**Abstract**

Autism spectrum disorders (ASDs) are characterized by deficits in social interactions, language development and repetitive behaviors. Because of its link to genetics and neural development, and the severe abnormalities in social interaction by which it is defined, autism offers the opportunity for scientists to study the neurobiological origin of social communication skills basic to human behavior. From a clinical point of view, understanding the genetic and neurobiological disease mechanisms is critical for developing treatment.

Animal models such as mice and flies are widely used as models for ASD due to the available genetic toolkit in these model systems. Flies, in particular, offer an excellent model system for studying the mechanisms of ASD due to their level of complexity. With ~100 K neurons, flies show a complex repertoire of social behaviors, including an elaborate mating ritual, and due to the available toolkit and knowledge in this model system, scientists are able to identify the circuits responsible for specific social behaviors down to a small number of well-defined cells.

Using recent tools for recording and fine characterization of social behaviors in flies, we aim to determine the effect of ASD-linked mutations on social behaviors in male and female flies. Focusing on acoustic communication, we will quantify how social communication is modulated by specific mutants in three different experimental setups: (1) a playback-assay, in which change in speed in response to courtship song playback is measured in solitary flies; (2) a courtship assay, in which acoustic communication is quantified in male and female dyads; and (3) a novel assay (‘multifly assay’), in which social communication over a food patch is monitored and quantified for a group of sex-mixed flies. Following the behavioral analysis, we will characterize how gene expression in specific cell types in the male and female central nervous systems are causally linked to specific social deficits.

This study aims to present a new framework for understanding disease mechanisms for ASD, building on recent advances in computational ethology and on the emerging understanding of the neural circuits controlling social behaviors in fruit flies.

**Keywords:** Social behaviors, acoustic communication, social deficits, autism spectrum disorders, ASD, neurogenetic disorder, neurodevelopment.

**Abstract in Lay Language**

Autistic spectrum disorders (ASD) are characterized largely by impairment in social interactions. Understanding the mechanisms underlying ASD are critical for the development of treatment. Animal models such as mice and flies are commonly used to study the circuits and mechanisms underlying behavioral control in healthy and unhealthy brains. In the case of ASD, studies in animal models such as mice and flies have revealed multiple mechanisms by which specific genetic mutations (that is, loss of function in specific genes) are linked to impaired animal physiology and behavior. Since atypical social communication is a hallmark of ASD, and given recent advances in the quantification of social behaviors in flies, we propose to measure how specific ASD-related genes modulate social communication in flies. We will characterize in detail social behaviors in male and female flies in the context of mating behavior. Then, based on our knowledge of the circuitry underlying mating behaviors in flies, we will causally link social abnormalities with specific genes and neurons.

This study takes advantage of computational tools for the quantification of social behaviors in flies to extend our understanding of the neural basis of ASD. Using this novel framework will facilitate future studies focusing on other genes and on other pathological conditions with a strong genetic basis, and which are characterized by impairment in social communication.

**Research Objectives**

Autism spectrum disorders (ASDs) are characterized by deficits in social interactions, language development and repetitive behaviors. *Drosophila melanogaster* is used as a common model system for studying the genetic basis of ASD. Here, we propose to characterize social deficits in *Drosophila* ASD-mutants. We will use existing and novel setups to characterize social deficits in males and females, ranging from solitary flies (responding to playback of courtship song) to complex environments with multiple individuals. We will use computational tools for tracking and parsing social behaviors, in both sexes. Once the social phenotypes are characterized, we will look at deficits in the formation and activity of specific neural populations, which were previously shown to have a role in the control of mating behaviors in flies. Based on previous literature [], we will focus on neurons expressing the sex determination genes *doublesex* and *fruitless*.

The specific aims of this proposal are to:

1. Establish a new paradigm for quantifying social deficits in *Drosophila* ASD models, using machine learning-based tools for the quantification of social communication.
2. Quantify social deficits in males and females, finding sex-shared and sexual dimorphic phenotypes.
3. Determine how the expression of specific ASD-linked genes in defined sexually dimorphic cells is causally linked to quantifiable social deficits in male and female flies.

The first year of this project will be dedicated to Aims 1 and 2: building the setups, preparing the flies, collecting and analyzing the behavioral data. In the second year of the project, we will focus on understanding the mechanisms underlying social deficits in males or females, focusing on 1-2 genes and on males or females, or both, depending on our findings in the behavioral screen.

The behavioral experiments and analyses involved in completing Aims 1 and 2 are described below in detail, while the experimental details involved in completing Aim 3 are explained in less detail.

**Clinical Relevance**

Autism spectrum disorder (ASD) is a complex group of multi-factorial developmental disorders that leads to communication and behavioral defects. Many genes predisposing an individual to ASD have been identified, and understanding the causal disease mechanism(s) is critical to developing treatment. Genetic models such as *Drosophila melanogaster* have been of paramount importance in deciphering the significance of specific alterations driving specific abnormalities both structurally and behaviorally. Many of the ASD-associated genes, such as *fmr1, Neurexin* and *Neuroligins*,encode proteins that have conserved functions in neurons and during synapse development, both in humans and in the fruit fly.

