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Hierarchical Spatial Search Strategies in *Drosophila*

Nicola Meda[1], Giulio M. Menti[1], Aram Megighian[1,3,*], Mauro A. Zordan[2,3]

[1]Department of Biomedical Sciences, University of Padova, via Ugo Bassi 58/B, 35131 Padova

[2]Department of Biology, University of Padova, via Ugo Bassi 58/B, 35131 Padova

[3]Padova Neuroscience Center, University of Padova, via Orus 2/B, 35131 Padova

*Correspondence: aram.megighian@unipd.it

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ABSTRACT

Animals rely on multiple sensory information systems to make decisions. The integration of information stemming from these systems is believed to result in a precise behavioural output. To what degree a single sensory system may override the others is unknown. Evidence for a hierarchical use of different systems to guide navigation is lacking. We used *Drosophila melanogaster* to investigate whether, in order to relieve an unpleasant stimulation, fruit flies employed an idiothetically-based local search strategy before making use of visual information, or viceversa. Fruit flies appear to initially resort to idiothetic information and only later, if the first strategy proves unsuccessful to relieve the unpleasant stimulation, make use of other information, such as visual cues. By leveraging on this innate preference for a hierarchical use of one strategy over another, we believe that *in vivo* recordings of brain activity during the navigation of fruit flies could provide mechanistic insights into how simultaneous information from multiple sensory modalities is evaluated, integrated, and motor responses elicited, thus shedding new light on the neural basis of decision-making.

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1. Introduction

Animals make use of different information to explore their surroundings. Olfactory [1], visual [2–4] cues and self-centred (idiothetic) path integration [5–9] can be used to navigate an environment in search for food [10], relief [11] or for the purpose of mating [12,13]. The reductionist approach to behavioural neuroscience has so far provided mechanistic insights into how each sensory modality drives specific behaviours [14], but the knowledge of the relationships between different sensory information systems, and how animals use these to reach their goals is just at its beginning [15–17]. To the best of our knowledge, there is no evidence for a hierarchical use of different information systems to guide navigation in simple animal models [18]. Invertebrates, particularly insects, are excellent models to study how a less complex brain produces behaviours [19,20]. Insects can be trained to pinpoint a location in space where an unpleasant stimulation ceases, where food may be present, or where a pleasant sensation may be experienced [2,21–23]. Insects explore their environment while relying on multiple sources of information, of which the most studied are visual cues and path integration. Visual stimuli can be associated with a stimulus of relevance to the animal, and having a positive valence, such as the obtainment of food, the relief from a negative stimulus or the possibility of mating. Path integration is the behavioural output of an “internal pacemaker”, the activity of which guides the locomotion of the animal in order to maximize its chance of reaching a relevant stimulus previously encountered while navigating. We used *Drosophila melanogaster* to investigate whether we could find evidence for the hierarchical use of different sources of information; specifically, we asked the question whether, in order to relieve an unpleasant stimulation, fruit flies employed an idiothetically-based local search strategy before making use of visual information, or viceversa.

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2. Materials and Methods

We obtained the experimental progeny, characterised by the expression of a red-shifted rhodopsin in bitter-sensing neurons, from crossing Blooming Stock Center (BSC) line 57670 (in which expression of Gal4 was restricted to bitter-sensing neurons), to BSC line 55135, a UAS-CsChrimson construct-bearing line. Thus, these transgenic flies are characterised by having bitter-sensing neurons that can be stimulated via brief red-light pulses [24,25]. For our behavioural experiments we opted to use only 6 to 10-day-old adult male flies. A single fly was loaded into a circular arena of 109mm diameter, in which the insect was free to walk, but not fly. The arena was loaded on a raised platform, which was illuminated from below by an infrared-emitting lamp. Optogenetic stimulation was delivered with a pair of 3 red-light (627nm) emitting LEDs placed on opposing sides of the arena and 30 cm above it. A cylinder composed of 48 panels, each consisting of an 8x8 grid of green (520nm) LEDs, was lowered around the arena. The LEDs were programmed to display two diametrically opposed black stripes, one vertical and the other horizontal, each spanning an equal area on an evenly green lit background. The vertical stripe was spatially-matched to a 6cm^2 “safe zone” (i.e. a zone where no optogenetic stimulation occurred). In order to trigger/shut off optogenetic stimulation according to the spatial location of the fly, we recorded fly movements inside the arena with a Chameleon 3 (FLIR, USA) camera equipped with an IR bandpass filter and live-tracked fly locomotion at 11 frames-per-second with a customised version of the Motion-Based Multiple Object Tracking script by MathWorks®. Real-time fly coordinates were used by the script to drive a Genuino board's output signals (and consequently the optogenetic stimulation) as follows: whenever the *xy* coordinates of the centroid representing the fly were within a "safe zone" in the arena, the optogenetic stimulus was turned off; as soon as the fly left the safe zone, the stimulus was turned on and the fly bitter-sensing neurons were stimulated. Behavioural experiments consisted of two parts, a training session and a probe session.

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Each training session was made up of 16 trials. We did not consider the first trial in our analysis, given that flies had not yet had any experience of a safe spatial location. During the first 30 seconds of each trial the fly was free to explore the arena in complete darkness; for the next 30 seconds the fly could explore the arena in the presence of the green LED visual patterns. During the last 2 minutes of each trial, the fly could be subjected to optogenetic stimulation according to its position in the arena, still in the presence of the green LED visual patterns. On alternate trials, the whole green LED panorama was rotated by 180°: during odd numbered trials (1st, 3rd,…,15th) the safe zone was adjacent to the 'southern end' of the arena and matched to the vertical stripe; during even numbered trials, the safe zone was adjacent to the 'northern end' of the arena and matched to the vertical stripe (figure 1A). If not otherwise specified, data from the analysis that follow were obtained from 140 experiments (flies N = 140). We analysed our data with a regression model selection-based approach. Full details on methods and statistical analyses are presented in electronic supplementary materials, methods S1 [26–33]. The procedures described herein had also been implemented in [34]. Detailed characteristics of each regression model can be found in electronic supplementary material, table S1.

