Optimisation of Locomotion Protocol on *Drosophila* Larvae and Study of *FoxP* Locus in *Drosophila melanogaster*

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Zusammenfassung

Das *Forkhead Box P2 (FoxP2)* Gen kodiert bei Menschen für einen Transkriptionsfaktor, der mit Sprachentwicklung assoziiert ist. Das Ortholog bei *Drosophila* ist das *dFoxP* Gen. Mutanten des *dFoxP* Gens zeigen Störungen des operanten Lernens und Bewegungslernens, jedoch ist die Rolle des *dFoxp* noch nicht vollständig verstanden. Ziel dieser Arbeit war eine weitere Aufklärung der Funktion des *dFoxP* Gens.

Es wurden Versuche zum konditionalen Knockout von *dFoxP* im embryonalen- und ersten larvalen Entwicklungszustand durchgeführt. Um die Auswirkungen des Knockouts auf die Bewegung der Larven zu dokumentieren, wurden Methoden zur Protokollierung der Fortbewegung von *Drosophila* Larven verifiziert und optimiert. Als Ergebnis hat sich gezeigt, dass ein Ausschalten des Gens im embryonalen und frühen larvalen Stadium eine Beeinträchtigung der Fortbewegung der Larven, sowie der Bewegung der adulten Fliegen verursacht.

Abstract

The *Forkhead Box P2 (FoxP2)* gene in humans encode a transcription factor that has been associated with verbal development. The *dFoxP* gene is the ortholog in *Drosophila*. Mutants of the *dFoxP* gene show defects in operant learning as well as movement learning, but the role of *dFoxP* genes in Drosophila still needs to be fully understood. This thesis aimed to gain deeper insight into the function of the *dFoxP* gene.

Experiments on a conditional knockout of *dFoxP* in embryonic and 1st instar larval developmental stage were carried out. To investigate the effects of the gene knockout on larval locomotion, methods of protocolling *Drosophila* larval locomotion were verified and optimised. It was found that knocking out the gene at the embryonic and 1st instar larval stage causes an impairment in larval locomotion as well as in the walking of adult flies.

1 Introduction

Drosophila melanogaster is a popular model organism due to its small size and short life cycle which facilitate raising a large number of individuals for experiments (Rubin 1988). In addition, considerable portions of the *Drosophila* genome have been found to be orthologous to that of mammals (Staats et al. 2018) making the study of this organism even more attractive.

One of these ortholog genes is the *Drosophila Forkhead Box P (dFoxP)* gene (Santos et al. 2011). The gene shows a fork-like structure in *dFoxP* mutant *Drosophila* embryos, which was the reason for its naming (Weigel et al. 1989). The Fox gene family is an evolutionarily conserved family, which encodes an array of transcription factors (Mazet et al. 2003; Banerjee-Basu and Baxevanis 2004; Santos et al. 2011). All *FoxP* family members have several conserved domains: a polyglutamine tract, a zinc finger domain, a leucine zipper, and a forkhead DNA binding domain (Lai et al. 2001; Carlsson and Mahlapuu 2002; Stroud et al. 2006). In humans, *FoxP2*, the transcription factor encoded by the *FoxP2* gene, causes speech and language disorders when mutated (Lai et al. 2001; Estruch et al. 2016). In *Drosophila*, a mutation of the gene affects operant learning (Mendoza et al. 2014) and motor coordination (Lawton et al. 2014; Palazzo et al. 2020).

A regulation of the expression of the gene of interest and the analysis of the resulting phenotype may lead to further understanding of the exact function of the gene (Barwell et al. 2017; Poirier und Seroude 2005). One possibility for targeted gene deletion is genome editing using CRISPR/Cas9 (Ma et al. 2014). For example, Andrew R. Bassett and colleagues used the CRISPR/Cas9 method for "mutagenesis and homologous recombination in Drosophila cell lines" (Bassett et al. 2014). The CRISPR/Cas9 system is an adaptive microbial immune system found in bacteria. It consists of CRISPR (clustered regularly interspaced short palindromic repeats) and the CRISPR-associated protein Cas9 (Barrangou et al. 2007; Barrangou and Marraffini 2014; Hryhorowicz et al. 2017). The mechanism of this specific kind of immunity consists of three steps. In the first step, the so-called *adaption phase*, the bacteria acquire new spacers by integrating short sequences of the invaders' genome into the CRISPR array. This process is assisted by the Cas proteins. During the second step the CRISPR-sequences are transcribed and processed to CRISPR RNAs (crRNAs). Each of the short crRNAs contains one spacer sequence. In the last phase, also called the *targeting phase*, the crRNAs are used as guides to direct the Cas endonucleases to the targeted sequence. The endonucleases then cut the invaders' genome, which results in its destruction and consequently in the immunity of the attacked cell (Charpentier and Marraffini 2014; Marraffini 2015).

A popular system for targeted gene expression in *Drosophila* is the Gal4/UAS (upstream activation sequence) system. It consists of a fly line with the yeast transcription factor Gal4, which is positioned downstream from a tissue-specific promoter and a second fly line with a UAS domain, with the gene

of interest downstream. By crossing these two fly lines, in the subsequent fly generation the gene of interest can be transcribed in cells in which Gal4 is expressed. With this method, the effects of the gene on development can be observed (Brand and Perrimon 1993; Hartley et al. 2002).

