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Supplemental Information

Olfactory Object Recognition Based on Fine-Scale Stimulus Timing in *Drosophila*

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Supplemental Information

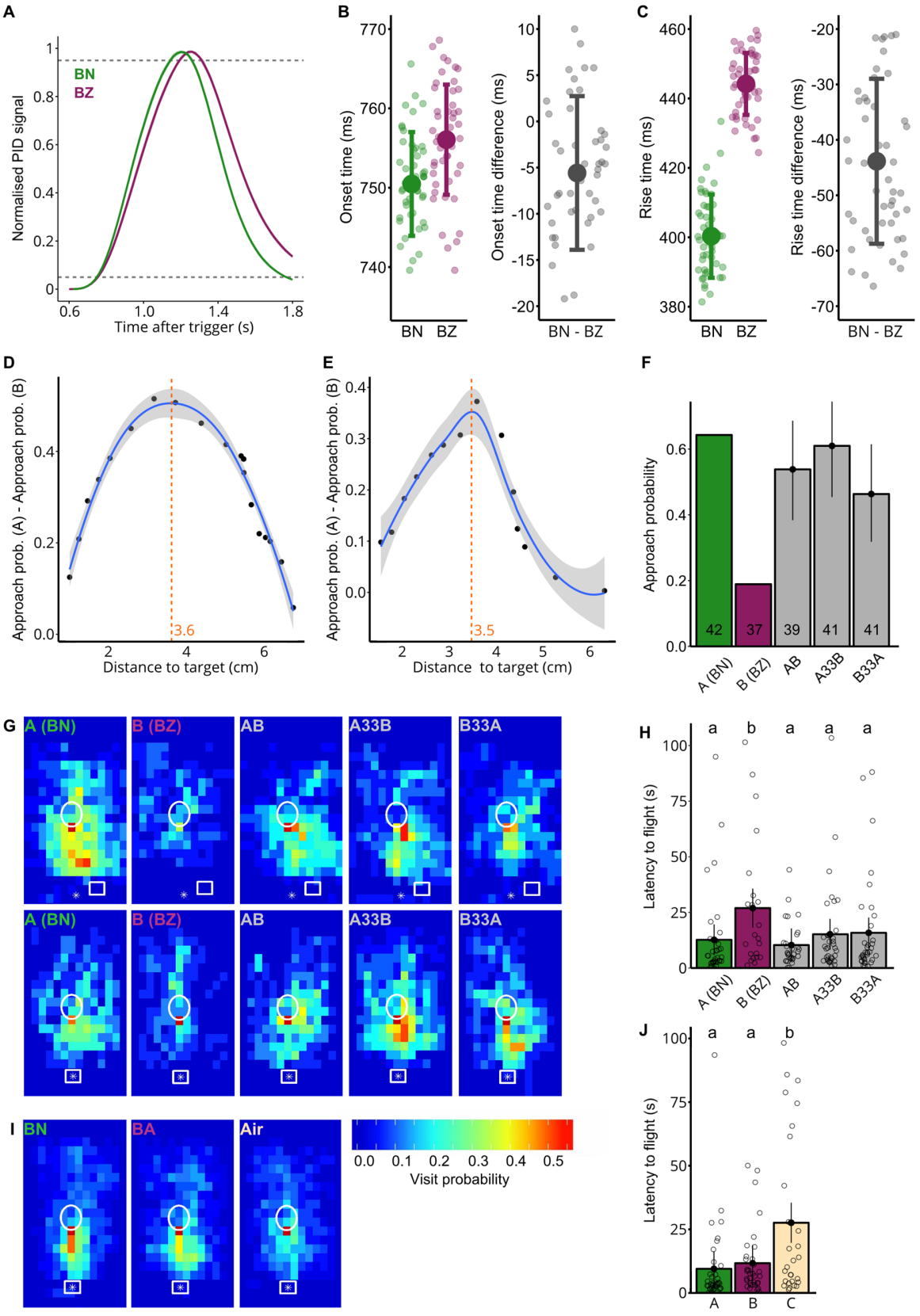

Figure S1. Stimulus timing and behavioral data, Related to Figure 1 and 2

(**A**) PID recordings of pulsed stimuli for the odorant pair with innate valence 2-butanone (BN, green) and benzaldehyde (BZ, magenta). Pulses were 500 ms long and with a 7 s interstimulus interval to allow the concentration to reach baseline again before the next pulse started (mean and SD over 50 pulses). Each PID signal was normalized to the maximum concentration reached. Grey dashed lines represent 5 and 95 % of the maximum.
(**B**) Left: Onset time (time taken to reach 5 % of maximum concentration after valve trigger) for BN and BZ (mean and SD over 50 pulses). Individual points represent the onsets for each pulse. Right: Onset time difference between pairs of BN and BZ pulses (mean and SD over 50 pulses).
(**C**) Left: Rise time (time take to reach 95 % of maximum concentration from the 5% onset time) for BN and BZ (mean and SD over 50 pulses). Individual points represent the rise times for each pulse. Right: Mean rise time difference between pairs of BN and BZ pulses (mean and SD over 50 Pulses).
(**D**) Thresholding method that uses the distance which separates flies' approach probabilities for (BN) A and (BZ) B best for set 1 (see "maximized A-B difference threshold" in Transparent Methods). Each point represents the proportion of A-stimulated flies that approached the target by the given minimum distance to the target minus the proportion of B-stimulated flies. The blue trend line was fitted using locally weighted scatterplot smoothing to avoid skewing by further away deviant points. The distance at the peak of the trend line was defined as threshold (orange dashed line and value)
(**E**) Same as (D) but for set 2 of BN and BZ.
(**F**) Approach probability for odorant mixtures with different asynchronies (maximized A-B difference threshold). Bars with vertical lines represent the mean and 95 % credible intervals. Since A and B are used to determine the threshold, they were not included in the statistical analysis.
(**G**) Visit probability maps of set 1 (Top) and set 2 (Bottom) of BN and BZ for single odorants and the mixtures. The take-off platform (white circle), landing platform (white rectangle) and odor source (white star) are indicated for position reference. n of set 1 = 24, 20, 22, 20 and 22; n of set 2 = 18, 17, 17, 21 and 19 for A, B, AB, A33B and B33A respectively.
(**H**) Response latency for odorants and mixtures, measured as the time taken from the fly entering the take-off platform to first flight. The lower case letters represent significantly different responses to the odorant treatments. Y axis limited to 110 s. n = 36, 25, 31, 37 and 37 for A, B, AB, A33B and B33A respectively.
(**I**) Same as (G) but for BN (A), BA (B) and blank air control (Air). n = 46, 45 and 41 for A, B, and C respectively.
(**J**) Same as (H) but for BN (A), BA (B) and blank air control (Air). n = 39, 34 and 29 for A, B and C respectively.

