

## CHAPTER IV

### TEMPORAL INDUCTION OF TRANSGENIC DMARR1 IN THE *ARR1* MUTANT BACKGROUND

#### Preface

The experiments performed and results obtained in this chapter are unpublished. All fly maintenance and electrophysiological assays have been performed by the author.

#### Introduction

As previously stated, an olfactory-based mutant phenotype is exhibited by *arr1* and *arr2* mutant *Drosophila* flies (Merrill et al., 2003; Merrill et al., 2005). In these studies, the EAG was utilized to record odor-evoked changes in extracellular voltage potentials in the antennae, and comparisons were made between the arrestin mutant flies and their wild-type counterparts. Perhaps the most striking, and unexpected, aspect of this phenotype is the observation that, no matter the odorant being tested, mutant responses registered at levels lower than wild-type. It is presumed that the EAG represents a measurement of the change of the summed receptor potential of olfactory neurons, in response to odorant stimulation, in proximity to the recording electrode relative to a reference electrode, where no odor-stimulated change is expected (Alcorta, 1991; Ayer and Carlson, 1992). Furthermore, it is presumed that Arr1 and Arr2, which are indeed expressed in the antennae (Merrill et al., 2002), function in the deactivation of olfactory signal transduction pathways. Given these presumptions, one might expect that disruption of a prominent deactivating agent, such as these arrestins, would actually be conducive to olfactory responses that are higher than wild-type levels. In this scenario, wherein wild-type levels of arrestin are lacking, ORs, now uninhibited by arrestin protein deactivation, may remain active for a longer duration, thus facilitating a greater

transduction potential and larger EAG response. In fact, olfactory signal transduction output is indeed enhanced in mammalian olfactory cell culture systems when peptide inhibition is utilized to acutely disrupt  $\beta$ -Arrestin function (Dawson et al., 1993).

Accordingly, it has been hypothesized (Merrill et al., 2005) that the observed olfactory deficits in arrestin mutant flies are the result of a progressive or chronic down-regulation of olfactory components in the antennae, as a means of compensation for the lack of arrestin-regulated deactivation of receptor-mediated signaling. A similar compensatory phenomena pertaining to olfactory signal transduction-mediated second messenger production is indeed observed in mammalian cell culture when another olfactory deactivating agent, the G protein coupled Receptor Kinase, GRK3, is constitutively absent (Peppel et al., 1997).

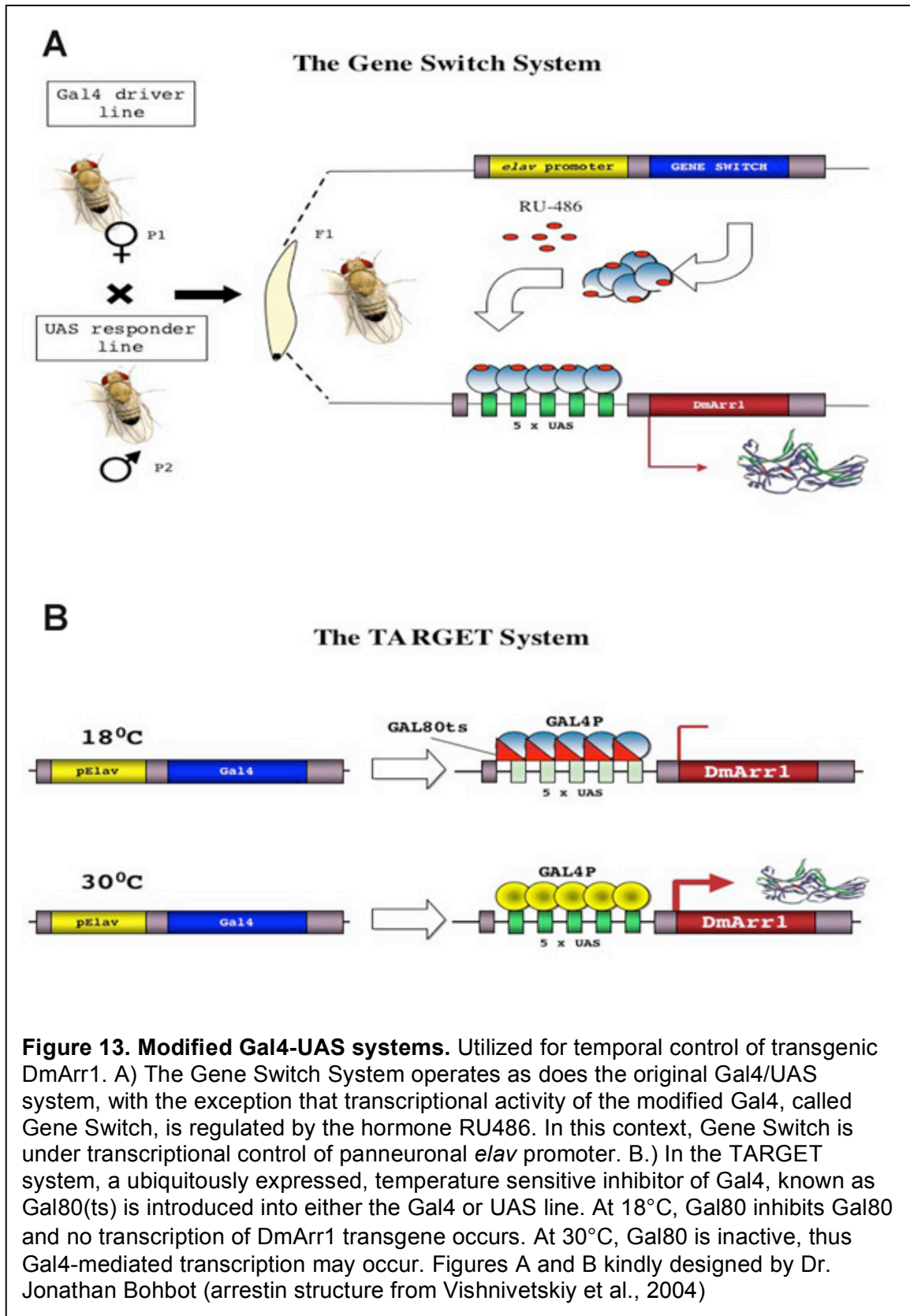
The genetic toolbox afforded by *D. melanogaster* allows for the opportunity to explore this hypothesis. One way, in particular, to do this is to utilize temporal transgene induction systems wherein transgenic arrestin can be induced at specific developmental stages or time-points in the adult fly lifespan. In fact, two convenient systems have been generated, both variations of the Gal4/UAS system (Brand and Perrimon, 1993), to spatially and temporally control transgene expression.

