Title: Voluntary passive movement in flies is play-like behavior

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Abstract: Play-like behaviour (PLB) is pervasive across the animal kingdom, especially in vertebrate species. Invertebrate PLB has been sparsely addressed and those studies have been restricted to social or object interaction. Here, we examined individual PLB in the vinegar fly *Drosophila melanogaster* by providing voluntary access to a spinning platform – a carousel. We demonstrate that the flies' idiosyncratic carousel interactions qualify as play-like behaviour: while some flies show spontaneous avoidance, others exhibit active stimulus seeking, engaging in repeated, prolonged visits. We propose that this self-exposure to external forces achieves intentional exafferent stimulation, which provides an efficient way to improve self-perception via internal model training and can shape multisensory integration.

One-Sentence Summary: Vinegar flies seek self-stimulation on a carousel.

Main Text:

Anecdotal accounts about animals repeatedly exposing themselves to sources of passive movement by engaging with swings, slides, or carousels are frequently shared on social media. Generally, such behavior is assumed to be 'play', which, according to primatologist Phyllis Dolhinow, is *"(...) hard to define, but I know it when I see it (...)*" (*1*). More reliable criteria for play-like behavior (PLB) require the activity to be 1) of no immediate relevance for survival, but also 2) voluntary, intentional, rewarding, 3) non-ethotypical, 4) repeated, but not stereotyped, and 5) free from stress (*2*, *3*). PLB following these rules has been found across the vertebrate subphylum (*3*); a recent study in rats even managed to identify involved brain regions (*4*). In invertebrates, the sparse reports so far addressed either social play in parasitoid wasps or spiders (*5*, *6*) or object play in bumblebees (*7*). Contrary to the former PLB categories, a convincing hypothesis of the adaptive value of voluntary passive movement PLB is lacking so far. Flies are highly sensitive to the direction of gravitational pull (*8*), hence such intentional passive motion would be sufficient to externally induce proprioceptive stimulation (*9*). Here, we identify voluntary spinning on a carousel as idiosyncratic PLB in the vinegar fly *Drosophila melanogaster*, and propose how this behavior represents a highly efficient way to train internal body models.

Results

We developed a behavioral setup to study voluntary passive PLB in flies, which consisted of an 'enriched environment', with access to food/water and a constantly spinning platform (a carousel, Fig. 1A). We exposed single flies to this experimental environment (ON) for several days and compared their behavior to that of flies exposed to the same arena with a stationary carousel (OFF). We restricted this study to male flies, given that they exhibit higher diurnal locomotor activity peaks (*10*, *11*) and more conserved sleeping pattern (*12*), which mostly seemed to occur on the food patch (Fig. 1B, C). Experiments lasted up to two weeks (3-4 days on average) without any further human interference, to provide a stress-free environment. For data comparability, and to allow for the novelty response to the unfamiliar environment to subside, we restricted the analysis to the first two complete consecutive days and nights. Both spinning platform and food spot were levelled with the rest of the arena to avoid negative geotaxis location preferences (*8*, *13*). Flies are capable of landmark and place learning (*14*) and can avoid unpleasant positions (*15*). Since we provide a high contrast landmark pattern around the arena, visits to the carousel's position only depend on intrinsic motivational drive. Hence this setup is different from forced passive movement, which evokes stress- and even depression-like symptoms in flies (*16*).

After an initial period of increased arena boundary exploration, both OFF control flies and ON experimental flies exhibited pronounced idiosyncratic place preference and locomotion, which followed overall diurnal activity (Fig. 1B, B', C, C'). As reported for idiosyncratic behavior (*17–22*), these individual patterns were conserved across days. Initial boundary exploration and diurnal activity patterns disappeared in both OFF and ON groups after randomly shuffling the order of individual region occupancies, which favors the interpretation that these observed patterns in the experimental data are not an artifact (Fig. S1). Temperature and humidity had a weak significant impact on general fly activity (Fig. S2) as reported previously (*23*), but not on place choice (Fig. S3).

