

The honeybee as a model for understanding the basis of cognition

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Abstract | Honeybees contradict the notion that insect behaviour tends to be relatively inflexible and stereotypical. Indeed, they live in colonies and exhibit complex social, navigational and communication behaviours, as well as a relatively rich cognitive repertoire. Because these relatively complex behaviours are controlled by a brain consisting of only 1 million or so neurons, honeybees offer an opportunity to study the relationship between behaviour and cognition in neural networks that are limited in size and complexity. Most recently, the honeybee has been used to model learning and memory formation, highlighting its utility for neuroscience research, in particular for understanding the basis of cognition.

Neuroethological research
Neuroethology recruits its questions and concepts predominantly from field studies that involve observing and analysing an animal's behaviour under natural conditions. An equivalent laboratory model is then devised that is compared and influenced by the results from the field, ensuring that appropriate paradigms are applied

Small brains, like those of insects, are thought to control behaviour by hard-wired neural connections determined by developmental programmes and triggered by external stimuli¹. Such an argument assumes that experience-dependent rewiring of networks during learning is more demanding and thus more 'neuron intensive'. That is, experience-dependent rewiring would require more extensive neuronal networks and larger numbers of neurons than are found in the insect brain. Although this might be the case for some insects, honeybees (*Apis mellifera*) seem to be an exception²⁻⁴.

The honeybee lives in a community whose cooperative actions are highly dependent on the experience of the individual members. Experience inside and outside the community creates memory traces in the bee brain that combine evaluations of temporal and spatial stimuli. This allows bees to develop expectancies about future events and to base their decisions and their communicative behaviour on the expected outcomes. Likewise, they evaluate the messages received from other community members in the context of their own experience. Furthermore, the cognitive dimensions of learning behaviour in bees reach far beyond simple stimulus associations and include learning about stimulus categories, their sequences and combinations, and trends in changing reward values. These memories are embedded in a complex spatial and temporal context and suggest that the honeybee could be used to model a variety of neurobiological concepts.

Indeed, ethological research of honeybee sensory physiology, navigation and communication has a long tradition. There is a rich body of literature dating back

100 years, including seminal work from the laboratory of Karl von Frisch⁵. Several important discoveries in ethology were first made in honeybees, such as visual detection of ultraviolet light, colour vision in an invertebrate, detection of linearly polarized light and communication with a symbolic behavioural routine. These important discoveries — validated by the awarding of the Nobel Prize to Karl von Frisch — have shaped ethology throughout the past century and paved the way for current neuroethological research.

For example, neural recordings from honeybee brains during learning, memory formation and retrieval activities are enabling researchers to investigate the neural correlates underlying these cognitive faculties⁶⁻⁸. Patterns of activity in synaptic ensembles and of single neurons that store components of a particular memory have been identified and characterized. The small size of the bee brain offers the opportunity to trace neural plasticity to specified neural circuits⁹ and to single neurons^{7,10}, an approach that will become even more fruitful as advances in electrophysiological and optophysiological recording technology facilitate more detailed recordings.

Despite this potential for the honeybee as a versatile model for cognitive neuroscience research, its use by the international research community remains limited. The rich behavioural repertoire of the bee that can be examined under laboratory conditions (including regulation of social components) and the accessibility of neural networks in the honeybee brain allow detailed resolution of synaptic circuit activity under behaviourally relevant *in vivo* conditions, options that should be attractive for neuroscientists in general.

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Here, I present examples of the power and current limitations of the honeybee as a model of cognitive neuroscience, focusing on studies that aim to unravel neural correlates of behavioural adaptation and social communication. This discussion is based on detailed knowledge of the structural organization of the bee brain and the learning conditions of the bee in both the laboratory and in the natural environment.

The insect brain

The nervous system of insects is composed of the brain and multiple segmental ganglia of the ventral chord in the thorax and abdomen. The brain processes second or higher order inputs from all sensory organs, and coordinates the behavioural output through descending premotor neurons or interneurons. Although the brain of the honeybee is small (about 0.4 to 0.6 mm³ with about 1 million neurons), it is large both in absolute and relative terms in comparison to other insect species. For example, the brain of common fruitflies (*Drosophila* spp.) is about 30 to 50-times smaller than the honeybee

brain and contains about 100,000 neurons (estimated by using data from REF. 11). Such comparisons are possible with high precision because standard atlases exist for both the *Drosophila*^{11–13} and the bee brain^{14,15}, allowing comparison also between absolute and relative volumes of brain parts.

The major differences relate to the neural organization of the visual system and the mushroom bodies, both of which are much more elaborate in the bee brain (whereas the olfactory system appears to differ less). The paired mushroom bodies are high-order integration centres for all sensory inputs. In honeybees, visual input predominates, and the mushroom bodies (containing more than 300,000 neurons) are many times larger than the mushroom bodies in *Drosophila* that process predominantly olfactory input and contain ~2,500 neurons. In relative terms, the mushroom body in the *Drosophila* brain comprises 2% of the total brain volume and that of the bee 20%. The differences in the size and organization of the mushroom body reflects global differences in brain organization between these two species, which

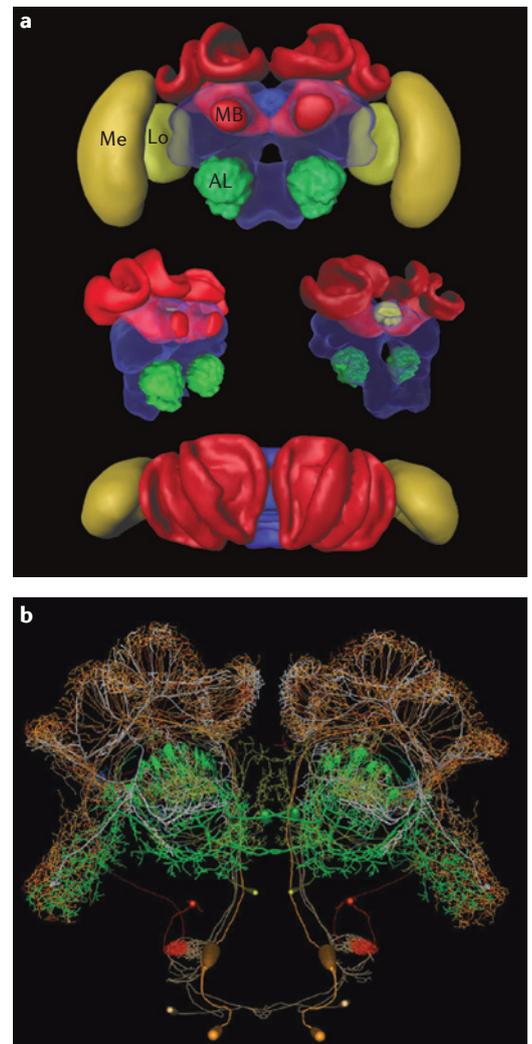
Box 1 | The Honeybee Standard Brain

The Honeybee Standard Brain serves as an interactive tool for relating morphologies of neurons in the bee brain and provides a reference system for functional and bibliographical information organized in an ontology of logical relations^{14,15} (see the [Virtual Atlas of the Honeybee Brain](#) website). The size of the bee brain allows confocal imaging of the whole brain, which is ideal for creating a standard atlas and has already led to a rich and versatile database.