The Gene Switch system (Osterwalder et al., 2001; Roman et al., 2001) relies upon a modified version of Gal4 that is expressed under the control of its upstream promoter, but is only transcriptionally activated in the presence of its hormonal activator, mifepristone. This compound, also known as RU486, can be added directly to melted fly food, such that adult flies can consume it during normal feeding behaviour. In this system, preexisting UAS-responder fly lines may be crossed to any number of Gene Switch Gal4 driver lines to investigate specific questions in the presence or absence of the RU486 drug (Figure 13A).

Alternatively, the temporal and regional gene expression targeting (TARGET) system (McGuire et al., 2003) may be used to conduct similar experiments. In this case, temperature serves as the induction agent. Namely, a temperature sensitive allele of the Gal4 inhibitor, Gal80(ts), is introduced into the standard Gal4/UAS system. At 30°C, Gal80(ts) is inhibited and Gal4 mediated transcription occurs unhindered. At 18°C however, Gal80(ts) is active and Gal4 mediated transcription is blocked. Thus, in this system, flies containing the Gal4, Gal80(ts), and UAS constructs can be shifted from 18°C to 30°C or vice versa to induce or block Gal4 mediated gene expression (Figure 13B).

Returning to the question of the arrestin mutant olfactory deficits, a series of predictions can be made based upon the specific relation between the life stage of the fly and the induction of the arrestin transgene in arrestin-mutant background. For the experiments described in this chapter, both the Gene Switch and TARGET systems have been used. Moreover, two different conditions of transgene induction have been explored. First, induction of DmArr1 in the *arr1<sup>1</sup>cn* mutant background during adulthood only has been considered. For these analyses, both the Gene Switch and TARGET systems have been utilized. In each, the flies were allowed to develop and eclose as mutants, without induced transgene present. At a fixed time-point, subsequent to eclosion, the flies were then shifted to conditions allowing for expression of the DmArr1 transgene. Here it is predicted that, if the stated hypothesis is correct, EAG responses will be smaller than mutant levels, as introduction of arrestin into a down-regulated olfactory system will only further diminish the olfactory response level. Moreover, it can be further predicted that as Arr1 is reintroduced into the olfactory system, the system will adapt and EAG levels will acclimatize to wild-type levels over time.

Conversely, EAG responses have been examined in flies wherein the DmArr1 transgene was induced during the larval and pupal developmental stages only;



subsequent to eclosion the flies were removed from the induction permissive temperature, and presuming complete degradation of present DmArr1 protein, the flies would be considered to be functional mutants after developing under wild-type conditions. As the Gene Switch system, by definition, requires supplementation with the RU486 drug, this system has not been considered for this phase of the experiment. During the pupal stage of development (which is several days in duration), the pupae have no access to RU486, and it has been reported that removal of larvae from an RU486 feeding source results in a gradual diminishment of GFP reporter gene presence over a period of 24 to 48 hours (Osterwalder et al., 2001), suggesting a loss in ability of transgene transcription in the absence of the regulatory drug. Thus, only the TARGET system has been considered for this developmental-induction paradigm.

Under the hypothesis being tested, it can be predicted that for flies in which DmArr1 is induced and present during development and removed in adulthood, EAG responses will be larger than wild-type levels after arrestin protein is eliminated. This would be the case due to the acute removal of a deactivating factor, arrestin, from an olfactory system that has developed as if it were wild-type. It can further be predicted that over time EAG responses will normalize to mutant levels as the olfactory system compensates for the lack of arrestin.

## **Materials and Methods**

### **Fly Cultures**

The wild-type flies used as controls in this experiment were Oregon R, obtained from Dr. C. Desai (Vanderbilt University). *w*, +; pUAS-DmArr1, henceforth UAS-DmArr1, flies were generated in the laboratory as previously described (Merrill et al., 2005). *w*, elavGal4<sup>c155</sup>; +; +, henceforth elav-Gal4, were used as described previously (Merrill et

al., 2005). *w*, (tub)pGal80(ts)/FM7c; +; +, henceforth pGal80, flies were obtained from the Bloomington Drosophila Stock Center (Stock #7016, Bloomington, Indiana). Flies carrying the pGal80 transgene ubiquitously express Gal80(ts) under control of the  $\alpha$ -tubulin promoter. Flies were grown in cylindrical plastic vials on a standard cornmeal, molasses, agar and sugar medium with yeast, with modifications for the Gene Switch experiments, as described below. All flies were cultured at various temperatures, as described below, on a 12-h: 12-h light: dark cycle.

