Developmental expression of *dFoxP* is required in motorneurons for operant self-learning in *Drosophila*

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Introduction

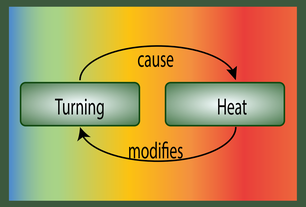
FoxP is a highly conserved transcription factor family with four different genes in vertebrates. *FoxP2* has a unique role as the so called “speech gene”. A defect causes a specific impairment in speech and articulation. In *Drosophila* on the other hand, only one gene is present, with three isoforms.

Speech learning can be characterized as a form of motor learning. The same concept can be found in the torque-learning task of tethered flies. Therefore, the *“Drosophila* flight simulator” can be used as a powerful tool to study the effects of d*FoxP* manipulations.

Operant learning can be split in to two main processes, operant self- and world-learning. In self-learning, the animal can only evaluate feedback from its own behavior. In world-learning, the animal can also learn about predictive environmental stimuli. While *dFoxP* mutants are still able to perform the world-learning task, their self-learning ability is greatly impaired.

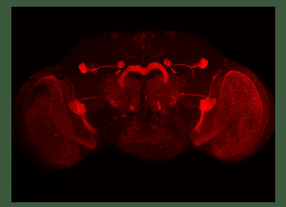
In addition to *FoxP*, Protein kinase C (*PKC*) has also been shown to be involved in self-learning. *PKCs* are serine/threonine kinases activated by diacylglycerol, lipid or Ca2+. In *Drosophila* five different *PKCs* are present.

Here, we probe the role of *FoxP* expression in the brain by using CRISPR/Cas9-based gene knock-out to eliminate *FoxP* expresssion from a range of brain neuropil areas. We also knock-out and overexpress atypical PKC (aPKC) in *FoxP*-positive neurons and motor neurons. Finally, we identify wing motor neurons where *FoxP* and aPKC are co-expressed.



Underlying mechanis

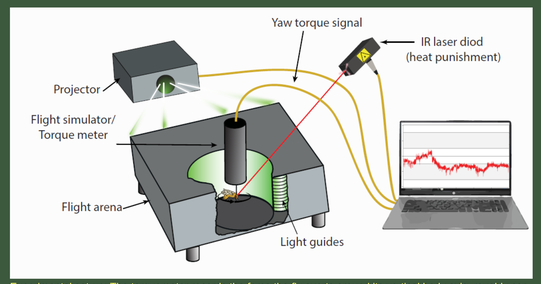
Schematic of tethered fly



FoxP expression pattern

Methods

Flight simulator setup



Experimental set-up: The torquemeter records the force the fly exerts around its vertical body axis, roughly corresponding to left or right turns, respectively, in free flight. The computer records and stores the torque data and controls the experimental consequences. For instance, left turning attempts by the fly may lead to unpleasant heat, generated by an infrared laser, focused on the head of the fly, without any other stimuli being present (operant self-learning). A projector serves to illuminate the environment of the fly (arena), both to adjust yaw torque before the experiment and to provide predictive stimuli in operant world-learning situations.

Experimental 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

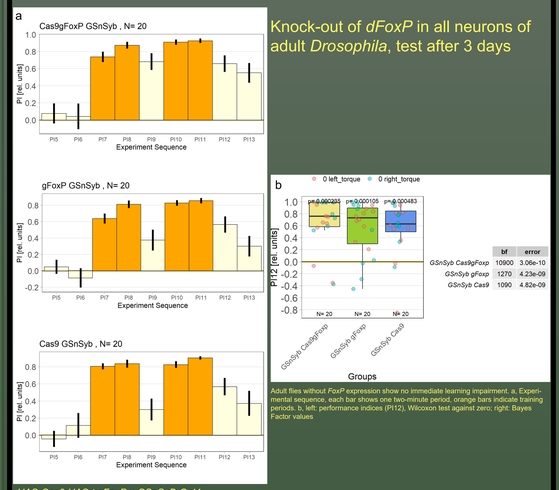
Local *FoxP* knock-out

Local *FoxP* knock-out in the brain did not disrupt learning:
- protocerebral bridge
- noduli
- dorsal neuron cluster (*ato*)
- fan-shaped body

Embryonic *FoxP* knock-out

Knock-out of *dFoxP* in the embryo leads to severe movement impairment. Flies are hardly able to walk and can not be tested in flight behavior.

