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Dissection of *Drosophila* Visual Circuits Implicative in Figure Motion

A thesis submitted in partial satisfaction  
of the requirements for the degree Master of Science  
in Physiological Science

By

Ross G Kelley

2013

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## ABSTRACT OF THE THESIS

Dissection of Visual Circuits Implicative in Figure Motion

By

Ross G Kelley

Master of Science in Integrative Biology and Physiology

University of California, Los Angeles, 2013

Professor Mark A Frye, Chair

The *Drosophila* visual system offers a model to study the foundations of how motion signals are computed from raw visual input and transformed into behavioral output. My studies focus on how specific cells in the *Drosophila* nervous system implement this input-output transformation. The individual cell types are known from classical studies using Golgi impregnations, but the assembly of motion processing circuits and the behavioral outputs remain poorly understood. Using an electronic flight simulator for flies and a white-noise analysis developed by Aptekar et al, I screen specific neurons in the optic lobes for behavioral ramifications. This approach produces wing responses to both the spatial and temporal dynamics of motion signals. The results of these experiments give Spatiotemporal Action Fields (STAFs)

across the entire visual panorama. Genetically inactivating a distinct grouping of cells in the third optic ganglion, the Lobula Plate, the Horizontal System (HS) cell group, produced a robust phenotype through STAF analysis. Using the Gal4-UAS transgene expression system, we selectively inactivated the HS cells by expressing in their membrane inward rectifying potassium channels (Kir2.1) to hyperpolarize these cells, preventing their role in synaptic signaling. The results of the experiments show mutants lose steering responses to several distinct categories of figure motion and reduced behavioral responses to figure motion set against a contrasting moving background, highlighting their role in figure tracking behavior. Finally, a synapse inactivating protein, tetanus toxin (TNT), expressed in the HS cell group, produces a different behavioral phenotype than overexpressing inward rectifier. TNT, a bacterial neurotoxin, cleaves SNARE proteins resulting in loss of synaptic output of the cell, but the dendrites are intact and signal normally, preserving dendro-dendritic interactions known to sculpt the visual receptive fields of these cells. The two distinct phenotypes to each genetically targeted silencer differentiate the functional role of dendritic integration versus axonal output in this important cell group.

The thesis of Ross G Kelley is approved.

David William Walker

Peter M Narins

Mark Arthur Frye, Committee Chair

University of California Los Angeles

2013

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## INTRODUCTION:

Navigation through the world requires the decomposition of a multitude of incoming sensory stimuli. The question of how these signals are transformed into behavioral responses is the central question of my research. Specifically, self-propelled movements cause the appearance of a moving visual background. Simultaneously, distinct visual objects attract our attention. We have a poor understanding of how the perception of smooth motion is computed from complex and noisy raw visual signals, in particular for perceiving a salient visual figure against a noisy moving background. In flying *Drosophila*, figure-background discrimination involves decomposing the figure into two discrete parallel subsystems that control flight steering efforts (Aptekar et al. 2012). Furthermore, there are distinct component responses in the fly's steering effort to both figure position and corresponding background motions (Fox et al. in review). The neuronal processes that coordinate these behavioral responses, as well as the associated cellular structures, are poorly understood in flies, or any other animal model. For my thesis, I studied the neuronal pathways and their behavioral function for figure-background discrimination. *Drosophila* is a genetic model system, and by genetically inactivating specific cell types in the visual system, I sought to discover which cells are involved in figure-background discrimination computations, and how. I used the following genetic and behavioral analytical techniques to resolve this insufficiency through the genetic inactivation of specific cells and a high throughput behavioral analysis.

Flies exhibit a vast array of remarkable complex flight behaviors over extraordinary brief time periods of milliseconds, from hovering to maneuverability in nearly every direction (Frye et al. 2004). The biomechanical flight dynamics of right-angled (yaw) turns in *Drosophila*, known

as saccades, occur on the scale of 50ms (Fry et al. 2003). Such remarkable response times makes the flies' flight a vigorous and adaptable system worthy of studying sensory motor transformations. To quantify visual perception and motor control, I used an electronic flight simulator to elicit opto-motor responses in flying flies (Figure 1). Opto-motor responses are visual reflexes with corresponding motor reactions to stabilize image slip across the eye, equivalent to human optokinetic eye movements (Götz 1968). The arena is composed of a cylindrical array of light-emitting diodes (LEDs) programmed to display arbitrary spatial patterns and temporal speeds to test various aspects of motion detection behavior, measured with an optical sensor of wing steering (Aptekar et al. 2012). Steering efforts are calculated using the differences between the right and left wing beat amplitudes (WBA) and are then correlated to the position of the figure on the cylindrical array. The heads of the flies in experiments are glued to isolate the visual response from proprioceptive, and allows for precise control of the visual motion stimulus input (Borst et al. 2010). To characterize behavior in a mathematically formal way, I use a white-noise system identification approach developed for figure detection by Aptekar et al., 2012 and expanded here. The stimulus consists of a randomly textured vertical bar moving against a randomly textured background with balanced contrast and luminance. Thus, the only distinguishing characteristic between the two components is their relative motion. The experiments measure wing responses to random visual stimuli, and quantify the steering opto-motor impulse response. The opto-motor responses to figure motion across the entire visual panorama give rise to Spatiotemporal Action Fields (STAFs). STAFs provide a quantitative map of the magnitude and temporal dynamics of wing responses to figure motion throughout the visual horizon (Figure 2).

The Gal4-UAS expression system remains the genetic chicanery of choice to manipulate populations of neurons in the fly brain (Duffy 2002). This genetic control system works through inserting Gal4, a transcription factor protein, into the genome under the endogenous genetic control of the host organism (in our case the fruit fly). This Gal4 insertion occurs at random as the insertion finds a promoter that will initiate transcription. Gal4 is ½ of a binary expression system that then turns on (or “drives”) the expression of a transgene-of-choice. This gives selective and specific expression of exogenous transgenes can then be used to manipulate neural circuits and enable us to observe any corresponding behavioral phenotypes. The UAS, upstream activating sequence, component corresponds to the other half of the binary expression system where Gal4 binds to induce the transcription of the exogenous gene of interest (GOI). Whenever the Gal4 is activated it will find the corresponding UAS sequence and therefore ‘drive’ the expression of the GOI.

The limitation of this method is determined by the specificity of expression within subgroups of neurons by the Gal4 driver, and the efficacy of the effector transgene. The strength of the promoter for the Gal4 insertions can also influence the amount of exogenous GOI expression. If the Gal4 is placed under the control of a weak promoter, then there will be fewer Gal4-UAS binding events and minimal expression of transgenes. Definitive isolation of cellular systems is paramount to making substantive claims about any resulting phenotypes. Due to the large number of available driver lines (those that express Gal4 in spatially restricted groups of neurons), it is possible to localize specific neurons through combinatorial constructions of transgenes. For example, if cells X, Y and Z are labeled and I believe the phenotype results from cell Y specifically, I can find another driver expressing cells V, W and Y to test for the observed phenotype. The driver line expression system I used involves applying a PhiC31 recombinase

vectors to enhance specificity of Gal4 insertions (Pfeiffer et al. 2008). The specificity of the driver can be directly observed through induction of green fluorescence protein (GFP) and in some cases there is GFP tagged to the desired transgene (Johns et al. 1999).

My work has also incorporated the use of inducible genetic reagents that have the ability to turn the Gal4/UAS expression system on and off. Through the use of inducible systems, one can control the temporal expression of the transgene (Klueg et al. 2002). For example, the induction system I used in my research was Gal80<sup>ts</sup>, another transcription factor that binds to the Gal4 and prevents expression from this promoter (McGuire et al. 2003). This protein product is shown to have temperature sensitivity; at 18°C there is suppression of the Gal4 promoter by Gal80<sup>ts</sup> and upon increasing to the permissible temperature, 30°C, Gal80<sup>ts</sup> is inactivated to release inhibition of Gal4. Through these temperature controls, one can have a better regulation of the genetic inactivation. This inducible expression system is also invaluable when ectopic expression of the transgene of choice results in death of the organism during development. In such events, the organism can be reared at the lower temperature to insure the transgene is suppressed due to Gal80<sup>ts</sup> inhibiting the transcription of Gal4. Once the flies are ready for experimentation, they can be kept at high temperature (29°C) to activate the transcription of Gal4 and the subsequent UAS-Gene.

The cellular inactivators used for my experiments were TNT and Kir2.1. They are characterized by different mechanisms of action on the control of neuronal communication, and therefore provide the potential for different observed behavioral phenotypes. UAS-TNT constructs crossed to the Gal4 driver line result in expression of tetanus neurotoxin light chain (Sweeney et al. 1996). This protein product cleaves the synaptic vesicle associated protein n-Synaptobrevin. As a result, there is complete blockage of chemical neurotransmitter release, yet

the cell is metabolically active and healthy. All electrical activity of the cell remains intact with administration of this inactivator. By contrast, UAS-Kir2.1 crossed into the corresponding Gal4 driver line results in the transcription of an inward rectifying potassium channel. This channel induces a potassium current, hyperpolarization, and subsequent reduced probability of firing action potentials leading to neurotransmitter release and postsynaptic communication (Baines et al. 2001).

