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Primer

The Dung Beetle Compass

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What do a burly rower, a backstroke swimmer and a hard-working South African dung beetle all have in common? The answer is: they all benefit from moving along a straight path, and do so moving backwards. This, however, is where the similarity ends. While the rower has solved this navigational challenge by handing the task of steering to the coxswain, who faces the direction of travel, and the swimmer is guided down her lane by colourful ropes, the beetle puts its faith in the sky. From here, it utilises a larger repertoire of celestial compass cues than is known to be used by any other animal studied to date. A robust internal compass, designed to interpret directional information, has evolved under the selective pressure of shifting today's lunch efficiently out of reach of competitors, also drawn to the common buffet. While this is a goal that beetles might share with the hungry athletes, they reach it with drastically different brain powers; the brain of the beetle is several times smaller than a match head, containing fewer than a million neurons.

One single dung pile can attract over 100 species of dung beetles, who gather there to eat and mate. To avoid leaving this feast empty-handed, some dung beetles sculpt a spherical food-package (ranging from the size of a pea to that of an orange, depending on the species). They then immediately set out with this ball of dung on a straight-line journey away from the intense competition at the dung pile. The direction of this journey is randomly chosen by each individual at the beginning of its departure, resulting in a radial pattern of outwardly-directed trajectories from the dung pile (Figure 1A).

A beetle's drive to adhere to its set course is so strong that it sticks to its path regardless of obstacles; over stones, through bushes and grass, across the hand of an experimenter or in an experimental arena. While rolling, a beetle can be removed from its ball at least 100 times, only to hold steadfastly to its initial bearing as soon as it gets back on its ball again. If a beetle is forced to make a new ball, however, the bearing information is reset in its brain and a new course is set. This unique and robust orientation behaviour, in combination with an accessible brain, make the dung beetle an ideal model system for understanding the fundamental visual and neural processes underlying straight-line orientation.

While few navigators are as single-minded as dung beetles, which lock onto one single bearing for the duration of their short journeys, the navigational movements of most animals, ranging from flies and butterflies to albatrosses, salmon and turtles, still require them to move along a stable course for at least parts of their travels. Herein, we provide an overview of recent behavioural, anatomical and physiological results showing how an insect brain has evolved to facilitate straight-line orientation.

A celestially driven snapshot compass

When lost in the desert at night, people tend to walk in circles. This is because the seemingly simple act of walking in a straight line involves a complex interplay of various sensory modalities, the motor system, and cognition. Interestingly, a dung beetle released in the same unchartered territory does *not* move in circles, but holds its chosen bearing until it encounters a suitable spot to bury its ball of dung (Figure 1B, grey paths). The key to the beetle's ascendency over humans in this particular skill lies

