Perspective

Insights from the past: the work of Hans von Alten on the evolution of brain structure, ecological adaptation, and cognition in hymenopteran species

Randolf Menzel[1] and Jürgen Rybak[2]

[1]*Department of Biology, Neurobiology, Freie Universität Berlin, 14195 Berlin, Germany;* [2]*Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, 07745 Jena, Germany*

In his treatise on arthropod brains, Hans von Alten (1910) focuses on a specific functional group of insects—the flying Hymenoptera—which exhibit a spectrum of lifestyles ranging from solitary to social. His work presents a distinctive comparative neuro-anatomical approach rooted in an eco-evolutionary and eco-behavioral background. We regard his publication as an exceptionally valuable source of information and seek to inspire the research community dedicated to the study of the insect brain to explore its insights further, even after more than 110 years. We have translated and annotated his work, expecting it to engage researchers not just with its remarkable drawings but also with its substantive content and exemplary research strategy. The present text is designed to complement von Alten's publication, situating it within the temporal context of nineteenth-century and early twentieth-century studies, and to draw connections to contemporary perspectives, especially concerning a central brain structure: the mushroom body.

Understanding the brain's architecture is paramount for grasping its phylogenetic and ontogenetic development, elucidating its intricate cellular and molecular components, deciphering its function as a driver of behavior and cognition, and unraveling the emergent properties that give rise to advanced cognitive abilities, self-awareness, and consciousness. This insight has driven neuroscience over the centuries, from the earliest fine-preparative and microscopic studies of Vesalius (1543) (see also Giménez-Roldán 2020), Harvey (1628), and Swammerdam (1737–1738) to the electron microscopic analyses used to study the connectomes of small brains (*Caenorhabditis elegans* [White et al. 1986]; *Drosophila* [Scheffer et al. 2020; Gruber et al. 2023]; zebrafish [Hildebrand et al. 2017]), as well as the digital brain atlases of invertebrates and vertebrates, which exist at different grades of resolution in mice and humans (e.g., https://alleninstitute.org/; https://www.frontiersin.org/research-topics/23/digital-brain-atlases). All these anatomical studies of the brain have been motivated by efforts to explore fundamental issues in the life sciences, including the phylogenetic history both of closely related species and across large phylogenetic scales, ontogenetic development, and the correlation between structure and function. The intricate complexity and considerable size of mammalian and human brains have spurred long-standing investigations into the brains of smaller animals, particularly social insects such as bees, wasps, and ants. These insects captivate researchers because of their impressive behavior. How is it possible for such small brains to accomplish extraordinary feats such as multiple forms of learning; the extraction of rules from sequential learning, counting, and number recognition; social communication, including symbolic coding and decoding (in the case of the honeybee); navigation; and lifelong memory? What evolutionary paths have led to these abilities and the underlying brain structures? Further, is it possible to derive phylogenetic relationships from comparative approaches at both the anatomical and the functional (behavioral) levels?

Historical background

In the seventeenth century, Swammerdam (1737–1738) was already posing these questions with his meticulous microscopic studies of entire brains. This was then echoed by numerous scholars in the eighteenth and nineteenth centuries, particularly in the context of social insects such as ants, wasps, and bees and using a comparative approach as the method of choice. The study by von Alten in 1910 provides an excellent culmination of this research over the eighteenth and nineteenth centuries and brings it into context within early twentieth-century thought.

These studies were incorporated into comparative approaches within the Darwin–Wallace theory of descent. Although nineteenth-century naturalists interested in insects were excellent observers (e.g., Fabre 1879), they were criticized for two inexcusable failures: reporting only single-case events and using unjustified anthropomorphic terminology. Indeed, repetitions under strict experimental control were rarely performed and, when they were, the conditions were not reported. Anthropomorphic terminology was not considered misleading or dangerous, because Darwin's gradualism guided the comparative approach in behavioral studies as in comparative morphology. Reading this literature gives the strong impression that authors such as Romanes (1883), Forel (1910), and Buttel-Reepen (1900), as well as Maeterlinck (1901) with his romantic style in praise of the honeybee, did not mean mental operations of a human kind when they used terms such as intelligence or mental power, but rather species-specific forms of such operations, although all these authors kept their meaning vague. Even so, the motivation arising from these approaches was highly productive in the striving to uncover the working of the brain.

The scientific context of the Hans von Alten study

Unlike most researchers into insect brains in the eighteenth and nineteenth centuries, Hans von Alten was a highly trained

Corresponding author: menzel@neurobiologie.fu-berlin.de

Published by Cold Spring Harbor Laboratory Press; ISSN 1549-5485/24
Article is online at http://www.learnmem.org/cgi/doi/10.1101/lm.053922.124.
Freely available online through the *Learning & Memory* Open Access option.

zoologist who worked in a famous research laboratory under the guidance of August Weismann (1834–1914), an eminent evolutionary biologist and head of the Zoology Institute at the University of Freiburg. In the second half of the nineteenth century, evolutionary biology in Germany had shifted from a Lamarckian view to Darwin–Wallace theory in a series of steps involving intense scientific battles. When Weismann became director of the Freiburg Zoology chair (1873), he studied freshwater and marine zooplankton and initially defended Lamarck's thesis of individual adaptation driven by external factors. When he shifted his research focus to ontogenetic development, he found no evidence for individual adaptation, and although he noticed that the evolutionary process as Darwin described it cannot be proven in laboratory experiments but only falsified, he became an enthusiastic defender of Darwin's theses. His *Aufsätze über Vererbung und verwandte biologische Fragen*, published in 1892 (Weismann 1892), provides an excellent record of the thought that led him to postulate the "germ plasm theory" and what was later called the "Weismann barrier" (for review, see Sabour and Schöler 2012). Following Hertwig (1895), the discoverer of meiosis in the sea urchin egg, he proposed that only changes in the germline would lead to evolutionary adaptations. He interpreted the germline as present only in egg and sperm cells, not somatic cells, and thus argued that only changes in these cells, but not the body cells, would contribute to the evolutionary process, precluding Lamarckian adaptation. Although this view was to be modified by the discovery of the identity of chromosomes in both germ cells and somatic cells, the idea of graded phylogenetic connections between anatomically and ecologically related species has provided and still provides the conceptual frame for comparative studies.

