The *Drosophila FoxP* gene is required for operant self-learning: Implications for the evolution of language acquisition

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1. Summary

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. These forms of vocal learning exhibit two common characteristics: 1. Spontaneous initiation of behavior ('trying out'); 2. Evaluation of sensory feedback shaping behavior. Using a torque learning essay in which both characteristics have been realized, we investigated the involvement of the fly orthologue, *dFoxP*, in operant self-learning in the fruit fly *Drosophila*. 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 isoform B of the *Drosophila* FoxP gene did not lead 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. Only this form of learning, operant self-learning, is conceptually similar to language acquisition. Neither the FoxP intron retention isoform nor isoform A appear to be involved in this form of learning. These results suggest a specific involvement of isoform B of the *Drosophila* FoxP gene in the neural plasticity underlying operant self-learning but not in other forms of learning. Further underscoring the conceptual parallels of operant self-learning with language acquisition, habit formation in flies is also affected by mutations in the *dFoxP* gene.

Perhaps not surprisingly, these results are consistent with the hypothesis that one of the evolutionary roots of language is the ability to directly modify the neural circuits controlling behavior. It is noteworthy that these roots can apparently be traced back to the Ur-bilaterian, the last common ancestor of vertebrates and invertebrates.

2. Conceptualizing language learning

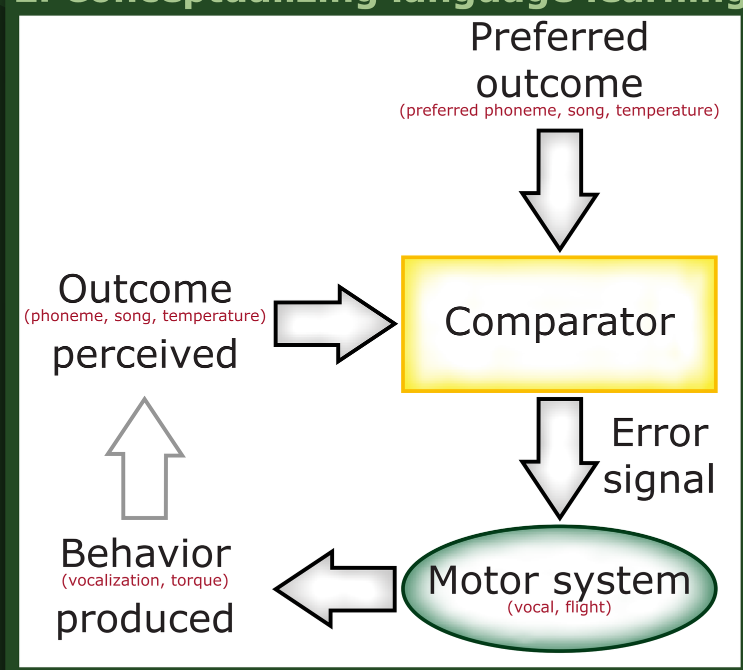

***Fig. 1:* Conceptual architecture of operant feedback loops.**
Given the operant nature of the learning procedure, vocal learning in songbirds and humans share some conceptual aspects with operant self-learning in Drosophila at the torque meter. The motor system (the vocal system in songbirds and humans, the flight system in flies) generates behavioral actions (vocalizations or torque) which lead to sensory feedback (phonemes, song or heat). This actual outcome is then evaluated with respect to the preferred outcome (intended phoneme, tutor song template or preferred temperature). Any deviation from the preferred outcome will lead to a teaching signal instructing the motor system to modify the generated behavior until the desired state of the animal is reached.