Part **a** of the figure shows the hierarchical organization of surface-based reconstructions of parts of the honeybee brain. Other experimental data (neurons) can be mapped onto the reconstructed surfaces, thus allowing a more complete understanding of the relationship between structure and function. The brain is shown from three sides, from the front, from the side by two oblique views and from above. The brain neuropils are depicted in colour: two visual ganglia in yellow (medulla: Me, lobula: Lo), the two olfactory neuropils, the antennal lobes (AL) in green, the two mushroom bodies (MB) in red, and the unstructured neuropile of the protocerebrum in light blue.

Part **b** of the figure shows examples of several paired neurons that have been registered into the standard atlas of the bee brain. The mushroom body extrinsic neuron PE1 is depicted in green, a mushroom body extrinsic neuron of the protocerebral calycal tract in white, a projection neuron of the median antenno-cerebralis tract in brown, and a projection neuron of the lateral antenno-cerebralis tract in red.

The currently developed ontology tools will play a central role in integrating data from multiple sources (for example, electrophysiology, imaging, immunocytochemistry and molecular biology). Ultimately, this will allow the user to specify a certain cell type in order to retrieve morphologies along with physiological characterizations. The anatomy of neural circuits can be composed from selected neurons registered into the Honeybee Standard Bee with a precision close to 3 µm, but higher resolutions can be reached with dual and triple staining⁷. Part **a** is reproduced, with permission, from REF. 93 © (2007) Cold Spring Harbor. Part **b** of the figure shows image courtesy of J. Rybak, Max Planck Institute for Chemical Ecology, Germany.



lead to richer crosstalk between sensory inputs and more centralized processing of higher order functions in the honeybee.

The digital three-dimensional standard atlas of the bee brain (BOX 1) provides a useful reference for identifying and classifying neurons, as well as for determining their contribution to neural networks. So far, about 40 individually identified neurons have been registered in the atlas, which were identified using intracellular recording and dye injection. Although this is a small number compared with the many neurons identified and sketched in camera lucida pictures over the past 40 years, the neurons registered in the atlas have been characterized spatially with high precision. Future high-resolution digital neuroanatomy research will continue to revolutionize our understanding of neural structures, particularly in the brains of insects.

The limited number of neurons in the insect brain allows the analyses of behavioural control and neural functions to be carried out at the level of the single neuron. This research strategy has been successfully applied in *Drosophila* using optophysiology techniques combined with molecular genetics techniques^{16–18}, and in the bee brain using intracellular recording and electrical and pharmacological manipulation. Indeed, single neurons have been shown to be sufficient for a cognitive function. For example, the octopaminergic ventral unpaired neuron 1 of the maxillary neuromere (VUMmx1) (FIG. 1) in honeybees uniquely mediates the reward value during reward learning⁶.

The dendrites of VUMmx1 arborize bilaterally into regions that are involved in the processing of olfactory information: antennal lobes, lip region of the mushroom bodies and lateral protocerebral lobes. VUMmx1 responds to sucrose stimulation, the rewarding stimulus in olfactory learning, and an intracellular stimulation of VUMmx1 replaces the reward during olfactory conditioning. Interestingly, VUMmx1 ‘learns’ about the odour and fails to respond to the reward if it follows the learned odour, whereas it does respond to an unpredicted reward¹⁹. Thus, the prediction error is coded in VUMmx1 and resembles properties of dopamine neurons in the mammalian ventral tegmentum²⁰. The multiple convergence sites of VUMmx1 with the olfactory pathway suggest that there are multiple sites of olfactory memory formation in honeybees, and indeed memory traces were found in the antennal lobe and the mushroom body (see below for more detail).

Learning and memory

Insects are traditionally used as models for the study of elemental forms of associative learning. In classical conditioning, an animal learns to associate an originally neutral stimulus (conditioned stimulus) with a biologically relevant stimulus (unconditioned stimulus). In operant conditioning, they evaluate their own behaviour with respect to the outcome of this behaviour. However, learning in honeybees extends beyond such elemental forms of learning.

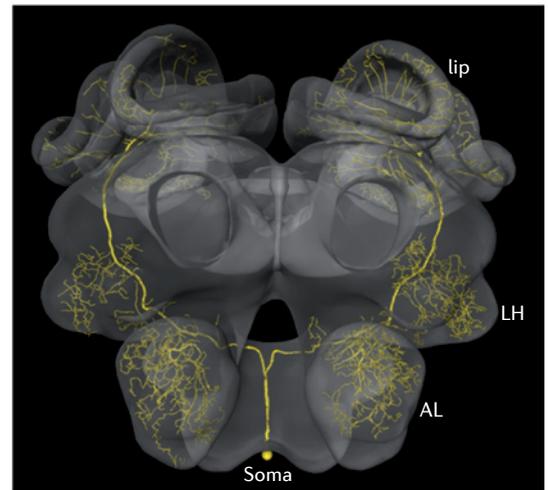


Figure 1 | Projection pattern of VUMmx1, which encodes reward in olfactory learning. The ventral unpaired median neuron 1 of the maxillary neuromere (VUMmx1), shown in yellow in the figure, is an ascending interneuron that responds to sucrose stimulation. VUMmx1 branches symmetrically into each half of the protocerebrum and converges with the three main neuropils of the olfactory pathway: the antennal lobes (AL), the lateral horns (LH) and the lip regions of the mushroom body calyces (lip). This neuron has been shown to be sufficient to support olfactory reward learning in the bee brain⁶. Similar to dopamine neurons in the ventral tegmentum of the mammalian brain, VUMmx1 codes for a prediction error: it decreases its response to an expected reward but increases its response to an unexpected reward²⁰. Figure is modified, with permission, from REF. 14 © (2005) Wiley.