### **Odorants and Chemicals**

Three Class I and Class II odorants that have been shown to elicit diminished responses in *arr1<sup>1</sup> cn* mutant flies (Merrill et al., 2005) were used for the physiological assays: heptanoic acid and octyl acetate (Aldrich, Milwaukee, WI), and 1-octanol (Sigma, St. Louis, Missouri). Odorants were diluted in mineral oil (Sigma, St. Louis, Missouri) to the concentrations indicated in figure legends. 10mg of RU486 (Sigma-Aldrich, St. Louis, Missouri) were obtained and diluted in 100% ethanol.

### **Electroantennogram Physiology**

The EAG responses of wild-type and all transgenic flies were recorded in a similar manner to those in previous studies (Alcorta, 1991; Riesgo-Escovar et al., 1994; Merrill et al., 2005). Briefly, 2- to 7-day-old adult female *Drosophila* were immobilized in the narrow end of a pipette tip, such that only the anterior portion of the head protruded. Flies were placed 5 mm from the tip of a constant stream of clean humidified air provided by a stimulus control device (CS-05, Syntech, The Netherlands). Odorants were delivered by passing 0.5-s pulses of air through a glass Pasteur pipette containing a 1.5-cm-diameter filter disk (VWR International, West Chester, Pennsylvania) saturated with 20  $\mu$ l-diluted odorant into the constant air stream. The stimulus control device

delivered a continuous flow rate of ~340 cc/min and a pulse rate of ~350 cc/min. The difference in air velocity between the continuous air stream and stimulus pulse did not produce a significant EAG response, as evidenced by the fact that odor-free (oil-alone) responses are consistently low and reflect only minor background noise. Glass microelectrodes filled with 0.1 M KCl transmitted electrical responses to odorant stimulation via silver-chloride wires to a signal acquisition system (IDAC232, Syntech, The Netherlands). Data were collected at 25 Hz, amplified 10X and converted from analog to digital, then displayed on a Gateway PC computer. EAG analysis was performed using EAG2000 software (Syntech, The Netherlands). The reference electrode was inserted into the back of the head while the recording electrode was placed on the anterior dorso-medial surface of the 3<sup>rd</sup> antennal segment to establish electrical contact. The amplitude of response, which represents the peak voltage deflection in response to odorant presentation, was recorded for analysis.

### **Gene Switch Experimental Setup**

As a basis for these experiments, a plasmid obtained from the laboratory of Haig Keshishian (Yale University), *w*, elav-Gene Switch (GS), containing the modified Gal4 Gene Switch transcription factor positioned downstream of pan-neuronal elav regulatory sequence, was utilized. This construct was used to generate multiple transgenic fly lines (by Rainbow Transgenic Flies, Inc. Newbury Park, California) containing the *w*, elav-GS constructs on the X chromosome. These flies, in which the Gene Switch Gal4 transcription factor may be expressed in all neurons, have the following genotype: *w*, elav-GS; +; +, henceforth “elav-GS”.

For all experiments, virgin elav-GS flies were crossed to male UAS-DmArr1 flies. Progeny elav-GS/UAS-DmArr1 flies were reared at 30°C on standard fly food (described above). 3 days post-eclosion, adults were transferred to fly food supplemented with 4%

final-volume ethanol, or 4% ethanol plus a specified final concentration of RU486. These flies were then allowed to feed for a specified amount of time. Parental UAS-DmArr1 flies were subjected to similar regimens, as described below, and where applicable, were allowed access to supplemented food for at least 48 hours before being tested.

### **TARGET Experimental Setup**

For these experiments, the UAS-DmArr1 chromosome was crossed into the pGal80 genotype, and elav-Gal4 virgins were crossed to pGal80/UAS-DmArr1 males, to generate pGal80/elav-Gal4/UAS-DmArr1 progeny. Four different rearing conditions were employed for the progeny flies: 1) Flies were reared at 18°C during pre-adult and post-eclosion stages. For these flies, no induction of DmArr1 transgene should occur. 2) Flies were reared at 18°C during pre-adult stages and shifted to 30°C within 24 hours post-eclosion. For these flies, transgene induction should only occur during the adult stage. 3) Flies were reared at 30°C during pre-adult and post-eclosion stages. These flies should phenocopy the previously reported DmArr1 transgenic rescue flies (Merrill et al., 2005), as induction of transgene should occur during all life stages. 4) Flies were reared at 30°C during the pre-adult stage and shifted to 18°C within 24 hours of eclosion. For these flies, induction of transgene expression should only occur largely during the pre-adult stage. For additional data collected from control flies, Oregon R flies were reared at 30°C throughout.



