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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: Compared to children in the general population, children with loss-of function mutations in the Neurofibromatosis type 1 (NF1) gene have greatly increased rates of autism spectrum disorder (ASD). Social and communicative disabilities in NF1 patients are among the greatest contributors to disease morbidity. Yet, the mechanisms by which loss of NF1 results in ASD and social deficits remain largely unknown, and no treatments effectively address these pervasive issues. Lack of progress in this area derives in part from a paucity of experimentally tractable animal models of social deficits in NF1. The *Drosophila melanogaster* model of NF1 recapitulates many features of the human disease; insights from the fly have led to important advances in NF1 biology and therapeutics. We find that *Drosophila* NF1 mutants display prominent impairments in social behaviors. We are poised to use this model towards a mechanistic understanding of how social deficits arise in NF1, and to define new treatment targets. Scope: Our data demonstrate that social impairments in NF1 mutant flies arise from a specific defect in peripheral sensory processing. The proposed studies will build on these data to establish the role of Nf1 in sensory gating within behaviorally relevant neural circuits. Experiments aim to determine the mechanism through which loss of Nf1 impairs sensory neuron function, define how impaired sensation translates to altered brain activity and disrupted behavioral output, and identify small molecules targets that can restore normal behavioral output. Major findings: We have made major progress towards each aim of the proposed work. This includes development of machine-learning approaches for automated behavioral annotation of social behaviors in flies, which will greatly accelerate all proposed aims. In addition, we have set the stage for initiation of the small molecule screen by defining the precise time window required for rescue of the Nf1 gene in order restore normal behaviors.

15. SUBJECT TERMS

Drosophila, social, behavior, autism, sensory processing, brain, circuits

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Introduction

Compared to children in the general population, children with loss-of function mutations in the Neurofibromatosis type 1 (NF1) gene have greatly increased rates of autism spectrum disorder (ASD). Studies suggest rates of ASD are 25-50% in NF1 (1-2% in general population), with NF1 patients being 13 times more likely to exhibit highly elevated ASD symptom burden. Social and communicative disabilities in NF1 patients are among the greatest contributors to disease morbidity. Yet, the mechanisms by which loss of NF1 results in ASD and social deficits remain largely unknown, and no treatments effectively address these pervasive issues. Lack of progress in this area derives in part from a paucity of experimentally tractable animal models of social deficits in NF1. The *Drosophila melanogaster* model of NF1 recapitulates many features of the human disease; insights from the fly have led to important advances in NF1 biology and therapeutics. We find that *Drosophila* NF1 mutants display prominent impairments in social behaviors. We are poised to use this model towards a mechanistic understanding of how social deficits arise in NF1, and to define new treatment targets.

Keywords

Drosophila, social, behavior, autism, sensory processing, brain, circuits

Accomplishments

What were the major goals of the project?

Major Task 1: Determine the mechanism through which NF1 regulates chemosensory neuronal function.
Major Task 2: Determine how sensory dysregulation in NF1 alters neural coding of social experience in the brain.
Major Task 3 Identify small molecule modifiers of NF1-associated social dysregulation.

What was accomplished under these goals?

1) Major activities: We report significant progress towards multiples goals of the funded project. Research has focused on determining the intracellular Nf1-dependent pathways utilized for coordinating social behaviors, and on launching the small molecule screen that will identify novel compounds to modulate social behavioral disturbances.

First, we have acquired all tools required for a complete understanding of intracellular Nf1 pathways related fly social behaviors. This includes numerous Nf1 deletion mutants, which are being re-expressed in an Nf1 mutant background. Specifically we have generated the following mutants:

pUAST-attB-C-3xFLAG-dNf1(RF) WT
pUAST-attB-C-3xFLAG-dNf1(RF) DN-term (D13-491)
pUAST-attB-C-3xFLAG-dNf1(RF) D Box 1 (D48-56)
pUAST-attB-C-3xFLAG-dNf1(RF) D N-IRA (D492-1093)
pUAST-attB-C-3xFLAG-dNf1(RF) DGRD (D1219-1580)
pUAST-attB-C-3xFLAG-dNf1(RF) DSec14 (D16111-1769)
pUAST-attB-C-3xFLAG-dNf1(RF) D C-term (D2266-2734)
pUAST-attB-C-3xFLAG-dNf1(RF) D N-IRA and DC-IRA del (D492-1093 + D1770-2263)
pUAST-attB-C-3xFLAG-dNf1(RF) D C-IRA (D1770-2263)