3. Results and Discussion

It has been extensively shown that *Drosophila melanogaster* can be trained to distinguish identical objects with different orientations [35–37]. We therefore trained flies to differentiate between a vertical black stripe, linked to relief from optogenetically-induced bitter taste, from a diametrically-opposed horizontal one (figure 1B-D, electronic supplementary material, figure S1). At the beginning of each new trial, the vertical stripe – safe zone match and the horizontal stripe positions were switched. In this way, at the start of each new trial and at the first onset of optogenetic stimulation, fruit flies could potentially

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experience two conflicting sources of information as to where relief could take place: either in the spatial location where flies previously (in the preceding trial) experienced relief (PreviousSafeZone – currently matched to the horizontal stripe) or in the current location of the vertical stripe (SafeZone). In this ambiguous situation, the animal is faced with the possibility of using a search strategy based either on local path integration or on visual information. To start with, we focused our attention on the behaviour of flies at the start of each new trial, 30'' before the first onset of optogenetic stimulation and 30'' after its onset. This 60-second period (from frame 330 to frame 990) was further divided into 6, 10-second sub-periods. As expected, this analysis revealed that in each new trial, before the onset of the first optogenetic stimulation, fruit flies were more numerous in the region where the previous safe zone was located than in the region where the current safe zone was located (figure 2A). As soon as the optogenetic stimulation started, fruit flies left the previous safe zone and began searching for relief on the opposite side of the arena (in the location corresponding to the current vertical stripe – safe zone match). As a consequence, following the onset of optogenetic stimulation, flies began to spend more time in the safe zone and less in the previous safe zone (figure 2B). This feature can also be seen in the density plots in figure 2D and 2E, as a reduction in the density of residency in the previous safe zone in favour of the current safe zone (demarcated by the square). One might expect that, following the onset of optogenetic stimulation, there should be an increase in the searching behaviour of the fruit flies in the vicinity of the current safe zone. Nonetheless, we show that throughout the whole training session, at the start of each new trial, at the first onset of the negative stimulation, fruit flies showed an equally elevated frequency of entries into either the previous safe zone or into the current safe zone (figure 2C), suggesting that some flies searched for relief in the previous safe zone. Furthermore, this behaviour does not appear to be affected by learning: in fact, training progression influenced neither the number of flies in both zones nor the number of visits to both zones

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between frames 330-990 (i.e. training progression is not a significant predictor of the aforementioned variables, electronic supplementary material, table S1). In order to identify the group of flies that, at the onset of the optogenetic stimulus entered the previous safe zone, we subset all the 140 flies object of our study into three subgroups: 1. flies which were inside the previous safe zone at the beginning of the stimulation (at frame 661); 2. flies that, in that same moment were inside the current safe zone; 3. flies that were in neither of the preceding zones. Since flies which were already inside the current safe zone at the onset of the optogenetic stimulation would not have experienced virtual bitter taste, we discarded this group of flies from this analysis, and considered only the two remaining groups. In figure 3A we show that the mean number of flies that were already inside the previous zone (blue pointrange) at frame 661 did not change in the 10 seconds after the onset of stimulation; on the other hand, flies that were outside the previous safe zone (orange pointrange) proceeded to enter the previous safe zone after the onset of stimulation, thus increasing the overall number of flies therein. This sudden entrance of flies from outside the previous safe zone is also seen in figure 3C, where an increase in the number of visits of this group of flies to the previous safe zone is evidenced. However, following the onset of optogenetic stimulation both groups of flies spend less time in the previous safe zone: this is because notwithstanding the increase in the number of visits to the previous zone, such visits are of short duration (since no relief is provided), suggesting that flies “attempt” to obtain relief from the bitter taste stimulation by entering the previous safe zone but, since this does not provide any relief, they quickly move away from that location. The density plots in figure 3D-F show the change in residency density for each of the two groups of flies separately, and for both groups considered together, respectively.

As evidenced by the data plotted in Figure 3, the group of flies that were outside of both zones at the onset of the optogenetic stimulation were responsible for the increase in the number of visits and in the mean number of flies in that same zone. However, as may be

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noticed in figure 3E, some of the fruit flies in this group entered the current safe zone, as evidenced by the increased residence density in the plots of the 10 seconds after the onset of the stimulation. We therefore investigated how many of the flies which were previously outside both zones then entered the safe zone or the previous safe zone. We found that flies belonging to this group increased their number of visits in the same measure to both zones (previous safe zone and safe zone) in the 10 seconds immediately following the start of stimulation (figure 4B), and this is reflected also on the mean number of flies that can be found in both zones during this period (figure 4A). The previous safe zone and the (current) safe zone were visited in the same measure by the same number of flies, suggesting that flies did not take into consideration which visual marker they were approaching. Given that this behavioural choice (choosing which zone to explore first) does not appear to depend upon learning (i.e. training progression, also for this subgroup of flies, does not influence the number of flies/number of visits to both zones, electronic supplementary material, table S1) and that fruit flies can differentiate between a vertical and a horizontal bar (electronic supplementary material, figure S1) [35–37], we wished to ascertain whether fruit flies entered preferentially into one of the two zones based on their spatial location in the arena (i.e. proximity to one of the two zones). Thus, we divided the group of flies outside of both zones at frame 661 according to which was the first zone entered. Figure 5A and 5C show the positions of the flies that entered the previous safe zone (marked by the horizontal bar) or the current safe zone respectively, during the trials in which the safe zone was located at the northern-end of the arena (marked by the vertical bar); whereas, Figure 5F and 5H, show the same information relative to the trials in which the safe zone was located at the southern-end of the arena. These density plots suggest that flies tend to enter the zone which was closest to them at the onset of the optogenetic stimulation. We thus tested whether the spatial distribution of each group of flies, for each set of trials (i.e. subdivided according to whether the safe zone was at the

