Freie Universität Berlin

Bachelor's Thesis

Testing *Drosophila* learning and memory mutants with and without methylphenidate treatment in Buridan's paradigm

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Declaration of self-reliance

I, Yasmine Graf, hereby declare that the work submitted is my own and that all passages and ideas that are not mine have been fully and properly acknowledged.

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Contents

1) Abstract

1.1) English version

Activity is one of the most important and complex traits animals have evolved. Exploration and foraging behavior are indispensable to life of all groups of insects and thus for the fruit fly *Drosophila melanogaster.* One very important component of the flies' activity are visual processes which are demonstrably affected by particular learning and memory mutations (van Swinderen et al., 2009). Interestingly recent studies showed that one of the mutants, *radish* can be rescued by a methylphenidate treatment whereas others cannot (van Swinderen & Brembs, 2010). To investigate which parts of activity are in detail affected by the mutations and/or the drug treatment Buridan's paradigm was used. As a result of this detailed activity analysis the present study gives evidence that it is not forcing activity in general that can be changed by this particular pharmacological treatment but one important part of it: time activity.

1.2) German version

Aktivität ist eines der wichtigsten und komplexesten Merkmale, das im Tierreich evolviert wurde. Exploration und aktive Futtersuche sind für alle Insektengruppen unabdinglich und somit auch für die Taufliege *Drosophila melanogaster*. Eine sehr wichtige Komponente in der Aktivität der Fliege sind visuelle Prozesse, die nachweislich von einigen Lern- und Erinnerungsmutationen beeinflusst werden können (van Swinderen et al., 2009). Interessanterweise konnte in einer kürzlich erschienenen Studie gezeigt werden, dass die betroffene Mutation des Gens *radish* durch Behandlung mit Methylphenidat ausgeglichen werden kann, wohingegen dies mit Mutationen anderer Gene nicht gelang (van Swinderen & Brembs, 2010). Um zu untersuchen, welche Teile der Aktivität im Einzelnen von der jeweiligen Mutation und/oder der Methylphenidatbehandlung beeinflusst werden, wurde das Buridan Paradigma angewandt. Als Resultat dieser detaillierten Aktivitätsanalyse kann die hier vorliegende Studie einen Anhaltspunkt dafür geben, dass die Behandlung mit Methylphenidat nicht zwangsläufig die gesamte Aktivität der Tiere beeinflusst, sondern nur einen wichtigen Teil: Die zeitliche Aktivität.

2) Introduction

It is well known that there are a lot of different learning and memory mutants of the fruit fly *Drosophila melanogaster* whose phenotypes are caused by single gene mutations (Dubnau & Tully, 1998).

One of these mutants is *dunce* (*dnc*), which shows an absolutely normal behavior in general, but a very low level of aversive olfactory learning (Dudai, Jan, Byers, Quinnt, & Benzer, 1976).

Another mutant is *rutabaga* (*rut*), which does not show any learning in the standard negatively reinforced task (Livingstone, Sziber, & Quinn, 1984).

Further there is the mutant *radish* (*rsh*) which behaves totally normal in most locomotive, olfactory or aversive learning assays, but seems not to remember the learned contexts, neither after long nor after short time periods (Folkers, Drain, & Quinn, 1993).

It was shown that there are two different types of memory formation in flies: The anesthesia-resistant memory (ARM), in which the gene *rsh* is involved and the long-term memory (LTM) which is dependent on the cAMP pathway and influenced by the genes *rut* and *dnc* (Davis, Cherry, Dauwalder, Han, & Skoulakis, 1995; Isabel, Pascual, & Preat, 2004).

The LTM impaired flies of the strains *rut* and *dnc* exhibited a different phenotype than the ARM impaired flies of *rsh* in some behavioral and brain-recording assays (van Swinderen & Brembs, 2010).

To research whether the two types of learning mutants also show diverse walking phenotypes the Buridan's paradigm was chosen. This paradigm allows the flies to walk around on the platform freely and makes it possible to document behavioral parameters like temporal and areal activity which are independent from choices the flies have to make or massive anthropological impacts on the flies.

Buridan's paradigm which was firstly invented by Karl Götz in 1980 (Götz, 1980) as a locomotion assay for *Drosophila* is based on the theory by the 14. Century philosopher Jean Buridan, that tells about a donkey, sitting between two hay stacks, being supposed to starve to death because it cannot decide from which stack to eat first (Buridan & King, 1985). In case of Götz' assay for the fruit fly the hay stacks are landmarks on each side of an arena. Whenever wild type flies notice that they cannot reach one of the landmarks they are supposed to turn around and try to reach the landmark on the other pole.

Many different researchers could show that wild type flies as well as mutant flies be-

have exactly the way Buridan predicted: Wild type Berlin females, for example, are known to show a significant attraction to the two dark objects on either pole of the arena in comparison to an arena without any objects. The same with the female mutant flies *optomotor blind* (*omb*^{H31}) (Bülthoff, Götz, & Herre, 1982). Wild type Berlin males as well showed a strong attraction to the landmarks (Roland Strauss, Hanesch, Kinkelin, Wolf, & Heisenberg, 1992). The very commonly as control groups for behavioral experiments used wild types Canton S and wild type Berlin exhibited an interesting phenomenon: It could be shown that even if the two lines are both wild types they behaved very different in Buridan's paradigm (R Strauss & Heisenberg, 1993).

A recent study found that the Canton S^{TP} females behave pretty similar to what they are supposed to do since they are wild type flies. They walk to and fro between the landmarks no matter whether these are narrow (11°) or wide (20°) (Blaszkiewicz, 2010). The same study showed that the here examined learning and memory mutants show different walking phenotypes in Buridan's paradigm: Some seemed to walk the same or similar traces as the wild type did, some seemed to walk around without any attention to the landmarks and some seemed to avoid the center of the arena (Blaszkiewicz, 2010).

One ambition of this study is to investigate whether these walking phenotypes are stable in the used learning and memory mutants. If so the results should be comparable to former findings.

To investigate whether the walking phenotypes of the learning and memory mutants *dnc*, *rut* and *rsh* can be approached to the CS^{TP} phenotype or even be completely rescued the drug methylphenidate (MPH) was used.

MPH is known as a psycho stimulant drug approved for treatment of psychological diseases, such as attention-deficit hyperactivity disorder (ADHD) in humans (Chiarello & Cole, 1987). For clinical purposes dl-threo-methylphenidate, a 50/50 mixture of the two threo enantiomers of MPH, is used (Gatley & Fowler, 1995). MPH generally affects the dopaminergic system in mammals (Iversen & Iversen, 2007). The effectiveness of MPH in mammalian brain cells is founded by its ability to bind to the presynaptic dopamine transporter, what was shown for mice (Gatley & Fowler, 1995) and baboons (Ding et al., 1994; Gatley & Fowler, 1995). In the rat brain it could be revealed that MPH derivates inhibit the presynaptic dopamine transporter what results in an increase of extracellular dopamine (Ritz, Lamb, Goldberg, & Kuhar, 1987). Furthermore it could be shown that MPH derivates increase the postsynaptic dopamine uptake

ration and the synaptic dopamine transmission in cells of the rat brain (Schweri et al., 1985). In a long-term study about boys who suffer from ADHD it was exhibited that a permanent MPH treatment in humans brings a significant down regulation of the post synaptic dopamine receptors and the presynaptic dopamine transporters (Vles et al., 2003).

Since dopamine is the only naturally occurring catecholaminergic neurotransmitter in insects (Sekeris, 1966) and so in the fruit fly, it is very likely that there are a lot of physiological and behavioral processes affected by dopamine pathways. Many studies could show that especially locomotion and stereotypic behaviors like grooming are activated and modulated by dopamine (Friggi-Grelin et al., 2003). A recent study already showed that MPH can rescue some attention-like defects in flies (van Swinderen & Brembs, 2010). The present study extends that approach to the flies' walking phenotype in Buridan's arena because it is assumed that the different mutants behave different to the wild type control. Additionally it was suspected that MPH treated flies behave different to the same strains without any drug treatment. The fact that Buridan's paradigm allows it to analyze a lot of different locomotive parameters from only one trial per fly makes it a very qualified method for examining a high number of different fragments of walking behavior in the flies and to see the particular influences of MPH on the single locomotive components.