A crucial criterium of PLB is its voluntariness; here, ON flies were always able to access and leave the moving platform on their own volition. Interestingly, the overall length of time flies spent on the carousel was significantly higher when the carousel was spinning compared to when it was not (Fig 1B, C, D). The duration of the average carousel visit also increased significantly, with a

massive increase in variance (Fig. 1E). Some ON flies chose to avoid the platform or spent large timespans immediately in front of it. Flies are known to respond to an unexpected, potentially threatening cue with evasive responses (*24–26*). This might explain carousel avoidance, but not voluntary, repeated exposure.

In order to further understand the idiosyncratic interactions the flies exhibited with the moving platform, we selected the ON flies with the highest probability of spending time on the carousel (>5% of time, 'seeker'), and those with the lowest probability of spending time on the carousel (up to 0.5% of time, 'avoider'). The environmental occupancy of these groups of ON flies differed substantially from each other and from control OFF flies (Fig. 2A-C). All groups spent most time on the food, but while OFF control flies showed considerable border exploration and no obvious differentiation of the carousel ROI, 'seeker' and 'avoider' were more drawn to the carousel and the food, respectively. We next explored how often the flies in these groups transition from the carousel, in relation to the time spent on the carousel ROI (Fig. 2A'-C'). As expected, seeker flies exhibited more and longer carousel visits compared to controls, whereas avoider flies showed few carousel visits of shorter durations. Interestingly, we observed differences in behavioral choices following carousel visits (Fig. 2A''-C''). We assessed which regions of interest (ROI) were frequented within 100 s after defined lengths of carousel visits. For the OFF control condition, ROI visit probabilities were roughly aligned with relative ROI size, independent of preceding disk visit durations. Carousel-seeking ON flies however kept returning to the moving platform at increasing rates (Fig. 2B''). In contrast, flies that mostly avoided the moving platform did so consistently after their few visits; instead they developed a subsequent preference for the border region (Fig. 2C''). Both OFF control and seeker flies showed an increasing preference for food visits the longer they stayed on the carousel ROI, similar to previous reports (*27*, *28*). Hunger drive is in antagonistic competition with other motivational drives like the positively evaluated possibility to mate (*25*, *29*), whereas thigmotactic border explorations are associated with fearful behavior in open field tests in flies, mice and humans (*30–32*). We therefore conclude that the interaction with a carousel enhances behavioral contrast between groups, suggesting idiosyncratic positive or negative association with this stimulus.

Finally, we addressed whether flies develop a positive association with voluntary passive movement. To do so we employed a spatial alteration paradigm with two carousels, spinning alternatingly for 5 minutes (Fig. 3A). We hypothesized that if flies positively value the exposure to voluntary passive movement they are more likely to remain on the moving carousel until it stops. Once again, we ranked flies according to their disk occupancy and found that the 33 flies that spent the most time on the moving disks remained significantly more on the disk until it became stationary as compared to transitioning from a stationary to a moving disk (Fig. S4). In line with our previous results, this suggests that the carousel movement was not perceived as aversive. This did not yet however allow us rule out that the flies felt trapped once they were on a moving disk, induced by the sensory experience of the visual flow or the centripetal force. Although the frequent revisits in the single carousel experiments (Fig. 2B'') render this possibility unlikely, we further hypothesized that if flies seek the experience of a moving carousel, they would display sequences of alternating ON carousel visits in the double carousel experiments. Such behavior would imply not only the active decision to go on a moving object, but to actively and repeatedly follow exposure to that stimulation. Indeed, such alternated sequences 'tracking' the ON carousel occurred at a significantly different rate to transitions between stationary carousels in an idiosyncratic fashion (Fig. S5); we even observed repetitive switching (Fig. 3B), strongly suggesting that the moving platform confers a situation-dependent positive value for individual flies - a key criterium of PLB.

Discussion

In this study we established a behavioral paradigm with a carousel as a means of studying voluntary passive movement, simulating a more natural setting of voluntary passive movement like sitting on swinging branches or leaves. Our data show that the flies' active exposure to the carousel fulfils all criteria of play-like behavior (PLB): it is of no immediate relevance for survival, the flies engage with the carousel voluntarily, repeatedly, in a stress-free environment and in a non-ethotypical manner, and the seeking behavior strongly implies a rewarding component of intentional visits. The novel identification of PLB in this insect now allows for further analysis of the neuronal underpinnings of this curious behavior, utilizing the full genetic arsenal available in the fly.

