Path integration: how details of the honeybee waggle dance and the foraging strategies of desert ants might help in understanding its mechanisms

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ABSTRACT

Path integration is a navigational strategy that gives an animal an estimate of its position relative to some starting point. For many decades, ingenious and probing behavioural experiments have been the only window onto the operation of path integration in arthropods. New methods have now made it possible to visualise the activity of neural circuits in Drosophila while they fly or walk in virtual reality. Studies of this kind, as well as electrophysiological recordings from single neurons in the brains of other insects, are revealing details of the neural mechanisms that control an insect’s direction of travel and other aspects of path integration. The aim here is first to review the major features of path integration in foraging desert ants and honeybees, the current champion path integrators of the insect world, and second consider how the elaborate behaviour of these insects might be accommodated within the framework of the newly understood neural circuits. The discussion focuses particularly on the ability of ants and honeybees to use a celestial compass to give direction in Earth-based coordinates, and of honeybees to use a landscape panorama to provide directional guidance for path integration. The possibility is raised that well-ordered behaviour might in some cases substitute for complex circuitry.

KEY WORDS: Central complex, Interactions between terrestrial and celestial cues, Time-compensated celestial navigation

Introduction

Path integration (PI) is the process by which an animal, when moving away from a start point, often its nest, cumulatively sums its path, generating an internal vector that specifies the line from the animal’s current position back to the start point, however circuitous the outward trip. PI can act over a small or a large spatial scale. For example, PI helps a Drosophila (Fig. 1A,B) return over a few centimetres to a drop of sugar that the fly has sampled (Kim and Dickinson, 2017). Over long distances, PI allows foraging insects like honeybees and desert ants to return straight home along a ‘home vector’, from hundreds of metres in walking ants (Fig. 1C) or kilometres in flying bees (von Frisch, 1967; Visscher and Seeley, 1982; Beekman and Ratnieks, 2000). These foragers can subsequently apply this information in reverse, using a ‘food vector’ to return from their nest to the same foraging location.

Several reviews on PI exist (e.g. Collett and Collett, 2000; Wehner and Srinivasan, 2003; Ronacher, 2008; Heinze et al., 2018). The major aim here is to discuss new and old behavioural findings, primarily in honeybees and desert ants, using new research on brain function in Drosophila as a background. One major advance is a detailed account of the operation of neural circuitry in a brain region known as the central complex (CX). This circuitry underpins the control of an insect’s direction of travel and is also involved in PI (Liis et al., 1994; Strausfeld, 1999; Strauss, 2002; Neuser et al., 2008; Martin et al., 2015; Stone et al., 2017). An understanding of this circuitry relies on techniques, applicable to Drosophila, for knocking out specific classes of neurons and imaging the activity of all the neurons of one type within the CX while flies move in virtual reality. The outcome of this research is an account of how flies control their path direction relative to terrestrial landmarks and celestial cues that is couched in terms of interactions between identified classes of cells (Seeilig and Jayaraman, 2015; Turner-Evans et al., 2017; Green et al., 2017; Giraldo et al., 2018).

PI in desert ants and bees is more elaborate than that of Drosophila, and raises intriguing questions that can be explored within the Drosophila framework. Thus, a recent paper (Stone et al., 2017) combines new data on neurons in bees that encode translational optic flow for path control (Fig. 3) with a computational model of PI based on neuroanatomy at a cellular level. Detailed consideration of food vectors and the honeybee waggle dance suggests possible constraints when modelling PI.

The waggle dance, which communicates the direction and distance of a honeybee’s foraging site to other foragers in the hive (Seeley, 1995), is especially helpful when considering the mechanisms underlying PI. The dance is performed on vertical honeycomb in the dark hive, with direction relative to the sun’s azimuth given by the angle between the vertical and the straight waggle portion of the dance (Fig. 2A). Distance is expressed as the number of waggles emitted at a constant 15 Hz during the waggle section of the dance or the duration of the waggle segment (Fig. 2A). The relationship between this duration and the distance to a foraging site is linear from tens of metres up to at least 5 km (von Frisch, 1967). It is an effective signal. Bees newly recruited by the dance, with a spatial array of ‘flower’ traps to choose between, tend to be found in traps near to the signalled position (Towne and Gould, 1988). As will be discussed later, the dance has been a powerful tool for investigating how bees learn to compensate for the sun’s diurnal path across the sky and so signal direction in terrestrial coordinates (Dyer and Dickinson, 1994).

Studying the dance has also revealed the ways in which celestial and terrestrial cues interact (Dyer, 1987; Towne and Moscrip, 2008). Before delving into the detail of these interactions and into the mechanisms underlying PI, it is helpful to consider some of the surprising ways in which insects rely on PI.