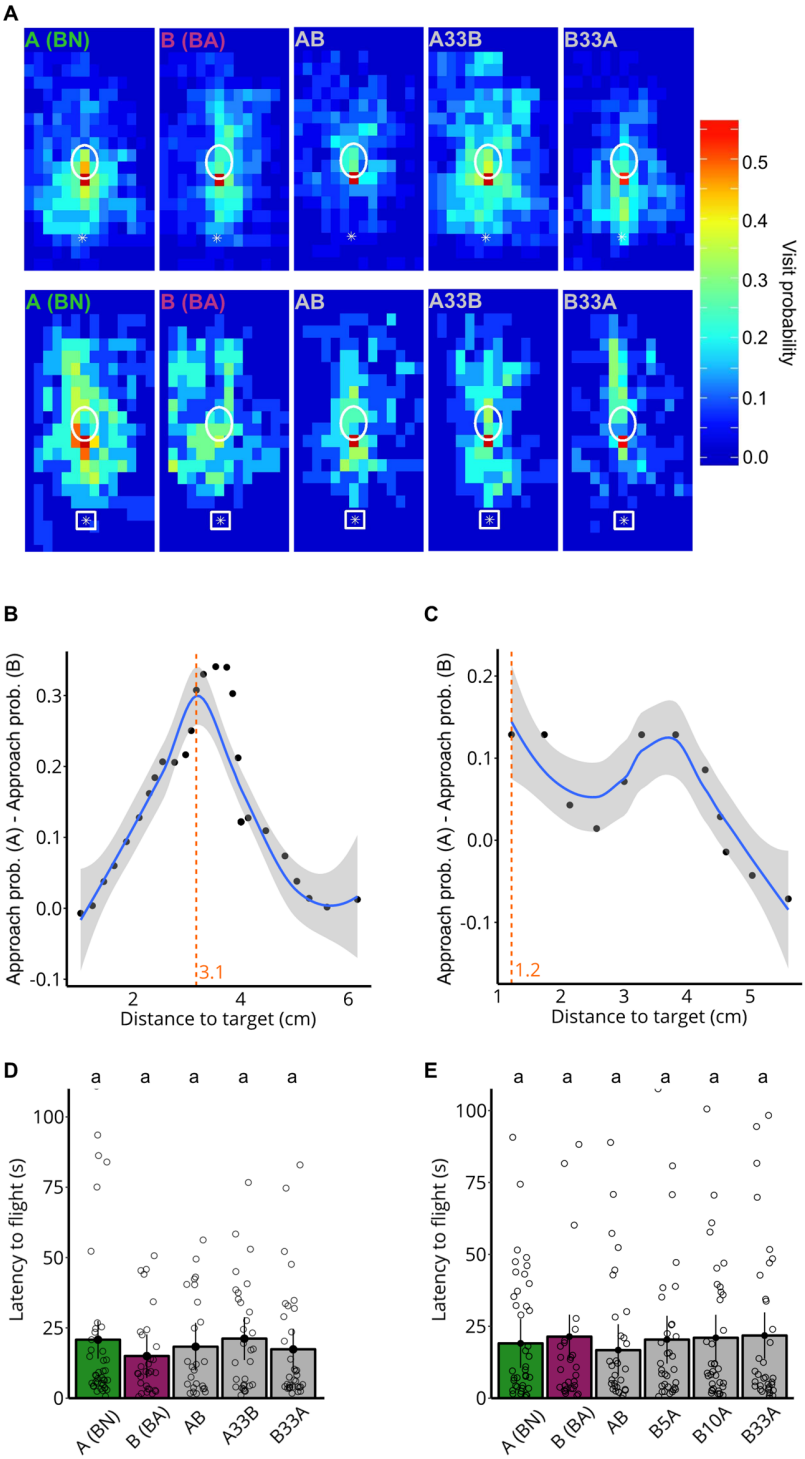

Figure S2. Behavioral data, Related to Figure 3B – E

(**A**) Visit probability maps of set 1 (Top) and set 2 (Bottom) of BN and BA for single odorants and the mixtures. The take-off platform (white circle), landing platform (white rectangle) and odor source (white star) are indicated for position reference. n for set 1 = 35, 34, 32, 35 and 33; n for set 2 = 14, 14, 12, 15 and 16 for A, B, AB, A33B and B33A respectively.

(**B**) Thresholding method that uses the distance which separates flies' approach probabilities for A and B best for set 1 of BN (A) and BA (B) (maximized A-B difference threshold).

(**C**) Same as (B) but for set 2 of BN and BA.

(**D**) Response latency for odorants and mixtures, measured as the time taken from the fly entering the take-off platform to first flight for the experiment shown in Figure 3E. The lower case letters represent significantly different responses to the odorant treatment. Y axis limited to 110 s. n = 42, 33, 28, 36 and 34 for A, B, AB, A33B and B33A respectively.

(**E**) Same as in (D) but for the experiment shown in Figure 3D. n = 42, 36, 33, 38, 36 and 39 for A, B, AB, B5A, B10A and B33A respectively.