But what could this PLB be good for, if for anything at all? On the one hand, we cannot completely rule out that this paradigm is too far removed from any natural situation and somewhat artifactual. On the other hand, some flies could seek the environmental enrichment and/or the sensory stimulation like individuals of many other species, which would allow for activity-dependent circuit refinement and would counter the negative effects of sensory deprivation (*33*). Below we will lay out how voluntary passive movement PLB is particularly suited to refine neuronal circuits for improved proprioceptive acuity and self representation in – at least - any bilaterian (*34*).

Our hypothesis is grounded on what sets active and passive movement apart: active movement involves i) an internal model representation of a predicted outcome of a planned action, which then in turn leads to ii) an efferent motor command. A corollary discharge represents a feedback loop of the internal model representation which is located further upstream of the action chain, while an efference copy represents a feedback loop of the efferent motor command (*35*). These feedback loops have been identified at multiple levels throughout the *Drosophila* larval connectome (*36*). The efference copy allows the organism to detect any mismatch between efferent motor command and executed motion - it cancels the proprioceptive reafference that was evoked by any active movement. Thus, only afferent signals evoked by external forces remain, like those created by passive movement. This sensory mismatch, or exafference, is then reported back to higher brain centers, where the exafferent signal is used together with the corollary discharge of the predicted motor outcome to update the internal model (*35*). Passive movement therefore creates a highly efficient exafferent signal due to the lack of an efference copy, which can lead to nausea (i.e. sea sickness) or depression-like states if forced upon the organism (*16*). If such passive movement is voluntary however, as in the PLB described here, the internal model of the planned exposure to passive motion will create a corollary discharge. This discharge predicts the exafferent passive motion signal and can direct the incoming activity into efficient refinement of internal models of self-perception and, as derivative, lead to improved vision compensation and generally improved sensorimotor control and intersensory coordination (*37–43*).

'Gamifying', and hence rewarding this training of internal models as in PLB might be one way to motivate organisms to engage in such tasks that improve self-perception. Value updates in the brain linked to rewarding associations are enabled by dopaminergic signaling; in Drosophila larvae, prominent corollary discharge onto dopaminergic neurons is actually functionally relevant in associative learning (*44*). Similarly, locomotion-sensitive dopaminergic neurons are involved in lateral line reafference and efference copy cancellation in zebrafish (*45*). This points to a common neuronal motif across organisms to utilize intentional exafferent proprioceptive signals like the described PLB here for dopamine-dependent self representation updates.

Ultimately, a realistic representation of one's own body (an embodied self) is a prerequisite for any organism to efficiently and intentionally interact with the world (*34*), and, unsurprisingly, somatosensory receptors are highly conserved across animal orders (*46*). Bumblebees fly sideways through narrow gaps if their wingspan wouldn't fit through otherwise (*47*), and flies can estimate their reach when climbing across gaps (*48*). Freshly hatched flies need to establish a realistic perception of their body by visually synchronizing their perceived body image with their actual size (*49*). This happens by integrating parallax motion information with step length, and leads to a permanent body size percept. Although there's no specific imprinting time window, once-established body size memory is extremely long-lasting and cannot be updated (*49*). Another example for exploiting mismatched body perception was demonstrated in adult foraging ants, where changing leg length (and hence step size) led to over- or underestimation of travelled distance (*50*). Similarly, a mismatch between landmark orientation and locomotor feedback leads to overcompensation of the internal heading direction representation in the ellipsoid body of the fly central complex (*39*). This is different from other body changes or modifications that can occur naturally: a hemimetabolous insect needs to relearn its size after molting (*51*), recovery of locomotion after injury relies on proprioceptive signaling (*52*), and impaired ability to fly due to damaged or glued wings will lead to immediate phototactic consequences (*53*). Similarly, hermit crabs adjust their movement in tight spaces to their embodied extended shell including modifications thereof (*54*). Interestingly, in humans, dissociative disorders like schizotypal personality disorder are associated with embodiment issues; positive schizotypal traits scale with a reduced ability to predict and process self-generated touch (*55*), and therefore problems with corollary discharge are thought to be linked to schizophrenia (*56*). Voluntary passive movement PLB might provide a useful approach to aide re-establishment of a robust body self representation.

