AWARD NUMBER: W81XWH-18-1-0777

TITLE: Autism-Associated Mutations in L-Type Ca2+ Channels

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CONTRACTING ORGANIZATION: Northwestern University

REPORT DATE: Oct 2019

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
Oct 2019	Annual Report	**09/30/2018 - 09/29/2019**

4. TITLE AND SUBTITLE

Autism-Associated Mutations in L-Type Ca2+ Channels

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0777

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development 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

Autism Spectrum Disorders (ASDs) are characterized by problems with social engagement and communication, as well as inappropriate restrictive and repetitive behaviors. It has been reported that as many as 1 in 70 children are diagnosed with autism; therefore it represents a major health problem that also profoundly impacts a sizeable number of military families. ASDs have a strong genetic heritability component, but only in a small proportion of cases has the genetic basis been identified, and there is large heterogeneity in the genetic causes. Recently several mutations were identified in individuals with ASDs in genes that code for important Ca^{2+} channels. These ion channels are known to affect neuronal and synaptic development, and therefore are likely causal to autism diagnosed in these patients. More specifically, because these mutations are known to cause a gain-of-function phenotype, increasing Ca^{2+} influx through the channel, they provide a unique opportunity to model the disorder in a mouse and establish a "molecules to behavior" understanding of how brain circuits are functionally altered in ASDs. The two partnering laboratories have collaborated to create a novel mutant mouse with the human mutation engineered into the genome. The mice display several aberrant repetitive and social behaviors that are correlates of the altered behaviors in the human disorder. Therefore, these mice are potentially valuable models for understanding the alterations in brain activity that underlie ASDs. In this proposal we will use these mice to determine the extent of the alteration in synapses, neural circuits and behavior and ask the following three questions: 1) how does the mutation in this ion channel affect the development of neurons in a region of the brain known to be important for repetitive and restricted behaviors? 2) what are the alterations in naturalistic behaviors in these mice that correlate with the symptoms of ASDs, and can we detect this by imaging activity of neurons as mice perform basic behaviors? 3) can we fix the problems in these mice by using drugs that target this ion channel? This proposal directly addresses one of the "Areas of Interest" by assessing novel therapeutics in valid preclinical models. These studies are designed to understand a critical problem in the ASD field, address important knowledge gaps, and ultimately will determine whether we can find ways to rectify the activity in brain circuits that contribute to the altered behaviors in ASDs. Our experimental design will employ cutting-edge techniques to record from neurons in regions of the brain associated with ASDs, and is designed to incorporate the complementary expertise of the partnering laboratories. The ultimate outcome will be in identifying the network basis for repetitive and restricted behaviors, which are a hallmark of ASDs, and will inform the future development of novel treatments.

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Introduction:

Autism Spectrum Disorders (ASDs) are a group of prevalent neurodevelopmental disorders. They are characterized by problems with social engagement and communication, inappropriate repetitive actions, perseverative behaviors, and a range of associated symptoms, including sensory and motor abnormalities, intellectual disability, and mood disorders (Delorme et al., 2013). Studies in families demonstrate that ASDs have a strong genetic heritability component (Folstein and Rosen-Sheidley, 2001; Miles, 2011). Single gene mutations are associated with approximately 5% of cases (De Rubeis et al., 2014) and approximately 10% of cases are associated with copy number variations (Matsunami et al., 2013); but in the vast majority of cases the genetics remain unknown (Miles, 2011). The application of whole exome sequencing of patient DNA has identified many rare *de novo* mutations associated with autism but establishing the effect of these mutations on brain development and function is still at an early stage. Many of these genetic mutations associated with autism converge upon synaptic and neuronal development abnormalities that are the basis for the aberrant behavioral phenotypes and other symptoms of the disorder (Delorme et al., 2013; De Rubeis et al., 2014).

Recently, a group of mutations in Cav1 Ca_{2+} channels have been linked to neurodevelopmental disorders including autism (Gargus, 2009; Pinggera et al., 2015). In particular, seven separate *de novo* missense mutations in *CACNA1D* have been discovered in individuals with autism (Iossifov et al., 2012; O'Roak et al., 2012; De Rubeis et al., 2014; Pinggera et al., 2017). All of these mutations occur within intracellular domains of the pore forming subunit of the ion channel (Pinggera and Striessnig, 2016). Three of these mutations have been functionally characterized in heterologous expression systems, including G407R, A749G, and V401L (Pinggera et al., 2015; Pinggera et al., 2017).

