, epinephrine causes a significant increase in SCC upon addition of the TRPV4 agonist, similar to previous experiments with the cAMP activator, forskolin (data not shown). No significant change in conductance was seen when treated with epinephrine (data not shown). Time point = 0 corresponds to TRPV4 agonist addition. Epinephrine was added to apical and basolateral membranes 10 minutes prior to adding TRPV4 agonist. (* = $p<0.05$)

Significance: Epinephrine is often used to combat inflammatory anaphylactic reactions. It is implicated in activation of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA). PKA phosphorylates and activates various ion channels, including Ca^{2+} channels such as TRPV4. The current data indicate that epinephrine may act as a sensitizing agent that potentiates TRPV4 activity.

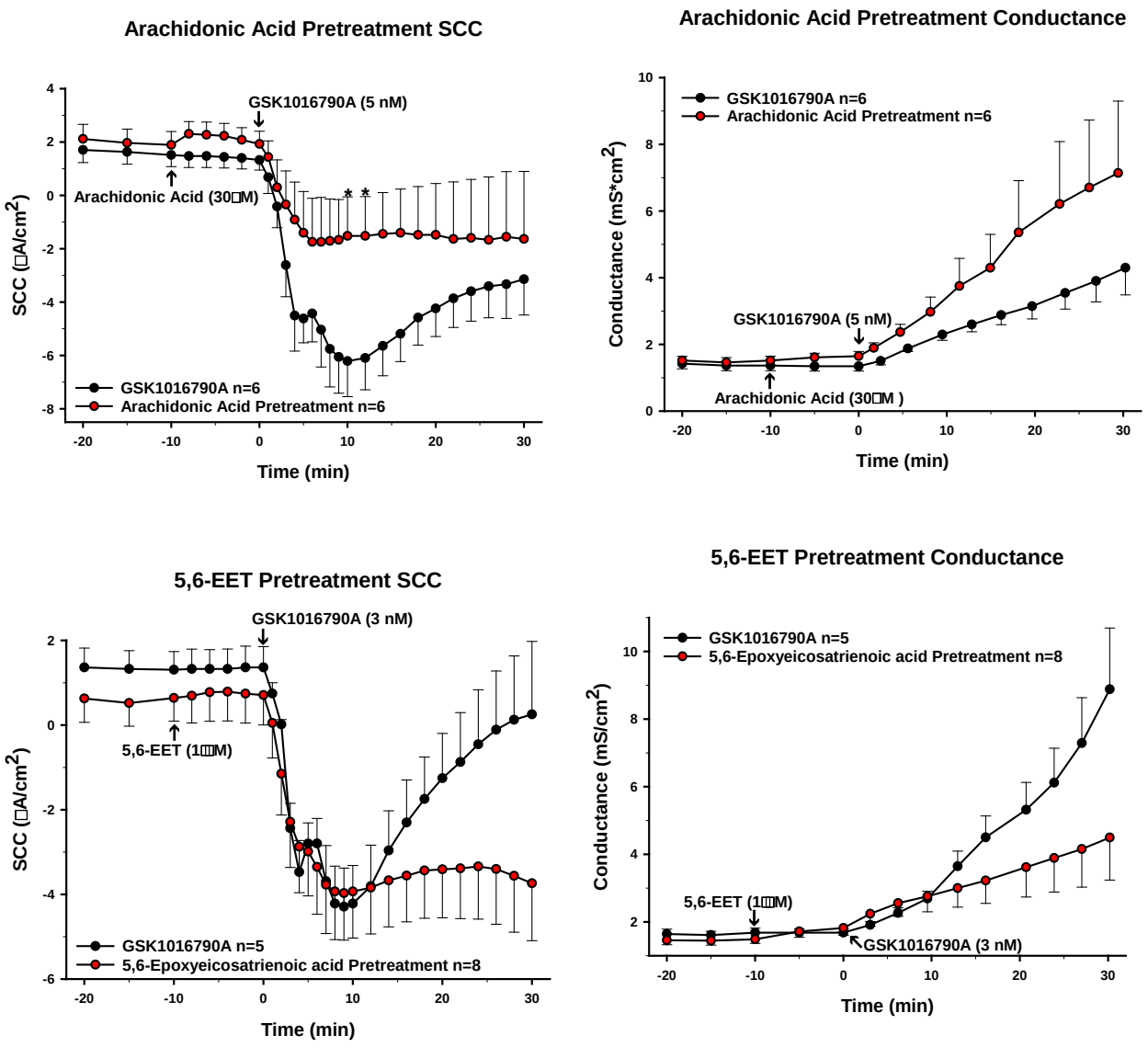


Figure 11: Arachidonic acid metabolites in PCP-R cells. Arachidonic acid (AA) and its metabolite, 5,6-EET, are known activators of TRPV4. However, TRPV4-mediated SCC and conductance changes were partially inhibited by AA in the PCP-R cells. 5,6-EET inhibited one of two phases of the stimulated ion flux. Both AA and 5,6-EET partially blocked the change in conductance, but not significantly. Time point 0 is the time of TRPV4 agonist addition. AA and 5,6-EET were added bilaterally 10 minutes prior to adding TRPV4 agonist. (* = $p<0.05$)

Significance: AA is largely considered to be a stimulator of TRPV4 activity. However, in our cell line, it is acting in an inhibitory fashion. This suggests that AA is being metabolized into a different compound that has inhibitory effects on TRPV4. 5,6-EET was tested as one of these potential compounds, but did not show the same inhibition. Therefore, we are currently working towards discovering which metabolite is responsible for this response.

Specific Aim 5

Major Task 1: Obtain tissue from all three animal models, section and identify the presence and polarization of identified transport proteins. No new animals – tissue used from Specific Aim 3

This Major Task is scheduled for years 2 and 3 of funding. We are collecting tissues during the experiments and anticipate that the studies will be conducted according to the scheduled timeframe listed in the SOW.

