3D reconstruction of the heading direction network in the dung beetle brain

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Abstract

For *Kheper lamarcki*, a species of dung beetle, the smell of dung means food. However, a dung pile can be a true battle arena. Since eating at the dung pile in peace is difficult, *K. lamarcki*'s solution is claiming a round-shape part of the dung pile and rolling it away. *K. lamarcki* has found the most effective way of rolling the ball, by travelling in a straight line. However, Maintaining a straight path is easier said than done. The answer to how *K. lamarcki* is capable of rolling straight, lies in the brain. External (and internal) cues, representing the beetle's current direction, are sent to the brain. This signal is then sent to a part of the brain called the Central Complex (CX) where the current direction is compared to the desired direction, thus correcting for errors in the beetle direction. **This circuitry, in the CX, has been highly conserved in insects, however not identical between species. With a novel protocol, we have obtained the data to describe in high resolution the current direction network of *K. lamarcki*. We aim to trace this network of *K. lamarcki* and to compare it with that of other insects.** Such insights could provide a better understanding of the neuronal basis of dung beetle navigation, the evolution of the heading network in insects and potential further applications in robotics and navigational AI.

Introduction

There is an ever increasing body of evidence that points to a retention of the neuronal map behind orientation in insects (Hokanen). Independent of the navigational strategy USED, the central complex(CX)(capitalized), the environmental cue integration centre, is highly conserved in insects (including, flies, locusts, bumblebees, sweet bees and ants (reference)).

At its fundamental core, orientating in ones environment relies on comparing one's current direction with one's desired direction (reference). Environmental cues, representing ones current direction, are carried from the sensory organs to the CX at the center of the insect's brain(Jundi et al 2018? or 2019?). FOR FLIES? Within the CX the environmental cues are sent to a neuropil called the ellipsoid body(EB) where a specified bump in activity along the EB translates to the FLY's current direction(Honkanen).

Mapping one's angle in physical space onto the EB requires each angle in physical space being represented within the EB. This is where columns come in, a specific

direction in physical space is represented as a spike in activity within one column of the EB(reference, fly paper with that figure). Certain types of cells behave in the same way, relaying information from a column of a neuropil to a column of another neuropil.

This bump in activity in one column (simplicfication) is relayed to the protocerebral bridge (PB) with columnar neurons called E-PGs. The recurrent circuit is closed with neurons named P-ENS. These neurons carrying optic flow infromation (whati s that) correct current direction based on variance in optic flow between eyes.

FOR FLIES This desired direction is relayed via the protocerebral bridge(PB) and stored in memory in another neuropil of the CX, the fan-shaped body (FB)(jundi?). Following the saving to memory, the desired direction needs to be continuously referenced throughout the journey with one's current direction. For the dung beetle, Kheper lamarcki, maintaining one desired direction is essential if one wants to eat in peace.

The smell of dung attracts thousands of dung beetles, all looking for a meal. To avoid the fierce competition at the dung pile, one species, K. lamarcki, has found an effective way to eat in peace – it shapes a piece of dung into a ball and escapes along a straight line(2002 paper?). The beetle performs straight line orientation thanks to several environmental cues, including the sun, polarized light and the wind(MAYBE Dacke et al., 2014; Jundi et al., 2014)(internal cues?).

Before commencing its journey the beetle must first decide its desired direction by taking a snapshot of its environment. This snapshot is taken when the beetle performs a little dance, a stereotypical behaviour observed where the beetle climbs onto its ball and rotates on top of it(refenrece). The environmental cues, OBSERVED during this dance, are carried from the sensory organs to the Central Complex(CX)(capitalized) at the center of the beetle's brain(Jundi et al 2018? or 2019?). It is hypothesized that for the beetle, at a random point in the dance, this bump in activity is decided as the desired direction for the beetle.

For the beetle, it is believed that with the snapshot now saved, it can commence its journey along a straight line. This step requires a continuous reference to the snapshot as the journey requires a continuous comparison to the desired direction with one's current direction.

