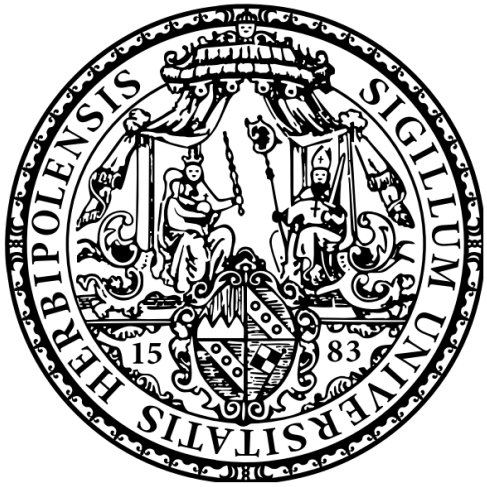
Spatially Selective Visual Attention

in *Drosophila melanogaster*

Räumlich selektive visuelle Aufmerksamkeit in *Drosophila melanogaster*



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1 Introduction

Die Natur schafft keine genera und species, sie schafft individua und unsere Kurzsichtigkeit muß sich Ähnlichkeiten aufsuchen um vieles auf einmal behalten zu können.

Georg Christoph Lichtenberg, Sudelbuch A

A hallmark of brains is the selection of one behavior out of many possible. At any moment in nature there are plenty of stimuli available but not all of them are important for an organism. Sometimes specific information or even complete sensory channels can be irrelevant in the current situation. The brain filters the input for significance for behavior and thereby improves the selection of a behaviorally adaptive response. Input to the brain includes all the available sensory information, but this study focuses on a mechanism of selection in the visual modality. Visual sceneries can be staggeringly complex. The abundance of visual information can serve as a basis of various behavioral responses, which are sometimes even mutually exclusive. Nevertheless, animals can successfully cope with this challenge and navigate their surroundings with impressive speed and accuracy. For example, the male hoverfly *Syritta pipiens* is able to chase its female conspecifics and hence to follow only a small fraction of the available stimuli while disregarding the rest (Collett and Land, 1975).

The selection of some stimuli and the suppression of others is subject of research on attentional processes. Several studies have shown that focusing attention on locations or objects improves the efficiency of their processing (Posner and Petersen, 1990; Theeuwes and Van der Burg, 2007). Attention has been compared to "[…] a spotlight that enhances the detection of an event within its beam" (Del Pezzo and Hoffman, 1980). Additionally, a possibly independent mechanism dampens the processing of stimuli that lie outside this spotlight (Munneke et al., 2008). Together these mechanisms allow an organism to select behaviorally relevant information from its surroundings.

Selective visual attention (SVA) is a property of higher visual systems that can separate the important from the irrelevant (e.g. the female from the rest in the case of *Syritta pipiens*). It possibly also assists processing as it adjusts the large visual input (Itti et al., 2001) to the limited capacities of a brain (Kahneman, 1973). SVA highlights subsets of visual input, which can be computed with near real-time performance. But more importantly, this highlighting provides a selection of stimuli for detailed scrutiny. There are probably several forms of SVA, depending on the criteria used for the selection such as color, motion or location in space. As a consequence of its inherent selection process, SVA is a powerful tool, which is able to modulate learning and memory (Mack and Rock, 1998) and even conscious perception (e.g. of a gorilla, Simons and Chabris, 1999). The metaphor of SVA as a spotlight fits one's subjective experience of shifting the focus of attention (FoA), which is usually coupled to the direction of gaze. Some of the observable effects of attentiveness by foveation are decreased response times, higher accuracy and lower thresholds for target detection. Other than a spotlight might suggest, what lies outside the FoA is not completely ignored. However, description of unattended objects is limited and less accurate (DeSchepper and Treisman, 1996).

1.1 Elements of Attention

An important prerequisite of attentional studies is a clear definition of the term attention. Even though intrinsically "[…] everyone knows what attention is" (James, 1890), subjects might not all describe the same of its manifold aspects when speaking about it. As an approach to categorize and organize those aspects, one can sort them with a seemingly simple taxonomy: Attention can be modulated volitionally (top-down) or by salience (bottom-up). Whether those provide separate mechanisms or just the two extremes of a continuum is debatable, however. Because top-down modulation is most likely a serial process, it is slower and susceptible to distractors, which require a share of the limited resources. Bottom-up attentional mechanisms are processing the environment in parallel on the basis of a salience map (Koch and Ullman, 1985), which determines the stimulus to attend to. The necessary favoring of novel stimuli and the avoidance of resampling of recently attended objects can be achieved either by a tagging mechanism, which marks the particular stimulus as irrelevant (Neill et al., 1992) or by inhibition of return to a previously attended location (Posner et al., 1985). Both would allow evaluation to proceed to the next salient stimulus.

As mentioned before, there are many criteria for the separation of stimuli and thus probably many forms of SVA. This study focuses on spatially selective visual attention (SVA). SVA can be cued to a certain location in space. The likelihood of a cue to catch attention depends on its salience, but the response can be overridden by top-down mechanisms (i.e. actively focusing elsewhere). There are many things that can attract visual attention in the real world, either because they are very obvious (e.g. a bull in a china store) or because the subject's internal state increases their salience (e.g. a bottle of water after sports). In the controlled environment of a lab, cues are by design in most of the cases less sophisticated and one-dimensional. Irrespective of their shape, color or kinetics, two generic types of cues can be distinguished. If a cue appears at the subsequent target location, it is called an exogenous cue. Correspondingly, endogenous cues are presented at a neutral position and indicate by their semantics where a target will appear (e.g. an arrow pointing towards the side at which the target will appear). In terms of paying attention, human subjects respond to a cue by either directing their gaze towards the cued location (overt attention) or by internally shifting their FoA there (covert attention). Helmholtz showed the latter in a seminal experiment for which he put up a poster with numerals written onto it in a dark room. He then lit the room with a small spark just long enough to see the poster, but not long enough to move his eyes. He found that he could read numerals in the periphery without directing his gaze there, if he decided to pay attention to this region before eliciting the spark (Helmholtz, 1866).

Attention has been described with several metaphors. Besides a spotlight, attention has been compared to a zoom lense (Eriksen and Murphy, 1987) or a filter (Broadbent, 1958). All these metaphors are useful, but none of them offers a complete and objective description of attention. Yet, this anthropocentric

perspective on attention might be already too specific and thus inadequate to characterize attention in simpler animals.

1.2 Attention in *Drosophila*

Taking a step back, one finds the focusing on one source of sensory inputs to the exclusion of others as a generic feature of SVA (Luck and Mangun, 1996). The demand for such a selection mechanism becomes apparent in the case of *Drosophila*, which possesses compound eyes that sample almost the entire visual space. During flight the visual input changes from moment to moment. A large fraction of it carries behaviorally irrelevant information and the fly has to extract the momentarily relevant from this flux. This study aims at examining how SVA is allocated in space and its temporal dynamics.

In the fly, SVA has been related to and described by processes like fixation behavior (Ye et al., 2004). Other studies made use of *Drosophila's* visual orientation behavior and related the effects of salience of a single target object on this behavior to attention (Xi et al., 2008). Another intriguing finding of these studies was the connection between dopamine, the mushroom bodies and attention. Ablation of the latter led to poor fixation of objects, if their salience was reduced by high background noise or low contrast (Xi et al., 2008). Mushroom bodies seemed to furthermore promote behavioral flexibility on the expense of habit formation (Brembs, 2009) thereby providing a prerequisite for attentional processes - the possibility of selection. After a prolonged blockade of dopamine release flies lost their ability to orient towards an object (Ye et al., 2004). The assumption that dopamine might be important for attention was strengthened by van Swinderen and Brembs (2010), who attributed various alterations in maze walking behavior of the learning and memory mutant rsh^1 to attention-like defects. They could revert the phenotype of rsh^1 to that of wild-type flies by application of methylphenidate. The drug is commonly administered to patients suffering from attention deficit and hyperactivity disorder (ADHD) and inhibits the re-uptake of dopamine from the synaptic cleft. In another experiment they measured changes in local field potentials (LFP) in response to novel visual stimuli and related those to attention-like processes. By attributing sustained LFP responses to particular objects, van Swinderen (2007) hypothesized a putative attention span of *Drosophila* of about 9-12s. Paulk et al. (2015) kept using electrophysiological measurements to investigate a possible expression of attention in the fly brain. They found an increase in local coherence in the central brain when the fly could actively move a visual panorama (closed-loop) in comparison to cases in which the fly's action had no effect on the visual surround (open-loop).

In order to learn about the properties of visual attention, it is a good start to move out of the brain and turn to its effects on behavior. Already decades ago, in the course of their detailed in-depth analysis of *Drosophila's* behavior in the flight simulator, Wolf and Heisenberg (1980) and Heisenberg and Wolf (1984) described a couple of experiments that addressed SVA. When a stripe was displaced front-to-back, a

stationary flying fly in most of the cases responded with a large yaw-torque spike, indicative of a body saccade. The saccade would have under natural conditions served to maintain a heading and would have stabilized the animal's flight. When a second stripe was added, but not displaced, the number of responses to the displaced stripe was reduced by about 50%. The stationary instead of the moving stripe seemed to occupy the fly's attention in one half of the cases, which thus produced no responses. One might expect that a rather simple animal like *Drosophila* would always respond to the most salient stimulus. The ability of the fly to not respond corroborates the employment of an active selection mechanism. This was also true for two identical stripes, which each had a characteristic yaw-torque pattern, when oscillating in phase. Interestingly, the fly switched between the corresponding responses of the equally salient stimuli (and no responses). Similar behavior could be seen when the stripes were replaced by random dot patterns. The fly alternated between responses that resembled those observed in the presence of a single pattern. These are examples of internally caused endogenous shifts of attention. However, similar to human attention, the fly's FoA can also be externally cued.

In the experiment just described, addition of an oscillating stripe in front of one of the two patterns cued the fly to preferentially follow the oscillation of this pattern. The cue did not have to be visual, it could even stem from a different sensory modality. An air puff with the scent of fermenting banana from one side for example prompted the fly to restrict its response to the information available at this side. Following up on the effects of visual cueing, Sareen et al. (2011) found that cue and displacement could be spatially separated by at least 20°, indicating a horizontal width of the FoA of about 40°. Additionally, cueing seemed to be more effective in the lower visual field, a finding that intuitively makes sense, as most of the objects of interest of *Drosophila* (e.g. food, mates, and predators) can be found in the lower visual field during flight. Furthermore, the cue could precede the displacement by up to 2s to still bias the fly's response towards a side.

The present study starts with a close analysis of the yaw-torque modulation in response to a displacement. The data indicate a clear separation of body saccades and responses and reveal two subgroups of responses. Next, examination moves on to the temporal properties of endogenous shifts of the FoA, the attention span. In the course of experiments it becomes obvious that *Drosophila* has an attention span and a first measurement of its duration is achieved. Making use of the shift in response frequencies after cueing, the work then investigates the temporal properties of the after-effect of cueing of covert attention as well as the different qualities of a cue in more detail. To see, if mutant phenotypes in SVA transfer to other behaviors, genetically and pharmacologically treated flies are tested not only while flying stationary but also while walking freely in an arena. Any transfer of phenotypes would strengthen their connection to a basal attentional mechanism. This study follows up on seminal work done by Wolf and Heisenberg (1980) and Sareen (2011) to improve the understanding of SVA in *Drosophila*.

2 Materials and Methods

Angesichts von Hindernissen mag die kürzeste Linie zwischen zwei Punkten die Krumme sein...

Bertolt Brecht, Leben des Galilei

2.1 Flies

Flies were cultured at 25°C on standard medium with 60% relative humidity under 12h light/dark cycle. Wild-type flies were of the CantonS strain and *radish* mutant flies were obtained from Josh Dubnau (Cold Spring Harbor Laboratory, Cold Spring Harbor, USA). *DopEcR* mutant flies were provided by Bertram Gerber (Leibniz Institute for Neurobiology, Magdeburg, Germany), the RNAi stocks were from VDRC (#106961 and #12082) and all *fumin* lines from Kazuhiko Kume (Nagoya University, Nagoya, Japan). For tethering, 2 to 4 days old female flies were anesthetized by cold and glued with dental composite (ESPE Sinfony™, DO3, 3M, Neuss, Germany) to a triangular-shaped holder made of copper wire (Ø = 0.05mm) using a micro-manipulator. The tip of the holder was positioned between the fly's head and thorax to prevent independent motion of the two body parts. The glue was then polymerized using a blue LED light source (10s pulse, < 0.5cm distance) and flies were kept in single vials with access to water for a minimum of 2h. For free walk experiments 2 to 4 days old group housed female flies were transferred from a food vial into the arena in the tip of a glass pipette.

2.2 Pharmacological treatment

2 days old rsh^1 or CantonS flies were put for 14h on 10ml of regular food that additionally contained 5mg of Methylphenidate hydrochloride (Sigma). To inhibit dDAT or DopR1 function, 2 days old CantonS flies were put for 14h on 10ml of regular food containing 30mg Desipramine hydrochloride or 1mg (+)-Butaclamol hydrochloride (Sigma), respectively. For a reduction of dopamine levels, freshly hatched CantonS flies were put on 20ml of regular food with 8mg α-Methyl-DL-tyrosine (Sigma) for 120h and to manipulate serotonin levels, rsh^1 flies were kept for 14h on regular food containing 10mg of Fluoxetine hydrochloride (Sigma). Uptake of food was verified by the addition of a non-hazardous blue dye, which stained the abdomen of the flies. To ablate the mushroom bodies, CantonS flies were treated with Hydroxyurea (Sigma, De Belle and Heisenberg, 1994). Prior to testing all flies were prepared as described above.

2.3 HPLC

3 days old female flies that were scheduled for measurement of dopamine and serotonin levels were sacrificed in a freezer at -18°C and after a couple of minutes transferred to liquid nitrogen for a few seconds. The frozen flies were then vortexed in order to separate the heads from the bodies and 20 heads were put into a small plastic vial (Eppendorf, 1.5ml) and stored in a freezer at -80°C until testing. To determine the levels of dopamine and serotonin in the heads, a HPLC was performed at the Department of Botany I of the University of Wuerzburg.

2.4 Setup

2.4.1 Light-guide arena

The fly was attached to the torque-meter and centered in a cylindrical arena (Ø = 90mm, h = 90mm). The inner surface of the arena incorporated the ends of 32 x 180 single light-guides, which connected it to a rectangular front plate that was penetrated by the other ends of the light-guides (see also Figure 1). The arrangement of connections conserved position information of the images that were projected onto the front plate. Thus, any visual stimuli shown on the front plate were transferred to the inner cylindrical surface and covered a 360° x ±45° panorama, which surrounded the fly. The apparatus was located in a dark chamber and its floor was covered with black cardboard to shield it from outside light. The fly was attached via a wire-hook and clamp to the torque-meter and centered in the arena (for further details: Wolf and Heisenberg, 1991). Position, timing and geometrical properties of the visual stimuli were controlled and updated at 300Hz using self-written software (VB.NET) and projected onto the device by a projector (120Hz, BenQ W770ST). A torque-meter was used to measure the generated yaw-torque and the values were stored on the controlling computer's hard disk at 100Hz. Experiments were performed under open-loop conditions, i.e. giving the fly no visual feedback of its generated yaw-torque.

2.4.2 Free walk arena

To investigate walking behavior, flies were walking freely on a sheet of white paper within a petri-dish arena (Ø = 86mm, h = 4mm). This arena was located on the bottom and in the center of an opaque acrylic glass cylinder (Ø = 125mm, h = 130mm), which had a black stripe in the center of every quadrant (h =130 mm, w = 15mm). Both, arena and cylinder were surrounded by another non-transparent black cylinder (Ø = 295mm, h = 240mm) to shield external stimuli (see also Figure 20). Experiments were performed in a dark room with the only source of light being three horizontally running rows of LEDs (Flex Strip, Synergy 21, Germering, Germany), which were attached to the inner surface of the outmost cylinder. The inner cylinder served as a diffusor, so that from the fly's perspective - with exception of the open ceiling - the panorama was homogenously lit. The movements of the fly were tracked and stored on a hard disk at 40Hz by self-written software (VB.NET) and a standard USB webcam (Logitech, C500). The position coordinates were post hoc analyzed using self-written software (VB.NET).

2.5 Stimulus conditions

2.5.1 Cueing experiments

Two black 18° wide stripes were presented on a white background, centered at $\psi_0 = \pm 45°$ in the fronto-lateral visual field of the fly. The stripes were displaced from front to back by $\Delta\psi = 30°$ at 150°/s and then slowly reset to their initial position at 20°/s. The inter-trial interval was set to 2s and prior to each displacement a cue, followed by a post cue pause (PCP) was added. The cue consisted of a 1s long 10Hz

oscillation of one of the stripes ($\Delta\psi = \pm 7.5°$) and thereafter the stripes remained stationary for the duration of the PCP. 6 different sets of PCP*s* (0, 1, 2, 3, 4 and 5s) were used in most of the experiments. A single set included 6 displacements of which for 3 consecutive displacements the left stripe and for the other 3 the right stripe was cued. The order of sets as well as the order of the cued sides within each set was randomized. For example, a test could have the following sequence: 1s wiggling of the left stripe (cueing), followed by 3s (PCP) during which the stripes remained stationary. Then a fast front-to-back displacement of both stripes and a slow resetting to the initial positions, after which the stripes remained stationary again for 2s (ITI). In experiments with a single stripe, the stripes were controlled by the same protocol, but only one stripe was shown in the panorama. The same protocol was also used in experiments, where only one of two stripes was displaced. Here, the only difference was that throughout the experiment one of the two stripes remained visible but stationary during the phase of displacement and resetting. To separately look at the effects of cueing in the lower and upper visual half field and to prevent overlap a gap (width = 20°) was inserted into both stripes at the level of the equator. This resulted in four stripes. Then one stripe in the LVF or UVF was cued and all four were displaced. In all experiments, a response was scored when yaw-torque modulation exceeded the range between maximum and minimum yaw-torque values recorded within 0.5s prior to displacement by more than 60% within 0.5s after onset of the displacement. Left (counterclockwise; ccw) and right (clockwise; cw) responses as seen from the position of the fly were scored separately. If no sufficiently large yaw-torque modulation was detected, a no response (nr) was scored.

2.5.2 Attention span experiments

Again, two black 18° wide stripes were presented on a white background and were displaced with the same parameters as described above, except the cueing. Each experiment consisted of a series of 60 displacements. During the inter-trial interval (ITI) the stripes remained stationary at the ψ_0 position for 1s or in some experiments for 3s or 4s or 5s. In most of the cases flies responded to the front-to-back motion with a phasic yaw-torque modulation, which during free flight would have caused a turn of the fly in the same direction as the movement of the stripe. Response detection was performed as described for the cueing experiments.

2.5.3 Free walk experiments

A black stripe was presented to the flies in every quadrant on a homogenously lit background. Because the cylinders were open on the upper end, from the inside a weakly illuminated circular section of the white ceiling and the grey webcam were visible to the fly. Single flies were put into the arena at room temperature and then tracked for 300s.

2.6 Data evaluation

2.6.1 Cueing experiments

To quantify the effect of cueing, a response index (RI) was used to show the distribution of responses towards the cued and the not cued stripe. It was calculated as (rf_{cued} - rf_{uncued}) / (rf_{cued} + rf_{uncued}) so that an equal number of responses towards and away from the cued stripe yielded a RI of 0. For every fly a separate RI was computed for each of the 6 different sets of PCPs. All single fly RIs were then averaged PCP-wise. The wild-type data consistently showed a tripartite pattern. The highest RI was found for PCP 0s, whereas for PCP 4s and 5s the RI was not significantly different from zero. PCP 1s through 3s showed only slightly decreasing values, which were significantly different from zero. Thus, for averaging, the data were grouped as PCP 0s (immediate cueing effect, ICE) and PCPs 1s, 2s and 3s (sustained cueing effect, SCE). In the experiments investigating the consequences of a reduced cueing duration or the efficacy of cueing in the upper or lower visual field, the short sustained cueing effect (sSCE) included only PCP 1s.

2.6.2 Attention span experiments

The experimentally amenable measure of a dwelling of the focus of attention (FoA) at a particular location in this paradigm was the length of chains of consecutive identical responses. Besides dwelling there is another mechanism that could influence the same parameter – sidedness. To see, if flies dwelled with their FoA at a particular location and to understand the corresponding properties, dwelling needed to be distinguished from sidedness. A side-preference in flies can be internally and/or externally caused. To minimize external influences flies have to be precisely aligned to the visual stimuli. Therefore, the center line of the arena was marked during mounting with a red laser beam and flies were thoroughly aligned to it. To measure the attention span, internal sidedness, i.e. the difference of the left and right mean response frequencies (rf_{ccw}; rf_{cw}) needed to be small. To clear the dataset of internally side-biased flies, some flies were excluded from evaluation. Three criteria were used: (1) An asymmetry index (AI) was calculated for each fly as AI = $|rf_{ccw}-rf_{cw}|$ / ($rf_{ccw}+rf_{cw}$). Flies with AI > 0.3 were not used for further evaluation. (2) The mean chain length of consecutive identical responses (cl_{ccw}; cl_{cw}) was derived from the data separately. Only flies with a small absolute difference (AD_{cl}) between both values were used (AD_{cl} = $|cl_{ccw} - cl_{cw}|$; $AD_{cl} < 0.6$). (3) Finally, flies with a low overall response rate (RR < 0.6) were also excluded from evaluation. These filtering steps yielded flies with a balanced number of rf_{ccw} and rf_{cw}, with the pooled response frequencies still matching those of the unfiltered data-set. To detect dwelling the fly data was compared to 100 simulated datasets, each consisting of 1000 repetitions computed with a certain dwelling factor (df, ranging from 1 (≙ no dwelling) to 2 with an increment of 0.01). Comparisons were carried out separately for both response polarities (ccw and cw) and the df for each fly was then calculated as the mean of the df_{ccw} and df_{cw} that each resulted in the best fit of simulated and fly data using the Gaussian least squares method. Remaining sidedness, by definition could only affect df_{ccw} or df_{cw} by

increasing the average chain length on that side. But at the same time the length of chains on the other side would be reduced by the same factor. Thus, the applied averaging of df_{ccw} and df_{cw} removed sidedness, leaving only the effects of dwelling.

2.6.3 Free walk experiments

For a detailed analysis of walking behavior of several fly strains, 6 parameters that could be derived from the stored XY-coordinates were chosen for evaluation. A fly was regarded as active whenever it walked for more than 4mm within 1s and activity was scored as the percentage of the overall experimental time. Only during those phases of activity the velocity [mm/s] of a fly was calculated. Furthermore, the total covered distance [mm] was extracted from the data as well as the number and the average duration [s] of idle events (pauses). An idle event was defined as a phase of inactivity that lasted for more than 1s. Finally, the percentage of total time spent in an outer rim (w = radius * 0.2) was calculated to check for potential centrophobism or wall following behavior.

2.6.4 Statistical analysis

All data were tested for normal distribution using a Kolmogorov-Smirnov test. If data were normally distributed, a one-sample t test was used to compare values with a random value and a two-sample t test was used to compare values with each other. Bonferroni corrections were used for multiple comparisons. If no normal distribution could be assumed, either a Wilcoxon Matched Pairs test for dependent pairwise comparisons or a Mann-Whitney test was used to test two groups against each other. Because not all data of the RIs of the cueing experiments were normally distributed, for all RIs a Wilcoxon-Signed-Rank test was used to compare values with zero and for the same reason a Mann-Whitney test was used to compare groups against each other in all free walk experiments. Comparison of more than two values was achieved by a one-way ANOVA with Holm-Sidak's multiple comparisons test, if the data were normally distributed and otherwise with a Kruskal-Wallis test with Dunn's test for multiple comparisons (* = $p < 0.05$, ** = $p < 0.01$, or *** = $p < 0.001$).

3 Results

Opposites are complementary.

Niels Bohr

3.1 Measuring the attention span

Earlier work (Sareen et al., 2011) has provided evidence, that the FoA of *Drosophila* can be cued and externally guided. This is in line with findings of so called exogenous cueing of attention in humans. However, there are also endogenous shifts of attention without external stimuli, representing an internally driven relocation of the FoA. While the cueing experiments were an example of bottom-up control of attention, in the following chapter this study focuses on top-down modulation of attention and investigates the temporal properties of internally driven shifts of attention.

3.1.1 Single stripe displacements elicit three types of response patterns

The yaw-torque response of a fly in the center of the light-guide arena to a front-to-back displacement of a black vertical stripe (height = 90°; width = 18°; azimuth: ψ_0 = + or − 45°; $\Delta\psi$ = 30° at a velocity of v = 150°/s; Figure 1A) was categorized by response polarity: Sometimes there was no detectable response to the motion at all (nr), but most often the fly produced a yaw-torque spike with the same polarity as the motion stimulus (Figure 1B, 'Single', syn-directional). In free flight or in the flight simulator (artificial closed-loop, Heisenberg and Wolf, 1979) this response would have brought the stripe back to about its initial position and would have corrected for the disturbance in flight direction. Remarkably, the fly sometimes also responded with a spike with opposite polarity (Figure 1B, 'Single', anti-directional). The phasic yaw-torque response of these anti-directional responses was shorter and also the latency was shorter (Figure 1C and D, 'Single'). Whereas in free flight the syn-directional responses would serve to maintain a certain flight direction, the anti-directional ones might be attempts to escape the attack of a predator.

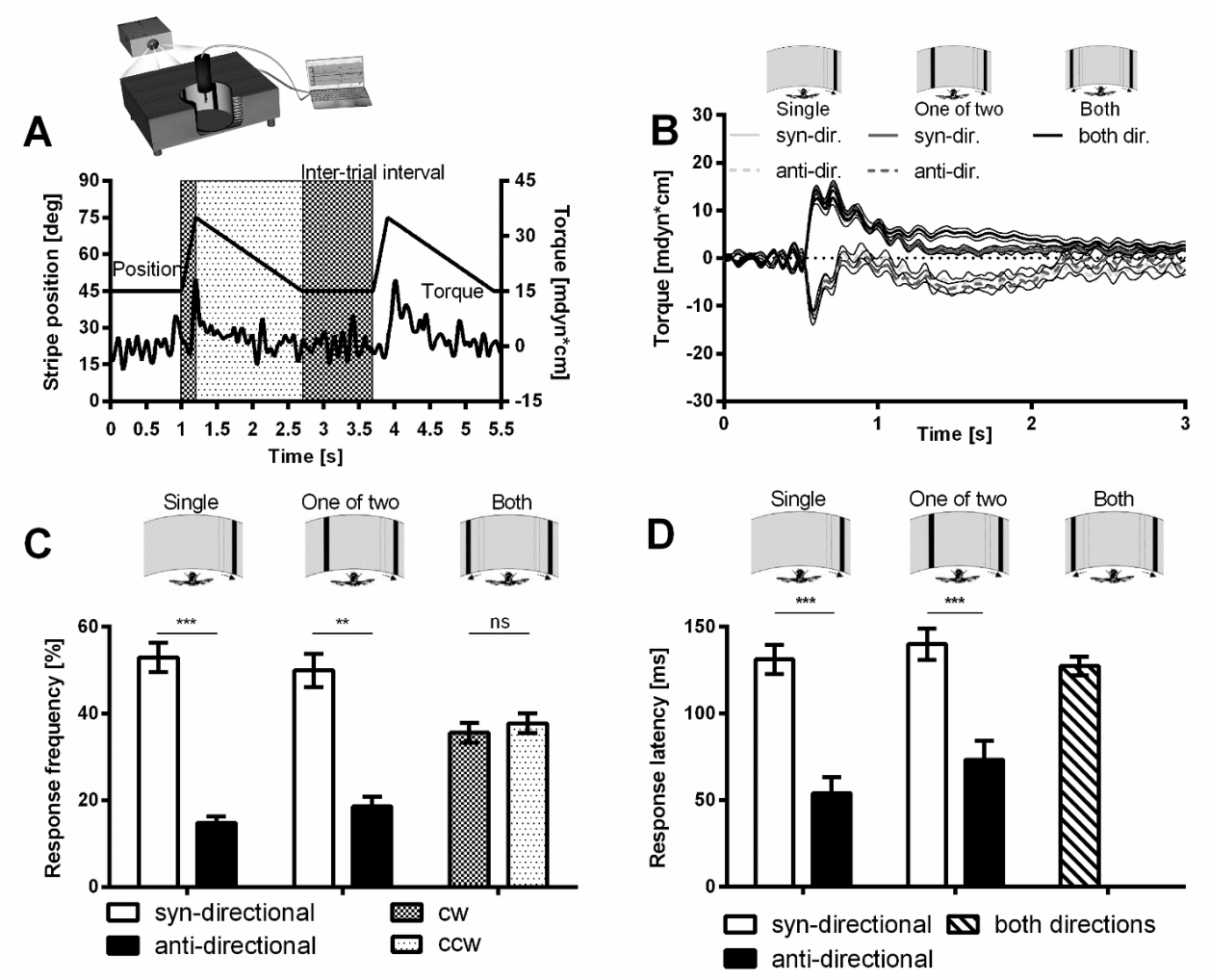

Figure 1: Characterization of yaw-torque responses to the displacement of one or two stripes. (A) Example trace of yaw-torque responses. The stripe is displaced with an inter-trial interval (ITI) of 1s. The fly responds to the fast displacements with a strong phasic modulation of yaw-torque. (B) Average yaw-torque responses to the displacement under three different experimental conditions. Responses to a single stripe ('Single', N = 21) can be syn-directional or anti-directional. Responses are very similar, if a second, stationary stripe is added ('One of two', N = 20). When both stripes are displaced at the same time, the responses look much like the syn-directional responses to a single stripe ('Both', N = 21). (C) Response frequencies. The majority of responses is syn-directional to the stripe. No such differentiation can be made in the 'Both' condition. (D) Response latencies. Anti-directional responses are elicited faster than the syn-directional ones. The responses in the 'Both' condition have the latencies of the syn-directional responses to a single stripe. All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

After the fast front-to-back displacement the stripe was slowly (v = 20°/s) reset to its initial position. After a syn-directional response the yaw-torque returned to the level from which it had started during the 1.5s of that phase. Anti-directional responses had a slightly smaller amplitude in the first phase of the response (Figure 1B, 'Single', anti-directional) and afterwards the fly generated a weak syn-directional response to the slow front-to-back motion. Its yaw-torque returned to base line only after the motion had stopped. If the fly was confronted with two stripes at ψ_0 = + and - 45° and only one of them was displaced, the responses were similar to the responses with a single stripe (Figure 1B, 'One of two'). The data favored the interpretation of the fast anti-directional responses as a putative attempt of the fly to evade the attack of a predator and of syn-directional ones as a contribution to stabilization of its orientation in space.

