Neuroarchitecture of the central complex of the desert locust: Tangential neurons

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Abstract
The central complex (CX) comprises a group of midline neuropils in the insect brain, consisting of the protocerebral bridge (PB), the upper (CBU) and lower division (CBL) of the central body and a pair of globular noduli. It receives prominent input from the visual system and plays a major role in spatial orientation of the animals. Vertical slices and horizontal layers of the CX are formed by columnar, tangential, and pontine neurons. While pontine and columnar neurons have been analyzed in detail, especially in the fruit fly and desert locust, understanding of the organization of tangential cells is still rudimentary. As a basis for future functional studies, we have studied the morphologies of tangential neurons of the CX of the desert locust Schistocerca gregaria. Intracellular dye injections revealed 43 different types of tangential neuron, 8 of the PB, 5 of the CBL, 24 of the CBU, 2 of the noduli, and 4 innervating multiple substructures. Cell bodies of these neurons were located in 11 different clusters in the cell body rind. Judging from the presence of fine versus beaded terminals, the vast majority of these neurons provide input into the CX, especially from the lateral complex (LX), the superior protocerebrum, the posterior slope, and other surrounding brain areas, but not directly from the mushroom bodies. Connections are largely subunit- and partly layer-specific. No direct connections were found between the CBU and the CBL. Instead, both subdivisions are connected in parallel with the PB and distinct layers of the noduli.

1 | INTRODUCTION

Insects show impressive abilities in spatial orientation that in many aspects come close to those of vertebrates. The multitude of navigational strategies of insects, ranging from landmark orientation (Collett & Zeil, 1997) to path integration (Wehner, Michel, &...
Antonsen, 1996) and even to map-like navigations (Menzel et al., 2005) reflects their adaptation to highly different habitats. The underlying mechanisms often work together as demonstrated in ants and bees which use visual landmarks, sky compass information, and olfactory cues to find back to their nest (Frost & Mouritsen, 2006; Steck, Hanson, & Knaden, 2011; Wehner et al., 1996). A sky compass, relying on the position of the sun, the sky polarization pattern, and other celestial cues is used by insects for maintaining navigational directions during food collection (Dacke, Byrne, Smolka, Warrant, & Baird, 2013; Dacke & el Jundi, 2018; Dacke, Nilsson, Scholtz, Byrne, & Warrant, 2003), homing (Wehner, 1997, 2003), and seasonal migrations (Frost & Mouritsen, 2006; Merlin, Heinze, & Reppert, 2012). Accumulating evidence from various insect species points to a key role of the central complex (CX), a group of neuropils in the central brain, in various navigational tasks, including sky compass orientation (Homberg, Heinze, Pfeiffer, Kinoshita, & el Jundi, 2011; Honkanen, Adden, da Silva Freitas, & Heinze, 2019; Merlin et al., 2012), spatial representation and recognition of objects (Liu et al., 2006; Neuser, Triphan, Mronz, Poeck, & Strauss, 2008; Seelig & Jayaraman, 2013; Triphan, Poecd, Neuser, & Strauss, 2010), coding of heading direction (Heinze & Homberg, 2007; Seelig & Jayaraman, 2015; Varga & Ritzmann, 2016; Turner-Evans et al., 2017), place learning (Ofstad, Zuker, & Reiser, 2011), control of walking trajectory (Martin, Guo, Mu, Harley, & Reiser, 2011), and path integration (Honkanen et al., 2019; Stone et al., 2017). Another line of evidence in the fly Drosophila points to a role of the CX in sleep control (Donlea et al., 2018; Donlea, Pimentel, & Miesenböck, 2014; Liu, Liu, Tabuchi, & Wu, 2016).

The CX spans the midline of the insect brain (Figure 1a,b). It consists of four subunits: the protocerebral bridge (PB), the upper and lower divisions of the central body (CBL, CBU), and the paired noduli (Hanesch, Fischbach, & Heisenberg, 1989; Heinze & Homberg, 2008; Müller, Homberg, & Kühn, 1997; Pfeiffer & Homberg, 2014; Williams, 1975). Internally, the PB, CBL, and CBU are subdivided by columnar neurons into vertical slices. In addition, the central body (CB) subdivisions are

**FIGURE 1**  Subdivisions of the locust central complex (CX), the lateral complex (LX), and major fiber tracts of tangential neurons to the central body and noduli (NO). (a) Frontal view, (b) cross section through the three-dimensional standard of the CX and LX of the desert locust (taken from el Jundi et al., 2010) illustrating the layering of the central body. The CX consists of the upper division (CBU) and lower division (CBL) of the central body, the protocerebral bridge (PB), and the paired noduli (NO). The LX consists of the upper (ULAL) and lower (LLAL) shell of the lateral accessory lobe, the medial (MBU) and the lateral bulb (LBU). The CBL consists of six layers labeled 1–6, the CBU of three layers labeled I–III. The NO can be divided into an upper unit (NOU), subdivided into three layers, and a lower unit (NOL). (c) Frontal view and (d) sagittal section at the level of the nodulus illustrating the trajectories of fiber tracts of tangential neurons. A, anterior; AB1–AB3, anterior bundles 1–3; ALI, anterior lip; d, dorsal; LBU, lateral bulb; IT1–IT6, isthmus tracts 1–6; NOL, lower unit of the nodulus; NOU, upper unit of the nodulus; p, posterior; pg, posterior groove; v, ventral; vg, ventral groove. Scale bars = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
composed of horizontal layers (Hanesch et al., 1989; Homberg, 1991; Ito et al., 2014; Pfeiffer & Homberg, 2014; Wolff, Iyer, & Rubin, 2015). Slices and layers of the CX are primarily built by three classes of neurons, columnar, tangential, and pontine neurons, studied most thoroughly in Drosophila and larger flies (Hanesch et al., 1989; Omoto et al., 2018; Strausfeld, 1976; Wolff et al., 2015; Wolff & Rubin, 2018), the desert locust Schistocerca gregaria (Heinze & Homberg, 2008; Müller et al., 1997; Vitzthum, Homberg, & Agricola, 1996), the monarch butterfly Danaus plexippus (Heinze, Florman, Asdokaraj, el Jundi, & Reppert, 2013), and dung beetles (el Jundi, Warrant, Pfeiffer, & Dacke, 2018).

Columnar neurons ramify in single slices of the PB, certain layers of the CBU or CBL, and in regions of the lateral accessory lobes (LALs) or noduli. They exhibit precise interhemispheric connections via chiasmal fiber crossings in an anterior or posterior chiasma (el Jundi et al., 2018; Hanesch et al., 1989; Heinze et al., 2013; Heinze & Homberg, 2008; Williams, 1975; Wolff et al., 2015) and are considered the principal output elements of the CX. Pontine neurons are intrinsic to the CBU and connect distinct slices and layers with one another (Andrade et al., 2019; el Jundi et al., 2018; Hanesch et al., 1989; Heinze et al., 2013; Heinze & Homberg, 2008; Homberg, 1985; Siegl, Schachter, Holstein, & Homberg, 2009). Tangential neurons are the principal input elements of the CX: they connect different regions of the brain to the PB or particular layers of the CB (el Jundi et al., 2018; Hanesch et al., 1989; Heinze et al., 2013; Heinze & Homberg, 2007; Müller et al., 1997; Omoto et al., 2018; Phillips-Portillo & Strausfeld, 2012).

For the desert locust, Müller et al. (1997) and Heinze and Homberg (2008) provided catalogues of pontine and columnar neurons of the CX. In contrast, our understanding of the diversity of tangential cell types, especially of the CBU, is still fragmentary and largely based on selected cell types (Beetz, el Jundi, Heinze, & Homberg, 2015; Heinze & Homberg, 2007; Müller et al., 1997; Omoto et al., 2018; Phillips-Portillo & Strausfeld, 2012).

To fill this gap, we analyzed the morphologies of tangential neurons of the CX in the desert locust, based on single-cell dye injections combined with fluorescent neuropil labeling. Some neurons were reconstructed in three dimensions (3D). We have mapped the cell body positions of tangential neurons in the brain and show that certain neuropil areas of the central brain are specifically connected to distinct CX subdivisions and, within subdivisions, to particular layers. The analysis of tangential neurons provides an essential basis for future functional studies on input pathways to the CX.

2 | MATERIALS AND METHODS

2.1 | Animals

Male and female adult desert locusts (Schistocerca gregaria) were obtained from crowded colonies at the Philipps-University of Marburg. The animals were kept at a light-dark cycle of 12:12 hr, a temperature of 28°C and 50% atmospheric humidity. All animal procedures were in conformity with the guidelines of the European Union (Directive 2010/63/EU) and the German Animal Welfare Act.

2.2 | Neurobiotin and Lucifer Yellow injections

Animals were cold-anesthetized to 4°C prior to preparation. Their head capsule was opened for single cell intracellular recordings with sharp glass microelectrodes as described by Vitzthum, Müller, and Homberg (2002) and Heinze and Homberg (2009). Following recording of neural activity during presentation of sensory stimuli, neurons were iontophoretically injected with Neurobiotin (Vector Laboratories) by applying constant depolarizing currents (0.5–4 nA; 1–10 min) or with Lucifer Yellow by applying constant hyperpolarizing currents (1–3 nA; 1–5 min). After dissection the brains were fixed overnight at 4°C in Neurobiotin fixative (4% paraformaldehyde, 0.25% glutaraldehyde, 2% saturated picric acid, in 0.1 M phosphate buffer) or 4% formaldehyde (Lucifer Yellow injections), followed by rinses in 0.1 M phosphate-buffered saline (PBS, 4 × 15 min). Neurobiotin injected brains were incubated with streptavidin-Cy3 conjugates (1:1,000; Jackson ImmunoResearch Labs, RRID: AB_2337244) for 3 days at 4°C. After washing in PBS with 0.3% Triton X-100 (PBT, 2 × 30 min) and in PBS (3 × 30 min) brains were dehydrated in an increasing ethanol series (30%, 50%, 70%, 90%, 95%, and 100%, 15 min each) and transferred to a mixture of methyl salicylate and ethanol (1:1; 15–20 min). Finally, the brains were cleared in methyl salicylate for about 35 minutes, before they were mounted in Permount (Fisher Scientific, Pittsburgh, PA) between two glass cover slides with spacing rings to avoid compression.