Some uses and limitations of PI

Among the most important benefits of PI is the ability to explore new terrain without becoming lost. An insect computing and using a
Fig. 1. Path integration – the short, the long and a possible precursor. (A) A female Drosophila exhibiting path integration (PI) in a tiny arena. The fly explores the arena for 10 min, sticking mostly to the arena’s edge (blue track). Once the fly reaches the centre of the arena, food-sensing neurons in its antennae are activated optogenetically. During this activation, the fly returns frequently to that central point to find the imagined food (red track). (B) Same track plotted against time. Adapted from Corfas et al. (2019), with permission. (C) Two long foraging tracks of desert ants monitored with GPS. The outward track is shown in black. Nests are indicated by red and blue squares. After reaching food, the ant is displaced (black arrow) and released. Its homeward track (yellow) ends with a search. Left: no visual features at the virtual nest position where search occurs. Right: the search is biased by visual features. From Huber and Knaden (2015), with permission. (D) Turn compensation. Millipedes forced to turn left by a channel (left) or kept to a path by a black shadow in an otherwise bright arena (right) turn in the opposite direction when the forced path ends. Dots (100 paths from 20 millipedes) show the direction of the straight path (γ is mean direction) (modified from Burger, 1971, with permission).
home or food vector needs no knowledge of the landscape in which it forages. A recent example of PI operating in unfamiliar surroundings comes from a study of desert ants (*Cataglyphis nodus*) embarking on a foraging career. When these novices leave their nest for the first time, they memorise the arrangement of surrounding visual features so that they can find their way back home (Fleischmann et al., 2018). They do so in what are called 'learning walks,' in which ants circle the nest, periodically turning towards and facing the nest entrance, presumably acquiring views that can guide their return. Experiments in which the direction of the magnetic field was shifted in the immediate vicinity of the nest showed that the ants tracked the direction of the nest using PI, with the Earth’s magnetic field as a compass (Fleischmann et al., 2018). These young ants may rely on magnetic cues, despite the presence of conflicting celestial cues, because they have not yet learnt how the sun moves through the sky (Grob et al., 2017).

By indicating when an individual is near its nest, an insect’s PI state can modulate behaviour that is quite distinct from navigation. The desert ant *Cataglyphis fortis* protects its nest from intruding conspecifics by responding aggressively to a stranger when its own PI state corresponds to an encounter near its nest, but it ignores another ant when far from home (Knaden and Wehner, 2004). Similarly, *C. fortis* can locate its nest hole by tracking the emerging plume of CO₂ to its source. An ant’s PI state indicates whether the

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**Fig. 2. The honeybee waggle dance.** (A) Left: the direction of the waggle segment of a honeybee dancing in its dark nest on vertical honeycomb replaces the sun by the gravity vector to signal the direction of food relative to the sun’s azimuth (redrawn from Seeley, 1995). Right: the distance of food from the hive is signalled by the duration of the waggle segment (simplified from Schürch et al., 2013). (B) Dances performed in overcast conditions after different experiences of the sun’s movements. Left: 540 dances from 46 bees that only experienced the sun when trained in the late afternoon and tested over the following days under overcast conditions. Open symbols: scatty bees with opposite behaviour from the rest. Right: bees trained with sun throughout the day. Thick line indicates local ephemeris function (adapted from Dyer and Dickinson, 1994; Copyright 1994 National Academy of Sciences).
plume comes from its own nest or from a strange one that it would be hazardous to enter (Buehlmann et al., 2012).

One likely limitation of PI, at least in ants, is that its operation is isolated from other navigational mechanisms. Thus, PI state is not reset by familiar visual scenes or transition points along a route (Collett et al., 2003; Knaden and Wehner, 2005; Collett and Collett, 2009). PI state also does not help an ant decide how to respond to a prominent visual feature that occurs twice at different distances along a route (Collett et al., 1992). These results indicate that PI and visual place or route learning do not combine to form a ‘cognitive map’, a point considered in more detail by Webb (2019). More elaborate interactions between PI and visual cues to location are found in rodents (Savelli and Knierim, 2019). Although PI and visual route circuitry are not intertwined centrally, their outputs come together in setting the direction of an ant’s path. If the two outputs indicate different directions, the ant’s path is a compromise (Collett, 2012; Wehner et al., 2016; Hoinville and Wehner, 2018). The resulting output is weighted towards the cue that gives the more reliable directional information: PI at the start of the path and visual cues towards the end (Collett, 2012; Wystrach et al., 2015).

### The mechanisms of arthropod PI as revealed by behaviour

#### Partitioning the PI problem

At an abstract level, the process of PI consists of adding up all the oriented lengths of segments along a path, or equivalently, of integrating an individual’s velocity vector over time. Thus, for PI to work, insects either rely on sensory cues to velocity, which could be delivered by vision or by proprioception, or use separate cues to distance and direction that are combined. Recent neurophysiological data on bees (Stone et al., 2017) suggest that they integrate the velocity vector. If distances travelled are short, velocity can be controlled sufficiently well with proprioceptive cues...
(Kim and Dickinson, 2017). But to minimise the accumulation of errors over longer journeys, velocity cues of all sorts must involve the use of a compass, with insects steering themselves relative to the sun or to the Earth’s magnetic field (Cheung et al., 2007; Heinze et al., 2018).