Adult *FoxP* knock-out, 3 days

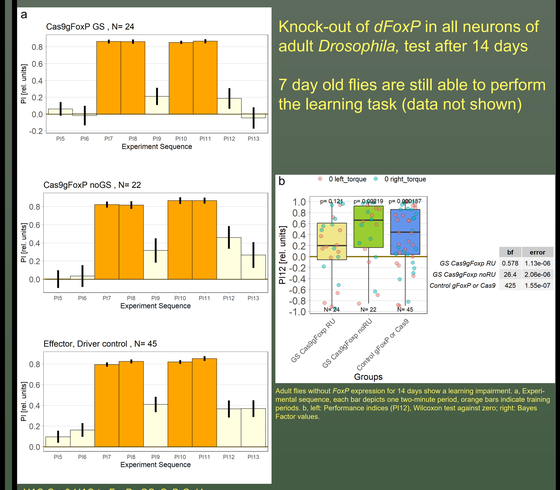


Knock-out of *dFoxP* in all neurons of adult *Drosophila*, test after 3 days

Adult flies without *FoxP* expression show no immediate learning impairment. a, Experimental sequence, each bar shows one two-minute period, orange bars indicate training periods. b, left: performance indices (PI12), Wilcoxon test against zero; right: Bayes Factor values

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

Adult *FoxP* knock-out, 14 days



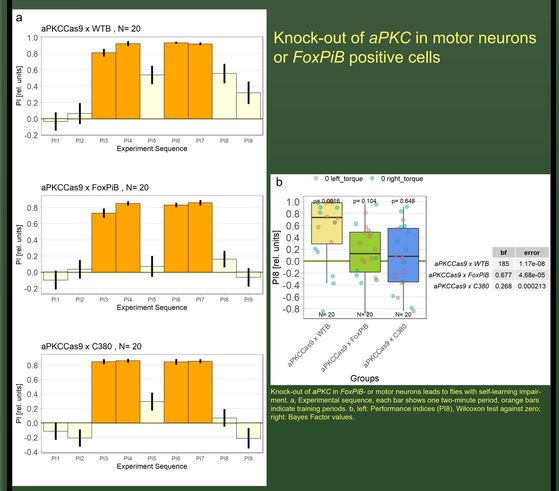
Knock-out of *dFoxP* in all neurons of adult *Drosophila,* test after 14 days

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

Adult flies without *FoxP* expression for 14 days show a learning impairment. a, Experimental sequence, each bar depicts one two-minute period, orange bars indicate training periods. b, left: Performance indices (PI12), Wilcoxon test against zero; right: Bayes Factor values.

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

aPKC knock-out



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

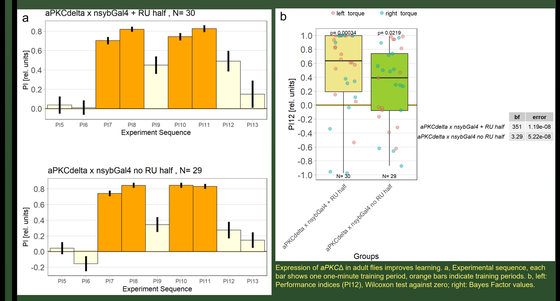
Knock-out of *aPKC* in *FoxPiB*- or motor neurons leads to flies with self-learning impairment. a, Experimental sequence, each bar shows one two-minute period, orange bars indicate training periods. b, left: Performance indices (PI8). Wilcoxon test against zero; right: Bayes Factor values.

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

aPKC overexpression

1 minute test periods

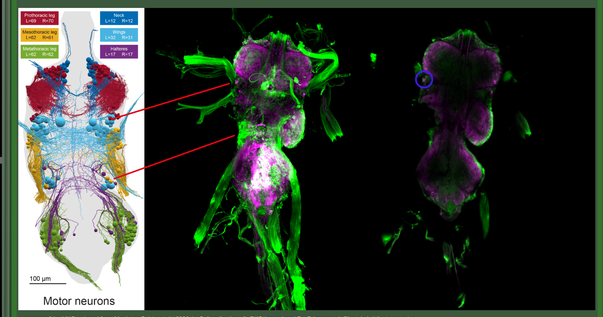
Expression of constitutively active *aPKCΔ* in all neurons



Expression of aPKCΔ in adult flies improves learning. a, Experimental sequence, each bar shows one one-minute training period, orange bars indicate training periods. b, left: Performance indices (PI12), Wilcoxon test against zero; right: Bayes Factor values.

UAS-aPKCΔ xGSnsyb-Gal4

FoxP aPKC colocalisation



a, neuron of the VNC, adaped from Maniates-Selvin et al., 2020. b, Collocalisation of aPKC (green) and FoxP (magenta). Blue circle idicates overlap.

Conclusions

FoxP shows developmental effects in *Drosophila*. A *FoxP* knock-out in the embryo leads to a severe movement defect. These flies cannot be tested in the *“Drosophila* flight simulator”. In contrast, knocking out *FoxP* in the adult fly shows no effects when tested until one week after the knock-out. These results indicate only a role of *FoxP* for operant self-learning during larval development, but not in the adult animal during the learning experiment. However, flies testet 14 days after the *FoxP* knock-out showed a learning impairment, indicating a role for a FoxP gene product also during the experient, but this gene product appears to have a long half-life.

Expression of *FoxP* is not required in the protocerebral bridge, noduli, dorsal neuron cluster or the fanshaped body.

Experiments knocking out *aPKC* in *FoxPiB*- or motor neurons and overexpressing the constitutively active *aPKCΔ* suggests aPKC operates in FoxP-positive motor neurons to mediate neuronal plasticity in operant self-learning.

References

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