Yaw-torque spikes were not exclusively produced in response to a displacement. Occasionally, a fly generated spontaneous body saccades while the stripes were stationary. However, the corresponding yaw-torque spikes could easily be distinguished from syn-directional responses by their smaller amplitude and slope, which made them more similar to anti-directional responses (Figure 2). Their dynamics differed between experiments with a single stripe and the 'One of two' condition, indicating that they were influenced by other stimulus parameters besides visual motion.

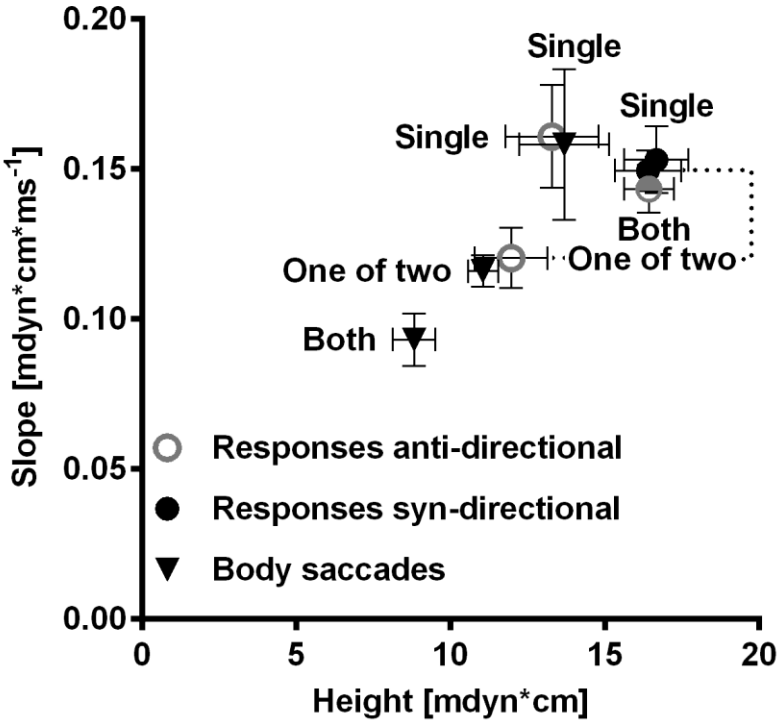

Figure 2: Height and slope of yaw-torque responses and body saccades during ITIs. For the conditions 'Single' (N = 21) and 'One of two' (N = 20) the data are split into 'syn' and 'anti' with regard to the response polarity. No such differentiation can be made for the 'Both' (N = 20) condition. These responses have a similar shape as the syn-directional ones generated when only one stripe is displaced. The body saccades differ in all three experimental conditions, but resemble the respective anti-directional responses. All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

3.1.2 Simultaneous displacement of two stripes

In the experiments with only one stripe getting displaced, there was no necessity for the fly to make use of SVA or at least the observed behavior could be explained without the employment of SVA. To characterize SVA, a situation of two identical competing visual stimuli was created, where two stripes were simultaneously displaced front-to-back, one at ψ_0 = +45°, the other at ψ_0 = -45° in front of the fly and then slowly shifted back to their initial positions. As shown before the responses to each of the two stripes alone were in majority syn-directional and thus incompatible with each other. The responses of the fly to the simultaneous displacement had a longer latency (Figure 1D, 'Both') and longer duration as well as a larger amplitude (Figure 1B, 'Both'), just like the syn-directional responses described above. Based on the response latency, the quick putative escape responses did not occur under these conditions. Because both

stripes were displaced simultaneously, it might have been expected that the total response frequencies added up to the sum of the response frequencies of the two single-stripe experiments. Alternatively, the response frequency might have also been expected to be zero, because the two stripes moved in opposite directions, yielding zero vector sum of the movements in the visual panorama. The data proved both assumptions to be wrong. With two simultaneously present motion stimuli the fly most often selected one of both to respond to. The overall frequency was only slightly higher and the response frequency for each stripe was lower than the frequency of syn-directional responses in the single-stripe experiments (Figure 1C, 'Both'). Thus, as a matter of fact the frequency of responses to the single stripes was reduced by the simultaneous displacement of the second stripe. Instead of SVA, two mutually inhibiting central pattern generators (CPGs) for cw and ccw turns would suffice to explain this suppression. This idea can be rejected however, because the ability to cue the FoA described in this study and in Sareen et al. (2011) favors SVA over mutual inhibition. The fact that the fly selected one out of two equally salient stimuli, which each required characteristic but incompatible responses allowed to measure a property of internally modulated SVA - the attention span. Defined as the time the FoA remained at the location to which it had been shifted, an attention span would add to the list of similarities to human attention and strengthen the concept of SVA in the fly. If the choice of one side had an after-effect biasing the choice in the next test, also another point against mutual inhibition could be made. An attention span would not be expected for a model based on mutual inhibition.

3.1.3 Is the choice of response polarity influenced by the previous choice?

The two stripes were displaced for 60 times with an inter-trial interval (ITI) of 1s. From this data, chains of consecutive responses with the same polarity (chains, e.g. cw-cw-cw…) were extracted (Figure 3).

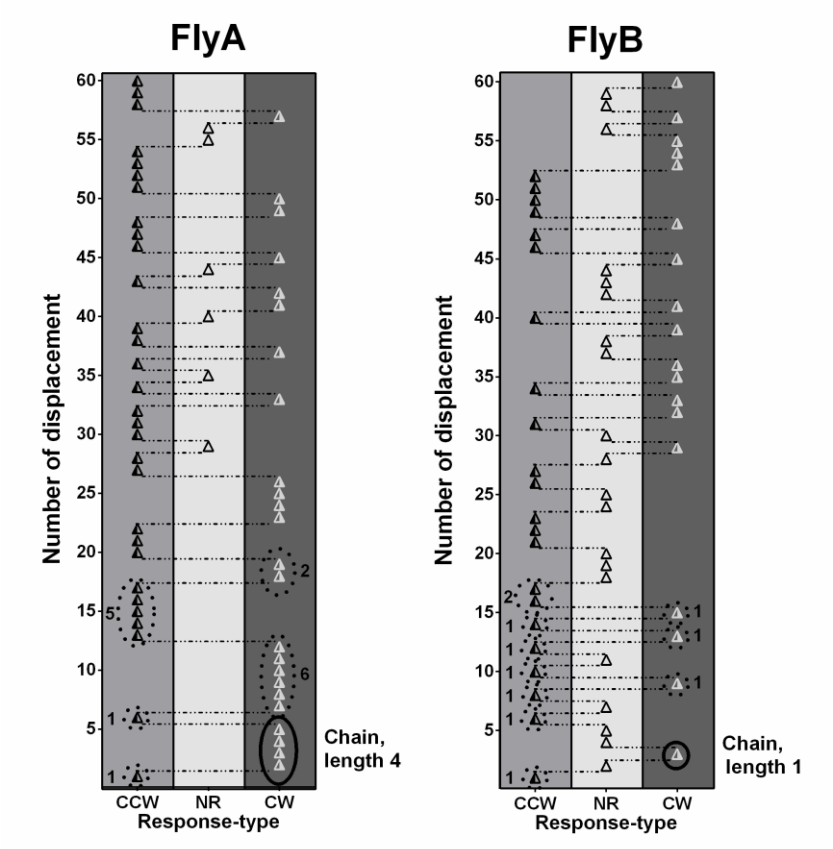

Figure 3: Examples of chains within the responses to 60 displacements of two stripes. Chains are defined as consecutive identical responses towards one side during which the fly's FoA is assumed to be on that side. FlyA frequently produces long chains, while FlyB often switches between the response types.

A fly with a response polarity that was constant over time produced long chains and a fly that frequently changed its response polarity was characterized by short chains. With increasing length the frequency of chains decreased (Figure 4A, 'Fly-data'), yielding an average chain length (CL) of 2.14 ±0.12 (Figure 4B). Following a model of mutually inhibiting CPGs, the response polarity would be expected to be exclusively determined by chance. If this was the case the distribution of CLs generated by the flies should be reproducible by a simulation of responses, based on the observed mean frequencies of cw, ccw or nr (rf_{ccw} = 0.33; rf_{cw} = 0.33; rf_{nr} = 0.33; Figure 4C). But neither the CL frequency nor the resulting average CL (CL = 1.49 ±0.01) of the calculated data did match fly data (Figure 4A 'Fly-data' and 'Simulation'). Thus, random selection of response polarity is not the way the choice of response type is realized in the fly. Instead it seemed to follow a mechanism that favors the formation of chains.

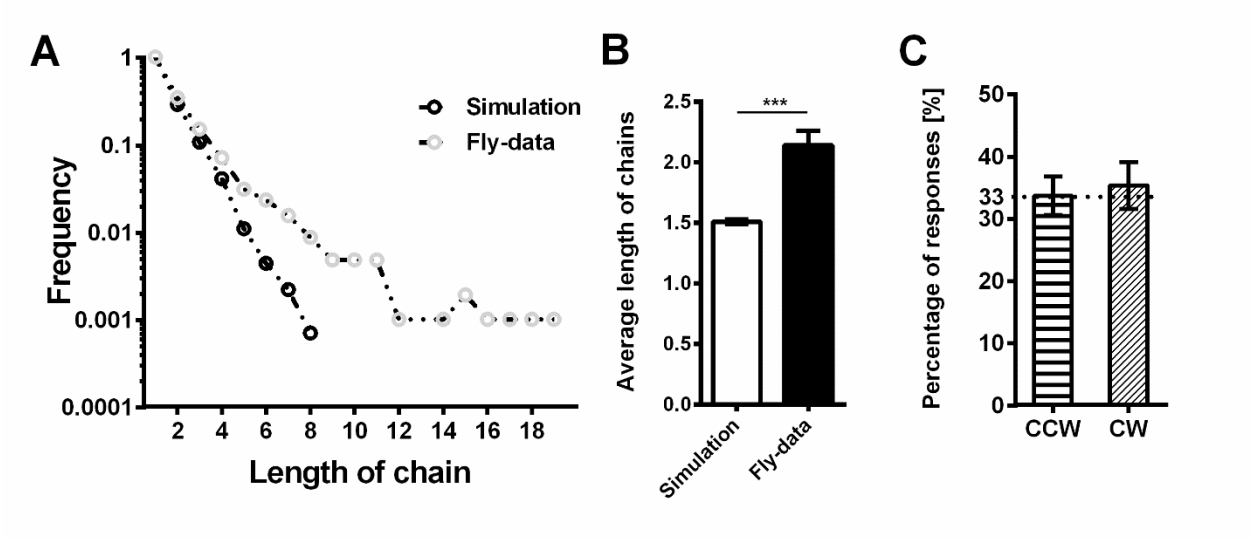

Figure 4: Chain lengths and response frequencies. Chains are consecutive identical responses. (A) Frequency of chain lengths. The mean chain length frequencies calculated from the mean response frequencies shown in (C) assuming random choices and the one of fly data differ. (B) Also the average length of chains differs for these two data-sets (N = 76, 76). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

3.1.4 Different mechanisms affect chain length

There are at least two basic mechanisms that both would lead to the formation of longer chains. In an attention span model, each response would be influenced by the preceding one, favoring an identical response by increasing its likelihood by a certain factor (dwelling factor, df). Alternatively, each fly could have an individual preference for responses towards one side (sidedness). Such a preference might be either endogenous to the fly or caused by external biases in the setup. In this paradigm, flies were very susceptible to external perturbations and already a small deviation of the longitudinal axis of the fly from the line of symmetry led to a preference for the stripe closer to the midline and resulted in sidedness (Figure 5).

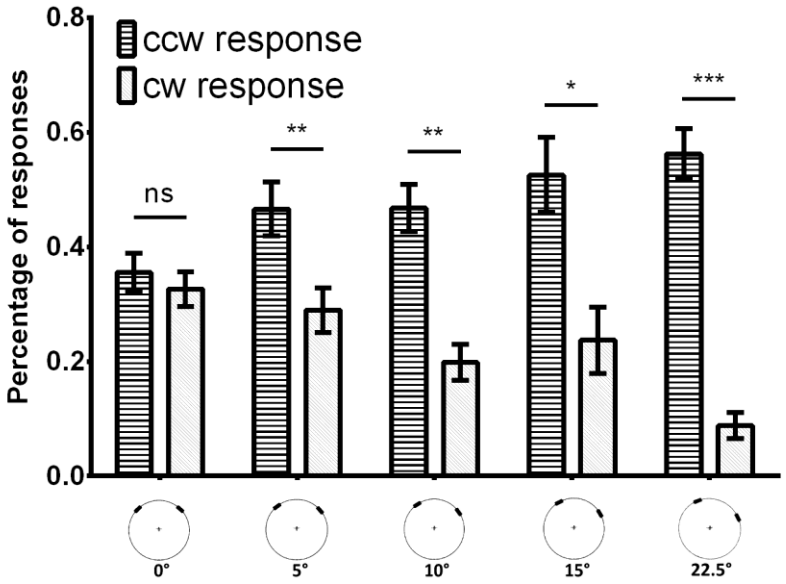

Figure 5: Effects of externally caused sidedness on response frequency. Equally frequent responses to either side are observed, if two stripes are displaced front-to-back from ±45°. If the longitudinal axis of the fly is shifted with regard to the stripes, the fly favors the stripe that is more in front (N = 72, 21, 20, 13, 18). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

A comparable influence of the azimuth of the stripe on the response frequencies had already become apparent in the work of Sareen (2011). To assess the attention span, sidedness needed to be controlled. Fortunately, within the series of 60 displacements sidedness could be calculated for each fly separately measuring the overall difference between cw and ccw responses (see Materials and Methods). With the main focus on understanding the dynamics of the changes in response polarity, flies with a strong sidedness were removed from the evaluation (see Materials and Methods). For the remaining flies a dwelling factor (df) that eliminated the influence of individual response asymmetries was calculated as the mean of the dwelling factors for cw and ccw responses. In contrast to responses, spontaneous body saccades [Heisenberg and Wolf, (1984); Wolf and Heisenberg, (1980)], which occurred while the stripes were not moving were not subject to sidedness. The ratio of response polarities was significantly altered after sidedness removal in the data, but a similar effect was not seen for body saccades, suggesting two different underlying behavioral patterns (Figure 6).

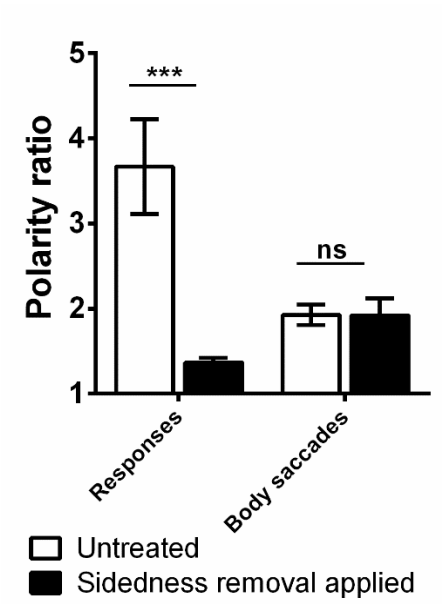

Figure 6: Polarity ratio of responses and body saccades. For each fly a polarity ratio is calculated as the number of responses of the more frequent polarity divided by the number of responses to the other side. The polarity ratio of responses (N = 76) gets close to 1 after sidedness removal (N = 21), but that of body saccades is not susceptible to the procedure. Error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

3.1.5 Duration of the attention span

A mean dwelling factor df = 1.36 was detected after removal of sidedness from further evaluation for CantonS wild-type. In other words, there was a by 36% increased probability of a repetition of response polarity as compared to the initial value (eg. after a response towards the left stripe the initial probabilities $p_{ccw} = p_{cw} = 0.33$ were set to $p_{ccw} = 0.448$ and $p_{cw} = 0.276$ for the next displacement). This tendency of the fly to repeat the previous response favored the formation of long chains and could thus explain the higher frequency of long chains in the fly data. Using the fly data corrected for sidedness for simulation of chain length distribution minimized the difference between this simulation and the both, observed chain length distribution and observed average chain length (Figure 7A and B, 'Fly-data' and 'Simulation df = 1.36'). Based on the findings of Sareen et al. (2011) and of this study one can assume that the fly had its FoA at the side it responded to. Thus, df > 1 did not only imply the tendency of the fly to repeat its previous response, but also indicated a prolonged dwelling of the FoA on the side to which it had been shifted. This in turn implies that SVA in flies has an attention span.

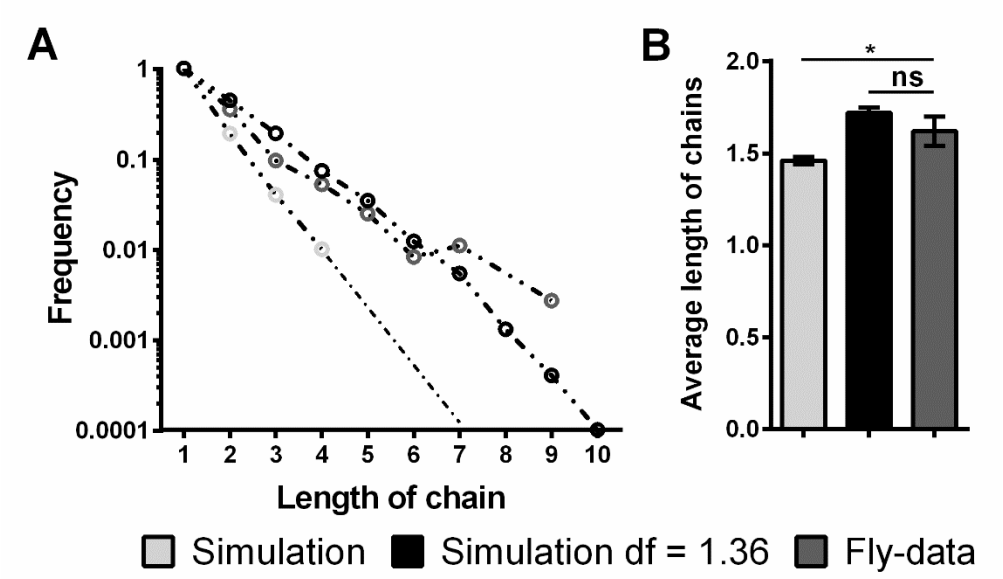

Figure 7: Chain lengths after removal of sidedness. (A) Frequencies of chain lengths. After removal of sidedness the calculated chain lengths ('Simulation') still differ from the measured ones ('Fly-data', N = 21). Introducing a dwelling factor (df = 1.36) gives the best fit between calculated and observed chain length frequency distributions. (B) Average chain lengths. Calculation after a simulation with a dwelling factor removes the significant difference to fly data. Error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

The df itself is not a measure of time and refers to the number of identical consecutive responses. But it can also be used to infer information about time because the inter-trial interval is known. Evidently, to be of adaptive value the FoA should not dwell on one side persistently. To determine the dwelling time of the FoA the ITI was prolonged iteratively and for each condition the df was computed. The longer intermissions had no substantial effect on the dynamics and frequencies of the responses (data not shown).

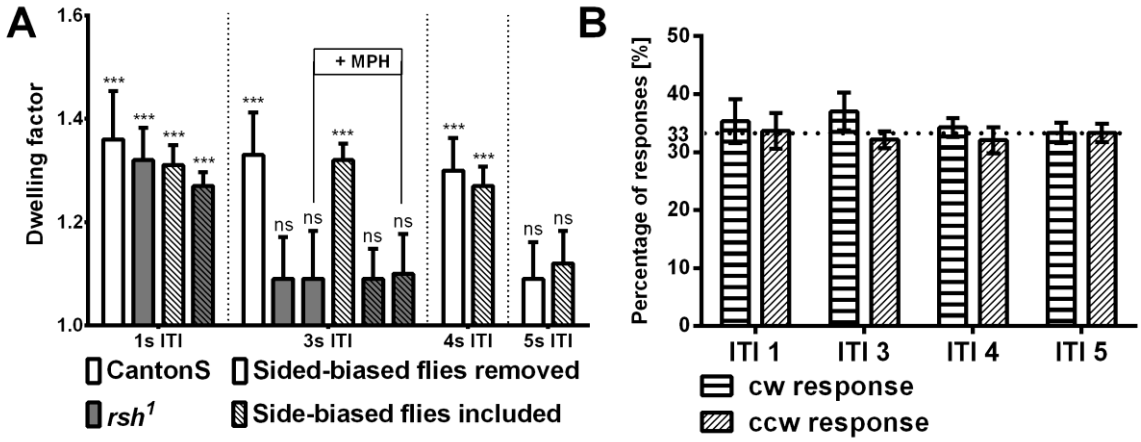

Figure 8: Temporal limitation of dwelling. (A) When the ITI is prolonged, the calculated dwelling factor does not decrease significantly during the first 4s in CantonS flies. However, the attention span lasts less than 5s (N = 21, 26, 26, 27). In *rsh*[1] flies dwelling can be detected for ITI 1s, but the attention span lasts less than 3s (N = 35, 21). The same results are obtained, if the sidedness removal procedure is not applied to the data. (B) Response polarity distributions. CantonS flies produce cw and ccw responses equally often with all tested ITIs. Different behavior of the batches of flies is thus unlikely to explain the observed differences (N = 76, 93, 76, 69). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

A non-significant trend of a decreasing df was found for ITI*s* of 1, 3 and 4s (Figure 8A, df_{1s} = 1.36; df_{4s} = 1.30). For ITI 5s a significantly lower dwelling factor was observed (df_{5s} = 1.09). Apparently, the FoA did dwell on one side for about 4s, because at ITI 5s the effect of dwelling waned and the probabilities of the response polarities returned to the initial level. Thus, the attention span of SVA amounted to between 4 and 5s under the conditions of this experiment. These results were not a consequence of the filtering steps applied to minimize the effects of externally caused sidedness. Without selection for flies with a minimum of sidedness, the same results were found. Also the response distribution and overall response frequencies were the same for all tested sets of flies, which excluded poor flight behavior of the flies tested with ITI 5s as an explanation for the observed reduction of the df (Figure 8B).

3.1.6 Attention span in *radish* mutant flies

Flies with a mutation in the *radish* gene showed attention-like deficits in stationary flight (van Swinderen and Brembs, 2010) and also mutant phenotypes in SVA and flight behavior in the scope of this study (e.g. Figure 32, lack of cued sustained shifts of attention and Figure 9C and Figure 31A, limited yaw-torque modifications). Hence it was interesting to see, whether this mutation also led to a mutant phenotype in the present paradigm, which employed a top-down modulation of attention. *rsh*[1] flies had no substantial

differences in response frequencies and dynamics in comparison to CantonS flies (Figure 9A and B), which made them suitable for the same kind of evaluation used previously.

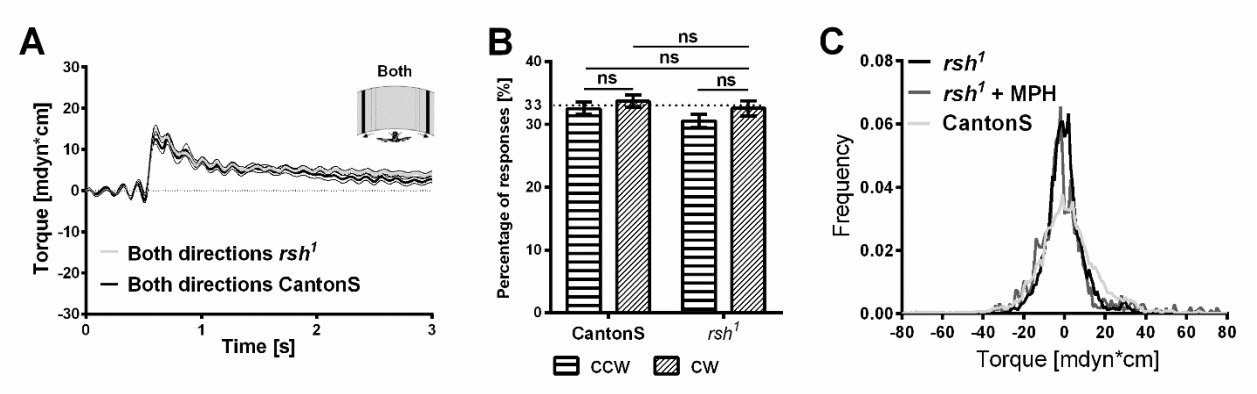

Figure 9: Characterization of *rsh*[1] flight behavior. (A) Average yaw-torque traces with two stripes being displaced simultaneously. No difference between CantonS and *rsh*[1] flies is found (N = 21, 35). (B) Response frequencies. CantonS and *rsh*[1] flies choose each response polarity equally often (N = 100, 56). (C) Yaw-torque histograms during 1s between test trials. Yaw-torque modulations are slightly reduced in *rsh*[1] flies as compared to CantonS flies. This reduction remains unaltered by MPH treatment of *rsh*[1] flies (N = 16). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

At ITI 1s no substantial difference to wild-type was observed (df = 1.32). Again, an incremental increase of the ITI was used to determine the dwelling time. Already at ITI 3s *rsh*[1] flies showed no more significant dwelling of the FoA (df = 1.09; Figure 8A). In their study, van Swinderen and Brembs (2010) discussed hyperactivity, manifesting also in altered yaw-torque behavior, as a possible reason for the defects they observed in the mutant and tried to connect the observed phenotype to attention-deficit and hyperactivity disorder (ADHD) in humans. They supported their claim by showing that methylphenidate ('Ritalin'), a drug used to treat ADHD in humans also rescued some of the defects in *rsh*[1] flies. However, MPH did not revert the attention span of *rsh*[1] flies to that of wild-type CantonS (Figure 8A). It was furthermore not possible to draw a direct connection of this reduced attention span phenotype to hyperactivity with regard to yaw-torque. Similar to data of *rsh*[1] flies in the cueing paradigm (see Figure 31A), the width of the yaw-torque modulation histogram was in fact narrower compared to wild-type (Figure 9C). These results argue that the *rsh*[1] attention defects found by van Swinderen and Brembs (2010) and defects of *rsh*[1] flies in cued shifts of attention could rely on the same mechanism, whereas the *rsh*[1] phenotype in the attention span of endogenously modulated SVA is most likely based on a different mechanism. As a consequence, they also indicate a substantial difference between the exogenously and endogenously modulated shifts of attention and a different involvement of the *radish* gene in the two.