Early results converge, as expected, on the relevance of the GAP related domain (GRD). Specifically, we suspect that deletion mutants of the GRD fail to restore normal behaviors. Importantly, rescue in these experiments is specific to the key sensory neurons defined by ppk23. To further investigate the GRD, we have obtained rescue transgenes containing only the GRD, as well as mutants with specific disruption to the catalytic domain, rendering it enzymatically inactive. In addition, we hypothesized that Ras signaling in ppk23+ neurons is required for normal social behaviors. A knockdown strategy of key molecules upstream of Nf1 is being pursued, targeting Alk and Alk ligands. We have obtained transgenic flies to test downstream Ras effectors as well, with experiments in progress.

Second, we have made major progress on high-throughput platforms for a small molecule screen. A major limiting factor for *Drosophila* social behavioral assays is analysis of social interactions. Previously, these interactions were scored manually. Over the funding period, we have utilized machine learning approaches to develop automated behavioral annotation of social interactions between flies. These approaches have dramatically improved our ability to rapidly analyze data, benefiting all aims of the proposal. Most notably, this platform sets the stage for the drug screen proposed in task 3. Another key issue for this screen is timing of

drug delivery. Over the past year, we have performed experiments aimed to defining the key window required for rescue of social behaviors, using both genetic and pharmacologic approaches. Our results provide crucial guidance for timing of drug delivery, setting the stage for results with translational potential.

2) Specific objectives: over the funding period, as described above, our objectives were to define the Nf1 protein domains required for coordinating social behaviors in flies, and to generate foundational work for a small molecule screen. We have been successful in both of these objectives over the funding period.

3) Significant results and key outcomes: As detailed above in section (1), we have made key progress on defining the domains of Nf1 that are required for its role in sensory neurons of the fly to coordinate social behaviors. We have obtained numerous fly lines that will facilitate this goal, and initial data supports the proposed hypothesis. We have also succeeding in automating the analysis of social interactions in flies, greatly accelerating our projects. Of note, we initially proposed a focus on Major tasks 1-2 during the first 12 months of the funding period, but due to COVID-related work restrictions, shifted our focus to tasks 1 and 3 (task 2 is more technically complicated and requires close work among a few different team members). Importantly, I have recruited two new team members to focus on the proposed work, a key step in the success of the project.

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?
Over the next funding period, I anticipate major progress on all aims of the proposed work.

1. We will complete the work outlined in task 1. We now have all transgenic flies in hand, and are rapidly performing behavioral assays to understand how distinct domains of the protein contribute to social behavioral restoration. We will also examine downstream signaling pathways using genetic approaches. The goal is to elucidate specific information regarding Nf1 function in a class of sensory neurons required for normal social behaviors. This approach will provide new insight into the intracellular functions of Nf1. The automated behavioral classifiers we have been developing should accelerate the pace of experiments.
2. We will begin work on task 2 of the proposal. A new postdoctoral fellow in the lab will lead these efforts. We hypothesize that P1 courtship “command” neurons in the brain of NF1 mutants are inappropriately active in the presence of a male fly due to loss of ppk23-dependent inhibitory input. To test this idea directly, we will monitor neural responses of P1 cells before and during social interactions *in vivo*. We will also assess P1 activity following a social interaction to define neural correlates of the altered behavioral state. We anticipate refining the in vivo assay during the next funding period, setting the stage for successful completion of this task by the end of the proposed 3 year timeline.
3. We made major progress on the foundation for a small molecule screen, leveraging the fly model to discover novel compounds to treat social differences in NF1. Over the next funding period, we will initiate the small molecule screen. This process will be facilitated by the automated behavioral classifiers we developed over the past year.

Impact

What was the impact on the development of the principal discipline(s) of the project?

