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FoxP Influences the Speed and Accuracy of a Perceptual Decision in *Drosophila*⁺

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Abstract

Decisions take time if information gradually accumulates to a response threshold, but the neural mechanisms of integration and thresholding are unknown. We characterized a decision process in *Drosophila* that bears the behavioral signature of evidence accumulation. As stimulus contrast in trained odor discriminations decreased, reaction times increased and perceptual accuracy declined, in quantitative agreement with a drift-diffusion model. *FoxP* mutants took longer than wild-type flies to form decisions of similar or reduced accuracy, especially in difficult, low-contrast tasks. RNAi knock-down of *FoxP* in αβ core Kenyon cells, or the overexpression of a potassium conductance in these neurons, recapitulated the *FoxP* mutant phenotype. A mushroom body subdomain whose development or function require the transcription factor FoxP thus supports the progression of a decision towards commitment.

Integrator models of perceptual decision-making (1–5) predict that reaction times will vary with the quality of sensory information: easy decisions, based on clear evidence, will be fast; difficult decisions, based on uncertain evidence, will be slow. We tested this prediction in fruit flies, using a reaction time version of an olfactory discrimination task (6–8). Flies were analyzed individually in narrow chambers, which were perfused with odor-air mixtures whose convergence defined a 7 mm wide decision zone (Fig. 1A). With odors present, the flies slowed upon entry into the decision zone, paused near the interface (Fig. 1B and fig. S1, A and B), and exited after committing to a choice (Fig. 1B and fig. S1). We quantified the time between entry and exit as the reaction time (Fig. 1A).

Flies were trained to avoid a specific concentration of 4-methylcyclohexanol (MCH) and had to distinguish the reinforced concentration from a lower concentration of the same odor. The difficulty of discrimination was titrated by varying the MCH concentration ratio during testing (Fig. 1). At a reinforced MCH intensity of ~12 ppb, corresponding to 10⁻⁴ volumes of saturated vapor per volume of air, flies achieved accuracies of nearly 100% at large concentration differences (concentration ratio of comparison to reinforced odor of 0.1–0.2) but performed randomly when the odor concentrations differed by only 10% (concentration ratio 0.9) (Fig. 1C). Reaction times increased as a function of difficulty (Fig. 1, D and F, and

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fig. S1C), suggesting that flies compensated for low stimulus contrast in difficult tasks by gathering information for longer. Because overall odor concentrations were highest in the low-contrast tasks that took the longest to complete (Fig. 1, D and F, and Fig. S1C), and because only relative stimulus contrast, not absolute stimulus intensity, affected reaction times (Fig. 2, B and G), our data favor integration over probability summation (9): if the probability of odor detection were rate-limiting, reaction time would be expected to correlate inversely with stimulus intensity (5, 9).

A drift-diffusion model of evidence integration (1, 3–5, 10) (Fig. 1E, see Materials and Methods) captured the empirical relationship between difficulty and performance. Drift-diffusion models decompose reaction times into decision and residual times. The residual time encompasses sensory and motor latencies, procrastination, and time required to indicate a choice. The decision time constitutes the integration period *per se*. Its duration depends on the mean rate at which evidence accrues (the drift rate) and the level of the decision criterion (the bound height) (Fig. 1E). Intuitively, the weaker the sensory evidence, the lower will be the drift rate, the longer the response time, and the poorer the decision accuracy. The model we use (10) formalizes this intuition by scaling the drift rate in proportion to stimulus contrast, which we quantify as $|\log(\text{odor concentration ratio})|$. Estimation of the three free model parameters (drift rate, bound height, and residual time) from reaction time measurements generates a prediction of the corresponding decision accuracies. The modeled chronometric and psychometric functions provided satisfying simultaneous fits to our performance data (Fig. 1, C and D).

At all difficulty levels, the reaction time distributions exhibited positive skew (Fig. 1F), a characteristic of information accumulation to threshold (1, 3, 5, 11). The drift-diffusion model explains the origin of the asymmetry: equal differences in drift rate generate unequal differences in reaction time at the intersection with the response bound (Fig. 1E). In contrast, transit times of non-decision zones located off center had nearly symmetrical distributions that did not vary with task difficulty (Fig. 1G).