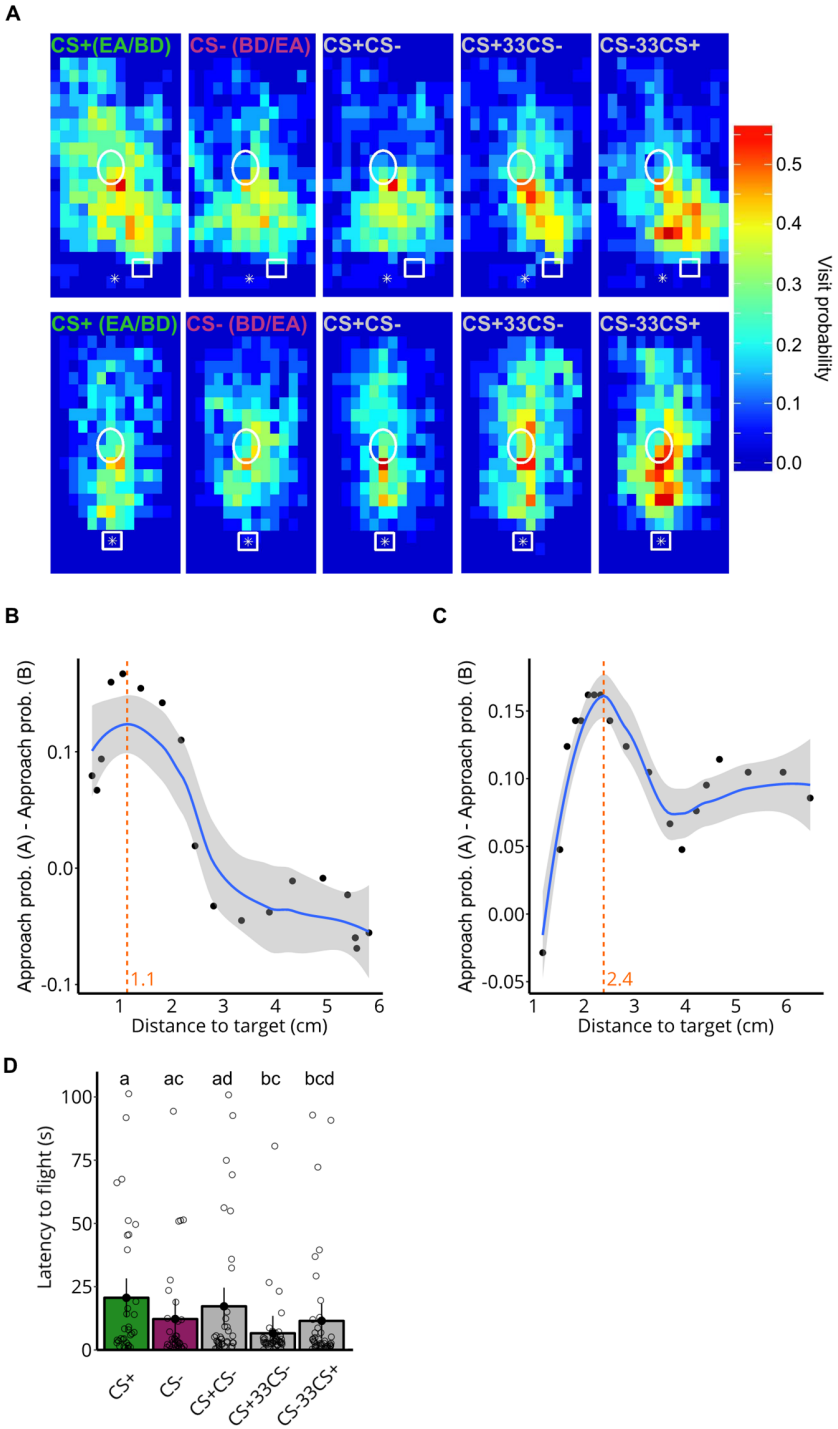

Figure S3. Behavioral data, Related to Figure 3G

(**A**) Visit probability maps of set 1 (Top) and set 2 (Bottom) of CS+ and CS- (either EA or BD) for single odorants and the mixtures. The take-off platform (white circle), landing platform (white rectangle) and odor source (white star) are indicated for position reference. n for set 1 = 19, 22, 23, 24 and 26; n for set 2 = 21, 21, 22, 20 and 24 for CS+, CS-, CS+CS-, CS+33CS- and CS-33CS+ respectively.

(**B**) Thresholding method that uses the distance which separates flies' approach probabilities for CS+ and CS- best for set 1 of EA and BD (maximized A-B difference threshold)

(**C**) Same as (B) but for set 2 of EA and BD.

(**D**) Response latency for odorants and mixtures, measured as the time taken from the fly entering the take-off platform to first flight. The lower case letters represent significantly different responses to the odorant treatments. Y axis limited to 110 s. n = 35, 37, 38, 43 and 46 for CS+, CS-, CS+CS-, CS+33CS- and CS-33CS+ respectively.

Table S1. Percentage of flies flying and their latency to flight, Related to Figure 2 and 3

Experiment	Stimulus	Total number of flies tested	Percentage of flies flying	Mean latency to flight (s)
Figure 2B, Figure S1D-H	A (BN)	42	86	13
	B (BZ)	37	68	27
	AB	39	80	10
	A33B	41	90	15
	B33A	41	90	16
Figure 2C	A (BN)	46	85	10
	B (BA)	45	76	12
	Air	41	71	28
Figure 3A	A (BN)	93	90	21
	B (BA)	91	76	16
	AB	85	72	19
	B33A	94	78	20
Figure 3D	A (BN)	44	96	19
	B (BA)	43	84	21
	AB	41	80	17
	B5A	43	88	20
	B10A	45	80	21
	B33A	45	87	22
