Disinhibition of a recurrent attractor gates a persistent goal signal for navigation

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Abstract

Recurrent attractor networks are widely thought to form the basis of working memory[1-3], but how stable attractor activity can be rapidly switched on and off is unclear[4-7]. Here we investigate how stability and rapid switching can be combined in a discrete recurrent circuit of the fly navigation center[8]. hΔK and PFG neurons are recurrently connected in a ring structure. Using *in vivo* imaging, we find that these two populations exhibit shared persistent bump activity that turns on with odor and terminates at the end of a goal-directed upwind run. Using whole-cell recordings, we show that persistence in hΔK depends on recurrent signalling, and that hΔK receives slow recurrent excitation and fast inhibition from its synaptic partners. Computational modeling reveals that this combination of slow excitation with fast inhibition yields stable and tuneable persistent attractor dynamics. Next we examine the mechanisms that allow this activity bump to be rapidly turned on and off. We find that while the PFG bump tracks heading continuously, the hΔK bump is suppressed during turns, and only becomes active during straight goal-directed runs. We can reproduce these dynamics in our model by using inhibition to dynamically uncouple activity in hΔK from PFG. When hΔK is inhibited, PFG neurons follow their inputs from the compass system; when hΔK is disinhibited, recurrent interactions lock this input into place, forming a heading memory. Consistent with this model, we find that inhibitory inputs onto hΔK increase during turns and are suppressed during odor input and goal-directed upwind runs. Our work reveals how disinhibition can serve as a gate to rapidly write an ongoing measurement to a recurrent memory circuit.

Introduction

Distributed networks of recurrently connected neurons are widely thought to form the substrate for short-term working memory[1-3]. During working memory tasks, neurons in the prefrontal cortex and elsewhere exhibit persistent activity that turns on with cue presentation and terminates with behavior[9-10]. The duration of persistence in these neurons is much longer than their intrinsic membrane time constants, arguing that persistence arises from network interactions[3]. Computational models that feature local recurrent excitation and global inhibition can recreate the types of persistent activity observed experimentally[1,11] (Fig. 1a). However, such networks exhibit a fundamental trade-off between stability and flexibility[1]. Networks configured to generate stable attractor dynamics are difficult to shut off once activated[5].

Computational models therefore often implement gates to control the timing of attractor dynamics[4,6,7,12]. How such gates might be implemented at a circuit and synaptic level is unclear.

The *Drosophila* brain offers a unique opportunity to study the function of recurrent circuits at a cellular level[13-15]. Connectomics describes the architecture of fly networks with synaptic and cellular precision[8,16,17], while precise genetic tools enable functional interrogation of the same networks[18]. The fan-shaped body (FB) is a highly recurrent part of the fly navigation center called the Central Complex, thought to compute and encode allocentric goals[8,19-25]. The FB contains ~30 types of local neurons that interact through complex patterns of feedforward and recurrent connectivity[8]. Several FB local neuron types have been shown to encode goal direction signals for navigation[22-24,26-28]. Outputs of the fan-shaped body compare these goal signals to an internal compass[29] to drive egocentric steering[19,24,25]. How goal signals in local neurons are dynamically regulated to generate moment-to-moment changes in behavior is not clear. The function of recurrent connections between local neuron types also remains unexplored.

Here we investigate how stable activity and rapid switching are combined in a discrete recurrent microcircuit within the FB. When flies navigate towards food, brief encounters with odor drive persistent goal-directed runs biased in the upwind direction[26,30]. In a previous study[26], we showed that a group of FB local neurons called hΔK exhibit a bump of activity that turns on with odor and persists while the fly maintains a stable heading after odor loss. Sparse activation of hΔK neurons caused flies to walk in repeatable directions relative to wind[22], while silencing led to less persistent trajectories after odor loss[26]. Together these data support the idea that bump activity in hΔK neurons represents a persistent goal signal for navigation.

hΔK neurons are tightly recurrently connected with a second group of FB local neurons, called PFGs, in a ring structure. This network receives spatially-structured inputs from the ellipsoid body compass, known to represent heading[13,29], and global inputs from several types of broadly-projecting tangential neurons (Fig 1b). The architecture of this circuit is highly reminiscent of classical models of working memory circuits[1] (Fig. 1a), and suggests that it might function to store heading representations over time. We therefore hypothesized that persistent activity in hΔK might arise from recurrent connections between hΔK and PFG. Consistent with this hypothesis we find that hΔK and PFG show similar persistent bump activity that is activated by odor and correlates with persistent goal-directed runs. We also show that persistent activity in hΔK can be driven by synaptic input but not current injection, and that it requires recurrent signaling from hΔK. We identify inhibitory tangential neurons that could provide the global inhibitory signals necessary for working memory circuit function, and show that excitation and inhibition onto hΔK have distinct dynamics that promote more stable and tuneable persistent attractor states.

Finally, we examine the mechanisms that allow hΔK activity to be rapidly turned on by odor and off when the fly turns. Surprisingly, we observe that even though hΔK and PFG are tightly anatomically coupled, they only show similar activity during goal directed runs, and their activity diverges during turning and rest. We show in our model that we can reproduce these dynamics by using feedforward inhibition to dynamically decouple hΔK from PFG in a stimulus and behavior-dependent manner. Imaging from inhibitory inputs onto hΔK supports this model,

showing that hΔK neurons are inhibited during turning and disinhibited during odor and goal-directed runs. Our data and models reveal a circuit motif— disinhibition of a recurrent half-center— that allows stable attractor dynamics to be rapidly switched on and off by either sensory input or ongoing behavior. We propose that this motif may represent a general solution to the problem of combining stability and flexibility in recurrent circuits.

Recurrently connected hΔK and PFG neurons show similar odor-evoked and goal-associated persistent activity

hΔK and PFG neurons are strongly and reciprocally connected in a ring structure with highly correlated forward and reverse connections (Fig. 1a; Ext Data Fig 1a,b). Reciprocal connections between hΔK and PFG are the most numerous of any recurrent connection in the Central Complex (Ext Data Fig 1c). To test the hypothesis that these recurrent connections contribute to persistent bump activity previously observed in a broad hΔK line[26] (VT062617-Gal4), we imaged from each population using cell-type specific split-Gal4 lines[18]. We recorded activity during closed-loop odor- and wind-guided navigation[26] (Fig 1c). In this paradigm, a tethered fly walks on an air-supported ball that controls the orientation of a wind stimulus in closed-loop. During each 50 second wind trial, we presented odor (0.5% apple cider vinegar) in open-loop as either a 15 s step, or as 1-10 500ms pulses at 1Hz. In response to odor, flies selected a heading trajectory that was biased upwind (Ext Data Fig. 1d-f) and persisted for several seconds after odor offset (Ext Data Fig. 1e,f).

We observed that both hΔK and PFG neurons showed a localized bump of activity that was preferentially activated during odor (Fig. 1d-g, Ext Data Fig. 1g,h) and persisted after odor offset while the fly maintained its trajectory (Fig. 1h-m). In both populations, the amplitude of bump activity grew similarly with pulse number (Ext Data Fig. 1g,h). In hΔK, the distribution of bump persistence times was very similar to the distribution of behavioral persistence times (Fig. 1i), while in PFG the neural distribution was slightly longer (Fig. 1l), a difference we address in detail below. To ask whether neural persistence predicts behavioral persistence, we detected all bump offsets that occurred after the odor stimulus, and compared trajectories associated with bump offset to those associated with maintained bump activity (Fig 1j,m). In both hΔK and PFG neurons, we found that bump offset was associated with a significant deviation of heading from the 'goal' trajectory adopted during odor, compared to periods when bump amplitude was maintained. Bump activity was also associated with higher forward velocities, and a greater probability of orienting upwind (Fig. 1j,m). Together, our results argue that hΔK and PFG neurons show similar odor response dynamics and similar persistent activity associated with maintaining an upwind-biased goal heading. Our imaging results thus support the hypothesis that recurrent connectivity between hΔK and PFG contributes to the persistent dynamics observed in both populations during goal-directed running.

Synaptic contributions to persistent activity in hΔK

We next asked whether persistent activity might arise in part from intrinsic properties of hΔK neurons. We performed whole-cell patch clamp recordings from hΔK neurons (labeled by VT062617-lexA) and injected positive or negative current. We observed rapid response kinetics

to current injection (Fig. 2a, Ext. Data Fig. 2a,b) with no persistent excitation. We conclude that intrinsic conductances do not contribute to persistent excitation in hΔK.

Since current injection did not drive persistent activity in hΔK neurons, we wondered whether synaptic input through tangential neurons could generate persistence. hΔK receives input from several tangential neuron types (Fig. 2b), with different connectivity patterns and predicted neurotransmitters[8,31]. To test the hypothesis that tangential inputs can drive persistent activity, we stimulated a group of tangential neurons that was previously shown to encode odor and drive upwind running[22] (65C03-Gal4) while recording from hΔK neurons using an orthogonal expression system (VT062617-lexA, Fig. 2c). 65C03-Gal4 labels 17 tangential neurons, at least two of which connected strongly and reciprocally with both hΔK and PFG (FB6A and D, Ext Data. Fig. 2c). We observed a brief inhibition that switched to slow and persistent excitation later in the stimulus (Fig. 2c). Persistent excitation grew with duty cycle (Ext Data Fig. 2d,e), and was strongly reduced by the nicotinic antagonists curare (10 μM) and imidacloprid (100 nM, Fig. 2d, Ext Data Fig. 2f)[32], arguing that it depends on cholinergic signaling. These neurons have been shown to express both cholinergic markers[18,22], and markers for glutamate[18] (an inhibitory transmitter in the fly brain[33]) which might account for the transient inhibition during stimulation. To ask whether recurrent signaling is required for persistent hΔK activity, we stimulated the same tangential neurons, and recorded from hΔK neurons while blocking recurrent signalling from hΔK using tetanus toxin (Fig. 2e). We found that blocking recurrent signaling abolished persistent excitation, and switched the response of hΔK from excitation to weak inhibition (Fig. 2e,f). We conclude that hΔK neurons can generate persistent activity in response to synaptic input, that this persistence requires cholinergic excitation, and that it depends on recurrent signaling by hΔK neurons.

Interestingly, we also observed slow persistent excitation in hΔK when we stimulated a second recurrently connected tangential neuron which expresses serotonin as its major neurotransmitter[34] (ExR3, Fig. 2b,g; Ext. Data Fig. 2g-h). This persistent excitation was reduced by the broad serotonin receptor antagonist methysergide (50 μM, Fig. 2h, Ext. Data Fig. 2i). Together these data suggest that hΔK generates persistent excitation in response to multiple recurrently-connected tangential neuron types expressing diverse transmitters and modulators.

In most computational models of recurrent attractor networks, broad inhibition is required together with local recurrent excitation to prevent runaway excitation[1,11,35]. We therefore wondered whether some tangential inputs provide global inhibition onto hΔK. Using connectomic predictions of neurotransmitter phenotype[16,31], we identified two putative inhibitory tangential neuron types that synapse onto hΔK, called FB5V and FB6M (Fig. 2b), and made or identified lines that label each type (Ext. Data Fig. 3a,b). To test whether these neurons provide functional inhibition onto hΔK, we stimulated each neuron type and recorded from hΔK using the same genetic strategy described above. In both lines we observed strong and rapid inhibition that depressed over time (Fig. 2i,k). For both neurons, stimulus-evoked inhibition was converted to excitation by 5 μM picrotoxin, a concentration known to block GABA-A receptors[33] (Fig. 2j,l). We conclude that some tangential inputs provide broad and rapid inhibition to the hΔK-PFG circuit, consistent with computational models of attractor network design.

Slow recurrent excitation increases the stability and tuneability of persistent activity in recurrent networks

Our imaging and physiology experiments, together with connectomics, argue that the hΔK-PFG circuit contains all the necessary ingredients to form an attractor network— local recurrent excitation and broad inhibition. However, our experiments also revealed an unexpected finding: synaptic excitation onto hΔK is slow, while inhibition is fast. To understand how these synaptic dynamics contribute to recurrent network function, we developed a connectome-based computational model (Fig. 3a, Ext Data Fig. 4a,b, Methods). In our model, connections between the 30 hΔK and 18 PFG neurons were specified by a Gaussian function matched to the structure of recurrent connectivity between these cell types, while two weights allowed us to tune the global strength of excitation and inhibition (Fig. 3b, Ext. Data Fig. 4a).

We first asked whether this network could reproduce the type of persistent bump activity we observed experimentally in hΔK and PFG neurons. With fixed weights, we found that any asymmetric input to the network was transformed into a localized bump of activity with a profile that closely matched those we recorded experimentally (Fig. 3c,d). Continuous attractors, that can generate a stable activity bump at any location, are difficult to build with noisy biological connectivity[36]. With Gaussian connectivity, we found that a bump of equal magnitude and duration could be generated anywhere around the ring (Ext. Data Fig. 5a). When we instead used real connectivity values from the hemibrain connectome[16], bump amplitude and duration varied, but we could still generate a bump at most locations (Ext. Data Fig. 5b). These simulations argue that the recurrent architecture of hΔK, PFG, and inhibitory tangential neurons can support persistent ring attractor dynamics even with real variation in connectivity strength.

Next, we asked how differential synaptic dynamics contribute to network function. Using second-order differential equations to generate fast and slow synaptic dynamics (Ext. Data Fig. 4b, Methods), we simulated network activity with either fast (Fig. 3e) or slow recurrent excitation (Fig. 3f). When all synapses were fast, we observed that small changes in global excitation or inhibition strength rapidly transitioned the network from a regime with transient responses (decay < 0.1 s) to a regime with non-decaying responses (Fig 3e). Slow decays similar to those we observed experimentally were only observed for a narrow band of excitation and inhibition weights, implying that these must be fine-tuned to produce realistic decays. In contrast, when recurrent excitation was slow, we observed a large regime of slowly decaying persistence (Fig 3f). Within this region, small changes in global excitation or inhibition strength gradually modulated the duration of persistence (Fig 3g,h). By incorporating trial-by-trial noise in excitation and inhibition, the slow model, but not the fast model, produced a distribution of decay times approaching the ones we observed experimentally (Fig. 3i). We conclude that the combination of fast inhibition with slow excitation allows the recurrent architecture of hΔK and PFG to generate stable and tuneable persistent activity.

In the model shown above, slow excitatory feedback was mediated by PFGs. When we stimulated PFGs and recorded from hΔK neurons, we observed excitation in some cells and inhibition in others (Ext Data Fig. 6a), likely because optogenetic stimulation produced an unrealistic depolarization of all members of the class, rather than a physiological bump. Cells that showed excitation showed slow and persistent dynamics, supporting the idea that PFG synapses are slow. However, we also wondered whether the slow dynamics we observed when stimulating excitatory tangential neurons could contribute to stabilization. To test this idea, we repeated our simulations with fast local excitation, but added a global excitatory neuron that could use fast or slow signaling (Ext. Data Fig. 6b). We found that slow feedback mediated by a

global excitatory tangential neuron could likewise stabilize persistent bump activity (Ext. Data Fig. 6c), at least in some regimes. In other regimes, we observed network oscillations (Ext. Data Fig. 6c, top left of parameter space). We conclude that slow recurrent excitation mediated by either PFGs or by excitatory tangential neurons is sufficient to stabilize network activity.

Finally we wondered whether the effects of slow excitation on stability and tuneability are specific to the hΔK-PFG circuit architecture, or a more general property of recurrent circuits. To address this question, we generated a simplified recurrent network with one self-recurrent excitatory unit and one bidirectionally connected inhibitory unit (Ext. Data Fig. 7). By assuming that the membrane time constant and synaptic onset kinetics were much faster than synaptic offset kinetics, we created a dynamical system with only two variables: recurrent excitation and inhibition (see Methods). Like our full model, this simple network showed persistent activity that depended on the speed of recurrent excitation: fast excitation led to a narrow band of excitation and inhibition strengths with stable persistent activity (Ext Data Fig. 7a), while slow excitation led to a much larger regime of tuneable decay durations (Ext Data Fig. 7b). We conclude that the ability of slow excitation to promote stable and tuneable persistence does not depend on a specific recurrent network configuration.

hΔK and PFG show uncoupled dynamics during turning and rest

Our imaging, electrophysiology, and modeling support the hypothesis that recurrent interactions between hΔK and PFG generate a shared persistent activity bump during odor-evoked goal-directed runs. However, this model does not address how the bump is turned on and off. Indeed, when we simulated the hΔK-PFG circuit described above with a heading input— a continuously varying sinusoidal compass input as suggested by the full connectome of the circuit (Fig. 1b, Ext Data Fig. 8a)— we observed a bump at most timepoints (Ext Data Fig. 8a). These observations suggest that some other mechanism must be responsible for turning the bump on with odor and off at the end of a goal-directed run, and led us to examine the dynamics of bump movement in our data more closely.

Close examination of our imaging data revealed that PFG and hΔK neurons showed distinct bump dynamics. In PFGs, a low amplitude bump was present at most times, and translated across columns during turns, becoming brighter during straight goal-directed runs (Fig. 4a). In contrast, hΔK only showed detectable bumps of activity during straight runs, and the bumps that appeared were generally fixed in place (Fig. 4b). To test whether these differences in bump dynamics held across trials and flies, we measured how far each continuous bump deviated laterally across the FB for both genotypes (Fig. 4c). We found that both the maximum and cumulative lateral deviation were significantly smaller in hΔK compared to PFG (Fig. 4d), supporting the idea that hΔK bumps are generally stable, while PFG bumps translate more readily across columns. To test the hypothesis that bump probability and amplitude depend on behavioral state, we divided trajectories into periods of rest, turns, and straight running (see Methods), and plotted the probability of observing a bump and its average amplitude during each behavior. We found that in both populations, bump probability and bump amplitude were highest during straight running (Fig. 4e,f). During turns and rest, PFGs showed a low amplitude bump, while hΔK rarely showed a bump (Fig. 4e,f). These data argue that hΔK and PFG show similar dynamics mostly during straight goal-directed runs, and that their activity must be functionally uncoupled at other times by some external mechanism.

In many parts of the Central Complex, lateral movement of a bump has been shown to encode a change in heading[13,29], while in some local neurons, bump movement reflects travelling direction[20,21].). To ask whether lateral shifts in PFG or hΔK bump position encode heading changes, we identified large shifts in heading (between 45-130°), and plotted bump position associated with these turns. We found that PFG bumps reliably shifted across columns during turns, although the gain of this shift was on average less than one (Fig. 4g). Such shifts tracked the turning direction, with left turns generating rightward shifts and right turns generating leftward shifts. In contrast, hΔK activity largely disappeared during turns, but could reemerge at a new position following the turn (Fig. 4h). Left and right turns similarly generated opposing direction shifts in hΔK. In single trails, we could observe a positive correlation of PFG bump position with heading (Fig. 4i,k). In contrast, hΔK neurons showed discrete jumps in bump position, with bumps appearing only during periods of relatively fixed heading (Fig. 5j,k). Correlations with travel direction were weaker than those with heading both populations (Ext. Data Fig. 8b,c). We conclude that both PFG and hΔK can track heading changes, but that PFG encodes continuous changes in heading, while hΔK exhibits punctate encoding only during straight runs.