2.3 | Texas Red application

For an analysis of cell clusters of tangential neurons, we inserted single crystals of dextran conjugated to Texas Red (MW 3,000; Life Technologies, Carlsbad, CA) into the CX of the locust. The head capsule of the harnessed animal was opened and fat and trachea covering the brain were removed. The neural sheath covering the brain was removed above the estimated position of the CX. Single crystals were manually inserted with the tip of a glass electrode into the region of the CX. The piece of head cuticle that was removed for injection was put back in place, and a piece of paper tissue soaked with locust saline solution was wrapped around the locust head to prevent desiccation. The dye was left to diffuse over night at 4°C. Afterward the brain was dissected out of the head capsule, fixed, rinsed in PBS, dehydrated and embedded as described for the Neurobiotin injected brains.

2.4 | Immunolabeling of rehydrated thick sections

Several mounted brains were selected for detailed analysis of neuronal morphologies and 3D reconstructions. Brains were incubated in
xylene (2-4 hr) to remove the embedding medium and were rehydrated in a decreasing ethanol series (100%, 95%, 90%, 70%, 50%, and 30%, 15 min each). After rinsing with PBS (4 x 20 min), they were embedded in albumin-gelatin (4.8% gelatin and 12% ovalbumin in demineralized water). The preparations were fixed over night at 4°C in 8% formaldehyde in 0.1 M phosphate buffer. On the following day the embedded brains were cut into 130 μm sections with a vibrating-blade microtome (Leica 1200S) and rinsed with PBS (4 x 15 min) before they were preincubated overnight in NGS (normal goat serum) (1:20) and PBT. Afterward the sections were incubated for 5 days with anti-synapsin (1:50), streptavidin-Cy3 (1:1,000) and NGS (1:100) in PBT at 4°C. Then the sections were washed in PBT (2 x 20 min) and PBS (3 x 20 min) before they were incubated in goat-anti-mouse-Cy5 (1:300) and streptavidin-Cy3 (1:1,000) in 1% NGS in PBT for 3 days at 4°C in darkness. The anti-synapsin antibody (RRID: AB_2315425), kindly supplied by Drs. E. Buchner and C. Wegener (Würzburg, Germany) was a monoclonal antibody raised in mouse against fusion proteins consisting of glutathione-S-transferase and the Drosophila SYN1 protein (Klages et al., 1996). It labels synaptic neuropils as shown in Drosophila (Klages et al., 1996), honeybees (Brandt et al., 2005), and locusts (Kurylas, Rohlfing, Krofczík, Jenett, & Homberg, 2008; Leitinger, Pabst, Rind, & Simmonds, 2004).

The sections were rinsed again in PBT (2 x 20 min) and PBS (3 x 20 min) and were dehydrated in an increasing ethanol series (30%, 50%, 70%, 90%, 95%, and 100%, 15 min each). The sections were transferred for 15 minutes to a mixture of 100% ethanol and xylene, and embedded in Entellan (Merck, Darmstadt, Germany). Epon embedded brains as described by Homberg, Würden, Dirksen, and Rao (1991) and Homberg and Würden (1997). Only injected neurons showed immunolabeling dem- onstrating the specificity of the anti-Lucifer antisem. Sections of Neurobiotin injected brains were incubated overnight at room tempera- ture with streptavidin conjugated to horseradish peroxidase (Amersham Buchler, Braunschweig, Germany) in PBT. After rinsing in PBS, the sec- tions were transferred to 0.05 M Tris-HCl buffer, pH 7.4. They were subsequently stained by incubation in 3,3′-diaminobenzidine tetrahydrochloride (0.3 mg/ml) with 0.3% nickel ammonium sulfate in 0.05 M Tris-HCl. The reaction was started by adding 0.015% H2O2 and was stopped by rinsing with 0.05 M Tris-HCl after staining intensity had reached a satisfactory level. The sections were mounted on chrome alum/gelatin-coated glass slides, dehydrated in ethanol, cleared in xylene, and embedded in Entellan (Merck, Darmstadt, Germany).

2.7 | Anatomical reconstructions

Fiber tracts used by tangential neurons to enter the CX were reconstructed from methylene blue stained 3-μm serial sections of Epon embedded brains as described by Homberg, Hofer, Pfeiffer, and Gebhardt (2003) using a camera lucida attachment on a Leitz compound microscope. Likewise, dye-injected, peroxidase-labeled neu- rons were reconstructed with a camera-lucida attachment to a Leitz compound microscope equipped with a 40x objective. Drawings were scanned and vectorized with Adobe Illustrator CS5 software (Adobe Illustrator CC, RRID: nlx_157287) to avoid loss of information during rescaling. In some cases, maximum projections of confocal data were used for reconstruction on a vector basis with Adobe Illustrator CS5 software to create a detailed image of the neuron. For an analysis of the cell clusters, cell body positions of tangential cells were mapped using a light microscope and Amira 5.33 software (Advanced 3D Visu- alization and Volume Modeling, RRID: nif-0000-00262). Cell clusters were registered and manually painted in Adobe Illustrator.

For 3D reconstructions, confocal data stacks were processed with Amira 5.33. For the reconstruction of neurons, we used the skeletonize plugin (Evers, Schmitt, Sibila, & Duch, 2005; Schmitt, Evers, Duch, Scholz, & Obermayer, 2004). To get a complete image stack, different image stacks of a brain were properly aligned in their x-, y-, and z-directions, before they were merged. The neurons were manually reconstructed based on brightness differences of the Cy3-immunofluorescence of the relevant image stacks. Neuropils were reconstructed based on the image stacks with Cy5-anti-synapsin staining. Characteristic vertices of the neuropils were marked on different levels of the image stacks and different directions for subsequent computation of the structures. The resulting structure of voxels was processed in high resolution (voxel size 2 x 2 x 2 μm or better). To present smooth struc- tures, polygonal surfaces were created and displayed with a filter.

2.6 | Peroxidase staining

Immunoperoxidase staining was performed on Lucifer Yellow and Neurobiotin injected and fixed brains. After rinsing with 0.1 M PBS (4 x 20 min) brains were embedded in albumin-gelatin and fixed over night at 4°C in 8% formaldehyde. On the following day the embedded brains were cut into 40 μm thick sections with a microtome (Leica 1200S) and rinsed with 0.1 M PBS (3 x 15 min). The Lucifer Yellow-

2.5 | Image acquisition and processing

Brain sections were scanned with a confocal laser scanning microscope (CLSM, TCS SP5, Leica Microsystems, Wetzlar, Germany) with a 20x objective (HCX PL APO lambda blue 20x/0.70 Imm UV, working distance: 260 mm, Leica). Most image stacks were generated with a scan velocity of 200 Hz, a resolution of 1,024 x 1,024 pixels in the xy-plane and a step size of 2 μm in the z-plane. A line average of 2 was used. The Cy3 signal was detected with a DPSS (561 nm) laser while Cy5-fluorescence was detected with a HeNe (633 nm) laser. For Texas Red injected whole mounts, a HeNe 2 mW laser (594 nm) was used.

2.4 | Section analysis

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Most of the 3D-reconstructed neurons were registered into a standard atlas of the CX of Schistocerca gregaria (el Jundi et al., 2010). To register individual neurons from different locusts into a standard, the individual neuropils and the neuropils of the standard were superimposed and adjusted by two modules. The first module, affine registration, overlays both neuropils by changing the position and the size of the individual neuropils while maintaining its proportions so it fits better into the standard neuropil. After the affine registration the resulting coordinates are the input for further computations. The second module, elastic registration, deforms the individual neuropils up to the point where both neuropils share the largest volume. The elastic registration ends in a data output in form of a vector field by which the corresponding neuron was inserted into the standard atlas. Some registrations were adjusted manually to correct minor errors of the algorithm.

3 | RESULTS

3.1 | Nomenclature and general anatomy

The naming of brain regions follows the nomenclature of Ito et al. (2014), as adapted for the locust by von Hadedl, Althaus, Häger, and Homberg (2018). The neuropil organization of the locust CX is outlined in Figure 1a,b. As published previously, the CBU consists of three layers termed I–III with layers I and II being further subdivided into layers la and b and IIa and b (Homberg, 1991). The CBL is subdivided into six layers (Müller et al., 1997). The noduli consist of an upper unit composed of three layers (I–III) and a lower unit (Heinze & Homberg, 2008). The anterior lip is a flat band of neuropil anterior to the CBL and not considered an integral part of the CX. It fuses laterally with the lateral complex (LX) and both areas are intimately connected to the CX. In contrast to columnar and pontine neurons that innervate and connect specific slices of CX subunits (Heinze & Homberg, 2008) tangential neurons ramify in many and often all slices of the PB or in particular layers of the CBU, CBL, or noduli. Their neurites enter the CX often but not always tangentially along one of the layers. Tangential neurons of the CX were named, following previous conventions (Heinze & Homberg, 2009; Müller et al., 1997) based on the innervated neuropil of the CX: TL neurons innervate the CBL, TU neurons the CBU, TB neurons the PB, and TN neurons innervate the noduli. One neuron termed TUN innervated the CBU and the noduli and three neurons, innervating several CX subunits were termed TCX neurons. For further distinction, subtypes of TL-, TB-, and TN neurons were numbered as TL1–TL6, TB1–TB8, and TN1 and TN2. TU neurons, in contrast, showed a large variety of subtypes, differing in cell body positions as well as in arborization areas outside the CX. They were, therefore, grouped based on the position of their cell bodies into TU_LAL neurons, having cell bodies near the LAL, TU_VES neurons with cell bodies near the vest, TU_CRE neurons with cell bodies near the crepine, TU_SLP neurons with somata near the superior lateral protocerebrum, and TU_P neurons with somata near the posterior slope. Based on the trajectories of cell body fibers, two subtypes of TU_CRE neurons (TU_CRE1, TU_CRE2) and at least three types of TU_P (TU_P1, TU_P2, TU_P3) were distinguished. Taken together, we identified 11 cell clusters in which different types of CX tangential neurons originated (Figure 2). Some neurons we included here have been described in previous studies, like tangential neurons of the CBL (Müller et al., 1997) or were revealed by immunolabeling using antisera against neuroactive substances (Table 1). For many of those cell types we present new data on their morphologies and have, therefore, included all types of tangential neurons of the locust CX known to date. Data on the immunoreactivity of tangential neurons using antisera against neuroactive substances (Table 1) provide information on their putative neurotransmitter as well as on the putative number of neurons of a given cell type.