1.1 Aim of the study

The aim of this study was to contribute to a better understanding of the function of the *dFoxP* gene in *Drosophila melanogaster*. Since the locomotion of adults had been previously already partially quantified (Palazzo et al. 2020), we decided to study larvae locomotion. Already existing protocols on *Drosophila* larvae locomotion were to be verified and optimised. This could be used to document the movement of larvae after the knockout of *dFoxp* in different developmental stages.

2 Materials

2.1 Fly line stocks

Table 1: Fly line stocks

Genotype	Use
ELAV-Gal4	Driver line
ELAV-Gal4;TubGal80	Driver line
ELAV-GeneSwitch	Driver line
UAS-gRNA	Responder line
UAS-gRNA-Cas9	Responder line
UAS-Cas9	Responder line
Canton-S	Wild type

2.2 Crosses

dFoxP knockout experiment

Table 2: Crosses made for dFoxP Knockout experiment

Female virgins	Males	Application
ELAV-Gal4	*UAS-Cas9-gRNA*	Experimental line
ELAV-Gal4	*UAS-gRNA*	Control line
ELAV-Gal4	*UAS-Cas9*	Control line

dFoxP knockout using Gal80^{ts}

Table 3: Crosses made for Gal80^{ts} experiment

Female virgins	Males	Application
ELAV-Gal4;TubGal80	*UAS-Cas9-gRNA*	Experimental line
ELAV-Gal4;TubGal80	*UAS-gRNA*	Control line
ELAV-Gal4;TubGal80	*UAS- Cas9*	Control line

dFoxP knockout using GeneSwitch

Table 4: Crosses made for GeneSwitch experiment

Female virgins	Males	Application
UAS-Cas9- gRNA	*ELAV-GeneSwitch*	Experimental line
UAS-gRNA	*ELAV-GeneSwitch*	Control line
UAS-Cas9	*ELAV-GeneSwitch*	Control line

2.3 Agar plates

Table 5: Quantity Agarplates

Volume	Composition
24.5 g	Agar Agar
700 ml	H_2O

3 Methods

3.1 Fly strains

All flies were obtained from the fly stocks in the laboratory in Regensburg. Fly stocks (**table 1**) were maintained at 25°C, in a 12/12 hours light/dark regime at 60 % relative humidity. For crossing, 10-15 virgin female flies and 4-5 male flies were placed together in a new vial. The crosses for the *dFoxP* knockout (**table 2**), and the crosses for the GeneSwitch (**table 4**) were raised at 25°C. The crosses with the temperature sensitive Gal4 inhibitor Gal80^{ts} (**table 3**) were raised at 18°C or 30°C. Before experimental use, flies were kept at 25°C. For the experiments 3rd instar larvae were tested and washed with H_2O before measuring locomotion.

To ensure transgenic expression the binary ELAV-Gal4/UAS system was used, for which both driver (Gal4) and responder (UAS) fly line are needed. The fly lines *ELAV-Gal4*, *ELAV-Gal4;TubGAl80* and *ELAV-GeneSwitch* were used as driver lines. ELAV is expressed in neurons (Pascale et al. 2008). The responder fly lines consisted of the experimental fly line *UAS-Cas9-gRNA* (both components of CRISPR/Cas9 system) and the control fly lines *UAS-Cas9* and *UAS-gRNA*, which contained just one of the two components of the CRISPR/Cas9 system. To obtain the experimental line, balancer chromosomes (Roote and Prokop 2013) were used to identify and select males with both Cas9 (UAS-Cas9/**Curly-O**) and gRNA (UAS-gRNA/**TM3**). Curly O leads to a deformation of the wings into a clearly curved shape, while TM3 expresses itself as short hair on the adult flies' backs. It was necessary to use homozygote experimental fly lines, because the experiments were done on larvae and the actual genotype cannot be seen in the phenotype (as larvae have neither wings nor hair). We therefore selected homozygous males without the balancers, which were phenotypically identifiable by their straight wings (no Curly-O) and long back-hair (no TM3).

3.2 Gal4-Gal80^{ts} system in Drosophila

The Gal4-Gal80^{ts} system is a modified GAL4/UAS system. Gal4;TubGal80^{ts} is a construct used to get temporal control over Gal4 by the temperature sensitive repressor Gal80^{ts}. At 19°C, the repressor Gal80^{ts} is inhibiting the activation domain of Gal4. The construct can bind to the upstream activation sequence (UAS), but cannot transcribe the construct, because its activation domain is masked by Gal80^{ts}. At 30°C the repressor is inactive and does not inhibit Gal4, and in consequence Gal4 binds to UAS and transcribes the construct. Therefore, a conditional knockout can be carried out (McGuire et al. 2003; Suster et al. 2004).

