

Behavioral modification in choice process of *Drosophila*

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Abstract In visual operant conditioning of *Drosophila* at the flight simulator, only motor output of flies—yaw torque—is recorded, which is involved in the conditioning process. The current study used a newly-designed data analysis method to study the torque distribution of *Drosophila*. Modification of torque distribution represents the effects of operant conditioning on flies' behavioral mode. Earlier works^[10] showed that, when facing contradictory visual cues, flies could make choices based upon the relative weightiness of different cues, and it was demonstrated that mushroom bodies might play an important role in such choice behavior. The new "torque-position map" method was used to explore the CS-US associative learning and choice behavior in *Drosophila* from the aspect of its behavioral mode. Finally, this work also discussed various possible neural bases involved in visual associative learning, choice processing and modification processing of the behavioral mode in the visual operant conditioning of *Drosophila*.

Keywords: visual operant conditioning, choice behavior, torque distribution, mushroom bodies.

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Because of its clear genetic and developmental background, diversity of behavioral paradigms and neuroanatomy of the brain, *Drosophila* has become an important animal model for studying genetic, molecular and cellular bases of learning and memory^[1]. Extensive research has explored the visual operant conditioning of *Drosophila* and related molecular bases^[2–8]; recently, researchers began to address cognition-like functions and involved neural substrates^[9–11]. In these studies, behavioral analysis focused on flies' ability to acquire or store the association between conditioned stimulus (CS) and unconditioned stimulus (US), while the properties of flies' behavioral output remained less understood. In visual operant conditioned behavior of *Drosophila*, the motor output (yaw torque) is directly involved in the feedback of conditioning (fig. 1(b)). Thus, to explore the yaw torque flies produce in conditioning may be useful for understanding the visual operant conditioning of *Drosophila*.

In visual operant conditioning, shape and color cues can be separately used as single visual cues or jointly used as combined visual cues to serve as conditioning stimulus. When double cues were coincident in training and became competitive in test, flies showed decision-like choice

behavior facing contradictory visual cues. Wild-type flies definitely chose flight pathways according to the cue that had relative higher weightiness^[10]; however, mutant flies with miniature mushroom bodies did not show such performance. The present study used a torque analysis method to examine torque distributions and behavioral modifications of *Drosophila* and to understand its function in choice behavior.

1 Materials and methods

1.1 Animal preparation

Drosophila melanogaster of wild-type strain WTB (wild-type Berlin) and mutant *mbm¹* (mushroom body miniature) were used. *mbm¹* flies have the same genetic background as WTB flies, and female *mbm¹* flies have almost no mushroom bodies (MBs) structure^[12]. Flies were maintained on standard corn meal/molasses medium^[3] at 25°C and 60% humidity with a 14 h/10 h light/dark photoperiod. Three- to four-day-old female flies were prepared according to standard procedures as described in earlier works^[2].

1.2 Flight simulator

The flight simulator (fig. 1) was a setup with negative feedback to simulate circumstance for flies' stable flight behavior^[13]. A torque meter was the core device of the flight simulator, on which individual fly was attached by glue at the head and thorax. The torque meter together with individual flies was placed in the center of a cylindrical panorama which could turn around its vertical axis. During stable flight, the fly had the solitary "behavioral module" to choose its horizontal orientation, and the only recorded component of such flight behavior was the yaw

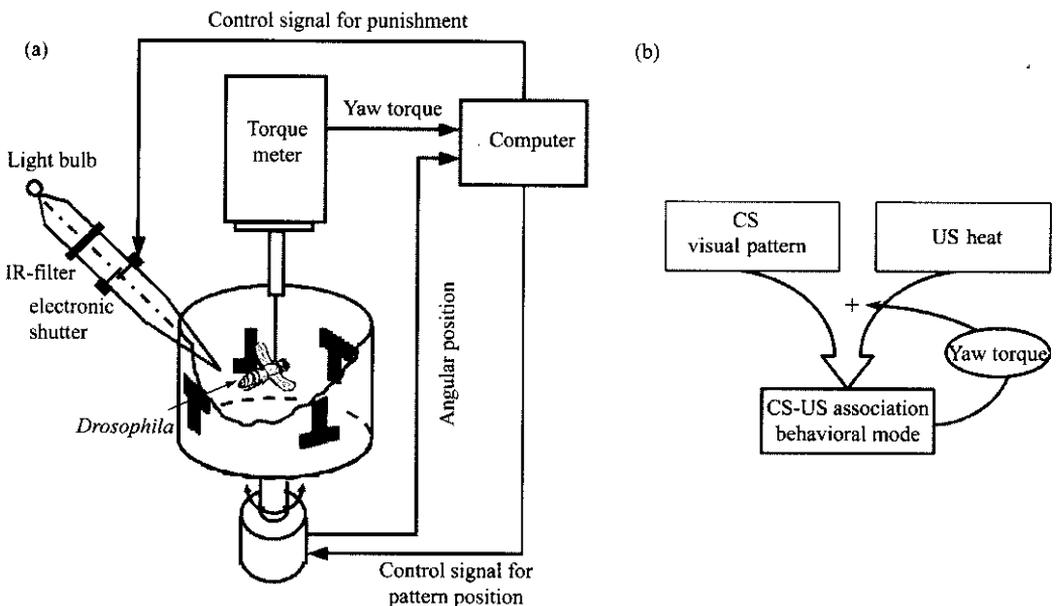


Fig. 1. (a) Flight simulator setup as used in experiments; (b) basic scheme of the visual operant conditioning paradigm. The "yaw torque" in the ellipse is the only motor output of *Drosophila* that is involved in the feedback in conditioning.