While most tangential neurons enter the PB from its lateral ends, tangential neurons of the CB and noduli send their fibers via two systems of tracts, the anterior bundles and the isthmus tracts, into these neuropils (Figure 1c,d). The anterior bundle originates in the superior protocerebrum and extends ventro-medially toward the fronto-lateral surface of the CB. Here the anterior bundle splits into three fascicles termed AB1–AB3. Fibers in anterior bundle 1 pass along the anterior surface of the CBU, giving off side branches into the CBU along that course. Fibers in anterior bundle 2 enter the ventral groove, the space between the anterior lip and CBL, sending side branches from here dorsally into the CBU. The anterior bundle 3, the smallest fascicle, projects ventrally around the CBL and sends processes from here into the CBU (Figure 1c,d). The isthmus tracts connect the LX with the CB and noduli. We have distinguished six isthmus tracts, termed IT1–IT6 that harbor neurites of tangential neurons. IT1–IT3 have been described previously (Kurylas et al., 2005). IT1 and IT4 have a common origin and are the most posterior isthmus tracts (Figure 1c). IT1 passes along the posterior dorsal face of the CBL and from here gives off processes into the CBL, the CBU or the noduli. IT2 is the largest isthmus tract. Most of its fibers run along the ventral face of the CBL and connect the bulbs to the CBU, but neurons projecting to the CBU take a more dorsal path through the ventral or posterior groove (Figure 1b,d). IT3 is, likewise, one of the larger tracts. It takes a path through the dorsal LAL and then enters the posterior groove innervating the CBU and CBL. IT4 crosses the LX posteriorly. Its fibers cross the midline of the brain dorsally from the noduli and, along that course, innervate the CBU. IT5 and IT6 contain small diameter fibers. IT5 passes most anteriorly through the LX in lateral-medial direction. Fibers enter the ventral groove and give rise to processes innervating the CBU. IT6 bypasses the medial bulb dorsally. Fibers enter the posterior groove and from here innervate the CBL (Figure 1c,d). The anterior bundles as well as the isthmus tracts cross the brain midline, innervating slices in the ipsi- and contralateral hemisphere of the CX. They can, therefore, be considered as commissures of the brain, which were mapped in detail by Boyan, Williams, and Meier (1993). A comparison of their nomenclature with the naming scheme used here suggests the following correspondence (nomenclature of...
Boyan et al., 1993 in brackets): AB1 (VCXI); AB2 (PCI); AB3 (PXIII); IT1, IT2/IT6 (M); IT2 (PCIII/IV); IT3 (PCXVI); IT4 (M); IT2/IT5 (PCV).

3.2 | Tangential neurons of the PB

The PB is innervated by eight types of tangential neuron termed TB1–TB8 (Figures 3 and 4). TB1-, TB2-, and TB3 neurons have distinct arborizations in several slices (Figure 3a) of the PB and ramifications in the posterior optic tubercle (POTU). TB4–TB8, in contrast, ramify widely and rather uniformly throughout all slices of the PB and, in addition, innervate various other parts of the protocerebrum. TB1- and TB2 neurons were already described by Heinze and Homberg (2007, 2009) and Beetz et al. (2015). TB1 neurons (Figure 3b) have varicose ramifications in two slices of the PB that are separated by seven slices in between and fine, smooth ramifications in most other slices except those that are adjacent to the varicociously innervated ones. Their terminals in the POTU have a partly varicose appearance. Four subtypes of TB1 neurons (TB1a, TB1b, TB1c, and TB1d) were distinguished based on innervated slices in the PB and layers in the POTU by Beetz et al. (2015). Accordingly, TB1a neurons of the left hemisphere have varicose ramifications in slices L8 and R1, TB1b in slices L7 and R2, TB1c in slices L6 and R3 and TB1d in slices L5 and R4. TB1 neurons of the right brain hemisphere are organized mirror symmetrically. As a result, a topographic organization is formed whereby all 16 slices are covered with varicose ramifications from the four subtypes and their mirror images (Beetz et al., 2015; Heinze & Homberg, 2007).

TB2 neurons (Figure 3c) have varicose ramifications in the ipsilateral outermost slice of the PB (L8 or R8) and in both innermost slices (L1 and R1). In contrast to ramifications in the outermost slices, those in R1 and L1 are narrower and cover only about one-half of each slice. Smooth ramifications are concentrated bilaterally in slices 4 and 5 while the other slices are free of ramifications (Beetz et al., 2015). TB3 neurons (Figure 3d) were encountered in two preparations, one showing a single TB3 neuron (Pegel, Pfeiffer, & Homberg, 2018) and a second showing a pair of TB3 neurons. TB3 neurons innervate only one hemisphere of the PB and the ipsilateral POTU. In all three neurons, varicose arborizations are in two innermost slices, probably slices 1, 2 and in the outermost slice 8. Smooth processes are present in slices 4–6, while slices 3 and 7 are free of arborizations (Figure 3d). Ramifications in the POTU are mostly varicose. One TB3 neuron has a process extending laterally from the POTU into the posterior slope (Figure 3d). The cell bodies of TB1–TB3 neurons are located in three cell clusters, one cluster dorso-lateral to the PB, Cluster 2 and 3 more ventrally, near the tubercle-PB fiber tract (Beetz et al., 2015). Because cluster 2 and 3 are in close proximity and difficult to identify relative to the cell body of single neurons, we distinguished here only two clusters, one cluster dorso-lateral to the PB, second, at the level of the tubercle PB tract (Figure 2b). Neurons TB4–TB7 share these positions. TB4-, TB5-, and TB7 neurons invade all slices of the PB with uniformly distributed processes. The TB4 neuron (Figure 3e) was found in a single preparation. It has beaded arborizations in all slices of the PB and regions of the ipsilateral ocellar root and fine, smooth ramifications along a small horizontal strip in the posterior slope. The cell body is located laterally to the PB. TB5 neurons (Figure 3f),
| Cell type | Putative neurotransmitter judged from immunostaining | Number of immunostained neurons per hemisphere | Input domains | Output domains | Reference |
|-----------|------------------------------------------------------|-----------------------------------------------|---------------|---------------|-----------|
| TB1/2     | Serotonin (S2), Dip-allatostatin (AST3), Mas-allatotropin (MT3), nitric oxide, orcokinin | 30\(^\text{c}\) | POTU, PB (certain slices) | POTU, PB (certain slices) | Homberg et al. (1991); Vitzthum et al. (1996); Homberg et al. (2004); Hofer, Dircksen, Tollbäck, and Homberg (2005); Kurylas et al. (2005); Beetz et al. (2015) |
| TB3       | No data                                             | 2                                             | POTU, PB (certain slices) | POTU, PB (certain slices) | Homberg et al. (2013); Vitzthum and Homberg (1998) |
| TB4/5     | No data                                             | No data                                       | PS             | PB, OR        | Homberg et al. (2013) |
| TB6/7     | Lom-tachykinin II (LTTI), tyramine (T5)             | 12–15\(^\text{a}\) | PS, WED, DAMMC | PB            | Homberg et al. (2013); Vitzthum and Homberg (1998) |
| TB8       | Octopamine (O2)                                     | 1                                             | PLP, WED, PS   | PB            | Homberg et al. (2013) |
| TL1       | Nitric oxide\(^\text{b}\)                          | 1                                             | LAL            | CBL (layers 1–5) | Kurylas et al. (2005) |
| TL2       | GABA, Lom-tachykinin II (LTTI), nitric oxide\(^\text{a}\) | −40\(^\text{d}\)                             | LBU            | CBL (layer 1, 2, 4, or 4/5) | Homberg et al. (1999); Vitzthum and Homberg (1998); Kurylas et al. (2005) |
| TL3       | GABA                                                | −40\(^\text{d}\)                             | MBU, LBU       | CBL (layer 4/5, 2, 2 + 6) | Homberg et al. (1999) |
| TL4       | GABA, orcokinin                                     | −10\(^\text{d}\)                             | LLAL, WED      | CBL (layer 1) | Homberg et al. (1999); Hofer et al. (2005) |
| TL5       | Dopamine                                            | 2                                             | PB, PS, LAL    | CBL (all layers) | Wendt and Homberg (1992) |
| T\(\text{UL}_{\text{AL}}\)1 | GABA                                                 | 2                                             | LAL            | CBU (layer IIb, ALI) | Homberg et al. (1999) |
| T\(\text{UL}_{\text{AL}}\)2 | GABA, Dip-allatostatin (AST2)                       | −15\(^\text{d}\)                             | LAL, WED, DAMMC, ICL | CBU (layer IIb) | Homberg et al. (1999); Vitzthum et al. (1996) |
| T\(\text{UL}_{\text{AL}}\)3/4 | No data                                              | No data                                       | ULAL, CRE      | CBU (layer II, III, ALI) | |
| T\(\text{UL}_{\text{AL}}\)4 | Dip-allatostatin (AST1)                             | 18                                            | CRE, VLP, PS, ULAL, LLAL | CBU (layer IIb) | Vitzthum et al. (1996) |
| T\(\text{UL}_{\text{RE}}\)1 | Mas-allatotropin (MT2)                              | 4                                             | CRE, SCL, SLP, SMP, ULAL | CBU (layer IIb) | Homberg et al. (2004) |
| T\(\text{UL}_{\text{RE}}\)2/1 | Serotonin (S3)                                     | 10\(^\text{d}\)                             | LX, CRE        | CBU (layer IIb) | Homberg et al. (1991) |
| T\(\text{UL}_{\text{RE}}\)2/3 | No data                                              | No data                                       | ULAL, CBU (layer IIa) | ALI | |
| T\(\text{UL}_{\text{LP}}\) | Dopamine (DC1, DC2); CCAP (cp7)                      | 19–24                                        | SIP, SLP, AVLP, CRE, SCL | CBU (layer IIa, IIb, or III) | Wendt and Homberg (1992); Dircksen and Homberg (1995) |
| T\(\text{UL}_{\text{PS}}\)1 | Mas-allatotropin (MT3), dopamine (DP2); CCAP (cp7) | 25–30                                        | LAL, PS, ICL, WED, DAMMC | CBU (layer IIa, IIb or IIb) | Wendt and Homberg (1992); Dircksen and Homberg (1995); Homberg et al. (2004) |
| T\(\text{UL}_{\text{PS}}\)2 | Serotonin (S5)                                      | 1                                             | SMP, SIP, SCL, CRE, AVLP | CBU (layer IIa, CRE, ULAL, ALI) | Homberg et al. (1991) |
| T\(\text{UL}_{\text{PS}}\)3/1 | No data                                              | No data                                       | SMP, SCL       | CBU (layer IIb) | |
| T\(\text{UL}_{\text{PS}}\)3/2 | Histamine (PMP)                                     | 6                                             | SIP            | CBU (layer IIa, ALI) | Gebhardt & Homberg et al. (2004) |
| T\(\text{UL}_{\text{PS}}\)3/3 | No data                                              | No data                                       | Not determined | CBU (layer IIb) | ULAL |
| T\(\text{UN}\) | Tyramine                                            | 4                                             | LLAL, WED, PVLP, PS | NOU (ipsilateral) | Homberg et al. (2013) |
| T\(\text{CX}\)1 | No data                                              | No data                                       | POTU, PB, OR, SCL, SIP | CBL, CBU, NO, MBU, LUB, ULAL | |
| T\(\text{CX}\)2 | No data                                              | No data                                       | PS, OR, POTU, ULAL, CBL, CBU (layer IIa, Ibb), CBL; all arborizations partly beaded | CBU (layer IIb) | |
| T\(\text{CX}\)3 | No data                                              | No data                                       | ICL, ALI, CBL, CBU, NO | LAL, ICL | |