To examine whether the relationship between task difficulty and reaction time generalized, we designed two tasks other than intensity discrimination. In a masking odor task (fig. S2A), shock-reinforced MCH was presented in a ubiquitous background of 3-octanol (OCT). Difficulty was adjusted by varying the level of masking odor while keeping constant the concentration of cue. Flies took longer to respond to cues hidden in a high level of background than to salient cues, and did so with lower accuracy (fig. S2A). In binary mixture discriminations (6, 7, 12) (fig. S2B), the closer the proportions of MCH and OCT, the lower the accuracy and speed of discrimination (fig. S2B).

A pilot analysis of 41 strains carrying candidate mutations implicated the transcription factor FoxP in the decision process. *FoxP*^{SZ-3955} mutants learned to distinguish the shock-reinforced concentration of MCH with the same accuracy as wild-type flies (Fig. 2, A and F) but took longer to decide (Fig. 2, B to D, G to I). The defect was subtle in easy discriminations (concentration ratio 0.1–0.4) but glaring in difficult tasks (concentration ratio 0.7–0.9).

Mutating *FoxP* might alter any one of several processes that affect performance in our assay: the abilities to learn from shock reinforcement, walk to and from the odor interface, detect olfactory cues, and decide. Learning and locomotor deficits could be ruled out by examining the accuracy scores (Fig. 2, A and F) and transit time distributions (Fig. 2, E and J), respectively; both were identical in *FoxP^Δ-SZ-3955* mutants and wild-type flies. Mutants detected odors with the same sensitivity as wild-type controls: diluting all odors 1,000-fold had similar effects on either genotype (compare Fig. 2, A to E, with Fig. 2, F to J). Where mutant and wild-type flies clearly differed, however, was in the dependence of reaction time on stimulus contrast: in mutants, narrowing the odor concentration difference caused disproportionate increases in reaction time (compare red and black curves in Fig. 2, B to D, and Fig. 2, G to I). A drift-diffusion model (10) (fig. S3, A and B) identified two changes that can account for this phenotype: a 38% drop in drift rate (fig. S3C) and a—perhaps compensatory—increase in the height of the response bound (fig. S3D). The reduction in drift rate suggests that *FoxP* mutants are impaired in the accumulation and/or retention of sensory information in the build-up to a choice.

We confirmed the *FoxP* mutant phenotype with two independently generated alleles (Fig. 2, K and L, and fig. S4). Heterozygous carriers of any one of these alleles performed like wild-type controls in easy discriminations (concentration ratio 0.1; Fig. 2, K and L) but displayed prolonged reaction times in difficult tasks (concentration ratio 0.7; Fig. 2L). Homozygous or transheterozygous carriers of two mutant alleles exhibited pronounced difficulty-dependent speed and, in some allelic combinations, also accuracy deficits (Fig. 2, K and L). The association of similar phenotypes with different mutant alleles, and the lack of complementation between alleles (Fig. 2, K and L), tie the defect in decision formation firmly to the *FoxP* locus.

To identify, label, and manipulate sites of *FoxP* action in the brain, we used a *FoxP* promoter fragment to direct the expression of GAL4. *FoxP-GAL4* driven transgene expression was confined to two subsets of Kenyon cells (KCs), the principal intrinsic neurons of the mushroom bodies (13, 14): ~80 KCs whose axons extend into the cores of the α and β lobes, and ~100 KCs innervating the γ lobes (Fig. 3, A and B). Given their positions as third-order olfactory neurons, the *FoxP-GAL4* expressing KCs could transmit sensory data to downstream integrators. Alternatively, the *FoxP-GAL4* positive KCs themselves could integrate olfactory signals, or the representations of momentary and accumulated sensory evidence might be entwined at population level. Because both representations require externally or recurrently evoked activity (15, 16), reducing the excitability of KCs is predicted to prolong reaction times.

We therefore targeted the inwardly-rectifying potassium channel Kir2.1 under *FoxP-GAL4* control to $\alpha\beta$ core ($\alpha\beta_c$) and γ KCs while tuning expression levels with temperature-sensitive GAL80^{ts} (17). Flies expressing low Kir2.1 levels behaved like homozygous *FoxP^Δ-SZ-3955* mutants: reaction times increased in a difficulty-dependent manner relative to parental controls (Fig. 3, D and E) but accuracy was maintained (Fig. 3C). Boosting the expression of Kir2.1 exacerbated this phenotype: despite a further increase in reaction times (Fig. 3, G and H), *FoxP>Kir2.1* flies now performed near chance level in difficult discriminations (Fig. 3F), echoing the accuracy defects of severe *FoxP* alleles (Fig. 2K).