We conclude that fly behavior exhibits insightful parallels to vertebrate action selection, which, in combination with previously demonstrated parallels in reward association (*57*) further supports the notion to pursue fundamental principles in neural circuit function using this accessible organism (*56*). The sustained boost in connectomic data availability for both the whole brain (*58*, *59*) and premotor circuits of the fly (*60–62*) will allow us to pinpoint these computational principles to identified cellular components (*63*), and to assess how internal models are neuronally implemented (*64*).

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Competing interests: Authors declare that they have no competing interests.

Data and materials availability: All data are available in the main text or the supplementary materials.

Supplementary Materials

Materials and Methods

Animals

CantonS animals ranged between 0 and 7 days in age. Flies were reared at 20-25°C and kept on standard fly food right before transfer into carousel arenas. Temperature and humidity were noted at the beginning of all experiments. Animal tracking lasted up to 346 hours or 2 weeks; most experiments were stopped after 3-4 days.

Carousel layout

The setup consists of a 10 cm diameter arena, an off-centered 4 cm diameter spinning platform and an off-centered circular food spot (1 cm diameter, Fig. 1). Both spinning platform and food spot were levelled with the rest of the arena to avoid negative geotaxis location preferences (*13*). The arena was covered by a Sigmacote-coated (Sigma-Aldrich SL2) glass dome to deter roof walking and lit from above by 12 white or 12 infrared (800 nm) LEDs in a 16:8 LD rhythm. To provide visual landmarks, the outer walls were covered by randomly patterned black/white squares.

Raspberry Pi recordings

A custom-written Python script automatically recorded batches of 30 min long videos with a resolution of 768x768 pixels at 5 fps as .h264 files, starting at the next half hour until manually interrupted. Lighting automatically changes to infrared LED light at 22:00 and back to white LED light at 6:00. Metadata (humidity, temperature, genotype, age, sex, food, date, time, additional information) is written into a .csv or .json datafile at the start of the experiment.

Data conversion and preparation

Raw .h264 files are copied from Raspberry Pis onto an analysis PC with a custom-written program that automatically converts .h264 files into .mp4 files.

Tracking

The fly position is assessed using DeepLabCut (*65*), which was manually customized to allow for batch processing. The network was trained on 10 frames from 40 flies each for 1,030,000 iterations, after marking the anterior and posterior end of the fly (or its center of gravity in later DeepLabCut versions) in each of these frames.

MATLAB analysis

Tracking data in the form of .h5 files and corresponding metadata are batch-loaded into MATLAB using a custom-written software. All subsequent analyses are performed using an expanded and improved version of code already described before (*28*). Only experiments with less than 5% undetected fly positions/frame (NaNs) were used in the analysis (306 of 349). To consolidate data consistency, we restricted further analysis to day/night 2 and 3, after initial boundary exploration settled (303 expts with less than 5% NaNs). Some analyses required a more rigid threshold for NaNs (88 expts with less than 0.005% NaNs). All MATLAB code is available online on Github (https://github.com/triphant/DrosoCarousel).

