Overexpression of Met and GCE in larval hemocytes is sufficient to increase lamellocyte formation in response to juvenile hormone mimic treatment in *Drosophila melanogaster*

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Overview

- Overexpression of the JH receptors are not enough for lamellocyte formation in larvae without Methoprene.
- Overexpression of Met or gce in hemocytes leads to increased lamellocyte formation in the presence of Methoprene.
- Mean lamellocyte count following treatment for overexpression of Met and gce were both greater than Gal4 alone.

Background

- With the aid of the juvenile hormone receptors, JH is able to postpone metamorphosis until it has reached the appropriate stage, third instar larvae. (Symkal 2014).
- In the final larval stage, there is a decrease in JH which then leads to metamorphosis (Jindra 2013).

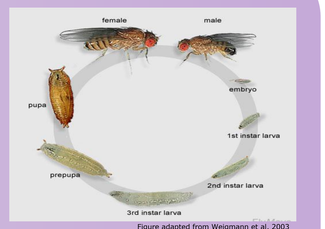


Figure adapted from Weigmann et al, 2003

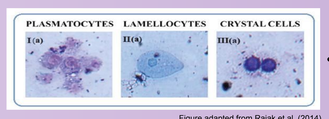


Figure adapted from Rajak et al. (2014)

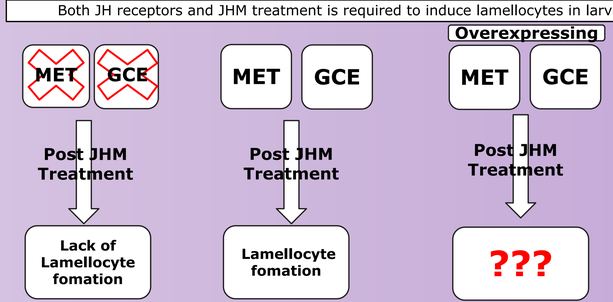
- The three hemocytes present in flies are plasmatocytes, lamellocytes and crystal cells.
- Lamellocytes only form as a immune response to wasp parasitization or by juvenile hormone mimic (JHM) treatment, Methoprene.

MET and GCE are genetically similar and code for the same protein

Figure adapted from Moore et al, 2000

- MET and GCE are juvenile hormone receptors that have 70% of the same amino acid sequence.
- MET and GCE are beta helix proteins where the helix binds to DNA.
- The DNA binding region is highly conserved illustrated by the protein alignment.

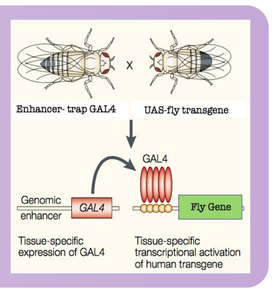
Both JH receptors and JHM treatment is required to induce lamellocytes in larvae



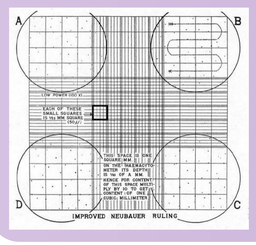
- In previous experiments of the lab, the absence of the JH receptors post hormonal treatment leads to zero lamellocytes being formed.
- The presence of the JH receptors post hormonal treatment leads to lamellocyte induction in larvae.
- We tested the hypothesis that overexpression of Met or gce in hemocytes would lead to increased lamellocyte formation in the presence of methoprene.

Materials & Methods

- Using the binary expression system, Gal4-UAS, the expression of either MET or gce was overexpressed in hemocytes. Hemese-Gal4 and UAS are in separate lines and when the two lines are crossed, the hemese-Gal4 protein binds to the UAS resulting in the expression of the reporter gene. (Zettervall et al. 2004)



- **Lethaltity Test:** 10 male and female prepupae were collected and isolated from the vials to observe if the crosses was lethal.
- **Hormone Treatment:** Two doses of 12.5 microliters of Methoprene in ethanol (1 ug/uL) were administered on top of the food to third instar larvae. For the control group, two doses of 12.5 microliters of 100% Ethanol were administered on top of the food.



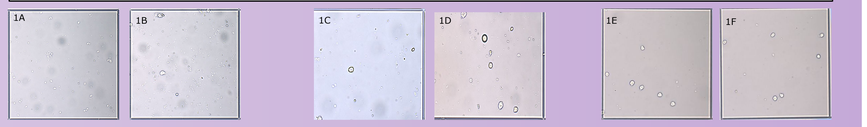
- **Lamellocyte Counting:** Third instar treated larvae was dissected in 20 microliters of PBS. The solution was transferred into a hemocytometer and counted under the magnification of 40x.
- **Whole Larva Imaging:** Minimum of five male larvae for each treatment were imaged using Qimaging system.