It is well accepted that social reciprocity deficits are a core feature of ASD. Detailed quantification of the social deficits associated with ASD models in flies is expected to contribute to our understanding of the neural basis of ASD in three ways. First, we will establish a novel experimental pipeline to quantify how ASD-associated genes modulate social communication in flies. The same framework could be similarly used to quantify the phenotypes of other genes that are associated with social deficits in humans. Second, for a small number of selected genes, we will have a rich and unbiased characterization of how their deficits impact innate social behaviors in both males and females. Third, we will determine how specific genetic deficits are associated with specific social abnormalities. Taking advantage of the existing knowledge and tools in *Drosophila*, together with novel advanced tools for behavioral quantification, this project is expected to contribute significantly to our understanding of the genetic and neural basis of social pathologies such as ASD.

**Scientific and Technological Background**

One of the main criteria for diagnosing ASD as stated in the latest Diagnostic and Statistical Manual of Mental Disorders (DSM-5) is ‘persistent deficits in social communication and social interaction across multiple contexts’. These can manifest as a wide variety of deficits: from social-emotional reciprocity to verbal and nonverbal communicative behaviors needed for social interactions, as well as deficits in establishing and understanding relationships (American Psychiatric Association, 2013). Due to the available genetic tools in these model systems, both mice and flies serve as important model systems for ASD [], and social abnormalities have been associated with ASD-linked mutations in both model systems [].

Studies of the phenotypes associated with ASD-linked genes in *Drosophila* are often focused on characterizing structural abnormalities such as in arborization patterns [] and the morphology of pre- and post-synaptic terminals []. At the behavioral level, abnormalities have been documented in learning and habituation [] (including in ‘courtship condition’ []), sleep [], general activity [] and social behaviors [].

While social abnormalities are a main characteristic of ASD in humans [], and while *Drosophila* has long been used as a model for ASD [(Coll-Tané *et al.* 2019; Bellosta and Soldano 2019)](https://paperpile.com/c/CTngrH/kvgv%2BcX4C), social deficits in fly ASD models are less well characterized. Inter-fly interval is often measured in a ‘*Social Space Assay*’ [], and male singing has been characterized in a very few studies of ASD-associated genes [].

Mating behavior in *Drosophila* is complex. Males chase and circle the female, tap her abdomen, sing, lick her genitalia and make copulation attempts []. Females slow down, turn, open their vaginal plates, extrude their ovipositor, show various rejecting behaviors including shoving and head-butting and accept copulation attempts []. Novel tools based on machine-learning enable the tracking and parsing of social behaviors in flies [(Anderson and Perona 2014; Pereira, Shaevitz, and Murthy 2020; Pereira *et al.* 2022; Steinfath *et al.* 2021)](https://paperpile.com/c/CTngrH/HKNN%2B5PtE%2BiAKL%2BehFJ), therefore allowing an automated quantification of social phenotypes, in the context of mating behaviors, between groups of flies (e.g., mutants and controls, sharing a similar genetic background).

Neurons controlling different aspects of mating behavior have been identified in both sexes []. It has been shown that much of the circuitry involved in the control of sexual behaviors such as mating and fighting involves cells expressing the sex determination genes *doublesex* and *fruitless*. Experimentally, this is important, as it allows us to focus our search for underlying circuits and mechanisms to a relatively small subset of neurons []. Importantly, many *dsx+/fru+* cells are sexually dimorphic and are involved in sexually dimorphic behaviors. For example, dsx+pC2l cells respond to similar song features, but drive sexually dimorphic behaviors. Therefore, in searching for abnormalities in the fly CNS that are associated with changes in mating behavior, we will first focus on *dsx+/fru+* in the central nervous system of males and females.

Some genetic mutations in *Drosophila* ASD models have been previously associated with social abnormalities in *Drosophila*. Among them are mutants for *dfmr1*, Dnlg, rugose and nf1 (see Table 1). Previous work has described social deficits with mutation of fly homologs of the fragile X mental retardation gene (*dfmr1*) and ASD candidate genes, such as *neurobeachin* (*rugose*) and neurologin (*Dnlg-2* and *Dnlg-4*) ([Bolduc *et al.*, 2010](https://www.sciencedirect.com/science/article/pii/S2211124720308378#bib12); [Hahn *et al.*, 2013](https://www.sciencedirect.com/science/article/pii/S2211124720308378#bib27); [Wise *et al.*, 2015](https://www.sciencedirect.com/science/article/pii/S2211124720308378#bib68); [Corthals *et al.*, 2017](https://www.sciencedirect.com/science/article/pii/S2211124720308378#bib16)). Neuroligins (Nlgs) are a family of phylogenetically conserved postsynaptic adhesion molecules present in nematodes, insects and mammals (among others). Impaired function of Nlgs (particularly of Nlg 3 and 4) has been associated with ASDs in humans and impaired social and communication behavior in mice.

Compared with the general population, children with NF1 have greatly increased rates of ASD ([Adviento](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib1) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib1)*[, 2014](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib1); [Plasschaert](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54)*[, 2015](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54); [Morris](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib47) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib47)*[, 2016](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib47)). Studies suggest that rates of ASD are 25%–50% in NF1 (1%–2% in general population), with NF1 patients being 13 times more likely to exhibit a highly elevated ASD symptom burden ([Morris](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib47) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib47)*[, 2016](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib47)). Social and communicative disabilities stemming from ASDs in NF1 patients are among the greatest contributors to disease morbidity ([Plasschaert](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54)*[, 2015](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54)). Children with NF1 experience increased isolation and bullying ([Noll](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib48) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib48)*[, 2007](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib48)), difficulties on social tasks, and poorer social outcomes ([Barton and North, 2004](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib8); [Huijbregts](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib34) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib34)*[, 2010](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib34); [Plasschaert](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54) *[et al.](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54)*[, 2015](https://www.sciencedirect.com/science/article/pii/S2211124720308378%22%20%5Cl%20%22bib54)). In *Drosophila*, males with mutations in nf1 show social behavior impairments, due at least in part from peripheral chemosensory neuron dysfunction [(Moscato](https://paperpile.com/c/CTngrH/eDuP) *[et al.](https://paperpile.com/c/CTngrH/eDuP)* [2020)](https://paperpile.com/c/CTngrH/eDuP).