Major Task 2: Obtain tissue from two tissue culture models, stain and identify the presence and polarization of transport proteins

The Major Task is scheduled for the second year of funding. The task will be altered because one of the two cell lines, the HIBCPP, was found to be unsuitable for these studies. As noted above, alternative experiments are being conducted in the PCP-R cell line and the immunohistochemical studies in the Major Task will be extended to examine the proteins involved in the cytokine responses.

Publications

It is anticipated that all publications noted in the SOW will be submitted in the timeframes indicated.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The project was not intended to provide training and professional development opportunities per se. However, it should be noted that the one-on-one training provided to the graduate students by the senior members of the experimental team as well as the opportunities to present at scientific meetings have been excellent during the first year. These activities are documented elsewhere in this report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Dr. Blazer-Yost and graduate student Alexandra Hochstetler attended a national meeting, HA Connect, June 28-30, 2018 in Orange County California. This meeting was sponsored by the Hydrocephalus Association and was designed to connect Health Care Professionals and Researchers with hydrocephalic patients and their parents/caregivers. Both Dr. Blazer-Yost and Ms. Hochstetler interacted with conference participants in multiple sessions and Ms. Hochstetler presented a poster on her research during the conference.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue to work with the Hydrocephalus Association to provide information regarding our on-going research to patients and their caregivers/parents. In that regard Dr. Blazer-Yost is already scheduled to do a webinar for the Hydrocephalus Association on Nov. 14th, 2018.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

In the first year of funding the team assembled for this project has made substantial progress toward proving the hypothesis that a channel protein called TRPV4 is important in the cells that produce cerebrospinal fluid. Our studies suggest that an inhibitor of this channel may be a target for drug development in the treatment of hydrocephalus in rodent models of the disease. Since there are no drugs available to treat hydrocephalus, these studies, if ultimately transferable to humans, could have a large clinical impact on disease treatment.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report. While there have been minor changes such as the inability to use one of the proposed cells lines, nothing has changed in the overall goals of the project or in its direction.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable – there are no human subject in the proposed experiments.

Significant changes in use or care of vertebrate animals

Whenever there are changes to the protocols involving the animals, they are first approved by our local IACUC committee and then submitted to ACURO for approval before the changes are instituted. We have had several of these amendments in both the rat and mouse protocols.

Significant changes in use of biohazards and/or select agents

Not applicable to these studies

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Oral Communications (no abstracts available)

2018 NIH Hydrocephalus Workshop: State-of-the-Art Science and Future Directions, April 19, 2018

Blazer-Yost, B.L., The TRPV4 Channel in Choroid Plexus: Implications for the Treatment of Hydrocephalus.

Experimental Biology, April 21- 25, 2018.

Hochstetler, A.E., Whitehouse, L., Antonellis, P., Berbari, N.F., Blazer-Yost, B.L. , Characterizing the Expression of TRPV4 in the Choroid Plexus Epithelia as a Prospective Component in the Development of Hydrocephalus in the $Gas8^{GT}$ Juvenile Mouse Model.

Lake Cumberland Biological Transport Meeting, Lake Cumberland, KT June 17-20, 2018

Hochstetler, A.E., Whitehouse, L., Antonellis, P., Berbari, N.F., Blazer-Yost, B.L. TRPV4 in Hydrocephalic Mice: Model Development for Proof-Of-Principle Studies.

Simpson, S., Preston, D., Blazer-Yost, B. TRPV4 Activation - Implications in Inflammation and Neurodegenerative Disease.

Preston, D., Blazer-Yost, B.L., Treatment of Hydrocephalus with TRPV4 antagonists: choroid plexus cultured cell studies

8^{th} Annual Meeting of the Indiana Physiological Society INPHYS – Taylor University, Upland IN - Feb. 17, 2018

Hochstetler, A.E., Whitehouse, L., Antonellis, P., Berbari, N.F., Blazer-Yost, B.L. , The $GAS8^{GT}$ Mutant Mouse as a Model of Hydrocephalus

Poster Presentations

Experimental Biology 2018 – San Diego, California – April 21-25, 2018

Danko, C., Preston, D, Simpson, S., Territo, P., Blazer-Yost, B.L. Ameliorating Ventriculomegaly in the Wpk Rat Model of Postnatal Hydrocephalus Abstract # 4079

Preston, D., Simpson, S., Schwerk, C., Schroten, H., Blazer-Yost, B.L. TRPV4 activation affects transepithelial ion transport in the choroid plexus epithelium Abstract # 3300

Hochstetler, A.E., Whitehouse, L., Antonellis, P., Berbari, N.F., Blazer-Yost, B.L. The GAS8$^{-/-}$ Mutant Mouse as a Model of Hydrocephalus" Abstract # 3176

Simpson, S., Preston, D., Blazer-Yost, B. TRPV4 Activation - Implications in Hydrocephalic Neurodegenerative Disease. Abstract # 3190

8th Annual Meeting of the Indiana Physiological Society INPHYS – Taylor University, Upland IN - Feb. 17, 2018

Preston, D., Simpson, S., Schwerk C., Schroten, H., Bonnie L. Blazer-Yost, B.L., TRPV4 Mediated Ion Transport in the Choroid Plexus

Simpson, S., Blazer-Yost, B. TRPV4 Activation - Implications in Hydrocephalic Neurodegenerative Disease.

Greater Indiana Society for Neuroscience – Purdue University - March 23, 2018

Preston, D., Simpson, S., Schwerk, C., Schroten, H., Blazer-Yost, B.L., TRPV4 Mediated Ion Transport in the Choroid Plexus

Simpson, S., Blazer-Yost, B. TRPV4 Activation - Implications in Hydrocephalic Neurodegenerative Disease.

