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PRINCIPAL INVESTIGATOR: D. James Surmeier

CONTRACTING ORGANIZATION: Northwestern University

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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 Ca2+ 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 Ca2+ 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
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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, 2013 #1063}. Studies in families demonstrate that ASDs have a strong genetic heritability component {Folstein, 2001 #1066;Miles, 2011 #1513}. Single gene mutations are associated with approximately 5% of cases {De Rubeis, 2014 #1501} and approximately 10% of cases are associated with copy number variations {Matsunami, 2013 #1514}; but in the vast majority of cases the genetics remain unknown {Miles, 2011 #1513}. 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, 2013 #1063;De Rubeis, 2014 #1501}.

Recently, a group of mutations in Cav1 Ca^{2+} channels have been linked to neurodevelopmental disorders including autism {Pinggera, 2015 #1385;Gargus, 2009 #1386}. In particular, seven separate de novo missense mutations in CACNA1D have been discovered in individuals with autism {Iossifov, 2012 #1499;O'Roak, 2012 #1500;De Rubeis, 2014 #1501;Pinggera, 2017 #1504}. All of these mutations occur within intracellular domains of the pore forming subunit of the ion channel {Pinggera, 2016 #1502}. Three of these mutations have been functionally characterized in heterologous expression systems, including G407R, A749G, and V401L {Pinggera, 2015 #1385;Pinggera, 2017 #1504}.

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.

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

Autism, Ca2+ Channels, behavior, synapses, striatum

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- Specific Aim 1: Determine the effects of the Cacna1d G407R mutation on striatal circuits and plasticity
  - Major Task 1: To determine the effects of a gain-of-function mutation on the formation and function of synaptic connections in the striatum
    - Subtask 1: Determine whether dendritic morphology and spine density are developmentally altered in dSPNs and iSPNs in G407R mutant mice – *Surmeier/Northwestern University*
    - Subtask 2: Determine whether synaptic transmission and dendritic excitability are altered in SPNs in G407R mice – *Surmeier/Northwestern University*
    - Subtask 3: Determine whether synaptic plasticity is altered in G407R mice – *Surmeier/Northwestern University*
- Specific Aim 2: Determine whether Cacna1d G407R mice display core features of autistic-like behavior
  - Major Task 2: Determine the alteration in naturalistic behaviors that correlate with ASD symptoms
    - Subtask 1: Determine whether there are disruption social interaction behaviors in G407R mice – *Contractor/Northwestern University*
    - Subtask 2: Determine whether there are alteration in repetitive behaviors and stereotypies in G407R mice – *Contractor/Northwestern University*
    - Subtask 3: Determine whether there are disruptions in motor behaviors and anxiety in G407R mice – *Contractor/Northwestern University*
    - Subtask 4: Determine whether chronic inhibition of Cav1.3 in G407R mice during early development reverses behavioral phenotypes – *Contractor-Surmeier/Northwestern University*
- Specific Aim 3: Determine whether imbalance in striatal circuit activity occurs in Cacna1d G407R mice
  - Major Task 3: Determine whether imbalances in activity of the striatal circuit correlate with altered behaviors
    - Subtask 1: Determine whether SPN activity in vivo is altered during action initiation and termination in G407R mice – *Contractor/Northwestern University*
    - Subtask 2: Determine whether SPN activity balance is altered during habitual actions in G407R mice – *Contractor/Northwestern University*

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.