**Progress Report (2nd year only)**

**Research Plan**

Aims 5.1-5.3 focus on the behavioral screening for social deficits in ASD mutants, while Aim 5.4 year 2 is focused on deciphering the circuit and molecular mechanisms underlying social deficits in flies.

**5.1. Building and testing behavioral setups for monitoring social communication in flies**

We will build three assays for monitoring social communication in flies:

1. **Playback assay**: Linear tracks and a speaker for measuring the responses of solitary males and females to courtship song.
2. **Courtship assay**: A chamber for monitoring social interactions in pairs of flies, based on a setup we used previously [], including an option for optogenetic activation and inhibition using red and green light, respectively.
3. **Multifly assay**: Building a novel setup for the characterization of social interactions over a food source in larger groups of flies.

The **playback assay** includes 12 linear tracks for measuring the responses of males and females to playback of courtship song. We will use the same chamber for measuring the change in speed in solitary males and females in response to pulse and sine song, varying the song parameters as done previously [(Deutsch](https://paperpile.com/c/CTngrH/gVLu) *[et al.](https://paperpile.com/c/CTngrH/gVLu)* [2019)](https://paperpile.com/c/CTngrH/gVLu), and for measuring chasing behaviors in males [(Zhou](https://paperpile.com/c/CTngrH/Gtrc) *[et al.](https://paperpile.com/c/CTngrH/Gtrc)* [2015)](https://paperpile.com/c/CTngrH/Gtrc). Experiments in this assay are aimed at measuring the responses of males and female to courtship song, isolated from other modalities (e.g., vision, olfaction), and confounds (male and female behavior interact with each other in closed loop during social interactions).

The **courtship assay** is based on a previous design I used and was involved in building as a post-doc in the Murthy lab at Princeton University. It is [] mm in diameter, tiled with 9 pressure microphones [] for recording courtship song, and equipped with a top-view camera. The acoustic signal will be sampled at 10K by a data acquisition card (DAQ) and the camera will acquire video at 150 frames per second. We will have six courtship assays, to allow high throughput screening. With 30 minutes per experiment, we can realistically, and based on previous experience, run 72 pairs of flies per working day.

The **multifly assay** is a novel assay which has a top camera, but covers 3 times more area at the same spatial resolution (of 30 pixels per mm) and at the same temporal resolution. For this assay, we will use a fast-speed camera, with 26.2 MP, equipped with a CMOS near-IR sensor. We will use a near-IR sensor with quantum efficiency of 30% (QEXFF) at 850 nm. We will illuminate at 850 nm, which is outside the fly visual spectrum [], and outside the excitation spectrum of the Channelrhodopsins csChrimson [] and grACR1 [] that that we will use for optogenetic activation and inhibition, respectively.

In both setups, temperature (25°C±0.5°C) and humidity will be controlled [], as mating behavior in insects is sensitive to both temperature and humidity []. In the multifly assay, we will record courtship activity in groups of 4 males and 4 females (4X4) for 1 hour. Running 6 rounds per day, we will have 24 flies for each sex per day. Optogenetic activation will be used in follow-up experiments (see Aim 5.6, 2nd year), but we will build and test them in the first year.The basis of the multifly assay will include a food patch, which will be replaced between experiments. The addition of food is motivated by two reasons. First, in their natural habitats, flies typically aggregate on food patches, where they feed, fight and mate []. Second, young children with autism were reported to pay less attention to other individuals and their actions, and focus their attention instead on non-social objects. We therefore chose to have both social and non-social stimuli in the multifly assay, and measure the time flies spend engaging in social activities in each experimental group.

We will collect and analyze (see Aim 5.3) 100 pairs in the wild-type flies and 12 groups on 4X4 flies in the courtship and multifly assay, respectively.

The advantage of the courtship assay over the multifly assay is that it is tiled with microphones, and therefore allows better discrimination of song types. The multifly assay also allows song detection, but song-type discrimination is less precise (see Aim 5.3). However, it allows the quantification of interactions in larger groups of flies, therefore testing other aspects of social communication, such as male-male competition, aggressive song [(Versteven *et al.* 2017)](https://paperpile.com/c/CTngrH/ZJ5I), target switching and female aggression [(Gaspar, Dias, and Vasconcelos 2022)](https://paperpile.com/c/CTngrH/OAUS).