3.3 GeneSwitch

Another method to get temporal control over Gal4 is GeneSwitch. This method is based on a Gal4 chimeric gene. Gal 4 is a yeast transcription factor, which encodes the GAL4 DNA-binding domain. As well as the use of the steroid RU486 (mifepristone), which activates the GAL4-progesterone-receptor proteins. After the Gal4 domain has been activated, the same process takes place as in the Gal4/UAS system and Gal4 binds to the UAS domain so that the transcript downstream of the UAS can be expressed. To induce knockout in the larvae to be tested, the hormone is administered through the food (Osterwalder et al. 2001; Roman et al. 2001). The steroid containing food was prepared using 20 mg of mifepristone (origin Thermo Fisher Scientific Inc.) solved in 1 ml of 99 % Ethanol, which was then diluted in 99 ml of $H_2O_{ddest.}$ (concentration of RU486: 200 µg/ml). *Drosophila* Instant food was mixed with this solution and the flies were placed on the fresh food until they had laid eggs. Then the flies were removed from the vials, the eggs were left on the food until 3rd instar larvae had developed.

3.4 Buridan's paradigm

To analyse the walking behaviour of the adult flies, the Buridan's paradigm was used. The construction regularly used to investigate the Buridan's paradigm consists of a circular raised area with a diameter of 117 mm surrounded by water, which is placed inside of a uniformly illuminated white cylinder, 313 mm in height and 293 mm in diameter. The Centroid Trajectory Analysis (CeTrAn) extracts a large number of parameters from the results of the fly movement and analyses them using the statistics project R The position of the flies, whose wings were removed before the start of the experiment in order to guarantee that only their walking behaviour was analysed (https://www.protocols.io/view/Preparing-flies-for-Buridan-s-Paradigm-c7vzn5), were documented using the software Buritrack (http://buridan.sourceforge.net). The data was recorded with the use of a camera (Logitech Quickcam Pro 9000) (Colomb et al. 2012, Palazzo 2020).

4 Results

4.1 Optimisation of locomotion protocol on Drosophila larvae

We compared three different methods quantifying larval locomotion in terms of their efficiency. Tests were carried out with wandering 3rd instar larvae of the *Canton-S* wildtype fly line (**table 1**). Larvae were washed before testing and placed on Petri dish containing the medium agar (**figure 1 A, table 5**). First, it was tested whether the use of a food source (yeast patch) was suitable to get information about locomotion ability of the larvae. A starting point in the middle of the petri dish was marked and surrounded with a ring of yeast at a distance of two centimetres (**figure 1 B method 1**). The time from the moment a larva (n = 12) was placed on the starting point until it reached the yeast ring was measured. Larvae needed an average of 130 (standard deviation, SD = 117.9) seconds to master the distance. In the second approach, the food source was reduced, and a short, one-centimetre food line was placed at two centimetres distance from the starting point (**figure 1 B method 2**). With this approach, tested larvae (n = 6) needed an average of 309 (SD = 233.9) seconds to reach the food patch. Finally, it was evaluated if the larvae would move around without the incentive of a food source. For this purpose, they were placed in the centre of the Petri dish (**figure 1 B method 3)**, and the time until they reached the outer edge of the dish was recorded. The tested larvae (n = 6) needed an average of 645 (SD = 456.7) seconds until they reached the edge of the dish. This showed that the larvae moved their bodies without the enticement of a food source. In addition, the direction of the movement of the larvae showed that they were not moving towards the yeast patch. They moved in an indeterminate direction and bumped into the food source unintentionally.

