Novelty choice in *Drosophila melanogaster*

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1. INTRODUCTION

1.1. Visual pattern recognition

To perform a task animal often must compute the sensory information from the environment. A highly sophisticated domain of sensory processing is visual pattern recognition. This is found throughout the animal kingdom from chordates like mammals to arthropods like the fruit fly *Drosophila melanogaster.* Visual images are stored as values for a set of parameters, such as size, luminance, contour length, orientation of edges, retinal position of the salient features, etc (Hertz M. 1933, Wehner R. 1975, Ronacher B. 1979 and Horridge and Zhang 1995). Visual pattern recognition in honeybee was already characterized in the first half of the 20th century (Von Frisch K: 1915, Hertz M: 1929, 1933 and Zerrahn G: 1933).

Insects often use landmarks to find an orientation or position in space. But how do they do that? One way would be to store the retinal coordinates of some of the memorized features of the scenery with respect to their own position and orientation. To find the orientation of interest the insect has to match the stored with the actual feature in a process called retinotopic matching. Wehner proposed (1969) and showed (1972) retinotopic matching to be involved in landmark learning in insects. Retinotopic matching has subsequently been postulated to be used for landmark learning in bee (Gould JL. 1985, Cartwright BA and Collett TS. 1979, 1983 and 1987) and ant (Wehner R and Räber F. 1979).

1.2. Visual pattern learning in *Drosophila melanogaster*

For the study of visual pattern recognition *Drosophila melanogaster* offers a special advantage (besides being a favorite genetic model organism). Tethered animals with head and eyes fixed in space can be trained to remember visual patterns. Most of these studies are performed in flight at a special sensor measuring the fly's yaw torque (torque meter; Fig. 1; Götz, 1964). During flight one of the sensory modalities is vision which in free flight would comprise of plants, animals, hills and mountains etc. For *Drosophila* at the torque meter these visual cues are replaced by artificial patterns. Mostly motion vision has been studied by measuring the attempted turns flies make to react appropriately to the presentation of a rotating striped drum or single stripe. The fly generates yaw torque in the same direction as movement of the patterns in an attempt to keep a straight course but

at the torque meter its yaw torque has no effect on its relative orientation with respect to the patterns. For this stimulus-response chain the feedback loop between the fly and the visual input from the world is said to be open. The behavior of the experimental animal has no effect on the stimulus condition. In open loop at the torque meter the tethered fly is unable to control the retinal movement of the visual surround.

1.3. Flight Simulator

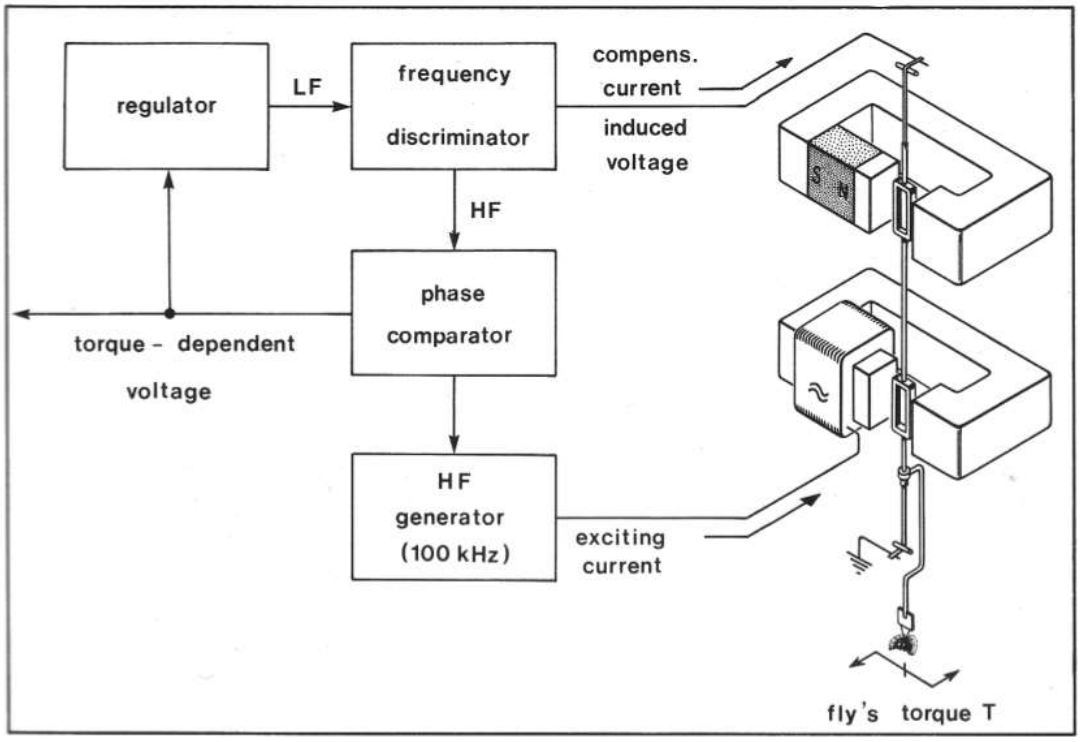


Figure 1. Torque compensator schematics. Redrawn from Götz 1964 (Heisenberg and Wolf, 1984).

The opposite of open loop is closed loop. It was an idea from Reichardt (1973; Reichardt and Wenking, 1969) to provide the fly *(Musca)* visual feedback by using the momentary yaw torque for driving the panorama so as to simulate turning in the horizontal plane in free flight. To achieve this, Reichardt had to determine the relation between the fly's yaw torque and angular velocity in free flight. He found that in free flight most of the torque is used to overcome air friction and only less than 1% is used to generate angular acceleration. To approximate this relation at the torque meter

Reichardt decided to make the fly's yaw torque inversely proportional to the angular velocity of the panorama (because of lack of air friction in tethered flight). He also calculated an optimal value for the relation between yaw torque and angular velocity (coupling coefficient, c) by calculating the air friction and the angular acceleration for a fly.

Reichardt's experimental design was used for *Drosophila* (Wolf and Heisernberg. 1990). For this the coupling coefficient had to be adjusted to fit the yaw torque and mass of *Drosophila.* The optimal coupling coefficient turned out to be ten times smaller than the natural coupling coefficient accommodating changes in fly's flight dynamics owing to the lack of mechanosensory feedback from the halters (Wolf and Heisernberg. 1990). However these artificial conditions seem not to substantially disturb the fly's visuo-motor behavior.

It has been shown that upon presentation of a single stripe or striped drum during flight in the flight simulator *Drosophila* shows optomotor balance (Wolf and Heisenberg. 1990). This is the ability of the fly to maintain a straight course irrespective of its orientation in relation to landmarks.

Flies in the flight simulator can turn towards or away from certain patterns. This means that they must recognize the presented patterns. Visual pattern recognition has also been shown in walking flies (Fischbach and Heisenberg. 1981).

1.4. Heat avoidance learning

Dill et al (1993) took the advantage of the fact that the flies could recognize the patterns for investigating their visual learning behavior. They showed that in the flight simulator flies could indeed learn to choose one of two patterns differing in the height of their centers of gravity (Dill et al. 1993, 1995). Later Ernst and Heisenberg (1999) showed that apart from height there are other pattern parameters that could be learn by the flies, such as contour orientation, vertical compactness and size. Early results had suggested that for visual pattern recognition retinotopic matching was used in *Drosophila* (1993), a postulated mechanism for pattern recognition in honeybees. The retinotopic matching concept was followed up by Tang et al (2004) who found the pattern recognition mechanism not to require retinotopic matching. They showed that flies recognize patterns even if these are shifted from their original position 20° upward or downward in the visual field (position invariance).

Visual pattern learning was shown to require the gene *rutabaga* (*rut*), coding an adenylyl cyclase which has originally been found to play a role for olfactory learning and memory in the mushroom bodies (Zars et al. 2000). Mutant *rut* flies could be rescued with respect to their phenotypes for height and contour orientation by the expression of wild-type cyclase (Rut^{+}) in neurons of the F5 and F1 layers of the fan-shaped body (FB) respectively (Liu et al. 2006). Furthermore, Pan et al (2009) showed that the *rut*-dependent mutant phenotype for height, contour orientation, vertical compactness and size could also be rescued in the ring neurons of the ellipsoid body (EB). In the EB non-specific rescue of the mutant phenotype was found contrary to the specific rescue shown by Liu et al (2006). Fig. 2 shows the brain of the *Drosophila.*

Another gene called *foraging* (*for*), which encodes a cyclic guanosine-3', 5'-monophosphate (cGMP)-dependent protein kinase (PKG) and has extensively been studied in food-search behavior in *Drosophila* is involved in visual pattern recognition. It has been reported by Wang et al (2008) that the for^{S} allele shows a mutant phenotype in visual learning and this phenotype can be rescued in the F5 layer of the fan-shaped body for the pattern parameter height, consistent with the studies from Liu et al (2006). In addition Wang et al (2008) also showed that the mutant phenotype for pattern parameters height and contour orientation can be rescued by expressing wild-type PKG in an otherwise for^{S} mutant background in ring neurons of the ellipsoid body.

A further gene playing a role in visual learning is *ignorant* (*ign*) coding p90S6KinaseII (Neuser et al. 2008). Flies remember the location of a landmark in the visual field after it has disappeared. Without this kinase the working memory of the landmark is lost. The kinase is required exclusively in ring neurons of the EB for this memory.

Intact mushroom bodies (MBs) are not required for visual pattern learning (Wolf et al. 1998). However, Liu et al (1999) showed that mushroom bodies are required to show visual learning when the context is changed between training and test (e.g. background illumination from green to blue or vice versa).

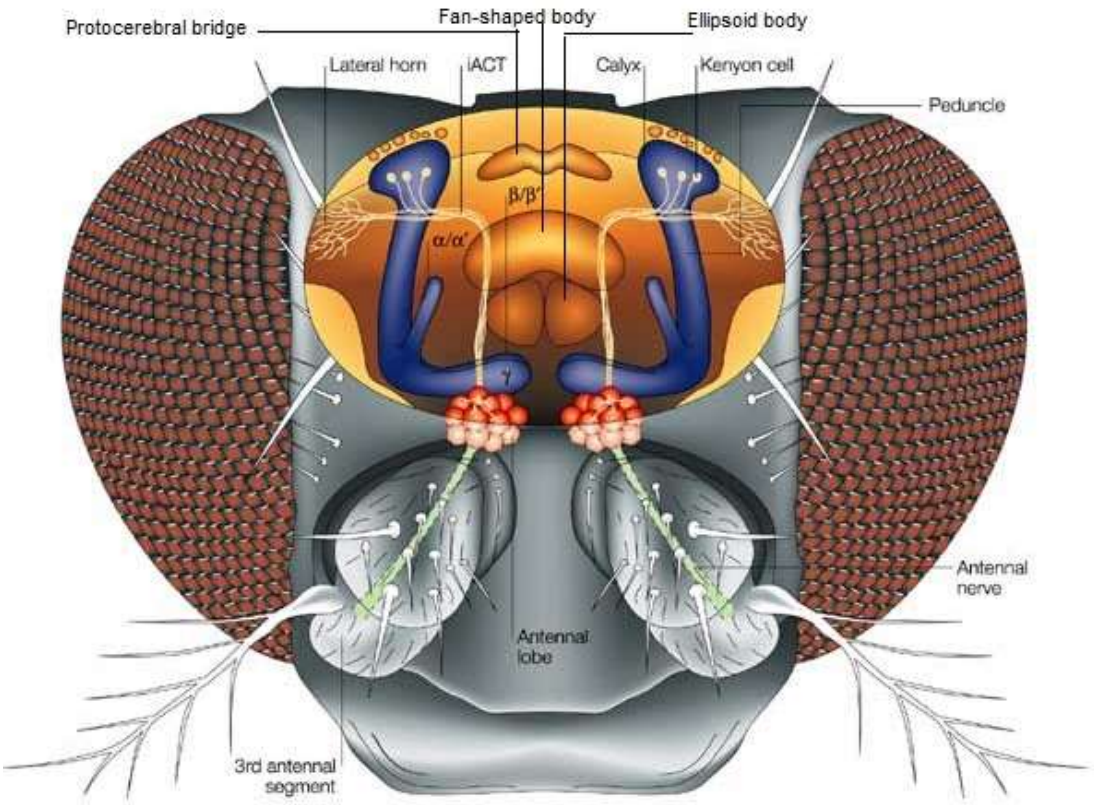


Figure 2. *Drosophila* brain. (modified from Heisenberg. 2003).

1.5. Novelty choice in *Drosophila melanogaster*

In addition to investigating the learning behavior Dill et al (1995) also studied novelty choice of flies for different pattern parameters. Novelty choice is a learning paradigm in which flies are trained/allowed to form a memory for a type of pattern (upright T) and then tested for their preference for the new pattern (inverted T) compared to the old pattern (upright T). The observed preference is called Novelty choice, which would be positive if the fly shows comparative preference for the new pattern and negative if the preference is towards the old pattern. Dill et al (1995) have shown that in the flight simulator flies show novelty choice for triangles differing in size and triangles with contrast change.

There is another novelty effect described for the fly visual system (Swinderen and Greenspan; 2003, Swinderen; 2007 and Swinderen et al. 2009), which might be related to the novelty choice just described. These authors measure a local field potential (LFP) in the brain. A 20-30 Hz oscillation in the LFP signals a new figure (cross or box) appearing in the fly's visual field after the fly has been exposed to the other figure. Whether this neuronal response is related to the novelty choice remains to be shown.

As evident from above not much is known about the novelty choice behavior. So, to further investigate it, the flight simulator that has been so effective in visual pattern recognition studies serves as an appropriate experimental setup.

In this thesis I want to further investigate the novelty choice behavior. I would like to ask whether flies show novelty choice for all the pattern parameters/combinations used in visual pattern learning. The absence of novelty choice for certain pattern parameters might help us understand the mechanism of novelty choice behavior. Furthermore I would like to know if *rut,* which plays a role in heat conditioning experiments also plays a role in novelty choice behavior. It would also be interesting to know if *ign*, which is involved in the spatial orientation (Neuser et al. 2008) memory, is also involved in the novelty choice behavior because novelty choice also seems to require short term working memory. As mentioned above, Rutabaga dependent memory for height and contour orientation parameters is shown to be located in the F5 and F1 layer of the fan-shaped body respectively in the heat conditioning experiment (Liu et al. 2006), while Rutabaga dependent memory for height, contour orientation, size and vertical compactness can also be rescued in the ring neurons of the ellopsoid body (Pan et al. 2009). Investigating the role of F layer neurons and ring neurons by blocking the neuronal output for novelty choice behavior would provide insight in the mechanism of fly pattern recognition in general and it would also help us understand the difference between the heat conditioning and the novelty choice behaviors if there are any.

2. RESULTS

2.1. Novelty choice paradigm

Novelty choice is a visual learning paradigm introduced by Dill et al in 1995. In this experiment a fly's pattern preference for a new pattern is measured in scenery of old patterns. These measurements are performed at a torque meter, which constitutes the core part of an experimental setup called flight simulator (Figure 3, Courtesy: R. Wolf). In the flight simulator a fly is tethered to the torque meter through its head and thorax. Hence, neither the whole fly nor its head can move in space. In the flight simulator the fly's yaw torque is recorded and transformed electronically into rotations of a virtual scenery surrounding the fly to simulate flight with a single degree of freedom i.e., rotations in a horizontal plane. Although the whole situation is unnatural for the fly, still it is effective for the experimenter to investigate visual learning behavior because of the experimental control the measurements of yaw torque provide. Upon exposure to patterns on a visual arena (LED-arena) that surrounds the fly at the torque meter, the fly modulates its yaw torque in an attempt to fly towards one of the patterns. The yaw torque measured by the torque meter is transformed into a DC-voltage and fed through a computer to the LED-controlling unit to rotate the visual pattern scenery in the horizontal plane so as to simulate the fly's rotation around its vertical axis. In this manner a fly can control the visual arena through its yaw torque. The loop between (intended) turning and the concomitant visual feed-back is closed (closed loop). The flight simulator has been shown to be a suitable experimental setup to investigate the organization of behavior (Liu et al. 2006).

As the name 'novelty choice' suggests, this behavior involves studying whether the fly makes a choice (attraction or avoidance) towards novel patterns or not (indifferent). For this choice to be made by the fly a pattern has to appear novel in comparison to a non-novel pattern. This is achieved by structuring the experiment in two parts. The first part is called conditioning phase and the second part is called test phase. For example, to display novelty choice behavior with respect to triangles differing in orientation (upright and inverted), the fly is exposed to four identical upright triangles positioned in the center of each of the four 90° quadrants of the arena. This phase, which may last from 1 to 18 minutes (Dill et al. 1995), enables the fly to form a memory for upright triangles. Then, in the subsequent test phase two opposing triangles are replaced by two inverted triangles the fly has not seen before. In this phase the fly must be able to distinguish the upright and inverted triangles.

Moreover, it must utilize the stored memory template of the upright triangles in order to make a choice towards the inverted triangles.

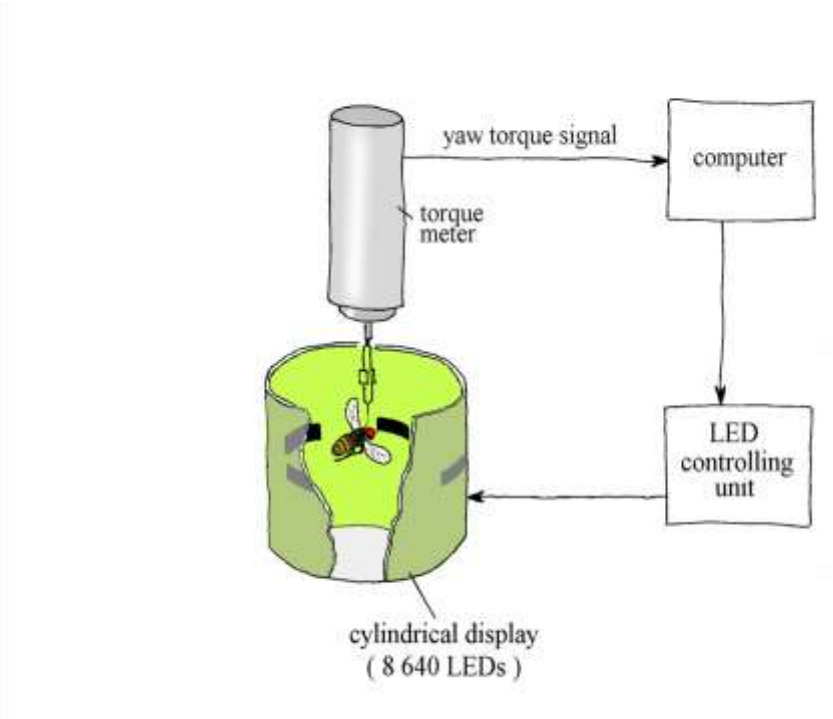


Figure 3: Experimental setup for novelty choice experiment.
A fly is tethered through its head and thorax to a torque meter, which measures the yaw torque produced by the fly. This yaw torque controls the angular velocity of the visual panorama surrounding the fly via a computer (Schematic courtesy: R. Wolf).

As mentioned, the prerequisite of novelty choice is the ability of the fly to distinguish the patterns used. Furthermore, the fly may have a spontaneous pattern preference, which in turn influences the novelty choice via fly's discrimination pattern preference (defined as SPP in Ernst and Heisenberg, 1999) in the choice between two patterns. Therefore, one cannot state that flies show novelty choice for triangles based only on the above-mentioned experiment. Novelty choice must be tested for both the patterns (upright and inverted triangles). Averaging these two novelty choice values yields the mean novelty choice score for the triangles by eliminating the discrimination pattern preference (to be called DPP here because it is calculated from PI_4). Subtracting one halfscore of PI_4 from the other provides the DPP. The two reciprocal experiments are performed alternatingly in order to have as similar experimental conditions (time of the day, temperature and humidity, etc) as possible.

2.2. NOVELTY CHOICE FOR DIFFERENT PATTERN PARAMETERS INVESTIGATED

2.2.1. Novelty choice for horizontal bars differing in height

The study by Dill et al. (1995) is further explored here with a wide array of pattern combinations. First of them consists of two horizontal bars (40°x12°) differing in the height of their centers of gravity (CsOG) by 23° with respect to the fly. The height difference (CsOG difference) is large enough for the fly to distinguish the two heights in the visual field. As explained, the novelty choice consists of two reciprocal experiments. Each of them has three two-minute memory formation periods followed by two two-minute novelty choice periods, forming six minutes of memory formation phase and four minutes of novelty choice phase. Mixing periods in which the visual arena is rotated clockwise for 2.5 seconds and counter clockwise for another 2.5 seconds at high speed separates each of the two-minute periods of the approximate ten-minute novelty choice experiment. This may induce the fly to choose a new flight direction in the novelty choice phase rather than keeping a steady course.

In one of the reciprocal experiments the memory formation phase begins with exposing flies to four identical horizontal bars located in the upper half of the visual field. Of these two opposing bars are shifted to the lower half of the visual field during the novelty choice phase. In the other experiment flies are exposed to four bars located in the lower half of the visual field during memory formation phase, two of these are shifted upward during the novelty choice phase. The performance of the flies is measured as preference index {(PI)=(t_{new}-t_{old}/t_{new}+t_{old}). t_{new}= Time spent flying towards new pattern, t_{old}= Time spent flying towards old pattern}. Scores range from 1 to -1. They are positive if a fly prefers the new pattern and negative if the preference is towards the old pattern. {No significant negative novelty choice score was observed throughout this study}.

Figure 4 shows the whole novelty choice experimental data of Wild-type *Berlin* flies (n=212) for height parameter. The near zero values of the columns 1-3 of the memory formation phase are expected because of the design of the experiment (only one type of pattern: no novel pattern). They will not be discussed further unless stated. Column 4-5 represent the novelty choice phase of the experiment and shows the novelty choice scores. As it is found that novelty choice scores normally decrease (Two-tailed t-test: P=0.0003) from column 4 to column 5, hence only column 4 $\{(PI_4)\}$ will be discussed for novelty choice unless stated. And it is in this phase (novelty choice) the DPP is

observed upon calculation. The error bars are standard errors of the mean (SEMs) throughout the results.

