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Learning, Memory, and Brain Plasticity in Cuttlefish (*Sepia officinalis*)

Ludovic Dickel[*,†]*, Anne-Sophie Darmaillacq*[*,†]*, Christelle Jozet-Alves*[*,†] and
Cécile Bellanger[*,†]

[*]Normandie Univ, UCBN, GMPc, EA 4259, F-14032 Caen, France [†]Centre de Recherches en Environnement Côtier, Station Marine de l'UCBN, 14530 Luc-sur-Mer, France

INTRODUCTION

It is important to remind behavioral neurobiologists and, more widely, experimental psychologists that some animals that are phylogenetically remote from mammals show particularly impressive learning and cognitive capabilities. Along with some other invertebrates, cephalopods are excellent representatives to support this assessment. They are well-known for their fascinating learning capabilities and their well-developed and centralized nervous system, which is arranged around the esophagus like an actual 'brain.' Cephalopod brains are by far the largest of all invertebrate nervous systems in their number of cells (520 million neurons in *Octopus*[1]). This molluskan brain can rival those of many vertebrates, including some bird species, in their brain-to-body weight ratio.[2] This may explain why cephalopods are so fascinating for those interested in the evolution of brain and behavior. They also provide original models for studying neural network bases of learning and memory. Of the 700 cephalopod species and despite the pioneering work on cuttlefish by Sanders and Young,[3] the common octopus (*Octopus vulgaris*) has been the most extensively studied species in this field, beginning in the second half of the 20th century (for review, see Wells[4]). There are various reasons for this. Octopuses are easy to keep under laboratory conditions; they live in a den and promptly catch any object moving near their home, which makes operant learning procedures easier to design. Moreover, octopuses successfully survive brain lesions, which can be carried out with a minimum of general tissue damage and blood loss.

The extraordinary amount of work by Young and collaborators on octopuses was undertaken at the Stazione Zoologica di Napoli (Italy), in the vicinity of which octopuses are abundant. Moreover, this marine station has extensive experience, facilities, and skills in maintaining octopuses under laboratory conditions. As a consequence, brain, learning, and memory capabilities in cuttlefish remained less known during most of the 20th century, despite the remarkable research of Wells[5,6] and Messenger[7−9] on both juveniles and adult animals. In the 1990s, a resurgence of interest for learning in cuttlefish occurred at the Marine Station of Luc-sur-Mer in Chichery's laboratory. It is located on the French coast of the English Channel, where the common cuttlefish (*Sepia officinalis*) comes to reproduce and lay eggs in spring and summer. Originally, the studies conducted in this laboratory followed the pioneering work of Sanders and Young[3] and Messenger.[7,8,10] The former investigated in detail the prey-catching behavior of cuttlefish. *Sepia officinalis* is an active predator, capturing large and very mobile prey such as crabs, fish, and shrimp. The attack is visually guided and consists of a precise sequence of events: (1) prey detection, (2) orientation of the head toward the prey, (3) pursuit of the prey when necessary, (4) positioning with ocular convergence, and (5) prey seizure.[7,11−13] The prey seizure stage can occur in two different ways—by rapidly shooting out the two long tentacles on fish, small crabs, and shrimp (Figure 25.1A)
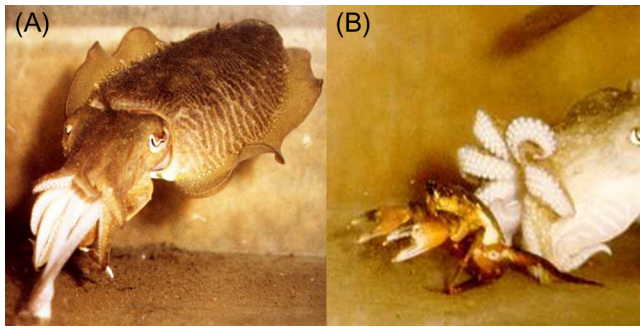
FIGURE 25.1 Prey-catching strategies in cuttlefish: (A) tentacle striking on a prawn and (B) jumping on a crab.
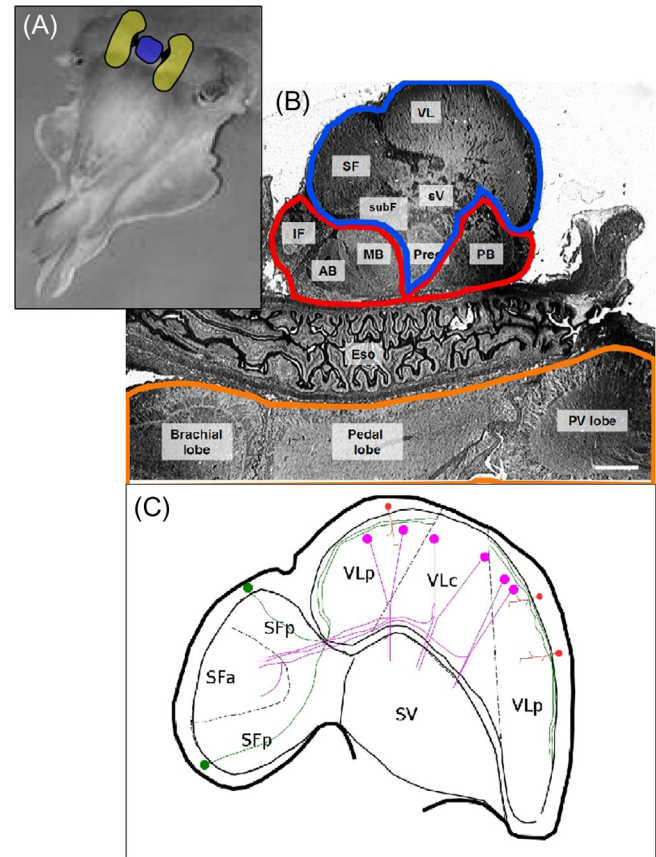