Dynamic inhibition of hΔK neurons allows for rapid gating of attractor dynamics

Our imaging results suggest that hΔK and PFG show similar stable high-amplitude bump activity during straight goal-directed runs but are functionally uncoupled during turns and rest. What mechanisms could perform this decoupling? To address this question, we revisited the connectome and our computational model. In the connectome, only PFGs receive direct input from the compass system that tracks fly heading (Fig. 5a), and this input is significantly weaker than the recurrent connections between hΔK and PFGs (Ext. Data Fig. 9a,b). We wondered whether this difference in input could be combined with gating of recurrent connectivity to produce the dynamics we observed in our imaging. If recurrent connections were shut off, PFGs might follow their compass input, while if recurrence were switched on, their activity would be locked in place at the location of the last compass input. To test this idea, we again implemented a shifting sinusoidal compass input to PFG neurons, and examined activity in the network when recurrent connections between hΔK and PFG were gated OFF or ON (Fig. 5b). Consistent with our prediction, we found that when recurrent excitation was gated OFF, PFG bump position followed the compass signal with low amplitude, while hΔK was silent. In contrast, when recurrent excitation was gated ON, both PFG and hΔK showed a localized high-amplitude bump that remained in place despite shifting input, similar to our experimental observations (Fig. 4a,b). We previously found that the hΔK bump did not shift when the wind position was shifted during odor[26].

Seeking a more physiological mechanism to rapidly gate connectivity between hΔK and PFG, we wondered whether strong global inhibition of hΔK could serve the same decoupling function. To test this hypothesis, we repeated our simulation but replaced gating of recurrent connections with strong inhibition of hΔK (Fig. 5c). Similar to our results with gated recurrence, we found that when hΔK was inhibited, PFGs followed the compass with low amplitude, while when hΔK was disinhibited, both populations showed a localized high-amplitude bump.

Finally, we wondered whether this disinhibitory mechanism could be used to rapidly write an ongoing heading signal into memory. We simulated time-varying heading trajectories and

removed inhibition when the fly was oriented in different directions (Fig. 5d). This mechanism could be used to rapidly write two different heading memories in quick succession (Fig. 5e). We observed that disinhibition stabilized bump position at the location of the compass input when inhibition was first removed (Fig 5f). We conclude that disinhibition could serve to functionally gate recurrent interactions between anatomically connected partners, rapidly switching the circuit from following an input to storing a pattern in memory.

Inhibitory inputs to hΔK show signatures of gating

Our model results suggest that disinhibition could support the divergent dynamics we observed in PFG and hΔK neurons, in which PFGs continuously track heading, while hΔK bumps are fixed in place and appear only during straight goal-directed runs. If this model is correct, we would expect inhibitory inputs onto hΔK to be tonically active, and to show inhibition during sensory stimuli that promote goal-directed running. We would also expect higher activity during turns, when we observe suppression of hΔK relative to PFG. To test these predictions, we imaged from the two inhibitory tangential neuron types we previously identified through electrophysiology as flies performed our olfactory navigation task.

Activity in these inhibitory inputs to hΔK strongly supports our disinhibition model. We first imaged from FB5V neurons (Fig. 6a), a group of 18 neurons that provides feedforward inhibition only onto to hΔK, similar to the inhibitory input we modeled above (Fig. 5c). In these neurons, we observed tonic activity that was strongly supressed during odor, and continued its suppression during maintained trajectories after odor offset (Fig. 6b). Across flies, we observed weak inhibition by wind and strong inhibition by odor (Fig. 6c, Ext. Data Fig. 10a). On a moment-to-moment basis, we found that FB5V activity was negatively correlated with upwind velocity, and positively correlated with angular speed, exactly as predicted by our disinhibition model (Fig. 6d,e). These encoding features might reflect the dendritic inputs to FB5V, which are found in regions encoding both odor (CRE[37]) and pre-motor activity (LAL[38-40], Fig. 6a). To examine the encoding of turns in more detail, we detected large turns (Δ heading > 130°) and plotted average activity associated with these (Fig. 6f). We observed strongly increasing activity during large heading changes (Fig. 6f,g), consistent with the idea that FB5V inhibits hΔK during large turns. To examine encoding of goal-directed walking, we detected deviations from the goal direction adopted during odor (> 90° from goal direction) and plotted activity associated with these deviations (Fig. 6h). We observed that FB5V activity increased when the fly deviated from its goal (Fig. 6h,i). Our imaging data thus support a role for FB5V suppression in enabling hΔK activity and goal-directed walking, and a role for FB5V elevation in decoupling hΔK from PFG during turns.

We also examined the activity of the second tangential neuron type we identified, called FB6M. This group of 4 neurons provides input to both hΔK and PFG and receives feedback from hΔK (Fig. 6j). In this cell type, we also observed tonic activity, with suppression only in response to wind (Fig. 6k,l, Ext Data. Fig. 10b). FB6M activity during wind was positively correlated with angular speed, but showed no correlation with upwind velocity (Fig. 6m,n). Like FB5V, FB6M showed increased activity during turns (Fig. 6o,p) and when the fly deviated from its goal direction (Fig 6q,r). The wind encoding of FB6M might reflect its inputs in mechanosensory regions, which are known to encode the presence of wind (WED[41-42], Fig. 6j). In our model, we find that inhibition of both hΔK and PFG does not decouple their activity but instead shuts off

both populations (Ext Data Fig. 9c). We conclude that FB6M also plays a disinhibitory role in enabling circuit activity during wind and straight walking, but may permissively regulate the activity of PFG and hΔK together, rather than serving to decouple these populations.

Discussion

Attractor networks composed of recurrently connected neurons contribute to many neural functions, including gaze stabilization[43-44], spatial working memory[9], motor control and decision making[10], navigation[13,45], and emotional states[46,47]. By forcing diverse patterns of input onto a limited set of stable states, attractor networks offer many advantages for computation[2], such as de-noising[48], evidence integration[49,50], and short-term information storage[1]. A major issue in building attractor networks is that network architecture alone cannot always produce the desired dynamics. In particular, recurrent networks with realistic fast units often fail to reproduce the kind of slow persistent activity dynamics observed in experiments[35,46]. A second issue is that attractor networks with fixed weights do not generate intrinsic dynamics that are restricted to particular behaviors, sensory inputs, or internal states[10,47]. How biological recurrent networks are structured to generate stable dynamics that can be rapidly switched on and off by sensory input or behavior is unclear.

One solution to generate stable slow dynamics is to make recurrent excitation slow[35,46]. The most common mechanism proposed for generating slow excitation is through NMDA receptors at excitatory glutamatergic synapses[35], although a variety of other slow biophysical mechanisms, such as synaptic facilitation[51] and peptide signaling[46] have also been proposed to play a role in stabilizing recurrent activity. Conversely, to control the onset and offset of attractor dynamics, many network models implement gates to globally scale the strength of recurrent connectivity[4,6,7]. A common motif for regulating the gain of neural activity in biological systems is disinhibition. In cortical circuits, a canonical disinhibitory circuit from VIP interneurons to PV interneurons to pyramidal neurons has been shown to regulate neuronal gain[52], contrast sensitivity[53], and learning[54]. In the basal ganglia, brief periods of disinhibition allow for the performance of skilled motor actions[55,56]. Disinhibition also gates visual responses in the retina[57], and both auditory responses and motor production in songbirds[58,59]. Despite the prevalence of disinhibition, the interaction of disinhibitory gating mechanisms with recurrent circuits has not been investigated in detail.

Here we took advantage of the complete connectome information available in the fly[8,16], combined with precise genetic access to neuronal populations[18], to investigate the relationship between anatomical connectivity and functional dynamics in a discrete recurrent circuit in the fly navigation center. Consistent with this circuit using reverberant excitation, we observed similar dynamics in both hΔK and PFG neurons in response to odor and during goal-directed behavior. We found that hΔK neurons exhibit persistent activity in response to synaptic input but not current injection, and we found that blockade of recurrent signaling in hΔK neurons abolished persistent activity. Computational modeling based on the connectome supports the idea that this circuit behaves as a ring attractor, capable of stabilizing activity at any point on the ring, even with the "noisy" connectivity observed in real connectomes, although some of the persistence we observed *in vivo* may also arise upstream, as inhibition in FB5V inputs to hΔK also outlasted the odor stimulus. The architecture of this network bears a striking resemblance to classical models proposed to underlie working memory in the prefrontal cortex[1,60]. However, the

accessibility of the fly circuit also reveals several design features that enable this circuit to combine stable representations with rapid gating.

First, slow excitation in the hΔK-PFG circuit appears to be achieved through multiple biophysical mechanisms. PFG neurons do not express any fast transmitter, and instead express only neuromodulators and peptides: tyramine, MIP, and Dh31[18]. Consistent with this expression profile, when we observed excitation in hΔK on stimulating PFGs, this excitation was slow and persistent (Ext Data Fig. 6). However, we also observed slow and persistent excitation when stimulating other tangential neurons that connect recurrently with PFG and hΔK. Our models indicate that recurrent excitation either from local PFG neurons, or through broadly-projecting tangential neurons, is sufficient to stabilize attractor dynamics and to make the duration of persistent activity tuneable. Our work expands on the classical idea that NMDA receptors are critical for building a stable working memory circuit[1,35], suggesting that any slow signaling mechanism, including through GPCRs, can serve to stabilize persistent activity. Our results are also consistent with recent findings in the hypothalamus arguing that slow signaling through neuropeptide receptors is required for a persistent aggressive state in mice[46,61,62]. Together, these studies support the idea that neuromodulatory neurons can form integral parts of recurrent circuits for information storage, in addition to providing global regulatory signals.

Second, the recurrent network is split into two "half-centers" composed of distinct neuron types: hΔK and PFG. In most attractor models, recurrent excitation occurs within a uniform population of excitatory neurons, parameterized by stimulus tuning, timing, or other features[1,11]. Here instead we find that the network is composed of two morphologically[8] and transcriptomically[18,63] distinct neuron types that receive distinct inputs. PFG neurons receive a heading direction signal from the compass system and form the content of memory, while hΔK neurons receive an inhibitory gating signal from FB5V and control memory formation. By separating the input of the network from the gating signal, we find in our models that we can rapidly gate recurrent activity on and off. hΔK and PFG also receive common input, for example from hΔB neurons that encode travel direction[20,21]. Although we did not observe encoding of travel direction in our paradigm, common input might allow this circuit to perform additional computations important for navigation. The separation of the circuit into two halves may also facilitate proper wiring of the network. Future studies may investigate the molecular signals that allow hΔK and PFG to wire together in a ring structure, that establish the proper ratio between recurrent and feedforward synapses, and that allow hΔK and PFG to wire to both distinct and common inputs.

Finally we show evidence that inhibition of hΔK allows the circuit to rapidly switch between two modes: information following and information storage. Our model circuit functions similar to a latch in digital logic[64]: during inhibition PFGs follow their compass inputs, while during disinhibition activity is locked into place. A critical feature of the network is that the recurrent synapses are much stronger than the feedforward synapses onto PFG, allowing recurrence to dominate when hΔK is disinhibited. In our paradigm, we observed that inhibition onto hΔK was supressed by odor and wind— two cues that promote straight upwind trajectories— and that activity of the circuit correlates with upwind-biased orientations. However, in principle this circuit could be used to store any heading direction, as long as the gate is turned on (i.e. inhibition turned off) under the correct conditions. In addition to suppression by sensory input, we also observed that inhibitory inputs onto hΔK are active during turning, suggesting that the circuit can be gated by motor features as well as sensory ones. The mechanism described here is

reminiscent of landmark learning in the compass system[65], where dopaminergic plasticity is gated by turning. However, disinhibition allows for near instantaneous— though short-lived— memory formation. Several previous models have used asymmetric synapses between tangential neurons and FB local neurons to store directional memories[27,66,67], although such a mechanism has not yet been demonstrated experimentally. A variety of plasticity mechanisms, operating at different timescales and gated by diverse sensory and motor features, are likely to underlie the complex dynamics of biological goal signals in the fly navigation center.

Gated recurrent networks have been theorized to initiate intrinsic dynamics, such as persistent activity, and to coordinate more complex forms of dynamics, such as the manipulation of information in working memory and motor preparation and control[3,6]. Similar types of gates are used extensively in machine learning[68], including as a solution to rapidly reset memory traces[69]. Here we describe a biological form of gating that allows a recurrent anatomical circuit to dynamically alter its function on fast behavioral timescales. If integrated into recurrent networks with different connectivity structures[2,70], disinhibition could be used to rapidly alter the function of other types of attractor networks, allowing for flexible use of line attractors, rotations, sequences, or bistable attractors in a stimulus- and behavior-dependent manner.

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Methods

Fly stocks

All experimental flies were raised on standard cornmeal-agar media at 25 °C on a 12-hour light cycle. For optogenetics experiments, the food was supplemented with 50 μL of 35 mM all-trans retinal (Sigma, R2500, dissolved in ethanol) mixed with ~1 teaspoon of hydrated potato flakes. Experimental flies were aged to 1-3 days for electrophysiology experiments or 9-12 days for imaging experiments. A list of fly genotypes is shown in **Table 1**.

Table 1: Fly genotypes

Genotype	Figures	Notes
VT062617AD; VT037223DBD > UAS-GCaMP7f, UAS-tdTomato	Fig. 1d,f,h,i,j Fig. 4b,d,e,f,h,j,k Ext. Data Fig. 1d,e,f,g Ext. Data Fig. 8b,c	Split Gal4 SS63158 targeting hΔK; gift of Tanya Wolff
SS52590 (VT017491AD; VT000355DBD) > UAS-GCaMP7f, UAS-tdTomato	Fig. 1e,g,k,l,m Fig. 4a,c,d,e,f,g,i,k Ext. Data Fig. 1d,e,f,h Ext. Data Fig. 8b,c	PFG split (BI: #86628)
VT062617-gal4 > UAS-GCaMP7f, UAS-tdTomato	Ext. Data Fig. 1d,e,f	Broad line targeting hΔK[26]
VT062617-lexA, 13xlexAop-GFP/+; UAS-Chrimson/65C03-gal4	Fig. 2a,c,d,f Ext. Data Fig. 2a,b,d,e,f	Recording from hΔK; activate 65C03 (BI: 41290) is a FB tangential line that drives upwind walking[22]
VT062617-lexA, 13xlexAop-GFP/lexAop-TNTe; UAS-Chrimson/65C03-gal4	Fig. 2e,f	Same as above with TNTe to block synapttic transmission from hΔK

VT062617-lexA, 13xlexAop-GFP/+; UAS-Chrimson/24C07-gal4	Fig. 2a,g,h Ext. Data Fig. 2a,b,g,h,i	Recording from hΔK; activate 24C07 (BI: 49074) is a FB tangential line targeting ExR3
VT062617-lexA, 13xlexAop-GFP/49B08AD; UAS-Chrimson/VT063626DBD	Fig. 2a,i,j Ext. Data Fig. 2a,b	Recording from hΔK; activate FB6M using 49B08AD (from BI: 86664); VT063626DBD (from BI: 73940)
49B08AD; VT063626DBD > GFP	Ext. Data Fig. 3a	Visualization of split used to target FB6M for activation
VT062617-lexA, 13xlexAop-GFP/+; UAS-Chrimson/36B06-gal4	Fig. 2a,k,l Ext. Data Fig. 2a,b	Recording from hΔK; activate 36B06 (BI: 49929) targeting FB5V
36B06-gal4 > GFP	Ext. Data Fig. 3b	Visualization of line used to target FB5V for activation
VT062617-lexA, 13xlexAop-GFP/+; UAS-Chrimson/21D07-gal4	Fig. 2a Ext. Data Fig. 2a,b	Recording from hΔK; activate 21D07 (BI: 48943) targeting FB5AB[22]
VT062617-lexA, 13xlexAop-GFP/ VT017491AD; UAS-Chrimson/ VT000355DBD	Fig. 2a Ext. Data Fig. 2a,b Ext. Data Fig. 6a	Recording from hΔK; activate PFG split (BI: #86628)
VT013602AD; 78H12DBD > UAS-GCaMP7f, UAS-tdTomato	Fig. 6b,c,d,e,f,g,h,i Ext. Data Fig. 10a	Split Gal-4 line SS88825 targeting FB5V
49B08AD; VT063626DBD > UAS-GCaMP7f, UAS-tdTomato	Fig. 6k,l,m,n,o,p,q,r Ext. Data Fig. 10b	Split Gal4 line targeting FB6M:49B08AD (from BI: 86664); VT063626DBD (from BI: 73940)

*BI refers to Bloomington Drosophila Stock Center

Two-photon calcium imaging

Calcium imaging was performed as described in our previous study[26]. Briefly, we anesthetized flies over ice and a chilled (4-10 °C) aluminum sarcophagus and then glued them onto custom 3D-printed holders. We minimized movement of the brain by stabilizing the proboscis and head, and glueing the eyes, head, and anterior tip of the thorax to the holder. To access the FB for imaging, we removed a small piece of the surface cuticle, trachea, and air sacs over the back of the brain using sharpened forceps. We positioned flies over an air-supported ball (9 mm diameter General plastics FR-7110, painted with black spots, supported by 0.4 L/s air) using two cameras (30fps, FLIR Blackfly, Computar 8.5mm 1:1.3 adjustable lens) at the front and side of the fly. A third camera (100 fps, Grasshopper, 94mm/0.5x InfiniStix Proximity Series lens, Edmund Optics Focal Length Extenders, working distance=15cm) was used to track the ball using Fictrac software[71]. We illuminated the fly and ball using two IR LEDs (M850F2) and T-cube LED Drivers (LEDD1B) coupled to fiber optic cables (Thorlabs M118L03 flat cleave patch

cables). We continuously perfused (1mL/m) the brain with extracellular saline (103 mM NaCl, 3 mM KCl, 5 mM TES, 8 mM trehalose dihydrate, 10 mM glucose, 26 mM $NaHCO_3$, 1 mM $NaH_2PO_4H_20$,1.5 mM $CaCl_22H_2O$, and 4 mM gCl_26H_2O, pH 7.1–7.4, osmolarity 270–274 mOsm) bubbled with carbogen (5% CO_2, 95% O_2) and warmed to 33°C.

We performed all imaging under a 20x water objective (Olympus Plan Fluorite XLUMP 20x/1.0) using an infrared laser (SpectraPhysics MaiTai HP or Coherent Axon 920) at 920 nM and 25-35 mM (measured at sample). Simultaneous fluorescence emission from GCaMP7f and tdTomato were separated by bandpass filters (Semrock, FF01-525/50-32 for green and FF01-607/70-32 for red) and detected using two GaAsP photomultiplier tubes. We captured volumes consisting of three 192 x 96 μm optical sections, separated by 15 μm, taken at 8 vol/s with a 1.2 μs dwell time.