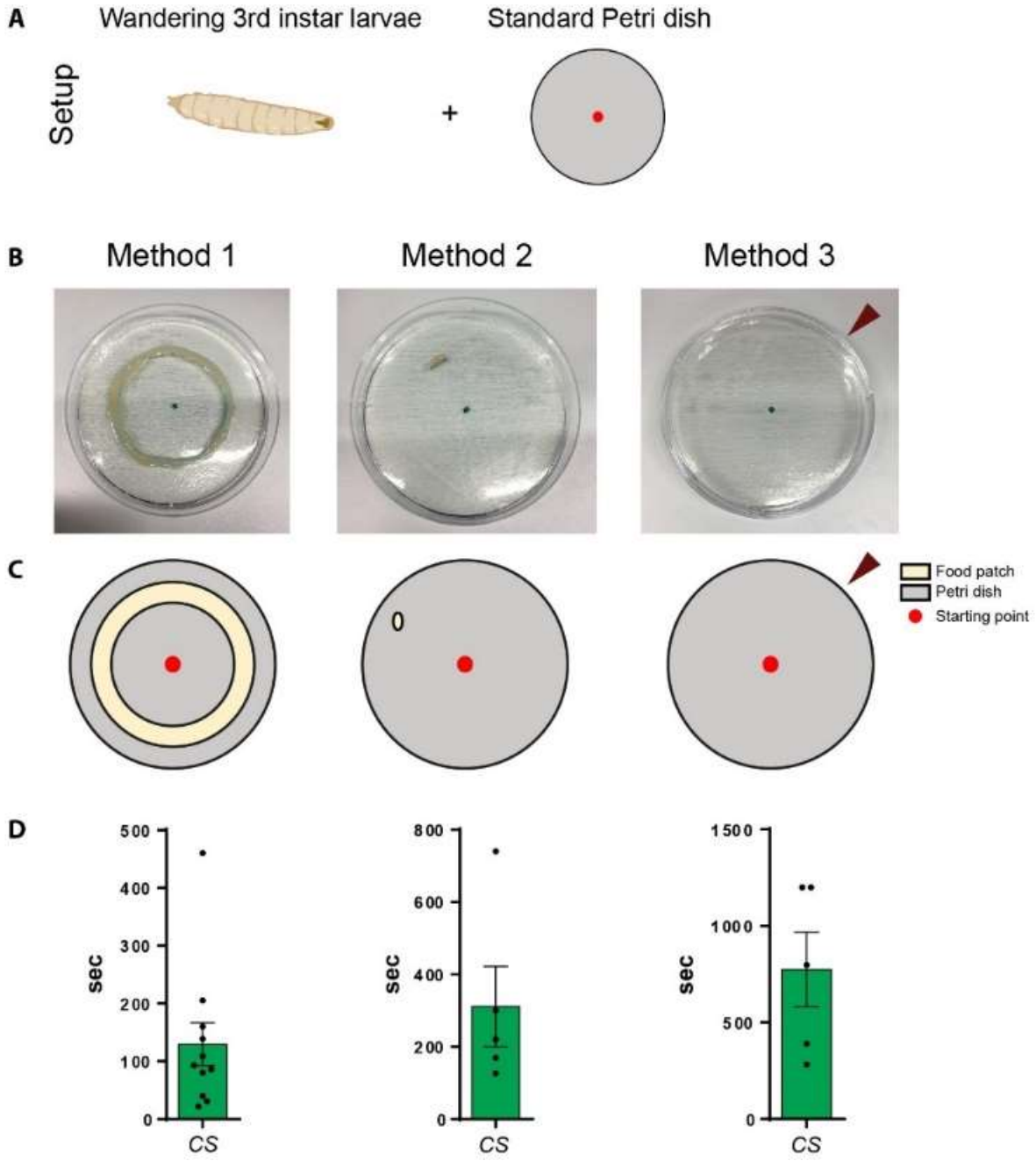

Figure 1 Locomotion protocol methods using Canton-S flyline. *A: Setup of the experiment. Using wandering 3rd instar larvae and a standard Petri dish for all experiments. B: All three methods photographed. C: Structure of the methods visualized. D: Results of all three methods in [s] with mean and standard deviation of time needed to reach destination by method.*

After we found that a food source had no impact on larval movement, we continued to work on method 3 to improve the measurement. To evaluate the efficiency of the method, the study was performed by two operators. By comparing their outcomes (the counting differences) the experimental setup was improved until the difference reached a minimum. Building on the already selected setup of method 3, we first used a 0.25 cm^2 grid to place under the Petri dish (**figure 2 A**) and count the boxes that the larvae migrated. The larvae (n = 20) were observed for one minute after they had been placed on the starting point. Every square they crossed with their entire length was counted. Operator 1

(Simon) counted, that they moved an average of 3.7 cm/min. Operator 2 (Sarah-Lynn) got the result of an average locomotion of 2 cm/min. Since the results still showed a sizable difference, we decided to use an even smaller squared pad to decrease the differences. The difference between the results of the two operators was reduced (**figure 2 B**) by using a 0.1 cm^2 grid to minimise the counting inaccuracy. The additional use of a microscope enabled precise observations of the experiment. Both operators counted a mean value of 4.0 cm/min. Hence, this method was suitable to efficiently document the locomotion of larvae.