NF1 is caused by mutations in the neurofibromin 1 gene, and resulting loss of function of the protein product. Neurofibromin 1 normally serves as a key regulator of another protein, Ras. Without neurofibromin to regulate Ras in NF1 patients, cells divide in an uncontrolled manner, leading to tumors. In contrast to the tumorigenic symptoms of NF1, little is known about the molecular and cellular basis of social behavioral differences, limiting the design of novel treatment strategies. The short- and long-term goals of this research proposal are to determine the mechanisms by which neurofibromin 1 regulates social function, and to identify new drugs to antagonize social deficits in an NF1 animal model. Our work will define how Nf1 acts to promote normal sensory neuron function (Aim 1). This will have a major impact as results (in combination with Aim 3) can potentially inform patient treatment options using FDA approved medications, based on the intracellular pathways required for Nf1 in sensory cells. Moreover, this work will likely trigger a crucial conceptual shift in the field to direct murine and human research towards detailed examination of sensory processing in children with NF1. Similarly, Aim 2 will provide the first explanation as to how sensory errors lead to behavioral errors at a

neural level, directing future work in murine and human research. Testing the possibility in flies that transient sensory experiences lead to persistent brain state changes will be particularly impactful, as this line of work would suggest behavioral interventions in humans must consider how the brain processes sensory experiences on longer time scales.

What was the impact on other disciplines?

Identification of available and novel bioactive small molecules that modify the social deficits in flies will impact the development of strategies to treat social deficits in NF1 patients and possibly other causes of ASD. Similar fly-based discovery paths have been successful in other neurodevelopmental diseases, and thus are very likely over the long-term to be highly relevant to humans.

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?

A deeper understanding of the specific defect we have discovered will set the stage for bench to bedside translation. Results from this work have potential to benefit NF1 patients with comorbid autism spectrum disorder by providing basic insights into how autistic features arise and thereby guiding the field to focus on sensory processing. This has potential to impact stigma associated with ASD in general at a societal level.

Changes/Problems
Nothing to report

Products
Nothing to report

Participants & Other Collaborating Organizations
What individuals have worked on the project?

Name:	*Matthew Kayser*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0003-2359-4967
Nearest person month worked:	3
Contribution to Project:	*Dr. Kayser oversaw the project and personnel, directed experiments, analyzed and interpreted data.*
Funding Support:	*N/A*

Name:	*Emilia Moscato*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.5
Contribution to Project:	*Dr. Moscato designed and conducted experiments, analyzed and interpreted data, and generated reagents.*
Funding Support:	*N/A*

Name:	*Benjamin Mainwaring*
Project Role:	*Research technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3.6
Contribution to Project:	*Mr. Mainwaring conducted experiments and developed automated behavioral classifiers.*
Funding Support:	*N/A*

Name:	*Cheuk Wong*
Project Role:	*Research technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Mr. Wong conducted experiments, analyzed and interpreted data, and worked on automated behavioral classifiers.*
Funding Support:	*This funding source and Burroughs Wellcome Fund*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Yes. During the funding period, the PI obtained 2 new NIH grants, neither of which have any overlap with DoD support or impact the percent effort spent on this DoD project.

R56AG071777 (Kayser, PI; Bonini, Co-PI) 04/01/2021 - 03/30/2022 1.8 cal
NIH/NIA
Deciphering the molecular interplay of sleep and neurodegeneration with Drosophila

The goal of the award is to use *Drosophila* to investigate key molecules that link sleep and degeneration of the brain. The critical cell types involved will be determined, and behavioral approaches to modifying sleep will be established.

Role: PI

R01NS120979 (Kayser) 09/01/2021 - 08/31/2025 2.0 cal
NIH/NINDS
Molecular and genetic analysis of the juvenile sleep state
The goals of the award are to investigate the genetic regulation of sleep maturation using *Drosophila*, with a focus on defining the early developmental mechanisms that establish normal sleep behaviors and circuits in juvenile adults.
Role: PI

What other organizations were involved as partners?
Nothing to report

Special Reporting Requirements
Nothing to report

Appendices
None