In all imaging experiments, flies walked on a floating ball and received wind (25 cm/s) and odor (0.5% apple cider vinegar, 0.3 L/min) stimuli controlled by custom Python code. The wind direction was set by a rotary union (Dynamic Sealing Technologies, # LT-2141-OF-ES12-F1-F2-C2) controlled by a stepper motor (Oriental PKP566FMN24A, with controller CVD524-K) and microcontroller (TeensyDuino +USB), and was controlled in closed loop by the flies' heading measured by ball movement as described in our previous paper[26]. The starting wind direction was randomly selected from 0°, 45°, -45°, 135°, or -135°. Wind was on from 5-60 seconds of each 65 second trial. The odor signal was randomly selected between five possible patterns on interleaved trials: odor step (15 s) or odor pulses of 1, 4, 7, or 10 pulses (1 Hz, 0.5 s pulse width). When the odor was turned off, a compensatory air valve was opened to maintain total air speed. Air to all lines (wind, ball support, odor, compensate) were passed through a pressure regulator (Cole-Parmer MIR2NA) and charcoal filters (Drierite 26800 Drying column, with desiccant replaced by activated charcoal). Wind and compensate were also humidified. Airflow for the wind line was controlled by a mass flow controller (Aalborg Model GFC17), while airflow for odor, compensate, and ball support were controlled by flowmeters (Cole-Parmer PMK1-010608). We controlled the timing of wind stimuli using a solenoid valve (Cole-Parmer Masterflex P/N 01540-11), and the timing of odor and compensate using high-speed three-way solenoid valves (Lee company, LHDA1233115HA).

Electrophysiology

For electrophysiology, we anesthetized flies over ice, glued them to custom 3D printed holders, and then removed their two front legs. To access dorsal cell bodies, we removed a large piece of the cuticle, trachea, and air sacs from the back of the brain, then manually detached the sheath using fine forceps. We minimized brain movements by removing the muscles on the brain and stabilizing the proboscis with glue. Starting at the dissection, and maintained throughout the entire experiment, we continuously perfused extracellular saline (same recipe as above), bubbled with carbogen, over the brain. Using a 40× objective (Olympus, LUMPLFLN40XW), an LED source (Cairn Research MONOLED), and a filter (U-N19002 AT-GFP/F LP C164404) we visualized GFP-positive cell bodies for recordings. Prior to recording,

we cleaned the area around the target cell using fine-tip glass pipettes filled with extracellular saline.

For patch clamp recordings, we pulled glass pipettes with a Sutter P-1000 puller, pressure polished the pipettes (final resistance of 3-5 MΩ), and then filled them with intracellular solution (140 mM KOH, 140 mM aspartic acid, 10 mM HEPES, 1 mM EGTA, 1 mM KCl, 4 mM MgATP, 0.5 mM Na_3GTP, and 13 mM biocytin hydrazide). We amplified the voltage signals using an Axonpatch 200B amplifier with a Brownlee Precision 410 preamplifier and then digitized the signals at 10 Kz. We delivered 73 $\mu W/mm^2$ red light (625 nm, measured at light source) for activating CsChrimson using a Thorlabs LED (M625F2) and T-Cube LED Driver (LEDD1B) coupled to optical fibers cables (Thorlabs M118L03 flat cleave patch cables) positioned below the head.

To better observe a mixture of excitation and inhibition, we depolarized cells to -38.1 ± 7.1 (mean + SD, N = 37 cells) using a small amount of positive current. Cells that did not spike were discarded. In all experiments, we randomly looped through 11 stimuli: seven optogenetic light stimuli (4 s of 12 $\mu W/mm^2$ low power; 4 s of 23 $\mu M/mm^2$ medium power; 4 s of 73 $\mu M/mm^2$ high power; 1 Hz pulses with 0.2 duty cycle; 1 Hz pulses with 0.5 duty cycle; 1 Hz pulses at 0.8 duty cycle; and a light plume stimulus), three current stimuli (4 s of -4 pA; 4 s of 0 pA; and 4 s of 4 pA), and one wind/odor stimulus (wind on for 20 s, 5% apple cider vinegar on for 4 s). We constructed the light plume stimulus by filtering the odor encounters of a fly navigating in a virtual olfactory plume[26] with a positive derivative filter. The stimuli were looped five times in all cells, and then five times per drug condition. While we only report the results of light pulse stimuli in this paper, the main electrophysiology findings hold across all optogenetic stimuli.

In several experiments, we perfused drugs onto the brain to block neurotransmitter receptors. In these experiments, drugs were mixed with extracellular saline and perfused onto the brain for at least five minutes before resuming stimuli presentation. Since picrotoxin generated large excitatory responses to synaptic input, we often hyperpolarized the cells to -50.3 ± 10.2 (mean + SD, N = 10 cells) to minimize depolarization block. If cells did enter depolarization block, we paused the experiment until the cell recovered. The list of drugs, their sources, and the final concentrations used are listed in **Table 2**.

Table 2. Drug sources and concentrations

Drug	Final concentration	Source	Identifier
Tubocurarine chloride (curare)	10 μM	Tocris	2820
Imidacloprid (IMI)	100 nM	Sigma	37894
Picrotoxin	5 μM	Tocris	1128
Methysergide	50 μM	Sigma	M137

Immunohistochemistry

To perform immunohistochemistry, dissected brains were fixed in 4% paraformaldehyde (dissolved in PBS) for 15 minutes, washed 3x in PBS, incubated in 5% normal goat serum (dissolved in PBST) for 60 minutes, incubated overnight in primary antibody solution (dissolved in 5% normal goat serum), washed 3x in PBST, incubated overnight in secondary antibody solution (dissolved in 5% normal goat serum), washed 3x in PBST, and washed 3x in PBS. The primary antibody solution contained chicken anti-GFP (Fisher Scientific RRID:AB_1074893) 1:50, mouse anti-nc82 (DSHB RRID:AB_2314866) 1:50, and rabbit anti-dsRed (Clontech 632496) 1:500. The secondary antibody solution contained Alexa488-conjugated goat anti-chicken (Fisher Scientific RRID:AB_2534096) 1:250, Alexa633-conjugated goat anti-mouse (Fisher Scientific RRID:AB_2535719) 1:250, and Alexa568-conjugated goat anti-rabbit (Fisher Scientific RRID:AB_2576217) 1:250. To image the brains, the brains were mounted on microscope slides, immersed in vectashield (Vector Labs H- 1000), sealed with coverslips, and then imaged using a 20× objective (Zeiss W Plan-Apochromat 20×/1.0 DIC CG 0.17 M27 75 mm) on a Zeiss LSM 800 confocal microscope at 1.25 µM depth resolution.

To compare hemibrain neuron morphology to R65C03-GAL4 (Ext. Data Fig. 2c), we warped a stain of R65C03 (from Flylight[72]) and hemibrain skeletons of layer 6 tangential neurons using the 2018 Janelia female template brain[73]. We overlayed the warped stain and warped skeletons and identified neurons with high overlap.

Data analysis

Connectomic analysis

Data from the hemibrain connectome[16] were obtained from neuprint explorer (http://neuprint.janelia.org, hemibrain:v1.2.1) and analyzed using custom Python code. Prior to visualizing the weight matrices (Ext. Data Fig. 1a; Fig 5a), we sorted each cell type by their FB or protocerebral bridge x position (i.e. columns), with hΔK positioning determined by their axons. To count the fraction of FB6M or FB5V synapses onto hΔK axons, we sorted synapses by their x-position on a hΔK cell-by-cell basis.

Calcium imaging and behavior analysis

We used fictrac software[71] to transform the x, y, and z rotations of a custom-made ball into 2D walking trajectories. The forward velocity and angular velocity were directly calculated as rotations around the x and z axes, respectively, which were then used to calculate heading (motor position, calculated as the integral of angular velocity), position (x = integral of forward velocity * sin(heading); y = integral of forward velocity * cos(heading)), and upwind velocity (vy = forward velocity * cos(heading)). The rotation around the y axis (side velocity) was additionally used to calculate travel direction (heading + $\tan^{-1}$(forward velocity / side velocity)). For data analysis, the position, angular velocity, angular speed, upwind velocity, and forward velocity were smoothed using a running average of 1 s width. Flies were only included for further analysis if they reliably oriented upwind on at least 50% of trials, which was identified by a

convergence of heading values towards the upwind direction (0°) and an increase in mean upwind velocity around wind or odor onset.

We used custom Matlab software to pre-process our calcium imaging data (see ref(26) for details), including motion correction, fluorescence extraction (across eight hand-drawn ROIs across the FB), and alignment with fictrac behavioral data. To detect activity bumps in hΔK neurons, PFG neurons, and neurons labeled by VT062617-gal4, we low-pass filtered bump amplitude data (butterworth filter (fs = 100 Hz, fc = 2 Hz)) and then established a threshold on a trial-by-trial basis according to the minimum ΔF/F value of that trial and the ΔF/F standard deviation across all trials. For most analyses, this threshold was set at minimum(ΔF/F) + 1* standard deviation, but to identify large PFG bump onsets and offsets for Fig. 1k-m, the threshold was increased to minimum(ΔF/F) + 1.5 * standard deviation. A minimum bump duration was set at 3 s, and any above-threshold activity within 3 s of bumps was merged. To calculate bump position, the raw ΔF/F values across all eight ROIs were smoothed (running average, width ~2 s) and then fit with a von Mises distribution at each time step using the Matlab function fminsearch. To aid fitting, we used the previous bump position or the ROI with the maximum ΔF/F (if the previous time step did not have a bump) as the initial search values.

During calcium imaging of FB5V and FB6M neurons, the calcium signal showed an artifactual decay from trial start to trial end. This artifact was removed by fitting an exponential decay using the beginning (first 5 s) and end (last 5 s) of the ΔF/F signal, and then subtracting this fit from the ΔF/F signal. Therefore, all ΔF/F signals reported for FB5V and FB6M are changes from this baseline.

In panels Fig. 1i,l, and Ext. Data Fig. 1f we calculated the persistence of walking direction following odor. To calculate the persistence of walking direction, we first defined a goal direction as the mean heading direction adopted in the first second after odor offset (post odor) or after trial start (baseline control), and then identified the time when flies stopped walking (forward velocity under 0.5 mm/s for at least 1.5 s) or deviated from the goal direction by at least 45°. In panels Fig 1h,i,k,l, we calculated the persistence of neural activity following odor. To calculate the neural persistence, we calculated the time between odor offset and bump offset. To better compare neural and behavioral persistence, we only considered trials where the bump activity turned on before odor offset and turned off after odor offset.

In panels Fig. 1j,m, we aligned bump amplitude and behavioral data to bump offset. We only included trials where the bump activity turned on before odor offset and turned off after odor offset, and we removed any data before bump onset. In control bump ON data, we took the same trials, but only included data between bump onset and bump offset, each aligned by the midpoint of the bump. For goal deviation, the 'goal' was defined as the mean heading during the first second of the bump ON or bump offset data.

In panel Fig 3d, we calculated the mean bump width of hΔK activity. To do this, we isolated periods with bumps, aligned the raw ΔF/F calcium signals (across 8 ROIs) to the column with the highest signal, and then normalized the activity.

In panel Fig. 4d, we calculated the maximum bump deviation and cumulative bump deviation. To quantify maximum bump deviation, we calculated the difference between the minimum and maximum unwrapped bump position between each bump onset and offset. To quantify cumulative bump deviation, we calculated how much the unwrapped bump position changed at each time point between bump onset and offset, summed the absolute value of these changes, and then divided the sum by the total length of the bump period.

In panels Fig. 4e-g and Fig. 6f,g,o,p, we identified discrete periods of turning. To identify turns, we identified segments of data where the unwrapped heading shifted by at least 90°. We then identified the beginning and end of each turn by searching backwards or forwards in time, respectively, until the heading stabilized (the maximum change in heading over 0.25 seconds was smaller than the 80th percentile of heading standard deviations across all bins of the same duration). Since this algorithm isolated periods of turning with different durations, when turns were aligned in time (Fig. 4g, Fig. 6f,o), the data were time warped such that all turns were equal in length. In cases where small turns were compared to large turns (Fig. 4g), turns were classified as small if the total change in heading was less than 130°, and large otherwise.

In panels Fig. 4e,f we further classified behavior as ‘rest’ or ‘straight running’. Rests were defined as periods of low movement ($v < 0.5$ mm/s) for at least 3 seconds. Straight running was defined as movements with high walking speed ($v >$ 50th quartile of speed for given fly) and stable heading (Δheading < 90°). Short straight running bouts (under 3 s) within 1 s of larger bouts were merged, and large bouts (over 3 s) within 3 s of each other were merged. Any turns that had overlapping sections with straight running bouts were removed.

In Fig. 4h, we calculated discrete shifts in hΔK bump position following turns. To do this, we identified pairs of hΔK bumps separated in time. For each bump pair, we calculated the change in wrapped heading from the end of the first bump to the beginning of the second bump, and then classified the pairs as left turns (0° > Δ heading) or right turns (0° < Δ heading). Since these data were wrapped, we centered the wrapping around the bump position or heading taken at the end of the first bump.

In panels Fig. 4k, Ext. Data Fig. 8b,c, we correlated bump position with heading or travel direction. Since individual bumps in hΔK (or VT062617) neurons exhibit stable position over time, we only included trials with at least two bumps separated in time.

In panels Fig. 6e,n, we cross correlated FB5V and FB6M $\Delta F/F$ signals with angular speed or upwind velocity. Prior to cross correlating, we mean subtracted the fluorescent and behavioral signals. To remove wind-coupled activity fluctuations in FB6M data, we only used data from 5 s after wind onset and 5 s before wind offset.

In panels Fig. 6h,q, we aligned FB6M and FB5V activity to deviations from goals. We first defined a goal direction as the mean heading adopted in the first second after odor offset, and then aligned neural and behavioral data to the time when flies deviated from this goal by at least 45°.

Electrophysiology analysis

For analysis of electrophysiology data, the voltage traces were low pass filtered using a butterworth filter (fs = 10000 Hz, fc = 5 Hz), downsampled by a factor of 10, and then baseline subtracted. We calculated persistent excitation (Fig. 2d,f,h; ExtData Fig. 2e,h) by summing the baseline-subtracted voltage signal between light offset and trial end, and then normalizing by the length of this period. For visualizing voltage signals across trials (e.g., Fig. 2c), the voltage signals were sorted by persistent excitation (Fig. 2a,c,e,g; ExtData Fig. 2a) or inhibition during the stimulus (Fig. 2i,k).

Statistics

All statistics were ran using GraphPad Prism 10 and reported in Tables 3 and 4. Non-parametric statistical tests were used if the data was not normally distributed (see Tables 3 and 4). An alpha value of 0.05 was used to determine significance, with *0.01<p<0.05, **0.001<p<0.01, ***0.0001<p<0.001, ****p<0.0001 reported on figures.

Modeling

Full FB model

To model the FB recurrent network, we created a rate-based dynamical system model consisting of 30 hΔK neurons, 18 PFG neurons, and 1 global inhibitory neuron. We approximated all dynamical systems using Euler's method of integration with a time step of 0.001 s. To generate ring recurrence between hΔK and PFG neurons, we gave each neuron a relative position in the FB, indicated by an angle from 0 to 2π, and then connected the populations with local excitation that was maximum at overlapping positions and decayed with distance. This was done explicitly using a gaussian equation ($\sigma_{PFG > h\Delta K} = 0.4$, $\sigma_{h\Delta K > PFG} = 0.3$, in radians) (Fig. 3; Fig. 5) or using the connectivity directly from the connectome (Ext. Data Fig. 5). Although real hΔK neurons have a 180° shift across the FB, for simplicity, we only show connectivity and activity simulations from the 'axons' of hΔK neurons. The weight matrices between hΔK or PFGs and the global inhibitory neuron were either one-to-all or all-to-one. To simulate the neural activity of each population, we used the following ordinary differential equations (ODEs):

$$\tau_{h\Delta K} * \frac{d\vec{u}_{h\Delta K}}{dt} = -\alpha_{h\Delta K} * \vec{u}_{h\Delta K} + exc * W_{PFG,h\Delta K}\vec{U}_{PFG} - W_{inh,h\Delta K}\vec{U}_{inh} + \vec{I}_{h\Delta K} \quad (1)$$

$$\tau_{PFG} * \frac{d\vec{u}_{PFG}}{dt} = -\alpha_{PFG} * \vec{u}_{PFG} + exc * W_{h\Delta K,PFG}\vec{U}_{h\Delta K} - W_{inh,PFG}\vec{U}_{inh} + \vec{I}_{PFG} \quad (2)$$

$$\tau_{inh} * \frac{d\vec{u}_{inh}}{dt} = -\alpha_{inh} * \vec{u}_{inh} + inh * (W_{h\Delta K,inh}\vec{U}_{h\Delta K} + W_{PFG,inh}\vec{U}_{PFG}) + \vec{I}_{inh} \quad (3)$$

Where τ_x is the time constant for population x, α_x is the leak of population x, $\vec{u}_x$ is the activity of population x, *exc* is the excitation strength, *inh* is the inhibition strength, $W_{x,y}$ is the weight matrix

from population x to population y, $\vec{U}_x$ is the synaptic output of population x, and $\vec{I}_x$ is the external input to population x. τ_x and α_x were set to 1 and 10, respectively, for all populations.

To simulate the synaptic output of each population, we used the following second order ODEs to simulate alpha functions:

$$\vec{U}_x{}'' = -(a_x + b_x) * \vec{U}_x' + a_x * b_x * (F_x(\vec{u}_x) - \vec{U}_x) \quad (4)$$

Where $1/a_x$ is the decay rate of the alpha function for population x, $1/b_x$ is the growth rate of the alpha function for population x, and F_x is the activation function of population x. For fast synapses, the values of a_x and b_x were 10 and 10.1, respectively; while, for slow synapses, the values of a_x and b_x were 0.8 and 1, respectively. We used the following activation functions:

$$F_{h\Delta k/PFG}(u_x) = \begin{cases} 0 \; if \; u_x < 0 \\ u_x \; if \; 0 < u_x < 1 \\ 1 \; if \; u_x > 1 \end{cases} \quad (5)$$

$$F_{inh}(u_x) = \begin{cases} 0 \; if \; u_x < 0 \\ u_x \; if \; 0 < u_x \end{cases} \quad (6)$$

During the simulations in Fig. 3, sinusoidal (direct offset = 1), Gaussian (normalized such that max = 1), or random input (gaussian distribution with μ = 1, σ = 0.5) were provided to the hΔK and PFG neurons for 4 or 10 seconds. During the simulations in Ext. Data Fig. 8a and Fig. 5, we only provided input to PFG neurons, which was sinusoidal with a phase that we randomly and stochastically updated over time. Phase shifts were stochastic, occurring with a 0.1% chance at each time step, and when they occurred, they were randomly drawn from a gaussian distribution with μ = 0 and σ = 50° and then smoothed over time according to:

$$\tau_{smooth} * \frac{dheading_{smooth}}{dt} = heading_{raw} - heading_{smooth} \quad (7)$$

Where τ_{smooth} equals 1. To account for the differences in synaptic strength between EPGs > PFG and hΔK <-> PFG synapses, we decreased the amplitude of the sinusoidal input to 1/6 of the recurrent excitation strength. We controlled the gate in Fig. 5 by either externally changing the excitation strength (*exc* in equations 1,2, increased from 0 to 6) or changing external input to hΔK ($\vec{I}_{h\Delta K}$ in equation 1, inhibition corresponded with a value of -5, and disinhibition corresponded with a value of 0). In Ext. Data Fig. 9, we additionally attempted to gate bump dynamics by adding external input to hΔK and PFG neurons ($\vec{I}_{h\Delta K}$ and $\vec{I}_{PFG}$ in equation 1, increased from -0.5 or -1.5 to 0).