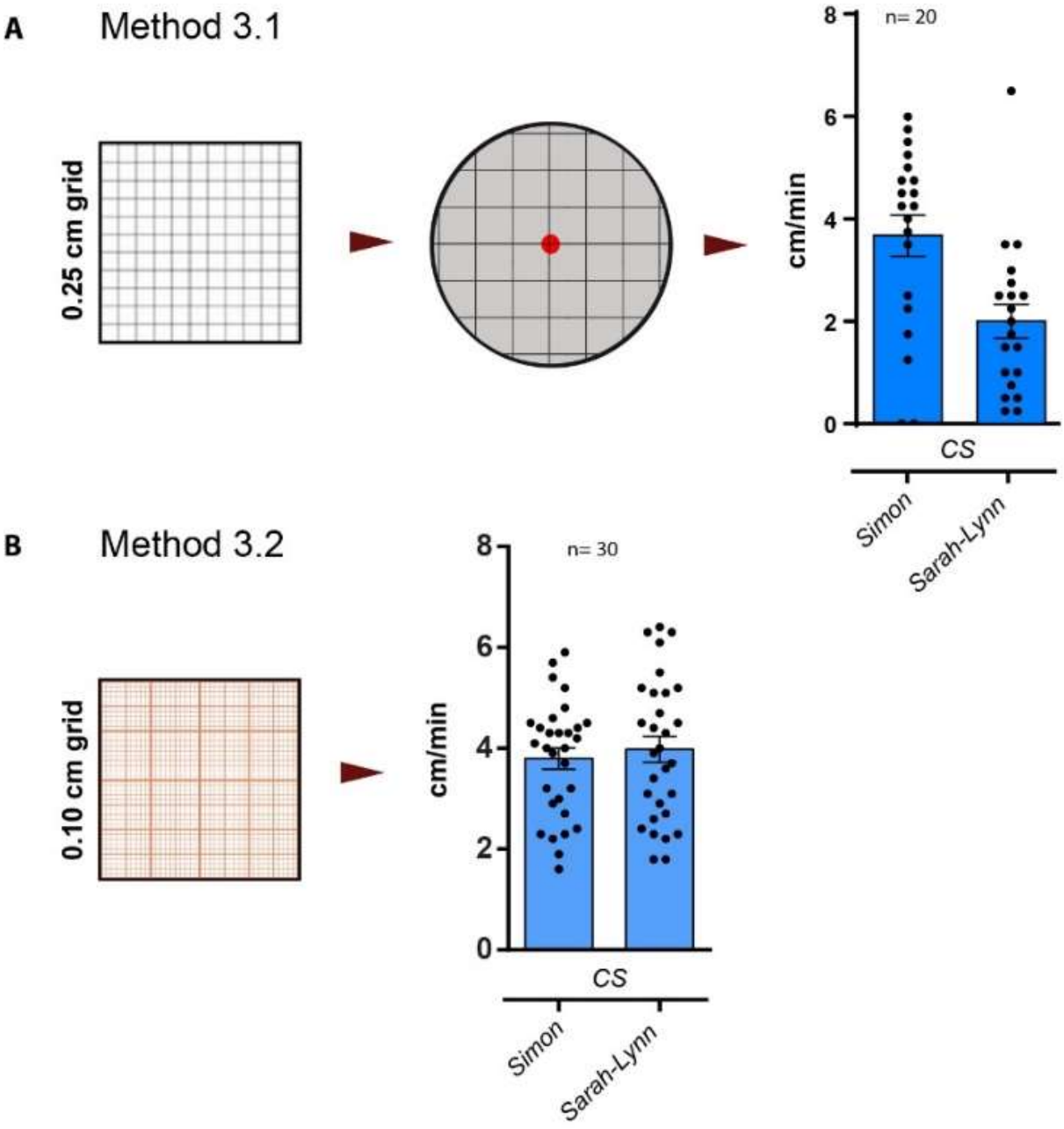

Figure 2: Improvements of method 3. *A: Method 3.1 using a 0.25 cm grid and results in [cm/min] with mean and standard error of speed. B: Method 3.2 using a 0.1 cm grid and results in [cm/min] with mean and standard error of speed.*

4.2 Knockout of dFoxP in different developmental stages

Different methods exist to determine the developmental stage at which the absence of the *dFoxP* gene affects larval locomotion. To achieve the knockout both components of the CRISPR/Cas9 system, Cas9 and gRNA, need to be present. The guide RNA (gRNA) is identical to the targeted sequence and can therefore detect this sequence and guide the endonuclease Cas9, which then cuts out the gene segment. If a fly line contains only a part of the system (either Cas9 or gRNA), there is no knockout of *dFoxP* and the locomotion of the larvae should not be effected.

<u>*dFoxP* gene knockout</u>

First, the *dFoxP* gene knockout was tested. For the experiment, 10-15 female virgins of the *ELAV-Gal4* driver fly line (**table 1**) were crossed (**table 2**) with 5 males each of the three used responder fly lines (**table 1**) in individual vials. The result of the knockout was that the larvae of both crosses with control lines moved fast. The gRNA Control larvae moved with a speed of about 4.0 (standard error, SE = 0.21) cm/min, the Cas9 control line about 4.75 (SE = 0.27) cm/min, while the larvae of the experimental CRISPR/Cas9 line did only move an average of 2.5 (SE = 0.27) cm/min (**figure 3**). The difference between the locomotion of control and experimental line was significant (p-value < 0.05).

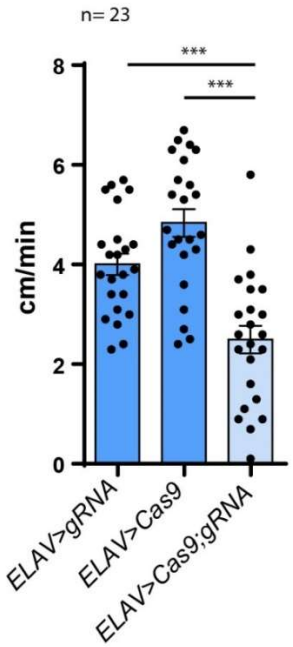

Figure 3: dFoxP knockout. *Speed of larvae locomotion in [cm/min] of experimental and control lines with mean and standard error.*

Gal80^{ts} experiment

In the next step, the knockout of the *dFoxP* gene was accomplished by using the Gal80^{ts} method (see section **3.2**). The temperature-sensitive Gal4 repressor Gal80^{ts} is inactivated at 30°C. This means, when Gal4 is active the *dFoxP* gene is muted and cannot perform its function.

Female virgins of the *ELAV-Gal4;TubGal80* driver line (**table 1**) were collected and crossed with males of the experimental *UAS-gRNA-Cas9* line, as well as *UAS-Cas9* and *UAS-gRNA* males, both control lines (**table 1**). After crossing (**table 3**), they were placed in 30°C environment for 2 days (36 hours). As Gal80^{ts} is inactive at this temperature, the knockout takes place in the embryonic developmental state. After 36 hours, the larvae were kept in 25°C until they were tested. This experiment should yield the same results as the already performed trial of the knockout in 25°C. This was shown to be the case, as the larvae of the control groups moved at an average of 4.5 cm/min (SE of gRNA control = 0.22 cm/min, SE of Cas9 control = 0.25 cm/min), while the larvae of the experimental cross were significantly slower (p-value < 0.05) with about 2.0 (SE=0.27) cm/ min (**figure 4 A**).