We have created a new mouse model in which we have engineered the G407R mutation in the alpha 1 subunit of Cav1.3 (*Cacna1d* G407R) providing a model with construct validity for autism. Using this model we propose to identify the synaptic and circuit basis for the core symptoms of autism that contribute to many of the aberrant behaviors, focusing primarily on alterations in function of the striatum.

Key Words:

Autism, Ca2+ Channels, behavior, synapses, striatum

Accomplishments:

What were the major goals of the project as stated in the approved SOW?

- Major Goal 1- *To determine the effects of a gain-of-function mutation on the formation and function of synaptic connections in the striatum*
- Major Goal 2 - *Determine the alteration in naturalistic behaviors that correlate with ASD symptoms*

What was accomplished?

The approved statement of work had two specific aims. **Aim 1** was to *Determine the effects of the Cacna1d G407R mutation on striatal circuits and plasticity* and ***major task 1*** in the **Statement of Work** was to: "determine the effects of a gain-of-function mutation on the formation and function of synaptic connections in the striatum" To determine the effects of a gain-of-function mutation on the formation and function of synaptic connections in the striatum.

Therefore, the major goals were to 1. Determine whether there were changes in the morphology of spiny projection neurons (SPNs) as part of Subtask 1. 2) Determine if there are functional changes in synapses and dendrites as part of Subtask 2.

We have made progress on both of these goals:

First: We have performed a morphological study in which we are filling defined populations of direct pathway SPNs (dSPNs) and indirect pathway (iSPNs) with intracellular dyes and analyzing the dendritic structure. These studies are still in process but up to date we have not observed significant differences between dSPNs in WT and G407R mice. This Subtask will be completed during the remainder of the project.

Second: We are characterizing functional effects in neurons of the G407R mutant mice. The goals were to determine whether the mutation affects synapses and excitability of neurons in the striatum using single cell electrophysiology and two-photon Ca2+ imaging in vitro. As an example of this ongoing work in Fig 1 we show

the results from Ca2+ imaging experiments of dendritic excitability. To determine whether the Cacna1dG407R mutation affected the Ca2+ transients in SPNs we patched neurons in slices and introduced Ca2+ indicators into the cell. Depolarization on the cells causing activation of Ca2+ channels which was quantified by the change in florescence (Figure 1A). We found a significantly larger Ca2+ transient in SPNs in mutant mice than WT littermates as would be expected by this mutation in the L-type Ca2+ channel. We elicited trains of action potentials and measured Ca2+ transients in the dendrites of SPNs, which is a correlate of backpropagating action potentials and can be used to determine whether the dendritic excitability is grossly altered in mutant mice (Figure 1B). We found no difference in this measure between WT and mutants, however we did see a small but significant difference in the peak Ca2+ transients in the proximal dendrites of SPN neurons (Figure 1D). Together these recordings demonstrate that there is an increase in the Ca2+ current in SPNs but that there is not a significant effect on dendritic excitability in these neurons (Figure 1). In ongoing work as part of this aim we are characterizing synaptic transmission onto dSPNs and iSPNs and will complete this work by the end of the award period.

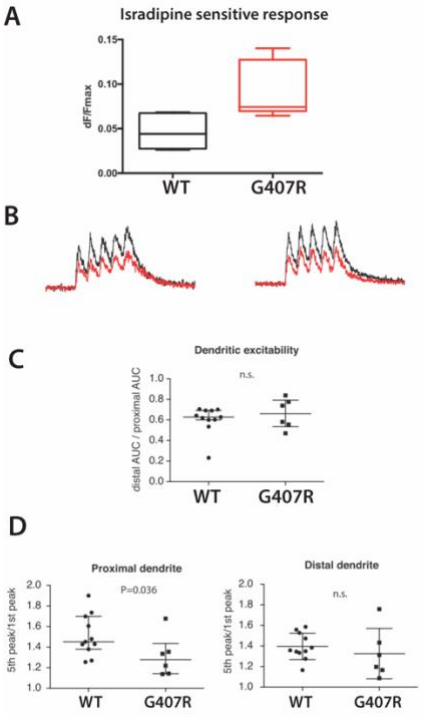

Figure 1 Ca2+ transients are increased in SPNs but dendritic excitability is not affected. (A) Ca2+ influx after depolarization of SPNs recorded by 2-P (B) Ca2+ transients evoked by a train of depolarization in the dendrites of SPNs (C) Relative Ca2+ response in proximal and distal dendrite as a measure of bAPs (D) Facilitation of Ca2+ transients in proximal and distal dendrites

Aim 2 was to ***Determine whether Cacna1d G407R mice display core features of autistic-like behavior*** and ***major task 2*** in the **Statement of Work** was to: "Determine the alteration in naturalistic behaviors that correlate with ASD symptoms". Thus, the major goal of this Aim is to analyze social (Subtask 1), repetitive (Subtask 2) and motor (Subtask 3) behaviors.