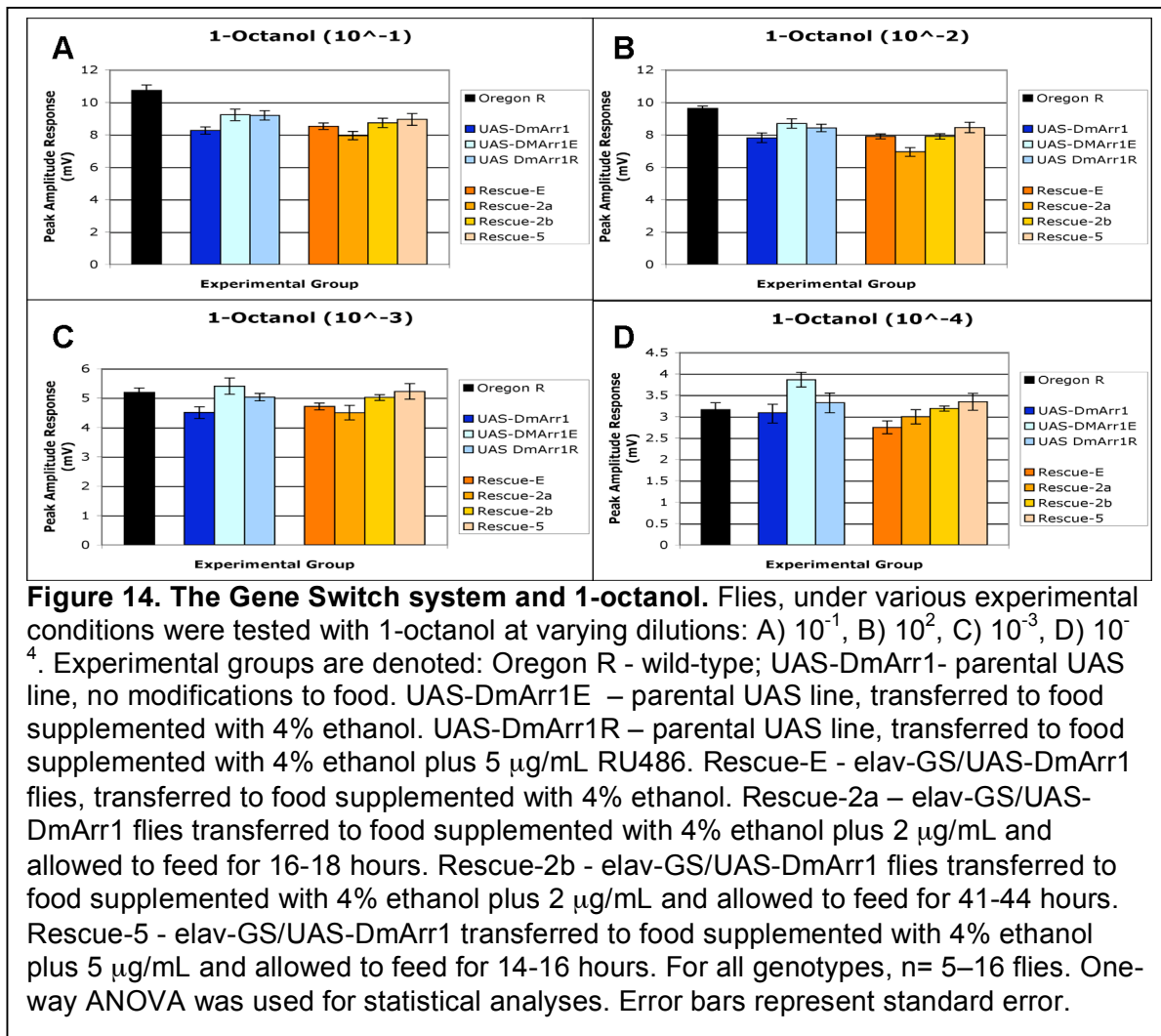
## Results

### The Gene Switch System – Induction During Adulthood

For this pilot study, Oregon R wild-type flies have been compared to parental UAS-DmArr1 mutant flies as well as elav-GS/UAS-DmArr1 rescue flies, all subjected to various conditions of induction. The data obtained from these experiments will be reported for each odorant independently. As an initial test of the elav-GS driver lines, elav-GS virgins were crossed to UAS-GFP males, and progeny adult flies were allowed access to food supplemented with 4% ethanol plus 8 $\mu$ g/mL RU486. After 48 hours of feeding, robust GFP was observed in the antennae and other neuronal tissues (data not shown).

*1-octanol*. Four dilutions of odorant have been tested ranging from 10<sup>-1</sup> to 10<sup>-4</sup> volume/volume dilution of odorant in mineral oil. At the 10<sup>-1</sup> and 10<sup>-3</sup> dilutions, for the parental responder fly-line, EAG responses were significantly higher in flies that had access to food treated with 4% ethanol only (Figure 14, UAS-DmArr1E) or 4% ethanol plus 5 $\mu$ g/mL RU486 (Figure 14, UAS-DmArr1R), as compared to flies reared on untreated food (Figure 14, UAS-DmArr1). At the two highest concentrations, for these flies, all responses were significantly lower than in wild-type flies (Figures 14A-14B). At 10<sup>-3</sup>, UAS-DmArr1E and UAS-DmArr1R flies responded no differently than wild-type, whereas UAS-DmArr1 flies responded at a significantly lower level than wild-type (Figure 14C). At 10<sup>-4</sup>, responses under all conditions in the parental line were no different than wild-type, with the exception of UAS-DmArr1E, for which the responses were significantly higher than the wild-type level (Figure 14D).