Abbreviations: ALI, anterior lip; AVLP, anterior ventro-lateral protocerebrum; CBL, lower division of the central body, CBU, upper division of the central body; CCAP, crustacean cardioactive peptide; CRE, crepine; DAMMC, dorsal antennal mechanosensory and motor center; GABA, \(\gamma\)-aminobutyric acid; ICL, inferior clamp; LAL, lateral accessory lobe; LLAL, lower shell of the LAL; LBU, lateral bulb, LX, lateral complex; M BU, medial bulb, NO, nodulus; NOL, lower unit of the nodulus; NOU, upper unit of the nodulus; OR, ocellar root; PB, protocerebral bridge; PLP, posterior lateral protocerebrum; POTU, posterior optic tubercle; PS, posterior slope; PVLP, posterior ventro-lateral protocerebrum; SCL, superior clamp; SIP, superior intermediate protocerebrum; SMP, superior medial protocerebrum; ULAL, upper shell of the LAL; VLP, ventro-lateral protocerebrum; WED, wedge.

\(^{a}\)Characters in parenthesis indicate code name for the immunolabeled neurons.

\(^{b}\)Nitric oxide revealed through NADPH-diaphorase histochemistry.

\(^{c}\)Cell numbers refer to combination of both cell types.

\(^{d}\)Cell numbers estimated from ensemble GABA immunostaining of TL2, TL3, TL4, T\(\text{UL}_{\text{AL}}\)2 neurons.
stained in two preparations, connect more ventral regions of the posterior slope to the PB. Their cell bodies are located posterior to the posterior slope-PB fiber bundle.

The TB6- and TB7-neurons (Figure 4a,b) were stained in single preparations. Both cell types have fine arborizations in large lateral and ventral areas of the posterior slope, partly extending into the wedge and dorsal antennal mechanosensory and motor center and invade the PB tangentially. The TB6 neuron invaded both hemispheres of the PB but its ramifications were not stained completely. In contrast, the TB7 neuron had uniform, finely beaded arborizations confined to the ipsilateral PB hemisphere. Judging from immunostaining data (Table 1) TB6- and TB7-neurons, taken together, comprise a small group of 12–15 neurons per brain hemisphere. The TB8 neuron (Figure 4b) was stained in one preparation. It connects ventrolateral and ventromedial neuropils of the posterior brain with the PB, but processes outside the PB were not stained completely. The cell body is located medial from the glomerular lobe near the esophageal foramen (Figure 2b). The cell body fiber projects dorsally along the posterior surface of the brain and gives rise to ramifications with fine processes in the posterior lateral protocerebrum, the wedge, and the posterior slope. Processes extend as far anteriorly as the posterior face of the LAL. A slender neurite extends to the posterior lateral protocerebrum, but terminal processes could not be revealed. The axon splits into three branches ventrally to the PB. One of these branches crosses the brain midline. All branches give rise to further collaterals which enter the PB ventrally at multiple sites. Processes throughout the PB are extremely fine and bear numerous beaded specializations. Judging from immunocytochemistry, the TB8 neuron may be a single octopaminergic neuron (Table 1).

3.3 | Tangential neurons of the CBL

The anatomical organization of the CBL has been described by Müller et al. (1997). The CBL is subdivided into six layers, and Müller et al. (1997) distinguished five types of tangential neuron, termed TL1–TL5. We provide 3D reconstructions of TL1–TL4 neurons and present novel details on their branching patterns (Figures 5a–e). Tangential neurons of the CBL have dendritic processes in the medial and lateral bulbs of the LX, the LAL, the PB, and inferior and superior neuropils of the brain. TL2–TL4 (Figure 5b–d) neurons share a common cell body position along the ventro-medial face of the LX (Figure 2a), and their fibers enter the CBL through isthmus tract 2 (Figure 1c,d). TL1- and TL5-neurons, in contrast, have cell type specific soma positions.
TL1 neurons send primary neurites along the posterior face of the antennal lobe and dorsally toward the LX. Neurons give rise to dense arborizations with fine endings around the lateral bulb and innervate more sparsely surrounding areas of the LAL. Axons project to the CBL through isthmus Tract 1, which bypasses the medial bulb dorsally, and enter the CBL along its posterior dorsal face (Figure 1c,d). The neurons give rise to prominently beaded dense processes throughout Layer 1–5 of the CBL (Figure 5a).

TL2 neurons (Figure 5b) have ramifications in areas of the lateral bulb and innervate certain layers of the CBL. Nerve endings in the lateral bulb have a bulky and irregular shape, when viewed at low magnification, but as shown by electron microscopy these consist of dense tangles of fine postsynaptic processes, which form the center of large microglomerular synaptic complexes (Träger, Wagner, Bausenwein, & Homberg, 2008). Axonal fibers run along the ventral face of the CBL and give off side branches sequentially which extend dorsally to terminals in specific layers. In addition to varicose terminals in layers 2, layers 3/4, and layers 4/5, already reported by Müller et al. (1997) we also found TL2 neurons with arborizations in layer 1. Neurons with arborizations in Layer 2 were encountered most frequently.

TL3 neurons have ramifications in the medial bulb (Figure 5c,d). Most neurons exclusively ramify in the medial bulb (TL3a, Figure 5c), while some have additional ramifications in the lateral bulb or along the isthmus tract (TL3b, Figure 5d). Ramifications in the bulbs are similar in appearance to those of TL2 neurons and again consist of fine tufts of processes forming the center of microglomerular complexes. Axons project to the ventral face of the CBL via isthmus tract 2. Near the brain midline, axonal fibers of most neurons bifurcate into two bilaterally symmetric collaterals, which again bifurcate symmetrically (Figure 5d) before entering their target layer. A single symmetric neuron of this type can be found in Figure 1C of Träger et al. (2008). In some neurons, side branches divide more irregularly (asymmetric) as processes project to a CBL layer (Figure 5c). TL3 neurons innervate certain layers of the CBL with beaded terminals. Ramifications in layer 4/5 were encountered more frequently than ramifications in layer 2 and layer 6. No neurons were found with ramifications in layer 3.

TL4 neurons (Figure 5e) were previously identified based on an ensemble Golgi-preparation (Müller et al., 1997) and a single intracellular dye fill (Pegel et al., 2018). Two additional intracellularly stained TL4 neurons confirm the earlier descriptions. TL4 neurons innervate a strip of neuropil in dorsal areas of the lower shell of the LAL (LLAL) and a small part of the wedge with almost exclusively fine ramifications. This presumably dendritic tree is connected to the primary
FIGURE 5  Tangential neurons of the lower division of the central body (CBL). Insets (a–e) show merged image stacks of the arborizations in the CBL. Schematic colored branching patterns are added to cell types in (b)–(e). Schematic sagittal views illustrate innervated layers (blue) of the CBL. TL2–TL4 were registered into the standard CX of the locust. (a) 3D-reconstruction of a TL1 neuron connecting areas around the lateral bulb with layers 1–5 of the CBL. (b) 3D-reconstruction of a TL2 neuron connecting the lateral bulb (LBU) with layer 1 of the CBL. The branching pattern in the CBL (blue scheme) is asymmetric. (c) 3D-reconstruction of a TL3a neuron connecting the MBU with layer 2 of the CBL. The branching pattern in the CBL is asymmetric. (d) 3D-reconstruction of two TL3b neurons connecting the LBU and small parts of the MBU with layer 4 and 5 of the CBL. The branching in the CBL is presented in red and shows a symmetric pattern. (e) 3D-reconstruction of a TL4 neuron connecting dorsal parts of the lower shell of the lateral accessory lobe (LLAL) and a small part of wedge (WED) with layer 1 of the CBL. The branching pattern in the CBL is symmetric. (f) Frontal reconstruction of two TL5 neurons connecting one hemisphere of the PB, parts of the posterior slope (PS) and parts of the lateral complex (LX) with all layers of the CBL (modified from Müller et al., 1997). (g) Registration of the arborizations of the TL2-, TL3a-, TL3b-, and TL4 neuron into the central-body standard illustrating layer specific arborizations. CBU, upper division of the central body. Scale bars = 100 μm, 50 μm (a inset) [Color figure can be viewed at wileyonlinelibrary.com]
neurite by a single fiber that runs via isthmus tract 6 dorsally around the medial bulb when joining the axon to the CBL. Cell bodies of TL4 neurons are among those of TL2- and TL3-neurons or, more laterally between the distal edge of the LAL and the antennal lobe (Figure 2a). Axonal fibers ascend near the brain midline along the posterior face of the CBL, bifurcate symmetrically and give rise to fine beaded terminals in layer 1 of the CBL. Their projections in layer 1 of the CBL have an umbrella-like appearance (Figure 5e).

