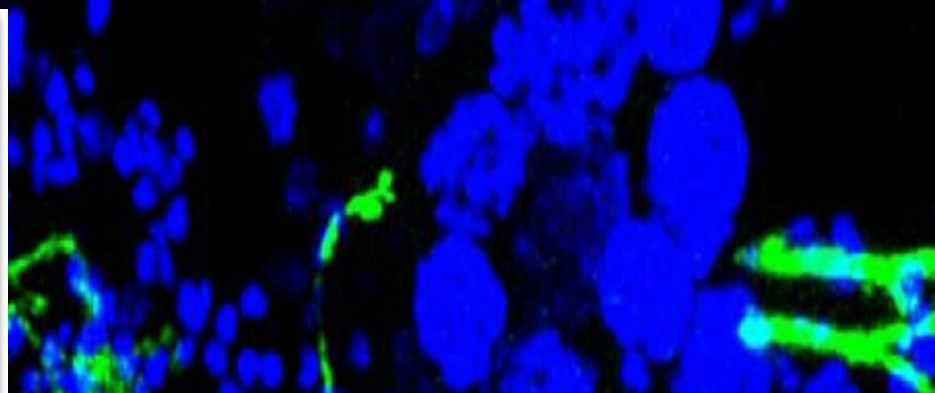
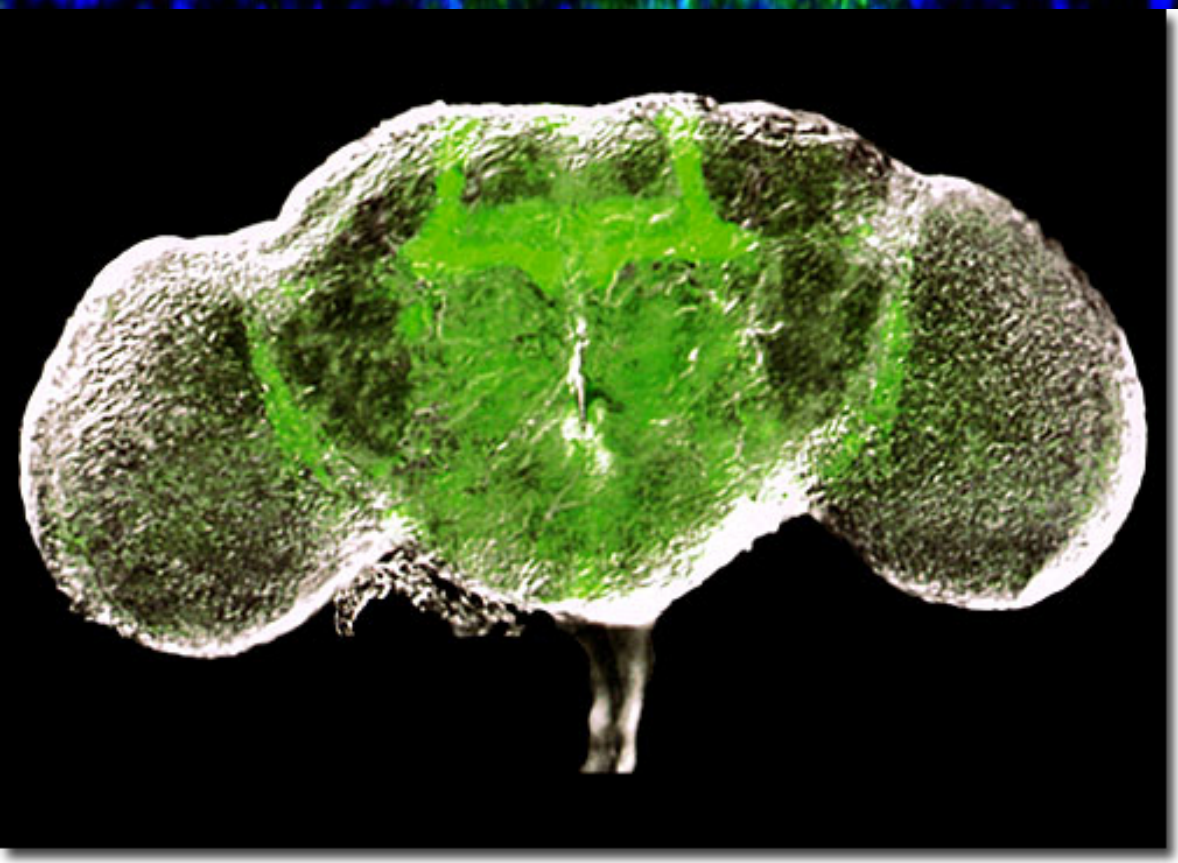


Pigment Dispersing Factor and Drosophila PER Gene Expression

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IS PIGMENT DISPERSING FACTOR EXPRESSED DIFFERENTLY IN PER WILD TYPE AND PER MUTANTS?