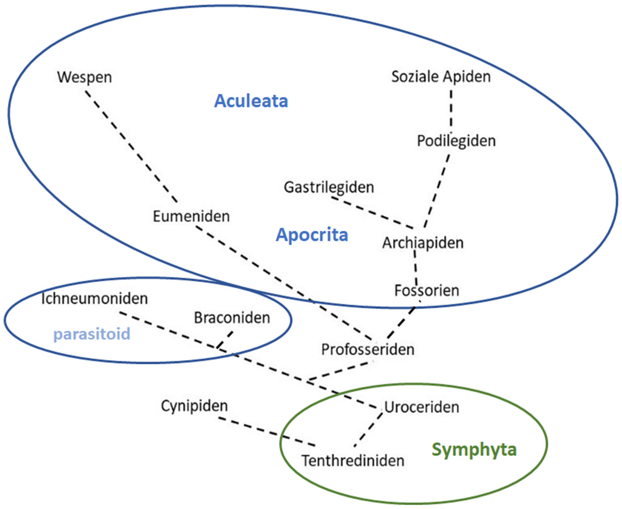

Figure 1. Phylogenetic tree proposed by von Alten based on his comparative brain studies of flying Hymenoptera (see his text, and Text Figure 19). Tenthrediniden = sawflies, Uroceriden = wood wasps, Cynipiden = gall wasps, Profosseriden = hypothetical group of ancestral forms leading to wasps, Ichneumoniden = parasitoid ichneumon wasps, Braconiden = parasitoid braconid wasps, Fossorien = sphecoid wasps, Archiapiden = e.g., *Colletes* and *Halictus* species, Gastrilegiden = e.g., megachilid bees, Podilegiden = e.g., Andrena and Anthophora species, Eumeniden = Vespoidea, Wespen = wasps, soziale Apiden = social bees (*Apis*, *Bombus*). Key evolutionary events such as the wasp waist and the stinger led to a strong radiation in the major lineages Apocrita and Aculeata, respectively.

Brain structure and phylogeny

Given this scientific context, it is not surprising that von Alten's comparative anatomical studies tempted him to build bridges to the study both of phylogenetic relationships and of behaviorally graded cognitive faculties. Fortunately, he turned to a rather closely related group of insects, the flying Hymenoptera. The lifestyle of these insects ranging from solitary to social bees and wasps provides an intuitive scale of graded behavioral complexity that he felt should be reflected in their corresponding brain structures. In Weismann's thinking, the somatic structure (here the brain) is a stable structure within each species, here prompting questions such as how brains differ between the sexes of the same species, how brains differ between the reproductive queens and sterile workers in the social Hymenoptera, and how brains vary according to the evolutionary adaptations that have led to different levels of performance (e.g., solitary and social lifestyle). The resulting approaches require (1) the selection of brain structures regarded as related to higher forms of behavior rather than basic sensory-motor performance, and the quantification of structural differences by absolute and relative measures, and (2) measures of graded complexity in behavior. von Alten solved the first problem by establishing distance values in selected sections of the brain (see his tables II–IV) that he considered representative of volume quantification. And he focused on distinct structures, predominantly the mushroom bodies (MBs), a paired brain structure that had been linked with more complex forms of behavior since Dujardin (1850), Kenyon (1896), and others (see below).

Using his measures of brain structures together with multiple qualitative estimates of brain structures available to him from earlier studies, he constructed a tree of phylogenetic relationships that is quite consistent with modern schemes of evolutionary relatedness in flying Hymenoptera (Fig. 1). An exception is the separation of Vespidae from Apoidea among the aculeate (stinging) Hymenoptera, and the introduction of hypothetical professial groups, which was already a matter of controversy among contemporary authors (e.g., Armbruster 1919). Modern phylogenetic analyses position Vespidae together with social bees (and ants) as monophyletic groups. The cynipids, as well as parasitoid groups of ichneumonids and braconids, belong to the apocritan Hymenoptera (Peters et al. 2017).

Histological methods available to von Alten

The work of von Alten was based on the contemporary state of the art and on the development of histological methods in neuroscience in general (for review, see Javaeed et al. 2021). In 1873, Camillo Golgi discovered the "black reaction," and in 1877 Paul Ehrlich had for the first time applied methylene blue staining to neuron morphology and putative connectivity, two methods still in use until recent times. Advances in histology in von Alten's time included the use of eosin for the staining of connective tissue and cytoplasm, and hematoxylin for cell nuclei. These techniques spurred advances in knowledge of the neuronal architecture of insects, exemplified by the work of Kenyon (1896) and Jonescu (1909), and provided an important backbone for von Alten's account of hymenopteran MBs and optic lobes.