It should be noted that because the life of an individual bee is relatively short but that of the colony is potentially unlimited, the individual animal cannot therefore be innately programmed for particular stimulus conditions that characterize feeding places, the place of the colony and potential new nest sites. Both observational (latent) learning and associative learning are highly developed in bees, surpassing what is known for other insect species²¹ (BOX 2).

Cognitive aspects of learning. Learning of visual, olfactory, gustatory and mechanosensory cues at a feeding site is a fast process in foraging bees. Foragers group visual patterns into categories (‘generalizing’) that do not necessarily resemble natural patterns²², indicating that visual perception and learning is not constrained by sensory filters but is characterized by general sensory coding strategies²¹. Delayed matching (or non-matching) to a sample of visual stimuli supports generalizing not only between different visual targets (for example, colours or patterns) but also across sensory modalities (for example, visual or olfactory)²³, thereby emphasizing the *trans*-sensory integration in the brain. The concept of symmetry (or non-symmetry) is learned, and a reversal of this rule is mastered soon afterwards²⁴, indicating that retrieval and abstraction of extracted sensory components is a cognitive ability available to the bee.

Camera lucida

An optical device that enables drawings of three-dimensional structures from two-dimensional images.

Octopaminergic

Neurons containing octopamine as the transmitter. Octopamine is closely related to noradrenaline.

Box 2 | Strengths and limitations of invertebrate model systems used in behavioural neuroscience

This table attempts to categorize the usefulness of invertebrate species in behavioural neuroscience with an emphasis on studies in learning and memory, and is based on qualitative assessment of the literature reviewed in REF. 21. The relative strengths of each species (rows) is judged on the basis of experimental accessibility and relates to the level of cognitive complexity as accessible in laboratory and field studies. Three different levels are distinguished: high and low usefulness, and no contribution. Definitions of the different technical approaches are outlined below.

Neuroanatomy. This refers to the complexity and accessibility of the nervous system of each animal species. If a connectome of the network as documented in virtual three-dimensional brain atlases is available, this will be reflected in a higher (that is, stronger) rating.

Biochemistry. This refers to the availability of data on intracellular signalling cascades underlying synaptic plasticity.

Molecular biology. The criteria used here are based on methods that allow manipulation of the signalling pathways that are involved in neural substrates of learning memory.

Electrophysiology. The accessibility of neurons to enable intracellular recordings and individual identification involved in processes of neural plasticity underlying learning and memory is taken as a criterion here.

Optophysiology. This refers to the possibility of tracing behavioural components underlying temporal and spatial patterns of neural activity by functional imaging of identified neural circuits under experimental conditions in which such behavioural components are performed.

Modelling of cellular pathways. This refers to the potential of modelling cellular pathways for quantifying and predicting the working of functional neural circuits.

Non-associative learning. The criterion here is the availability of laboratory tests of non-associative plasticity (such as habituation, sensitization and their neural correlates of synaptic depression and facilitation) that provide tools of relating cellular and neural network properties to elemental components of behavioural plasticity.

Associative learning. This refers to the availability of laboratory tests of classical conditioning that offer the potential to trace essential components of associative learning to neural processes. The repertoire and the robustness of these behavioural paradigms under laboratory conditions are used as an important component in selecting the respective species for model studies.

Operant learning. The availability of operant forms of learning (another form of associative learning) under laboratory conditions is used as a criterion here.

Natural learning. This refers to the richness of learning under natural conditions (for example, latent learning, observational learning, learning in the social context, learning during exploration and play) of the respective animal species. The availability of such studies and the potential to transfer essential components into the laboratory has been taken as an additional criterion for estimating the usefulness of the respective species as a model system.

Modelling at the systems level. This refers to the analysis of neural processes across levels of integration, which requires both bottom-up and top-down approaches. The coordination of these approaches is used as an additional argument that provides the potential of modelling lower level processes in such a way that higher level functions may be predicted.

Common name	Species	Neuroanatomy	Biochemistry	Molecular biology	Electrophysiology	Optophysiology	Modelling of cellular pathways	Non-associative learning	Associative learning	Operant learning	Natural learning	Modelling at the systems level
Nematoda												
Eelworm	<i>Caenorhabditis elegans</i>	High	Low	High	Low	Low	High	High	None	None	None	None
Mollusca												
Sea hare	<i>Aplysia californica</i>	Low	High	High	High	None	High	High	Low	Low	None	Low
Opalescent sea slug	<i>Hermisenda crassicornis</i>	Low	Low	Low	High	None	Low	Low	None	None	None	None
Freshwater snail	<i>Lymnea stagnalis</i>	High	High	Low	High	None	Low	High	High	Low	None	Low
Land slug	<i>Limax maximus</i>	Low	Low	Low	High	Low	Low	Low	High	Low	None	None
Octopus	<i>Octopus vulgaris</i>	Low	Low	None	Low	None	Low	None	Low	High	High	Low
Arthropoda												
Cockroach	<i>Periplaneta americana</i>	Low	Low	None	Low	None	Low	Low	High	Low	None	Low
Locust	<i>Locusta migratoria</i>	High	None	None	High	None	High	None	Low	None	None	None
Cricket	<i>Gryllus bimaculatus</i>	Low	None	None	High	None	None	Low	High	High	None	None
Hawkmoth	<i>Manduca sexta</i>	High	Low	None	High	None	Low	None	Low	Low	None	None
Fruitfly	<i>Drosophila melanogaster</i>	High	High	High	Low	High	High	Low	High	High	Low	Low
Honeybee	<i>Apis mellifera</i>	High	High	Low	High	High	None	Low	High	High	High	Low
Arthropoda												
Mud-flat crab	<i>Chasmognathus granulata</i>	None	Low	None	None	None	Low	Low	Low	High	High	None

See REF. 21 for more information on the usefulness of invertebrate models for the study of cognition.

Summer bees

Bees that emerge in spring and summer, live for 4–6 weeks and die before the colony prepares for the winter cluster.

Winter bees

Bees that emerge in autumn and survive by forming a winter cluster that keeps the temperature well above 30 °C inside the cluster. These are the bees that start foraging in early spring time.