To bolster and refine our identification of *FoxP-GAL4* positive KCs as sites of FoxP action, we compared the consequences of introducing Kir2.1 with those of reducing *FoxP* expression, using a panel of *GAL4* lines whose expression domains included all or parts of the *FoxP-GAL4* pattern: *OK107-GAL4* targets all KCs (18), *NP6024-GAL4* and *NP7175-GAL4* label $\alpha\beta_c$ neurons (14, 19), and *NP1131-GAL4* marks γ KCs (14, 20) (Fig. 4, A and B). Knock-down of *FoxP* in $\alpha\beta_c$, but not γ , KCs prolonged reaction times in difficult discriminations (Fig. 4, C and D, and fig. S4B), mirroring the differential impact of Kir2.1 on these neuronal populations (Fig. 4, E and F). Attempts to disrupt *FoxP* expression with the help of *FoxP-GAL4* itself produced marginal effects (fig. S5A), probably due to inadequate *FoxP*^{RNAi} levels. Consistent with this interpretation, significant decision phenotypes were seen with all three of the three *GAL4* drivers capable of expressing high levels of *FoxP*^{RNAi} in $\alpha\beta_c$ KCs (Fig. 4C and fig. S5, B and C).

The evolution of a decision towards commitment requires the progression of neural activity from a choice-neutral to a choice-specific state. Mutations in *FoxP* evidently slow this progression, at least in part by interfering with the function of $\alpha\beta_c$ neurons. The same neurons have been implicated in value-based decisions, such as choices between odors associated with punishments of differing severity (19). It remains untested whether value judgments also incur a difficulty-dependent cost of decision time. Nonetheless, the available evidence suggests that ~80 *FoxP-GAL4* positive $\alpha\beta_c$ KCs form part of a versatile decision circuit that processes sensory information in one context and remembered value in another.

As a transcription factor (21, 22), FoxP could act during development to specify synaptic connections and/or throughout life to regulate neuronal function. Vertebrate *FoxP* homologs have been linked to both types of processes (23–26) and been attributed critical roles in cognitive development (24, 27, 28), vocal communication (21, 26, 29), and motor control (23, 25). A potential commonality between these processes and decision-making is their unfolding over time: neurons representing trains of thought, strings of syllables, chains of motor commands, or accumulating evidence must all step through ordered activity sequences. It is therefore tempting to speculate that understanding the function of an ancestral *FoxP* gene (22) might reveal fundamentals of temporal processing (30).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Analysis of decision accuracies and reaction times

(A) Movement trajectory (red) near the odor interface. Orange color indicates the 7 mm wide decision zone and gray shading on the left the aversively reinforced odor MCH, which must be distinguished from a lower MCH concentration on the right. The kymograph shows position on the long chamber axis over time. Because the centroid of the fly's silhouette rather than the position of the antennae is monitored, the trajectory stops short of the odor interface. (B) Example kymographs of wild-type flies discriminating different MCH intensities. (C) Accuracy (mean $\pm$ SEM; $N=142$ –173 flies per data point) vs. difficulty. Asterisks indicate significant differences from concentration ratio 0.1 ($P<0.0001$). The red line depicts the psychometric function predicted by a drift-diffusion model (10). (D) Reaction time (mean $\pm$ SEM; $N=592$ –828 decisions per data point) vs. difficulty. Asterisks indicate significant differences from concentration ratio 0.1 ($P<0.0005$). The red line depicts the chronometric function predicted by the drift-diffusion model (10). (E) Drift-diffusion model of evidence accumulation to response bound A. (F, G) Frequency (top) and cumulative frequency distributions (bottom) of reaction (F) and transit times (G) at odor concentration ratios corresponding to easy (0.1), intermediate (0.5), and hard (0.9) difficulty levels. Reaction time distributions differ between difficulty levels ($P<0.0001$) but transit time distributions do not ($P>0.3158$).