I focused my work on a well-studied visual processing center in flies called the lobula plate. This region is part of the optic lobes of the brain and responsible for motion processing in both larger flies and *Drosophila* (Scott et al. 2002). The most intriguing and well-studied group are the Horizontal System (HS) cells (Figure 3), part of the giant lobula plate tangential cells (LPTC), that are known to show a directionally tuned response to horizontal motion (Borst et al. 2002). For visual motion oriented in the preferred direction, moving medially to laterally (front-to-back), HS responds with robust depolarization and shows inhibition in the non-preferred direction (back-to-front). These cells have also been shown to modify their response characteristics according to different arousal states (Maimon et al. 2010). The basis for the observed increase in gain is thought to be a form of selective attention during flight whereby competing incoming stimuli are filtered to direct behavioral decisions. These attributes of the LPTCs illustrate the complex physiology attainable in this set of cells. There are three distinct HS cells, HS North (HSN), HS Equatorial (HSE) and HS South (HSS), moving dorsally to ventrally, respectively (Schnell, et al 2010). Each cell contains arborized dendrites and as a result there are large regions of morphological and receptive field overlap among the cells. The dendrites of these cells express both GABA and cholinergic receptors (Raghu et al. 2009).

LPTCs such as HS are thought to implement a canonical model for detecting motion. The “Reichardt” model of motion detection has formed the computational basis for describing motion detection from insects to vertebrates (Gabbiani et al 2012). The model describes how two mirror-symmetrical adjacent luminance detecting cells can detect motion through a delay imposed by a low pass filter, creating an asymmetric temporal filtering and multiplication of the inputs (Eichner et al 2011). The LPTCs are shown to spatially integrate incoming motion cues across the eye through their highly arborized dendrites and are believed to provide the output for motion behavior (Schnell, et al. 2012). The LPTCs have outputs to either the thoracic ganglion for wing or neck muscles for control of head movements (Huston and Krapp 2008). Despite the massive attention its physiological properties have received, the behavioral significance of this cell class has remained enigmatic. Using a combination of genetically targeted inactivation of HS and quantified performance of visually guided flight, I tested the hypothesis that these cells play an important role in figure tracking.

By understanding the behaviors and underlying neural circuitry of a simpler organism, we can begin to work towards comprehending the more complex arena of mammals. The fly is a dynamic model organism with the potential to lend tremendous insights into how numerically compact circuits assemble into observed behaviors. With this knowledge we can begin to better build vehicles better equipped for self-sustained flight.

## MATERIALS AND METHODS:

### *Drosophila melanogaster*

Flies were maintained in standard media that was housed in a temperature- and humidity-controlled room at 25°C. For the temperature-sensitive mutants, crosses were housed at 18°C to insure inhibition of the desired inactivation transgene. Temperature-sensitive mutants were kept at 29°C for a minimal of 48 hours before being used in experiments to ensure the activation of transcription. The remaining stocks and genetic crosses were housed at 25°C. The light:dark cycle was maintained on a strict 16:8 schedule.

### *Behavioral Assay*

Female flies aged 3-5 post-eclosion were used for all experiments. Flies were cold anesthetized and then glued to a rigid tungsten wire using UV-activated glue. The wire was fixed on the dorsal thorax and head, and retained into a temperature-controlled chamber for approximately one hour before being placed in the flight arena for experiments. Flight arenas were used for all experiments. Flies are suspended in the center of the arena, composed of a cylindrical array of light emitting diodes (96 x 32) wrapping 330° along the horizontal axis and 120° along the vertical axis (Theobald et al. 2010). Each pixel subtends 3.75° on a fly's retina. Measurements of wing beats are made using an infrared light-emitting diode projecting a shadow of the wings onto photodiodes that measure the actual wing beat amplitudes. The differences between the right and left wing beats signify the direction in which the fly intends to turn. For example, if a fly is intending to turn to the right there will be a greater WBA in the left than the right. Flies were presented experimental trials one at a time with different types of stimuli presented random orders. Between individual trials, flies were presented a six pixel (22.5°) wide vertical bar for 4 s where they had complete control under closed-loop conditions. Only flies that completed a



minimum of 30 trials were used for data analysis.

### *Statistical Analysis*

P-value maps were made to statistically compare the STAFs between intact and genetic inactivation experiments. The method conducts a Student's T-test for the individual pixels found on the STAF analysis. The areas of lowest correlations,  $p < 0.05$ , are highlighted with red color indicating regions of the STAF, containing statistically significant areas of difference. The Benjamini Hockberg correction was used to reject false discoveries. By comparing every point on the STAF we observe any differences in the behavioral responses between the compared genetic backgrounds and produce a map similar to the STAF. This makes the comparisons easier to visualize.

### *Genetic Crosses*

All crosses were housed in conditions similar to those described in aforementioned methods under *Drosophila melanogaster*. The results of the crosses produced heterozygous genotypes as seen in the sample cross in Figure 3. For the crosses that were lethal to the progeny, temperature-sensitive Kir2.1 was used to suppress the expression of transgenes until necessary for experiments, after adult eclosion. In some cases, the insertion of a transgene into the genome creates lethality in the offspring that are homozygous for the insertion and therefore stocks need to be kept as heterozygotes. In these instances, balancer chromosomes are used to create a screening method for the progeny to have the appropriate genotype for experimentation (Casso et al. 2000).

## RESULTS:

The R27B03 Gal4 driver selectively labels all three HS cells in the Lobula plate with some other scattered neuronal expression elsewhere in the optic lobes and central brain (Figure 4). This line was crossed to both TNT and Kir2.1, each giving unique phenotypes (Figures 5, 6, and 7). The results of the experiments gave entirely different phenotypes. When inactivating Gal4-R27B03 with Kir2.1, there are robust background responses across the entire STAF with diminished figure, EM and FM responses (Figure 5). There is also an absence of figure impulse inhibition at the midline of the background STAF, as seen in wildtype, thus strengthening the background response. Conversely, when Gal4-R27B03 driver was crossed with UAS-TNT, there is a clear diminished response to background motion and the remaining figure, EM and FM responses were all intact. One of the limitations to obtaining definite results is the expression of the Gal4 in extraneous cells. In the R27B03 cell line, some cells in the line stained outside the HS cells. Therefore, we decided to run the experiments in another cell line that has been used to study the HS system, 3A. This line has been used in the past to study both HS and VS cells of the Lobula Plate (Scott et al. 2002). When inactivated with Kir2.1, there appeared to be no loss of function seen in the figure-background responses. The result of this experiment also led me to further test the controls of the inactivators to ensure there was the appropriate inhibition occurring with Kir2.1.

I used the W11 Gal4 driver line that expresses in laminar neurons to test the fidelity of the Kir2.1 inactivator (Zhu et al. 2009). When inactivated with TNT, these mutant flies were completely motion blind leading to the idea that motion signals were sent through the lamina and medulla for conditioning early motion signals. It was thought that inactivation of these cells would lead to no motion responses in the mutants and therefore give a verifiable control test of

the efficacy of the inactivation. When crossed with UAS-Kir2.1, these mutants displayed completely functional responses to all aspects of figure-background discrimination. This result led me to believe there may be an issue with the expression of the Kir2.1 due to it having the Gal80 temperature-sensitive component and I am currently working to resolve this insufficiency.

## DISCUSSION

The results of the STAF analysis on different cells in the *Drosophila* nervous system led to some interesting findings for the understanding of figure background discrimination. There are also interesting conclusions to draw from the results of using different inactivators for experimentation. There appears to be some indication for a role of the HS cells in responses to figure-background discrimination. Due to the directional specificity and physiology of these cells, it was postulated that HS would have an observable output function in this aspect of visual motion detection. The results of the experiments gave conflicting evidence for the precise aspect of figure-background these cells were playing. When crossed with the TNT inactivator, the background response was lost and when inactivated with Kir2.1 the background response was the only aspect of behavioral response still intact. One explanation why the two different phenotypes were observed is based on the subcellular differences of the inactivation method used (Figure 6 & 7). Due to TNT only working on the output of the cells, the remainder of the cell is intact and fully operational. It has been previously found that the HS cells in *Drosophila* are electrically coupled to one another via dendro-dendritic gap junctions to one another and other LPTCs (Schnell et al. 2010). Through these connections, HSs are thought to give rise to the ipsilateral and contralateral motion response. As a result, the administration of TNT to these cells would keep signaling between coupled cells intact and therefore not disrupt the overall signaling. When these cells are inactivated with Kir2.1, there is a subsequent hyperpolarization that keeps the cell at or near its resting potential, resulting in an electrically silent cell. Therefore, the Kir2.1 inactivation could be the true phenotype observed from the experiments conducted.

The results brought about another interesting point of consideration, the importance in choosing the appropriate inactivator for experimentation. There appears to be a variety of cellular

properties to consider when deciding to ablate a cell. Characteristics such as postsynaptic targets, electrical properties, neurotransmitters released and strength of the driver line expression are all viable considerations beforehand. The high degree of intricacy in any system leads to difficulty in controlling all the necessary parameters. Furthermore, the added complexity of analyzing behavior makes the selection process all the more complex. With any behavioral paradigm, there are a multitude of incoming signals and computations responsible for the output. Accordingly, the ability to discover the significance of any individual cell or even a group of cells makes the process difficult. The ability of these networks to modify their excitability makes the conclusions to be drawn even more transient.