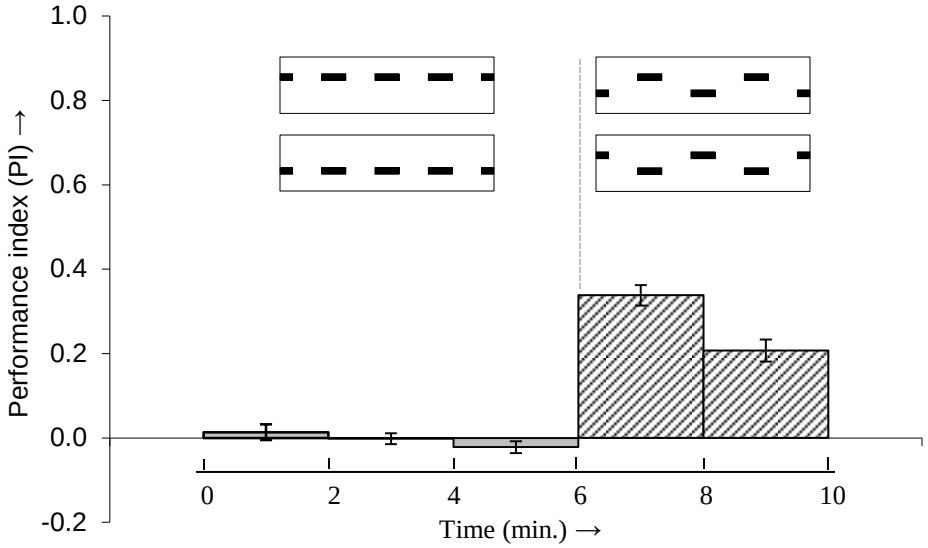


Figure 4: Novelty choice for horizontal bars differing in height.
For the first 6-minutes flies are exposed to four identical horizontal bars (40°x12°) located in the upper/lower visual field in each quadrant of an arena. In the subsequent 4-minute period two opposing bars are shifted to the lower/upper visual field respectively. The performance of the flies is measured as preference index {(PI)=(t_{new}-t_{old}/t_{new}+t_{old}), with t_{new}= Time spent flying towards new pattern, t_{old}= Time spent flying towards old pattern}. Equal numbers of experiments start with the bars in the upper and the lower visual field. WT-*Berlin* flies (n=212). Error bars are SEMs throughout the results.

2.2.2. Novelty choice for the pattern parameters 'Vertical compactness' and 'Size'

After finding that the WT-*Berlin* flies show novelty choice for two horizontal bars differing in height, novelty choice for other pattern parameters like vertical compactness and size that are learned by the flies in heat conditioning experiments (Ernst and Heisenberg. 1999) is investigated.

In one of the reciprocal experiments to study the novelty choice for vertical compactness, flies (WT-*Berlin*) are exposed to four identical horizontal bars (40°x20°) located at the centers of the quadrants during memory formation phase. For the novelty choice phase two of these horizontal bars (40°x20°) in opposing quadrants are each replaced with a pair of horizontal bars (40°x10°) located above and below (CsOG 29° apart with respect to the fly) the centers of the quadrants. In the

reciprocal experiments flies are exposed to these pairs of horizontal bars (40°x10°) in all four quadrants during memory formation phase, of which two opposing once are replaced by single bars (40°x20°) at the centers of the quadrants in the novelty choice phase. Fig. 5 (left) shows the average novelty choice of WT- *Berlin* flies for the pattern parameter 'vertical compactness' (Two-tailed t-test: P<0.0001).

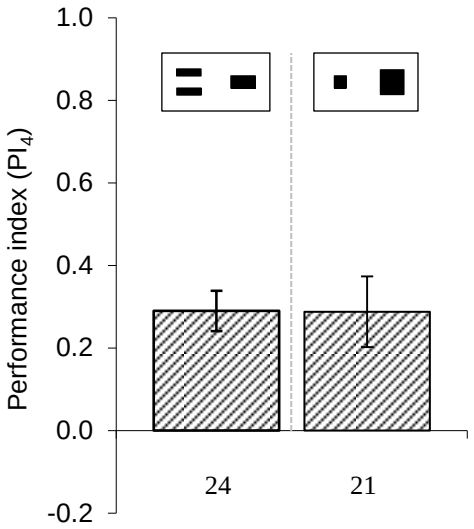


Figure 5: Vertical compactness and size parameters elicit novelty choice.
For vertical compactness half of the flies are exposed to four identical horizontal bars (40°x20°) located at the centers of the four quadrants during memory formation phase. The other half are exposed to four identical horizontal bar pairs (40°x10°) located above and below (CsOG 29° apart) the centers of the quadrants. During novelty choice phase two of the opposing patterns (horizontal bars/horizontal bar pairs) are replaced with the other patterns (horizontal bar pairs/horizontal bars) respectively.
Novelty choice for size is measured with large (40°x40°) and small (20°x20°) squares. Here wild-type *Berlin* flies show average novelty choice for both vertical compactness (P<0.0001) and size (P=0.003) parameter. The patterns investigated and the numbers of flies are represented above and below the novelty choice score columns: This scheme of representation is followed onwards. Statistics: Two-tailed test (will be used onwards too, unless stated).

Dill et al. (1995) have also shown the novelty choice for triangles of different sizes. This study was followed here with squares differing in size (40°x40° and 20°x20°). The right column of Fig. 5 shows the average novelty choice of wild-type *Berlin* flies for size (Two-tailed t-test: P=0.003). The patterns investigated and numbers of flies (n) are shown above and below the novelty choice score columns: This scheme of representation is followed onwards along with the genotype of flies used.

2.2.3. No novelty choice for contour orientation

Another pattern parameter that can be learned by the flies in the heat conditioning experiment is contour orientation (Tang et al. 2004; Liu et al. 2006). Novelty choice for contour orientation is measured with oblique bars oriented at +45° and -45°. Here we measure novelty choice for oblique bars in parallel with novelty choice for height. WT-*Berlin* flies show no average novelty choice for contour orientation (Fig. 6): Two-tailed test: P=0.72. There are several possible reasons why a fly would not show a novelty choice effect with a particular pattern pair. For one, the fly might not be able to distinguish the two patterns. Secondly, patterns might elicit a strong spontaneous attraction or avoidance, which could be extracted from the difference of the two halfscores of the novelty choice measurement (DPP). A positive or negative DPP value would show that the patterns are distinguishable while zero DPP value would suggest that the patterns are not distinguishable. The flies show DPP value of 0.01 for oblique bars oriented at +45° and -45° suggesting that they might not be distinguishable.

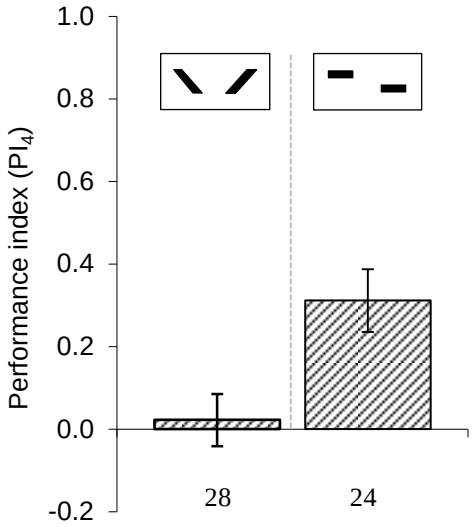


Figure 6: A change in contour orientation does not elicit novelty choice.
Novelty choice for contour orientation is measured with oblique bars oriented at +/-45° in parallel with height novelty choice.
WT-*Berlin* flies show novelty choice for height (P=0.0004) but no novelty choice for contour orientation (P=0.72). Novelty choice for height and contour orientation differ significantly (P=0.005).

As the flies can learn contour orientation in heat conditioning experiments (Tang et al. 2004, Liu et al. 2006) the finding that they show no novelty choice for contour orientation is unexpected. So

to investigate this negative finding, novelty choice is measured for two other pattern combinations differing in contour orientation, horizontal vs vertical bars and horizontal vs oblique bars. These pattern combinations are selected because the two patterns in each pair differ not only in contour orientation but also in vertical extent. If differences in contour orientation would suppress novelty choice, these pairs should not show a novelty choice effect either. All these experiments are again performed in parallel with height novelty choice (serving as control).

Consistent with the findings from Fig. 6 WT-*Berlin* flies show no novelty choice for the two oblique bars (Fig. 7): P=0.43, with a DPP value of 0.10. In conclusion oblique bars oriented at +/- 45° show no novelty choice. Flies however show novelty choice for horizontal vs oblique bars (Fig. 7): P=0.0002, for horizontal vs vertical bars {(Fig. 7): P=0.02}. {Significance is calculated by Unpaired t-test}. These findings show that patterns differing in contour orientation do not suppress novelty choice for a second pattern parameter (vertical extent).

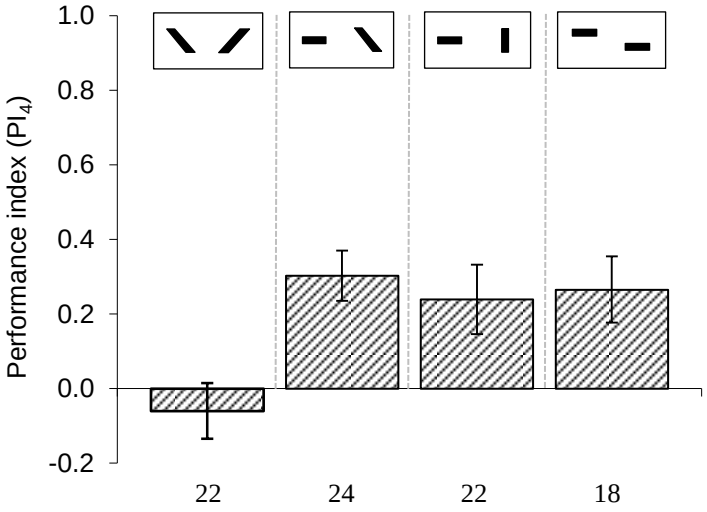


Figure 7: Contour orientation does not dominantly suppress novelty choice.
WT-*Berlin* flies show novelty choice for height (P=0.008), horizontal vs oblique bars (P=0.0002) and for horizontal vs vertical bars (P=0.02) but they show no novelty choice for two oblique bars (P=0.43). All the measurements are performed in parallel.

2.2.4. Circle and cross do not elicit novelty choice

One would assume that circle and cross would be distinguished by the fly and show novelty choice. But the lack of understanding of the novelty choice behavior, prompted to inspect the

aforementioned assumption. This pattern combination (circle and cross) is used here to get an insight in the mechanism of the novelty choice behavior.

However contrary to the assumption WT-*Berlin* flies show no average novelty choice for circle and cross pattern combination (Fig. 8): Two-tailed t-test: P=0.46, while they show novelty choice for height measured in parallel. The DPP value of 0.09 for circle and cross show that the patterns might not be distinguished resulting in close to zero novelty choice scores. Therefore to measure novelty choice for this pattern combination they need to be modified to make them distinguishable.

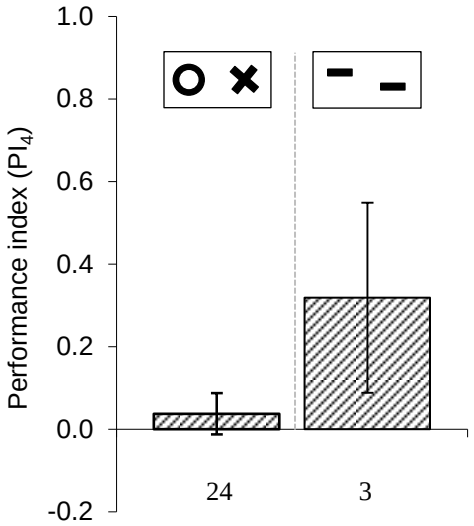


Figure 8: No novelty choice with circle and cross.
Here WT-*Berlin* flies show no novelty choice for circle and cross (P=0.46) but novelty choice for height seems to work as usual (n=3).

2.2.5. No novelty choice for standard T patterns

Another pattern pair that can be learned by the flies in the heat conditioning experiment is the upright/inverted-T (Dill et al. 1993, Tang et al. 2004, Liu et al. 2006). In this pattern pair the upright and inverted Ts are positioned at the center of each quadrant. Their CsOG difference with respect to the fly (13°) is large enough to be distinguished by the flies in the heat conditioning studies. If the CsOG difference was reduced to zero in triangles and composite patterns, no heat conditioning was observable (Ernst and Heisenberg, 1999). Here the same pattern pair of Ts (CsOG 13° apart) is used to investigate the novelty choice and to compare it with the novelty choice for horizontal bars (CsOG 23° apart used consistently to measure height novelty choice).

Fig. 9 show that WT-*Berlin* flies show no average novelty choice for Ts {(CsOG 13° apart): P=0.72} while they show novelty choice for height {(CsOG 23° apart): P=0.0001} as expected. However flies do show a DPP of 0.55 for inverted Ts which might be responsible for the novelty choice outcome. In other words the strong spontaneous preference for inverted Ts might eclipse the average novelty choice scores for Ts. Novelty choice for Ts and horizontal bars differ significantly (P=0.003; Unpaired t-test). One might attribute this result to the difference in CsOG of the patterns used for the study. Therefore the novelty choice for height needs to be further investigated.

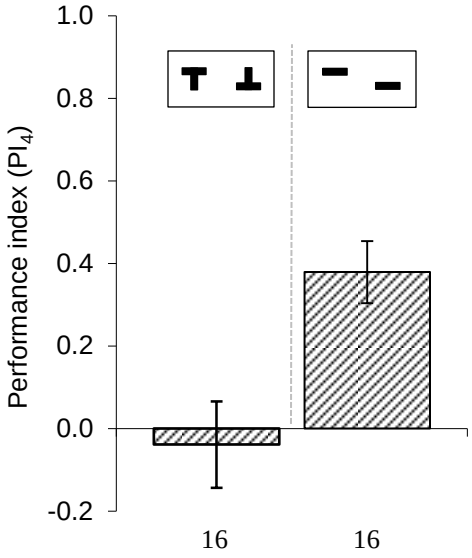


Figure 9: No novelty choice with standard T patterns.
Wild-type *Berlin* flies show no novelty choice for Ts {(COG 13° apart): P=0.72} while they show novelty choice for height {(COG 23° apart): P=0.0001} (positive control). Novelty choice for Ts and height differ significantly (P=0.003).

2.3. NOVELTY CHOICE FOR HEIGHT

2.3.1. Fly's vertical position in arena for height

Fig. 9 indicates that flies show no novelty choice for Ts (CsOG 13° apart) which might be due to high DPP of the fly for inverted Ts. This strong pattern preference might depend upon where in fly's visual field patterns are presented, which in turn is govern by the fly's vertical position in the visual arena (normally 4.9 cm from lower margin of panorama). Therefore to examine this effect and possibly to eliminate it, the fly's vertical position is systematically varied.

For this 'titration' of vertical position in the visual arena (12.0 cm high) flies are fixed at three positions: 4.8 cm, 5.4 cm and 6.0 cm from the bottom the arena for the novelty choice measurements. At all these positions the same T patterns (CsOG 13° apart) and horizontal bars (CsOG 23° apart) are used so as to vary only one parameter in the experiment, the height of the patterns in the fly's visual field. For the same reason all the experimental (at three positions) are measured in parallel. It must be noted that the CsOG difference is not adjusted to the same value at the three heights.

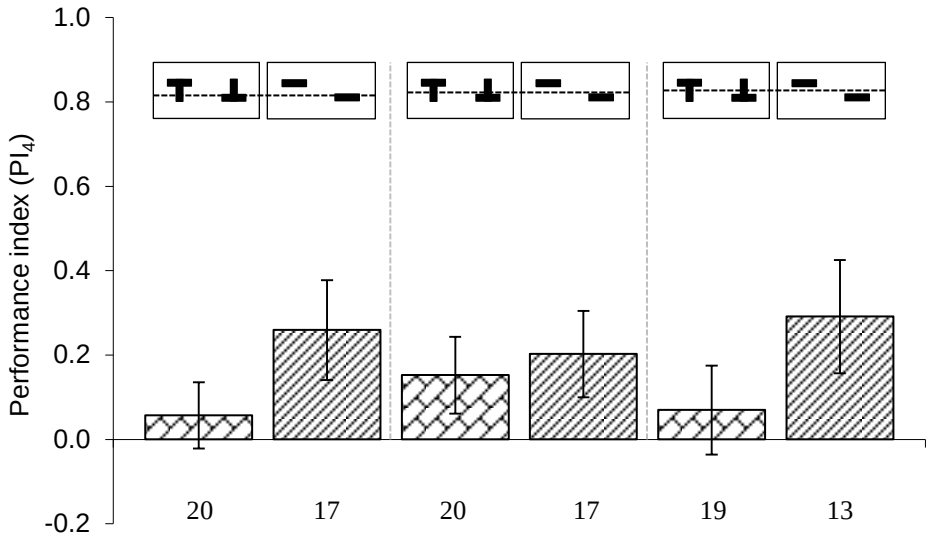


Figure 10: Novelty choice for Ts (CsOG 13° apart) is not improved by changing fly's vertical position.

WT-*Berlin* flies show no significant novelty choice for Ts {(CsOG 13° apart): P=0.48, 0.11 and 0.52 respectively} but they show novelty choice for height (CsOG 23° apart) measured at 4.8 cm (left pair), at 5.4 cm (middle pair) and at 6.0 cm (right pair) from the bottom of the arena.

------- Dashed black lines on patterns represent the fly's vertical position.

Furthermore the fly's longitudinal axis (see Methods) is also kept constant, i.e 30° upward. In horizontal bars experiment with this angle (30°) the COG of upper bars is 18°, 14° and 10° below fly's horizon and the COG of the lower bars is 42°, 37° and 32° below fly's horizon at 6.0, 5.4 and 4.8 cm respectively. Although the CsOG difference between horizontal bars is 23°, with respect to fly the CsOG difference is 24°, 23° and 22° at 6.0, 5.4 and 4.8 cm respectively. In addition the COG of upright Ts is 23°, 19° and 14° below fly's horizon and the COG of the inverted Ts is 37°, 32° and 27° below fly's horizon at 6.0, 5.4 and 4.8 cm respectively in Ts experiment. And the CsOG difference (13°) with respect to fly is 14°, 13° and 13° at 6.0, 5.4 and 4.8 cm respectively.

Consistent with the results from Fig. 9 WT-*Berlin* flies show no significant novelty choice for Ts (CsOG 13° apart) measured at 4.8 cm (left pair: P=0.48), at 5.4 cm (middle pair: P=0.11) and at 6.0 cm (right pair: P=0.52) {Fig. 10}. At all three vertical positions they do show normal novelty choice for horizontal bars (CsOG 23° apart). Significance is calculated by Two-tailed t-test. And the flies show DPP values for upper horizontal bars as 0.33, 0.21 and 0.52 at 4.8, 5.4 and 6.0 cm respectively. In Ts novelty choice experiment at 4.8 cm flies show DPP value of 0.23 for inverted Ts, at 5.4 cm DPP value of 0.12 for upright Ts and at 6.0 cm DPP value of 0.05 for inverted Ts.

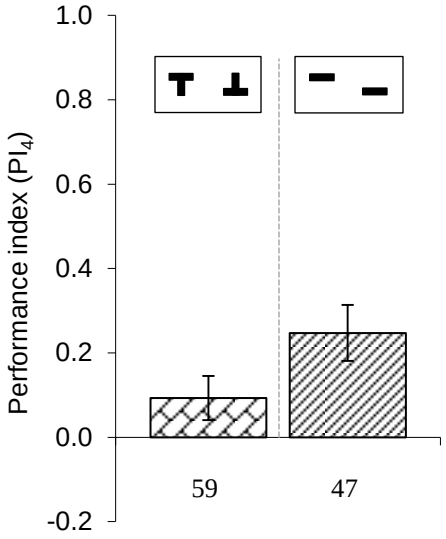


Figure 11: Fly's vertical position for height novelty choice.
Novelty choice of WT- *Berlin* flies is not improved even after averaging all the measurements from 4.8, 5.4 and 6.0 cm (Fig. 8 data) for Ts (CsOG 13° apart).

In conclusion WT- *Berlin* flies show no novelty choice for Ts (CsOG 13° apart): P=0.0805, irrespective of the fly's vertical fixation position in the arena. In addition high DPP values do not necessarily decrease the novelty choice score.

As mentioned above the investigated T patterns (CsOG 13° apart) can be learned by the flies in the heat conditioning experiment. The findings from figure 9 and 10 suggest a difference in pattern discrimination mechanism between heat conditioning and the novelty choice behavior. This claim could be strengthened by further insight in the pattern parameters eliciting novelty choice behavior.

Would an increase in the CsOG difference of the upright and inverted Ts lead to a novelty choice effect? Second: is it only the CsOG difference in the Ts or some other/additional features that are evaluated by the flies for the novelty choice behavior?

2.3.2. Criteria for novelty choice behavior with T patterns

The issue of Ts eliciting novelty choice behavior or not is addressed by moving them apart so that their CsOG difference (13°) coincides with that of the horizontal bars (23°). Interestingly, under these conditions WT-*Berlin* flies show novelty choice for Ts (Fig. 12; second column: P=0.005). One of the criteria seems to be that the CsOG difference must be large enough (in this case 23°).

