# Appendix A: An overview of brain organization in CC and non-CC lineages

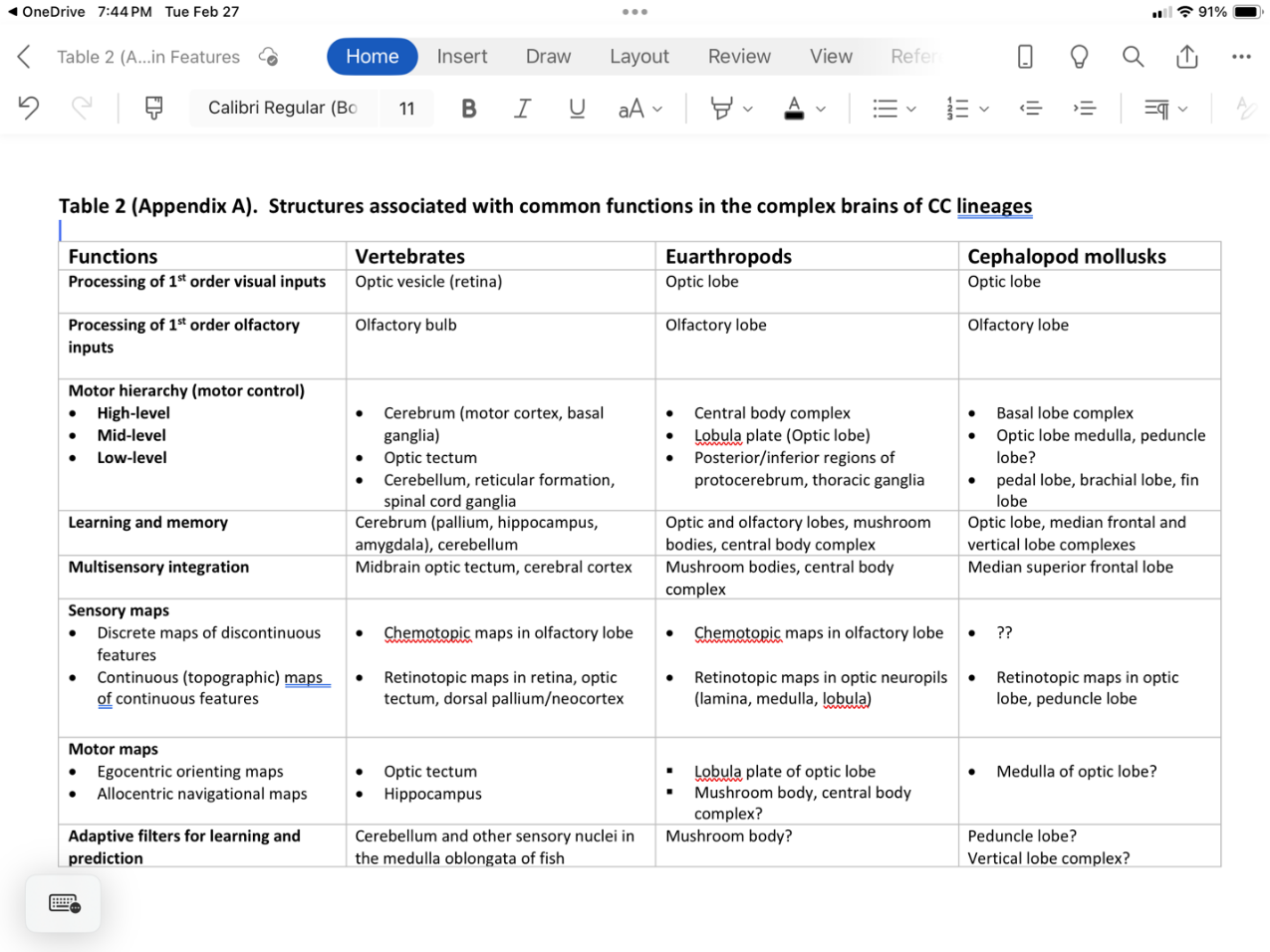
Direct comparison of brain regions across widely divergent taxa is a daunting task, made more difficult by the fact that the terminology used to describe different invertebrate brain regions is extremely varied and often confusing, even within the same phylum (Ito et al., 2014; Richter et al., 2010). To simplify comparisons, we divided bilaterian phyla (the only phyla with brains) into deuterostomes (vertebrates and other chordates) and protostomes (euarthropods, mollusks and other invertebrates)(see Figs 1 and 2 in the main paper for examples in a phylogenetic context).