The composition and location of the cell in the circuit can also have an effect on the observed phenotype. The response properties of the LPTC have led them to be proposed as Reichardt detectors (Gilbert 2008). The spatial and temporal integration of different excitatory and inhibitory neurotransmitters onto the dendrites of these cells certainly give compelling evidence for an ability to sum incoming sensory signals. The output phenotypes of the LPTCs are known for the vertical system but remain unidentified for the HS system. There is evidence for the VS cells signaling with inhibitory GABA synapses. With the neural underpinnings of the cell, there could be important ramifications as a result of removing their output from the circuit.

The comparisons of the two inactivators to parental controls showed interesting results as well. Our statistical analysis shows that there is only significance seen in the Kir2.1 inactivation when compared to parental controls when using a Student's T-test (Figure 8). The TNT inactivation shows no significances when compared to parental controls when using a Student's T-test (Figure 9). This could be a result of the figure detecting system being more easily perturbed. The highly detailed and complex computations required to track figures could make it

more prone to being ablated. Accordingly, the background responses could have more inputs giving it the ability to accommodate any perturbations of the system. This would explain why the TNT background disruption did not show any statistical significance for Figure 9.

In total, the results of the experiments conducted also lend credibility to the STAF analysis methodology. The STAF technique developed by Aptekar et al. 2013 (pending submission) allows for a strong screening tool to look for neurons that are implicit in figure-tracking behaviors. Through the course of my master's research, I performed over twenty inactivation experiments of specific cells in the *Drosophila* nervous system. There were a total of six crosses that produced interesting phenotypes. The finding of my research lends credibility to the selectivity of the STAF analysis because it does not appear to produce an exceedingly large number of significant results. Therefore, the STAF analysis is a viable and proficient method for testing the behavioral flight responses in *Drosophila* visual experiments.

It appears from the results of my experiments that the UAS-TNT construct gave the most definitive results. I intend to run a Gal4-3A; UAS-TNT inactivation experiment in hopes to obtain similar results as the Gal4R27B03; UAS-TNT. This will further validate the conclusions for the role of the HS cell in figure-background discrimination behavior and finally solve the riddle of behavioral significance in the HS cells of the Lobula Plate.

FIGURES:

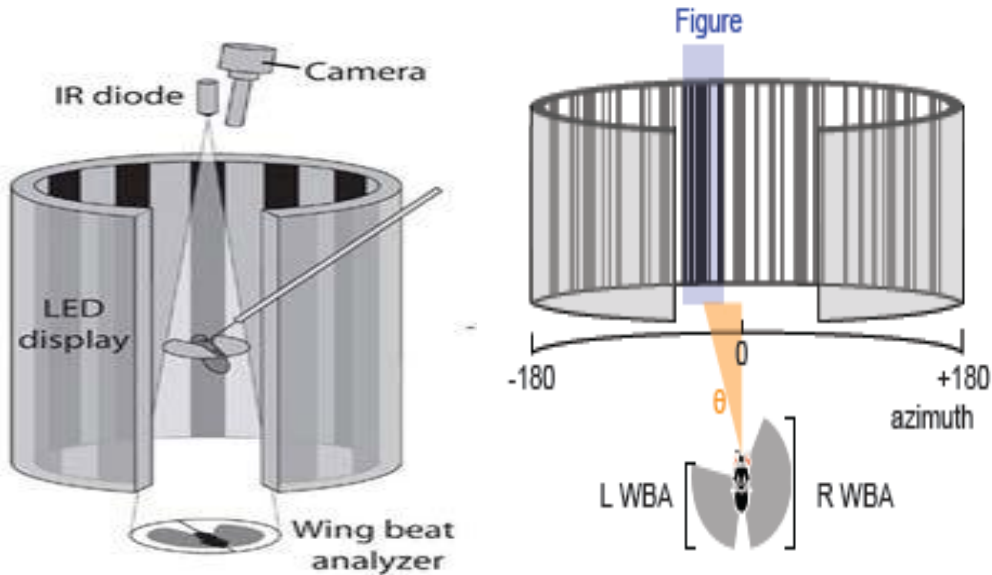
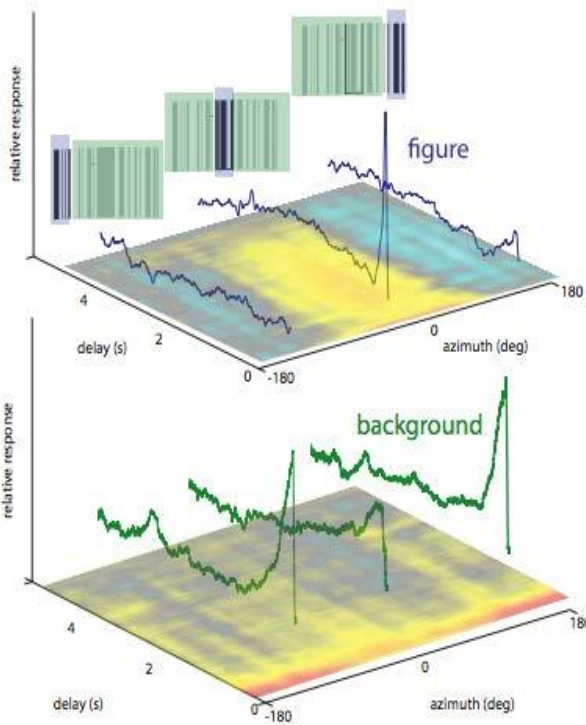


Figure 1: Electronic Flight Simulator animation. Flight arena set-up showing fly suspending in the middle of a flight arena composed of LED displays with IR diode casting the shadows of the wings onto photodiodes that detects changes in wing beat amplitudes (Left panel). When a fly attempts to steer towards a presented object, there is a change in WBA. As in the right figure, the fly is steering to the left in the yaw plane. To achieve such an acrobatic maneuver, the fly increases the wing output on the right side and decreases the output on the left side leading to an imbalance of forces creating a torque about its perpendicular (y-axis) center of gravity. The patterns displayed around the arena are composed of randomly striped patterns comprising both the background and the figure window (Right panel). These randomly striped patterns result in the figure being defined only by its relative motion and therefore only tests the visual motion responses in flies.

A



B

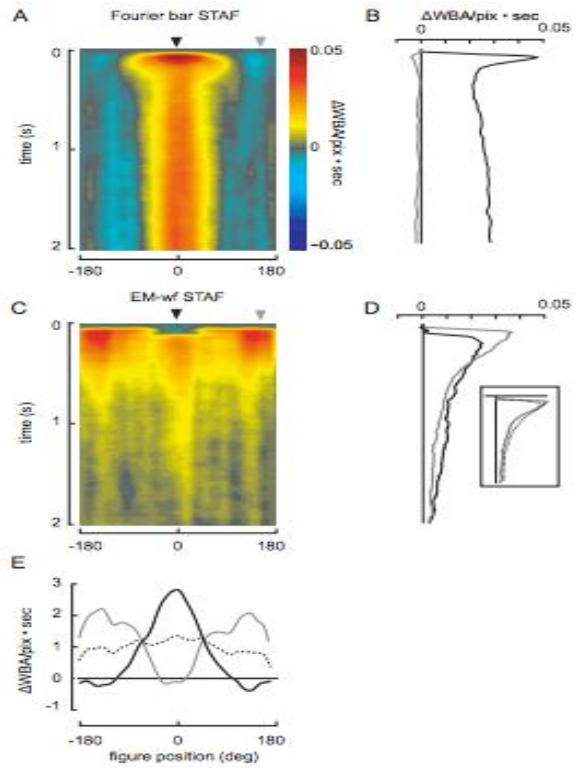


Figure 2: STAF (Spatiotemporal Action Fields) analysis. The method looks at the impulse response of a fly to figure and background visual motion cues in time (A & B, B&D) and collects all these responses across the visual panorama to create the STAF (B, A&C). There are characteristic responses to both moving figures and background. The responses are subdivided such that the two subsystems are shared in the total wing output (B, E). The STAF figures represent the composite response of a population of flies for a given phenotype. The red indicates the highest degree of correlation between wing responses and stimulus location. The blue indicates the areas of the least amount of correlation between the wing responses and stimulus location.



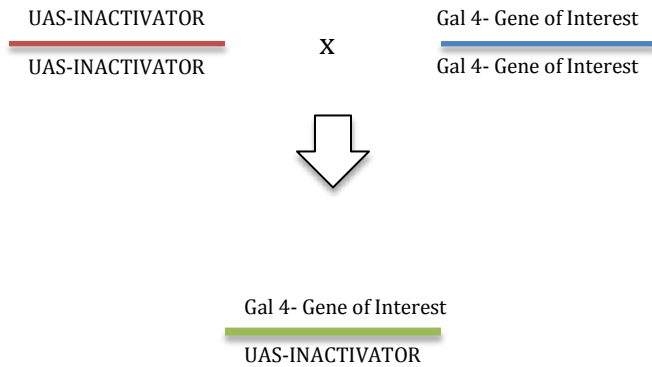


Figure 3: Sample genetic cross. The schematic of a genetic cross producing the desired combination for the Gal4-UAS expression system. The F1 progeny contains both elements in a heterozygote.

