Sample Personal Statement – NSF

All research begins with an initial observation followed by persistent curiosity and the desire to unveil a specific pathway, or effect. For me, these observations always have revolved around animal behavior. Why do some fish hover at the surface of the tank while others swim swiftly from one side to the other? How does an insect pick up on a subtle scent out of so many odors that are present in the environment? What factors determine when an animal mates? Eats? Sleeps? Behavioral questions are boundless, but the current information on this subject does not begin to reveal the complexity of the molecular and neuronal pathways that lead to these behaviors. My undergraduate exposure to research and a solid background in molecular biology have provided me with the skills to focus on one of these questions in my graduate studies. My curiosity, diligence and joy for teaching have led to the opportunities that influenced my decision to obtain a doctorate in neurobiology and animal behavior.

I had no idea what getting a PhD would entail until I was exposed to my first laboratory experience, through the Amgen program at Columbia University, in summer 2009. The program was crucial to my development as a scientist because it was tailored to students who were interested in a research career and culminated with a presentation and a paper in the format of a scientific article. It was encouraging to meet many students who shared my passion for science, as well as my uncertainty about how to turn that passion into a career. In the Woolley lab's nurturing environment I honed my research skills and explored an independent project. My project dealt with determining the effects of rearing conditions on female song preference in Zebra finches (*Taeniopygia guttata*). Although I did not obtain conclusive results, the skills acquired in that lab were influential in my subsequent work and can be applied to my graduate-school research on zebra fish (*Danio rerio*). I learned not only how to handle zebra finches, but also how to address a scientific question effectively and how to adjust an experimental setup. I became even more interested in studying behavior, although I also learned that such research requires a great deal of patience since the model organism is not always receptive to the experimental setup. But getting results is so gratifying that it compensates for the hard work and inevitable initial frustrations.

My laboratory experience in the Woolley lab reinforced my decision to get my PhD in behavior. It is not only behavior that intrigues me, but also the way that genes lead to behavior through the activation of various downstream pathways, both neural and molecular. For almost a year, I have been conducting research in the Blau lab at New York University, which studies how genes generate circadian rhythms of behavior in *Drosophila.* I have been determining the effects of the Rho family of GTPases (proteins that are activated when bound to GTP and inactivated when bound to GDP) in sustaining circadian rhythms of behavior. Although I conduct independent research, I work closely with a graduate student who has shown me what to expect while working towards my PhD and who has demonstrated the commitment and organization skills needed to succeed. At the Blau lab, I have acquired many basic laboratory skills (i.e. qPCR, electrophoresis, building a construct) that will be useful in my intended project on aggression in zebra fish.

I wish to get my PhD also because of my great joy in teaching. I relish breaking up science into non-intimidating bits teaching it, which is why I work as a Biology tutor at my school and formerly as a Biology teaching assistant (TA). I currently lead Biology review sessions for which I formulate lesson plans and teach three times a week. I feel

that too many students are discouraged by the complexity of the subject and never get a chance to appreciate the subject as innovative and fascinating. My goal as a professor is to focus on the research that has resulted in the textbook facts, to provide a deeper and more meaningful understanding of the subject.

My work as a Biology TA, through the Higher Opportunities Program at New York University was particularly rewarding because it allowed me to work with students of a minority background. This program provides minority and low-income students with opportunities to succeed at NYU and in their later careers. I coached the students through a difficult subject while identifying with them. I think it is important that successful figures from all ethnic backgrounds provide a wide variety of role models. In my own life, there have been no prominent influential female figures. I have yet to meet a Hispanic female scientist, but I hope one day to be that role model for young women. I feel it my duty to be a strong presence in my community and lead by example. This is one reason I decided to join the club Women and Youth Supporting Each other (WYSE) at NYU. This curriculum-based mentor program goes weekly to a middle school in Brooklyn to provide Black and Latina girls with the resources and information to make informed decisions about their bodies, their relationships, and their options for the future. After being a part of the club for three years, and serving as a board member for two, I was elected president. WYSE has been an integral part of my college experience. I would love to start a similar program for students interested in science, at schools that have minimal resources and which might lack the appropriate forum to discuss and expand on that interest. The program could focus on discussing relevant topics, pairing students with mentors, and providing them with possible career options attainable with a Biology degree.