3.1.7 The focus of attention is shifted independent of yaw-torque

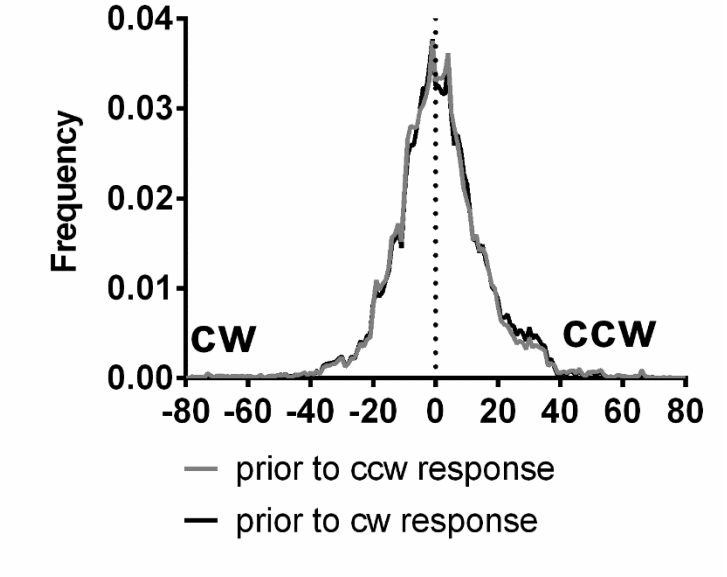

Figure 10: Yaw-torque does not influence response polarity. A histogram of CantonS yaw-torque generated during 1s between the test trials is split into 'cw' and 'ccw' according to the subsequent response polarity. No differences can be found (N = 100).

To exclude the possibility that activation of a CPG for flight-direction also led to the corresponding response polarity, it was necessary to ensure that yaw-torque levels prior to the displacement and response polarity were not depending on each other. The yaw-torque generated by the flies during 1s before the onset of a displacement, categorized with regard to the subsequent response polarity ('cw' or 'ccw'), was almost identical in both categories (Figure 10). Hence, in this paradigm the flies were able to shift their FoA independently of yaw-torque and the dynamics measured in *rsh*^{1} and CantonS flies were reflecting the dynamics of the FoA instead of CPGs for cw and ccw rotation.

3.1.8 Beyond dynamics: What makes the FoA dwell on one side?

Much like for mammalian covert attention, where reaction times towards a target in a recently examined area were increased and those towards a target at a new location were decreased [Klein, (1988); Posner and Cohen, (1984); Tipper et al., (1991)], here the FoA might not have been sticking to one side, but rather have been repelled by the other side (Inhibition of Return; IoR).

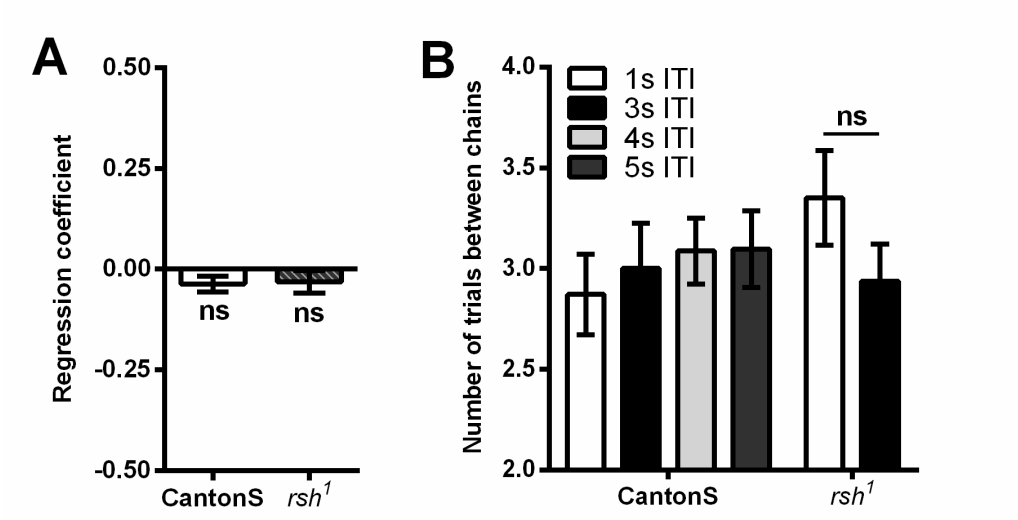

Figure 11: Inhibition of return does not explain the attention span. (A) Neither in CantonS nor in *rsh*[1] flies a correlation is found between chain length on one side and subsequent number of responses to the other side and no responses (N = 21, 35). (B) After a chain on one side, the number of displacements it takes a fly to return with its response polarity to that side is independent of the duration of the ITI (N = 21, 26, 26, 27, 35, 21). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

Two tests were performed to evaluate the data for possible indications of IoR. The first test assumed a gradual build-up of inhibition at the location of dwelling. This would manifest in a positive correlation of CL (e.g. cw) and the number of test trials (ccw + nr) until the next chain on the same side (cw) would be initiated. Because this correlation was neither found in CantonS nor in *rsh*[1] flies (Figure 11A), the first test rejected the hypothesis of IoR as the cause for the observed dynamics. To strengthen this finding, a second test was performed, which assumed a constant duration of IoR. The duration of putative IoR in the data was derived in two steps. First, the number of test trials that occurred between chains on the same side was obtained. It turned out to be around 3 for all tested ITIs (Figure 11B). Second, this number was converted to time ($3 * [T_{Disp} + T_{ITI}]$). This time should give the duration of a putative IoR effect. However, according to these calculations pauses of 3 test trials would last from 8.1s (ITI = 1s) to up to 20.1s (ITI = 5s), giving no fixed value for IoR. Together, the results of both tests speak against the hypothesis that IoR is part of a mechanism for dwelling.

3.2 Cued shifts of attention

The FoA can be shifted endogenously. If this shift takes place without redirection of gaze, or in the case of a fly of its body axis, it is a shift of so called covert attention (Warren and Warren, 1986). Trapped at the torque-meter, the fly can only express shifts of this form of attention, which can be externally guided by means of a non-visual or visual cue [Heisenberg and Wolf, (1984); Sareen et al., (2011)]. This study investigates the effects of cueing in a situation, which requires SVA as the fly has to choose between two equally salient stimuli which are presented to it at the same time. The focus lies on the after-effect of cueing as well as on the localization of circuits and neurotransmitters involved in SVA in the *Drosophila* brain. Some new observations are opposite to reports of an earlier study on the subject. Their closer investigation helps to complement our understanding of SVA in the fly.

3.2.1 Displacement of a single stripe may elicit different response patterns

To better understand the flies' behavior in the cueing situation, first their responses to the displacement of a single black vertical stripe were analyzed (Figure 12). Besides the presence of a cue the following experiments were performed analog to the experiments described in chapter 3.1.1. The stripe was quickly moved front-to-back (height = 90°; width = 18°; azimuth: ψ_0 = + or − 45°; $\Delta\psi$ = 30° at a velocity of v = 150°/s), after it had oscillated prior to the displacement in half of the cases (cueing; $\Delta\psi_{cue}$ = 15°, f_{cue} = 10Hz and dur_{cue} = 1s).

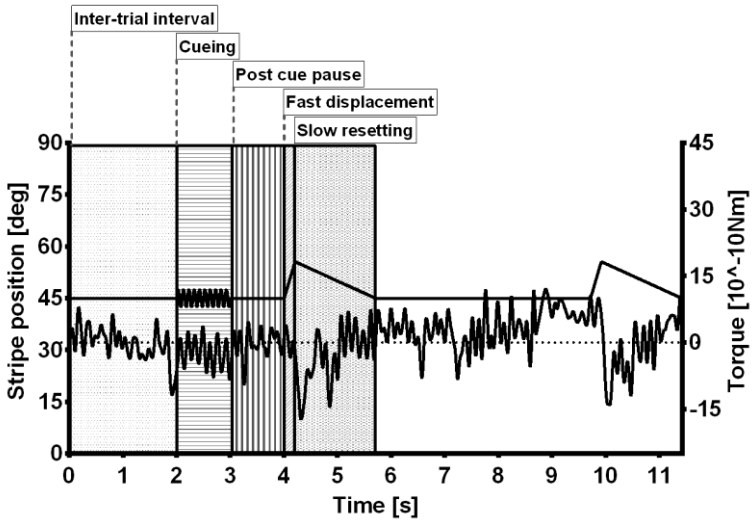

Figure 12: Example trace of yaw-torque responses in the experimental setup. The fly is attached to a torque-meter and centered in a light-guide arena (see Materials and Methods). A single black vertical stripe is presented on a white background at the azimuthal position ψ = 45°. After 2s it is oscillated for 1s (cueing; $\Delta\psi$ = 15°, f_{cue} = 10Hz). It then remains stationary for 1s (post cue pause, PCP), before it gets displaced from front to back ($\Delta\psi$ = 30°, v = 150°/s) and then slowly reset to its initial position (v = 20°/s). It follows an inter-trial interval (ITI), in this case without cueing.

The yaw-torque responses of a fly in the center of the light-guide arena were recorded and could be categorized into three groups: Sometimes there was no detectable response to the motion at all (nr), but most often the fly produced a yaw-torque spike with the same polarity as the motion stimulus (Figure 13A, 'Single', syn-directional). Such a response would have under natural feedback conditions (closed-loop, Heisenberg and Wolf, 1979) counter-balanced the motion of the stripe. Occasionally, the fly generated a yaw-torque spike of opposite polarity in response to the displacement (Figure 13A, 'Single', anti-directional), but these responses happened only rarely (Figure 13C, 'Single', 'No cue' and 'Stripe cued'). However, their latency was only about half of the latency of the syn-directional ones (Figure 13B, 'Single'). The probability of the different response types did not differ with regard to the presence or absence of a preceding cue (Figure 13C, 'Single', 'No cue' and 'Stripe cued').

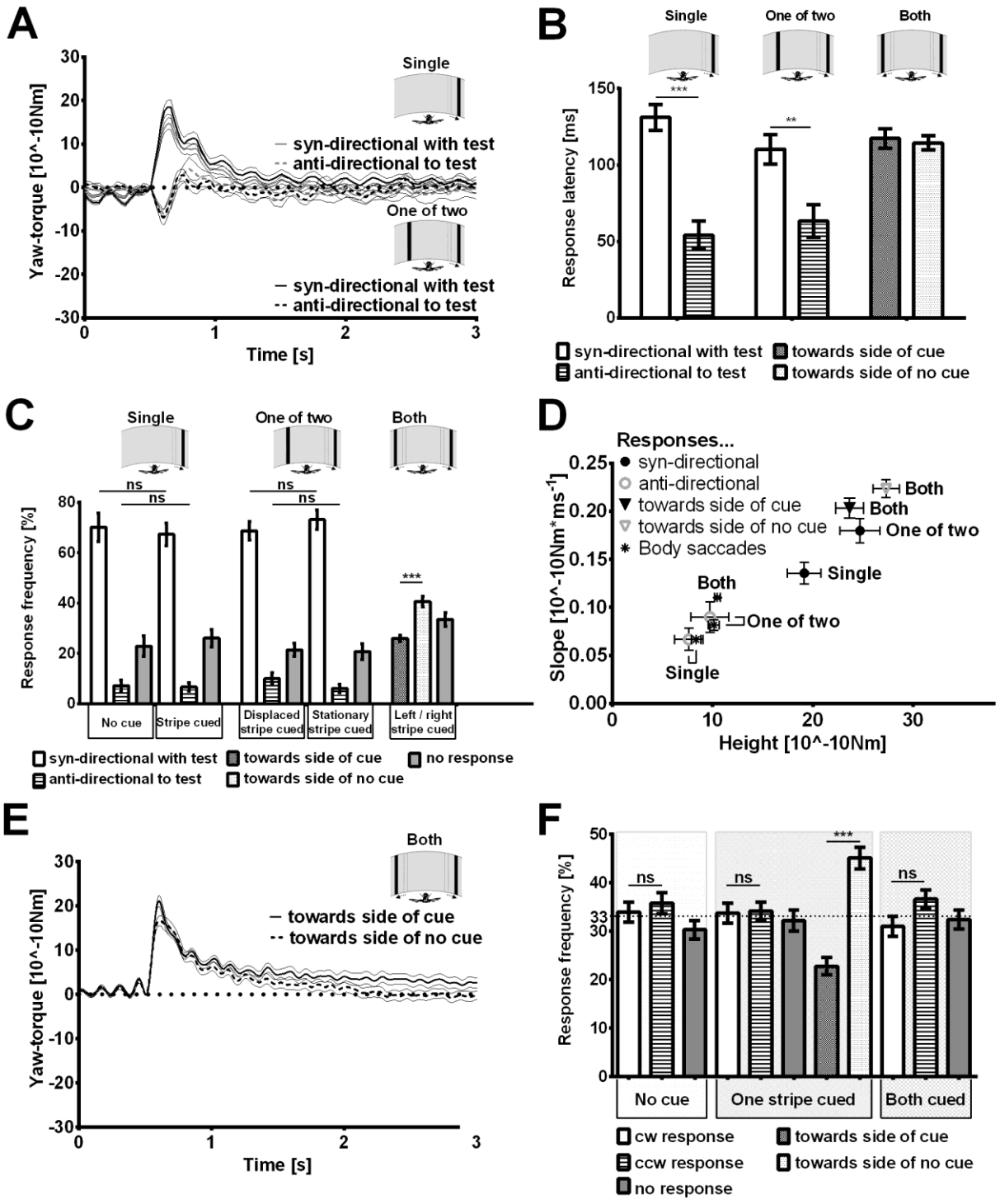

Figure 13: Yaw-torque responses to front-to-back displacements of one or two stripes. (A) Average yaw-torque traces of wild-type flies in response to the displacement of one stripe. Responses to a single stripe can be syn-directional or anti-directional ('Single', N = 25). If a second, stationary stripe is present ('One of two', N = 35), the responses are similar. (B) Response latencies. The anti-directional responses are elicited faster. No differences between the two response types can be seen, when both stripes are displaced ('Both', N = 52). (C) Response

frequencies. If only one stripe is displaced, the majority of responses is towards the direction of motion of this stripe. Anti-directional responses happen only rarely. The simultaneous displacement of two stripes increases the no response rate and the influence of the cue becomes apparent as a bias in the responses towards the not cued side. (D) Slope and height of the rising phase of yaw-torque responses and spontaneous body saccades during ITIs. The responses are grouped as syn- and anti-directional and towards cued or not cued side, respectively. They differ from each other with regard to the analyzed parameters. Anti-directional responses and responses towards the not cued side are comparable to body saccades. (E) Average yaw-torque traces of wild-type flies in response to the simultaneous displacement of two stripes. Both response types resemble the syn-directional responses to a single stripe. (F) Cueing does not reduce the overall response rate. The no response rate remains constant in same flies tested without cueing or with one or both stripes cued (N = 71). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

This does not imply that flies generally ignored the stimulus, because a peak was found at the 10Hz oscillating frequency of the stripe in the power spectrum of a Fourier analysis of yaw-torque during the cue (Figure 14).

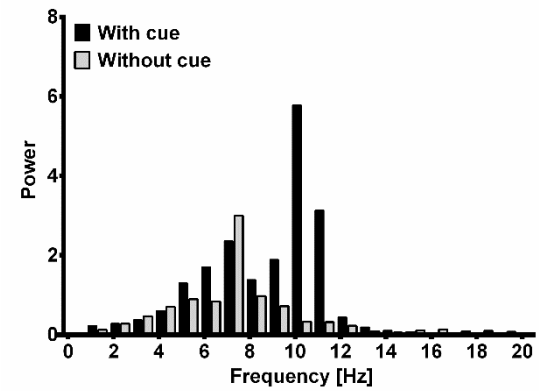

Figure 14: Power spectrum of the yaw-torque produced by the flies during the cueing of the stripe. A peak at the 10Hz component of the spectrum can be seen. This matches the oscillation frequency of the cued stripe (N = 52).

The fast front-to-back displacement was followed by 1.5s of slowly (v = 20°/s) resetting the stripe to its initial position (Figure 12). After a syn-directional response during that phase the yaw-torque returned to the level it had started from. Anti-directional responses had a smaller amplitude and during the resetting of the stripe the fly generated a weak syn-directional response before its yaw-torque reached the base line again (Figure 13A, 'Single'). Similar yaw-torque patterns could be found, if the fly faced two stripes at ψ_0 = + and − 45° and only one of them was displaced (Figure 13A, 'One of two'). The presence of the second stationary stripe did not alter the overall response frequencies (Figure 13B, 'One of two'), nor the response frequencies with or without a preceding cue in comparison to the single stripe experiments (Figure 13C, 'One of two', 'Displaced stripe cued' and 'Stationary stripe cued'). The response latency of anti-directional responses was about the same for both conditions, too, while syn-directional responses were elicited somewhat faster (Figure 13B, 'One of two'). These data suggest a substantial difference between anti- and syn-directional responses. Anti-directional responses were quite

rare and were initiated earlier after the displacement than the syn-directional ones. Furthermore, they could also be distinguished by the yaw-torque trace during resetting of the stripes and the listed properties seemed to be stable, as they were independent of the presence of a second stationary stripe. While anti-directional responses might be interpreted as escape responses, syn-directional ones would contribute to a stabilization of orientation in space.

While the stripes were not moving the fly nevertheless occasionally generated body saccades (Heisenberg and Wolf, 1979). However, they had different parameters when compared to syn-directional responses or to both response types in the 'Both' condition. Their smaller amplitude and slope of the rising phase made them more similar to anti-directional responses (Figure 13D). Because their dynamics nevertheless differed between experiments with a single stripe and the 'One of two' condition, it is likely that they were influenced by other stimulus parameters besides motion.

Increasing resolution of the analysis of complex behavior often reveals smaller, stereotyped subunits of behavior. The responses to the displacement of a stripe comprised such fixed action patterns. They were highly stereotypical and distinguishable from optomotor responses, because they were not elicited by large field motion and were not always in phase with the stimulus motion.

3.2.2 Simultaneous displacement of two stripes

To assess attention, two stripes were simultaneously displaced front-to-back, one at $\psi_0 = +45°$, the other at $\psi_0 = -45°$ in front of the fly with the same parameters as described for the single stripe. Prior to the displacement one of the two stripes was cued and the other remained stationary. A typical stimulus sequence consisted of an inter-trial interval (ITI) of 2s during which the stripes remained stationary, followed by a cueing of one of the two stripes and then by a post cue pause (PCP) of 1s, before the displacement and subsequent resetting of the stripes took place (Figure 13E). Each of the two simultaneous motion stimuli alone would have caused mostly syn-directional responses, incompatible with each other. The response patterns of responses towards and away from the cued side bared great similarity and resembled the one of syn-directional responses of the single stripe experiments. It is noticeable that the absolute values for height and slope had increased in comparison to the displacement of two stripes without prior cueing (Figure 2). Possibly, this reflects an influence of cueing on the arousal state of the animal. For both polarities the response latency was now in the range of the latencies of syn-directional responses to only one stripe. Additionally, the fly increased the number of no responses, indicating on the one hand that the quick escape responses were absent under this condition, on the other hand maybe reflecting the challenge for the fly of finding the appropriate response to this conflicting stimulus condition (Figure 13C, 'Both', 'Left/right stripe cued'). Strikingly, the responses towards the not cued side now represented the majority of responses. This finding already falsified two possible hypotheses, but it also added another aspect to the behavior that needed to be explained. The response frequencies in the

two stripes experiment again might have been the sum of two experiments with single stripe conditions. Or one might have expected no responses exclusively, because the vector sum of the two stripes being displaced with opposite polarity is zero. Neither was the case. The frequency of responses towards or away from the cued side was lower than the frequency of syn-directional responses in the single stripe experiments. Also, the overall response frequency was lower when two stripes were displaced. Put another way, this implied that the simultaneous displacement of two stripes reduced the frequency of responses to the single stripes.

3.2.3 Positive and negative cueing

Comparable experiments were described in Sareen et al. (2011), who reported a majority of responses towards the side of the cue after cueing (positive cueing). Interestingly, now the polarity of the majority of responses was away from that side (negative cueing), while the overall response rate remained constant. Even if both stripes were cued, the response rate stayed the same (Figure 13F). It is thus unlikely, that the cue simply suppressed responses to the cued stripe. The responses away from the cued side in the 'Both' condition could not be compared to anti-directional responses. They were no fast escape responses. Instead, their pattern of yaw-torque modulation resembled syn-directional responses to single stripes. Also, responses towards and away from the side of cue could hardly be distinguished from each other with regard to their yaw-torque pattern, latency or slope and amplitude (Figure 13E, B and D). Despite the similarity of patterns of both response types, responses towards the side of no cue prevailed under a variety of stimulus conditions (Figure 15).

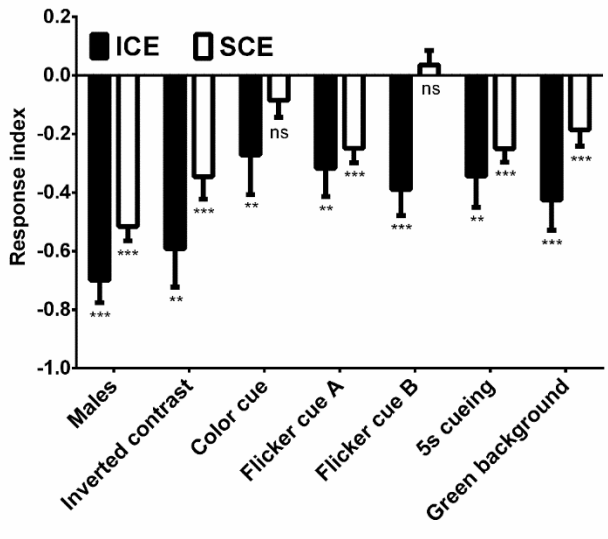

Figure 15: Many cueing stimuli and experimental conditions lead to negative cueing. Male flies show the same cueing effect as female flies (N = 18). All tested stimuli elicit a negative ICE (N = 9, 19, 31, 22, 22, 23). With inverted contrast the cueing has an after-effect. Changing the background color on one side of the panorama from white to green or blue for 1s works as a cue, but has no after-effect. After flickering black (cue A) or grey (cue B) stripes for 1s at 10Hz the cueing effect is only sustained for cue A. Increasing the duration of cueing as well as performing the experiment on a green background leads to a negative SCE. All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

There were a variety of differences between the setup used in Sareen et al. (2011) and the one used here, ranging from luminance (17.5µW/cm^2 instead of 127.0µ/cm^2) and hence contrast to the dimensions of the arena. Experimental context can have substantial influence on the outcome of experiments and thus it came as no surprise that already minor changes of the setup did influence the response frequencies. In this study some effort was put into defining a parameter that had changed the way that flies evaluated the experimental conditions and that caused them to respond mostly towards the not cued side. In a repetition of the experiment performed by Sareen et al. (2011) with the same device (LED arena) the positive cueing was reproduced, arguing against a genetic drift in the stock as an explanation for the change in response polarity. Furthermore, even in the new device (light-guide arena) a combination of parameters was found, which led to significantly positive cueing (Figure 16A). It included the same width and oscillation frequency of the stripes (w = 6° and f = 10Hz) that had been used by Sareen et al. (2011) in the LED arena. However, the oscillation amplitude had to be changed from 4° to 2° in order to get positive cueing. The same flies tested first for responses to cueing with one and then with the other condition showed a significantly negative immediate and sustained cueing effect at the 4° condition, but could be positively cued with a 2° amplitude. Interestingly, due to the different dimensions of the devices the 4° oscillation amplitude in the LED arena and the 2° oscillation amplitude in the light-guide arena had in common a maximum of 50% luminance modulation of only one row of adjacent LEDs or light-guides, respectively on each side of the stripe. Presumably, the intensity modulation during cueing has to be rather weak for a cue to be attractive. The higher luminance in the light-guide arena may have therefore yielded a comparable modulation only with the reduced oscillation amplitude. How the various parameters determine the quality of a cue in detail still remains to be understood, as intensity modulation by flickering grey stripes between black and white as a cue led to a negative immediate cueing effect, but had no after-effect, while flickering of black stripes led to ICE and SCE (Figure 15, Flicker cue A and Flicker cue B).

It is remarkable, that all of the tested cueing stimuli were sufficient to cue the FoA to one side. This speaks in favor of SVA being a stimulus independent generic process. Interestingly, all of these cueing stimuli biased the distribution of response polarity towards the not cued side. Sareen (2011) had found only a bias towards the cued side with the tested cueing stimuli. It can not be excluded that the tested parameters were a random selection of stimuli that by chance all led to the same cueing effects. However, it is more likely that the two different experimental setups rendered a setting, which added to the quality of the presented cues. The consistency of the sign of the cueing effect in each setup indicates a strong influence of the experimental context on the quality of a cue.

3.2.4 Dynamics of the cueing effect

What happened to the FoA during cueing? First, in average the oscillation frequency of the cue was represented in the corresponding yaw-torque of the fly (Figure 14). Second, the parameters of the response action patterns during the test resembled responses to a single stripe. That suggests that the fly perceived the cue and then produced a yaw-torque modulation in response to the movement of a stripe. Judged by the polarity of the response it was towards the not cued stripe and not away from the cued one. However, cueing never resulted exclusively in responses towards or away from the cued side. Obviously a cue could be both, attractive and repellent. The cue biased the response ratio towards one or the other stripe, depending on a yet not known variable. One could assume that a shortened negative cue might be less repellent or that the repulsion might be preceded by a short phase of attraction. Hence it was checked, if after a shortened cue duration (CD) the FoA might still be on the cued side. Experiments were performed to measure effects (ICE, PCP 0s and sSCE, PCP 1s) of cues lasting between 0 and 1s.

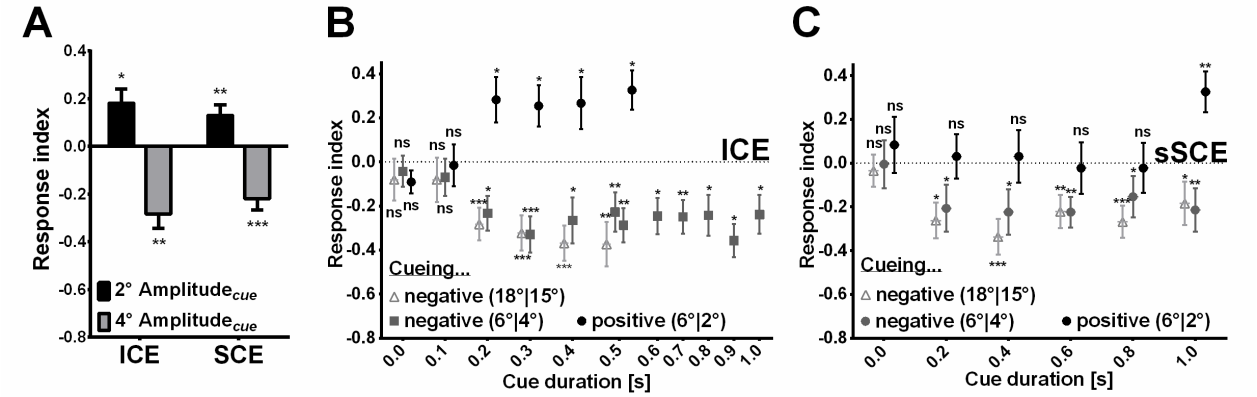

Figure 16: Quality and dynamics of cueing. (A) The same flies tested with cues with 2° or 4° oscillation amplitude respond towards the cued stripe (width = 6°) in the test, respectively away from it. Both types of cue elicit an immediate as well as a sustained cueing effect (N = 22). (B) Influence of a shortened cueing duration on the ICE. Shorter cueing durations lead to negative cueing for two combinations of stripe width and cueing amplitude, that both elicit negative cueing in the standard experiment ('18°|15°', N = 33; '6°|4°', N = 29, 23). No cueing can be observed, if the cue is shorter than 0.2s. The same is true for positive cueing ('6°|2°', N = 21). (C) Influence of a shortened cueing duration on the sSCE. 0.2s of cueing are sufficient to maintain a negative cueing effect for at least 1s ('18°|15°', N = 32; '6°|4°, N = 19). A minimum of 1s of cueing is required to lead to an effect after 1s ('6°|2°', N = 22). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

To cover a broad spectrum of cue properties, two combinations of oscillation amplitude and stripe width that both yielded negative cueing were used, along with the combination for positive cueing. In summary, no reversed cueing effect was found for the shortened cueing duration. At the earliest moment an ICE

could be observed, it was negative or positive, depending on the experimental conditions. Cueing occurred already with two cycles of the oscillation (CD = 0.2s). No immediate cueing effect was found for CD = 0.1s and – serving as a control – for CD = 0s (Figure 16B). Results were quite similar for the sSCE after negative cueing (Figure 16C). No sSCE was found for CD = 0s, i.e. without cue, but negative cueing became evident with CD >= 0.2s. Varying stripe width and wiggle amplitude failed to alter the quality of the cue in this regard. All values were significantly negative for CD > 0s. Interestingly, in the case of positive cueing the cue had to last for more than 0.8s to elicit an after-effect (sSCE). The results did not completely falsify the above hypothesis, that (1) with a reduced CD the FoA could still be at the cued side at the moment of the displacement or (2) that a short CD in combination with thinner stripes and a smaller amplitude could make the cue less repellent. What happened to the FoA before a cueing effect became apparent remains unclear. It is still possible that short cues might attract the FoA, but this attraction would not show, since the response would not be activated before the FoA was shifted from the cued to the not cued side. Also, there might exist a combination of parameters, which results in a less repellent cue. However, thick stripes and a large oscillation amplitude elicited a cueing effect of about the same strength as the smaller stripes with a smaller oscillation amplitude. Thus, there is no obvious linear relationship between stripe width and oscillation amplitude and strength or sign of the cue. This indicates the occurrence of cueing effects to follow an all or nothing principle.