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northern end of the arena or at the southern end) was more aggregated than the distribution expected under the hypotheses of a random distribution. To do this, we applied Marcon and Peuch's M function [29–31] and tested if the observed spatial distribution of flies presented evidence of a greater aggregation than the random expectation represented by 10.000 simulated random distributions. For both sets of trials (i.e. north/south according to where the safe zone was located), and for both groups (i.e. which zone was entered first), flies showed evidence for significantly greater aggregation than expected under the null hypothesis of random distribution (figure 5B and 5D and 5G and 5I). Moreover, if at the time of onset of the optogenetic stimulation, the position of a fly in the arena may be considered a predictor of which will be the first zone to be visited, the positions of the flies which are predicted to enter the previous safe zone should not show any overlap with the positions of the flies which are predicted to enter the current safe zone (i.e. the two groups should be spatially segregated). We found that the two groups of flies show significant segregation in both sets of trials (figure 5E and 5L). We also conducted a further set of experiments, with 40 more flies, in which the visual environment presented two identical and diametrically opposed vertical stripes, with only one of them matched to a safe zone. With this set of experiments we reproduced the results previously described herein, suggesting that the initial search strategy employed by the flies object of the present work appears to be independent of visual cues (electronic supplementary material, figure S2 and S3), is not a consequence of learning and shows evidence of being guided by the spatial location of the fly at the onset of the negative stimulation much in the same way as occurs in the case of an idiothetically-based local search strategy (path integration) [6,8,38]. In fact, at the beginning of a new trial, before the optogenetic stimulation begins, the fruit flies are free to explore the environment without being punished: as flies experience the first instance of bitter-taste (at frame 661), the closest spatial location that yielded no punishment is in the vicinity of the fly itself (namely, where

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the fly was at frame 660). Thus, it is straightforward for an animal to search for relief in the vicinity of its current position.

4. Conclusions

With the paradigm employed in these experiments and the large sample of flies tested, we provide evidence for a consistent hierarchical use of information during spatial navigation in *D. melanogaster*. Our experiments show that fruit flies, when forced to choose between relying on self-centred or visual cues to guide their local search, appear to initially resort to idiothetic information and only later, if the first strategy proves unsuccessful, make use of other information, such as visual cues. The reasons for such a consistent hierarchical use of one informational system over another are at present unknown, but we advance the hypothesis that employing path integration as the first spatial search strategy possibly requires less computation, thus yielding faster behavioural responses, than the evaluation and sensory integration of external (i.e. visual) information. By leveraging on this innate preference of a hierarchical use of one strategy over another, *in vivo* recordings of brain activity during the navigation of fruit flies could provide mechanistic insights into how simultaneous information from multiple sensory modalities is evaluated, integrated and motor responses elicited, thus shedding new light on the neural basis of decision-making.

Figure captions

Figure 1. Paradigm and fruit flies training

A) brief summary of the behavioural paradigm and experimental question; B) time spent (s) in the safe zone, marked by the vertical stripe, compared to the time spent in the non-safe zone (marked by the horizontal stripe) during 16 trials of training. Flies spent

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significantly more time in the safe zone during the time when optogenetic stimulation may occur than in a homologous zone where no relief is provided (number of observations = 280, linear mixed-effects (lme) model with zone as explanatory variable compared to the null model χ^2 = 309.67, df = 1, p = 0.000, ΔAIC = 307.6, ΔBIC = 304); C) Velocity profile of fruit flies after entering a zone where relief is provided (yellow line) compared to the profile (grey line) after entering the non-safe zone (number of observed velocities = 624637, lme model with interaction between frame after entrance into one of the zones and specific zone as explanatory variable compared to a model with only frame after entrance as explanatory variable, χ^2 = 16688, df = 1, p = 0.000, ΔAIC > 10^4, ΔBIC > 10^4); D) density plots describing the residency of flies during training session, while optogenetic stimulation is triggered if flies leave the safe zone (squared). The plot on the left describes the residency of flies when the safe zone is at the southern end (during odd numbered trials) of the arena, the right one when the safe zone is at the northern-end.

Figure 2. Distribution of flies in the two zones before and after the onset of optogenetic stimulation.

We subdivided the 660 frames (60 seconds) time window considered into 6 identical periods of 10 seconds each (from frame 330 to frame 440, from 440 to 550 etc.). The green bar represents the 30 seconds of visual pattern display, without optogenetic stimulation, the red bar marks the half-minute during which optogenetic is triggered according to fly position in the arena. For each 10 second period we computed the mean of the observed values of the y-variables later explained. Pointrange represents the mean and the confidence interval around the mean. A) Mean number of flies for each period considered, divided according to the zone considered. The number of fruit flies in the previous safe zone (matched to the horizontal stripe) decreases after the onset of