Figure 3E, Figure S2	A (BN)	49	86	21
	B (BA)	48	70	15
	AB	44	64	18
	A33B	50	72	21
	B33A	49	70	17
Figure 3G, Figure S3	CS+ (EA/BD)	40	88	21
	CS- (BD/EA)	43	86	12
	CS+CS-	45	84	17
	CS+33CS-	44	92	7
	CS-33CS+	50	98	12

Table S2. Additional statistical analysis of approach probabilities using the exact binomial test, Related to Figure 2 and 3

P-values and corrected alpha values are given for the exact binomial test and Bayesian probabilities are given for the Bayesian analysis. Red values indicate significant differences.

Experiment	Odorants	Comparison	P-value	Corrected alpha	Bayesian probability
Figure 2B	BN (A), BZ (B)	A vs B	<0.001	0.05	>0.999
Figure 2C	BN (A), BA (B), Air	A vs B	<0.01	0.025	0.962
		A vs Air	<0.001	0.025	>0.999
Figure 3A	BN (A), BA (B)	A vs AB	<0.001	0.017	0.993
		B vs AB	0.62	0.017	0.338
		B33A vs AB	<0.001	0.017	0.996
Figure 3D	BN (A), BA (B)	B5A vs AB	0.1	0.017	0.894
		B10A vs AB	1.0	0.017	0.5
		B33A vs AB	<0.001	0.017	0.995
Figure 3E	BN (A), BA (B)	A33B vs AB	0.21	0.025	0.793
		B33A vs AB	<0.01	0.025	0.957
Figure 3G	EA/BD (CS+), BD/EA (CS-)	CS+33CS- vs CS+CS-	<0.05	0.025	0.965
		CS-33CS+ vs CS+CS-	<0.01	0.025	0.981

TRANSPARENT METHODS

Animals

Wild-type Canton S *Drosophila melanogaster* were reared on standard medium (100 mL contain 7.1 g cornmeal, 6.7 g fructose; 2.4 g dry yeast, 2.1 g sugar beet syrup, 0.7 g agar, 0.61 ml propionic acid, and 0.282 g ethyl paraben) under a 12:12 hours light:dark cycle (light from 09:00 to 21:00), at 25 °C and 60% relative humidity. All flies used in the experiments were female, aged between four and eight days old.