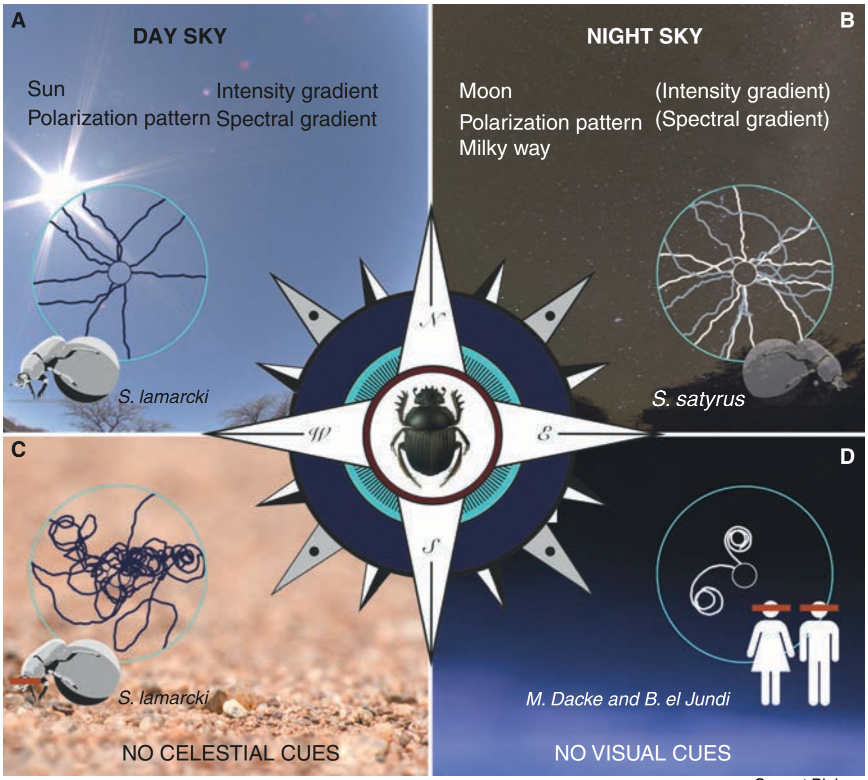

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Figure 1. Compass input and performance.
(A,B) The dung beetle snapshot compass utilizes a large repertoire of celestial cues to guide their straight-line journey away from the intense competition at the dung pile (A and B). The circular diagrams show the straight paths taken by the beetles from centre of a 3 metre wide arena placed outdoors under the sunlit sky (A, black paths), a moonlit sky (B, white paths) or a starry sky (B, grey paths). (C) With the celestial input to the compass removed by the introduction of beetle-tailored caps, the beetles start to roll in circles. (D) When blindfolded (and without auditory input) and asked to walk in a straight line backwards away from the centre of a hockey-pitch, the authors of this Primer failed to deliver and instead walked in circles.

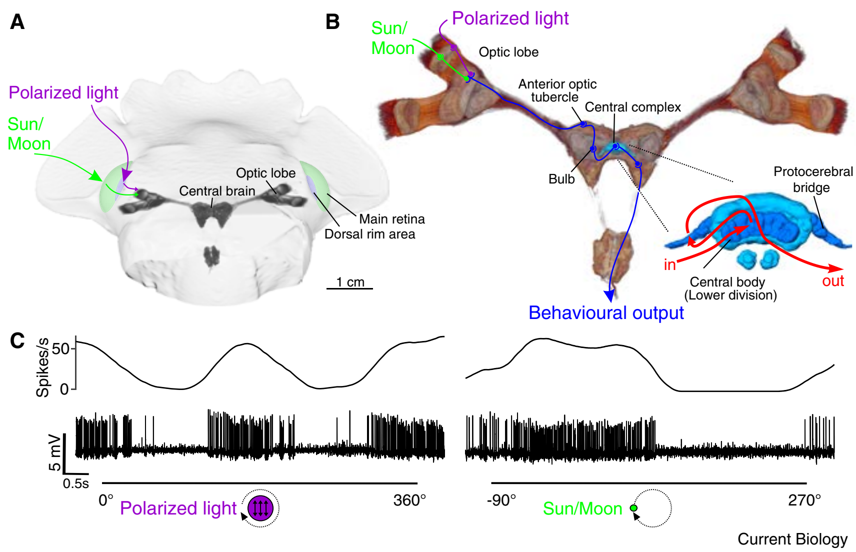

Figure 2. The neural substrate of celestial compass orientation in the dung beetle brain.
(A) Posterodorsal view of the dung beetle brain within the animal's head capsule (MicroCT-image stack of the beetle's head kindly provided by Emily Baird and Martin Bech). The brain receives polarized skylight information via a specialized eye region, the dorsal rim area (purple), located at the dorsal edge of each dorsal compound eye. Other celestial cues, such as the sun or moon are detected via the main retina (green). (B) The sky compass pathway in the dung beetle brain. Skylight information is transferred from the optic lobes to the central brain, where it is processed by two brain areas (anterior optic tubercle, bulb) before entering the central complex. Celestial cues are mainly encoded by two brain areas in the central complex, the lower division of the central body (also called ellipsoid body in flies), and the protocerebral bridge. The output signal of the central complex is finally transferred via descending neurons to the motor control centres of the thoracic ganglia. (C) Neural tuning (intracellular recording) of the same central-complex input neuron (TL cell) during stimulation with a dorsally rotated polarizer (left) and a green light spot moving on a circular path around the beetle's head (right). Upper trace: sliding average; lower trace: firing activity of the cell. Bar indicates the rotation of the polarization stimulus (left) and the position of the green light spot (right) in relation to the beetle's head.

