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The *Drosophila FoxP* gene is necessary for operant self-learning: Implications for the evolutionary origins of language

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8. No obvious brain defects in FoxP^{3955} mutants

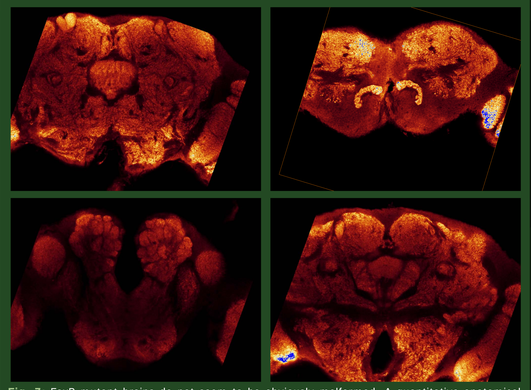


Fig. 7: FoxP mutant brains do not seem to be obviously malformed. A quantitative anatomical analysis searching for more subtle defects is currently under way.

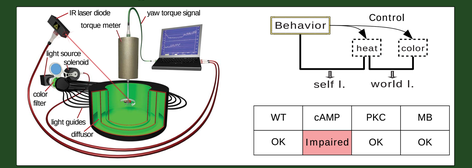
1. Abstract

In humans, mutations of the transcription factor Forkhead box protein P2 (FOXP2) cause a severe speech and language disorder. Downregulating the Zebrafinch FOXP2 orthologue in development results in incomplete and inaccurate song imitation. Because both language and song learning can be seen as instances of operant trial-and-error learning, we investigated the involvement of the fly orthologue, FoxP, in operant self-learning in the fly. The experiments were performed using stationary flying *Drosophila* at the torque compensator with heat as punishment. Both a P-Element insertion and RNAi-mediated knockdown of the last exon of the *Drosophila* FoxP gene did not lead to alterations of the gross brain anatomy, nor to an impairment in operant world-learning, i.e., color-learning, compared to control flies. However, both fly strains were impaired in operant self-learning, i.e., yaw-torque learning without any environmental predictors. These results suggest a specific involvement of the *Drosophila* FoxP gene in the neural plasticity underlying operant self-learning but not other forms of learning. To investigate the effects of RNAi knockdown and P-Element insertion on FoxP abundance and localization in the fly central nervous system, we have generated polyclonal chicken antibodies against four different regions of the putative FoxP protein. ELISA results show specific detection of two of the peptides by their respective antibodies. Analysis of FoxP expression patterns on the mRNA as well as on the protein level shows differential FoxP expression in the different fly strains.
Perhaps not surprisingly, these results suggest that one of the evolutionary roots of language is the ability to directly modify behavioral circuits. It is noteworthy, however, that these roots can apparently be traced back to the Urbilaterian, the last common ancestor of vertebrates and invertebrates.

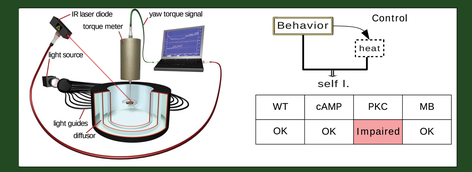
2. The FoxP gene family tree

***Fig. 1:* The insect FoxP orthologues fit right into the FoxP family tree**
The bilaterian FoxP gene family arose from a single FoxP gene. The ancestral variant, conserved in the invertebrate lineage, later underwent two subsequent duplicatiuons, leading to the four vertebrate genes, FoxP1, FoxP2, FoxP3 and FoxP4.