To calculate the network decay rate in Fig. 3, we calculated the maximum difference in hΔK output at each time step following external input offset and then fit this difference with an exponential decay function.

In Fig. 5f and Ext. Data Fig. 5, we calculated the bump position by finding the relative position (as an angle) of the neuron with the maximum activity.

FB model with global excitation

To add global excitation to the FB model (Ext. Data Fig. 6), we included an additional excitatory neuron with one-to-all and all-to-one connections with the hΔK and PFG neurons, and one-to-one connections with the global inhibitory neuron:

$$\tau_{h\Delta K} * \frac{d\vec{u}_{h\Delta K}}{dt} = -\vec{u}_{h\Delta K} + exc_{local} * W_{PFG,h\Delta K}\vec{U}_{PFG} + exc_{global} * W_{global,h\Delta K}\vec{U}_{global} - W_{inh,h\Delta K}\vec{U}_{inh} + \vec{I}_{h\Delta K} \quad (8)$$

$$\tau_{PFG} * \frac{d\vec{u}_{PFG}}{dt} = -\vec{u}_{PFG} + exc_{local} * W_{h\Delta K,PFG}\vec{U}_{h\Delta K} + exc_{global} * W_{global,PFG}\vec{U}_{global} - W_{inh,PFG}\vec{U}_{inh} + \vec{I}_{PFG} \quad (9)$$

$$\tau_{inh} * \frac{d\vec{u}_{inh}}{dt} = -\vec{u}_{inh} + inh * (W_{h\Delta K,inh}\vec{U}_{h\Delta K} + W_{PFG,inh}\vec{U}_{PFG} + W_{global,inh}\vec{U}_{global}) + \vec{I}_{inh} \quad (10)$$

$$\tau_{global} * \frac{d\vec{u}_{global}}{dt} = -\vec{u}_{global} + exc_{global} * W_{h\Delta K,global}\vec{U}_{h\Delta K} + exc_{global} * W_{PFG,global}\vec{U}_{PFG} - W_{inh,global}\vec{U}_{inh} + \vec{I}_{global} \quad (11)$$

Where exc_{local} is the excitation strength between local neurons (hΔK and PFGs) and exc_{global} is the excitation strength between global excitatory neuron and local neurons. We simulated the synaptic output of the global excitation neuron using the same second order ODEs as the full model (equation 4) and the same activation function as those used for hΔK and PFG neurons (equation 5). During the stimulus period, the global excitatory neuron received an external input equal to 1. When we combined slow global excitation and fast global inhibition, the network often displayed global network oscillations (top left of parameter space in Ext. Data Fig 6b). When calculating the network decay rates, we removed parameter combinations that lead to oscillations.

Simple recurrent network model

To simplify the recurrent network to two dynamic variables, we began by reducing the hΔK and PFG populations to one global excitatory neuron. This led to the following 6 variable dynamical system:

$$\tau_{exc} * \frac{du_{exc}}{dt} = -u_{exc} + W_{exc,exc}\, U_{exc} - W_{inh,exc}U_{inh} + I_{exc} \quad (12)$$

$$\tau_{inh} * \frac{du_{inh}}{dt} = -u_{inh} + W_{exc,inh}\, U_{exc} + I_{inh} \quad (13)$$

$$U_{exc}'' = -(a_{exc} + b_{exc}) * U_{exc}' + a_{exc} * b_{exc} * (F_{exc}(u_{exc}) - U_{exc}) \quad (14)$$

$$U_{inh}'' = -(a_{inh} + b_{inh}) * U_{inh}' + a_{inh} * b_{inh} * (F_{inh}(u_{inh}) - U_{inh}) \quad (15)$$

We made two simplifying assumptions. First, we assumed that the synaptic rise and decay constants ($1/a_x$, $1/b_x$) were much slower than the membrane time constants (τ_x). This means that the variables u_{exc} and u_{inh} instantaneously follow the synaptic dynamics. Second, we assumed that the synaptic decay time constant (a_x) was much slower than the synaptic rise time constant (b_x). These assumptions lead to the following 2 variable dynamical system:

$$U_{exc}' = b_{exc} * (F_{exc}(W_{exc,exc}U_{exc} - W_{inh,exc}U_{inh} + I_{exc}) - U_{exc}) \quad (16)$$

$$U_{inh}' = b_{inh} * (F_{inh}(W_{exc,inh}U_{exc} + I_{inh}) - U_{inh}) \quad (17)$$

We used the same activation functions as those used for the excitatory and inhibitory neurons in the full model (equations 5,6). During simulations, we kept $W_{exc,inh}$ constant at 1, whereas we varied $W_{exc,exc}$ and $W_{inh,exc}$ with excitation strength and inhibition strength, respectively. During input, the excitatory unit received an external input equal to 1, whereas the inhibitory unit received no input.

Data Availability Statement

All data will be made available on Zenodo upon publication.

Code Availability Statement

All code will be made available at Github upon publication.

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Author Contributions

A.J.D. and K.I.N designed the study. A.J.D. performed all electrophysiology and imaging experiments, analyzed all data, and performed all computational modeling. N.D.K. provided

assistance and training on the 2P imaging protocol and contributed to data analysis. E.H performed confocal imaging. B.E. provided advice and feedback on computational modeling and developed the minimal recurrent circuit model. A.J.D. and K.I.N. wrote the paper with feedback from all authors.

Competing Interest Declaration

The authors declare no competing interests.

Materials and Correspondence

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Figure and Legends

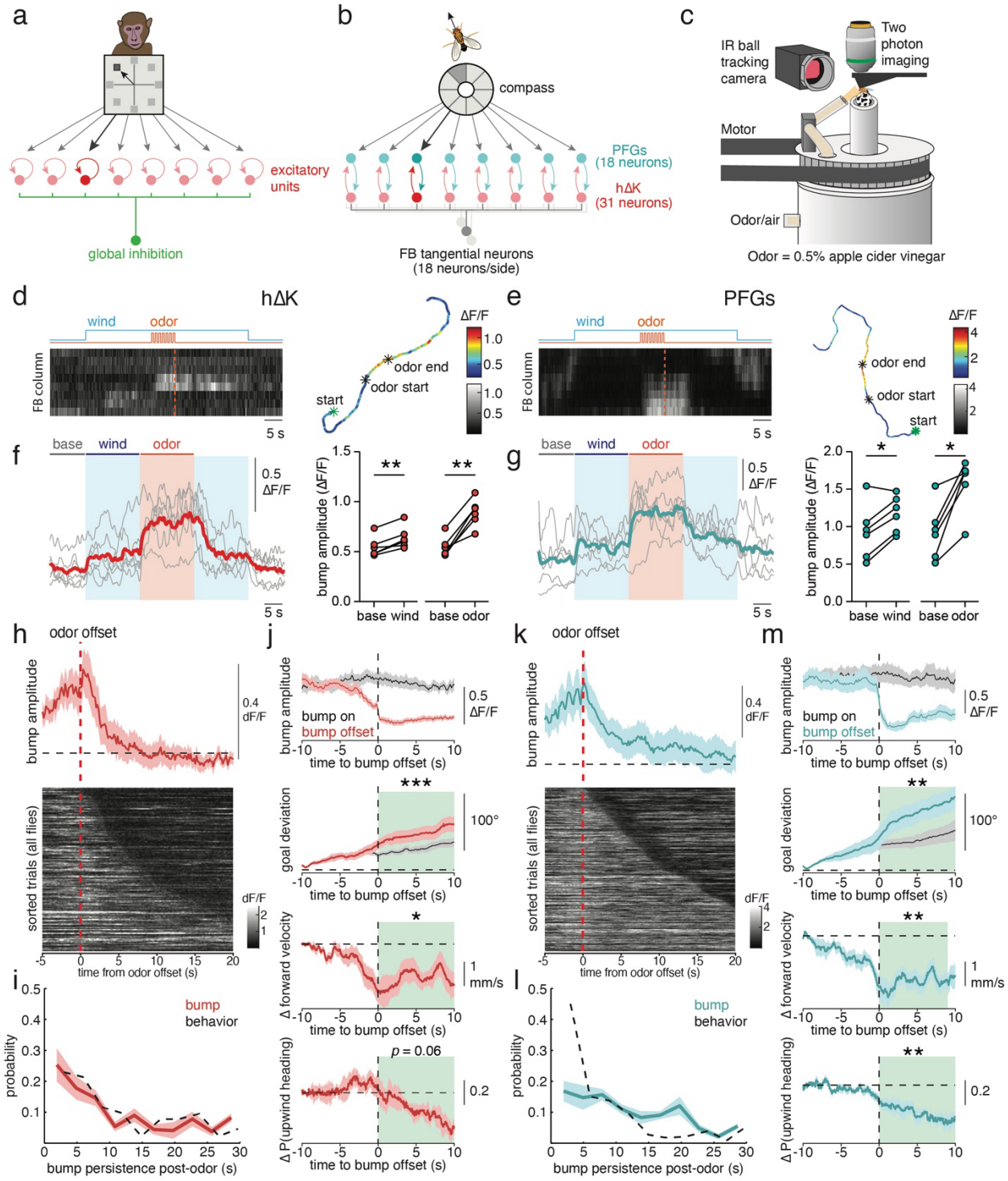

Fig 1: Recurrently connected hΔK and PFG neurons show similar encoding of odor and goal-directed behavior

Figure 1: Recurrently connected hΔK and PFG neurons show similar encoding of odor and goal-directed behavior.

a, Schematic of a recurrent network model developed to describe working memory activity in macaque prefrontal cortex (adapted from Compte et al.[1]). The recurrent network is composed of local recurrent excitation and global inhibition, and is structured to maintain a spatially-tuned direction signal over time. **b,** Schematic of the recurrent network architecture between hΔK and PFG neurons in the FB. hΔK and PFG neurons display local recurrent excitation along the FB, PFG neurons receive spatially structured inputs from the compass, and both hΔK and PFG neurons receive global inputs from ~18 FB tangential neurons that each provide at least 5% of tangential input to either hΔK or PFG neurons. **c,** Schematic of the *in vivo* imaging paradigm. Fly movement on an air-supported ball is tracked using IR light and an IR-sensitive camera. A tube that can deliver wind and 0.5% ACV rotates around the fly in closed-loop with the fly's heading. **d,** Example of hΔK bump activity in response to 7 pulses of ACV at 1 Hz. Left: activity shown as raw ΔF/F across FB columns as a function of time. Right: fictive 2D trajectory of the fly color-coded by bump amplitude. Note activity bump that persists after odor offset. **e,** Same as **d** but for PFG neurons. **f,** Mean bump amplitude of hΔK neurons during baseline, wind, and a 15s pulse of ACV. Left: mean activity for each fly (grey) and across flies (red) as a function of time. Right: average activity for each fly as a function of phase. Colored bars on the left plot indicate the windows for averaging for the right plot. **g,** Same as **f** but for PFG neurons. Individual flies in grey and average in blue. **h,** Bump amplitude of hΔK neurons aligned to odor offset for all stimuli. Top: mean bump amplitude aligned to odor offset (error = mean ± SEM); bottom: individual trials sorted by duration of persistent activity. Red dashed line indicates odor offset and black dashed line indicates mean bump amplitude before odor onset. **i,** Distributions of hΔK bump persistence time (red, error = mean ± SEM) and goal directed walking (black) following odor offset. **j,** Mean bump amplitude, goal deviation, change in forward velocity, and change in probability of upwind heading aligned to bump offset (red) or the center of bump periods (grey) (error = mean ± SEM). Green bars indicate the time bin for statistical evaluation: goal deviation compares red to gray curves, forward velocity and probability of upwind heading are compared to zero. **k-m,** Same as **h-j** but for PFG neurons.

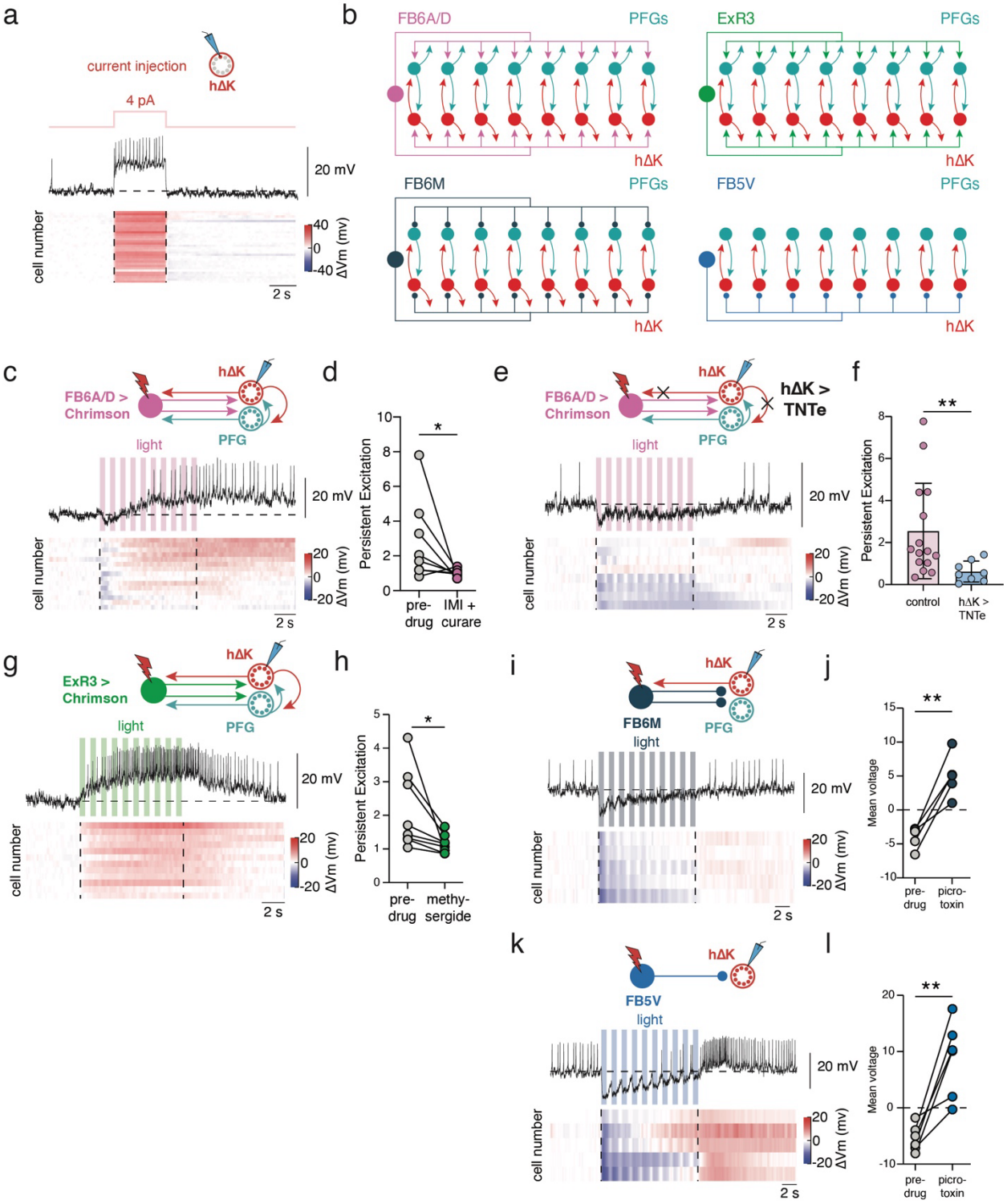

Fig 2: hΔK neurons receive slow excitation and fast inhibition from their tangential partners

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a, Spiking and membrane potential response of hΔK neurons (VT062617-lexA) during a 4 pA current injection. Top: example trace; bottom: heat map of the mean membrane potential response with each cell as a row. **b,** Schematic of connectivity between tangential inputs (pink: FB6A/D, green: ExR3, dark blue: FB6M, light blue: FB5V) and hΔK and PFG neurons. **c,** Voltage response of hΔK neurons to optogenetic activation of FB6A/D (65C03-Gal4). Same data format as **a**. **d,** Persistent excitation (see Methods) following activation of FB6A/D before and after application of the nicotinic acetylcholine receptor blockers IMI (100 nM) and curare (10 μM). **e,** Spiking and membrane potential response of hΔK neurons following optogenetic activation of FB6A/D when all hΔK neurons express tetanus toxin (TNTe) to block synaptic transmission. Same data format as **a**. **f,** Persistent excitation following activation of FB6A/D in control hΔK neurons (same data as **c**) and neurons expressing TNTe. **g,** Spiking and membrane potential response of hΔK neurons following optogenetic activation of ExR3 (24C07-gal4). Same data format as **a**. **h,** Persistent excitation following activation of ExR3 before and after application of the general serotonin receptor antagonist methysergide (50 μM). **i,** Spiking and membrane potential response of hΔK neurons following optogenetic activation of FB6M (49B08AD; VT063626DBD). Same data format as **a**. **j,** Mean voltage response of hΔK neurons during optogenetic stimulation of FB6M before and after adding picrotoxin (5 μM). **k,** Spiking and membrane potential responses of hΔK neurons following optogenetic activation of FB5V (36B06-gal4). Same data format as **a**. **j,** Mean voltage response of hΔK neurons during optogenetic stimulation of FB5V before and after adding picrotoxin (5 μM).