torque around the vertical axis. Two patterns and two patterns on the screen were equally sized and located at the center of each quadrant, and the same patterns were located in opposite quadrants. White light illuminated the panorama from behind. Monochromatic filters can be added on the screen to adjust the colors of the patterns (blue/green) and intensity of colors (CI: color intensity). The fly's yaw torque was continuously measured by the torque meter. Through a feedback circuit the fly maneuvers the turning movement of the panorama with its yaw torque (coupling coefficient: $k = -11^\circ (\text{s} \cdot 10^{-10} \text{ Nm})^{-1}$). Actually, the fly could not turn around to change its orientation during stable flight, but it could change the angular position of the panorama with its torque output, and so it could choose its flight direction referring to visual patterns on the screen. An infrared light beam was focused on the fly's rear from above, which functioned as a negative reinforcer in conditioning and could be intercepted by a computer-controlled shutter. The computer continuously recorded on-line the torque of the fly and the angular position of the panorama at a sampling frequency of 20 Hz. Fig. 2 shows the experimental traces for recorded yaw torque and angular position, with the shaded area showing where the fly was heated by the infrared light beam.

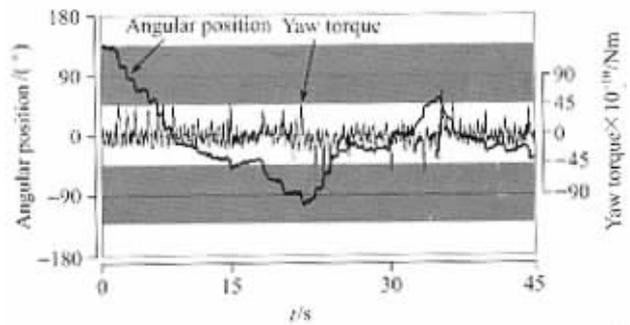


Fig. 2. Experimental traces from a 45-s experimental course. The angular position varies between -180° and 180° and the yaw torque variable fluctuates around zero.

1.3 Experimental procedure

The experimental procedure for the choice behavior paradigm was divided into successive periods (PTE, TR1, TR2, TE1 and TE2) (table 1). All periods were 4 min long with exception of TE1, which was 2 min. Before each trial, two kinds of visual patterns were matched using different shapes (and) and different colors (black, blue and green). For example, blue and green represented two kinds of patterns. From these, one kind of pattern was set as the conditioned stimulus (CS+) negatively reinforced by heat punishment. In the pre-test period (PTE) the preference of the fly towards the two kinds of patterns was tested; in training periods (TR1 and TR2) the fly was trained to avoid the CS+ patterns; in the memory-test period (TE1) the memory retention of the training was checked. After TE1 the matching of shape and color cues was reversed and the fly was tested to make a choice between contradictory visual cues in the choice-test period (TE2). During training periods, heat was projected on the fly whenever the fly was heading into a quadrant containing the CS+ patterns. Through such training the fly was able to form the association between visual patterns and heat punishment, and the fly learned to discriminate different patterns according to shape or color cues and to choose the non-heated

pattern to decide flight direction. During the pre-test period, memory-test and choice-test periods, the heat was switched off permanently.

Table 1 Experimental procedure to examine choice behavior in *Drosophila*

Pre-test PTE	Training TR1	Training TR2	Memory-test TE1	Choice-test TE2
	pattern 1 (CS+): color 1 + shape 1			pattern A: color 1 + shape 2
	pattern 2 (CS-): color 2 + shape 2			pattern B: color 2 + shape 1

1.4 Data analysis

1.4.1 Performance index (PI). In earlier works a performance index (PI) was introduced to estimate the fly's learning and memory abilities^[2]. The performance index is also called preference index in PTE, avoidance index in TR1 and TR2, memory index in TE1 and choice index in TE2. Four quadrants of the panorama were divided into two groups: one group was the CS+ domains with the punished visual patterns, and the other group was the CS- domains. The time a fly spent in each group was calculated from recorded data sequence of the angular position of the panorama; the times in the CS- and CS+ domains were recorded as t_1 and t_2 respectively. The calculation for the performance index was $PI = (t_1 - t_2) / (t_1 + t_2)$; $-1 \leq PI \leq 1$. $PI = 1$ meant the fly completely avoided the heat punishment; $PI = -1$ meant the fly was staying in the punished domains all the time; and $PI = 0$ meant the fly stayed in the CS+ and CS- domains equally.

1.4.2 Torque-position map. The calculation of PI only depended on the variable of the angular position of the panorama, which did not take into account the fly's yaw torque. Obviously, the PI evaluation method is insufficient to explore the dynamics of the conditioning process. Therefore, a new data analysis method was needed to enhance understanding of the fly's operant conditioned behavior, which would take into consideration both the angular position and yaw torque.

The newly designed torque-position map method was used to explore the torque flies produced at each angular position during conditioning. The polarity of the yaw torque is related to the turning direction of flies in the flight simulator (positive torques represent clockwise turning actions and *vice versa*). Analysis revealed no significant correlation between the torque polarity (i.e., the turning direction) and the angular position. So the torque-position map method was only used to study the relationship between the torque amplitude and the angular position. The analysis included a transformation of the angular position variable. The true angular position variable changes in the range of -180° to 180° . Torque-position map analysis used angular distances between the fly's orientation and the visual patterns to represent the fly's relative angular position (fig. 3(a)). Thus, when the fly was heading towards CS+ patterns, the position of the fly was set as -45° ; when heading towards the CS- patterns, the position was 45° ; and when heading towards the boundary between the CS+ and CS- domains, the position was 0° . The newly defined angular

position enabled the torque-position map method to statistically delineate changes of torque distribution of flies in conditioning. After the angular position transformation, the 360-degree panorama was divided into four equal domains (all ranging from -45° to 45°). The four domains were superimposed, and then the torque amplitude was averaged at each angular position and plotted on the torque-position map (fig. 3(b)).