My laboratory experiences both at Columbia and NYU have elucidated the realities of conducting research, my teaching skills have been strengthened through my work as a tutor and TA, and my desire to spread knowledge to younger generations has been unveiled through my work as a mentor with WYSE. These experiences have defined my determination and given me the skills to take on the arduous challenge of obtaining a doctoral degree in neurobiology and behavior. I believe that these skills coupled with my patience, optimism, and passion for neurobiology and behavior will contribute to my success as a PhD student. The NSF fellowship would allow me to disseminate both my research and my enthusiasm for science to an international audience. This fellowship would give me the opportunity to make an impact my research not only in my field, but in my community and globally. This is why I consider receiving the fellowship imperative to my success as PhD student, as well as a scientist in academia.

Sample Research Statement – NSF

Characterizing the serotonergic brain regions and neuronal pathways implicated in zebrafish aggression

Keywords: *Danio rerio*, Aggression, Serotonin (5-HT) system, Vivo-morpholino (VMO), GAL4/UAS

As a prospective PhD student, I can only propose what I would like to study, given the opportunity. I am interested in characterizing the connection between the serotonin (5-HT) system and aggression in zebrafish (*Danio rerio*). More specifically, I would like to know what components of the serotonin system are most significant in aggression, and which serotonergic brain regions and neural pathways are behaviorally relevant to aggression. I am fascinated by the fact that altering a single gene can have resonating implications on downstream molecular and neuronal targets, and can sometimes be enough to completely alter a behavior. I have spent almost a year investigating the role of the Rho family of proteins in circadian rhythms using *Drosophila*. I would like to pursue my graduate studies in aggression because it is a nearly universal behavior in the animal kingdom that has not been extensively studied and has broad social relevance in our society [1]. In the last decade, zebrafish has emerged as a promising model for studying behavior [2]. As a model organism, zebrafish are prolific breeders, cheap, and easy to maintain. In addition, they share 70-80% homology with human genes [3] meaning that any genes studied in this system are likely to have a homolog in the human genome. The zebrafish brain has been considerably characterized, which would facilitate connecting aggression with a brain region or a neuronal pathway.

In general, the serotonin system has four major components: (1) serotonin synthesis via tryptophan hydroxylase (TPH), (2) serotonin degradation by monoamine oxidase (MAO), (3) serotonin reuptake from the synaptic cleft via a transporter (SERT), and (4) serotonin 5-HT receptors [1,4]. The serotonin system has generated interest because studies in rats and humans have shown a link between the serotonin system and aggression [1,5]. In addition, the genes *tph* and *htr1a* (codes for one of the receptors for serotonin) have been shown to be overexpressed in aggressive male zebrafish [4]. Although previous research supports the involvement of the serotonin system in aggression, the link has not been completely established in zebrafish and the behaviorally relevant brain regions and neuronal pathways have not been characterized.

I want to begin substantiating the link between 5-HT system and aggression by knocking down different components of the serotonin system and then testing their effect on zebrafish aggression. Whereas previous experiments [4] used pharmacological agents to disrupt the serotonin system, I would use vivo- morpholino (VMO), antisense oligonucleotides that knockdown gene expression in adult zebrafish [6]. I would introduce VMO in adulthood because serotonin has an essential developmental role and disrupting its expression in larvae could cause developmental defects. I could verify whether or not VMO has led to a significant decrease in protein levels by performing immunocytochemistry (ICC). I would test aggression by using the mirror image test [2].

Once I determine which components of the serotonin system are most closely linked to aggression, I would assess how their expression varies in aggressive v. nonaggressive zebrafish in order to identify brain regions pertinent to aggression. Using

in situ hybrization, I would compare levels of expression and localization for genes of interest and perform ICC to verify my in situ results. I would use the mirror image test to select for aggressive and nonaggressive adult zebrafish (3-4 months old).

Once I know which brain regions are most likely to be implicated in aggression, I can begin manipulating the subpopulation of serotonergic neurons in these regions using the Gal4/UAS system. The yeast-derived transcriptional activator Gal4 under the control of a cell or tissue-specific promoter/enhancer drives expression of an upstream activating sequence (UAS) transgenic construct and targets expression in specific cell populations [7]. I am familiar with this system because I have been using it for almost a year in *Drosophila* to induce expression of RNAi in specific neural groups that are involved in generating circadian rhythms of activity. ZTrap is a database that would allow me to identify transgenic fish that target cell populations of interest [8]. I would be able to inhibit activity and ablate specific populations of neurons by expressing tetanus neurotoxin tagged with CFP and *nsfB-mCherry* (causes DNA cross-linking), respectively [7]. I would use GAL4 under the control of a heat inducible promoter [7], so that I could try activating transcription of the UAS transgene in adulthood. Since I would be able to visualize GAL4 expression, I would concurrently stain for serotonergic neurons, using antibodies for SERT or TPH [9], to make sure that the GAL4/UAS targeted cells were serotonergic. Following these manipulations, the zebrafish would be tested for aggression in hopes of directly linking specific neural groups to aggressive behavior. 5-HT also has a role in neurogenesis, so it could be affecting aggression via changes in neural connectivity [9]. Identifying these serotonergic neurons and visualizing their connectivity using immunocytochemistry would begin to uncover neuronal pathways and associated brain regions involved in zebrafish aggression.