3.2.5 Cueing in the lower and upper visual field does not add up

One of the features of SVA discovered by Sareen et al. (2011) was that the FoA could be cued in the LVF but not in the UVF. With the settings for positive cueing this effect was reproduced in the light-guide arena (Figure 17A). The modified experimental protocol and the discovery of negative cueing allowed to investigate advantages of parts of the visual field in cueing beyond an attractive ICE, i.e. with negative cueing and for a sSCE. A positive cue did not lead to a sSCE in the LVF and UVF. To be effective, cueing had to occur in both fields. This result indicates that for the fly a cue in both half fields is more than the sum of inputs received in each field alone. Instead, it seems to be evaluated as a separate condition. Negative cueing was less sensitive to the location of cue presentation. Only in the UVF there was, like for positive cueing, no ICE. Besides that, negative cueing led to ICE and sSCE in both visual half fields (Figure 17B).

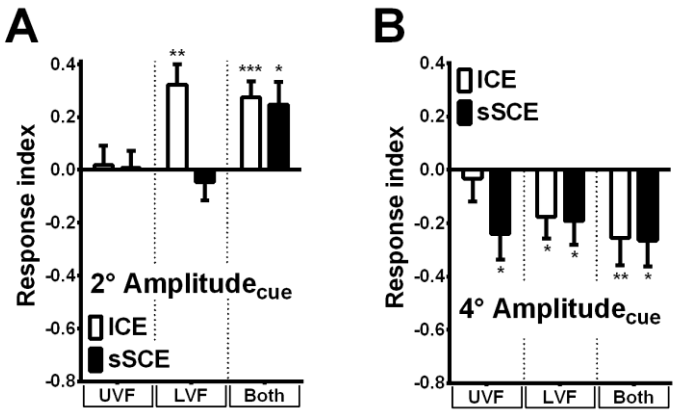

Figure 17: Cueing effects are different in the lower and upper visual field. (A) Positive cueing is effective, if the cue is present in both visual half fields. In the lower visual field it is restricted to the ICE (N = 32). (B) Negative cueing works mostly irrespective of whether the cue is presented in the LVF or UVF. Only in the UVF an ICE is missing (N = 23). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

3.2.6 Response polarity is independent of yaw-torque

Using a visual learning task in the flight simulator Tang et al. (2004) provided evidence that yaw-torque and the location in the visual field a fly attends to might be connected. Additionally, Tang and Juusola (2010) found higher levels of activity at the same side of the brain the fly tried to turn to. If the FoA and/or such brain activity were coupled with yaw-torque the influence of the cue on response polarity could be explained – in addition to a shift of the FoA - by deploying central pattern generators for flight direction. This issue was addressed by an analysis of the yaw-torque during 1s before the displacement. The yaw-torque was categorized by four groups ('cw' and 'ccw', each for responses towards and away from the side of cue) according to the subsequent response polarity. A putative influence of the preceding yaw-torque level on response polarity should have manifested as a difference of the yaw-torque histograms of the four groups.

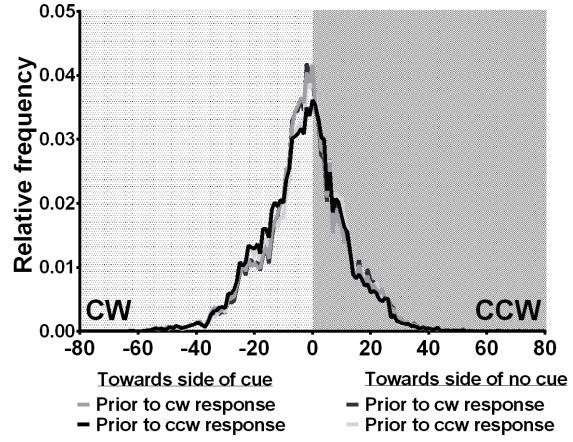

Figure 18: No influence of yaw-torque on response polarity. The yaw-torque of wild-type flies during 1s before the displacement is grouped with regard to the subsequent response polarity as 'towards side of cue, cw', 'towards side of cue, ccw', 'towards side of no cue, cw' and 'towards side of no cue, ccw'. The histograms of the four groups show no difference (N = 138).

Because there was no such difference (Figure 18), the flies most likely were able to shift their FoA independent of yaw-torque in the paradigm.

3.2.7 Temporal properties of the cueing effect

Sareen et al. (2011) showed that a cue can be used to guide the FoA to one side and that the after-effect of cueing lasts for at least 2s. To measure the duration of the after-effect they used various PCPs (phases of stationary stripes between cue and test, lasting 0s, 0.5s, 1s, 2s or 5s). The cueing effect diminished over time, but with PCP 2s still biased the responses. With PCP 5s response polarity appeared to be randomly chosen. Because the after-effect was lost between 2s and 5s, emphasis was put on that phase in the experiments of this study and PCP 0.5s was removed from the experiment. Instead, two new PCPs– 3s and 4s – were added and it showed that the effect lasted up to 3s but was no longer detectable after 4s (Figure 19).

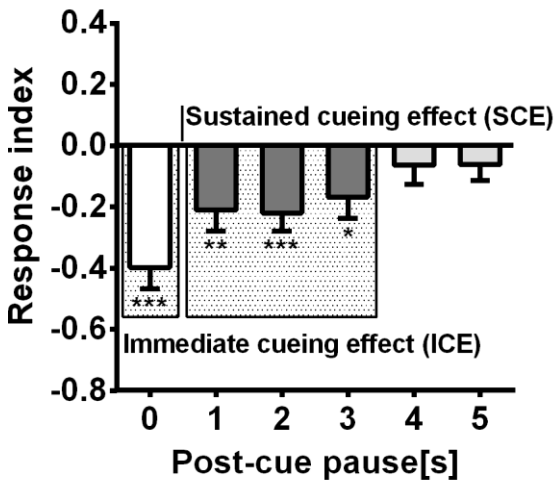

Figure 19: Temporal dynamics of cueing. The effect of cueing is the strongest, if the test takes place immediately after the cueing (ICE). It is slightly reduced, if a PCP of 1, 2 or 3s is introduced (SCE). If test and cue are separated by 4 or 5s no more effect of cueing can be seen (N = 52). Error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

In line with the data shown by Sareen (2011), the bias of the response distribution towards a side was the strongest immediately after the cue and then remained at a lower level between 1s and 3s after the cue. This pattern consistently showed in the data, thus the evaluation was simplified by distinguishing between an immediate cueing effect (ICE, comprising PCP 0s) and a sustained cueing effect (SCE, grouped PCPs 1s, 2s and 3s).

A lot of work has been spent on the characterization of the guidance of the FoA and many of its properties have been described in Sareen (2011) (see also chapter 4.1.1). In summary, chapter 3.2 presented data showing that cueing influences the distribution of responses, but does not change the overall response frequency. It became clear that cueing does not exclusively lead to one response type, but only shifts the ratio in favor of one. Besides salience, visual field position and light intensity there are further unknown

features of the cue that determine its after-effect and sign. However, positive as well as negative cues both had the same latency, required the same minimum cueing duration and were not influenced by torque-levels prior to the displacement. Thus, the specific effects might vary, but cueing itself seems to be a key feature of SVA.

3.3 Neuronal underpinnings of cued shifts of attention

Making use of negative cueing the next chapters of this study will focus on the neuronal underpinnings of the cueing effect to gain insights into circuits and neurotransmitter systems required for this attentional mechanism. In the course of this investigation, manipulations that led to defects of SVA during tethered flight were also checked for possible influences on the characteristics of walking. SVA might be behavior specific, but it might also affect other behaviors. In humans an attentional deficit is often associated with hyperactivity and the same has been suggested for *Drosophila*. However, it is a difficult task to extract symptoms like hyperactivity from yaw-torque traces of flies in the flight simulator. Histograms of all observed yaw-torque values, as well as Fourier analysis of yaw-torque signals are helpful means to gain information about the characteristics of flight in the flight simulator. But combinations of different flight behaviors might lead to the same results. Therefore the results can be ambiguously interpreted and are hence insufficient to accept or reject the hypothesis that hyperactivity can be found in flies expressing attentional deficits. Specifically, in rsh^1 flies a peak in the power spectrum of a Fourier analysis was termed hyperactivity (van Swinderen and Brembs, 2010). To investigate, whether attentional deficits of rsh^1, but also of *fmn* flies and of CantonS flies after αMT and MPH treatment manifested in symptoms like hyperactivity, flies were tested in a free walk arena, where their movements were tracked and then analyzed for activity, covered distance, velocity, idle events and distribution across the arena (Figure 20). The setup was used to see, if mutant phenotypes in SVA and the consequences of pharmacologically compromising SVA become apparent in more than one behavior or if they are behavior specific. Altered characteristics of walking could be explained by recruitment of the specific gene product or drug target within various mechanisms, but also as a consequence of abnormal attentional processes. The results gathered during walking add to the understanding of attention in *Drosophila*, but require further research to be linked closer to attention.

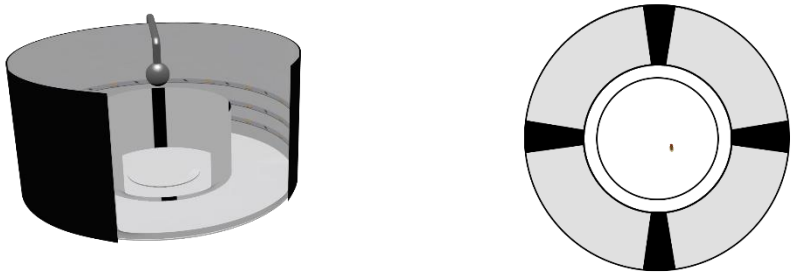

Figure 20: Free walk arena. An outer cylinder holds a row of LEDs and blocks stray light from reaching the inner cylinder. The top view shows the only landmarks visible to the fly. One black stripe is presented per quadrant of the inner panorama. The fly is contained by the lid of a petri dish within the inner cylinder. A webcam above the arena tracks the movements of the fly.

3.3.1 Compromising dopamine synthesis via αMT abolishes the sustained cueing effect

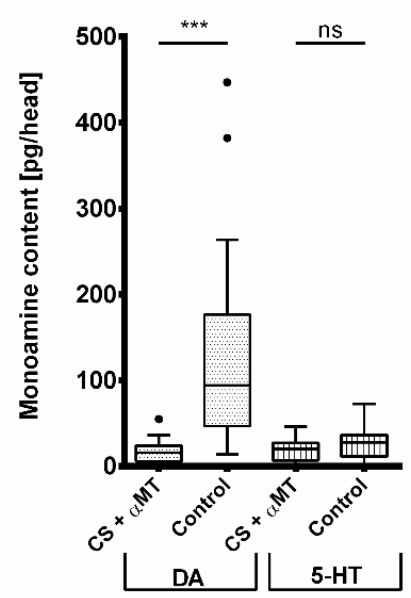

Figure 21: Reducing systemic dopamine by inhibition of its synthesis. Feeding αMT to wild-type flies reduces systemic levels of dopamine, whithout influencing serotonin levels (N = 19, 18, 22, 19 samples à 20 heads).

Dopamine is a neurotransmitter that in many cases has been associated with attention in mammals (e.g. Nieoullon, 2002) and insects (e.g. van Swinderen and Andretic, 2011) and is thus an interesting candidate to check for its involvement in SVA in *Drosophila*. Two rate-limiting enzymes provide the synthesis of dopamine: tyrosin hydroxylase (TH) converts tyrosine into L-DOPA, which is further modified by the dopa decarboxylase (DDC) to become dopamine. CantonS flies were fed with α-methyl-DL-tyrosine (αMT), a TH-inhibitor, which effectively reduced dopamine at a systemic level (Figure 21, 'DA'). Another set of CantonS flies was treated identically, but not fed with

the drug. It was quite remarkable, that αMT-treated flies survived and even beyond that still managed to fly in the flight simulator. Obviously, the brain can partially cope with a deficiency in one of its major parts, like the dopaminergic system. There is some indication towards an interplay between dopamine and serotonin levels [see Kapur and Remington, (1996) for humans, Chaouloff et al., (1987) for rats and Gainetdinov et al., (1999) for mice], but here the serotonin levels remained unaltered (Figure 21, '5-HT'). After the severe reduction of dopamine levels the flies were not unscathed, though. Both, control and αMT treated flies had a wild-type like ICE, but only the control showed a wild-type like significant SCE (Figure 22). The absence of the SCE in αMT-treated flies indicated that sufficient amounts of dopamine are crucial to maintain SVA in *Drosophila*. Additionally, the fact that the ICE remained intact indicated, that ICE and SCE may deploy different systems or pathways.

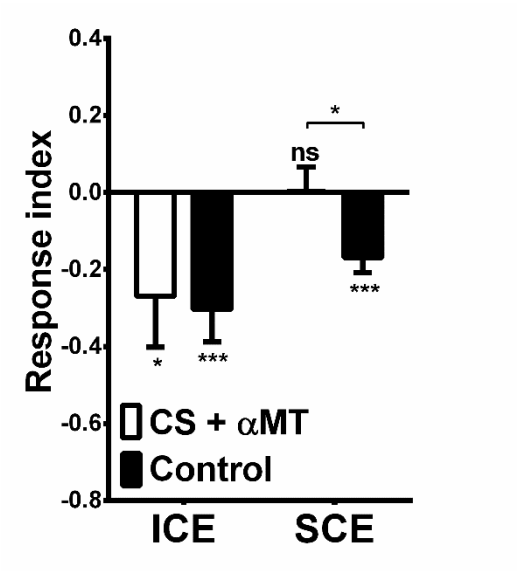

Figure 22: Reduced systemic dopamine levels lead to a loss of SCE in wild-type flies. (N = 23, 44). Error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

3.3.2 Pharmacological suppression of dopamine synthesis also affects free walk behavior

Lowering the level of dopamine abolished the sustained response to a cue in tethered flight and in order to see, if it also had an effect on walking behavior, CantonS flies were again fed with α-Methyl-DL-tyrosine. This procedure led to a severe reduction of dopamine levels. Control flies were of the same age to exclude possible age effects. As a result of the treatment, flies showed less walking activity as well as less covered distance (Figure 23A and B). The reason for the difference in covered distance was an interplay of the lowered activity, as well as decreased velocity (Figure 23C) and the same amount of idle events, which were longer after treatment (Figure 23D and E). Interestingly, the reduction of dopamine levels did also alter the time the flies spent at the periphery of the arena. It was lowered in comparison to control flies (Figure 23F).

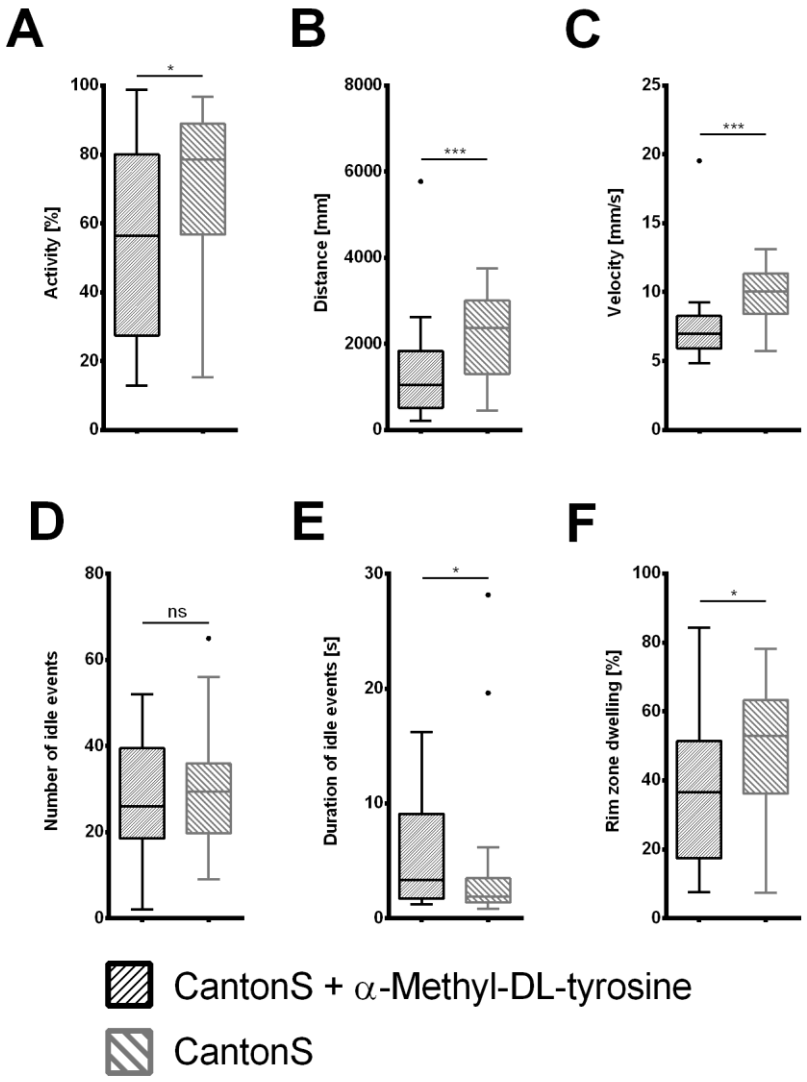

Figure 23: Inhibition of dopamine synthesis affects characteristics of walking. Except in the number of idle events, CantonS flies treated with the dopamine synthesis inhibitor αMT differ from untreated flies (N = 29, 30). (A) Activity. (B) Distance. (C) Velocity. (D) Number of idle events. (E) Duration of idle events. (F) Rim zone dwelling.

3.3.3 Compromising regulation of dopamine levels at the synapse via interference with dDAT

Fumin (dDAT^{fmn} or *fmn*) flies have a transposon insertion in the dopamine transporter (DAT) gene, which leads to a loss of function (Pörzgen et al., 2001). They show alterations in their activity/rest pattern as well as hyperactivity and based on these findings Kume et al. (2005) suggested a role of dopamine in arousal in *Drosophila*. Seugnet et al. (2009) and Kong et al. (2010) further provided support for the connection between hyperactivity and increased dopamine signaling, which is possibly the result of the defective dopamine re-uptake of dDAT^{fmn} flies. Testing dDAT^{fmn} flies for effects of cueing on SVA showed an intact ICE, but no SCE (Figure 24A), which again emphasized the independence of those two effects. Furthermore the lack of SCE in dDAT^{fmn} flies stressed the necessity of balanced dopamine signaling in SVA and the

role of dDAT in it in particular. The latter seems to be a crucial part of a system that maintains a balanced and functional dopamine level, because dDAT^{fmn}/+ flies showed the same defects as the homozygous mutant animals. This state of haplo-insufficiency, in which a single copy of the gene is incapable of producing sufficient amounts of a protein (here dDAT) to avoid phenotypicial defects, further pointed out the importance of dDAT in SVA.

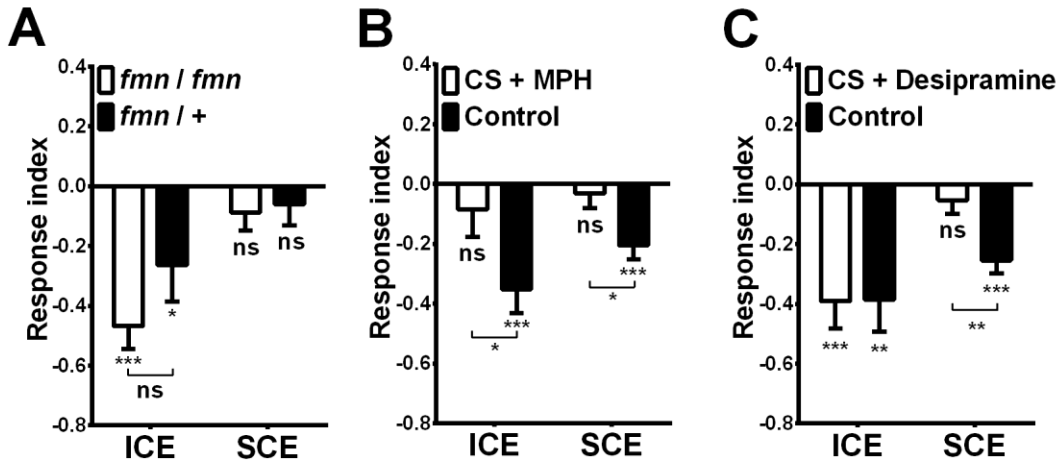

Figure 24: Manipulation of the dopamine transporter dDAT abolishes the SCE. (A) Flies with a lesion in the dDAT gene (*fmn*) show no sustained response to cueing. The same defect is apparent in flies homozygous for this mutation (N = 26, 26). (B and C) Pharmacological manipulation of dDAT function. Both MPH (N = 41, 42) and Desipramine (N = 26, 29) are known to inhibit re-uptake of dopamine via dDAT and lead to a loss of the SCE. MPH additionally causes a loss of the ICE. All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

The results were confirmed pharmacologically by treating CantonS flies with methylphenidate hydrochloride (MPH), a drug which is marketed as 'Ritalin' and which is frequently prescribed to children suffering from attention deficit and hyperactivity disorder (ADHD). It targets the dopaminergic system (Iversen and Iversen, 2007) and the recycling of dopamine via the DAT in particular. MPH treatment abolished the SCE (Figure 24B). Desipramine is a dDAT inhibitor with high affinity for dDAT (Pörzgen et al., 2001), already known to cause poor memory retention in an olfactory memory task (Zhang et al., 2008). The cueing phenotype of flies treated with Desipramine resembled that of *fmn* mutant flies (Figure 24C). Interestingly, only MPH additionally abolished the ICE. This surprising impairment could have been a result of effects on the serotonergic system, as MPH also has a low affinity for serotonin transporters (Volkow et al., 2000). The occurrence of an ICE after the more selective inhibition of dDAT with Desipramine indeed argued for a weak contribution of the serotonergic system. However, the loss of ICE might also be due to potentially stronger effects of MPH on the dDAT. Thus the role of the serotonergic

system can not be finally determined based on these data. Taken together, the pharmacological treatments as well as genetic modification both attributed great importance to the maintenance of SVA to dDAT and in consequence to a balanced dopamine level. Because dDAT dependent increase of dopamine signaling as well as inhibition of dopamine synthesis impaired SVA, it seems that proper SVA requires a balance between too much and too little dopamine signaling. The observed effects of altered dopamine signaling are in line with the hypothesis of an inverted U-shaped relationship between dopamine levels and function (Cools and Robbins, 2004).

3.3.4 Effects of increased local dopamine signaling on free walk behavior

The consequence of the loss of function mutation in the dDAT in *fmn* flies could be pharmacologically mimicked by feeding CantonS flies MPH. MPH is a dopamine re-uptake inhibitor and thus also increases the duration dopamine remains in the synaptic cleft. As a result of such treatment, flies showed a reduced activity (Figure 25A). Similar to the results of a drastic reduction of overall dopamine levels, a decrease in covered distance was found (Figure 25B) as a combined consequence of the decrease in activity as well as slower walking (Fig. 35C) and the same amount of idle events, which were longer after treatment (Figure 25D and E). The inhibition of dopamine re-uptake did not alter the distribution of positions across the arena (Figure 25F).

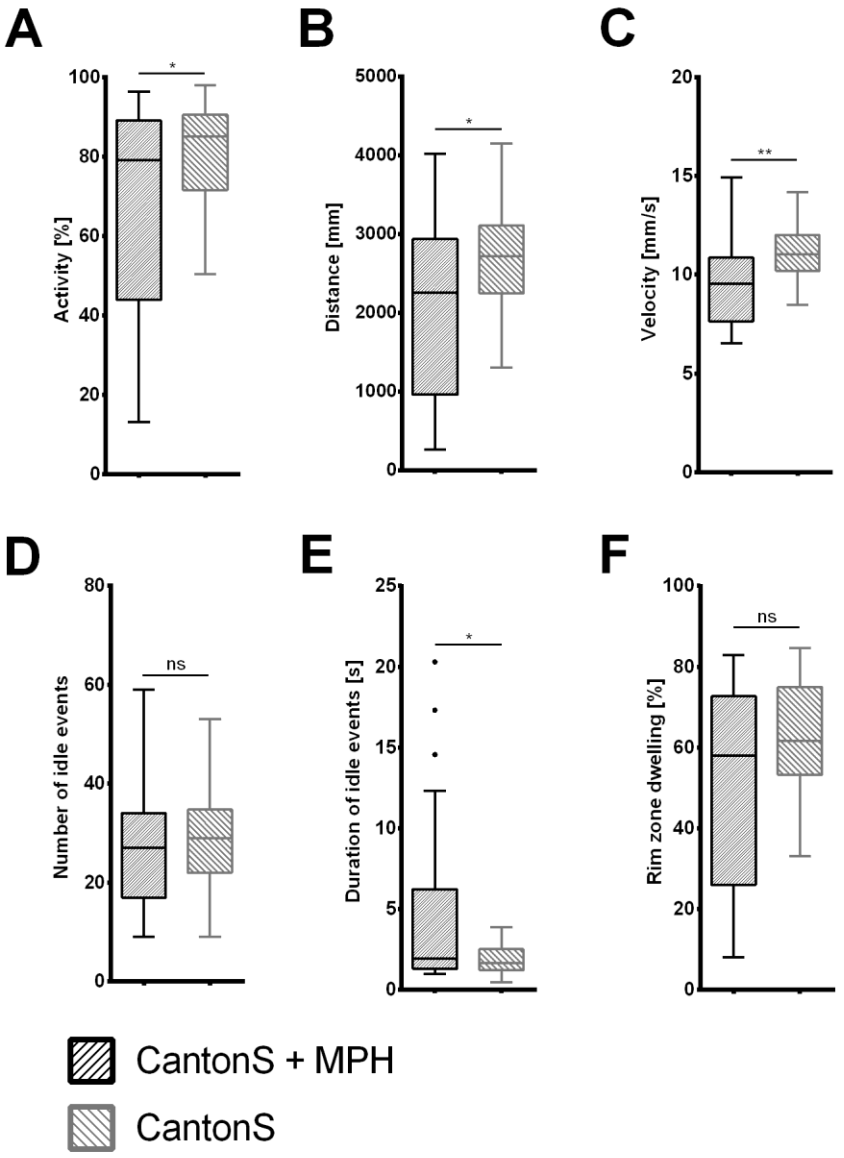

Figure 25: Compromising dDAT function affects characteristics of walking. Except in the number of idle events and in the time spent in the outer parts of the arena, CantonS flies treated with the dopamine re-uptake inhibitor MPH differ from untreated flies (N = 32, 47). (A) Activity. (B) Distance. (C) Velocity. (D) Number of idle events. (E) Duration of idle events. (F) Rim zone dwelling.