FIGURE 25.2 (A) Front view of a cuttlefish showing the central nervous system: optic lobes (yellow) and central mass (blue). (B) Sagittal section of an adult cuttlefish brain showing the supra- and subesophageal masses. The lower motor centers are surrounded in orange, the intermediate and higher motor centers in red, and the sensory association centers (electrically silent areas) in blue. AB, anterior basal lobe; Eso, esophagus; IF, inferior frontal lobe; MB, median basal lobe; PB, posterior basal lobe; Prec, precommissural lobe; PV lobe, palleovisceral lobe; SF, superior frontal lobe; subF, subfrontal lobe; sV, subvertical lobe; VL, vertical lobe. Scale bar = 450 μm. (C) Diagram of the vertical lobe connections. SFa, anterior part of the superior frontal lobe; SFp, posterior part of the superior frontal lobe; SV, subvertical lobe; VLc, central vertical lobe; VLp, peripheral vertical lobe.

or by jumping on the prey and seizing it with the eight arms (Figure 25.1B).[14] After the prey has been caught, it is manipulated and ingested. These prey-catching behaviors are 'all-or-none' events. They are easily identifiable and quantifiable so that they were advantageously used in assessing learning capabilities in cuttlefish.

Although cuttlefish are very efficient swimmers (with the use of both funnel and fins), they are nectobenthic and spend most of their time on the bottom of their tanks in captivity, hiding in dark shelters when available, digging into the sand, or concealing in their environment. They possess spectacular capabilities in displaying a great variety of neurally controlled body and color patterns that are adapted to the color and the texture of the background (for a review, see Hanlon and Messenger[15]).

In this chapter, we summarize the main data available concerning the anatomical and functional organization of the cuttlefish brain. Then, we review some of different learning procedures that are used in adult and juvenile cuttlefish and, when possible, their neural correlates. Finally, we highlight some of the most promising avenues to follow in the future to better understand the extraordinary behavioral plasticity of these sophisticated invertebrates.

THE CUTTLEFISH BRAIN

The cephalopod central nervous system, lying between the eyes, consists of a central circumesophageal brain connected to two large optic lobes by short optic tracts (Figure 25.2A). The anatomy of the brain of *Sepia* has been described in great detail (for review, see Nixon and Young[16]). Each brain structure, called a lobe, consists of cell layers (cortex) surrounding a neuropil (networks of fibers). Based on the behavioral responses induced by electrical stimulation of different lobes,[17–19] the cuttlefish brain was functionally subdivided into lower, intermediate, and higher motor centers and silent areas, hence considered as sensory association centers involved in the integration of multisensory inputs.

The optic lobes integrate visual inputs from the retina and are involved in the control of motor programs (higher motor centers).[17–19] Moreover, the optic lobes have been suggested to store memory in *Octopus*,[20] particularly in the case of associative learning.

The central mass of the brain (Figure 25.2B) comprises a supra- and a subesophageal mass connected by periesophageal lobes. The sub- and periesophageal lobes, classified as lower and intermediate motor

centers, control swimming, escape behavior, ink ejection, and arms and tentacle movements. The ventral part of the supraesophageal mass comprises some higher centers implicated in the control of motor programs; their electrical stimulation induces combined movements of different groups of effectors.[17]

