Dopamine–Mushroom Body Circuit Regulates Saliency-Based Decision-Making in *Drosophila*

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Drosophila melanogaster can make appropriate choices among alternative flight options on the basis of the relative salience of competing visual cues. We show that this choice behavior consists of early and late phases; the former requires activation of the dopaminergic system and mushroom bodies, whereas the latter is independent of these activities. Immunohistological analysis showed that mushroom bodies are densely innervated by dopaminergic axons. Thus, the circuit from the dopamine system to mushroom bodies is crucial for choice behavior in *Drosophila.*

Value-based decision-making is a complex behavior controlled, in part, by the dopamine system (*1*, *2*). Primates make choices among many available options to produce an advantageous response (*3*). The complexity of the mammalian brain has made it difficult to fully understand the neural circuits underlying value-based decision-making. To discern these circuits, we studied this phenomenon in *Drosophila*, because the functions of dopamine neurons are largely conserved evolutionarily (*4*–*6*). For example, forming aversive olfactory memories in *Drosophila* requires dopamine, allows punishment prediction, and involves neural activities that are similar to primates and rodents during conditioning (*1*, *7*).

To explore the circuitry mediating value-based choice behavior of *Drosophila* (*8*), we developed a novel paradigm involving relative saliency evaluation of contradictory cues (Fig. 1A). Flies were trained in a flight simulator to associate heat punishment with one of two bars (*9*) with compound cues, position (upper and lower) and color (blue and green). After training with one bar (e.g., upper and blue), flies were confronted with conflicting cues (e.g. upper-green and lower-blue) and had to decide whether to follow the position or color cue depending on their relative saliency. Position and color saliency were quantified by vertical separation between the bar center of gravity (ΔCOG) (*10*) and color intensity (CI) (*8*), respectively. Amount of time spent in the conditioned quadrants was quantified as a preference index (PI) over 2-min blocks (*11*). *Wild-type Berlin* (*WTB*), *Canton-S* (*CS*), and mutant *mushroom body miniature*[1] (*mbm*[1]) flies were trained with an upper-blue bar (CI = 1.0 and ΔCOG = 60°). They were then tested for choice behavior by changing both the color (blue to green; CI unchanged) and position cue saliency (ΔCOG from 60° to 40°). Wild types preferentially chose the position cue and followed the upper-green bar (Fig. 1A), whereas mutants could not decide which bar to follow, as evidenced by substantially reduced PIs.

To further characterize choice behavior, *WTB*, *CS* and *mbm*[1] flies were tested by using a wide range of position cue saliencies (ΔCOG: 0° to 60° in 5° or 10° increments) without changing the color cue CI. Figure 1B depicts the percentage of time spent following the position cue as a function of ΔCOG. The choice curve of wild types (*WTB* and *CS*) exhibited a distinct transition in the preference for position cues, as a function of relative salience (position versus color), at ΔCOG = 30° and could be fit by a sigmoid function (Boltzmann fit, r^2 = 0.97 for *WTB* and *CS*). In contrast,