Pigment Dispersing Factor (PDF):

- A light sensitive oscillating neuropeptide
- Controls fly locomotion and behavioral rhythmicity in constant conditions
- Expressed in lateral and central neurons
- Related to clock gene expression: PDF mutants do not express the same PER nuclear translocation amplitude and phase as PDF wild type flies

Experimental Procedure

IS PIGMENT DISPERSING FACTOR EXPRESSED DIFFERENTLY IN PER WILD TYPE AND PER MUTANTS?

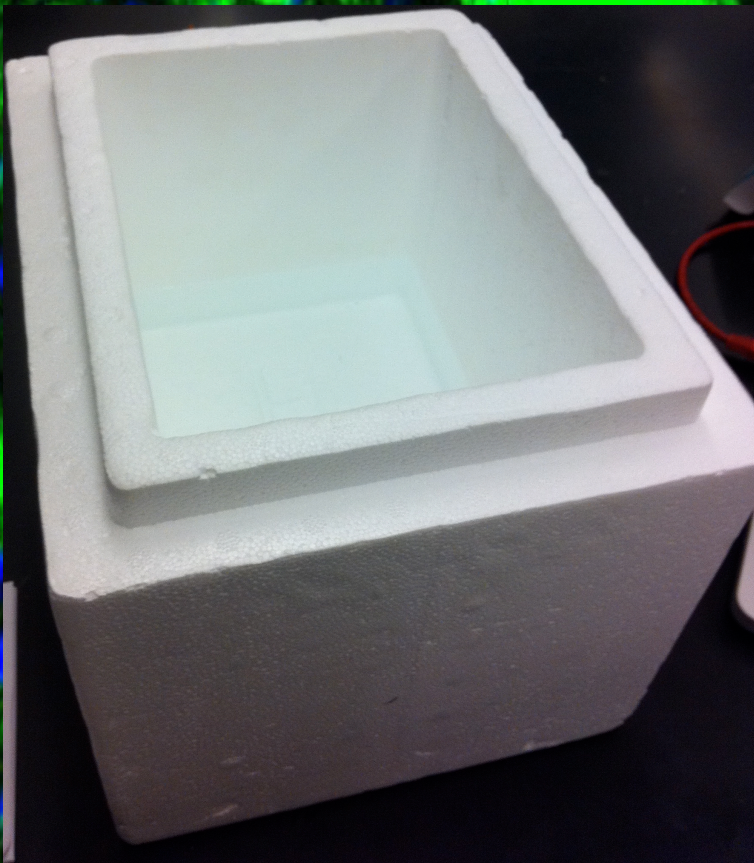
Hypothesis: PER short mutants display less PDF expression at the time of shift in their cycles than PER wild type flies.

Design:

1. PER short and PER wild type *Drosophila* procured and allowed to acclimatize for 10 days
2. Flies knocked out with CO₂ (N=9 each group), put on ice, and micro-dissected in PBS with 1% Triton at the time of change in each cycle (11 am and 1pm respectively).
3. Fix in 4% Para in 1X PBS, rinse with PBS several times (15 min each)
4. Soak in Block and incubate
5. Soak in primary antibody, an anti-PDF that was made in rabbit, and incubate at 4 degrees
6. Rinse in PBS several times
7. Repeat 5-6 for secondary antibody
8. Soak in 70% glycerol at 4 degrees overnight
9. Mount the tissues in "Vectashield" with lifted coverslip
10. Image brains using confocal microscope



Figures 1-4 (from left top to right bottom). Dissecting and staining set- glass disposable pipettes, sharpened forceps, brain tray with wells and foil. CO2 tank. Styrofoam ice box. Microscope and stage used for dissection.



