SUMMARY STATEMENT

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(Privileged Communication) ***Release Date:*** **07/31/2014**

Application Number: **1 R21 HD082747-01**

Principal Investigator

CHAUDHURY, ARUN

Applicant Organization: HARVARD UNIVERSITY (MEDICAL SCHOOL)

Review Group: **ZRG1 MDCN-P (57)**
Center for Scientific Review Special Emphasis Panel
RFA Panel: Molecular and Cellular Substrates of Complex Brain Disorders

Meeting Date: **07/14/2014** ***RFA/PA:*** **PA13-303**
Council: **OCT 2014** ***PCC:*** **IDDB -AK**
Requested Start: **12/01/2014**

Project Title: **Role of shank in pathophysiology of gastrointestinal motility disorders in autism**

SRG Action: **++**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**

Project Year	Direct Costs Requested
1	150,000
2	125,000
TOTAL	275,000

++NOTE TO APPLICANT: Members of the Scientific Review Group (SRG) were asked to identify those applications with the highest scientific merit, generally the top half. Written comments, criterion scores, and preliminary impact scores were submitted by the assigned reviewers prior to the SRG meeting. At the meeting, the more meritorious applications were discussed and given final impact scores; by concurrence of the full SRG, the remaining applications, including this application, were not discussed or scored. The reviewers' comments (largely unedited by NIH staff) and criterion scores for this application are provided below. Because applications deemed by the SRG to have the highest scientific merit generally are considered for funding first, it is highly unlikely that an application with an ND recommendation will be funded. Each applicant should read the written critiques carefully and, if there are questions about the review or future options for the project, discuss them with the Program Contact listed above.

1R21HD082747-01 CHAUDHURY, ARUN

DESCRIPTION (provided by applicant): PSD proteins in postsynaptic densities of excitatory synapses are relatively immobile components and there is a structured organization of mobile scaffolding proteins lying beneath the PSDs. For example, shank proteins are located further away from the membrane in the cytosolic faces of the PSDs, facing the actin cytoskeleton. The rationale of this organization may be related to important roles of these proteins as "exchange hubs" for the signaling proteins for their migration from the subcortical cytosol to the membrane. Notably, PSD95 have also been demonstrated in prejunctional nerve terminals of the nitrergic neuronal processes traversing the gastrointestinal smooth muscle bundles. It has been recently reported that motor proteins like myosin Va play important role in transcytosis of nNOS. In this proposal, we hypothesize that nNOS requires important interactions with scaffolding proteins in the cortical cytoskeleton of the nerve terminal prior to docking at the membrane. In this context, we propose to examine the role of "shank", named for SRC homology (SH3) and multiple ankyrin repeat domains, in nitric oxide synthesis. We hypothesize that dynein light chain LC8-nNOS from acto-myosin Va is exchanged with shank, which thereafter facilitates transposition of nNOS for binding with palmitoyl-PSD95 at the nerve terminal membrane. We plan to examine these protein interactions in enteric nerve terminals by the powerful imaging technique of proximity ligation assay. We will also examine in vitro nitric oxide production of electrically stimulated enteric nerve terminals. We plan to compare nitric oxide production in wild type mice with shank3 knockout mice with an aim to directly establish the role of shank in nitric oxide production. We anticipate significant reduction of nitric oxide production in shank3 knockout mice. In a separate set of experiments, we propose to inject poly-I:C to pregnant female wild type mice to mimic viral infection. Pups born to these mice are known animal models of autism spectrum disorders and display behavioral abnormalities that recapitulate features of human patients with these pervasive neurodevelopmental disorders. Deletion of shank3 in humans is a monogenic cause of autism called Phelan-Mcdermid syndrome. Numerous gastrointestinal motility disorders are identified in these patients, including chronic constipation and cyclical vomiting disorder. The current proposal is significant and novel from the perspective of the first-level investigations into these complex GI motility problems that may result from defective nitric oxide synthesis in the enteric nerve-smooth muscle junctions and lays groundwork for development of rational pharmacotherapy for functional bowel disorders in autism.