3. The FoxP gene family tree

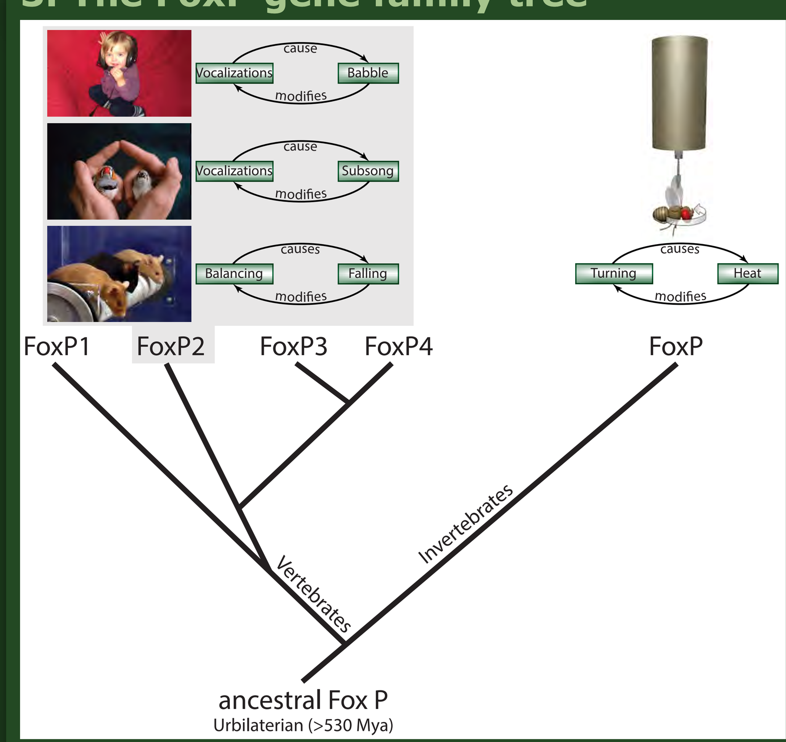

***Fig. 2:* The insect FoxP orthologues suggest the ancestral form**
The bilaterian FoxP gene family arose from a single FoxP gene. The ancestral variant, conserved in the invertebrate lineage, later underwent subsequent tendem duplications, leading to the four vertebrate genes, FoxP1, FoxP2, FoxP3 and FoxP4.

4. The *Drosophila* FoxP gene locus

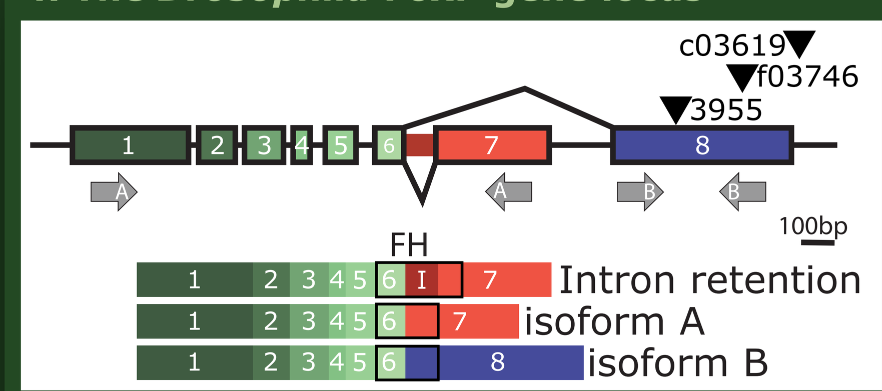

***Fig. 3:* The Drosophila FoxP gene locus and putative isoform mRNA structure.**
Location of the three P-Element insertions (black triangles) and RT-PCR primer pairs (grey arrows) on the genomic structure of the dFoxP gene (above). Structure of the three cloned transcripts (below). FH: Forkhead-Box Domain.

5. Characterizing three insertion lines

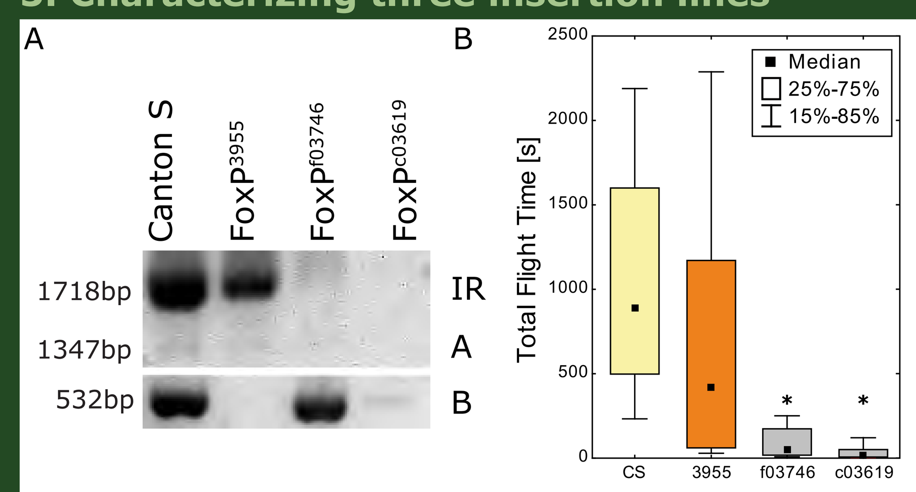

***Fig. 4:* The three insertion mutants differ in isoform expression patterns and only one insetion line shows normal flight performance.**
A - rtPCR results using the primers as described in Fig. 3. The three lines show marked differences in the expression patterns of the three isoforms. B - Flight performace tests show that only line 3955 is suitable for behavioral experiments at the torque meter. Number of animals: CS: 18, 3955: 30, f03746: 34, c03619: 37