TL5 neurons (Figure 5f) innervate the ipsilateral hemisphere of the PB and small areas of the dorsal posterior slope with fine branches. These ramifications are connected through the w-bundles (Williams, 1975) with fine arborizations in the LAL. The innervations in the LAL are located dorso-ventrally and slightly more concentrated in or around the lateral bulb. Beaded and varicose arborizations innervate all layers of the CBL. The cell bodies of TL5 neurons are located together with those of TUPES1 neurons (see below) in a cluster dorsal to the lateral ends of the PB (Figure 2b). Reconstruction of a single TL5 neuron closely resembling the double impalement of Figure 5f can be found in Vitzthum et al. (2002).

3.4 | Tangential neurons of the CBU

Tangential neurons of the CBU (TU neurons) are the largest and most diverse group of tangential neurons with a large variety of ramifications outside the CBU. Within the CBU, most neurons have varicose or beaded terminals and innervate one of the three layers of the CBU. Their ramifications outside the CX are, with few exceptions, fine and, therefore, likely dendritic and are concentrated in subfields of the LAL, areas in the superior, ventrolateral, inferior, and ventromedial protocerebrum. TU neurons were grouped based on soma positions, which were clustered in seven different cell groups. To differentiate between these groups, TU neurons were named after the neuropil closest to their cell body cluster.

3.5 | TULAL neurons

TULAL neurons (Figure 6) share their soma positions with those of TL2-TL4 neurons in the region ventral to the LAL. TULAL neurons have fine processes in both shells of the LAL, send axonal fibers through isthmus tract 2 to the CX and innervate the CBU with beaded ramifications. Two subtypes of TULAL neurons could be distinguished. Judging from GABA immunostaining, TULAL1 neurons occur as a pair of neurons per brain hemisphere, whereas TULAL2 neurons are a small group of 15 or more neurons (Homberg et al., 1999; Table 1).

TULAL1 neurons (Figure 6a) have ramifications in large parts of the LAL (Figure 6b). Their axons split medial into two main collaterals, which enter the CBU from the ventral groove in front of the CBL and spread like wings in layer Ib of the CBU, while a small side branch innervates the anterior lip. The neurons innervate layer Ib of the CBU and, more sparsely, the anterior lip in a varicose manner. TULAL2 neurons (Figure 6c-f) have fine arborizations in the LAL, concentrated in dorsal aspects, and in the wedge (Figure 6d,e). One TULAL2 neuron had additional fine ramifications in the dorsal antennal mechanosensory and motor center and a small branch in the inferior clamp (Figure 6e,f). The axons of TULAL2 neurons (Figure 6c-f) enter the CBU along the posterior face of the CBL. They split at the midline of the brain into 3–5 main branches, which further ramify into smaller branches and innervate layer Iib of the CBU with varicose terminals.

3.6 | TUVES neurons

TUVES neurons have somata near the ventricle. They share their soma position as well as the trajectory of their cell body fiber with those of TL1 neurons (Figure 2). TUVES neurons connect areas in the superior intermediate protocerebrum, ventrolateral protocerebrum, crepine, posterior slope, and LAL to layers I, II, or III of the CBU. Axons enter the CBU via isthmus tract 1 or 4. Four subtypes of TUVES neurons could be distinguished (Figure 7a-f). TUVES1–3 neurons have large axon diameters and likely occur only as a single bilateral pair of neurons, while TUVES4 neurons are a small group of at least 18 neurons per hemisphere (Table 1). TUVES-L is the largest neuron of the locust CX and was termed accordingly “giant fan-shaped neuron” by Williams (1972), Homberg (1994), and el Jundi et al. (2010). TUVES1 and TUVES2 neurons share similar innervation domains outside the CX. Both neurons ramify widely in the upper shell of the LAL (ULAL) and send prominent side branches into the crepine, medial, and anterior of the medial lobe of the mushroom body (Figure 7b). Large axons enter the ventral groove of the CB and give rise to fan-like ramifications in layer II of the CBU (TUVES1; Figure 7a–c) and layer III of the CBU (TUVES2; Figure 7d). In the CBU, the tangential fibers of the TUVES1 neurons give rise to eight major side branches arranged like the staves of a fan. The side branches from the bilateral pair of neurons fasciculate and densely innervate layer II (Figure 7c). The TUVES3 neuron (Figure 7e) has a complex branching pattern with fine arborizations in the posterior slope, the ventro-lateral protocerebrum, the ULAL and LLAL (Figure 7e, lower inset). It has varicose fan-like arborizations in layer Iib of the CBU originating from eight main side branches (Figure 7e, upper inset). TUVES4 neurons have fine branches in the crepine and the superior intermediate protocerebrum, sparse branches in the anterior lip, but no ramifications in the LAL (Figure 7f). Their major neurites cross the brain midline via isthmus tract 4 dorsally from the noduli and give rise to small processes extending through layers III and II to fine beaded terminals in layer Ia of the CBU.

3.7 | TUCRE1 neurons

TUCRE1 neurons (Figure 8a–c) have somata in the anterior protocerebrum near the crepine (Figure 2a). Judging from Mas-allatotropin immunostaining (Homberg et al., 2004) at least four bilateral pairs of TUCRE1 neurons exist. They connect parts of the lateral and superior protocerebrum with the CBU. The neurons have fine branches in the crepine, the superior clamp, the superior intermediate protocerebrum, the superior medial protocerebrum, and medial parts of the ULAL. The
main fiber of TUCRE1 runs from the soma posteriorly around the vertical lobe, where it joins the anterior bundle, while the cell body fiber of TUCRE12 passes anteriorly around the vertical lobe to the anterior bundle (Figure 8c). Axonal fibers of both subtypes project toward the brain midline via anterior bundle 3 and give rise to side branches that ascend along the posterior face of the CBL to the CBU. The varicose ramifications in layer IIb of the CBU can be divided into eight subregions, illustrated in Figure 8a.

### 3.8 | TUCRE2 neurons

TUCRE2 neurons have cell bodies clustered near the crepine, but more lateral than TUCRE1 neurons (Figure 2a). Three subtypes, TUCRE21–3, could be distinguished. TUCRE21/2 neurons, taken together, consist of at least 10 neurons as suggested by 5-HT immunostaining (Table 1; Homberg, 1991). TUCRE2 neurons have exceedingly fine primary neurites that share a similar trajectory via isthmus tract 5 anteriorly through the LX. TUCRE21/2 innervate parts of the LX and layer Ib of the CBU. Their small axons enter the ventral groove and send fine beaded rim-like ramifications into layer Ib of the CBU. The TUCRE21 neuron (Figure 9a) arborizes in the crepine and sparsely in the LX; fine branches give rise to a small rim of varicose ramifications in layer Ib of the CBU. The TUCRE22 neuron (Figure 9b) has fine ramifications in dorsal parts of the ULAL and several branches innervate layer Ib of the CBU. The TUCRE23 neuron (Figure 9c,d) has fine processes in a small dorsal strip in the ULAL and in parts of layer Ia of the CBU. An axonal process originating at the lateral edge of the CBU gives rise to prominent varicose ramifications throughout the anterior lip. Another neuron of the CRE2 cluster, however without projections to the CBU (Figure 9e–g), has fine ramifications in the anterior lip and dorsal ULAL (Figure 9e,g) and sends varicose, likely presynaptic, processes in a wing-like manner (Figure 9e,f) to wide areas of the superior medial, intermediate and lateral protocerebrum of both hemispheres.

### 3.9 | TUSLP neurons

TUSLP neurons have somata near the superior lateral protocerebrum, ventro-lateral from the calyx of the mushroom body (Figures 2a, 10, and 11). TUSLP neurons connect parts of the superior and lateral
FIGURE 7  Tangential neurons of the CBU with somata in the VES cluster. (a) 3-D reconstruction of a TUVES1 neuron with fine ramifications in the crepine (CRE), the ULAL, and varicose branches in layer IIa and IIb of the CBU. (b) Ventral view of the TUVES1 neuron. Note ramifications in the CRE extending around the medial lobe (ML) of the mushroom body. (c) Merged image stack of fan-shaped projections of the bilateral pair of TUVES1 neurons in the CBU. Eight major side branches from the right and left neuron fasciculate as they project into layer II of the CBU (circles). (d) 3D-reconstruction of a TUVES2 neuron with fine ramifications in the CRE, branches concentrated in the ULAL and beaded terminals in layer III of the CBU. (e) Reconstruction of a TUVES3 neuron with fine branches in large fields of the posterior slope (PS; lower inset), the lateral complex (LX), the ventro-lateral protocerebrum (VLP) and the crepine (CRE). Eight branches innervate layer IIb of the CBU (upper inset). (f) TUVES4 neuron with fine ramifications in the superior intermediate protocerebrum (SIP) and the crepine (CRE). In the CBU (layer Ia) and the anterior lip (ALI) varicose branches emerge. Schematic sagittal views illustrate innervated areas (blue) of the CX and ALI. (a) and (b) modified from el Jundi et al. (2010). Scale bars = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
protocerebrum via the anterior bundle to certain layers of the CBU (Figures 10 and 11). The axons of TUSLP neurons with ramifications in layers I and III of the CBU run via anterior bundle 1 along the anterior face of the CB (Figures 10b and 11c). In contrast, TUSLP neurons that innervate layer II of the CBU continue via anterior bundle 2, enter the ventral groove and project side branches from there to the CBU (Figures 10a,c, and 11a). TUSLP neurons are diverse in morphology and some neurons of that cluster do not target layers of the CBU but innervate the anterior lip (Figure 10d) or the contralateral LAL (Figure 10d). TUSLP1–3 neurons and a fourth neuron not innervating the CX closely resemble each other. They have dendritic ramifications in the superior intermediate protocerebrum and axons in the anterior bundle, but differ in their axonal targets within or near the CBU that are innervated with beaded terminals. The TUSLP1 neuron (Figure 10a) has ramifications in layer IIb of the CBU, TUSLP2 (Figure 10b) in layer Ia of the CBU and adjacent areas of the ULALs, TUSLP3 (Figure 10c) in layer Ila of the CBU, while a fourth neuron does not innervate the CX but the anterior lip (Figure 10d).