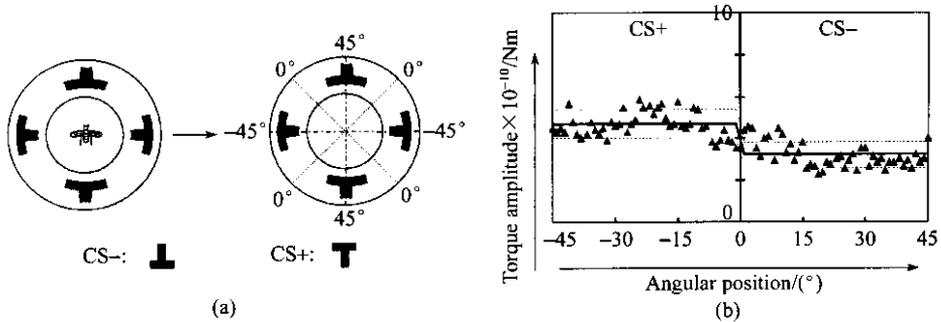


Fig. 3. (a) The 360-degree panorama was divided into four 90-degree domains (ranging from -45° to 45°). One of the two patterns (or) was set to be punished by heat for each trial, two domains with the punished patterns were CS+ domains and the others were CS- domains. (b) An example for the torque-position map and domain specific torque distributions. The dots represent the average torque amplitudes flies produced at each angular position. The horizontal “solid lines \pm dashed lines” show the average amplitude levels (Mean \pm S.D.) for the CS+ and CS- domains. Data shown are from one WTB fly during the experimental period of TR1.

1.4.3 Domain specific torque distribution. In addition to the analysis of torque production at each angular position, torque distributions in the CS+ and CS- domains were also studied and quantitatively compared. In conditioning, the reinforcer (heat) was presented throughout the CS+ domain and canceled throughout the CS- domain, and it did not depend on specific angular positions in the CS+ domain. Thus, the torque distributions in both CS+ and CS- domains could be computed, and the ANOVA-test method was used to compare both torque distributions. Torque distribution of the fly was regarded as symmetric between CS+ and CS- domains when ANOVA-test results had no significant difference, otherwise the torque distribution was asymmetric. As shown in fig. 3(b), the horizontal solid lines indicate the average torque amplitude levels in each domain and the dashed lines represent the deviations (S.D.) of torque distribution in each domain.

2 Results and analysis

2.1 Torque distributions of *Drosophila* facing a single visual cue

The performance indices and torque distributions of WTB flies ($n=12$) were studied in conditioned learning with only shape cue. The two kinds of visual patterns for conditioned stimulus in learning paradigm were different only in aspect of shape (black and on white background). Because only the shape cue was used in conditioning, the memory retention was tested in TE2, which was different from that in choice paradigm as described in sec. 1.3. In the

training period, flies were trained to choose their flight direction in response to the shapes. Before each trial or patterns were set as punished objects (CS+). PIs (fig. 4(a)) revealed that flies exposed to training period for such learning tasks chose their orientation toward the CS- patterns and avoided the CS+ patterns (positive PI_{TR1} , PI_{TR2}), and such preference was still detectable in

