CRISPR/Cas9-based genome editing of the FoxP locus in Drosophila melanogaster

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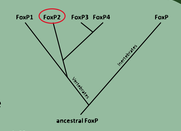
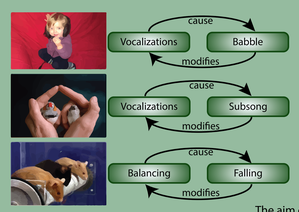
1. Introduction

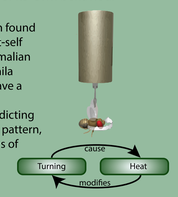


1.1 The family of Fox genes groups a large number of transcription factors that share a conserved DNA-binding domain, called the Forkhead domain.
In vertebrates the FoxP subfamily comprises four genes differentially expressed in different tissues. In *Drosophila melanogaster* on the contrary, only one gene of the FoxP subfamily has been identified (*dFoxP*).
The Foxp2 gene sequence is highly conserved in vertebrates and has been etensively shown to have importance in vocal learning and the modulation of the interconnected neural circuits. One mutation in the FOXP2 gene appears to affect language acquisition in afflicted patients, without other obvious impairments.

1.2
- operant world-learning, in which the subject is able to learn from casual cues in the environment
- operant self-learning, in which the subject is able to learn from the outcome of its own behaviour



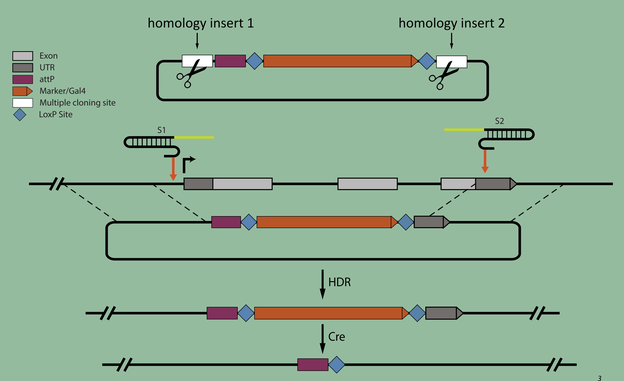
1.3 The FoxP2 gene has been found to be involved in operant-self learning in various mammalian species, and the Drosophila homologous seems to have a conserved function.
However, due to contraddicting evidence, the expression pattern, function and mechanisms of action remain still to be elucidated.



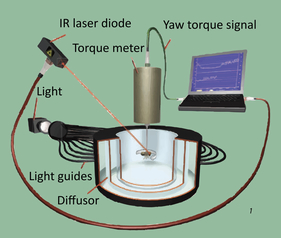
The aim of this work is thus to shed light on this controversial gene.

2. Experimental strategy

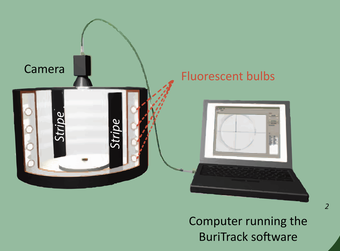
Homology recombination by CRISPR/Cas9



Flight simulator

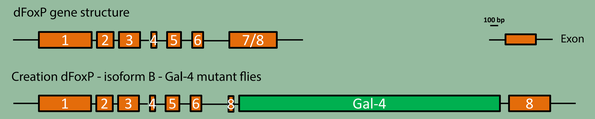


Buridan´s paradigm



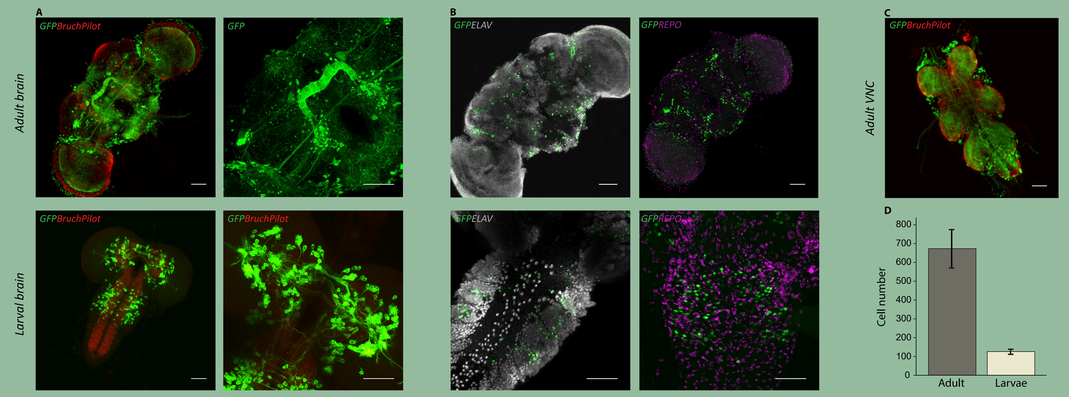
3. Results

Creation of the construct by CRISPR/Cas9



Using the CRISPR/Cas9 technique we created a fly with a Gal-4 constructs inserted directly into the exon 8 of the *dFoxP* - isoform B sequence. This construct has both the advantages of the high fidelity reproduction of *dFoxP* - isoform B expression via the Gal-4 protein, and a total Knock Out background.