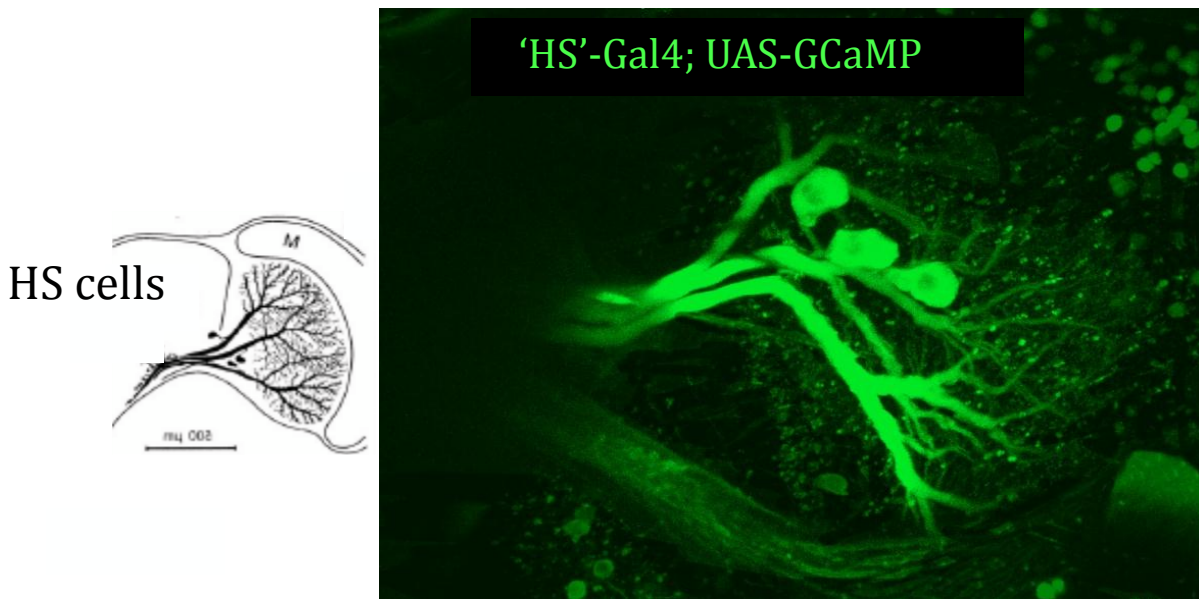
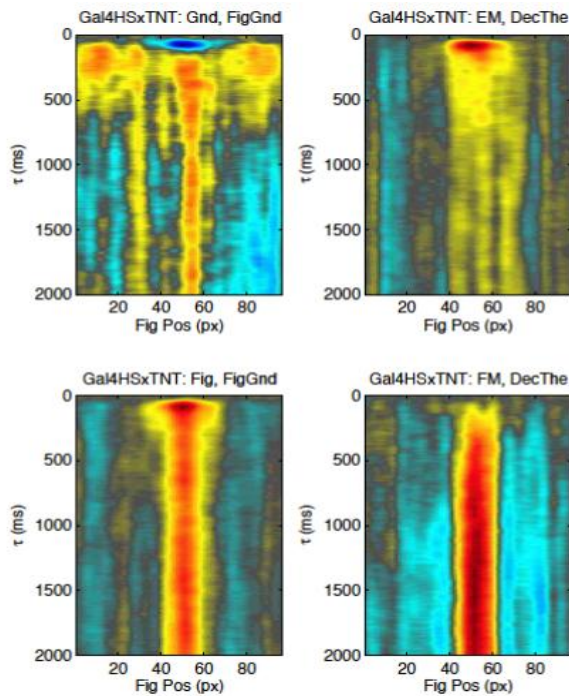


Figure 4: Gal4-R27B03; UAS-GCaMP histology. This is an image of the HS cells in the Lobula Plate of the *Drosophila* the optic lobe. The three cell bodies of the HS cells are clearly shown as the large circular shapes in middle of the image. The dendrites are seen on the right.

A.



B.

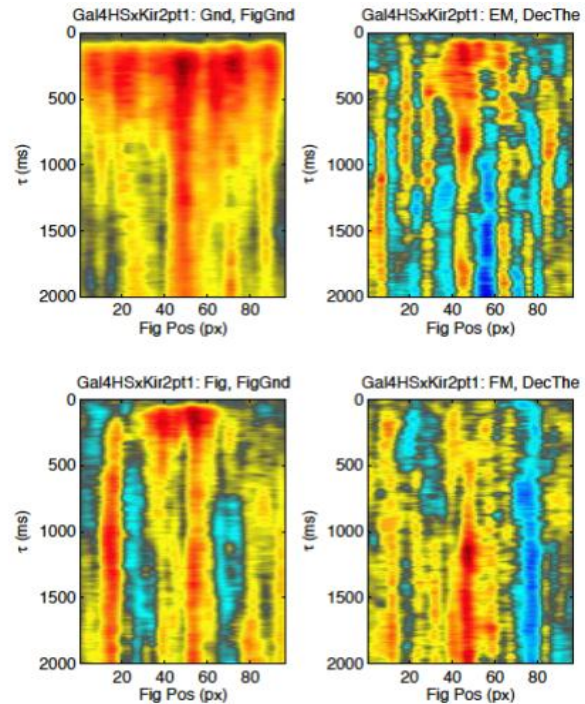


Figure 5: Gal4-R27B03 genetic inactivation and STAF analysis (TNTxR27B03 (A), Kir2.1xR2703 (B) STAF). The results show a clear difference between the two inactivators (A $\leftrightarrow$ B). The TNT inactivation clearly shows a diminishing of the background response system but nearly flawless figure, EM and FM responses. When the same cells were inactivated with Kir2.1, only the background responses remain intact and EM, FM and figure show complete disruption. In the control experiment, one can clearly see the prototypical STAF phenotype observed in wild-type behaving *Drosophila*.