Hydrocephalus Association meeting – HA Connect June 28-30, 2018

Hochstetler, A.E., Whitehouse, L., Antonellis, P., Berbari, N.F., Blazer-Yost, B.L. TRPV4 in Rodent Hydrocephalus.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Bonnie Blazer-Yost
Project Role: PI
No change

Name: Nick Berbari
Project Role: Co-investigator
No change

Name: Charley Goodlett
Project Role: Co-investigator
No change

Name: Paul Territo
Project Role: Co-investigator
No change

Name: Daniel Fulkerson
Project Role: Co-investigator
Nearest person month worked: 1
Dr. Fulkerson left the project to enter private practice in July 2018

Name: Karl Balsara
Project Role: Co-investigator
Nearest person month worked: 0
Dr. Balsara is a neurosurgeon who is replacing Dr. Fulkerson on the grant. This personnel change has been previously reported.

Name: Amanda Riley
Project Role: Imaging tech
No change

Name: Lei Jiang
Project Role: Post-doc, imaging center
No change

Name: Scott Persohn
Project Role: Image Analyst
No change

Name: Daniel Preston
Project Role: Graduate student
No change

Name: Listed as TBA on grant – now Alexandra Hochstetler
Project Role: Graduate student
No change

Name: Stefanie Simpson
Project Role: Graduate Student
Change: Ms. Simpson was originally listed as a graduate student on the grant with 6 months effort. This effort was reduced to 3 months because she obtained a graduate fellowship from the university. She will still be working on the project but is no longer receiving a stipend or fee remission.

Name: Caleb Danko
Project Role: Graduate Student
Nearest person month worked: 3
Contribution to the project: Mr. Danko was a Master's student who was involved in the maintenance of the animal colony, genotyping of the animals and collating data during the drug treatment protocols. Mr. Danko left the project and the Master's program due to health issues.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

New Awards amongst the Senior Personnel

New Awards: Bonnie Blazer-Yost

Mayfield Education and Research Foundation $59,318 Role: PI
Pilot Research Grant 03/01/18 – 08/30/19 0 month
Title: Development of Pharmacotherapy for the Treatment of Hydrocephalus
Goal: Pilot studies of preclinical testing of SGK1 inhibitors for the treatment of hydrocephalus

Indiana University Collaborative Research Grant 07/01/18 – 06/30/19 $75,000
Title: Advanced Ultrasound Imaging of the Brain in Neonatal Hydrocephalus Using a Rat Hydrocephalus Model
Corresponding PI: Monica Forbes-Amrhein; Co-PIs Bonnie Blazer-Yost, Jeffrey Raskin, Mark Holland and Rupa Radhakrishnan
Goal: Test new ultrasound techniques in a rodent model of hydrocephalus with a view to developing bed-side imaging techniques in neonatal humans with hydrocephalus.

New Awards: Charles Goodlett
None to report

New awards: Karl Balsara
None to Report

New Awards: Nick Berbari

R01DK114008 (Berbari PI) 04/01/2018 – 03/31/2023 1.0 summer month
NIH-NIDDK $1,948,508 2.0 academic months
Ciliary Mchr1 Signaling in Feeding Behavior and Obesity
The goal of this project is to assess if changes in Mchr1 signaling contribute to increased feeding behavior and obesity observed in mouse models of ciliopathies.

New Awards: Paul Territo

Research Project – Internal 1/1/18 – 12/31/21 1.2 cal. months
PHI-PHGM-PED PRE SARCOMA $1,021,255 Role: Co-I (Clapp)
Grant officer: Heather Daniel Email: hdaniel@iu.edu
Title: Pediatric Hematology Oncology Precision Genomics: 2nd Generation Ask for Expansion of our of Efforts in Pediatric Precision Genomics, Pre-Sarcoma and Sarcoma
This is the approach utilized in the pediatric pre-sarcoma/sarcoma precision oncology research group. Additional investment in this program will increase the capability of this research program, thereby allowing for more efficient translation of novel, potentially curative therapeutic approaches to children with aggressive cancers.
Specific Aims
1. Clinical Care (Precision Genomics).
2. Preclinical Research and Target/Biomarker Validation.

Award # - X81XWH-18-10433 9/1/18 – 8/31/21 3.0 cal. months
U.S. Department of Defense $2,376,437 Role: Co-I (White)
Grant officer: N/A
Title: Chronic Headache Due to Mild Traumatic Brain Injury in Adults: Alterations of Brain Function, Central Sensitization and Inflammatory Processes
The purpose of the proposed study is to identify risk factors that predict which patients following mTBI will experience chronic PTHs (i.e., lasting more than 3 months), with a focus on dysfunctional pain processing in the central nervous system, brain abnormalities following mTBI, and inflammation.
Specific Aims
1. a) Determine whether single and repeated mTBI enhances central sensitization of the trigeminal pain network and impairs descending pain inhibition measured by quantitative sensory tests compared to control participants at 1-week and 6-months post injury, and b) Determine whether central sensitization and descending pain inhibitory capacity predict the frequency and intensity of post-traumatic headaches at 1-week, 1-month, and 6-months post-injury in mTBI patients.
2. a) Determine whether regional metabolic, structural, and functional neuroimaging metrics (e.g. T1-weighted and T2-weighted scans for grey matter and white matter, glucose metabolism via 18F-FDG PET, tissue perfusion via pCASL MRI, white matter tractography via DTI, resting-state functional connectivity via BOLD fMRI, and tissue diffusion via Intra-Voxel Incoherent Motion diffusion MRI) are altered in single mTBI and repeated mTBI patients compared to control participants, b) Determine whether regional metabolic, structural, and functional neuroimaging metrics predict the frequency and intensity of post-traumatic headaches.
3. a) Determine whether monocytes derived from patients with mTBI primes the innate immune system to chronically produce proinflammatory cytokines/chemokines at 1-week, 1-month, and 6-months post-injury and b) Determine whether changes in proinflammatory cytokines/chemokines production at 1-week, 1-month, and 6-months post-injury predicts the frequency and intensity of post-traumatic headaches.