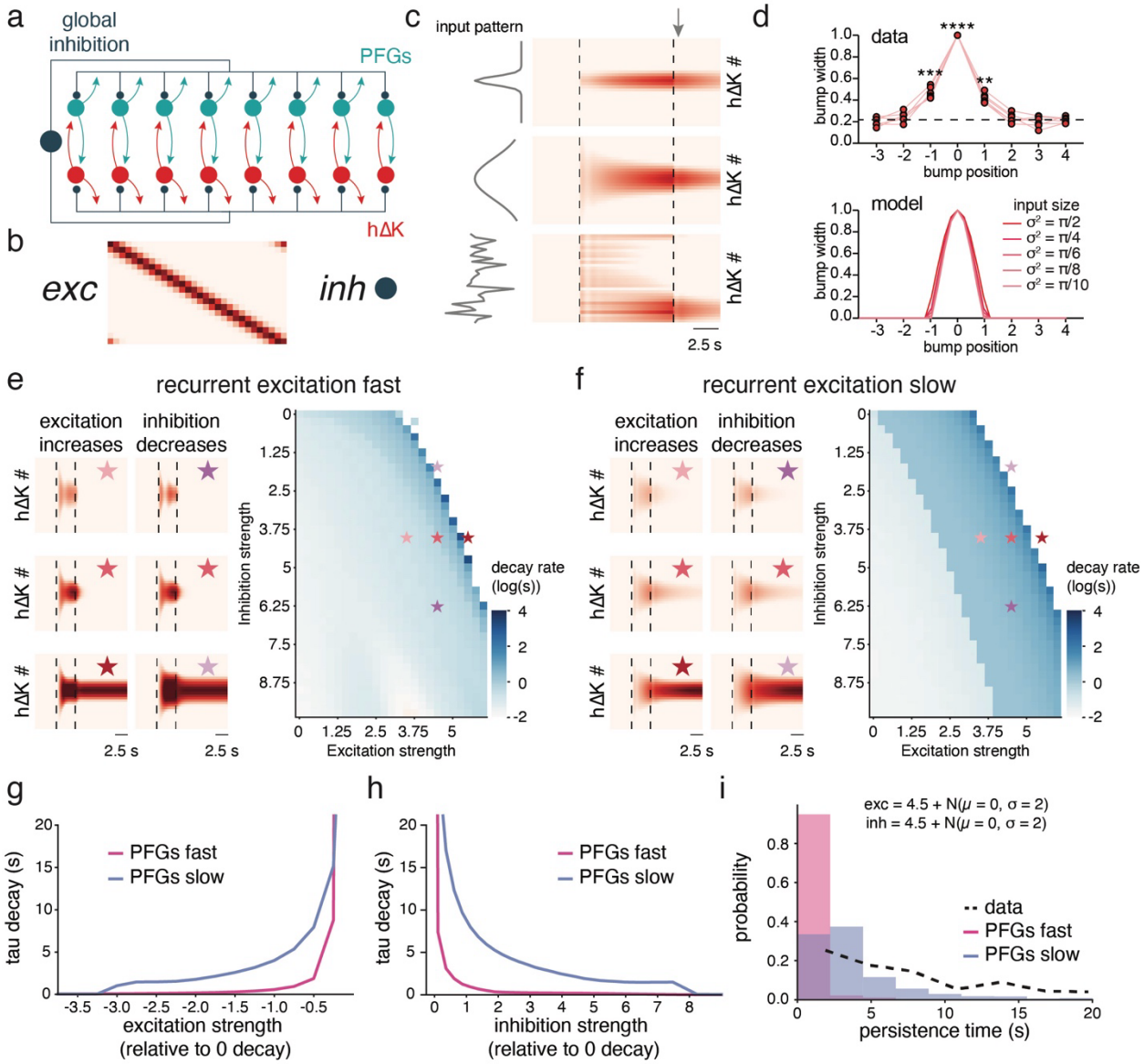

Fig 3: Slow recurrent excitation promotes more stable and tuneable attractor dynamics

Figure 3: Slow recurrent excitation promotes more stable and tuneable attractor dynamics.

a, Schematic of connectivity used in simulations. hΔK and PFG neurons are recurrently connected through ring recurrence and both populations are recurrently connected to a global inhibitory neuron. **b,** Schematic showing how the weight matrices are implemented in the model. The weight matrices between hΔK and PFG neurons are scaled by the parameter *exc* (excitation strength), while the connectivity of hΔK and PFG with the global inhibitory neuron are scaled by the parameter *inh* (inhibition strength). **c,** Simulations of hΔK activity in response to narrow Gaussian input ($\sigma^2 = \pi/2$), wide Gaussian input ($\sigma^2 = \pi/10$), or random input. Black dashed lines indicate when the inputs are on. Note the similarity in bump width following input

offset across all input shapes (arrow). **d,** hΔK bump width from calcium imaging data (top) or model simulations (bottom). Model simulations used Gaussian inputs with various widths. **e,** Simulations of hΔK activity using fast recurrent excitation across various excitation and inhibition strengths *exc* and *inh*. Right: decay rate ($\log_{10}$ scale) of the network following input offset as a function of *exc* and *inh*. White indicates zero network decay. Left: Example dynamics at various locations in parameter space (stars). **f,** Same as **e** but using slow recurrent excitation in PFG neurons (see Methods). **g,** Mean decay time (tau) of the network as a function of excitation strength using fast (pink) or slow (blue) recurrent excitation. Averages are composed of rows of **e** and **f**, respectively, each aligned to the smallest excitation strength that generated non-decaying persistence. **h,** Mean decay time (tau) of the network as a function of inhibition strength using fast (pink) or slow (blue) recurrent excitation. Averages are composed of the columns of **e** and **f** , respectively, each aligned to the largest inhibition strength that generated non-decaying persistence. **i,** Distributions of persistence times in the fast (pink) and slow (blue) models when the excitation and inhibition strengths are randomly drawn from a Gaussian distribution. Simulations that generated non-decaying persistence were excluded. For comparison, the persistence distribution of hΔK activity following odor offset from Fig. 1i is shown in black.

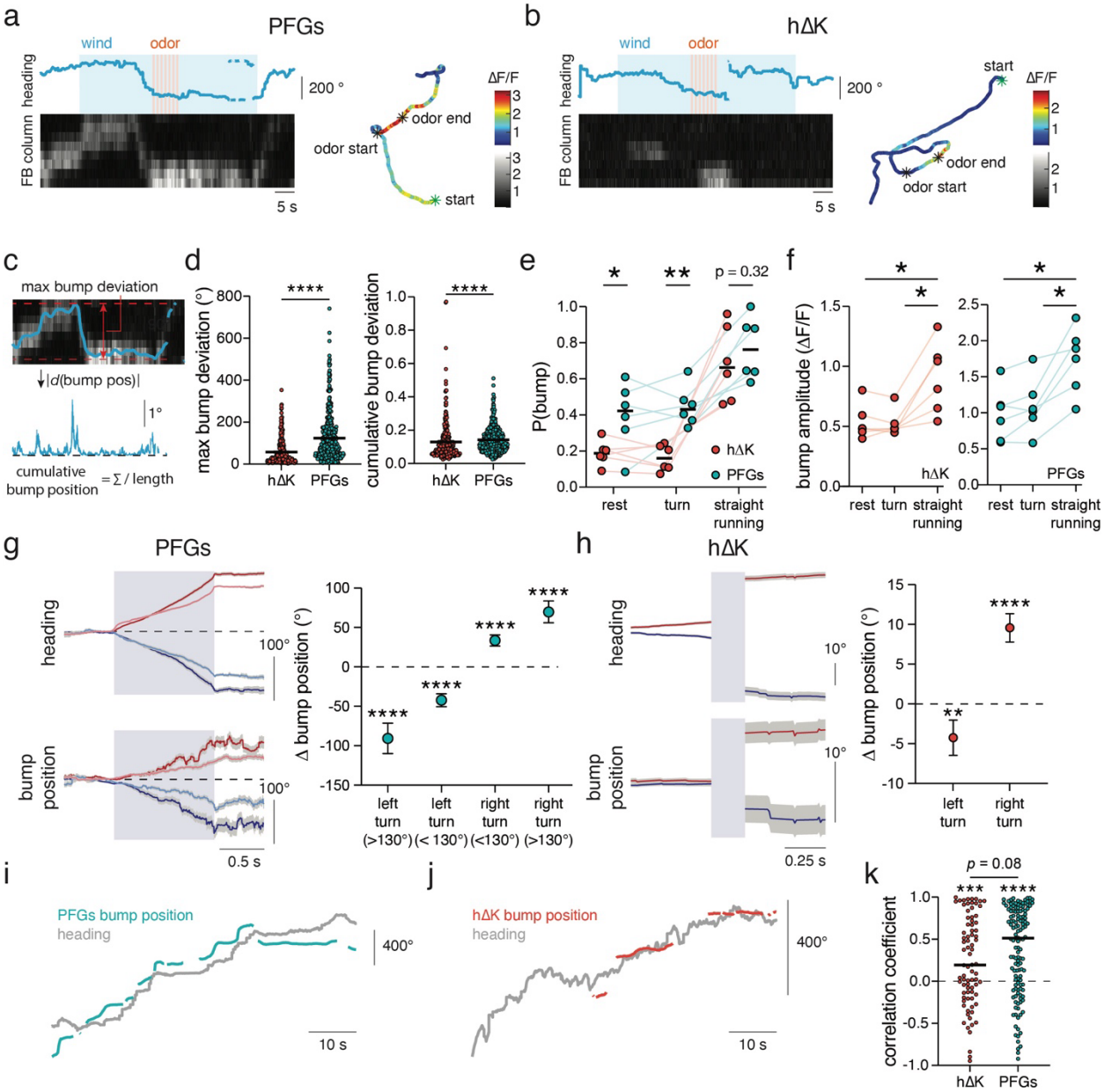

Fig 4: PFG and hΔK show divergent bump position dynamics

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a, Example PFG bump activity in response to 7 pulses of odor at 1 Hz. Left: activity shown as raw ΔF/F across FB columns as a function of time. Right: fictive 2D trajectory of the fly color-coded by bump amplitude. **b,** Same as **a** but for hΔK neurons. Note lateral bump movement in PFG but not in hΔK. **c,** Schematic computation of max and cumulative bump deviation. For max, bump position is unwrapped and maximum difference in bump position is calculated. For cumulative, total change in bump position is summed across time steps and normalized by the bump duration. **d,** Max bump deviation (left) and cumulative bump deviation (right) for all hΔK bumps (red) and PFG bumps (blue). **e,** Bump probability for hΔK (red) and PFG (blue) neurons

across rests, turns, and straight runs (see Methods). **f,** Bump amplitude for hΔK (left) and PFG (right) neurons across rests, turns, and straight runs. **g,** Left, mean heading and PFG bump position during large right turns (dark red, Δ heading > 130°), small right turns (light red, 130° > Δ heading > 90°), small left turns (light blue, -90° > Δ heading > -130°), or large left turns (dark blue, -130° < Δ heading). Right, average shifts in PFG bump position across these turns. Positive shifts in bump position correspond with leftward shifts across the FB, and vice versa. **h,** Left, mean heading and hΔK bump position across subsequent bumps separated by right (red, 0° < Δ heading) or left (blue, 0° > Δ heading) turns. Right, average shifts in hΔK bump position across these turns. **i,** Example of the PFG bump position aligned to the corresponding heading of the fly. **j,** Same as **i** but for hΔK neurons. **k,** Correlation coefficients between heading and hΔK (red) or PFG (blue) bump position across trials.

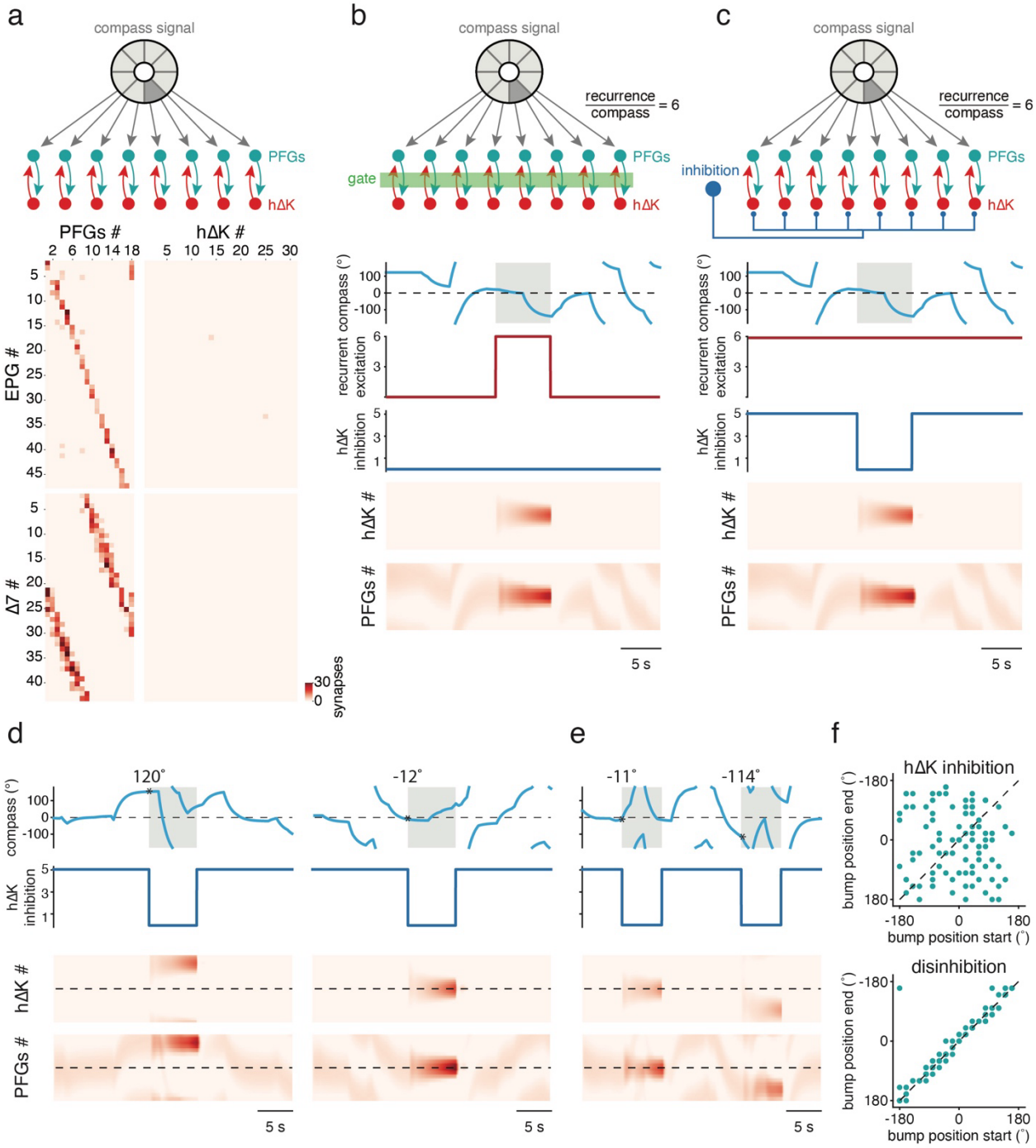


Fig. 5: Disinhibition of hΔK allows for rapid gating of recurrent attractor dynamics

Figure 5: Disinhibition of hΔK allows for rapid gating of recurrent attractor dynamics.

a, Top, schematic of the connectivity between the compass system of the ellipsoid body and the hΔK-PFG recurrent network. Bottom, synaptic weights from heading-tuned EPG and Δ7 neurons to PFG (left) and hΔK (right) taken from the hemibrain connectome. hΔK and PFG neurons are sorted by their position along the FB, while EPGs are sorted by their position along the EB and

Δ7 neurons are sorted by their position along the protocerebral bridge. **b,** Simulations of hΔK and PFG population activity when the EPG compass signal (light blue) is randomly and stochastically updated and recurrent excitation strength (red) is transiently gated from OFF to ON (top). In these simulations, the strength of recurrent synapses relative to compass inputs is 6:1. Note higher amplitude stable bump in the presence of recurrent excitation. **c,** Same as **b** but instead of directly modulating recurrent excitation, external global inhibition of hΔK neurons (dark blue) is transiently shut off. Note higher amplitude stable bump during disinhibition. **d,** Further examples of the simulation shown in **c** but where the compass signal at the start of disinhibition varies. Note that the stable hΔK and PFG bump positions depend on these initial compass signals (left: 120°, right: -12°). **e,** Same type of simulation as **c** and **d** but with two bouts of disinhibition separated by a short time interval. Note that each disinhibition bout stabilizes the hΔK and PFG bumps to a different position that depend on the location of the compass input at the time of disinhibition (first: -11°, second: -114°). **f,** Bump position at the beginning and end of a period of hΔK inhibition (top) or disinhibition (bottom) as the compass input randomly fluctuates.

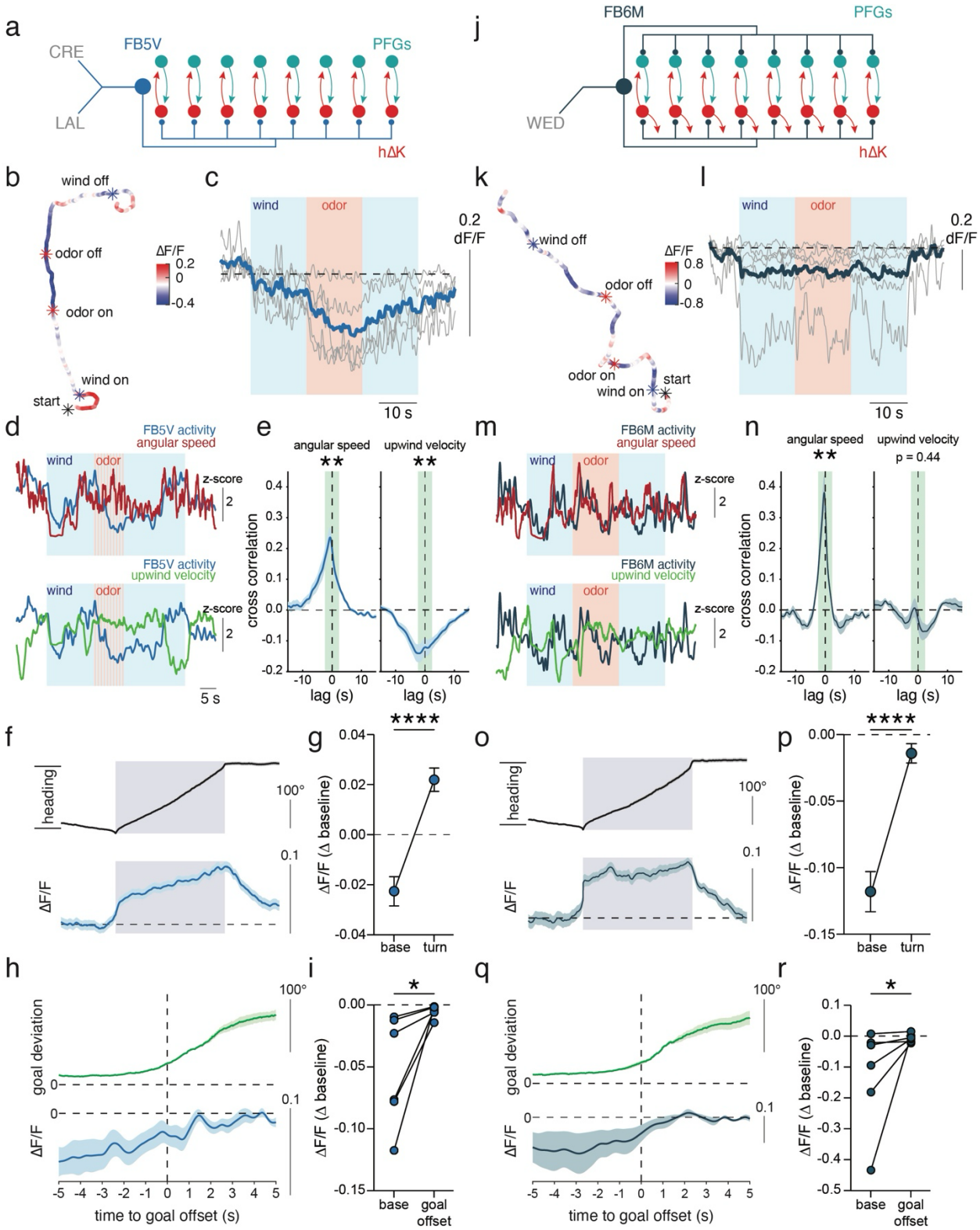


Fig. 6: Inhibitory inputs to hΔK show signatures of gating

Figure 6: Inhibitory inputs to hΔK show signatures of gating.

a, Schematic of connectivity between FB5V neurons and the hΔK-PFG recurrent network. Major brain regions innervated by dendrites of FB5V are shown in grey. **b,** Example walking trajectory color coded by activity in FB5V ΔF/F (baseline subtracted). Note inhibition during odor that persists during continued upwind walking. **c,** Mean ΔF/F (baseline subtracted) of FB5V during baseline, wind, and 15s of ACV. Individual flies in grey, average across flies in blue. **d,** Example of FB5V ΔF/F (blue), angular speed (red), and upwind velocity (green) in response to wind and 10 pulses of ACV at 1 Hz. All data z-scored. **e,** Cross correlation between FB5V ΔF/F and angular speed (left) or upwind velocity (right). Statistics correspond to the average cross correlation from -2 to 2 s, shown in green. **f,** Mean heading (absolute value and baseline subtracted) and FB5V ΔF/F during large (>130°) turns. Error = mean ± SEM. **g,** Mean FB5V ΔF/F before and at the end of the turns isolated in **f** (error = mean ± SEM). **h,** Deviation from goal direction and FB5V ΔF/F aligned to the offset of goal-directed walking following odor offset (see Methods, error = mean ± SEM). **i,** Mean FB5V ΔF/F during goal-directed walking (base) and following the offset of goal-directed walking. **j-r,** Same as **a-i**, respectively, but for FB6M.