The vertical lobe (VL) system, also called the VL complex by Young,[21] corresponds to the dorsal part of the central mass. It contains associative structures (electrically 'silent areas'[17]) receiving inputs from different sensory systems and is known to be involved in learning and memory (for reviews, see[22,23]). The VL system includes the precommissural, subfrontal, superior frontal, subvertical, and vertical lobes (Figure 25.2B). Young[21] has described in detail this system in squids, but the general organization is strikingly similar in the cuttlefish. At the front of the VL system, the superior frontal lobe shows two distinct parts: the anterior superior frontal lobe, which receives inputs from the VL, and the posterior superior frontal lobe, which sends all its axons to the VL (Figure 25.2C). Each part is recruited at specific stages of learning.[24] The subvertical lobe is positioned behind the superior frontal lobe and below the VL. This structure receives axons from and sends axons to the VL and the superior frontal lobe. Both the superior frontal lobe and the subvertical lobe are centers of sensory multiconvergence, sending pretreated visual and tactile information to the VL. The VL is located in the dorsal part of the supraesophageal mass. Young[21] described two parts in the VL: the central VL corresponding to the whole middle part of the dome and, surrounding it, the peripheral VL (Figure 25.2C). Ablation or partial lesion of the VL induces learning and long-term memory impairments.[3,25] In the dorsal part of the VL, a large tract of fibers coming from the posterior superior frontal lobe is situated just below the cortex (Figure 25.2C). This VL−superior frontal lobe tract may be involved in the regulation of locomotor activity level and in memory.[25] Small cells scattered throughout the neuropil of the VL send axons ending in the subvertical lobe (Figure 25.2C). These VL−subvertical lobe tracts are the sites of long-term potentiation,[26] and their appearance during development is concomitant with the maturation of short-term memory capabilities in early juveniles.[27] Young[28] described a series of intersected matrices within the VL system in *Octopus* and emphasized the similarity of their organization to that of the hippocampus of vertebrates. He distinguished distinct memory matrices for visual and tactile learning.[29] Despite similar anatomical organization, the VL system of cuttlefish seems to show different functional and neural network properties compared to the VL system of octopuses.[26] Young suggested that if a system of matrices for tactile memory exists in the cuttlefish, it would differ from that of octopuses.[16] Finally, as in *Octopus*, VL damage or removal impairs learning and memory but never completely; this suggests a synergistic involvement of other structures (e.g., optic lobes).

BRAIN AND BEHAVIORAL PLASTICITY IN ADULTS

The 'Prawn in a Tube' Procedure

In behavioral neuroscience, one of the most used learning paradigms in cuttlefish is the 'prawn in a tube' (PT) procedure. In this procedure, prawns are enclosed in a transparent tube (made of clear Perspex or glass) in the middle of the cuttlefish tank (Figure 25.3). Under these conditions, a cuttlefish attempts to catch the unreachable prey by rapidly shooting out its two long tentacles, subsequently referred to as a 'strike.'

The number of strikes decreases with time during a continuous presentation (Figure 25.4) or in the course of eight successive 3-min presentations of the apparatus (with an interval of 30 min between presentations).[8] This learning is apparently very simple and

FIGURE 25.3   **Cuttlefish pointing at prawns enclosed in a glass tube.**
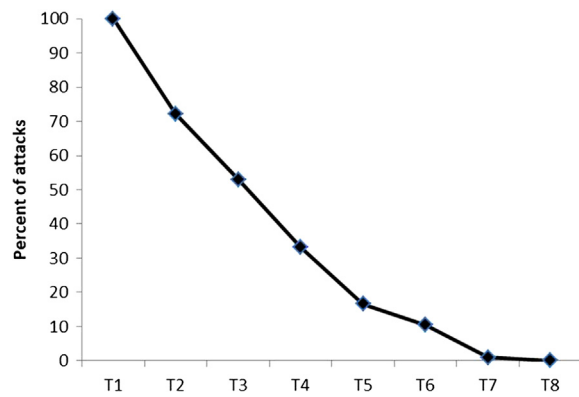
FIGURE 25.4 **Percentage of attacks during the presentation of the glass tube containing shrimp.** The number of attacks was counted by 3-min blocks and then expressed as a percentage of the number of attacks at T1. The training session was stopped when the cuttlefish reached the learning criterion of less than two attacks during a 3-min block.

FIGURE 25.5 Mean number of attacks $\pm$ SEM during the first 3 min (T1) and the last 3 min of the training session (T8) and the first 3 min of the retention test (R1) in the three conditions: Cr−Cr, crabs were used in both training and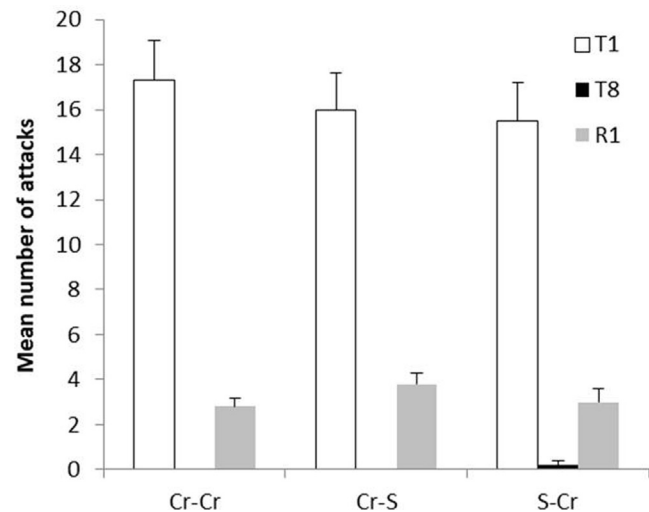 the retention test; Cr−S, crabs were used during training and shrimp during the retention test; and S−Cr, shrimp were used during training and crabs during the retention test.