Aim of this study was to examine the different fly mutant's walking phenotypes in Buridan's arena with and without MPH treatment. It was asked whether flies of the various mutants show different walking phenotypes with special regards to single activity aspects and if the flies appear to behave differently after an MPH treatment. It was supposed that the mutants behave differently in the arena (Blaszkiewicz, 2010). Furthermore it was supposed that these differences might be only measurable in some activity aspects. Another assumption was that flies of the lines Canton S^{TP} and *radish* behave differently after an MPH treatment (van Swinderen & Brembs, 2010) and these differences affect some locomotive facets more than others. If this is the case the flies' walking parameters should be different from each other without an MPH treatment and the parameters of the wild type and *radish* should show a difference between before and after treatment.

3) Material & Methods

3.1) Drosophila melanogaster strains and stocks

Flies of the strains *rutabaga*^{1} (*rut*^{1}), *radish* (*rsh*), *dunce*^{1} (*dnc*^{1}), *rutabaga*^{2080} (*rut*^{2080}) and wild type Canton S^{TP} (CS^{TP}) were cultured at 25° C, with 60 % humidity, on a 12 h light/dark cycle. They were bred on standard cornmeal-molasses medium with a blot of yeast on top, in small fly vials. The density in the vials was controlled by housing four times as much females together with a number of males (approx. 20 females with five males).

All used fly strains were provided by Dr. Thomas Preat (ESPCI, Paris, France). The mutant *rsh* was outcrossed in 2010; *rut*^{2080} in 2007. The mutations *rut*^{1} and *dnc*^{1} were generated from the Canton S^{TP} line with the mutagen Ethyl methanesulfonate (EMS) and identified by a PCR-Screen. Hence all of the tested lines had the same genetic background.

Only 2- to 6-d-old females were phenotyped. Therefore 0- to 3-d-old females were collected, incubated for another 1- to 2-days and got their wings shortened under CO_2 anesthesia exactly one day before the experiment. For the experiment the flies were starved for 2 hours in small vials without any food or water (Fig. 2).

The experimental preparations always started early in the morning, so that it was possible to begin the experiments in the early afternoon, always at about the same time. The flies were tested in a randomly rotating system; consequently flies of the five different strains and the two different treatments were examined at all possible experimental times and under all possible experimental conditions.

36 flies of the mutant *dnc*^{1} could be investigated. Of lines CS^{TP} and *rut*^{2080} it have been 48 individuals each, and 50 of both *rsh* and *rut*^{1}. One half of each line was used to examine the locomotive behavior with MPH and the other half was used as a control without MPH.

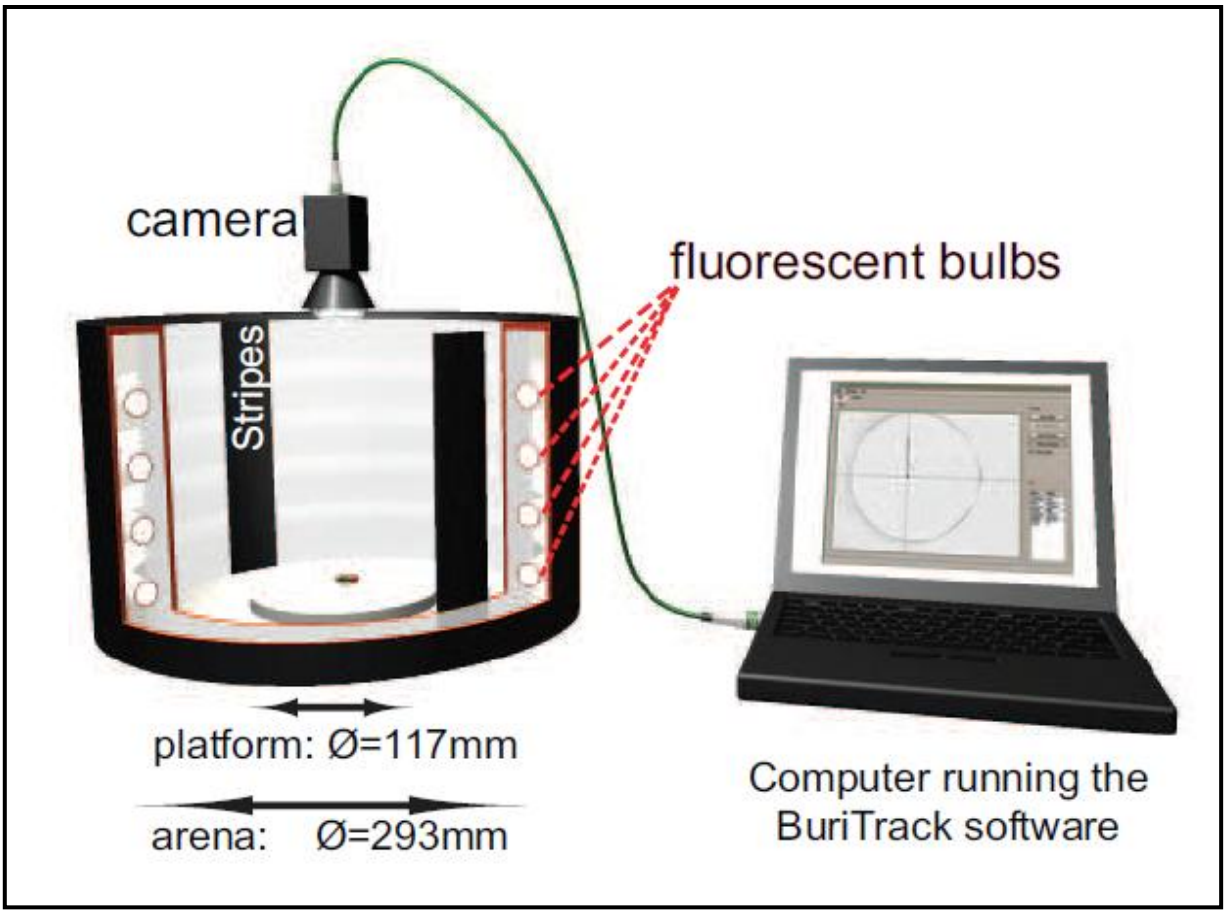


Fig. 1: From Colomb et al., 2012: Installation of the experimental set up: Buridan's arena. On the left hand side the arena with its components as a longitudinal section is shown: Visible are the arena with the platform in the center, the two stripes on both poles of the arena (Stripes), the light source, hidden behind a diffusing wall and the camera on top of the arena which sends the video signal to a computer equipped with the Buritrack software.

3.2) Buridan's paradigm

For phenotyping the different fly strains Buridan's paradigm was used.

Buridan's paradigm is a free choice assay for adult flies in which they can walk around freely, on a round platform with a diameter of 117 mm, surrounded by water.

The platform is located in the middle of a uniformly illuminated white cylinder, the arena, 313 mm in height and 293 mm breadth wise, so the center of the platform and so of the arena has a distance to the arena walls of 146.5 mm.

of >1000 Hz and so do not flicker for the fly's eye (Shields, 1989), hidden behind the cylindrical, diffusing arena wall. There were two stripes of black cardboard, 30 mm x 313 mm x 1 mm, taped on two opponent poles on the inside of the arena wall, so that the flies could see them, but never reach them because of the water around the platform (Fig. 1).

For the experiment the arena was aligned every single time so that it was warranted that the platform was perfectly horizontal and the flies were not influenced in their walking behavior by a possible decline.

When the lights were switched on the inside of the arena reached a temperature of approx. 24° C. All other possible light sources with the exception of the screen of the recording computer were switched off so that a potential reaction of the flies to stray light could be excluded.

With the help of a plastic hose appareled with a filter the single flies were sucked out of the vials and positioned in the middle of the platform. The walking flies were observed for each with 5 minutes.

