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

Ottavia Palazzo[1,2], Anders Eriksson[1], Mathias Raß[1], Nicole Schäfer[2], Diana Pauly[2], Björn Brembs[1]

[1] Institute of Zoology – Neurogenetics, University of Regensburg, Regensburg, Germany
[2] Experimental Ophthalmology - University Hospital, Regensburg, Germany

SCAN ME

1. Introduction

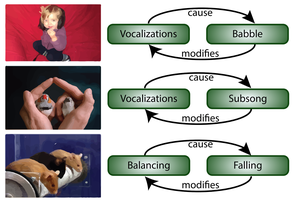
FoxP

The family of Fox genes groups a large number of transcription factors that share a conserved DNA-binding domain, 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*).

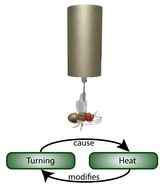
The Foxp2 gene sequence is highly conserved in vertebrates and has been shown to be important in vocal learning and the modulation of the underlyiong neural circuits. One mutation in the FOXP2 human gene affects language acquisition in afflicted patients, without other obvious impairments.

Learning

- operant world-learning, in which the subject is learns from relations between the environment
- operant self-learning, in which the subject learns from the outcome of its own behavior

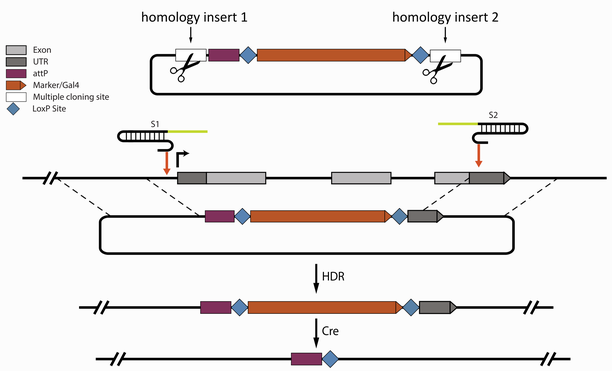


The FoxP2 gene has been found to be involved in operant-self learning in various mammalian species, and the Drosophila homologous seems to have a conserved function.
However, due to contraddicting evidence, the expression pattern, function and mechanisms of action remain still to be elucidated).

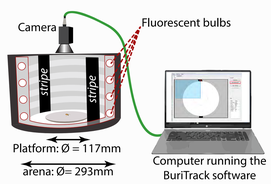


2. Methods

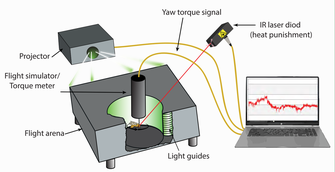
Homology recombination by CRISPR/Cas9



Buridan´s paradigm



Flight simulator

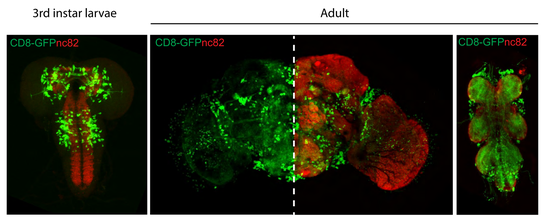


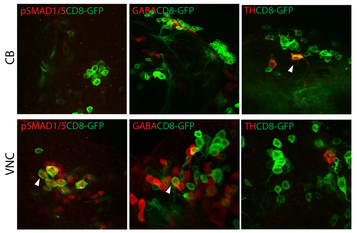
3. Results

WT gene locus

IsoB-specific driver line

Isoform unspecific driver line

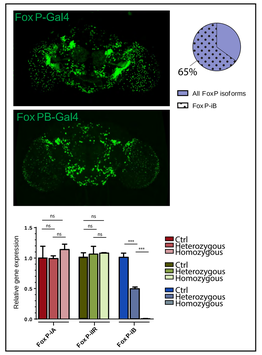




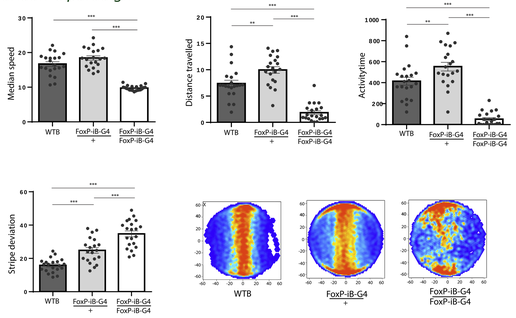
- FoxP-iB is expressed at every time point during development

- FoxP-iB is expressed only in nerons and not in glia

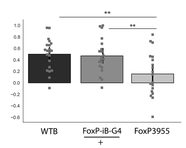
- FoxP-iB is expressed in various types of neuronal cells, like: motorneurons, GABA- ergic, cholinergic and dopaminergic neurons



Buridan' s paradigm



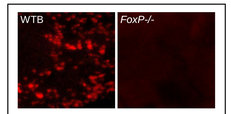
Flight simulator



* p<0.05; ** p<0.01; *** p<0.001 - all graphs are represented with standard error of the mean.