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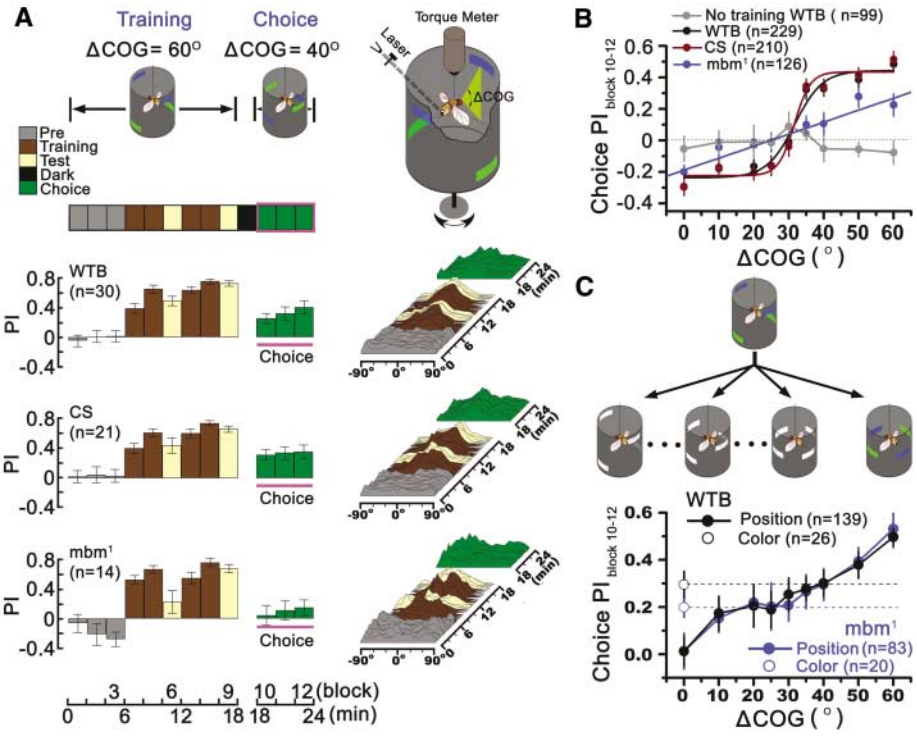

Fig. 1. Visual choice test using position-color dilemma. (**A**) Horizontal bars of different colors and positions (COG) were used as visual cues in varying arena quadrants. Flies were suspended from a torque meter and trained (blocks 4 to 8) at CI = 1.0 and ΔCOG = 60° to prefer upper-blue bars by pairing infrared laser beam punishment with lower-green bars. After training, flies with a preference index (PI) > 0.3 (block 9) were transferred to a "dilemma" with a new ΔCOG (40°) and reversed colors; choice behavior was tested during blocks 10 to 12. *WTB* and *CS* show significant choice PI ($P < 0.01$), whereas *mbm*[1] does not ($P > 0.05$). *P* values are based on one-sample *t* tests. Images on the right show the relative flight times of flies in directions between −90° and +90°. (**B**) Choice PI of flies as a function of relative saliency of ΔCOG between 0° to 60°; controls were untrained *WTB* flies. (**C**) Simple choice tests for color (CI = 1.0) and position cues (ΔCOG from 0° to 60° in 5° or 10° increments). Data presented as means ± SEM. *n* indicates the total number of flies examined.

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position cue preference in *mbm*^{1} flies climbed up progressively (linear fit, $r^2 = 0.92$) (Fig. 1B). Mushroom bodies (MBs) are essential for olfactory (*12*, *13*) but not visual reinforcement learning (*14*), and, in the visual choice paradigm, *mbm*^{1} flies could not distinguish pertinent position or color cues when their saliencies varied. This is consistent with previous findings that MBs participate in decision-making when *Drosophila* confronts a shape-color dilemma (*8*). Flies could interpret cue saliency as a representation of punishment probability and alter their choice strategy accordingly. Along these lines, without prior training wild types randomly chose all saliency cues.

Primate studies suggest two general categories of decision-making: simple perceptual and value-based (*2*). The former is based on simple linear subtraction of alternative sensory inputs (*15*), and the latter on nonlinear calculation of the relative values of stimuli. We investigated which decision-making type *Drosophila* used when faced with conflicting visual cues. For this purpose, flies were trained with both color and position cues (CI = 1.0 and ΔCOG = 60°), and then their preference for a single cue (each tested separately) was assessed during the posttraining session (Fig. 1C). When position cue saliency was varied (ΔCOG from 0° to 60°), a sigmoid retrieval curve was not evident. Wild-type and *mbm*^{1} flies performed similarly under these conditions indicating that retrieval of single visual cues is not MB dependent. We then asked how visual perception of separated cues after compound training contributes to decision-making. The choice curve predicted by subtracting the PI at CI = 1.0 from the PIs of position cues (ΔCOG from 0° to 60°) was linear and similar to the performance of *mbm*^{1} flies in the position-color dilemma (Fig. 1B). Thus, *mbm*^{1} flies make perceptual decisions in conflict situations by a simple subtraction mechanism, which is thought a general mechanism for perceptual decision-making in the human brain (*2*). In contrast to *mbm*^{1}, wild-type flies performed according to a sigmoid choice curve, and the mechanism underlying should be beyond simple comparison of the different cues perception.

