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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

15. SUBJECT TERMS

None Listed

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1. 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.

2. Key Words:

Autism, Ca2+ Channels, behavior, synapses, striatum

3. Accomplishments:

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

The project had two major goals outlined in the SOW:

1. ***Determine the effects of the Cacna1d G407R mutation on striatal circuits and plasticity.***

This goal was further divided into two subaims to compare the 1) morphological and 2) functional changes in synapses in the G407R mouse.

1. Morphology: As we reported in the prior report when comparing spine morphological properties in the striatum of G407R mutant mice and their littermate controls we found there to be no significant difference in spine density or gross changes in spine morphology in the mutant mice in either indirect pathway spiny projection neurons (iSPNs) and direct pathway neurons (dSPNs).

2. Functional studies: In the current cycle we have performed further functional studies to determine how this mutation in Cacna1d affects the functional and synaptic properties of SPNs. We highlight two additional electrophysiological experiments that we performed in acute sections of the dorsolateral striatum in Fig 1. In the first we performed Ca2+ imaging experiments in the dendrites of SPNs in the dorsolateral striatum. Whole-cell patch clamp experiments were performed and SPNs filled with a ratiometric Ca2+ indicator Fluo4 (Fig 1A). Neurons were depolarized in current clamp to elicit action potentials and the Ca2+ signal was imaged in the dendrites. To isolate the Ca2+ signal that originates from the Cav1.3 L-type Ca2+ channels, isradipine (a specific antagonist of L-type channels) was applied and the relative isradipine sensitive signal calculated. In comparisons across genotypes there was a significantly larger isradipine sensitive Ca2+ signal in the Cacna1d G407R het mice than in the littermate controls, as would be expected with this channel mutation.

Cav1.3 Ca2+ channels are known to be involved in the induction of plasticity in the striatum. The most common form of synaptic plasticity is long term depression (LTD). Multiple forms of LTD have been demonstrated to co-exist in SPNs including an mGluR mediated form of presynaptic endocannabinoid LTD