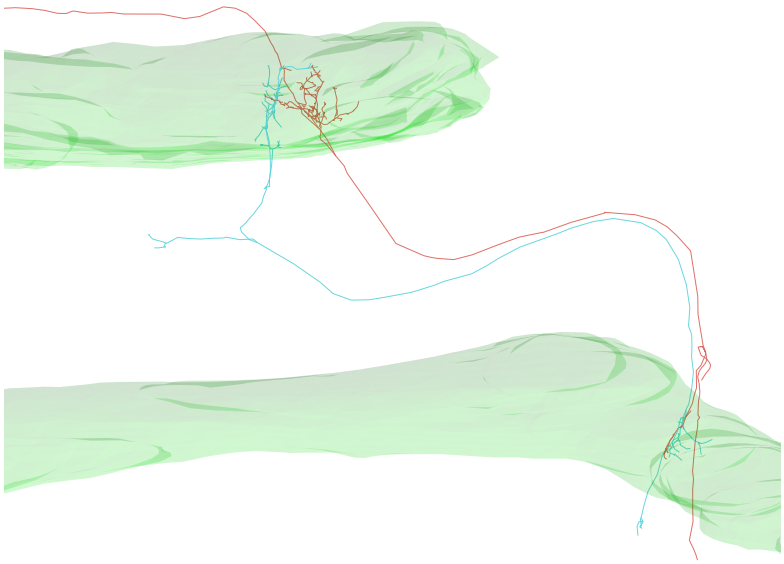
Most of this is based off beahvioural research, not having jumped into the inner workings of the brain. Similarly to other insects a CX is present along with the neuropils and even E-PG and P-EN neurons have been located, however beyond this many of stipulations remain unconfirmed at the neuronal level. neuropil of the CX, the fan-shaped body (FB)(jundi?). This project aims to elaborate on previous dung beetle research about the EB, PB and the E-PG, and P-EN neurons as the current direction/heading network in the dung beetle brain.

figure of flow of information to central complex and central complex in all comparison species

Materials and methods

Beetle to image stack

Following the protocol established by UNKNOWN the brain from a dung beetle of the species K. lamarcki was preserved for serial block-face electron microscopy. Subsequently, another protocol established by UNKNOWN was followed for finding the area of interest (CX) and to commence the microscopy. Pictures were taken at UNKWON nm intervals, (where intervals were created by a diamond knife) at a resolution of UNKOWN. Equally, higher (UNKNWON) resolution photos were taken of



S1 Fig. Bold the title sentence. Looking at PEN_1 and EPG_1 we see overlap within the PB and an offset within the EB, the only thing worth noting is the presence of one brance veering off for the PEN neuron entering into the same column as the EPG.

the neuropils of the central complex, for the LEFT neuropil, LEFT-SIDE of the EB and FB and the RIGHT side of the PB. [1].

Image stack processing

The stack of photos went through a pre-processing phase of merging, aligning and downgrading to allow use of the Amira(VERSION) and catmaid (VERSION) softwares. Once pre-processed, neuropil boundaries were segmented manually for all neuropils of the central complex, with Amira to form 3D reconstructions of the neuropils. Once E-PG and P-EN neurons were localized, the neurons could be traced manually in Catmaid.

Visualization

A python script using pymaid was used to plot the neurons (SUPPORTING INFO.

Results

Finding E-PGS & P-ENs

Number of EPGs found in Column 4 of the PB was 6, this was confirmed when tracing the number of neurons in column 3, which also contained 4 E-PGs. E-PG neurons indeed arborized in the EB and PB neuropils while all trailing off in the same direction, mostly likely to the Gall, although not confirmed. The identification as E-PG was strengthened as all other traced E-PGs also headed in the same direction.