**Monitoring translational optic flow for PI**

Before the 1990s it was unclear how bees assessed the distance that they covered on their flight to or from a foraging site. The answer came from changing the distance to visual features that bees encounter along their flight path, thereby demonstrating the involvement of translational optic flow (Esch and Burns, 1996; Srinivasan et al., 1996). In one telling experiment, bees with their hive on the ground were trained to collect sugar solution from a tethered balloon that gained height when the tether was lengthened. As height increased, the bees flew further, but experienced less optic flow from the ground plane and signalled shorter distances (Esch and Burns, 1996). This result also implies that bees lack a mechanism to adjust their estimate of flow according to their height above ground.

A variant of the previous experiment was to place a hive on the roof of one skyscraper (50 m high) and a feeder at the top of another (34 m high) at a distance of 228 m. Dancing bees signalled only 128 m. As signalers and recruiets were exposed to the same optic flow indicating the same metric distance, the dance recruited bees to the feeder (Esch and Burns, 1996). In other experiments, bees flew through narrow channels, in which optic flow increased relative to the bees’ normal surroundings (Srinivasan et al., 1996, 1997). Bees then signalled greater distances than they had actually traversed (Esch et al., 2001).

These experiments emphasise that the reliability of a translational flow signal as a measure of a bee’s velocity depends, among other things, on bees maintaining a standard height (e.g. Portelli et al., 2010), and also that the pitch of the head is constant. Despite its shortcomings, optic flow has an important advantage as a means of estimating velocity: the same flow is generated whether a bee’s movement is produced actively by its wing beats or through transport by wind.

**Translational optic flow detectors for PI**

The different patterns of optic flow impinging on an insect’s retina while it translates (Fig. 3A) or rotates (Gibson, 1950) are detected by movement detectors with large receptive fields. These neurons take their inputs from many neurons with smaller receptive fields that are selective for a particular direction. In flies, the encoding of rotational flow by wide-field detectors is well understood (Krapp and Hengstenberg, 1996; Borst et al., 2010), but detailed studies of translational flow detectors are rare.

The direction of motion through space to which a translational flow detector is tuned depends on the retinal position of its focus of expansion – the region of the retina from which the flow of motion originates and where image speed is low (Fig. 3A,B). Translational flow detectors that probably provide input for PI have recently been recorded in bees in an area of the CX called the nodulus (Stone et al., 2017). There seem to be just four neurons in the nodulus with their foci of expansion set roughly 90 deg apart and at 45 deg relative to the bee’s longitudinal axis. The span of directions to which each neuron responds is broad, so that the bee’s travel direction can be decoded from the combined outputs of the neurons (Fig. 3C,D). The directional signals from the bee’s flow detectors are initially in egocentric coordinates (i.e. relative to the bee’s longitudinal axis) and are presumably transformed continuously into celestial coordinates before integration (Stone et al., 2017). An additional complication to this transformation is that many insects can fly stably in a controlled direction, whether forwards, sideways or backwards.

**Holonomic control of insect paths**

‘Holonomic’ is a term from robotics that refers to the ability of an agent to control its path, irrespective of the orientation of the agent’s body relative to its direction of travel. Consider a bee flying north and maintaining its direction of travel with respect to the sun, first in still air and second when a steady wind begins to blow from the east. We can assume that in still air the bee faces forward and keeps the sun at a fixed angle to its travel direction along its longitudinal axis. Thus, the focus of expansion of translational flow and the bee’s body axis are aligned. When the wind starts blowing from the east, the wind velocity will add to the bee’s velocity through the air so that the bee’s velocity vector will rotate westward. Unless the bee itself rotates, the sun’s position on the retina will not shift. The only visual clue to the bee’s change of travel direction is the angle between the focus of expansion of translational flow and the direction of the sun. The bee, in response, needs to change its flight direction to counter the wind and restore the position of the focus of expansion relative to the sun (Collett et al., 1993; Riley et al., 1999). When it does so, flight direction and body orientation will no longer be aligned.

Holonomic control also occurs in ants walking backwards while dragging heavy prey to the nest. Part of their strategy for keeping on course and updating PI state is to stop occasionally and face in their usual direction of travel (Pfeffer and Wittlinger, 2016a). Facing forwards seems to improve the precision of the path when they return to moving backwards (Pfeffer and Wittlinger, 2016a). Nonetheless, rotating the sun using a mirror while the ants walk backwards (Schwarz et al., 2017) changes the ants’ direction of travel, indicating that backward-walking ants are guided by the sun. Again, the most plausible mechanism is that ants align the focus of expansion of translational flow with their desired compass-controlled travel direction. The blind area at the back of the eye (Zollikofer et al., 1995) would probably make backward steering less precise and so encourage occasional checks.