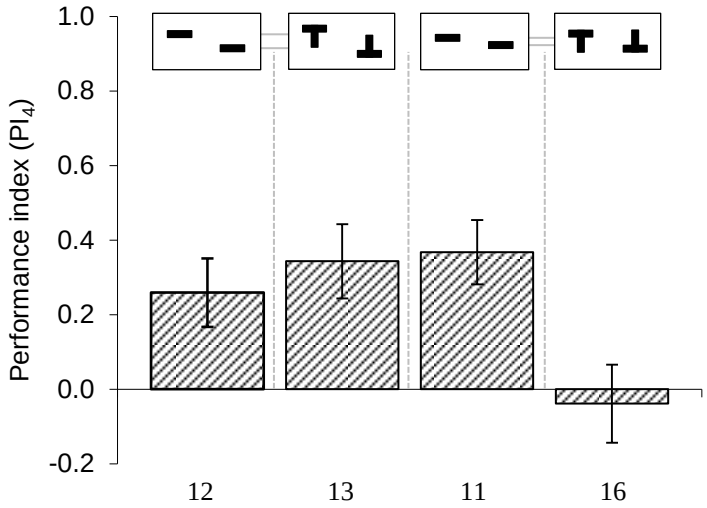


Figure 12: Novelty choice for Ts requires larger COG difference than for horizontal bars.
WT-*Berlin* flies show novelty choice for Ts only when the CsOG difference is large (23°): (second column: P=0.005), however they show novelty choice for horizontal bars even with smaller CsOG difference (13°) (third column: P=0.002).
——— Full grey lines between patterns represent the CsOG of the patterns.

If the T patterns are discriminated only be the height of their COG one would also expect horizontal bars not to elicit novelty choice behavior at a smaller CsOG difference (13°). The result of this experiment is shown in the third column of Fig. 12: P=0.002. A full novelty choice effect is observed. The vertical parts of the T patterns seem to affect the height discrimination of the Ts more than what is accounted for by the COG calculation.

These two experiments are performed in parallel with Ts (CsOG 13° apart) and horizontal bars (CsOG 23° apart). The fly is positioned at 6.0 cm from the bottom in the arena. Although no change is observed in the behavior at different vertical fixed positions of the fly, from this point onward all the measurements will be performed at the vertical fixed position of 6.0 cm to keep conditions uniform. Significance is calculated by Two-tailed t-test.

These findings show that for Ts to elicit novelty choice behavior the CsOG difference has to be larger than in the heat conditioning experiments in which Ts with CsOG 13° apart can be learned successfully. This in turn indicates a difference in pattern discrimination mechanisms between novelty choice and heat conditioning.

2.4. ROLE OF DIFFERENENT GENES IN THE NOVELTY CHOICE

2.4.1. *rutabaga* gene is not involved in the novelty choice

To understand how distinct the two behaviors are role of the genes involved in the heat conditioning experiment is investigated for novelty choice. One such gene studied extensively in olfactory learning called *rutabaga (rut) encodes* the Rutabaga protein-a type 1 adenylyl cyclase.

This protein is considered to be a coincidence detector between the conditional stimulus and unconditional stimulus in olfactory associative learning (Dudai et al. 1988, Levin et al. 1992, Abrams et al. 1998, Renger et al. 2000, Tomchik and Davis. 2009 and Gervasi et al. 2010) and it is shown to selectively rescue the olfactory associative learning defect in the mutant (Zars et al. 2000, McGuire et al. 2001). In addition in visual learning the protein also rescues the Rutabaga dependent memory for pattern parameter height (Ts and HB), vertical compactness, size and contour orientation in heat conditioning experiment (Liu et al. 2006, Pan et al. 2009).

The role of *rut* gene in novelty choice behavior would provide some clues on whether or not these behaviors share a common pathway. For this study two mutant allele of *rut*: rut^{2080} and rut^{1} are used to measure the novelty choice for horizontal bars in parallel with WT-*Berlin* (WTB) flies (serving as control).

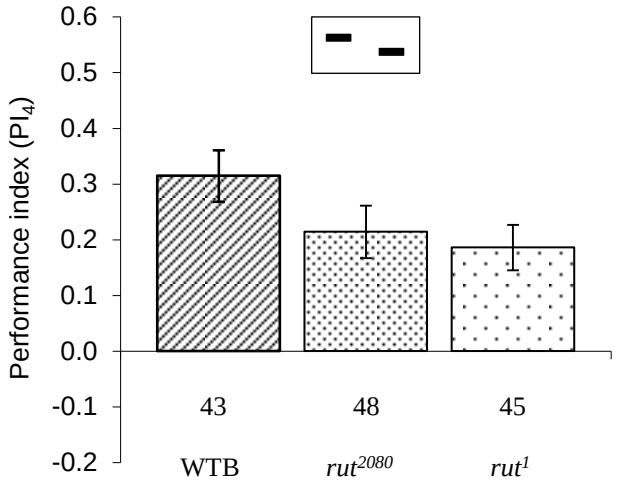


Figure 13: Rutabaga is not involved in novelty choice.
rut^{2080} *and* rut^{1} flies show wild-type like novelty choice for horizontal bars: P<0.0001, <0.0001 and <0.0001 for WTB, rut^{2080} and rut^{1} respectively).

Fig. 13 show that both the mutant alleles of *rut*: rut^{2080} and rut^{1} show novelty choice for horizontal bars (Two-tailed t-test: P<0.0001, <0.0001 and <0.0001 for WTB, rut^{2080} and rut^{1} respectively). This finding indicates that *rut* is not involved in the novelty choice behavior. It is in line with the absence of an obvious reinforcer in novelty choice behavior. Furthermore the Rutabaga cAMP pathway involved in the heat conditioning experiment seems to be dispensable for novelty choice behavior.

2.4.2. Dopamine is not involved in the novelty choice

In aversive and appetitive olfactory learning the Rut cyclase is shown to be stimulated by dopamine via the dopamine receptor Dumb (Kim et al. 2007). As the Rut cAMP pathway seems not to be involved in novelty choice, one might expect dopamine to be dispensable too.

The role of dopamine in novelty choice can be studied by controlling its excretion from dopaminergic neurons. For this the UAS/GAL4 expression system (Brand and Perrimon. 1993) is used, which consists of the UAS effector (construct) and the Gal4 driver (construct). The effector here is Shibire^{ts1} (UAS-*Shi*^{ts1}): a temperature-sensitive dynamin that regulates the vesicle release at synapse terminals (Kitamoto et al. 2002). At restrictive temperature (T = 31°C) the endocytic vesicles are unable to separate from the parent membranes and this results in the depletion of vesicles in synaptic terminals. However the effect is reversed at the permissive temperature T = 25°C). The driver used is TH-Gal4, which encodes the rate-limiting enzyme for dopamine synthesis: Tyrosine Hydroxylase (TH). So the dopamine synthesis can be regulated by regulating the concentration of tyrosine hydroxylase during dopamine synthesis.

By combining UAS-*shi*^{ts1} with the TH-Gal4, Shibire^{ts1} is expressed at all the TH sites for regulating dopamine synthesis. The progenies containing both the UAS-*shi*^{ts1} and TH-Gal4 are used to measure the novelty choice for horizontal bars, vertical compactness and size at permissive temperature (T = 25°C), which serves as control. Experimental flies are incubated for 10-12 minutes at 31°C and soon after measured at restrictive temperature (T = 31°C). Both experimental and controls are measured in parallel.

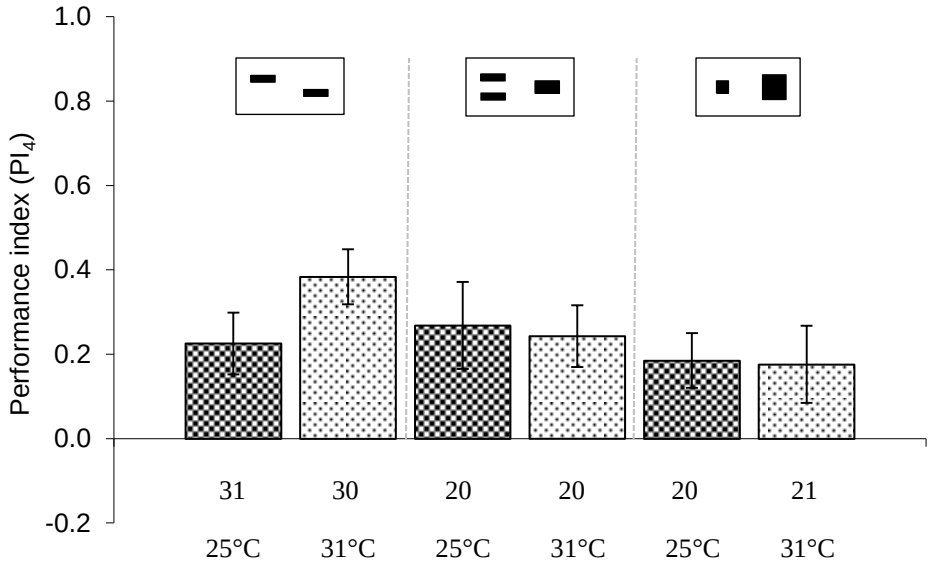


Figure 14: Dopamine is not involved in novelty choice.
At both restrictive and permissive temperatures flies (UAS-*shi*^{ts1}/TH-Gal4) show novelty choice for horizontal bars, vertical compactness and size (P=0.11, 0.84 and 0.94 for horizontal bars, vertical compactness and size respectively).

Fig. 14 shows that even after blocking the dopamine output (31°C) flies (UAS-*shi*^{ts1}/TH-Gal4) show novelty choice for horizontal bars, vertical compactness and size. Controls and experimentals do not differ significantly: Unpaired t-test: P=0.11, 0.84 and 0.94 for horizontal bars, vertical compactness and size respectively. These results indicate that dopamine does not play a role in novelty choice behavior.

2.4.3. The gene *ignorant (ign)* is required for novelty choice

The *ignorant* gene encodes a protein of serine-threonine kinase family called ribosomal S6 kinase II (S6KII), which is involve in operant place learning as well as in classical olfactory conditioning (Putz et al. 2004). S6KII is required for the formation of spatial orientation memory during locomotion and it is required in a subset of ring neurons to show this memory (Neuser et al. 2008). As discussed earlier flies must form a memory to show novelty choice. This working memory (short term memory) seems quite similar to spatial orientation memory and might be worked upon by the same circuitry. To investigate this hypothesis the mutant allele of the gene, which is a null allele (*ign*$^{58/1}$) is used.

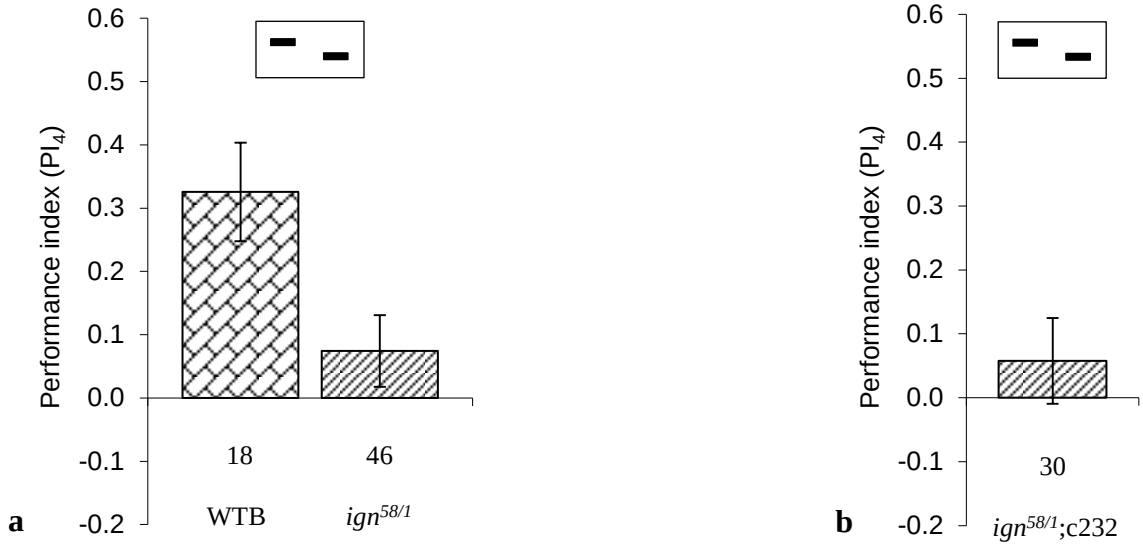


Figure 15: *ign* is required for novelty choice.
a) *ign*$^{58/1}$ flies show no novelty choice for horizontal bars. Differ significantly from WT-*Berlin*: P= 0.02. **b)** *ign*$^{58/1}$;c232 flies obtained by combining *ign*$^{58/1}$ with c232 Gal4 also show no novelty choice for horizontal bars in addition (P= 0.40).

Interestingly *ign*$^{58/1}$ flies show no novelty choice for horizontal bars (Fig. 15a) while the WT-*Berlin* flies behave normally (serving as control). They differ significantly (P=0.02). In addition the *ign*$^{58/1}$;c232 Gal4 flies obtained by combining *ign*$^{58/1}$ with c232 Gal4 also show no novelty choice for horizontal bars (Fig. 15b: P=0.40).

Furthermore to investigate whether the mutant phenotype shown by *ign*$^{58/1}$ flies is pattern specific, novelty choice is also investigated for vertical compactness and size. The following experiments are done 24 months later, using the same *ign*$^{58/1}$ flies stock. Unexpectedly, *ign*$^{58/1}$ flies show novelty choice for horizontal bars and vertical compactness but not for size (Fig. 16: P=0.057). Significance: horizontal bars and size: P=0.001; vertical compactness and size: P=0.040. This loss of mutant phenotype for horizontal bars might be attributed to the presence of modifiers in the fly's genome. These might have accumulated over a period of time (in this case two years) and might now rescue the mutant phenotype. Alternatively it could be due to contamination of the *ign*$^{58/1}$ fly stock.

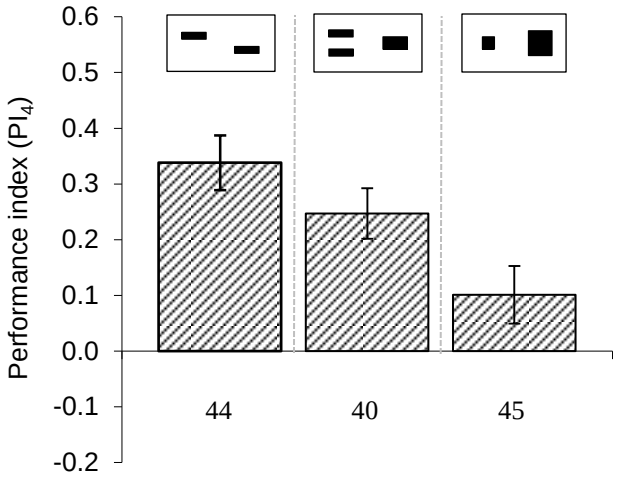


Figure 16: Loss of mutant *ign*$^{58/1}$ phenotype.
ign$^{58/1}$ flies, when used after a period of two years show novelty choice for horizontal bars and vertical compactness but not for size. Significance: horizontal bars and size: P=0.001; vertical compactness and size: P=0.040.

Therefore fresh *ign*$^{58/1}$ fly stock is obtained from other labs (courtesy: Prof. R. Strauss and Prof. T. Raaba) and then flies from these stocks are used to measure novelty choice for the above three pattern parameters.

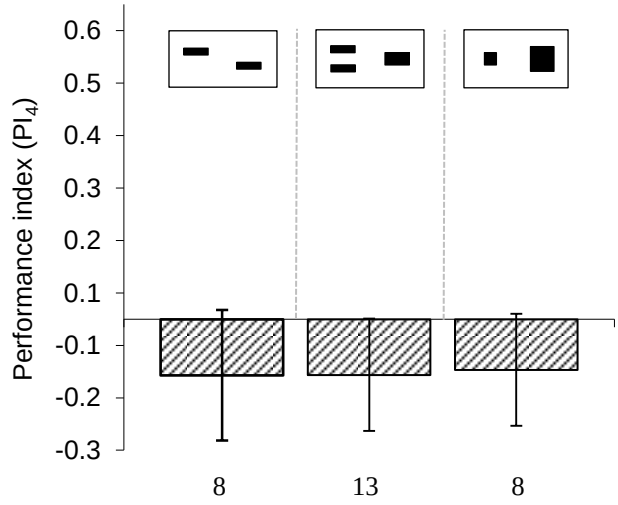


Figure 17: *ign* is required in novelty choice for vertical compactness and size.
ign$^{58/1}$ flies show no novelty choice for horizontal bars, vertical compactness and size: P=0.42, 0.34 and 0.39 respectively.

In agreement with Fig. 15 these results indicate that *ign*$^{58/1}$ flies show no novelty choice for any of the pattern parameters (Fig. 17: P=0.42, 0.34 and 0.39 for horizontal bars, vertical compactness and size respectively; for significance Unpaired t-test is used). Irrespective of the patterns used the *ign* gene is needed in novelty choice.

2.4.4. The *ignorant* mutant phenotype can be rescued by the *ign*$^{+}$ transgene

As a further proof that S6KII is required for novelty choice WT-S6KII cDNA is expressed ubiquitously in an *ign* mutant background by combining *ign*$^{58/1}$;;UAS-*ign* with *act*-Gal4. The male progeny of this cross (*ign*$^{58/1}$;;UAS-*ign*/*act*-Gal4) expressing wild-type S6KII throughout in an *ign* mutant background is used to measure the novelty choice for height, vertical compactness and size. The effector (*ign*$^{58/1}$;;UAS-*ign*/+) and the driver (*act*-Gal4/+) controls are measured in parallel with the experimental (*ign*$^{58/1}$;;UAS-*ign*/*act*-Gal4) flies.

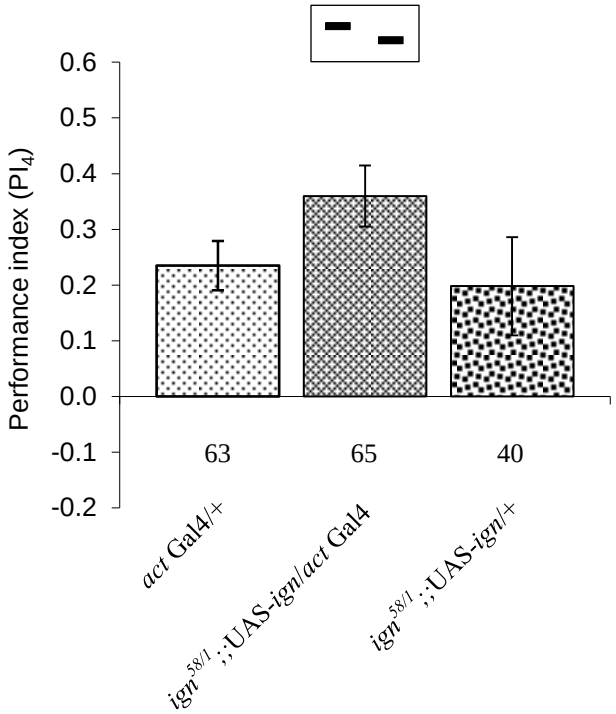


Figure 18: Ubiquitous expression of WT-S6KII in *ign* mutant background
As a tendency *ign*$^{58/1}$;;UAS-*ign*/*act*-Gal4 flies show a slightly larger novelty choice effect for horizontal bars then the respective control (*ign*$^{58/1}$;;UAS-*ign*/+*)* flies.

The experiment of Fig. 18 intends to show that the experimentals expressing WT-S6KII ubiquitously have normal novelty choice for horizontal bars. However, as I used the ign mutant stock of Fig. 16 to construct the effector/mutant flies used here, they already show a substantial novelty choice effect.

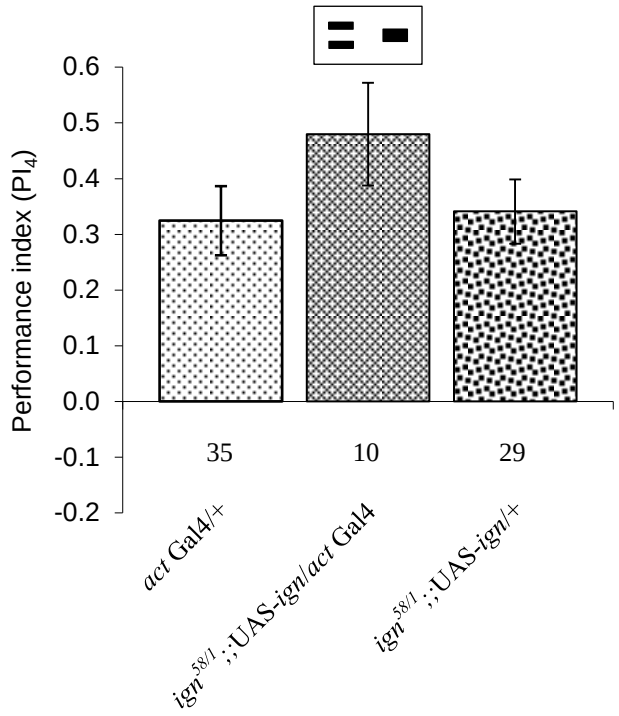


Figure 19: Ubiquitous expression of WT-S6KII in *ign* mutant background for vertical compactness.
Here to the *ign*$^{58/1}$;;UAS-*ign*/*act*-Gal4 flies show a slightly larger novelty choice effect for vertical compactness then the respective control (*ign*$^{58/1}$;;UAS-*ign*/+*)* flies. However none of the groups differ significantly.

Similar outcome is observed when rescuing the mutant phenotype for vertical compactness (Fig. 19) and size (Fig. 20). Here too the experimental and the effector/mutant control groups do not differ significantly. Genotypes are compared with Unpaired t-test.

Based on the effector control data these results indicate that the *ign* mutant phenotype cannot be rescued. However, as discussed earlier the effector control is deteriorating, added by lower number of flies would result in wild-type like novelty choice. Therefore despite being insignificant, these results can be taken as a tendency towards the rescue effect of the mutant phenotype, which needs to be further investigated with different Gal4 drivers/effector.