Only 1 PEN neuron was found in column 6 of the PB. The PEN neuron identification was deduced from the arborization into the PB, EB and noduli. Projection density of the E-PGs and P-ENs as well as layer projection were not confirmed due to having access only to the overview, lower-resolution image stack.

Presence of an offset

Within the 6th column of the PB, 4 E-PGs and 1 P-EN shared the same column. However, when following these neurons into the EB, the arborization was no longer in the same column. Although not confirmed by how many columns the E-PG and P-EN are offset, it seems as though the P-ENs are offset by 1 column from the E-PGs.

(FIGURE) one figure showing PB EB and noduli as well as offset for all neurons of same PB column, and hopefully the presence of 4 epgs and 1 pen

Discussion

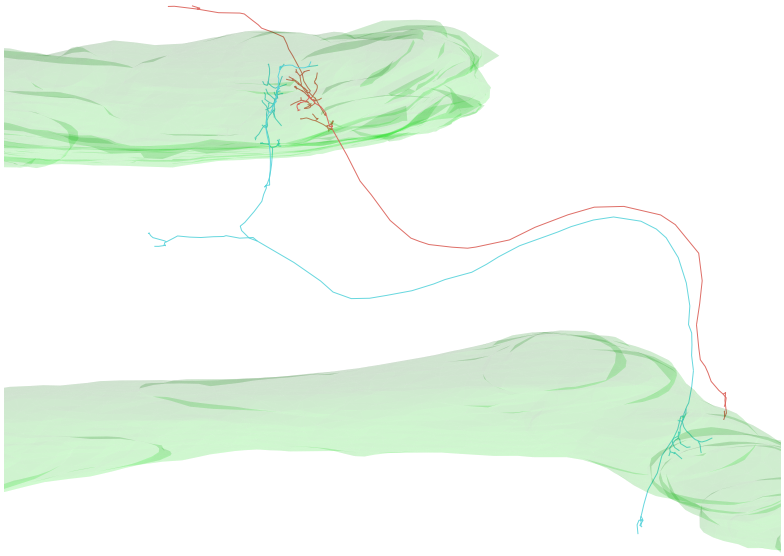
The offset

Indeed it seems as though the the current direction network is well conserved within insects. Mapping one's angle in physical space onto the EB requires each angle in physical space being represented within the EB. This is where columns come in, a specific direction in physical space is represented as a spike in activity within one column of the EB(reference, fly paper with that figure). Certain types of cells behave in the same way, relaying information from a column of a neuropil to a column of another neuropil. E-PG and P-EN neurons are indeed columnar, each E-PG and P-EN falling within a specific column within the EB and PB, thus confirming the findings of columns within the PB and EB of K. lamarcki (Immonen 2017).

el. jundi 2018 first described E-PG and P-EN neurons within the CX of K. lamarcki, and now the presence of an offset has been observed between these two neurons. This indeed follows the larger trend within insects as such an offset has been found in a growing number of insects (including, flies, locusts, bumblebees, sweet bees and ants (reference)).

future directions

However, it needs to be considered that without higher-resolution, neuropil-specific images, a recurrent loop (communication) between E-PGs and P-ENs cannot be confirmed. Finding post and pre synaptic regions (arborization overlap) between E-PGs and P-ENs should be conducted to confirm the current direction network in K. lamarcki.



S1 Fig. Bold the title sentence. Confirmation of the statement above with EPG_2.

(and in the same breadth, determining the presence of layers within the EB, to shed light on.)