After each tested fly the platform was cleaned with an ethanol saturated wipe to avoid potentially olfactory or other marks that could have been left on the plastic surface.

To document the flies' walking tracks the software Buritrack (Colomb et al., 2012) was used. For this purpose a webcam, added above the arena and linked to a computer, was necessary. The camera sent the picture of the inside of the arena to the computer and the software analyzed the flies' movements. Recorded were: Median speed (in mm/s), walking distance (in mm), turning angle (in degrees), meander (in degrees × s/mm), centrophobism indices, stripe deviation (in degrees), number of walks and activity metrics. Additionally a Principle Components Analysis (PCA) was generated.

All data were saved as digital tables what made it possible to analyze them with the rggrunner software.

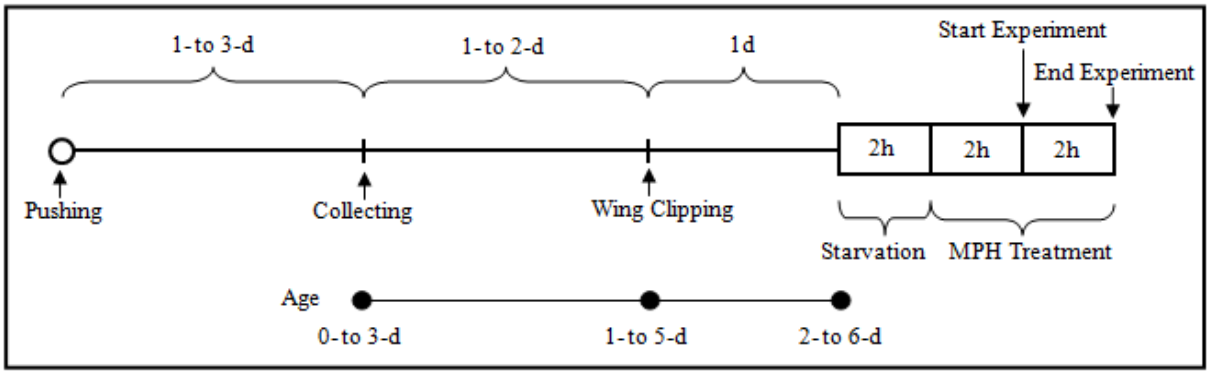


Fig. 2: Time flow of the experimental preparations and the experiment itself. The arrows show the distinct points of the preparation steps: Pushing was the point when the breeding vials were cleaned from flies at undefined ages. Collecting means the point when flies for the experiment were separated and put into new vials. Wing Clipping was when the collected flies were anesthetized and got their wings shortened. The start and the end point of the experiment are marked with arrows as well. The brackets mark the particular interims between the preparation steps and their duration and the experimental periods of the flies starving or the flies feeding on the drug. The boxes highlight the defined durations of the experimental periods. The lower line shows the age of the experimental flies: Dependent on the particular interims the flies were at different ages on the experimental day.

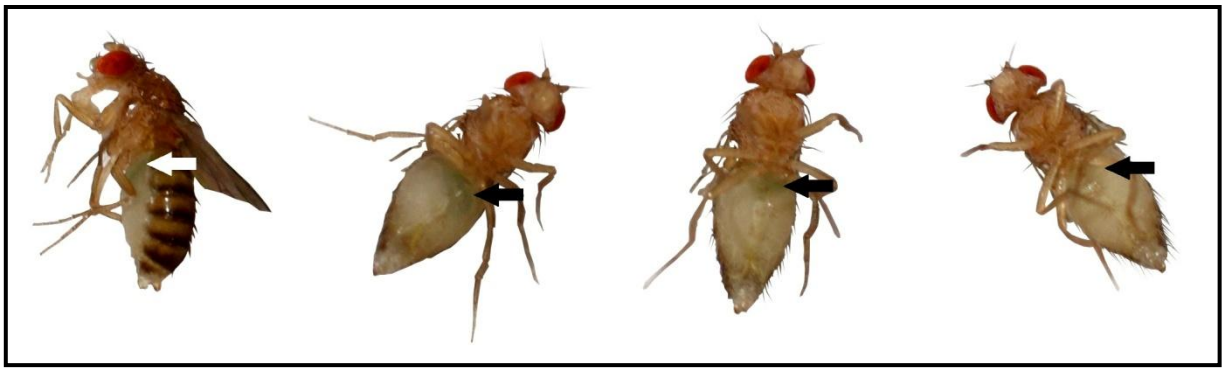


Fig. 3: Female flies after treatment with colored food in contrast to a fly which fed on regular fly food. The three left flies fed on colored food and hence they have blue colored stomach and gut regions (arrows). The fly on the right hand side of the figure fed on regular fly food and does not show any blue spots shining through the cuticle of the abdomen (arrow).

3.3) Pharmacology

The flies were treated with methylphenidate hydrochloride (MPH) (M2892 Sigma – Sigma-Aldrich, United States).

The solid methylphenidate hydrochloride was solubilized in aqua dest. at a concentration of 20 mg/ml.

The dilution was then mixed into blue colored standard medium. Therefore the cornmeal/molasses medium was heated in the microwave and thus liquefied. Blue, sugar free food coloring (Patentblau V, E 131 Indigotin I E 132, Ruth Gmbh & Co.KG, Bochum, Germany) was added (approx. 15 µl/g food) as soon as the medium had cooled down. Small, empty vials were filled with 100 µl of the MPH-dilution respectively 100 µl of aqua dest. and 3.9 g of the colored standard medium. The two substances were mixed well and put into the refrigerator overnight.

The 2 h starved flies were put into the prewarmed food vials; ed (Armstrong, Texada, Munjaal, Baker, & Beckingham, 2006), i.e. the vials with the experimental food were turned around for the whole feeding period. The flies fed on the medium for at least 2 h before testing and not more than 4 h. (Fig. 2) Because of the coloring it was possible to use only the flies which actually fed on the MPH respectively the control food for the experiment; the flies which fed on the medium had blue guts which shined through the cuticle (Fig. 3).

3.4) Analysis and statistics

In order to analyze the recorded data from Buritrack the program rggrunner with the R Script CeTrAn (Colomb et al., 2012) was used. This program is able to conclude all measured data in Excel sheets (Microsoft Corporation, Redmond, WA, USA) and visualize them as charts. Therefore the generated files were imported into the program and also a previously drawn table with every fly's file name and the group to which it was supposed to be allocated, assigned with a tab character between the file name and the group. With this information the rggrunner could calculate the averages and standard errors of all documented walking parameters from the Buritrack.

Principal components analysis (PCA) was used to transform the data and extract components that explain more of the variation of locomotive behavior of the different genotypes and differently treated flies than any single walking parameter does.

For checking whether the used genotypes (CS^{TP}, *rsh*, dnc^{1}, rut^{1}, rut^{2080}) or the applied treatments (MPH, control) or both had an effect on the measured parameters an analysis of variance (ANOVA) was done. For the calculated p-values from the ANOVA it is: $p > 0.05$ – not significant (-); $0.05 > p > 0.01$ – significant (*); $0.01 > p > 0.001$ – highly significant (**); $0.001 > p > 0$ – extremely significant (***).