3.3.5 Dopamine receptors and their recruitment in SVA

To exert its effect as a neurotransmitter, dopamine needs to bind to a receptor. Based on amino acid sequence homology, sensitivity to class specific agonist and antagonist drugs as well as signal transduction pathways mammalian dopamine receptors can be divided into the two families of D1 and D2 receptors. The respective *Drosophila* homologs DopR1 (dumb) and DopR2 (DAMB) are D1-like and DD2R is a D2-like receptor. They are involved in a range of behaviors including learning, wakefulness, arousal and locomotion [Draper et al., (2007); Kim et al., (2007); Lebestky et al., (2009); Seugnet et al., (2009)]. Both D1-like receptors are strongly expressed in the MBs [Kim et al., (2003); Han et al., (1996)], whereas DD2R is only expressed in distinct cells in the CNS (Draper et al., 2007). Additionally, *Drosophila* has a β-adrenergic-like G-protein coupled receptor called DopEcR, with high expression in the CNS, which

responds to dopamine as well as to ecdyson (Srivastava et al., 2005). Its expression in the MBs is critical for DopEcR dependent processing of courtship memory (Ishimoto et al., 2013). With flies mutant for the respective gene, three of the four receptors were tested to see, which receptor could mediate the observed effects of dopamine manipulation on SVA.

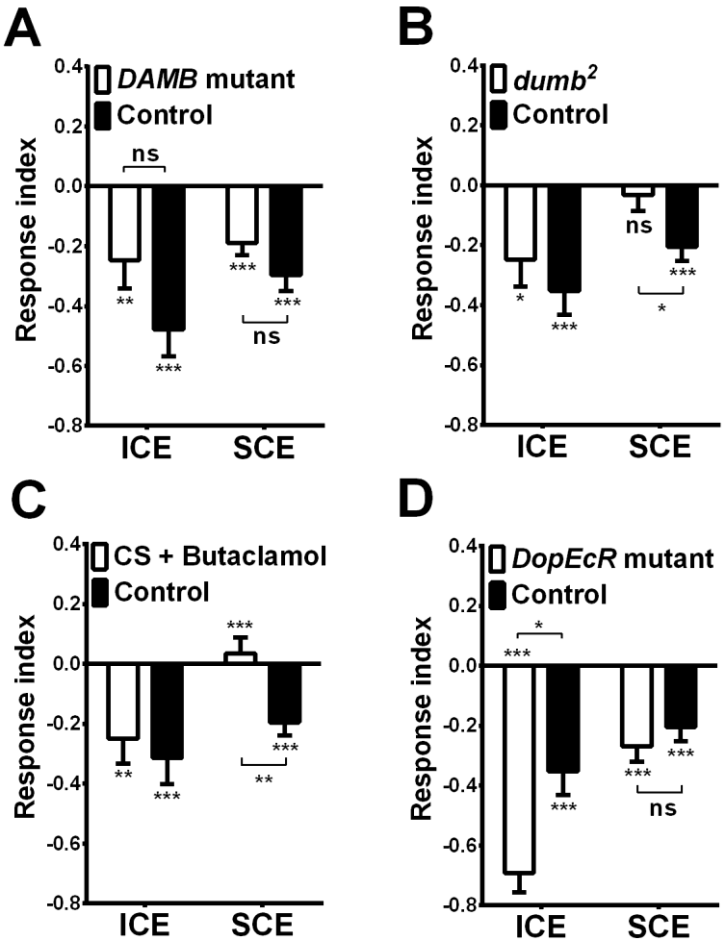

Figure 26: Recruitment of dopamine receptors for SVA. (A) The receptor DAMB is dispensable for SVA (N = 27, 30). (B and C) *DopR1* mutant flies (*dumb*^{2}) are defective in the maintenance of SVA (N = 29, 35). Pharmacological manipulation of DopR1 by its antagonist Butaclamol mimics the genetic results (N = 26, 26). (D) The DopEcR is not required for SVA (N = 26, 35). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

3.3.5.1 The dopamine receptor in the mushroom bodies (DAMB) is dispensable for SVA

In rhesus monkeys injections of D1 receptor agonists into the prefrontal cortex are associated with defects in working memory (Sawaguchi and Rakic, 1991). This type of memory is in close relationship to attention (Fougnie, 2008) and in the case of *Drosophila* might be required to maintain SVA. As will be shown in chapter 3.4, the MBs are important for SVA. Thus, strong expression in the MBs and spatial co-expression with the *rutabaga* encoded adenylylcyclase (Han et al., 1996), together with the capability to mediate a dopamine induced increase in cyclic adenosine monophosphate (cAMP) levels made DAMB an interesting candidate to test for effects on SVA. Surprisingly, *DAMB* mutant flies showed no defects in SVA and

displayed a wild-type like ICE and SCE (Figure 26A). Thus, examination of *Drosophila's* second D1 like receptor became of interest.

3.3.5.2 Cueing has no after-effect in *DopR1* mutant flies

dumb^2 is a hypomorphic DopR1 allele, whose name is an acronym of D1 (uno) in mushroom bodies. As the name suggests it has strong expression in the MBs (Kim et al., 2007) and similar to DAMB it is able to mediate an increase in cAMP levels (Sugamori et al., 1995). The *dumb*^{2} flies had a light eye color and Sareen (2011) reported insufficient amounts of yaw-torque responses of these flies. Possibly the reduced optical separation of single ommatidia led to an altered visual perception, which interfered with the normal response frequency. Thus, the *dumb*^{2} line was crossed to CantonS to regain a wild-type eye color. When tested after crossing for immediate and sustained effects of cueing, there was a normal ICE, but no SCE (Figure 26B). In other words, even though the flies now responded to the displacements at the same rate as wild-type, they did not express a SCE like wild-type. To independently verify this finding, flies were put for 14h on food enriched with 1mg/mL Butaclamol, a DopR1 antagonist with higher affinity for DopR1 than for DopR2 [Karpova et al., (2012); Chen et al., (2012)]. Effects of this treatment resembled the *dumb*^{2} flies' phenotype of normal ICE, but no significant SCE (Figure 26C). Because DopR2 mutant flies showed no defect in SVA, DopR1 seems to be the relevant target of Butaclamol. This finding points towards DopR1 as a likely candidate for mediating the dopamine effects on SVA. Interestingly, genetic as well as pharmacological manipulation did not compromise the ICE, a peculiar fact that once again suggested a basic difference between ICE and SCE, possibly caused by different recruitment of working memory. The ICE occurs directly after the cue has ceased and hence does not require any storage of the cued side. Because cue and test are temporally separated by several seconds, the SCE can only occur, if the side of the cue is still stored in a working memory at the time of the test.

3.3.5.3 Cued shifts of attention are independent of the DopEcR

Finally, flies with a mutation in the DopEcR were analyzed. Expression of this receptor is predominant in the MBs and its function is behaviorally linked to the MBs. Nonetheless, neither ICE nor SCE were compromised by the lack of functional DopEcR (Figure 26D). Hence the observed effects of dopamine on SVA were most likely not mediated by this receptor.

3.4 The mushroom bodies are required for SVA

The mushroom bodies (MBs) are a prominent paired neuropil of the *Drosophila* central brain (Figure 27). In each hemisphere the MB consists of ~2000 Kenyon cells [KC, Aso et al., (2009)], whose cell bodies cluster dorso-posteriorly in the brain. They receive input via dendritic branches at the so called calyx and extend axons, which along the way form the peduncle and the α, β and γ lobes. Based on the different lobes, 3 compartments can be distinguished: α/β, α'/β' and γ. γ, β and β' KC axons lie horizontally within

the brain and α and α' extend vertically. The α/β compartment can be further subdivided into α/β_s (surface), α/β_c (core) and α/β_p (posterior). Unlike the rest, the about 90 α/β_p KCs form no dendritic branches in the calyx, the main source of olfactory input. They bypass it and arborize in the accessory calyx, instead. This thin bundle at the anterior dorsal edge of the calyx receives no olfactory input (Tanaka et al., 2008) except, possibly, a single olfactory projection neuron (Lin et al., 2007). Because α/β_p cells do not only have a different pattern of connectivity, but also a different, mesh-like morphology in the lobes (Tanaka et al., 2008), it is assumed that the neurons of α/β_p serve a different function and receive input from other sensory modalities.

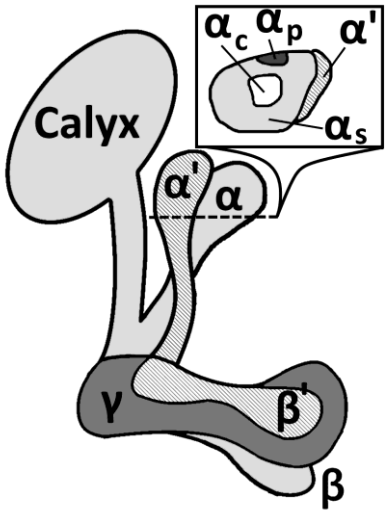

Figure 27: Scheme of the adult mushroom body. Kenyon cells receive input in the calyx and run axons, which form the peduncle and the different lobe systems. The αβ-lobes can be further subdivided into $\alpha\beta_c$, $\alpha\beta_s$ and $\alpha\beta_p$ (separation shown for the α-lobe).

MBs in general are involved in a variety of complex brain functions such as learning and memory, sleep regulation, decision-making, context generalization and higher order motor control [Pascual and Préat, (2001); Joiner et al., (2006); Xi et al., (2008); Liu et al., (1999); Martin et al., (1998)]. With the knowledge about the crucial role of dopamine in SVA, the intimate linking of the dopamine system to the MBs suggests an involvement of this structure in SVA and in particular in cueing. Additionally, several of the genes used in this study are preferentially expressed in the MBs. In fact, in contrast to Sareen (2011) this study found an important role of the mushroom bodies in selective visual attention. Newly hatched CantonS flies were fed with hydroxyurea [HU, De Belle and Heisenberg, (1994)] to obtain flies with only embryonic MB KCs. As a control, another set of flies was treated identically, except that they were not fed the drug. Despite the wild-type like behavior of the control flies, HU flies showed neither significant ICE, nor SCE when tested (Figure 28A). Also for positive cueing the MBs proved to be necessary for SVA, as HU treated flies again showed neither ICE nor SCE, while the expected positive cueing effect was observed in the control flies (Figure 28B).

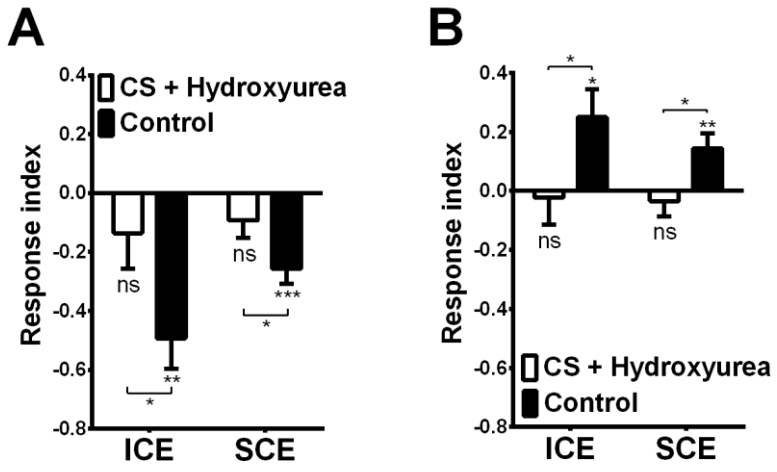

Figure 28: The mushroom bodies are required for SVA. After chemical ablation of the MBs with hydroxyurea, only embryonic MBs are left in adult flies. These flies show neither ICE nor SCE after negative (A) as well as after positive (B) cueing (N =22, 24 and 29, 20). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

Hence, a different recruitment of the MBs in positive and negative cueing could not serve as an explanation for the different finding in the present study in that of Sareen (2011). Sareen, however, did not check the structure of the mushroom bodies in the HU flies tested behaviorally. Possibly, in those experiments the HU treatment had not been effective. Whatever the explanation may be, the present study clearly links SVA to the MBs.

3.4.1 Regulation of dopamine re-uptake at the MBs

Recently it has been shown, that for olfactory memory, cholinergic KCs are required within the MBs (Barnstedt et al., 2016). It remains elusive, though, whether dopamine is used as a neurotransmitter by MB intrinsic neurons. However, it is known that the MBs are strongly innervated by dopaminergic neurons [Liu et al., (2012); Mao and Davis, (2009)]. Marking dDAT with GFP revealed regular presynaptic expression at the MBs (Vogt et al., 2014). The MB intrinsic neurons might themselves not be dopaminergic, but still possess the relevant machinery to regulate dopamine levels at the synaptic cleft. This raised the question, whether the roles of the MBs and dDAT in SVA are not separate, but in fact two sides of the same coin, namely dDAT expression at the MBs. Ueno and Kume (2014) rescued a short sleep phenotype of dDAT^{fmn} by expressing dDAT in the MBs and speculated that ectopically expressed dDAT gets transported to the synaptic site. Thus, the MBs might in fact contribute to dDAT dependent re-uptake and recycling of dopamine at the site of dopaminergic synaptic input.

3.4.2 Necessity and sufficiency of dDAT for sustained cueing in the mushroom body compartments

Pharmacological ablation of the MBs was a good first step to reveal a contribution of MBs to SVA. To probe single compartments of the MBs for necessity, the Gal4/UAS-system (Brand and Perrimon, 1993)

was used next to achieve cell-specific dDAT expression in the MBs in a dDAT^{fmn}/+ background. Then RNAi against dDAT mRNA was expressed in wild-type background to see, if impairing dDAT function in the same sets of cells also interfered with the susceptibility to cueing. In this study so far it has been shown, that proper dopamine signaling, the MBs and dDAT each play a pivotal role in SVA. The manipulation of dDAT in the MBs embraced these factors.

3.4.3 Site specific dDAT expression and knockdown in dDAT^{fmn}/+ or wild-type background

First, OK107-Gal4 was used, which strongly labels the complete MBs. Providing dDAT function at all MB cells rescued the SCE defect that was apparent in the *fmn* mutant and control flies (Figure 29A). Reciprocally, knocking down dDAT in the same cells with RNAi suppressed both ICE and SCE (Figure 29B). These findings confirmed the results so far, which had indicated that for proper cueing dDAT influences dopamine signaling at the MBs. Next, dDAT expression was driven by MB247-Gal4, a line that also labels α, β and γ-lobes (including the accessory calyx), but has no or only marginal expression in the prime lobes. The results showed a wild-type like ICE and a rescue of the SCE (Figure 29C). Again, the knockdown of dDAT in MB247-Gal4 labeled cells removed the SCE (Figure 29D). In combination with the OK107-Gal4 results, the MB247-Gal4 results gave first evidence that the prime system might not be required to maintain SVA. However, given that both lines' expression patterns included the γ-lobe, further controls were needed to examine the requirement of the MB substructures in more detail. c739-Gal4 and c305a-Gal4 label the αβ and the α'β'-lobes, respectively, but not the γ-lobe. Thus, the roles of the αβ and α'β'-lobes in SVA could be addressed separately with these lines. Because c739-Gal4 but not c305a-Gal4 driven dDAT expression rescued the SCE defect in dDAT^{fmn}/+ flies (Figure 29E and G), dDAT function at the αβ-lobes of the MBs is sufficient to maintain SVA. A successful knockdown in c739-Gal4 labeled cells furthermore showed the necessity of dDAT function in these cells (Figure 29F). dDAT expression driven in the γ–lobes by NP1131-Gal4 (Figure 29H) failed to rescue the SCE. Taken together, the KCs that were targeted by NP1131-Gal4, c305a-Gal4 and c739-Gal4 refined the picture showing requirement of dDAT expression at the MBs, which was initially gained by comparing expression patterns of OK107-Gal4 and MB247-Gal4. Because NP1131-Gal4 did not rescue the dDAT^{fmn}/+ phenotype, the rescues of OK107-Gal4 and MB247-Gal4 were due to their expression in the αβ-lobes, and not in the γ-lobe. The combined results of the MB247-Gal4 and c305a-Gal4 rescue experiments also showed that dDAT at the α'β'-lobes is neither necessary (MB247-Gal4), nor sufficient (c305a-Gal4) to see an after-effect of cueing. In summary, expression of dDAT in the αβ-, not in the α'β' or γ-lobes is necessary and sufficient for a lasting cueing of SVA. Because unlike dDAT^{fmn}/+ flies, flies that were heterozygous for *fmn* and OK107-Gal4 lacked an ICE, it is a caveat that some properties like for example the insertion site of the OK107-Gal4 line instead of the RNAi could be responsible for the lack of ICE in the dDAT knockdown. A comparable issue emerged

for MB247-Gal4. Here the flies heterozygous for MB247-Gal4 and *fmn* also lacked an ICE, which surprisingly showed again, when knocking down dDAT with RNAi. However, animals homozygous for either line responded to the cueing like the wild-type. Also speaking in favor of the generic finding of both experiments - the importance of dDAT function at the MBs -rescue of the phenotype worked with c739-Gal4, which has an expression pattern that is included in MB247-Gal4 as well as in OK107-Gal4. Here the heterozygous flies showed an ICE.

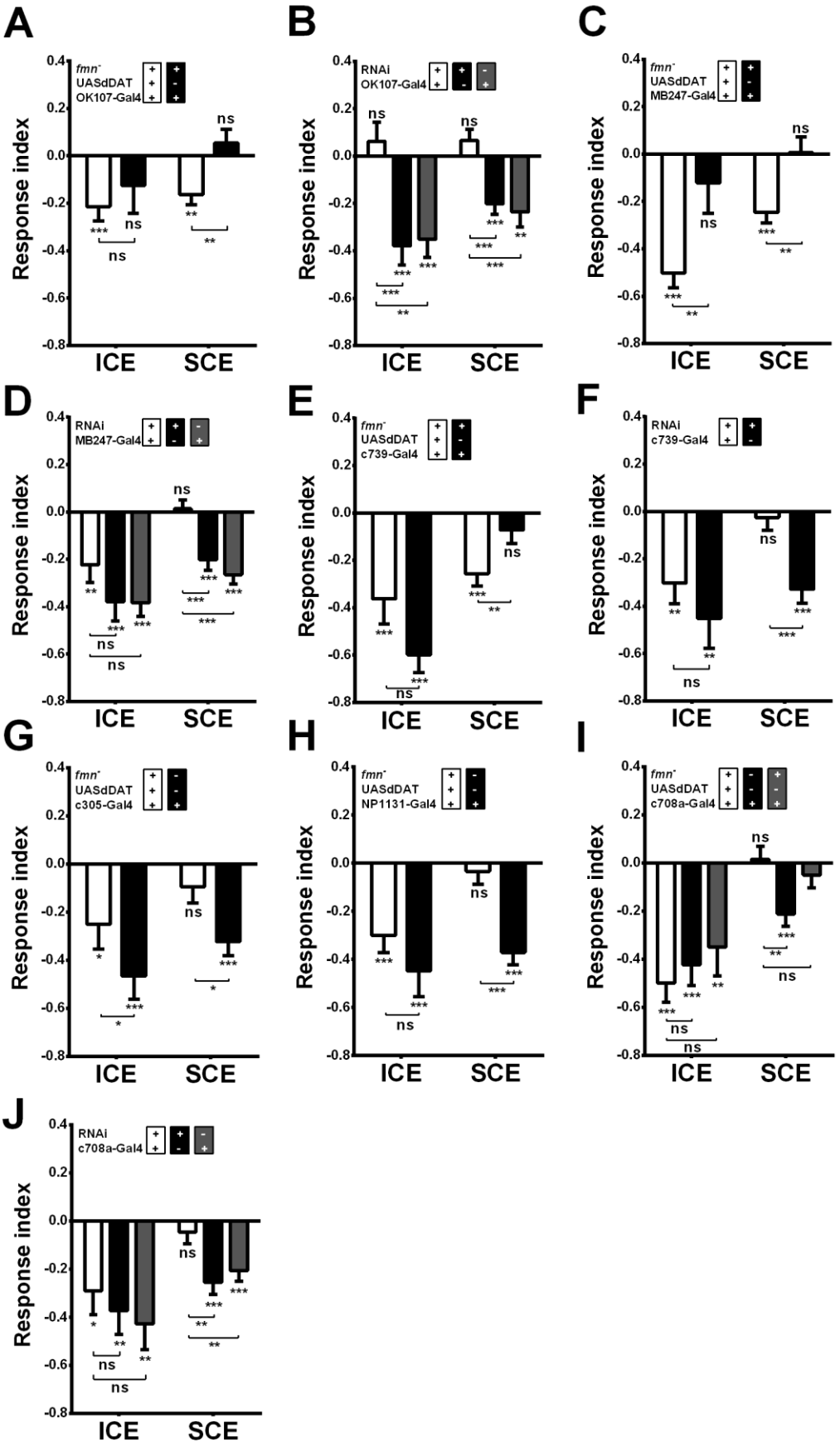
A
fmn-
UASdDAT
OK107-Gal4
B
RNAi
OK107-Gal4
C
fmn-
UASdDAT
MB247-Gal4
D
RNAi
MB247-Gal4
E
fmn-
UASdDAT
c739-Gal4
F
RNAi
c739-Gal4
G
fmn-
UASdDAT
c305-Gal4
H
fmn-
UASdDAT
NP1131-Gal4
I
fmn-
UASdDAT
c708a-Gal4
J
RNAi
c708a-Gal4
Response index
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
ICE
SCE
ns
*
**
***

Figure 29: Rescue and knockdown of dDAT function in specific mushroom body compartments. (A) OK107-Gal4 labels the complete MBs and rescues the phenotype of dDAT^{fmn}/+ flies (N = 38, 25). (B) Knockdown of dDAT function in the same set of cells abolishes ICE and SCE (N = 32, 29, 19). (C and D) Besides its only marginal expression in the α'β'-lobes, MB247-Gal4 labels the same KCs as OK107-Gal4. Providing dDAT function in these cells rescues the SCE, while knocking it down leads to a dDAT^{fmn}-like phenotype (N = 35, 17 and 47, 29, 18). (E and F) Expression of dDAT in the αβ-lobes, driven by c739-Gal4, is sufficient to rescue the phenotype. Knockdown of dDAT function in the same set of cells abolishes the SCE and demonstrates necessity of the αβ-lobes for sustained SVA (N = 24, 27 and 27, 21). (G and H) Expression of dDAT in the α'β'-lobes (c305-Gal4) or the γ-lobes (NP1131-Gal4) is insufficient to rescue the phenotype (N = 20, 25 and 26, 19). (I and J) c708a-Gal4 is expressed only in $\alpha\beta_{posterior}$ KCs. Restoring dDAT function in these cells is not enough to restore a wild-type like SCE, but knocking down dDAT function there is sufficient to cause a dDAT^{fmn}-like phenotype (N = 29, 23, 20 and 23, 21). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

c708a-Gal4 labels the KCs of $\alpha\beta_p$ exclusively. It allowed for a more detailed investigation of the sub-compartments of the αβ-lobes, amongst which the different anatomy and wiring suggest a special role for the $\alpha\beta_p$. Interestingly, a rescue in those approximately 90 cells did not restore a wild-type phenotype (Figure 29I). However, knocking down the dDAT protein in these cells abolished the SCE (Figure 29J), ascribing a significant role for cueing in SVA to this small subset of αβ KCs.

3.4.4 The dDAT^{fmn} phenotype includes free walk behavior and can be rescued in the MBs

fmn is a loss of function mutation of the dopamine transporter dDAT and leads to an augmentation of dopamine signaling. Heterozygous dDAT^{fmn}/+ flies had a reduced activity (Figure 30A), covered distance (Figure 30B), velocity (Figure 30C) and an increased number (Figure 30D) and duration of idle events (Figure 30E). Re-establishing dDAT function at the MBs driven by OK107-Gal4 not only rescued the sustained response to a cue during flight as described before, but also rescued the phenotype in the mentioned parameters.

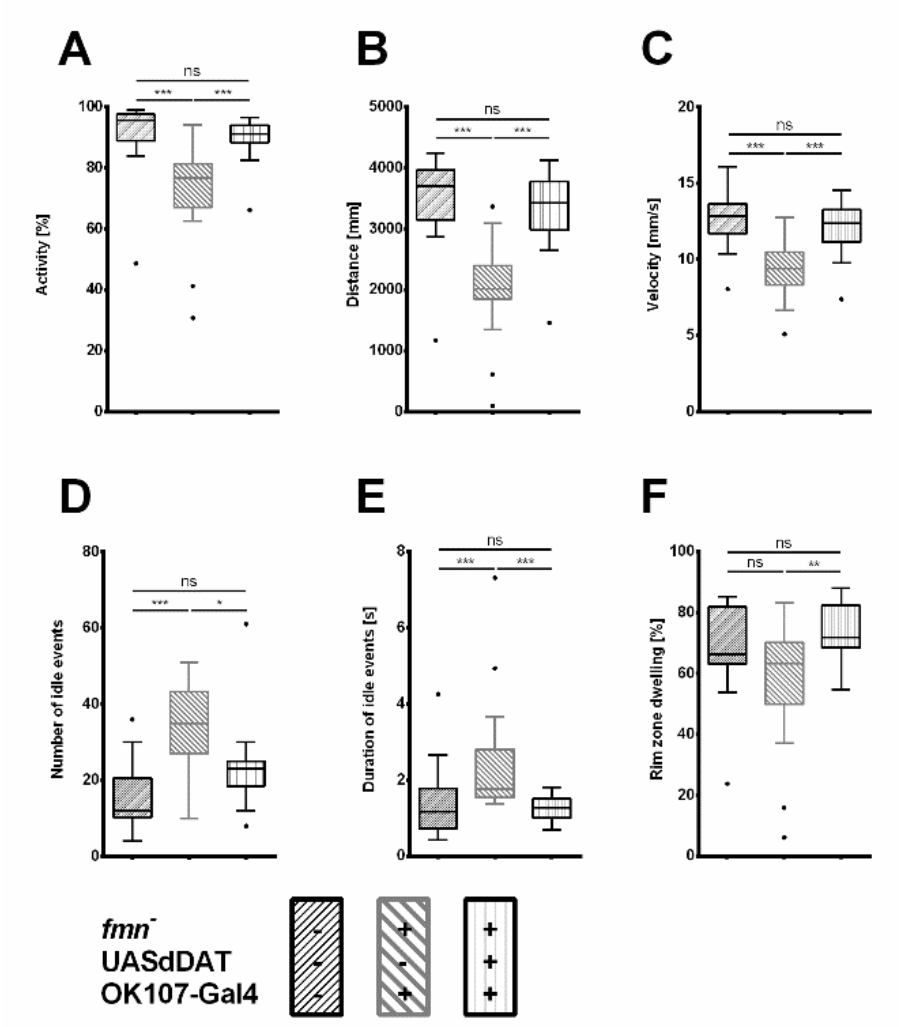

Figure 30: Analysis of dDAT^{fmn}/+ flies' walking behavior. CantonS and dDAT^{fmn}/+ flies differ with regard to the investigated parameters. In almost all cases, rescue of dDAT function in the MBs in a dDAT^{fmn}/+ background removes the difference (N = 20, 21, 17). (A) Activity. (B) Distance. (C) Velocity. (D) Number of idle events. (E) Duration of idle events. (F) Rim zone dwelling.

Analysis of positions of the flies in the arena revealed that *fmn* flies spent less, yet not significantly less, time at the periphery of the arena (Figure 30F) than the controls. Expression of dDAT in the MBs increased this duration. The summarized phenotype of *fmn* flies during free walk included less covered distance as a result of lower velocity and lower activity. The lowered activity was a consequence of more and longer idle events of the mutant flies. Finally, there was at least a trend towards a less centrophobic behavior or less wall following. The mutant phenotype could be rescued with regard to these parameters by expression of dDAT at the MBs using OK107-Gal4 as a driver line.