For the elav-GS/UAS-DmArr1 rescue flies, conditions of testing included flies that were transferred to food supplemented with 4% ethanol and allowed to feed for at least 48 hours (Figure 14, Rescue-E), flies that were transferred to food supplemented with

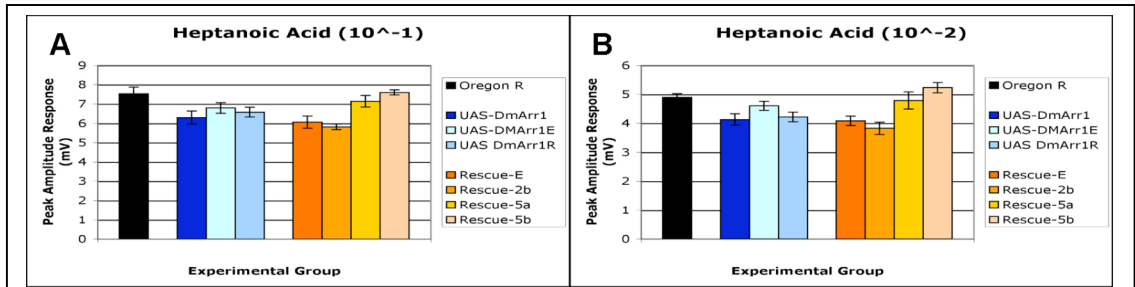


4% ethanol plus 2 $\mu$ g/mL RU486 and tested after 14-16 or 41-44 hours of feeding (Figure 14, Rescue-2a and Rescue-2b, respectively), and flies that were transferred to food supplemented with 4% ethanol plus 5 $\mu$ g/mL RU486 and tested after 14-16 hours of feeding (Figure 14, Rescue-5). At the two highest dilutions tested, EAG responses were not significantly different amongst all four conditions examined within the different rescue fly groups, with the exception that the Rescue-2a group responded significantly lower than all rescue fly groups at the 10<sup>-2</sup> dilution. Moreover, all four groups responded at levels significantly lower than those of the wild-type flies (Figures 14A-14B). At the 10<sup>-3</sup> dilution, Rescue-2b and Rescue-5 flies responded at levels statistically similar to wild-type flies, whereas Rescue-E and Rescue-2a flies at levels significantly lower than wild-type (Figure 14C). At the 10<sup>-4</sup> dilution, responses among all four groups were statistically indifferent and were also similar to responses of wild-type flies, with the exception that Rescue-5 flies responded at a significantly higher level than Rescue-E flies (Figure 14D).

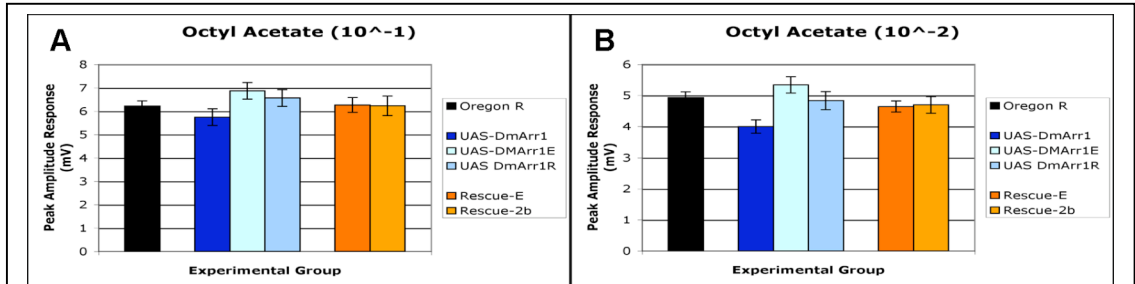
All groups were also tested with the mineral oil solvent prior to 1-octanol presentations (data not shown). All parental and rescue groups responded to the oil at levels statistically similar to wild-type. In consideration of pair-wise comparisons, statistically different response levels were observed on occasion, but no single group responded to oil at significantly greater or lesser levels than all other groups.

*Heptanoic acid.* Two dilutions of odorant have been tested, namely 10<sup>-1</sup> and 10<sup>-2</sup> volume/volume dilution of odorant in mineral oil. For the parental responder line, flies were tested after access to fly food treated with 4% ethanol (Figure 15, UAS-DmArr1E), 4% ethanol plus 5 $\mu$ g/mL RU486 (Figure 15, UAS-DmArr1R), or untreated food (Figure 15, UAS-DmArr1). For both dilutions tested, all groups responded at levels both statistically similar to each other and statistically lower than wild-type (Figures 15A-15B).

For the elav-GS/UAS DmArr1 rescue flies, four conditions were examined: food supplemented with either 4% ethanol and flies tested after at least 48 hours of feeding



**Figure 15. The Gene Switch system and heptanoic acid.** Flies, under various experimental conditions were tested with 1-octanol at varying dilutions: A)  $10^{-1}$ , B)  $10^{-2}$ . Experimental groups are denoted: Oregon R - wild-type; UAS-DmArr1 - parental UAS line, no modifications to food. UAS-DmArr1E – parental UAS line, transferred to food supplemented with 4% ethanol. UAS-DmArr1R – parental UAS line, transferred to food supplemented with 4% ethanol plus 5  $\mu\text{g}/\text{mL}$  RU486. Rescue-E - elav-GS/UAS-DmArr1 flies, transferred to food supplemented with 4% ethanol. Rescue-2b - elav-GS/UAS-DmArr1 flies transferred to food supplemented with 4% ethanol plus 2  $\mu\text{g}/\text{mL}$  and allowed to feed for 41-44 hours. Rescue-5a - elav-GS/UAS-DmArr1 transferred to food supplemented with 4% ethanol plus 5  $\mu\text{g}/\text{mL}$  and allowed to feed for 14-16 hours. Rescue-5b - elav-GS/UAS-DmArr1 transferred to food supplemented with 4% ethanol plus 5  $\mu\text{g}/\text{mL}$  and allowed to feed for 20-22 hours. For all genotypes,  $n = 6-15$  flies. One-way ANOVA was used for statistical analyses. Error bars represent standard error.



