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Gαi and G¥30A act downstream of Tre1 in Drosophila courtship

Emily Mu

San Jose State University

Emily Mu

Major:
Biological Sciences, Molecular Biology

Mentor:
Dr. Rachael French,
Dr. Maria E. Cruz

Co-Author:
Cac Tran

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Biography

As a first-generation American and college student, Emily's fascination with the life sciences and passion for service drove her to work in a field that contributes to scientific and human advancement. Outside of her research in the French Laboratory and the McNair Scholars Program, she has been an active member of her campus community; having served as chapter president of Global Medical Brigades, acquiring alumni status in Alpha Omicron Pi women's fraternity, and participating in the San José State University NSF-REU, Research by Undergraduates Using Molecular Biology Techniques (RUMBA). Emily has led one of the three medical brigades she has participated in, providing her with a worldview that has stretched her research interests beyond the scope of biology. She is interested in human genetics, biochemistry, and global health. After earning her bachelor's degree, Emily will continue on to graduate school in pursuit of these research interests with the goal of one day mentoring, empowering, and advocating for students in academia.

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ABSTRACT

The role of genes in morphological development is well understood for a variety of model organisms, but there remains a gap in our understanding of how genetics mediate behavior. Are there master genes that regulate behavior? Answering this question will lead to a better understanding of the development and function of the central nervous system, eventually allowing us to map out the pathways that regulate specific behaviors. We are using *Drosophila melanogaster* as a model organism and the male courtship ritual as the behavior of interest to study the relationships between genes, neural development, and behavior. *Trapped in endoderm 1* (*Tre1*), a gene encoding an orphan G-protein coupled receptor (GPCR), is required for normal courtship behavior in fruit flies, but how this receptor regulates behavior is not yet understood. Here, we characterize the signaling cascade downstream of Tre1 by testing mutations in the *Drosophila* G-proteins for courtship defects similar to those seen in *Tre1*. Our results demonstrate that Gαi is a candidate downstream effector for Tre1, while also implicating Gγ30A in courtship behavior. Future goals include completing the characterization of the G-protein mutations and conducting experiments to explore the complex interaction between G-protein signaling and courtship initiation.

INTRODUCTION

Genes are responsible for the development of the central nervous system (CNS), which, in turn, mediates behavior. Many of the genetic pathways underlying morphological development in animals have been identified and well-characterized. In contrast, there is still much to be understood about how a gene or genes specify behavior (Demir and Dickson 2005). Elucidating the roles of genes with regard to particular behaviors has the potential to be a powerful set of tools to map out the molecular pathways that direct the wiring of the CNS.

Courtship behavior is observable and well characterized in *Drosophila melanogaster*, making it a practical model for the purposes of this study. In addition, the behavior is innate – it is "hard wired" into the brain. Only males perform the courtship ritual – and they are able to perform it even if they have been socially isolated since "birth." The courtship ritual is a stereotyped set of six distinct steps that must be executed correctly, and in the proper order, to ensure reproductive success (Baker *et al.* 2001). Because innate behaviors such as this do not have to be learned, they are ideal models for the study of how genes pattern the nervous system to elicit specific behavioral responses. We seek to expand our knowledge on this particular trait as it is so evolutionarily favored it is programmed into the *Drosophila* nervous system.

We previously demonstrated that the gene *Trapped in endoderm-1* (*Tre1)* is required for normal courtship behavior in fruit flies. Specifically, male flies in which Tre1-expressing cells are silenced, or with loss of function mutations in *Tre1*, initiate courtship much more rapidly than wild-type males (Luu *et al.* 2016). *Tre1* encodes an orphan G-protein coupled receptor (GPCR). In this work, we aimed to identify components of the G-protein signaling cascade downstream of *Tre1*. To accomplish this goal, we tested mutations disrupting subunits of heterotrimeric G-proteins for courtship initiation defects. Here, we show that both Gαi and Gγ30A are involved in courtship initiation, and that these genes may reveal a complex role for G-protein signaling in the programming of this behavior.

METHODS

Fly stocks

Fly stocks were maintained in a 25℃ incubator and grown on standard cornmeal/molasses medium. All strains of flies were acquired from the Bloomington *Drosophila* Stock Center at the University of Indiana. Fly stocks used were: *w;wild type Berline (*our laboratory wild type strain, used as the control genetic background), $GɣA^{EY11766}$(Bloomington Stock 20695), $GɣA^{e0084}$ (Bloomington Stock 17891), $GαO^{KG01266}$(Bloomington Stock 13714), $GαO^{MB00893}$ (Bloomington Stock 22924), $GαF^{MB10810}$ (Bloomington Stock 29157), and $GαI^{EY039776}$(Bloomington Stock 15698).