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Sample Previous Research – NSF

The most important lesson I have learned while conducting research is that failure is inevitable. Even the most meticulously planned out experiment can run into unforeseen problems. It is important to accept failure, learn from it and move on because in the end it is the anecdotes of initial failures that enrich every success story. I have also learned that it is crucial that I trust in my abilities as a scientist and the potential significance of my research in order to conduct research efficiently. Self-doubt only hinders productivity and the ability to think clearly about the research question at hand. My undergraduate research experiences have prepared me for the difficulties associated with research, but also have revealed how rewarding research can be and how gratifying it is to share results with peers both in and out of the scientific field.

My first research experience was through the Amgen scholars summer research program at Columbia University during summer 2009. That summer, I worked in a neuroscience and behavior lab under the guidance of Sarah M. N. Woolley, PhD, studying the effects of developmental experience on female song preference using zebra finches (*Taeniopygia guttata*). Zebra finches are sexually dimorphic, with only males being able to produce song [1], so the modes of song acquisition have been well characterized, but the mechanisms with which females acquire song preference have not. I was interested in female song preference because they are providing evolutionary pressures for certain types of songs and are the ones choosing the genes that will make it to the next generation. In order to determine how rearing conditions affected female song preference, I tested three groups of females for song preference: isolated females (no exposure to male song during development), cross-fostered females (raised by another species of finches, the blackheart finches), and normal females (exposed to male song during development). Females from each group were tested for preference for normal song, blackheart song and cross-fostered (males raised by blackheart finches) song. A great deal of time went into adjusting the experimental set up, so I was not able to obtain conclusive results from my study. However, the few trials that I conducted showed that cross-fostered females did not show a strong preference for normal zebra finch song. I worked independently on this project, and was responsible for all aspects of this experiment from song recording to preference testing.

The following year, I began conducting research at New York University in a neurobiology lab studying circadian activity in *Drosophila melanogaster* led by Justin Blau, PhD. Last summer I received a Dean Undergraduate Research Fellowship (DURF) to conduct research, and I am currently doing a 4-credit independent study continuing my research that will culminate in an honors thesis. I have been determining the role of the Rho family of GTPases [2] (proteins that are activated when bound to GTP and inactivated when bound to GDP) in sustaining circadian rhythms of behavior. The molecular oscillations that generate circadian rhythms have been well characterized [3], but the pathways activated downstream of the molecular clock have not. The Rho pathway is a potential downstream pathway because preliminary experiments show that two GEFs (Guanine exchange factor, activates GTPases by exchanging GDP for GTP) are differentially expressed (i.e. higher expression during the day v. night or vice versa), suggesting GTPase involvement in circadian rhythms. Also, knocking down expression of these GEFs leads to arrythmiticity (flies with no sustainable rhythm of activity). I am using RNAi to knockdown expression of specific GTPases (i.e. Rho1, Rac1, cdc42) in clock neurons (characterized by molecular oscillations that generate circadian rhythms)

and then testing their circadian activity in behavioral monitors [4] to determine in which clock cell groups GTPases are most significant. I have also been performing immunocytochemistry on flies that have yielded an interesting phenotype (i.e. deviation from 24 hr rhythms of activity) in order to gain insight on the effects of the GTPase knockdown on the expression and localization of clock proteins. So far, my results suggest that Rho1 has a role in sustaining circadian rhythms via PDF cells (master oscillators that help synchronize clock cells) [3] because knocking down its expression yields arrhythmic flies. Finally, I have also been developing an assay to visualize synaptic vesicles using FM1-43 dye [5] because GTPases are involved in vesicle trafficking and could be affecting circadian rhythms by altering vesicle release to target neurons. Since this dye has never been used to visualize PDF cells, I have spent the last few months troubleshooting this assay. At the Blau lab, I have learned many basic laboratory skills (i.e. qPCR, electrophoresis, *Drosophila* genetics) needed to conduct research in neurobiology. Although I conduct research independently, I work closely with a PhD student, Afroditi Petsakou who provides me with guidance in my research.

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