**Figure 16. The Gene Switch system and octyl acetate.** Flies, under various experimental conditions were tested with 1-octanol at varying dilutions: A)  $10^{-1}$ , B)  $10^{-2}$ . Experimental groups are denoted: Oregon R - wild-type; UAS-DmArr1 - parental UAS line, no modifications to food. UAS-DmArr1E – parental UAS line, transferred to food supplemented with 4% ethanol. UAS-DmArr1R – parental UAS line, transferred to food supplemented with 4% ethanol plus 5  $\mu\text{g}/\text{mL}$  RU486. Rescue-E - elav-GS/UAS-DmArr1 flies, transferred to food supplemented with 4% ethanol. Rescue-2b - elav-GS/UAS-DmArr1 flies transferred to food supplemented with 4% ethanol plus 2  $\mu\text{g}/\text{mL}$  and allowed to feed for 41-44 hours. For all genotypes,  $n = 6-15$  flies. One-way ANOVA was used for statistical analyses. Error bars represent standard error.

(Figure 15, Rescue-E), 4% ethanol plus 2 $\mu$ g/mL RU486 and flies tested after 41-44 hours of feeding (Figure 15, Rescue-2b), or 4% ethanol plus 5 $\mu$ g/mL RU486 and flies tested after 14-16 or 20-22 hours of feeding (Figure 15, Rescue-5a and Rescue-5b, respectively). For both dilutions, Rescue-5a and Rescue-5b flies responded at levels statistically similar to wild-type. Rescue-E, Rescue-2a and Rescue-2b flies responded at levels significantly lower than wild-type and statistically similar to the parental groups (Figures 15A-15B).

All groups were also tested with the mineral oil solvent prior to heptanoic acid presentations (data not shown). While most groups responded to oil at levels similar to each other collectively, there were exceptions. The UAS-DmArr1 group displayed statistically diminished responses compared to all except the Rescue-2b group. On the other hand, the Rescue-5b group responded at levels significantly greater than several other groups.

*Octyl acetate*. Two dilutions of odorant have been tested, 10<sup>-1</sup> and 10<sup>-2</sup> volume/volume odorant diluted in mineral oil. For the parental line, flies were tested after access to food treated with 4% ethanol (Figure 16, UAS-DmArr1E), 4% ethanol plus 5 $\mu$ g/mL RU486 (Figure 16, UAS-DmArr1R), or untreated food (Figure 16, UAS-DmArr1). For the 10<sup>-1</sup> dilution, all parental groups responded at levels statistically similar to each other and wild-type, with the exception of the UAS-DmArr1-E group, which responded at a significantly greater level than the UAS-DmArr1 group (Figure 16A). For the 10<sup>-2</sup> dilution, UAS-DmArr1E and UAS-DmArr1R flies responded at levels statistically similar to each other and wild-type, and significantly higher than UAS-DmArr1 flies (Figure 16B).

For the *elav-GS/UAS-DmArr1* rescue flies, two conditions were examined: food supplemented with 4% ethanol and flies tested after at least 48 hours of feeding (Figure 16, Rescue-E), and 4% ethanol plus 2 $\mu$ g/mL RU486 and tested after 41-44 hours of

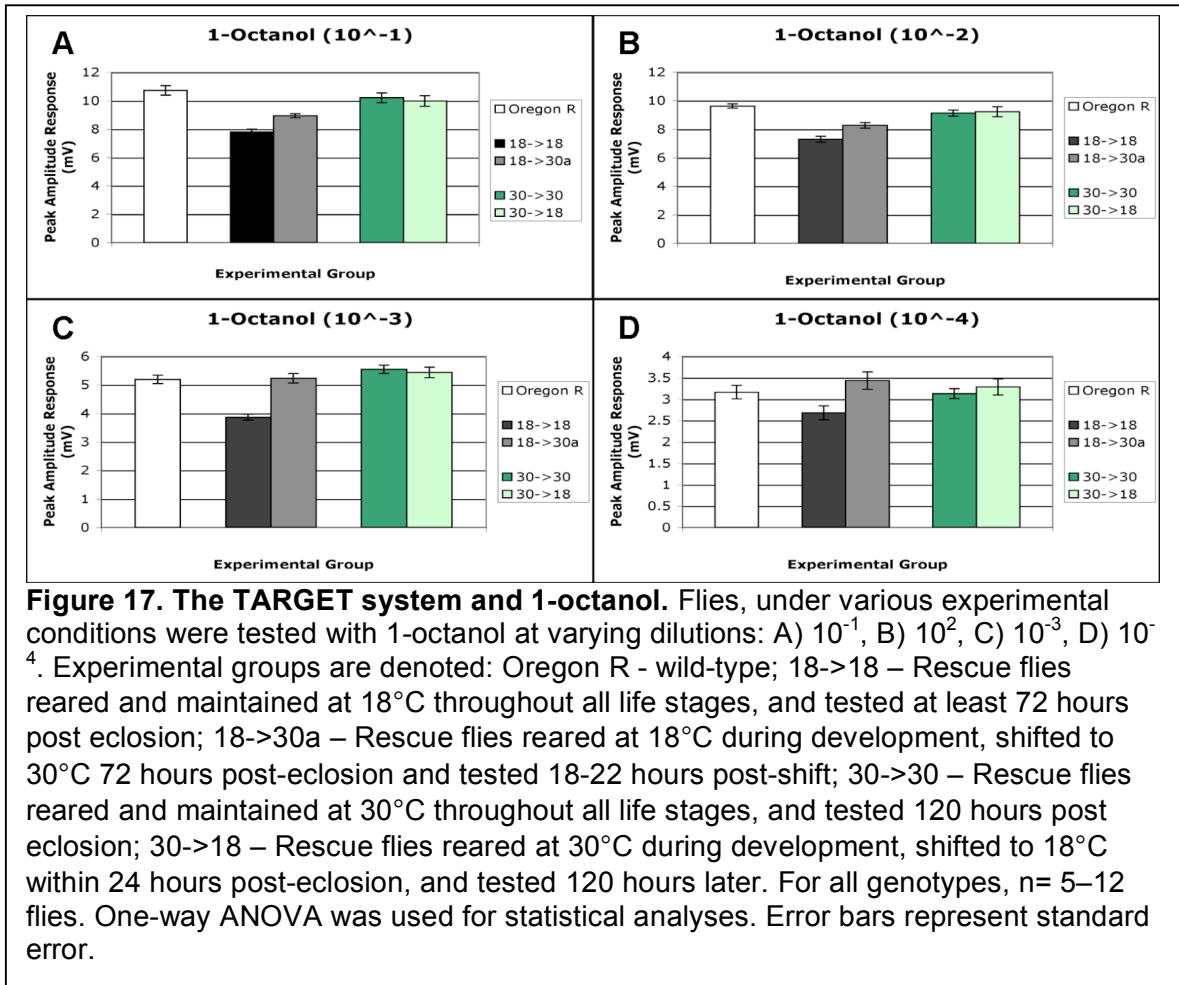
feeding (Figure 16, Rescue-2b). Under both conditions, and at both dilutions examined, flies responded at levels statistically similar to each other and wild-type (Figures 16A-16B).