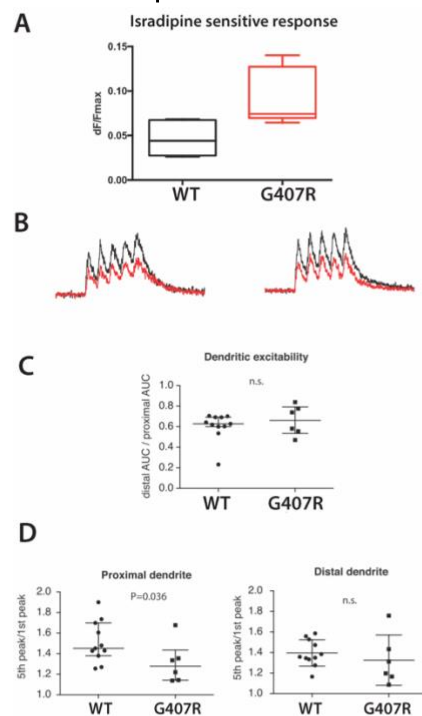

Figure 1 Ca2+ transients are increased in SPNs but dendritic excitability is not affected. (A) Ca2+ influx after depolarization of SPNs recorded by 2-P (B) Ca2+ transients evoked by a train of depolarization in the dendrites of SPNs (C) Relative Ca2+ response in proximal and distal dendrite as a measure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

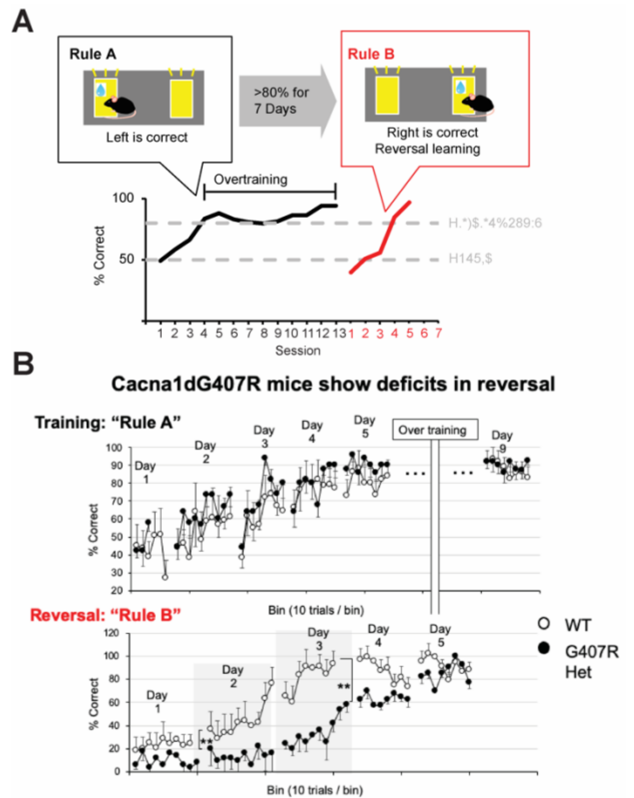

Figure 2 Behavioral flexibility is impaired in Cacna1dG407R mice (A) Schematic of the reversal learning experiments in the touch screen operant chamber (B) Top: initial learning and overtraining period in Cacna1dG407R mice and littermate controls. Bottom: Learning curve after reversal of the rule.

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 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.

Citations:

De Rubeis S et al. (2014) Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515:209-215.

Delorme R, Ey E, Toro R, Leboyer M, Gillberg C, Bourgeron T (2013) Progress toward treatments for synaptic defects in autism. Nat Med 19:685-694.

Folstein SE, Rosen-Sheidley B (2001) Genetics of autism: complex aetiology for a heterogeneous disorder. Nature reviews Genetics 2:943-955.

Gargus JJ (2009) Genetic calcium signaling abnormalities in the central nervous system: seizures, migraine, and autism. Ann N Y Acad Sci 1151:133-156.

Iossifov I et al. (2012) De novo gene disruptions in children on the autistic spectrum. Neuron 74:285-299.

Matsunami N, Hadley D, Hensel CH, Christensen GB, Kim C, Frackelton E, Thomas K, da Silva RP, Stevens

J, Baird L, Otterud B, Ho K, Varvil T, Leppert T, Lambert CG, Leppert M, Hakonarson H (2013) Identification of rare recurrent copy number variants in high-risk autism families and their prevalence in a large ASD population. PLoS One 8:e52239.
Miles JH (2011) Autism spectrum disorders--a genetics review. Genet Med 13:278-294.
O'Roak BJ et al. (2012) Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485:246-250.
Pinggera A, Striessnig J (2016) Cav 1.3 (CACNA1D) L-type Ca2+ channel dysfunction in CNS disorders. J Physiol 594:5839-5849.
Pinggera A, Mackenroth L, Rump A, Schallner J, Beleggia F, Wollnik B, Striessnig J (2017) New gain-of-function mutation shows CACNA1D as recurrently mutated gene in autism spectrum disorders and epilepsy. Hum Mol Genet 26:2923-2932.
Pinggera A, Lieb A, Benedetti B, Lampert M, Monteleone S, Liedl KR, Tuluc P, Striessnig J (2015) CACNA1D de novo mutations in autism spectrum disorders activate Cav1.3 L-type calcium channels. Biol Psychiatry 77:816-822.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

We are next looking at other perseverative behaviors before we test the mice for social interaction behaviors.