We investigated how and when MBs contribute to the decision-making process by selectively disrupting their function at different stages of choice behavior with *shibire*^{ts1} (*shi*^{ts1}), a temperature-sensitive mutant form of *dynamin*. In *shi*^{ts1} mutants (*16*–*18*), synaptic transmission is normal at permissive temperature (PT, below 30°C) and blocked at restrictive temperature (RT, above 30°C). Transgenic 247/upstream activation sequence (UAS)-*shi*^{ts1} flies, with restricted *shi*^{ts1} expression in MBs, were trained to follow bars with compound cues (CI = 1.0 and ΔCOG = 60°) at PT (24°C) then tested at RT (30°C) for 6 min of choice performance with conflicting cues (Fig. 2A) (*11*). They showed a sigmoid choice curve at PT, but a linear one at RT, which is similar to *mbm*^{1} flies (Fig. 2B); wild types were unaffected by the temperature shift (fig. S1).

Dopamine plays a crucial role in the motivation to acquire a reward or avoid a punishment (*19*, *20*). In *Drosophila*, dopaminergic transmission also mediates punishment prediction and associates punishment with a conditioned stimulus (*7*). Expression of *shi*^{ts1} in dopaminergic neurons is triggered by tyrosinse hydroxylase (TH)-Gal4 (*21*) and dopa decarboxylase (Ddc)-Gal4 (*22*). Ddc/UAS-*shi*^{ts1} flies express *shi*^{ts1} in both dopaminergic and serotoninergic neurons, whereas TH-Gal4/UAS-*shi*^{ts1} flies express it only in the former. Both types of transgenic flies were tested for choice behavior (Fig. 2A) and exhibited a sigmoid choice curve at PT, similar to wild types (Fig. 1B). However, their choice behavior was severely impaired at RT (Fig. 2, C and D), as evidenced by a linear choice curve, indicating that dopamine deprivation was sufficient to disturb decision-making based on relative cue saliency.

Because dopaminergic synaptic activity is necessary for memory acquisition in aversive olfactory conditioning (*4*), blocking it could impair visual memory required for decision-making rather than the process itself. To address this issue, we trained flies at PT and tested their preference for conditioned cues at RT, which required memory retrieval. Flies of all genotypes (CS, 247/UAS-*shi*^{ts1}, Ddc/UAS-*shi*^{ts1}, and TH/UAS-*shi*^{ts1}) performed similarly at both temperatures (Fig. 2F). Therefore, reduced dopaminergic transmission specifically disrupts saliency-based decision-making.