**5.2 Establishing a pipeline for unbiased quantification of social deficits in flies**

We will establish computational pipelines for the analysis of mating behaviors in both assays (see Fig. XX) based on our previous work [(Deutsch *et al.* 2020)](https://paperpile.com/c/CTngrH/0teW), using novel tools for pose estimation in multiple flies [(Pereira *et al.* 2022)](https://paperpile.com/c/CTngrH/iAKL) and song segmentation [(Steinfath *et al.* 2021)](https://paperpile.com/c/CTngrH/ehFJ). We will establish a similar pipeline analysis for the ‘multifly assay’, except that we will use video for inferring fly singing (see Figure xx). Using video instead of microphones makes it straightforward to assign song to the singing fly, even when two neighbor males are singing near each other. Also, not having microphones under the arena makes the surface more homogenous, therefore allowing better tracking of flies, at a lower rate of identity flips.

Identity flips occur occasionally when flies are interacting and their bodies overlap. In these cases, manual proofreading is needed. My lab is actively collaborating with Dr. Talmo Pereira [(Pereira *et al.* 2019, 2022)](https://paperpile.com/c/CTngrH/qoji%2BiAKL) from the Salk institute (San Diego), aiming to minimize identity flips in large groups of flies. This will be one of the aims for the first year of this project. Manual proofreading will still be needed, and will be done in my lab using SLEAP [(Pereira *et al.* 2022)](https://paperpile.com/c/CTngrH/iAKL).

Lastly, after tracking the flies, calculating kinematic features [] and inferring some dynamics for each fly (Fig xx), we will quantify the group dynamics, at the level of individuals, pairs and the whole group. For example, we will measure what triggers partner switching, using a generalized linear model, based on relative measures such as pairwise distances and relative angles between the flies.

**5.3 Creating genetic lines for the screening of social deficits in ASD mutants**

Based on previous literature, we chose four candidate ASD mutants for behavioral screening (see Table 1). We will outcross all mutants to a wild-type (WT) Canton S background for seven generations []. This will allow us to compare groups of flies with shared genetic backgrounds, therefore testing the net effect of the mutation.

**5.4 Screening ASD mutants for social deficits**

Using the behavioral assays we built (Aim 5.1), the computational pipelines we established (Aim 5.2) and the mutant flies we outcrossed to WT background, we will screen the four candidate mutants (see Table 1), looking for phenotypes that differentiate them from the WT controls, in both behavioral assays.

In the courtship and multifly assays, only the males or females will be mutated, while the other-sex flies will be wild-type. All the mutant flies will be in a wild-type background (see Aim 5.3).

In the playback assay, we will run 12 flies in parallel as before [(Deutsch *et al.* 2019)](https://paperpile.com/c/CTngrH/gVLu), scoring change in speed in response to pulse and sine song in solitary males and females (Fig. xx). We will vary the inter-pulse interval (see Fig. XX) of pulse-song (in the range 16-96 ms) and the frequency of sine song (in the range 100-350 Hz)

We will also measure the chasing index in groups of 8 males in the playback assay, in response to pulse song (16-96 ms) as done previously [(Zhou *et al.* 2015)](https://paperpile.com/c/CTngrH/Gtrc), see Fig xx.

In the courtship assay, we will have either a mutant male or mutant female, the other fly being wild-type. Wild-type pairs will be used as controls. We will measure both male singing (see Fig. XX) and the female responses to male singing (Fig. xx).

In the multifly assay, either the males or females will be mutated. The phenotypes in the multifly assay may be more complicated and require follow-up experiments to reveal the underlying circuitry. For example, if we identify differences in partner-switching probability by males in the presence of a female target in the vicinity of a courting male, we will test if this effect depends on vision, by repeating the experiment in the dark.

A satisfactory outcome of this aim will be identifying specific social deficits in one or more of the tested mutants. Based on the behavioral quantification of the identified deficits in the three behavioral assays, varying their level of complexity, we will choose which mutants to focus on, to reveal underlying mechanisms in the second year of the project.

**5.5 Deciphering the mechanism underlying social deficits in ASD fly mutants (year 2)**

Upon completion of the behavioral screen, we aim to reveal the mechanism(s) underlying detected social phenotypes in male and/or female brains. While social impairments could arise at both the periphery and in the central nervous system, we focus here on the role of neurons specifically in the fly brain. As a first step, we will measure changes in the number and morphology of *doublesex (dsx)-*expressing neurons in control and mutant flies. To stain *dsx+* cells, we will either use a monoclonal antibody that recognizes the male and female proteins DSXM and DSXF [(Mellert, Robinett, and Baker 2012)](https://paperpile.com/c/CTngrH/wJlL) or the UAS-Gal4 system as we have done previously (Fig xx [(Deutsch *et al.* 2019)](https://paperpile.com/c/CTngrH/gVLu)). We will examine expression of the mutated gene/protein in the same flies, (see Table 1).

We will image fly brains from each group using high-resolution confocal microscopy, and compare dsx+ cell number and morphology between control and mutated flies who share a similar genetic background. We will also verify that the protein product of the mutated gene is not detected in the mutant flies, using available antibodies. In follow-up experiments, we will measure fine morphological differences between mutated flies and controls using the UAS/GAL4 system, focusing on specific populations (dsx+pC1, dsx+pC2 and dsx+pCd).

Next, to test if the observed behavioral phenotypes are causally linked to the tested gene in specific neural populations, we will use the UAS/GAL4 system to perform rescue experiments as well as for cell-specific knockdown by RNAi for the tested genes (see Table 1).

Follow-up experiments will be conducted based on the experimental findings.