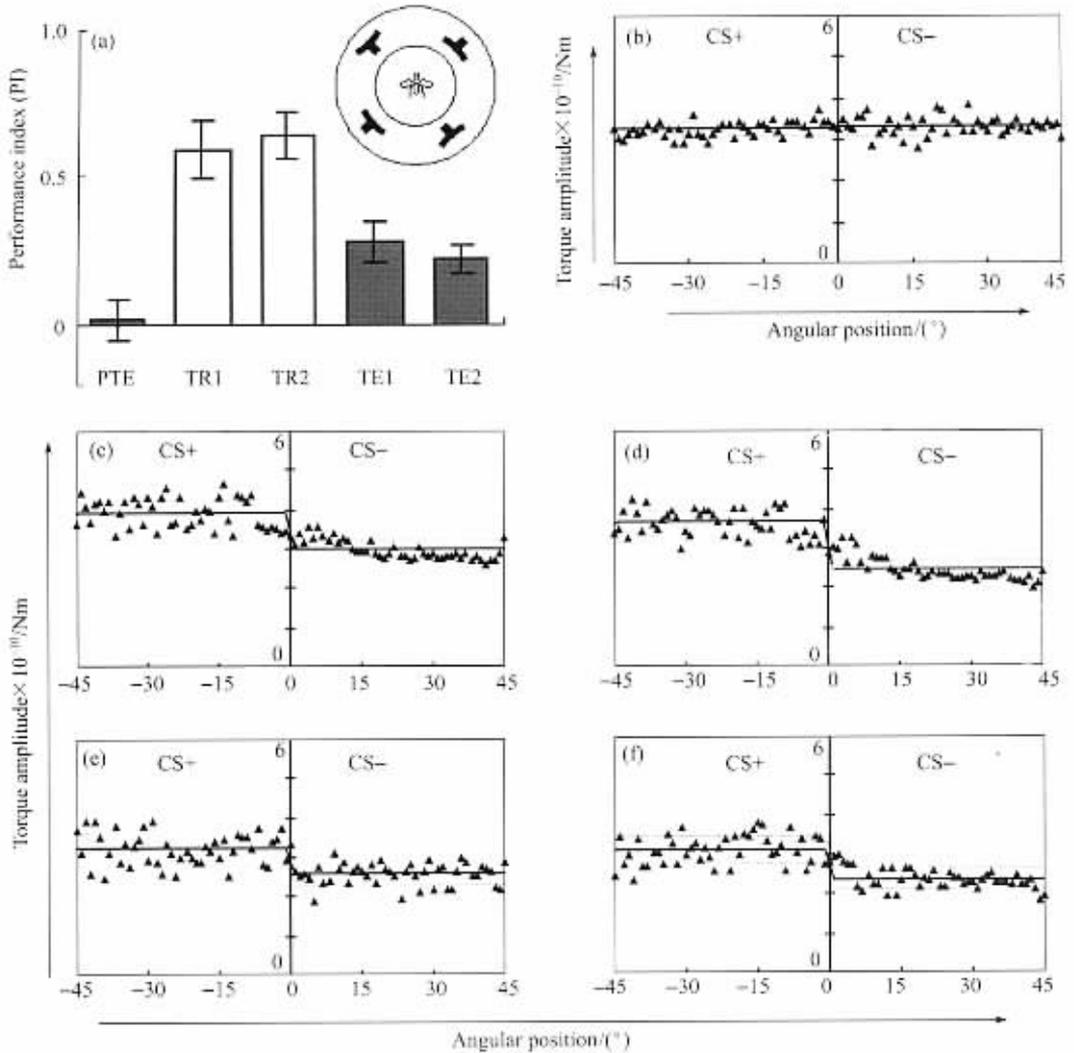


Fig. 4. The performance indices and torque distributions of WTB flies ($n=12$) during conditioning with the pattern shape cue. (a) Performance indices flies gained during each experimental period. During the pre-training period (PTE), flies had no preference for different pattern shapes; during training periods (TR1 and TR2), flies learned to avoid the CS+ patterns and inclined to the CS- patterns and the tendency persisted during the memory test periods (TE1 and TE2). (b) In the PTE period, WTB flies produced approximately equal torque amplitudes at all angular positions, and the torque distributions between CS+ and CS- domains were symmetrical. (c) and (d) In the TR1 and TR2 periods, WTB flies produced significantly higher torque amplitudes in the CS+ domain than in the CS- domain, which persisted during TE1 and TE2 ((e) and (f)). Among the 12 flies, 6 flies were punished for the shape and the others were punished for the shape.

memory-test periods after the training (positive PI_{TE1} , PI_{TE2}). At the same time, results from torque-position map analysis showed that torque distributions in the CS+ and CS- domains seemed to be symmetric before training and flies produced approximately equal torque amplitudes at each angular position (fig. 4(b)). In contrast, torque distributions came to be asymmetric in training periods and the torque level in the CS+ domain was measurably higher than in the CS- domain (fig. 4(c) and (d)). Finally, the asymmetric torque distributions between CS+ and CS- domains persisted in the memory-test periods after training (fig. 4(e) and (f)).

Qualitative descriptions of torque-position map analysis revealed the first data on the modification of flies' torque distributions in conditioning, which indicated the flies' process of behavioral modification caused by the operant conditioning. Additional quantitative analysis was conducted for torque distributions of WTB flies for the same learning task, in which the ANOVA-test was used to determine the difference of torque distributions between CS+ and CS- domains (table 2). In PTE, there was no significant difference of torque distributions between CS+ and CS- domains (average \pm S.D. for CS+ and CS- domains were 3.29 ± 0.19 , 3.36 ± 0.22 (10^{-10} Nm) respectively; $F_{[1, 88]} = 2.75$, $P = 0.10$). In the training periods (TR1 and TR2), results from the ANOVA-test showed a significant difference of torque distributions between CS+ and CS- domains (for TR1: $F_{[1, 88]} = 187.15$, $P < 0.001$; and for TR2: $F_{[1, 88]} = 143.85$, $P < 0.001$); the averaged torque amplitude level in the CS+ domain was higher than in the CS- domain. In memory-test periods after training (TE1 and TE2), a significant difference in torque distributions between CS+ and CS- domains still existed (for TE1: $F_{[1, 88]} = 88.01$, $P < 0.001$; and for TE2: $F_{[1, 88]} = 107.03$, $P < 0.001$).

Table 2 Torque distributions (average \pm S.D.) ($\times 10^{-10}$ Nm) of WTB flies ($n=12$) in experiments with only shape cue

Experimental period	CS+ domain	CS- domain	ANOVA-test results	
			$F_{[1, 88]}$	P
PTE	3.29 ± 0.19	3.36 ± 0.22	2.75	0.10
TR1	3.92 ± 0.38	2.79 ± 0.25	187.15	<0.001**
TR2	3.66 ± 0.32	2.56 ± 0.28	143.85	<0.001**
TE1	3.21 ± 0.39	2.58 ± 0.23	88.01	<0.001**
TE2	3.11 ± 0.18	2.43 ± 0.26	107.03	<0.001**

*** indicates level of significance $P < 0.001$.

The torque-position map analysis was used to reveal changes of torque distributions from symmetric status to asymmetric status in operant conditioning, and the ANOVA-test was used to quantitatively detect the significance of such modification. Changes in flies' torque distributions actually represented the behavioral modifications of flies in response to the operant training. Similar experiments also revealed such behavioral modifications in mutant *mbm¹* flies in the same learning tasks with only shape cue.