To test the effect of the knockout of the *dFoxP* gene in larval development on larval locomotion, the same crosses were carried out as in the temperature switch experiment in the embryonic stage described above. The crosses were then kept at 18°C until 1st instar larvae were visible. Gal80^{ts} is active at this temperature, and (as it did not inhibit Gal4) *dFoxP* was expressed. The larvae were transferred to 30°C (i.e. the temperature that facilitates the knock-out of *dFoxP*) for 18-36 hours, and then transferred to 25°C until they were tested. The results showed that there is a significant difference (p-value < 0.05) in the locomotion of the larvae as both control groups moved an average of 4.0 (SE of both control groups = 0.25) cm/min and the larvae of the experimental group moved about 3.5 (SE = 0.24) cm/min. The difference of control and experimental group in the 1st instar larvae knockout was less strong (**figure 4 B**), compared to the difference of movement between control and experimental groups in the embryonic knockout experiment (**figure 4 A**). Accordingly, it was concluded that the knockout of *dFoxP* in larval development has an effect on larval locomotion, even though the effect is less pronounced than during the embryonic stage.

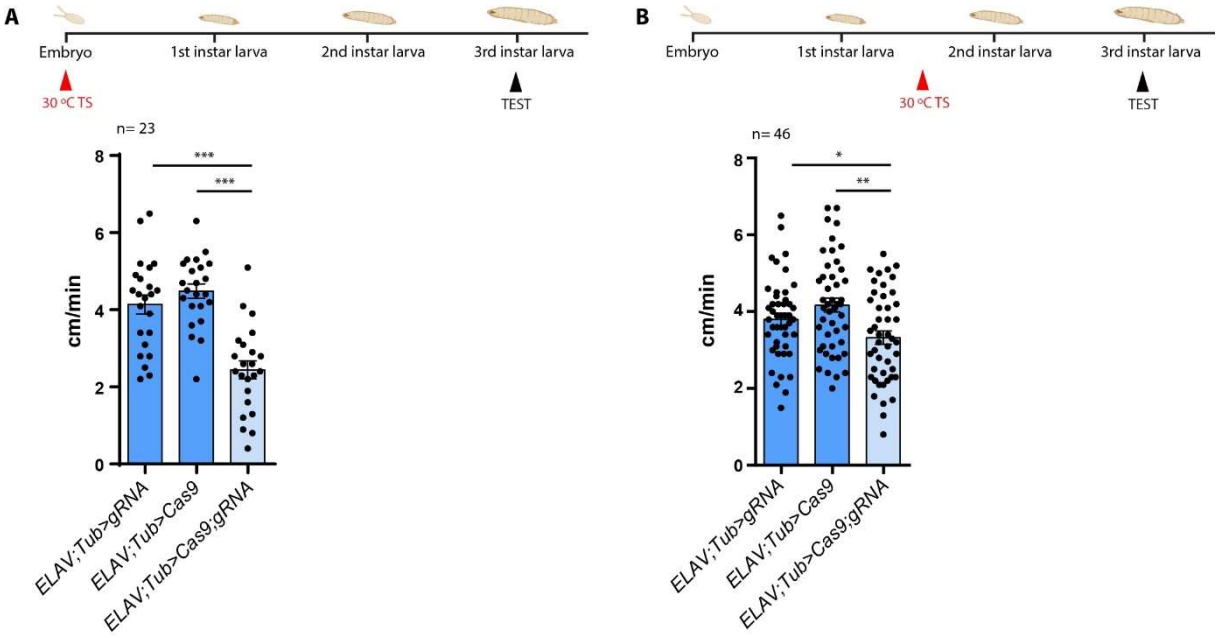

Figure 4: dFoxP knockout using the Gal80^{ts} method. *A: Larval locomotion in [cm/min] when knockout happens at embryonic stage with mean and standard error of speed. B: Larval locomotion [cm/min] when knockout happens at 1st instar larva stage with mean and standard error of speed.*

GeneSwitch experiment

The GeneSwitch method (see section **3.3**) was performed to verify the previous result. The larvae receive the steroid containing food as soon as they hatch, therefore the knockout of *dFoxP* occurs in the first hours of 1st instar larval stage.

For the experimental line female virgins of the of the three responder fly lines (*UAS-Cas9-gRNA*, *UAS-Cas9*, *UAS-gRNA*) (**table 1**) were crossed with males of the *ELAV-GeneSwitch* line (**table 4**). The crosses were kept in a vial with standard *Drosophila* food, and after one day were raised on RU486 containing food, until the 3rd instar larvae that were to be tested had developed. We found that the experimental line was significantly slower than both control lines (**figure 5**), with a difference of about 1 cm/min (control lines moved an average of 3.5 cm in one minute, SE = 0.14 cm/min, whereas the experimental line moved an average of 2.5 cm in one minute, SE = 0.15 cm/min). Thus, it was shown that a knockout of *dFoxP* in the early 1st larval stage influences the locomotion of the larvae.

