Developmental expression of *dFoxP* is required in motorneurons for operant selflearning in *Drosophila*

Andreas Ehweiner, Björn Brembs

Institute of Zoology – Neurogenetics, University of Regensburg, Regensburg, Germany

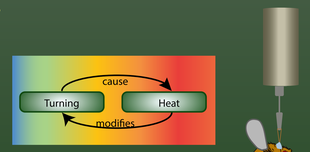
Introduction

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 on 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 toque-learning task of tethered flies. Therefor the “Drosophila flight simulator” (DTS) can be used as a powerful tool to study the effects of dFoxP manipulations.

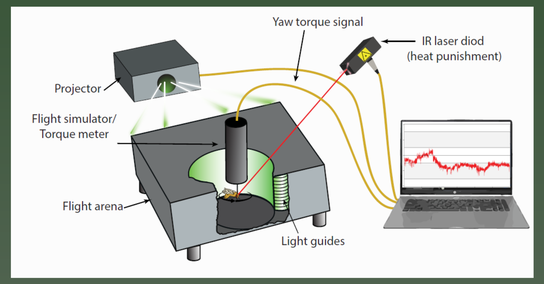
Operant learning behavior can be split in to two main forms, operant self and world learning. In self-learning, the animal is getting only information about its own behavior. In world learning on the other hand information about the environment can be perceived. While dFoxP mutants are still able to perform the world learning task, their self-learning ability is greatly impaired.

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.



Methods

Flight simulator setup



Training protocol

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

1 period = 2 min

Results

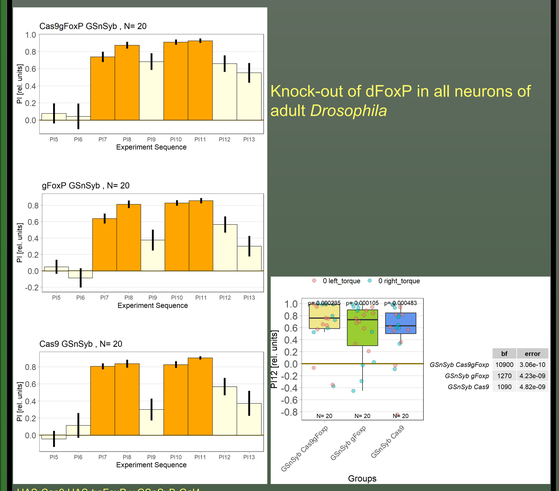
Local FoxP knock-out

Local knock-out showed no learning defects:
- protocerebral bridge
- noduli
- ato cluster
- fanshape body

Embryonic FoxP knock-out

Knock-out of dFoxP in the embryo is leading to a sever movement impairment. Flies are hardly able to walk and couldn’t be tested for flying behavior.

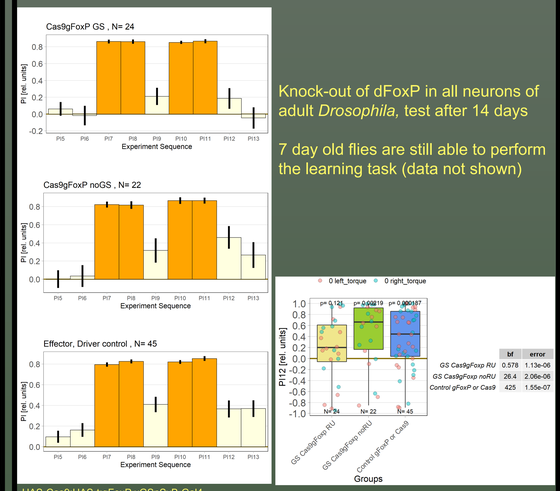
Conditional FoxP knock-out



Knock-out of dFoxP in all neurons of adult *Drosophila*

UAS-Cas9;UAS-t:gFoxP x GSnSyB-Gal4
UAS-t:gFoxP x GSnSyB-Gal4
UAS-Cas9 x GSnSyB-Gal4

FoxP knock-out, test after 14 days

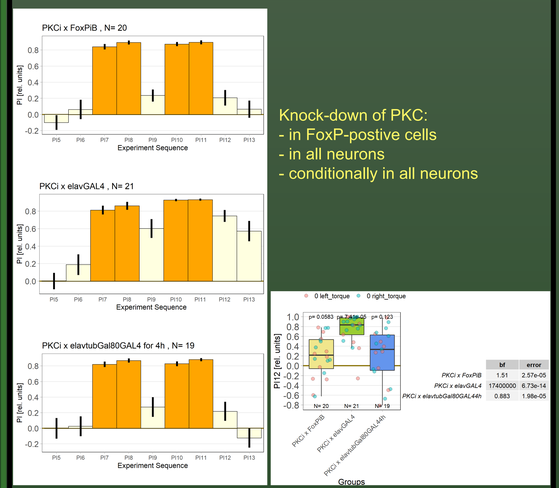


Knock-out of dFoxP in all neurons of adult *Drosophila*, test after 14 days

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

UAS-Cas9;UAS-t:gFoxP xGSnSyB-Gal4
UAS-t:gFoxP x GSnSyB-Gal4
UAS-Cas9 xGSnSyB-Gal4

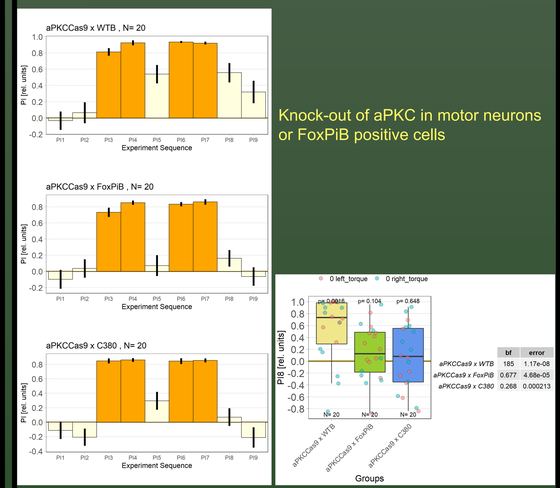
PKC knock-down



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

UAS-PKCi x FoxPiB-Gal4
UAS-PKCi x elav-Gal4
UAS-PKCi x elav-Gal4;tubGal80

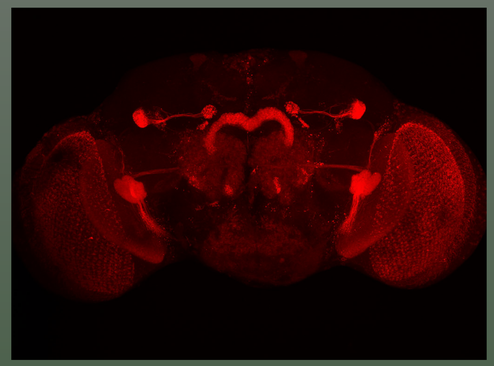
aPKC knock-out



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

g-aPKC;UAS-Cas9 x WTB
g-aPKC;UAS-Cas9 x FoxPiB-Gal4
g-aPKC;UAS-Cas9 x C380-Gal4

Conclusion



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

Knocking 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 dFoxPiB 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 knockout of aPKC in all FoxPiB positive neurons was sufficient to also prevent self-learning.

References

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