The strength of von Alten, however, lay in his comparative approach, which focused on the group Hymenoptera using basic, though superbly implemented, histology. He was also one of the first (see, e.g., Flögel 1878) to apply features of the brain to phylogenetic arguments. Initiated by von Alten and continued by Holmgren (1916) and in more modern times by Howse (1974), this line of research thus laid the foundations for neurophylogeny, a term coined to refer to a field that has been reinvented today (e.g., Hanström 1928; Strausfeld and Meinertzhagen 1998; Harzsch 2006; Richter et al. 2010). Hanström (1928), for example, provided a rich data set of comparative data in a large number of different hexapod species using volumetric measures. He confirmed the picture already developed by Flögel (1878) and von Alten (1910), concluding that MB size correlates with the number of connections to all the sensory centers (see below). Apidae and Formicidae were discovered to possess the largest and most intricately structured MBs, suggesting that these structures not only facilitate sensory integration but also function as association centers, controlling complex behaviors (see below). Hanström (1928) and later Howse (1974) quantified the observations made by nineteenth-century insect anatomists with respect to the central complex, which they found to be relatively uniform in structure across insect orders.

Focus on the mushroom body

The question of the brain–behavior relationship has deep roots in philosophical argument. How can the unity of perception, memory, and feeling emerge from symmetrical brains composed of two seemingly identical structures (e.g., the cerebral lobes in humans)? The arguments go in two directions, from brain to behavior, and from behavior to brain. In the first case, early speculations on unique brain structures for higher forms of cognition focused on an unpaired brain structure. In the thinking of Descartes (1662; republication 1969), the epiphysis cerebri or pineal gland, which lies on the midline of the otherwise symmetrically organized human brain, appeared as a unique meeting point of the two epistemic worlds: the soul and the brain as a machine. The body is bilaterally symmetrical, so the brain is too. Lower forms of cognition (sensory and motor functions) are, therefore, bilaterally organized. Unique, undivided thoughts and memories may thus have to come from unpaired parts of the brain.

The epistemological dimension of these arguments applies to all animals with a centralized brain, naturally in graded fashion. The uniqueness of perception and cognition as the essential mechanisms controlling the animal's own body requires neural processes that create a unity of central command. The arthropod brain houses only one unpaired, centrally located structure, the central complex. The nineteenth-century anatomists interested in the brain–behavior relationship of arthropods noticed one fact that must have surprised them and that was also confirmed by von Alten. The central complex is the most stable structure across species and environmental adaptations (Hanström 1928; Howse 1974). The central complex was, therefore, dismissed by nineteenth-century anatomists as a candidate for higher brain function. Given these conditions, it was obvious for them to look both for brain structures that were structurally impressive and variable in size and for subcompartments indicating structure–function relationships that reflected ecological adaptations along with graded phylogenetic relationships. Because only paired structures were available for consideration, other arguments needed to be incorporated.

The MBs drew the attention of the first anatomist intrigued by the arthropod brain, as is evident in the work of Dujardin (1850). He called them *lobes à convolutions* by analogy with the folded nature of the mammalian cortex. From the very discovery of these impressive, paired structures in a few species of Hymenoptera and Orthoptera, the MBs were thus linked to insect intelligence. In a sense, the erroneous description of its structure (*lobes à convolutions*) and the incorrect comparison with what was believed to be the site of mammalian intelligence (cortical convolutions) laid the foundation for the ideas about the relationship between the MB and hexapod intelligence. Even after the sectioning of the brain by late-nineteenth-century anatomists verified that MBs are not "convolute" but rather compact structures, the idea of graded structural complexity related to graded cognitive faculties in insects was retained. Relevant here are the studies by Leydig (1864) (studying ants, bees, and wasps), who called them *gestielte Körper*, by Rabl-Rückhard (1875) (*Gryllus*, *Locusta*, *Dycticus*), who corrected the term "convolutions" and called them cups (*ingförmige Körper*), and by Dietl (1876), who coined the term *pilzhutförmiger Körper* (mushroom body). Although Dietl described them as more complex in Hymenoptera than in Orthoptera, he hesitated to associate them with cognitive functions (for citations, see Strausfeld and Meinertzhagen 1998). Kenyon (1896), who provided the most informative structural analysis of the brain before von Alten, summarized the most up-to-date arguments in terms of a relationship between structural complexity and cognitive faculties. We thus want to cite two paragraphs, one from Kenyon (1896) and one from von Alten, which reflect most distinctively the kind of thinking on the MB that existed at the turn of the twentieth century:

Kenyon (1896), p. 644: "As to the intellectual function of the bodies, not all of the early writers supported Dujardin's inference. They were supposed to be connected with sight; but Rabl-Rückhard (1875) showed that they are fully developed in a blind African ant (*Typhlopone*). Dietl (1876) was loth to acknowledge an intellectual function, even though he found the organs more highly developed in Hymenoptera than in Orthoptera. But Forel (1874) adhered to Dujardin's supposition, and showed that among Hymenoptera even of the same species the bodies are most prominent where one usually recognizes most intelligence, as in the worker bees and ants, while they are small in the females (queens) and the males. Brandt (1876), two years later in a note on the brain of Hymenoptera makes the same observations as to the differences in the same species, while Berger (1878) considered the structures as 'organs of projection of the first order.'"

von Alten (1910), p. 516: "On the basis of these physiological and comparative anatomical, as well as on the basis of the following findings, as I would like to note right here, one will probably not to go too far if one addresses the MBs as the main reflex and association centers of the hymenopteran brain. They could also be described as 'organs of intelligence' if one assumes that bees possess psychic abilities in the sense of Bethe (1897) (sentience, ability to form new associations on the basis of memory and experience), which is as vividly disputed (Bethe 1897) on the one hand as it is claimed on the other (Buttel-Reepen 1900; Romanes 1883; Forel 1874; Wasmann 1905, 1909)."