Wind tunnel

We carried out experiments in two wind tunnels, referred to here as wind tunnel 1 (WT 1, data shown in Figure 3D) and wind tunnel 2 (WT 2 data shown in all other figures). We filmed each experiment using Raspberry Pi cameras (Raspberry Pi Camera Module v2; Raspberry Pi 3 model B) for 2 or 3 minutes with a resolution of 640 x 480 pixels and 90 frames s^{-1}; the first 10 seconds of flight duration were used for the analysis.

Both wind tunnels were constructed from clear Plexiglas. The inner side walls and floor were covered by a random checker board pattern (grey on white paper). The dimension of WT 1 was 1.2 m x 0.19 m x 0.19 m and of WT 2 was 2 m x 0.40 m x 0.40 m. The exhaust took in room air (28 °C, 60 % relative humidity) through the tunnel and removed it from the setup building via a ventilation shaft. An aluminum honeycomb grid (hole diameter x length: 0.53 cm x 3 cm, WT 1; 0.32 cm x 9.7 cm, WT 2) at the inlet and a grid at the outlet of the tunnel created a non-turbulent flow throughout. The wind speed was 40 cm/s. We injected odorants into the inlet of the wind tunnel with an olfactory stimulator (Raiser et al., 2016). The outlet of the olfactory stimulator was 1 cm in diameter and was placed just outside of the honey comb grid, creating a non-turbulent air-stream within the tunnel that allowed us to control the timing of the odorant stimuli. Flies entered the tunnel through a glass tube that was connected to a take-off platform whose center was 7.5 cm (WT 1) or 6 cm (WT 2) downstream from the inner side of the honeycomb grid.

In the course of most of the experimental protocols (see below) we added a black landing platform at the honey comb grid near the entry site of the odorant plume. We introduced this landing platform in an attempt to increase flies approach behavior for attractive odorants, because recent studies have demonstrated that *Drosophila* approach dark spots when stimulated by an attractive odorant (Breugel et al., 2017; Saxena et al., 2018). Experimental protocols that differ with respect to the existence or position of a landing platform are referred to as “set 1”or “set 2” respectively. In WT 1 we used two cameras to film the flies. One camera was placed above the wind tunnel to capture the x-y plane of movement, whereas the other was placed at the side of the wind tunnel (90° to the other camera), thus capturing the movement of the fly within the z-y plane. The volume filmed measured 17.3 cm x 13.0 cm x 17.3 cm (x, y, z). In WT 2 we used a single camera placed above the wind tunnel to record the fly trajectories in the x-y plane. In order to capture the z-y plane of the flight track, we positioned a mirror at a 45° angle to the camera inside of the wind tunnel. The volume filmed measured 13.7 cm x 10.3 cm x 9.5 cm (x, y, z). Both wind tunnels were illuminated with indirect, homogeneous, white light with a color

temperature of 6500 K (WT 1: compact fluorescent light, tageslichtlampe24.de; WT 2: LEDs, led-konzept.de). Additionally, we used 850 nm backlight illumination (96 LED IR Lamp, Conrad; plus 2 cm thick polyethylene foam as diffusor) to get contrast-rich images of the flies.

Odorant delivery

Odorants were delivered into the wind tunnels using a custom-made multichannel olfactory stimulator (Raiser et al., 2016). All odorants were supplied by Sigma Aldrich. Pure odorants were stored in 20 ml glass vials (Schmidlin) sealed with a Teflon septum. The cross section of the odorant surface was 3.1 cm^2. The headspace of odorized air was permanently drawn into the air dilution system using flowmeters (112-02GL, Analyt-MTC) and an electronic pressure control (35898; Analyt-MTC). The stimulator had three channels: one for each odorant and one for blank air. The odorant vials were constantly flushed with clean air throughout the experiment, so that the headspace concentration reached a steady state of odorant evaporation into the air and odorant removal by the air flush. Note that due to the permanent air stream the headspace odorant concentration never saturated. The total flow per odorant channel was always 300 ml min^{-1}. In WT 1, BN was released at 50 ml min^{-1} and added to 250 ml min^{-1} air, and BA was released at 30 ml min^{-1} and added to 270 ml min^{-1} air (experiments in Figure 3). In WT 2, BN, BA and BZ were released at 50 ml min^{-1} and were added to 250 ml min^{-1} air (experiments in Figures 2 and 3). For the conditioned odorants we used the PID to determine the head space concentrations in the conditioning tubes (see below) by moving the PID needle rapidly into the conditioning tubes to prevent dilution in odorant concentration due to air suction of the PID. These concentrations from the conditioning paradigm were then adjusted in the odor delivery device by measuring the odorant concentration just above the take-off platform with the PID. EA was released at 4 ml min^{-1} and added to 296 ml min^{-1} air, and BD was released at 1.84 ml min^{-1} and added to 298.16 ml min^{-1} air (experiments in Figure 3).