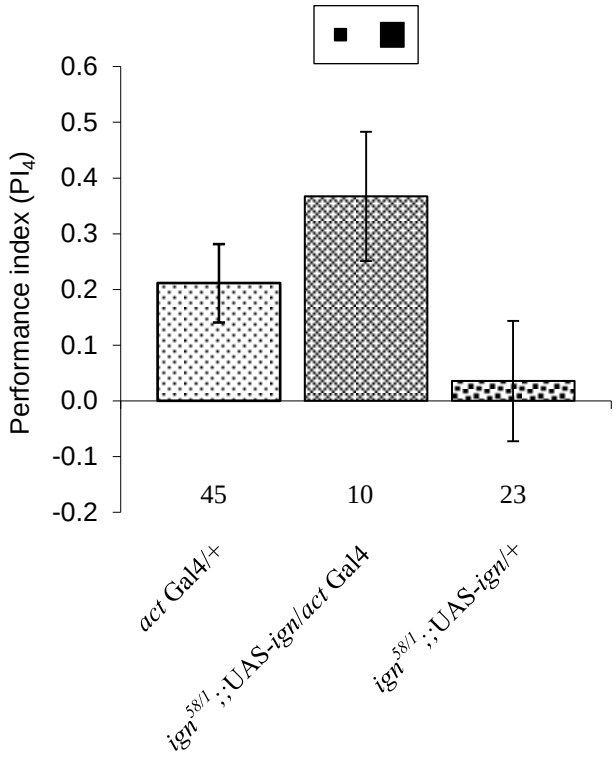


Figure 20: *ign* dependent rescued for size.
Here *ign*$^{58/1}$;;UAS-*ign*/*act*-Gal4 flies seem to rescues the novelty choice for size. However none of the groups differ significantly.

The data of Fig.s 18-20 suggest that the *ign* gene may play a special role in novelty choice for size. The *ign::UAS-ign* flies appear to be phenotypically reverted for novelty choice using horizontal bars and vertical compactness, while they are still mutant for size. It could be that the parameter size requires S6KII in yet other parts of the circuitry than the other two parameters, i. e. in neurons where the modifier proteins replacing S6KII are not expressed.

2.5. NOVELTY CHOICE CIRCUITRY

2.5.1. RING NEURONS OF ELLIPSOID BODY

2.5.1.1. RESCUE OF *IGNORANT* PHENOTYPE

2.5.1.1.1. S6KII in ring neurons is sufficient for height novelty choice in the *ign*$^{58/1}$ mutant

In 2008 Wang et al. have shown the involvement of ellipsoid body in the heat conditioning studies. Pan et al (2009) have shown that in *rut* mutants wild-type Rut adenylyl cyclase is sufficient in GABAergic neuronal cells of the ellipsoid body called ring neurons to allow for normal heat conditioning for height, vertical compactness, size and contour orientation parameters.

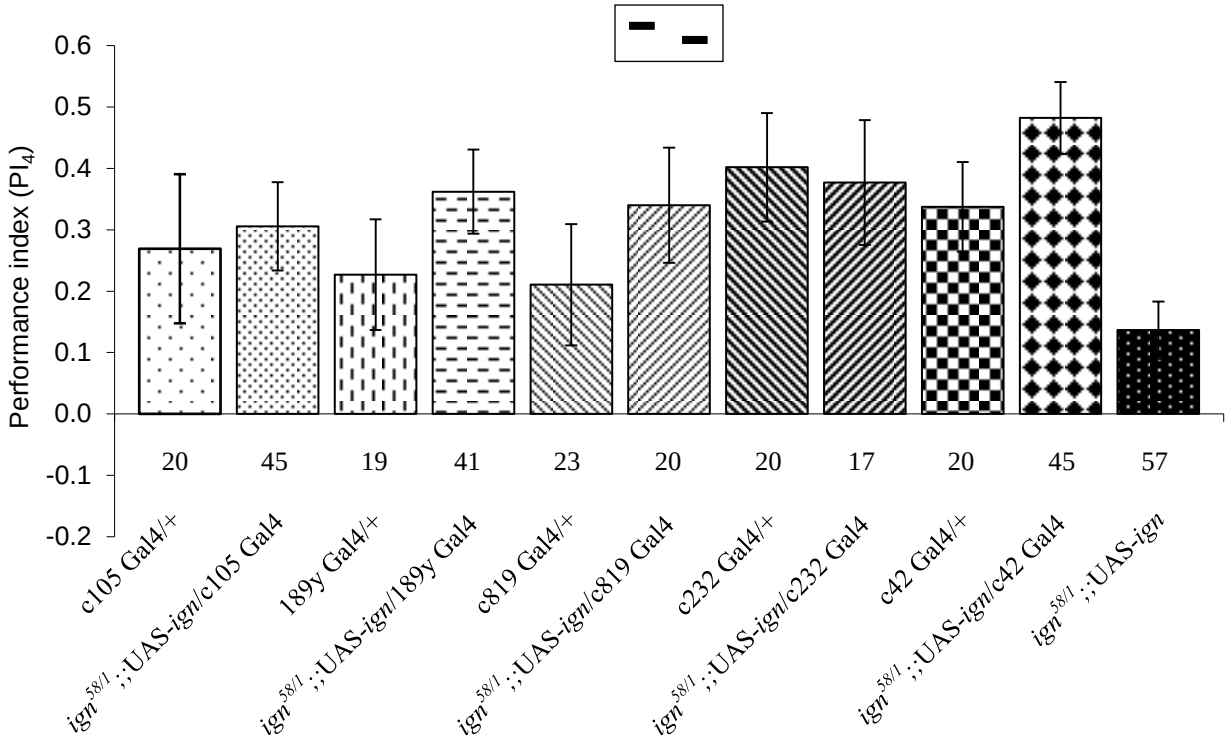


Figure 21: S6KII in R1, R3, R3/R4d or R2/R4m neurons is sufficient for height novelty choice.

WT-S6KII expression in only R1, R3, R3/R4d or R2/R4m ring neurons of the ellipsoid body restore the mutant phenotype for height novelty choice. The experimental and effector control differ significantly.

In addition ring neurons of the ellipsoid body are involved in a functional spatial orientation memory (Neuser et al. 2008). The ring neuron's involvement in the heat conditioning and in short term working memory makes them a promising candidate for novelty choice behavior.

Their role in novelty choice behavior is investigated by expressing WT-S6KII (UAS-*ign*) in an *ign* mutant background only in R1, R3, R2/R4m or in R3/R4d ring neurons of the ellipsoid body. This is achieved by combining $ign^{58/1}$;;UAS-*ign* with R1 driver line c105-Gal4, R3 driver line 189y-Gal4, R2/R4m driver line c819-Gal4 and c42-Gal4 and R3/R4d driver line c232-Gal4. The progenies ($ign^{58/1}$;;UAS-*ign*/c105-Gal4, $ign^{58/1}$;;UAS-*ign*/189y-Gal4, $ign^{58/1}$;;UAS-*ign*/c819-Gal4, $ign^{58/1}$;;UAS-*ign*/c232-Gal4 and $ign^{58/1}$;;UAS-*ign*/c42-Gal4) expressing wild-type S6KII in ring neurons in an otherwise *ign* mutant background are used to measure the novelty choice for height parameter. c105-Gal4/+, 189y-Gal4/+, c819-Gal4/+, c232-Gal4/+ and c42-Gal4/+ flies are measured as driver controls in parallel with experimental flies. $ign^{58/1}$;;UAS-*ign* flies are used as effector/mutant controls.

The experimental progenies expressing WT-S6KII in R1, R3, R3/R4d or R2/R4m ring neurons restore the S6KII mutant phenotype (statistically compared with effector controls) for height (Fig. 21). The experimental and effector control differ significantly: S6KII expressed in R1 neurons ($ign^{58/1}$;;UAS-*ign*/c105-Gal4) $p = 0.04$; in R3 neurons ($ign^{58/1}$;;UAS-*ign*/189y-Gal4) $p = 0.006$; R3/R4d ($ign^{58/1}$;;UAS-*ign*/c232-Gal4) $p = 0.02$ or in R2/R4m neurons ($ign^{58/1}$;;UAS-*ign*/c232-Gal4) $p = 0.03$, ($ign^{58/1}$;;UAS-*ign*/c42-Gal4) $p < 0.0001$. Expectedly none of the driver control differs significantly from their respective experimental. Statistical test: Unpaired t-test.

Genotypes are again compared with Mann-Whitney U-tests, the threshold for significance is adapted for three tests to alpha = 0.0167, 0.0033 and 0.00033 for *, ** and *** respectively using Bonferroni correction. The P values 0.01, 0.009, 0.0001, 0.09 and 0.07 are observed for $ign^{58/1}$;;UAS-*ign*/c105-Gal 4, $ign^{58/1}$;;UAS-*ign*/189y-Gal 4, $ign^{58/1}$;;UAS-*ign*/c42-Gal 4, $ign^{58/1}$;;UAS-*ign*/c232-Gal 4 and $ign^{58/1}$;;UAS-*ign*/c819-Gal 4 respectively.

Furthermore Kruskal-wallis followed by Dunn's muliple comparision test shows that none of the five groups differ significantly from each other.

These findings demonstrate that the expression of WT-S6KII cDNA only in R1, R3, R3/R4d or R2/R4m ring neurons of the ellipsoid body restores the S6KII mutant phenotype for height parameter, indicating that WT-S6KII protein in R1, R3, R3/R4d or in R2/R4m ring neurons of the ellipsoid in an *ign* mutant background is sufficient to fully restore the S6KII mutant phenotype for the height parameter.

2.5.1.1.2. Ring neurons are not sufficient for vertical compactness

Owing to the presumed similarities in the pattern parameter and behavior display with respect to mutants and wild-type fly's alike, vertical compactness might be perceived as an extension of height parameter. But as is always one needs experimental data supporting such an assumption to make aforementioned claim. Therefore if vertical compactness and height parameters were to be similar, one would get a similar rescue effect for both the pattern parameters. The ellipsoid body ring neurons driver lines expressing in R1 (c105-Gal4), R3 (189y-Gal4) and R2/R4m (c819-Gal4; c42-Gal4) used for height novelty choice are utilize to investigate the novelty choice for vertical compactness. For driver control c105-Gal4/+, 189y-Gal4/+, c819-Gal4/+ and c42-Gal4/+ flies are used and *ign*$^{58/1}$;;UAS-*ign* flies are used as effector/mutant control. Experimental, driver and effector/mutant controls are measured in parallel.

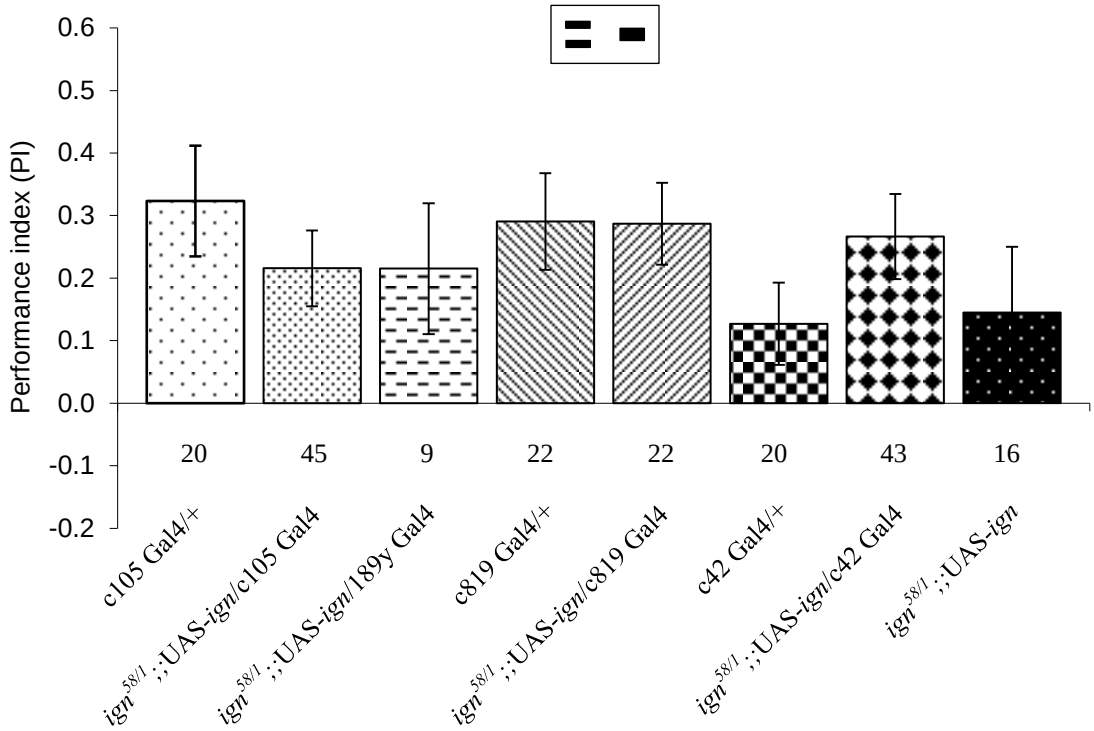


Figure 22: Ellipsoid body ring neurons are not sufficient for vertical compactness. WT-S6KII in R1, R3 or R2/R4d ring neurons of the ellipsoid body do not restore the S6KII mutant phenotype for vertical compactness unlike height parameter. P=0.56, 0.67, 0.24 and 0.35 for *ign*$^{58/1}$;;UAS-*ign*/c105-Gal4, *ign*$^{58/1}$;;UAS-*ign*/189y-Gal4, *ign*$^{58/1}$;;UAS-*ign*/c819-Gal4 and *ign*$^{58/1}$;;UAS-*ign*/c42-Gal4 respectively.

The experimental progenies expressing WT-S6KII in R1, R3 or R2/R4d ring neurons fail to restore the S6KII mutant phenotype for vertical compactness (Fig. 22) in contrast to their behavior for height novelty choice (Fig. 21). None of the experimental differ significantly from the effector control: (*ign*$^{58/1}$;;UAS-*ign*/c105-Gal4) p = 0.56, (*ign*$^{58/1}$;;UAS-*ign*/189y-Gal4) p = 0.67, (*ign*$^{58/1}$;;UAS-*ign*/c819-Gal4) p = 0.24 and (*ign*$^{58/1}$;;UAS-*ign*/c42-Gal4) p = 0.35. As expected none of the driver control differs significantly from their respective experimental. Statistical test: Unpaired t-test.

Fig. 22 shows that WT-S6KII in ellipsoid body ring neurons is not sufficient to restore the novelty choice for vertical compactness unlike height novelty choice. These results suggest that the height and vertical compactness are indeed two different pattern parameters.

2.5.1.1.3. Role of ellipsoid body ring neurons in the novelty choice for size

Another pattern parameter that requires WT-S6KII is size. As ring neurons of the ellipsoid body are required for height, their role in size novelty choice is investigated here.

The driver lines (R1; c105-Gal4, R3; 189y-Gal4, R3/R4d; c232-Gal4 and R2/R4m; c819-Gal4/c42-Gal4) that rescues the *ign* dependent mutant phenotype for height are used for this study. c105-Gal4/+, 189y-Gal4/+, c819-Gal4/+, c232-Gal4/+ and c42-Gal4/+ flies are used as driver control. Experimental and driver control are measured in parallel.

The experimental progenies expressing WT-S6KII in R1, R3, R3/R4d or R2/R4m ring neurons of the ellipsoid body fail to restore the S6KII mutant phenotype for size (Fig. 23). Although the experimental flies do not show novelty choice for size, a clear conclusion can't be drawn from the results as the driver controls themselves show no novelty choice for size. Therefore these results need to be scrutinized further. However the same driver control flies (same stock batch) behave normally for height and vertical compactness, suggesting size parameter to be critical for the visual pattern discrimination in novelty choice behavior.

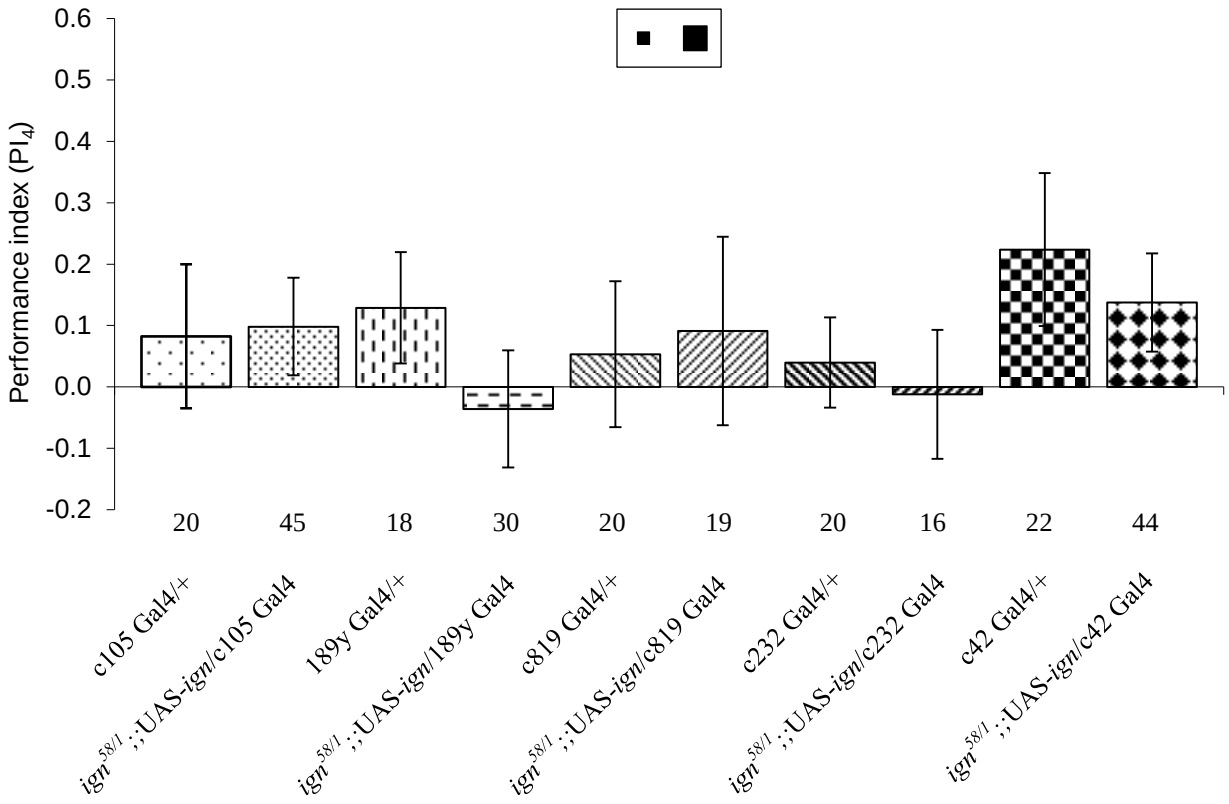
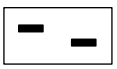


Figure 23: Role of ellipsoid body ring neurons in novelty choice for size.
The experimental flies expressing WT-S6KII in R1, R3, R3/R4d or R2/R4m ring neurons of the ellipsoid body show not novelty choice for size unlike height parameter. Driver controls themselves show no novelty choice for size.

2.5.1.1.4. Role of ellipsoid body ring neurons further investigated

To investigate the role of ellipsoid body ring neurons in the novelty choice further, additional ring neuron driver lines are used. These are: c547 (R2/R4m), R28D01 (R1), R38H02 (R4) and R14G08 (unknown). Furthermore the Gal4 driver lines c105 (R1), 189y (R3) and c42 (R2/R4m) are also used to test the rescue of novelty choice with horizontal/oblique bars.

Table 1: Investigating role of ring neurons in the novelty choice behavior.

Gal4 driver lines				
c105	——	——	——	0.0177^{ns}
189y	——	——	——	0.0922^{ns}
c42	——	——	——	0.0759^{ns}
c547	0.1226^{ns}	——	——	——
R14G08	0.1431^{ns}	0.2921*	0.1051^{ns}	——
R28D01	0.1403^{ns}	0.13^{ns}	0.0719^{ns}	-0.1588^{ns}
R38H02	0.0191^{ns}	0.0023^{ns}	0.068^{ns}	-0.0578^{ns}

The experimental progenies expressing WT-S6KII in R1, R3, R2/R4m and R4 ring neurons fail to restore the S6KII mutant phenotype for height, vertical compactness [one exception], size and horizontal-oblique bar combination (Table 1). Consistent with the above findings these results show that WT-S6KII in ring neurons is not sufficient to restore the mutant phenotype for size and vertical compactness. In addition the negative rescue effect for height suggests that not all groups of ring neurons might be sufficient to rescue the height novelty choice effect. Statistical test: Unpaired t-test.

2.5.1.2. BLOCKING RING NEURONS

2.5.1.2.1. Blocking by RNA-interference with ign for height

It is shown in Fig. 21 that WT-S6KII in ring neurons of the ellipsoid body is sufficient to support novelty choice for height in the *ign* mutant. So the question to be answered is whether these ring neurons are necessary for height. To investigate this, the *ign* gene expression in the progenies is interfered by RNA-interference method by driving the expression of *ignRNAi* (UAS *ignRNAi*) in the ring neurons: (R1, R3, R3/R4d and R2/R4m; c105-Gal4, 189y-Gal4, c232-Gal4 and c42-Gal4 respectively). c105-Gal4/+, 189y-Gal4/+, c819-Gal4/+, c232-Gal4/+ and c42-Gal4/+ flies are used as driver control and measured in parallel with the experimental flies.