So which cognitive functions are served by the MBs? In flying Hymenoptera, graded behavioral performances appear intuitively rather obviously given the existing solitary and social lifestyles. The intuition was, and to some extent still is, that social life is more complex and thus may require larger brains in an absolute or allometric sense, dedicated brain structures, and possibly unique neurons (intellective cells). These differences should be expressed particularly in those brain parts that correlate with more complex behavior. Indeed, the phylogenetic tree that von Alten derives from his anatomical measures (Fig. 1) roughly reflects a hierarchy that has the social Hymenoptera (social bees, wasps) on top, the solitary, nest-dwelling bees in the middle (gastrilegids and podilegids), and solitary Hymenoptera (Symphyta) without brood care at the bottom.

However, von Alten did not distinguish between reflexes and learned behavior, suggesting that both innate behavioral routines and experience-dependent behavior reflect graded behavioral complexity. Social life in insects—the lifestyle inside the colony—is in general mostly controlled by stereotypical and innate sensory-response routines that depend predominantly on peripheral specializations both at the sensory and motor level rather than on central processing in the brain. Exceptions exist (e.g., the waggle dance in honeybees), but the rule appears to apply well at least for hymenopteran species, including ants.

Interestingly, no correlation has so far been found in ants between the complexity of their social life and measures of the MBs. It is thus likely that life outside the nest is more closely related to MB size and structure.

MB-related studies over the last 50 years

Eco-evolutionary and neurobiological approaches over the last 50 years have shifted the focus to learning, memory formation, and neural processes possibly involved in foraging at the food site, at the nest site, and during navigation (Howse 1975; Giurfa 2003; Menzel 2012, 2014; Farris 2016).

Here is a short overview.

Learning: Elementary forms of olfactory learning are unaffected if partial lesions or anesthetic treatments are performed on the MB, but complex olfactory learning tasks require both functional MBs and their interactions (Malun et al. 2002; Komischke et al. 2005; Devaud et al. 2007; for review, see Menzel et al. 2007). If an olfactory stimulus is trained only via one antenna the memory can also be recalled via the other antenna, indicating information transfer between the two sides, most likely at the level of the MB. If, however, the two input sides are specifically trained to different olfactory tasks, the memory recall is side-specific (Sandoz and Menzel 2001). A preference for one side of olfactory input over the other may exist in the honeybee, but not in the solitary bee *Osmia* (Frasnelli et al. 2012). However, it is not known whether this is a property of the MB or the peripheral olfactory pathway.

Memory: Local cooling of the MB on one side during one-trial olfactory conditioning via the antenna on the same side uncovered the role of the MB in turning an erasable short-term memory into a stable long-term memory (Erber et al. 1980). The temporal dynamic of this consolidation process is completed after 5 min, whereas a sensory memory can be blocked in the olfactory lobe on the same side only within the first minute after a single learning trial. Structural correlates of long-term olfactory memory have been documented for the microglomeruli in the MB calyx (Hourcade et al. 2010).

Navigation: Solitary flying hymenopterans build nests to which, like social hymenopterans, they have to return from their foraging flights. The foraging flights of solitary bees are usually more stereotypical, particularly when pollen is collected. In contrast, they are highly variable in social bees, which live as a biological unit over different yearly seasons and in much more variable habitats, forcing the individual animals continuously to learn both the nest site and food sources anew. Intuitively, more specialist solitary hymenopterans (e.g., oligolectic species) would be expected to express less complex behavioral performances and have smaller/less complex MBs, but systematic studies are lacking. The search for neural correlates has focused on the volume and structure of the MB calyx during the transition between nest duties and foraging. Total volume was found to depend on age and environment (not only inside the nest, in isolation, but also outside the nest, foraging) both in worker bees (Maleszka et al. 2009) and drones (Fahrbach et al. 1997). Age- and experience-dependent structural changes were located in the microglomerular complexes (Krofczik et al. 2008; Rybak 2013), and the dendritic branches of the MB intrinsic neurons, the Kenyon cells (Farris et al. 2001). In the study by Krofczik et al. (2008), the number of microglomerular complexes and the bouton volumes increased during maturation, independent of environmental conditions. In contrast, manipulations of behavior and sensory experience caused a decrease in the number of microglomerular complexes, but an increase in bouton volume. In the study by Farris et al. (2001), the branching and length of dendrites were strongly correlated with worker age, but when age-matched bees were directly compared, those with foraging experience had longer, more branched dendrites than those that had foraged less or not at all. The density of Kenyon cell dendritic spines remained constant regardless of age or behavioral state. Older and more experienced foragers, therefore, have a greater total number of dendritic spines in the MB neuropil, corroborating the findings mentioned above on the structural correlates of olfactory memory (Hourcade et al. 2010).

Evolutionary driving forces of brain structure in hymenopteran insects

The intuitive theory of the relationship between brain structure and sociality in Hymenoptera has been termed the "social brain hypothesis," a concept originally developed for primates (Dunbar 1998). The essence of this hypothesis is that social behavior requires more sophisticated neural processing, particularly in those brain centers that deal with cognitive processes. As we have seen, nineteenth- and twentieth-century research on the MB substantially supported the idea that flying Hymenoptera with a social lifestyle (bees and wasps) have a larger and more structured MB. However, we have argued above that social life inside the colony is predominantly organized via innate and rather stereotypical processes, leading to the proposal that social life is controlled by sensory-motor routines independent of higher-order neural mechanisms.