4. 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

5. CHANGES/PROBLEMS:

There have been no changes to the Specific Aims.

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**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

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**

Nothing to Report

- **Other Products**

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:	D. James Surmeier
Project Role:	PD/PI
eRA Commons Username:	JSurmeier
Nearest person month worked:	1

Contribution to Project:
Dr. Surmeier has provided expertise in studying basal ganglia circuits, single cell electrophysiology, and high-resolution imaging. He provided oversight of these experiments in Aim 1 and provided advice and mentorship to the postdoctoral fellow. He is involved in all aspects of the study ensuring quality control of the experiments and rigorous methodologyand constrained coding.
Funding Support:
NIH; CHDI Foundation; JPB Foundation; Michael J. Fox Foundation; William N. & Bernice E. Bumpus Foundation; Department of the Army

Name:	Weixing Shen
Project Role:	Research Associate Professor
eRA Commons Username:	WEIXINGSHEN
Nearest person month worked:	1

Contribution to Project:
Dr. Shen has been involved in all aspects of the study including writing articles and presenting data at relevant scientific meetings.
Funding Support:
JPB Foundation; Department of the Army

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Completed Awards:
NIH P50 NS047085; ADAMAS Pharmaceuticals Contract dated 2/1/17 has ended

New Awards:
P50DA044121 (Apkarian) 9/1/18-6/30/23 15% calendar
NINDS $488,960
Point of Contact: James Washington, NINDS - Neuroscience Center, Division of Extramural Activities
6001 Executive Boulevard Suite 3309, Bethesda, MD 20892- 9531
Title: Center for chronic pain and drug abuse
Goals: Our overarching hypothesis is that the chronic pain state primes limbic circuitry for opiate abuse, and also that associated adaptations depend on the duration and dose of both chronic pain and opioid exposure.
Specific Aims: 1) To determine whether morphine reinforcement and seeking behavior is enhanced in SNI mice trained to self-administer morphine; 2) To determine whether SNI differentially affects VTA DA neurons innervating the medial shell and core of the NAc and whether these effects are modulated by morphine self-administration (MSA); 3) To determine whether short-term (5d) morphine self-administration alters SNI-induced adaptations in specific NAc circuits; and 4) To determine whether long-term (14 d) morphine self-administration and withdrawal alters SNI induced adaptations in specific NAc circuits.
Overlap: None

R35GM131788 (Silverman) 5/1/19- 4/30/24 1% calendar
NIH $215,195
Point of Contact: James Washington, NINDS - Neuroscience Center, Division of Extramural Activities
6001 Executive Boulevard Suite 3309, Bethesda, MD 20892- 9531
Title: Selective inhibition of nitric oxide synthase for multiple indications
Goals: This proposal is a continuation of R01 GM049725, "Selective Inhibition of Nitric Oxide Synthase for Multiple Indications". Nitric oxide synthase (NOS) is a remarkable target, as we have found that neuronal nitric oxide synthase (nNOS) inhibitors are applicable to the potential treatment of neurodegenerative diseases (e.g., Parkinson's, Alzheimer's, cerebral palsy), bacterial infection, and melanoma. Inhibitors of nNOS block the excess NO that can cause degeneration of neurons.
Specific Aims: This award type, NIH R35, do not include specific aims.
Overlap: None

What other organizations were involved as partners?

Nothing toReport

8. **SPECIAL REPORTING REQUIREMENTS COLLABORATIVE AWARDS:** N/A

9. **QUAD CHARTS:** N/A

10. **APPENDICES:** Nothing to Report