Courtship assays

For courtship assays, virgin males were kept in isolation for 2-3 days after eclosion. Each male was then presented with a single 1-2-day-old w^{1118}*; WTB* virgin female. Single male and female pairs were placed into custom plexiglass chambers 10 mm in diameter and 6 mm in height, separated by plastic transparencies. Contact between pairs was initiated by removal of the transparencies. Courtship behavior was recorded in infrared light for 20 minutes.

Statistical Analyses

An α level of 0.05 was used in all experiments. Statistics were performed on log-transformed means of wing song latency. Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD *post hoc* test. No statistical tests were used to predetermine sample sizes, but our sample sizes are consistent with those reported in previous publications (Tran *et al.* 2014; Luu *et al.* 2015). Data in figures are back-transformed.

RESULTS

Flies mutant for *Gαi* mutants phenocopy *Tre1*

In order to identify the G-proteins that transduce the *Tre1* signal, we began a systematic survey of courtship phenotypes in male flies mutant for all of the *Drosophila* heterotrimeric G-protein genes. The *Drosophila* genome encodes 8 predicted Gα subunits (*concertina* (*cta*), *Gαf, Gαi, Gαo, Gαq, Gαs, CG17760,* and *CG30054*). To date, we have tested loss-of-function alleles in *cta*, *Gαf, Gαi, Gαo,* and *Gαq*. We find that mutation of

cta, Gαf, Gαo, and *Gαq* have no consistent effect on courtship behavior (data not shown). However, mutation of *Gαi* consistently phenocopies the *Tre1* "rapid courtship" phenotype (Figure 1). While control animals initiated courtship in 108 seconds, on average, male flies homozygous for $G\alpha I^{EY03976b}$, a loss-of-function mutation in *Gαi*, initiate courtship approximately three times as fast, in an average time of 32 seconds ($P < 0.01$, student's T test, N = 24 males). These data are consistent with *Gαi* functioning downstream of the Tre1 receptor in courtship initiation.

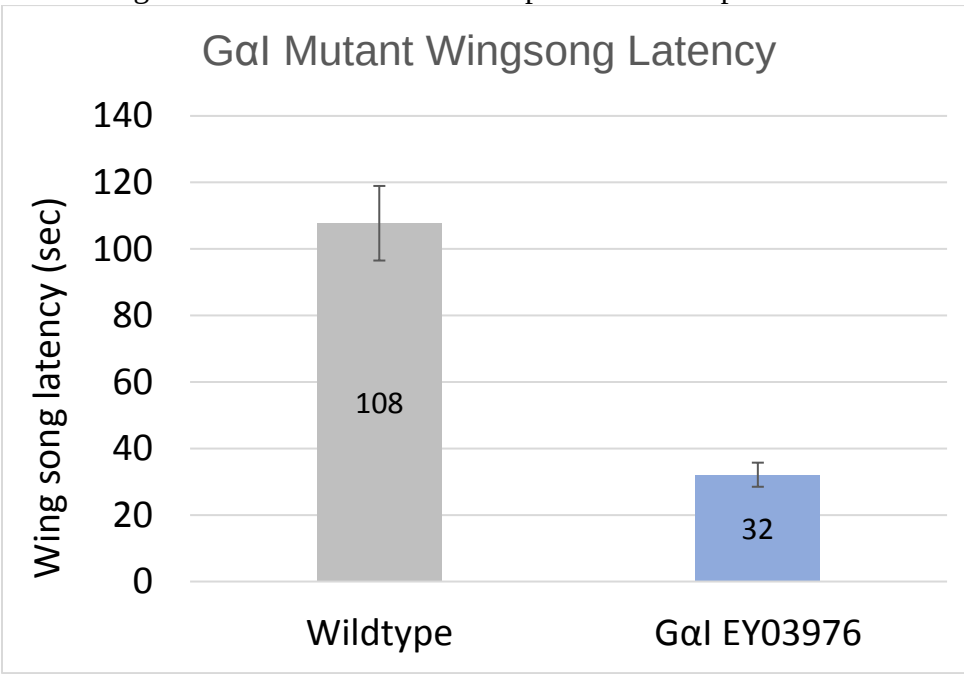

Figure 1. Flies mutant for *GαI* display rapid courtship initiation. $G\alpha I^{EY03976b}$ mutant flies initiate courtship in 32 seconds, compared to 108 seconds in wild type controls (*w; WTB)*. (p = 8.22991E-10, student's T-test, N = 24 males for each genotype)

GƳ30A mutants demonstrate slower courtship initiation

The Drosophila genome encodes two Gγ subunits, *Gγ1* and *Gγ30A*. We have tested loss-of-function mutations in both of these genes, and find that, while Gγ1 does not appear to affect courtship initiation, mutation of

Gγ30A consistently results in a longer time to courtship initiation. Figure 2 shows the average time to courtship initiation for two alleles of Gγ30A. Male flies homozygous for either allele display an increase in courtship initiation (N = 24 males, p = 0.123). Though these results do not achieve statistical significance, the consistency of the phenotype over multiple trials in two independent alleles suggests that Gγ30A is involved in courtship initiation. Additional experiments will be needed to confirm these results.