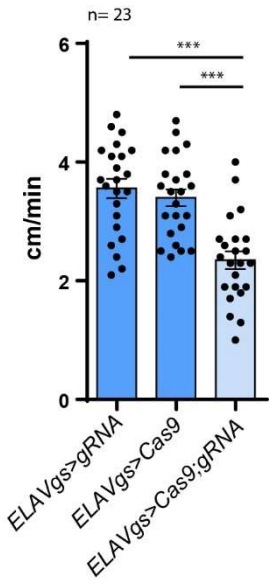

Figure 5: Larvae locomotion of GeneSwitch experiment. *Results of larvae locomotion in [cm/min] of experimental and control lines with mean and standard error of speed.*

4.3 Analysing adult flies of dFoxP Knockout in embryonic and 1st instar larvae stage

To analyse the walking behaviour of adult flies, the Buridan's paradigm was used (see section **3.4**). Therefore, the same experimental fly lines as in the Gal80^{ts} experiment (**table 3**) were used. For the *dFoxP* knockout at the embryonic stage flies were raised at 30°C for 36 hours and then kept at 25°C until adulthood. To achieve the knockout at 1st instar larvae stage the cross was raised at 18°C until 1st instar larvae were visible, then switched to 30°C for 36 hours and subsequently left at 25°C for the rest of the development. Two days-old female flies (homozygous) were collected, and their wings were clipped under CO_2 anaesthesia. After three hours of recovery at 25°C they were tested in Buridan's paradigm for 10 minutes. The results showed that both groups (embryonic and 1st instar larvae knockout) did not walk properly, with a very low activity, i.e. the time in which they show any movement of their bodies as well as the low median speed, which means their maximal travel distance divided by two (**figure 6**). The results of the knockout in the embryonic and 1st instar larval developmental stages were compared with the results of a previous done knockout of *dFoxp* in the adult stage (Palazzo 2020) to illustrate the different walking behaviour.

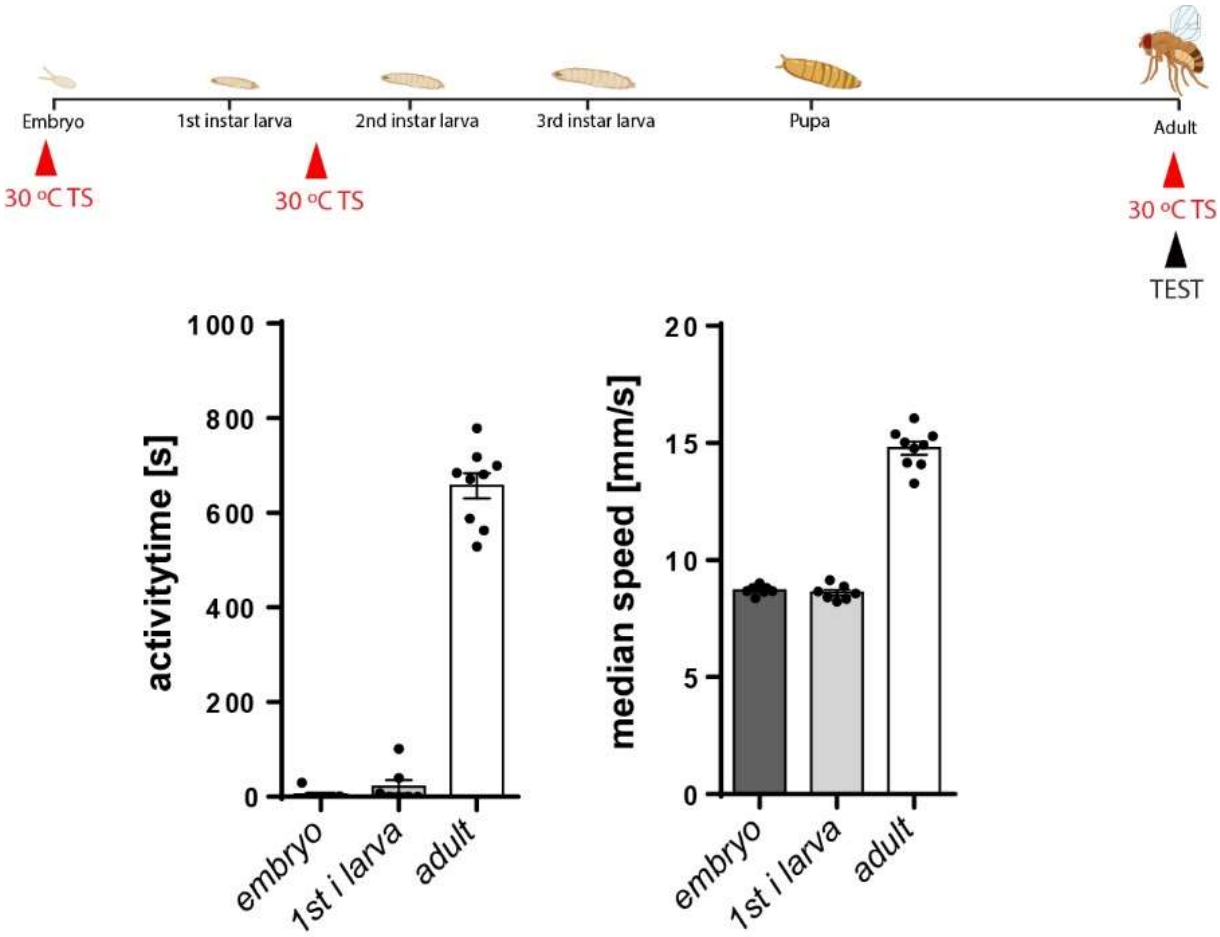

Figure 6: Analysing the walking behaviour if adult flies with Buridan's paradigm. *Comparing the effects of dFoxP knockout in embryo stage, 1st instar larva stage and adult stage on activitytime in [s] and median speed [mm/s]*