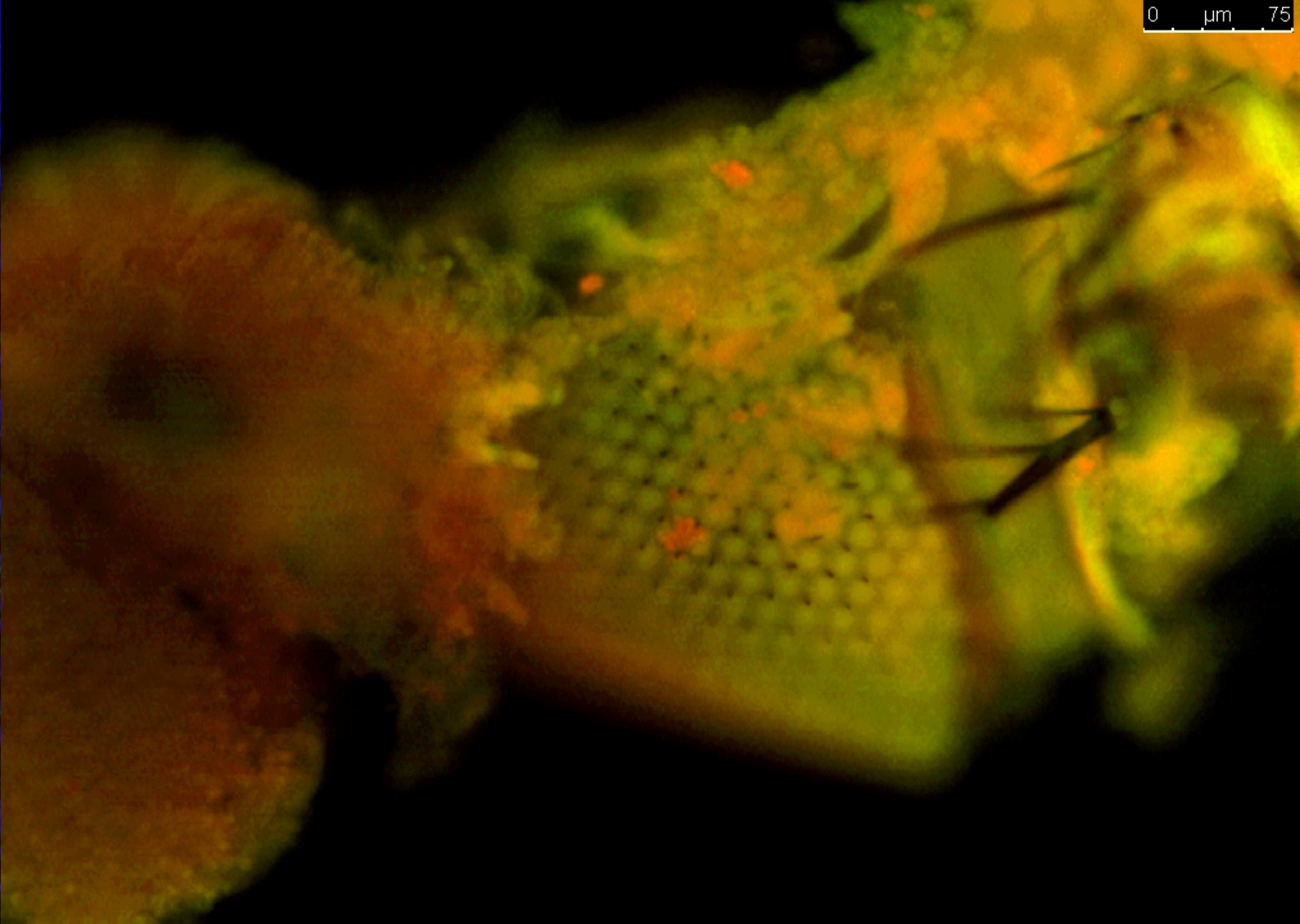
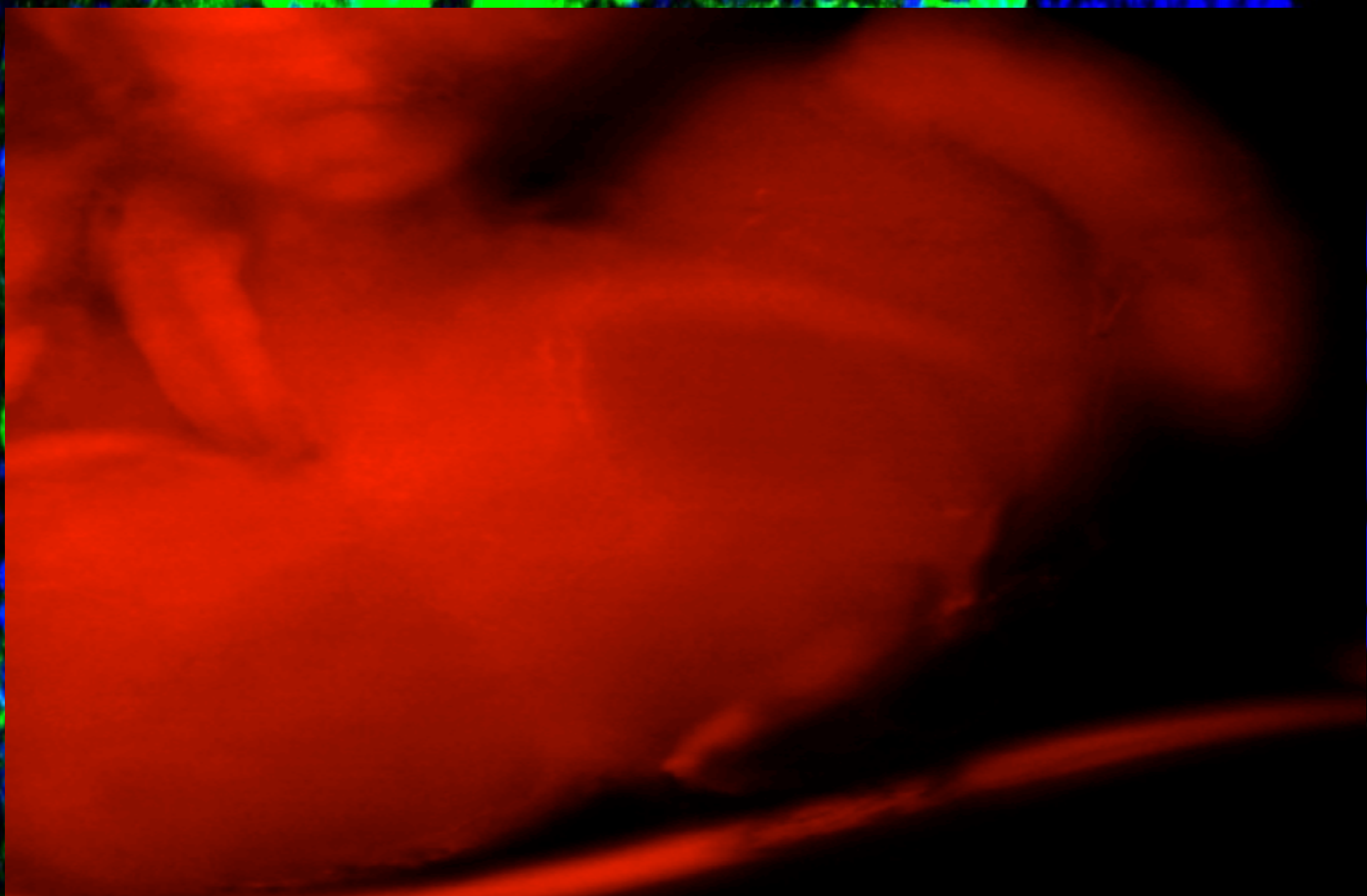