3.5 Attentional deficits of *rsh*^{1} flies

radish is a gene that plays an important role in the formation of anesthesia resistant memory (ARM), a memory phase which develops within hours after learning. Flies with a mutation in the *radish* gene are unable to form an aversive olfactory memory that is resistant to cold-shock induced anesthesia (Folkers

et al., 2006). Additional implication of *radish* in appetitive learning has been stated by Krashes and Waddell (2008). Whether the *radish* gene product is also involved in the modulation of long-term memory (LTM) is not finally resolved, yet [Tully et al., (1994); Isabel et al., (2004)]. *radish* is known to be highly expressed in the MBs, in particular in the αβ-lobes (Wu et al., 2013) – one of the reasons for it to show up in the scope of this study - but structure and function of the actual protein remain elusive. Chiang et al. (2004) reported identification of the phospholipase A2 gene as *radish*, but a more recent study rejected this finding and led to a retraction of the paper (Folkers et al., 2006). The *radish* sequence contains several cAMP dependent protein kinase (PKA) phosphorylation sites, which might be a link to cAMP pathways. Formstecher et al. (2005) reported binding of radish to a GTPase, which amongst others regulates synaptic morphology, thereby providing a possible mechanistic link between *radish* and learning and memory. Blockage of the αβ-lobes of the MBs, but not the γ-lobes, can reduce ARM (Isabel et al., 2004), a finding which maps behavioral data to the expression pattern of *radish*. Similar to the work here, van Swinderen et al. (2009) established a connection of the MBs and attention by rescuing attention defects in *dunce* mutant flies. *dunce* is a classical learning and memory gene, which encodes a phosphodiesterase and the authors found wild-type like performance after expression of the functional protein in the MBs of *dunce* mutant flies.

However, the main reason to investigate the role of *radish* in SVA was not only its expression in the MB sub-compartment that has been shown to be necessary and sufficient for sustained SVA with regard to dDAT expression, but also its implication in attentional processes by the work of van Swinderen and Brembs (2010). Using multiple paradigms, they consistently found clues of defective short-term choice processes. Walking in a multiple y-maze, with the floor displaying a perpendicularly moving grating, rsh^1 flies were easily visually distractible. Wild-type flies' choices were strongly influenced by the moving grating and caused them to distribute with a bias to the side the grating was moving to. Analog to this, rsh^1 flies also differed from wild-type flies in their response to two distinct fields of coherently moving circles or dots, moving in opposing directions. Instead of showing prolonged epochs of following a single field, they ended up forming a distribution, which suggested that they ignored the motion. The authors, however assigned this defect to an increased alternation rate between the usages of the two fields as reference for walking straight, as it turned out that rsh^1 flies seemed to follow one or the other motion for only very short periods of time. These results so far were gathered in walking flies, a behavioral state, which accounts for different responses as compared to flying and might therefore also contain specific defects. However, the authors moved on to investigate the mutant during tethered flight as well and thereby resembled the basic conditions provided to a fly in the setup used in this study. When given the choice between an already familiarized object and a novel one, flies tended to prefer the novel one [e.g. measured as increased fixation time by Solanki et al. (2015)]. Exploiting the novelty behavior, van Swinderen and Brembs (2010) found a modulation in the 20-30Hz frequency band of local field potentials

(LFP) in response to the novel stimulus. This particular modulation was less sustained in *rsh*^{1} flies, indicating again a defect in short-term choice processes. Another peculiarity of *rsh*^{1} flies was a peak at ~1.6Hz in the power spectrum of a Fourier analysis of yaw-torque generated during closed-loop tethered flight. This peak, termed by the authors oscillatory hyperactivity, appeared only in the presence of two conflicting stimuli. The conflict was thought to arise from the distribution of the stimuli into four quadrants of the visual field, allowing the fly to keep only one stimulus at a time in its preferred part of the visual field. Without any feedback or visual stimuli, the peak changed into a broader distribution of higher values in the power spectrum between 0.5 and 3Hz, which was not significantly different from wild-type. In summary, the *rsh*^{1} mutation, besides causing defects in ARM, led to phenotypes that can be described as hyperactivity (e.g. peak in the yaw-torque power spectrum), high distractibility (e.g. reduced response to a moving grating in a y-maze in the presence of a distractor) and increased alternations between competing stimuli (e.g. shortened LFP response to a novel stimulus). Because attention requires selection as well as suppression of stimuli, impaired short-term choice processes might be a manifestation of impaired attentional mechanisms.

3.5.1 Analysis of *rsh*^{1} yaw-torque

There are at least two distinct and prominent ways how hyperactivity might be reflected in yaw-torque histograms. It might show as larger amplitudes, which would use a broad spectrum of yaw-torque values, resulting in wide histograms. In contrast, it is also plausible to assume, that hyperactivity may cause flies to produce small amplitudes at a high frequency, resulting in a narrow yaw-torque histogram. The latter seemed to be the case (Figure 31A). Unfortunately, there are many combinations of intermediate parameters for frequency and amplitude of yaw-torque modulation that can lead to identical yaw-torque histograms, thus the information regarding composition of frequencies and amplitudes of yaw-torque modulation within a yaw-torque histogram was ambiguous and limited. What the data showed, however, is that *rsh*^{1} flies only had a weak modulation of yaw-torque, even though in principle they were capable of producing the same amount of yaw-torque as wild-type flies did, measured in form of an optomotor response (Figure 31B). A Fourier analysis, which provides means to decompose a signal into its basic oscillations, revealed that the mutant flies did not differ from wild-type with regard to the frequencies that underlay their yaw-torque. The power spectrum of the yaw-torque produced during epochs with stationary stripes was indistinguishable for *rsh*^{1} and CantonS (Figure 31C). A peak at ~1.6Hz, as described in van Swinderen and Brembs (2010) was not found in the data.

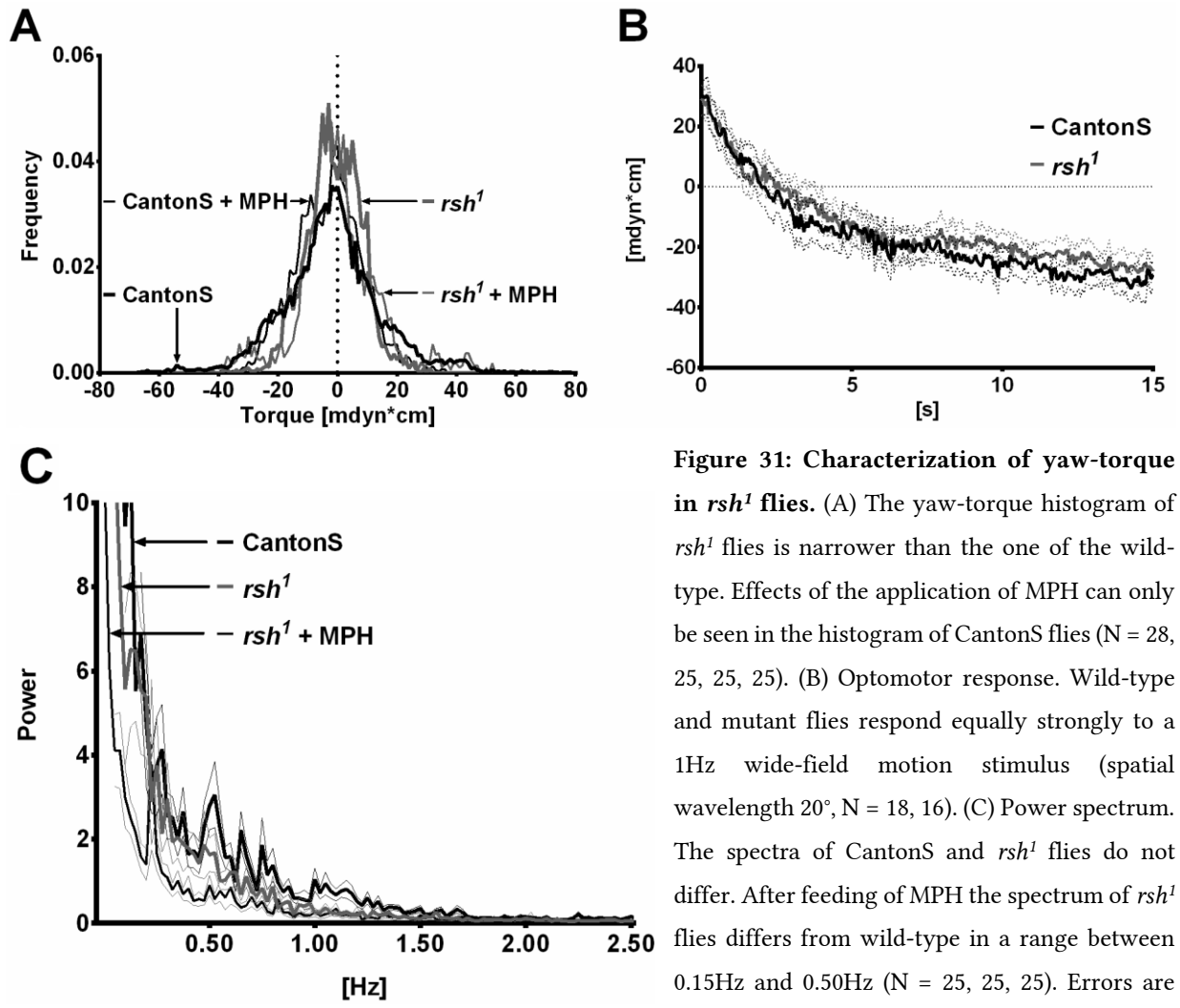

Figure 31: Characterization of yaw-torque in *rsh*[1] flies. (A) The yaw-torque histogram of *rsh*[1] flies is narrower than the one of the wild-type. Effects of the application of MPH can only be seen in the histogram of CantonS flies (N = 28, 25, 25, 25). (B) Optomotor response. Wild-type and mutant flies respond equally strongly to a 1Hz wide-field motion stimulus (spatial wavelength 20°, N = 18, 16). (C) Power spectrum. The spectra of CantonS and *rsh*[1] flies do not differ. After feeding of MPH the spectrum of *rsh*[1] flies differs from wild-type in a range between 0.15Hz and 0.50Hz (N = 25, 25, 25). Errors are SEM.

3.5.2 *rsh*[1] flies are impaired in SVA

Throughout the range of experimental conditions *rsh*[1] flies most often had no significant ICE (Figure 32). What was consistently missing was a SCE. Due to the lack of a reliable UAS construct, it was not possible to genetically address the role of *rsh*[1] in the MBs specifically. van Swinderen and Brembs (2010) presented compelling evidence, that feeding flies with MPH rescued defects in selection/suppression dynamics in those flies. As already explained before, they attributed the defects to attention-like mechanisms in the fly brain. To validate this interpretation, several pharmacological rescue experiments were performed. Speaking in favor of defects in basic attentional mechanisms in *rsh*[1] flies, they showed mutant phenotypes in independent studies (van Swinderen and Brembs, (2010) and present study), and in both cases these phenotypes could be ameliorated by application of MPH via food (Figure 32, '*rsh*[1] + MPH').

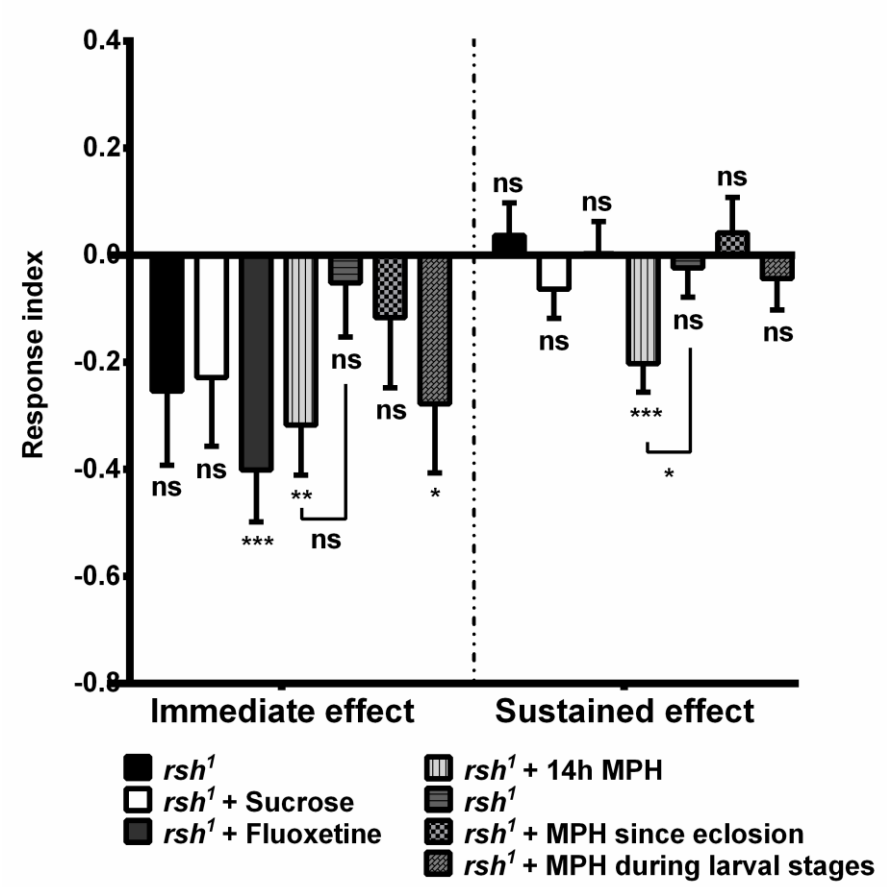

Figure 32: Pharmacological manipulation of the *rsh*[1] phenotype. Flies with a mutation in the *radish* gene are not susceptible to cueing. After 14h treatment with the serotonin re-uptake inhibitor Fluoxetine or the dopamine re-uptake inhibitor MPH, these flies respond to cueing again, but only 14h of feeding the adults with MPH rescues the after-effect of cueing (N = 21, 24, 28, 21, 25, 20, 24). All error bars are SEMs (*P < 0.05, **P < 0.01, ***P < 0.001).

Mutant flies that were exposed to MPH behaved much like the wild-type in terms of having a significant ICE, which was slightly higher than the also significant SCE. Interestingly, *radish* seemed to be differently involved in the phenotypes seen as a narrower yaw-torque histogram and a lack of cueing, because MPH did only change the shape of the histogram of CantonS, but not of *rsh*[1] flies (Figure 31A). Astonishingly, MPH did alter the power spectrum of yaw-torque of *rsh*[1] flies, but in a way that it after application differed from that of CantonS (Figure 31C). Nevertheless, it seemed that increased dopamine signaling could compensate for the yet to be discovered reason for the attentional impairment of *rsh*[1]. But maybe the course for these defects was already set earlier during development. As a matter of fact, Calcagno et al. (2013) described a critical window during larval development, during which altered dopamine signaling could lead to permanent impairment of arousal states in the adult. To test for potentially permanent effects of MPH treatment on ICE and SCE, some flies were chronically exposed to food enriched with MPH throughout larval life and others for all of larval and adult life until they were tested in the paradigm. In both cases there was no rescue of the SCE. This was intriguing, because it suggested a negative effect of chronic in comparison to temporally limited MPH application. Feeding of the drug for 14h prior to the experiment did rescue the phenotype, but either the prolonged exposure during the adult stage or during larval stage or maybe an interaction of both prevented a successful rescue (Figure 32, '*rsh*[1] + MPH since

eclosion'). MPH exclusively during larval development did not rescue the phenotype either. Under these conditions the ICE was significant, which it had not been in the untreated mutant flies (Figure 32, '*rsh*^{1} + MPH during larval stages'). The absolute values for both groups differed only very little, though, so that this finding merely did more than to attribute statistical significance to a trend, that could already be seen in the untreated *rsh*^{1} flies. Amphetamine-like stimulants like MPH interfere with the serotonergic system, although they mainly act on the dopaminergic system and their affinity for binding 5-HT receptors or transporters is very weak (Gatley et al., 1996). Nevertheless, based on this finding Gainetdinov et al. (1999) conceptualized a role of serotonin in ADHD. This posed the question, whether it could have actually been the effect of MPH on the serotonergic system that rescued the phenotype. To test this, flies were fed for 14h with the 5-HT re-uptake inhibitor Fluoxetine (Wood et al., 1999). As a result, *rsh*^{1} flies expressed a strong ICE, but again no SCE (Figure 32, '*rsh*^{1} + Fluoxetine'). This finding suggested that the effect of MPH on the SCE in *rsh*^{1} flies is mediated via the dopaminergic, rather than the serotonergic system. Finally, there is evidence that in *rsh*^{1} larvae defects in aversive olfactory conditioning can be compensated by feeding the animal sucrose 1h prior to the experiment (Thum A., personal communication). With the idea, that a brief exposure to sucrose might be able – possibly via the connection of reward and the dopaminergic system – to also rescue the cued shifts of attention, adult *rsh*^{1} flies were treated accordingly before testing. With ICE and SCE being comparable to untreated *rsh*^{1} flies the procedure turned out to be ineffective in this paradigm (Figure 32, '*rsh*^{1} + Sucrose').

3.5.3 The *rsh*^{1} phenotype includes free walk behavior

Addressing the question of hyperactivity in *radish* mutant flies by observing walking behavior proved that *rsh*^{1} flies were indeed hyperactive in comparison to CantonS flies (Figure 33A). Because both groups of flies walked with the same average velocity (Figure 33C), the higher activity allowed *rsh*^{1} flies to cover a greater distance than CantonS (Figure 33B). Interestingly, MPH did not only rescue the attentional deficit that became apparent after cueing the FoA, but also the hyperactivity phenotype in freely walking flies and as a consequence also reduced the covered distance. The latter effect may have been based partially on the reduced velocity (Figure 33C, *rsh*^{1} vs. *rsh*^{1} + MPH), albeit this reduction was not enough to become significant in comparison to CantonS. Analog to this, MPH treatment increased the number of idle events only in comparison to *rsh*^{1} flies. CantonS and *rsh*^{1} flies did not differ significantly in this parameter (Figure 33D).

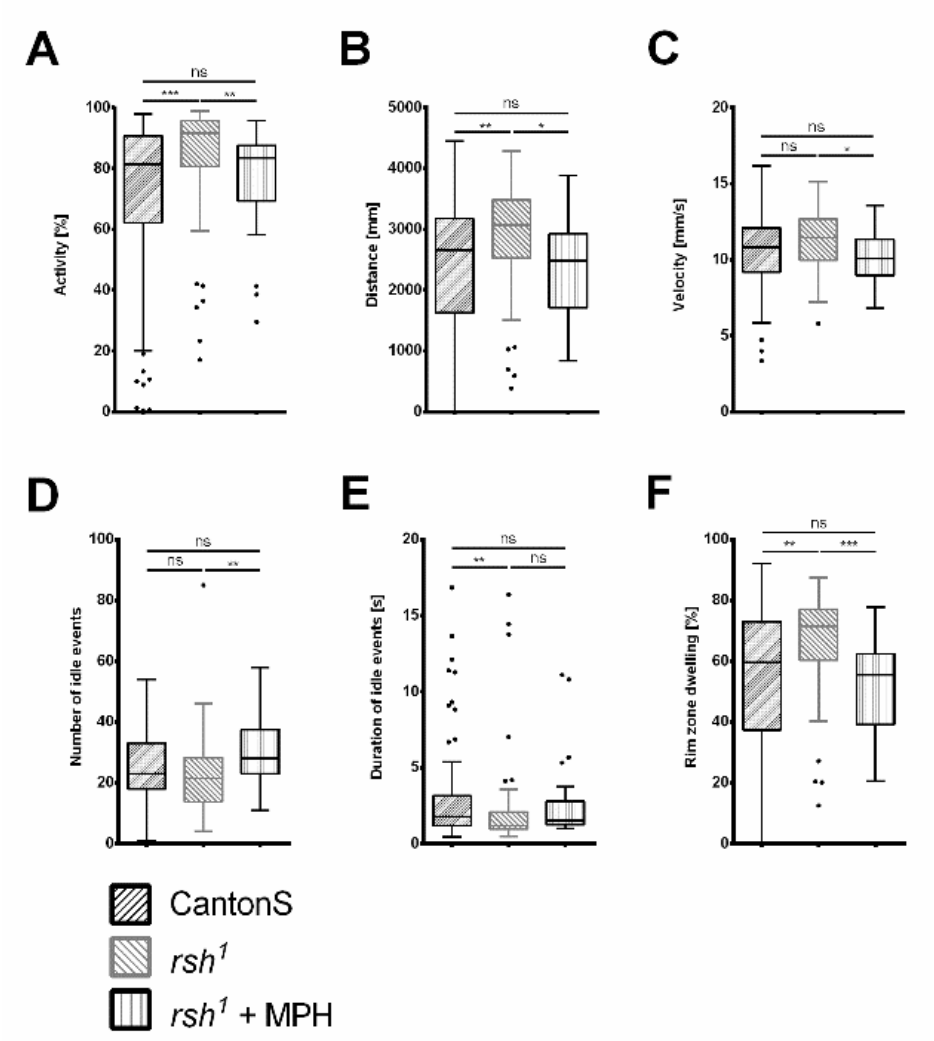

Figure 33: Analysis of *rsh*[1] flies' walking behavior. CantonS and *rsh*[1] flies differ with regard to the investigated parameters. Often, treatment with MPH removes the difference (N = 83, 58, 25). (A) Activity. (B) Distance. (C) Velocity. (D) Number of idle events. (E) Duration of idle events. (F) Rim zone dwelling.

Nevertheless, in both cases the MPH treatment countered a weak trend in the data. A similar effect of MPH on *rsh*[1] flies could be observed for the duration of the idle events. *rsh*[1] flies made shorter stops than the wild-type (Figure 33E) and feeding of MPH ameliorated but did not significantly increase this duration in comparison to *rsh*[1] while at the same time it removed the difference to wild-type. Finally, a strong effect was seen with regard to the distribution of positions across the arena (Figure 33F). *rsh*[1] flies spent a great portion of time at the periphery of the arena, while CantonS and *rsh*[1] + MPH flies did so for less time of the experiment. It is a striking finding that the phenotype caused by a mutation in the *radish* gene was contrary to the phenotype of a defect in the dDAT. This became apparent in activity, covered distance and the duration of idle events. In the other measured parameters trends in opposite directions were found and the rescues in these cases countered the trends.

3.6 ICE and SCE – two distinct effects

The majority of findings presented here showed a striking similarity with regard to the relation of ICE and SCE. With only a few exceptions, the ICE was stronger than the SCE (Figure 34).

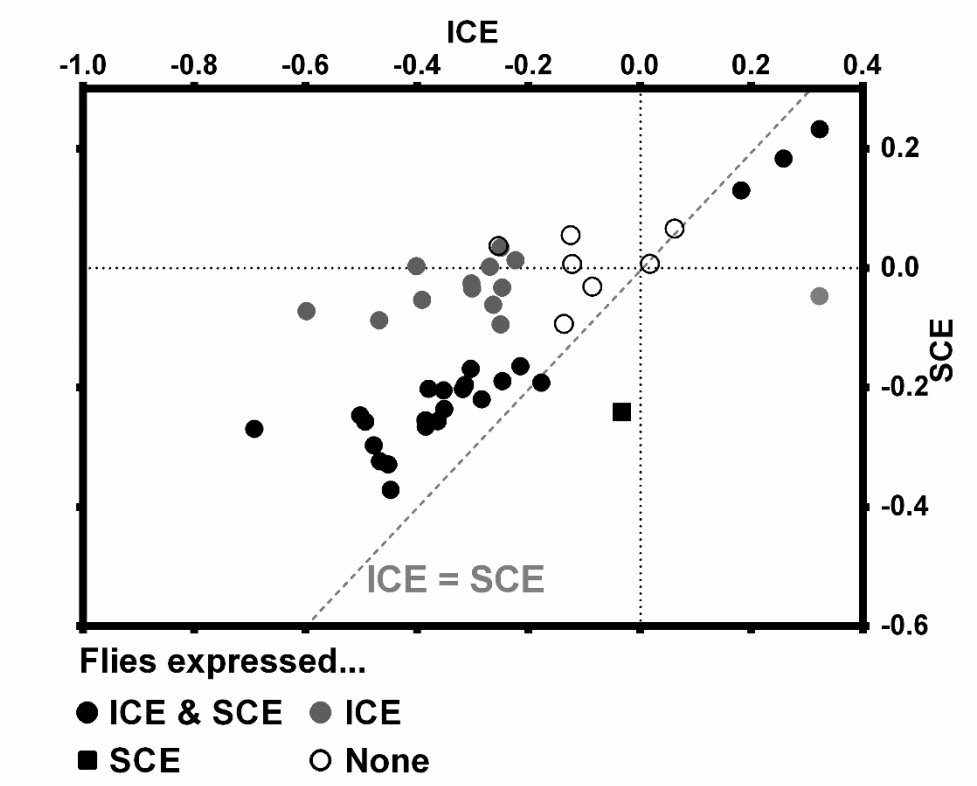

Figure 34: ICE and SCE scatterplot of response indices of various experiments. The data include experiments with wild-type and genetically as well as pharmacologically manipulated flies. In most cases SCE and ICE occur together. After manipulation sometimes the SCE is lost, but the ICE remains. Often both effects are lost. In only one single experiment a SCE is found without ICE.

This decline in strength of the cueing effect was still visible, if one looked at the data at a finer resolution, comparing the values of ICE and PCP 1s versus the values of PCP 1s, PCP2s and PCP 3s (Figure 19). There was a pronounced decline between the first two values and a rather weak one between the second, third and fourth value. Thus, the difference between ICE and SCE was not solely due to the overall decline. Instead, it pointed out that the effect of cueing was especially strong when there was no pause between cue and displacement. Adding a pause decreased the after-effect, but the pause duration did not influence it strongly. Besides in absolute values, ICE and SCE did also differ in responsiveness to pharmacologic and genetic treatment. It became apparent, that except in one experiment, there were no results with an SCE exclusively. In plenty experiments the flies displayed an ICE and SCE, an ICE only or even no SVA at all. Obviously defects in ICE were either more severe or generic, such that they at the same time

abolished the SCE. In contrast, several pharmacologic and genetic rescue and knockdown experiments proved, that the SCE could be manipulated without affecting the ICE. Both effects require a response to the cueing, but only the SCE requires a stability of this response over a certain period of time and thus possibly relies on some sort of working memory. This feature is unique to the SCE and could serve as an explanation for the finding that only the SCE could be addressed separately, while manipulation of the ICE almost every time affected the SCE, as well. The result of the experiment, which tested negative cueing in the UVF does not fit to that explanation. Assuming that the ICE represents a fly's ability to express SVA, one would expect no SCE without ICE. Because this is the only finding of an independent SCE, it might be a false negative and one can assume that SVA was not compromised in these flies. However, some factor in the environment may have caused the flies not to express it.

3.7 Levels of dopamine and serotonin in CantonS, *rsh*^{1} and dDAT^{fmn} flies

In the scope of this study dopamine and its involvement in attention was investigated. Thus, dopamine levels in fly heads were measured via HPLC in the three lines CantonS, *rsh*^{1} and *fmn* and also after treatment with MPH of CantonS and *rsh*^{1}. As mentioned earlier, there is some evidence that manipulation of dopamine can also affect the serotonergic system [see Kapur and Remington, (1996) for humans, Chaouloff et al., (1987) for rats and Gainetdinov et al., (1999) for mice]. To exclude serotonergic side-effects, serotonin levels were also measured. All lines showed no significant deviation in serotonin levels from the CantonS control (Figure 35B). Interestingly, also no significant differences in dopamine levels could be found in fly heads (Figure 35A).

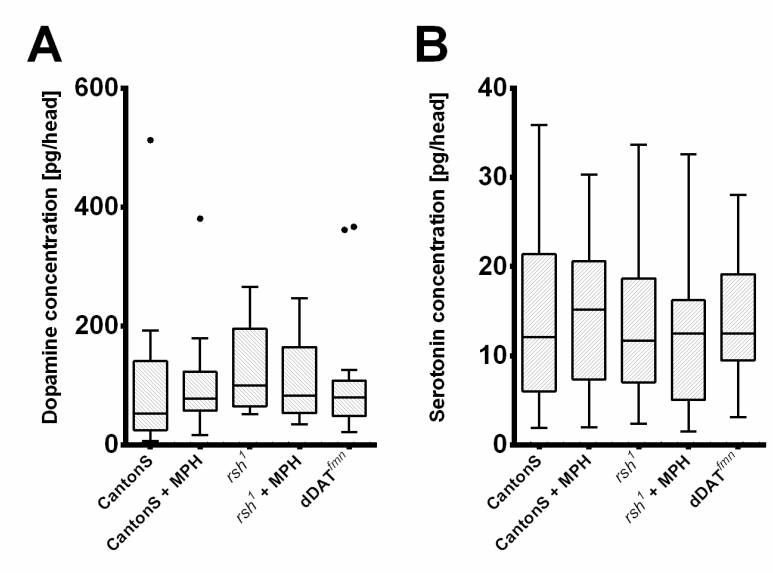

Figure 35: Manipulation of the dDAT does not alter systemic dopamine or serotonin levels. (A) The systemic dopamine levels in CantonS, *rsh*^{1} and dDAT^{fmn} flies do not differ significantly (N = 21, 18, 23). MPH inhibits the re-uptake of dopamine and leads to behavioral phenotypes, but does not change the dopamine level systemically (N = 22, 18). (B) No difference can be seen in the systemic serotonin levels of the tested flies.