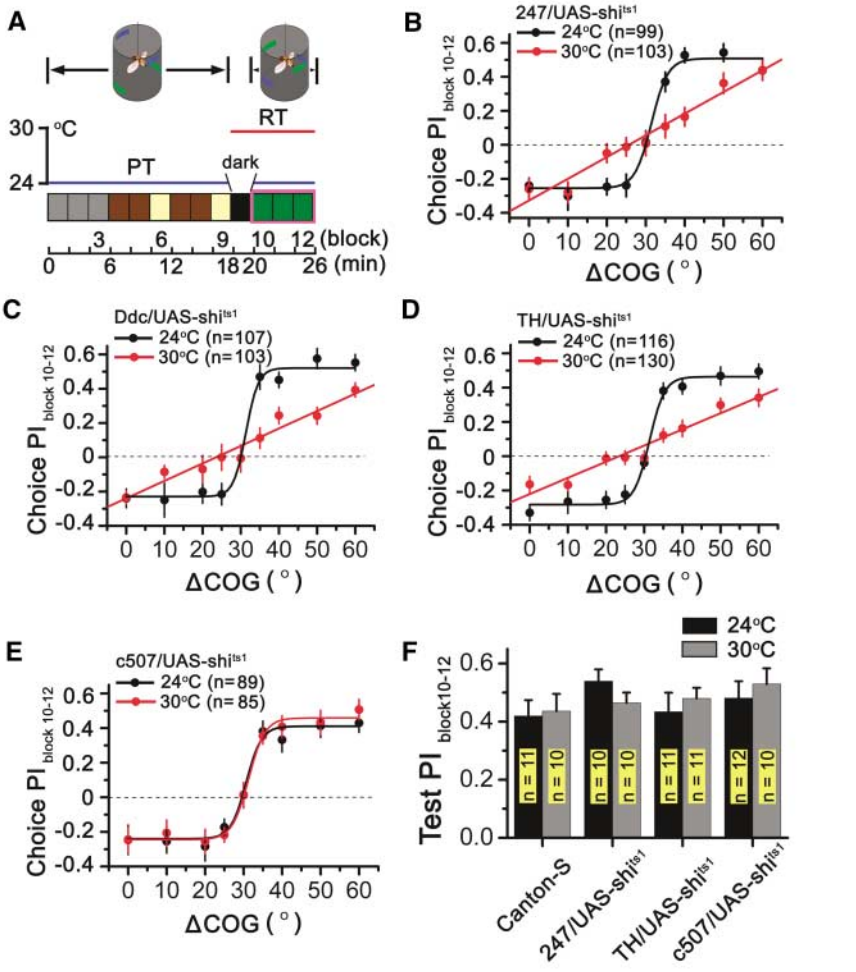

Fig. 2. Choice behavior depends on dopamine and MBs. (**A**) Choice behavior–temperature shift paradigm involved training flies at PT (24°C) and testing at RT (30°C). (**B**) Choice PI in 247/UAS-*shi*^{ts1} flies fit a sigmoid curve at PT (Boltzmann fit, $r^2 = 0.99$). In contrast, RT resulted in genetic silencing of MB function and defective choice performance (linear fit, $r^2 = 0.97$). (**C** and **D**) Choice behaviors in Ddc-Gal4/UAS-*shi*^{ts1} flies (PT, Boltzmann fit, $r^2 = 0.99$; RT, linear fit, $r^2 = 0.95$) and TH-Gal4/UAS-*shi*^{ts1} flies (PT, Boltzmann fit, $r^2 = 0.99$; RT, linear fit, $r^2 = 0.95$). (**E**) Choice behavior in c507/UAS-*shi*^{ts1} flies (Boltzmann fit, $r^2 = 0.99$ at PT and RT). (**F**) Transgenic and control (*CS*) flies showed normal memory retrieval at both RT and PT. Error bars indicate mean ± SEM for (B) to (F).

In addition to MBs, the ellipsoid body (EB) in the *Drosophila* central complex (*23*) was examined for its potential contribution to decision-making. Transgenic flies c507/UAS-*shi*^{ts1} expressing *shi*^{ts1} specifically in the EB showed normal sigmoid choice behavior at both temperatures (Fig. 2E), indicating that the EB is not critical for this behavior.

Both dopamine and MBs are involved in saliency-based decision-making, and D1-type dopamine receptors are densely distributed in MB lobes (*24*, *25*). To determine how dopamine and MBs interact, we examined the anatomical relation between them by simultaneously expressing a red fluorescent protein (RFP), driven by 247-Gal4, specifically in MB neurons and visualizing dopaminergic neurons with immunostaining for TH, an enzyme specifically used in dopamine synthesis. Dopaminergic fibers were broadly distributed in *Drosophila* brain, with the highest density around MBs (Fig. 3A). Higher magnification showed that TH staining was concentrated in MB lobes rather than calyces or peduncles (Fig. 3B); thus, dopaminergic processes occupy MB lobes containing Kenyon cell axons, as confirmed by labeling dopaminergic neurons with green fluorescent protein (GFP)–tagged synaptic vesicle protein *Synaptotagmin I* (*Syt I*) (*26*) (Fig. 3C). Furthermore, dopaminergic axons, not dendrites, invade MB lobes, because the dendrite-specific *Drosophila Down Syndrome Cell Adhesion Molecule* conjugated to GFP (*Dscam[17.1]-GFP*) (*27*) in dopaminergic neurons did not colocalize with immunostaining for the MB marker Fasciclin II (Fas II) (*28*, *29*) (Fig. 3D). Dopaminergic axons specifically innervate MB lobes, because the prominent lobe-like profile of dopaminergic fibers (Fig. 3, E and G) was largely abolished (Fig. 3, H and J) in flies treated with hydroxyurea (HU) to ablate MBs. Their absence in calyces suggests that dopamine regulates MBs by acting on Kenyon cell output.