## 1. A comparison of brain development and organization in deuterostomes and protostomes

The brains of deuterostomes and protostomes develop in fundamentally different ways. Brains in deuterostomes develop from an elongated, hollow tube along the anterior-posterior body axis. As the hollow tube develops, it divides into three compartments, the prosencephalon, mesencephalon and rhombencephalon and together these form the basic scaffold of the vertebrate brain (Sugahara et al. 2017; Table 1). Northcutt (2002) recognizes five morphologically distinct structures within this scaffold: (1) the olfactory bulbs and (2) cerebral hemispheres (prosencephalon), (3) the optic tectum (mesencephalon), and (4) the cerebellum and (5) medulla oblongata (rhombencephalon) (Table 1). We also recognize the laminated retina of the optic vesicle as a distinct brain structure that is comparable to the optic lobes of euarthropods and mollusks.

In contrast, euarthropod, mollusk and other protostome brains develop from a ring of connectives with ganglia (collection of neuron cell bodies) around the mouth and esophagus (Young 1971, Roth and Dicke 2013). In euarthropods, fusion of the ganglia results in a tripartite organization along the anterior posterior axis similar to that in vertebrates. Moreover, at least five structures are readily recognized within the three major neuromeres in most euarthropods – the (1) optic lobes, (2) mushroom bodies and (3) central body complex (protocerebrum), (4) olfactory lobes (aka antennal lobes in insects)(deuterocerebrum) and (5) mechanosensory neuropils from secondary antenna (tritocerebrum) (Loesel et al. 2013; refs)(Table 1).

The organization of ganglionic masses in cephalopod mollusk brains along the dorsal-ventral (Young 1971, Grasso and Basil, 2009) rather than anterior-posterior body axis make comparisons with vertebrates and euarthropod brains more difficult. The supraoesophageal mass, which is the largest, is connected to the large optic lobes outside the cranial cavity (see Fig. 2D in the main paper), as is the anterior most segment of the supraoesophageal mass in euarthropods (the protocerebrum)(Fig.2B in main paper). The supraesophageal mass of cephalopod mollusks houses several other large structures, including the vertical, frontal and basal lobe complexes, whereas small brachial and pedal lobes are part A screenshot of a computer

Description automatically generatedof the suboesophageal mass (Young, 1971; Budelmann 1994; Yamazaki et al. 2002) (Table 1).

Large lobes or structures in the brains of all three CC lineages are further subdivided into different functional regions (Tables 1 and 2). For example, the cerebral hemispheres of most vertebrate brains are partitioned into at least three functional regions, principally along the dorsal-ventral axis: (1) a dorsal (surface) part known as the pallium (non-mammals) or neocortex (mammals), which has numerous higher-order sensory, motor and associative functions (see below), (2) an internal dorsomedial pallial structure called the hippocampus (mammals), best known for its involvement in spatial learning and memory (Broglio et al, 2005; Eichenbaum, 2017; Nyberg et al., 2022), and (3) a highly conserved ventral region known as the subpallium (non-mammals) or basal ganglia (mammals), involved in motor control and motor learning (Striedter and Northcutt, 2020). The pallium/neocortex, the structure most frequently associated with executive function and cognitive abilities, is even further subdivided into anatomically and physiologically defined areas with distinct inputs and outputs. These include primary sensory areas for higher-order processing, motor areas for controlling vocalizations and other motor behaviors, associative areas and areas dedicated to higher mental functions (Kaas 2008,2017).