practical, but to be efficiently used in functional neurobiology, it was of crucial importance to better characterize the nature of this learning and of the reinforcements that are possibly involved as well as the different cues cuttlefish can rely on to solve and memorize the task. As a consequence, the PT procedure has been extensively investigated in several studies, all based on the remarkable set of observations made by Messenger.[8]

PT Procedure: Habituation or Associative Learning?

When cuttlefish's tentacles were cut, the waning of 'pseudo-strikes' during the tube presentation was significantly lower than the waning of tentacle strikes in the non-operated animals placed in the same conditions. Furthermore, when a crab was offered after the learning, it was rapidly captured by the jumping or striking strategy.[8] This suggests that the waning of attempted captures in this learning was not the result of motor fatigue or of temporary incapacity to make tentacle strikes. Agin et al.[30] and Purdy et al.[31] assessed whether the decrease in the PT procedure was better interpreted as habituation or associative learning by testing whether the response could be reinstated through dishabituation. Agin et al. used a flashing light or a free-moving crab as 'dishabituating' stimulus, whereas Purdy et al. used a free-swimming shrimp (these authors used fish in the tube during training). In neither of these works did the authors demonstrate any increase in attempted captures (i.e., any 'dishabituation') on the glass during the PT presentation following the dishabituating stimulus. These works confirmed the original hypothesis of Sanders and Young[3] and Messenger,[8] according to which this

learning was considered to be associative, with each rapid shot of the tentacles (occurring in less than 32 msec at 25°C[8]) being associated with the 'pain' inflicted to the cuttlefish when tentacle clubs hit the transparent wall.

Nature of the Reinforcement of PT Learning

Several authors[3,8,10,25,32−35] noted that the lack of food reward at each attempted capture could also potentially play a role in the inhibition of predatory behavior. To better balance these two types of potential negative reinforcements, Cartron et al.[36] demonstrated that a rapid waning of attempted captures also occurs when large crabs (instead of shrimp) are placed in the glass tube (Figure 25.5). As mentioned previously, large crabs are generally caught with cuttlefish's eight arms (jumping strategy) instead of tentacle tips. The distance of attack (distance between the cuttlefish arms and the prey at the end of the positioning phase) is comparable in the striking and jumping strategies, but the attack duration is much longer for the latter (mean duration of jumping, 248 msec).[37] In the 'crab in the tube' procedure, jumps are even reduced in strength by the opening of the arm crown when cuttlefish reach the transparent tube. As a consequence, the 'pain,' if any, inflicted to the cuttlefish when it jumps on a transparent tube is probably largely attenuated at each attempted capture. As a consequence, pain is probably not strongly involved in the learning not to attack a crab in a transparent tube. Interestingly,

Cartron *et al.* observed a small number of tentacle strikes on the glass during training in the 'crab in the tube' procedure (~10% of the total number of attempted captures at the beginning of training), but their numbers did not decrease with time except at the very end of training, when the predatory behavior was totally inhibited. Thus, strikes on the glass may play a role in learning but are not necessary to explain the waning of attempted captures. It is thus unlikely that 'pain' is mainly responsible of the inhibition of the predatory behavior in the PT procedure.

This idea is supported by Dickel *et al.*,[34] who have shown that the higher the number of tentacle strikes at the beginning of the PT learning, the longer the time needed for the animal to inhibit its predatory behavior. Thus, a high number of tentacle impacts on the glass (i.e., the number of 'strike-contingent pain') does not make the animal learn faster. Because it is unlikely that 'pain' is mainly responsible for the inhibition of the predatory behavior in the PT procedure, one can hypothesize that the lack of food reward at each attempted capture serves as a negative reinforcement in this learning in cuttlefish. This is also in agreement with the waning of the number of 'pseudo-strikes' observed in the PT procedure (or on free-swimming prawns) when the cuttlefish's tentacles are removed.[7,8] Finally, note that before the inhibition of their predatory behavior in the PT procedure, cuttlefish alternately switch their prey-capture strategy from striking to jumping. Unfortunately, although this has been mentioned in the literature,[32] the phenomenon has never been further investigated.