Comparison of STAFS:R27B03xTNT v R27B03xKir2pt1

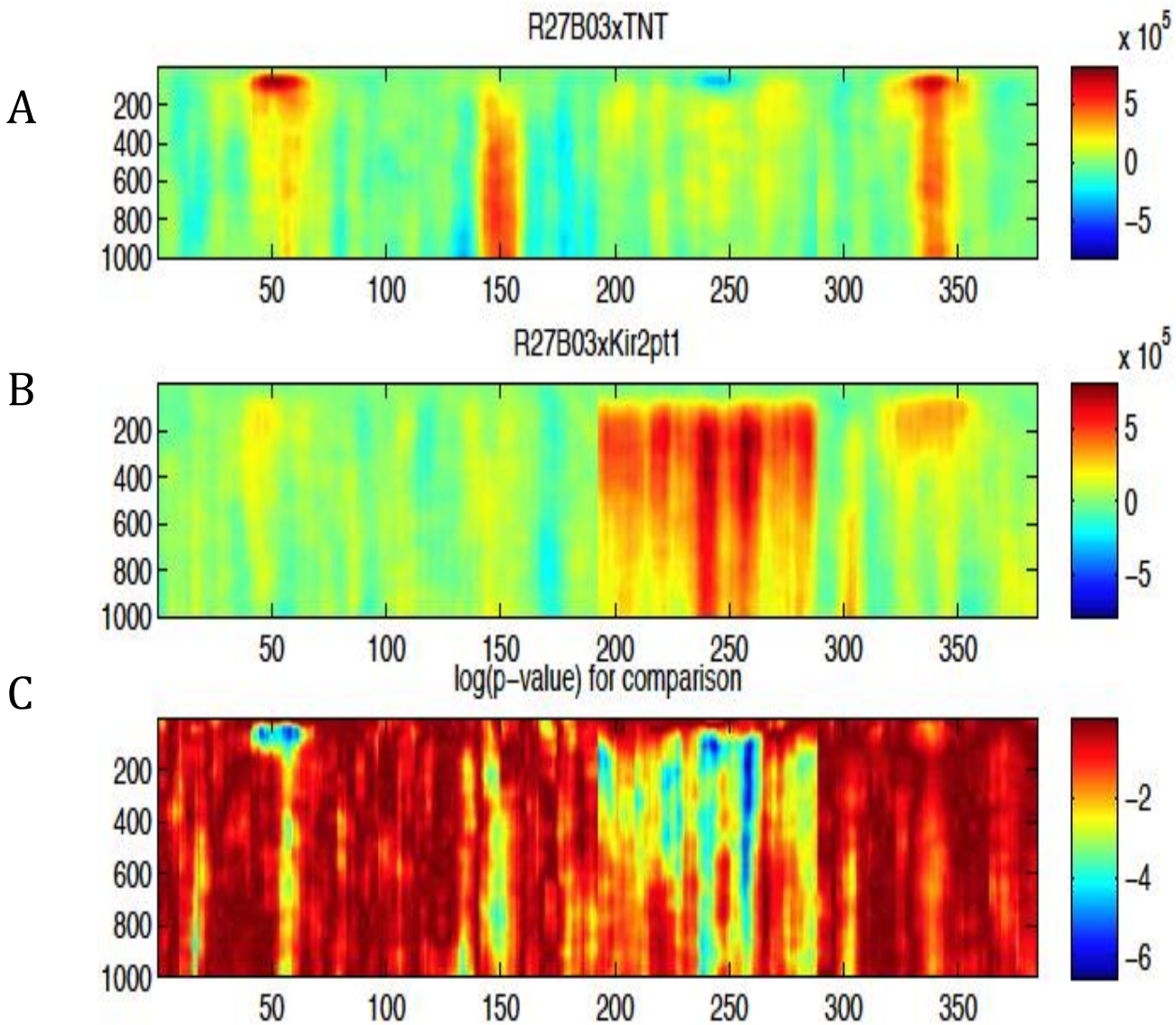


Figure 6: Gal4-R27B03 statistics comparing the TNT (A) and Kir2.1 (B) inactivations. A and B are the composite STAF analyses from Figure 4 placed side-by-side for simplicity of viewing and comparing TNT to Kir2.1 inactivation. C is the results from the Student's T-test comparing each pixel of the STAFs in A and B. The areas in blue highlight the areas of highest statistical significance ( $p < 0.000001$ ). The greatest differences are seen in the EM response and background response. There is also a strong statistical difference between the figure responses ( $p < 0.001$ ).

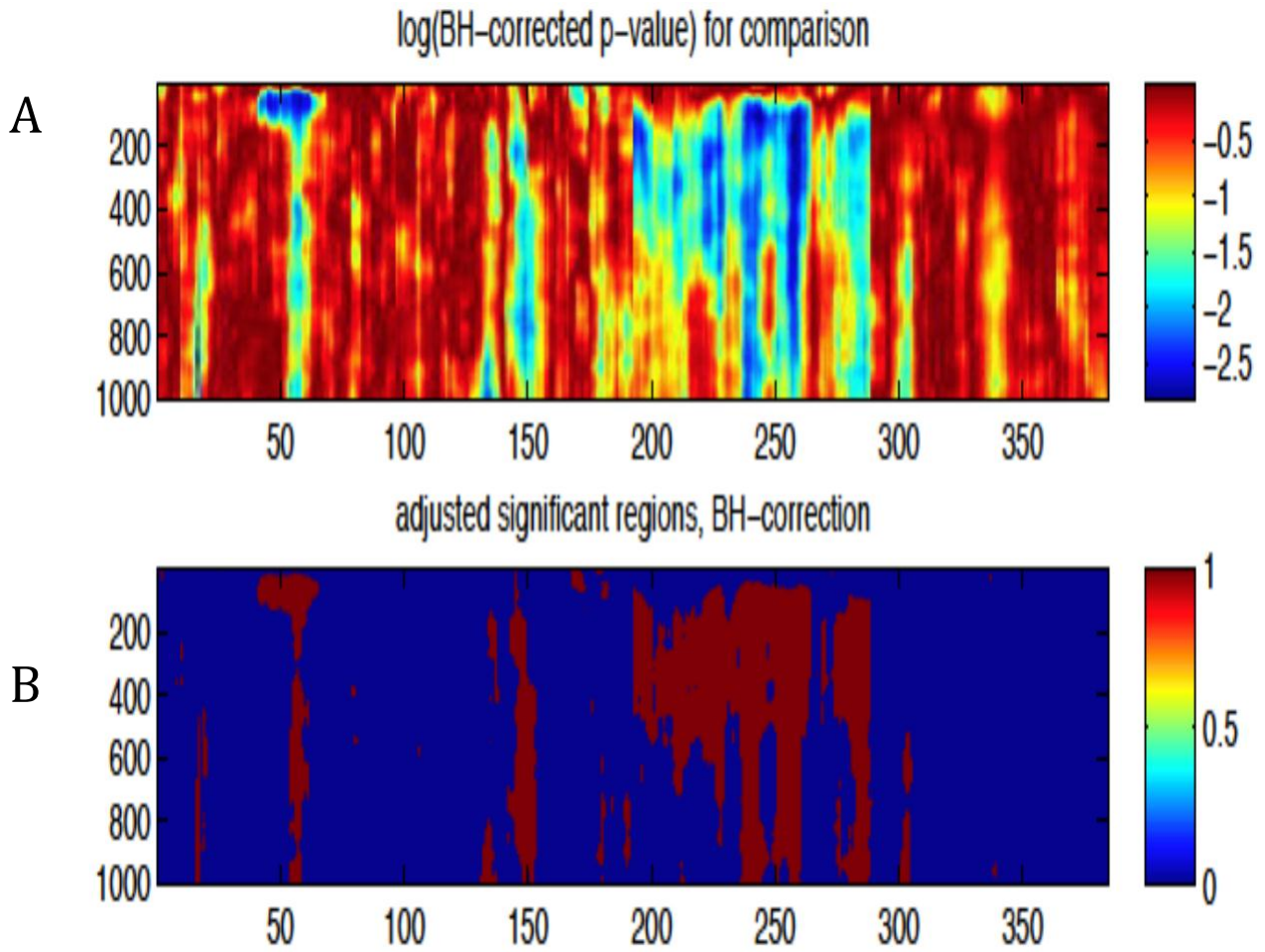
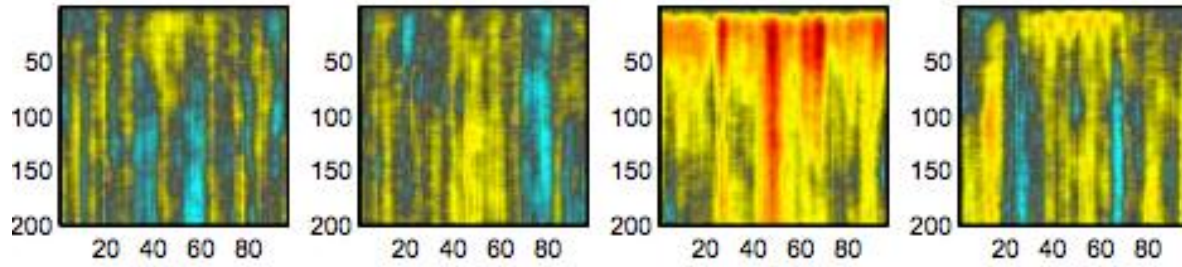


Figure 7: Comparison statistics for Gal4-R27B03 inactivation with Benjamini Hockberg. Panel A shows the Benjamini Hockberg correction that was used to reject false discoveries. After this correction, there are still regions of significance in the EM and background responses. This correction also highlighted statistical differences in figure responses. Panel B shows the adjusted significant regions for all values that show statistical significance. These results clearly show the strong differences between the two inactivators used in the same Gal4 driver line.