We have made progress in this aim by performing experiments to address social behaviors and repetitive or perseverative behaviors in *Cacna1d G407R* mice. These experiments are ongoing and in this report we show as an example a task in which we test behavioral flexibility as an assessment of perseveration in *Cacna1d G407R* mice. For this task we have designed and built custom touch screen operant chambers in which we can train mice to touch a lit panel. Animals are trained to touch a panel on one side (e.g. left) and receive a water reward for each correct touch (Figure 2A). Once they reach criterion they are overtrained for 7 days and then the rule for correct response is reversed (eg. Right panel)(Figure 2 A). The mice are then assessed for how quickly they learn the new rule which is a measure of behavioral flexibility. Many autism mouse models display perseverative behaviors and we had hypothesized that the Cacna1dG407R mice would also have perseverative behaviors and therefore might be impaired in their ability to perform reversal learning. We found that WT and G407R mice learned the task at similar rates during the sessions on the first few days reaching criterion at the same time (Figure 2 B Top panel). Mice were then overtrained and the rule reversed. In this case we saw a significant divergence in the learning curves of the two genotypes (Figure 2 bottom panel). WT mice reached criterion with the new rule within session on the 3rd day whereas G407R mice required up to 5

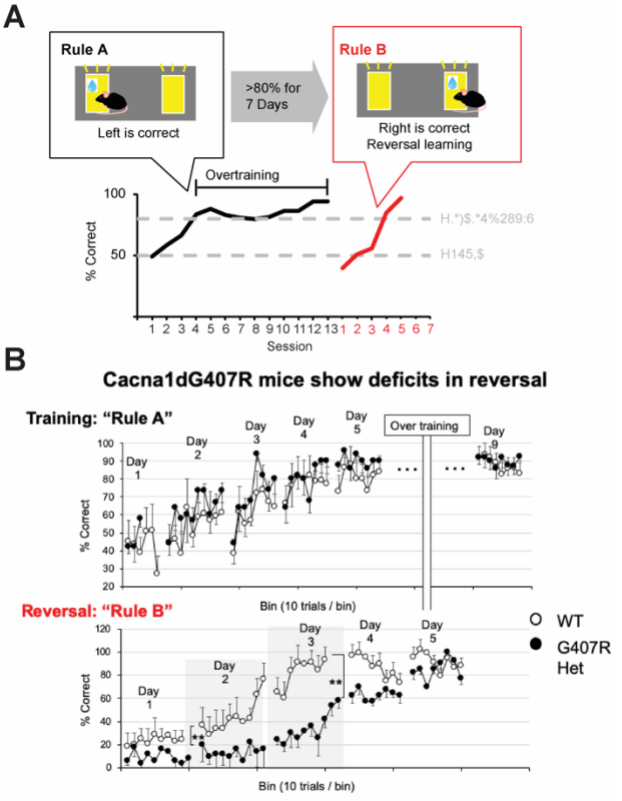

Figure 2 Behavioral flexibility is impaired in Cacna1dG407R mice (A) Schematic of the reversal learning experiments in the touch screen operant chamber (B) Top: initial learning and overtraining period in Cacna1dG407R mice and littermate controls. Bottom: Learning curve after reversal of the rule.

days to reach criterion (figure 2B). These results suggest a major disruption in reversal learning that reflects a lack of behavioral flexibility in the Cacna1dG407R mice. We are next looking at other perseverative behaviors before we test the mice for social interaction behaviors.

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Impact:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

Changes/Problems:
There have not been any problems and no changes to the Aims.

Products:
Nothing to report

7. PARTICIPANTS:

Name: Anis Contractor
Project Role: PI
Researcher Identifier (e.g. ORCID ID) :
Nearest person month worked: 0.6
Contribution to Project: Overall lead for the project, provides scientific direction, mentors postdocs, analyses data and performs administrative duties
Funding Support: None (Complete only if the funding support is provided from other than this award.)

Name: Jian Xu
Project Role: Research Assistant Professor
Researcher Identifier (e.g. ORCID ID) :
Nearest person month worked: 0.6
Contribution to Project: Performed experiments and analyzed data
Funding Support: None (Complete only if the funding support is provided from other than this award.)

Special Reporting Requirements:

Nothing to report

Appendices

Nothing to report