Methods

Introduction

FoxP is a highly conserved transcription factor, with four different genes in vertebrates. FoxP2 has a unique role as the so-called "speech gene." A defect causes impairment in speech and vocalization. In Drosophila, the other hand only one gene is present, with three isoforms.

Speech learning is characterized as a form of motor learning. The same mechanism can be found in the touch-learning task of larval flies. Therefore, "Drosophila Flight Simulator" (DTS) can be used as a powerful tool to study the effects of dFoxP manipulations.

Operant learning behavior can be split into two main forms, operant self and world learning. In self-learning, the animal is getting only information about its own behavior. In world learning, the animal is getting only information about the environment. In Drosophila, the animal is getting only information about the environment.

Protein kinase C (PKC) has also been shown to be involved in learning behavior. PKC are serine/threonine kinases activated by diacylglycerol (DAG), lipid or Ca2+. In Drosophila, five different PKC are present.

Results

Embryonic FoxP knock-out

Knock-out of dFoxP in the embryos is leading to a severe movement defect. Flies are hardly able to walk and couldn't be tested for flying behavior.

Local FoxP knock-out

Local knock-out showed no learning defects:
- protocerebral bridge
- notal
- ato cluster
- lamelica body.

Conditional FoxP knock-out

Knock-out of dFoxP in all neurons of adult Drosophila

PKC knock-down

Knock-down of PKC:
- in FoxP-positive cells
- in all neurons
- conditionally in all neurons

aPKC knock-out

Knock-out of aPKC in motor neurons or FoxP2-positive cells

Embryonic FoxP knock-out

FoxP knock-out, test after 14 days

7 day old flies are still able to perform the learning task (data not shown)

References

3. Bjoörn Brembs and Plendl (2008), Double Dissociation of PKC and AC Manipulations on Operant and Classical Learning in Drosophila

Conclusion

FoxP is showing a clear developmental effect in Drosophila. A knock-out in the embryo is leading to a severe movement defect. These flies cannot be tested in the DTS.

Knock-out FoxP in the adult fly shows no immediate effect. These flies perform equal to the genetic controls. This would indicate only a developmental role of FoxP for operant self-learning. But testing the flies later after the knock-out is indicating an additional role of FoxP. 14 day old flies without FoxP showed a learning impairment compared to the genetic controls.

Expressing the protein kinase C (PKC) inhibitor PKCi in dFoxP-positive neurons showed a learning impairment. Since it was not known which of the five PKC is involved in this learning process we performed a knock-out of the atypical PKC (aPKC). A knock-out of aPKC in all motor neurons showed a learning defect, suggesting that aPKC is the gene responsible for operant self-learning in Drosophila. Interestingly a knock-out of aPKC in all FoxP-positive neurons was sufficient to also prevent self-learning.

Training protocol

- Period 1: Pretest
- Period 2: Training
- Period 3: Punishment
- Period 4: Training
- Period 5: Test
- Period 6: Training
- Period 7: Test
- Period 8: Training
- Period 9: Test

1 period = 2 min