These may include:

1. Testing functional connectivity between *dsx+* cells, or between specific *dsx+* populations and known upstream/downstream targets, using csChrimson for activation and GCaMP for imaging of brain activity under a two-photon microscope as done previously [(Deutsch *et al.* 2020)](https://paperpile.com/c/CTngrH/0teW).
2. Single-cell RNAseq in specific *dsx+* cells to determine up- and downregulation of specific genes in mutant flies.
3. Two-photon calcium imaging of functional responses of *dsx+* neurons to courtship song in mutated versus control flies [(Deutsch *et al.* 2019)](https://paperpile.com/c/CTngrH/gVLu).

Our end goal is to link specific mechanisms in specific neurons in the fly CNS to specific social deficits. If the same gene causes social deficits in both sexes, by running the morphological and functional tests described above, we will reveal how sex-specific social deficits are linked to sexual dimorphic modulations of male and female neural circuits.

**Time schedule (Year 1)**

|  |
| --- |
| **Project Month** |
| **1-3** | **1-4** | **4-8** | **9-12** |
| Aims 5.1, 5.2: Building and testing behavioral setups for monitoring social communication in flies and establishing a pipeline for unbiased quantification of social deficits in flies | Aim 5.3: Ordering flies and creating genetic lines for the screening of social deficits in ASD mutants | Aim 5.4: Screening ASD mutants for social deficits – data collection | Aim 5.4: screening ASD mutants for social deficits – data analysis |

**Expected results and significance**

By the end of the first year we aim to complete the characterization of social traits in ASD-linked mutants that will serve as a basis for circuit and mechanism analysis in the second and following years. Specifically, we will quantify how selected mutants modulate the responses of male and female flies to courtship song (in solitary males and females, and in courted females), and how mating behaviors in complex, ethologically relevant settings are modulated by the mutation. This includes a characterization of the amount of time male and female flies are engaged in social (mating, fighting) versus non-social (e.g., grooming, eating) activities.

By the end of the project we hope to reveal mechanisms underlying social deficits in fly models for ASD, at the resolution of specific neurons and molecular mechanisms. This resolution is enabled by the relative simplicity of the fly brain (compared to rodent models) and the existing tools in the fly model system.

By accomplishing the objectives defined for this project, we hope to establish a new framework for the study of social communication deficits in ASD-linked mutations, as well as in other mutants whose associated pathology includes a major social deficit. We will use two established setups and one novel setup, and will share details about the experimental setups and open-source code for the behavioral analyses, allowing other groups to use this framework in future studies.

**Previous experience of applicant**

Dr. Deutsch completed his B.Sc. and M.Sc. in electrical engineering at Tel Aviv University. He earned his Ph.D. from the Weizmann Institute under the supervision or Prof. Ehud Ahissar and Prof. Elad Schneidman, studying ‘active sensing’ in the rat whisker system.

His post-doctoral studies were in the lab of Prof. Mala Murthy at the Princeton Neuroscience Institute, Princeton University, where he studied the neural basis of social communication in the fruit fly *Drosophila melanogaster*. During his post-doc, Dr. Deutsch contributed to building hardware and software for manipulating and measuring mating behavior and brain activity in sexually dimorphic cells. Specifically, Dr. Deutsch found shared and sexually dimorphic circuits for acoustic communication in flies, and revealed circuit mechanisms for the control of persistent social stated of aggression and receptivity in female flies. Dr. Deutsch has extensive experience in behavioral analysis, optogenetic activation, neural tracing and two-photon imaging.

Publications relevant to the current application:

1. Tomomi Karigo., **Deutsch D.**, Flexibility in neural circuits regulating mating behaviors in mice and flies. Front. Neural circuits 08 (2022).

2. Pereira T., Tabris N., Matsliah A., Turner D., Li J., Ravindranath S., Papadoyannis E., Normand E., **Deutsch D.**, Wang Z. Y., McKenzie-Smith G., … Shaevitz J., Murthy M. SLEAP: a deep learning system for multi-animal pose tracking. Nature Methods 19, 486-495 (2022).

3. Dorkenwald S.\*, McKellar C.\*, …., **Deutsch D.**, … Murthy M., Seung H. S. (\*Equal contribution). Online community for whole-brain connectomics. Nature Methods 19, 119-128 (2021).

4. **Deutsch D.**, Pacheco DA., Encarnacion-Rivera LJ., Pereira T., Fathy R., Clemens J., Girardin C., Calhouln AJ., Ireland EC., Burke AT., Dorkenwald S., McLellar C., Macrina T., Lu R., Lee K., Kemnitz N., Ih D., Castro M., Halageri A., Jordan C., Silversmith W., Wu J., Seung HS., Murthy M. The neural basis for a persistent internal state in *Drosophila* females. bioRxiv: https://doi.org/10.1101/2020.02.13.947952 (2020). eLife 9:e59502 DOI: 10.7554/eLife.59502 (2020).

5. **Deutsch D**.\*, Clemens J.\*, Thiberge SY., Guan G., Murthy M. (\*Equal contribution). Shared song detector neurons in *Drosophila* male and female brains drive sex-specific behaviors. bioRxiv: https://doi.org/10.1101/366765 (2018). Current Biology 29, 3200-3215 (2019).