All groups were also tested with the mineral oil solvent prior to octyl acetate presentations (data not shown). All groups responded to oil at levels statistically similar to each other, with the exception that the UAS-DmArr1 group displayed significantly lower responses compared to all other groups.

### **The TARGET System- Induction During Adulthood**

Within this system of induction, 1-octanol and heptanoic acid have been tested. EAG responses from wild-type flies were compared to those of Gal80/elav-Gal4/UAS-DmArr1 rescue flies under conditions of no induction throughout the life of the fly and induction during adulthood.

*1-octanol.* All flies were tested at four different dilutions of 1-octanol, ranging from  $10^{-1}$  to  $10^{-4}$  volume/volume dilution in mineral oil. For flies that were reared in pre-adult stages at 18°C and shifted to 30°C in the adult stage (Figure 17, 18->30a), physiological assays were performed over a range of 18-22 hours post-shift. Flies that were held at 18°C throughout adulthood (Figure 17, 18->18) were tested at least 72 hours post-eclosion. At the two highest dilutions of 1-octanol tested, 18->18 and 18->30a flies responded at levels significantly lower than wild-type. That said, 18->30a flies responded at significantly higher levels than 18->18 flies (Figures 17A-17B). For the  $10^{-3}$  and  $10^{-4}$  dilutions, 18->30a flies responded at levels statistically similar to wild-type and significantly greater levels than 18->18 flies (Figures 17C-17D). All groups were also tested with the mineral oil solvent prior to 1-octanol presentations (data not shown). While 18->30a and wild-type flies responded to oil at statistically similar levels, 18->18 flies responded at levels significantly lower than both of these groups.



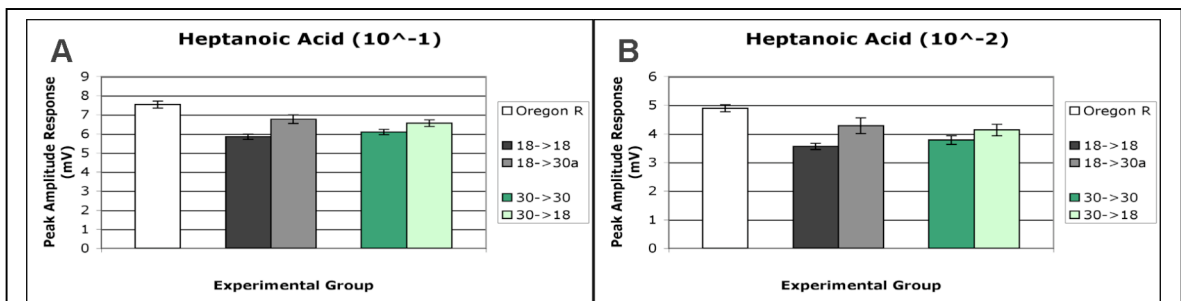
*Heptanoic acid*- All flies were tested at  $10^{-1}$  and  $10^{-2}$  volume/volume dilution of odorant in mineral oil. For flies shifted to a condition of induction in adulthood (Figure 18, 18->30a), EAG assays were performed across a time range of 18-22 hours, post-shift. Flies that were held at 18°C throughout adulthood (Figure 18, 18->18) were tested at least 72 hours post-eclosion. For both dilutions and groups tested, flies responded at levels significantly lower than wild-type. At the same time, 18->30a flies responded at significantly higher levels than 18->18 flies (Figures 18A-18B). All groups were also tested with the mineral oil solvent prior to heptanoic acid presentations (data not shown). Both 18->18 and 18->30a flies responded to oil at levels significantly lower than wild-type.