To determine whether choice behavior is time dependent, we examined decision-making at different times after flies encountered conflicting visual cues (Fig. 1A and fig. S2A). During the first 30 s of conflict cues presentation (fig. S2B, C), wild types (*WTB* and *CS*) showed linear choice performance according to position cue saliency; however, sigmoid choice behavior was evident at 90 to 120 and 330 to 360 s. These results suggest that decisive choices are time dependent and that the early test phase likely involved simple perceptual decision-making. To explore the circuits involved, we selectively disrupted MBs and dopaminergic function at varying times after choice behavior testing began with temperature-sensitive 247/UAS-*shi*^{ts1} and TH/UAS-*shi*^{ts1} flies. Flies were given a choice test using a ΔCOG shift of 60° to 40° with CI = 1.0 because these parameters caused the largest difference in choice behavior between mutants and wild types. After testing started, flies were kept at PT for 1, 2, or 4 min before exposure to RT (Fig. 4A). Both 247/UAS-*shi*^{ts1} and TH/UAS-*shi*^{ts1} flies executed clear choices at PT; however, those kept at PT for 1 or 2 min, but not 4 min, performed worse at RT (Fig. 4, B and C). These findings indicate that MB dopaminergic activity is only required during the first 4 min after encountering conflicting cues and not after stable choice behavior is established.

The above results suggest that choice behavior of flies requires two phases: an initial involving dopaminergic and MBs activities and a later executing phase that is independent of these activities. Accordingly, we hypothesized that, if flies were presented with a second set of conflicting cues, then dopamine system and MB would be reactivated. We tested this hypothesis by first determining whether wild types correctly discern the salient cue after sequential transition of cue positions (ΔCOG shift from 60° to 40° then to 20°, at CI = 1.0); their choice was not significantly different from that seen after a direct transition (ΔCOG shift from 60° to 20°) (Fig. 4D). Next, TH/UAS-*shi*^{ts1} and 247/UAS-*shi*^{ts1} flies were exposed to two sequential sets of conflicting position-color cues (Fig. 4, F and G) and exhibited normal choice behavior with notable PIs for the first choice test at PT (ΔCOG = 40°, upper-green bar, and CI = 1.0). However, when these flies were tested for the second cue set (ΔCOG = 20°, upper-green bar, and CI = 1.0), they followed the color rather than the position cue, resulting in negative PIs. Both transgenic fly strains performed correctly at PT but incorrectly (PIs near zero) when the second choice test was performed at RT (Fig. 4, F and G), whereas their visual perception to ΔCOG = 20° was still normal (fig. S3). Flies were also tested with a shape-color dilemma (*8*) as the second choice (fig. S4) and acted similarly to the performance in position-color dilemma. Thus, the dopamine- and MB-independent execution of a decision is specific for an established choice condition; a new conflicting set again requires dopamine and MB activities for decision-making.

This study demonstrated two distinct decision-making processes in *Drosophila*: one that is