**FIGURE 8** Tangential neurons of the CBU with somata in CRE cluster 1. (a) 3D-reconstruction of a TU_{CRE1} neuron with fine branches in the crepine (CRE), the superior clamp (SCL), the superior intermediate protocerebrum (SIP), the superior medial protocerebrum (SMP) and small parts of the ULAL and beaded terminals in layer IIb of the CBU (modified from Rosner & Homberg, 2013). Eight major domains of arborization can be distinguished in layer IIb (red circles). (b) Frontal reconstruction of the same cell type from a different preparation. (c) Stack of confocal images of fine arborizations of a TU_{CRE2} neuron in the CRE (left) and frontal reconstruction of the neuron (right). Its cell body got lost during histological processing, its likely position is indicated by a dotted circle. Like TU_{CRE1}, the neuron has fine ramifications in the CRE, SIP, SMP, SCL, and ULAL. Axonal fibers of both subtypes enter the central body via anterior bundle 3 and give rise to varicose arborizations in layer IIb of the CBU, illustrated in blue in the sagittal inset. CA, calyx; ML, medial lobe; PED, pedunculus; VL, vertical lobe. Scale bars = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
Three other neurons, TUSLP4 and 5, and a neuron not innervating the CX, differ from the first group in their dendritic fields of arborization which, in addition to extensive ramifications in the superior medial and intermediate protocerebrum, include wide projections in the anterior ventro-lateral protocerebrum (Figure 11) and, in TUSLP4, the crepine and superior clamp. TUSLP4 (Figure 11a) has varicose ramifications in layer Iib of the CBU (Figure 11b), TUSLP5 (Figure 11c) in layer III of the CBU, and the third neuron of that subtype (Figure 11d)
sends an axon via anterior bundle 1 along the anterior face of the CB to beaded arborizations in the contralateral LAL.

3.10 | TUPS1 neurons

TUPS1 neurons have their somata near the posterior slope lateral to the PB together with TL5 neurons (Figures 2b and 12). They connect areas in the posterior slope, the inferior clamp, the LAL, the wedge, and the dorsal antennal mechanosensory and motor center to layer II of the CBU. Axonal fibers join the w-bundle between the PB and CB and enter the CBU ventrally through the posterior groove. The neurons have varicose processes in layer IIb (Figure 12a,c), IIa (Figure 12b), or Ib (not shown) while arborizations outside the CBU are fine. TUPS1 neurons are dopamine-immunoreactive (DP2 neurons, Wendt & Homberg, 1992) and thus, may consist of as many as 28 neurons per hemisphere including TL5 cells of the CBL. Four TUPS1 neurons were stained individually (Figure 12). All neurons ramify widely in the LAL, most densely in dorsal aspects of the ULAL. TUPS13 has a small additional area of ramifications in the inferior clamp (Figure 12a). TUPS1 in the inferior clamp and the superior lateral protocerebrum (Figure 12c).

**FIGURE 10** Tangential neurons of the CBU with somata in the SLP cluster. (a) 3D-reconstruction of a TUSLP1 neuron with fine ramifications in the superior intermediate protocerebrum (SIP) and varicose arborizations in the CBU (IIb). Inset shows fine branches in the SIP. (b) 2D-reconstruction of a TUSLP2 neuron with fine arborizations in the SIP and varicose ramifications in the CBU (Ia). (c) 2D-reconstruction of a TUSLP3 neuron with fine branches in the SIP and varicose arborizations in the CBU (Ia). (d) 2D-reconstruction of a neuron with fine ramifications in the SIP and arborizations in the anterior lip (ALI). Schematic sagittal views illustrate innervated areas (blue) of the CB and ALI. CA, calyx; CBL, lower division of the central body; PED, pedunculus. Scale bars = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
3.11 | TUPS2 neurons

TUPS2 neurons have somata in the posterior brain adjacent to the posterior slope (Figure 2b). All neurons innervate layer I of the CBU and/or the anterior lip, and all neurons have wide ramifications in areas outside the CX. Their axons enter the CB via anterior bundle 1. The TUPS21 neuron (Figure 13a) has fine branches in the superior medial protocerebrum, in the superior clamp and wide beaded terminals in layer la of the CBU, in parts of both ULAL, and in the anterior lip. The TUPS22 neuron (Figure 13b) has fine ramifications in the superior intermediate protocerebrum and the superior medial protocerebrum around the vertical lobe, the crepine, and the anterior ventro-lateral protocerebrum, and invades layer I of the CBU and the anterior lip with dense beaded terminals. A third neuron of that group (Figure 13c) ramifies in the anterior lip, parts of both LX and the superior protocerebrum but not within the CX. Arborizations in the superior protocerebrum consist of fine fibers, while the branches in parts of both ULAL and the anterior lip are of varicose nature.
3.12 | **TUPS3 neurons**

TUPS3 neurons are diverse in morphology, with somata in different positions in the posterior medial soma rind, near the posterior slope. They are probably not related clonally and were, therefore, not included in Figure 2. The TUPS1 neuron (Figure 14a) has fine arborizations in the superior medial protocerebrum and superior clamp and likely axonal projections to layer Ib of the CBU with sparse ramifications in the

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**FIGURE 12** Tangential neurons of the CBU with somata in the PS cluster 1. (a–c) Frontal reconstructions (a,c) and confocal image (b) of TUPS1–3 neurons. All neurons have somata in a cell cluster near the posterior slope (PS) dorsally from the PB. Cell body fibers project to extensive fine arborizations concentrated in the ULAL. Axonal fibers enter the ventral groove of the CB and give rise to beaded processes innervating the CBU, illustrated in blue in the schematic sagittal insets. (a) The TUPS1 neuron has additional fine ramifications in the inferior clamp (ICL) and arborizes in layer IIb of the CBU. (b) The TUPS2 has fine ramifications in the dorsal part of the LAL and beaded terminals in layer IIa of the CBU. (c) The TUPS3 neuron has additional fine arborizations in the ICL, the posterior slope (PS), the wedge (WED), the dorsal antennal mechanosensory and motor center (DAMMC) and varicose branches in layer IIb of the CBU. (d) Merged image stack of the CBU of the TUPS1–3 neuron. CBL, lower division of the central body; LX, lateral complex; PB, protocerebral bridge; PED, pedunculus. Scale bars = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]
anterior lip. The soma lies dorsally near the posterior face of the calyx. The TUPS2 neuron has a small soma near the posterior slope but further ventrally. Its primary neurite projects to the superior intermediate protocerebrum (SIP), the superior medial protocerebrum (SMP), and the superior clamp (SCL), and varicose branches in the CBU (Ia) and the anterior lip (ALI). Its primary neurite projects to the superior intermediate protocerebrum and gives rise to a rather focussed field of dendritic processes. An axonal fiber enters the anterior bundle 1 and gives rise to processes in layer Ia of the CBU and sparse branches in the anterior lip. The stained cell resembles six pairs of histamine-immunoreactive PMP neurons reconstructed by Gebhardt and Homberg (2004) suggesting that TUPS2 neurons occur as a small group of neurons with highly similar ramifications in the superior intermediate protocerebrum and layer Ia of the CBU. The TUPS3 neuron (Figure 14c) is the only TU neuron that does not enter the CBU via the isthmus tracts or anterior bundle but enters the CBU at its dorsal face. From the cell body in the posterior pars intercerebrali near the posterior slope, the primary neurite gives rise to a characteristic rim-like pattern of varicose branches in the outermost part of layer Ia of the CBU (Figure 14d) with fine beaded processes extending bilaterally to the ULAL. No dendritic ramifications outside the CBU/LX could be identified.
Several types of tangential neuron of the noduli were encountered. All neurons have cell bodies together with TL1 and TUVES neurons and a cell body fiber running through isthmus tract 1 to the NO and CBU (Figure 15). One neuron, termed tangential neuron of the CBU/NO (TUN1) innervates both noduli and the CBU (Figure 15a,b). It enters the CX along the posterior groove. The neuron has fine ramifications in the ULAL and LLAL and axonal projections with beaded fiber terminals in layer Ib of the CBU and layer I of the upper units of the NO.

The TN1 neuron (Figure 15c) innervates layer II of the upper unit of the ipsilateral nodulus in a dense and varicose manner. Fine processes are concentrated in an arc-like dorsal domain of the LLAL and parts of the wedge. Some of those arborizations reach the posterior ventro-lateral protocerebrum lateral to the LAL, and a small fiber near the soma innervates the posterior slope. The TN2 neuron (Figure 15d) innervates layer III of the upper subunit of the ipsilateral nodulus. Fine processes in the LAL are adjacent to but spatially largely distinct from the ramifications of TN1. Fine branches also innervate parts of the wedge. Again, fine side branches near the soma extend to the posterior slope.

TCX neurons have complex arborization patterns and innervate several or all neuropils of the CX. Their cell bodies are clustered in the...
posterior pars intercerebralis near the mushroom body calyx (Figure 2b). Each TCX neuron had a highly unique morphology. The TCX1 neuron connects all CX neuropils involved in the processing of sky compass signals (Figure 16a). The neuron was originally described as tangential neuron of the CBL and CBU (TLU1) by Bockhorst and Homberg (2015), but because it also arborizes in the noduli and PB, the neuron was renamed TCX1. Smooth input ramifications are in the ipsilateral POTU and the ipsilateral hemisphere of the PB and the ocellar root. In addition, a small fiber with fine arborizations extends ipsilaterally to the superior clamp and the superior intermediate...
protocerebrum. An axonal fiber descends from the PB to the CB and gives rise to beaded ramifications throughout the CBL, all layers of the CBU (concentrated in layer I, II), the lower units of both noduli, the medial and lateral bulbs, and parts of the ULAL of both LX.