The Transparent Tube as a Learning Cue in the PT Procedure

The glass tube is not 'invisible' to cuttlefish, as was originally thought. Cuttlefish are sensitive to the linear polarization of light; this sensitivity has been shown to help cuttlefish detect transparent prey.[38] Therefore, it seems likely that cuttlefish can perceive other transparent objects, such as glass or Perspex tubes, based on this sensitivity. Thus, cuttlefish can use the sight of the transparent apparatus in front of the prawns as an important cue to learn and memorize the PT situation. In agreement with this hypothesis, even just after the learning session, cuttlefish rapidly capture a shrimp[34,35] or another prey type[8,31] that freely swims outside the glass tube in the testing tank.

Does PT Learning Rely on Tactile Information?

Tactile and visual learning systems are considered as 'separated' in cephalopod brain (at least in *Octopus*)[28,29]; thus, it is an important question to ask about the role of tactile inputs in PT learning in cuttlefish. This question was first tackled by Sanders and Young.[3] Although the decision to attack or not a prey is based on visual inputs coming from the prawns, the cuttlefish 'feels' the transparent tube with its tentacles and/or arms at each strike/jump. Does this mean that learning mainly relies on visual information or does it involve the integration of both visual and tactile inputs? Two sets of information clarified this question. First, Messenger[8] has shown that learning occurs even when the cuttlefish has no tentacle—that is, when it has no tactile cues from the tube. As a consequence, it is clear that a cuttlefish can learn even when only visual cues are available. On the other hand, when the tube is made 'invisible' to the cuttlefish (by covering the glass tube with a depolarizing filter), learning is still possible, at a rate comparable to that observed in the PT procedure without a depolarizing filter.[36] With an 'invisible' tube, learning is probably largely based on tactile cues, so in principle both types of cues can be used in the learning task. It is interesting to note that the suppression of tactile cues from the 'transparent' tube, using cuttlefish with no tentacles,[8] is more deleterious for the learning rate than the reduction of visual cues from the tube.[36] One can therefore hypothesize that cuttlefish also use tactile information from the glass tube in the priming of the initial training. Note that during a retention test, cuttlefish that previously had totally inhibited their predatory behavior during the training still attempt to catch the prawns, even if less often than at the beginning of the training.[8,30,32,34,35,39,40] Thus, it is unlikely that cuttlefish only rely on visual information to remember the task, with tactile memory processes probably largely involved in the priming of the retention task. Different authors have also mentioned that in the presence of the PT apparatus, cuttlefish often display slight touches of the glass tube with their arm tips or grasp on it after attempted jumping. By increasing the amount of tactile information from the tube, these behaviors could play a role in facilitating learning in some individuals. This purely observational study has yet to be precisely investigated by quantitative experiments.

Stimulus Specificity of PT Learning

The last important point concerns the stimulus-specificity of this learning. In the PT procedure, does a cuttlefish learn not to capture 'shrimp when enclosed in a tube' (in this case, the cues are both prey type and the transparent tube) or 'any prey enclosed in the tube' (in this case, the learning cue is the transparent tube)? The number of attempted captures during a retention test is not significantly affected by a change of prey type in the transparent tube between the learning and a 1-hr retention test (shrimp vs. crabs or crabs vs. shrimp; Figure 25.5).[36] These observations highlight the crucial importance of the glass tube as a learning cue in this paradigm. However, Purdy *et al.*[31] suggested more selectivity in the learning responses to

prey species in another cuttlefish species (*S. pharaonis*). By using fish or shrimp as prey in the tube and daily sessions as training; cuttlefish that switched from fish to shrimp seemed to slightly, but significantly, increase their attack rates, whereas cuttlefish that switched from shrimp to fish did not. The authors interpreted this asymmetrical stimulus specificity as effects of salience or appetence of the two prey types (cuttlefish rely on linear polarization of light to detect silvery fish[41]), which could suggest that fish and shrimp placed in a Perspex apparatus induce a different motivational state in cuttlefish. These interesting data in *S. pharaonis* probably have to be further investigated by using larger samples and a standardized learning protocol in *S. officinalis*.

In summary, the PT learning procedure is probably largely associative, with the presence of the tube (perceived by both visual and tactile cues) being largely associated with the absence of food reward at each attempted capture. It is of crucial importance to highlight that PT learning in cuttlefish is likely to involve both tactile and visual memory systems of the brain.