Figure 5-6 (top to bottom). Drosophila eye, cuticle, and photoreceptors imaged with confocal microscopy using red and green overlay. Drosophila brain and antenna imaged in red with confocal microscopy.



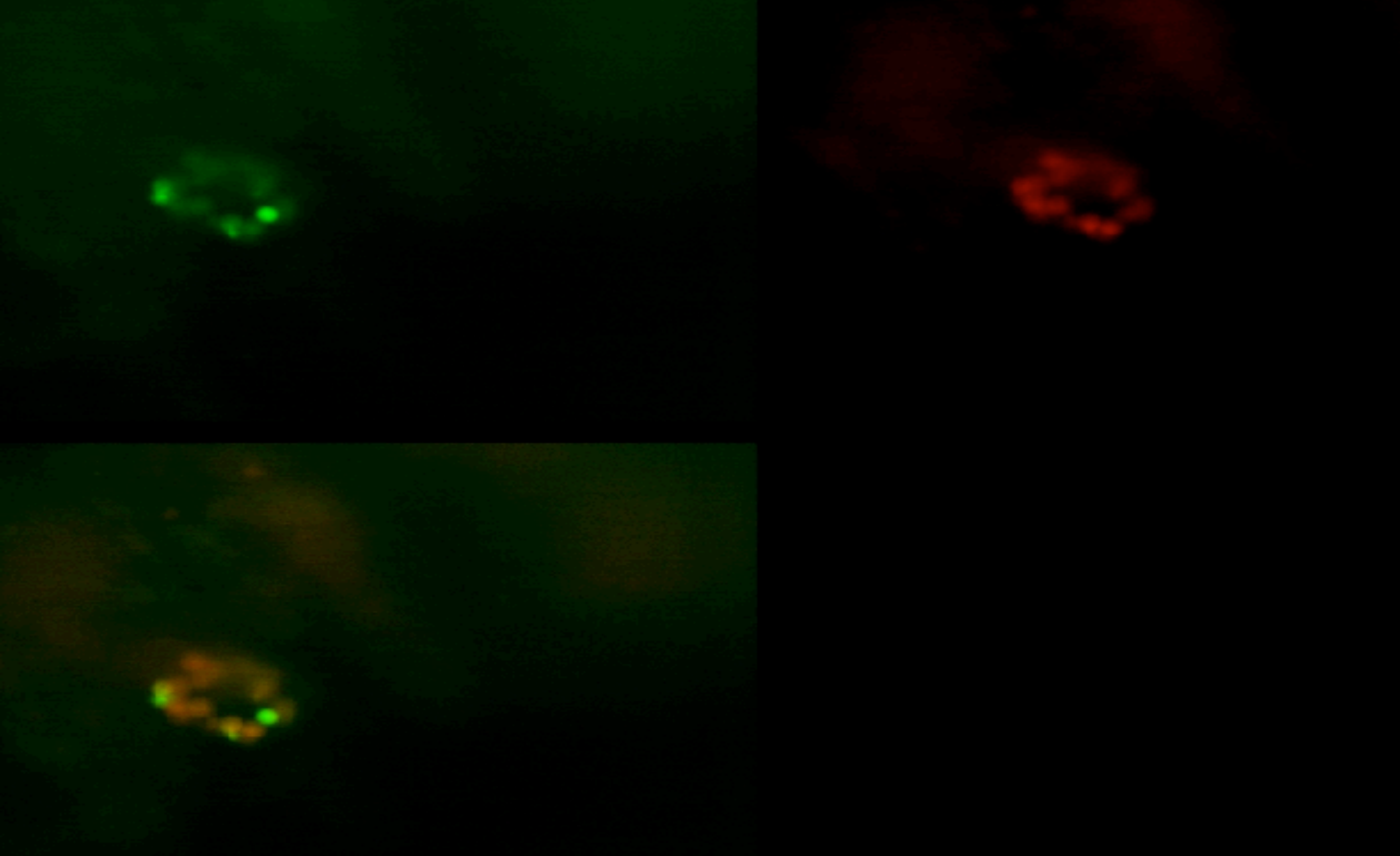
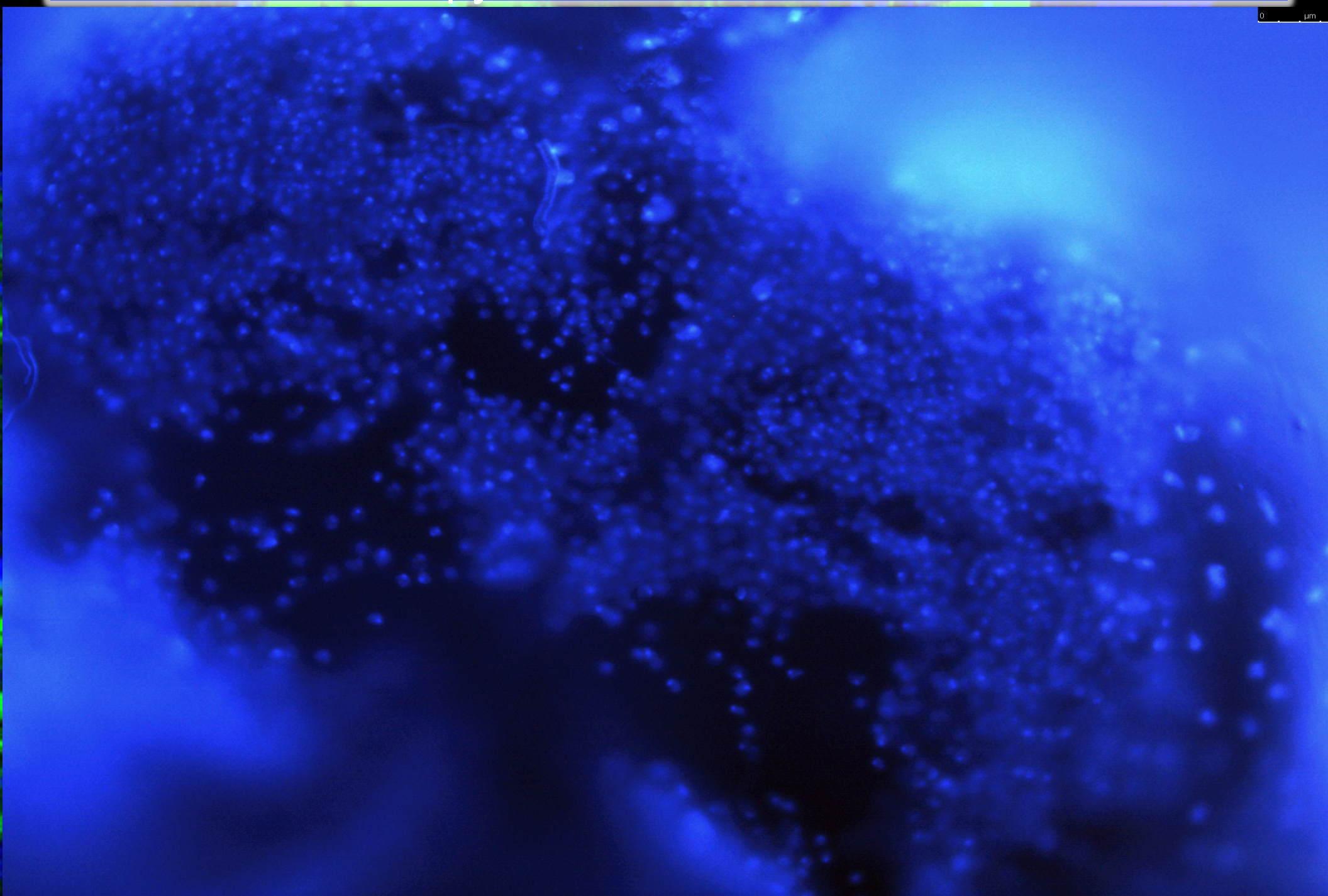