in its rare ability to detect and orient via the weak light intensity differences provided by the Milky Way. On moonlit nights, courses steered by the beetles become more stable (Figure 1B, white paths). This is because the beetle's compass, like those of ants, sandhoppers and frogs, is now also able to extract additional directional information from this bright point of reference in the night sky. But even before the moon rises, the beetles can gain directional information from an accompanying pattern of polarized light that forms across the sky, produced as rays of moonlight reflected from below the horizon strike the upper atmosphere.

As night transitions into day, diurnal beetles emerge from the safety of the ground to forage. Not unexpectedly, it is the sun that guides these, and many other animals, along the paths and routes they aim to travel (Figure 1A). On occasions when the sun is hidden behind clouds, or shielded from view by the thick canopy of an acacia, the beetles steer their balls by the bright pattern of polarized light that now surrounds the sun, or the spectral and intensity gradients that form across the sunlit sky. This also holds true for other insects, such as ants and bees, that engage their internal compasses with high precision when plotting the shortest (and often straightest) routes back to their nests. But dung beetles use this information for departure rather than returning.

The critical role of sky-based input to the beetle compass can be demonstrated by the introduction of a beetle-tailored cap, that effectively blocks the view of the sky from the wearer. With the celestial input to the compass removed, beetles are no longer able to correct for the noise that unavoidably accumulates in their navigational system and they start to roll in circles (Figure 1C). When blindfolded and asked to 'escape' along one single bearing from the centre of a hockey pitch, the researchers carrying out this study fared no better (Figure 1D). In contrast to the beetles, however, the researchers — and most likely the majority of our navigating fauna — need a blindfold rather than a cap to lose their way. This is because most compass systems also make use of individual landmarks, or the visual panorama, to support navigation in well-known locations. Interestingly, the beetle does not seem to use visual landmarks for navigation.

But never failing to impress, dung beetles compensate for this earthly ignorance with a dance. Before the beetle departs, it climbs on top of its ball, holds out its head and rotates around its own axis. It was recently discovered that this dance signifies the moment when beetles record a snapshot of visual cues in the sky, a stored template to which they match their current view of the sky as they travel (Figure 3A,C).

While other insects, such as bees and ants, employ an innate prediction of the relationship between the sky polarization pattern and the position of the sun (for example), the beetles accept any combination of these cues. Ball-rolling dung beetles are found in dense forests, deserts and savannahs on all continents (excluding Antarctica) and this navigational technique, which is simultaneously straightforward and flexible, could potentially support straight-line orientation in all these habitats.

The dung beetle compass is nothing special

The processing of celestial compass cues from these different environments starts at the level of the beetles' eyes, more specifically in the dorsal pair of eyes (dung beetles have four eyes, two on each side of the head). As in many other insects, a small region of the beetle's eye, termed the dorsal rim area, holds UV-sensitive photoreceptors, which have evolved to respond to the polarization plane of light (Figure 2A). Other directional cues, such as the sun or moon, the intensity and spectral gradients of the sky, and the Milky Way, are detected via the main retina of the dorsal eye.

Behind the eyes, well protected within the heavily armoured shovel-shaped head, lies the dung beetle brain (Figure 2A). The central brain itself is surprisingly small and connected via long optic stalks to the optic lobes and the retinas of the eyes (Figure 2A). By tracing the neural tracts from the eyes into the brain, the wiring network behind the compass orientation system can systematically be exposed. Such tracing experiments have been performed in several insects, including dung beetles (Figure 2B), and reveal a highly conserved sky compass pathway.

Even though it is still not known where polarization information and, for example, solar information are first combined in the beetle brain, studies in locusts show that this already takes place in the optic lobes. From here, the information package is transferred to the anterior optic tubercle in the central brain. Sky-compass neurons from this relay station target a region called the bulb. Next, compass information is transmitted to a midline brain region termed the central complex. Skylight information passes through this enigmatic brain area mainly via two neuropils; the lower division of the central body (in flies called ellipsoid body) and the protocerebral bridge (Figure 2B).