Table 3: Statistics for main figures. Significant p values are bolded.

Figure	Comparison	Sample size	Statistical test	p value
Fig. 1f	base vs wind	N=6 flies n = 4-9 trials/fly	Paired t-test	**0.0027**
	base vs odor	N=6 flies n = 4-9 trials/fly	Paired t-test	**0.0006**
Fig. 1g	base vs wind	N=6 flies n = 6-11 trials/fly	Paired t-test	**0.0128**
	base vs odor	N=6 flies n = 6-11 trials/fly	Paired t-test	**0.0053**
Fig. 1h	Average bump amplitude	N = 6 flies n = 39-49 trials/fly	N/A	N/A
Fig. 1i	persistence distributions	N = 6 flies n = 13-25 trials/fly	N/A	N/A
Fig. 1j	goal deviation bump vs offset	N = 6 flies n = 13-23 trials/fly	Paired t-test	**0.0005**
	forward velocity compared to 0	N = 6 flies n = 13-23 trials/fly	One-sample t-test	**0.0367**
	P(upwind heading) compared to 0	N = 6 flies n = 13-23 trials/fly	One-sample t-test	0.0614
Fig. 1k	Average bump amplitude	N = 6 flies n = 49-59 trials/fly	N/A	N/A
Fig. 1l	persistence distributions	N = 6 flies n = 11-48 trials/fly	N/A	N/A
Fig. 1m	goal deviation bump vs offset	N = 6 flies n = 10-48 trials/fly	Paired t-test	**0.0091**
	forward velocity compared to 0	N = 6 flies n = 10-48 trials/fly	One-sample t-test	**0.0030**
	P(upwind heading) compared to 0	N = 6 flies n = 10-48 trials/fly	One-sample t-test	**0.0091**
Fig. 2d	pre-drug vs IMI + curare	N = 8 cells $n_{pre\text{-}drug}$ = 6-10 trials/cell n_{IMI_curare} = 1-16 trials/cell	Wilcoxon matched-pairs signed rank test	**0.0391**
Fig. 2f	control vs hΔK>TNTe	$N_{control}$ = 15 cells $n_{control}$ = 5-10 trials/cell N_{TNTe} = 8 cells n_{TNTe} = 1-9 trials/cell	Mann Whitney signed-rank test	**0.0032**
Fig. 2h	pre-drug vs methysergide	N = 8 cells $n_{pre\text{-}drug}$ = 5-8 trials/cell $n_{methysergide}$ = 5-9 trials/cell	Paired t-test	**0.0367**

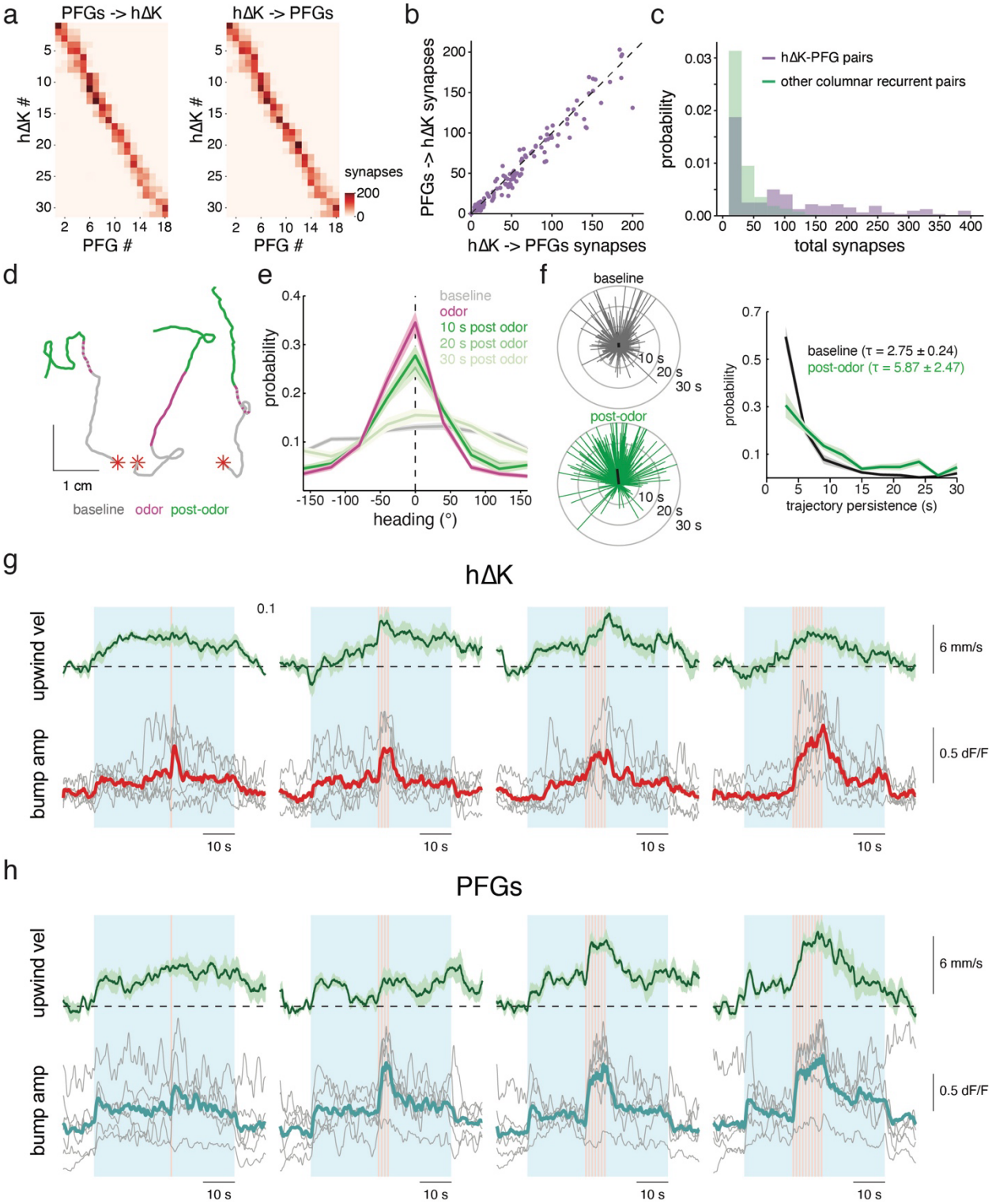
Fig. 2j	pre-drug vs picrotoxin	N = 5 cells $n_{pre\text{-}drug}$ = 5-10 trials/cell $n_{picrotoxin}$ = 2-6 trials/cell	Paired t-test	**0.0039**
Fig. 2l	pre-drug vs picrotoxin	N = 6 cells $n_{pre\text{-}drug}$ = 5-7 trials/cell $n_{picrotoxin}$ = 3-13 trials/cell	Paired t-test	**0.0049**
Fig. 3d	Columns vs 0	N = 6 flies n = 39-49 trials/fly	One-way ANOVA with multiple comparisons	**Main effect of column:** ***p* < 0.0001** **Column 4 vs -3:** ***p* = 0.9410** **Column 4 vs -2:** ***p* = 0.7244** **Column 4 vs -1:** ***p* = 0.0006** **Column 4 vs -0:** ***p* < 0.0001** **Column 4 vs 1:** ***p* = 0.0013** **Column 4 vs 2:** ***p* = 0.9018** **Column 4 vs 3:** ***p* = 0.9588**
Fig. 4d	Max bump deviation hΔK vs PFGs	$N_{h\Delta K}$ = 281 bumps N_{PFG} = 434 bumps	Mann Whitney signed-rank test	**< 0.0001**
	Cumulative bump deviation hΔK vs PFGs	$N_{h\Delta K}$ = 281 bumps N_{PFG} = 434 bumps	Mann Whitney signed-rank test	**< 0.0001**
Fig. 4e	hΔK vs PFGs	hΔK: N = 6 flies n_{rest} = 6-73 rests/fly n_{turn} = 57-76 turns/fly $n_{straight_run}$ = 2-93 runs/fly	Two-way analysis of variance with multiple comparisons	**Main effect of behavioral stage:** ***p* < 0.0001** **Main effect of genotype:** ***p* = 0.0004**

		PFGs: N = 6 flies n_{rest} = 47-87 rests/fly n_{turn} = 24-181 turns/fly $n_{straight_run}$ = 7-75 runs/fly		Interaction: p = 0.2578 **Rest hΔK vs PFGs:** **p = 0.0197** **Turn hΔK vs PFGs:** **p = 0.0020** Surge hΔK vs PFGs: p = 0.3155
Fig. 4f	hΔK across behavioral stage	N = 6 flies n_{rest} = 6-73 rests/fly n_{turn} = 57-76 turns/fly $n_{straight_run}$ = 2-93 runs/fly	Friedman test with multiple comparisons	**Main effect of behavioral stage:** **p = 0.0081** rest vs turn: $p > 0.9999$ **rest vs straight running:** **p = 0.0281** **turn vs straight running:** **p = 0.0281**
	PFGs across behavioral stage	N = 6 flies n_{rest} = 47-87 rests/fly n_{turn} = 24-181 turns/fly $n_{straight_run}$ = 7-75 runs/fly	One-way analysis of variance with multiple comparisons	**Main effect of behavioral stage:** **p = 0.0038** rest vs turn: $p > 0.0986$ **rest vs straight running:** **p = 0.0149** **turn vs straight running:** **p = 0.0176**
Fig. 4g	PFG across turns	Left turn (>130°) N = 71 turns Left turn (<130°) N = 50 turns	Wilcoxon signed rank test	**Left turn (>130°):** **$p < 0.0001$** **Left turn (<130°):** **$p < 0.0001$**

		Right turn (<130°) N = 140 turns Right turn (>130°) N = 117 turns		**Right turn (<130°):** ***p* < 0.0001** **Right turn (>130°):** ***p* < 0.0001**
Fig. 4h	hΔK across turns	Left turns: N = 476 turns Right turns: N = 696 turns	circ_medtest from Circular Statistics Toolbox (test significance from median = 0)	**Left turns:** ***p* = 0.0089** **Right turns:** ***p* < 0.0001**
Fig. 4k	hΔK vs PFGs vs 0	$N_{h\Delta K}$ = 80 trials N_{PFGs} = 133 trials	Mann Whitney signed-rank test and Wilcoxon signed rank test	hΔK vs PFGs: *p* = 0.0837 **hΔK vs 0:** ***p* < 0.0003** **PFGs vs 0:** ***p* < 0.0001**
Fig. 6c	mean ΔF/F	N = 6 flies n = 8-13 trials/fly	N/A	N/A
Fig. 6e	angular speed cross correlation	N = 6 flies n = 39-65 trials/fly	One-sample t-test	**0.0019**
	upwind velocity cross correlation	N = 6 flies n = 39-65 trials/fly	One-sample t-test	**0.0081**
Fig. 6f	mean lheadingl and ΔF/F	N = 322 turns	N/A	N/A
Fig. 6g	base vs turn	N = 322 turns	Wilcoxon matched-pairs signed rank test	**< 0.0001**
Fig. 6h	mean goal deviation and ΔF/F	N = 6 flies n = 39-65 trials/fly	N/A	N/A
Fig. 6i	base vs goal offset	N = 6 flies n = 39-65 trials/fly	Paired t-test	**0.0423**
Fig. 6l	mean ΔF/F	N = 6 flies n = 4-12 trials/fly	N/A	N/A
Fig. 6n	angular speed cross correlation	N = 6 flies n = 26-56 trials/fly	One-sample t-test	**0.0039**
	upwind velocity cross correlation	N = 6 flies n = 26-56 trials/fly	One-sample t-test	0.4382
Fig. 6o	mean lheadingl and ΔF/F	N = 322 turns	N/A	N/A
Fig. 6p	base vs turn	N = 180 turns	Wilcoxon matched-pairs signed rank test	**< 0.0001**

Fig. 6q	mean goal deviation and ΔF/F	N = 6 flies n = 26-56 trials/fly	N/A	N/A
Fig. 6r	base vs goal offset	N = 6 flies n = 26-56 trials/fly	Wilcoxon matched-pairs signed rank test	**0.0312**

Extended Data



Ext. Data Fig. 1: Additional data on connectomics, behavior and neural responses of hΔK and PFG

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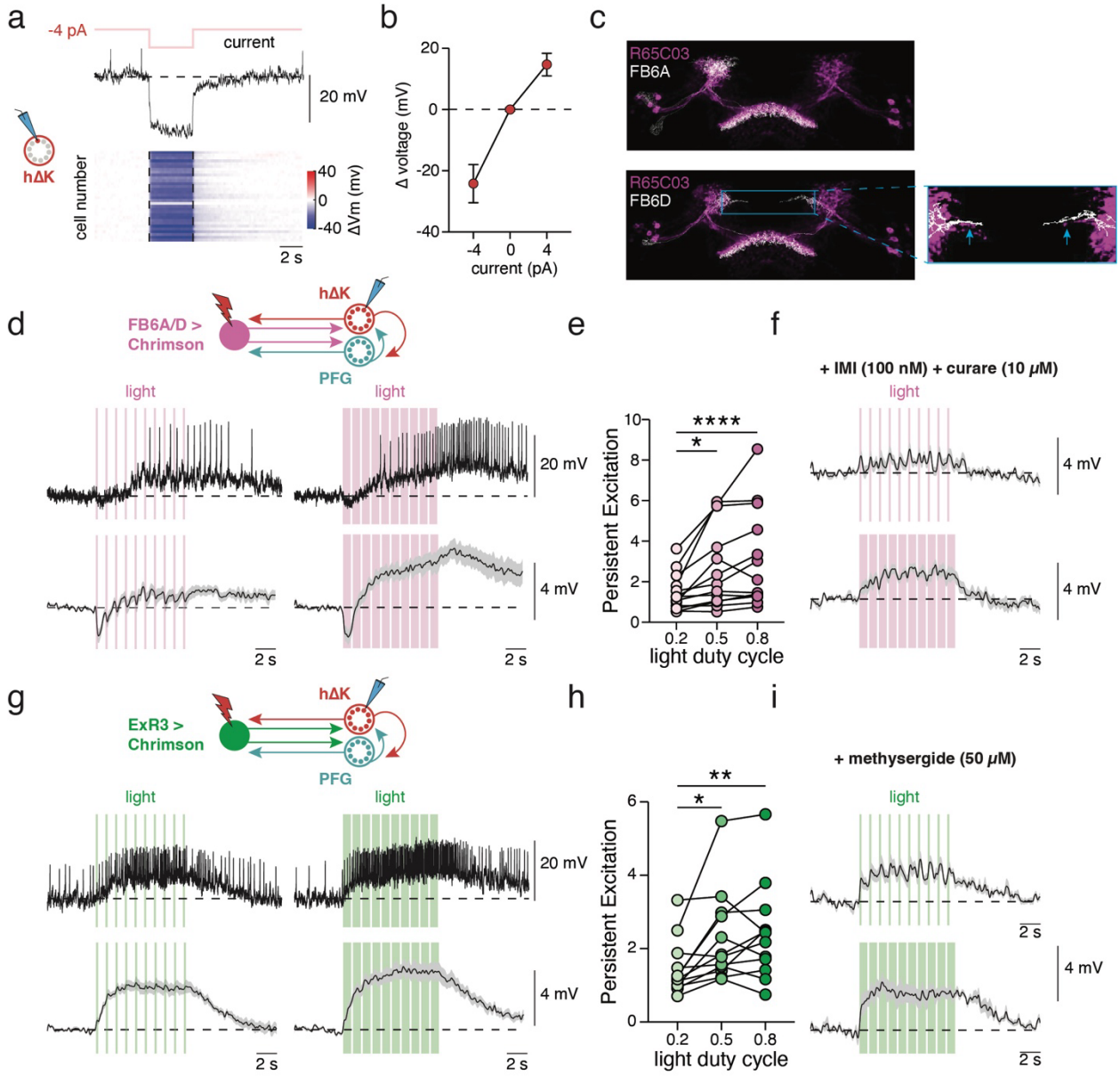
a, Synaptic weights from PFG to hΔK neurons (left) and hΔK to PFG neurons (right) taken from the hemibrain connectome. Both hΔK and PFG neurons are sorted by their axon position along the FB. **b,** Comparison of the reciprocal synaptic weights between hΔK and PFG neurons. Each dot represents a pair of hΔK and PFG neurons. **c,** Distributions of the total synaptic weight between pairs of recurrently-connected columnar neurons in the central complex. Data is separated for hΔK-PFG pairs (purple) and other columnar pairs (green), which includes all EPG, Δ7, PFN, PFL, PFR, FC, FS, FR, hΔ, and vΔ neurons. **d,** Example virtual trajectories of walking flies before (grey), during (pink), and following (green) odor. Note how flies often maintain their walking direction following odor offset (middle, right). **e,** Distribution of heading before wind/odor (baseline), during odor, 10 s after odor, 20 s after odor, and 30 s after odor (error = mean ± SEM). **f,** Left, vectors representing the direction (angle) and duration (length) flies walk following trial start (top, baseline) or odor offset (bottom). Right, distributions of the persistence of walking trajectories following trial start (baseline) or odor offset. Note, if flies maintained their direction for long enough following trial start, wind and odor were turned on, leading to the slight bias towards upwind in baseline. **g,** Mean upwind velocity and hΔK bump amplitude during baseline (no stimuli), wind, and pulses (1, 4, 7, or 10) of odor. Individual fly averages in grey; average across flies in red. Shaded regions are mean ± SEM**. h,** Same as **g** but for PFG neurons. Individual fly averages in grey; average across flies in blue.