The two odorant channels and a blank channel (each with an airstream of 300 ml min^{-1}) were combined and injected into a carrier air stream of 410 ml min^{-1} and, resulting in a total air flow at the outlet of the stimulator of 1.31 L min^{-1}, and a wind speed of 0.4 ms^{-1}.

Stimuli were presented either as single odorants (either A or B), as a synchronous mixture of odorants presented simultaneously (AB) or as an asynchronous mixture, with different time delays between the release of the odorants. In BΔtA, B starts before A, with Δt being either 5 ms, 10 ms or 33 ms. In AΔtB, A starts before B, with Δt being 33 ms (Figure 1C). Note that the trailing odorant ended at the same time as the preceding odorant. Stimuli were delivered in odorant pulses of 500 ms, and the interstimulus interval was 2 s. To exclude that differences in flies' approach behavior towards the asynchronous and synchronous mixture reflected responses to mechanical cues produced by valve switching, we applied the single odorants together with a 33 ms delayed blank stimulus (both stimuli ended at the same time).

During experiments, all odorants were removed from the wind tunnel via an exhaust into the outside atmosphere. Between experimental sessions using different odorants, the stimulator valves were flushed out over night to remove any residual odorants. Valves were controlled by compact RIO systems equipped with digital I/O modules Ni-9403 and odorant delivery was controlled by software written by Stefanie Neupert in LabVIEW 2011 SP1 (National Instruments).

Experimental protocol for odorants with innate valence

Day 1: Between 13:00 and 16:00, approximately 100 adult flies were removed from standard corn meal agar food and were subjected to food and water starvation for 24 hours in a cage (30×30×30 cm, BugDorm-1, BugDorm) that allowed them to move around freely, in a room with an approximate relative humidity of 60%, a temperature of 25 - 28 °C and 12 hour daylight cycle.

Day 2: Between 15:00 and 20:00, individual, flying female flies were removed from the cage and placed into a PVC tube through which they could walk freely to enter the wind tunnel and reach the take-off platform. For each experimental trial we used a single fly. Once the fly reached the take-off platform, odorant stimulation started. Each fly was stimulated repeatedly with the same odorant stimulus. After each experimental trial we removed and discarded the fly. During one experimental session (for a data set shown in a given panel of a figure), an equal number of flies were stimulated with the different stimuli so that between-session variability would affect the behavior to all stimuli equally. The order of stimuli was alternated.

Most of the experimental paradigms were made up of different sets, depending on the presence and location of the black landing platform. In the experiment shown in Figure 2A, 2B and S3, set 1 placed the landing platform 1.5 cm to the right of the odor source, whereas set 2 placed the platform at the odor source directly. In the experiment shown in Figure 2C, S1I and S1J, there was only one set, with the landing platform placed centrally at the location of the odor source. In the experiment shown in Figure 3B and 3D, there was only one set, where

the black platform was located 0.5 cm to the right of the odor source. For the experiments shown in Figure 3E, S2A-C, set 1 contained no landing platform, whereas set 2 contained the landing platform at the location of the odor source.

Differential conditioning

Day 1: Between 15:00 and 16:00, approximately 100 adult flies were removed from standard corn meal agar food and put into a cage (30×30×30 cm, BugDorm-1, BugDorm) that contained a differential conditioning apparatus (Figure 3F). Flies could move around freely at an approximate relative humidity of 30%, a temperature of 25 - 28 °C and normal 12 hour daylight cycle for 24 h.

We trained flies in a differential conditioning paradigm to associate one odorant (positively conditioned stimulus, CS+) with 1 M sucrose solution as the positive reinforcer and to associate another odorant (negatively conditioned stimulus, CS-) with saturated NaCl solution as negative reinforcer (Figure 3F). We used BD and EA as conditioned odorants. We balanced the experiments so that in half of the experiments we used BD as CS+ and EA as CS- and vice versa. CS+ and sucrose solution and CS- and NaCl solution were applied via two horizontally positioned plastic tubes (15 ml, 120 x 17 mm; Sarstedt). Each tube contained 10 ml of either sucrose or NaCl solution and were plugged with a cotton wool to avoid spillage. The frontal 2 cm of each tube remained empty. The odorant was delivered into this empty space via diffusion through a shortened head of a needle (1.2 x 40 mm, Sterican) which ended 1.5 cm inside the empty space of the tube. The needle was connected with a 20 ml glass vial (Schmidlin) that contained the pure odorant and was sealed with a Teflon septum. Thus, to reach the sucrose or NaCl solution, flies had to move through odorized air inside the plastic tube.