**Sensing velocity during walking**

Experiments in which the legs of desert ants were artificially shortened or lengthened indicate that ants monitor the number and amplitude of steps that they take and transmit that proprioceptive input to an integrator (Wittlinger et al., 2007). Ants, as do honeybees, discount the vertical component of their movements when updating the integrator; their home vectors are the same length, whether the ant walks a given horizontal distance over flat ground, or over a sequence of hillocks to reach its nest or feeding site (Wohlgemuth et al., 2002).

It is unclear whether proprioceptive cues reaching the integrator encode velocity or just distance. Cue conflict experiments in desert ants show that the directional components of the home vector are dominated by celestial cues (Lebhardt et al., 2012). Indeed, the integrator only accepts distance information when the sky is visible (Sommer and Wehner, 2005). These experiments do not necessarily mean that proprioceptive velocity is ignored. It could be that signals in proprioceptive coordinates are transformed into celestial coordinates before the integration stage and that the sky must be visible for the transformation to occur.

The contribution of optic flow to PI in ants has only recently become clear. Ants that are carried by nest mates between one nest site and another rely on optic flow from the ground to assess how far they have been carried. When they are placed on the ground, they
travel roughly the correct distance to reach the start of the transport (Pfeffer and Wittlinger, 2016b). They fail to do so if their ventral visual field is obscured before the return trip, indicating that proprioceptive and visual integrators are separate and do not accept each other’s inputs.

The operation of these separate integrators has been analysed by covering the ventral visual field during different phases of a normal foraging trip (Wolf et al., 2018). The foraging ants moved through open-topped channels that excluded lateral optic flow, but allowed a view of the sky to give compass information and had a textured floor to generate ventral optic flow. Eye caps, which obscured the ventral visual field, were applied and removed at different stages of the foraging trip. The distance walked on the homeward trip was normal when ventral vision was excluded throughout the trip. The key manipulation was to allow ventral vision during the outward leg and apply eye caps just before the homeward leg. The caps caused the ants to search for their nest at about 60% of the expected distance. This shortfall is not caused by manipulating the ants: they ran the full distance if eye caps were applied and then immediately removed before the homeward trip. When the eye caps were removed at the start of searching, the ants continued further in the direction of the nest, overshooting it by ca. 50% of the feeder-to-nest distance.

These observations are explained (Wolf et al., 2018) as follows: separate optic flow and proprioceptive integrators are ‘charged’ in parallel on a normal food-ward trip. The two integrators contribute to the home vector with different and adjustable weights. When eyes are capped on the food-ward leg, the weight of the optic flow integrator is zero and that of the proprioceptive integrator is one. When both integrators are charged on the food-ward leg, their weights are more equal and sum to one. If the eyes are capped at the feeder, the weight of the proprioceptive integrator remains less than one, causing the ant to stop and search well before the nest location. The visual integrator was incapacitated by the eye cap, but retained its charge, so when the ant could see again, it discharged the flow integrator by continuing its nest-ward path. It is unclear why ants overshoot the nest. What this work makes very clear is that the optic flow and proprioceptive integrators work in tandem, and are likely to be implemented in similar ways.

**Food vectors**

After returning from a successful foraging trip, ants and bees can perform an outward trip from their nest directly to where they had previously found food, guided by a food vector. A variety of evidence shows that desert ants store the vector’s distance and direction. First, vectors were monitored under conditions in which there were no landmarks to navigate by (Collett et al., 1999; Bošek et al., 2012). Second, when ants were forced to deviate from the direct path to food, they turned towards the food site once they became free to choose their direction of travel (Collett et al., 1999), just as they do during home vectors (Schmidt et al., 1992). Additionally, desert ants familiar with a route performed a food vector after being taken from the feeder and placed directly in the nest, showing that, once a food vector is acquired, its expression is independent of the home vector (Knaden and Wehner, 2006).

Food and home vectors are closely related, being the same length, but pointing in opposite directions. This relationship is emphasised by experiments that examine how ants adapt to a foraging route in which they are always carried over part of the route so that the food-ward and nest-ward portions differ in direction. In one study (Collett et al., 1999), the ants’ routes in both directions were prescribed by having the ants run in two open-topped channels that obscured the landscape, but allowed a view of the sky (Fig. 4). The first channel led from the nest to the feeder. From there, ants were carried to the start of the second channel that led them back to the nest. Tests after several days of this training showed that the home and food vectors had rotated. They remained parallel and lay roughly midway between the two channels, pointing in opposite directions. This rotation partially corrected the home vector, but induced a corresponding error in the food vector.

In a later study (Wehner et al., 2002) with a similar geometry and ants carried from the feeder to a release site, the triangular route was in the open so that ants were free to choose their path, making it possible to record the two vectors on every trial. A detectable and equal rotation of both vectors occurred close to the start of training. In contrast to routes in channels, the change in direction was small even after 20 or so displacements. The ants, instead of changing the vector direction further, learnt to bias their search in the direction of the nest (cf. Cheng and Wehner, 2002).