3. PKC activity is required specifically for self-learning



WT	cAMP	PKC	MB
OK	Impaired	OK	OK



WT	cAMP	PKC	MB
OK	OK	Impaired	OK

Fig. 2: Two operant conditioning experiments, distinguished by the presence or absence of predictive stimuli. Above: Flies learn to avoid the heat associated with one of two colors and left or right turning, respectively. Manipulating cAMP levels abolishes learning in this task. Below: Removing the color stimuli leaves the animal with only its behavior as predictor of heat punishment. Manipulating PKC abolishes learning in this task. *Brembs & Plendl, Curr. Biol. 2008*

4. Insertion 3955 in the FoxP gene affects self-learning

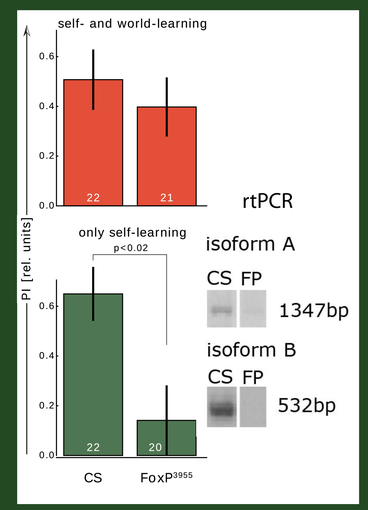


Fig. 3: FoxP function dissociates between self- and world-learning. Canton S wild-type flies perform well in both learning situations, whereas a FoxP insertion mutant line (3955) how significantly reduced learning scores specifically in the self-learning task.
Reverse transcriptase PCR shows that the insertion affects both FoxP isoforms, but while small amounts of isoform A can still be detected, isoform B appears to be entirely absent

5. The Drosophila FoxP gene locus

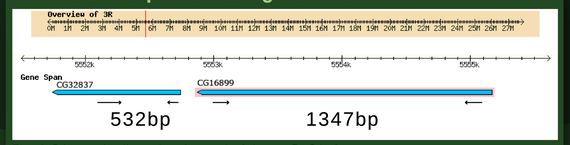


Fig. 4: Primer pairs directed against each of the two FoxP isoforms.

6. *Drosophila* FoxP isoform B is required for self-learning

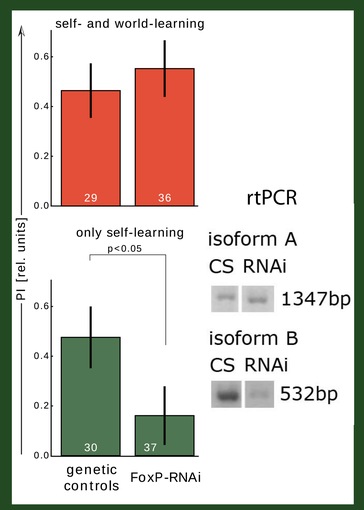


Fig. 5: Targeting isoform with with an RNAi construct directed against the last exon of the FoxP gene yields a phenocopy of the FoxP^{3955} insertion.

7. FoxP protein expression

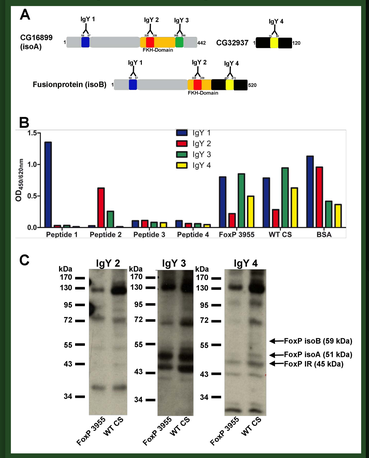


***Fig. 6:* Raising polyclonal chicken IgY antibodies against Drosophila FoxP protein.**
A Peptide regions used for BSA-conjugates to immunize chicken. Peptide 1 (IgY1), peptide 2 (IgY2) and peptide 3 (IgY) are sequences of CG16899 (isoform A) and peptide 4 is located in CG32937. All IgY except IgY 3 could bind to a putative fusionprotein of CG16899 and CG32937 (isoform B). **B** Indirect ELISA-Titer after eight boosts. Only IgY 1 and IgY 2 specficially detect their peptide. All IgY bind to extracts of *Drosophila* heads from FoxP^{3955} or wildtype Canton S. The detection of BSA is shown as a positive control. **C** Immunoblot using IgY2, IgY3 and IgY4 binding to head extracts from FoxP^{3955} or wildtype Canton S. Different polyclonal antibodies show different positive protein bands.

Presented at the annual meeting of the Society for Neuroscience in San Diego, Ca, November, 2010