Memory Trace of PT Learning

Because of its relative convenience, this learning paradigm has been principally used to investigate the formation of memory trace in cuttlefish. One of the most famous pieces of research in this field was published by Messenger,[39] who determined a rate of recovery of predatory behavior as a function of different rest intervals after initial 20-min training. Using a large sample of individuals, the mean number of tentacle strikes observed during a 5-min presentation of the PT during the retention test was compared to the one observed during the first 5 min of the learning. The results are remarkable because the recovery curve was not smooth. There was no recovery of the response (i.e., good retention) in retention tests performed within the 15 min following the training session. Then the response began to recover and then declined until it almost disappeared at 60 min. It then recovered again and, by 90 min, reached an asymptote until 24 hr. Note that the retention of such learning is still considerable at 24 hr. This biphasic retention curve may reflect the presence of more than one memory system with different time courses (short-term and long-term memory systems in cuttlefish) that also has been observed in *Octopus*.[42]

The long-term retention of PT learning is, at least partially, protein synthesis-dependent.[43] Cycloheximide (CMX) injections (translation blocker and inhibitor of protein synthesis) impair recall after a 24-hr retention delay. Amnesia was observed when CMX was injected 1−4 hr after the initial training but not when it was administrated immediately or 6 hr after training. This suggests that *de novo* protein synthesis involved in long-term consolidation occurs during a sensitive window opening within the first hour post-learning and closing during the fifth hour post-learning. However, CXM did not completely abolish the long-term retention of PT learning. This may be due to a moderate degree of cerebral protein synthesis inhibition. A second hypothesis is that consolidation could occur as a multiple 'waves' process starting during the training session.[44] Two main interdependent parameters may be relevant in the process of long-term storage—the time course of the training session and the amount of sensory information received (especially for tactile stimuli)—but their respective importance remains to be determined experimentally.

Brain Correlates of PT Learning

The neural correlates of PT learning have been investigated using two main approaches, lesion and pharmacological, but most studies have focused on the VL system. Brain lesion approaches were used first; the removal of the vertical and superior frontal lobes (Figure 25.2B) had no apparent effect on feeding behavior toward a prawn.[3] Ablation of the superior frontal lobe after the training had no effect on the retention performances, whereas ablation of the VL induced long-term retention deficits (18 hr). Nevertheless, VL-less animals were still capable of relearning. This study suggested the involvement of alternative learning centers such as the optic lobes. A few animals with restricted brain damage (excitotoxic lesions) were tested with the PT procedure in our laboratory (unpublished data). An animal with a lesion in the posterior part of the VL inhibits its tentacle strikes more slowly than a sham animal and shows no 24-hr retention of the task. A lesion in the anterior part of the superior frontal lobe has no effect on the acquisition performance but induces 24-hr retention impairments. These results slightly differ from those of ablation experiments. In both brain lesion experiments (ablation and excitotoxic lesion), local neurons, inputs and outputs, are deleted but ablation disrupts the fibers that only run across the lesioned region to end in another part of the brain, whereas excitotoxic lesion does not. Although these data are preliminary, they suggest that specific attention has to be paid to very precise neural pathways within the VL system. Surprisingly, cytochrome oxidase (CO) activity, commonly used as a marker of neuronal activity, remains unchanged in the VL during consolidation or long-term retention of PT learning, whereas modulations in frontal superior lobe activity have been evidenced in its posterior part (corresponding to outputs to the VL) during consolidation and in its anterior part (corresponding to both inputs from VL, inferior frontal, subvertical, and optic lobes and outputs to the subvertical lobe) after a 24-hr retention test.[24] There is no direct evidence for the involvement of a 'non-VL system' lobe in the PT procedure. However, only a subset of the

structures of the VL system (VL, subvertical, and superior frontal lobes; Figure 25.2C) have been investigated as possible structures of interest as neural substrates of learning and memory in cuttlefish. Because there is evidence that tactile information is involved in PT learning, the inferior frontal lobe (Figure 25.2B) should also be considered as a structure of interest. Indeed, this lobe receives tactile proprioceptive inputs and sends axons to the subvertical and superior frontal (probably the anterior part) lobes. One can consider those lobes receiving inputs from the optic lobes as a network of multimodal integration that may integrate visual and tactile information in PT learning. Common neurotransmitters and neuromodulators (acetylcholine, serotonin, GABA, glutamate, octopamine, and oxytocin/vasopressin[45–47]) have been described in the cuttlefish brain, but very little is known about their functional involvement, especially in memory processes. However, it is known that cholinergic neurons are the sites of synaptic long-term potentiation in the VL in cuttlefish.[26] Furthermore, acetylcholine catabolism is increased in the optic lobes after a 24-hr retention delay but not after 2- and 60-min retention delays.[45] This is a further argument for involvement of the optic lobes in long-term retention of PT learning. Two vasopressin/oxytocin superfamily peptides, octopressin and cephalotocin, are also implicated in long-term retention (24 hr) of PT learning.[46] These peptides are widely expressed in cuttlefish brain, particularly in the vertical, optic, and inferior frontal lobes. Finally, different sets of evidence are in agreement with the 'visuo-tactile' matrices suggested in the literature, but numerous gaps remain to be filled (spatiotemporal implication of the actors). In complement with the approaches cited previously, electrophysiological and molecular markers of neuronal activity should be further developed.