Ito et al. (2014) recognize as many as 47 different subdivisions (their level 2 designations corresponding to synaptic neuropils) among major brain lobes in euarthropod hexapods (insects). For example, the mushroom body (MB) common to most, if not all euarthropods, is associated with learning and memory, and is structurally differentiated into several regions, including a basal pedunculus and an apical calyx (Strausfeld 1998). In species with some of the largest MBs (e.g., honeybees), the MBs receive multiple sensory inputs and have highly elaborated (gyrencephalic) calyces, which are functionally segregated into input areas that are modal-specific (reviewed in Farris, 2005; 2008; 2013). The major lobes of the octopus brain are likewise subdivided into another 15 or so minor lobes, 5 of which form the vertical lobe complex, which is associated with learning and memory (Young, 1971). A final example is the 1st- order visual centers that receive direct inputs from the eyes in all three CC lineages. These are all differentiated into distinct layers and columns that function in serial and parallel processing of the visual scene (Sanes and Zipursky, 2010)(see also Brain traits in Appendix B).

## 2. Deuterostome variations in brain complexity

Within deuterostomes, vertebrate brains clearly show more regional specializations than those in non-vertebrate phyla. Hemichordata (acorn worms) and Echinodermata (starfish, brittle stars), for example, have diffuse nervous systems with no clear centralization of neurons that could qualify as a brain (Smith, 1937; Lowe et al., 2006; Fritsch and Glover, 2009; Hoekstra et al., 2012). Within the third deuterostome phylum (Chordata), the CNS of free-swimming larval tunicates (sea squirts, subphylum Urochordata) consists of a rostral ganglion with a single light-detecting organ (ocellus) and a caudal ganglion and nerve cord that are reportedly homologous to the vertebrate diencephalic forebrain, hindbrain and spinal cord (Fritzsch and Glover 2009). Similarly, amphioxus (lancelets, subphylum Cephalochordata) brains can be differentiated into a rudimentary rostral region (cerebral vesicle) that receives sensory inputs from a single frontal eye and more posterior regions that are homologous to the vertebrate diencephalon and midbrain and/or hindbrain, respectively (Wicht and Lacalli, 2005). Despite regional homologies with parts of the vertebrate brain, brains in non-vertebrate chordates are not divided into five distinct structures within a well-delineated tripartite brain, as they are in vertebrates.

With a few notable exceptions (e.g., the absence of a cerebellum in hagfish), the five main vertebrate brain structures, as well as many of their functional subdivisions (e.g. the ventral motor region of the cerebral hemispheres, Table 1, Row 2) are generally conserved across taxa (Northcutt, 2002). However, some regions have undergone relatively dramatic evolutionary changes in terms of the size and number of subdivisions. Of particular relevance to cognitive abilities, are those associated with the olfactory bulb and pallial regions of the telencephalon (Streidter and Northcutt, 2020). In mammals, especially primates, the olfactory bulb is substantially reduced compared to non-mammalian vertebrates (fish, birds, amphibians and reptiles) (Streidter and Northcutt, 2020). Concomitant with the reduction in the olfactory bulb is an increase in the size of the pallium/neocortex and the number of its subdivisions – from ~ 10 – 15 in non-mammalian vertebrates to as many as 200 in humans (Streidter and Northcutt, 2020; Kaas 2008, 2013). This represents a major evolutionary shift from an olfactory- to visually- dominated telencephalon that accompanied the land-to-water transition of vertebrates (MacIver and Findlay, 2022).

## 3. Protostome variations in brain complexity

Among protostomes, brain complexity varies considerably within CC lineages of each clade (Fig. 1 and 3, main paper). Mushroom bodies in ecdysozoan euarthropods vary in both size and complexity, depending in part on the number of sensory modalities that provide input (Gronenberg 2001; Farris, 2013, 2015; Wolff and Strausfeld, 2015). Similarly, the size (and differentiation) of the optic lobe is variable, with the optic lobe often reduced in size or absent in some Myriopods (centipedes and millipedes). In general, myriopods have a much simpler ground pattern organization of the brain than other euarthropods (Strausfeld and Andrew, 2011). Mollusks in the spiralian protostome clade exhibit the widest range in both body plan (Wanninger and Wollesen, 2019) and brain diversity (Hochner and Glantzman, 2016), from hardly any brain at all in subphyla with no heads or limbs and sessile lifestyles (e.g., bivalves) to reportedly the most complex brains of all invertebrates – those of cephalopod mollusks. This subphylum is characterized by high levels of mobility and behavioral flexibility (Grasso and Basil, 2009; Faller et al., 2012; Hochner and Glanzman, 2016), made possible by the addition of limbs and the reduction (and internalization) of the shell (Kroger et al.). Even within this ‘super brain’ subphylum, the octopus has many more (~ 40 total) brain lobes than the less-mobile nautilus, which has an external shell and only ~ 13 brain lobes (Grasso and Basil, 2009).