R01DK116963 9/1/18 – 8/31/21 2.4 cal. months
NIH R01 $6,605,931 Role: Co-I (Fogel)
Grant officer: N/A

Title: Magnetic Resonance Imaging as a Non-Invasive Method for Assessment of Pancreatic Fibrosis (MINIMAP): A Pilot Study

We hypothesize that MRI can serve as a valuable non- invasive tool to detect Chronic Pancreatitis (CP), even in the early stages of the disease.
Specific Aims
1. As the primary endpoint in this study, we will evaluate the role of T1 relaxation properties of the pancreatic parenchyma in the assessment of CP using quantitative T1 mapping imaging technique.
2. We will combine the results from the primary and secondary endpoints to generate a composite scoring system. We will demonstrate that an increased number of features will correlate with a diagnosis
of advanced or definite CP with higher sensitivity and specificity.

U54AG054345-03S2 9/15/18 – 8/31/21 0.24 cal. months
NIH-NIA $124,395 Role: PI (Lamb and Territo)
Grant officer: Suzana Petanceska Email: petanceskas@nia.nih.gov
Title: The IU/JAX Alzheimer's Disease Precision Models Center: Drug Selection Criteria

Disease Precision Models Center (IU/JAX ADPMC) seeks to generate and characterize novel animal models of AD, assess the relevance of these to model to human disease and to develop a preclinical testing pipeline through which novel therapies can be tested to greatly accelerate the process by which therapies are successfully moved forward to human AD clinical trials.
Specific Aims
1. Maximize Human Datasets to Identify Putative Variants, Genes and Biomarkers for AD.
2. Generate and Characterize the Next Generation of Mouse Models of AD.
3. Validate the Next Generation of Mouse Models of AD and Develop a Preclinical Testing Pipeline.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)
- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc.,*

available to project staff);

- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Appendix: Copies of Abstracts Presented at Regional and National Meetings

Experimental Biology 2018 – San Diego, California – April 21-25, 2018

Ameliorating Ventriculomegaly in the Wpk Rat Model of Postnatal Hydrocephalus

Caleb Danko[1], Daniel Preston[1], Stefanie Simpson[1], Paul Territo[2], Bonnie Blazer-Yost[1]. [1]Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, [2]Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN

Abstract # 4079 Poster presentation

In the pediatric population, hydrocephalus is a disease that affects approximately 1:1000 births. This disease has multiple causes that can result in a dysfunction of the homeostatic mechanisms of cerebrospinal fluid (CSF) secretion or absorption, or a blockage of CSF circulation, any of which can lead to an over accumulation of CSF within the ventricles of the brain, resulting in ventriculomegaly. In pediatric patients this results in enlarged cranial dimensions. Current therapeutic treatments for hydrocephalus include ventriculoperitoneal shunt implantation to create an artificial drainage mechanism for the CSF or an endoscopic third ventriculostomy, ablating a portion of the choroid plexus (CP) of the third ventricle. While the current treatment options are effective in treating the ventriculomegaly of hydrocephalus, both pose serious risks to individuals, notably high shunt failure rates and/or ablation of a tissue critical to cerebral homeostasis. The CP epithelium is a critical barrier tissue which forms the blood-CSF barrier, maintaining a similar function to that of the blood brain barrier. The CP also produces the majority of the CSF. However, the regulatory mechanisms controlling CSF production and composition are poorly defined. Developing a better understanding of these regulatory mechanisms could lead to novel drug treatments for hydrocephalus. The Wpk rat model of Meckel Gruber Syndrome exhibits severe hydrocephalus in the homozygous animals (Wpk -/-) which causes terminal disease by postnatal (P) day 21. The Wpk heterozygous (Wpk +/-) animals exhibit a mild form of hydrocephalus and live to approximately 1 year of age. Previously we reported that treatment with RN1734, an antagonist of the transient receptor potential vanilloid-4 (TRPV4) channel reduces cranial dimensions of Wpk -/- hydrocephalic rats. TRPV4 is an osmo- and mechano-sensitive Ca^{2+} influx channel located in the CP epithelium. For the present studies the rats were treated with either RN1734 or a vehicle by daily intraperitoneal injection from P7 through P14. Pre-treatment MRIs were taken on P7 and post-treatment MRIs on P15 in order to quantify the volume of the ventricles. Wpk -/- rats treated with RN1734 had a significantly reduced ventricular volume compared to their vehicle treated counterparts. Wild-type and Wpk +/- rats had reduced ventricular volumes but with a limited number of animals this did not reach statistical significance. These data suggest that TRPV4 plays a role in the production of CSF, and may provide insight into new pharmaceutical targets to treat hydrocephalus.

Support or Funding Information
Hydrocephalus Association Innovator Award; Indiana University Collaborative Research Grant and Indiana Clinical and Translational Sciences Institute CTR Award; Department of Defense Office of the Congressionally Directed Medical Research Programs (CDMRP).