The expression of learned behaviour in bees is dependent on context; for example, social conditions, time of the day, location and on which stimuli characterize the target. This allows for the possibility that bees store the what, where and when of memory items as a compound (that is, combined and integrated) memory²⁵, a property that is well documented in birds and mammals²⁶. However, it is unknown whether it is justified to assume that honeybees are capable of planning their actions according to the what, where and when categories of memory. This is because in all experiments conducted so far that assess the cognitive aspects of learning in bees, the sensory cues controlling behaviour were available to the animal.

For example, the flight path along sequentially experienced landmarks guide the way through simple²⁷ and complex mazes²⁸, which allow the bees to perform right or left decision-making actions according to the patterns of visual stimuli experienced at the respective location. Such decisions can be made to be dependent on the number of sequentially experienced stimuli, leading to an estimate of three to four learned sequential items²⁹.

This property of bees has been interpreted as a form of numerosity, a simple form of counting that has not been described for any other invertebrate so far.

Working memory. The size and duration of working memory is considered to be an essential component of intelligence both in animals and in humans^{30,31}. As intelligence correlates to some extent with brain size³² it is particularly interesting to ask whether bees possess working memory for making decisions according to past experience.

In test procedures developed for laboratory mammals but applied to bees, short-term working memory (in the seconds range) was observed in maze learning²⁸ and in matching-to-sample tests³³. Longer term working memory (in the range of several minutes) was reported in tests in which the quantitative reward conditions were made contingent on the animals' own behaviour³⁴. Memory in the hour and day range was found in tests that involved learning of incentive gradients³⁵ and navigational tasks (see below). Lastly, bees are able to retrieve consolidated, remote memories successfully over their lifetime (a matter of weeks in summer bees, or months in winter bees)³⁶.

Box 3 | Proboscis extension response conditioning



Associative learning and memory formation in honeybees can be studied in the laboratory with a robust paradigm: the reward conditioning of the proboscis extension response^{81–84}. Harnessed (see the figure) bees learn to associate a conditioned stimulus (which can be an odour, a mechanical stimulus applied to the antennae or a change in CO₂ concentration, humidity, temperature and so on) with a rewarding unconditioned sucrose stimulus presented a few seconds afterwards. Hungry bees extend the proboscis (tongue) when the sucrose receptors on the antennae are stimulated.

After conditioning, bees respond with proboscis extension to the conditioned stimulus but not to control stimuli, which are not followed by the unconditioned stimulus. As in most forms of Pavlovian classical conditioning of other animal species, the optimal time interval between onset of the conditioned stimulus and the unconditioned stimulus in bees is 2–4 seconds. Associative learning under these conditions is fast (within a few trials) and robust, as demonstrated by high retention scores over several days. Multiple conditioning procedures have been tested²¹, including trial spacing effects, second-order conditioning, conditioned inhibition, extinction learning and spontaneous recovery from extinction, compound processing, occasion setting and others. They each demonstrate that associative learning under these laboratory conditions resembles well-established forms of classical conditioning in mammals. Neural activity in single or multiple neurons measured by Ca²⁺ imaging, or intracellular or extracellular recordings enables the neural correlates of multiple forms of associative learning to be determined. Such an approach has been successfully applied in the past (for examples, see the main text: 'In search of the engram' and 'Memory encoding in the mushroom body') and is likely to yield further insights of the neurological basis of learning and memory in honeybees in the future.

Neural and cellular correlates of learning and memory. It is generally agreed in neuroscience that learning leaves physical memory traces as changes in neural activity and communication between neurons. Neurons identified on the basis of structure (morphology or molecular markers) and well-characterized neural circuits should allow localization of these changes and therefore help to uncover their spatial and temporal pattern of learning-related neural changes. Ideally, such attempts should be combined with online (simultaneous) monitoring of behavioural changes.

The search for neural and cellular correlates of learning and memory in honeybees has been facilitated by the use of the classical insect model of reward: the proboscis extension response paradigm, responses of harnessed bees to olfactory stimuli are measured^{37,38} (BOX 3), and four stages of memory formation following the acquisition process (besides a sensory memory in the seconds range) have been distinguished: short-term memory, middle-term memory and two types of long-term memory. The four stages of memory formation are dependent on the respective time courses controlling behaviour, the sensitivity of the bees to procedures that induce retrograde amnesia (such as cooling, mild electric shocks, anaesthesia or pharmacological treatments), and the molecular pathways involved²⁵. In this way, memory formation in bees resembles the general structure of memory dynamics in other invertebrates and mammals¹⁶ (BOX 2; FIG. 2).

Consolidation of middle-term memory from short-term memory requires ongoing neural activity in the minute range and activation of protein kinase M (PKM), which is mediated by the proteolytic cleavage of protein kinase C (PKC) in the hour range³⁹. By contrast, consolidation of short-term to long-term memory requires

Zinc finger nuclease technology

Zinc finger nucleases are synthetic enzymes engineered to break and modify genomic DNA at any selected sites.

The zinc finger nuclease or its mRNA is transported into the cell by transfection or by direct injection in embryos.

the activity the cyclic AMP-dependent protein kinase A (PKA) in the antennal lobes⁴⁰. Long-term memories can be further divided into translation-dependent early long-term memory (1–2 days after conditioning) and translation-dependent and transcription-dependent late long-term memory (>3 days after conditioning), which can occur in parallel⁴¹. Interfering with the PKC system in the antennal lobe impairs middle-term memory without disturbing long-term memory³⁹, which indicates that the formation of middle-term memory occurs in parallel to the consolidation processes that convert short-term to long-term memory.

Both the antennal lobes and the mushroom body, two neuropils in which the olfactory and the reward pathways converge, have specific roles in the formation of these memory stages⁴². For example, formation of early long-term memory but not late long-term memory requires glutamatergic neurotransmission in the mushroom body^{43,44}. In the course of specific olfactory long-term memory formation, transcription-dependent processes lead to an increase of the density of synaptic aggregates (microglomeruli) in the lip region of the mushroom body, whereas the volume of the neuropil remains constant⁴⁵.

Recently, the effects of post-translational protein modifications (mostly acetylation of histones) and of

DNA methylation on learning and memory consolidation has attracted considerable interest in mammals⁴⁶. The honeybee (but not *Drosophila*) uses a conserved family of enzymes (DNA methyltransferases) to mark their genes with methyl tags. This process is similar to what occurs in mammals, which makes the bee highly suitable for study by researchers searching for DNA modifications during ontogenetic development (for example, programming the development of a queen⁴⁷) and neural plasticity⁴⁸.