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optogenetic stimulation in favour of an increase in the number of flies in the current safe zone (matched to the vertical stripe). The mean number of fruit flies inside each zone depends on the zone itself (whether it is providing relief or not) and its interaction with the period of time considered (number of observations = 179, generalised linear mixed-model (glmm) with zone and period interaction as explanatory variable compared to only zone model χ^2 = 83.47, df = 1, p = 0.000, ΔAIC = 81.5, ΔBIC = 78.3). This result is independent from the trial in which flies are counted (the best glmm is a null model), suggesting there is no change as the training progresses; B) The time fruit flies spend in the zones depends on the zone considered and its interaction with the progression of time (number of observations = 3527, lme with zone and period interaction as explanatory variables when compared to a model with only zone as the explanatory variable χ^2 = 174.88, df = 1, p = .000, ΔAIC = 173, ΔBIC = 167). In this case a significant role is played by the progression of training, since flies, as expected, improve their learning (number of observations = 3527, lme with interaction between zone and trial as explanatory variables, when compared to a model with only zone as the explanatory variable χ^2 = 10.14, df = 1, p = 0.001, ΔAIC = 9, ΔBIC = 2); C) Mean number of visits to the two zones throughout the whole training period is a function of the interaction between each zone and the period of time considered (number of observations = 1504, generalised linear mixed-model (glmm) with zone and period interaction as explanatory variable compared to a model with only zone as explanatory χ^2 = 67.66, df = 1, p = 0.000, ΔAIC = 65.6, ΔBIC = 60.4). Zone alone is not a good predictor of the number of visits, suggesting that there is no difference between the number of visits to the two zones, suggesting that flies search within both zones in the same measure during the time period considered (when a model with zone as the explanatory variable is compared to a null model, there's no significant difference between the two models, number of observations = 1504, χ^2 = 0.001, df = 1, p = 0.96); D) Density plots of the spatial distributions of flies before the onset of optogenetic stimulation

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(higher row) and during the stimulation (lower row) when the safe zone is located at the northern-end of the arena. Fruit flies are aggregated within the previous safe zone in the first 10 seconds after the visual stimuli are displayed (440, representing the period of time from frame 330 to frame 440), in the following two periods they begin to scatter throughout the whole arena, and since optogenetic stimulation is delivered according to the position of the flies (770), flies start to aggregate in the safe zone. E) As in D), but when the safe zone is located at the southern-end of the arena.

Figure 3. Some flies return to the previous safe zone at the onset of optogenetic stimulation

Subsetting of periods was performed as described in the caption to Figure 2. Pointrange represents the mean and the confidence interval around the mean. We subdivided the flies into two groups: one consisting in flies which at frame 661 (when the optogenetic stimulation starts) which are already inside the previous safe zone and another consisting in flies which are outside both the previous safe zone and the current safe zone. A) The mean number of flies that are already inside the previous safe zone (blue point range) at the onset of the stimulation remains stable during the first 10 seconds of stimulation; Some of the flies that were outside both zones at frame 661 (orange point range) return to the previous safe zone (number of observations = 180, generalised linear mixed-model (glmm) with the interaction of fly group and period as the explanatory variables compared to a model with only fly group as the predictor $\chi^2 = 39.43$, df = 1, $p < 0.001$, $\Delta AIC = 37.5$, $\Delta BIC = 34.3$). This result is independent from the trial in which flies are counted (the best glmm is a null model), suggesting there is no change as the training progresses (i.e. independence from learning). A model with only group as the explanatory variable is better than a null model (electronic supplementary material, table S1), suggesting there is also a difference between the two groups of flies independently from the period of time B) The

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time fruit flies spend in the previous safe zone depends on the zone considered and its interaction with the progression of time (number of observations = 4232, lme with interaction between group and time as explanatory variables when compared to a model with only zone as the explanatory variable χ^2 = 220.19, df = 1, p = 0.000, ΔAIC = 218, ΔBIC = 212). In this case the progression of training is not significant, suggesting that this effect is not affected by learning. C) Mean number of visits to the previous safe zone throughout the whole training session is a function of the interaction between each group of flies and the period of time considered, with no effect due to the progression of training (number of observations = 1082, generalised linear mixed-model (glmm) with the interaction between group and period as explanatory variables compared to a model with only group as the explanatory variable (χ^2 = 10.3, df = 1, p = 0.001, ΔAIC = 8.3, ΔBIC = 3.3); D) Density plots of flies already inside the previous safe zone in the 10-second-period before the onset of stimulation (660) and in the following 10 seconds (770), when the safe zone is at the northern-end (left) or at southern-end (right). In both cases the flies quickly spread away from the previous safe zone; E) As in D), but in this case the group of flies which are outside the previous safe zone at frame 661 are represented; F) superimposition of the density plots shown in D) and E)

Figure 4. Fruit flies which are initially outside the two zones, equally distribute to either of the two zones soon after optogenetic stimulus onset

Subdivision of periods as described in the caption to figure 2. Pointrange represents the mean and the confidence interval around the mean. A) Mean number of fruit flies, which are initially outside both zones and that at the onset of optogenetic stimulation (at frame 661), then populate the two zones. In the 10-second-period after the onset of optogenetic stimulation (770), an approximately equal number of flies enters into either the previous safe zone or the current safe zone. Thus the difference between the number of flies that

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approach either one of the two zones is not significant (number of observations = 180, generalised linear mixed-model (glmm) with zone and period interaction as explanatory variable compared to a model with only zone as the predictor χ^2 = 205.43, df = 1, p = 0.000, ΔAIC = 203.4, ΔBIC = 200.2); B) Mean number of visits to the zones throughout the whole training session is a function of the interaction between zone and the period of time considered, with no effect due to the progression of training (number of observations = 1392, generalised linear mixed-model (glmm) with the interaction between zone and period as the explanatory variables compared to a model with only group as the predictor (χ^2 = 67.88, df = 1, p = 0.000, ΔAIC = 65.9, ΔBIC = 60.7). Zone alone is not a good predictor of the number of visits, suggesting that there is no difference between the number of visits to the two zones, suggesting that flies search both zones in the same measure (when a model with zone as the explanatory variable is compared to a null model, there's no significant difference between the two models; number of observations = 1392, χ^2 = 1.26, df = 1, p = 0.26).