Because neither pharmacological nor genetic manipulation of the dopamine transporter (i.e. MPH and dDAT^{fmn}) led to altered overall dopamine levels, it can be assumed that only the temporal and spatial properties of dopamine signaling were altered and led to the observed phenotypes. However, with the same rationale effects on 5-HT signaling can not be excluded either. As shown before, the defect in *rsh*^{1} flies was susceptible to MPH, which increases dopamine signaling. Hence, it seemed plausible to expect lowered levels of dopamine in these flies. This was not the case. Analog to the wild-type, again application of MPH did not alter the systemic dopamine levels. Because SVA was nevertheless impaired in dDAT^{fmn}, *rsh*^{1} and CantonS + MPH flies, in conclusion the observed phenotypes could be ascribed to an altered local signaling.

4 Discussion

It is actually impossible in theory to determine exactly what the hidden mechanism is without opening the box, since there are always many different mechanisms with identical behavior.

Valentino Braitenberg, Vehicles: Experiments in synthetic psychology.

4.1 Cued shifts of attention

4.1.1 Earlier findings

SVA is a significant component of vision in *Drosophila*. The task to explore it starts with a description of its behavioral manifestations. Eventually, its characterization may serve as a foundation to find possible similarities and common phylogenetic origins of visual attention in insects and mammals. In flies SVA has been studied entirely during stationary flight (Wolf and Heisenberg, 1980). Sareen et al. (2011) introduced the present paradigm focusing on external cueing. They spatially and temporally separated the cueing and test stimuli, thereby excluding alternative explanations to SVA, like interference between cue and test. Besides showing the possibility to guide shifts of attention they also started a first approach to measure how long the after-effect of cueing persisted. In the course of that study, various interesting findings appeared, which might help to understand the diversity of results gained in this study. It is in this regard probably helpful to start with a conclusion the author made: '[…] even slight changes in the experimental conditions have major effects on the responses of the flies.' The results of the present work lead to the same conclusion. Besides the fact that in principle cueing works reliably, the details about which variables determine the quality of cue or the distribution of responses between two stripes and how they do so remain yet elusive.

In line with the present data Sareen (2011) found that the cue only shifted the distribution of responses in favor of one stripe, because the overall response rate stayed the same in comparison to responses without cueing. In response to the stimuli, so called landing responses (which can also be observed, even if a fly is not flying and should thus better be called collision avoidance responses, CAR) occurred, i.e. the fly lifted its forelegs above the head and lowered the other legs. The author stated that the percentages of CARs for responses towards and away from the side of cue were similar to the ones, when both stripes were displaced without prior cueing. They were also similar to the percentage of CARs after syn-directional responses when only one stripe was displaced. That supports the claim that responses towards and away from the side of cue in the 'Both' conditions are the same kind of response but with opposite sign and that they are different from the anti-directional responses in the 'Single' conditions. CAR occurrence in the 'Both' condition also proved that stimuli outside of the FoA were still processed. In contrast to the yaw-torque response, which followed the motion of one stripe, the CAR required evaluation of both stripes.

Sareen analyzed the different requirement of the UVF and LVF for cueing, and found cueing to be effective in the LVF only. However, the results were gained with a set of specific parameters. The displacement had to take place at a certain azimuth, the height of the arena was not allowed to be increased and the contrast was also not allowed to be inverted. If these parameters were changed, the responses towards a single stripe in the LVF were no longer balanced and the syn-directional ones prevailed. Interestingly, in the present study a higher response rate to a single stripe was found compared to Sareen (2011). But if the

author inverted the contrast, the response rate was strongly increased and the difference between the two experiments was reduced. When the author displaced a single stripe in the UVF, responses were predominantly syn-directional. However, cueing of the stripe strongly reduced the syn-directional responses. This showed, that even though in general findings like the lack of influence of the cue on the overall response rate can be reproduced in various experimental conditions, there are still conditions, in which the opposite is true. Further studies will be needed to understand how a fly perceives a cue and to identify the variables that change the quality of a cue for it. It is also not understood yet, why reportedly a flicker stimulus was sufficient to elicit cueing, but an even stronger flicker stimulus with a slightly lower frequency was not. It is unknown, how such a highly salient stimulus could escape the attentional system.

Making use of the positive cueing conditions in the LVF, Sareen (2011) found that cue and test could be spatially separated by at least 20°, which is indicative of a horizontal size of 40° of the FoA. Transfer of the cueing effect from the UVF to the LVF did not work. Also the stripes, which were later on displaced needed to be continuously visible to the fly to carry the cueing effect. Cueing was effective for various azimuthal positions, but could not be tested at lateral positions >74°, because then responses to a single stripe displacement were no longer balanced and anti-directional responses prevailed. In summary, cueing seems to be a key feature of SVA, but detailed knowledge about the crucial features of a cue and their perceptual consequences is still lacking.

The present study extended this research with a modified version of the setup used in Sareen et al. (2011). Even though unintended, experimenters might bias data due to a subconscious influence of expectancy (Sackett, 1979). To address this issue the classification of responses was changed from manual to automated response detection. Additionally, the speed of stimulus presentation as well as data storage was increased. The setup was then used to gain more insights into the structure and properties of the yaw-torque responses and to start dissection of the neuronal architecture of SVA in flies. As described before, the simultaneous front-to-back displacement of two stripes challenged SVA. As the vector sum of the motion was zero, one might have expected the flies not to respond at all. Also in the new setup, this was not the case. Instead the flies responded to either one of the stripes as if it was the only stimulus present. By this selection/suppression mechanism a basic definition of attention was met.

It is assumed that the response polarity is set by shifts of the focus of attention (FoA) to either one or the other side of the visual panorama. At this point, however, mutually inhibiting networks of central pattern generators (CPGs) for left or right turns could have also produced the behavior. If so, the responses should have been distributed stochastically. As shown in chapter 3.1 they were not, thus the idea of shifts of the FoA underlying these restricted responses was supported. But attention serves more than just to avoid the activation of behaviorally incompatible responses. In humans it is known that objects within the FoA are preferentially and in more detail processed and it will be an important task for future studies on the

subject of SVA to find alterations of processing within the FoA in flies, too. It is assumed, that the fly always responded to the stripe within its FoA. The ability to bias response frequencies towards one side by means of a cue showed that the FoA can be guided.

4.1.2 Properties of cueing

Finding positive as well as negative cueing (i.e. towards the side of the cue or away from that side) revealed a more elaborate internal choice process than initially expected. One can speculate that the cue was perceived after it appeared but did not immediately attract the FoA. The lack of observable cueing effects after a short cueing duration supports this idea. In a next step a decision would have been made on where the FoA would be established or moved to. This idea is intriguing, because if the decision about the relocation of the FoA were an internal one and under the control of the fly, this would suggest that bottom-up modulation of attention were not a separate process. Instead, eventually the response to external cues would be a top-down modulation of attention. It could thus be away from or towards the side of the cue or even suppressed. However, there might also be no decision at all, as the quality of the cue in combination with the internal state of the fly could already determine how the cue is perceived and where the FoA will be shifted to.

An intermediate step of decision making was especially suggested by the new finding of negative cueing. Even though negative cues exerted influence on the response (i.e. the stimulus received attention), also in the case of very short durations of the cue no positive bias in the response distribution was observed. Two explanations come to mind. Either the short cue did move the FoA, but the response was only activated once the FoA had been shifted to the other side. Or, a short cue did not leave enough time for the shift of the FoA to take place and the cued stripe was never highlighted by the FoA. For instance, in macaques it has been shown that the allocation of endogenous attention requires time [120ms to see a response to a cue in the recording of single neurons, which levels off after additional 70ms, Busse et al., (2008)].

Once the FoA was established on one side it remained there for 3-4s even after the cue was no longer present. A direct interaction of cue and test could thus be excluded. Also, this finding contradicted the model of mutually inhibiting CPGs, because the FoA was established before the response, i.e. before the activation of a CPG took place. The idea of an intermediate stage of decision making was substantialized by the fact that especially in the case of positive cueing there were different effects of cueing with two stripes spanning both the upper and lower visual field (UVF, LVF) compared to cueing only in the UVF or LVF. A cueing effect in both visual hemispheres was observed, even though cueing exclusively in the LVF or the UVF was not effective. The responses of the fly could not simply be explained as a summation of UVF and LVF effects alone, but the fly rather evaluated the situation discretely and answered it specifically.

In general, cueing worked in both visual half fields, but some of its properties could be found in the LVF exclusively. Different prerequisites for attentional tasks in the LVF and UVF were also subject to discussion in studies about human attention [Carrasco et al., (2001); Fecteau et al., (2000); Kraft et al., (2007)], because the outcome of experiments strongly depends on the paradigm and stimuli used. Some studies proposed that shifts of attention are more effective in the UVF [e.g. Danckert and Goodale, (2003); Previc, (1990)]. The advantage of the LVF in *Drosophila* is probably due to the different environmental demands of flies and humans. Predators like robber flies and dragon flies usually attack from below and also objects of interest like food or spots to land on can be found in the LVF during flight. The LVF in human vision is peripersonal (Previc, 1990), which means it contains the visual space below eye level where reaching and grasping movements are performed. It is thus specialized for processing visual information for the control of one's own actions. The extrapersonal space (i.e. the UVF) however includes the visual scenery and is thus better adapted to tasks which require attentional selection mechanisms like for example visual search.

Generally, it is important to note that a cue never shifted the response ratio all the way. Finding the same overall frequency of responses in experiments with and without cueing showed that the cueing only modulated the frequency of occurrence of responses towards one or the other side. This suggests two possible interpretations. Either, for a certain stimulus condition the cue was always negative or always positive but it was recognized only in part of the trials. Alternatively, in what has been called positive or negative cueing, the cues on a trial to trial basis were ambiguous and could have had an attractive or repulsive effect. In both cases, the observed effects of cueing would not lead to one response type (towards or away from the side of cue) exclusively, but rather shift the response ratio in favor of the side of the cued or not cued stripe. With the available data it remains hidden, which of the possibilities resembles the implementation of cue evaluation in the fly brain.

Taking a closer look at the effects of cueing, namely ICE and SCE, revealed that they were differently affected by the visual stimuli as well as by the genetic and pharmacological manipulations. Usually, the ICE was stronger than the SCE and – with one single exception – there was no SCE without ICE. Most likely the exception represents a false negative finding where the ICE was existent, but for some unknown reason was not expressed as a bias in the distribution of responses. All the other data show that ICE and SCE are separate processes and at the same time suggest that the ICE represents the flies' ability to apply SVA, whereas the SCE is a specific feature of that SVA. Whenever the ICE was abolished, SVA could not be observed at all. In contrast, a lack of SCE was not necessarily coupled to a lack of ICE or SVA. Other than the ICE, the SCE occurs after the cue has already ceased, but is still in reference to the cue. Hence it requires the cued side to be stored in a short working memory, which is likely to reside in the MBs (see chapter 3.4).

Looking more closely at the yaw-torque patterns of syn- and anti-directional responses revealed fundamental differences between the two. Syn-directional responses were some kind of object response where the fly possibly interpreted the motion of the stripe as unintended self-rotation and elicited a phasic yaw-torque spike to counter-balance this disturbance. They could easily be distinguished from anti-directional ones by their slope of the rising phase, amplitude and latency until onset. Anti-directional responses were much faster, had a less steep slope and a smaller amplitude. They resembled body saccades in the latter two parameters. Taken together, anti-directional responses presumably were escape or startle responses as a consequence of the displacement, possibly because the FoA had not been at the displaced stripe at the onset of the displacement. The responses towards or away from the side of cue were characterized by the same properties as syn-directional responses.

Finally, the data indicate that stimuli which are outside the FoA are still being processed to some extent in *Drosophila*. Two findings lead to this assumption. First, flies occasionally showed collision avoidance responses (CARs; i.e. they lifted their forelegs) in addition to a phasic yaw-torque response. These CARs are typically elicited by looming stimuli, like in this case the two stripes moving front-to-back. Obviously, the fly ignored one stripe in terms of response polarity, but still responded to both in terms of the CAR. The optomotor pathways for the two kinds of responses are not yet separated at the level of the retina and lamina (Rister et al., 2007), thus the selection/suppression mechanism of visual attention must either operate at a later stage in stimulus processing or be behaviorally selective. Second, in the 'One of two' condition the majority of responses was syn-directional. Based on experiments where two stripes were displaced without prior cueing one would have expected the FoA to be on each stripe equally often. As a consequence, the FoA would have been expected to reside on the side of the stationary stripe in 50% of the tests and thus the syn-directional responses should have been fewer than in the 'Single' condition. They were not, showing that even though the displaced stripe was likely to be outside the FoA in many cases, the fly still responded to it. This finding is in line with the anecdotal observation that in cases of sensory deprivation a fly often responds to whatever stimulus it can get. Sometimes this reveals formerly unknown properties of fly behavior, which remain concealed for the cursory observer. Here it provides a further example showing that the fly is not blind outside the FoA.

4.1.3 Dopamine signaling

This study aims at improving our understanding of SVA. After dealing with the 'what' and 'how' by describing and analyzing some of the dynamics and properties of the guidance of the FoA, questions about circuits and mechanisms arise. The neurotransmitter dopamine is known to be involved in mammalian attention [Nieoullon, (2002); Swanson et al., (2007); van Swinderen and Andretic, (2011)]. Sareen (2011) tested flies with defects in the dopaminergic system for SVA related phenotypes, but could not get any results as the flies did not produce a sufficient amount of yaw-torque responses in the paradigm.

Interestingly, flies with the same mutation but a more wild-type like eye color, as well as different lines with mutations associated with the dopaminergic system performed fine in the recent setup. They proved the involvement of dopamine in SVA in flies, which in turn helped to reveal similarities between the basic principles of visual attention in mammals and *Drosophila*. First, dopamine levels need to be precisely regulated for effective guidance of the FoA. Either to little or too much dopamine signaling led to a loss of wild-type like responses to the cueing and sometimes to a loss of SVA at all. Too much signaling can be achieved by inhibition of the dDAT by MPH or Desipramine or by a loss of function mutation in the *fumin* gene which codes for dDAT. The state of haplo-insufficiency of *fmn*, i.e. a single functional copy of the gene is not enough to warrant wild-type like dDAT function, further suggested, that the equilibrium of dopamine signaling lies within a narrow range. It is hypothesized that human ADHD is associated with an increased density of DAT and thus a reduced dopamine signaling (Swanson et al., 2007). This leads to the speculation that such an aberration might also be present in *radish* mutant flies, which profited from inhibition of the DAT by MPH. Interestingly, these flies as well as human subjects with ADHD expressed impaired performance in covert visual-spatial orienting (Nigg and Casey, 2005). The same inhibition by MPH or Desipramine in wild-type flies shifted the dopamine signaling out of the normal range and caused a mutant phenotype in SVA. Creating an imbalance towards the opposite side via inhibition of dopamine synthesis or a mutation in the DopR1 also led to a mutant phenotype.

4.1.4 Towards a localization of the SVA network in the brain

The specific level of dopamine signaling which seems to be required for normal SVA does not have to be maintained ubiquitously in the brain. Avoiding excessive signaling at a substructure of the MBs, the αβ-lobes, was sufficient and necessary for the sustained guidance of SVA. In olfactory conditioning another part of the MBs, the γ-lobes, are known to contribute to short-term memory [Isabel et al., (2004); Zars, (2000)], but are also important for visual memories (Vogt et al., 2014). The authors suggested them as a possible center for visual attention. The results shown in this study reject this hypothesis, as a rescue in the γ-lobes did not rescue the maintenance SVA in dDAT^{fmn}/+ flies. This does not rule out the possibility, that the γ–lobes might be involved in other forms of visual attention.

A substructure of the αβ-lobes, $\alpha\beta_p$, seems to be important for SVA. So far, not much is known about the role of $\alpha\beta_p$. The approximately 90 cells can be distinguished by their different anatomy e.g. a mesh-like arrangement of their fibers in the lobes [Aso et al., (2014); Tanaka et al., (2008)]. Pai et al. (2013) and Perisse et al. (2013) found that blocking these cells led to an impairment in 24h olfactory memory retrieval, while for 3h olfactory memory, aversive as well as appetitive, they were dispensable. In the visual task here, despite a failed phenotypical rescue via dDAT expression specifically in these cells, a phenotype caused by a knockdown of dDAT proved their crucial role in upholding SVA. Because they bypass the main olfactory input center of the MBs, the calyx, and terminate in the accessory calyx, they are a promising

candidate for involvement in SVA, because they might receive visual input, which is obviously needed for the task. Their small number could have been insufficient to shift the level of dopamine into the functional range in the dDAT^{fmn}/+ flies, thereby explaining the failed rescue within these cells.

4.2 Attention span

4.2.1 The duration might vary in a natural environment

The selectivity of responses of *Drosophila* in the presence of two equally salient stimuli represents ongoing selection/suppression mechanisms in the fly. Analysis of their dynamics revealed an attention span that consolidates the finding of spatially selective visual attention. Lacking external cues, flies endogenously shifted their FoA during stationary flight at the torque-meter. The position of the FoA could be derived from the polarity of responses, because the flies presumably responded to the stripe which was within their FoA. Once it was shifted to one side it stayed there for several seconds. The flight simulator provides some of the natural feedback conditions a fly would perceive during free flight, but it does not fully resemble free flight. Trapped at the torque-meter, the fly is presumably in a state of stress. Still, it has been shown extensively that flies can easily learn to control the visual panorama in closed-loop with various sets of behaviors (Wolf et al., 1992). But besides yaw-torque a fly would normally also make use of roll and pitch while navigating through 3D space, movements which are prevented in this setup. In addition, it lacks the mechanosensory feedback of the halters. The lack of this feedback can be observed as an attenuation of its wing steering range (Bartussek and Lehmann, 2015). The experiments in this study were performed in open-loop, meaning that the fly's response to the displacement additionally failed to produce the expected visual feedback. Due to these reasons the attention span in *Drosophila* in free flight under more natural conditions might vary from the <5s reported here. But why is an attention span useful at all? It takes time to detect and evaluate the behavioral relevance of a motion stimulus at a certain position in the visual field. The experiments in chapter 3.2.4 suggested that the initial detection of a stimulus does not necessarily need to be combined with the establishment of the FoA. But once the FoA is on one side, further information on the stimulus is more likely to be gathered at the same spot than elsewhere in the surround. Therefore, the fly kept its FoA at this location either to scrutinize the area or to wait for something else to happen. The small number of distractors in the uniformly lit arena might have favored the long duration of the measured attention span. However, neither did this nor the lack of visual feedback of yaw-torque modulation lead to a habituation of the response rate. In the course of the 60 displacements the flies' motivation to answer the stimuli seemed to be constant as the no response rate did not increase.

4.2.2 Another measurement of an attention span

Some years ago van Swinderen (2007) used a different approach to measure an attention span in *Drosophila*. He recorded local field potentials (LFP) and computed power spectra of a small frequency

range. The fly was tethered and two visual patterns were rotated around it at 0.33Hz. When he introduced a novel pattern, the power spectrum peaked at a certain frequency range whenever the pattern was in the frontal part. No such peak could be detected for the familiar pattern. This effect was said to show the allocation of the fly's attention to one or the other pattern. The average number of rotations the fly continuously showed the same preference (3-4) was defined as the attention span. Converted to seconds he suggested a relatively long attention span of 9-12s.

How does this fit to the shorter duration of the attention span found here? A rotation of the patterns with 0.33Hz leads to a reappearance of a pattern in the frontal part of the visual field every 3s. In the framework of this study's experiments this can be translated to a protocol with an ITI of 3s, because in both cases the stimulus attracting attention was refreshed every 3s, either by a displacement or by reappearance. Differences arise in the definition of the attention span. Here it was measured as the time during which the fly maintained its FoA at a certain position without any further stimuli. During the 9-12s suggested by van Swinderen (2007), the stimulus was refreshed 3 to 4 times. Looking at the data from this angle, finding several cycles of sustained increased LFP responses for one of the patterns is in line with the present finding of an attention span of 4-5s. Regarding the average length of chains (~2) observed here as the number of stimulus refreshments, one needs to multiply it with the 4-5s of the attention span to make both definitions logically comparable. In conclusion, both paradigms used slightly different ways to define the attention span, but arrived at comparable results when logics are adjusted. Nevertheless, future experiments should aim at consolidating the interpretation of changes in LFPs as an attentional mechanism. However, the requirements of the kind of visual attention investigated by van Swinderen (2007) were distinct from those of SVA. There was no need for the FoA to be shifted within the visual field to give an attention span. van Swinderen and Brembs (2010) also reported the implication of visual attention in experiments with flies walking through a multiple y-maze. But these experiments again had different requirements, e.g. they did not involve pattern recognition and novelty choice. This is important, because different forms of visual stimuli might recruit different forms of visual attention in *Drosophila* (e.g. spatial, feature-based or object-based attention). Before speculating about possible relations or interactions of those forms, further research should be undertaken to characterize them separately. It will also be crucial to find modifications of visual processing which are modified in accordance with the position of the FoA in the visual field and which persist as well.

Furthermore, it will be exciting to learn more about the attention span besides its duration. Can it be influenced by properties of the stimulus like size or shape? Where in the brain are its neuronal substrates – are the MBs part of it? It reveals that the fly has a tendency to repeat a response polarity as long as the interval between the two tests is shorter than 5s. Together with the finding that the FoA could be guided, this supports the hypothesis that the responses to the test stimulus on one side of the visual field are

associated with the position of the FoA. In comparison to the single stripe experiments, the pattern of responses when two stripes were displaced showed a longer phase of returning to base line. This was possibly caused by the fact that rare startle responses (i.e. anti-directional ones) could not be distinguished from normal responses during analysis. Thus, their typical yaw-torque pattern modified the shape of the average responses, increasing the duration it took the average yaw-torque to reach base line again.

4.3 Attentional phenotypes transfer to walking behavior

Attention deficit and hyperactivity disorder (ADHD) is a human disease which symptomatically connects attention and hyperactivity. It is commonly treated with MPH ('Ritalin'). The drug failed to rescue the attention span phenotype in *rsh*^{1} flies during tethered flight, but it effectively cured the cueing defects of these flies. In closed-loop experiments van Swinderen and Brembs (2010) found a frequent switching of *rsh*^{1} flies between different patterns at different positions in the visual field. Wild-type flies tended to do so less often. They traced this behavioral hyperactivity back to an oscillatory hyperactivity (~1.6Hz) in the power spectrum after a Fourier analysis of yaw-torque traces. As part of a second experiment they rotated two different patterns around the fly in open-loop and used sustained changes in LFP associated with a novel pattern as an indication of an attention span. *rsh*^{1} flies had a significantly shortened attention span in this paradigm (see also chapter 3.1.6). Application of MPH did reset the oscillatory hyperactivity of *rsh*^{1} flies to wild-type levels, but – similar to the results described in this study - failed to rescue the defects in attention span. In search of hyperactivity in *rsh*^{1}, in this study the flies were tracked during free walk in a small arena. *fmn* flies and rescues of dDAT function in a dDAT^{fmn}/+ background in the MBs as well as pharmacological manipulations of the dopaminergic system were also tested to see, if a connection between attention, dopamine and hyperactivity generalized. Because they had the same amount of pauses, however significantly shorter ones, *rsh*^{1} flies showed increased activity and covered a longer distance than wild-type. The hyperactivity of these flies can thus not only be seen in the assumed attentional mechanisms within the brain, but it also manifested in locomotor behavior. It is tempting to assume a causality, but so far the results do not allow such conclusions.

Interestingly, MPH was able to fully reset the activity to wild-type levels by increasing the number of pauses. It is a challenge that some ambiguity in the data remains. The rescue was sometimes significantly different from the test group, but not from the control, while control and test did not differ. Trends in the data led to the expectation that an increase of tested animals would most likely remove those incongruities. The effect of MPH on the wild-type was comparable to its effect on *rsh*^{1} flies. It reduced activity, but different from *rsh*^{1}, in CantonS flies the number of pauses remained unaltered while their duration increased. The decrease in activity as well as the reduced velocity both contributed to the reduction of covered distance. Explorative behavior was affected differently. After being put into the unfamiliar environment *rsh*^{1} flies spent significantly more time at the outer parts of the arena than the

wild-type did. MPH led to loss of such centrophobism or wall-following behavior (Soibam et al., 2012) in *rsh*^{1} flies but did not affect this behavior in the wild-type.

Besides MPH, also αMT affects the dopaminergic system. It blocks the synthesis of dopamine and limits the overall amount of systemic dopamine. One might therefore expect it to have contrary effects on behavior. This was not the case. Both, augmentation of dopamine signaling and a reduction of overall dopamine levels similarly influenced behavior. The finding seems puzzling at first glance, but in fact it nicely fits with the findings in chapter 3.3, which also support the idea of an inverted U-shaped relation of dopamine levels and function. The susceptibility of *rsh*^{1} flies to augmentation of dopamine signaling via MPH and its wild-type like levels of systemic dopamine suggest a reduced base level of dopamine signaling in the mutant. The *fmn* flies possibly represent the opposite signaling defect. The experiments in this study are a first approach towards an understanding of how the attentional phenotypes influence other behaviors like walking. It will be fruitful to study this in more detail to unravel potential causalities and underlying mechanisms. *fmn* and *rsh*^{1} will be of particular interest, as their phenotypes during free walk are to a large extent opposite. Perhaps a gradual increase of dopamine signaling in *rsh*^{1} flies could first rescue their defects, but eventually lead to a *fmn* like phenotype. On the other hand, feeding MPH to *fmn* flies should not rescue, but rather exacerbate the phenotype.

This study showed that expression of dDAT in the MBs of *Drosophila* can rescue the defects of dDAT^{fmn}/+. This is an exciting finding, because the mechanisms underlying the flies' attentional phenotype as well as their walking phenotype seem to overlap not only in the neurotransmitter and protein, but also spatially. Further experiments are required to see, if the αβ-lobes are also crucial for the walking task. One would also have to exclude locomotor defects as a possible source of explanation, as the MBs are well known to be involved in locomotion (Martin et al., 1998). The observed aberrations of walking behavior, specifically the decrease in activity in *fmn* flies seem to contradict an earlier study (Kume et al., 2005), which found more activity bouts in those flies than in the wild-type. However, in a more recent study (Ueno et al., 2012) they revised their findings and reported fewer long lasting rest bouts, but no alterations in activity bouts. They attributed the initial false result to the tracking system, which offered only gross temporal resolution. For the new experiments they resorted to a new way of tracking flies. As a consequence, the results of their study and of this study are shedding light on different aspects of behavior of *fmn* flies. Here the flies were tracked for 300s at 40Hz, there for 24h at 1Hz. Presumably due to the considerably shorter observation time the modifications of long rest bouts did not show in this study, but at the same time it offered analysis at high temporal resolution. Thus, the micro-behavior of *fmn* flies turns out to be hypoactive at high temporal resolution, whereas these flies were hyperactive in the range of days. This conclusion needs to be further substantiated, because two types of experimental setups were used. Future

studies should make use of the best of both and track the flies' behavior at a high temporal resolution for long durations.

5 Synopsis

Building on earlier studies this study characterized spatially selective visual attention in *Drosophila*. It confirmed the finding that shifts of the FoA can be externally guided by visual cues. As shown, shifts of the FoA are independent of yaw-torque. However, Tang et al. (2004) provided evidence that the position of the FoA is nevertheless coupled to yaw-torque. Taken together these results suggest that the FoA can be moved independently of yaw-torque, but once a fly turns in one direction the FoA is more likely to be shifted towards this side. In other words, the FoA follows yaw-torque, but yaw-torque does not follow the FoA.

The present study contributed a determination of the duration of the after-effect of cueing, which lasts up to 4s. A new experimental setup provided the basis for a detailed investigation of yaw-torque responses of the fly. It was shown that response polarity is identical to the motion direction of the displaced stripe. Analysis of yaw-torque revealed that responses away from the displaced stripe represent a different kind of behavior, probably fast escape attempts. It needs to be mentioned, that similar to Sareen (2011) this study finds the specific properties of the fly behavior in response to the displacements strongly depending on the quality of the cue and the location of the stripe. The guidance and maintenance of SVA has many different aspects. It becomes evident across a wide range of parameters. This study gives first evidence that a cue can not only be attractive but also repellent to the FoA. Linking the necessity of proper regulation of dopamine signaling to a sub-compartment of the mushroom bodies and possibly to only 90 Kenyon cells within, which are required to maintain SVA, opens a door for future research. It can provide new insight into the mechanisms and presumably the connection patterns required to equip the fly with a complex ability like SVA.

