Monitoring Calcium Levels in the Drosophila Brain Blood Barrier

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Background

The fruit fly, *Drosophila melanogaster*, is a primary model organism used for genetic research. *Drosophila* has given insight into the roles of genes in behavior, some which have proven to have a compatible connection to humans. Our lab has found that the blood brain barrier (bbb), besides its important function as a barrier, plays an important role in regard to the degree of male courtship. It has been found that the dopamine receptor (D2R) is male preferentially expressed in the bbb and its required in the bbb for normal courtship. Calcium is known to be a vital component in cell signaling and we hypothesize that it may play a vital role in male courtship behavior by being dependent on D2R.

We used the Gal4-UAS system to conditionally express the calcium sensor NFAT (nuclear factors of activated T cells). When this sensor is activated by calcium it changes its conformation and regulates a process that generates green fluorescent protein (GFP) expression. Therefore, we can visualize GFP and make conclusions about the calcium concentrations in the bbb cells.

Results

NFAT was crossed into both wildtype and D2R mutant (D2RM) to test whether the presence of D2R affects calcium levels. We observed significant differences in the levels of GFP expression between the two genotypes **(Fig 1).**

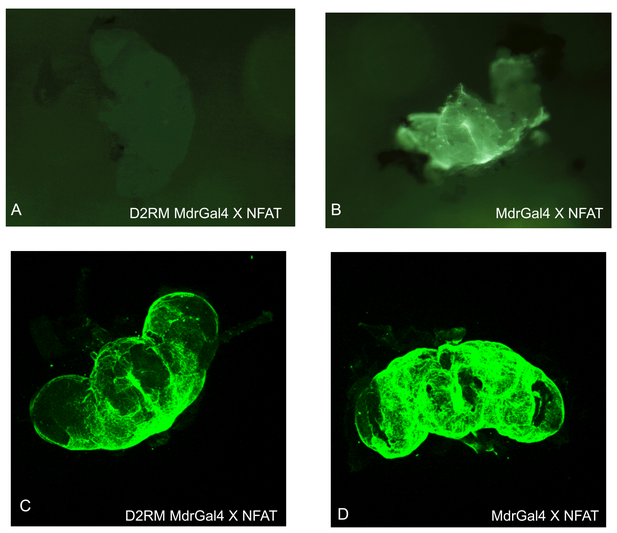


Figure 1. GFP Expression in D2R mutants and wildtype.
A difference in GFP between the mutant and wildtype can be observed under both direct observation and confocal microscope. **A.** Direct observation of D2R mutant showed no GFP expression. **B.** Direct observation of wildtype showed a high signal of GFP. **C.** Confocal image of D2R mutant showing small quantity of GFP. **D.** Confocal image of wildtype Gal4 showing much larger amount of GFP signal than in the mutant.

We next tested a conditional expression system of NFAT **(Fig. 2**). This would allow us to visualize only the calcium produced in mature adult males. We used the *Gal4/UAS/Gal80^{ts}* system as shown in the methods. We expect expression only at 32ºC. When imaged, we can see an expression of GFP in the flies moved to 32ºC (**Fig 2.A and C**). This is because at this temperature the Gal80 inhibitor was inactivated, allowing NFAT to bind to calcium, thus signaling GFP. The brains from the males kept at 18ºC (**Fig 2. B and D**) did not show any amount of GFP, this is due to Gal80 inhibiting Gal4.

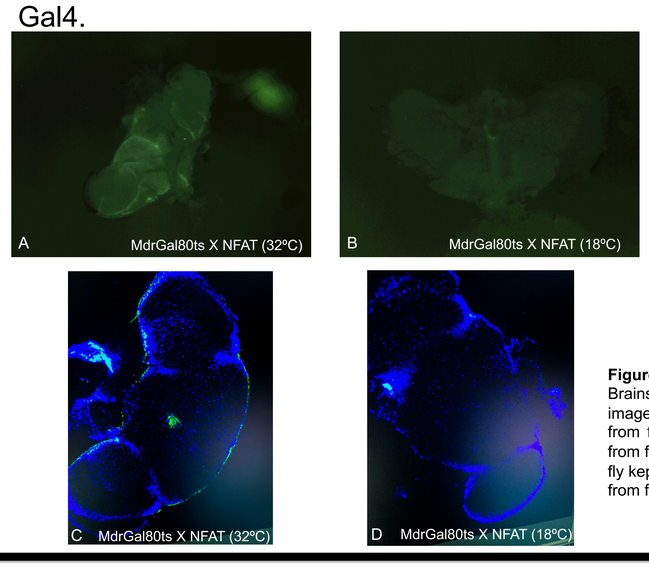


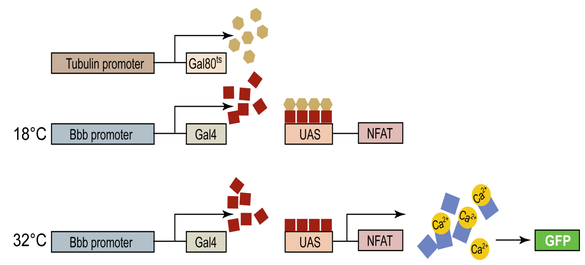
Figure 2. GFP Expression in MdrGal80ts
Brains where seen by direct observation under dissecting microscope and imaged under Leica SP8 confocal microscope. **A.** Direct observation of brain from fly kept at 32ºC shows signals of GFP. **B.** Direct observation of brain from fly kept at 18ºC shows no signal of GFP. **C.** Confocal image of brain from fly kept at 32ºC shows thin line of GFP expression. **D**. Confocal image of brain from fly kept at 18ºC shows no expression of GFP.

The presence of the NFAT transgene in wildtype does not affect courtship (**Graph 1**).

Graph1. MdrGal80ts/NFAT Courtship
Courtship Index (CI) is equal to the fraction of time the male spends courting during the 10 min observation period.

Methodology

- D2R hypomorphic mutants and wildtype flies containing the bbb specific Gal4 driver were crossed with a fly strain that contains the calcium sensor NFAT.
- The crosses were kept at 18ºC which resulted in Gal80ts inhibiting Gal4.
- After a waiting period of 7-9 days, half of the male progeny were moved and kept at 32ºC for 24 hours. At this temperature the Gal80ts inhibitor is not active, and the system can produce NFAT.
- The flies were then moved to 25ºC for 2 hours before courtship testing.
- The flies were kept overnight at 25ºC; they were then dissected in 1X PBS.
- Expression of GFP was examined by direct observation under the microscope and by staining brains using antibodies against GFP using immuhistochemistry.
- The primary antibody used was Chicken anti GFP. The secondary antibody used was Goat anti chicken, coupled to Alexa 488.
- Brains were then mounted using mounting media and then imaged under a Leica SP8 confocal microscope.



The GAl4/UAS/Gal80ts sytem that allows conditional expression of NFAT

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Thank you to Mike de la Flor for the graph showing conditional NFAT expression and to Joseph L Reeve for helping with the fixing and staining process of the brains.

Conclusions

This system is well suited to detect calcium in the bbb. The Gal80 experiments demonstrates that this system allows us to visualize and monitor calcium levels conditionally in the adult bbb. We have known how important D2R is for courtship and with the results showing less calcium in D2RM than in wildtype we can also conclude that D2R in the bbb affects signaling through calcium. Future experiments will aim to further explore the role of calcium and its importance in the bbb.

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