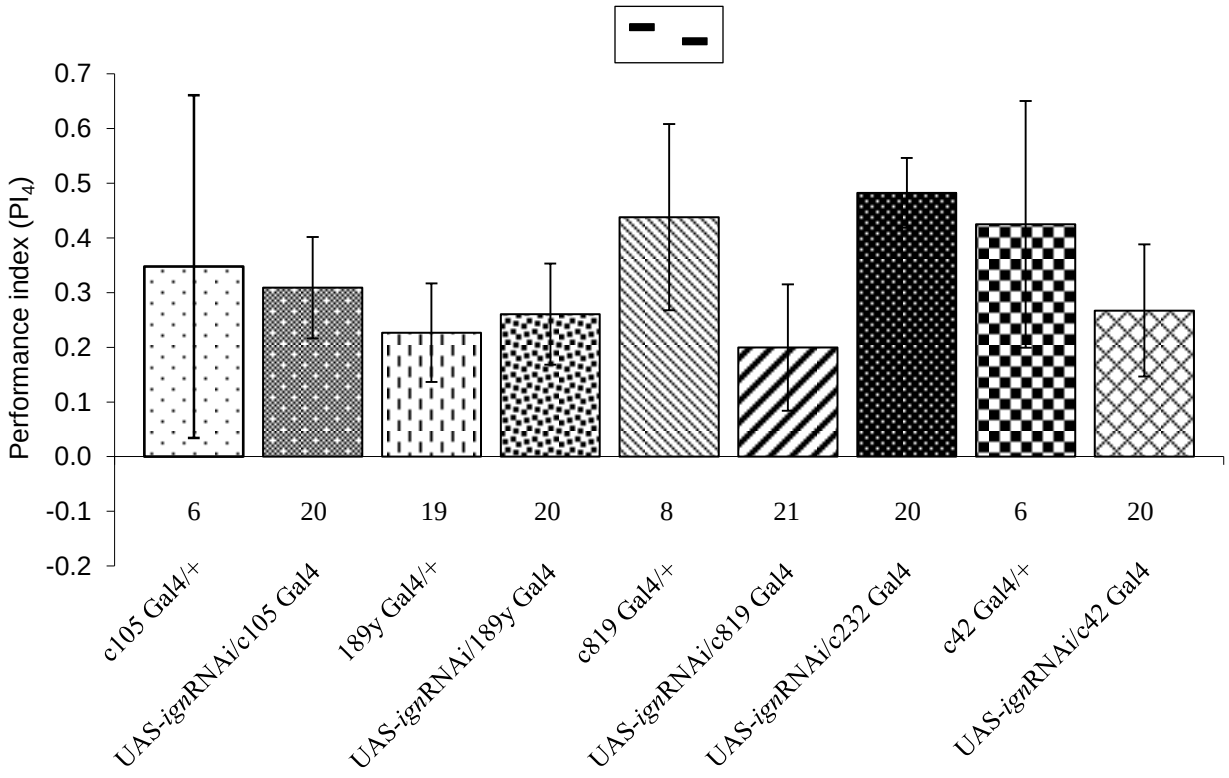


Figure 24: RNA interference of ring neurons for height.
If RNAi would suppress the WT-S6KII in R1, R3, R3/R4d and R2/R4m ring neurons of ellipsoid body by c105-Gal4, 189y-Gal4, c232-Gal4 and c42-Gal4 respectively. Then WT-S6KII is not required to show novelty choice for height.

The experimental progenies that should have reduced WT-S6KII in R1, R3, R3/R4d or R2/R4m ring neurons are not affected in novelty choice for height (Fig. 24). These results suggest that the ring neurons are redundant for novelty choice for height. Such a result would be in line with the results of Fig. 21 because these also suggest that the ellipsoid body is redundant with respect to the ring neurons regarding novelty choice for height. Alternatively it could be that the RNAi is now working effectively therefore these findings need to be confirmed by other blocking methods.

2.5.1.2.2. Blocking by RNA-interference with ign for size

From above results it can be assumed that novelty choice for size involves a different circuitry. And the lack of its understanding prompted me to investigate the necessity of ring neurons for size, despite the findings that driver controls show near zero novelty choice (Fig. 23), which could be attributed to number of things; first: bad-batch of flies, second: Gal4 driver lines being problematic for size and so on.

The *ign* gene expression is silenced in R1, R3, R3/R4d and R2/R4m ring neurons by driving *ignRNAi* (UAS *ignRNAi*) with Gal4 driver lines: (R1; c105, R3; 189y, R3/R4d; c232 and R2/R4m; c819/c42). c105-Gal4/+, 189y-Gal4/+, c819-Gal4/+, c232-Gal4/+ and c42-Gal4/+ flies; used as driver control are measured in parallel with the experimental flies.

The experimental progenies without WT S6KII in R1, R3, R3/R4d or R2/R4m ring neurons do not show wild-type phenotype for size (Fig. 25). However, the driver controls themselves show no novelty choice for size consistent with the Fig. 23 results. It seems that Gal4 in ring neurons interferes with novelty choice for size.

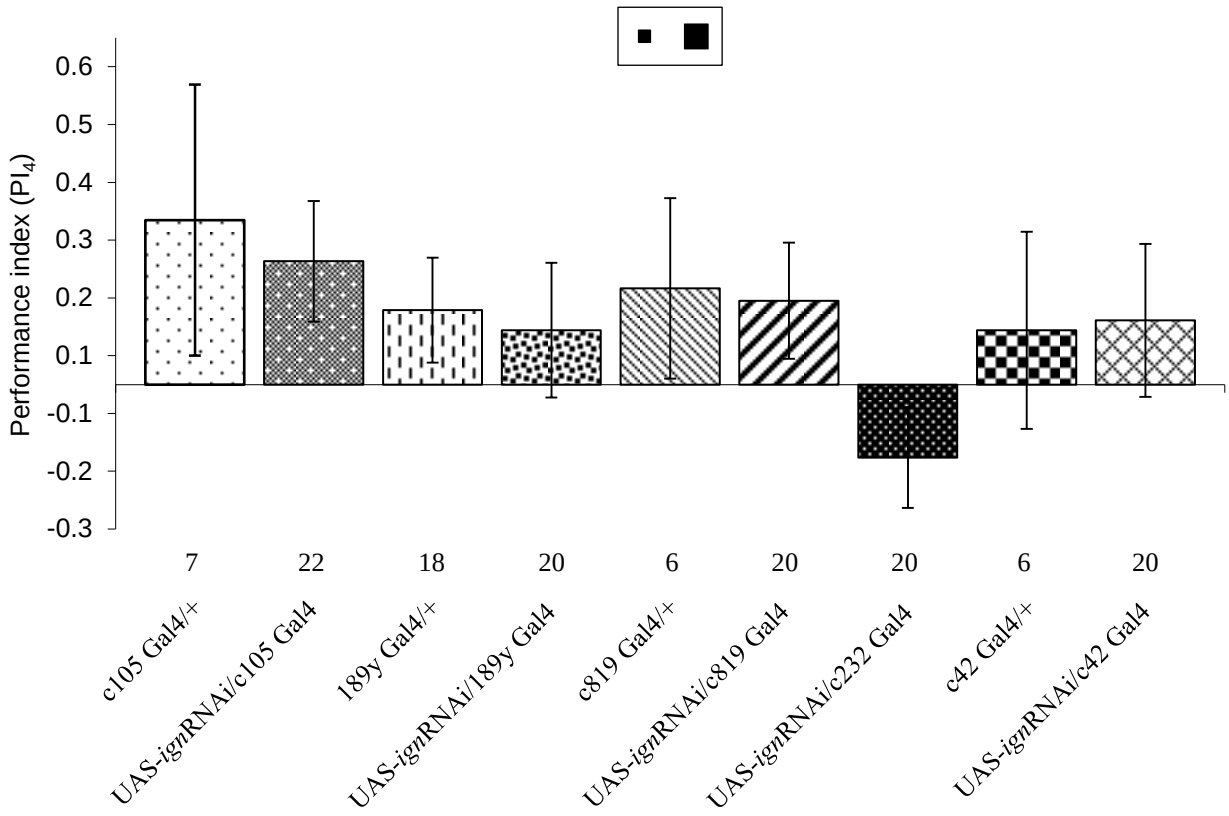


Figure 25: RNA interference of ring neurons for size.
Blocking the WT-S6KII output in R1, R3, R3/R4d and R2/R4m ring neurons of ellipsoid body by c105-Gal4, 189y-Gal4, c232-Gal4 and c42-Gal4 respectively with RNAi, suppresses the wild-type phenotype for size. However the driver controls show no novelty choice.

2.5.1.2.3. Blocking by Shibire^{ts1} for height

To confirm that small groups of ring neurons are dispensible for height (Fig. 24), the ineffectiveness of the silencing of ring neurons in novelty choice needs to be shown by other methods as mentioned above. Here Shibire^{ts1} is used to block the ring neuron output. Advantageously this temperature-sensitive regulation of Shibire^{ts1} allows examining whether the observed mutant phenotype is an adult or a developmental one in addition.

The expression of Shibire^{ts1} is driven in R1, R3, R3/R4d and R2/R4m ring neurons by combining UAS-*shi*^{ts1} with the respective Gal4 driver lines: (R1; c105, R3; 189y, R3/R4d; c232 and R2/R4m; c819/c42). The progenies containing both the UAS-*shi*^{ts1} and c105, 189y, c232, c819 or c42 Gal4 constructs measured at permissive temperature (T = 25°C) act as controls. As experimentals the

flies are incubated for 10-12 minutes at 31°C and soon after measured at the same restrictive temperature (T = 31°C). Experimentals and controls are measured in parallel.

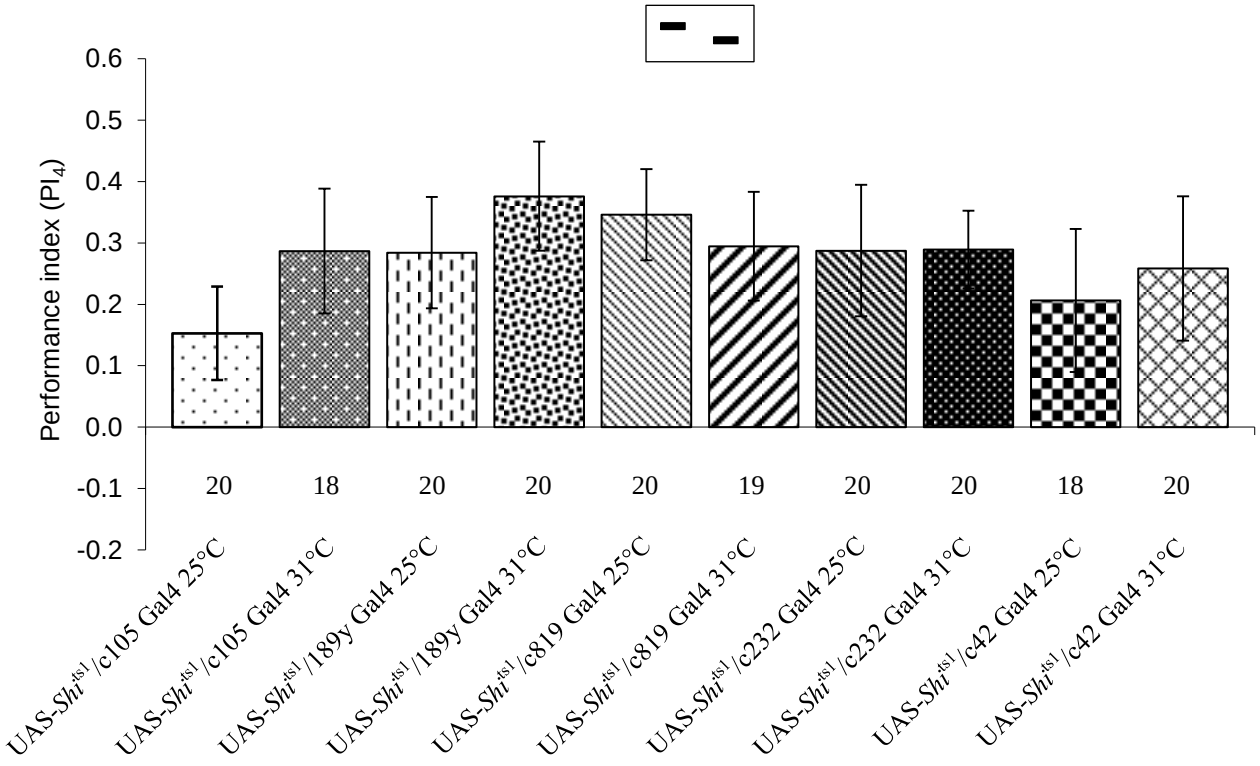


Figure 26: Silencing ring neurons by Shibire^{ts1} do not affect height.
Silencing R1, R3, R3/R4d and R2/R4m ring neurons by Shibire^{ts1} do not affect the wild-type phenotype for height novelty choice at both permissive (25°C) and restrictive (31°C) temperatures.

Fig. 26 shows that at both permissive and restrictive temperatures flies show novelty choice (wild-type phenotype unaffected) for height, suggesting that small groups of ring neurons might be dispensable for height.

2.5.1.2.4. Blocking by TNT for height

To consolidate the speculated redundancy of ring neuron function in the ellipsoid body for height novelty choice, Tetanus neurotoxin (TNT) is used to block groups of ring neurons. The Gal4 driver lines: 189y, c105, c819 and c232 are used to drive the expression of TNT by UAS-TNTE in R3,

R1, R2/R4m and R3/R4d respectively. UAS-TNTE/+ flies, used as effector control are measured in parallel.

Blocking R3 ring neuron function with TNT expression (UAS-TNTE/189y Gal4) blocks the novelty choice for height (Fig. 26; P=0.28). However the wild-type phenotype is unaffected when TNT is expressed in R1 (UAS-TNTE/c105 Gal4), R2/R4m (UAS-TNTE/c819 Gal4) and R3/R4d (UAS-TNTE/c232 Gal4). In addition the effector control shows normal (wild-type) novelty choice behavior.

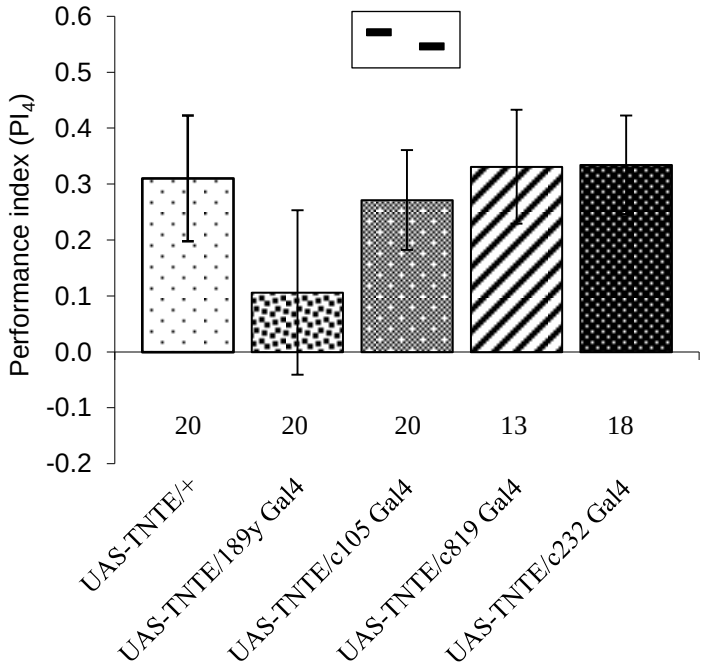


Figure 27: R3 ring neurons are necessary for height.
Silencing the R3 ring neurons of the ellipsoid body blocks the novelty choice for height (P=0.28), while blocking R1, R2/R4m or R3/R4d has no effect on height novelty choice.

Although not significant blocking R3 ring neurons with TNT expression (UAS-TNTE/189y Gal4) blocks the novelty choice for height (Fig. 27). Over a large number of flies this tendency (blocking novelty choice) would stabilize and the novelty choice would be abolished. As a result the R3 ring neurons of the ellipsoid body would be the only neurons necessary (Fig. 27) and sufficient (Fig. 21) for height novelty choice.

2.5.1.2.5. Blocking by TNT for size

It is evident from the above results that novelty choice for size needs to be addressed more aggressively to get an understanding of the behavior. The necessity of different ring neurons is further investigated here by driving the expressing TNT in R1 and R3 neurons. c105 and 189y Gal4 driver lines are used to drive the expression of TNT in R1 and R3 neurons respectively. UAS-TNTE/+ flies, used as effector control are measured in parallel with the experiments.

Blocking the R1 and R3 neuron output with TNT expression (UAS-TNTE/c105 or 189y Gal4) blocks the novelty choice for size (Fig. 28). In addition the effector control shows normal (wild-type) behavior. However it must be noted that the driver controls for these Gal4 lines also show no novelty choice for size (Fig. 23 & 25). Therefore to state that R1 and R3 neurons are necessary for size these findings need to be scrutinized.

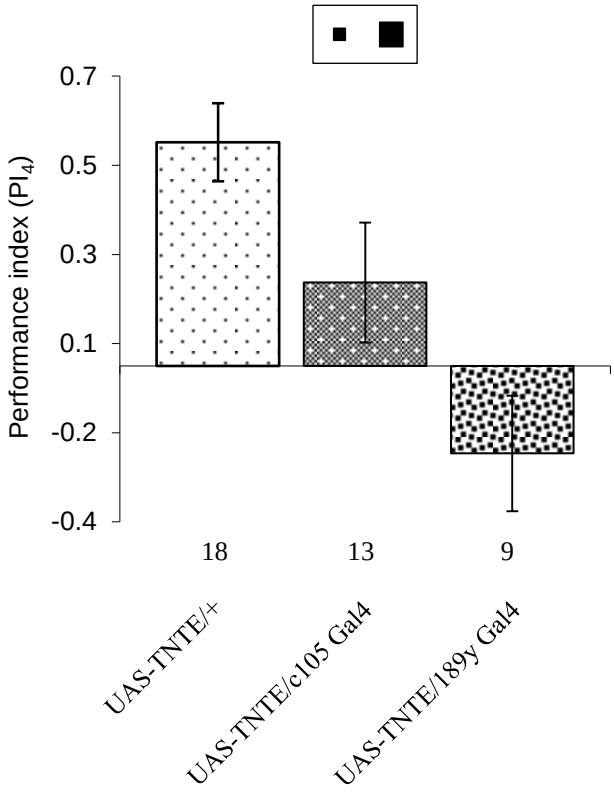


Figure 28: Investigating necessity of ring neurons for size.
Blocking R1 and R3 neurons output by TNT expression block the novelty choice for size. However the driver control shows no novelty choice while the effector control shows normal novelty choice for size.

2.5.1.3. ROLE OF ELLIPSOID BODY IN THE NOVELTY CHOICE

2.5.1.3.1. An intact ellipsoid body is required for the novelty choice

The ellipsoid body is a part of the central complex of the *Drosophila* brain. It is reported by Ilius et al (1994) that it is involved in the visual flight control at the torque meter. Recent studies have also shown that ring neurons of the ellipsoid body are sufficient to restore the mutant phenotypes of *rut* for height, vertical compactness and size in heat conditioning experiments (Pan et al. 2009) and are also involved in functional spatial orientation memory (Neuser et al. 2008).

To further consolidate the role of ellipsoid body ring neurons in the novelty choice behavior, I investigated mutants of the *ellipsoid body open (ebo)* gene. In these mutants the ellipsoid body is deformed; as a result the ellipsoid body function is altered (Ilius et al. 1994). Three alleles of the gene: ebo^{1041}, ebo^{678} and ebo^{KS263} are used in parallel with WT-*Berlin.*

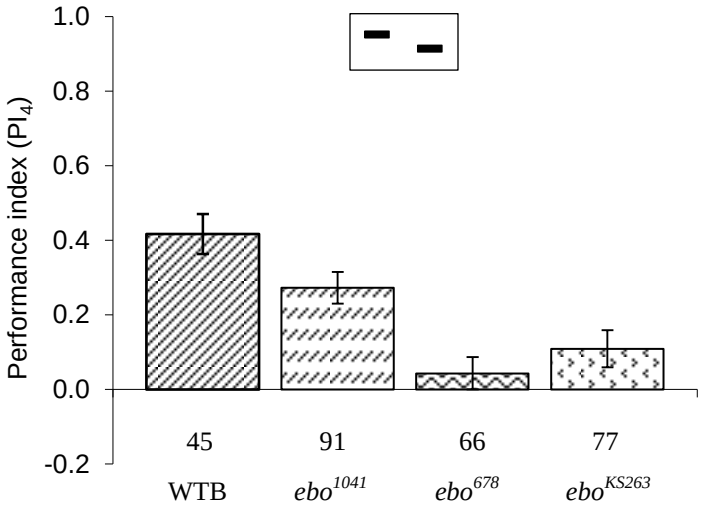


Figure 29: An intact ellipsoid body is required for height novelty choice.
ebo^{1041} flies show novelty choice for horizontal bars, but ebo^{678} and ebo^{KS263} show no novelty choice. The ebo^{1041} and ebo^{678}/ebo^{KS263} differ significantly: P=0.03 and 0.01 respectively for ebo^{678} and ebo^{KS263}.

Fig. 29 shows that the *ebo* allele ebo^{1041} shows novelty choice for horizontal bars (wild-type like), however the other two alleles: ebo^{678} and ebo^{KS263}, show no novelty choice. These two alleles

ebo^{678} (P=0.03) and *ebo*^{KS263} (P=0.01) differ significantly from *ebo*^{1041}. The structural defects in these flies are confirmed by paraffin sectioning.

Here *ebo* alleles *ebo*^{678} and *ebo*^{KS263}, show no novelty choice for horizontal bars, emphasizing the importance of ellipsoid body for the novelty choice behavior consistent with the findings from Fig. 21. The data obtained above speak only for horizontal bars, so the next question is whether *ebo* plays a role in novelty choice for other pattern parameters. To answer this question, only one allele of *ebo*: *ebo*^{KS263} is used to measure the novelty choice for horizontal bars, vertical compactness and size in parallel.

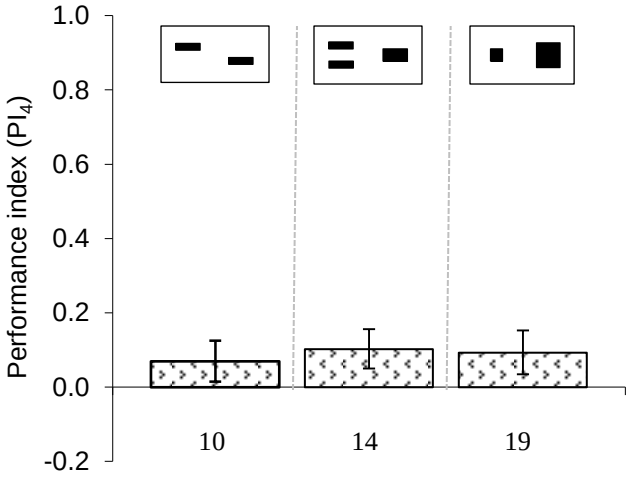


Figure 30: Intact ellipsoid body is required for novelty choice.
ebo^{KS263} show no novelty choice for horizontal bars, vertical compactness and size: P=0.24, 0.07 and 0.13 respectively.