Experimental Biology 2018 – San Diego, California – April 21-25, 2018

TRPV4 activation affects transepithelial ion transport in the choroid plexus epithelium

Daniel Preston[1], Stefanie Simpson[1], Christian Schwerk[2], Horst Schroten[2], Bonnie Blazer-Yost[1]. [1]Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, [2]Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany

Abstract # 3300 Poster presentation

Cerebrospinal fluid (CSF) is thought to be produced primarily by the Choroid Plexus (CP), which is found in the lateral, third and fourth ventricles of the brain. The CP is composed of a high resistance monolayer of ciliated cuboidal epithelial cells surrounding an underlying capillary network. The epithelium acts as a barrier, regulating the trafficking of small molecules, ions and water between the CSF and blood. Transient Receptor Potential Vanilloid-4 (TRPV4) is a membrane-bound cation channel expressed in a wide variety of tissues including the CP. TRPV4 is activated by various mechanisms including fluid sheer stress, temperature, hypotonicity, among others. When activated TRPV4 allows the influx of Ca^{2+}, which can secondarily stimulate Ca^{2+}-activated ion channels. We utilized the high-resistance porcine choroid plexus cell line (PCP-R) for Ussing chamber electrophysiology studies of TRPV4 activation and its role in transepithelial ion transport. Treatment with GSK1016790A, a TRPV4 agonist, stimulated a change in short circuit current (SCC), consistent with either cation secretion or anion absorption. The two most likely families of channels responsible for this transepithelial ion movement are the Ca^{2+}-activated K^+ channels, including the large, intermediate, and small conductance potassium channels (BK, IK, and SK1, SK2 and SK3, respectively) as well as Ca^{2+}-activated Cl^- channels such as TMEM16A. Iberiotoxin, a specific inhibitor of the BK channel, did not have an effect on TRPV4-mediated ion flux. We previously demonstrated that fluoxetine, a non-specific inhibitor of all three SK channels, blocked the SCC produced in response to GSK1016790A. However, this effect was not replicated when Apamin was utilized at a concentration high enough to inhibit all three SK channels. High dose TRAM34, a specific inhibitor of IK also prevented GSK1016790A-stimulated transepithelial ion transport. Treatment with T16Ainh-a01, a TMEM16A-specific inhibitor also prevented the short circuit current change observed with TRPV4 activation. RT-PCR was used to determine the presence of channels in the PCP-R cell line. Present were TRPV4, SK2, IK, and TMEM16A, while SK1, SK3, and BK were shown to be absent and verified using additional primers. Using a WPK rat model of hydrocephalus, we have also previously demonstrated that treatment with the TRPV4 antagonist RN-1734 was capable of diminishing the ventriculomegaly associated with acute hydrocephalus. These data suggest that the TRPV4-induced transepithelial ion flux is mediated by both Ca^{2+}-activated K^+ channels as well as Ca^{2+}-activated Cl^- channels. This further implies that TRPV4 may be involved in the control of CSF production. These data, provide intriguing new targets to explore in the treatment of hydrocephalus.

Support or Funding Information

Funding: Hydrocephalus Association Innovator Award; Indiana University Collaborative Research Grant and Indiana Clinical and Translational Sciences Institute CTR Award.

Experimental Biology 2018 – San Diego, California – April 21-25, 2018

TRPV4 Activation - Implications in Hydrocephalic Neurodegenerative Disease

Stefanie Simpson, Bonnie Blazer-Yost. Biology, Indiana University - Purdue University Indianapolis, Indianapolis, IN

Abstract # 3190 – poster presentation

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury, often share similar pathologies, one of which is hydrocephalus. Hydrocephalus is characterized by an imbalance in cerebrospinal fluid (CSF) production and/or reabsorption or by a blockage in the CSF circulation resulting in enlarged ventricles and an increase in brain hydrostatic pressure leading to neuronal destruction. The choroid plexus (CP) is the main producer of CSF and is composed of a high resistance barrier epithelium that surrounds a network of capillaries. The CP epithelium regulates the transport of ions and water between the ventricles and capillaries, thus controlling the production of CSF. Transient Receptor Potential Vanilloid-4 (TRPV4) is a mechano- and osmo-sensitive, cation channel present in CP epithelia. TRPV4 activation leads to an influx of calcium, which can stimulate calcium activated ion channels leading to the potential for significant transepithelial ion flux. If TRPV4 is overstimulated, it is possible that excess ion movement can cause an increase in CSF volume, resulting in hydrocephalus. Current research in our laboratory has shown that treatment with a TRPV4 antagonist ameliorates hydrocephalus in the *Wpk* rodent model. We have also shown that TRPV4 expression is increased in the late-stage hydrocephalic animal compared to normal. TRPV4 can be activated through a number of stimuli, including physical means osmotic changes, increases in temperature and chemical activators, such as cytokines and inflammatory mediators. In particular, the latter activators can be seen in the symptomologies of neurodegenerative diseases exhibiting hydrocephalus. Utilizing a high resistance porcine choroid plexus (PCP-R) cell line, we investigated the effects of various cytokines, inflammatory mediators, and inflammation pathways on TRPV4 activity using immunohistochemistry and electrophysiology techniques. We found that pro-inflammatory cytokines, such as Tumor Necrosis Factor (TNF)-α and interleukin (IL)-1β, had no effect on TRPV4 stimulated ion flux *in vitro*; pro-inflammatory mediators, arachidonic acid and its metabolite 5,6-epoxyeicosatrienoic acid (EET), had inhibitory effects. Furthermore, the hormone epinephrine, used to combat inflammatory anaphylactic reactions, potentiated TRPV4 activity. Immunofluorescence of the kinase SPAK, which plays a role in Nuclear Factor (NF)-κB – dependent inflammation and epithelial barrier function, was increased in the CP of hydrocephalic rats compared to the wild type, and inhibitors of NF-kB altered TRPV4-mediated ion flux. Together these data imply that selected components of an inflammatory response can play a role in TRPV4 activation and can potentially contribute to the subsequent development of hydrocephalus.