**Resources**

Dr. Deutsch is a new faculty member in the Department of Neurobiology, University of Haifa, Israel. He was appointed senior lecturer starting Feb 2022, and arrived in Israel in August 2022 following my post-doctoral studies at Princeton University. His lab has a functional two-photon microscope. The rest of the lab, including dedicated rooms for the behavioral experiments described in this proposal, are under construction, and are expected to be ready in August 2022. Amplifiers for the behavioral rigs have already been built, and cameras have been ordered, such that he will be able to assemble the behavioral rigs as soon as the rooms are ready. The first three months of this project (Sep 2023–Nov 2023) will be dedicated to building the behavioral setups as stated above.

**Curriculum Vitae and List of publications**

**Current Position**

Senior lecturer University of Haifa, Israel 2022 - current

**Education**

Postdoctoral research associate Princeton University, USA 2014 - 2022

Ph.D. student, Neurobiology Weizmann Institute of Science, Israel 2008 - 2013

M.Sc., Electrical Engineering\* Tel Aviv University, Israel 2007

B.Sc., Electrical Engineering Tel Aviv University, Israel 2006

\* Direct MSc path for outstanding BSc students

**Funding**

1. Zuckerman STEM leadership program (October 2022-September 2026)

2. Contributed data and figures for two funded NIH BRAIN initiative projects: 1) ‘Population neural activity mediating sensory perception across modalities’ (1R01NS110060, 2019-2023) and 2) ‘Dissecting Sensorimotor Pathways Underlying Social Interactions: Models, Circuits, and Behavior’(1RO1NS104899, 2018-2022)

3. Recording from the barrel cortex of freely moving rats during a localization task. Collaboration with Prof. Anton Sirota, Germany, Berlin Family Foundation New Scientist Fund, WIS Project Number: 720664 (with Prof. Ehud Ahissar, 2012)

4. Whisking in a natural-like environment: A quantitative comparison between rodent strains, Kahn Family Center for Systems Biology (student research proposal, 2011)

5. Partial on-line guidance of surgical brain implants, Yeda-Sela Center of the Weizmann Institute of Technology (2011)

**Technical Contributions**

1. Developed a multisensory virtual reality setup for monitoring brain activity under a two-photon microscope (with Stephan Thiberge, Bezos Center, Princeton Neuroscience Institute), including Python interface for fly motion tracking and control of multisensory stimuli (<https://github.com/murthylab/fly-vr>).

2. Contributed to the development of an automated tool for neural segmentation in an Electron Microscopy dataset of an entire adult *Drosophila* brain (<https://flywire.ai/> 3).

3. Contributed to the development of SLEAP, a framework for multi-animal pose tracking via deep learning.

**Publications**

1. Tomomi Karigo., **Deutsch D.**, Flexibility in neural circuits regulating mating behaviors in mice and flies. Front. Neural Circuits 08 (2022).

2. Pereira T., Tabris N., Matsliah A., Turner D., Li J., Ravindranath S., Papadoyannis E., Normand E., **Deutsch D.**, Wang Z. Y., McKenzie-Smith G., Mitelut C., Castro M., D’Uva J., Kislin M., Sanes D, Sa D. Kocher S., Wang S., Falkner A., Shaevitz J., Murthy M. SLEAP: a deep learning system for multi-animal pose tracking. Nature Methods 19, 486-495 (2022).

3. Dorkenwald S.\*, McKellar C.\*, Macrina T.\*, Kemnitz N.\*, Lee K.\*, Lu R.\*, Wu J.\*, Popovych S., Mitchell E., Nehoran B., Jia Z., Bae J. A., Mu S., Ih D., Castro M., Ogedengbe O., Halageri A., Ashwood Z., Zung J., Collman F., Schneider-Mizell C., Jordan C., Silversmith W., Baker C., **Deutsch D.**, Encarnacion-Rivera L., Kumar S., Burke A., Gager J., Hebditch J., Koolman S., Moore M., Morejohn S., Silverman B., Willie K., Willie R., Yu SC., Li K., Murthy M., Seung H. S. (\*Equal contribution). Online community for whole-brain connectomics. Nature Methods 19, 119-128 (2021).

4. **Deutsch D.**, Pacheco DA., Encarnacion-Rivera LJ., Pereira T., Fathy R., Clemens J., Girardin C., Calhouln AJ., Ireland EC., Burke AT., Dorkenwald S., McLellar C., Macrina T., Lu R., Lee K., Kemnitz N., Ih D., Castro M., Halageri A., Jordan C., Silversmith W., Wu J., Seung HS., Murthy M. The neural basis for a persistent internal state in *Drosophila* females. bioRxiv: https://doi.org/10.1101/2020.02.13.947952 (2020). eLife 9:e59502 DOI: 10.7554/eLife.59502 (2020).

5. **Deutsch D**.\*, Clemens J.\*, Thiberge SY., Guan G., Murthy M. (\*Equal contribution). Shared song detector neurons in *Drosophila* male and female brains drive sex-specific behaviors. bioRxiv: https://doi.org/10.1101/366765 (2018). Current Biology 29, 3200-3215 (2019).