Figure 5. Spatial dependence of the choice of the first zone

A) Spatial distribution at frame 661 (when the first pulse of optogenetic stimulation is delivered) of fruit flies which will then approach the horizontal stripe, thus entering the previous safe zone first, during trials in which this is found at the southern-end of the arena; B) Marcon and Peuch M's function value represents the number of observed flies at each distance from a single fly (radius) compared to the expected values from a random distribution (red dashed line, grey shading represent the envelope built on 10.000 simulations of random distributions). A value greater than 1 suggests aggregation. A goodness-of-fit test of the observed data reveals that flies are significantly more aggregated than expected (number of observations = 277, p = 0.000); C) Spatial distribution at frame 661 of fruit flies which will approach the vertical stripe, thus entering

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the safe zone first; D) M function value is significantly greater than expected under the null hypothesis (number of observations = 256, p = 0.000) suggesting spatial aggregation of flies; E) M function value assessing whether the two distributions of flies reported in A) and C) actually consist of two distinct aggregates. An M function value < 1 suggests that spatial repulsion between the two groups of flies (number of observations = 533, p = 0.000), indicating that flies which will enter the current safe zone are spatially segregated from flies which will enter the previous safe zone; F) Spatial distribution of fruit flies at frame 661 which will approach the horizontal stripe, thus entering the previous safe zone first, located at the northern-end of the arena. G) Goodness-of-fit of observed data suggests that flies are more spatially aggregated than expected, as seen in B) (number of observations = 229, p = 0.000); H) Spatial distribution of fruit flies at frame 661 which will approach the vertical stripe, thus entering the safe zone first; I) Goodness-of-fit of observed data suggests that flies are more spatially aggregated than expected (number of observations = 209, p = 0.000); L) M function value assessing whether the two distributions of flies reported in F) and H) actually represent two distinct aggregates. A goodness-of-fit test suggests spatial repulsion between the two groups of flies (number of observations = 438, p = 0.000), indicating that flies which will enter the safe zone are spatially segregated from flies which will enter the previous safe zone, thus supporting the hypotheses that spatial location is a predictor of which zone is explored first;

CRediT authorship contribution statement Nicola Meda Conceptualization, Methodology, Data acquisition, Formal analysis, Visualization, Data curation, Writing - original draft, Writing - review & editing. **Giulio M. Menti** Methodology, Data acquisition, Writing - review & editing **Aram Megighian** Conceptualization, Methodology, Writing - review & editing **Mauro A. Zordan** Conceptualization, Methodology, Software, Writing - review & editing

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Competing interests the authors declare no conflict of interests