Day 2: Between 15:00 and 16:00, the conditioning apparatus was removed and flies were subjected to food and water starvation for the following 24 h in a room with an approximate relative humidity of 60%, a temperature of 25 - 28 °C and normal 12 hour daylight cycle.

Day 3: Flies were tested in the wind tunnel as described above in the section “Experimental protocol for odorants with innate valence” (Day 2).

The conditioning experiments (Figure 3G, S3) also had two sets, depending on the location of the black landing platform. In set 1, the black platform was located 1.5 cm to the right of the odor source, and in set 2, the black platform was at the location of the odor source.

Stimulus dynamics

To assess the dynamics and precision of the different stimuli, we used a photoionization detector (PID; miniPID model 200B; Aurora Scientific) to record the concentration change of pulses of each of the odorant pairs (BN and BA, BN and BZ, BD and EA) within the wind tunnel. Each pulse had a duration of 500 ms, and an interstimulus interval of 7 s to allow the odorant to clear from the odor delivery device and/or PID and to allow the PID signal to return to baseline before the following pulse was given. We gave a sequence of 100 pulses, alternating between odorant A and odorant B (7 s interval between A and B), thus 50 pulses of each odorant. For each odorant pulse, we calculated the onset time as the time it took to reach 5 % of the maximum PID signal, and the rise time as the time it took for the PID signal to reach from 5 % to 95 % of its maximum. We also calculated the difference in both the onset times and in the rise times between each of the 50 pairs of successive pulses (A – B) (we compared pairs of successive pulses to reduce the variability due to minor changes in wind speed in the wind tunnel which was affected by the wind speed outside).

Calculating flies’ distance to the target

To calculate the Euclidean distance to the source, we obtained the x, y and z coordinates of the fly for the first 10 s of flight of the recording. For the experiments shown in Fig. 3D, if a fly did not take off, we calculated its closest distance to the target. For all other experiments, if a fly did not take off we took the closest distance between the take-off platform and the target.

For WT 1, we used two cameras. Both cameras were triggered simultaneously with a TTL pulse, however to ensure that they did not go out of sync, all videos were aligned by first frame of flight. We calculated the Euclidean distance of the fly to the target:

Euclidean distance = $\sqrt{(x - x_0)^2 + (y - y_0)^2 + (z - z_0)^2}$

Where x, y and z are the coordinates of the fly’s location in a particular frame, and x0, y0 and z0 are the coordinates of the target.

For WT 2, a single camera was used to film the fly trajectories in the x and y plane. In order to record the movement in the z plane simultaneously, a mirror was placed at 45° to the x-y plane. Thus on the right half of the video recordings, the x-y plane was recorded, and on the left half of the video, the mirrored z-y plane was recorded. However, this resulted in the image in the left half shrinking to 1.3 times smaller than the original objects on the right half. Therefore, we calculated the fly’s distance to the target in WT 2 by:

Euclidean distance = $\sqrt{(x - x_0)^2 + (y - y_0)^2 + ((z - z_0) * 1.3)^2}$

Where x, y and z are the coordinates of the fly's location in a particular frame, and x0, y0 and z0 are the coordinates of the target

Quantifying flies' approach with the "half-distance threshold"

In order to measure approach behavior, we used the halfway distance between the frontal border of take-off platform and the target to determine the circular approach area around the target. In WT 1, we used a value of 117 pixels (3.2 cm) for the radius and in WT 2 a value of 71 pixels (2.7 cm).

Quantifying flies' approach with the "maximized A-B difference threshold"

To make the quantification of flies' approach behavior less arbitrary and to account for the fact that flies distributed differently in the two different wind tunnels and experimental sets, we calculated an approach area that segregated flies' approach probabilities for the attractive odorant A (or CS+) and the aversive odorant B (or CS-) the most. To determine the radius of this area, we took the Euclidean distance to target for each fly that was exposed to the attractive odorant A (or CS+) alone or the aversive odorant B (or CS-) alone; those flies that encountered mixtures of odorants were not incorporated in this process. The minimum distances were arranged in ascending order, and at each distance, we counted the number of flies from treatment A and treatment B that were included within this threshold distance. Thus for each of these distances, we calculated the difference in approach probabilities by:

Difference in approach probabilities = $\frac{A_{in}}{A_{in}+A_{out}} - \frac{B_{in}}{B_{in}+B_{out}}$

Where A_{in} represents the number of flies that were presented with odorant A and were included below the threshold, A_{out} is the number of flies presented with A but excluded above the threshold. B_{in} and B_{out} were the same measures for the flies that were presented with odorant B. We then plotted the thresholding index against the vector of minimum distances, and fitted a curve using locally weighted scatterplot smoothing using the R function "geom_smooth" with default parameters from the package "ggplot2", as this method avoids deviant points at further away regions in the scatterplot from affecting the local fit, and it highlights trends in data that may be unclear with a parametric fitting (Figure 3C and S1D, S1E, S2B, S2C, S3B and S3C). We took the distance that corresponded to the maximum peak of the curve as the radius of the approach area, as this point indicates the greatest separation between the two treatment groups. Since we used treatments A and B in defining the approach areas, we did not include these flies in the statistical analyses and restrict the comparisons to the mixtures.