The honeybee genome sequence⁴⁹ provides an outstanding opportunity for studying the molecular mechanisms of honeybee cognition. A major obstacle to overcome, however, is the absence of techniques to specifically interfere with gene expression and protein function in bees. Although antisense and RNAi techniques^{50,51} have been successfully applied in bees, so far these manipulations do not allow the modification of spatial and temporal features of the cellular processes involved in honeybee cognition with the resolution and specification necessary for meaningful conclusions to be drawn.

The generation of transgenic bees might overcome this problem. All the key steps for the generation of transgenic animals are established. Embryos can be injected and reared in the laboratory until the emergence of adults⁵². Techniques that allow the stable integration of foreign DNA into the genome, such as viral-based expression systems or the zinc finger nuclease technology, are emerging. Thus, it is theoretically possible to generate transgenic honeybee queens and transgenic offspring. Unfortunately, however, bee colonies that are removed from their natural environment and housed in large cages do not survive for more than a few months. The most interesting behavioural experiments can be carried out only with free-flying bees that are kept in their natural environment. Thus, it is likely that transgenic animals will not be suitable models for examining the most complex aspect of the bee's cognitive capacity because the risk that they would spread into the environment and mix with native bee populations is too high.

In search of the engram

An engram imaged. Learning leads to a memory engram, which is a representation of stored knowledge that can be retrieved for mental operations and behavioural control. Together with the original ideas of Ramon y Cajal^{53,54}, contemporary neuroscience considers the engram to be stored in the lasting changes in synaptic strength at specific locations within the neural circuits that are involved in all aspects of perception, integration, mental processing and motor control.

Ideally, one would like to elucidate the whole engram during memory formation and activation, which requires resolving activity simultaneously within multiple brain structures with subcellular resolution. This might seem a demanding task for higher organisms, but it is not out of reach for insect brains. Such an approach would allow the monitoring of activity over time, thereby revealing differences in neural excitation patterns before, during and after learning.

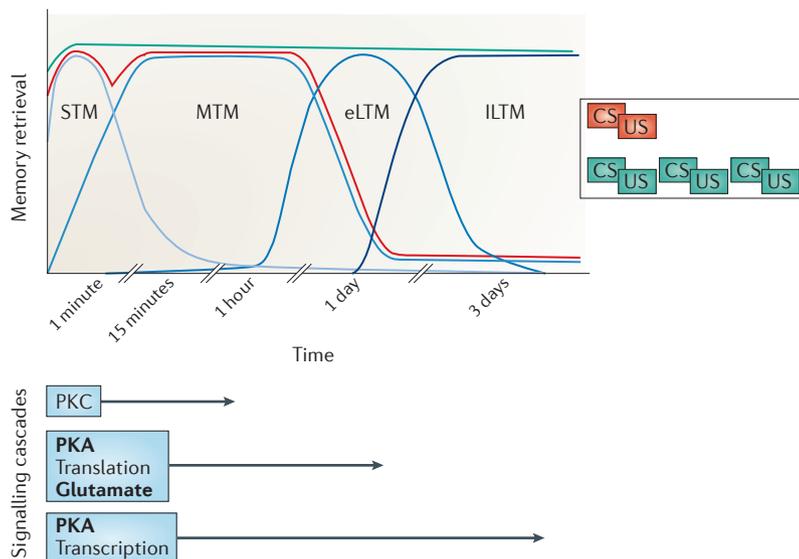


Figure 2 | Memory formation is a multi-phase process in honeybees. In proboscis extension response (PER) olfactory conditioning, a single learning trial leads to short-term (STM) and middle-term (MTM) memory, and multiple learning trials lead to early long-term (eLTM) and late long-term memory (ILTM). Nicotinic acetylcholine receptors in the olfactory pathway are activated by an odorant (the conditioned stimulus (CS)). Shortly afterwards metabotropic octopamine receptors in postsynaptic neurons of the reward pathway — the ventral unpaired median neuron 1 of the maxillary neuromere (VUMmx1) (FIG. 1) — are activated by a sucrose reward, which leads to the initiation of signalling pathways as indicated in the lower part of the panel. The transition from STM to MTM depends on the formation of constitutively activated protein kinase C (PKC). A parallel molecular signalling pathway during STM requires prolonged activation of protein kinase A (PKA), a process that depends also on NMDA receptors and leads to translation-dependent eLTM. Transcription-dependent ILTM may be initiated either consecutively or in parallel to eLTM^{39–41,43,91}. Figure is modified, with permission, from REF. 38 © (2002) Elsevier.