Support or Funding Information

Department of Defense office of the Congressionally Directed Medical Research Programs (CDMRP) and an Indiana Clinical and Translational Sciences Award

Experimental Biology 2018 – San Diego, California – April 21-25, 2018

Characterizing the Expression of TRPV4 in the Choroid Plexus Epithelia as a Prospective Component in the Development of Hydrocephalus in the Gas8^{GT} Juvenile Mouse Model

Alexandra Elizabeth Hochstetler[1], Logan Whitehouse[1], Patrick Antonellis[1], Nicolas F Berbari[2], Bonnie L Blazer-Yost[2]. [1]Biology, IUPUI, Indianapolis, IN, [2]Biology, IUPUI, Indianapolis, IN

Abstract #3176 – oral and poster presentations

Maintenance of homeostatic cerebrospinal fluid (CSF) secretion and absorption is essential for basic neurologic function. The choroid plexus epithelia, which is thought to produce the majority of the CSF, are among the most secretory of all epithelia. However, the control of this secretory process is poorly described. In diseased states such as hydrocephalus, where this homeostasis is disrupted, patients often experience symptoms such as cognitive impairment, motor/stability issues, and incontinence, among others. Current treatment for hydrocephalus is limited to surgically invasive shunting procedures which often fail and need revising – particularly in pediatric cases. In order to study the mechanism of this disease, a knock out mouse line of the Growth Arrest Specific 8 (Gas8) allele was generated. Loss of function of the Gas8 allele induces ciliopathy similar to Primary Ciliary Dyskinesia (PCD) in humans, with one of the symptoms being severe perinatal hydrocephalus. The disease progression in the mutant (-/-) mice is from postnatal day 0 (P0) to postnatal day 12-16 (P12-16), which makes this an excellent model to study pediatric/juvenile hydrocephalus. It is believed that one of the mechanisms of pediatric hydrocephalus is the overproduction of CSF by the choroid plexus (CP) epithelial cells that line the ventricles of the brain. The protein of interest to this research is the non-specific cation channel, Transient Receptor Potential Vanilloid 4 (TRPV4), which has been shown to be activated by multiple stimuli including osmotic and pressure changes as well as by prostanoid metabolites. Activation of TRPV4 results in Ca^{2+} influx through the channel resulting in changes in intracellular signaling including the secondary activation of Ca^{2+}-stimulated ion transporters. Consistent with other studies, TRPV4 is localized to the apical membrane of the CP epithelial tissue in the Wild Type Gas8 mice. Preliminary data on the juvenile Gas8 mice indicate that TRPV4 is overexpressed in CP epithelia, ependymal cells and in the sub-ventricular zone in the mutants as their hydrocephalus progresses as compared to the wild type (+/+) and heterozygous (+/-) animals. This suggests that antagonistic compounds of the TRPV4 channel have the potential to reduce hydrocephalus in the Gas8 model. Successfully targeting the molecular mechanisms for hydrocephalus in rodent models can provide a promising base for preclinical studies aimed at developing pharmaceutical agents to treat this disease.

Support or Funding Information
Funding: Grants from the Hydrocephalus Association and the Department of Defense Office of the Congressionally Directed Medical Research Programs (CDMRP).

8th Annual meeting of the Indiana Physiological Society Meeting, Taylor University, Upland IN, February 17, 2018

TRPV4 Mediated Ion Transport in the Choroid Plexus

Daniel Preston, Stefanie Simpson, Christian Schwerk, Horst Schroten and Bonnie L. Blazer-Yost

Cerebrospinal fluid (CSF) is thought to be produced primarily by the Choroid Plexus (CP), which is found in the lateral, third and fourth ventricles of the brain. The CP is composed of a high resistance monolayer of ciliated cuboidal epithelial cells surrounding an underlying capillary network. The epithelium acts as a barrier, regulating the trafficking of small molecules, ions and water between the CSF and blood. Transient Receptor Potential Vanilloid-4 (TRPV4) is a membrane-bound cation channel expressed in a wide variety of tissues including the CP. TRPV4 is activated by various mechanisms including fluid sheer stress, temperature, hypotonicity, among others. When activated TRPV4 allows the influx of Ca^{2+}, which can secondarily stimulate Ca^{2+}-activated ion channels. We utilized the high-resistance porcine choroid plexus cell line (PCP-R) for Ussing chamber electrophysiology studies of TRPV4 activation and its role in transepithelial ion transport. Treatment with GSK1016790A, a TRPV4 agonist, stimulated a change in short circuit current (SCC), consistent with either cation secretion or anion absorption. The two most likely families of channels responsible for this transepithelial ion movement are the Ca^{2+}-activated K^+ channels, including the large, intermediate, and small conductance potassium channels (BK, IK, and SK1, SK2 and SK3, respectively) as well as Ca^{2+}-activated Cl^- channels such as TMEM16A. Iberiotoxin, a specific inhibitor of the BK channel, did not have an effect on TRPV4-mediated ion flux. We previously demonstrated that fluoxetine, a non-specific inhibitor of all three SK channels, blocked the SCC produced in response to GSK1016790A. However, this effect was not replicated when Apamin was utilized at a concentration high enough to inhibit all three SK channels. High dose TRAM34, a specific inhibitor of IK also prevented GSK1016790A-stimulated transepithelial ion transport. Treatment with T16Ainh-A01, a TMEM16A-specific inhibitor also prevented the short circuit current change observed with TRPV4 activation. RT-PCR was used to determine the presence of channels in the PCP-R cell line. Present were TRPV4, SK2, IK, and TMEM16A, while SK1, SK3, and BK were shown to be absent and verified using additional primers. Using a WPK rat model of hydrocephalus, we have also previously demonstrated that treatment with the TRPV4 antagonist RN-1734 was capable of diminishing the ventriculomegaly associated with acute hydrocephalus. These data suggest that the TRPV4-induced transepithelial ion flux is mediated by both Ca^{2+}-activated K^+ channels as well as Ca^{2+}-activated Cl^- channels. This further implies that TRPV4 may be involved in the control of CSF production. These data, provide intriguing new targets to explore in the treatment of hydrocephalus.