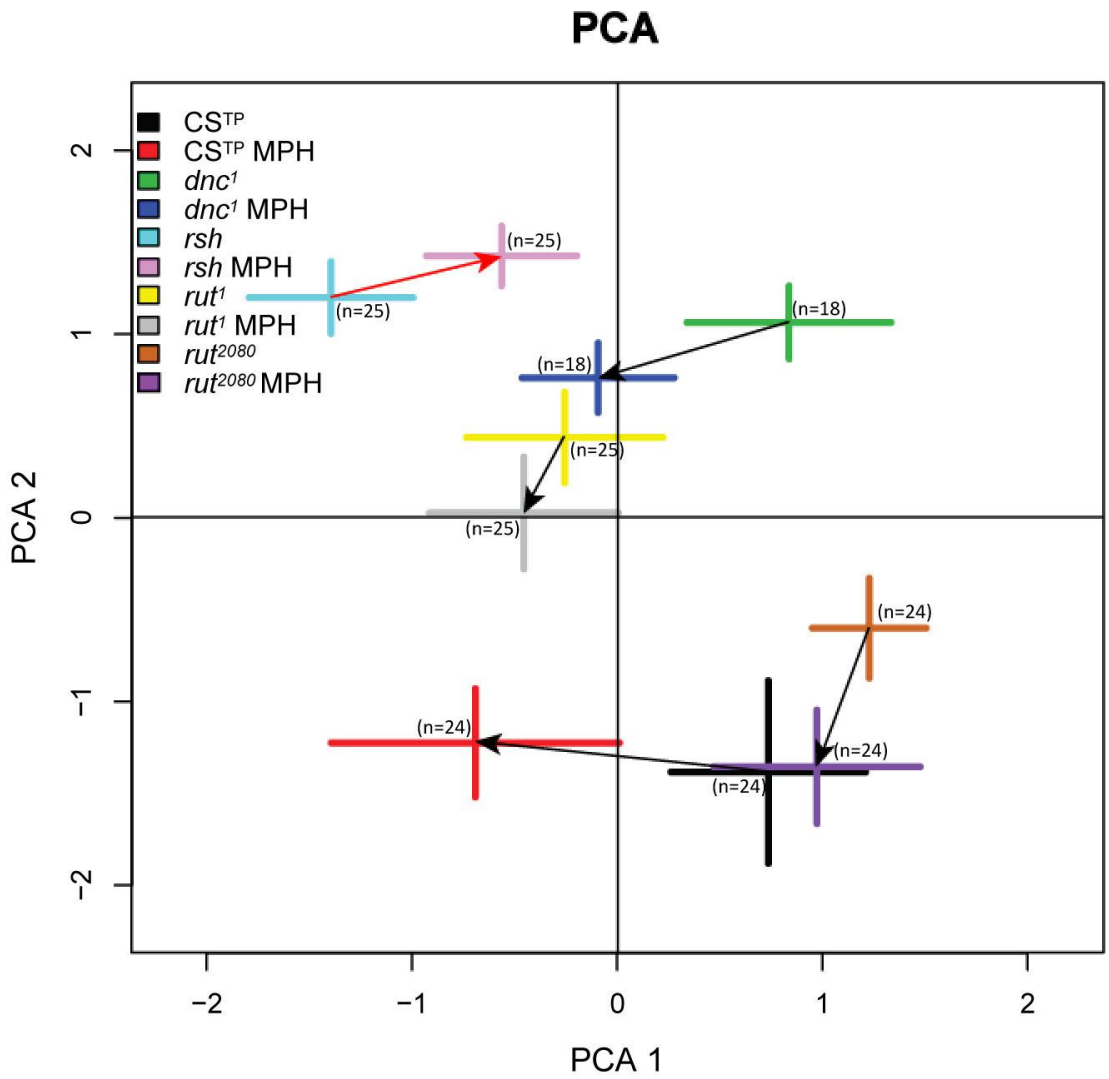
Furthermore the post hoc test Tukey-HSD was used to quantify the calculated effects. The hence generated adjusted p-values determine the respective levels of significance for the single effects. Whenever the post hoc test was done the adjusted p-values for each combination of genotypes and treatments were summed up in form of a cross tabulation and marked red if significant.

4) Results

Principle components analysis found that approx. 70% of the variation of the measured data is explained by the first three generated components. The first component (PC1) alone explains over 35% of the variation and loaded on preferentially temporal parameters. It loaded strongly negatively on the number of pauses the flies made and strongly positively on the activity time, the number of walks and less strongly positively on the speed of the flies. There are few, rather areal parameters loading on PC1: turning angle (slightly negatively), meander (slightly negatively) and distance traveled (strongly positively). The second principle component (PC2) loaded negatively on space parameters like stripe deviation and thigmotaxis (sitting and moving) and on the temporal parameter pause length. It explained approx. another 20% of the variation. The third PC cannot be clearly allocated to either time or space activity because it loaded on both types of parameters with approx. the same power. The strongest loadings on PC3 were turning angle and meander (both strongly positively) and pause length (negatively). PC3 explains another 15% of the data variation.

It could be shown that the examined fly mutants differ in both PC1 and PC2. This result is significant for rut^{2080} and dnc^{1} (adjusted $p < 0.001$) and rut^{2080} and rut^{1} (adjusted $p < 0.05$) in matters of PC1 and most of the cases in matters of PC2 (Fig. 4, tables).

The findings for MPH effects are not significant for any mutant but it is conspicuous that all of the mutant flies got a lower PC1 score after the MPH treatment (Fig. 4, black arrows) aside *rsh*, which got a higher PC1 score after the drug treatment (Fig. 4, red arrow). No mutant exhibited a strong MPH-dependent shift in matters of PC2.



PCA 1

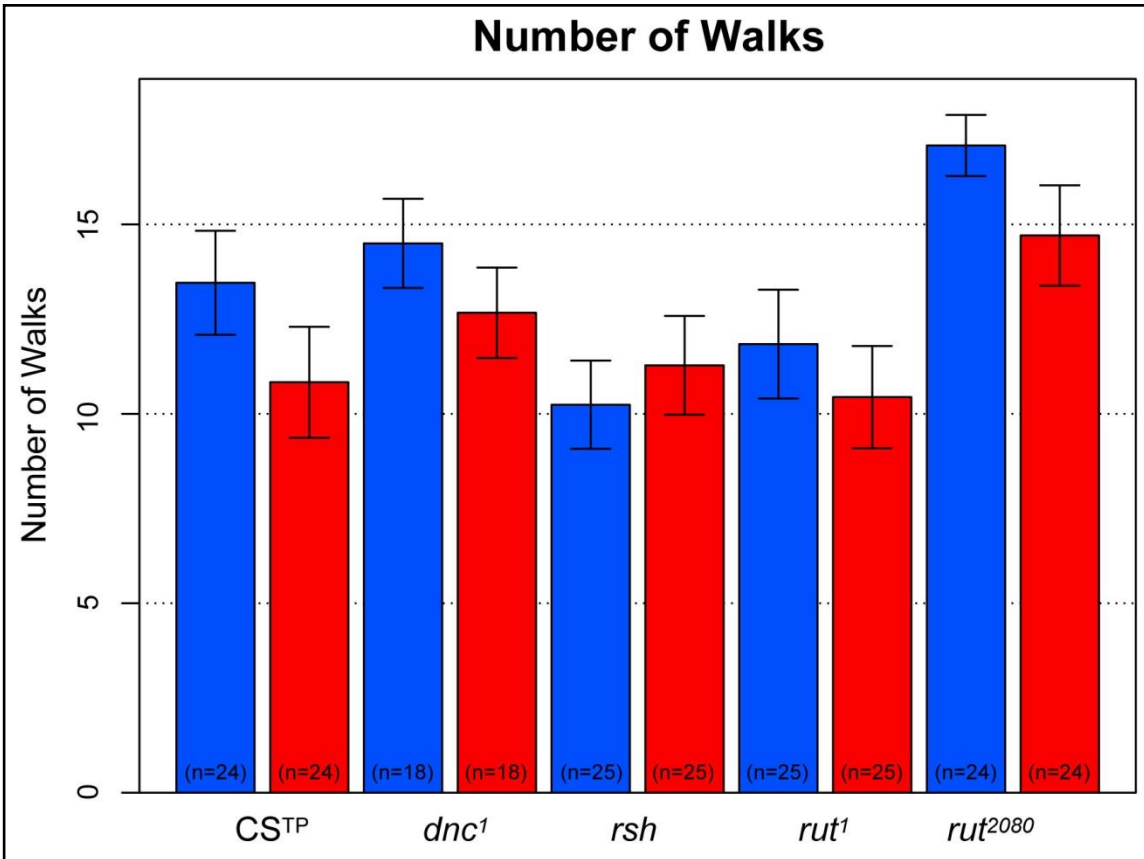
genotype	CS^{TP}	*dnc*^{1}	*rsh*	*rut*^{1}	*rut*^{2080}
CS^{TP}	-	0.957893	0.191706	0.925168	0.141771
dnc^{1}	0.957893	-	0.053278	0.591114	0.598878
rsh	0.191706	0.053278	-	0.650575	0.000081
rut^{1}	0.925168	0.591114	0.650575	-	0.014084
rut^{2080}	0.141771	0.598878	0.000081	0.014084	-