PUBLIC HEALTH RELEVANCE: Complex protein organization in nerve terminals controls precise release of gaseous neurotransmitters like nitric oxide to facilitate passage of food through the intestine. Based on previous studies, rational hypothesis is forwarded to perform rigorous and systematic animal studies to examine the role of shank protein in nitric oxide synthesis. Shank protein is deleted in Phelan-McDermid syndrome and may be the basis for cyclical vomiting and chronic constipation seen in these patients and others with autism spectrum disorders.

CRITIQUE 1:

Significance: 6
Investigator(s): 3
Innovation: 3
Approach: 6
Environment: 2

Overall Impact: The aim of the study is to shed light on the molecular mechanisms underlying the gastrointestinal motility disorders that are often diagnosed in children with a pervasive developmental disorder. In order to better understand these mechanisms the investigators propose to investigate two different mouse models: Shank3 knockout and maternal immune activation mice. The study has a number of strengths. It addresses an area that urgently needs more attention. The relationship between

gastrointestinal problems and the behavioral phenotypes that characterize autism spectrum disorders is poorly understood. The investigator has a strong research record in the area and is well qualified for a study of this kind. The hypotheses to be tested are supported by preliminary data and the methods adequate for addressing these. However, there are also concerns. The gastrointestinal problems of the mice are not adequately discussed and more importantly how the results will be linked to these issues. No data analysis plan is presented and statistical power is nowhere addressed. The mouse models chosen are not justified and it is questionable to what extent the maternal immune activation mice are the best choice to follow-up the results obtained from experiments in the shank3(exon4-9) knockout mice. The two sets of models are not well integrated, and it is not clear how the results will inform each other. More thought needs also to be given about the next steps should the studies be successful. Overall, the concerns outweigh the strengths.

1. Significance:

Strengths

- Feeding and gastrointestinal (GI) problems are reported in a large number of patients with an Autism Spectrum Disorder (ASD). GI problems in this population are poorly understood. Addressing GI problems in children with an ASD was recently raised to priority status by the National Institutes of Health Interagency Autism Coordinating Committee (http://iacc.hhs.gov/). So, the study addresses an important and under-researched problem.

Weaknesses

- The proposal is centered on the role of shank3, which is deleted in patients with Phelan McDermid syndrome; it is not clear how the results will generalize.
- The proposal is stated to shed light on the pathophysiology of GI problems in ASDs. However, it is really a study of Phelan McDermid Syndrome.
- It is unclear how the outcome of this study will inform future studies.

2. Investigator(s):

Strengths

- The PI of the study, Dr. Chaudhury, is Instructor in the Department of Surgery at Brigham Women's Hospital. He obtained his MD in 2004 at the All India Institute of Medical Sciences. His postgraduate education included a fellowship in Nutritional Neurosciences at the University of Pennsylvania and Neurogastroenterology at Harvard Medical School. His background is well suited for the proposed study.
- The PI has a longstanding research interest and a good publication record in the area.

Weaknesses

- The track record in relation to grants is weak.

3. Innovation:

Strengths

- The notion that defects in the neuromuscular junctional transmission underlie the GI symptoms in ASD and that the pathophysiological mechanisms are shared with those that lead to the behavioral symptoms is interesting and novel.

Weaknesses

- Most of the methods proposed are conventional and lack innovation.

4. Approach:

Strengths

- Experiments will be conducted in two different mouse models of autism spectrum disorders –the shank(exon4-9) knockout mice and the maternal immune activation model mice.

Weaknesses

- The choice of mouse models is not entirely clear and needs better justification. There are many different murine models of ASD and several of PMD (i.e., Shank deficiency) and why the two specific ones will be investigated is unclear.
- More thought could be given as to how the results will be linked back to gastrointestinal symptoms. Showing differences between wild-type and knockout mice will not be sufficient to link the differences causally to GI problems observed.
- The GI problems in the mice are not adequately discussed and described.
- The number of mice to be use needs justification.
- No statistical analysis plan is presented. What are the outcome measures and how are the data analyzed? (There is some cursory discussion in the Animal Section, but it is insufficient).
- The two sets of studies are not integrated.
- The size of the deletion in PMD varies from less than 100 kb to more than 9 Mb; while SHANK3 is considered the strongest candidate for the neurobehavioral symptoms, the role of other genes deleted needs to be determined.