Fig. 2. Decision accuracies and reaction times of *FoxP* mutants

(A–J) Wild-type (black) and *FoxP*^{δ-SZ-3955} mutant flies (red) performed intensity discrimination at high (10^{-1} , left) and low (10^{-4} , right) MCH concentrations. (A, F) Accuracies (mean±SEM; $N=129$ –182 flies per data point) vs. difficulty. (B, G) Reaction times (mean±SEM; $N=442$ –802 decisions per data point) vs. difficulty. Asterisks indicate significant differences between wild-type and *FoxP*^{δ-SZ-3955} mutant flies ($P<0.0014$). (C–J) Reaction (C, H), cumulative reaction (D, I), and transit time distributions (E, J) of wild-type (black) and *FoxP*^{δ-SZ-3955} mutant flies (red) at easy and hard difficulty levels. Reaction time distributions differ between genotypes ($P<0.0014$) but transit time distributions do not ($P>0.0685$). (K, L) Flies heterozygous (open symbols), homozygous (filled symbols), or transheterozygous (mixed symbols) for the indicated *FoxP* alleles performed MCH intensity discrimination. (K) Accuracies (mean±SEM; $N=124$ –190 flies per genotype) and (L) reaction times (mean±SEM; $N=394$ –841 decisions per genotype) vs. difficulty. Asterisks indicate significant differences from wild-type performance (accuracy: $P<0.0001$; reaction time: $P<0.005$).

Fig. 3. Identification and manipulation of neurons expressing *FoxP-GAL4*

(A, B) *FoxP-GAL4* drives mCD8-GFP expression in $\alpha\beta_c$ and weakly in γ KCs. A cross-section through the α lobe (B, inset) shows confinement of *FoxP-GAL4* expression (red) to the core; nc82 staining in gray. Scale bars, 20 μ m. (C–H) Flies expressing Kir2.1 under *FoxP-GAL4* and *tubP-GAL80^{ts}* control (*FoxP>Kir2.1*, red) and parental controls (*FoxP-GAL4*, dark gray; *UAS-Kir2.1*, light gray) were maintained at the indicated temperatures for 18 h before performing MCH intensity discrimination. (C, F) Accuracy (mean $\pm$ SEM; $N=112$ –152 flies per genotype) vs. difficulty. The asterisk indicates a significant genotype effect ($P<0.0033$). (D, G) Reaction times (mean $\pm$ SEM; $N=261$ –720 decisions per genotype) vs. difficulty. Asterisks indicate significant genotype effects ($P<0.0005$). (E, H) Cumulative reaction time distributions of flies maintained at 25 °C (E) and 30 °C (H). Irrespective of temperature, the distributions differ between experimental flies (red) and parental controls (gray) at the hard ($P<0.0005$) but not the easy difficulty level ($P>0.2139$).

Fig. 4. Manipulation of *FoxP* expression and excitability in KC subsets

(A, B) *NP6024-GAL4* (A) labels $\alpha\beta_c$ KCs; *NP1131-GAL4* (B) labels γ and some $\alpha'\beta'$ KCs. Insets show cross-sections through the α lobes: *GAL4*-driven GFP expression in red; mushroom body staining with *mb247-LexA:LexAop-rCD2::RFP* in gray. Scale bars, 10 μ m. (C, D) Flies expressing *FoxP^{RNAi}* in $\alpha\beta_c$ (C) or γ KCs (D) performed MCH intensity discrimination. Accuracies (mean $\pm$ SEM; $N=119$ –178 flies per genotype) and reaction times (mean $\pm$ SEM; $N=410$ –803 decisions per genotype) at easy and hard difficulty levels. Red and gray symbols denote experimental flies and parental controls, respectively (*X-GAL4*, dark gray; *UAS-FoxP^{RNAi}*, light gray). Asterisks indicate significant genotype effects on reaction times ($P<0.0031$). (E, F) Flies expressing *Kir2.1* in $\alpha\beta_c$ (E) or γ KCs (F) performed MCH intensity discrimination. Accuracies (mean $\pm$ SEM; $N=51$ –149 flies per genotype) and reaction times (mean $\pm$ SEM; $N=136$ –414 decisions per genotype) at easy and hard difficulty levels. Red and gray symbols denote experimental flies and parental controls, respectively (*X-GAL4*, dark gray; *UAS-Kir2.1*, light gray). Asterisks indicate significant genotype effects on accuracies ($P<0.032$) and reaction times ($P<0.0007$).