The central complex not only receives compass information, but amongst other tasks also serves as a key control station for motor control of the wings and legs. Accordingly, the central-complex output-signal becomes a combination of visual and motor-commands, transferred via descending neurons to three ganglia in the thorax, where the information is finally converted into a motor pattern. Taken together, and put into the perspective of other navigating insects, the beetles seem to rely on the same neural network to hold their single bearing as do path-integrating and migratory insects (which use it for their long-distance travels).

If a dung beetle with its ball is picked up during its directed journey over the sun-lit or moon-lit savanna, and subsequently released in an arena lit by a single green light spot, the beetle will keep the same heading with respect to this artificial cue as it did to the celestial body

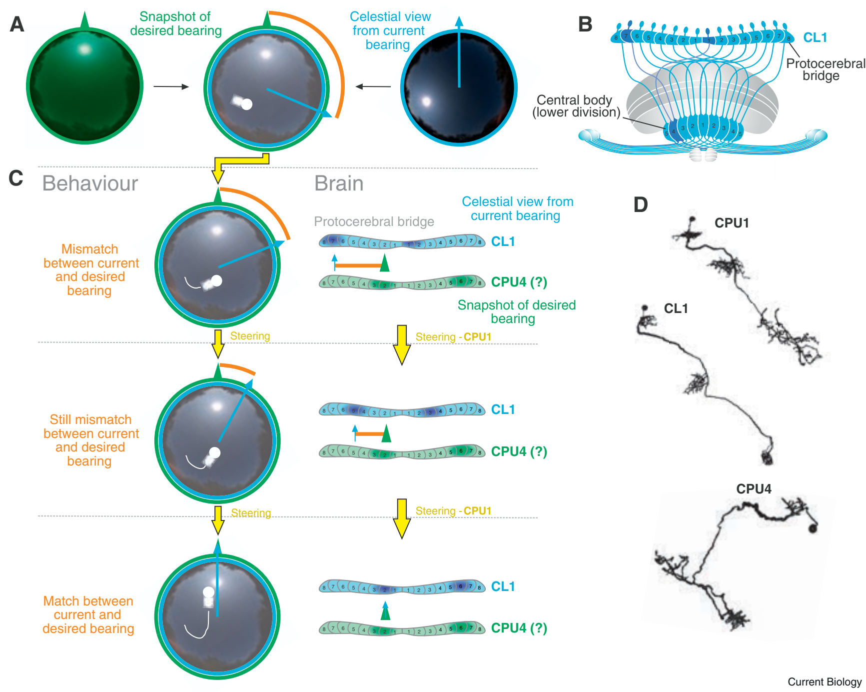

Figure 3. Decoding straight-line orientation in the dung beetle's central complex.
(A) To maintain a constant bearing, a dung beetle can compare a celestial snapshot - taken when oriented along the desired bearing (left green circle) - with the sky as observed when orienting along its current bearing (right blue circle). The middle circle is an overlay of the celestial views from the desired and the current bearing. The blue arrow indicates the current rolling direction of the beetle, the green triangle shows the desired bearing. The orange sector indicates the angular difference between desired and current bearing. (B) Neuroarchitecture of the central complex. The central complex consists of layers and slices. Individual slices of the lower division of the central body are interconnected with slices of the protocerebral bridge via CL1 neurons. If for instance the CL1 neurons in slice 4 are maximally activated, this causes the activity of two slices in the protocerebral bridge (left slice 1 and right slice 7, dark blue). (C) Schematic overview of the behaviour (left) and possible neural tuning in the protocerebral bridge (right) that would allow a dung beetle to correct its course by the use of a celestial snapshot compass. CL1 neurons could encode the current celestial scene, while CPU4 could function as the memorized celestial snapshot. The animal steers (controlled putatively by CPU1 cells) until the current and desired directions match. While turning, the activity of the CL1 cells changes until the activity pattern matches that of the memorized celestial snapshot. For explanation of symbols see (A). (D) Anatomy of three types of central-complex neurons in the dung beetle brain. The dung beetle central complex houses all the neuron types that have been described in other insects, and which are known to be crucially involved in establishing the functionality of this brain region. Image of the South African lunar sky in (A) and (C) kindly provided by James J. Foster.