Comparison of STAFS:Kir2pt1xR27B03 v PCFxR27B03

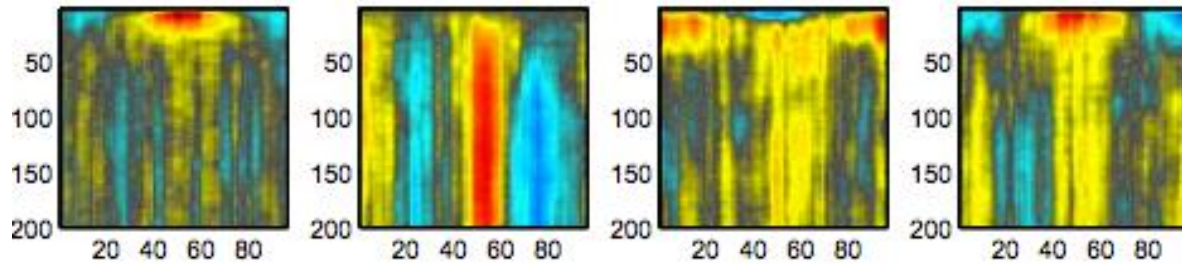
Kir2pt1xR27B03

**A**



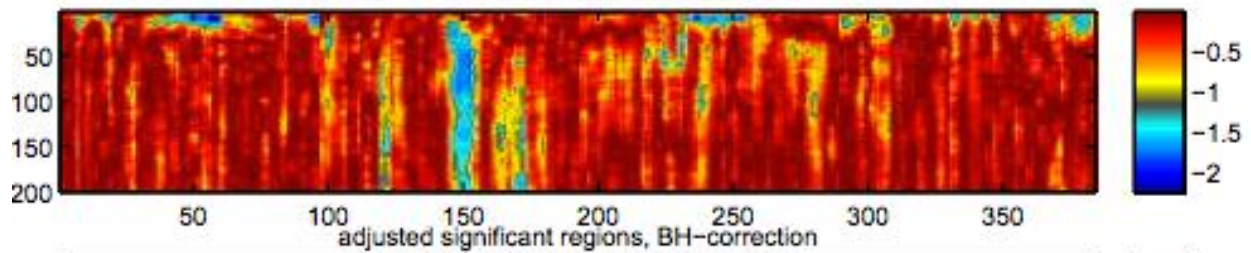
PCFxR27B03

**B**



log(BH-corrected p-value) for comparison

**C**



adjusted significant regions, BH-correction

**D**

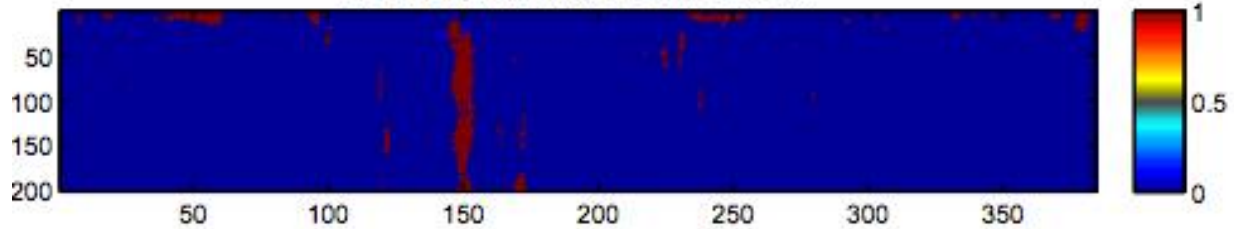


Figure 8: Statistical analysis for the Kir2.1 inactivation of R27B03 compared to Gal4-R27B03 parental control (Panels A & B). The most significant loss in terms of statistical significance is in the figure tracking system (Panel C & D).

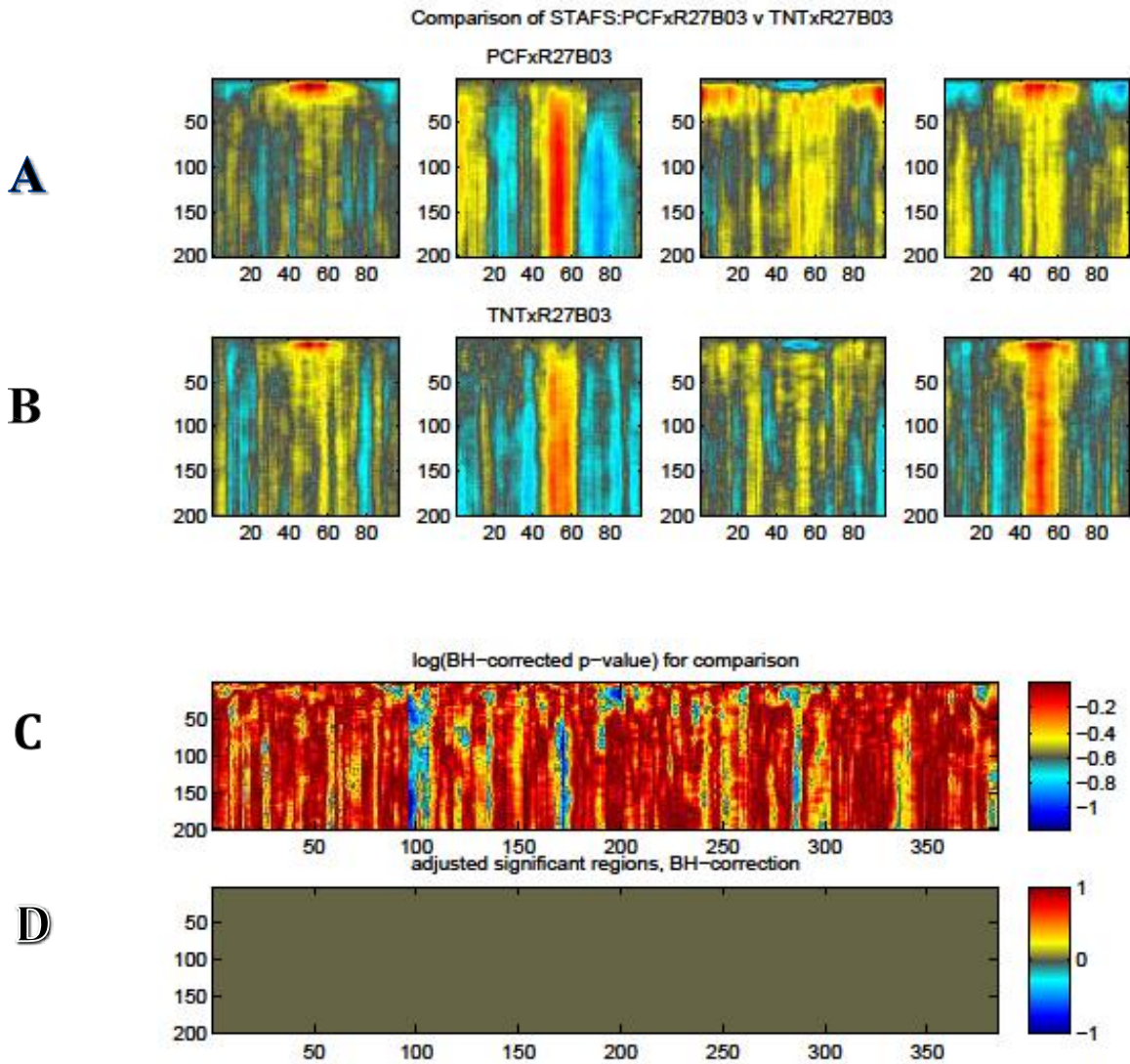


Figure 9: Statistical comparisons of the Gal4-R27B03 inactivation with TNT. Panels A and B are the reformatted STAFs aligned horizontally for comparison purposes. Panel C shows the results of the Student's T-test, with Benjimini Hockberg correction, for the STAFs in panels A and B. The most striking differences appear to be in the figure responses , although these do not appear to surpass any level of significantly different areas on the STAF analysis (Panel D).

## APPENDIX:

This contains additional experiments I conducted throughout the course of the Masters Program.

### B-line Results:

The next set of experiments involved looking into the motion responses through STAF analysis of lobula specific pathways. These lines show expression patterns that connect between the optic lobes and the central brain (Otsuana et al. 2006). The cell line B145-313 (NP5006), this line selectively labels the LT10 in the optic lobe (Otsuna et al 2006). This neuron is found in the anterior dorsal area of the lateral cell body region and projects to the dorsal portion of the lobula. The results of the STAF analysis show there are diminished background responses when inactivated with TNT and intact figure, EM and FM responses (Figure 9). However, when the cells were inactivated with Kir2.1, all the responses appeared intact and regular. This was another indication that the Kir2.1 inactivator may be corrupted. The cell line B123-405 (NP1047), this driver specifically labels a single neuron with its cell body also found in the anterior-dorsal region of the lateral cell body (Otsuana et al. 2006). This neuron appears to collect information from distinct layers of the lobula and transmit impulses to the ventrolateral protocerebellum (VLPR). The STAF analysis with the TNT inactivator shows a slightly diminished response to backgrounds with the remainder of the STAF criteria intact. When this driver line was crossed with UAS-Kir2.1, there were no discrepancies observed in the STAF analysis.

The cell line B123-319 (NP1035), this line labels the LT10 and LT11 cells mentioned previously. Interestingly, there is also a slight background deficit with intact figure, EM and FM responses when inactivated with TNT. The cell line B154-319 (NP 7121), in addition to labeling the LT10 cell, this line is found to have expression in glial cells and other regions of the nervous

system. The inactivation with TNT produced the previously observed diminished background responses and preserved figure, FM and EM behaviors.

The results of the B-lines indicate for a potential coding for background motion responses for the lines inactivated. Upon visual comparison, there appears to be a small discrepancy in all of the background responses of the lines. This finding could shed light on a potential behavioral role of lobula specific projection pathways in the processing of motion signals. The morphology of the cells indicated that there could be a component of motion detection passing through these cell types. The observed minor diminishing of background responses observed could be a result of the background system being a much more robust and adaptable subsystem. Accordingly, there could be more components as a part of the overall circuit that can accommodate the loss some of the individual components. If this were the case, then the observed results would fit nicely into such a system. A future experiment to test this idea would be to find a driver line that expresses in all the aforementioned cells. If the discrepancies in the background responses are amplified, then there would be more proof that these cells are implicit in the response.