One explanation of these findings is that the two vectors are encoded as one, with motivational state setting the sign of the direction (Dyer et al., 2002). The two vectors would then necessarily adapt together. In the displacement experiments in which the two vectors cannot both be correct, the outcome is a compromise. Do these results suggest the existence of a long-term memory of a home vector, as may be the case in wood ants (Fernandes et al., 2015)? The question is hard to answer in desert ants because an ant’s immediate response, should it be startled or unexpectedly find food, is to guide its homeward path by its current PI state (Cheng et al., 2005), thus masking the expression of any long-term memory.

Good evidence for food vectors in honeybees comes from the bee waggle dance. It is remarkable that although the waggle dance signals direction relative to the current position of the sun (Fig. 2A), honeybees familiar with a landscape can signal the correct compass direction when cloud cover prevents any input from celestial cues (Dyer, 1987; Towne and Moscrip, 2008). Bees may then rely on a memory of the sun’s changing position relative to the visual panorama over the course of a day (see Boxes 1 and 2 for evidence and a possible mechanism).

**Time compensation of celestial cues**

Honeybees and desert ants generate food and home vectors that are in terrestrial rather than celestial coordinates, which they do by adjusting
constant foraging direction in Earth-based coordinates. The direction of dance relative to the sun (Lindauer, 1961), and from their dances when foraging in overcast conditions (Dyer, 1987). In both cases, the direction of the sun during the day (time compensation). Evidence that PI in desert ants is controlled by time-compensated celestial cues comes from foragers that were held for 3 h after they had reached their foraging site. Their home vector expressed on a test ground after this delay was in Earth-based rather than celestial coordinates – an angular difference of about 50 deg (Wehner and Lanfranconi, 1981). The evidence for time compensation in honeybees is even more compelling. It comes from the ‘marathon’ waggle dances that bees perform over several hours while remaining in the hive with no view of the sun (Lindauer, 1961), and from their dances when foraging in overcast conditions (Dyer, 1987). In both cases, the direction of the dance relative to the sun’s position rotates, such that the bees signal a constant foraging direction in Earth-based coordinates.

Time compensation probably involves learning because the sun’s changing azimuthal position, its ephemeris function, depends on latitude. At the equator, the sun is in the East all morning and shifts rapidly to the West at midday. With increasing latitude, the change from East to West occurs more slowly. Honeybees and desert ants have an inbuilt ephemeris function seemingly adapted for the tropics (Dyer and Dickinson, 1994; Wehner and Muller, 1993), with the expected position of the sun remaining in the East during the morning and changing to the West around the middle of the day (Fig. 2B, left). This innate ephemeris function was demonstrated in bees that had previously not seen the sun and were first exposed to it late one afternoon while learning a foraging route (Dyer and Dickinson, 1994). On a subsequent cloudy morning, bees were for the first time allowed to fly throughout the day. Early on, their waggle dance signalled that the sun was in the East. The direction of the dance was the same until a little before midday, when it abruptly switched to the West. This step function adjusts to the local ephemeris as the insect experiences the sun’s daily path in the area around the hive (Fig. 2B, right). For that to happen, a circadian time signal needs to be linked to the sun’s azimuth relative to some Earth-based reference direction. The reference could come from a magnetic compass (Fleischmann et al., 2018) or from the visual panorama. If the reference direction is magnetic, then the insect will record the sun’s azimuth relative to, say, East. If insects rely on the panorama, then the reference direction could be the sun’s position within the panorama at a fixed time of day (e.g. Box 2) or some other favoured direction relative to the panorama.

Possible mechanistic explanations of how bees might learn to compensate for the sun’s diurnal path across the sky or generate a dance relative to a sun hidden under cloud cover and remember the sun’s position relative to the landscape (see Boxes 1 and 2) emerge from a growing understanding of the interacting roles of the mushroom body (MB) in route learning and of the CX in controlling an insect’s direction of travel.