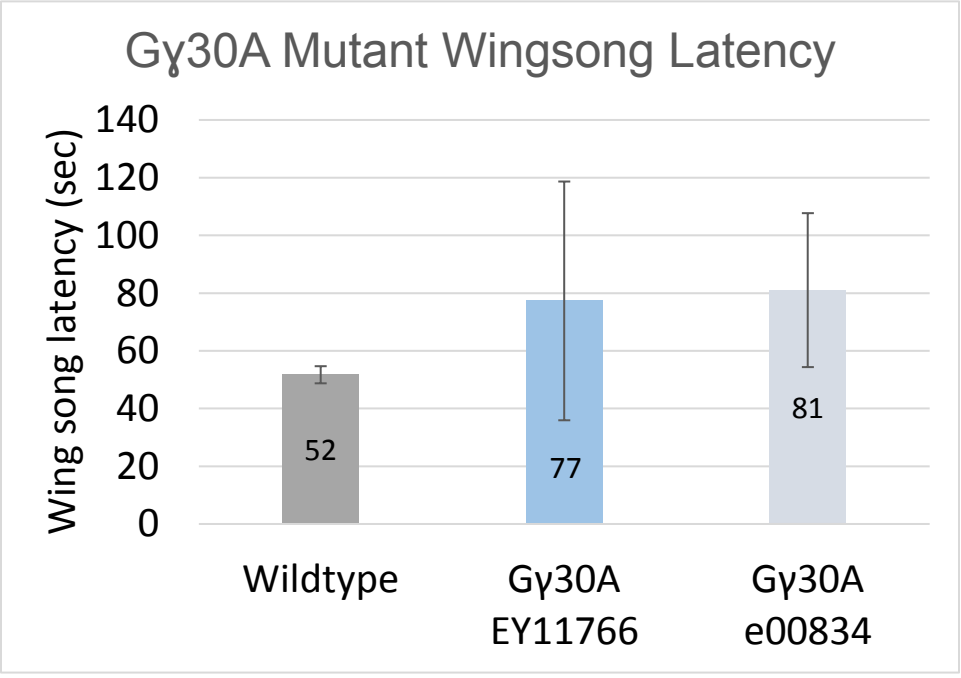

Figure 2. *GY30A* mutant males display slower courtship initiation. *GY30A* mutant males initiate courtship in 77-81 seconds compared with 52 seconds in wild type controls (w; WTB). (p = 0.123, one-way ANOVA, N = 24 males for each genotype).

DISCUSSION

We have begun a systematic analysis of the roles of all *Drosophila* G-protein genes in courtship initiation, in order to elucidate the signal transduction cascade downstream of Tre1. To date, we have screened 5 of

the 8 Gα subunits, and both Gγ subunits. We find that mutation of Gαi phenocopies the Tre1 rapid-courtship effect (Figure 1), strongly suggesting that Gαi functions downstream of the Tre1 GPCR. When activated, Gαi inhibits the activity of adenylate cyclase (reviewed in Birnbaumer, 2007). In mammals, Gαi is coupled to histamine H3 and H4 receptors (Interpro, http://www.ebi.ac.uk/interpro/entry/IPR003980). This is particularly interesting because we have evidence that Tre1 may function as a histamine receptor, and this would be the first example of a metabotropic histamine receptor in *Drosophila*, and only the second in any invertebrate (Zaki et al., 2017).

We also show evidence that Gγ30A is involved in courtship initiation, but the phenotype of Gγ30A mutants is the opposite of Gαi mutants (Figure 2). This difference in can be explained in at least two ways. First, this result is consistent with the function of Gβγ subunits as inhibitors of Gα activity (Clapham and Neer, 1997). Second, it may be that the function of Gγ30A is independent of Gαi, and the regulation of courtship initiation by G-protein signaling is more complex. In order to distinguish between these possibilities, we will begin testing signal transduction pathways downstream of both Gγ30A and Gαi, including mutations disrupting adenylate cyclase (downstream of both Gαi and Gγ), as well as PI3K (inhibited by Gγ, so expected to phenocopy Gγ30A) and phospholipase C β (activated by Gγ, so expected to have rapid courtship).

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