outdoors. This is because it now interprets the bright green light as the sun or the moon. Likewise, a beetle keeps a similar heading under a large polarization filter as it does under the sky-wide polarization patterns of the high African skies. These important findings allow us to present biologically relevant, but easily manipulated, compass cues to a beetle in our experimental set-ups, while simultaneously recording the electrical activity in its central-complex neurons (Figure 2C). But instead of allowing the beetle to perform a rotational dance on top of its ball, which would make delicate intracellular recordings impossible to obtain, the green light spot is moved on a circular path around the head of the immobilised beetle to simulate the rapid shift in solar or lunar azimuth that the beetle would have experienced while dancing. Similarly, the polarizer can be rotated by 360° in the animal's zenith.

The electrical responses obtained from the central-complex cells in response to these manipulations typically show one maximum to the mimicked celestial body and two maxima (180° apart) to the polarization stimulus (Figure 2C). This clearly demonstrates that the central complex of dung beetles, like that of all other species of insects investigated, holds the neuronal substrate for orientation. Moreover, when recording from the very same central-complex neuron while simultaneously rotating the ersatz sun/moon, and then the polarizer above the beetle's head, it becomes apparent that a single neuron in this part of the brain combines the directional input from both of these tightly linked celestial cues. Interestingly, the neural tuning does not encode the inherent natural 90° spatial distribution of these cues. This observation is well in line with the flexible coding of these celestial cues demonstrated behaviourally, where beetles reliably orient to any physically possible, or naturally impossible, combination of these cues.

The neural needle of the celestial snapshot compass

How a beetle initially decides the direction to move its spherical treasure away from the dung pat is still not known. The neurons that drive this decision could possibly be the same as the ones that also allow other animals, such as butterflies and moths, to disperse in different directions — a strategy that is crucial for animals in general to maintain or extend their ecological niche. But irrespective of how the initial exit bearing from the dung pat is set, the celestial snapshot taken at the start of the journey — which from that moment defines the bearing that the beetle should travel (Figure 3A, green) — needs to be stored somewhere in the beetle brain. When negotiating dips, twigs or tufts of grass in its path, a beetle can then simply steer according to the relative fit (Figure 3A, middle) between the stored celestial snapshot and the current celestial view (Figure 3A, blue). As long as the snapshot and the current view match, the beetle can continue to push its ball backwards. But as soon as the snapshot and the current view no longer match, the animal has to turn with its ball until they line up again (Figure 3C). These comparisons between a memorized snapshot and a current celestial view, and the resulting steering commands, are most likely also being executed in the navigational cockpit of the beetle — the central complex.

The central complex consists of four brain areas: the protocerebral bridge; the upper and lower divisions of the central body; and the paired noduli. Each of these can further be subdivided into vertical slices and/or horizontal layers (Figure 3B). The protocerebral bridge can be divided into 18 slices, each interconnected with a slice of the lower division of the central body via a type of neuron called CL1. This results in a highly organized branching pattern between the protocerebral bridge and the central body (Figure 3B). CL1 neurons have recently been described as head-direction cells in fruit flies, which implies that these cells combine visual information with self-motion cues. Moreover, each CL1 neuron has a different visual receptive field, and together, the full set of CL1 cells cover the entire visual field of the animal, their mapping and connectivity within the central body forming a ring attractor network. This means that the activity of the entire population of CL1 neurons represents the visual environment as one single activity bump, much as the needle of the magnetic compass on a ship that outputs only one reading for each direction the vessel is steered.

Because of the branching pattern of the CL1 cells, a single activity bump in the central body will give rise to two activity bumps, eight slices apart, in the protocerebral bridge (Figure 3B). Consequently, the positions of these bumps on the protocerebral bridge are intimately connected to the heading that an animal chooses to travel, resulting in a dynamic 'compass needle' in each hemisphere of the protocerebral bridge. In dung beetles, it is well documented that the CL1 neurons encode the position of skylight cues (polarized light and sun/moon). This makes these cells ideal candidates for the decoder of the current celestial view (Figures 3C, blue). But where is the orientation reference, the celestial snapshot, stored in the brain? Currently, we have no physiological evidence to conclusively answer this question, but a recent model put forward by Stone *et al.* (2017) of the central complex in path-integrating insects, suggests that the CPU4 neurons could provide the beetles with the ideal neuronal substrate to memorize this type of directional information.