Box 1. Waggle dances under overcast conditions are directed by the visual panorama
The ability of bees to signal a direction in terms of celestial cues when the sun is invisible is important because it suggests that there is some linkage between celestial cues and the visual features of a landscape. Any doubts about this ability, or any suspicions that magnetic cues might provide a substitute for the missing celestial cues, are dispelled by experiments in which a colony was moved from its familiar surroundings to a second landscape that resembled the first, apart from a large rotation of the most prominent visual feature: a forest edge. The hive (black rectangle) in the familiar site was close to the forest edge, and bees learnt a route to a feeder (small open square) along it. Dances recorded in sunlight signalled the route to the feeder (left panel: open circles represent individual dance direction and the triangle shows mean direction). The trained bees were then tested when the sun was invisible in an unfamiliar landscape with the forest edge and the hive and feeder (open circle) oriented ca. 150 deg relative to the training landscape. The bees flew in the new hive-to-feeder direction, but danced as though they were still in the training landscape (right panel: filled circles represent individual dance direction and the triangle shows mean direction; open square gives predicted dance direction). Adapted from Dyer (1987). This finding is not limited to paths learnt in familiar surroundings or to a route parallel to the forest edge. When bees in a rotated unfamiliar landscape were trained to a feeder in a new location under dense cloud, their dance again signalled a direction appropriate to flying the newly trained route at the familiar site (Towne and Moscrip, 2008). In both sets of experiments, as soon as the clouds dispersed, the dances were directed by the sun and signalled the correct direction of the route in the rotated landscape. Bees can indeed use features of a familiar panorama to direct their dances.
The current consensus is that insects learn a visually guided route as the set of panoramic views that they see when facing along a route (Collett, 2010; Baddeley et al., 2012). To perform the route, they turn until the panorama on their retina matches a view from the set and then move forwards. Such ‘view memories’ are likely to be stored within the MB (Webb and Wystrach, 2016; Ardin et al., 2016). The MB is a complex central brain structure that has long been thought to be concerned with spatial learning (Strausfeld, 2012). In Hymenoptera, the MB first enlarged in solitary parasitic wasps that must often navigate to their nest or to the known locations of hosts (Rosenheim, 1987; Van Nouhuys and Kaartinen, 2008), suggesting that the MB grew to support spatial learning and the wasp’s need for flexible navigation (Farris and Schulmeister, 2010). The MB of several insects, including bees and ants, receive input from areas of the optic lobe concerned with terrestrial and celestial cues (Strausfeld, 2012; honeybees: Mobbs, 1982; ants: Ehmer and Gronenberg, 2002; bumblebees: Paulk and Gronenberg, 2008). Thus, the sun’s position relative to a visual panorama at a particular time of day could, in principle, be encoded within the MB.

Studies on the learning of attractive and aversive odours in Drosophila indicate how information about views might be transmitted to the CX. Whereas complex sensory patterns (e.g. odours or a visual panorama) are encoded in the MB, the majority of the sparse outputs from the MB signal only whether to approach or avoid a learnt sensory pattern (Aso et al., 2014; Cognigni et al., 2018); the output neurons communicate nothing about the nature of the pattern evoking that output. In the case of a remembered view along a route, the MB output may only report that a current view is attractive and that the insect should continue to travel in its current facing direction.

Anatomical studies in monarch butterflies (Heinze et al., 2013) and Drosophila (Aso et al., 2014) suggest that there are indirect connections from the MB projecting to a major component of the CX, the fan-shaped body (FB) (Fig. 5A). The CX has its own inputs...
from the visual system, enabling an insect to control its direction of travel relative to terrestrial or celestial cues (Locust: Heinze et al., 2009; Pegel et al., 2018; Drosophila: Liu et al., 2006; Bausenwein et al., 1994; Seelig and Jayaraman, 2013; Omoto et al., 2017; Giraldo et al., 2018). Thus, when an ant or a bee faces along its route, an attractive output signal from the MB could place route guidance under the immediate control of the CX by triggering the CX to use its current encoding of the retinal position of one or more prominent visual features to guide the insect’s path (Collett and Collett, 2018).

Regions of the CX that include the FB and the ellipsoid body (EB) enlarge when young desert ants (C. nodus) perform their learning walks under a normal sky, but there is no enlargement if filters above the ant distort the sky pattern in various ways (Grob et al., 2017), suggesting that the diurnally changing position of the sun might be encoded in the CX. How might this occur? One possibility is that the sun’s position is mapped spatially across an array of neurons, with the appropriate activity level across the map activated by a circadian signal. Another suggestion is that position and time are both encoded spatially in a 2D array. However it is done, the format must allow celestial and Earth-based coordinates to remain separable, as is necessary for generating the waggle dance. The same visual conditions that were needed for an enlargement of visual input synapses to the CX during the learning walks...
walks of *C. noda* also led to enlargement of the synapses made by visual inputs to the MB, encouraging the notion that ants (and bees) may learn the changing position of the sun within a panorama during learning walks (or flights).

**The integrator and its coordinates**

The four nodulus neurons transmitting translational velocity signals in bees project widely within the FB (Stone et al., 2017), where studies on *Drosophila* indicate that the integrator is located (Neuser et al., 2008). Although the integrator’s neural circuitry is unknown, there are several computational models of how an integrator might operate, which share the assumption that integration occurs across a spatial array of ‘accumulators’ with different preferred heading directions, each updating according to the insect’s current direction of travel. The first of these models (Mittelstaedt and Mittelstaedt, 1982) considered the simplest case of two accumulators, one integrating components of travel in, say, the East–West direction, the other in the North–South direction. During any outward path, the two accumulators sum their independent components. The integrator has no history of how the end state was reached, but the vector sum of the contents of the two accumulators generates a home vector. Later models contained more accumulators (Wittmann and Schwegler, 1995; Haferlach et al., 2007).

Assuming that each accumulator extracts the component of travel in its preferred direction, the activity pattern across all accumulators, when an end point is reached, could be read in two ways. The distance and direction of the home vector are given by the vector sum of the outputs of all the accumulators. The sum of the outputs, ignoring direction, gives the total path length. Such a dual output might explain an intriguing finding (von Frisch, 1967) that deserves further research; namely, bees, detouring around a mountain to reach a food source, signal the hive-to-food direction, but the distance signalled in the waggle dance is the detour from hive to food.