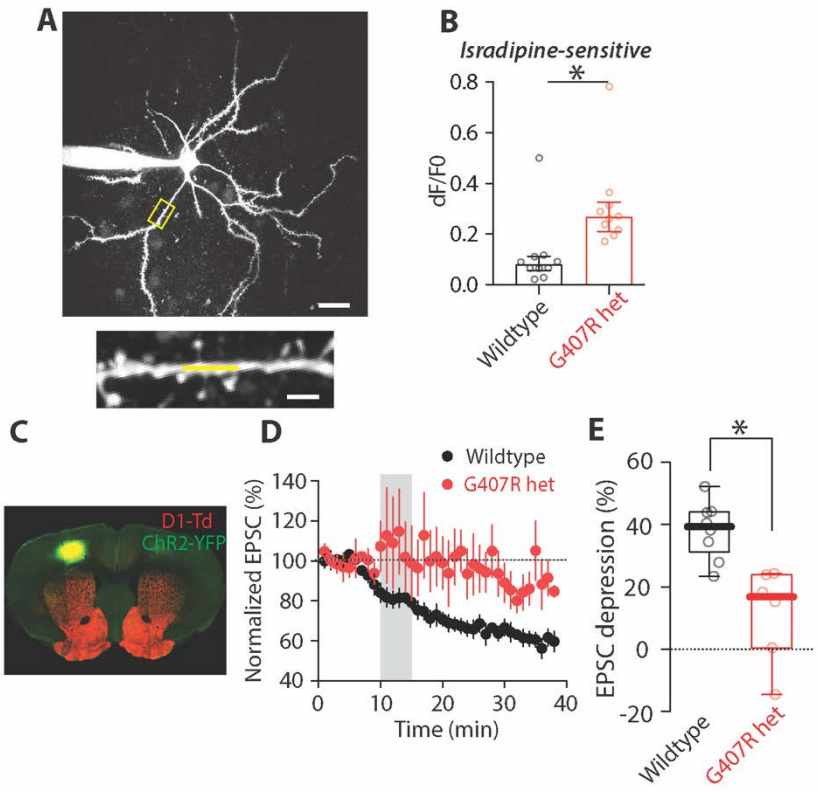

Figure 1: Dendritic Ca^{2+} is enhanced while NO-LTD is impaired in G407R mutant.
(A) Schematic depicting the calcium imaging assay. An SPN was patched in the whole-cell configuration, dialyzed with Ca^{2+}-insensitive Alexa 568 and Ca^{2+}-sensitive Fluo4. Dendritic Ca^{2+} transients were triggered by somatic depolarization (-60 mV to -40 mV for 1s) through the patch pipette. Two-photon line-scan imaging was performed at segments of dendrites ~45 μm from the soma (yellow box). Scale bars, 20 μm (top) and 2 μm (bottom).
(B) Box plots showing the effect of G407R mutation on the isradipine-sensitive component of dendritic Ca^{2+} transients. G407R mutation significantly increases depolarization-evoked Ca^{2+} transients in the dendrites. *, $P < 0.05$, Mann-Whitney test. n =10 cells.
(C) Confocal image confirming that ChR2-YFP (green) was virally expressed in the M1 cortex of D1-Tdtomato mouse.
(D) EPSC was evoked by wide-filed blue LED illumination (0.3 ms duration). LTD was induced bath application of SNAP (5 μM) for 5 min (indicated by a grey bar). Plot shows EPSC amplitude as a function of time in wildtype (black) and G407R mutant (red). Data are mean ± SEM. n = 8 dSPNs from wildtype and 6 dSPNs from G407R heterozygous mice.
(E) Box plot quantifying the last 10 min of LTD. In G407R mutant mice, SNAP-induced LTD was significantly impaired. *, $P < 0.05$, Mann-Whitney test.

(eCB-LTD) which requires L-type Ca2+ channels. This form of LTD is disrupted by a postsynaptic form of LTD that is dependent upon nitric oxide (NO-LTD) (Rafalovich et al., 2015). Thus we had hypothesized that the G407R mutation in Cav1.3 would disrupt the induction/expression of NO-LTD. To test this we used slices of the dorsolateral cortex. In these experiments D1SPNs were labeled with tdTom (red fluorescent protein) to enable us to identify the D1SPNs in acute slices. To activate synaptic inputs to these neurons a viral construct expressing ChR2 (channelrhodopsin) was stereotaxically introduced into the primary motor cortex (M1) (Figure 1C). This enabled us to precisely optically activate corticostriatal synapses to D1SPNs. We thus activated recorded optically evoked EPSCs in D1SPNs and applied the NO donor (*S*)-nitroso-*N*-acetyl-D,L-penicillamine (SNAP, 100 μM) to induce NO-LTD (Figure 1D). In recordings from control mice SNAP induced a long-lasting depression of corticostriatal EPSCs. In contrast in the interleaved recordings from G407R mice there was much smaller depression induced by SNAP (Figure 1D & E). These experiments demonstrate that that NO-LTD is disrupted in G407R mutant mice and furthermore suggest that striatal dependent behaviors that are driven by synaptic plasticity mechanisms could be disrupted in G407R mice.

2. ***Determine whether Cacna1d G407R mice display core features of autistic-like behavior*** We had proposed to determine whether there were changes in in naturalistic behaviors in the mutant mice that correlated with ASD symptoms. In the prior progress report we had focused on reversal tasks which can be used in mice to model perseverative and repetitive behaviors (Subtask 2) that are also a hallmark of ASDs. We have now performed further behavioral experiments on these mice and report here the first that are linked to altered social behaviors (Subtask 1). Thus in this paradigm we monitored mice for observational fear responses which are a measure of social behaviors. In this task mice are placed into two chambers separated by a clear perspex panel (Figure 2 A). After acclimation one of the mice (demonstrator) receives foot shocks, while the other mouse is monitored for freezing responses (observer). (Figure 2A & B). We found that in this simple social behavioral task that WT observers had clear freezing responses when demonstrator mice were shocked. In contrast when the observer mice was a G407R mutant, the observer mice had significantly less freezing in response to the demonstrator being shocked (Figure 2 C & D). These results are the first to demonstrate a social deficits in the G407R mutant mice and will be used as a basis to investigate more social behaviors in the G407R mutant mice. Thus far we have evidence that in both

perseverative (subtask 2 of the SOW) and social tasks (subtask 1 of the SOW) the mutant mice show significant alterations in behaviors. Current work is also directed towards assessment of motor behaviors (subtask 3 of the SOW).