Farris and Schulmeister (2011) offer an interesting alternative view based on comparisons of MB structure and volume across Hymenoptera, including solitary parasitoid wasps (Orussoidea/Apocrita). These species appear to have developed large and highly structured MBs as early as 90 Myr before the development of sociality in Aculeata. Elaborate MBs with distinct morphological subcompartments were thus evolved at the base of Euhymenoptera, together with the acquisition of parasitoid behavioral ecology. This discovery implies that the neural requirements involved in consistently tracking and relocating prey over substantial distances serve as preadaptations for central-place foraging. The wiring of the MB and its intrinsic structure provide the essential anatomical and functional conditions for navigation over greater distances. The MB receives highly preprocessed sensory input of all modalities, preserves aminergic neurons from evaluating circuits, sends feedback neurons to the sensory neuropils, and feeds into premotor centers (Hammer 1993; Rybak and Menzel 1993; Grünewald 1999; Abel et al. 2001; Gronenberg 2001; Strausfeld 2002; Menzel 2012; Devaud et al. 2015). These conditions allow for flexible and adaptive processing, learning, and memory, as has been well documented for highly evolved flying Hymenoptera but also at a lower level of complexity for other insects.

Molecular studies of the hymenopteran MB

von Alten found the concurrent elaboration of the MB calyces with distinct subcompartments throughout the evolution of the aculeate Hymenoptera, leading to the differentiation and diversity of the Kenyon cells. These observations are corroborated by recent molecular studies that have uncovered distinctive genetic profiles of Kenyon cell types. von Alten documented the evolution from the chalice typus of Tenthredinidae (sawflies) to the cup type of aculeate species (*Bombus*/*Apis*). He also showed that Kenyon cells are structurally diverse. Specifically, he identified medial large Kenyon cells (LKCs) as the typical Kenyon cells of the aculeate hymenopteran species.

Molecular genetic studies targeting MB-connected genes provide valuable information on the differentiation of Kenyon cells across the lineage of hymenopteran species. Such studies are still rather rare. Oya et al. (2017) identified the gene of a tachykinin-related peptide that is differentially expressed in Kenyon cell types, applying in situ hybridization experiments with MB tissue from four species (as a reference they used the known cell types in *Apis mellifera*: three type K-I, and one type K-II): the eusocial *Vespa mandarinia* (Vespidae) and the solitary scoliid flower wasp *Campsomeris prismatica* (both: Aculeata, Apocrita), the solitary parasitic wasp *Ascogaster reticulatus* (Braconidae, Ichneumonidae, Apocrita), and the ancestral hymenopteran the phytophagous sawfly *Arge similis* (Tenthredinidae). They found three K-I cell types in both aculeate wasp species (as in honeybees), only two K-I subtypes in the parasitic braconid wasp, and only one K-I type in the sawfly (Tenthredinidae). They interpret their findings as indicative of visual input primarily to the MB calyces exclusively in apocritan Hymenoptera, specifically for the purpose of host finding among parasitoid wasps (see also Ehmer and Gronenberg 2002; Farris and Schulmeister 2011). This interpretation potentially lends support to the hypothesis of the role of central-place foraging in the evolutionary development of the MB, which serves as a preadaptation for eusociality.

In a recent extension of these studies, the same research group, Kuwabara et al. (2023), performed comparative transcriptome analyses on Kenyon cell types in the honeybee and the turnip sawfly (*Athalia rosae*, Tenthredinidae), a primitive flying Hymenoptera without central-place foraging habits. These analyses revealed both similar and different gene expression between these two species. Unique gene expression profiles were found both in different honeybee Kenyon cell types and in that of the sawfly. The sawfly contains only one Kenyon cell type, which shares some of the gene expression profile of the different Kenyon cell types found in the honeybee. Furthermore, specific gene expression profiles in each of the honeybee Kenyon cell types were not found in the Kenyon cells in the sawfly. In particular, LKCs of type I known to be involved in learning and memory, probably acquired by specific gene expression and subsequent functional diversification, increased learning and memory abilities. Most interestingly, two specific sawfly genes appear to be related to learning and memory, and elementary forms of learning are documented in the study of Kuwabara et al. (2023).

Several immediate early genes (IEGs) have been identified as specifically expressed in Kenyon cells under behavioral conditions that simulate learning in navigational tasks. Ugajin et al. (2018) identified three genes, the *Src homology 3* (*SH3*) *domain-binding kinase* (*Sbk*), the *family with sequence similarity 46* (*Fam46*), and *GB47136*, as novel neural IEGs in the honeybee. Foragers and/or orientating bees, which fly around their hives to memorize positional information, showed induced expression of these IEGs in the MB, indicating a possible role for the novel IEGs in foraging-related learning and memory processes in the honeybee. Geng et al. (2022) focused on three IEGs (*kakusei*, *Hr38*, and *Egr1*) that are known to be related to foraging and orientation, and compared their relative expression in the calyces of the MB, the optic lobes, and the rest of the brain after color discrimination learning. Successful learners exhibited an *Egr1* up-regulation only in the calyces of the MB.

The study of IEGs in Hymenoptera will be particularly informative if combined with developmental studies comparable to those on the expansion of the neocortex in mammals in an evolutionary context. Heide et al. (2020) had previously examined the effect of overexpression of the cortical human-specific gene *ARHGAP11B* in developing mouse and ferret neocortex. They found that *ARHGAP11B* expressed in the fetal neocortex of the marmoset under the control of the human gene promoter enlarged the neocortex and induced its folding. It will be exciting to see whether any of the identified hymenopteran IEGs play a comparable role in the MB and drive changes in its development across other flying hymenopteran species, and whether overexpression has functional consequences.