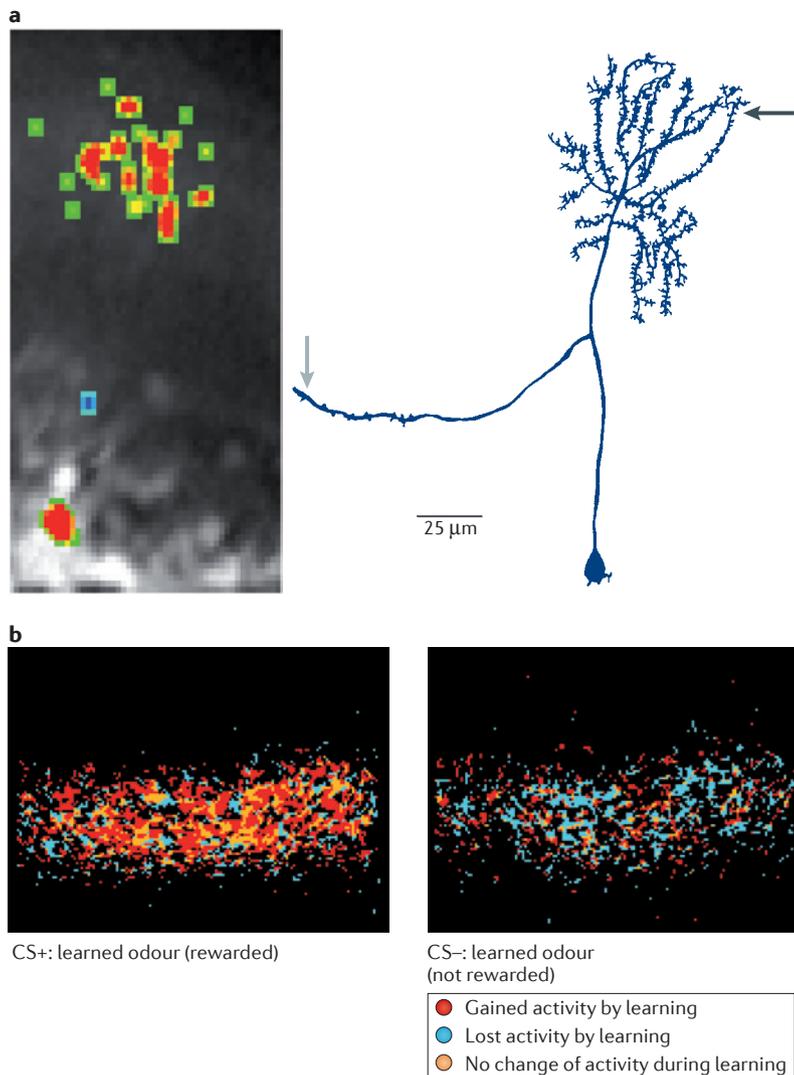


Figure 3 | Visualizing the engram of learned odours at the input side of the mushroom body. **a** | The mushroom body contains a large number of densely packed intrinsic neurons. A single intrinsic neuron (a Kenyon cell type K II with dendrites in the collar of the mushroom body), revealed using Golgi staining, is shown on the right of the panel and consists of spiny dendrites (black arrow), soma and two axon collaterals. A pseudocolour image of an intrinsic neuron filled with the Ca^{2+} indicator dye Fura 2 via its collateral projecting to the alpha lobe (grey arrow) is shown on the left. Ca^{2+} activity is enhanced in the dendrites and the soma when the animal is stimulated with an odour (red and orange colouration represents higher Ca^{2+} concentrations). **b** | Ca^{2+} activity in the spiny dendrites' many intrinsic neurons in the lip region of the mushroom body was imaged during odour learning. The bee was trained to an odour (conditioned stimulus; CS+) by presenting the odour shortly before the sucrose reward, and to a different odour without reward (CS-). Pseudocolour images of the changes in Ca^{2+} activity during learning of CS+ and CS- are shown (Fura 2 protocol as in panel a, with areas of higher Ca^{2+} concentrations represented by red and orange colours). CS+ responses after learning are dominated by gained activity and CS- responses by lost activity, but opposite changes are also found for both learned odours. Calculated from data reported in REF. 56. The right panel of **a** is modified, with permission, from REF. 92 © (1982) The Royal Society. Part **b** is modified from REF. 56.

exciting aspect of this approach lies in the fact that it reaches beyond correlations between neural events and behavioural changes and allows for interpretation on the causal level. Although such an approach is not yet possible for the honeybee (see above), a first glimpse into a limited part of the engram for olfactory learning has been obtained by imaging the Ca^{2+} activity of dendrites in neurons of the lip region of the mushroom body, a central structure of the insect brain known to be involved in memory storage^{9,56} (FIG. 3).

During the learning phase, in which bees are trained to associate a particular odour as a conditioning stimulus with a reward (unconditioned stimulus), more of the responding intrinsic neurons in the mushroom body exhibited an increase of activity in dendritic postsynaptic sites, including spines. By contrast, when the bees are exposed to an odour that was not accompanied by a reward most of these structures exhibited a decrease in activity. It should be noted, however, that opposite changes also occur for both learned odours, and many postsynaptic structures stayed unchanged. As expected, the small part of the engram imaged for even a rather simple odour discrimination task involves extensive changes in synaptic transmission and synaptic strength. As we can record whether the animals have learned the odours and have successfully retrieved the memory of them, improved methods will allow us to access the formation and use of the engram.

Memory encoding in the mushroom body. The 150,000 or so mushroom body intrinsic neurons synapse onto a smaller number of extrinsic neurons⁵⁷ (a few hundred), and it is at these synapses that the value of the learned stimulus combinations (rather than the stimuli themselves) is encoded. One of the extrinsic mushroom body neurons, the PE1 neuron, has been shown to specifically reduce its response under rewarding conditions⁷. Other extrinsic neurons are recruited to represent the rewarding stimuli, and those that are not lose their responsiveness^{8,58}. In all cases, the range of multi-sensory responses that are characteristic for the respective neuron is broad and rather unspecific before learning and becomes more specific after learning.

The mushroom body as a whole seems to act as a re-coding device, converting sensory information to value-based information. In this respect the mushroom body shares properties with the mammalian hippocampus⁵⁹⁻⁶¹ and prefrontal cortex^{62,63}. A summary of our current picture of the distributed memory trace with its multiple aspects is shown in FIG. 4. Primary and higher order integration centres work together on the network level to orchestrate a memory trace that develops over time, enhances learned stimuli, couples retrieval on actual context conditions, creates specific expectations and evaluates the current conditions with the expected ones.

Both forward and recurrent loops of neural connections distribute the memory trace, and descending premotor commands originate from multiple levels of integration. As in any other neural system we do not

In *Drosophila*, combinations of targeted expression of activity-indicating dyes and light-sensitive ion channels in selected neurons are already used as tools to monitor and manipulate neural activity that constitute traces of the engram during its formation^{16,18,55}. The

yet understand the logic of the multiple-faceted coding and storing schemes that comprise the memory trace, but again the limited number and the unique structures of these neurons in the honeybee brain offers an opportunity to gain insight into the value-based engram in insects.

Collective cognition in the social context

The individual bee and the community. In a honeybee colony, up to 60,000 workers (daughters of one queen) coordinate multiple tasks, adapting to changing environmental conditions of the colony. Allocation of tasks to the individual worker is an emergent property that arises from genetic dispositions, highly plastic age-dependent developmental programs, epigenetic regulation, and pheromone-dependent processes and learning. Ageing in bees is accompanied by cognitive decline and reduced resilience to stress⁶⁴. Cognitive decline and reduced resilience to stress are reversed under certain social conditions; for example, when the colony divides by

swarming or when either foragers or young nurse bees are removed from the colony. Each of these circumstances causes old bees to become cognitively young again and younger bees exhibit a faster age-related cognitive decline.