2.2 Torque distributions of flies facing contradictory visual cues

Recently, Tang and Guo^[10] developed the choice paradigm to study the choice behavior of *Drosophila* facing contradictory visual cues: two kinds of visual patterns used as conditioned stimulus had different shapes (or) and colors (blue or green), and before each trial one kind was set as the punished object (e.g., blue patterns were punished by heat, while green patterns were not). In the memory-test period (TE1), flies' ability to remember the pattern-heat association was tested; but in choice-test period (TE2), the matching of shape and color cues was reversed, which made the two visual cues become competitive (e.g., blue and green patterns). Tang and Guo^[10] found that the choice behavior of flies depended on the relative salience of shape and color cues, and demonstrated that mushroom bodies might be involved in such processing. In their work they defined an index to represent the intensity of color (CI), when the CI value was changed the relative weightiness of color vs. shape was altered: at CI = 1.0 the color had greatest intensity and weightiness of color was greater than that of shape; at CI = 0 the color had the least intensity and weightiness of color was less than that of shape. Given different CI values, flies showed distinct choice behaviors. The present work used the torque analysis method and the ANOVA-test to carry out behavioral studies on torque distributions of WTB and *mbm*¹ flies in choice processing.

Heat punishment was assigned to blue patterns in periods of PTE, TR1, TR2 and TE1 in choice experiments, WTB and *mbm*¹ flies were trained to prefer green patterns and avoid blue patterns. According to PIs, the conditioned avoidance behavior resembled those shown in learning tasks with only shape cue, and did not depend on CI values. Torque distribution analyses showed that torque distributions in such experimental periods were similar to those produced in relevant periods described in sec. 2.1: in the PTE period, the averaged torque amplitude levels in the domain with a blue pattern and the domain with a green pattern were similar, and torque distributions between the two domains seemed to be symmetric; in periods of TR1, TR2 and TE1, torque distributions between the blue and green domains were asymmetric, and the averaged torque amplitude level in the punished blue domain was significantly higher than in the non-punished green domain. After TE1, the matching of shape and color cues was reversed, and novel blue and green patterns were presented. In the choice-test period (TE2) following the reversing of shape-color matching, flies had to choose which pattern to fly toward and which to avoid, avoiding blue patterns if they made the choice in response to color, or avoiding green patterns if they made the choice in response to shape. The following sections presented results of torque distribution studies for choice processing at typical CI values. CI=1.0, CI=0.7 and CI=0.8 represented choices on bases of shape, color and disability in choice-

making.

2.2.1 Torque distributions of flies at CI = 1.0. The first torque analysis for choice experiments was conducted using WTB ($n = 9$) and *mbm*¹ ($n = 9$) flies at CI = 1.0 (table 3). WTB flies showed positive performance indices in both TE1 and TE2 (positive PI_{TE1} , PI_{TE2}), which indicated that WTB flies made choices in TE2 according to color cues at CI = 1.0. Torque analysis results showed that the averaged torque amplitude level of WTB flies in the blue domain was significantly higher than in the green domain for both TE1 and TE2 (for TE1: $F_{[1,88]} = 68.01$, $P < 0.001$; TE2: $F_{[1,88]} = 30.79$, $P < 0.01$). Torque analysis also revealed WTB flies' choice behavior in response to color cues. Thus, flies' torque distribution modifications had common properties with PIs in such choice processing: they all connected to the color cue that was used for choices. At CI = 1.0 *mbm*¹ flies also showed choice behavior similar to WTB flies. Unlike the performance of WTB flies in TE1 and TE2, the PI of *mbm*¹ flies in TE2 was lower than in TE1 ($PI_{TE1} = 0.28 \pm 0.05$, $PI_{TE2} = 0.15 \pm 0.06$; $P < 0.01$), and the significance level of torque distributions between blue and green domains was also reduced (for TE1: $F_{[1,88]} = 52.08$, $P < 0.001$; for TE2: $F_{[1,88]} = 6.95$, $P < 0.01$). But analysis still demonstrated that *mbm*¹ flies made definite choices according to color cue when facing contradictory shape and color cues.

Table 3 Performance indices and torque distributions (average \pm S.D.) ($\times 10^{-10}$ Nm) of WTB and *mbm*¹ flies during choice processes

CI	Fly strain	Experimental period	PIs \pm S.E.M.	Domain 1		Domain 2		ANOVA-test results	
								$F_{[1,88]}$	P
CI = 1.0	WTB ($n = 9$)	TE1	0.35 ± 0.05	blue	3.10 ± 0.34	green	2.56 ± 0.21	68.01	< 0.001
		TE2	0.33 ± 0.06	blue	3.03 ± 0.37	green	2.51 ± 0.27	30.79	< 0.001
	<i>mbm</i> ¹ ($n = 9$)	TE1	0.28 ± 0.05	blue	2.81 ± 0.29	green	2.33 ± 0.22	52.08	< 0.001
		TE2	0.15 ± 0.06	blue	2.66 ± 0.33	green	2.26 ± 0.25	6.95	< 0.01
CI = 0.8	WTB ($n = 10$)	TE1	0.37 ± 0.06	blue	3.32 ± 0.31	green	2.76 ± 0.22	89.67	< 0.001
		TE2	-0.04 ± 0.07	blue	2.93 ± 0.40	green	2.81 ± 0.34	4.09	0.05
	<i>mbm</i> ¹ ($n = 12$)	TE1	0.22 ± 0.04	blue	2.86 ± 0.35	green	2.40 ± 0.24	29.20	< 0.001
		TE2	0.02 ± 0.05	blue	2.66 ± 0.38	green	2.57 ± 0.35	3.01	0.09
CI = 0.7	WTB ($n = 11$)	TE1	0.41 ± 0.03	blue	3.21 ± 0.32	green	2.67 ± 0.20	76.01	< 0.001
		TE2	-0.34 ± 0.07	blue	2.73 ± 0.29	green	3.12 ± 0.37	16.91	< 0.001
	<i>mbm</i> ¹ ($n = 13$)	TE1	0.31 ± 0.06	blue	2.87 ± 0.33	green	2.37 ± 0.28	32.20	< 0.001
		TE2	-0.02 ± 0.04	blue	2.60 ± 0.30	Green	2.53 ± 0.25	2.26	0.14

“**” and “***” indicate levels of significance $P < 0.01$ and $P < 0.001$ respectively.