Brains in non-CC invertebrate phyla generally consist of fewer, less-differentiated lobes (or ganglia) compared to CC lineages. For example, brains in protostome (Spiralia) phyla, such as Platyhelminthes (Morris et al., 2007; Hartenstein 2015), Brachiopoda (Kuzmina and Temereva 2021) and Nemertea (Beckers and von Dohren, 2015) typically consist of no more than one pair (often unfused) of cerebral ganglia (Fig. 3D). Brains in ecdysozoan protostome phyla, such as Nematoda (Telford et al., 2008) and Tardigrada (Zantke et al., 2008; Mayer et al. 2013a,b), are likewise reduced in having only one or two segments of the tripartite brain normally present in euarthropods. Nevertheless, signs of regional differentiation in the form of mushroom bodies have been identified in several non-CC phyla, including both spiralian (Annelida, Platyhelminthes and Nemertea) and ecdysozoan (Tardigrada and Onychophora) protostome phyla (Heuer and Loesel 2008; Strausfeld et al., 2006; 2020; Martin et al., 2022).

Among non-CC phyla, annelid brains are the most diverse (Bullock and Horridge, 1965, Heuer et al, 2010; Purschke 2016; Beckers et al 2019a, b), with two polyphyletic clades (Sedentaria and Errantia) being the main source of diversity (Weigert and Bleidorn, 2016). These two clades show adaptations for either an errant (= mobile) or sedentary lifestyle, with several modifications of body and sensory traits, such as complex eyes, to guide mobility in Errantia (Struck et al. 2011; Purschke & Nowak, 2015; Harley and Asplen, 2018). In addition, animals in the sedentary clade (e.g., lugworms, Capitellidae) have simple brains (two unfused, undifferentiated ganglia) like those found in nematodes, whereas those in the errant clade (e.g., ragworms, Nereididae) have more complex brains like those found in euarthropods. The latter consist of fused and differentiated ganglia with distinct neuropils - e.g., 1st -order optic and/or olfactory lobes, mushroom bodies and unpaired midline neuropils (Heuer et al, 2010; Purschke 2015).

3. Summary and conclusions

Complex brains in the three CC lineages are similar in having (1) a differentiation of the brain into discrete lobes or structures, principally along the anterior-posterior body axis, but also along the dorsal-ventral axis, (2) a bilaterally symmetric pairing of lobes/structures in the medio-lateral (left-right) axis and (3) further internal differentiation of lobes/structures into functionally specialized subdivisions (Fig. 2). Shared functional and organizational themes are at least partly, if not largely due to the role of shared gene regulatory networks in brain development (Tomer et al., 2010; Strausfeld and Hirth, 2013; Farris, 2015; Wolf and Strausfeld, 2015; Bridi et al., 2020). Brain features shared among CC lineages include (1) first-order brain regions that process inputs from sense organs, most notably visual and olfactory senses, (2) motor hierarchies that consist of low, mid and high-level brain regions that control motor actions, (3) structures associated with learning and memory that are distributed along the motor hierarchy and finally, (4) multisensory integration areas, also distributed along the motor hierarchy (see Brain traits in Appendix B).

Regional differentiation of the brain reaches its highest levels in CC lineages and its lowest levels in non-CC phyla. However, the degree of differentiation can vary widely within a given phylum, especially among mollusks and annelids, in which high degrees of regional complexity are associated with increased mobility.

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