Figure 1

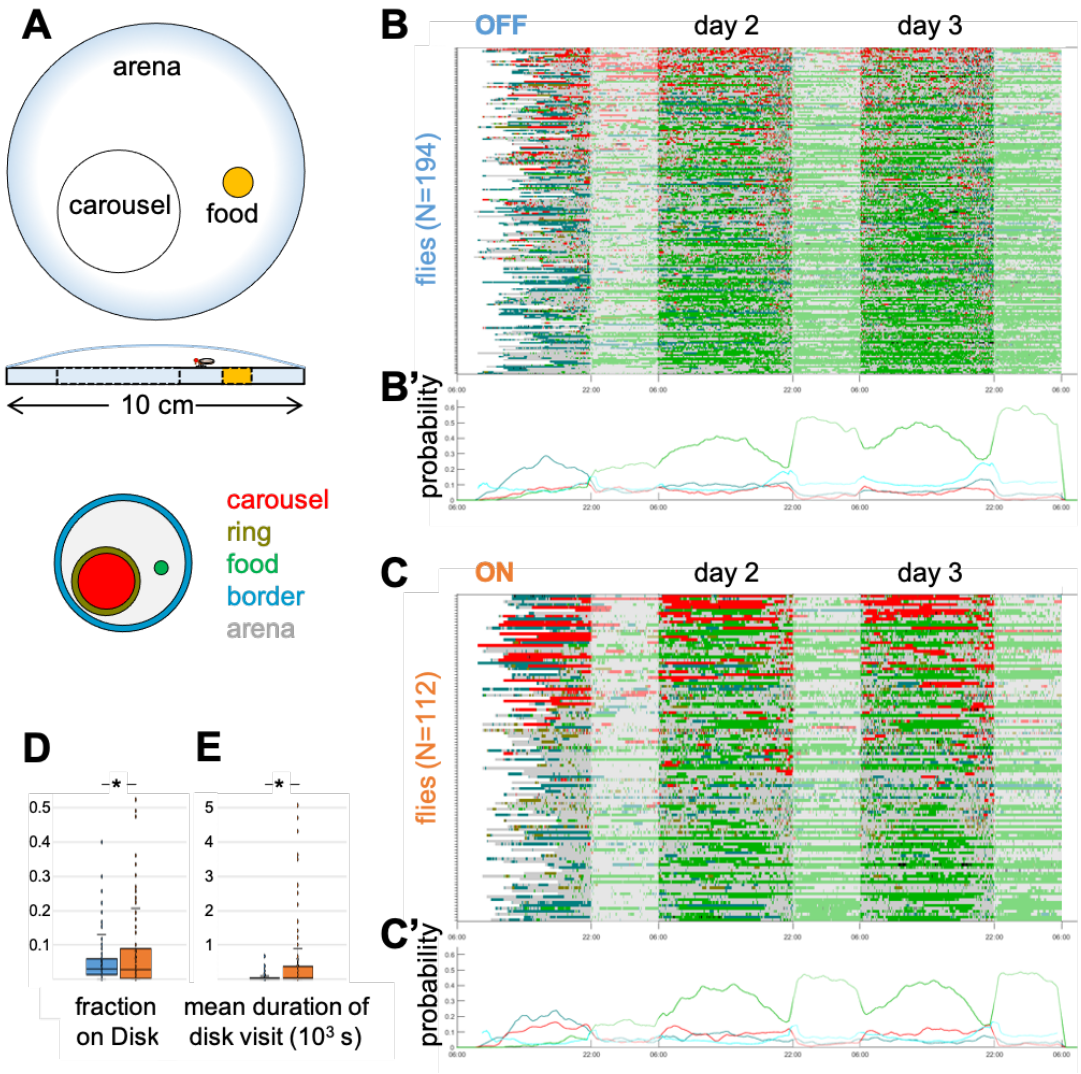

Fig. 1: Carousel setup and idiosyncratic interaction

A The setup arena (10 cm diameter) contains a continuously spinning platform (4 cm diameter, 360°/s) and a food spot (1 cm diameter). The arena is covered by a Sigmacote-coated glass dome, a 16:8 LD rhythm is provided by IR and white light LEDs. Regions of interest (carousel, ring, food, border, arena) are defined for further analysis. **B, C** Experimental ethograms and **B', C'** occupancy probability graphs (rolling window smoothing with 18.000 frames) over three nights (shaded) and two consecutive days of control flies (carousel OFF) and experimental flies (carousel ON). Elevated border exploration occurs at the beginning in both groups (teal). Circadian behavior settles from the second day onwards, e.g. increased food visits at night and during mid-day (green) and rhythmic activity (cyan). The 'ring' ROI was omitted from occupancy probability graphs for clarity reasons. Carousel occupancies in experimental flies are more biased towards either less fragmented and prolonged visits or avoidance, mostly during day periods (red). **D** ON flies spend significantly more time on the carousel than OFF controls. Two-tailed t-test, $p < 0{,}01$. **E** Individual mean carousel visits are significantly longer in ON flies as compared to OFF controls, with a massive increase in variance ($s^2_{OFF} = 1{,}8 \times 10^5$, $s^2_{ON} = 2{,}7 \times 10^7$). Two-tailed t-test, $p < 0{,}001$. Data in D & E originates from clipped 48 h, beginning with day 2.