The TCX2 neuron innervates the posterior slope, the POTU, the ocellar root, and more sparsely, the superior intermediate protocerebrum (SIP). Three major fibers give rise to ramifications in the ipsi- and contralateral upper shell of the LAL (ULAL) dorsally from isthmus tract 1 and wide ramifications in the central body, concentrated in the CBL and layers la and Ila of the CBU. All processes are fine with partly beaded appearance. Scale bars = 100 μm [Color figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

We have morphologically characterized 43 types of tangential neuron of the locust CX, complementing earlier studies on neurons of the CBL (Müller et al., 1997), the PB (Beetz et al., 2015), and columnar
and pontine neurons of the CX (Heinze & Homberg, 2008). The large majority of tangential neurons invade single substructures of the CX, the PB (8 cell types), the CBL (5 cell types), the CBU (24 cell types), or the noduli (2 cell types), and only four neurons innervated two (TUN, TCX2), three (TCX3), or all four subdivisions of the CX (TCX1). Cell bodies of tangential neurons were distributed in at least 11 different cell clusters in the central brain (Figure 2). Four of these clusters contained neurons targeting different CX substructures suggesting that these neurons are clonally related and may share the same neurotransmitter, such as GABA in TL2–TL4 and TULAL neurons and dopamine in TL5 and TU_{PS1} neurons (Table 1, Figure 2). Analysis of neurons labeled by antisera against neuroactive substances allows estimates of the minimal number of individual neurons of a given cell type (Table 1). While some neurons were found in groups of up to 40 individuals (TL2, TL3), other cell types might be individuals (TL1, TB8). Although the number of different cell types described here is already high, it is likely that additional cell types or subtypes exist, especially those that occur in small numbers and/or have small fiber diameter that were unlikely to be penetrated by our glass microcapillaries. Among these are a pair of serotonin-immunolabeled S4 neurons (Homberg, 1991) that project along the posterior face of the CB and innervate layer Ib of the CBU and two pairs of SFamide-immunolabeled neurons that arborize in nearly all brain areas including the CX (Gellerer et al., 2015).

4.1 | Homologous cell types in *Drosophila*

Tangential neurons of the CX have been studied particularly well in *Drosophila*, notably neurons of the PB (Wolff et al., 2015) and the ellipsoid body, the equivalent of the CBL (Omoto et al., 2018). This allows for detailed comparison of likely homologous cell types in the fly and the locust, as well as identification of species-specific differences. The *Drosophila* equivalents of TL neurons are six types of ring neuron, termed R1–R6, and four types of extrinsic ring neurons, termed ExR1–ExR4 (Omoto et al., 2018). R1–R6 neurons derive from a common lineage (DALv2/EBa1) and most closely resemble TL2–TL4 neurons of the locust (Omoto et al., 2018). Of these, only R1 and TL4 do not invade the bulbs, but dorsal aspects of the LAL and may thus be homologous. R2–R4 neurons, like TL2/3 neurons, are GABAergic (Kottler et al., 2017; Table 1). Based on the layer of innervation in the ellipsoid body/CBL, R2 neurons resemble TL2-, and subtypes of R3, TL3 neurons, but whether there is full correspondence between the *Drosophila* and locust cell types, remains to be seen. R4–R6 neurons penetrate the ellipsoid body from the outside (corresponding to dorsal face of the CBL) which was not found in the locust. Of the four types of ExR neurons, ExR2 shows striking similarity to TL5 neurons both in terms of cell body position and neurotransmitter (dopamine, Table 1). ExR2 neurons are part of the PPM3 group, some members of which innervate the fan-shaped body (Omoto et al., 2018), like the dopamine-immunostained TU_{PS1} neurons. ExR4 neurons have somata ventrally of the antennal lobe, like TL1 neurons. ExR4 neurons are members of the BAmv1 lineage, also termed LALv1, which contributes tangential neurons to the ellipsoid body/CBL, fan-shaped body/CBU and noduli like the cluster of TL1-, TN-, TUN-, and TU_{VES} neurons (Figure 2b).

Members of the TU_{SLP} neurons with principle fibers entering the CBU via the anterior bundle are likely homologous to Fm1/Fm3 neurons (Hanesch et al., 1989) and ExFl2 neurons (Young & Armstrong, 2010) that are clustered together ventro-laterally from the calyces. Like
TU\textsubscript{SLP} neurons, they enter the fan-shaped body through one of two fascicles, corresponding to anterior bundles 1 and 2 (Figure 1c). The neurons are members of the CP2 lineage (Wong et al., 2013) and include dopaminergic DL1 neurons (Hartenstein, Cruz, Lovick, & Guo, 2017), corresponding to dopamine-immunoreactive members of the TU\textsubscript{SLP} neurons (Table 1). Tangential neurons of the noduli (TN neurons) have been described by Wolff and Rubin (2018) in Drosophila. As in the locust, these neurons have ventro-medially located cell bodies, wide ramifications in subfields of the LAL and invade a particular layer of the ipsilateral nodulus (Figure 4 of Wolff & Rubin, 2018).

Tangential neurons of the PB, resembling TB1–TB3 neurons are also present in Drosophila (Wolff et al., 2015), but in contrast to the locust counterparts, they are intrinsic to the PB, that is, they lack a projection to the POTU. A neuron similar to TB7 has also been reported (Figure 3r in Wolff et al., 2015). Like the locust TB7 cell it has wide dendrites in the posterior slope and axonal terminals throughout the ipsilateral hemisphere of the PB. Finally, the octopaminergic OA-AL2i1 cell in Drosophila (Busch, Selcho, Ito, & Tanimoto, 2009). Both share similar cell body positions and a highly similar way of entry into the PB. Taken together, these data are beginning to provide insights into modifications of the CX network during insect evolution and support the clonal relationship of tangential neurons in the locust brain that form a common cell cluster.

### 4.2 | Topographically distinct connections of CX subunits

With a few notable exceptions (TU\textsubscript{CRE2}–3, TCX3), the distribution of fine vs. beaded or varicose ramifications suggests that tangential neurons have dendritic ramifications outside the CX (fine processes) and axonal terminals within the CX (beaded or varicose specializations). This is in agreement with data from the monarch butterfly (Heinze et al., 2013), dung beetles (el Jundi et al., 2018), and, partly supported through synaptic markers, the fly Drosophila (Hanesch et al., 1989; Li et al., 2009; Omoto et al., 2018; Wolff et al., 2015; Wolff & Rubin, 2018). This, however, does not rule out that axo-axonal synapses within the CX may exist as proposed for TL neurons in the locust CBL (Homberg & Müller, 2016) and R neurons in the fruit fly ellipsoid body (Omoto et al., 2018). TB1–TB3 neurons differ from all other tangential neurons that innervate certain layers homogeneously, in that their arborizations in the PB are slice-specific: two to three slices that are 7 or 8 slices apart receive varicose processes and other slices in between receive smooth, fine processes (Figure 3b–d; Beetz et al., 2015). Ramifications of tangential neurons outside the CX are largely subdivision-specific, i.e. different brain areas are largely connected to the PB, CBU, CBL, and noduli.

The PB is specifically connected with the POTUs by at least 30 neurons per hemisphere (TB1–TB3). Neurons homologous to TB1/2 have been reported from the monarch butterfly (Heinze et al., 2013) and the dung beetle (el Jundi et al., 2018), and similar neurons are also present in Drosophila (Wolff et al., 2015). Like in the fly, these neurons in the dung beetle lack connections with the POTU, which may have been lost in both taxa. Another major input to the locust PB is provided by at least 15–18 TB4–TB8 neurons, all of which have ramifications in the posterior slope area. TB4–TB8 neurons branch uniformly throughout all slices of the ipsilateral (TB7) or bilateral PB (TB4, TB6, TB8). The multicolumnar TB1–TB3 neurons are key elements of the sky compass network of the CX (Heinze et al., 2009; Heinze & Homberg, 2008) and might contribute to establishing the compass-like representation of heading directions in the PB through a commissure connecting the right and left tubercle (Beetz et al., 2015). The role of TB4–TB8 neurons remains to be established. The posterior slope is a major site of synaptic connections between outputs from the lobula complex signaling visual flow fields and descending neurons but also receives substantial ascending input (flies: Strausfeld, Bassemir, Singh, & Boyan, 1984; Strausfeld & Bassemir, 1985; Haag, Wertz, & Borst, 2007; moths: Namiki, Wada, & Kanzaki, 2018; locusts: Rind, 1990; Gewecke & Hou, 1992). Projections to the PB may, therefore, provide information related to ego-motion in space.

The CBL is specifically connected by about 80 TL2 and TL3 neurons per hemisphere to the medial and lateral bulbs providing sun and sky compass input to the CX (Heinze et al., 2009; Homberg et al., 1999; Pegel et al., 2018; Vitzthum et al., 2002). In contrast, TL1 and TL4 neurons innervate the dorsal LLAL (TL4) and areas surrounding the lateral bulb (TL1), that are not directly targeted by neurons from the anterior optic tubercle. Nevertheless, TL1 and TL4 neurons are, likewise, sensitive to E-vector angle and solar azimuth (Pegel et al., 2018). An analysis of branching patterns within the CBL revealed asymmetric (TL2, some TL3) and symmetric (most TL3, TL4) innervation patterns in the CBL. In functional terms, asymmetric neurons would provide signals with time delays to adjacent slices of the CBL, while symmetric neurons provide signals simultaneously to all slices. This will have consequences for temporal integration of head-direction coding in postsynaptic CL neurons during turns of the animal, which might allow for calculating turning direction and speed. TL5 neurons, which branch throughout all layers of the CBL, one hemisphere of the PB, and in parts of the LX, are insensitive to E-vector angle (Vitzthum et al., 2002) but could play a modulatory role.