PCA 2

genotype	CS^{TP}	*dnc*^{1}	*rsh*	*rut*^{1}	*rut*^{2080}
CS^{TP}	-	0.000017	0.000017	0.000018	0.789854
dnc^{1}	0.000017	-	0.686993	0.173669	0.000017
rsh	0.000017	0.686993	-	0.001126	0.000017
rut^{1}	0.000018	0.173669	0.001126	-	0.000189
rut^{2080}	0.789854	0.000017	0.000017	0.000189	-

Fig. 4: PCA with the first two components PC1 and PC2 witch explain approx. 55% of the variation of the data. The centers of the colored crosses show the mean PCA scores for all the fly genotypes with and without MPH treatment (see legend) and their standard deviation (arms of the crosses). The two tables list the adjusted p-values for all possible combinations of fly genotypes for PC1 (upper table) and PC2 (lower table); the red highlighted values show significances of the genotypic differences. Black arrows demonstrate the non-significant shift of the mean PC1 scores of the fly strains to lower values after MPH treatment. The red arrow shows the non-significant shift of the mean PC1 score of *rsh* to a higher score after MPH treatment.

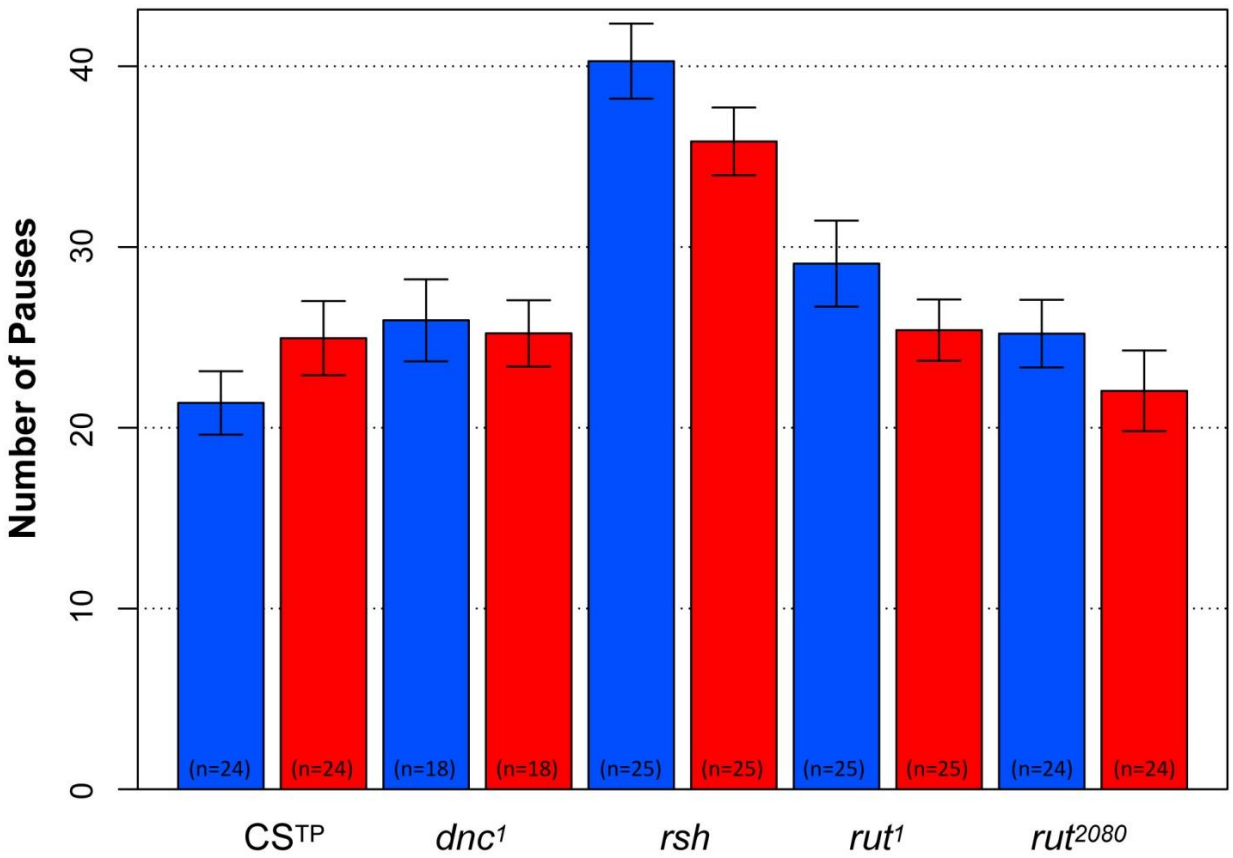
As an example for the differences between fly genotypes respectively temporal activity serves the number of walks between the two stripes of Buridan's arena: With more than 15 walks per trial *rut*^{2080} clearly stands out from the other mutants and the wild type. This is significant for all genotypes but *dnc*^{1} (Fig. 5, table). Another interesting observation is the fact that all fly strains walk less after the MPH treatment except from *rsh* which walks approx. two bouts more per trail than without the drug treatment (Fig. 5). No change in the number of walks after MPH treatment was significant.



genotype	CS^{TP}	*dnc*^{1}	*rsh*	*rut*^{1}	*rut*^{2080}
CS^{TP}	-	0.835592	0.808442	0.931906	0.027405
dnc^{1}	0.835592	-	0.235309	0.380980	0.448263
rsh	0.808442	0.235309	-	0.998152	0.000475
rut^{1}	0.931906	0.380980	0.998152	-	0.001584
rut^{2080}	0.027405	0.448263	0.000475	0.001584	-

Fig. 5: Number of walks. It is shown how often (y-axis) flies of the different strains (x-axis) walked back and fro the two poles of the arena during one trial (five minutes) on average. Blue bars indicate fly strains without MPH treatment, red ones with MPH treatment; Error bars: standard error. The table lists the adjusted p-values for the genotypes without drug treatment and shows that *rut*^{2080} walks significantly more between the poles than CS^{TP}, *rsh* and *rut*^{1} (red values).