Figure 2

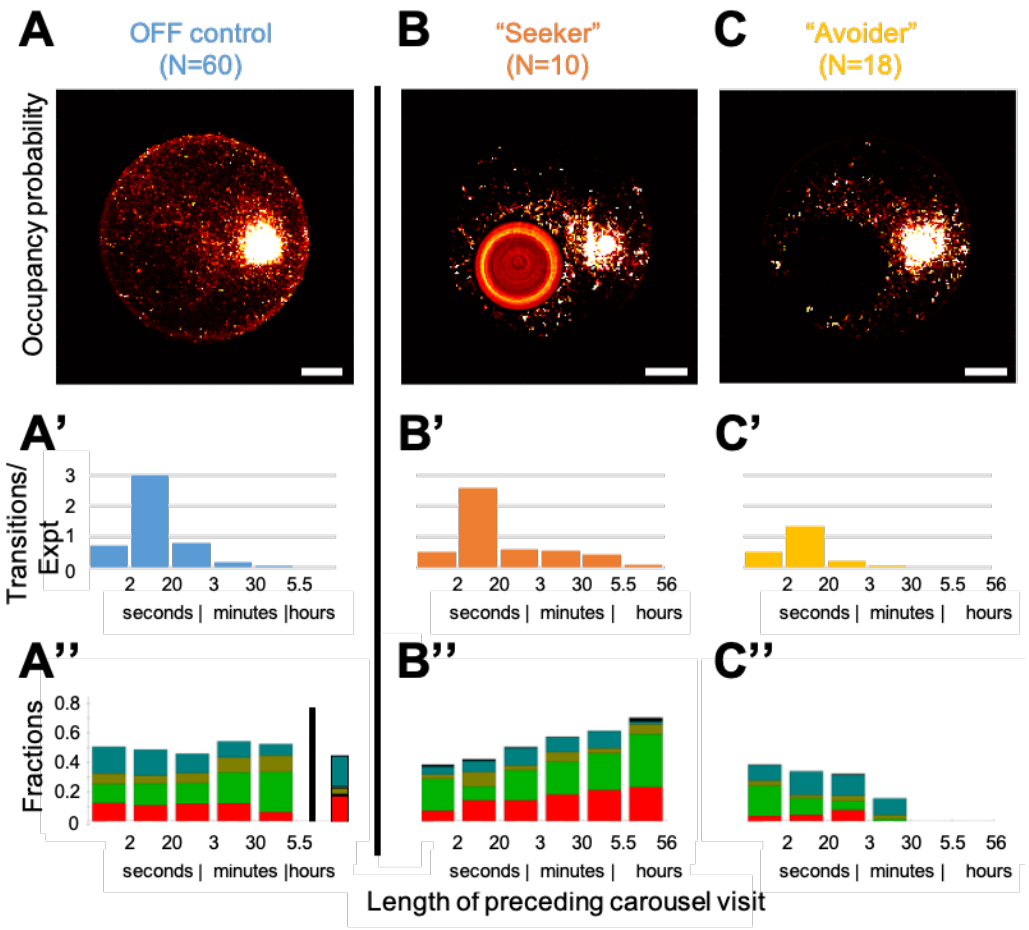

Fig. 2: Carousel visits have divergent effects on subsequent behavior in idiosyncratically sorted fly groups

A Occupancy preferences in OFF control flies reveal preferred visits to the food and border region, while the stationary carousel remains inconspicuous in comparison to the remaining arena. Scale bar in A-C: 2 cm. **A'** OFF control flies rarely spent more than 3 minutes on the carousel ROI. **A''** Stationary carousel stays of varying lengths have no major influence on subsequent arena region visits, except for an increasing preference for food visits. The leftmost stacked bar represents ROI fractions according to area size for reference (disk, red; food, green; ring, olive; border, teal). **B-B''** Carousel-seeking flies exhibit an increasing probability of recurring carousel visits, dependent on length of stay, mostly at the expense of visits to the remaining arena. **C-C''** Carousel-avoiding flies show the highest food occupancy. They rarely return to the carousel after accidental < 30 min visits, which are followed by increased border region visits. All N are selected for <0,005% unidentified positions/frame (NaNs, black fraction in B'').