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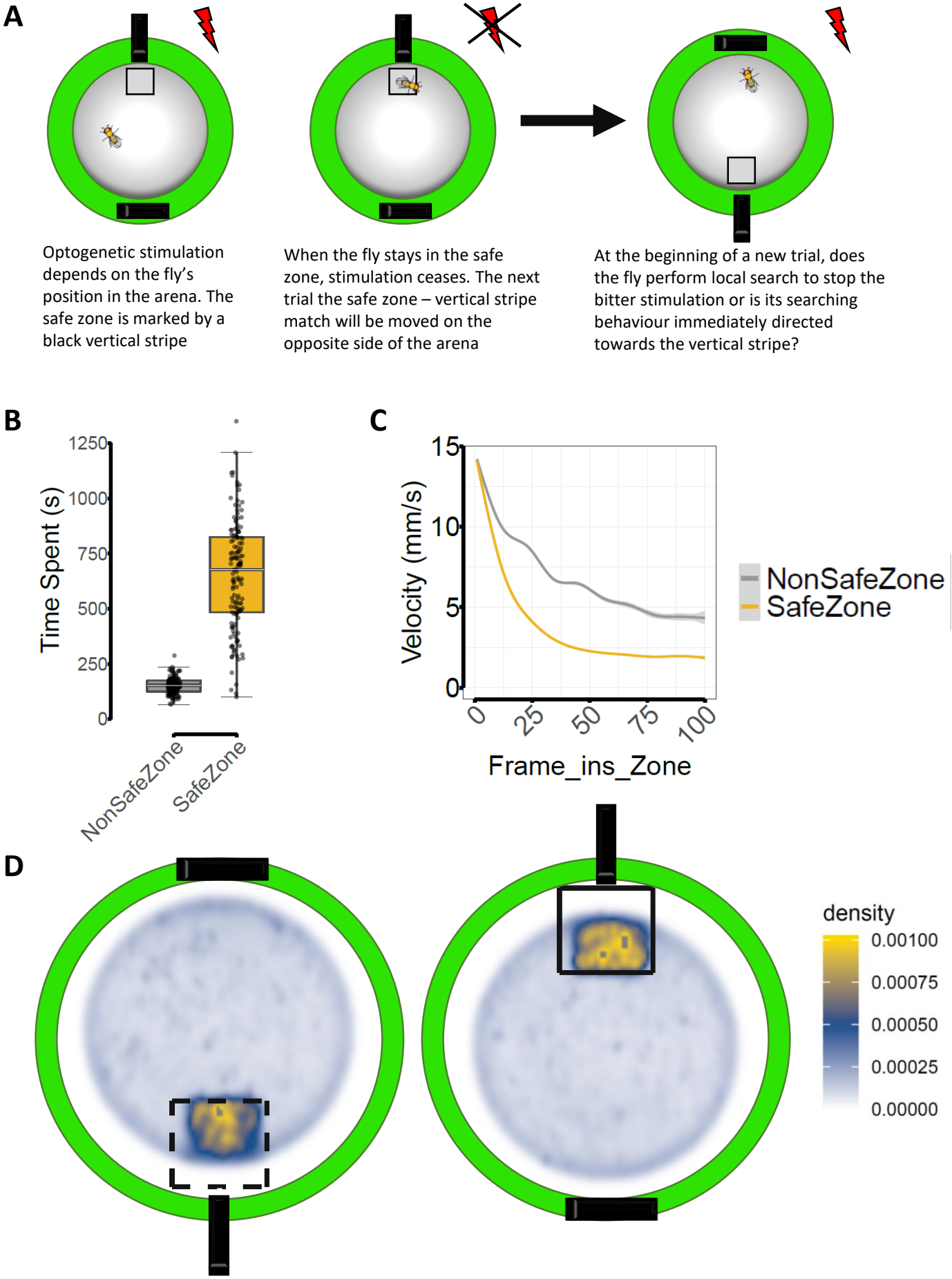
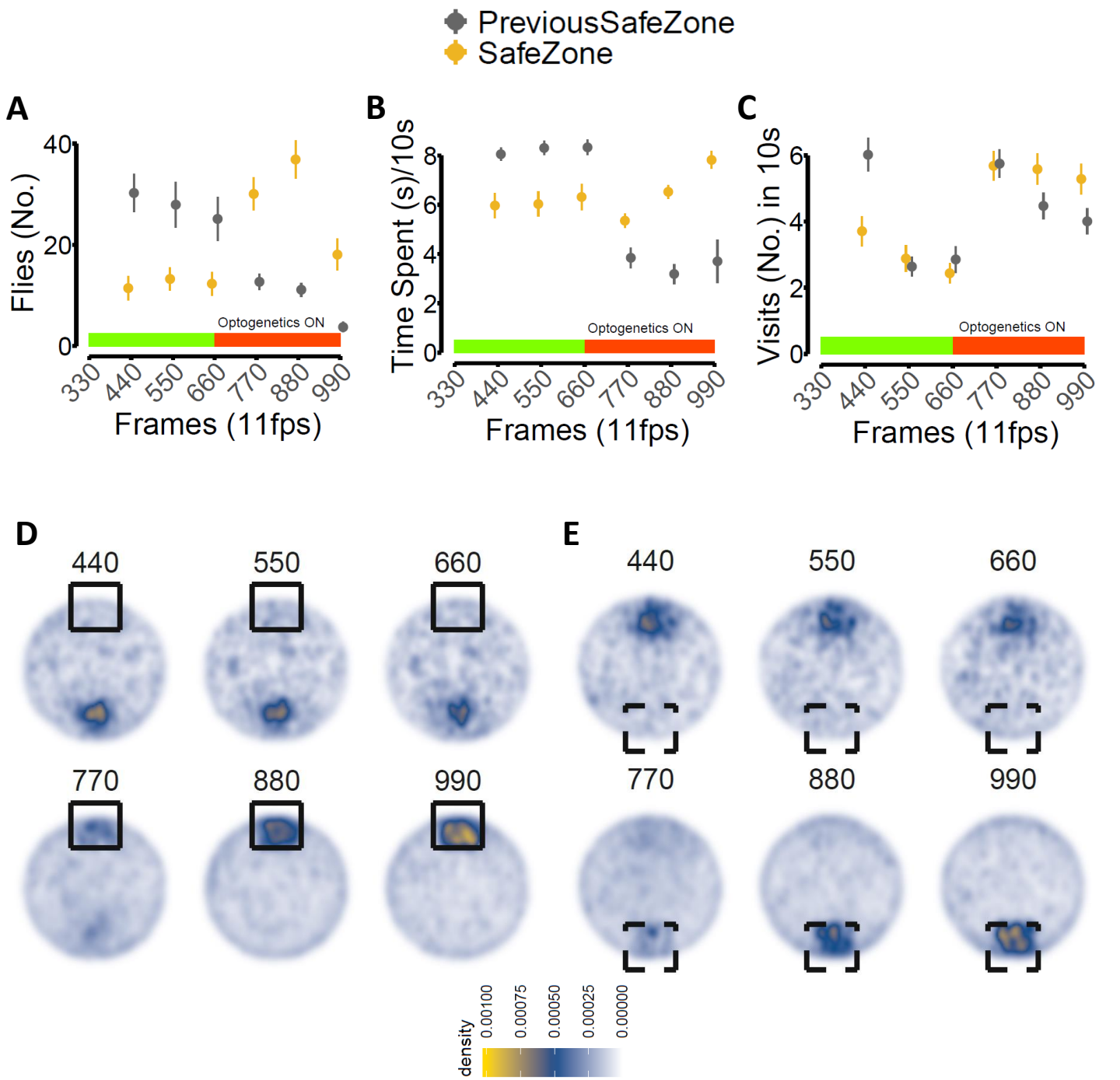