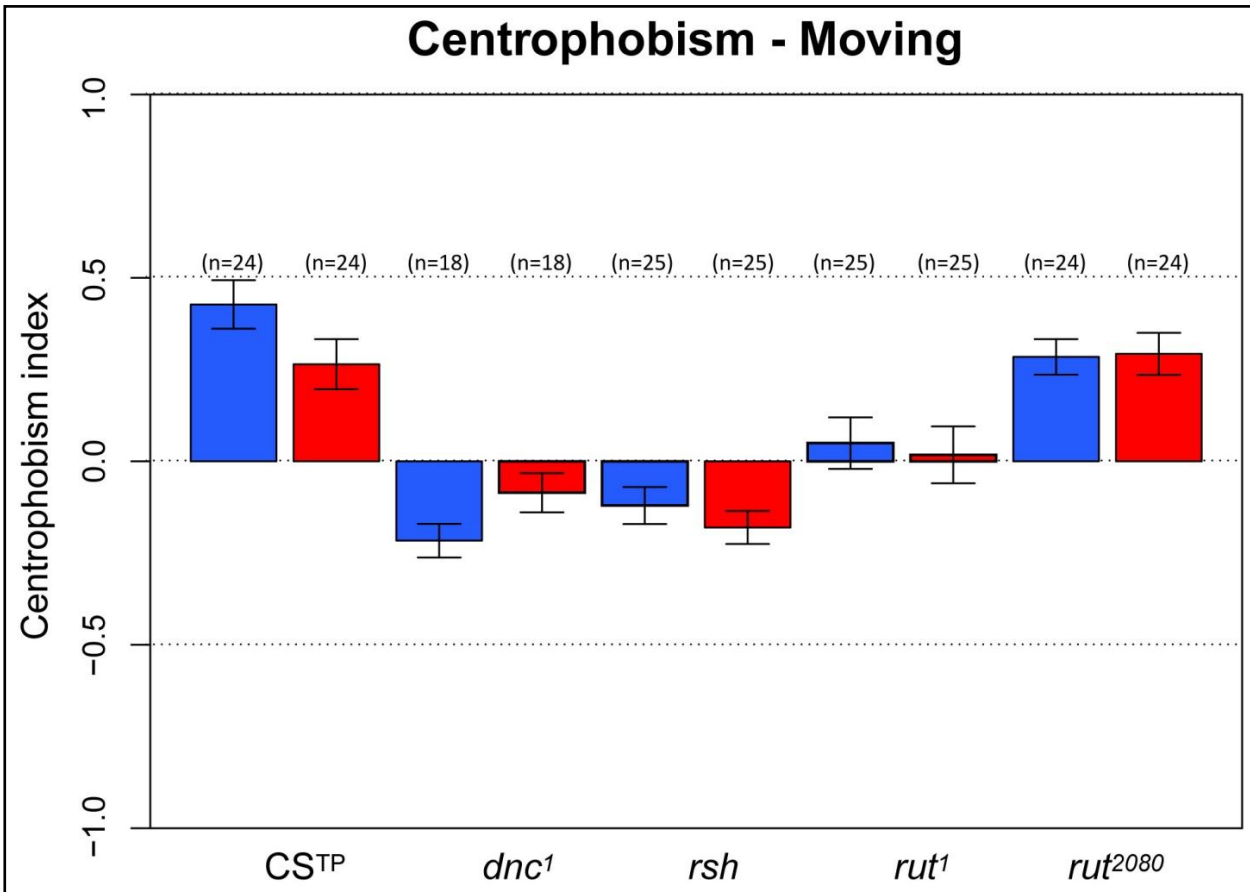
Another example for differences in temporal activity of the flies is the number of pauses they made (Fig. 6). It could clearly be exhibited that *rsh* flies make more pauses than all of the other fly strains. This result is significant for all strains (Fig. 6, table). Furthermore there is the tendency of *rsh* flies after MPH treatment to make fewer pauses; this tendency can also be shown for the two *rut* mutants and is not significant.



genotype	CS^{TP}	dnc^{1}	*rsh*	rut^{1}	rut^{2080}
CS^{TP}	-	0.800727	0.000017	0.245182	0.999406
dnc^{1}	0.800727	-	0.000017	0.939689	0.896817
rsh	0.000017	0.000017	-	0.000018	0.000017
rut^{1}	0.245182	0.939689	0.000018	-	0.365540
rut^{2080}	0.999406	0.896817	0.000017	0.365540	-

Fig. 6: Number of pauses. The mean number of pauses (y-axis) the flies (x-axis) made per trial (five minutes) is demonstrated for each fly strain without MPH treatment (blue bars) and with MPH treatment (red bars). Error bars represent standard error. The table shows the adjusted p-values for each possible comparison of genotypes without MPH treatment.

Different fly strains showed very variable centrophobic behavior while walking around in the arena. Because centrophobism is directly linked to thigmotaxis (Besson & Martin, 2005), an element of the PC2, it is a suitable example for the differing space activity in the various fly lines. It arises that CS^{TP} and *rut*^{2080} flies prefer to walk at the edges of the arena whereas *dnc*^{1}, *rsh* and *rut*^{1} show a less strong centrophobic behavior (Fig. 7). The mutant *rut*^{1} seems not to show any preference for the inner or outer region of the arena since its centrophism index is lower than the ones of CS^{TP} and *rut*^{2080} (adjusted $p < 0.001$) and higher than the ones of *dnc*^{1}



genotype	CS^{TP}	*dnc*^{1}	*rsh*	*rut*^{1}	*rut*^{2080}
CS^{TP}	-	0.000017	0.000017	0.000018	0.875021
dnc^{1}	0.000017	-	1.000000	0.032897	0.000017
rsh	0.000017	1.000000	-	0.014810	0.000017
rut^{1}	0.000018	0.032897	0.014810	-	0.000177
rut^{2080}	0.875021	0.000017	0.000017	0.000177	-

Fig. 7: Centrophobism index. The Graph shows the different mean centrophobism indices (y-axis) of the fly lines (x-axis) walking around in the arena. The scale of the index goes from -1.0 (moving only in the center of the arena = a radius √2 times smaller than the platform radius) to +1.0 (moving only at the edges of the arena = arena area – center of the arena). Blue bars represent the flies without MPH treatment, red bars with MPH treatment, error bars the standard error. The table lists the adjusted p-values for the fly genotypes without MPH treatment.

and *rsh* (adjusted $p < 0.05$) (Fig. 7, table). A clear tendency of MPH affecting the centrophobism index is not distinguishable. An alternative way to visualize the different centrophobic behaviors are the transition plots. These displays underline the calculated indices for centrophobism in the fly lines. The transition displays of CS^{TP} and rut^{2080} clarify the fact that both lines rather walk around in the lateral parts of the arena (Fig. 8 and 10 B, left). It made no conspicuous difference whether the flies got MPH treatment or not, the walking paths look fairly the same (Fig. 8 and 10 B, right). For dnc^{1} and *rsh* it could be shown that these flies cross the center of the arena very often and did not move at the edges for longer times (Fig. 9 A and B, left). Also here no obvious effects of MPH were detectable (Fig. 9 A and B, right). The mutant rut^{1} spend almost the same amounts of time moving in the center of the arena and in the lateral regions (Fig. 10 A, left). No apparent change in MPH treated flies here either (Fig. 10 A, right). The transition plots only show the walking paths of the flies and are not statistically testable.

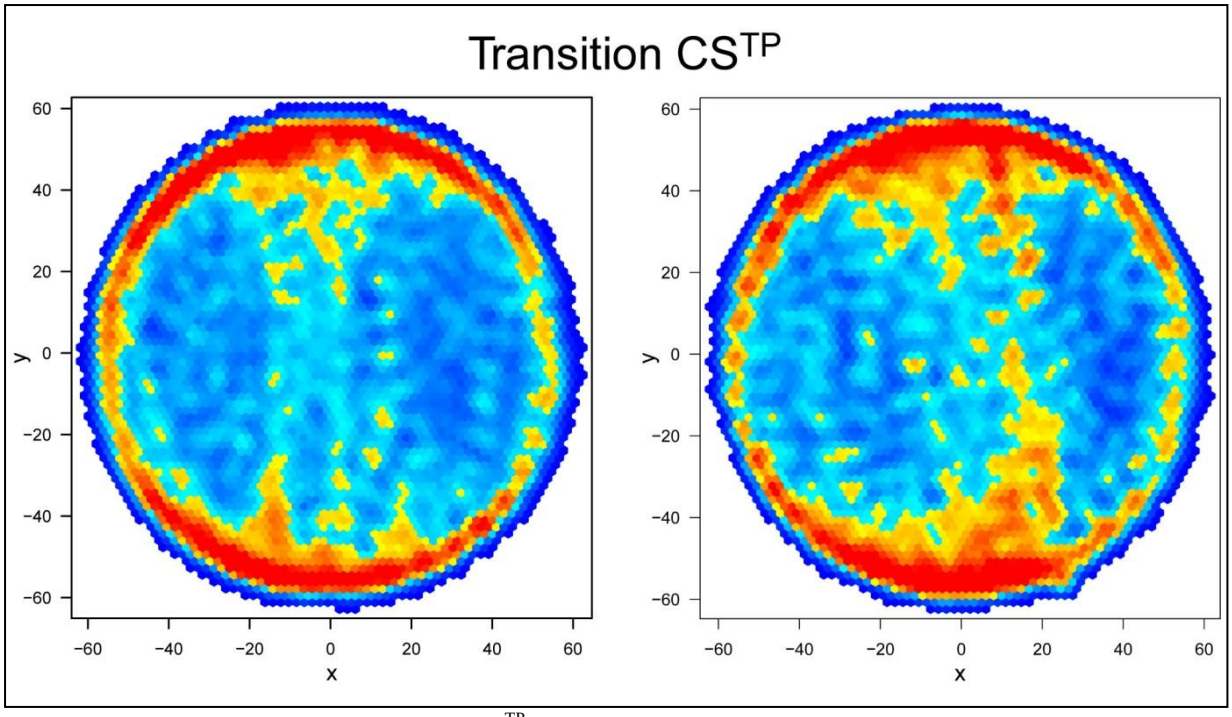


Fig. 8: Transition display for the wild type CS^{TP}. The transition display shows a map of the platform in Buridan's arena from above (x-axis: width of the platform in mm; y-axis: length of the platform in mm); beyond the platform, at an imaginary extension of the y-axis the stripes were added. The display only shows where on the platform the flies walked, not where they stayed. The scale of the movements goes from blue (rarely crossed areas) to red (> 95% of the testing time crossed areas); white areas haven't been crossed. On the left hand side the display for CS^{TP} flies without MPH treatment is shown and on the right hand side the diagram for same flies with MPH treatment is shown. Obviously the average of the CS^{TP} flies avoided the center of the arena and preferred it to