Figure 3

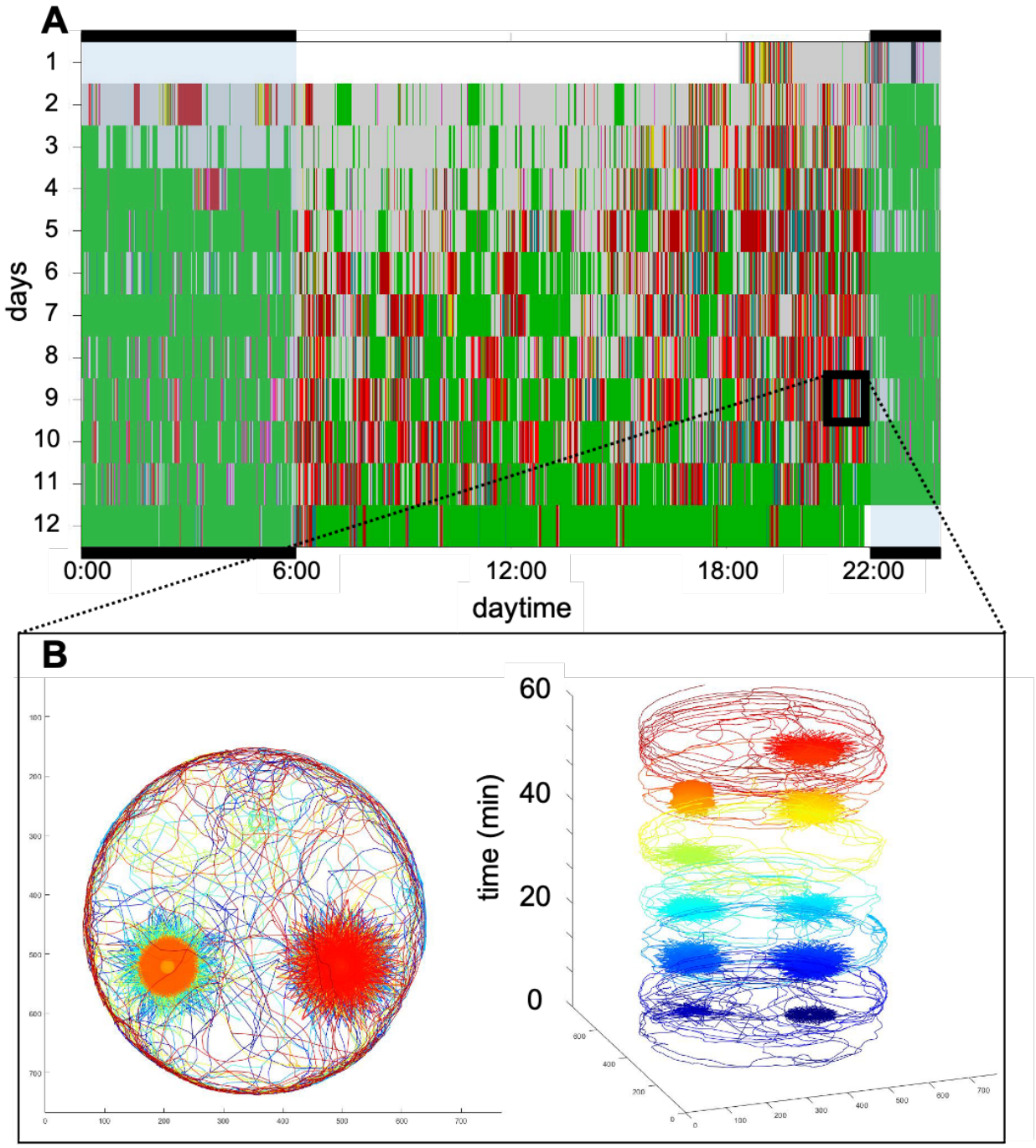

Fig. 3: Flies seek spinning carousels

A Representative experimental ethogram for a single flies' behavior over 12 days in a double carousel setup. Carousel visits preferentially occur during the evening activity peak and show no obvious pattern across days. **B** Walking traces during 1 h of the evening activity peak of day 9 reveal repeated, alternating visits to the respective moving carousel.