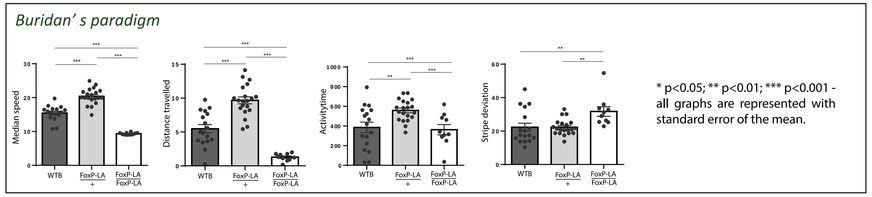
The homozygous phenotype has a low flight performance, which was insufficient for the flight simulator test.

FoxP-KO



The KO construct is working well.

Buridan' s paradigm



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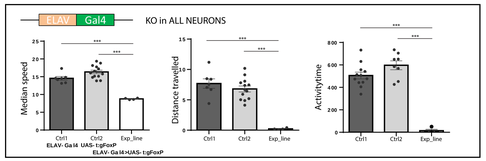
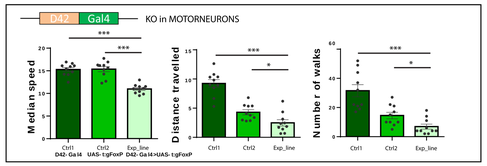
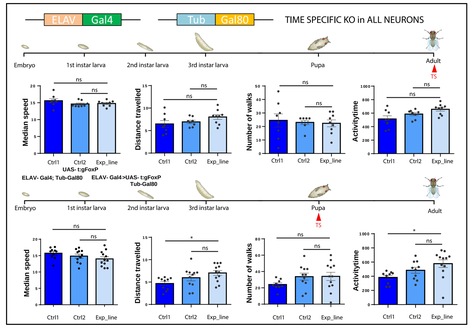
FoxP-conditional KO — UAS | tRNA | gRNA | tRNA | gRNA | tRNA | gRNA | tRNA | gRNA — *Buridan' s paradigm*







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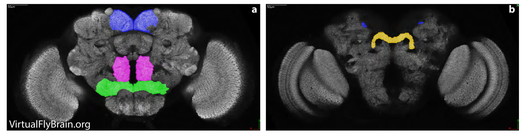
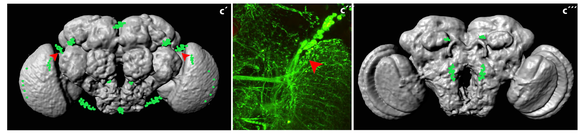
The time specific conditional KO was done as follows: flies were grown at 18 °C, and once hatched the virgins were put at 30 °C for 48 hrs. Afterwards the wings were clipped and the flies recovered at 25 °C for 12 hrs before the test.

4. Conclusions



The neuropil regions that result to be mostly marked by 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.

From the behavioral data we can extrapolate four major conclusions:

- comparing 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***. Differential dominance is a situation in which the dominance effects at a pleiotropic locus vary between traits. The FoxP-iB heterozygous mutant flies in fact, despite of a WT 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 temporal measures performed in Buridan's.

- Knocking out all FoxP isoforms does not seem to have a stronger effect on general locomotor behaviour if compared to knocking out only isoform B.

- If FoxP is knocked out only in ***motorneurons*** we can already see a phenotype, suggesting that motorneurons are heavily involved. However, if FoxP is removed in every neuron exclusively during ***adulthood*** (or pupal stage), no effect is observed, suggesting a developmental function of this transcription factor.

5. References

1 Ezequiel Mendoza, Julien Colomb, Jürgen Rybak, Hans-Joachim Pflüger, Troy Zars, Constance Scharff., Björn Brembs. (2014) Drosophila FoxP Mutants Are Deficient in Operant Self-Learning.

2 Julien Colomb, Lutz Reiter, Jedrzej Blaszkiewicz, Jan Wessnitzer, Bjoern Brembs. (2012) Open Source Tracking and Analysis of Adult Drosophila Locomotion in Buridan's Paradigm with and without Visual Targets.

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.