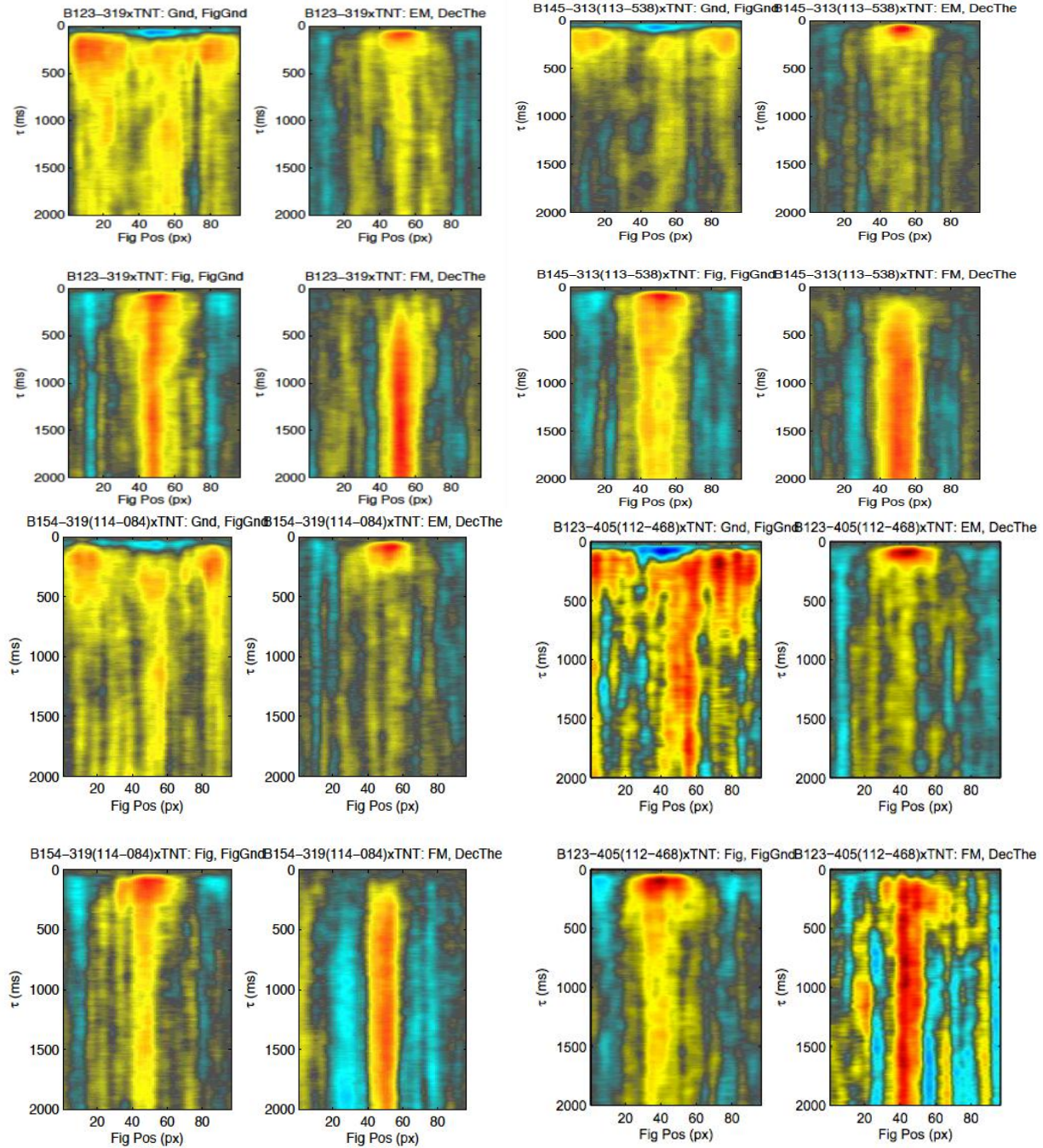
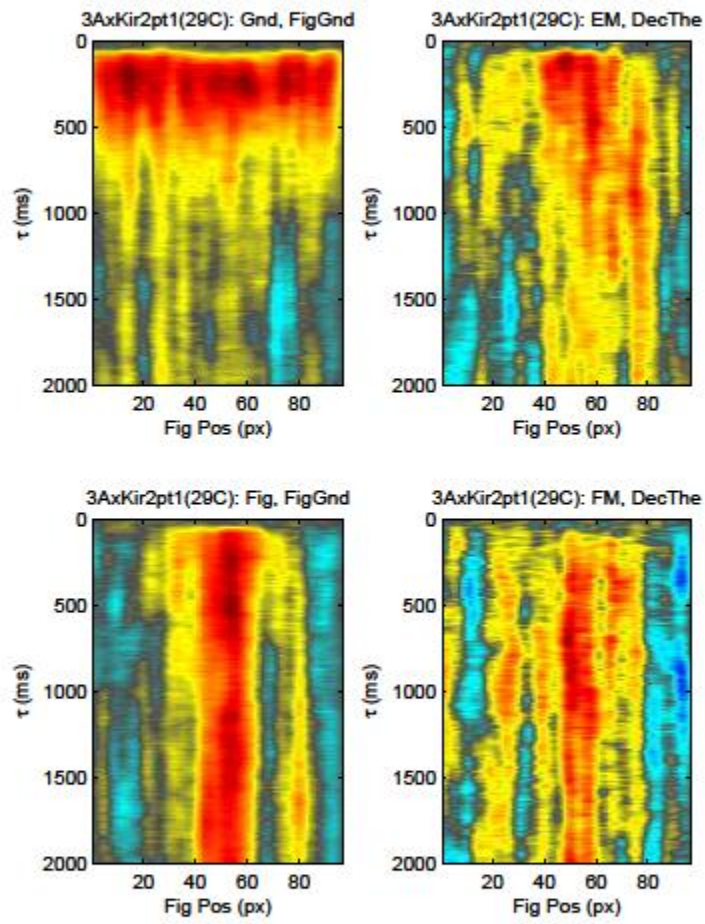
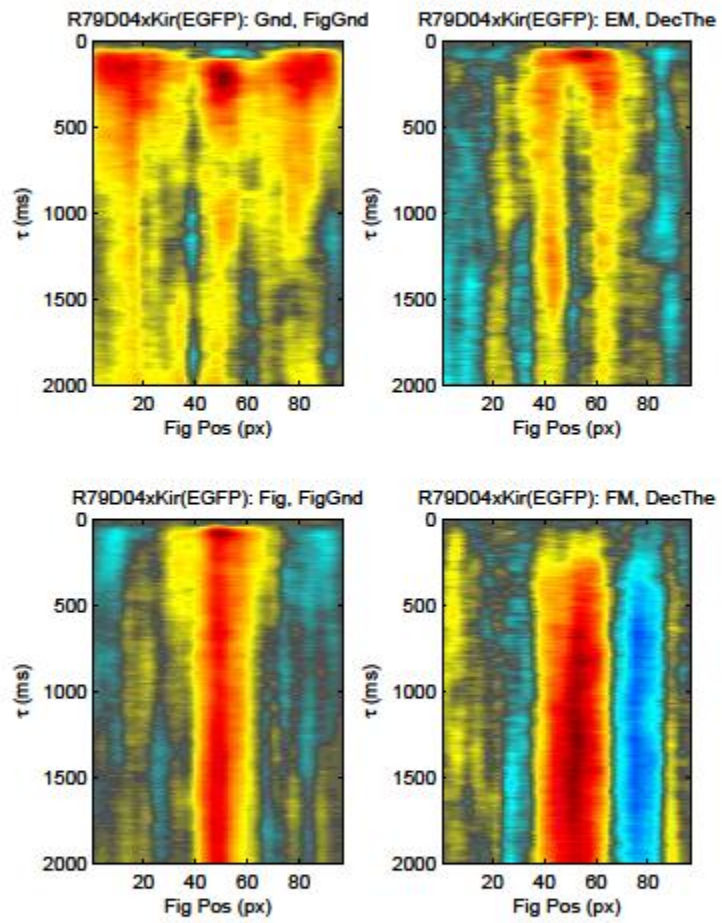
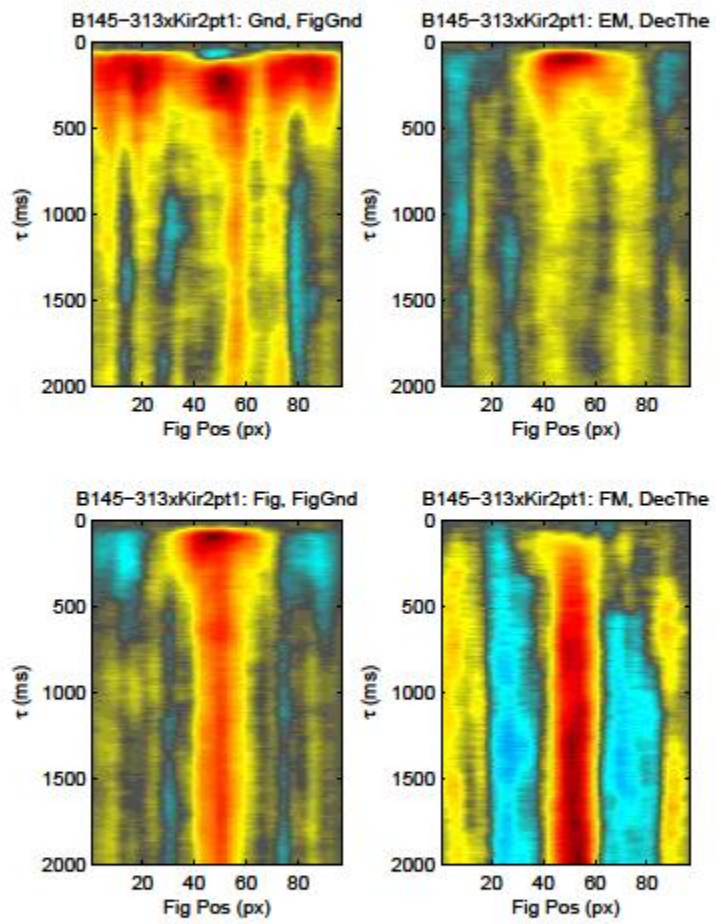
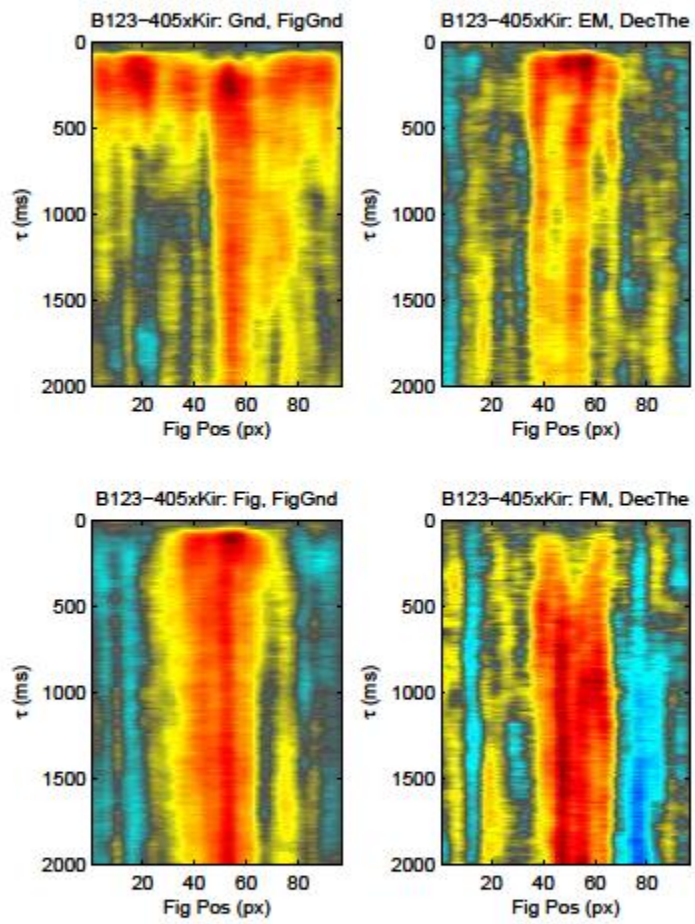