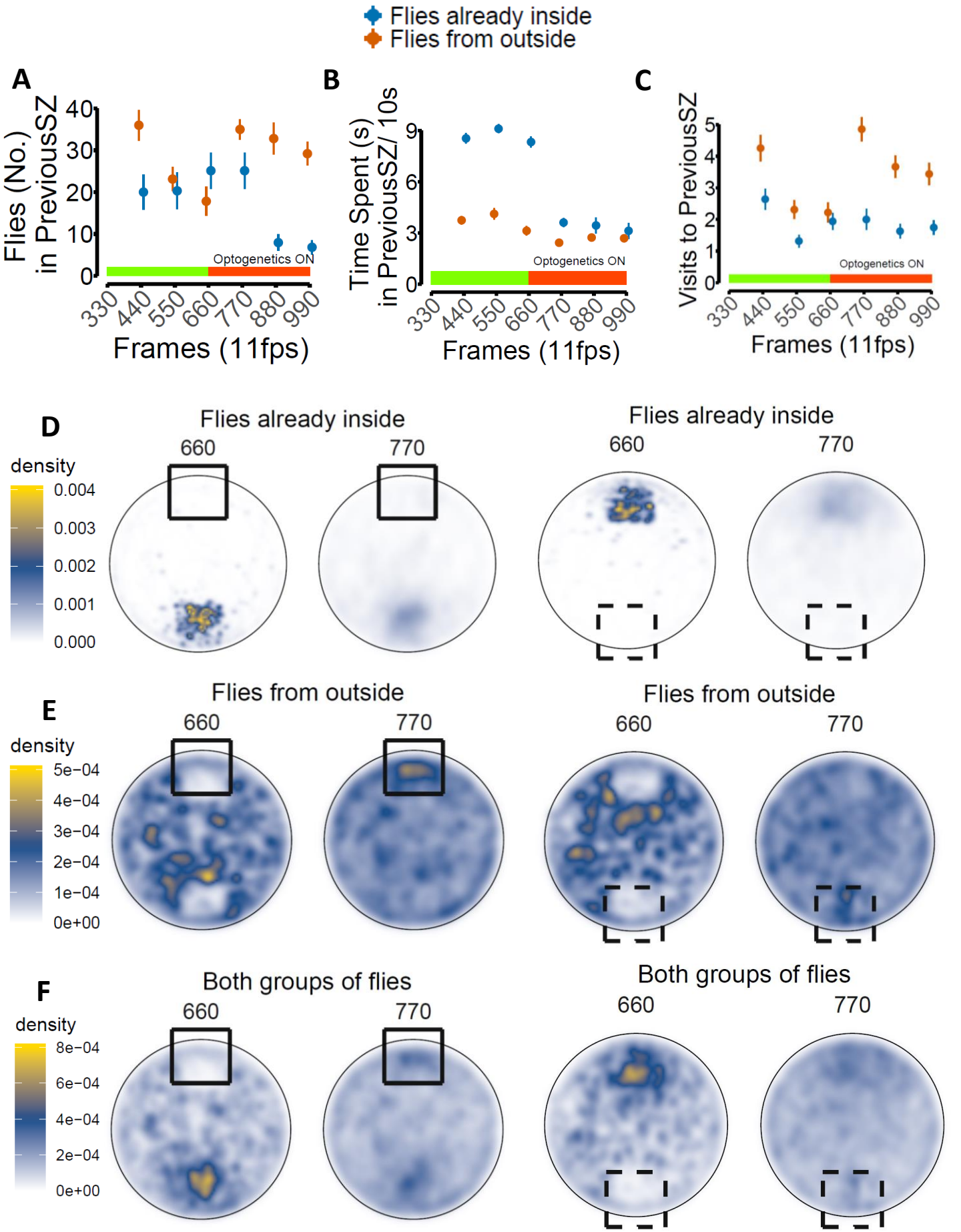
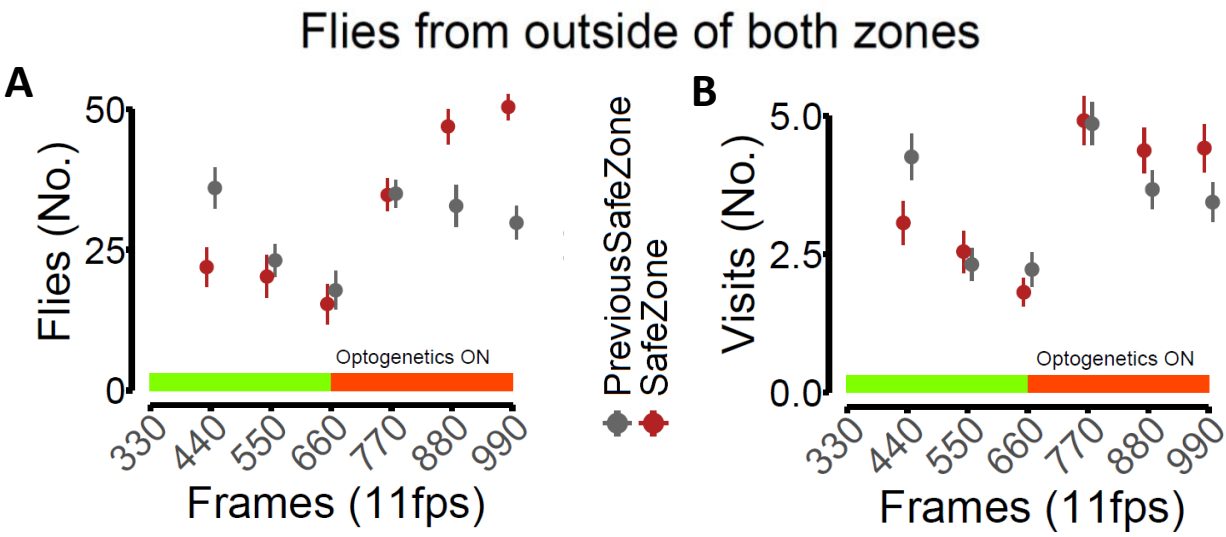
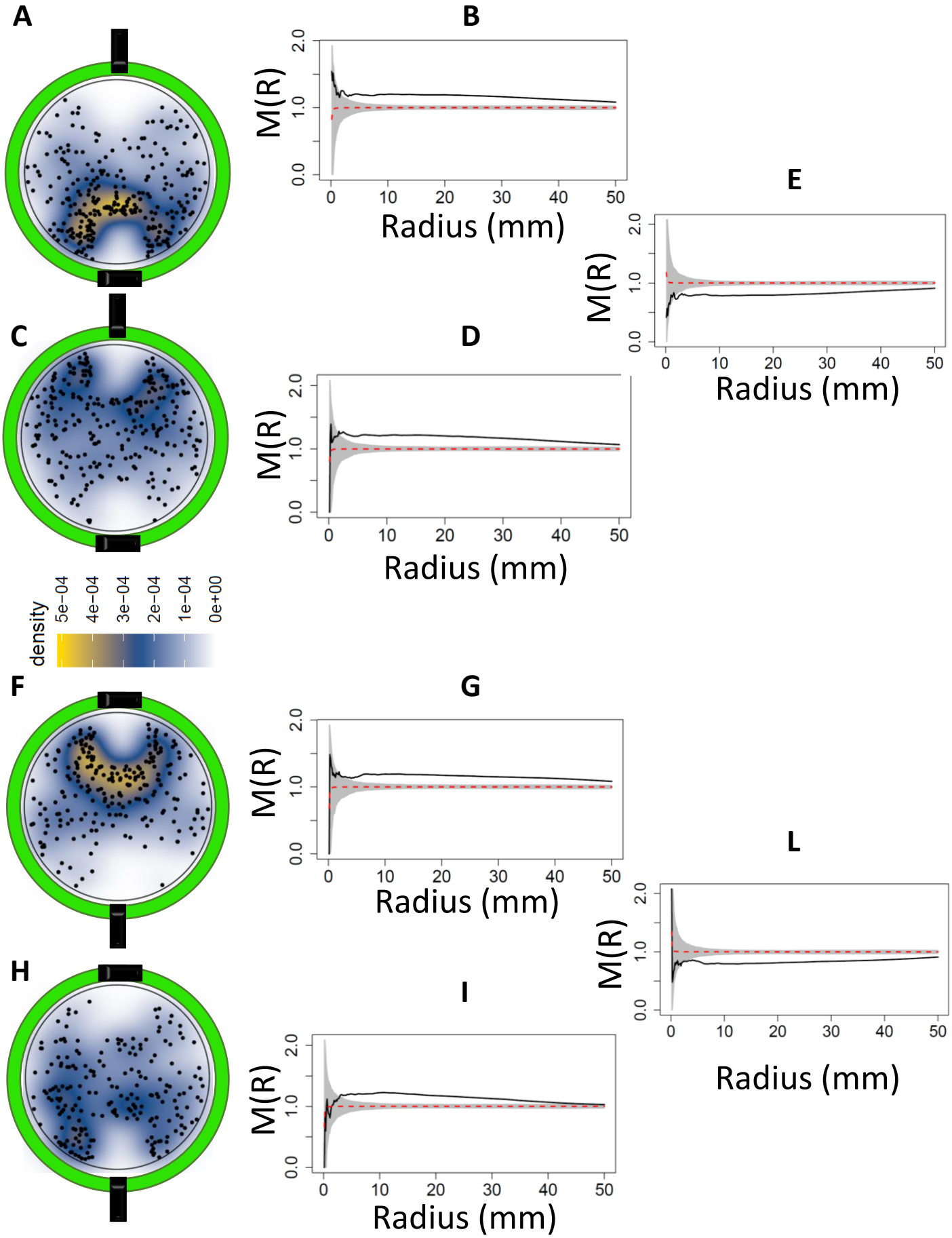