Funding: Department of Defense, Hydrocephalus Association Innovator Award; Indiana Clinical and Translational Sciences Institute CTR Award

8th Annual meeting of the Indiana Physiological Society Meeting, Taylor University, Upland IN, February 17, 2018

TRPV4 Activation - Implications in Hydrocephalic Neurodegenerative Disease

Stefanie Simpson, Bonnie Blazer-Yost. Biology, Indiana University - Purdue University Indianapolis, Indianapolis, IN

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury, often share similar pathologies, one of which is hydrocephalus. Hydrocephalus is characterized by an imbalance in cerebrospinal fluid (CSF) production and/or reabsorption or by a blockage in the CSF circulation resulting in enlarged ventricles and an increase in brain hydrostatic pressure leading to neuronal destruction. The choroid plexus (CP) is the main producer of CSF and is composed of a high resistance barrier epithelium that surrounds a network of capillaries. The CP epithelium regulates the transport of ions and water between the ventricles and capillaries, thus controlling the production of CSF. Transient Receptor Potential Vanilloid-4 (TRPV4) is a mechano- and osmo-sensitive, cation channel present in CP epithelia. TRPV4 activation leads to an influx of calcium, which can stimulate calcium activated ion channels leading to the potential for significant transepithelial ion flux. If TRPV4 is overstimulated, it is possible that excess ion movement can cause an increase in CSF volume, resulting in hydrocephalus. Current research in our laboratory has shown that treatment with a TRPV4 antagonist ameliorates hydrocephalus in the *Wpk* rodent model. We have also shown that TRPV4 expression is increased in the late-stage hydrocephalic animal compared to normal. TRPV4 can be activated through a number of stimuli, including physical means osmotic changes, increases in temperature and chemical activators, such as cytokines and inflammatory mediators. In particular, the latter activators can be seen in the symptomologies of neurodegenerative diseases exhibiting hydrocephalus. Utilizing a high resistance porcine choroid plexus (PCP-R) cell line, we investigated the effects of various cytokines, inflammatory mediators, and inflammation pathways on TRPV4 activity using immunohistochemistry and electrophysiology techniques. We found that pro-inflammatory cytokines, such as Tumor Necrosis Factor (TNF)-α and interleukin (IL)-1β, had no effect on TRPV4 stimulated ion flux *in vitro*; pro-inflammatory mediators, arachidonic acid and its metabolite 5,6-epoxyeicosatrienoic acid (EET), had inhibitory effects. Furthermore, the hormone epinephrine, used to combat inflammatory anaphylactic reactions, potentiated TRPV4 activity. Immunofluorescence of the kinase SPAK, which plays a role in Nuclear Factor (NF)-κB – dependent inflammation and epithelial barrier function, was increased in the CP of hydrocephalic rats compared to the wild type, and inhibitors of NF-kB altered TRPV4-mediated ion flux. Together these data imply that selected components of an inflammatory response can play a role in TRPV4 activation and can potentially contribute to the subsequent development of hydrocephalus.

Support or Funding Information
Department of Defense office of the Congressionally Directed Medical Research Programs (CDMRP) and an Indiana Clinical and Translational Sciences Award

Greater Indiana Society for Neuroscience – Purdue University March 23, 2018

TRPV4 Mediated Ion Transport in the Choroid Plexus

Daniel Preston[1], Stefanie Simpson[1], Christian Schwerk[2], Horst Schroten[2] and Bonnie L. Blazer-Yost[1]
[1]Department of Biology, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana, United States; [2]Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany; [3]Department of Neurosurgery, Indiana University School of Medicine, Indianapolis, Indiana, United States

Cerebrospinal fluid (CSF) is produced primarily by the choroid plexus (CP), found in the ventricles of the brain. Composed of a high resistance epithelium surrounding a capillary network, the CP epithelium acts as a barrier, regulating ion transport between the CSF and blood. Transient Receptor Potential Vanilloid-4 (TRPV4) is a cation channel expressed in the CP. Activated TRPV4 stimulates Ca^{2+}-activated ion channels. We utilized the high-resistance porcine choroid plexus cell line (PCP-R) for Ussing chamber electrophysiology of TRPV4 activation and its role in transepithelial ion transport. Treatment with GSK1016790A, a TRPV4 agonist, stimulated a decrease in short circuit current (SCC), consistent with either cation secretion or anion absorption. Families of channels likely responsible for this ion movement are the Ca^{2+}-activated K^+ channels, including the large, intermediate, and small conductance potassium channels (BK, IK, and SK1, SK2 and SK3, respectively) as well as Ca^{2+}-activated Cl^- channels such as TMEM16A. Iberiotoxin, a specific inhibitor of the BK channel, did not have an effect on TRPV4-mediated ion flux. Fluoxetine, a non-specific inhibitor of all three SK channels, blocked the SCC produced in response to GSK1016790A. This effect was not replicated when Apamin was added. High dose TRAM34, a specific inhibitor of IK also prevented GSK1016790A-stimulated transepithelial ion transport. Treatment with T16Ainh-A01, a TMEM16A-specific inhibitor also prevented the short circuit current change observed with TRPV4 activation. These data suggest that Ca2+-activated K+ and Cl- channels mediates TRPV4-induced transepithelial ion flux. This further implies that TRPV4 may be involved in the control of CSF production.