In parallel to the CL1 neurons described above, visual input to the CPU4 neurons could give rise to two activity bumps in the protocerebral bridge (Figure 3C) and one in the upper division of the central body. If the CPU4 neurons indeed function as memory cells — as the model suggests — the positions of these bumps should not to be linked to the rotatory movements of the insect but would rather 'lock' to given slices within the protocerebral bridge and the central body. In this way, this type of neuron could potentially represent the relatively stable celestial snapshot taken prior to rolling. According to this theory, the current celestial view (coded by the CL1 neurons) and the celestial snapshot that represents the desired heading (coded by the CPU4 neurons) would then be compared and matched along the neuropils of the central complex. From here, the activity of CL1 and CPU4 cells could either directly or indirectly affect the neural activity of a downstream neuron to control steering. Such a neuron, which guides the animal's next manoeuvre, has been detected in cockroaches, but the identity of this cell is not yet known. One likely candidate is the CPU1 neuron, which has been suggested in bees and flies to transmit steering information from the protocerebral bridge and upper division of the central body to descending pathways.

Taken together, the central complex provides a neural substrate that can cover the main components of a celestial snapshot network, but do all these neurons exist in dung beetles? When tracing individual neurons of the dung beetle's central complex, we can indeed find all three cell types mentioned above (Figure 3D). The neural compass needle for straight-line orientation is therefore likely to be located in this central area of the brain.

A dynamic compass network supports reliable orientation at all times of day

Fresh dung is produced around the clock. Dung beetles potentially need to follow their internal celestial compasses under the baking sun as well as under dark skies. To support reliable orientation 24/7 within many species active at different times, the dung beetle compass relies on a large repertoire of compass cues (Figure 1A,B), dynamically adjusting the relative weights of these cues according to their availability and the time of day. Behaviourally, this can most easily be demonstrated by the use of a mirror. When positioned so as to change the position of the sun, or the moon, by 180° (while the experimenter simultaneously shields the real sun or moon from the beetle's view), diurnal beetles swiftly change their bearings by about 180°. Nocturnal beetles, on the other hand, carry on as if nothing has changed.

The explanation for these contrasting behaviours can be found by simulating the same situation in the lab, while simultaneously recording from the TL (Figure 2C) and CL1 neurons in the central complex of either of the two species of beetles. From these recordings, it is obvious that the diurnal beetle's sky-compass network codes the sun as the main reference for straight-line orientation. In their nocturnal cousins, the same types of compass neurons are primarily tuned to the polarization pattern of the moon, rather than to the moon itself. Consequently, as the sky-wide polarization pattern remains unaffected by the mirror in our experiment above, the beetles do not follow the 'displaced moon'. But if the nocturnal beetles are instead made to roll during the day (which is possible through their abrupt exhumation from the ground followed by the addition of some tempting, fresh dung) they can now be observed to quickly and accurately change their bearing in response to the mirrored sun.

An identical switch, from broad polarization tuning to point source tuning, can be recorded from their central-complex neurons as we raise the light levels in the lab from dim to bright. This dynamic modulation of the relative impact of different compass cues most likely serves to allow the animals to follow the most reliable compass cue at any moment in time. On moonless nights, the beetles fall back on the Milky Way for directional guidance (Figure 1B). Even though we do not yet know how this celestial cue is encoded, we expect it to be processed by the same compass network as presented above (Figure 2B).

Taken together, dung beetles rely on the same neural network to stabilise their bearings as path-integrating and migratory insects do, with the central complex as the main cockpit for steering. For tenacious dung beetles that push their balls through highly challenging terrains at all hours of the day, a dynamic and straightforward snapshot compass system seems to be the most efficient way to roll as the crow flies.

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