6. Wallach A.\*, **Deutsch D.\***, Oram T.\*, Ahissar E. (\*Equal contribution). Predictive whisker kinematics reveal context dependent attentional shifts. PLoS Biol 18(5) (2020)

7. **Deutsch D.**, Schneidman E., Ahissar E. Generalization of object localization from whiskers to other body parts in freely-moving rats. Front. Integr. Neurosci. (2019)

8. ShermanD., OramT., **DeutschD**., GordonG., Ahissar E., and Harel D. Tactile modulation of

whisking via the brainstem loop: Statechart modeling and experimental validation. PLoS ONE 8, e79831 (2013)

9. Bagdasarian K\*., SzwedM.\*,Knutsen PM.\*, **Deutsch** **D**.\*, Derdikman D., Pietr M.

SimonyE.and Ahissar E. (\*Equal contribution). Pre-neuronal morphological processing of

object location by individual whiskers. Nat. Neurosci. 16, 622–631 (2013)

10. **Deutsch D.**, Pietr, M., Knutsen P.M., Ahissar E. and Schneidman E. Fast feedback in active

sensing: touch-induced changes to whisker-object interaction. PLoS ONE **7**, e44272 (2012)

11. **Deutsch D.**, Natan A., Shapira Y. and Kronik L.,Electrostatic Properties of Adsorbed Polar Molecules: Opposite Behavior of a Single Molecule and a Molecular Monolayer. J. AM. CHEM. SOC. 129, 2989-2997 (2007)

**Manuscripts in progress**

1. Normand E., Wang M., Murthy M., **Deutsch D.**, Visual and auditory cues shape fast social response in *Drosophila* females. In preparation.

2. **Deutsch D.**\*, Wang K.\*, Matsliah A., Dickson B., Murthy M., (\*Equal contribution). The connectome of sexually dimorphic cells in the *Drosophila* brain. In preparation.

**Selected Oral Presentations**

1. **Deutsch D.**, Neural circuits for mating and aggressive behaviors in *Drosophila.* Seminar at Ben Gurion University (2022)

2. **Deutsch D.**, Sexually dimorphic circuits for mating and aggressive behaviors in *Drosophila*. A research workshop: Sexual dimorphism of neuronal circuits and behavior. Weizmann Institute, Israel (2022)

3. **Deutsch D.** Neural circuits for flexible social behaviors (virtual). Department of neurobiology, University of Haifa, Israel (2021)

4. **Deutsch D.** Neural circuits for flexible social behaviors (virtual). Department of medical neurobiology and department of cognition, Hebrew university, Israel (2020)

5. **Deutsch D.** Neural circuits for flexible social behaviors (virtual), Faculty of Medicine, Technion, Israel (2021)

6. **Deutsch D.**, Pacheco D., Encarnacion-Rivera L., Pereira T., Fathy R., Calhoun A., Ireland E. FlyWire Team and Murthy M. Internal brain states shape social communication in *Drosophila* females. ISFN (virtual), Eilat, Israel (2021)

7. **Deutsch D.**, Pacheco D., Encarnacion-Rivera L., Pereira T., Fathy R., Calhoun A., Ireland E. FlyWire Team and Murthy M. The neural basis for persistent internal state in *Drosophila* females. Neural Circuits. CSHL (virtual), NY, USA (2020)

8. **Deutsch D.**, Pacheco D., Encarnacion-Rivera L., Pereira T., Fathy R., Seung S., Murthy M. Social communication and sensorimotor transformation. Department of medical neurobiology, the Hebrew university Hadassah medical school, Israel (2020)

9. **Deutsch D.**, Pacheco D., Encarnacion-Rivera L., Pereira T., Fathy R., Seung S., Murthy M. Social communication and sensorimotor transformation. ISFN, Eilat, Israel (2020)

10. **Deutsch D.**, Clemens J., Murthy M. Connecting neural and behavioral tuning for acoustic communication signals. ISFN, Eilat, Israel (2017)

11. **Deutsch D.**, Clemens J., Murthy M. From courtship song processing to behavior in *Drosophila*. Sense to Synapse, NYU university, USA (2017)

12. **Deutsch D.**, Clemens J., Murthy M., From courtship song to behavior in *Drosophila*. Neurobiology of Drosophila. CSHL, NY, USA (2017)

13. **Deutsch D.**, Schneidman E., Ahissar E., The use of whiskers and body in object localization. ISFN, Eilat, Israel (2011)

14. **Deutsch D.**, Pietr PM., Knutsen PM., Ahissar E., Schneidman E., Closed loop whisking – effects of contact. Barrels meeting, San-Diego, USA (2010)

15. **Deutsch D.**, Pietr PM., Knutsen PM., Ahissar E., Schneidman E., Contact-induced feedback on rat whisking. BIOTACT consortium meeting, Garmisch-Partenkirchen, Germany (2010)

16. **Deutsch D.**, Pietr PM., Knutsen PM., Ahissar E., Schneidman E., Contact-induced feedback during whisking. ISFN meeting, Eilat, Israel (2009)

**Teaching Experience**

Python for Biologists, University of Haifa, Israel 2022

International Summer School: Physics of Life (virtual) 2020

Summer course: Neurotechnologies for Analysis of Neural Dynamics. Princeton, NJ, USA 2017

**Military Service**

F-16 Co-pilot in the Israeli Air Force. Rank: Major 1994 – 2000

**Budget**

**\*Salaries $15,400**

**\*Consumable Supplies $2400**

**\*Animals $1,000**

**\*\* Equipment -**

**\*Other expenses $1,200**

 **Overhead $5,000**

 **Total $25,000**

**\* Please specify.**

**\*\* Please specify and justify. Will be granted in special cases only.**

**If you are applying for a Charles E. Smith Fellowship in honor of Prof. Joel Elkes, please describe the use of the additional Budget that will be available. (see page 2)**

**Budget Justification**

**Salaries:**

75% of a PhD student time will be devoted to this project. With a scholarship of 6000 NIS/month, $ 15,400 will cover ~75% of the scholarship.