Fig. 30 shows that *ebo*^{KS263} flies show no novelty choice for horizontal bars, vertical compactness and size (0.24, 0.07 and 0.13 respectively). These findings indicate that ellipsoid body is indeed a crucial structure for novelty choice and therefore it needs to be further investigated. Significance is calculated by two-tailed t-test.

2.5.2. FAN-SHAPED BODY CIRCUITRY

2.5.2.1. RESCUE OF *IGNORANT* PHENOTYPE IN F1 NEURONS

2.5.2.1.1. Role of F1 neurons of the fan-shaped body in the novelty choice for height

As shown in Fig. 21 WT-S6KII in R2/R4m ring neurons rescue the S6KII mutant phenotype for the height parameter by using c42-Gal4 driver line. This Gal4 driver line in addition has an expression in some of the F1 neurons of the fan-shaped body. It is shown by Liu et al (2006) that F1 neurons of the fan-shaped body are a site of rescue of Rutabaga dependent memory for the pattern parameter contour orientation in the heat conditioning study. These two findings: sufficiency of Rut in F1 neurons for contour orientation in the heat conditioning study and their possible involvement in the novelty choice for height, prompted me to investigate whether S6KII in F1 neurons contributes to the novelty choice for height. The Gal4 driver lines NP 6510 and NP 6561 are used for this experiment.

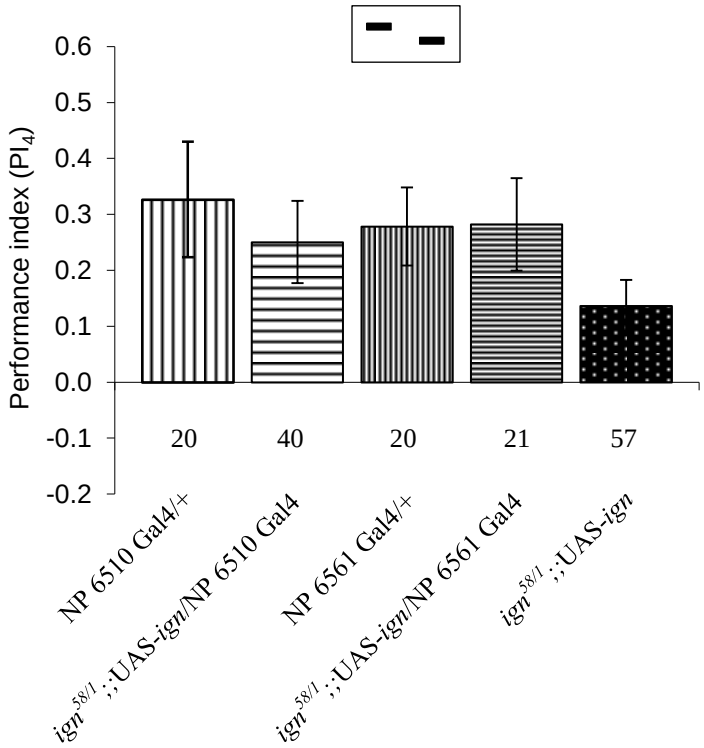


Figure 31: F1 neurons are not sufficient to rescue height novelty choice.
WT-S6KII expression in F1 neurons of the fan-shaped body does not restore the mutant phenotype for height novelty choice. The experimental and effector control do not differ significantly: P=0.17 and 0.12 respectively for *ign*$^{58/1}$;;UAS-*ign*/NP 6510 Gal4 and *ign*$^{58/1}$;;UAS-*ign*/NP 6561 Gal4 compared to *ign*$^{58/1}$;;UAS-*ign* effector.

The progenies expressing WT- S6KII in F1 neurons in an otherwise *ign* mutant background do not restore (Fig. 31) the S6KII mutant phenotype for the height parameter. The experimental and

effector control do not differ significantly: (*ign*$^{58/1}$;;UAS-*ign*/NP 6510 Gal4) p = 0.17 and (*ign*$^{58/1}$;;UAS-*ign*/NP 6561 Gal4) p = 0.12. The driver control and experimental expectedly show no significant difference between each other. Statistical test: Unpaired t-test.

This does not prove that novelty choice for the height parameter can be rescued in F1 neurons of the fan-shaped body. Importantly WT-S6KII protein in R1, R3, R3/R4d or in R2/R4m ring neurons in an *ign* mutant background is sufficient to fully restore the S6KII mutant phenotype for the height parameter.

2.5.2.1.2. F1 neurons of the fan-shaped body are not sufficient for vertical compactness

In the same line the role of F1 neurons of fan-shaped body are investigated in the novelty choice for vertical compactness with the Gal4 driver lines NP 6510 and NP 6561.

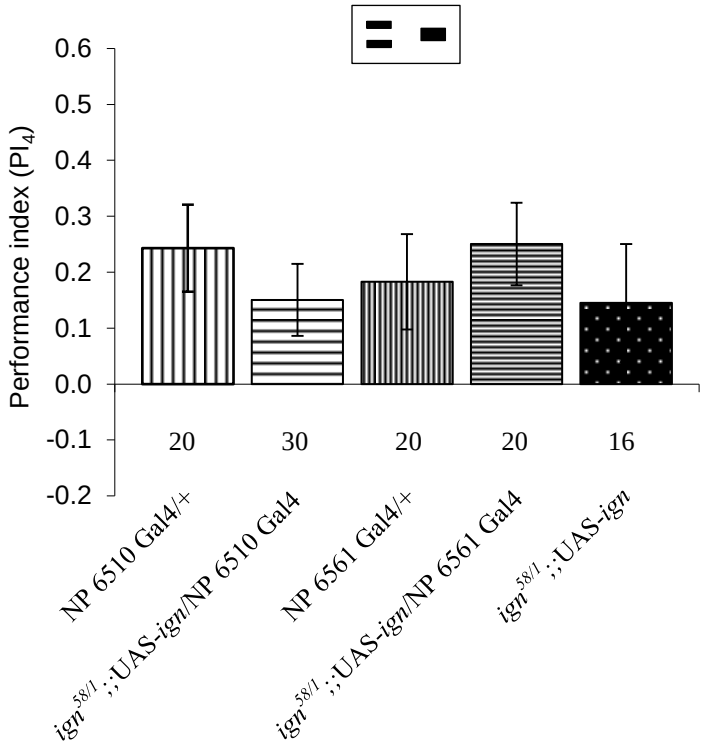


Figure 32: F1 neurons of the fan-shaped body are not sufficient for vertical compactness. WT-S6KII in F1 neurons of the fan-shaped body fail to restore the S6KII mutant phenotype for vertical compactness. P=0.96 and 0.40 for *ign*$^{58/1}$;;UAS-*ign*/NP 6510-Gal4 and *ign*$^{58/1}$;;UAS-*ign*/NP 6561-Gal4 respectively.

Again, the progenies expressing WT-S6KII protein in F1 layer neurons in an otherwise *ign* mutant background do not restore (Fig. 32) the S6KII mutant phenotype for vertical compactness. The experimental and effector controls do not differ significantly: ($ign^{58/1}$;;UAS-*ign*/NP 6510 Gal4) p = 0.96 and ($ign^{58/1}$;;UAS-*ign*/NP 6561 Gal4) p = 0.40. The driver controls and experimentals do not differ significantly as well. These results indicate that F1 layer neurons of the fan-shaped body expressing Gal4 are not sufficient to restore the mutant phenotype for vertical compactness novelty choice.

2.5.2.1.3. Role of F1 neurons of the fan-shaped body for size

The role of F1 layer neurons is also investigated for size with the Gal4 driver lines NP 6510 and NP 6561. NP 6510/+ and NP 6561/+ flies are used as driver control. Experimentals and driver controls are measured in parallel.

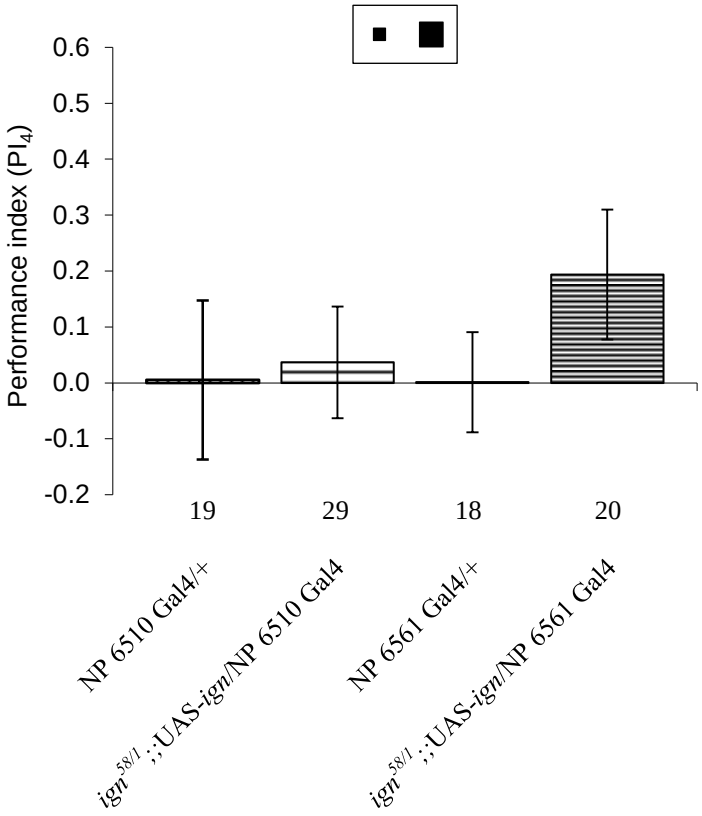


Figure 33: Role of F1 neurons of the fan-shaped body for size.
Flies expressing WT-S6KII in F1 neurons of the fan-shaped body do not show novelty choice for size. Furthermore the driver controls themselves shows no novelty choice for size.

The experimental progenies expressing WT-S6KII protein in F1 layer neurons in an otherwise *ign* mutant background are unable to repair (Fig. 33) the S6KII mutant phenotype for size. Furthermore as observed in Fig. 23 and 25 here too the driver controls do not show novelty choice for size. Therefore the negative results again might be attributed to the driver lines.

2.5.2.2. BLOCKING THE F1 NEURONS

2.5.2.2.1. Blocking of F1 neurons by Shibire^{ts1} for height

To investigate the possible necessity of F1 neurons in the novelty choice for height, the Gal4 driver lines NP 6560 and NP 6561 are used to drive the expression of Shibire^{ts1} by UAS-*shi*^{ts1}. The progenies (UAS-*shi*^{ts1}/NP 65610 Gal4 and UAS-*shi*^{ts1}/NP 6561 Gal4) measured at permissive temperature (T = 25°C) act as controls. In parallel the experimental flies are measured at restrictive temperature (T = 31°C) soon after they are incubated for 10-12 minutes at 31°C.

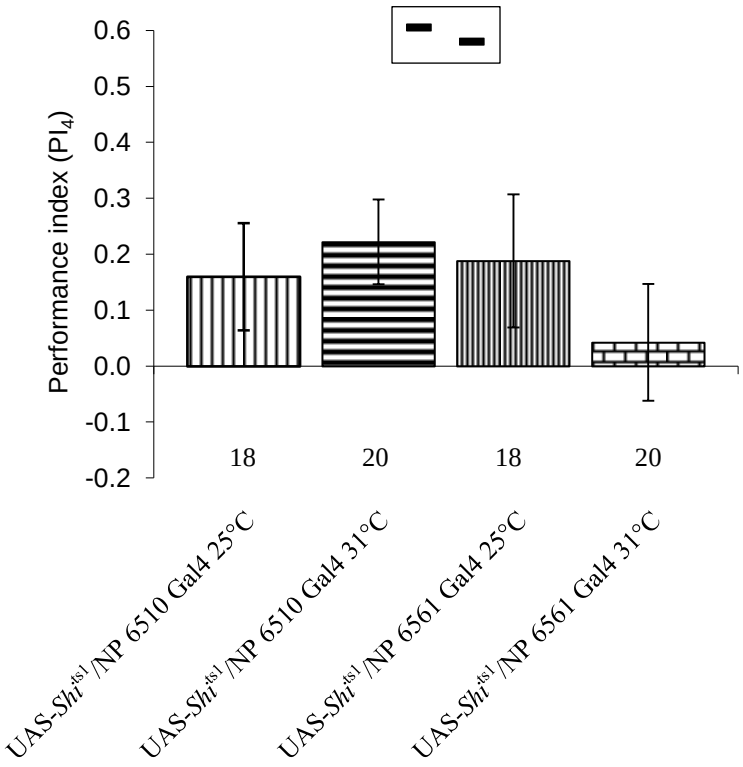


Figure 34: Novelty choice for height is blocked in F1 neurons with Shibire^{ts1}.
Expressing Shibire^{ts1} in F1 neurons of the fan-shaped body significantly abolishes the novelty choice for height at both restrictive (31°C) and permissive (25°C) temperatures.

The UAS-*shi*^{ts1}/NP 6510 Gal4 flies show novelty choice for height only at the restrictive, but not at the permissive temperature. UAS-*shi*^{ts1}/NP 6561 Gal4 flies show novelty choice for height neither at permissive nor at restrictive temperature. The four responses do not differ significantly from each other. However, the results for the Gal4 line NP6561 point at a possible suppression of height novelty choice at the restrictive temperature.

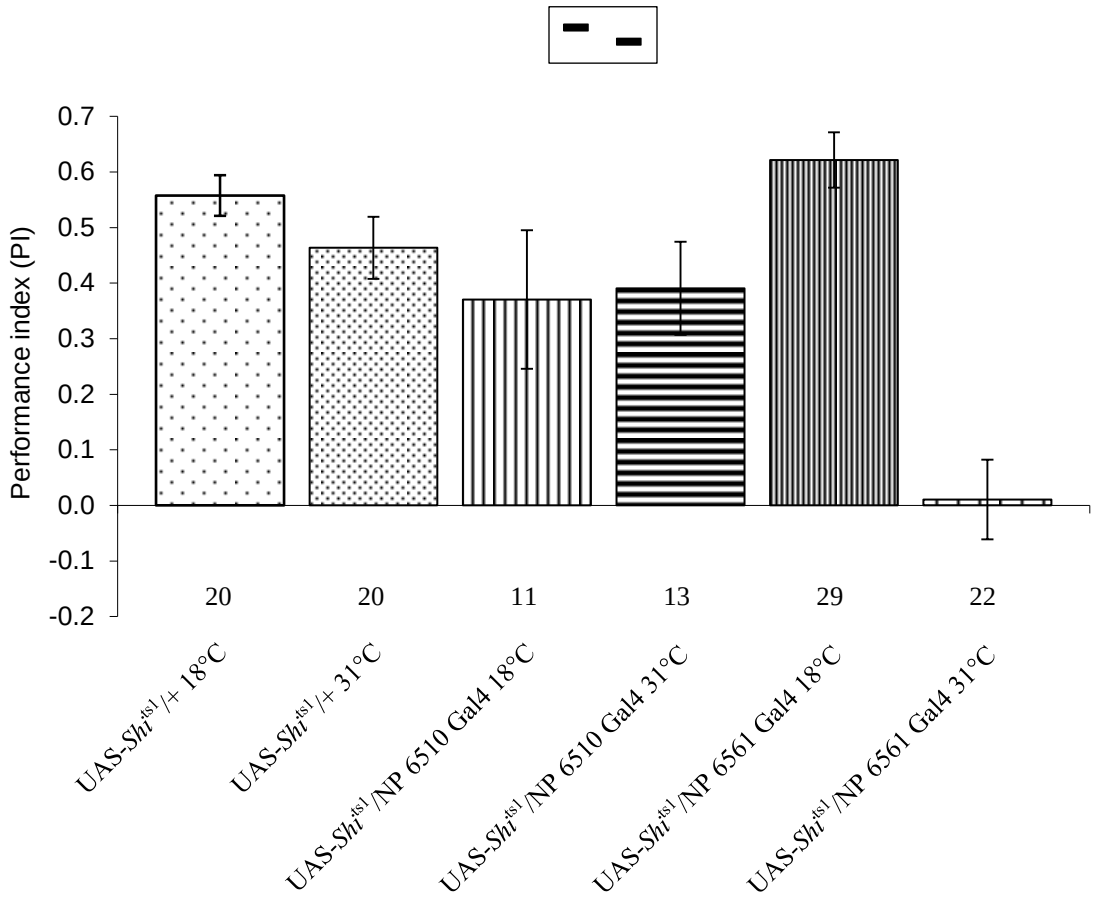


Figure 35: F1 neurons in adult are necessary for height novelty choice.
Expressing Shibire^{ts1} in F1 neurons of the fan-shaped body in NP6561 abolishes the novelty choice for height at restrictive (31°C) but not at permissive (18°C) temperatures (P<0-0001), while the controls show normal novelty choice at both permissive and restrictive temperatures. Furthermore NP6510 flies show normal novelty choice at both the temperatures.

Both the Gal4 lines (NP6510 and NP6561) have an expression in the F1 layer, but it cannot be excluded that the Gal4 is expressed in different neurons of the F1 layer, resulting in differential behavior (2nd and 4th column in Fig.34). To follow up on this possibility the experiment is repeated under conditions in which the Shibire^{ts1} should be fully in the permissive state. This is achieved by

maintaining the progenies at 18°C throughout to exclude any negative effect that might occur at 25°C. Control flies (UAS-*shi*^{ts1}/NP 6510 Gal4 and UAS-*shi*^{ts1}/NP 6561 Gal4) are measured at permissive temperature (T = 18°C), whereas the experimental flies are measured at restrictive temperature (T = 31°C) soon after they have been incubated for 10-12 minutes at 31°C. UAS-*shi*^{ts1}/+ flies are measured as effector control both at permissive (T = 18°C) and restrictive temperatures (T = 31°C).

Fig. 35 shows that UAS-*shi*^{ts1}/NP 6510 Gal4 flies show novelty choice for height at both the temperatures. Consistent with the tendency in Fig. 34, UAS-*shi*^{ts1}/NP 6561 Gal4 flies shows novelty choice only at permissive but no novelty choice at restrictive temperature indicating the necessity of some of the neurons labeled only by NP 6561 Gal4 line for height (Unpaired t-test: P<0-0001). The effector control (UAS-*shi*^{ts1}/+ flies) shows novelty choice for height at both permissive (T = 18°C) and restrictive temperatures (T = 31°C). Importantly, the results demonstrate that the phenotype is an adult phenotype not developmental.

2.5.2.2.2. Blocking F1 neurons output by TNT for height novelty choice

F1 neurons of the fan-shaped body are necessary for height novelty choice, shown above in Fig. 35. This effect is only observed for the NP 6561 Gal4 driver line. To consolidate this finding a different effector construct, tetanus toxin light chain (UAS-TNTE) is used to block the F1 neurons' output. For this, UAS-TNTE is combined with NP 6510 and NP6561 Gal4 to express TNT in the respective F1 neurons of the fan-shaped body. In this experiment TNT expression is not restricted to the adult stage, because in the two driver lines Gal4 might also be expressed in the larval and embryonic stages.

Consistent with the Shibire^{ts1} findings of the Fig. 35, NP6561 flies with silenced F1 neurons show no novelty choice for height (Fig. 36; P=0.45 and 0.08 for NP6510 and NP6561 respectively), demonstrating that F1 neurons of the fan-shaped body are necessary for height. The phenotype observed here by UAS-TNTE/NP 6510 flies could be attributed to the expression of TNT during the developmental phase, but this needs to be investigated further.

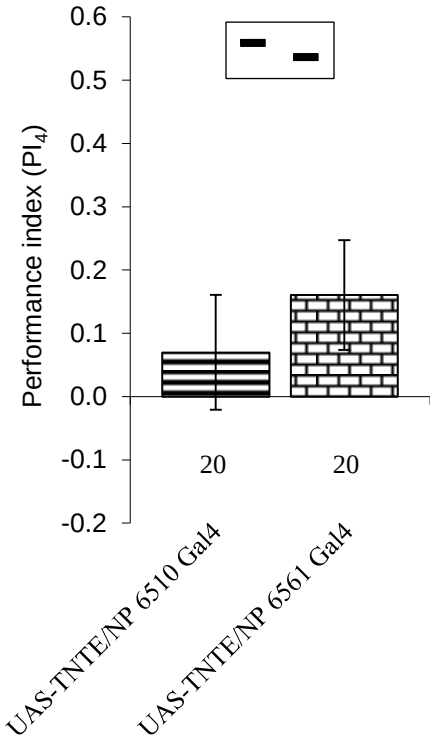


Figure 36: TNT in F1 layer neurons suppresses height novelty choice.
Expressing TNT in F1 layer neurons of the fan-shaped body blocks the novelty choice for height using NP 6510 and NP 6561 Gal4 driver lines. Two-tailed t-test; P=0.45 and 0.08 for NP6510 and NP6561 respectively.

2.5.2.2.3. Blocking F1 neurons output by TNT for vertical compactness

Although F1 neurons of the NP 6510 driver line appear not to rescue novelty choice for vertical compactness by WT S6KII expression in the *ign* mutant (Fig. 32), it still is interesting to investigate whether F1 neurons might be necessary. Here UAS-TNTE is combined with NP 6510 Gal4 to express TNT in F1 neurons of the fan-shaped body.

Fig. 37 shows that blocking F1 neurons with TNT does not affect the novelty choice for vertical compactness (Two-tailed t-test; P=0.04), suggesting that these F1 neurons of the fan-shaped body might not be necessary for vertical compactness.