Results

The lethal phase analysis revealed there was no difference in eclosion rate for the genotypes, n=60

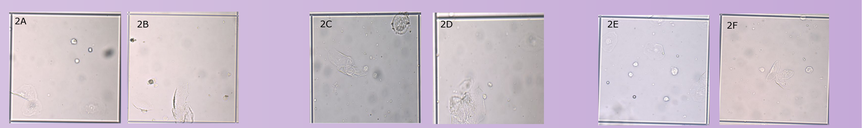
Control Hemese-Gal4	100% Enclosing
Hemese-Gal4>UAS-Met	100% Enclosing
Hemese-Gal4>UAS-gce	100% Enclosing

Ethanol Treated Larva had zero lamellocyte induction, n=30



Control | Overexpressing gce | Overexpressing MET

Methoprene Treated Larva had lamellocyte induction, n=40



Control | Overexpressing gce | Overexpressing MET

Figure 1: Larvae only had plasmatocytes after ethanol treatment
Figure 2: Larvae had both lamellocytes and plasmatocytes post JHM

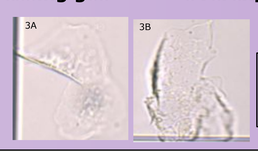


Figure 3: Magnified picture of lamellocytes

hemese-Gal4>Met had more lamellocytes than hemese-Gal4

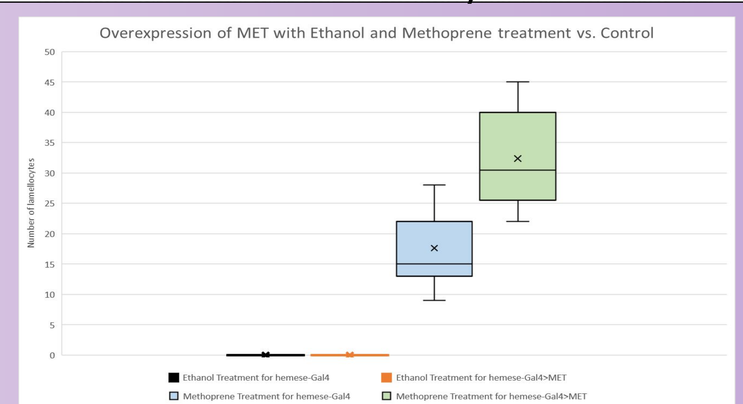


Figure 3: The overexpression of MET post JHM treatment had a greater lamellocyte induction in larvae versus the control

Overexpression of the GCE induced a greater number of lamellocytes than the control

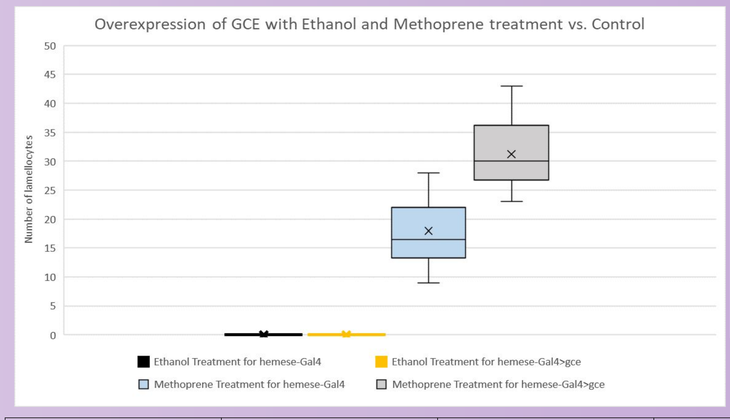


Figure 4: The overexpression of GCE post JHM treatment had a greater lamellocyte induction in larvae versus the control

	Control	Overexpressing gce	Overexpressing MET
Ethanol Mean	0	0	0
Methoprene Mean	18	32	31
T-value		5.7192	7.3094
P-value		**0.0003**	**< 0.0001**

Figure 5: We ran a paired t-test on our data in order to determine the statistical significance of the n-values for each genotype, hoping to have a better idea of how overexpression of JH receptors effected lamellocyte induction. Based on a significance level of .05, both the overexpression of gce and MET were statistically significant with a p-value of 0.0003 and <0.0001.

Discussion

- Our initial hypothesis was correct as through overexpressing either GCE and Met lead to increased llamellocyte induction. The average of both MET overexpression and GCE overexpression are nearly identical possibly due to gene similarities.
- The results indicated that overexpression of the JH receptors are not enough for lamellocyte formation without methoprene as all of the ethanol treated larvae had zero lamellocyte induction. Overexpression of either Met or Gce was sufficient to increase lamellocyte production in response to Methoprene
- Mean lamellocyte count following treatment for Gal4>Met and Gal4>gce were both greater than Gal4 alone, ($p=0.0003$, $p<0.0001$), respectively. These results are consistent with the mutant results showing that lack of JHR leads to a lack of response, indicating that the lamellocyte induction is caused by the methoprene exposure.

Future Directions

- We hypothesize that the fat body also plays a role in this response. The next steps include conducting experiments to study the overexpression of UAS-Met or UAS-gce using Gal4 in the fat body.

References

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Acknowledgements

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