**Animals:**

Ordering and shipping the flies, both for the experiments that we will conduct during the first year, and flies we will get during the first year, and prepare for the next year (this process – specifically backcrossing - takes several months, see the body of the proposal).

**Consumable Supplies:**

$200/month X 12 months for fly supply (food, vials/vial-stops, tapes)

**Other expenses:**

cover abstract submission, student participation in meetings and publication fees – all associated with this project.

**Salaries:

Other expenses:**

(publication fees)

**Table 1:** **Mutants to be Tested in this Study**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Mutant | References(*Drosophila*) | Associated disorder | Reported social phenotypes in flies | Antibody | UAS-RNAi |
| *dfmr1* | [(Dockendorff](https://paperpile.com/c/CTngrH/fJFP%2BqH4Y) *[et al.](https://paperpile.com/c/CTngrH/fJFP%2BqH4Y)* [2002; McBride](https://paperpile.com/c/CTngrH/fJFP%2BqH4Y) *[et al.](https://paperpile.com/c/CTngrH/fJFP%2BqH4Y)* [2005)](https://paperpile.com/c/CTngrH/fJFP%2BqH4Y) | Fragile X | Reduced wing vibrations and copulation attempt rate | +monoclonal | Bloomington#27484 |
| *nf1* | [(Dyson](https://paperpile.com/c/CTngrH/TT9x) *[et al.](https://paperpile.com/c/CTngrH/TT9x)* [2022)](https://paperpile.com/c/CTngrH/TT9x) | Neuro- fibromatosis Type 1 ([(Garg](https://paperpile.com/c/CTngrH/Mahv) *[et al.](https://paperpile.com/c/CTngrH/Mahv)* [2013)](https://paperpile.com/c/CTngrH/Mahv)) | Pheromone detection by contact [(Moscato](https://paperpile.com/c/CTngrH/eDuP) *[et al.](https://paperpile.com/c/CTngrH/eDuP)* [2020)](https://paperpile.com/c/CTngrH/eDuP) | +Monoclonal[(The](https://paperpile.com/c/CTngrH/Khnc) *[et al.](https://paperpile.com/c/CTngrH/Khnc)* [1997)](https://paperpile.com/c/CTngrH/Khnc) | VDRC #109637 |
| *dnlg2, dnlg4* | [(Hahn](https://paperpile.com/c/CTngrH/OiUN) *[et al.](https://paperpile.com/c/CTngrH/OiUN)* [2013)](https://paperpile.com/c/CTngrH/OiUN) | Nlg3,4 associated with autism [(Jamain](https://paperpile.com/c/CTngrH/BLMb) *[et al.](https://paperpile.com/c/CTngrH/BLMb)* [2003)](https://paperpile.com/c/CTngrH/BLMb) | Modulated courtship song [(Hahn](https://paperpile.com/c/CTngrH/OiUN) *[et al.](https://paperpile.com/c/CTngrH/OiUN)* [2013)](https://paperpile.com/c/CTngrH/OiUN); inter-fly- distance [(Corthals](https://paperpile.com/c/CTngrH/w8OX) *[et al.](https://paperpile.com/c/CTngrH/w8OX)* [2017)](https://paperpile.com/c/CTngrH/w8OX) | dnlg2[(Chen](https://paperpile.com/c/CTngrH/KlEv) *[et al.](https://paperpile.com/c/CTngrH/KlEv)* [2012)](https://paperpile.com/c/CTngrH/KlEv) dnlg4[(Li](https://paperpile.com/c/CTngrH/N5w9) *[et al.](https://paperpile.com/c/CTngrH/N5w9)* [2013)](https://paperpile.com/c/CTngrH/N5w9) | dnlg4-VDRC#V6791 |
| *rugose* | [(Wise](https://paperpile.com/c/CTngrH/cWIP) *[et al.](https://paperpile.com/c/CTngrH/cWIP)* [2015)](https://paperpile.com/c/CTngrH/cWIP) | Human homolog NBEA is ASD candidate gene [(Mulhern](https://paperpile.com/c/CTngrH/Izjj) *[et al.](https://paperpile.com/c/CTngrH/Izjj)* [2018)](https://paperpile.com/c/CTngrH/Izjj) | Inter-fly- distance[(Wise](https://paperpile.com/c/CTngrH/cWIP) *[et al.](https://paperpile.com/c/CTngrH/cWIP)* [2015)](https://paperpile.com/c/CTngrH/cWIP) | [(Volders](https://paperpile.com/c/CTngrH/B0QM) *[et al.](https://paperpile.com/c/CTngrH/B0QM)* [2012)](https://paperpile.com/c/CTngrH/B0QM) | DBSC#57703[(Zhao](https://paperpile.com/c/CTngrH/ZKVB%2BoC00) *[et al.](https://paperpile.com/c/CTngrH/ZKVB%2BoC00)* [2013; Perkins](https://paperpile.com/c/CTngrH/ZKVB%2BoC00) *[et al.](https://paperpile.com/c/CTngrH/ZKVB%2BoC00)* [2015)](https://paperpile.com/c/CTngrH/ZKVB%2BoC00) |

Mutants associated with ASD to be tested in this study.

**Missing Figure 1-3**

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