6. PKC activity is required specifically for self-learning

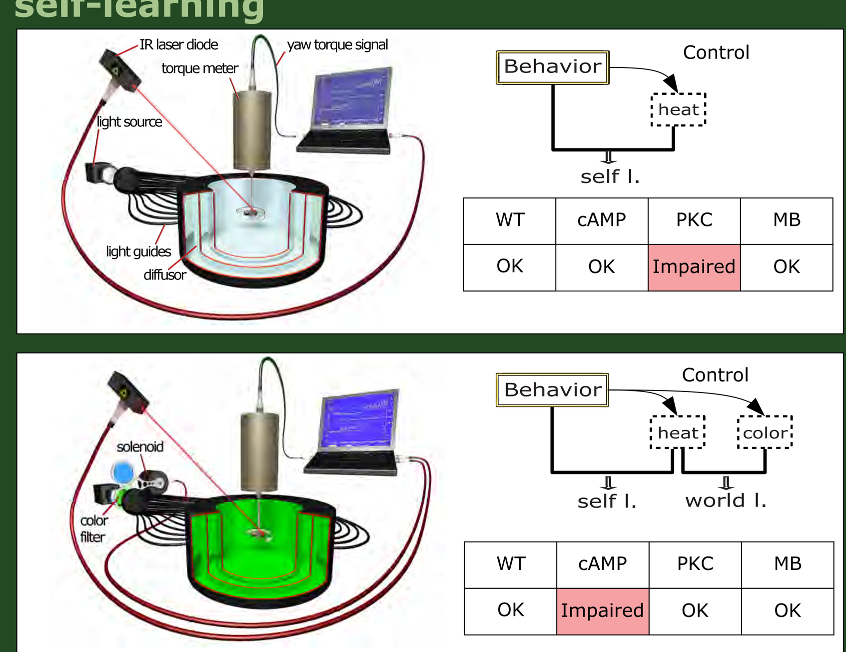

***Fig. 5:* Two operant conditioning experiments, distinguished by the presence or absence of predictive stimuli.**
Above: Flies learn to avoid the heat by trying out different behavioral programs and evaluating the resulting sensory feedback. No sensory predictors are present. Manipulating PKC activity, but not cAMP levels abolishes learning in this task. Below: Adding predictive color stimuli allows the animal to also learn which colors are predicting the heat punishment. Manipulating cAMP levels abolishes learning in this task, while reducing PKC activity has no effect. *Brembs & Plendl, Curr. Biol. 2008*

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

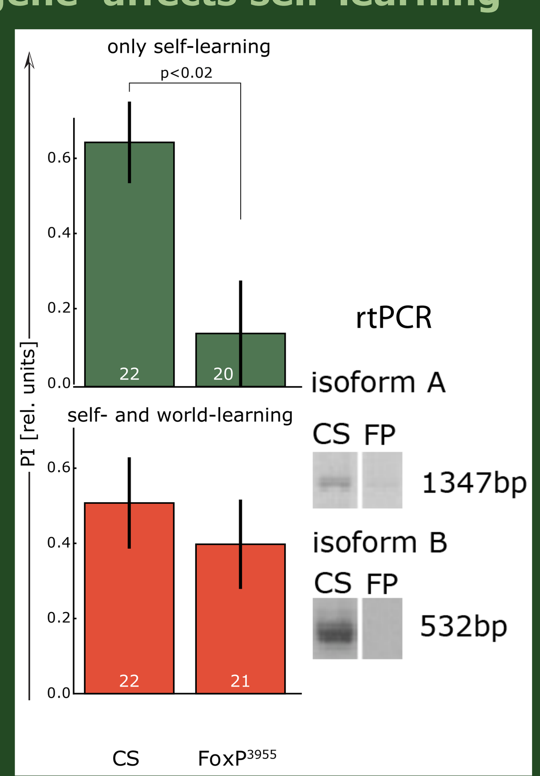

***Fig. 6:* FoxP function dissociates between self- and world-learning.**
Canton S wild-type flies perform well in both learning situations, whereas a FoxP insertion mutant line (3955) how significantly reduced learning scores specifically in the self-learning task.
Reverse transcriptase PCR shows that the insertion affects both FoxP isoforms, but while small amounts of isoform A can still be detected, isoform B appears to be entirely absent