Interestingly, cognitive decline is lacking in winter bees when they live for nearly a year without foraging, and indeed foraging is one action apart from social signals that leads to ageing. Reversal of age-dependent cognitive functions by social signals is a unique and exciting property of the honeybee colony, and eight related proteins have been identified that are highly expressed in the central brain are upregulated during these processes⁶⁵. It will be exciting to search for the role of these proteins and their related genes in the mammalian brain.

DNA methylation may also be involved in social regulation of cognitive age, as methylation sites correlate more closely with a task (for example, nursing or foraging) than age⁶⁶. As epigenetic mechanisms in bees are mechanistically similar to those of mammals (see above), these findings are particularly interesting from a comparative point of view.

Through her pheromones, the queen manipulates the reproductive status of her daughters and the value-coding dopaminergic system of young workers to keep them involved in their duties as caretakers for the larvae and the colony as a whole⁶⁷. Foraging bees may switch between food collection and information collection. This becomes particularly important when a colony starves or prepares for swarming. A subset of food foragers act as scouts (information foragers that search for new feeding grounds or a new nest)^{68,69}. Social decision-making processes, for example, in bee swarms, have been compared with an oligarchic ruling system in which a few informed members, the scouts, decide upon a new nest site by sensing the majority of votes⁷⁰. On an elementary basis the processes involved in the social decision-making of a bee colony have been modelled based on an analogy to brain processes potentially involved in neural decision-making^{71,72}.

The attractiveness of such models has gained considerable value by the recent discovery of cross-inhibitory signals between scouts voting for different goals⁷³. Such cross inhibition parallels the mutual inhibition between integrating neurons, leading to a decision process between close options. The interplay between genetic predisposition, epigenetic regulation, experience and social signals offer an exciting opportunity to study how an individual's experience determines the neural conditions that influence a socially controlled novel behaviour⁷⁴.

The waggle dance and navigation. Communication between foragers about locations in the explored environment involves ritualized movements, the so-called waggle dance⁵ (BOX 4), which encodes distance, direction, qualitative and quantitative aspects of the goal. This symbolic form of communication adapts to the needs of the colony and is influenced by the responses of the receiving bees (the recruits).

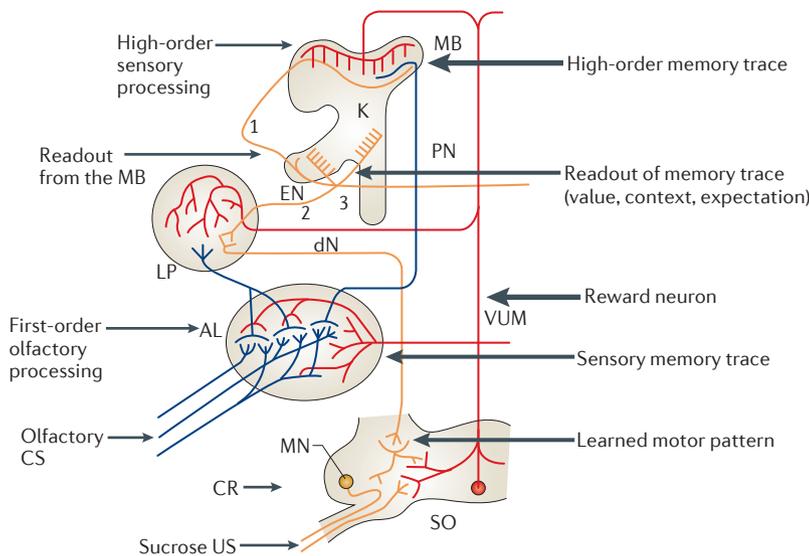
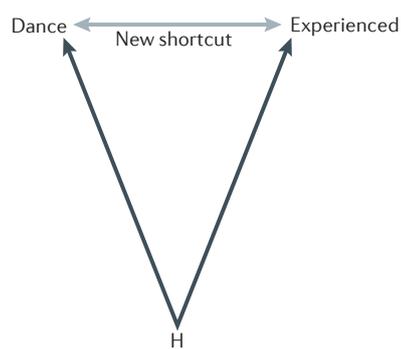
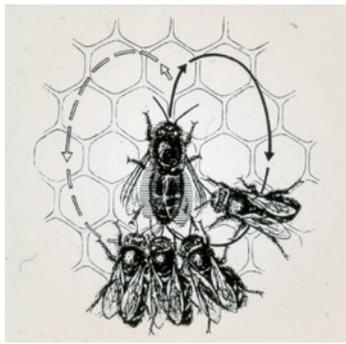


Figure 4 | Brain structures and their connections housing the olfactory memory trace. The diagram shows the major circuitry components of half of the bee brain. All structures are mirror symmetric in the other half of the brain. The olfactory conditioned stimulus (CS) is received by the olfactory receptor neurons in the antennae whose axons (blue lines on the left) project to the first order processing neuropil, antennal lobe (AL). Second-order olfactory interneurons (blue lines in the middle, projection neurons (PN), and above the AL) project to the input site of the mushroom body (MB). The MB consists of >150,000 densely packed intrinsic neurons, the Kenyon cells (K). Extrinsic neurons (EN) of the MB project to multiple sites in the brain, some (label 1) in a recurrent loop to the MB input, some (label 2) to the lateral protocerebrum (LP) and some to the other side of the brain (label 3). Descending interneurons (dN) carry premotor commands to the subesophageal ganglion (SO), whose motor neurons (MN) control the movements of the mouth parts leading to the conditioned response (CR). A single identified neuron, the ventral unpaired median neuron 1 of the maxillary neuromere (VUM, red line) has been identified as a neural structure that is sufficient for the rewarding function in olfactory learning. The different components of the memory trace are tentatively related to the different brain structures (black arrows on the right side). A sensory memory trace is considered to reside in the AL, a high-order memory trace storing the multiplicity of stimulus and context-dependent parameters in the input site of the MB, a value-based categorizing memory trace may control learned expectation under appropriate context conditions, and the descending neurons together with the premotor network of the SO ganglion may store learned motor patterns. US, unconditioned stimulus.

Box 4 | The cognitive map of the honeybee



The neural substrate of navigation is unknown in any invertebrate, but the two main hypotheses or concepts under consideration by those working in the field are the multiple isolated functions concept and the integrated cognitive map concept. In the concept of multiple isolated functions, animals find their way around the environment by applying different and independent strategies according to the particular task to be solved; for example, path integration, image matching and piloting by beacon orientation^{85,86}.