If integration occurs in the FB, there would probably be eight accumulator channels (Fig. 5A) with their preferred directions 45° apart, one for each of the FB’s columns (Williams, 1975; Hanesch et al., 1989; Stone et al., 2017). It is supposed that in bees, translational velocity, initially in egocentric coordinates, is first transformed into celestial coordinates (Stone et al., 2017). The behavioural data on honeybees and desert ants suggest that it is then converted into Earth-based coordinates. Because in desert ants a PI state can be held stably for at least a day (Ziegler and Wehner, 1997), one might expect that each accumulator monitors direction in Earth-based coordinates. In terms of circuits and evolution, the transformation from celestial to terrestrial coordinates might be accomplished most simply by adding/subtracting the current ephemeris angle to the output of the translational flow detectors when or before the output reaches the FB accumulators. This arrangement also has the benefit of accommodating the sun-related directional signal of the honeybee waggle dance (Fig. 2A).

How food vectors are encoded is unknown, but a reasonable guess is that they are stored separately in the FB in the same format as the information encoded by the integrator. A detailed computational model of food vectors (Le Moël et al., 2019) with a similar encoding to that suggested here mimics successfully many features of these vectors. On this assumption, the direction of the waggle dance on vertical comb (Fig. 2A) can be derived by taking the output of the food vector in Earth-based coordinates and subtracting/adding the current ephemeris angle from/to the vector direction. The waggle dance also requires travel direction and body orientation to be aligned, tempting the suggestion that this property applies generally to the FB integrator. In one respect, the dance differs from PI home or food vectors: its performance does not affect the state of the PI integrator.

**Guiding return trips**

How do the contents of the integrator guide return trips? At least three ways can be envisaged. The first and simplest is that the integrator fills up on the outward trip and empties on the return, when it guides the homeward path directly. This scheme does not account for the generation of food vectors or for the search that occurs at the end of a home vector, should an insect not encounter its nest. In desert ants, the search consists of loops of increasing size that take ants away from and back to the PI-determined nest position (Wehner and Srinivasan, 1981). The state of the integrator at the start of the home vector influences the search pattern, such that ants produce a wider search pattern for the nest at the end of a long home vector than after a short one (Merkle et al., 2006). In the Australian desert ant *Melophorus bagoti*, the length of the roughly linear segments that comprise the search increases and the amplitude of the inward turns decreases when the distance between feeder and nest increases (Schultheiss and Cheng, 2011). These PI state-dependent changes in the search pattern suggest that signs of the initial length of the home vector persist after the home vector has been performed. This persistence is not easily explained by a single integrator filling and emptying.

A second suggestion is that the integrator fills up from zero on both the outward and return trips (Collett et al., 1999; Knaden and Wehner, 2006). A home vector or food vector is formed by taking the vector sum of the outputs of the accumulators and inverting compass direction, after which the integrator is zeroed. The vector then guides the journey home or to the food, during which the integrator re-fills. If all goes well, vector and PI state correspond when the insect reaches its destination. Two variants of this possibility can be distinguished: (1) home and food vectors are separate entities or (2) they are the same, but their direction inverts, pointing either at the food or at the nest. The linkage between food and home vectors during adaptation experiments (Fig. 4) favours the second variant.

A third suggestion is that the integrator fills up from zero on the outward trip and continues to fill up on the homeward trip, until all the accumulators are in equilibrium so that there is no tendency to move (Stone et al., 2017). This model supposes that the integrator drives steering directly and that the balance between two sides provides a turning signal. When signals are balanced, the insect is facing the nest. This model ignores food vectors, but it can be expanded to include them (Heinze et al., 2018). All three possibilities are consistent with the finding that ants reset the integrator to zero while in the nest (Knaden and Wehner, 2006).

**CX and the control of path direction**

The way that the output of the integrator is likely to set an insect’s travel direction can be explained in terms of the known activity and connections of identified neurons within the CX of *Drosophila*. The natural dispersal flight of *Drosophila* provides a particularly nice example as these flights are guided by celestial cues that can be mimicked in the laboratory. Marking and release experiments show that flies from one natal site disperse in many directions. In just a few hours they can fly ∼10 km from the site, presumably taking an almost straight path (Coyne et al., 1982; Dickinson, 2014). The close laboratory parallel is that during tethered flight, a fly will keep an artificial sun stably in one position on its retina for several hours (Fig. 5B), with different individuals keeping the sun in different retinal positions (Warren et al., 2019; Giraldo et al., 2018).

The process of stabilising a moving image on the retina or controlling the retinal position of a terrestrial landmark or the sun
can be seen in the activity of neurons connecting two closely interacting structures of the CX (Seeig and Jayaraman, 2015; Turner-Evans et al., 2017; Green et al., 2017). The first is a ring-like structure, the EB. The second is a linear structure, the protocerebral bridge (PB) (Fig. 5A). The left side of the PB is involved in the control of turns to the left, and the right side with turns to the right. The EB and each half of the PB are formed of eight functioning compartments, subdividing 360 deg of horizontal directions into 45 deg sectors.