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

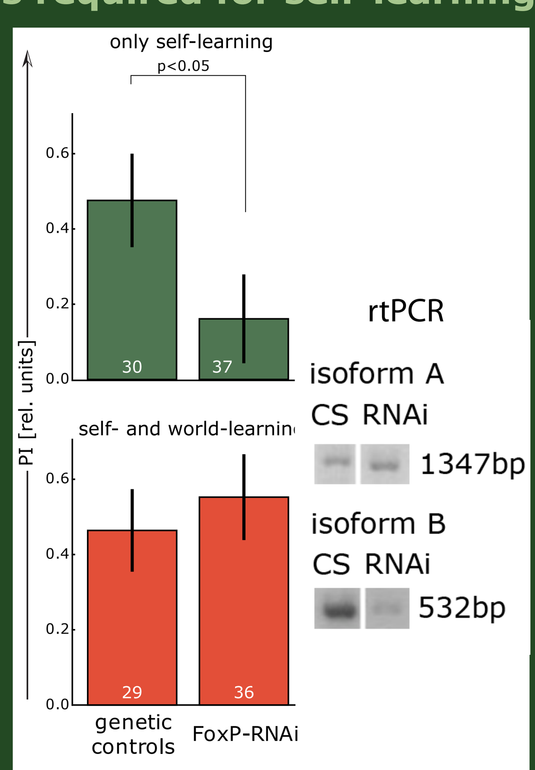

***Fig. 7:* Self-learning requires isoform B.**
Targeting isoform B with with an RNAi construct directed against the last exon of the FoxP gene yields a phenocopy of the FoxP3955 insertion: self-learning is abolished, while world-learning is unaffected.

9. *dFoxP* is required for habit-formation

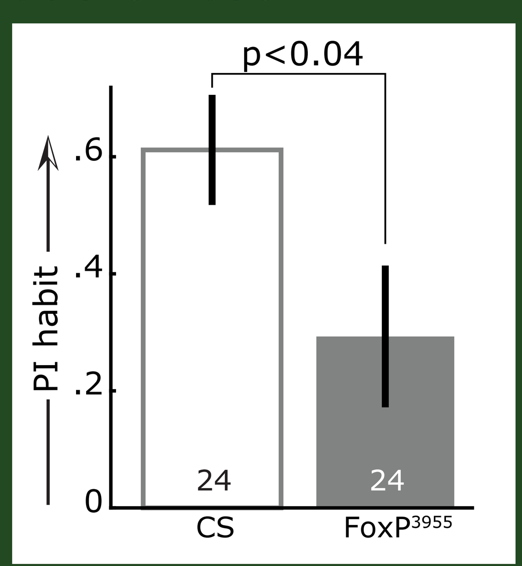

***Fig. 8:* Habit formation requires intact dFoxP.**
, Mutant FoxP3955 flies were impaired in habit formation. In a 2-minute self-learning test (i.e. without colors) after 16 minutes of training in world-learning (i.e. with colors), FoxP3955 flies showed a significantly reduced preference for the previously unpunished turning-maneuvers, compared to wild type control animals (Mann-Whitney U-Test, U=186.0, p<0.04).