Tangential neurons of the CBU show the largest variety of cell types. Although at least six widely separated cell clusters contribute to TU neurons, most of these connect the superior medial and/or intermediate protocerebrum (>50 neurons per hemisphere: TU\textsubscript{VES4}, TU\textsubscript{CRE1}, TU\textsubscript{SLP}, TU\textsubscript{PS2}, TU\textsubscript{PS3}; Table 1) and/or the LAL to the CBU (>50 neurons: TU\textsubscript{LAL}, TU\textsubscript{VES1–3}, TU\textsubscript{CRE2}, TU\textsubscript{PS1}). Individual neurons usually innervate single CBU layers (Ia, Ib, Ia, Ib, II, III); an exception is the TU\textsubscript{VES1} neuron, arborizing in layers Ia and Ib (Figure 7a). Both the superior medial/intermediate protocerebrum and the LAL receive strong excitatory input from ascending neurons associated with flight motor activity (Homberg, 1994). Interestingly, calcium imaging data from Drosophila showed that visual stimuli elicit activity in the fan-shaped body (equivalent to CBU) only during flight, but not when the animal is at rest (Weir & Dickinson, 2015) suggesting that visual input to the CBU is gated by motor activity. Although motor activity related input to the CX is, likewise, important in the locust (Rosner, Pegel, & Homberg, 2019), pontine as well
as tangential neurons of the CBU in restrained animals were strongly sensitive to motion stimuli (looming disc), partly in an azimuth-dependent way (Rosner & Homberg, 2013).

The superior protocerebrum is connected to all layers of the CBU, but most neurons target layer I. Connections from the LAL are most numerous to layer II but all other layers except layer I are also innervated. Prominent connections from the superior protocerebrum and LAL to the CBU were, likewise, found in the house cricket (Schildberger, 1983), flies (Hanesch et al., 1989; Li et al., 2009; Phillips-Portillo & Strausfeld, 2012), the monarch butterfly (Heinze et al., 2013), and dung beetles (el Junidi et al., 2018). The connections of the superior protocerebrum are poorly understood. In Drosophila the superior protocerebrum is targeted by output neurons of the mushroom body, encoding learned olfactory and perhaps other sensory associations (Aso et al., 2014; Aso et al., 2014; Wu et al., 2017).

These inputs might bias behavioral decisions mediated by the CX based on prior experience and memory.

The LALs are subdivided into two major divisions, the LLAL and ULAL. TU neurons have ramifications in both subdivisions, however, more often concentrated in dorsal parts of the ULAL/LLAL (e.g., Figures 6c–f, 7a,d, 9a,b, and 12a–c). This contrasts with the projections of columnar outputs of the CX that project more densely to ventral parts of the LALs (Heinze & Homberg, 2008). The spatial separation between CX outputs and TU input areas, however, is not absolute, because some TU neurons do have substantial ramifications in ventral aspects of the LAL (e.g., Figures 6a,b and 7a) while certain columnar outputs such as CU2 neurons (Heinze & Homberg, 2008) ramify in dorsal parts of the LALs. This allows feedback connections between certain CBU outputs and inputs in the LAL (el Junidi et al., 2010). The LAL receives massive input from visual neurons of the upper unit of the anterior optic tubercle (Homberg et al., 2003; Pfeiffer, Kinoshita, & Homberg, 2005) as well as ascending input from the ventral nerve cord (Homberg, 1994). The visual signals through the upper units of the anterior optic tubercle are not well understood. Whereas calcium imaging in bees suggested an involvement in coding chromatic signals (Mota, Gronenberg, Giurfa, & Sandoz, 2013), studies in the privet hawk moth and Drosophila provide evidence for a role in figure-ground discrimination (Aptekar, Keleş, Lu, Zolotova, & Frye, 2015; Collett, 1972). The proposed role in object detection corresponds to strong responses to horizontally moving bars or squares in neurons of the fan-shaped body (equivalent to the CBU in locusts) of the fly (Weir & Dickinson, 2015).

Both TU neurons innervating the noduli (Figure 15c,d) arborize in the LX, the wedge and the posterior slope. In bees, TU neurons showed strong responses to optic flow stimulation and were proposed to play a role in distance estimation during flight used for path integration in the CX network (Stone et al., 2017). No functional data exist on these neurons in other species.

### 4.3 Anterior lip

The anterior lip is a small horizontal band of diffuse neuropil anterior to the ventral groove (Figure 1). It is particularly prominent in cockroaches, highly reduced in bees and apparently not present in flies. In the locust, it is closely connected to the CX network and receives input both, from CBU columnar neurons (type CU1; Heinze & Homberg, 2008) and tangential cells (Figures 6a, 7f, 9d, and 13a,b; Table 1). In most cases, neurons have arborizations in the anterior lip and layer I of the CBU. In addition to TU neurons with axonal terminals in layer I and the lip, some variants of TU neurons target the anterior lip exclusively (Figures 10d and 13c). Only one neuron has been identified as a possible postsynaptic partner of the columnar and tangential inputs (Figure 9e–g). It has dendrites in the anterior lip and terminals in the superior protocerebrum in both hemispheres, enabling the possibility of feedback loops, which are vital, when for example, aligning mental snapshots with the surrounding terrain (Seelig & Jayaraman, 2015; Wiederman, Fabian, Dunbier, & O’Carroll, 2017).

### 4.4 Wedge, antennal mechanosensory and motor center, posterior slope

In addition to the cell-type specific neuropils providing input to the CX, various neurons additionally arborize in the wedge and/or the dorsal antennal mechanosensory and motor center (Table 1). The wedge is innervated by TB6-, TB7-, and TB8 neurons (Figure 4), both TN neurons (Figure 15c,d), the TU₁₅₂2 neuron (Figure 6c–f), and the TU₅₅₃3 neuron (Figure 12c), the dorsal antennal mechanosensory and motor center by the TB6-, TB7-, TU₁₅₂2- and TU₅₅₃3 neurons. In Drosophila, the wedge receives direct input from antennal mechanosensory neurons and input from secondary neurons of the antennal mechanosensory and motor center (Ito et al., 2014). Patch-clamp recordings showed that wedge neurons in Drosophila are sensitive to wind direction by integrating mechanosensory input from both antennae (Suver et al., 2019). Extracellular recordings in the cockroach Blaberus suggest that CX neurons receive prominent mechanosensory input from the antennae, but whether these responses, likewise, reflect spatial tactile information, remains to be seen (Ritzmann, Ridgel, & Pollak, 2008). Taken together, these data point to a major contribution of antennal mechanosensory input to the encoding of space in the CX.

The posterior slope is the major input site of TB4–TB7 neurons to the PB, but certain neurons to the CBL (TL5, Figure 5f), the CBU (TUᵥₑ₅₃3, Figure 7e; TU₅₅₃3, Figure 12c) and the noduli (TN, Figure 15) also ramify in the posterior slope, which thus provides input to all CX subunits. Based on the strong innervation by intersegmental interneurons, these inputs might serve to monitor the behavioral state of the animal, which strongly influences neural excitation in the CX at all levels (Homberg, 1994; Rosner et al., 2019), likely to prioritize navigation relevant information input during walking and flight (Pfeiffer & Homberg, 2014).

### 4.5 Circadian clock and sleep–wake control

Long range migrations, controlled by a sun compass, require compensation for the apparent movement of the sun. Time compensation can be achieved by an internal circadian clock, which is associated with...
the accessory medulla in the optic lobe of *Drosophila* and the cockroach *Rhyparobia* (Helfrich-Förster, Stengl, & Homberg, 1998). In many insects, neurons immunolabeled for pigment dispersing hormone (PDH) provide circadian control over wide brain areas. In the locust, PDH-immunoreactive neurons of the accessory medulla densely innervate the POTUs and the superior clamp between the vertical lobe and the pedunculus of the mushroom body (Homberg et al., 1991). Circadian signals might, therefore, provide time compensation to the internal sky compass in the CX via TB neurons in the POTUs and/or via TUCRE1 neurons that have dense dendritic arbors in the superior clamp, but physiological evidence supporting these hypotheses is still lacking.

In *Drosophila*, several pathways leading to R neurons of the ellipsoid body (corresponding to locust TL neurons) are involved in circadian control of locomotor activity and sleep homeostasis. PPM3-EB neurons of the fly (likely homologous to locust TL5 neurons), are involved in circadian locomotor control (Liang et al., 2019), neurons of the anterior optic tubercle–bulb pathway (Guo, Holla, Díaz, & Rosbash, 2018) and a pathway via dorsal fan-shaped body neurons (dFB neurons, corresponding to locust TuSyp neurons), helicon cells (no equivalents known in locusts) and R neurons (Donlea et al., 2018) participate in sleep–wake control. Unfortunately, no corresponding data exist for any other insect species, but neurons anatomically resembling those fly neurons are largely present in the locust.

### 4.6 TCX neurons

With the exception of the multi-center TCX neurons, we found no direct connections between the CBU and CBL, and even in the three TCX neurons, the CBL and CBU seem to be innervated in parallel rather than having inputs in one neuropil and outputs in the other. Except for their multi-center branching pattern and apparent clonal relation, each TCX neuron seems to be unique in connectivity and function. TCX1 and TCX3 are sensitive to E-vector angle of a zenithal light stimulus suggesting involvement in sky compass computations (Bockhorst & Homberg, 2015). TCX1 might be suited to receive circadian signals in the POTU and PB for distribution to all sky-compass processing areas of the CX (CBL, CBU, lateral and medial bulbs). TCX3 is the only tangential neuron with fine, presumably postsynaptic terminals in the CBL. Its prominently beaded terminals in both LALs could serve as an output signal from the CBL in parallel to those from columnar neurons of the CBU. The TCX2 neuron had fine beaded terminals throughout all of its endings (Figure 16b) and might have a general role in synchronizing several brain areas including the CX.

### 4.7 Conclusions

The CX can be regarded as a brain area that integrates sensory inputs to generate goal-directed behavior. As such, it receives sensory information related to heading directions and their changes (solar azimuth, sky-compass related, rotational flow fields), distance information (translational and rotational optic flow), spatial object information (collision relevant, goal) as well as internal or reafferent signals on behavioral state (flight, walking, turning direction) and circadian input (rest, sleep–wake). These inputs are used by the CX for decisions on oriented behaviors based on the animal’s need and spatial memories, essential e.g. for path integration. The multitude of tangential neurons reported here provides these inputs. Some of these are fairly well understood (e.g., sky compass input), others such as inputs signaling behavioral state are more elusive. How these different inputs interact within the CX is just beginning to be understood. The CBU and CBL may perform particular subroutines on their own, while parallel connections with the PB and noduli as well as the overarching CX neurons may serve to fine tune the priorities in navigational decisions based on the availability of different inputs as well as internal states. Our atlas of tangential neurons in the locust further specifies the modular assembly of the CX and might pave the way for future functional analyses on the nature and integration of inputs in the CX.

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## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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