Spatial Learning

Evidence for Spatial Skills in Cuttlefish

Aitken et al.[48] found high site fidelity and quite small home ranges in the giant Australian cuttlefish (S. apama; between 5300 and 27,300 m^2). Another interesting observation is that after a few days of monitoring, cuttlefish seem to have settled into a den (rock crevice, no signal), and thereafter only occasional foraging excursions were observed (<5% of the day).[48] We can hypothesize that cuttlefish may have spatial knowledge of their surroundings at least to optimize their hunting time, and to quickly return to safe places. Karson et al.[49] undertook preliminary observations to determine whether S. officinalis would explore a completely novel environment—a large artificial pond. After several hours of observation, the authors closed a hole

previously allowing cuttlefish to travel from one side to the other side of the pond. This elicited investigation of the closed hole; it suggests that cuttlefish are able to learn where they can find openings in vertical barriers. Cuttlefish orientation has the added complexity of navigating in a three-dimensional environment. Karson et al. then designed a wall maze with two holes allowing cuttlefish (S. officinalis) to access their home tank—one on the left side (10 cm above the bottom of the tank) and one on the right side (60 cm above the bottom of the tank)—with both holes remaining open throughout the experiment. The testing arena had no horizontal surface on which the cuttlefish could settle, providing high motivation to escape. Cuttlefish demonstrated a significant decrease in exit time within 10 trials.

Karson et al.[49] designed another spatial task apparatus: They trained cuttlefish to exit a circular testing arena with two exit holes cut on opposite sides. The exit holes were surrounded by visual cues (striped or spotted panels of fabric), with only one exit hole opened to a resting tank. Cuttlefish demonstrated their ability to solve the task and showed a marked performance improvement over serial reversals (once the cuttlefish reached the acquisition criterion, the open exit hole was closed and the opposite hole was opened). Hvorecny et al.[50] tested S. officinalis and S. pharaonis in a modified version of Karson et al.'s spatial learning apparatus. Two distinct testing arenas were used—one with a brick and the other with algae inside. The open exit hole was indicated by different visual cues in the two mazes (i.e., a striped panel of fabric indicating the open hole in the arena with a brick and a spotted panel of fabric indicating the open hole in the arena with algae). The authors showed that some cuttlefish learned to select the correct exit hole in the two different mazes when trials were intermixed. This experiment demonstrates that cuttlefish are capable of conditional discrimination. All these experiments showed that spatial learning is possible in cuttlefish, but none have studied which sensory senses and spatial mechanisms cuttlefish use to orient.

The complexity of their visual system (for review, see Nixon and Young[16]) and their high performance in visual discrimination tasks[51] suggest that cuttlefish are able to rely on visual landmarks to orient.

Strategy Used to Solve a Spatial Task

The circular testing arena (described previously) has been used successfully to test cuttlefish spatial capabilities.[49,50] Nevertheless, other learning tasks have been designed to ask very precise questions about information cuttlefish attend to and learn to solve a spatial problem. For example, do cuttlefish learn to use visual cues within a maze and/or in the laboratory environment? Alternatively, do cuttlefish use a motor sequence

to solve a spatial task? In the studies described previously, the authors trained animals to solve a spatial task and then modified their environment (e.g., by displacing landmarks) to determine which strategy the animals were using to solve the task (e.g., using landmarks or learning a motor sequence).

Alves et al.[52] designed a cross-maze apparatus to explore cuttlefish (S. officinalis) spatial strategies, a task extensively used in a wide range of models (rats[53] and fish[54]). In this experiment, cuttlefish were rewarded for solving the task with time in the dark on the sandy bottom of a goal compartment located at the end of one arm of the maze. Locating the goal compartment required the cuttlefish to either learn a motor sequence (e.g., the need to turn left to find the entrance of the goal compartment) or orient using visual cues. In a first experiment, only distal visual cues were provided around the maze (water-pipes and sets of shelves of the laboratory room).[52] When the animals reached an acquisition criterion, cuttlefish were again placed in the cross-maze but in the start arm opposite to the one used during training. Nine of 10 cuttlefish swam in the opposite direction of the rewarded goal compartment, indicating that cuttlefish used the previously rewarded motor sequence. In a second experiment,[52] the cross-maze was surrounded by black curtains, and two visual cues were provided just above the water (striped and spotted PVC panels). By changing the right/left location of the two visual cues at the end of acquisition, the authors showed that half of the cuttlefish consistently swam in the opposite direction of the rewarded goal compartment; this result indicates that cuttlefish were using visual cues to solve the task. The other cuttlefish consistently swam in the correct direction of the rewarded goal compartment; this result means that these cuttlefish were using a motor sequence to solve the task. These two experiments successfully demonstrated that cuttlefish can use either visual cues or motor sequence to solve the same spatial task. Moreover, the availability and salience of visual cues seemed to determine whether the cuttlefish used the visual cues or a motor sequence as shown in vertebrates.[53,55] In this experiment, the choice made by a cuttlefish during the probe tests does not exclude the possibility that both strategies occurred in parallel during training (as in Cartron et al.'s experiment[56]).