5 Discussion

5.1 Optimisation of locomotion protocol on Drosophila larvae

We refined the protocol on larvae locomotion by using a 0.1 cm^2 grid under a microscope to achieve the most accurate results. There are already protocols that can be used to test larval locomotion, for example Charles D. Nichols, Jaime Becnel and Udai B. Pandey tested larvae on a 15 cm Petri dish containing 2% agarose on a graph paper with a 0.2 cm^2 grid and counted the number of grid lines crossed in 1 minute (Nichols et al. 2012). Our goal was to improve this protocol by investigating if extrinsic factors, in this case a food source, influence the behaviour of larvae. It was shown that neither the direction of the movement nor the locomotion of the larvae is dependent on a food source. After confirming that there is no need for an extrinsic factor to drive locomotion, the already used techniques of protocolling larvae locomotion were optimised by using a 0.1 cm^2 grid under a microscope. By measuring the walking distance in a set period (1 minute) it is also possible to simultaneously calculate the speed of the larvae. This technique offers accurate results and is easily applicable and therefore it is efficient for use for larvae locomotion experiments. Although there are computer programs for tracking *Drosophila* larvae locomotion (Aleman-Meza et al. 2015) that can be used especially for more extensive measuring as well as to avoid human error, the manual protocols are a suitable and uncomplicated alternative to acquire data on a smaller scale.

5.2 Knockout of dFoxP in different developmental stages

Larvae behaviour analysis

There are several studies on the function of *dFoxP* in adult flies (Mendoza et al. 2014; Castells-Nobau et al. 2019; Palazzo et al. 2020). Adult *Drosophila FoxP* mutant flies show deficiency in operant self-learning (Mendoza et al. 2014), as well as motor coordination (Lawton et al. 2014; Palazzo et al. 2020). It has also been shown that *FoxP* is active throughout the development and especially important during pupal development (Schatton and Scharff 2017; Castells-Nobau et al. 2019). We found, that by knocking out *dFoxP* in the embryonic stage, the locomotion of larvae is significantly (p-value < 0.05) reduced from an average moving distance of 4 cm per minute in the control lines to an average of 2 cm per minute in the experimental line. The knockout of the gene in later larvae development stage (1st instar larvae stage) still has an effect on the locomotion. But it was visible that the knockout of *dFoxP* in the early 1st instar larval stage influences the locomotion of the larvae more strongly than a knockout of the gene in the later hours 1st instar larval stage, as the significant difference of their movement behaviour gets less pronounced. There is need for further research on the knockout of

dFoxP in the 2nd and 3rd instar larvae stage and its effects on the larval locomotion, as well as on adult flies.

Adult flies behaviour analysis

When testing the adult flies with the *dFoxP* knockout in embryonic as well as in 1st instar larvae stage, we found that both groups did not walk properly, as they showed low activity and median speed. Though flies with a knockout of *dFoxP* in 1st instar larvae stage showed higher activity than flies with a knockout of *dFoxP* in embyonic stage, the difference was not significant, possibly because of the low sample size. The increased activity of the adult flies of the knockout in the 1st instar larvae stage can also be explained by the fact that the larvae already had a less pronounced difference of movement between experimental and control line compared to the results of the embryonic knockout. We then compared this to the results of the walking behaviour of adult flies from a previous experiment (Palazzo 2020), in which *dFoxP* was knocked out in adult fly stage to investigate the difference of their walking behaviour. We found that the knockout in earlier developmental stages has an effect on the walking behaviour of adult flies, while it was already known that the later knockout in the adult fly no longer has an effect on its movement. As expected, the results also show, that the phenotype is more pronounced in the adult flies than in larvae. Since this preliminary experiment tested only seven flies per group, it would be worth expanding the sample size of the experiment.

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6.3 Dissertation

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6.4 List of tables

6.5 List of figures

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6.7 Declaration of Authorship

The submitted printed copies and the submitted electronic version of the thesis are identical. I have written the thesis independently, have not used any sources or aids other than those indicated and have not already submitted the thesis to another university for the purpose of obtaining an academic degree. Furthermore, I confirm that I am aware of the legal consequences provided in § 27 (3) of the current examination regulations.

Die vorgelegten Druckexemplare und die vorgelegte elektronische Version der Arbeit sind identisch. Ich habe die Arbeit selbstständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und die Arbeit nicht bereits an einer anderen Hochschule zur Erlangung eines akademischen Grades eingereicht. Weiterhin bestätige ich, dass ich von den in § 27 Abs. 3 der geltenden Prüfungsordnung vorgesehenen Rechtsfolgen Kenntnis habe.

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