5. Environment:

Strengths

- Harvard Medical School and Brigham Hospital have outstanding facilities. The research environment is among the best in the world.

Weaknesses

- More information is required to judge how much access the applicant will have to these facilities.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

Vertebrate Animals:

Acceptable

- Use of species and number is justified; the number of mice is required to obtain adequate statistical power.
- Pain will be minimized and an adequate euthanasia protocol is in place.
- Adequate veterinary care is provided.

Biohazards:

Not Applicable (No Biohazards)

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 2
Investigator(s): 2
Innovation: 3
Approach: 2
Environment: 4

Overall Impact: This proposal addresses an understudied aspect of some autistic syndromes (as well as other neurological/neurodegenerative diseases), which are autonomic nervous system effects on GI function. The hypothesis is bold and unconventional and put forth by a junior investigator with a long-standing interest and publication record on neural mechanisms of gut motility. Largely stemming from the investigators own discoveries, but also some supporting literature in field, the hypothesis aims to bridge gaps in our understanding of the molecular mechanisms regulating neuromuscular transmission at gut smooth muscle. At a synapse in which a role for Myosin and nNOS-membrane translocation dependent nitrergic neurotransmission plays a role in gut contraction, the hypothesis is to determine whether the PSD scaffold protein Shank3 is required. This hypothesis will be tested Aim 1 by examining the integrity of NOS in enteric nerve terminals from KO (exon 4-9) mice. Aim 2 will test whether causes of autism more generally also affect NOS gut transmission, using a maternal immune activation model. The two Aims are relatively straightforward and will achieve the critical supporting or refuting data necessary to launch more complex investigations.

1. Significance:

Strengths

- Success has the potential to take an unconventional idea and elucidate molecular mechanisms for an important symptomatology in autism and other neurological disorders affecting the autonomic nervous system.
- Mechanisms revealed herein may also inform putative mechanisms for disrupted brain CNS processes driving other symptomatology.
- Results may end up relating to GI dysmotility in PD as well, cholinergic transmission/autonomic effects.
- nNOS deletion in mice causes autism behaviors (Walton 2013) lending further support of pathway/hypothesis.

2. Investigator(s):

Strengths

- The PI is a clinically trained MD in India and a recent instructor in surgery with GI interest finishing postdoc in 6/13.
- The PI has many publications on this topic starting in 2004 “neurogastroenterol motil” and good reviews on mechanism of GI motility in general.
- There is translational interest and a patent on “myosin activators in gaseous neurotransmission.”
- The PI is well poised as an investigator to make an unconventional leap from autism gene to associated symptoms, and in the process may identify pathways and processes with brain implications as well.
- There is opportunity for disparate fields/perspectives to be engaged in autism research.

3. Innovation:

Strengths

- Innovation is conceptual mostly.

4. Approach:

Strengths

- Multiple and appropriate assays to view significance of interaction and test proposed mechanism are employed including: terminal purification co-IP, whole mount confocal, DAF loaded nerve terminals to monitor KCl depolarization release; subcellular fractionation of cytosolic and membrane bound nNOS, and pharmacological disruption of shank/gkap interaction through PDZ domain ligand.
- Anticipated results are clear and thought through. Includes a good discussion of potential caveat that there are redundant functions with other isoforms, or that another isoform, such as Shank2 may in fact be the functionally important isoform in gut. This is an R21 exploratory grant mechanism; therefore, risk is appropriate.
- Awareness of data supporting shank1/shank2 in gut nmj is explicit and will serve to maximize meaningful interpretation of results, both positive and negative.
- Although unusual, the maternal immune activation model was recently featured in a Cell paper. Gut pathophysiology recently described in this model makes it a good choice among models to test generalizability of hypothesis between gut neurotransmission and autistic syndromes.
- Second Aim methodologies are similar to first Aim, feasible.
- Thoughtful discussion of feasibility and outcome significance shows maturity of thinking by this junior investigator.
- A number of alternative mechanistic hypotheses are recognized and testable by the investigator if initial hypothesis is refuted by experimentation.