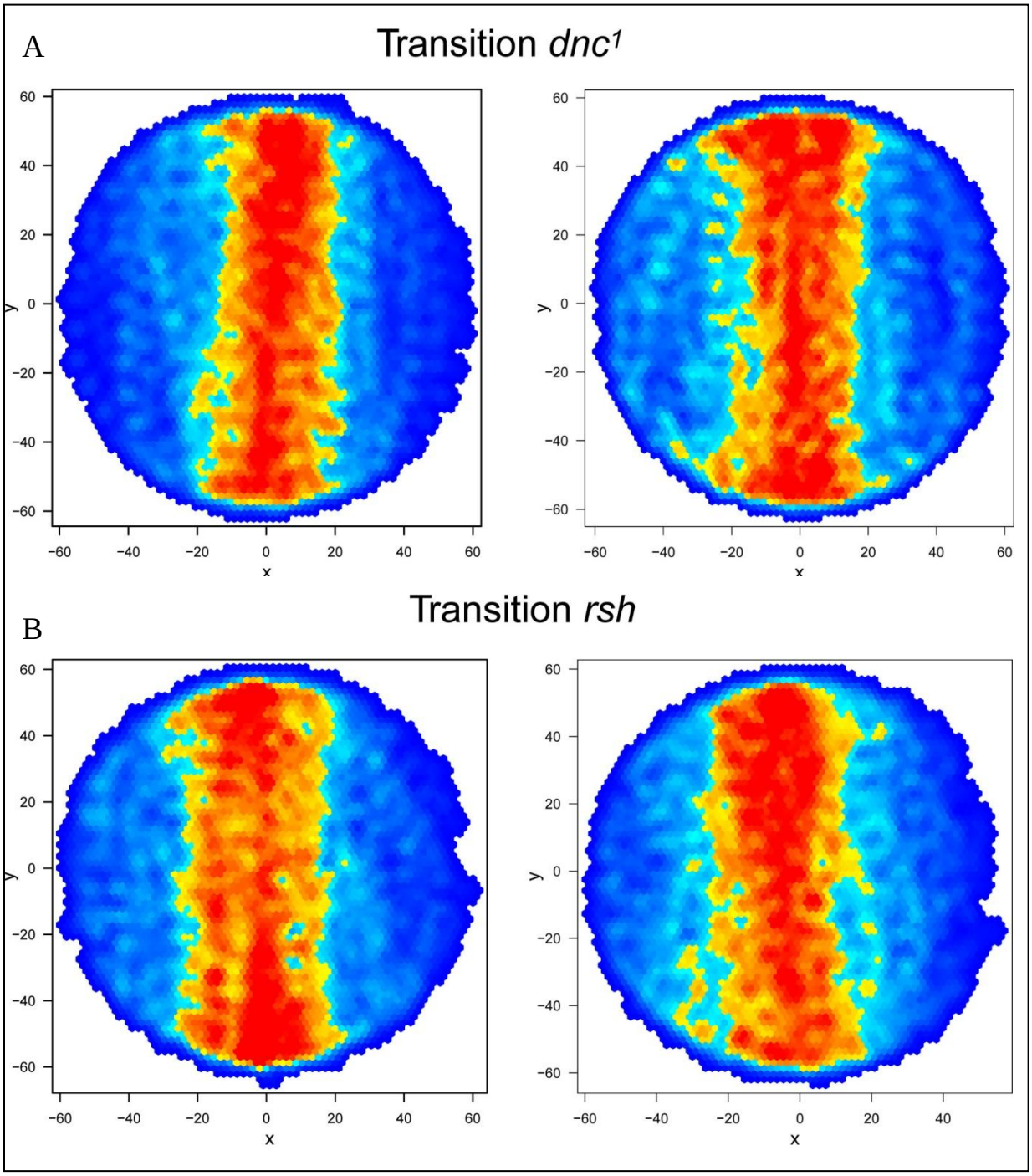


Fig. 9: Transition displays for the mutants *dnc*[1](A) and *rsh* (B). The transition display shows a map of the platform in Buridan's arena from above (x-axis: width of the platform in mm; y-axis: length of the platform in mm); beyond the platform, at an imaginary extension of the y-axis the stripes were added. The display only shows where on the platform the flies walked, not where they stayed. The scale of the movements goes from blue (rarely crossed areas) to red (> 95% of the testing time crossed areas); white areas haven't been crossed. On the left hand side the displays for *dnc*[1](A) and *rsh* (B) flies without MPH treatment is shown and on the right hand side the diagram for same flies with MPH treatment is shown. Matching with the data from the centrophobism plot *dnc*[1] (A) and *rsh* (B) very often cross the central area of the platform while walking back and fro the two stripes.