Thus, at $CI = 1.0$ both WTB and mbm^1 flies could make choices according to color cue on the basis of its greater weightiness. In choice processing, modifications of flies' torque distributions depended on the color cue. Choice behaviors of WTB and mbm^1 flies in experiments indicated that the knowledge of “visual pattern (CS)-heat (US)” association acquired using combined visual cues of shape and color could be detected through a single cue in the choice-test period, although the reversed visual cues were competitive. Modifications of torque distributions in choice behavior were connected with the color cue. This fact together with the earlier results that the choice indices of flies were connected to the color cue at $CI = 1.0$ ^[10] elucidated that choice behaviors in flies could be observed through both performance indices and torque distributions.

2.2.2 Torque distribution of flies at $CI = 0.8$. At $CI = 0.8$, memory and choice indices and torque distributions of WTB ($n = 10$) and mbm^1 ($n = 12$) flies in TE1 and TE2 were revealed (table 3). Both WTB and mbm^1 flies showed similar performances in TE1 as at $CI = 1.0$. They both had positive memory indices (WTB, $PI = 0.37 \pm 0.06$; mbm^1 , $PI = 0.22 \pm 0.04$). This fact indicated that both strains had normal associative learning and memory in training and memory-test periods. Torque analysis for both strains in TE1 indicated they had a significantly higher level of averaged torque amplitudes in the blue domain than in the green domain (WTB: $F_{[1,88]} = 89.67$, $P < 0.001$; mbm^1 : $F_{[1,88]} = 29.20$, $P < 0.001$). However, in the choice-test period (TE2), performances of both WTB and mbm^1 flies were distinctly different from those of $CI = 1.0$. Choice indices of both strains were near zero (WTB, $PI = -0.04 \pm 0.07$; mbm^1 , $PI = 0.02 \pm 0.06$), which indicated that they could not decide which pattern to pursue and which pattern to escape from, and they chose their flight direction on the basis of color or shape equally. At the same time, torque analysis showed that for both strains in TE2 the difference of torque distributions between the blue and the green domains was not significant (WTB: $F_{[1,88]} = 4.09$, $P = 0.05$; mbm^1 : $F_{[1,88]} = 3.01$, $P = 0.09$), and the torque distribution in the two domains was symmetric.

Color and shape cues have equal relative weightiness at $CI = 0.8$ ^[10]. The results showed that both WTB and mbm^1 flies failed to make choices when facing contradictory visual cues that had equal relative weightiness, and this disability in choice processing was confirmed by PI and torque distribution data.

2.2.3 Torque distribution of flies at $CI = 0.7$. Choice behaviors of WTB ($n = 11$) and mbm^1 ($n = 13$) flies at $CI = 0.7$ (table 3) were also studied. In contrast to choice behavior at $CI = 1.0$, WTB flies displayed choice behavior relying on shape at $CI = 0.7$, while mbm^1 flies were unable to make definite choices when facing contradictory visual cues.

In the memory-test period (TE1), both WTB and mbm^1 flies showed similar PIs and torque distributions at $CI = 0.7$ as at $CI = 1.0$ and $CI = 0.7$. In the choice-test period (TE2), WTB flies showed negative PI ($PI_{TE2} = -0.34 \pm 0.07$), which indicated that WTB flies chose to avoid green

patterns and had preferences toward blue patterns. Torque analysis showed that the torque distribution of WTB flies in TE2 was also asymmetric, but the averaged torque amplitude level in the green domain was distinctly higher than in the blue domain, which was different from the situation in TE1. Thus, due to the greater relative weightiness of shape cue at CI = 0.7, the choice behavior of WTB flies was based upon the shape but not color, and the choice behavior of WTB flies at CI = 0.7 was revealed from aspects of PIs and torque distributions. Compared to the choice behavior of WTB flies, mutant *mbm*¹ flies' PI in TE2 was near zero ($PI_{TE2} = -0.02 \pm 0.04$), and the difference of torque distributions between green and blue domains was not significant ($F_{[1,88]} = 2.26, P = 0.14$). The results indicated that although shape had relative salience to color, *mbm*¹ flies could not make definite choices, and this disability was manifested in both choice indices and torque distributions.

In conclusion, at CI=0.7 WTB and *mbm*¹ flies showed different choice behaviors that were apparent both in PIs and in torque distributions: WTB flies exhibited the ability to discriminate small differences of relative salience between competitive color-shape cues and to make definite choices. In choice processing, the asymmetry in torque distribution persisted and modifications of WTB flies' torque distributions were connected to shape cue. In contrast, *mbm*¹ flies lacked this decision-making ability, and could not make distinct choices when facing competitive visual cues; their torque distributions appeared to be symmetric in the choice-test period and there was no noticeable modification of torque distributions.

2.2.4 Torque distribution of flies around the choice point (CI=0.8). At CI = 0.8, color and shape cues have close relative salience and they affected flies' choice behavior equally. To further understand flies' choice behavior depending on CI and the difference of choice behaviors between WTB and *mbm*¹ strains, this study explored the choice behaviors of WTB and *mbm*¹ flies around the choice point (CI = 0.8).