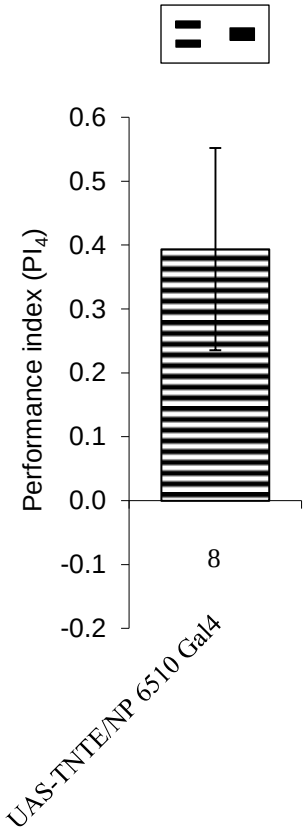


Figure 37: F1 neurons might not be necessary for vertical compactness.
Blocking the F1 neurons of the fan-shaped body by TNT does not affect the novelty choice for vertical compactness. P=0.04.

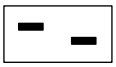
2.5.2.3. CAN THE *IGNORANT* PHENOTYPE BE RESCUED IN F5 LAYER NEURONS?

2.5.2.3.1. Role of F5 neurons of the fan-shaped body in the novelty choice

It is shown above that the F1 neurons of the fan-shaped body play a role in novelty choice. In this chapter it is investigated whether neurons in the F5 layer of the fan-shaped body are also involved. In 2006 Liu et al. have shown that the Rutabaga dependent memory defect for the height parameter in the heat conditioning experiment is located in some of the F5 neurons of the fan-shaped body. This in turn raises the question whether F5 layer neurons are involved in the novelty choice for height too.

For this investigation WT-S6KII is expressed in some of the F5 neurons in an *ign* mutant background by combining $ign^{58/1}$;;UAS-*ign* with Gal4 driver lines (c5, c205 and 104y). The progenies ($ign^{58/1}$;;UAS-*ign*/c5, c205 or 104y -Gal4) expressing WT-S6KII in F5 neurons in an *ign* mutant background are used to measure the novelty choice for horizontal bars, vertical compactness, size parameters and also for horizontal-oblique pattern combination.

Table 2: No rescue of novelty choice in F5 neurons of the fan-shaped body.

Gal4 driver lines				
c5	0.1687^{ns}	——	0.1559^{ns}	——
104y	0.1644^{ns}	——	——	0.1095^{ns}
c205	0.0889^{ns}	0.0751^{ns}	——	0.1424^{ns}

The data in Table 2 show that with all three driver lines, flies expressing WT-S6KII in some of the F5 neurons show no significant novelty choice for height, vertical compactness and size parameter. In addition they also show no novelty choice for horizontal-oblique pattern combination. The lack of driver controls makes it difficult to draw a final conclusion but these findings indicate that F5 neurons of the fan-shaped body might not be involved in the novelty choice behavior. Statistical test: Unpaired t-test.

2.5.3. MUSHROOM BODIES IN THE NOVELTY CHOICE

2.5.3.1. *mushroom body miniature (mbm)* gene is required for size novelty choice

Drosophila mushroom bodies (MBs), one of the central brain structures are involved in several cognitive behaviors, such as olfactory learning (Heisenberg et al. 1985, de Belle et al. 1994, Connolly et al. 1996), visual context generalization (Liu et al. 1999) and choice behavior facing conflicting cues (Tang and Guo. 2001; Zang et al. 2007) . They also modulate salience-based selective fixation behavior (Xi et al. 2008). This makes the investigation of the mushroom bodies' involvement in novelty choice behavior an interesting question.

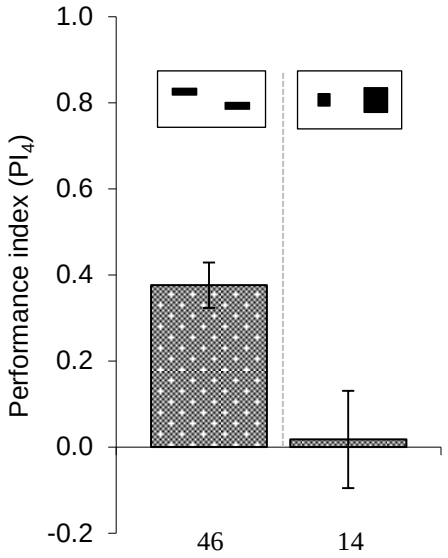


Figure 38: A mutation in the *mbm* gene impairs novelty choice for size.
Mutant *mbm*^{1} females show novelty choice for horizontal bars while interestingly they lack novelty choice for size. The responses to the two pairs of pattern differ significantly (P=0.003).

The autosomal *mushroom body miniature* (*mbm*) gene which encodes a zinc-finger protein implicated in nucleic acid binding and brain development is used here. The mutant *mbm*^{1} has an anatomical defect of the MBs observed only in females. In *mbm*^{1} females the Kenyon cell fibers start to degenerate in the third larval instar and their regeneration is not observed during metamorphosis. As a result adult *mbm*^{1} females lack most Kenyon cell fibers and are impaired in MB-mediated associative odor learning (de Belle and Heisenberg. 1996). Therefore to investigate the role of mushroom bodies in the novelty choice for horizontal bars and size *mbm*^{1} females are used, contrary to *ign*$^{58/1}$ males used in previous sections because of X-chromosome deletion in the *ign* mutant.

Interestingly Fig. 38 shows that in *mbm*[1] females novelty choice for size is impaired. However, consistent with the findings for visual learning, MBs are not required for novelty choice for horizontal bars. The two responses differ significantly (Unpaired t-test: P=0.003). These results indicate that the neural processing underlying novelty choice for size and for horizontal bars is different.

Unexpectedly, *mbm*[1] males which have normal-looking MBs, are also impaired in novelty choice for size and unaffected in novelty choice for horizontal bars and vertical compactness (Fig. 39). This finding raises the possibility that the impairment in novelty choice for size in females and males of *mbm*[1] is not related to the MBs. Therefore the role of mushroom bodies in the novelty choice for size needs to be further investigated by additional methods.

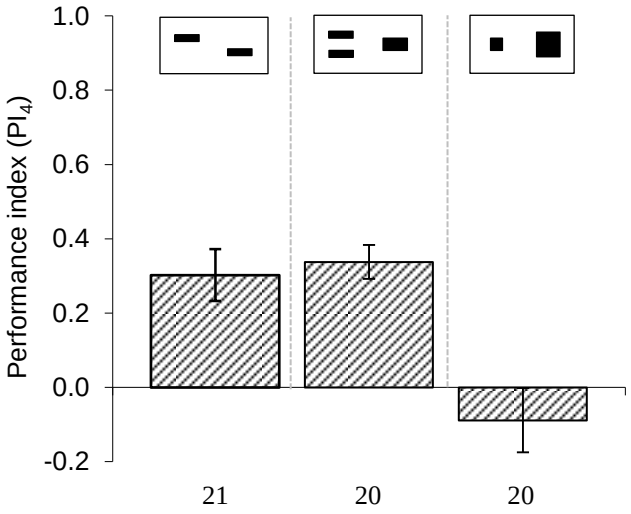


Figure 39: No novelty choice for size in *mbm*[1] males.
mbm[1] males show novelty choice for horizontal bars and vertical compactness but they lack novelty choice for size. Size vs horizontal bars; P=0.001 and size vs vertical compactness; P<0.0001 (Unpaired t-test).

2.5.3.2. Hydroxyurea

The mutant phenotype in *mbm*[1] needs not be only structural and can be anywhere. Hence the results of Figs. 38 and 39 do not prove the involvement of the MBs in novelty choice for size. To confirm an involvement of the MBs (Fig. 38) another method of blocking the mushroom body function, hydroxyurea treatment is applied here. The hydroxyurea treatment ablates the mushroom body

neuroblasts during the early first instar larval stage (de Belle and Heisenberg. 1994, Prokop and Technau. 1994), which in turn leaves the adult flies with only the embryonic mushroom bodies. These mushroom body-ablated flies are used to measure the novelty choice for height, vertical compactness and size parameter in parallel with control flies (which go through the same treatment except hydoxyurea: see Methods).

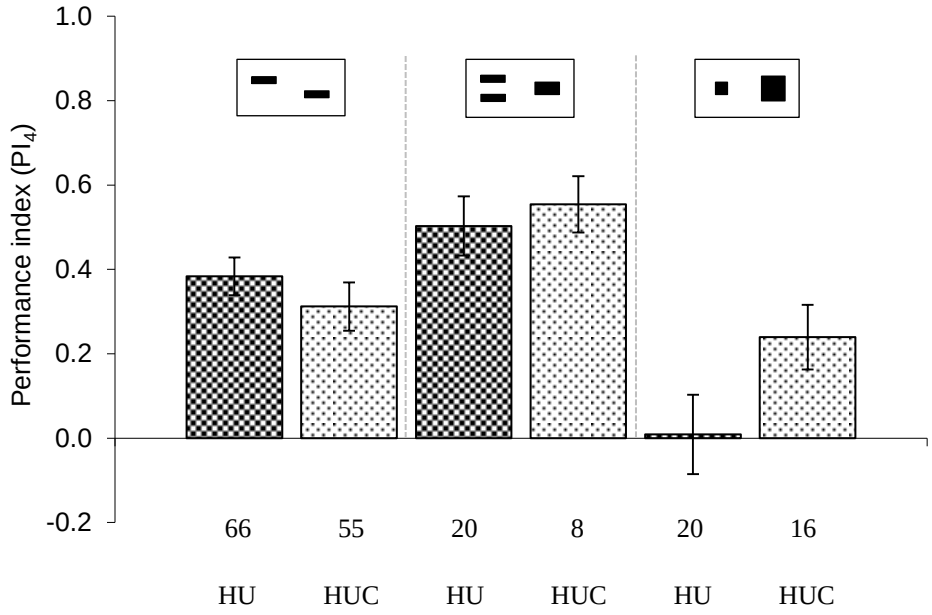


Figure 40: Flies with reduced mushroom bodies are impaired in size novelty choice. Mushroom body-ablated flies (HU) show novelty choice for height and vertical compactness, but not for size. The respective control flies (HUC) show novelty choice for height, vertical compactness as well as for size.

Consistent with results from Fig. 38 here HU flies show novelty choice for height and vertical compactness but they show no novelty choice for size (Fig. 40). The HU-Control (HUC) flies, which go through the same treatment as HU flies except for hydroxyurea, show novelty choice for all the three pattern parameters. The mushroom body ablation in HU flies is confirmed by paraffin sectioning (Fig. 41). These findings confirm the earlier conclusion that *Drosophila* mushroom bodies are dispensable for novelty choice for height and vertical compactness and they strengthen the assumption that MBs are indispensable for size novelty choice.

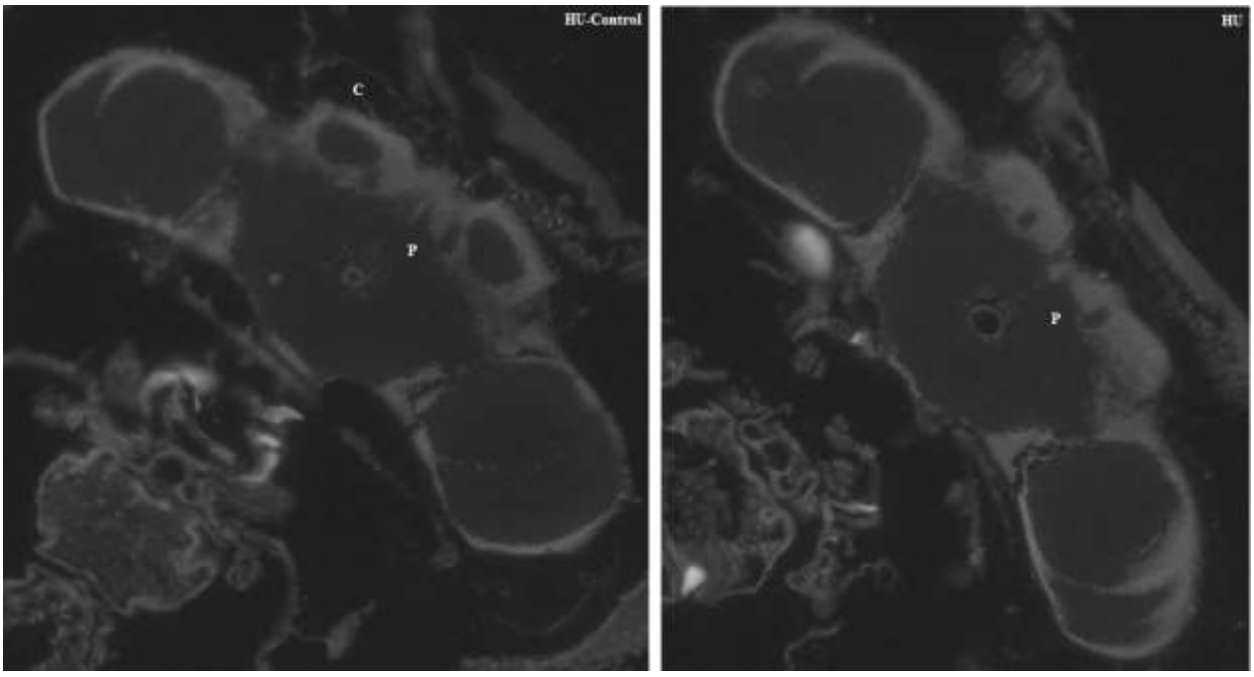


Figure 41: Paraffin sectioning of HU and HUC fly brain.
HU-Control (HUC) brain shows calyx (C) and protocerebral bridge (P) but HU fly brain only shows protocerebral bridge.

2.5.3.3. Blocking mushroom body output

A further method for blocking the mushroom bodies is by using the GAL4/UAS expression system. Here I use the driver line mb247 Gal4 which selectively expresses Gal4 in α/β and γ Kenyon cells. As effectors I use tetanus neurotoxin (UAS-TNTE) and the conditional blocker (UAS *Shi*^{ts1}).

Consistent with the results from Fig. 40 flies with non-functional α/β and γ Kenyon cells show no novelty choice for size (Fig. 42). The UAS TNTE/+ and mb247 Gal4/+ controls expectedly behave like wild type. To investigate whether the phenotype is developmental or adult UAS *Shi*^{ts1} is used to temporally regulate the function of the α/β and γ Kenyon cells via temperature by combining UAS *Shi*^{ts1} with the mb247 Gal4 driver. The cross and the progenies are maintained at 18° C throughout, experimental flies (UAS *Shi*^{ts1}/mb 247 Gal4) are given a heat shock (31° C) for 15 minutes before experiment which is then followed by the novelty choice measurement for size at 31° C. The control flies (UAS *Shi*^{ts1}/mb 247 Gal4) are not given any heat shock and the measurement is performed at 18° C. The experimental flies show no novelty choice for size (Fig. 42) whereas the control flies show

novelty choice for size indicating that the phenotype is adult, not developmental. All the driver and effecter controls (UAS *Shi*^{ts1}/+ at 18° C, UAS *Shi*^{ts1}/+ at 31° C and mb247 Gal4/+ at 31° C) expectedly show novelty choice for size. Importantly, the experimental flies (UAS *Shi*^{ts1}/mb247 Gal4 at 31° C) show wild-type like novelty choice for height (Fig. 42). These findings support those obtained with *mbm*^{1} females (Fig. 38), hydroxyurea (Fig. 40) and TNT (Fig. 42).

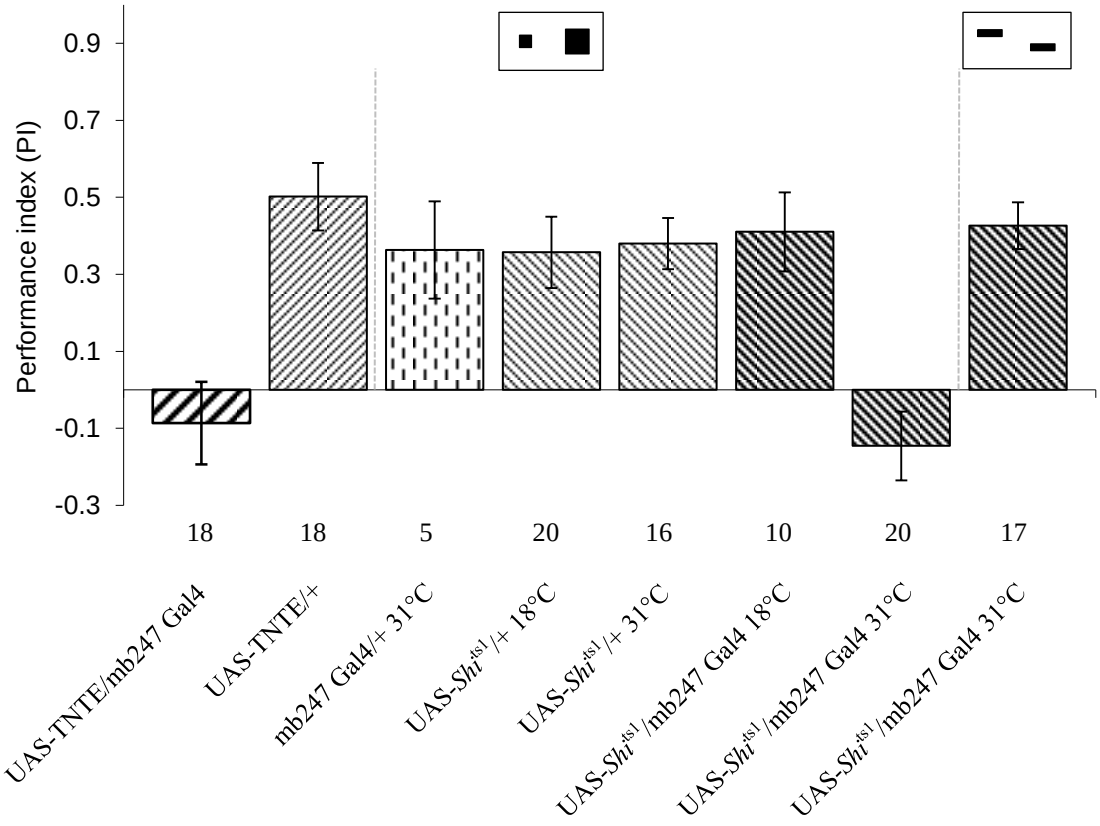


Figure 42: Mushroom bodies are necessary for size.
Flies expressing TNT in α/β and γ Kenyon cells (UAS TNTE/mb247 Gal4) show no novelty choice for size, while the control flies UAS TNTE/+ and mb247 Gal4/+ expectedly behave wild-type like. Flies expressing *Shi*^{ts1} in α/β and γ Kenyon cells (UAS *Shi*^{ts1}/mb 247 Gal4) again are impaired for size novelty choice at the restrictive temperature (31° C) but normal at the permissive temperature (18° C). Interestingly, novelty choice for height is normal at both temperatures.

Umpaired t-test show that UAS TNTE/mb247 Gal4 flies differ significantly from the UAS TNTE/+ control flies; P=0.0002, the control and experimental UAS *Shi*^{ts1}/mb 247 Gal4 flies measured for size differ significantly; P=0.0007, furthermore the experimental UAS *Shi*^{ts1}/mb 247 Gal4 flies measured for size and height differ significantly; P<0.0001.

Taking all these results together it is evident that MBs are necessary for size but not for height novelty choice. This result is in line with the earlier findings in the experiments on the central complex pointing at a special processing for size novelty choice. Also the phenotypic reversion of the mutant *ign*$^{58/1}$ which spares the defect in size novelty choice (Fig. 16) points in this direction. It would be interesting to investigate whether size also plays a special role in heat conditioning.

3. DISCUSSION

In this study I show that flies show novelty choice for height (HB), vertical compactness and size parameters but not for contour orientation, a parameter that can be learned by the flies at the torque meter in heat conditioning experiments (Liu et al. 2006, Pan et al. 2009). Another pattern pair used to study height parameter in heat conditioning experiment is an upright and inverted T at the same height (called standard T). The flies can learn standard Ts in the heat conditioning experiment but they fail to show novelty choice for standard Ts.

In visual learning the mutant memory phenotype for height parameter (Ts and HB) can be rescued in the F5 layer of fan-shaped body and ring neurons of the ellipsoid body with wild-type Rutabaga (Liu et al. 2006, Pan et al. 2009). Here I show that novelty choice is independent of Rutabaga. Also I find that novelty choice is independent of dopamine. Furthermore I show that S6KII a protein shown to be involved in spatial orientation memory by Neuser et al (2008) is required in the novelty choice behavior.

The S6KII mutant phenotype for horizontal bars can be rescued in the ring neurons of the ellipsoid body but not in F5 layer of the fan-shaped body where the memory for height parameter is shown to be rescue in heat conditioning experiment (Liu et al. 2006). In addition I show that ellipsoid body mutants ebo^{678} and ebo^{KS263} show no novelty for horizontal bars, supporting the above finding. Furthermore I find that neurons of the F1 layer of the fan-shaped body where the memory for pattern parameter contour orientation can be rescued in heat conditioning experiments (Liu et al. 2006) are also required for novelty choice with horizontal bars.

In addition I find that mushroom bodies are required to show novelty choice for size but they are dispensable for horizontal bars. This is independently found in flies in which mushroom body development has been blocked by hydroxyurea and by genetically blocking mushroom body function or development.

3.1. Novelty choice for different pattern parameters

The range of pattern parameters studied by Dill et al. (1995) in novelty choice is expanded here. I show that out of four pattern parameters that can be learned by the flies in heat conditioning (Ernst and Heisenberg 1999), novelty choice is elicited for horizontal bars, vertical compactness and

size but not for contour orientation (measured with oblique bars oriented at +/- 45°). The lack of novelty choice with oblique bars is unexpected.

As mentioned above, another pattern pair that can be learned by the flies in the heat conditioning experiment and not in novelty choice is the standard Ts pair (Dill et al. 1993, Tang et al. 2004, Liu et al. 2006). Both the standard Ts and the oblique bars pair contain bars differing in orientation. So, could it be the different orientations that negatively influence the novelty choice? Measuring the novelty choice for pattern combinations differing in orientation (horizontal vs vertical bars and horizontal vs oblique bars) tests this hypothesis. I find that the horizontal vs vertical bars and horizontal vs oblique bars elicit novelty choice. Hence it is not the different orientations that interfere with the novelty choice.