Ext. Data Fig. 2: Additional data on excitatory inputs to hΔK

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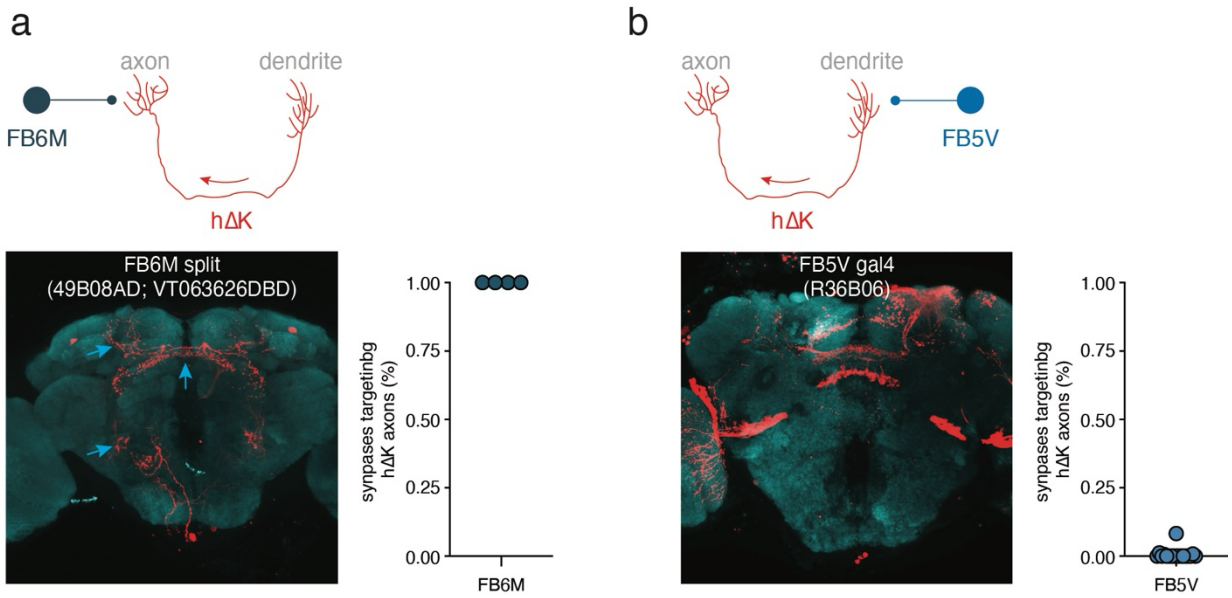
a, Voltage response of hΔK neurons (VT062617-lexA) during a -4 pA current injection. Top: example trace, bottom: heat map of mean membrane potential responses with each cell as a row. **b,** Mean membrane potential responses of hΔK neurons during -4pA, 0pA, or 4pA current injections. **c,** Overlay of R65C03 GAL4 line (image from Flylight[72]) and FB6A (top) or FB6D (bottom) skeletons from the hemibrain connectome, all warped to the Janelia female template brain[73]. The dendrites of FB6A and FB6D overlap strongly with the R65C03 stain. The right shows that R65C03 contains the medial-projecting processes that are unique to FB6D (blue arrows). Note that R65C03 contains multiple tangential neurons (around 17 per side), which overlaps with several other layer 6 neurons, such as FB6C_a, FB6C_b, FB6E, FB6G, FB6I, and

FB6Z. **d,** Spiking and membrane potential responses of hΔK neurons to optogenetic activation of FB6A/D (65C03-gal4) using pulsed light (1 Hz) at 0.2 (left) or 0.8 (right) duty cycle. **e,** Mean persistent excitation (see Methods) following activation of FB6A/D at 0.2, 0.5, or 0.8 duty cycle. Data for 0.5 duty cycle are from Fig. 2c. **f,** Mean membrane potential responses of hΔK neurons in response to optogenetic activation of FB6A/D using pulsed light (1 Hz) at 0.2 (top) or 0.8 (bottom) duty cycle in the presence of IMI (100 nM) and curare (10 μM). **g,** Same as **d** but in response to activating ExR3 neurons (24C07-gal4). **h,** Same as **e**, but in response to activating ExR3 neurons. Data for 0.5 duty cycle are from Fig. 2g. **i,** Mean membrane potential of hΔK neurons in response to optogenetic activation of ExR3 using pulsed light (1 Hz) at 0.2 (top) or 0.8 (bottom) duty cycle in the presence of the general serotonin receptor antagonist methysergide (50 μM).



Ext. Data Fig. 3: Additional data on inhibitory inputs to hΔK

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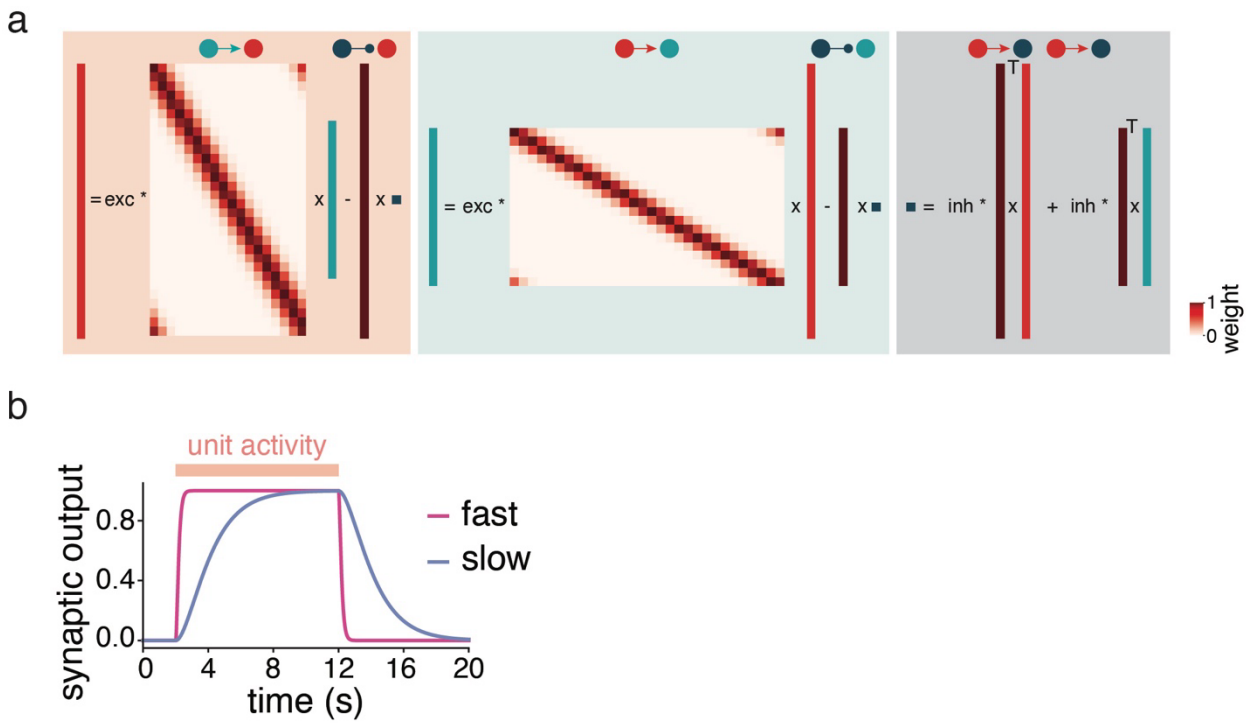
a, Top, schematic showing axo-axonic connectivity between FB6M and hΔK neurons. Bottom, left, GFP staining of the line 49B08AD; VT063626DBD, which targets FB6M. Arrows indicate characteristic morphological features of FB6M, including FB axons, ventral WED dendrites, and dorsal SMP dendrites. Bottom, right, percent of FB6M synapses targeting hΔK axons. Each data point corresponds with one FB6M neuron. **b,** Top, schematic showing axo-dendritic connectivity between FB5V and hΔK neurons. Bottom, left, GFP staining of the line R36B06, which targets FB5V, among other neurons. Bottom, right, percent of FB5V synapses targeting the hΔK axons. Each data point corresponds with one FB5V neuron.



Ext. Data Fig. 4: Methods details for recurrent model of hΔK and PFG

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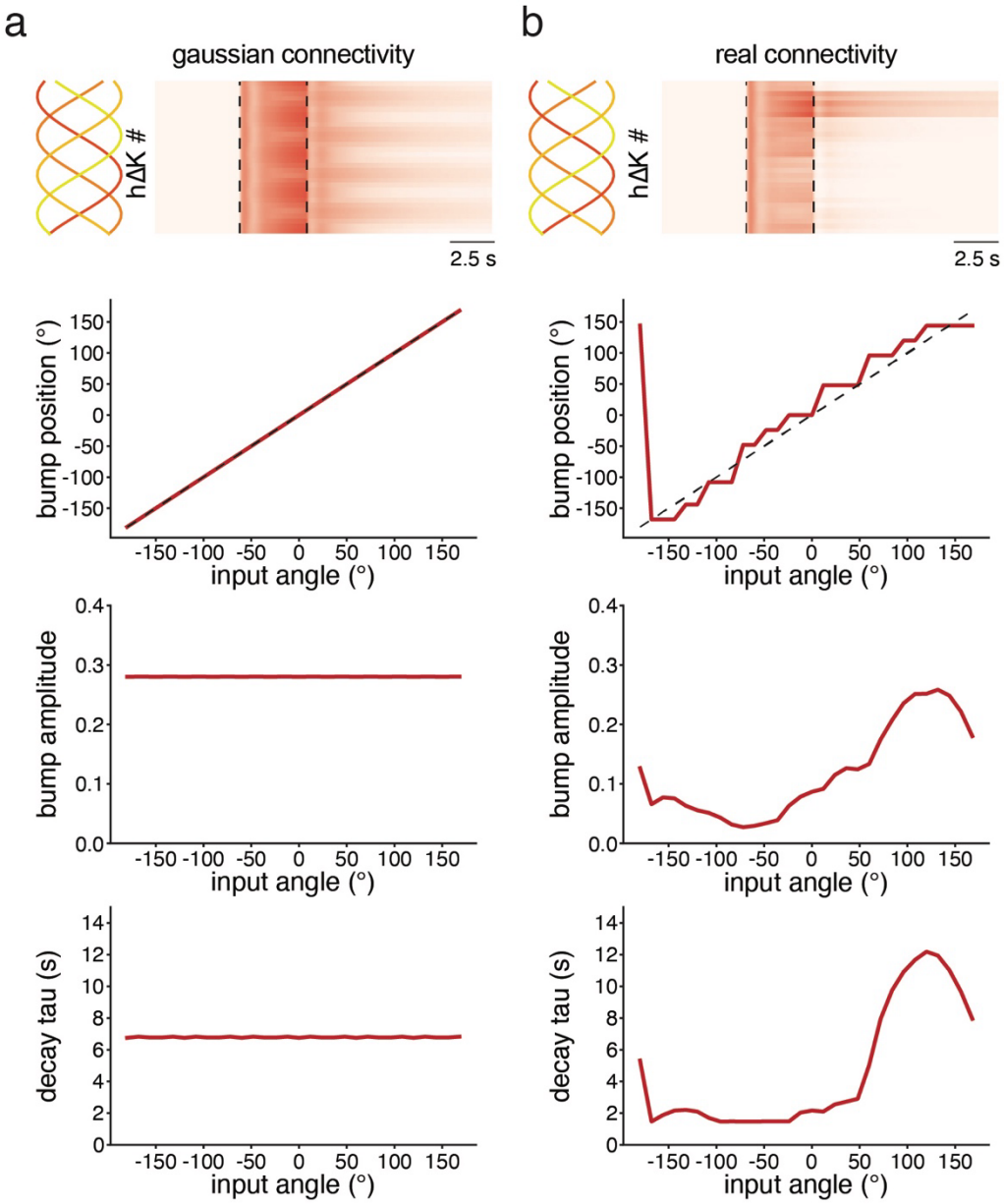
a, Schematic demonstrating how activity of hΔK (red), PFGs (turquoise blue), and global inhibition (dark blue) neurons interact in the model. hΔK activity is the sum of the PFG activity matrix multiplied by the PFG > hΔK weight matrix, scaled by the excitation strength *E*, and the global inhibition activity matrix multiplied by the inhibition>hΔK weight matrix. Similarly, PFG activity is the sum of the hΔK activity matrix multiplied by the hΔK>PFG weight matrix, scaled by the excitation strength *E*, and the global inhibition activity matrix multiplied with the inhibition>PFG weight matrix. Finally, global inhibition is the sum of the hΔK activity matrix multiplied with the hΔK>inhibition weight matrix and the PFG activity matrix multiplied with the PFG>inhibition weight matrix, all scaled by the inhibition strength *I*. **b,** Synaptic output of model neurons using fast (pink) or slow (blue) synaptic speeds in response to a prolonged step of activity.

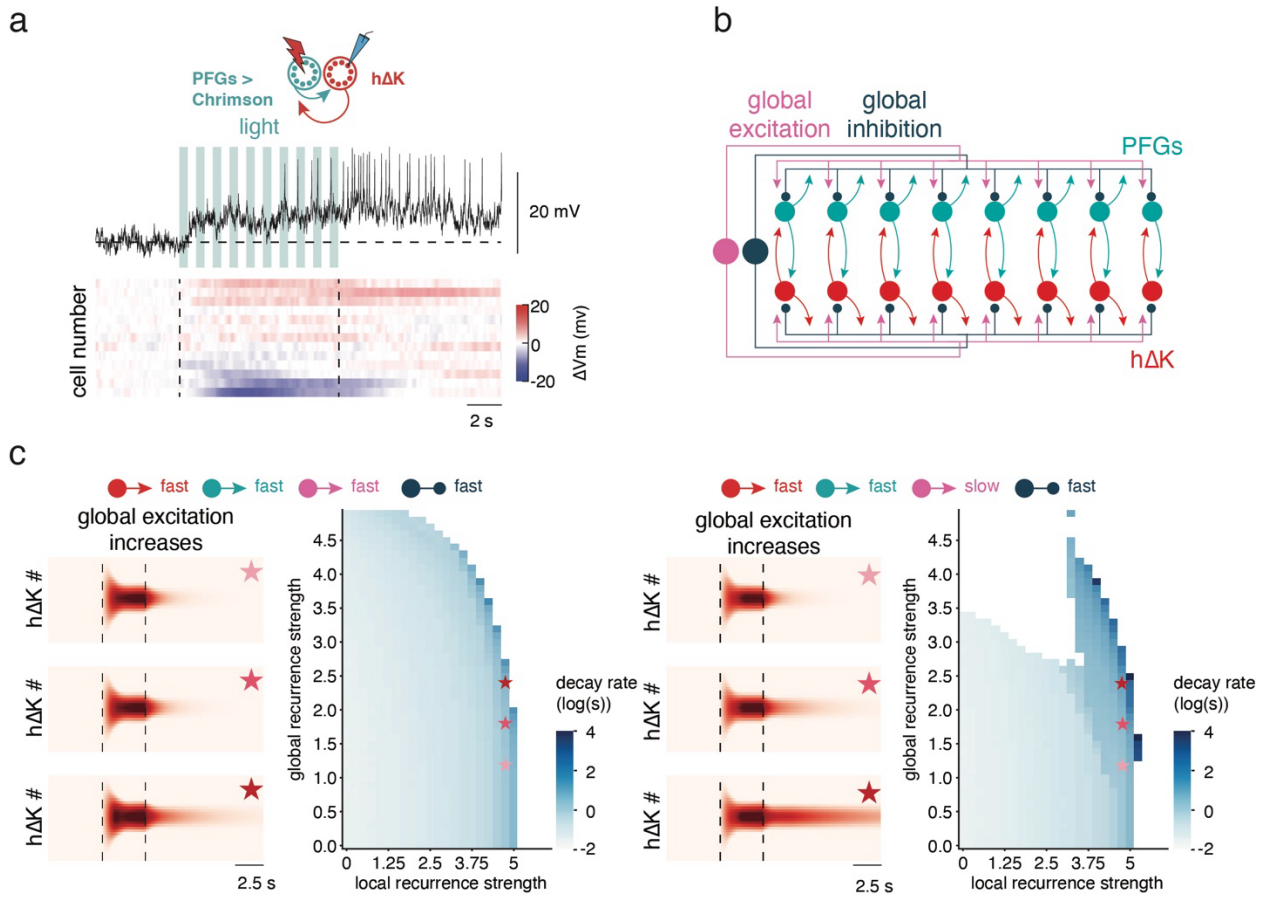


Ext. Data Fig. 5: A model with real connectivity between PFG and hΔK shows inhomogeneity in bump amplitude and duration

Ext. Data Fig. 5: A model with real connectivity between PFG and hΔK can form a bump at most locations but shows inhomogeneity in bump amplitude and duration.

a, hΔK bump position, bump amplitude, and decay rate (tau) in response to sinusoidal input across all possible phases when the connectivity between hΔK and PFG neurons is approximated by a Gaussian model. Top heatmap shows four separate simulations, all overlayed, in response to the sinusoidal inputs shown. **b,** Same as **a** but using the real connectivity between hΔK and PFG neurons from the hemibrain connectome (see Ext. Data Fig. 1a). Note that bump position can be stabilized at most of the same position as with the

Gaussian model, however bump amplitude and decay time constant depend strongly on position.