Conclusion

von Alten's (1910) publication constitutes a highly valuable resource for studying brain architecture across a diverse and extensive collection of hymenopteran species. It particularly emphasizes the MB in the context of eco-evolutionary and eco-behavioral considerations. von Alten provides an enlightening summary of the literature of the nineteenth century and imparts information that remains highly significant even after more than 110 years. The research strategy applied by von Alten is exemplary, particularly with respect to his focus on the flying Hymenoptera, and, if combined with the methods now available to us, will lead to great insights. Today it is possible to establish volumetric brain atlases that allow for sophisticated quantitative comparison (e.g., https://insectbraindb.org/app/). Currently, phylogenetic relationships between species can be established at a much higher level of sophistication, crucially allowing researchers to test the value of comparative studies on the level of brain structures, ecological adaptation, and behavioral performance. Quantifications of behavior under laboratory and natural conditions are now possible that will lead to a deeper understanding of ecological adaptations. The extensive array of molecular tools at our disposal will enable us to conduct more rigorous testing than has been undertaken thus far, especially when integrated with comparative developmental studies.

We have taken on the task of translating and annotating the original publication by von Alten, in the anticipation that it will inspire researchers focusing on the insect brain to undertake a thorough exploration of structure–function relationships and their evolutionary roots.

Acknowledgments

The work was supported by funds from the Deutsche Forschungsgemeinschaft (DFG) (grant no. 430592330), in the Priority Program "Evolutionary Optimization of Neuronal Processing" (DFG-SPP 2205), and by the Max Planck Society.

References

Abel R, Rybak J, Menzel R. 2001. Structure and response patterns of olfactory interneurons in the honeybee, *Apis mellifera*. *J Comp Neurol* **437:** 363–383. doi:10.1002/cne.1289

Armbruster L. 1919. Bienen und Wespengehirne - Messbare phaenotypische und genotypische Instinktveränderungen. *Arch Bienenk* **1:** 145–184.

Berger E. 1878. Untersuchungen über den Bau des Gehirnes und der Retina der Arthropoden. *Arb Zool Inst Wien, Bd* **1:** 2.

Bethe A. 1897. Vergleichende Untersuchungen über die Functionen des Centralnervensystems der Arthropoden. *Pflüger Arch Gesamte Physiol Menschen Tiere* **68:** 449–545. doi:10.1007/BF01647367

Brandt ME. 1876. Anatomical and morphological researches on the nervous system of Hymenopterous insects. *Ann Mag Nat Hist* **18:** 504–506. doi:10.1080/00222937608682085

Buttel-Reepen H. 1900. *Sind die Bienen Reflexmaschinen? Experimentelle Beiträge zur Biologie der Honigbiene*. Arthur Georgi, Leipzig.

Descartes R. 1969. De Homine/Traité de l'Homme. In *Über den Menschen sowie Beschreibung des menschlichen Körpers*. Springer, Heidelberg (Erstveröffentlichung 1662).

Devaud JM, Blunk A, Podufall J, Giurfa M, Grünewald B. 2007. Using local anaesthetics to block neuronal activity and map specific learning tasks to the mushroom bodies of an insect brain. *Eur J Neurosci* **26:** 3193–3206. doi:10.1111/j.1460-9568.2007.05904.x

Devaud J-M, Papouin T, Carcaud J, Sandoz J-C, Grünewald B, Giurfa M. 2015. Neural substrate for higher-order learning in an insect: mushroom bodies are necessary for configural discriminations. *Proc Natl Acad Sci* **112:** E5854–E5862. doi:10.1073/pnas.1508422112

Dietl MJ. 1876. Die Organisation des Arthropodengehirns. *Z Wiss Zool* **27:** 488–517.

Dujardin. 1850. Memoire sur le systeme nerveux des insects. *Ann Sci Nat Zool* **14:** 195–206.

Dunbar RI. 1998. The social brain hypothesis. *Evol Anthropol* **6:** 178–190. doi:10.1002/(SICI)1520-6505(1998)6:5<178::AID-EVAN5>3.0.CO;2-8

Ehmer B, Gronenberg W. 2002. Segregation of visual input to the mushroom bodies in the honeybee (*Apis mellifera*). *J Comp Neurol* **451:** 362–373. doi:10.1002/cne.10355

Ehrlich P. 1877. Beiträge zur Kenntniss der Anilinfärbungen und ihrer Verwendung in der mikroskopischen Technik. *Arch Mikrosk Anat* **13:** 263–277. doi:10.1007/BF02933937

Erber J, Masuhr T, Menzel R. 1980. Localization of short-term memory in the brain of the bee, *Apis mellifera*. *Physiol Entomol* **5:** 343–358. doi:10.1111/j.1365-3032.1980.tb00244.x

Fabre. 1879. *Souvenirs entomologiques*. C. Delagrave, Paris.

Fahrbach SE, Giray T, Farris SM, Robinson GE. 1997. Expansion of the neuropil of the mushroom bodies in male honey bees is coincident with initiation of flight. *Neurosci Lett* **236:** 135–138. doi:10.1016/S0304-3940(97)00772-6

Farris SM. 2016. Insect societies and the social brain. *Curr Opin Insect Sci* **15:** 1–8. doi:10.1016/j.cois.2016.01.010

Farris SM, Schulmeister S. 2011. Parasitoidism, not sociality, is associated with the evolution of elaborate mushroom bodies in the brains of hymenopteran insects. *Proc R Soc Lond B* **278:** 940–951. doi:10.1098/rspb.2010.2161

Farris SM, Robinson GE, Fahrbach SE. 2001. Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honeybee. *J Neurosci* **21:** 6395–6404. doi:10.1523/JNEUROSCI.21-16-06395.2001

Flögel J. 1878. Über den einheitlichen Bau des Gehirns in den verschiedenen Insektenordnungen. *Z Wiss Zool* **30:** 556–592.

Forel A. 1874. Fourmis de la Suisse. In *Nouveaux mémoires de la société Helvétique*. Schweizerische Naturforschende Gesellschaft, Zuerich.