In training and memory-test periods CI was set as 0.8, after which the matching of color and shape cues was reversed and CI was changed to 0.84 or 0.76. Thus the relative weightiness of color and shape was changed. As shown in plate I, the PIs and torque distributions of two strains in TE1 and TE2 were studied. In TE1, both kinds of flies showed preferences for green patterns (positive PI_{TE1}). ANOVA-test results indicated that in TE1 both strains had significantly higher torque amplitude levels in the green domain ($P < 0.001$). When the CI was changed to 0.84 in TE2, WTB flies made choices according to color cue and had preferences for green patterns, whereas *mbm*¹ flies flew toward two patterns equally (WTB, $PI_{TE2} = 0.19 \pm 0.07$; *mbm*¹: $PI_{TE2} = 0.03 \pm 0.06$). Torque analysis results showed that in TE2, WTB flies produced significantly higher torque level in the blue domain than in green domain, whereas the torque distributions of *mbm*¹ flies in the two domains had no significant difference (WTB: $F_{[1,88]} = 32.60$,

$P < 0.001$; mbm^1 : $F_{[1,88]} = 2.44$, $P = 0.12$). When the CI was reduced to 0.76 in TE2, WTB flies began to make choices according to shape cue and had preferences for blue patterns; however, mbm^1 flies chose green and blue patterns equally (WTB: $PI_{TE2} = -0.15 \pm 0.06$; mbm^1 : $PI_{TE2} = -0.05 \pm 0.07$; negative PI_{TE2} indicated a preference for blue color). Torque analysis showed that WTB flies' level of torque amplitude was significantly higher in the green domain than in the blue domain, whereas mbm^1 flies produced approximately equal torque amplitude levels in both domains (WTB: $F_{[1,88]} = 13.79$, $P < 0.001$; mbm^1 : $F_{[1,88]} = 0.26$, $P = 0.62$).

From the results of the present analysis of WTB and mbm^1 flies and the choice behaviors of both strains at CI = 0.8, we can conclude that when the matching of shape and color cues was changed and the CI value was not altered (CI = 0.8), WTB and mbm^1 flies both showed disabilities in making choices when facing competitive visual cues. When the matching of shape and color cues was changed and the CI was altered (from 0.8 to 0.84 or 0.76), WTB flies could discriminate the slightly different salience between competitive visual cues and choose the noticeable one for making choices, but mbm^1 flies lacked the acute ability to fulfill the choice behavior. In all cases, the differences of choice behavior between WTB and mbm^1 flies were confirmed by both aspects of PIs and torque distributions.

3 Discussion

A new torque analysis method was used to study the conditioned behavior of *Drosophila* in visual operant conditioning at the flight simulator. Results showed that with the formation of a CS-US association, a process relating to flies' behavioral mode modifications was involved in conditioning. The torque output was the only behavioral output recorded in the flight simulator, as the torque distribution mode represents the fly's behavioral mode. Before operant training, the behavioral mode appeared to be similar torque amplitudes produced at each angular position. Through operant training, the behavioral mode was modified and data exhibited a significant difference between the CS+ and CS- domains. Flies produced distinctly higher torque amplitudes in the CS+ domain than in the CS- domain. Such behavioral modifications could help the flies to efficiently avoid the punishment pathway and pursue the unpunished flight pathway, which can be useful for the formation of CS-US association. Due to the motor output variables of flies recorded on-line in the flight simulator, this work tried to study the operant conditioned behavior and choice behavior of flies through focusing on a novel aspect of the behavioral mode. Unlike PIs, the traditional method used to describe flies' behavior, the torque analysis method first presented in this paper offered a new method for dynamic behavioral study of flies' conditioned behavior.

The most marked difference between operant conditioning and classical conditioning is that an animal's motor output is actively involved in operant conditioning to form the CS-US association, whereas in classical conditioning no motor output is involved and the CS-US association is formed passively. Heisenberg et al.^[7,8] explored both operant and classical

conditioned behavior of flies at the flight simulator and found that, because of the involvement of the torque output in operant conditioning, flies had better learning performance in operant conditioning than in classical conditioning. The theoretical explanation was also proposed that classical conditioning was based upon a two-term contingency of CS-US or Behavior-US, while operant conditioning was based upon a three-term contingency of CS-US-Behavior. Therefore, studying flies' torque output in operant conditioning can increase knowledge about their conditioned behavior. The results of the present study showed that the behavioral modification process was closely related with the CS-US association process in the learning paradigm or with the choice process in the choice paradigm. Modifications of torque distributions actually revealed the direct effects of operant training on flies' behavior, while CS-US association ability and cognition-like choice ability were shown to be learned "knowledge" or "experience" accumulated through repetition^[4, 14].

Scientists recently have begun to explore choice behavior of *Drosophila* in facing contradictory visual cues^[10]. The newly designed torque analysis method offers new ways to advance understanding of such choice behavior. The torque analysis of the choice behavior of flies indicated that the decision made choice behavior and the process of behavioral modifications were closely related, both for wild-type WTB flies and for mutant *mbm¹* flies. When flies could not make definite decisions in choice behavior, the torque distributions looked the same as before operant training. An example of this is the *mbm¹* flies' disability at CI = 0.7 and both WTB and *mbm¹* flies' disabilities at CI = 0.8. In contrast, the torque distributions showed a significantly asymmetry tendency when flies made definite decisions in the choice processes.

Milner et al.^[15] theorized that nervous systems contained multi-memory systems and animals could have kinds of learning and memory behavior in different behavioral paradigms; studies on vertebrates demonstrated that different forms or different orders of behavioral performance in learning paradigms might be connected with different neural substrates or signal systems. With a combination of behavioral studies and molecular genetics and other research methods, learning and memory of *Drosophila* in olfactory classical conditioning has been widely investigated^[16]. Results have shown that mushroom bodies played an important role in such learning task, and neurotransmission in mushroom bodies was necessary for memory retrieval but not for acquisition or storage of memory^[17, 18]. Works on visual operant conditioning of *Drosophila* proved that mushroom bodies were dispensable for visual associative learning^[5] but were necessary for some cognition-like functions^[9, 10], while evidence is still lacking to elucidate the cellular and molecular mechanisms in mushroom bodies and some other brain structures that are involved in visual associative learning or cognition-like behaviors. Similarly, the present behavioral studies with wild-type and mutant flies were insufficient to elucidate the neural bases underlying the behavioral modifications of flies. Martin et al.^[19] revealed that mushroom bodies could suppress the locomotor activities. In contrast, the torque analysis in this study showed that the loss of mushroom bodies in flies did not noticeably interfere in their torque production or other

behavioral aspects; it did not affect the CS-US association, but ultimately affected their choice ability. Thus, mushroom bodies seem to serve as control units in flies' nervous systems to participate in the integration process of competitive multi information pathways; in choice behavior between contradictory visual cues, its function may be to emphasize one cue but suppress another.