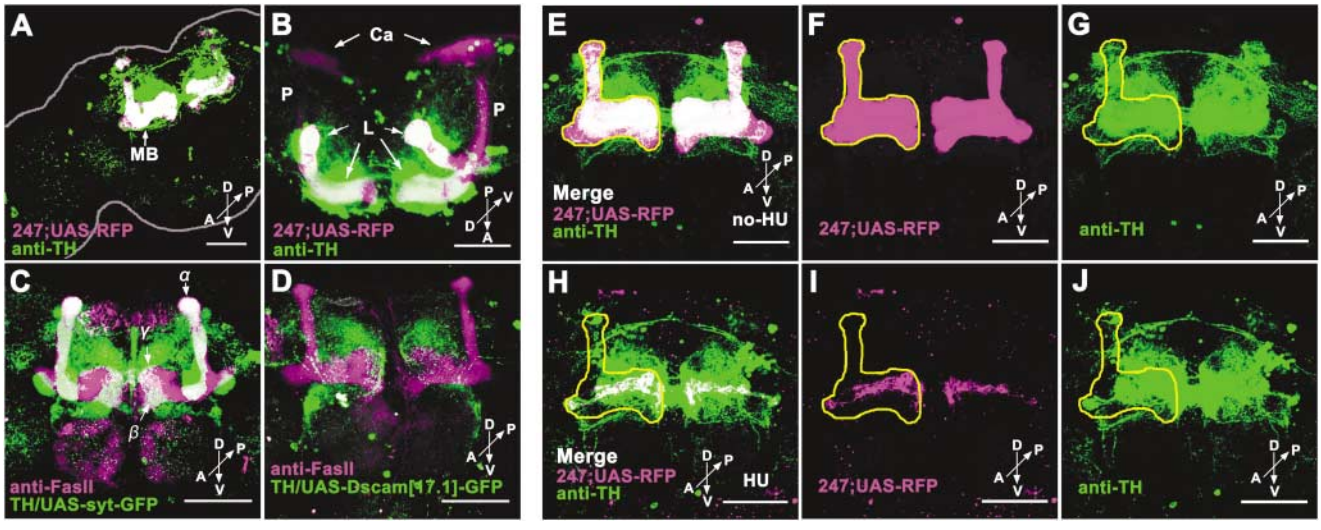

Fig. 3. Dopaminergic neurons project to MB lobes. D, dorsal; V, ventral; A, anterior; P, posterior. (**A**) Overlay of fly brain expressing RFP in MBs and immunostained with a TH antibody. (**B**) MB lobes (L), but not calyx (Ca) or peduncle (P), contained TH immunostaining. (**C**) Overlay of *Syt-GFP* expression in dopaminergic neurons and FasII immunostaining in MBs. α, β, and γ denote MB lobes. (**D**) *Dscam[17.1]-GFP* expressed in dopaminergic dendrites showed little colocalization with FasII immunostaining in MBs. (**E** to **J**) A comparison of the MBs of HU-treated with those of control flies showed that dopaminergic innervation axons depend on intact MB lobes. (E), (F), and (G) are wild types; (H), (I), and (J) are HU-treated. All images are superimposed confocal sections. Scale bars indicate 50 μm.