Figure 7-8 (top to bottom). Drosophila neural cell cluster imaged with confocal microscopy using red and green and overlay. The three green spots on the overlay that do not show any red are neural clusters expressing PDF. Drosophila neural nuclei imaged in blue with DAPI with confocal microscopy.



Conclusions

- No significant difference could be analyzed between PER mutants and PER wild type flies.
- One neural cluster found in PER wild type fly that showed PDF expression.
- Better dissection experience needed and more time for imaging in order to carry out study with larger sample size, better timing.
- Learned techniques of confocal microscopy, Drosophila brain dissections, and fluorescent staining and antibody labeling.

Future Directions

- Perform same experiment with practiced dissectors and with better time precision
- Could be coupled with DAPI for clearer view of cells for contrast
- Could be better localized to optic neurons
- Need more controls- need fluorescent stain with no PDF, need different times of day to compare for each group

References

- Helfrich-Forster, Charlotte, Marcus Tauber, Jae H. Park, Max Muhlig-Versen, Stephen Schneuwly, and Alois Hofbauer. "Ectopic Expression of the Neuropeptide Pigment-dispersing Factor Alters Behavioral Rhythms in Drosophila Melanogaster." *The Journal of Neuroscience* 20.9 (2000): 3339-353. Print.
- Lin, Y. "The Neuropeptide Pigment-Dispersing Factor Coordinates Pacemaker Interactions in the Drosophila Circadian System." *Journal of Neuroscience* 24.36 (2004): 7951-957. Print.
- Sheeba, Vasu, Vijay K. Sharma, Hualyu H. Gu, Tu-Ting Chou, Diane K. O'Dowd, and Todd C. Holmes. "Pigment Dispersing Factor-dependent and -independent Circadian Locomotor Behavioral Rhythms." *The Journal of Neuroscience* 28.1 (2008): 217-27. Print.
- Zhou, X. "Drosophila Olfactory Response Rhythms Require Clock Genes but Not Pigment Dispersing Factor or Lateral Neurons." *Journal of Biological Rhythms* 20.3 (2005): 237-44. Print.
- <http://ibp.med.uth.tmc.edu/crb/Assets/images/innovative-program-technology/05-fly-larva-serotonin-large.jpg>
- [http://upload.wikimedia.org/wikipedia/commons/4/4c/Drosophila_melanogaster_-_side_\(aka\).jpg](http://upload.wikimedia.org/wikipedia/commons/4/4c/Drosophila_melanogaster_-_side_(aka).jpg)

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