Figure 9: B-line TNT inactivation. All the inactivations appear to show a diminishing of the background responses. The most profound effect on the background response was seen in the B145-313 line.

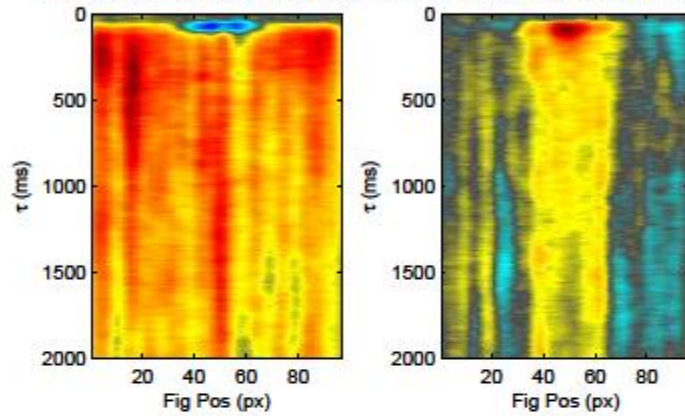




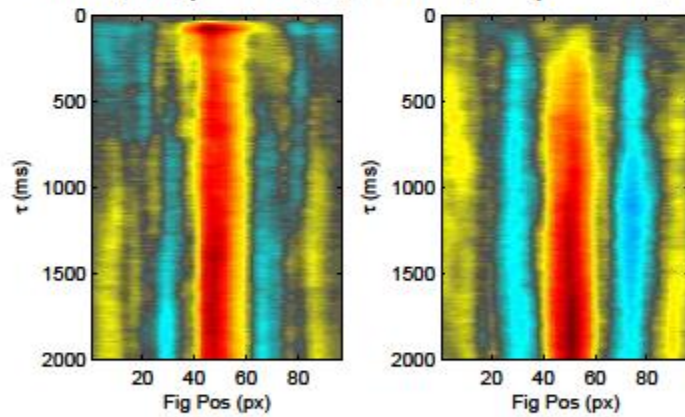


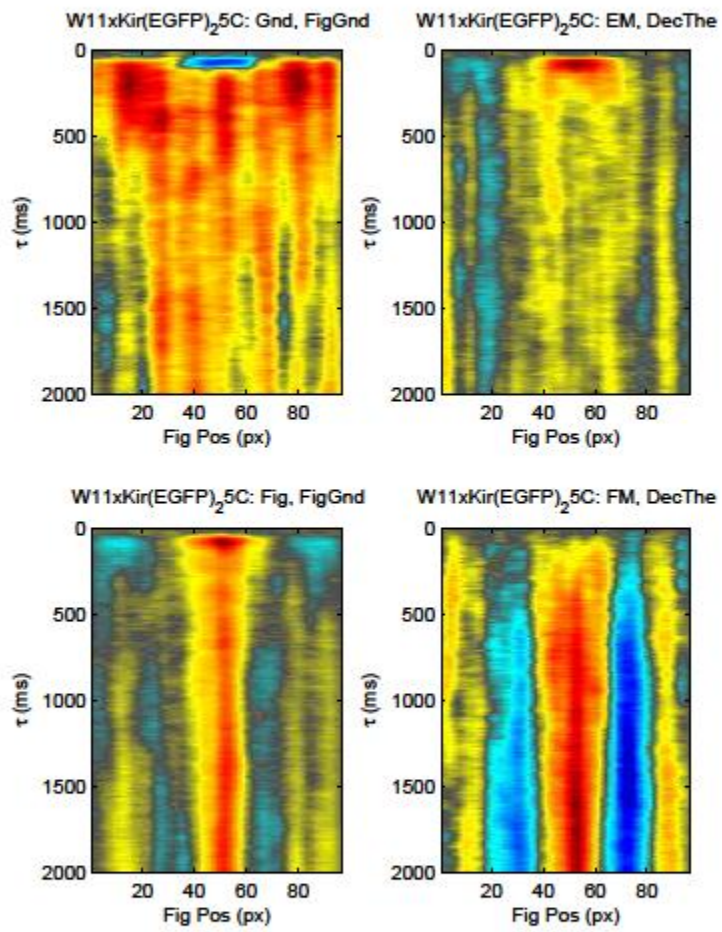


W11xKir(EGFP)<sub>1</sub>8Craised: Gnd, FigGnd W11xKir(EGFP)<sub>1</sub>8Craised: EM, DecThe

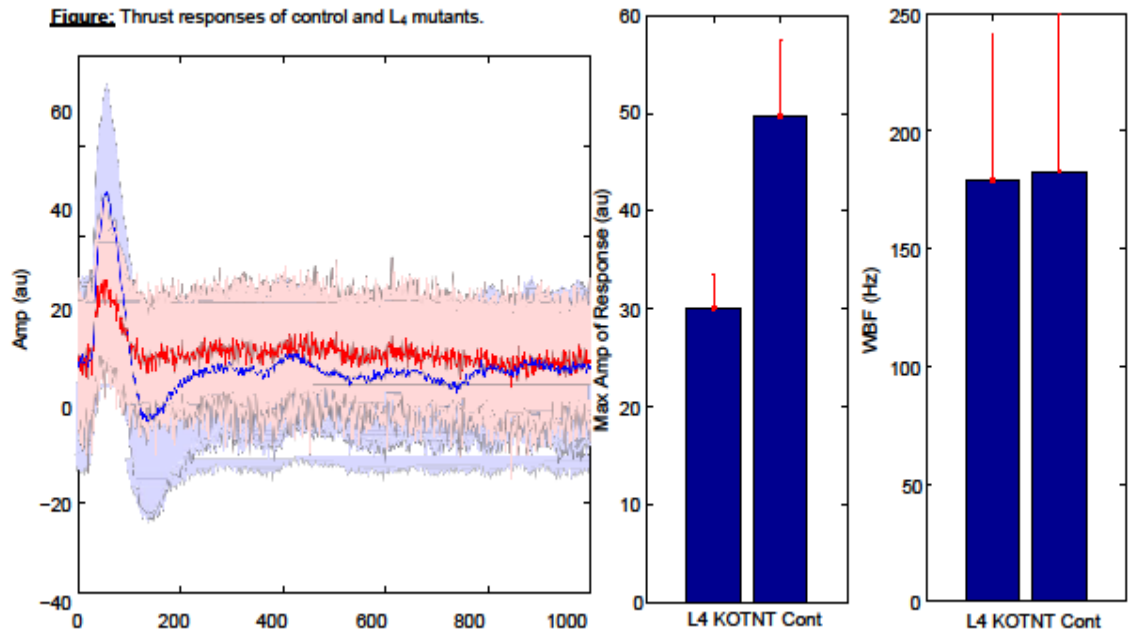


W11xKir(EGFP)<sub>1</sub>8Craised: Fig, FigGnd W11xKir(EGFP)<sub>1</sub>8Craised: FM, DecThe





**Figure:** Thrust responses of control and L<sub>4</sub> mutants.





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