So far the data collected has pointed towards confirming the current direction network in K. lamarcki, however a more complete mapping of these two types of neurons could show subtle differences of the curerent direction network that exist between k. lamarcki and other insects. The largest question with regards to E-PGs and P-ENs is how the 360 field is actually represented in the EB. Yes we know that each angle is physical space is covered by a column in the EB, however the EB is not a circle but an ovoid shape with two ends. How does the beetle cover this 360 field without a complete circle? Research on other species have pointed towards overlap in neurons, indeed projecting into two EB columns, which may be the case for K. lamarcki, but poses an interesting question to try and solve.

is aborization density the same in each column
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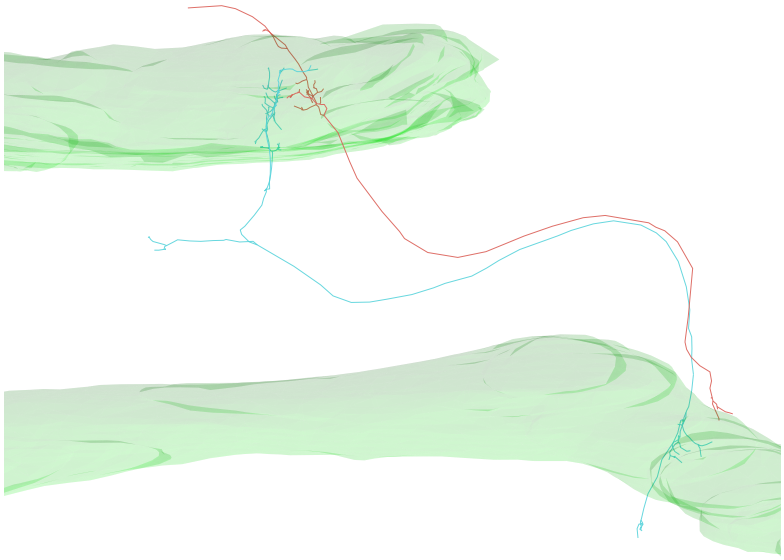

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Conclusion

Within the CX we can theorize that following this confirmation with these twos type of columnar neuron, columnar neurons within the CX most likely follow the same destiny doubling down on the theory of high evolutionary retention current direction network and the greater CX network.

Supporting information

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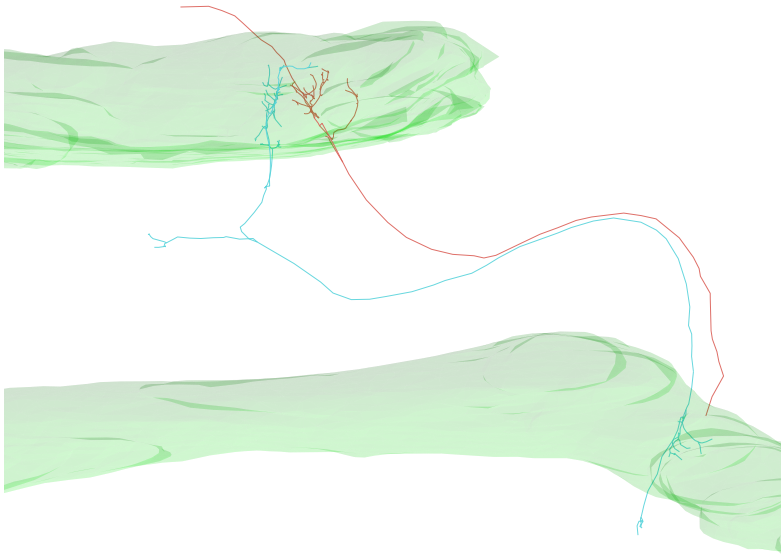

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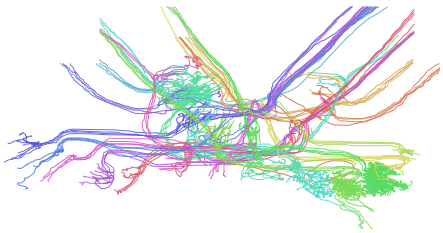

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Acknowledgments

Marcel Sayre? Valentin Gillet? Saroja? Sherry?

References

1. Stone T, Webb B, Adden A, Weddig NB, Honkanen A, Templin R, et al. An Anatomically Constrained Model for Path Integration in the Bee Brain. Current Biology. 2017;27(20):3069–3085.e11. doi:10.1016/j.cub.2017.08.052.