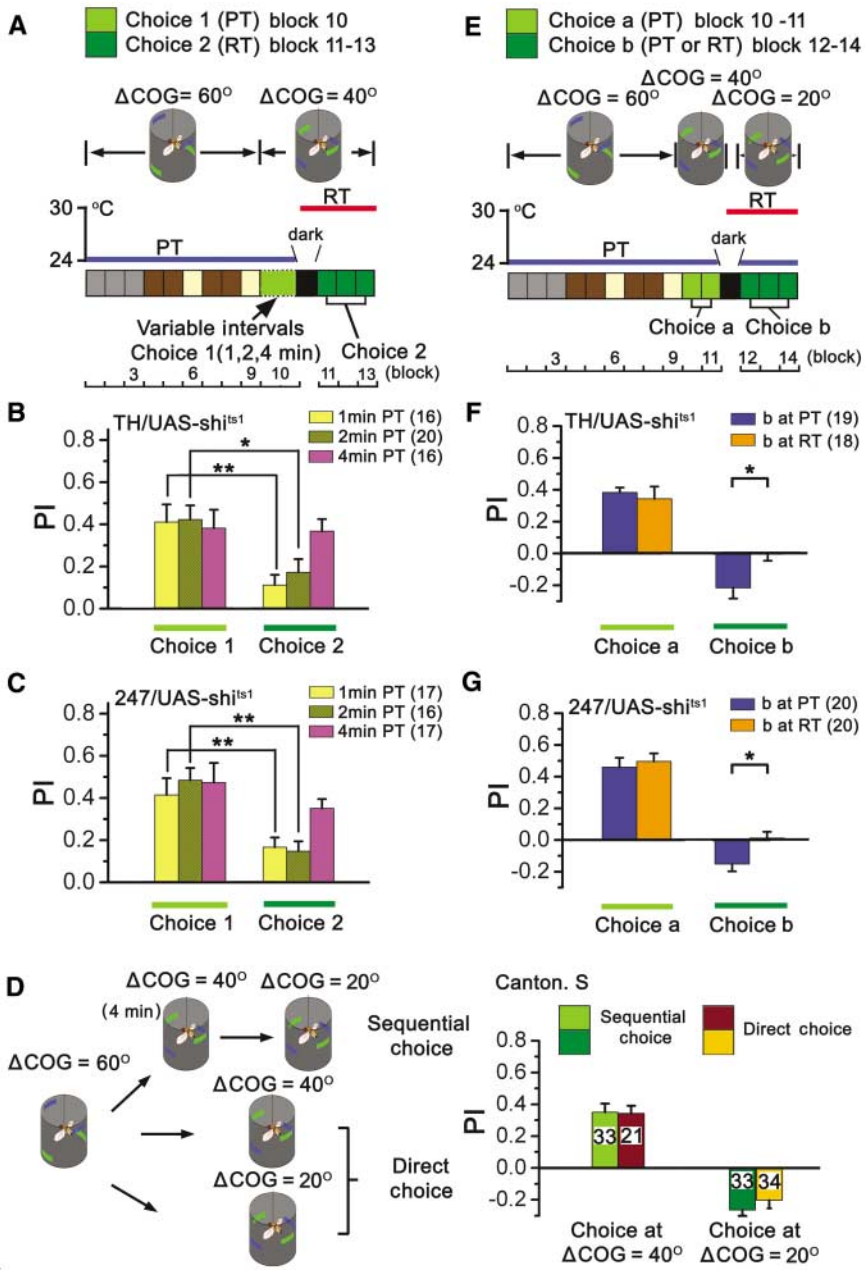

Fig. 4. Dopamine and MBs are required to form novel decisions but not to execute them. (**A**) Two-phase choice test involving time intervals (1, 2, and 4 min) during which a choice is made at PT, then at RT. (**B** and **C**) After 1- and 2-min intervals of normal choice behavior at PT (choice 1), TH/UAS-*shi*^{ts1} and 247/UAS-*shi*^{ts1} flies exhibited low PI at RT (choice 2); PIs were normal after 4 min at PT. (**D**) Comparison of sequential and direct choice paradigms. (**E**) Sequential choice performance. Flies were trained (as in Fig. 1A) and then subjected to two sequential choice tests: choice a, blocks 10 and 11, CI = 1.0, ΔCOG = 40°, and PT. Choice b, blocks 12 to 14, CI = 1.0, ΔCOG = 20°, and PT or RT. (**F** and **G**) TH/UAS-*shi* ^{ts1} and 247/UAS-*shi*^{ts1} flies showed significant PIs for choice b at PT compared with RT. *P < 0.05; **P < 0.01. P values are based on two-tailed Student's t test. Error bars indicate mean ± SEM for (B) to (D), (F), and (G).

nonlinear and saliency-based and the other that is linear, simple perceptual. The latter process could be performed in the absence of dopaminergic-MB circuits by subtracting the saliency of conflicting cues, but the ability to amplify the difference at crucial points was compromised. Thus, linear choice performance was displayed instead of the sigmoid pattern of wild types. We propose that changing from linear to nonlinear decision-making depends on a gating mechanism of the dopaminergic-MB circuit whereby only the stronger "winner" signal is transmitted to the MB while other weaker inputs are inhibited. Thus, flies implementing the gating function in MBs and the amplification effects of dopamine can accomplish a winner-takes-all decision. Two different phases, namely formation and execution, are involved in saliency-based decision-making in *Drosophila*, and a dynamic balance must be established between maintaining an existing choice and switching to a new decision.

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Figs. S1 to S4
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