By imaging calcium dynamics, it becomes possible to ‘see’ the neural activity of the EB or PB while a fly walks or flies. Doing so reveals a single pulse or ‘bump’ of activity within the EB (Seeig and Jayaraman, 2015) and one on each side of the PB. The bumps are closely correlated with the fly’s heading (or travel) direction relative to a visual cue (Green et al., 2017; Turner-Evans et al., 2017; Seeig and Jayaraman, 2015) (Fig. 5C). The bumps shift with the visual feature and maintain a constant angular distance from it. Knocking out this circuitry disrupts the fly’s control of travel direction relative to an artificial sun (Fig. 5B). Instead of facing in a variety of directions, all flies face roughly towards the sun (Giraldo et al., 2018).

A variety of input signals reach the EB and PB. The bump seems not to be tied to a particular directional system, but to be ‘free-floating’, at the service of the system currently engaging the insect. It is supposed that the position of the bumps of activity in the EB and the PB assumes an alignment of facing and travel direction. This assumption is hard to test because the fly’s head must be fixed for imaging. Perhaps something might be learnt from experiments combining an artificial sun with and without side winds.

The argument so far implies that in honeybees and ants navigating with PI, the controlling directional signals are time-compensated celestial cues. To control the bump with such a signal, it seems likely that the current desired direction is fed from the accumulators in the PB via fixed connections to corresponding compartments in the PB, where in locusts there is a spatial map of celestial coordinates (Heinze, 2014; Bockhorst and Homberg, 2015; Heinze and Homberg, 2007, 2009). If the mapping in the PB is time compensated, then the FB and PB are ‘in phase’ and can communicate directly, tying the bump to the desired direction set by the FB, much like the control of the bump by terrestrial features (Seeig and Jayaraman, 2015). If the mapping is not time compensated, then one way to utilise fixed connections between corresponding compartments of the FB and PB would be for an insect to turn to face the sun, or, on overcast days, the position of the sun in a time-stamped memorised view of the panorama. The possibility of such a behaviourally mediated shift register could be investigated in desert ants when they select the direction of their home vector after displacement from a food site.

Conclusion

This account of arthropod PI has concentrated on desert ants and honeybees, relating behavioural studies to current knowledge of the operation of the MB and CX of Drosophila and other insects. Studies of the honeybee waggle dance have been particularly helpful in raising questions about transformations between celestial and terrestrial coordinates. The waggle dance also poses different problems: how can bees perform PI when the sun is obscured and they can no longer rely on celestial cues? And how do bees learn the position of the sun relative to a visual panorama? It is possible that some circuit elaboration is avoided through appropriate behaviour. For instance, insects might set the initial state of a neural circuit and simplify its operation by turning to face the sun or its remembered position relative to a point in the landscape. Although such speculation helps in articulating questions, the tentative answers are like skittles waiting for facts to knock them down.

The research on the CX surveyed here emphasises what an elaborate and sophisticated structure the CX can be in certain insects, raising the question of how it got that way. One must look to other invertebrates for clues to its putative ancestral functions. The number of compartments in the CX and their elaboration within the arthropods varies phylogenetically and to match the complexity of the behaviour that the CX supports (Strausfeld, 2012). An early and ubiquitous form of sensorimotor control that could involve a less elaborate CX is an ability to travel in a fixed direction (Honkanen et al., 2019) when approaching a target or dispersing or escaping. Moving in a straight line is known to involve the CX of various insects (cockroaches: Martin et al., 2015; dung-beetles: el Jundi et al., 2015; Drosophila: Warren et al., 2019), and relies on guidance cues from several sensory modalities that provide input to the CX. Of special interest is that keeping to a fixed direction of travel involves a memory that stabilises the current direction of travel. Properties of this memory have been explored by forcing an animal off its chosen course (Dingle, 1965; Kupfermann, 1966). If a woodlouse or millipede is forced from a straight path to turn left, it will, when it is free to choose its direction, turn right and take a path that is offset and often parallel to its initial direction (Fig. 1D). This short-term memory for direction is malleable and may adjust to an animal’s changing path: the longer the enforced straight path, the smaller the counter-turn when the animal selects its own direction (Dingle, 1964). This brief memory might thus encode an animal’s starting and current directions spatially within a structure like the FB, with perhaps fewer columns, pre-adapting such a structure for the longer-lasting and more elaborate memories involved in PI. Similarly, individual FB columns might expand to accommodate extra neurons needed for the food vectors of desert ants and honeybees.

Acknowledgements

Many thanks to Matthew Collett, Stanley Heinze, Jeremy Niven, Will Towne and an anonymous reviewer for comments that hugely improved this paper.

Competing interests

The author declares no competing or financial interests.

Funding

Financial support came from a Leverhulme Trust Emeritus Fellowship.

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