Behavioral analysis and results from molecular genetic and neuroscience studies are still insufficient to clarify the differences or relationships among behavioral modification process, visual associative learning and choice behavior in *Drosophila* visual operant conditioning. In-depth and thorough studies of their molecular, cellular and neural bases for them are still necessary. The present work is the first behavioral study on torque distributions of flies in visual operant conditioning. Results from torque analysis of wild-type WTB and mutant *mbm¹* flies showed that the behavioral modification process was closely related to the CS-US associative learning process and choice process, which indicated that they might have related neural bases.

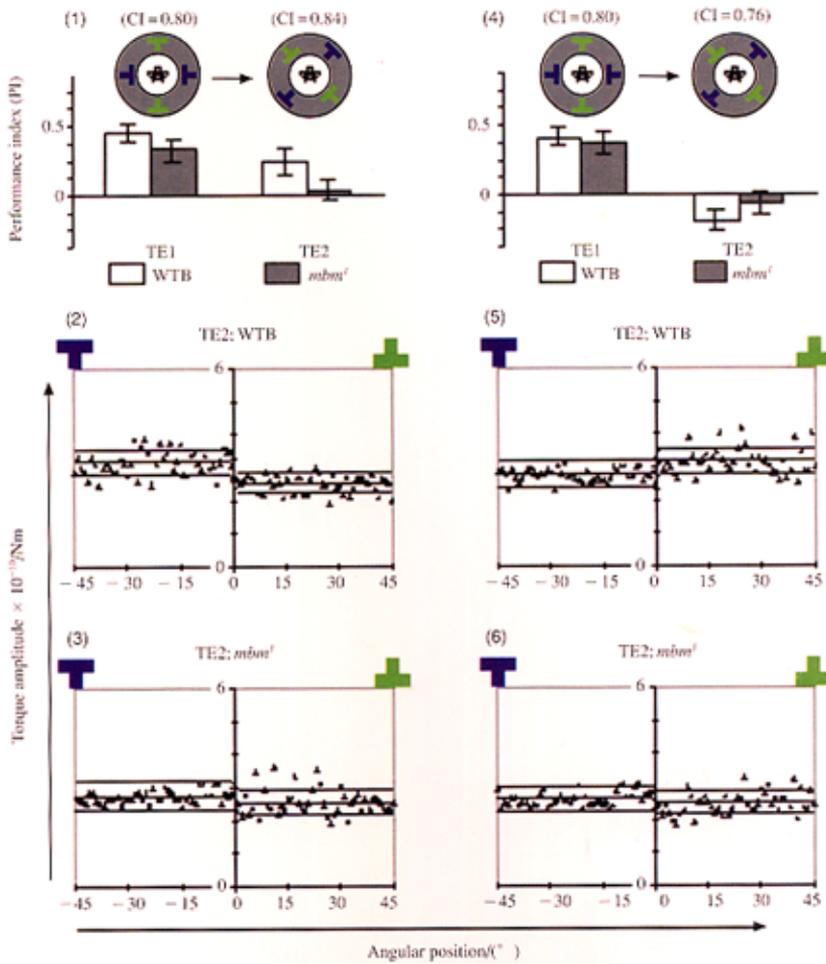
The clear genetic and developmental background of *Drosophila* and modern molecular genetic techniques have led to the practice of obtaining single gene mutants of *Drosophila* that have certain functional phenotype or behavioral phenotypes^[1], making them an important model in neuroscience studies of the route of "Gene-Brain-Behavior". Multi-behavioral paradigms and analysis methods enable the study learning/memory and cognition-like functions from various aspects, which undoubtedly will lead to an understanding of neural functions in *Drosophila* and even in mammals.

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When the shape and color cues were reversed and the value of CI was changed during TE2, WTB and *mbm*¹ flies showed different choice behavior when facing contradictory visual cues. For training (TR1 and TR2) and memory test (TE1) periods CI=0.8, flies were punished for the visual pattern of blue and flies definitely flew toward the green pattern. When the CI was changed to 0.84 in the choice test period (TE2), the color cue had relatively high weight: WTB flies ($n=16$) made the choice according to color cue (positive PI_{TE2} indicates the preference for the green pattern), but the *mbm*¹ flies ($n=16$) did not make a definite choice ($PI_{TE2} \sim 0$) (1). During the choice test period (TE2), the average torque amplitude level in the blue domain was noticeably higher than in the green domain for WTB flies (2), but the average torque amplitude levels of *mbm*¹ flies in blue and green domains were close to each other (3). When the CI was changed to 0.76 during TE2, the shape cue had relatively high weight: WTB flies ($n=14$) made the choice according to shape cue (negative PI_{TE2} means a preference for the blue pattern), however, the *mbm*¹ flies ($n=13$) had no definite preference ($PI_{TE2} \sim 0$) (4). During TE2, the average torque amplitude level of WTB flies in the green domain was measurably higher than in the blue domain (5); whereas, the averaged torque amplitude levels of *mbm*¹ flies in the blue and green domains were close to each other (6).