Ext. Data Fig. 6: Slow feedback through tangential neurons can also stabilize recurrent attractor dynamics

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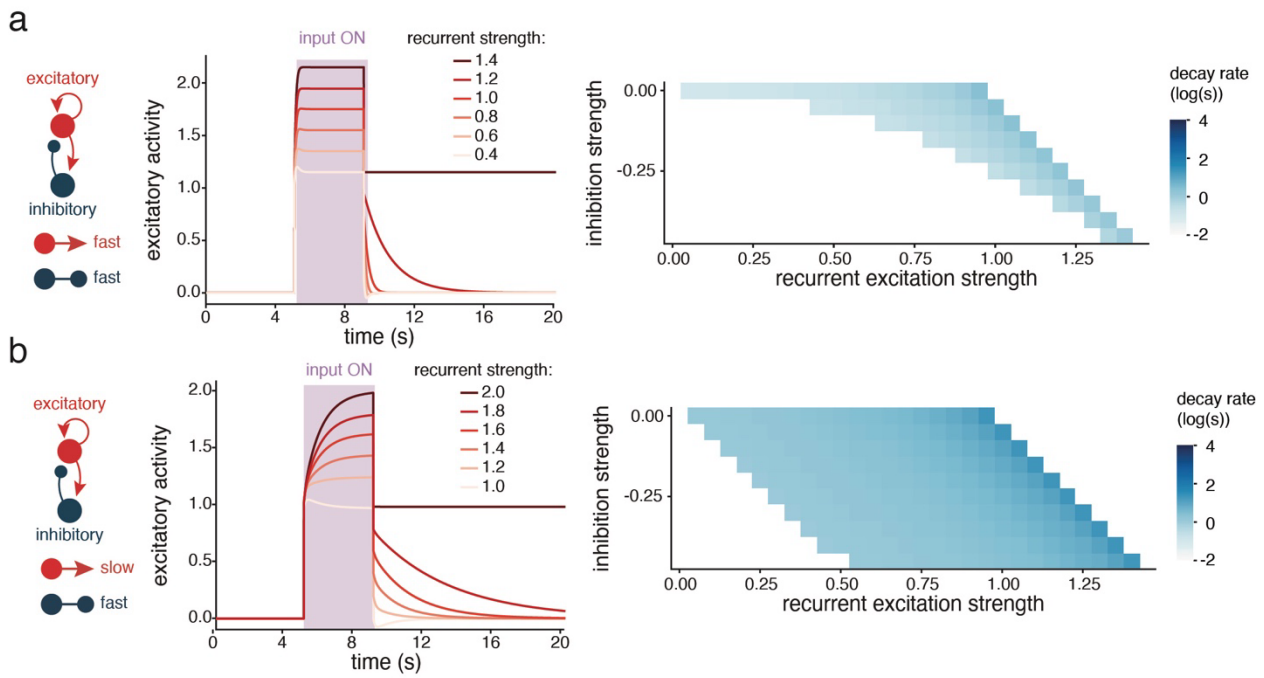
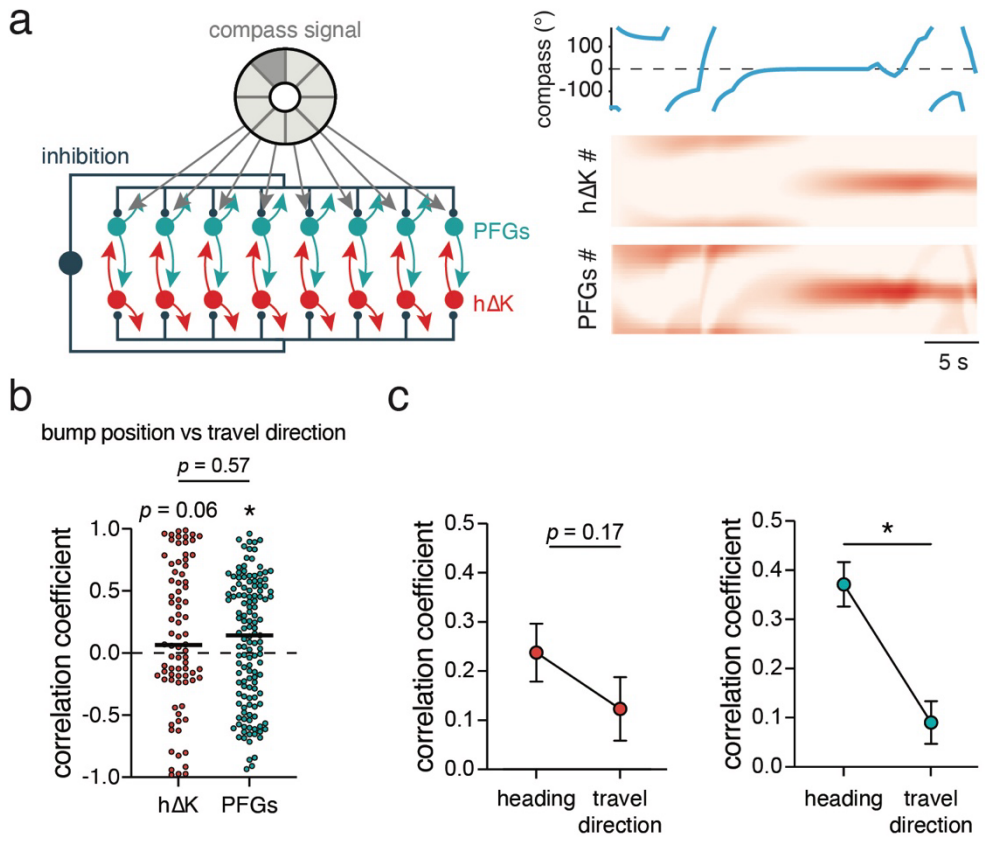
a, Voltage responses of hΔK neurons to optogenetic activation of all PFG neurons (SS52590 split) using pulsed light (1 Hz, 0.5 duty cycle). Top: example trace, bottom: heat map of the mean membrane potential responses with each cell as a row. **b,** Schematic of connectivity with a recurrent excitatory tangential neuron. hΔK and PFG neurons are recurrently connected through ring recurrence and both populations are recurrently connected to a global inhibitory neuron and a global excitatory neuron. **c,** Simulations of hΔK activity using fast recurrent excitation and fast global excitation (left) or fast recurrent excitation and slow global excitation (right) across various local and global excitation strengths. The parameter spaces show the decay rate ($\log_{10}$ scale) of the network following input offset as a function of local and global excitation strength. White indicates zero network decay or oscillations. Specific examples from the parameter space are shown at left (stars). Note, the white chunk missing in the slow global excitation parameter space (right) corresponds with parameter combinations that lead to strong oscillations in hΔK and PFG neurons.



Ext. Data Fig. 7: Slow excitation stabilizes persistent activity in simple recurrent networks.

Ext. Data Fig. 7: Slow excitation stabilizes persistent activity in a minimal recurrent network.

a, Left, schematic of a simple two-unit recurrent network using fast excitation and slow excitation. Middle, activity of excitatory unit in response to inputs as a function of recurrent excitation strength. Right, parameter space showing the decay rate ($\log_{10}$ scale) of the excitatory unit following input offset as a function of recurrent excitation and inhibition strength. **b,** Same as **a** but the excitatory unit uses slow synaptic signaling.



Ext. Data Fig. 8: Additional data on differences in bump dynamics between PFG and hΔK

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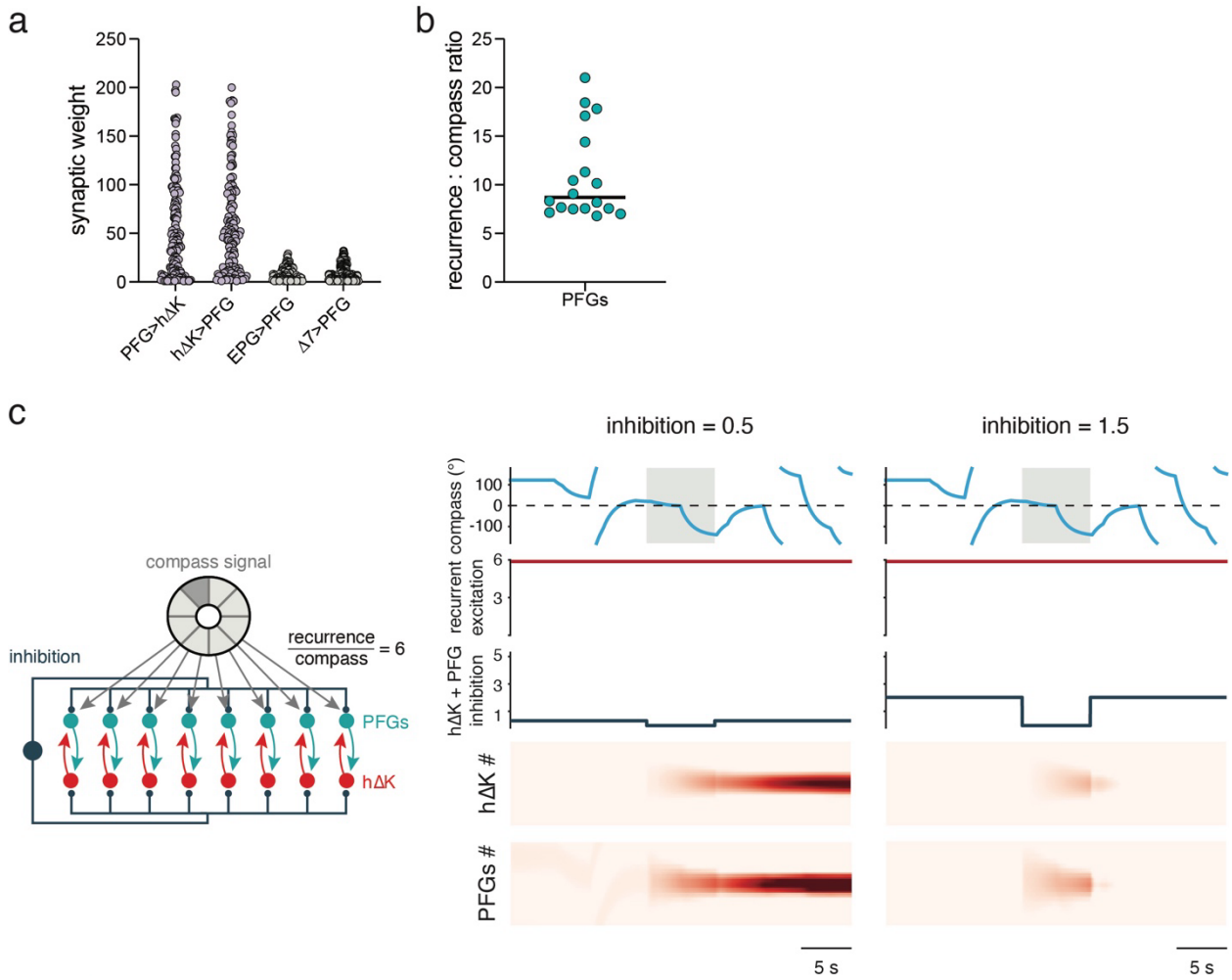
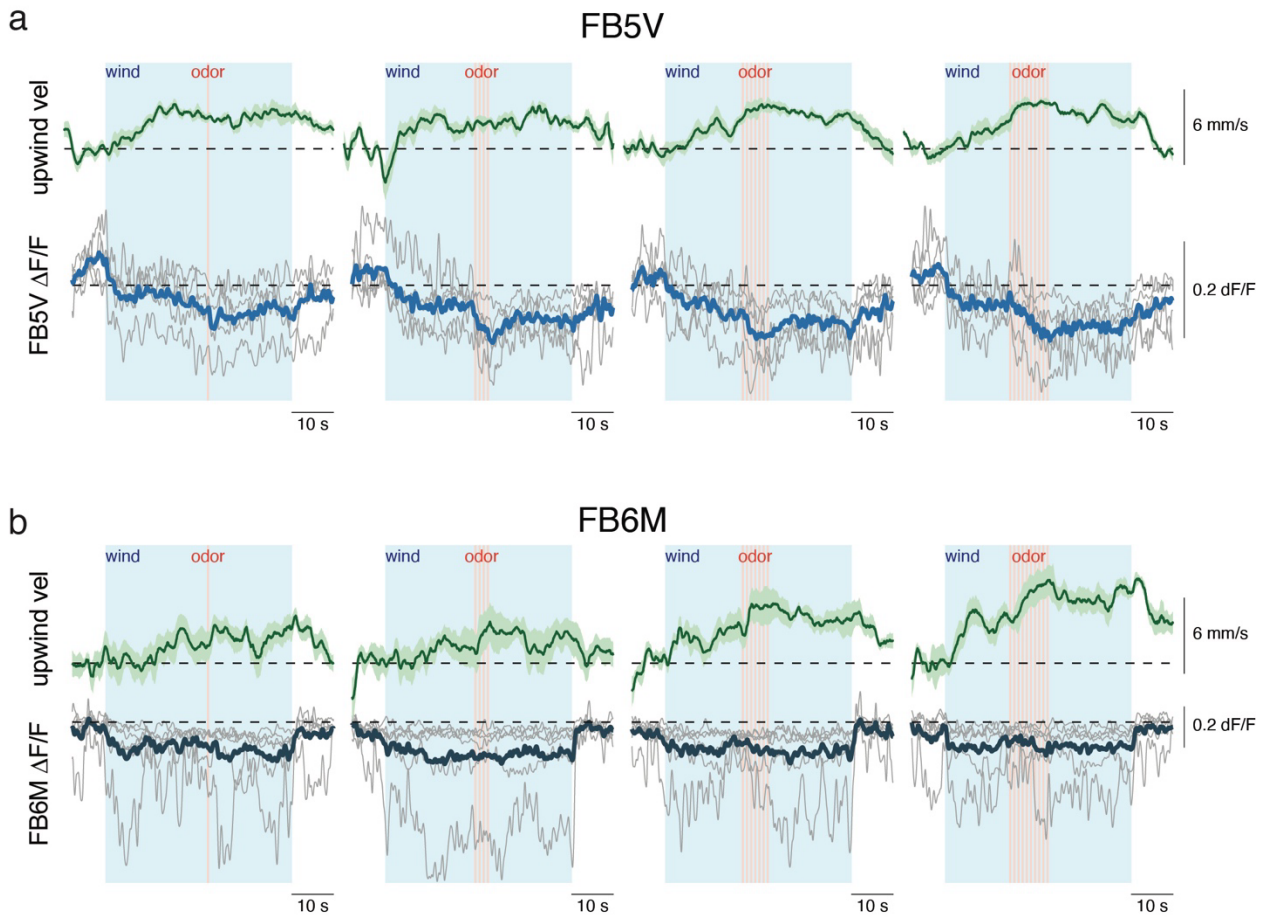
a, Simulations of hΔK and PFG population activity when the EPG compass signal (light blue) is randomly and stochastically updated and PFG neurons receive direct sinusoidal inputs from the compass. Left shows a schematic of the network configuration for the simulation. Right shows the simulation output. Note presence of a bump at all times in both populations. **b,** Correlation coefficients between travel direction and hΔK (red) or PFG (blue) bump positions across trials. **c,** Average correlation coefficients of hΔK (left) or PFG (right) bump position with heading direction versus travel direction (error = mean ± SEM).



Ext. Data Fig. 9: Additional data on disinhibition model

Ext. Data Fig. 9: Additional data on disinhibition model

a, Synaptic weights between pairs of PFG and hΔK neurons, as well as from EPG and Δ7 neurons to PFG neurons. **b,** The ratio of total synaptic input from hΔK neurons (recurrent) to total synaptic input from EPGs (compass), for each PFG neuron. **c,** Simulation of hΔK and PFG population activity with inhibition to both PFG and hΔK. As in Fig. 5, EPG compass signal (light blue) is randomly and stochastically updated and global inhibition to hΔK and PFG neurons is transiently shut off. The global inhibition strength was set to 0.5 (left) or 1.5 (right), showing how the simulations gate activity in both hΔK and PFG neurons but do not permit continuous compass representations in PFG neurons during inhibition.



Ext. Data Fig. 10: Additional data on encoding of sensory input by FB5V and FB6M

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a, Mean upwind velocity and FB5V ΔF/F during baseline (no stimuli), wind, and pulses (1, 4, 7, or 10) of odor. Individual fly averages in grey; average across flies in blue. Shaded regions are mean ± SEM. **b,** Same as **a** but for FB6M neurons. Individual fly averages in grey; average across flies in blue.

Table 4: Statistics for Extended Data figures. Significant p values are bolded.

Figure	Comparison	Sample size	Statistical test	p value
Ext. Data Fig. 1e	heading distributions	N = 18 flies $n_{baseline}$ = 935 trials n_{odor} = 935 trials n_{10s} = 935 trials n_{20s} = 935 trials n_{30s} = 724 trials	N/A	N/A
Ext. Data Fig. 1f	trajectory persistence distributions	N = 18 flies n = 10-48 trials/fly	N/A	N/A
Ext. Data Fig. 1g	mean upwind velocity and bump amplitude	N = 6 flies n_1 = 3-7 trials/fly n_2 = 2-8 trials/fly n_3 = 2-9 trials/fly n_4 = 4-8 trials/fly	N/A	N/A
Ext. Data Fig. 1h	mean upwind velocity and bump amplitude	N = 6 flies n_1 = 6-12 trials/fly n_2 = 5-12 trials/fly n_3 = 7-12 trials/fly n_4 = 5-12 trials/fly	N/A	N/A
Ext. Data Fig. 2b	mean Δvoltage across current steps	N = 32 cells n_{-4pA} = 3-10 trials/cell n_{0pA} = 3-10 trials/cell n_{4pA} = 4-9 trials/cell	N/A	N/A
Ext. Data Fig. 2d	mean voltage over time	N = 13 cells $n_{0.2}$ = 4-9 trials/cell $n_{0.8}$ = 5-9 trials/cell	N/A	N/A
Ext. Data Fig. 2e	light duty cycle	N = 13 cells n = 4-9 trials/cell $n_{0.5}$ = 4-9 trials/cell $n_{0.8}$ = 5-9 trials/cell	Friedman test with multiple comparisons	**Main effect of duty cycle: $p < 0.0001$** **0.2 vs 0.5: $p = 0.0181$** **0.2 vs 0.8: $p < 0.0001$** 0.5 vs 0.8: $p = 0.3500$
Ext. Data Fig. 2f	mean voltage over time	N = 8 cells $n_{0.2}$ = 1-7 trials/cell $n_{0.8}$ = 2-7 trials/cell	N/A	N/A
Ext. Data Fig. 2g	mean voltage over time	N = 13 cells $n_{0.2}$ = 5-8 trials/cell $n_{0.8}$ = 5-9 trials/cell	N/A	N/A

Ext. Data Fig. 2h	light duty cycle	N = 13 cells $n_{0.2}$ = 5-8 trials/cell $n_{0.5}$ = 4-8 trials/cell $n_{0.8}$ = 5-9 trials/cell	Friedman test with multiple comparisons	**Main effect of duty cycle: *p* = 0.0014** **0.2 vs 0.5: *p* = 0.0239** **0.2 vs 0.8: *p* = 0016** 0.5 vs 0.8: $p > 0.9999$
Ext. Data Fig. 2i	mean voltage over time	N = 8 cells $n_{0.2}$ = 3-5 trials/cell $n_{0.8}$ = 3-5 trials/cell	N/A	N/A
Ext. Data Fig. 8b	hΔK vs PFGs	$N_{h\Delta K}$ = 80 trials N_{PFGs} = 133 trials	Mann Whitney signed-rank test and Wilcoxon signed rank test	hΔK vs PFGs: $p = 0.5723$ hΔK vs 0: $p = 0.0938$ **PFGs vs 0: *p* = 0.0430**
Ext. Data Fig. 8c	hΔK heading vs travel direction	N = 80 trials	Wilcoxon matched-pairs signed rank test	0.1733
	PFGs heading vs travel direction	N = 133 trials	Wilcoxon matched-pairs signed rank test	**< 0.0001**
Ext. Data Fig. 10a	mean ΔF/F over time	N = 6 flies n_1 = 8-14 trials/fly n_2 = 7-12 trials/fly n_3 = 8-14 trials/fly n_4 = 8-12 trials/fly	N/A	N/A
Ext. Data Fig. 10b	mean ΔF/F over time	N = 6 flies n_1 = 5-11 trials/fly n_2 = 5-11 trials/fly n_3 = 5-11 trials/fly n_4 = 5-11 trials/fly	N/A	N/A