Approach probability

In both WT 1 and WT 2 we filmed two angles of the flight area. Thus in each wind tunnel, there were two separate areas of approach, one for each of the two cameras for WT 1, and one for each side of the video screen for WT 2 (mirrored and original view). To calculate the approach probability, we gave each fly a binary score. The coordinate of each fly in every frame was recorded and tested as to whether it fell within the approach area boundaries. If a fly entered the approach area at any frame within 10 seconds after take-off, the fly was given a score of 1; if not, was given a score of 0. This was done for each camera (WT 1) or video side (WT 2), and then the results were combined so that only if a fly was in both areas of approach at the same time point, would it be given a score of 1. Finally, we calculated the proportion of flies in each treatment that entered the approach area to get the approach probability.

Visit probability maps

We extracted the x-y coordinates of the fly during the first ten seconds of flight. We divided the recording image into 20 x 20 pixel bins to create two visit probability maps. Each bin was represented by a cell in the map. We then plotted each coordinate point onto the visit map, giving the cell a score of 1 if one or more points fell into the bin, or a 0 if no points fell into the bin. For the flies that did not fly, a matrix of zeros was generated, with a score of 1 for the cell that represented the closest point on the take-off platform to the odor source. We calculated the mean for each pixel bin across all of the flies in a treatment group.

Response latency

We selected the flies that started flying within 10 000 frames after entering the take-off platform (111 s, corresponding to approximately 50 odorant pulses). We defined the individual response latency for each fly as the time point of flight minus the time point of entry onto the take-off platform.

Statistical Analysis

For all data analysis, R version 3.5.0 was used (R Core Team, 2012). All statistics were performed using Bayesian data analysis, based on (Korner-Nievergelt et al., 2015).

To investigate the effect of the synchronous and asynchronous mixtures on approach probability, we used a binomial generalized linear model (GLM), with approach probability as the binary response variable (1 = approach, 0 = no approach). We used the logistic regression (logit) link function. The synchronous and asynchronous mixtures were used as explanatory variables. We used an improper prior distribution (flat prior) and simulated 100 000 values from the posterior distribution of the model parameters using the function "sim" from the package "arm". The means of the simulated values from the posterior distributions of the model parameters were used as estimates, and the 2.5 % and 97.5 % quantiles as the lower and upper limits of the 95 % credible intervals. To test for differences between approach probabilities for the asynchronous and synchronous mixtures, we calculated the proportion of simulated values from the posterior distribution that were larger for asynchronous than for synchronous mixtures. We declared an effect to be significant if the proportion was greater than 0.95.

In the figures, we used different letters for comparisons between all stimuli and we used stars for comparisons between the synchronous mixture AB and the asynchronous mixtures. Posterior probabilities above 0.95 were indicated by "*" and above 0.99 by "**". A posterior probability of, for example, 0.96 for the comparison between the asynchronous mixture B33A and the synchronous mixture AB ($p(B33A > AB) = 0.96$) means that one can be 96% certain that flies' approach probability is greater for B33A than for AB.

To compare the response latencies across treatment groups, we modeled the response latency to the mixtures using a linear model with the synchronous mixture AB as the reference level. As in the previous model, the synchronous and asynchronous mixtures were used as categorical explanatory variables. Note that in this model we compared all combinations of mixtures within an experiment. We used the same methodology as before to simulate values from the posterior distribution and generate the means and the 95 % credible intervals. To test for differences, we calculated the proportion of draws from the posterior distribution for which the mean of each draw was smaller in one mixture than the mean of each draw of another mixture. We declared an effect to be significant if the proportion was greater than 0.95. If the posterior probability was higher than 0.95, it was deemed significantly different (with letters).

We also analyzed the behavioral data using frequentist statistics (Table S2). To see whether the asynchronous mixtures induced a significantly different approach probability than the synchronous mixture, we used an exact binomial test, using the R function "binom.test" from the R package "stats". We did the same comparisons as with the Bayesian analysis. For the experiment comparing BN, BA and blank air, we used the mean approach probability to BN (A) as the hypothesized probability of success. For each experiment involving synchronous mixtures, we used the mean approach probability to the synchronous mixture as the hypothesized probability of success. To compensate for multiple comparisons, we applied a Bonferroni correction by dividing the alpha level (0.05) by the number of comparisons within each experiment. We determined the result to be significant if the p value was smaller than the corrected alpha level.

SUPPLEMENTAL REFERENCES

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