Forel A. 1910. *Das Sinnesleben der Insekten*. Reinhardt, Muenchen.

Frasnelli E, Vallortigara G, Rogers LJ. 2012. Left–right asymmetries of behaviour and nervous system in invertebrates. *Neurosci Biobehav Rev* **36:** 1273–1291. doi:10.1016/j.neubiorev.2012.02.006

Geng H, Lafon G, Avarguès-Weber A, Buatois A, Massou I, Giurfa M. 2022. Visual learning in a virtual reality environment upregulates immediate early gene expression in the mushroom bodies of honey bees. *Commun Biol* **5:** 130. doi:10.1038/s42003-022-03075-8

Giménez-Roldán S. 2020. Andreas Vesalius and the brain: limitations of *De humani corporis fabrica libri septem* and some comments on the matter. *Neurosci Hist* **8:** 76–86.

Giurfa M. 2003. Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain. *Curr Opin Neurobiol* **13:** 726–735. doi:10.1016/j.conb.2003.10.015

Gronenberg W. 2001. Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *J Comp Neurol* **435:** 474–489. doi:10.1002/cne.1045

Gruber L, Cantera R, Pleijzier MW, Steinert M, Pertsch T, Hansson BS, Rybak J. 2023. The unique synaptic circuitry of specialized olfactory glomeruli in *Drosophila melanogaster*. *eLife* **12:** e88824. doi:10.7554/elife.88824.1

Grünewald B. 1999. Physiological properties and response modulations of mushroom body feedback neurons during olfactory learning in the honeybee *Apis mellifera*. *J Comp Physiol A* **185:** 565–576. doi:10.1007/s003590050417

Hammer M. 1993. An identified neuron mediates the unconditioned stimulus in associative olfactory learning in honeybees. *Nature* **366:** 59–63. doi:10.1038/366059a0

Hanström B. 1928. *Vergleichende Anatomie des Nervensystems der wirbellosen Tiere: Unter Berücksichtigung seiner Funktion*. Springer, Heidelberg.

Harvey W. 1628. Exercitatio anatomica de motu cordis et sanguinis in animalibus. *Frankfurt am Main* **1628:** 17.

Harzsch S. 2006. Neurophylogeny: architecture of the nervous system and a fresh view on arthropod phyologeny. *Integr Comp Biol* **46:** 162–194. doi:10.1093/icb/icj011

Heide M, Haffner C, Murayama A, Kurotaki Y, Shinohara H, Okano H, Sasaki E, Huttner WB. 2020. Human-specific ARHGAP11B increases size and folding of primate neocortex in the fetal marmoset. *Science* **369:** 546–550. doi:10.1126/science.abb2401

Hertwig O. 1895. *The cell: outlines of general anatomy and physiology*. Sonnenschein, London.

Hildebrand DGC, Cicconet M, Torres RM, Choi W, Quan TM, Moon J, Wetzel AW, Scott Champion A, Graham BJ, Randlett O, et al. 2017. Whole-brain serial-section electron microscopy in larval zebrafish. *Nature* **545:** 345–349. doi:10.1038/nature22356

Holmgren NF. 1916. *Zur vergleichenden Anatomie des Gehirns von Polychaeten, Onychophoren, Xiphosuren, Arachniden, Crustaceen, Myriapoden, und Insekten*. Almqvist & Wiksells, Stockholm.

Hourcade B, Muenz TS, Sandoz JC, Rössler W, Devaud JM. 2010. Long-term memory leads to synaptic reorganization in the mushroom bodies: a memory trace in the insect brain? *J Neurosci* **30:** 6461–6465. doi:10.1523/JNEUROSCI.0841-10.2010

Howse P. 1974. Design and function in the insect brain. In *Experimental analysis of insect behaviour* (ed. Browne LB), pp. 180–194. Springer, Berlin.

Howse P. 1975. Brain structure and behavior in insects. *Annu Rev Entomol* **20:** 359–379. doi:10.1146/annurev.en.20.010175.002043

Javaeed A, Qamar S, Ali S, Mustafa MAT, Nusrat A, Ghauri SK. 2021. Histological stains in the past, present, and future. *Cureus* **13:** e18486. doi:10.7759/cureus.18486

Jonescu CN. 1909. Vergleichende Untersuchungen über das Gehirn der Honigbiene. *Z Naturwiss* **45:** 111–180.

Kenyon FC. 1896. The brain of the bee - a preliminary contribution to the morphology of the nervous system of the Arthropoda. *J Comp Neurol* **6:** 133–210. doi:10.1002/cne.910060302

Komischke B, Sandoz JC, Malun D, Giurfa M. 2005. Partial unilateral lesions of the mushroom bodies affect olfactory learning in honeybees *Apis mellifera* L. *Eur J Neurosci* **21:** 477–485. doi:10.1111/j.1460-9568.2005.03879.x

Krofczik S, Khojasteh U, de Ibarra NH, Menzel R. 2008. Adaptation of microglomerular complexes in the honeybee mushroom body lip to manipulations of behavioral maturation and sensory experience. *Dev Neurobiol* **68:** 1007–1017. doi:10.1002/dneu.20640

Kuwabara T, Kohno H, Hatakeyama M, Kubo T. 2023. Evolutionary dynamics of mushroom body Kenyon cell types in hymenopteran brains from multifunctional type to functionally specialized types. *Sci Adv* **9:** eadd4201. doi:10.1126/sciadv.add4201

Leydig F. 1864. *Vom Bau des thierischen Körpers: Tafeln zur vergleichenden Anatomie*. H. Laupp, Tübingen.

