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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. Recent findings implicate specific ion channels acting downstream of Nf1 to effect sensory neuron excitability. In addition, we have made progress on refining *in vivo* recording of brain activity in Nf1 mutant flies during social interactions.

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?

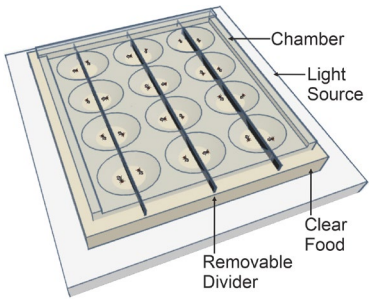
Major Task 1: Determine the mechanism through which NF1 regulates chemosensory neuronal function.
Major Task 2: Determine how sensory dysregulation in NF1 alters neural coding of social experience in the brain.
Major Task 3 Identify small molecule modifiers of NF1-associated social dysregulation.

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, refining automated behavioral annotation of social behaviors to facilitate high-throughput behavioral analysis, and obtaining *in vivo* measures of brain activity in Nf1 mutant flies during social interactions.

First, regarding the intracellular role for Nf1 in sensory neurons excitability, our results support the anticipated role of the GAP related domain (GRD). Specifically, deletion mutants of the GRD appears to 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 will utilize 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. Finally, existing results suggest a role for specific ion channels downstream of Nf1, which are poised to impinge upon cellular excitability. Specifically, small conductance Ca^{2+}-activated K^+ (SK) channels and Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are known to be influenced by Nf1, and we are examining a causal role for these channels in ppk23 neuron excitability changes in the NF1 model.

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. Moreover, animals had to be individually manipulated and handled numerous times over their lifespan prior to running the behavioral assay. We have developed a new behavior chamber based on existing models in the field. Instead of isolating newly hatched flies and maintaining them in individual vials, flies are placed directly into the chamber in which behavior will be assessed. The chamber sits on a layer of clear food, and flies remain separated by removable dividers (see Figure). After reaching mature adulthood, dividers are removed, and pairs of flies interact while their behavior is recorded. This advance dramatically increases throughput by eliminating daily tasks, reduces variability and error related to fly manipulations/handling, and facilitates automated analysis of fly behavior.



Finally, a postdoctoral fellow in the lab, Dr. Jadwiga Bilchak, has gained expertise and refined our ability to pursue Aim 2 (recording of brain activity in awake, behaving NF1 mutants). Dr. Bilchak has begun collecting data towards this Aim, and we are on track to successfully complete these research endeavors as proposed.

2) Specific objectives: over the funding period, as described above, our objectives were to define the Nf1 protein domains and downstream intracellular signals required for coordinating social behaviors in flies, to pursue a small molecule screen, and to begin determining how neuronal activity of social behavioral output loci are altered in Nf1 mutants. We have been successful in 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 begun to identify specific proteins downstream of Nf1 that directly impact neuronal excitability. A new postdoctoral fellow, Dr. Jadwiga Bilchak, began in the lab January 2022 and is leading our research efforts on NF1. Dr. Bilchak is supported by an institutional NIH T32 as she works on this 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. In addition to what we proposed, we anticipate defining specific ion channels whose function is disrupted with loss of Nf1. 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 developed and new behavioral chambers will accelerate the pace of experiments.
2. We will continue work on task 2 of the proposal. Dr. Jadwiga Bilchak is leading 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.
3. We made major progress on the small molecule screen, leveraging the fly model to discover novel compounds to treat social differences in NF1. Over the next funding period, we will continue to pursue this screen. This process will be facilitated by the automated behavioral classifiers and new chambers 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:	2.4
Contribution to Project:	*Dr. Kayser oversaw the project and personnel, directed experiments, analyzed and interpreted data.*
Funding Support:	*N/A*

Name:	*Jenny Luong*
Project Role:	*Senior Research Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	*Dr. Luong 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:	4
Contribution to Project:	*Mr. Mainwaring conducted experiments and developed automated behavioral classifiers.*
Funding Support:	*N/A*

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 1 new NIH grant, which has no overlap with DoD support or impact the percent effort spent on this DoD project.

R01AG071777 (Kayser, PI; Bonini, Co-PI) 02/01/2022 - 01/31/2027 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

What other organizations were involved as partners?
Nothing to report

Special Reporting Requirements
Nothing to report

Appendices
None