On the account of supporting the concept of SVA in relatively simple organisms like *Drosophila* and even relating it to human SVA, this study also discovers an attention span in spatially selective visual attention. The *radish* gene, which has been linked to attentional mechanisms before (van Swinderen and Brembs, 2010), leads to a shortened attention span when defective (rsh^{1}) and rsh^{1} flies are impaired with regard to cued shifts of attention. This indicates a possible overlap of the mechanisms and circuitry for internal and external modulation of visual attention. However, at this point it needs to be made clear that the attention span and the after-effect of cueing are not to be confused. The cue is a signal from the outside world, which might dis- and re-appear. The attention span, however, is a state maintained internally by the fly. With increasing knowledge about the function of *radish* and networks involved, the gene will help to reveal characteristics and molecular substrates of SVA. Similar to human attentional deficits, the cueing

phenotype of *rsh*^{1} flies can be ameliorated by MPH. This represents another similarity between human and *Drosophila* SVA. It is intriguing to regard the latter as a rudimentary version of the first. Of course, if one takes a closer look differences arise. But this is not surprising, because the different visual systems and ecological requirements may have shaped SVA in humans and *Drosophila* to fit the respective needs. In flies one can now get at the underlying physiological and circuit mechanisms.

Especially in the visual modality attention is a crucial ability to select the relevant from the irrelevant. The consequences of this selection affect features like learning and memory, choice behavior and novelty choice. To see, whether the effects on SVA observed in this study are task specific, the experimental conditions were changed from stationary flight to freely walking. Flies that had mutant phenotypes during flight also expressed aberrations during walking. Rescues and pharmacological treatments which were successful during flight did also work during walking. Because these manipulations affected the mushroom bodies, which are known to be involved in the regulation of locomotion, further controls are required to pinpoint the observed walking phenotypes to impaired SVA. Just like sleep, learning or fear, SVA needs to be described via its influence on behavior. These experiments are a first glimpse to see, how attentional deficits generalize.

6 References

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7 Summary

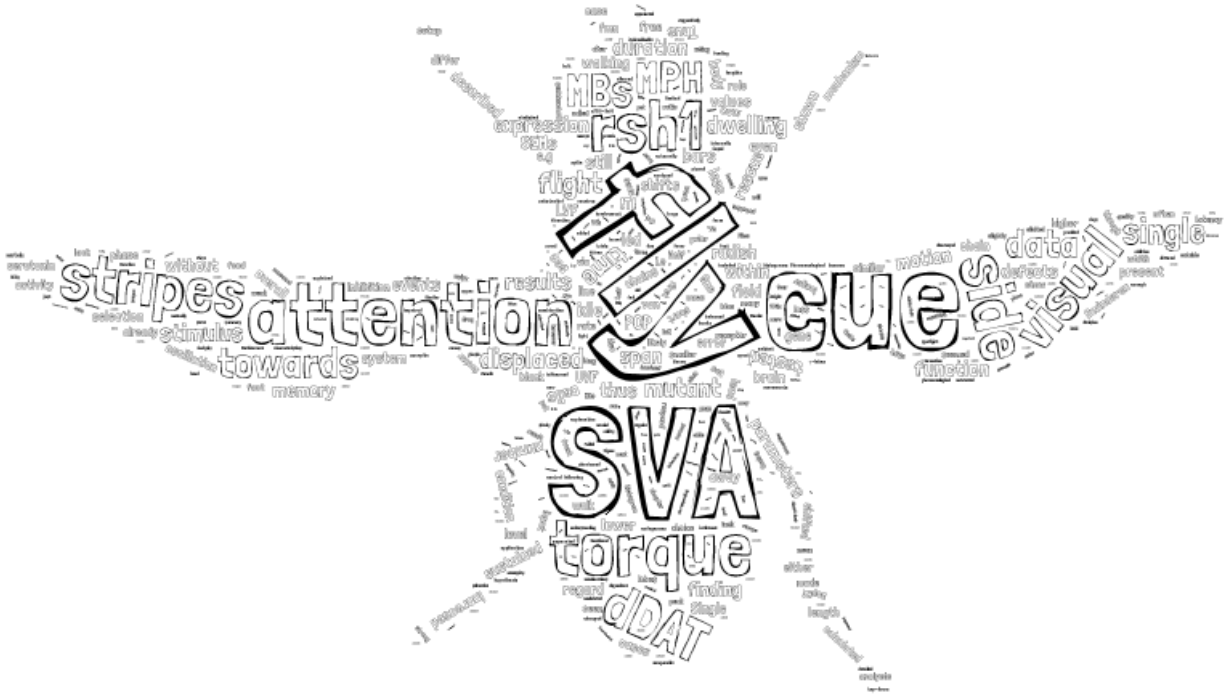

Figure 36: Graphic summary. Boldness and size of words indicate the frequency of occurrence in this study and in consequence their importance for the description of selective visual attention in *Drosophila*. Association and arrangement of headwords is of reader's choice and may hence not fully resemble the author's conclusion. Thus, it is recommended to additionally read the written summary.

Finding the right behavior at the right time is one of the major tasks of brains. In a natural scenery there is often an abundance of stimuli present and the brain has to separate the relevant from the irrelevant ones. Selective visual attention (SVA) is a property of higher visual systems that achieves this separation, as it allows to '[…] focus on one source of sensory input to the exclusion of others' (Luck and Mangun, 1996). There are probably several forms of SVA depending upon the criteria used for the separation, such as salience, color, location in space, novelty, or motion. Many studies have investigated SVA in humans and non-human primates. However, complex functions like attention were initially not expected to be already implemented in the brains of simple organisms like *Drosophila*. After a first demonstration of selective attention in the fly (Wolf and Heisenberg, 1980), it took some time until other studies included attentional mechanisms in their argumentation to explain certain behaviors of *Drosophila*. However, their definition and characterization of attention differed and often was ambiguous.

Here, one particular form, spatially selective visual attention in the fly *Drosophila* is investigated. It has been shown earlier that the fly spontaneously may restrict its behavioral responses in stationary flight to the visual stimuli on one side of the visual field. On the basis of experiments of Sareen et al., (2011) it has been conjectured that the fly has a focus of attention (FoA) and that the fly responds to the visual stimuli within this area of the visual field. Whether the FoA is the adequate concept for this spatial property of SVA in the fly needs to be further discussed and is a subject also of the present study. At this stage, the concept will be used in the description of the new results expanding the characterization of SVA.

This study continued the investigation of SVA during tethered flight with variable but controlled visual input and an automated primary data evaluation. This standardized paradigm allowed for analysis of wild-type behavior as well as for a comparison of several mutant and pharmacologically manipulated strains to the wild-type. Some properties of human SVA like the occurrence of externally as well as internally caused shifts of attention were found in *Drosophila* and it could be shown, that SVA in the fly can be externally guided and has an attention span. Additionally, a neurotransmitter and proteins, which play a significant role in SVA were discovered. Based on this, the genetic tools available for *Drosophila* provided the means to a first examination of cells and circuits involved in SVA. Finally, the free walk behavior of flies that had been shown to have compromised SVA was characterized. The results suggested that the observed phenotypes of SVA were not behavior specific.

Covert shifts of the FoA were investigated. The FoA can be externally guided by visual cues to one or the other side of the visual field and even after the cue has disappeared it remains there for <4s. An intriguing finding of this study is the fact, that the quality of the cue determines whether it is attractive or repellent. For example a cue can be changed from being repellent (negative) to being attractive (positive) by changing its oscillation amplitude from 4° to 2°. Testing the effectiveness of cues in the upper and lower visual field separately, revealed that the perception of a cue by the fly is not exclusively based on a sum of its specifications. Because positive cueing did not have an after-effect in each of the two half-fields alone, but did so if the cue was shown in both, the fly seems to evaluate the cue for each combination of parameters specifically. Whether this evaluation of the cue changed on a trial-to-trial basis or if the cue in some cases failed to shift the FoA can at this point not be determined.

Looking at the responses of the fly to the displacement of a black vertical stripe showed that they can be categorized as no responses, syn-directional responses (following the direction of motion of the stripe) and anti-directional responses (in the opposite direction of the motion of the stripe). The yaw-torque patterns of the latter bared similarities with spontaneous body saccades and they most likely represented escape attempts of the fly. Syn-directional responses, however, were genuine object responses, distinguishable by a longer latency until they were elicited and a larger amplitude. These properties as well as the distribution of response polarities were not influenced by the presence or absence of a cue.

When two stripes were displaced simultaneously in opposite directions the rate of no responses increased in comparison to the displacement of a single stripe. If one of the stripes was cued, both, the responses towards and away from the side of cue resembled the syn-directional responses.

Significant progress was made with the elucidation of the neuronal underpinnings of SVA. Ablation of the mushroom bodies (MB) demonstrated their requirement for SVA. Furthermore, it was shown that dopamine signaling has to be balanced between too much and too little. Either inhibiting the synthesis of dopamine or its re-uptake at the synapse via the dDAT impaired the flies' susceptibility to cueing. Using the Gal4/UAS system, cell specific expression or knockdown of the dDAT was used to scrutinize the role of MB sub-compartments in SVA. The αβ-lobes turned out to be necessary and sufficient to maintain SVA. The Gal4-line c708a labels only a subset of Kenyon cells (KC) within the αβ-lobes, $\alpha\beta_{posterior}$. These cells stand out, because of (A) the mesh-like arrangement of their fibers within the lobes and (B) the fact that unlike the other KCs they bypass the calyx and thereby the main source of olfactory input to the MBs, forming connections only in the posterior accessory calyx (Tanaka et al., 2008). This structure receives no or only marginal olfactory input, suggesting for it a role in tasks other than olfaction. This study shows their requirement in a visual task by demonstrating that they are necessary to uphold SVA. Restoring dDAT function in these approximately only 90 cells was probably insufficient to lower the dopamine concentration at the relevant synapses and hence a rescue failed. Alternatively, the processes mediating SVA at the αβ-lobes might require an interplay between all of their KCs. In conclusion, the results provide an initial point for future research to fully understand the localization of and circuitry required for SVA in the brain.

In the experiments described so far, attention has been externally guided. However, flies are also able to internally shift their FoA without any cues from the outside world. In a set of 60 consecutive simultaneous displacements of two stripes, they were more likely to produce a response with the same polarity as the preceding one than a random polarity selection predicted. This suggested a dwelling of the FoA on one side of the visual field. Assuming that each response was influenced by the previous one in a way that the probability to repeat the response polarity was increased by a certain factor (dwelling factor, df), a random selection of response type including a df was computed. Implementation of the df removed the difference between observed probability of polarity repetition and the one suggested by random selection. When the interval between displacements was iteratively increased to 5s, no significant df could be detected anymore for pauses longer than 4s. In conclusion, *Drosophila* has an attention span of approximately 4s. Flies with a mutation in the *radish* gene expressed no after-effect of cueing and had a shortened attention span of about 1s. The dDAT inhibitor methylphenidate is able to rescue the first, but does not affect the latter phenotype. Probably, *radish* is differently involved in the two mechanisms.

This study showed, that endogenous (covert) shifts of spatially selective visual attention in the fly *Drosophila* can be internally and externally guided. The variables determining the quality of a cue turned out to be multifaceted and a more systematic approach is needed for a better understanding of what property or feature of the cue changes the way it is evaluated by the fly. A first step has been made to demonstrate that SVA is a fundamental process and compromising it can influence the characteristics of other behaviors like walking. The existence of an attention span, the dependence of SVA on dopamine as well as the susceptibility to pharmacological manipulations, which in humans are used to treat respective diseases, point towards striking similarities between SVA in humans and *Drosophila*.

8 Zusammenfassung

Eine der Hauptaufgaben eines Gehirns ist es, das richtige Verhalten zur richtigen Zeit zu finden. In einer natürlichen Umgebung gibt es eine Vielzahl visueller Reize, die das Gehirn unterteilen muss in solche, die irrelevant und solche, die bedeutsam sind. Selektive visuelle Aufmerksamkeit (SVA) ist eine Eigenschaft hoch entwickelter visueller Systeme, die diese Unterteilung erzielt, indem sie es erlaubt „[…] eine Quelle sensorischen Inputs zu fokussieren und dabei andere auszuschließen“ (Luck and Mangun, 1996). In Abhängigkeit der Kriterien (z.B. Salienz, Farbe, Lage im Raum, Neuartigkeit oder Bewegung), die für die Aufteilung herangezogen werden, existieren wahrscheinlich mehrere Formen von SVA. Viele Studien haben sich mit SVA in Menschen und in Primaten beschäftigt, ohne jedoch zu erwarten, dass eine komplexe Funktion wie Aufmerksamkeit bereits in den Gehirnen von einfachen Organismen wie *Drosophila* implementiert zu finden. Erst einige Zeit nachdem selektive Aufmerksamkeit ein erstes Mal in der Fliege gezeigt worden war (Wolf, Heisenberg, 1980) begannen auch andere Studien Aufmerksamkeit in ihrer Argumentation als Erklärung für bestimmte Verhaltensweisen von *Drosophila* heranzuziehen. Definition und Charakterisierung des Begriffes Aufmerksamkeit waren jedoch oft mehrdeutig und unterschieden sich von Studie zu Studie.

In dieser Arbeit wird eine ganz bestimmte Form von Aufmerksamkeit – räumlich selektive visuelle Aufmerksamkeit - anhand der Fliege *Drosophila* untersucht. Es wurde bereits gezeigt, dass die Fliege im stationären Flug ihre Verhaltensantworten spontan auf visuelle Reize einer Seite des visuellen Feldes beschränken kann. Basierend auf Experimenten von Sareen et al. (2011) wurde vermutet, dass die Fliege einen Aufmerksamkeitsfokus (FoA) besitzt und auf Reize, die innerhalb dieses Teils des visuellen Feldes liegen antwortet. Ob der FoA ein angemessenes Konzept für diese räumliche Eigenschaft von SVA in der Fliege ist, steht zur Debatte und ist auch ein Thema dieser Studie. Vorerst soll dieses Konzept jedoch für die Beschreibung der Ergebnisse, die die Charakterisierung von SVA vorantreiben, genutzt werden.

Die vorliegende Arbeit führt die Untersuchung von SVA mit variablem aber kontrolliertem visuellem Input im stationären Flug fort und nutzt dazu eine automatisierte Datenerfassung. Dieses standardisierte Paradigma ermöglicht eine Analyse von Verhalten im Wildtyp aber auch einen Vergleich mit verschiedenen mutanten und pharmakologisch manipulierten Fliegenstämmen. Einige im Menschen auftretende Eigenschaften von SVA wurden auch in *Drosophila* gefunden. Dazu zählt das Auftreten von extern und intern verursachten Aufmerksamkeitsverlagerungen. Es konnte gezeigt werden, dass SVA in der Fliege extern gelenkt werden kann und eine Aufmerksamkeitsspanne aufweist. Zusätzlich wurden ein Neurotransmitter und einige Proteine entdeckt, die eine wichtige Rolle in SVA einnehmen. Darauf basierend ermöglichten es die verfügbaren genetischen Werkzeuge mit einer ersten Untersuchung der an SVA beteiligten Zellen und Netzwerke zu beginnen. Des Weiteren wurde das Laufverhalten von Fliegen,

die Einschränkungen in SVA aufwiesen charakterisiert. Die Ergebnisse lassen vermuten, dass die beobachteten Phänotypen von SVA nicht verhaltensspezifisch sind.

Als nächstes wurden interne Bewegungen des Aufmerksamkeitskegels (FoA) betrachtet. Der FoA kann durch visuelle Reize von außerhalb zu der einen oder der anderen Seite des visuellen Feldes gelenkt werden. Er verweilt dort für >4s nachdem der lenkende Reiz verschwunden ist. Es ist ein spannender Befund dieser Arbeit, dass dieser Reiz in Abhängigkeit seiner Beschaffenheit abstoßend oder anziehend sein kann. So kann ein abstoßender (negativer) Reiz auf einmal anziehend (positiv) werden, wenn seine Oszillationsamplitude von 4° auf 2° reduziert wird. Eine Überprüfung der Wirksamkeit von Aufmerksamkeitslenkung durch Reize im oberen und unteren Teil des visuellen Feldes ergab, dass die Wahrnehmung eines Reizes durch die Fliege sich nicht ausschließlich aus der Summe seiner Spezifikationen ergibt. Da positive Aufmerksamkeitslenkung in keinem der beiden Halbfelder einen Nacheffekt hatte, ein solcher aber bei der Präsentation von Reizen in beiden Felder gleichzeitig auftrat, kann vermutet werden, dass die Fliege den Reiz für jede Kombination von Parametern spezifisch bewertet. Ob sich diese Bewertung in jedem einzelnen Durchgang änderte oder ob der Reiz in manchen Fällen den FoA nicht auf eine Seite lenkte kann mit dem jetzigen Kenntnisstand nicht bestimmt werden.

Betrachtet man die Antworten der Fliege auf eine Versetzung eines schwarzen vertikalen Streifens, so zeigt sich eine mögliche Unterteilung in die Kategorien „keine Antwort“, „syn-direktionale Antwort“ (der Bewegungsrichtung des Streifens folgend) und „anti-direktionale Antwort“ (entgegengesetzt zur Bewegungsrichtung des Streifens). Die Drehmomentmuster der letzteren Kategorie wiesen starke Ähnlichkeit zu spontanen Körpersakkaden auf und es handelte sich bei ihnen sehr wahrscheinlich um Fluchtversuche der Fliege. Syn-direktionale Antworten waren hingegen reine Objekt-Bewegungsantworten, erkennbar an einer längeren Latenz bis zu ihrer Auslösung und einer größeren Amplitude. Diese Eigenschaften und auch die Verteilung der Antworten auf die beiden Kategorien wurden durch die An- oder Abwesenheit eines vorhergehenden Reizes nicht beeinflusst. Wurden zwei Streifen gleichzeitig gegenläufig versetzt, so blieben die Antworten im Vergleich zur Versetzung eines einzelnen Streifens häufiger aus. Wurde der FoA zuvor auf eine Seite gelenkt, so entsprachen die Drehmomentmuster der Antworten auf diese Seite und auch die der Antworten auf die andere Seite denen der syn-direktionalen Antworten.

Die Aufklärung der SVA zu Grunde liegenden neuronalen Strukturen konnte bedeutend vorangetrieben werden. Eine Ablation der Pilzkörper (MB) zeigte, dass diese für SVA benötigt werden. Außerdem konnte gezeigt werden, dass die von Dopamin übermittelte Signalstärke weder zu stark, noch zu schwach sein darf. Wurde die Synthese von Dopamin inhibiert oder seine Wiederaufnahme aus dem synaptischen Spalt mittels dDAT blockiert, führte dies dazu, dass die Aufmerksamkeit dieser Fliegen nicht mehr extern gelenkt werden konnte. Mithilfe des Gal4/UAS-Systems und zellspezifischer Expression oder

Unterdrückung der Bildung von dDAT wurde die Rolle einzelner Strukturen der Pilzkörper in SVA genauer untersucht. Es zeigte sich, dass die αβ-Loben sowohl ausreichend als auch notwendig sind, um SVA nachhaltig zu lenken. Die Gal4-Linie c708a markiert einen Teil der Kenyonzellen (KC) innerhalb der αβ-Loben, $\alpha\beta_{posterior}$. Diese Zellen sind besonders, da (A) ihre Fasern innerhalb der Loben eine netzartige Anordnung aufweisen und (B) da sie anders als die anderen KCs nicht mit der Kalyx, der größten Quelle olfaktorischen Inputs in die MBs, verknüpft sind, sondern nur in der posterioren akzessorischen Kalyx Verbindungen ausbilden (Tanaka et al., 2008). Diese Struktur erhält keinen oder zumindest nur marginalen olfaktorischen Input und es ist anzunehmen, dass sie eher an Aufgaben aus anderen sensorischen Modalitäten beteiligt ist. In dieser Arbeit wird die Beteiligung dieser Zellen an einem visuellen Task gezeigt, genauer ihre Notwendigkeit für einen Nacheffekt der Lenkung von SVA. Eine Wiederherstellung der Funktion von dDAT in diesen ca. 90 Zellen war erfolglos, da die geringe Anzahl möglicherweise nicht ausreichte, um die Konzentration von Dopamin an den relevanten Synapsen zu senken. Es ist jedoch auch möglich, dass die Prozesse, die SVA über die αβ-Loben vermitteln ein Zusammenspiel aller dortigen KCs erfordern. Zusammen bilden die gesammelten Ergebnisse einen Ausgangspunkt für zukünftige Bestrebungen, die für SVA erforderlichen neuronalen Strukturen und deren Verortung komplett zu verstehen.

In den bisher beschriebenen Experimenten wurde die Aufmerksamkeit extern gelenkt. Fliegen können ihren FoA aber auch ganz ohne äußerliche Reize intern verlagern. In einer Reihe von 60 aufeinanderfolgenden gleichzeitigen Versetzungen zweier Streifen zeigte sich, dass die Fliegen häufiger Antworten mit der gleichen Polarität wie die vorausgegangene produzierten, als dies eine zufällige Auswahl der Polarität vorhersagte. Dies ließ vermuten, dass der FoA auf einer Seite des visuellen Feldes verweilt. Es wurde angenommen, dass jede Antwort von der vorhergehenden beeinflusst wird, sodass die Wahrscheinlichkeit die Polarität dieser Antwort zu wiederholen um einen gewissen Faktor erhöht wird (dwelling factor, df). Deswegen wurde eine zufällige Verteilung der Antwortpolaritäten unter Berücksichtigung des df berechnet. Dadurch verschwand der Unterschied zwischen der beobachteten Wiederholungswahrscheinlichkeit einer Antwortpolarität und derer einer rein zufälligen Wahl der Antwort. Als das Intervall zwischen den einzelnen Versetzungen schrittweise auf 5s erhöht wurde, konnte bereits bei Pausen über 4s kein signifikanter df mehr festgestellt werden. Als Schlussfolgerung ergibt sich, dass *Drosophila* eine Aufmerksamkeitsspanne von etwa 4s besitzt. Fliegen mit einer Mutation im *radish* Gen zeigten keine anhaltende Lenkung von SVA und hatten zudem eine verkürzte Aufmerksamkeitsspanne von ungefähr 1s. Der dDAT-Inhibitor Methylphenidat beseitigte den zuerst erwähnten Phänotyp, verlängerte jedoch nicht die Aufmerksamkeitsspanne. Es ist anzunehmen, dass *radish* auf unterschiedliche Art und Weise an beiden Mechanismen beteiligt ist.

Im Zuge dieser Arbeit wurde gezeigt, dass endogene (covert) Verlagerungen von räumlich selektiver visueller Aufmerksamkeit in der Fliege *Drosophila* intern und extern gelenkt werden können. Vielfältige Variablen bestimmen die Beschaffenheit eines Reizes. Es bedarf eines systematischeren Ansatzes, um die Eigenschaften eines Reizes genauer zu verstehen, die dessen Wahrnehmung durch die Fliege verändern. Es konnte bereits grundlegend gezeigt werden, dass SVA ein fundamentaler Prozess ist, dessen Fehlfunktion auch die Eigenschaften anderer Verhaltensweisen wie z.B. Laufen beeinflusst. Die Existenz einer Aufmerksamkeitsspanne, die Abhängigkeit von SVA von Dopamin sowie deren Zugänglichkeit für pharmakologische Manipulationen, deren Nutzen für den Menschen in der Behandlung aufmerksamkeitsbezogener Erkrankungen liegt, deuten auf starke Ähnlichkeiten zwischen SVA in Menschen und in *Drosophila* hin.

9 Appendix

9.1 Affidavit

I hereby declare that my thesis entitled: „**Spatially selective visual attention in *Drosophila melanogaster***" is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore I verify that the thesis has not been submitted as part of another examination process neither in identical nor in similar form.

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation: „**Räumlich selektive visuelle Aufmerksamkeit in *Drosophila melanogaster***", eigenständig, d. h. insbesondere selbständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen, als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Augsburg, den 02. April 2016

_____

9.2 Curriculum Vitae

9.3 Publications and conference contributions

Publications

Koenig S, Wolf R, Heisenberg M (2016) Vision in Flies: Measuring the Attention Span. PLoS One 11:e0148208 Available at: http://dx.plos.org/10.1371/journal.pone.0148208.

Koenig S, Wolf R, Heisenberg M (2016) Visual attention in flies – Dopamine in the mushroom bodies mediates the after-effect of cueing (under review).

Conference contributions

Koenig S, Sareen P, Wolf R, Heisenberg M (2012) Selective visual attention in Drosophila – How long is the attention-span? Talk presented at the Learning and Memory Symposium, University of Konstanz, Germany.

Koenig S, Sareen P, Wolf R, Heisenberg M (2012) Selective visual attention in Drosophila – How long is the attention-span? Poster presented at the European *Drosophila* Neurobiology Conference, Padua, Italy.

Koenig S, Wolf R, Heisenberg M (2013) External guidance of the Focus of Attention. Poster presented at the Neurobiology of *Drosophila* Conference, Cold Spring Harbor Laboratory, NY, USA.

Koenig S, Sareen P, Wolf R, Heisenberg M (2013) Selective visual attention in Drosophila – How long is the attention-span? Poster presented at the Meeting of the German Neuroscience Society, Göttingen, Germany.

Koenig S, Wolf R, Heisenberg M (2014) Guidance of the Focus of Attention. Poster presented at the 79th CSHL Symposium: Cognition, Cold Spring Harbor Laboratory, NY, USA.

Koenig S, Wolf R, Heisenberg M (2014) Guidance of the Focus of Attention. Poster presented at the European *Drosophila* Neurobiology Conference, Hersonissos, Greece.

Koenig S, Wolf R, Heisenberg M (2015) Selective Visual Attention Requires Dopamine and the Mushroom Body. Poster presented at the Gordon Research Conference 'Neuroethology: Behavior, Evolution & Neurobiology', Lucca, Italy.

Koenig S, Wolf R, Heisenberg M (2015) Selective Visual Attention Requires Dopamine and the Mushroom Body. Poster presented at the Meeting of the German Neuroscience Society, Göttingen, Germany.

Koenig S, Wolf R, Heisenberg M (2015) Selective Visual Attention Requires Dopamine and the Mushroom Body. Poster presented at the conference 'Building the brain: from genes to circuits and cognition', Royal Society, London UK.

Koenig S, Wolf R, Heisenberg M (2016) Spatial Visual Attention – Cueing the Focus of Attention and Measuring the Attention Span of *Drosophila*. Talk presented at the Kolloquium der Entwicklungs- und Neurobiology, University of Mainz, Germany.

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First and foremost I want to thank my supervisor Prof. Heisenberg for the great opportunity to be part of his group. He introduced me to the world of flies, flight simulators and genetics and his support and influence are the basis of this thesis. I enjoyed the freedom to pursue my own ideas but found guidance when needed. Many insightful discussions and occasional challenges of my point of view encouraged me to often perform analysis after analysis after analysis after analysis...

I owe a lot to Reinhard Wolf, who supervised me already during my early times as a student. He raised my interest in the techniques required to investigate the rich behavioral repertoire of *Drosophila*. He also assisted my first steps into programming and after a while we mutually promoted our coding skills. I am very aware that the level of his support can not be taken for granted. Working with him was a great motivation.

I want to thank the former and present members of my lab, too. They tolerated and hopefully sometimes even enjoyed my entertainment. Only on rare occasion colleagues turn into friends and I am happy to have witnessed such a thing. I am especially grateful to Anne Haberberger, who helped me with innumerable experiments.

Konrad Öchsner and Hans Kaderschabek provided important technical support and were always available when I needed assistance. Susanne Clemens-Richter supported me with the HU experiments. Dr. Markus Krischke measured HPLC contents in fly heads and Katherina Beck spent a lot of time introducing me to antibody staining and microscopy. I am very thankful to the mentioned persons and to everybody, who supported my work. This spirit of cooperation and support is outstanding and benefits science and those working in the field. I also want to thank the Rudolf-Virchow-Center for hosting our lab.

Last but not least I am very grateful to my parents for their unconditional support. Without them I would not have been able to follow my interests in Wuerzburg. During my time there, Lara was the person who kept my life outside of work running and universally supported me.