Weaknesses

- Article not available regarding compound binding shank3 PDZ domain, may have non-specific interactions with other PDZ domain containing compounds as alluded to in abstract. Positive result here may be supportive, but non-specific. (Some specificity might be hinted at if difference in drug effect when target, shank3, not present.)
- Important control was not explicitly mentioned, which is that specificity of commercially available antibodies should be tested in KO mouse tissue early on in process.

- (Although it is unconventional to invoke psd95 and shank in the PRE synaptic terminal, this is not a possibility that can be excluded from our current understanding.)
- Note: Rubinstein and Mayer ref was not found in pubmed or in references. Lehtonen reference is for a myopathy? Role of neurons.

5. Environment:

Weaknesses

- Facilities and Equipment statement did not cover description of location of laboratory and overall research community that enhances activities of this junior investigator.

Vertebrate Animals:

Acceptable

Budget and Period of Support:

Recommend as Requested

Additional Comments to Applicant (Optional):

- If not funded by nichd, ninds, nimh, and niddk may be interested. New investigator may not have been experienced enough to know to suggest this.

CRITIQUE 3:

Significance: 2
Investigator(s): 5
Innovation: 3
Approach: 6
Environment: 2

Overall Impact: This is a new proposal to study a molecular hypothesis of gastrointestinal disorders associated with autism spectrum disorder (ASD). The PI presents an interesting model that proposes a role for shank proteins in the transport of nNOS across the actin cortex in nerve terminals of the enteric nervous system. Concerns about the scientific approach and interpretation reduce enthusiasm.

1. Significance:

Strengths

- ASD is known to be associated with problems of bowel motility. This proposal will examine a hypothesized role for Shank3 in targeting of nNOS to myenteric nerve terminals.

2. Investigator(s):

Strengths

- Dr. Chaudhury is an Instructor in the Department of Surgery at Harvard Medical School. The PI received his MD and residency training in All India Institute for Medical Sciences and postdoctoral training at the Monell Chemical Senses Center in Philadelphia (2007) and at Harvard Medical School. Since 2007 he has been a member of the Harvard Clinical and translational science center. He became an Instructor in 2013. The PI has published in the field of nitrergic signaling in the GI with Dr. R. Goyal.

Weakness

- There is no track record of independent scientific productivity.

3. Innovation:

Strengths

- The hypothetical model is interesting.

Weaknesses

- The methods used to assess the hypothesis are not as selective or powerful as likely needed to address the hypothesis.

4. Approach:

Strengths

- Studies will use a “ligated enteric” preparation and biochemical fractionation to generate a preparation to study NO generation. The PI is familiar with this preparation, and biochemical results can be efficiently obtained.

Weaknesses

- It is not known if the Shank3 KO mice possess relevant GI phenotypes. It is also not known that Shank3 is localized to the presynaptic terminal. This is important for the model that will be tested. It is not clear if Shank1/3 bind nNOS (as predicted in Figure 2). It is not clear if the chemically induced autism mouse model has GI phenotypes that can be related to nNOS. The PI does not have experience with Shank Abs that will be necessary to test the hypothesis. Localization studies rely on biochemical preparations that may not be entirely pure. A single chemical (Tetrahydroquinoline carboxylate) is used to inhibit Shank3 PDZ domain binding- this is not convincingly specific.

5. Environment:

Strengths

- The environment is appropriate.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Acceptable

Biohazards:

Not Applicable (No Biohazards)

Resource Sharing Plans:

- This reviewer did not see a plan for resource sharing.

Budget and Period of Support:

Recommend as Requested

MEETING ROSTER

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CENTER FOR SCIENTIFIC REVIEW
RFA Panel: Molecular and Cellular Substrates of Complex Brain Disorders
ZRG1 MDCN-P (57) R
July 14, 2014

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.