10. Subtle brain defects in FoxP mutants

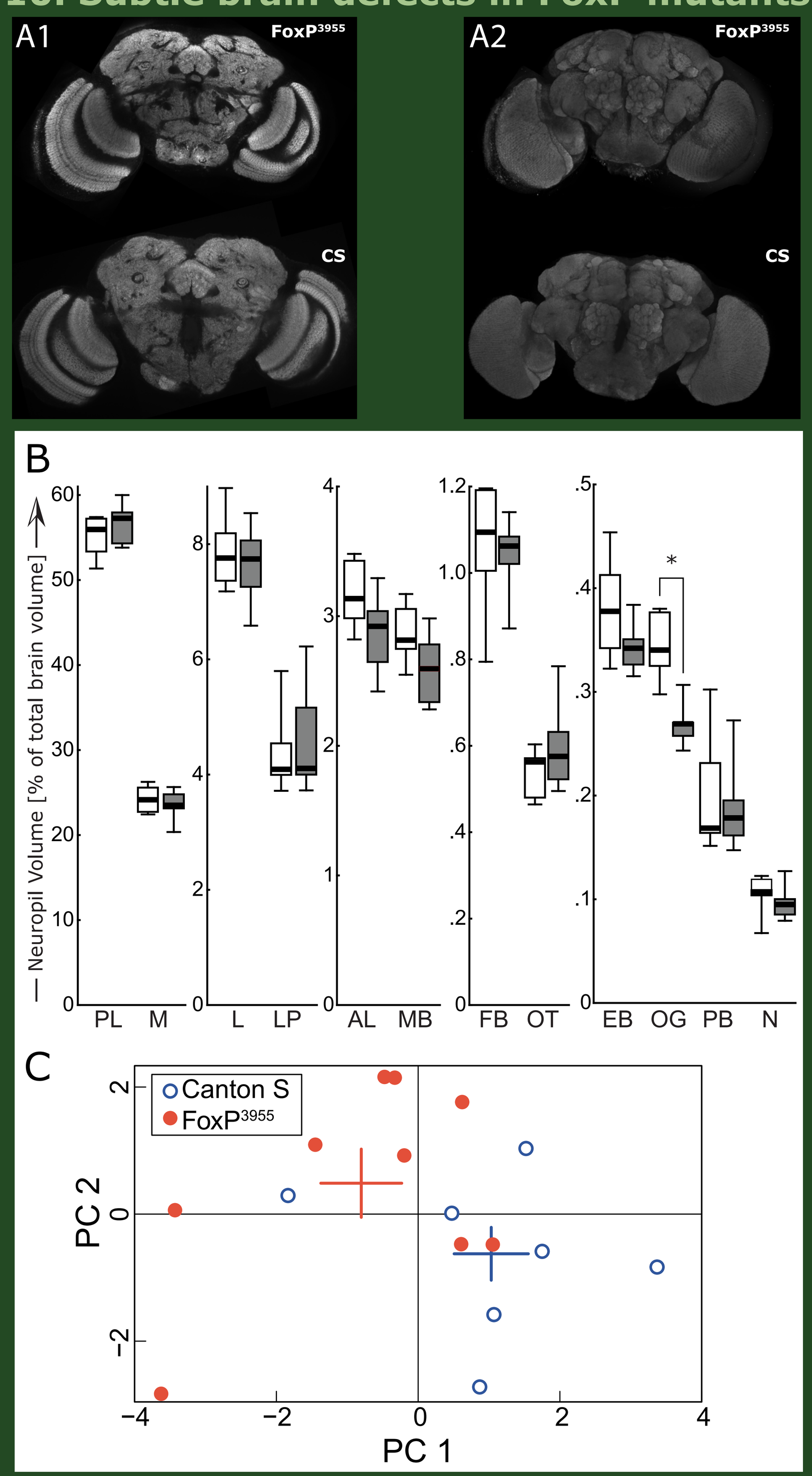

***Fig. 9:* Subtle morphological alterations in the brains of FoxP3955 mutants.**
A1 - Frontal sections of one typical wildtype and mutant fly brain, respectively. A2 - Volume rendering of a wildtype and a mutant fly brain. B, Quantitative volumetric analysis of eleven major neuropils (M – medulla, L – lobula, LP – lobula plate, MB – mushroom bodies, AL – antennal lobes, FB – fan-shaped body, OT – optic tubercle, EB – ellipsoid body, OG – optic glomeruli (purple in a), PB – protocerebral bridge, N – noduli) revealed a significant reduction in the volume of the optic glomeruli in FoxP3955 flies (Mann-Whitney U-Test, U=2.0, p<0.002). The volume of the remaining neuropils (denoted PL – protocerebral lobes) did not differ significantly. Asterisk – significant difference with a Bonferroni-corrected level of p<0.004. Black stripes – median, boxes – 25-75% percentiles, whiskers – total range. Grey boxes indicate FoxP3955, white boxes Canton S. C, Principal Components Analysis of the volumetric data. Plotted are the factor loadings of the individual flies on the two first components. Colored bars indicate means and standard errors (PC). Factor loadings are significantly different between Canton S and Foxp3955 for PC1 (Mann-Whitney U-Test, U=52.0, p<0.04), but fail to reach significance for PC2. Number of brains analyzed: 7 (Canton S) and 9 (Foxp3955).

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