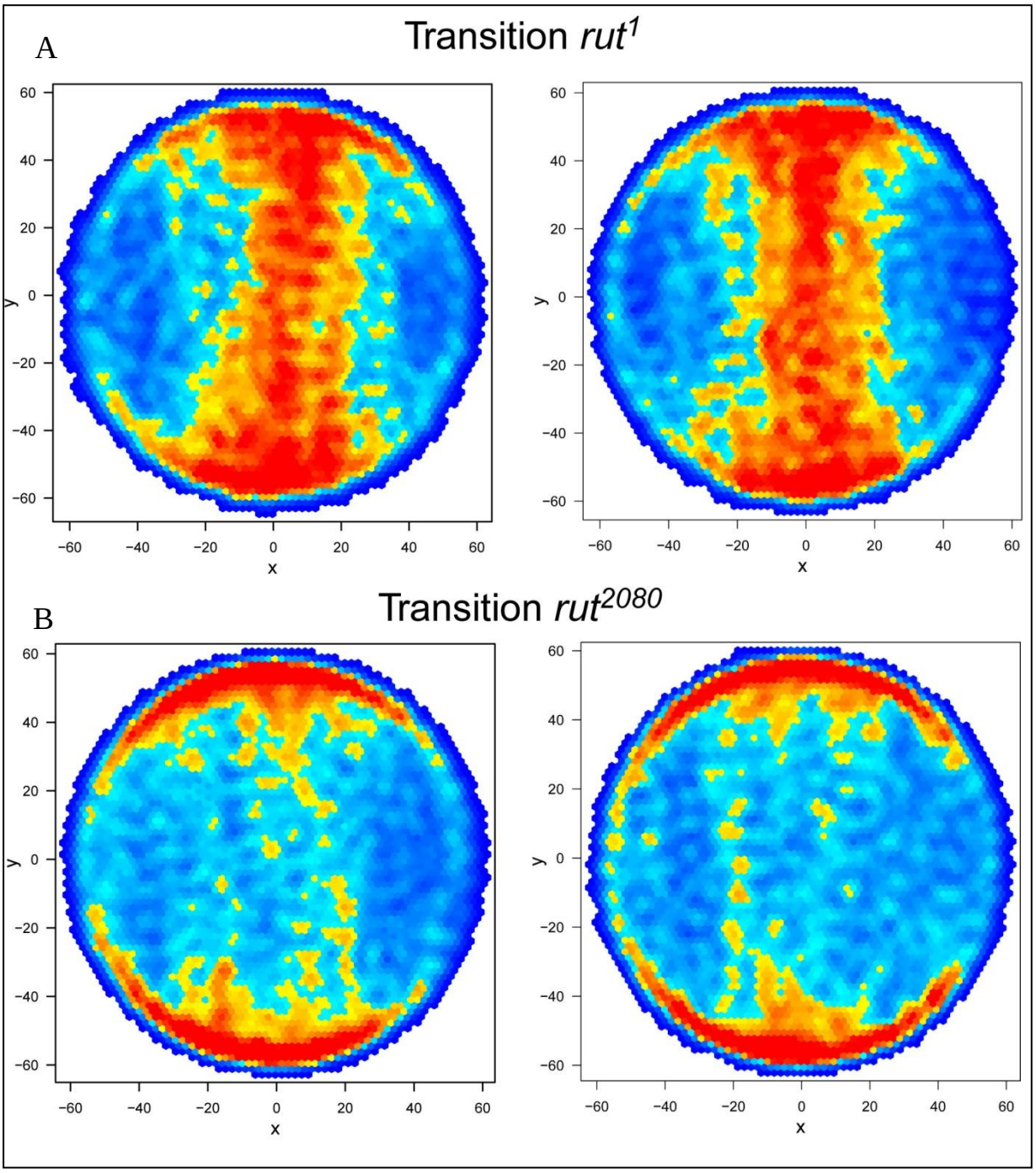


Fig. 10: Transition displays for the mutants *rut*[1] (A) and *rut*[2080] (B). The transition display shows a map of the platform in Buridan's arena from above (x-axis: width of the platform in mm; y-axis: length of the platform in mm); beyond the platform, at an imaginary extension of the y-axis the stripes were added. The display only shows where on the platform the flies walked, not where they stayed. The scale of the movements goes from blue (rarely crossed areas) to red (> 95% of the testing time crossed areas); white areas haven't been crossed. On the left hand side the displays for *rut*[1] (A) and *rut*[2080] (B) flies without MPH treatment is shown and on the right hand side the diagrams for the same flies with MPH treatment is shown. Matching with the data from the centrophobism plot it can be shown that *rut*[1] flies (A) spend approx. the same portions of time moving in the center of the arena and in the outer areas of the arena. This is shown by the approx. same amounts of red in the center and in the periphery of the map. *rut*[2080] flies (B) spend more time moving near the edges of the arena than in the center.

5) Discussion

The here presented work could clearly confirm previous findings of selected *Drosophila melanogaster* learning and memory mutants showing different locomotive phenotypes. The results give evidence of the mutants differing in rather areal fractions of their locomotion.

Since the calculated PC2 was preferentially loaded with space parameters of movement, like centrophobism which exhibited distinct differences between the fly lines (Fig. 4 and 7), it is assumable that the mutant flies use their space and explore their environment in a different way than wild type flies do. It could also be shown that the mutant rut^{2080} uses space in Buridan's arena very similar to CS^{TP} (Fig. 8 and 10 B, left), but seems to distinguish from the wild type respective the temporal parts of movement (Fig. 4 and 5). Flies of the line dnc^{1} seem to be as temporally active as the wild type, but completely differ with regard to areal activity (Fig. 4). The mutants *rsh* and rut^{1} appear to differ from the wild type relating to both time and space parameters.

It can consequently be assumed that there is a distinction between different aspects of activity in the fruit fly and those manipulations like pharmacological treatments need to be analyzed with reference to these distinct aspects.

This investigation for example showed that an MPH treatment has the tendency to affect rather time activity in the flies but space parameters seem to stay uninfluenced by the drug (Fig. 4). In reference to former results (van Swinderen & Brembs, 2010) it is imaginable that changed results in locomotion and attention assays after an MPH treatment are caused by an increase of the temporal activity and therefore a higher attention-like level for some mutants. Since time activity of *rsh* flies with MPH treatment appears to develop exactly in the contrary direction to the other mutants and the wild type flies with the same treatment it is assumable that MPH which affects the dopamine pathway has a different influence on *rsh* because the mutation affects exactly these pathways in some way. Consequently only mechanisms which concern the time activity are supposed to be affected by the mutation and space activity should then be regulated separately.

Another explanation is the possible separation of dopaminergic effects on temporal and areal activity processes in the fly. Given that dopamine affects time aspects of activity in a different way from space aspects it is assumable that these two and

maybe more variables of activity are regulated in different ways. Conceivable where mechanisms like interactions of different dopaminergic receptor groups (D1 – D5) (Seeman & Van Tol, 1993), different second messenger pathways, a chronologically shifted function of dopamine effects or even different neurons being responsible for the various aspects of activity. This assumption also matches with the fact that there are many different learning and memory mutants with impaired dopamine-linked processes in *Drosophila melanogaster* (Bolduc & Tully, 2009; Martin Heisenberg Alexander Borst, Sibylle Wagner and Duncan, 1984; Skoulakis & Grammenoudi, 2006). It is likely that some of these mutants can be rescued by particular pharmacological treatments whereas others won't show any change in behavior just because the treatment affects only those parts of the behavior which are not impaired. In this case the behavior of *rsh* could have been changed to a nearly wild type level (Fig. 5) with respect to time activity because MPH changed the part of the dopamine cascade which is responsible for just this fragment of activity.

To confirm this theory it will be necessary to increase the sample sizes of the experimental groups and to analyze where exactly the limits in the concentration of the drug are. It is possible that higher concentrations of MPH increase the effect on the walking behavior of *rsh* and CS^{TP} such that it becomes significant. Even if the here used 20 mg / ml already is very highly concentrated, it might be that even higher drug concentrations affect the activity of the flies more strongly.

Another interesting point is that *rsh* flies seem to manage their foraging time completely different from how the other examined flies are doing it. The mutant takes noticeably more breaks than the other lines while walking in the arena. These pauses might be used for new orientation between the two landmarks and therefore might be a hint for an attention-like deficit in these flies (van Swinderen & Brembs, 2010). When treated with MPH *rsh*'s numbers of pauses appears to decrease and thus approaches to the pause levels of the other lines (Fig. 6). This effect is an example for the changed temporal activity in the mutant after MPH treatment and could indicate a higher attention level after the drug treatment.

Since MPH only appears to affect time activity it is not very surprising that the walking paths of the flies evidently do not change after the drug treatment (Fig. 8 – 10, right). It is clearly visible that every single mutant walks nearly the same paths with and without MPH treatment (Fig. 8 - 10, right). The analysis of the centrophobism indices also showed that there are no differences in *rsh*, rut^1 and

rut^{2080} and slight, not significant differences in dnc^{1} and CS^{TP} with and without the drug. This again points to an effectiveness of MPH only on time activity in flies.
Further investigations of activity in flies being split into different aspects and how this is realized will be necessary. In addition the exact effectiveness of MPH in the flies' cells should be examined.
It is likely that other substances which affect the dopamine pathways have the potential to change other activity aspects and thus change the behavior of other learning and memory mutants or even rescue them.
Cocaine for example is known to affect locomotion in the fruit fly (Hardie, Zhang, & Hirsh, 2007; Li, Chaney, Roberts, Forte, & Hirsh, 2000) and also nicotine and ethanol are capable to change the walking behavior of *Drosophila* (Bainton et al., 2000). The connections between all these effects and the presumably plenty of different activity aspects need to be examined in further studies.

6) Conclusion

Different learning and memory mutants of *Drosophila melanogaster* show different walking phenotypes in Buridan's arena. The walking behavior of the flies appears to be divided into at least two different fragments: time and space activity. MPH seems not to rescue any of the mutants' walking phenotypes but shows a slight trend to change the behavior of the mutant *rsh* respectively time activity. Future studies will be necessary to examine the exact effects of MPH on the flies' walking behavior and how the distinction between time and space parameters of activity can be affected by the drug differently.

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