The integrated cognitive map concept assumes an integration of multiple neural processes that lead to a memory structure organized as a geometric map^{87,88}. A cognitive map allows the animal to travel along new shortcuts between locations. Behavioural observations of navigation and communication allow characterization of the cognitive complexity of navigation in the honeybee. Before bees start their foraging activities, they explore the environment learning about the local conditions of the sun compass and the spatial relations between landmarks and their hive⁸⁹. Multiple cues and multiple forms of learning lead to a spatial memory that guides them during extended trips to feeding places.

Honeybees communicate about a location of a food site or a new nest site by a ritualized movement, the waggle dance (left side of the figure), performed in the dark hive on the vertical surface of the comb. Distance as measured visually⁹⁰ is encoded in the speed of the dance rounds, and direction of the outbound flight, as measured relative to the sun compass, is transferred to the angle of the waggle run relative to gravity. Recruits receive the information by closely following the dancing bee. The recruits integrate this information into a spatial memory that allows them to navigate along new shortcuts (grey line in the right side of the figure), a condition that is considered to reflect a form of cognitive map⁸⁷ if certain more simple explanations (such as the use of a beacon at the goal or the structure of the panorama) can be excluded^{76,77}. Bees first learned to forage at the experienced location. When food is no longer available the bees are motivated to follow dancers and receive information about the dance communicated location. In the experiment depicted here, both locations had a distance of 650 m from the hive (H in the figure), and the angle between them as seen from the hive was 30°. New shortcut flights (marked with the grey arrow) were performed between the two locations. These and other findings indicate a common frame of spatial reference for experienced and communicated locations that is best conceptualized as cognitive map⁷⁶.

These findings might help attempts to unravel the neural organization of spatial memory as a substrate for navigation and spatial communication. Indeed, it might be possible to draw meaningful comparisons between the search for representations of geometric structure in the honeybee brain and research into place and head direction cells in the hippocampus of mammals⁷⁹. The figure on the left is reproduced, with permission, from REF. 5 © (1967) The Belknap Press of Harvard University Press. The figure on the right is reproduced, with permission, from REF. 76 © (2011) Cell Press.

The bee dance is a unique communication system about remote spatial locations, and it is not known to exist in any other animal species in such an advanced form. The communication process involves multiple forms of learning both by the recruited bee and the dancer. The recruited bee learns the odour on the body of the dancer from the indicated food source, and as it may have experienced the same odour during its

foraging the activation of the respective memory will also guide it to the formerly visited place⁷⁵.

Under such conditions the recruit will attend the dance only for a short period collecting less information about the spatial parameters, and leaves the hive for inspection of its former forage. If the dancer does not communicate any odour from the indicated goal and recruits attend the dance for multiple rounds it learns the spatial parameters of the goal, and this message is not just about the flight vector towards the goal but also indicates the dance-communicated place in spatial relation to those places learned by the recruit before. This allows the recruits to make decisions according to their own experience in the environment. The bee will fly either directly to the location indicated by the dance, or its formerly visited place. After arriving there it can take a direct shortcut between these two places: that is, the formerly experienced place and the dance indicated place⁷⁶.

These and additional data^{77,78} are taken as evidence that the multiple navigational mechanisms used by bees are integrated and form a map-like representation of the environment, which allows them to navigate along new shortcuts between multiple locations without having to deploy basic navigational tools such as orientation to a beacon or matching with picture memories (BOX 4). Also dancing bees learn about the importance of their communicated location for the colony by the feedback from the recruits. In the context of a swarm they also attend other dances and may send a stop signal to another dancing bee if it advertises for a different nest site that is considered to be less attractive (see above).

Taken together, the complexity of dance communication and the richness of information transfer proves that even a tiny brain like that of the bee allows for cognitive processes, including extraction of spatial relations, comparison of the values of represented goals and decision-making on representations. As the neural code of space is unknown for any invertebrate, comparison between, for example, the hippocampus of vertebrates with its place, grid and head direction cells⁷⁹ and its interaction with the prefrontal cortex⁸⁰, is not possible yet. It may turn out that these cognitive processes can be acquired by very different neural strategies in the bee. Furthermore, we may have to ask which additional cognitive processes characterize hippocampal–prefrontal cortex function not available to the tiny bee brain, an approach that may require more natural settings of experimentation.

Conclusions

Honeybees are one of several invertebrate models that provide unique opportunities to dissect the molecular, cellular and neural processes that lead to the generation and modification of certain behaviours (BOX 2). Ideally, a model species should possess rich sensory, motor and cognitive faculties, easily accessible neurons and neural circuits for long-lasting recordings under close-to-natural conditions, and a cellular composition that allows for effective molecular and genetic manipulations.

Perhaps, surprisingly, none of the currently used invertebrate or vertebrate model systems combine all

these ideal conditions and it is therefore necessary to study many different model species. This is also rewarding because general rules of neural function — and similarities and differences — can be discovered only by comparison across species, thanks to the evolutionary links between animals. Honeybees are attractive for such a comparative approach because they exhibit relatively complex cognitive functions with a relatively small brain, causing us to reflect on whether the previously identified determinants of cognitive differences in higher organisms (such as number and size of neurons, local and far-ranging circuit connectivity)³² are indeed necessary to explain how the diverse and complex cognitive behaviours described above are achieved by the relatively small brain of the honeybee.

Although the honeybee does not (yet) offer the elegance of molecular genetics that are available in species such as *Caenorhabditis elegans* and *Drosophila*, these

limitations could be overcome by the application of technologies developed for use in other model organisms, such as the design of targeted somatic transfection with viruses combined with expression switches. Furthermore, although electrophysiological studies are currently more difficult in bees than in molluscs, moths and locusts (BOX 2), recordings from selected neurons is likely to become easier with the availability of the three-dimensional virtual brain atlases and devices that guide recording electrodes to preselected stereotaxic coordinates. Moreover, imaging of neural activity in the honeybee brain is likely to be enhanced by the application of multiphoton microscopy combined with somatic transfections of targeted neurons. With these and future technological advances combined with the diverse behavioural and cognitive repertoire of the honeybee, it seems likely that this organism will continue to provide important insights into nervous system function.

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Competing interests statement

The author declares no competing financial interests.

FURTHER INFORMATION

Randolf Menzel's homepage:
<http://www.neurobiologie.fu-berlin.de/menzel/menzel.html>
 The Virtual Atlas of the Honeybee Brain:
<http://www.neurobiologie.fu-berlin.de/beebrain>
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