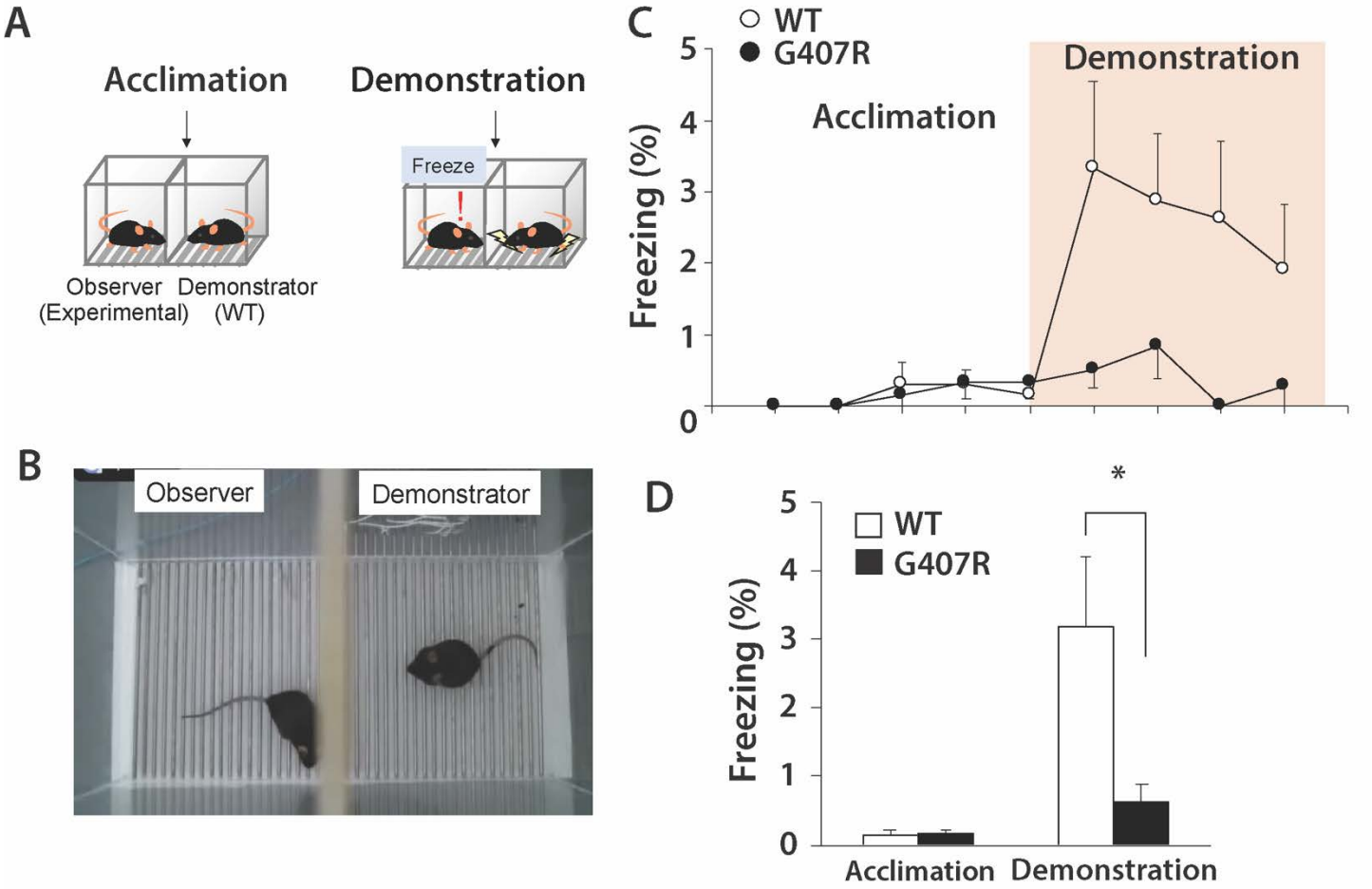

Figure 2: Observational fear responses are disrupted in G407R mutant mice.
(A) Cartoon representation of the behavioral paradigm. Mice are placed in two chambers and can observe one another during acclimation. During the demonstration phase demonstrator receives shocks (2s, 1mA every 10s) and the freezing behavior of the observer is quantified
(B) Picture of mice in the behavioral apparatus during observational fear task
(C) Freezing response of the observer mice grouped from all experiments. WT mice demonstrate normal freezing in response to observation of the demonstrator. G407R mice show little or no freezing when observing the demonstrator
(D) Grouped data from all experiments demonstrating that G407R mice are impaired in observational fear responses.

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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 not been any problems and no changes to the Aims.

6. Products:
Nothing to report

7. PARTICIPANTS:

Name: Anis Contractor
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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.)

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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
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Special Reporting Requirements:

Nothing to report

Appendices

Nothing to report