Funding:
Department of Defense, Hydrocephalus Association Innovator Award; Indiana Clinical and Translational Sciences Institute CTR Award

TRPV4 Activation - Implications in Hydrocephalic Neurodegenerative Disease

Stefanie Simpson, Bonnie Blazer-Yost. Biology, Indiana University - Purdue University Indianapolis, Indianapolis, IN

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury, often share similar pathologies, one of which is hydrocephalus. Hydrocephalus is characterized by an imbalance in cerebrospinal fluid (CSF) production and/or reabsorption or by a blockage in the CSF circulation resulting in enlarged ventricles and an increase in brain hydrostatic pressure leading to neuronal destruction. The choroid plexus (CP) is the main producer of CSF and is composed of a high resistance barrier epithelium that surrounds a network of capillaries. The CP epithelium regulates the transport of ions and water between the ventricles and capillaries, thus controlling the production of CSF. Transient Receptor Potential Vanilloid-4 (TRPV4) is a mechano- and osmo-sensitive, cation channel present in CP epithelia. TRPV4 activation leads to an influx of calcium, which can stimulate calcium activated ion channels leading to the potential for significant transepithelial ion flux. If TRPV4 is overstimulated, it is possible that excess ion movement can cause an increase in CSF volume, resulting in hydrocephalus. Current research in our laboratory has shown that treatment with a TRPV4 antagonist ameliorates hydrocephalus in the *Wpk* rodent model. We have also shown that TRPV4 expression is increased in the late-stage hydrocephalic animal compared to normal. TRPV4 can be activated through a number of stimuli, including physical means osmotic changes, increases in temperature and chemical activators, such as cytokines and inflammatory mediators. In particular, the latter activators can be seen in the symptomologies of neurodegenerative diseases exhibiting hydrocephalus. Utilizing a high resistance porcine choroid plexus (PCP-R) cell line, we investigated the effects of various cytokines, inflammatory mediators, and inflammation pathways on TRPV4 activity using immunohistochemistry and electrophysiology techniques. We found that pro-inflammatory cytokines, such as Tumor Necrosis Factor (TNF)-α and interleukin (IL)-1β, had no effect on TRPV4 stimulated ion flux *in vitro*; pro-inflammatory mediators, arachidonic acid and its metabolite 5,6-epoxyeicosatrienoic acid (EET), had inhibitory effects. Furthermore, the hormone epinephrine, used to combat inflammatory anaphylactic reactions, potentiated TRPV4 activity. Immunofluorescence of the kinase SPAK, which plays a role in Nuclear Factor (NF)-κB – dependent inflammation and epithelial barrier function, was increased in the CP of hydrocephalic rats compared to the wild type, and inhibitors of NF-kB altered TRPV4-mediated ion flux. Together these data imply that selected components of an inflammatory response can play a role in TRPV4 activation and can potentially contribute to the subsequent development of hydrocephalus.

Support or Funding Information

Department of Defense office of the Congressionally Directed Medical Research Programs (CDMRP) and an Indiana Clinical and Translational Sciences Award

Characterizing the Expression of TRPV4 in the Choroid Plexus Epithelia as a Prospective Component in the Development of Hydrocephalus in the Gas8^{GT} Juvenile Mouse Model

Alexandra Elizabeth Hochstetler[1], Logan Whitehouse[1], Patrick Antonellis[1], Nicolas F Berbari[2], Bonnie L Blazer-Yost[2]. [1]Biology, IUPUI, Indianapolis, IN, [2]Biology, IUPUI, Indianapolis, IN

Maintenance of homeostatic cerebrospinal fluid (CSF) secretion and absorption is essential for basic neurologic function. The choroid plexus epithelia, which is thought to produce the majority of the CSF, are among the most secretory of all epithelia. However, the control of this secretory process is poorly described. In diseased states such as hydrocephalus, where this homeostasis is disrupted, patients often experience symptoms such as cognitive impairment, motor/stability issues, and incontinence, among others. Current treatment for hydrocephalus is limited to surgically invasive shunting procedures which often fail and need revising – particularly in pediatric cases. In order to study the mechanism of this disease, a knock out mouse line of the Growth Arrest Specific 8 (Gas8) allele was generated. Loss of function of the Gas8 allele induces ciliopathy similar to Primary Ciliary Dyskinesia (PCD) in humans, with one of the symptoms being severe perinatal hydrocephalus. The disease progression in the mutant (-/-) mice is from postnatal day 0 (P0) to postnatal day 12-16 (P12-16), which makes this an excellent model to study pediatric/juvenile hydrocephalus. It is believed that one of the mechanisms of pediatric hydrocephalus is the overproduction of CSF by the choroid plexus (CP) epithelial cells that line the ventricles of the brain. The protein of interest to this research is the non-specific cation channel, Transient Receptor Potential Vanilloid 4 (TRPV4), which has been shown to be activated by multiple stimuli including osmotic and pressure changes as well as by prostanoid metabolites. Activation of TRPV4 results in Ca^{2+} influx through the channel resulting in changes in intracellular signaling including the secondary activation of Ca^{2+}-stimulated ion transporters. Consistent with other studies, TRPV4 is localized to the apical membrane of the CP epithelial tissue in the Wild Type Gas8 mice. Preliminary data on the juvenile Gas8 mice indicate that TRPV4 is overexpressed in CP epithelia, ependymal cells and in the sub-ventricular zone in the mutants as their hydrocephalus progresses as compared to the wild type (+/+) and heterozygous (+/-) animals. This suggests that antagonistic compounds of the TRPV4 channel have the potential to reduce hydrocephalus in the Gas8 model. Successfully targeting the molecular mechanisms for hydrocephalus in rodent models can provide a promising base for preclinical studies aimed at developing pharmaceutical agents to treat this disease.

Support or Funding Information
Funding: Grants from the Hydrocephalus Association and the Department of Defense Office of the Congressionally Directed Medical Research Programs (CDMRP).