Maeterlinck M. 1901. *The life of the bee* (trans. Sutro A). Dodd, Mead, New York.

Maleszka J, Barron AB, Helliwell PG, Maleszka R. 2009. Effect of age, behaviour and social environment on honey bee brain plasticity. *J Comp Physiol A* **195:** 733–740. doi:10.1007/s00359-009-0449-0

Malun D, Giurfa M, Galizia CG, Plath N, Brandt R, Gerber B, Eisermann B. 2002. Hydroxyurea-induced partial mushroom body ablation does not affect acquisition and retention of olfactory differential conditioning in honeybees. *J Neurobiol* **53:** 343–360. doi:10.1002/neu.10119

Menzel R. 2012. The honeybee as a model for understanding the basis of cognition. *Nat Rev Neurosci* **13:** 758–768. doi:10.1038/nrn3357

Menzel R. 2014. The insect mushroom body, an experience-dependent recoding device. *J Physiol* **108:** 84–95. doi:10.1016/j.jphysparis.2014.07.004

Menzel R, Brembs B, Giurfa M. 2007. Cognition in invertebrates. In *Evolution of nervous systems, vol II: evolution of nervous systems in invertebrates* (ed. Kaas JH), pp. 403–422. Academic, Oxford.

Oya S, Kohno H, Kainoh Y, Ono M, Kubo T. 2017. Increased complexity of mushroom body Kenyon cell subtypes in the brain is associated with behavioral evolution in hymenopteran insects. *Sci Rep* **7:** 13785. doi:10.1038/s41598-017-14174-6

Peters RS, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, Kozlov A, Podsiadlowski L, Petersen M, Lanfear R, et al. 2017. Evolutionary history of the hymenoptera. *Curr Biol* **27:** 1013–1018. doi:10.1016/j.cub.2017.01.027

Rabl-Rückhard. 1875. Studien ueber Insektengehirne. *Arch Anat Physiol Wiss Med* 480–499.

Richter S, Loesel R, Purschke G, Schmidt-Rhaesa A, Scholtz G, Stach T, Vogt L, Wanninger A, Brenneis G, Döring C, et al. 2010. Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Front Zool* **7:** 29–36. doi:10.1186/1742-9994-7-29

Romanes GJ. 1883. *Animal intelligence*. D. Appleton, New York.

Rybak J. 2013. Exploring brain connectivity in insect model systems of learning and memory. In *Invertebrate learning and memory. Handbooks of behavioral neuroscience* (ed. Menzel R, Benjamin P), pp. 26–40. Academic, San Diego.

Rybak J, Menzel R. 1993. Anatomy of the mushroom bodies in the honey bee brain: the neuronal connections of the α-lobe. *J Comp Neurol* **334:** 444–465. doi:10.1002/cne.903340309

Sabour D, Schöler HR. 2012. Reprogramming and the mammalian germline: the Weismann barrier revisited. *Curr Opin Cell Biol* **24:** 716–723. doi:10.1016/j.ceb.2012.08.006

Sandoz J-C, Menzel R. 2001. Side-specificity of olfactory learning in the honeybee: generalization between odors and sides. *Learn Mem* **8:** 286–294. doi:10.1101/lm.41401

Scheffer LK, Xu CS, Januszewski M, Lu Z, Takemura S-y, Hayworth KJ, Huang GB, Shinomiya K, Maitlin-Shepard J, Berg S, et al. 2020. A connectome and analysis of the adult *Drosophila* central brain. *eLife* **9:** e57443. doi:10.7554/eLife.57443

Strausfeld NJ. 2002. Organization of the honey bee mushroom body: representation of the calyx within the vertical and γ lobes. *J Comp Neurol* **450:** 4–33. doi:10.1002/cne.10285

Strausfeld NJ, Meinertzhagen IA. 1998. The insect neuron: types, morphologies, fine structure, and relationship to the architectonics of the insect nervous system. In *Microscopic anatomy of invertebrates* (ed. Harrison F, Locke M), Vol. 11B, pp. 487–538. Wiley, Hoboken, NJ.

Swammerdam SJ. 1737–1738. *Bybel der natuure*. R. Röwer, Leyden, Netherlands.

Ugajin A, Uchiyama H, Miyata T, Sasaki T, Yajima S, Ono M. 2018. Identification and initial characterization of novel neural immediate early genes possibly differentially contributing to foraging-related learning and memory processes in the honeybee. *Insect Mol Biol* **27:** 154–165. doi:10.1111/imb.12355

Vesalius A. 1543. *De Humanis Corporis Fabrica Libri Septem*. J Oporinus, Basel.

von Alten H. 1910. Zur Phylogenie des Hymenopterengehirns. *Z Naturwiss* **46:** 511–590.

Wasmann E. 1905. *Instinkt und Intelligenz im Tierreich: ein kritischer Beitrag zur modernen Tierpsychologie*. Herder.

Wasmann E. 1909. *Die psychischen Fähigkeiten der Ameisen: mit einem Ausblick auf die vergleichende Tierpsychologie*. E. Schweizerbartsche Verlagsbuchhandlung (E. Nägele).

Weismann A. 1892. *Aufsätze über Vererbung und verwandte biologische Fragen*. Gustav Fischer.

White JG, Southgate E, Thomson JN, Brenner S. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* **314:** 1–340. doi:10.1098/rstb.1986.0056

Received January 15, 2024; accepted in revised form March 12, 2024.

Insights from the past: the work of Hans von Alten on the evolution of brain structure, ecological adaptation, and cognition in hymenopteran species

Randolf Menzel and Jürgen Rybak

Learn. Mem. 2024, **31:** a053922
Access the most recent version at doi:10.1101/lm.053922.124