A
Optogenetic stimulation depends on the fly's position in the arena. The safe zone is marked by a black vertical stripe
When the fly stays in the safe zone, stimulation ceases. The next trial the safe zone – vertical stripe match will be moved on the opposite side of the arena
At the beginning of a new trial, does the fly perform local search to stop the bitter stimulation or is its searching behaviour immediately directed towards the vertical stripe?
B
Time Spent (s)
1250
1000
750
500
250
0
NonSafeZone
SafeZone
C
Velocity (mm/s)
15
10
5
0
0
25
50
75
100
Frame_ins_Zone
NonSafeZone
SafeZone
D
density
0.00100
0.00075
0.00050
0.00025
0.00000


PreviousSafeZone
SafeZone
A
Flies (No.)
40
20
0
Optogenetics ON
330
440
550
660
770
880
990
Frames (11fps)
B
Time Spent (s)/10s
8
6
4
2
0
Optogenetics ON
330
440
550
660
770
880
990
Frames (11fps)
C
Visits (No.) in 10s
6
4
2
0
Optogenetics ON
330
440
550
660
770
880
990
Frames (11fps)
D
440
550
660
770
880
990
E
440
550
660
770
880
990
density
0.00100
0.00075
0.00050
0.00025
0.00000


Flies already inside
Flies from outside
A
Flies (No.) in PreviousSZ
Optogenetics ON
Frames (11fps)
B
Time Spent (s) in PreviousSZ/ 10s
Optogenetics ON
Frames (11fps)
C
Visits to PreviousSZ
Optogenetics ON
Frames (11fps)
D
Flies already inside
660
770
density
0.004
0.003
0.002
0.001
0.000
E
Flies from outside
660
770
density
5e−04
4e−04
3e−04
2e−04
1e−04
0e+00
F
Both groups of flies
660
770
density
8e−04
6e−04
4e−04
2e−04
0e+00


Flies from outside of both zones
A
Flies (No.)
50
25
0
Optogenetics ON
330
440
550
660
770
880
990
Frames (11fps)
PreviousSafeZone
SafeZone
B
Visits (No.)
5.0
2.5
0.0
Optogenetics ON
330
440
550
660
770
880
990
Frames (11fps)


A
B
C
D
E
F
G
H
I
L
M(R)
Radius (mm)
density
5e−04
4e−04
3e−04
2e−04
1e−04
0e+00