3.2. Difference in evaluation of height parameter in horizontal bars and Ts

In the standard T pattern pair that has been used extensively in heat conditioning experiments the two Ts differ in the height of their CsOG by 13° whereas the horizontal bars differ in the height of their CsOG by 23° with respect to the fly. One would assume that because of the smaller CsOG difference in the Ts pair, the Ts might fail to elicit novelty choice. This assumption is tested by measuring the novelty choice for Ts differing in their CsOG by 23°. I find that although the standard Ts pair does not elicit novelty choice, the Ts pair with CsOG 23° apart does. Interestingly, I also find that the horizontal bars with CsOG 13° apart elicit novelty choice. In other words, a 13° height difference in the CsOG is enough to elicit the novelty choice effect. Hence, in the standard Ts pair it is not the different heights of the CsOG alone that matter for the novelty choice effect. The vertical bars of the Ts might influence the flight performance in the flight simulator. In fact, we do not know whether the flies evaluate the CsOG at all.

These findings point at the possibility that Ts are evaluated differently in novelty choice and heat conditioning. Furthermore they reason that smaller differences in height can be learned in heat conditioning than in novelty choice.

3.3. Role of different molecules in the novelty choice

The Rutabaga protein, a type 1 adenylyl cyclase encoded by *rutabaga (rut)* has been shown to selectively rescue the olfactory associative learning defect in the mutant (Zars et al. 2000, McGuire et al. 2001). In visual learning (heat conditioning) the protein also rescues the Rutabaga dependent memory for standard Ts, horizontal bars, vertical compactness, size and contour orientation (Liu et al. 2006, Pan et al. 2009). The finding that novelty choice is independent of Rutabaga, which is known to act as a coincidence detector in associative olfactory learning (Abrams and Kandel. 1988) is in line with the notion that no reinforcement is involved in novelty choice.

The biogenic amine dopamine regulates the Rut cyclase via the dopamine receptor Dumb (Kim et al. 2007) in aversive and appetitive olfactory learning. Consistent with the above result I find that dopamine is not involved in novelty choice. These findings indicate that the Rut cAMP pathway involved in visual heat conditioning is not involved in novelty choice.

Furthermore I find that a protein of serine-threonine kinase family called ribosomal S6 kinase II (S6KII) encoded by the *ignorant* gene, which is shown to be involved in operant place learning, classical olfactory conditioning (Putz et al. 2004) and also in spatial orientation memory by Neuser et al. (2008) is required for novelty choice. As explained above, to show a preference for the novel pattern in the test, the fly has to remember the non-novel pattern. For this the fly needs a short term working memory, like the one utilized for spatial orientation (Neuser et al. 2008).

Many experiments show that the *ign* mutant phenotype can be rescued by expression of the ign^{+} transgene. In the course of the experiments the effector control in combination with the *ign* mutant ($ign^{58/1}$;;UAS-*ign*) did not always differ significantly from the experimentals. This was tentatively attributed to the accumulation of genetic modifiers in the effector stock leading to a partial loss of the mutant phenotype. Interestingly the mutant phenotype for size was more persistent than that for height and vertical compactness.

3.4. Rescue of S6KII mutant phenotype for height parameter

It has been shown that the Rutabaga dependent memory for height, vertical compactness, size and contour orientation can be rescued in the ring neurons of the ellipsoid body (Pan et al. 2009). Upon examining these ring neurons I find that the *ign* dependent mutant phenotype for height can be rescued by expressing wild-type *ign* in only subsets of ring neurons such as R1, R3, R3/R4d orR2/R4m. The independent rescue of the mutant phenotype in different subsets of ring neurons points to the redundancy of ring neuron function in novelty choice. This is also observed for the role of the ring neurons in visual orientation in walking behavior (R. Strauss, personal communication).

It has been proposed that vertical compactness and size might be measured by the same feature detectors as height if height was measured upward and downward from the average height of all the patterns in the panorama. If so, one would expect the same rescue effect for all three pattern parameters. However, here I show that the *ign* dependent mutant phenotypes for vertical compactness and size cannot be rescued in the ring neurons contrary to the height parameter. In addition the driver controls for size show near zero novelty choice, which could indicate that the size parameter in general is a difficult parameter and that Gal4 protein in ring neurons has a deleterious effect. On the other hand the *act*-Gal4 driver shows normal novelty choice for size although it should express Gal4 protein in the ring neurons. This problem needs further investigation.

Expression of RNAi for *ign* in ring neurons does not block novelty choice for height, which could be attributed to the ineffectiveness/weakness of the RNAi stock. Therefore the ring neurons function is silenced by expression of Shibire^{ts1} and TNT, but here too the novelty choice is unaffected and remains wild-type like. These findings suggest that the novelty choice circuitry remains functional despite silencing subsets of ring neurons. This again supports the redundancy hypothesis discussed earlier.

In conclusion I showed that the ring neurons are part of novelty choice circuitry from the fact that wild-type S6KII in ring neurons rescues the mutant phenotype. This finding is supported by the ellipsoid body mutant data showing that *ebo*^{678} and *ebo*^{KS263} flies show a mutant phenotype for novelty choice.

Interestingly, blocking the output of neurons in the F1 layer of the fan-shaped body by the expression of Shibire^{ts1} or TNT abolishes novelty choice for height. However, wild-type S6KII in these neurons in the *ign* mutant does not rescue the mutant phenotype. If they need S6KII at all, they cannot substitute for S6KII in ring neurons. Nevertheless, they are part of the novelty choice circuitry.

3.5. Size but not height requires mushroom bodies

Drosohila mushroom bodies (MBs) are one of the central brain structures that are extensively studied and shown to be involved in olfactory learning (Heisenberg et al. 1985, de Belle et al. 1994, Connolly et al. 1996). In visual behavior they have been shown to be involve in visual context generalization (Liu et al. 1999) and choice behavior facing conflicting cues (Tang and Guo. 2001; Zang et al. 2007). They also modulate salience-based selective fixation behavior (Xi et al. 2008). As mushroom bodies are not studied as extensively for their role in visual behavior as in olfactory learning, any insight in the role of mushroom bodies in visual behavior would be interesting. I find that mushroom bodies are required to show novelty choice for size but they are not required for height. Their role in novelty choice is examined by blocking the mushroom body function. Various techniques like HU treatment, genetic silencing and also the mushroom body mutant *mushroom body miniature^{1} (mbm^{1})* are used. By all the three methods I find that mushroom bodies are dispensable for height but not for size novelty choice. *mbm^{1}* is an autosomal gene causing an anatomical defect of the MBs observed only in females. Therefore only females were originally used for experiments. Interestingly, *mbm^{1}* males, which have normal looking mushroom bodies and normal olfactory learning, also show the mutant phenotype for size and are normal for height and vertical compactness novelty choice. The males must have some defect that causes the mutant phenotype specifically for size, presumably in the mushroom bodies. This differential behavior for size on the one hand and height and vertical compactness on the other also rejects the hypothesis of only one feature detector evaluating all three pattern parameters.

In summary I have shown that out of four pattern parameters that can be learned by the flies, novelty choice is elicited by height (horizontal bars), size and vertical compactness but not by oblique bars. Furthermore flies do not show novelty choice for standard Ts. I also find that novelty choice is independent of Rutabaga which is supported by the finding that dopamine is not required for novelty

choice either. Importantly I find that S6KII is required for novelty choice and the S6KII mutant phenotype for height parameter can be rescued in the ring neurons of the ellipsoid body. The importance of ellipsoid body for novelty choice is also shown by the finding that ellipsoid body mutants ebo^{678} and ebo^{KS263} show no novelty choice. Interestingly I find that F1 layer of the fan-shaped body is required for height novelty choice. Additionally I show that mushroom bodies are required to show novelty choice for size but not for height.

4. MATERIAL AND METHODS

4.1. Flies

Fly strains were reared on standard cornmeal molasses medium at 25°C (or at 18°C for temperature-shift experiments) and 60% relative humidity under 14h/10h light/dark cycle. Wild-type *Berlin* flies were used for all wild-type behavior experiments.

Gal4 driver strains: R28D01, R38H02 and R14G08 were obtained from G. M. Rubin, Janelia Farm Research Campus, USA. TH-Gal4 (second chromosome insertion) was obtained from H. Tanimoto, Munich.

UAS effector strains: UAS-*shi*^{ts1} (second chromosome insertion) was obtained from H. Tanimoto,Munich. UAS*ign*RNAi (second chromosome insertion) was obtained from Vienna Stock Center (CG 17596).

Mutant strains: One line of *ign*$^{58/1}$ was provided by R. Strauss, Mainz and another line by T. Raabe, Würzburg. All other strains came from from Wuerzburg fly stock collection, Department of Neurobiology and Genetics.

For *ign* rescue experimental animals were obtained by crossing *ign*$^{58/1}$;;UAS-*ign* virgins with Gal4 males. The male progenies (carrying *ign*$^{58/1}$ on the X chromosome) were used. Heterozygous control genotypes were obtained by crossing the Gal4 drivers and UAS-effector strains to wild-type Canton S. *ign*$^{58/1}$;;UAS-*ign* as well as *ign*$^{58/1}$;;UAS-*ign*/+ flies were used as effector/mutant controls.

UAS-*Shi*^{ts1} flies were combined with Gal4 drivers flies to obtain the UAS-*Shi*^{ts1}/Gal4 experimental flies.

UAS-*ign*RNAi flies were crossed to the respective Gal4 drivers to obtain UAS-*ign*RNAi/Gal4 experimental flies. The insertion in the driver lines used was either on 2nd or on 3rd chromosome.

4.2. Hydroxyurea (HU) treatment

Early first instar larvae [1 hour after larval hatching (ALH)] were collected and fed HU in a heat-killed yeast suspension (50-60 mg HU/ml yeast) for 4 hours at 25°C. Another group of larvae 1 hour ALH is fed only the yeast suspension without any HU, which serves as control. Both HU-treated

and control larvae were then washed with water and transferred to standard cornmeal molasses medium for further development at 25°C and 60% relative humidity under 14H/10h light/dark cycle (de Belle and Heisenberg. 1994 and Sweeney et al. 2012).

4.3. Temperature-shift experiments with Shibire^{ts1}

For Shibire^{ts1} experiments, UAS-*Shi*^{ts1}/Gal4 flies were raised at 25°C or at 18°C and, if serving as controls, were subsequently measured at 25°C and 18°C respectively. The experimental animals were shifted to 31°C 10-15 minutes before the experiment and were measured also at this temperature.

4.4. Visual stimuli

The visual stimuli used in the novelty choice experiment were black patterns on white background (18mm 5x7 White Dot Matrix Display from Forge Europe; Electro/Optical Characteristics – I_F = 29mA, T_a = 25°C). Sizes of patterns as seen from the fly were as follows: In the horizontal bars pattern bars measured 40°x12°; in the squares pattern pair these measured 40°x38° and 20°x20°; in the vertical compactness pattern pair the single bars measured 40°x20° and the two bars 40°x10° with CsOG shifted +/-15° vertically with respect to the CsOG of the single bars. Patterns used for contour orientation: oblique bars measure 38° in their long axis and 16° in their short axis; horizontal bars measure 12° in height and 40° in width, vertical bars measure 38° in height and 12° in width. T patterns measure 34° in height and 40° in width. The bars in the Ts were 12° in width. In the circle/cross pattern pair the circle measured 42° in the outer and 22° in the inner diameter. The crosses measured 40° in height and 44° in width. The width of their arms was 8°.

4.5. Experimental procedure

For behavioral experiments 3-5 days old flies were used. They were first anesthetized (cold) and then a small triangular copper wire hook is glued on their head and thorax joining the two. They were left in a transparent plastic vial each with sucrose and water overnight followed by the measurements next day. The angle between the hook and the fly's longitudinal body axis was kept

approximately constant (about 60°) throughout the study. In the experiment the long axis of the fly points 30° upward to the front.

The fly was attached to the clamp via the hook (copper wire), which in turn was attached to the torque meter. Head and thorax have to be joined because head (and eye) movements would disturb the visuo-motor experiments at the torque meter (Heisenberg and Wolf 1988). For the experiments the fly at the torque meter was placed in the center of a cylindrical LED arena (180 x 48 green LEDs, Luminance during experiment 2.5 μW cm^{-2}), which was used to present the patterns. Open and closed loop experiments were performed at the torque meter as described in the Introduction. For closed-loop experiments zero torque, the torque at which the panorama is at rest, needs to be adjusted. Independent of visual stimulation *Drosophila* continuously modulates its yaw torque in a nonrandom manner. In addition the torque meter has a different zero for each fly at rest and in flight. Although the zero torque is not critical for closed loop experiments, an appropriate value can be found for each fly by measuring the optomotor response in open loop for clockwise and counterclockwise rotation of a pattern before the start of the closed loop experiment. For further details of fly preparation and experimental set-up, see Heisenberg and Wolf (1988).

The novelty choice experiment lasts for approximately ten minutes. It begins with adjusting the zero torque in open loop. This phase was followed by the presentation of one type of pattern (e.g. horizontal bar in upper visual field in each quadrant) in the closed loop condition, so that the fly can maneuver the visual scenery and form a memory for the pattern displayed. This stimulus condition was presented for six minutes in two minute bins. From the seventh minute onwards the novelty choice phase starts lasting till the tenth minute, during which the fly was presented with the old patterns and the new patterns in closed loop condition. The choice phase was also divided in two two-minute bins. Each two-minute bin was separated from the next by a so-called mixing period of 5-seconds. The mixing period consists of fast open-loop rotation of the visual scenery, first clockwise for 2.5 seconds, then counterclockwise for again 2.5 seconds. This may induce the fly to choose a new flight direction after each two minute period.

4.6. Statistical analysis

The significance of single and double group was calculated with one sample and two sample Unpaired t-test respectively. For comparison between *ign*$^{58/1}$;;UAS-*ign*/c105-Gal4, *ign*$^{58/1}$;;UAS-*ign*/189y-Gal4, *ign*$^{58/1}$;;UAS-*ign*/c819-Gal4, *ign*$^{58/1}$;;UAS-*ign*/c232-Gal4, *ign*$^{58/1}$;;UAS-*ign*/c42-Gal4 and *ign*$^{58/1}$;;UAS-*ign* for height; Mann-Whitney U-test followed by Bonferroni correction was performed, in addition to Unpaired t-test. To show the redundancy of the ring neurons for height novelty choice; *ign*$^{58/1}$;;UAS-*ign*/c105-Gal4, *ign*$^{58/1}$;;UAS-*ign*/189y-Gal4, *ign*$^{58/1}$;;UAS-*ign*/c819-Gal4, *ign*$^{58/1}$;;UAS-*ign*/c232-Gal4 and *ign*$^{58/1}$;;UAS-*ign*/c42-Gal4 are compared with Kruskal-Wallis test followed by Dunn's multiple comparision test. Data are represented as means +/- S.E.M.

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6. SUMMARY

This study explores novelty choice, a behavioral paradigm for the investigation of visual pattern recognition and learning of the fly *Drosophila melanogaster* in the flight simulator. Pattern recognition in novelty choice differs significantly from pattern recognition studied by heat conditioning, although both paradigms use the same test. Out of the four pattern parameters that the flies can learn in heat conditioning, novelty choice can be shown for height (horizontal bars differing in height), size and vertical compactness but not for oblique bars oriented at +/- 45°. Upright and inverted Ts [differing in their centers of gravity (CsOG) by 13°] that have been extensively used for heat conditioning experiments, do not elicit novelty choice. In contrast, horizontal bars differing in their CsOG by 13° do elicit novelty choice; so do the Ts after increasing their CsOG difference from 13° to 23°. This indicates that in the Ts the heights of the CsOG are not the only pattern parameters that matter for the novelty choice behavior.

The novelty choice and heat conditioning paradigms are further differentiated using the gene *rutabaga* (*rut*) coding for a type 1 adenylyl cyclase. This protein had been shown to be involved in memory formation in the heat conditioning paradigm. Novelty choice is not affected by mutations in the *rut* gene. This is in line with the finding that dopamine, which in olfactory learning is known to regulate Rutabaga via the dopamine receptor Dumb in the mushroom bodies, is dispensable for novelty choice. It is concluded that in novelty choice the Rut cAMP pathway is not involved.

Novelty choice requires short term working memory, as has been described in spatial orientation during locomotion. The protein S6KII that has been shown to be involved in visual orientation memory in walking flies is found here to be also required for novelty choice.

As in heat conditioning the central complex plays a major role in novelty choice. The S6KII mutant phenotype for height can be rescued in some subsets of the ring neurons of the ellipsoid body. In addition the finding that the ellipsoid body mutants ebo^{678} and ebo^{KS263} also show a mutant phenotype for height confirm the importance of ellipsoid body for height novelty choice. Interestingly some neurons in the F1 layer of the fan-shaped body are necessary for height novelty choice.

Furthermore, different novelty choice phenotypes for different pattern parameters are found with and without mushroom bodies. Mushroom bodies are required in novelty choice for size but they are dispensable for height and vertical compactness. This special circuit requirement for the size parameter in novelty choice is found using various means of interference with mushroom body function during development or adulthood.

7. ZUSAMMENFASSUNG

Diese Studie untersucht Novelty Choice, ein Verhaltens-Paradigma für die Untersuchung der visuellen Mustererkennung und des Lernens der Fliege *Drosophila melanogaster* im Flugsimulator. Mustererkennung in Novelty Choice unterscheidet sich deutlich von Mustererkennung durch heat conditioning, obwohl beide Paradigmen den gleichen Test verwenden. Von den vier Muster-Parametern, die die Fliegen im heat conditioning für die Musterunterscheidung lernen kann, lernt sie in Novelty Choice nur die Höhe (horizontale Balken in unterschiedlicher Höhe), Größe und vertikale Kompaktheit, nicht dagegen die schrägen Balken im Winkel von +/- 45°. Aufrechte und umgekehrte Ts [hinsichtlich ihrer Schwerpunkte (CsOG) um 13° voneinander verschieden], die bisher weitgehend für heat conditioning Experimente verwendet werden, lösen kein Novelty Choice aus. Im Gegensatz dazu reagiert die Fliege auf horizontale Balken, die sich in ihren CsOG um 13° unterscheiden, mit Novelty Choice. Auch die Ts lösen Novelty Choice aus, wenn ihre CsOG-Differenzen von 13° auf 23° erhöht wird. Dies deutet darauf hin, dass in den Ts die Höhen der CsOG nicht die einzigen relevanten Musterparameter für Novelty Choice Verhalten sind.

Die Novelty Choice und heat conditioning Paradigmen unterscheiden sich darüber hinaus in der Rolle des Gens *rutabaga* (*rut*), das eine Typ-1-Adenylylcyclase codiert. Für dieses Protein wurde gezeigt, dass es bei der Gedächtnisbildung in der heat conditioning beteiligt ist. Novelty Choice wird nicht durch Mutationen im Gen *rut* beeinflusst. Dies steht im Einklang mit der Erkenntnis, dass Dopamin, das bei olfaktorischem Lernen bekanntermaßen Rutabaga über den Dopamin-Rezeptor Dumb in den Pilzkörpern reguliert, entbehrlich für die Novelty Choice ist. Die Schlussfolgerung ist, dass der Rut cAMP Signalweg bei der Novelty Choice nicht beteiligt ist.

Novelty choice erfordert kurzfristigen Arbeitsgedächtnisspeicher, wie in der räumlichen Orientierung während der Fortbewegung beschrieben wurde. Das Protein S6KII, für welches gezeigt wurde, dass es am visuellen Orientierungsgedächtnis laufender Fliegen beteiligt ist, wird hier als ebenso notwendig für Novelty Choice entdeckt.

Wie in heat conditioning spielt der Zentralkomplex eine wichtige Rolle in Novelty Choice. Der S6KII Mutantenphänotyp für Höhe kann in einigen Untergruppen der Ring-Neuronen des

Ellipsoidkörpers gerettet werden. Weiterhin kann festgestellt werden, dass die Ellipsoidkörper-Mutanten *ebo*^{678} und *ebo*^{KS263}, welche ebenfalls einen Mutantenphänotyp für Höhe zeigen, die Bedeutung des Ellipsoidkörpers für die Novelty Choice hinsichtlich des Höheparameters bestätigen. Interessanterweise sind einige Neuronen in der F1-Schicht des Fächerförmigen Körpers notwendig für Novelty Choice (für Höhe).

Darüber hinaus werden mit und ohne Pilzkörper unterschiedliche Phänotypen für verschiedene Musterparameter bei Novelty Choice gefunden. Die Pilzkörper sind in der Novelty Choice für Größe erforderlich, aber für Höhe und vertikale Kompaktheit sind sie entbehrlich. Diese spezielle Schaltungsvoraussetzung für den Größenparameter in Novelty Choice wird unter Verwendung verschiedener Mittel der Interferenz mit Pilzkörperfunktionen während der Entwicklung oder im Erwachsenenalter gefunden.

8. ANNEX

8.1. Affidavit

I hereby confirm that my thesis entitled "Novelty choice in *Drosophila melanogaster*" is the result of my own work. I did not receive any help or support from any commercial consultants. All sources and/or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Wuerzburg: 04.06.2013

Erklärung

Hiermit bestätige ich, dass meine Arbeit mit dem Titel "Novelty Choice in *Drosophila melanogaster*" das Ergebnis meiner eigenen Arbeit ist. Ich erhielt keine Hilfe oder Unterstützung einer kommerziellen Organisation. Alle verwendeten Quellen und / oder Materialien sind aufgelistet und in der Dissertation angegeben.

Darüber hinaus bestätige ich, dass diese Arbeit noch nicht als Teil eines anderen Prüfungsprozesses weder in identischer noch in ähnlicher Form vorgelegt wurde."

Würzburg: 04.06.2013

Narendra Solanki

8.2. Curriculum vitae

Wuerzburg, 04.06.2013

Narendra Solanki

8.3. Selected conference contributions

Mar 2011	"Vision in Flies", Janelia Farm-HHMI, Washington DC, United States.
Aug 2010	"9th International Congress of Neuroethology", University of Salamanca, Spain.
Sep-Oct 2009	"Neurobiology of *Drosophila*", CSHL, NY, United States.
Sep 2008	"12th European *Drosophila* Neurobiology Conference", University of Wuerzuburg, Germany.

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