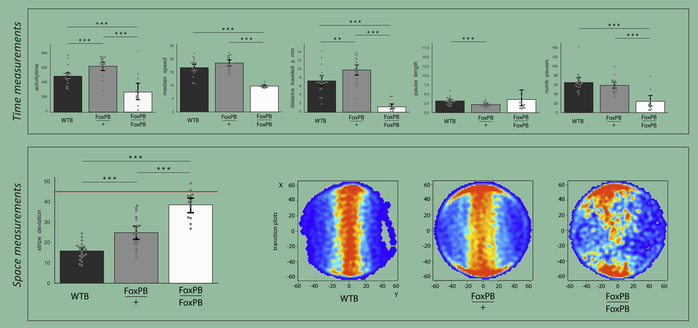
Expression pattern analysis



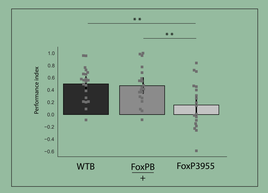
In this pictures we can see the expression pattern of the *dFoxP* - isoform B protein in both larval brain and adult brain. A) and C) show the expression of CD8-GFP (which is expressed in neuron´s pyrenophore and projections)in brain and VNC, B) shows the expression of Stinger-GFP (which is expressed in cell nucleai). BruchPilot stains the synapses, ELAV is a neuron-specific protein, REPO is a transcription factor specifically expressed in glial cells. The graph in D) shows a Stinger-GFP cell count performed in both adult and larvae brains. (scale bars: 50 µm)

Behavioral analysis

Buridan´s paradigm

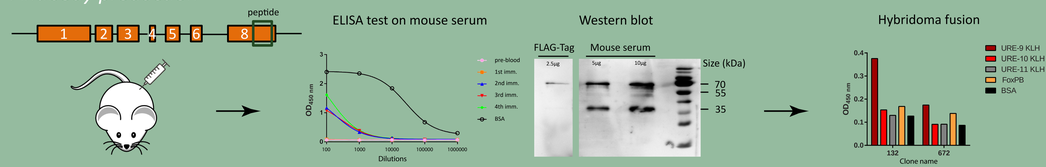


Flight simulator



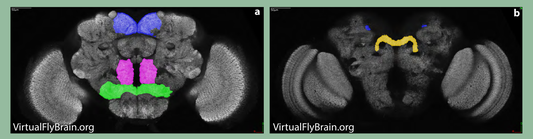
* p<0,05; ** p<0,01; *** p<0,001
all graphs are represented with standard error of the mean.
In the Flight simulator experiment we weren´t able to test the homozygous FoxPB/FoxPB genotype due to its impossibility to perform flight.

Antibody production

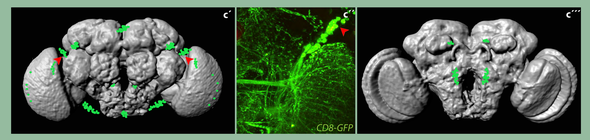


4x peptide injections in BALB/c mice — ELISA and WB are conducted on a FLAG-tagged protein grown and purified from HEK cells — positive clones from fusion

4. Conclusions



4.1 The neuropil regions that result to be mostly marked by the CD8-GFP expression are highlighted in a and in b: Saddle, Vest, Superior medial protocerebrum (a), and the Protocerebral bridge (b).



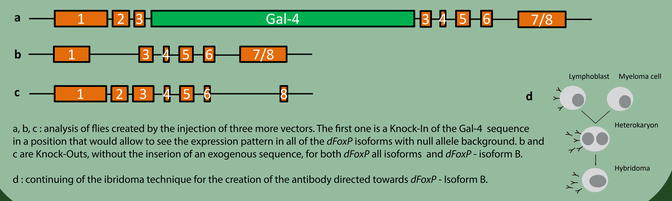
This scheme of the Stinger-GFP expression pattern reveal mostly a *dFoxP*-isoB clustered expression in the cortex, where we can identify four major bilateral clusters and two central ones (c´ and c´´´, green dots).
The red arrowheads in c´ and c´´ indicate the dorsal cluster neurons, with projections spanning the entire central brain.

4.2 From the behavioral data we can extrapolate two major information:

when we compare the two different set-ups (Buridan´s paradigm and Flight simulator), we notice that the heterozygous flies have two different outcomes: a dominant behavior in Buridan´s paradigm and a recessive one in the Flight simulator. This phenomenon is called ***Differential Dominance***.
The differential dominance is a situation in which the dominance effects at a pleiotropic locus vary between traits. The FoxPB heterozigous mutant flies in fact, despite of an unvaried learning ability, have a significant change in activity measurements.

when we compare instead the results obtained from only one set-up (Buridan´s paradigm), we observe a clear phenomenon of ***Overdominance***, which is a condition in genetics where the phenotype of the heterozygous lies outside the phenotypical range of both homozygous parents, and this can be seen in all of the time measurements performed in Buridan.

5. Future perspectives



a, b, c : analysis of flies created by the injection of three more vectors. The first one is a Knock-In of the Gal-4 sequence in a position that would allow to see the expression pattern in all of the *dFoxP* isoforms with null allele background. b and c are Knock-Outs, without the inserion of an exogenous sequence, for both *dFoxP* all isoforms and *dFoxP* - isoform B.

d : continuing of the ibridoma technique for the creation of the antibody directed towards *dFoxP* - Isoform B.

6. References

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3 Scott J. Gratz,1 Fiona P. Ukken, C. Dustin Rubinstein, Gene Thiede, Laura K. Donohue, Alexander M. Cummings, and Kate M. O'Connor-Giles. (2014) Highly Specific and Efficient CRISPR/Cas9-Catalyzed Homology-Directed Repair in Drosophila.