### **The TARGET System- Induction During Development**

Under this paradigm, Gal80/elav-Gal4/UAS-DmArr1 flies were raised at the induction permissive temperature throughout pre-adult stages and then either shifted to the induction repressive temperature (Figures 17 and 18, 30->18) or maintained at the induction permissive temperature (Figures 17 and 18, 30->30) until testing. In both cases, flies were held at the appropriate temperature for 120 hours, post-eclosion, and then tested.

*1-octanol*. For this odorant, dilutions ranging from  $10^{-1}$  to  $10^{-4}$  were used to test the flies. At all concentrations tested, both 30->30 and 30->18 flies responded at levels statistically similar to wild-type and significantly greater than 18->18 flies (Figures 17A-17D). These groups were also tested with the mineral oil solvent prior to 1-octanol presentations (data not shown). While 30->18 flies responded to oil at levels statistically similar to wild-type, 30->30 flies' responses were significantly lower than wild-type.





**Figure 18. The TARGET system and heptanoic acid.** Flies, under various experimental conditions were tested with 1-octanol at varying dilutions: A)  $10^{-1}$ , B)  $10^{-2}$ , C)  $10^{-3}$ , D)  $10^{-4}$ . Experimental groups are denoted: Oregon R - wild-type; 18->18 – Rescue flies reared and maintained at 18°C throughout all life stages, and tested at least 72 hours post eclosion; 18->30a – Rescue flies reared at 18°C during development, shifted to 30°C 72 hours post-eclosion and tested 18-22 hours post-shift; 30->30 – Rescue flies reared and maintained at 30°C throughout all life stages, and tested 120 hours post eclosion; 30->18 – Rescue flies reared at 30°C during development, shifted to 18°C within 24 hours post-eclosion, and tested 120 hours later. For all genotypes, n= 5–12 flies. One-way ANOVA was used for statistical analyses. Error bars represent standard error.

*Heptanoic acid*. For this odorant,  $10^{-1}$  and  $10^{-2}$  dilutions were used to test the flies. At both concentrations, both 30->18 and 30->30 flies responded at levels significantly lower than wild-type. 30->18 flies responded at levels significantly greater than 18->18 flies, whereas 30->30 flies responded at levels statistically similar to 18->18 flies (Figures 18A-18B). These groups were also tested with the mineral oil solvent prior to heptanoic acid presentations (data not shown). While 30->18 flies responded to oil at levels statistically similar to wild-type, 30->30 flies' responses were significantly lower than wild-type.

### **Discussion**

The available data generated in the aforementioned experiments is ambiguous, and, moreover, uninterpretable without further experimentation. A necessary first step would be to correlate physiological response levels to transgene RNA and protein expression levels for the various conditions of transgene expression being considered.

At the very least, it can be surmised that inconsistencies are observed regarding predictions made in consideration of the stated hypothesis. Under no conditions of transgene induction during adulthood, for any of the odorants tested, were EAG responses observed to be smaller than mutant levels. Nor at any time were EAG responses ever observed to be higher than wild-type levels when transgene induction occurred during pre-adult stages followed by removal of induction (and presumed removal of protein) during the adult life of the fly. To be sure, it must be reemphasized that transgene expression levels must be considered if any definitive conclusions are to be made. There is no available knowledge of the turnover rate of arrestin proteins in the fly, and as such, transgenically expressed arrestin protein levels must be monitored subsequent to cessation of induction.

One unexpected, although not entirely surprising, observation from these studies pertains to the fact that under similar conditions of transgene induction, or removal of induction, different categories of response levels were observed when the flies were presented with different odorants. For example, in using the TARGET system to induce expression of DmArr1 during pre-adult stages only, EAG responses to 1-octanol were statistically similar to wild-type levels, however, EAG responses generated in response to heptanoic acid were significantly lower than wild-type levels. While results such as these are unexpected, they are not surprising considering that a precedence for differential odorant- and mutant- dependent physiological phenotypes have been reported for single and double *arr1* and *arr2* mutant flies (Merrill et al., 2005).

Important caveats must be mentioned regarding the Gene Switch system in this experimental context. First, for two of three odorants used in these studies it must be noted that for parental UAS flies, responses were significantly higher in flies given access to food supplemented with ethanol or ethanol plus RU-486 as compared to genetically identical flies that had access to untreated fly food. These observations imply that ethanol may have a modulatory effect on olfactory physiology in the fly, thus raising the potential for confounding effects pertaining to interpretation of the data. This problem may be overcome, however, via utilization of other solvents such as sucrose (Mao et al., 2004). Another consideration that must be controlled for is the actual time of induction in each fly that is tested. This issue becomes problematic when one considers that in this system, induction is regulated by feeding behaviours that cannot be well controlled for. Theoretically, if different experimental animals do not express identical feeding patterns, this may result in highly variable data points.

Given these concerns with the Gene Switch system, the TARGET system may indeed be a more reliable transgene induction paradigm to provide for the study of the effects of the temporal relevance of arrestin function in the olfactory antennae. Using

TARGET, a more comprehensive analysis, including consideration of quantitative transgene RNA and protein expression levels, as well as a more inclusive range of induction periods, odorants, and appropriate control groups, may yield valuable information allowing for proper evaluation of the hypothesis being tested. Conversely, the data generated may lend itself more aptly to alternative hypotheses that might also account for the observed physiological olfactory deficit phenotypes in sensory arrestin mutant fruit flies.