Cartron et al.[56] examined the ability of cuttlefish (S. officinalis) to solve a Y-maze with two kinds of visual cues: the e-vector of a polarized light and two PVC panels (one striped and one spotted PVC panel) placed just above the water surface. During training, both visual cues were available. At the end of training, one kind of cue was randomly eliminated by the experimenters (i.e., either the PVC panels or the filter linearly polarizing the light). All cuttlefish tested were still able to orient when one of the visual cues became unavailable. Cuttlefish were also given one probe trial with conflicting information: The e-vector of the polarized light indicated one arm as rewarded, whereas the PVC panels indicated the other arm as rewarded. The latency to choose an arm was significantly greater when cuttlefish had to deal with conflicting information than when the two types of cue (e-vector and PVC panels) were congruent. This study showed for the first time the ability of cuttlefish to orient either parallel or perpendicular to the e-vector of a polarized light to find a goal compartment. Moreover, it clearly indicates that redundant spatial information is acquired simultaneously in cuttlefish. Such simultaneous learning has been demonstrated in mammals[57] and insects.[58]

Spatial Cognition and Sexual Maturation

Jozet-Alves et al.[59] assessed spatial learning performances of male and female cuttlefish (S. officinalis), either before or after sexual maturation, in a T-maze (procedure described previously[52]). Sexually mature males were more likely to attend to the visual cues provided above the apparatus to solve the maze compared to sexually mature females and immature cuttlefish. In contrast, sexually mature females have been shown to preferentially rely on a motor sequence (right vs. left turn). However, this difference in strategy did not lead to a sex difference in overall performance: Males and females did not differ in the time they took to learn the spatial task. This study demonstrated for the first time a cognitive dimorphism between sexes in an invertebrate. Several evolutionary hypotheses have been proposed to explain such differences. One of the best supported hypotheses suggests that the sex differences in spatial capabilities will evolve only in species in which range expansion is significantly different between males and females.[60,61] Jozet-Alves et al.[59] also showed that sexually mature males traveled a longer distance when placed in an open field compared with the other tested cuttlefish. These results are consistent with the range-size hypothesis.

Brain Correlates of Spatial Learning

Graindorge et al.[25] have made electrolytic lesions restricted either to the ventral or to the dorsal part of the VL in S. officinalis. Sham-operated and VL lesioned cuttlefish were trained in a modified version of Alves et al.'s[52] spatial learning procedure (the cuttlefish were given only three sessions of trials), and their locomotor activity was assessed in an open field. The results showed that ventral lesions of the VL led to impairment in the acquisition of spatial tasks, whereas dorsal lesions increased locomotor activity in an open field. These data highlight direct functional analogies

between the VL of cuttlefish and the vertebrate's hippocampus.[25]

Other Learning

Classical Conditioning

Following Thomas's[62] classification of learning, some authors tried to undertake a systematic and comparative analysis of learning in cuttlefish. They began with the demonstration of signal learning. In classical conditioning, a neutral stimulus (the conditioned stimulus (CS)) is presented to an animal just before the presentation of an unconditioned stimulus (US) that elicits an unconditioned response. After repeated presentations of the CS followed by the US, some animals begin to behave toward the CS as if it were the US. In appetitive conditioning, in which the US is food, animals either try to 'eat' the CS, which is called auto-shaping, or just approach it using a behavior called sign tracking.[63] Purdy et al.[64] showed evidence of sign tracking in S. officinalis. In the paired condition, they presented a light for 30 sec followed by food delivery to the side of the tank; this occurred four times a day for 30 days, with an intertrial interval of 20 min. Control cuttlefish (unpaired condition) were presented the light and food with a delay of 2 min. The cuttlefish in the paired condition oriented and positioned themselves toward the light significantly more often than did the control cuttlefish. The cuttlefish performed an anticipatory response, which suggests that they are capable of sign tracking. From an ecological perspective, such learning makes sense. Cuttlefish predate upon prey that often hide in crevices or within algae. One way for a cuttlefish to find food is to orient toward places or stimuli where it has previously successfully caught prey. Unfortunately, only a few cuttlefish were used in this study (six and three in the paired and unpaired conditions, respectively). As a consequence, even if the results appear clear-cut, one should interpret these conclusions with caution. However, they are supported by another study that used a classical conditioning procedure.[51] In this study, the CS was plastic spheres of different brightness paired or not with food (US). Even if cuttlefish were not required to make any particular response, in the paired condition they tended to catch the sphere instead of orienting toward it (a phenomenon known as autoshaping). The difference observed between the experiments by Purdy et al.[64] and Cole and Adamo[51] may be due to (1) the nature of the US (the spheres having more features in common with prey than a light) and (2) the shorter delay between CS and US in presentation.[64] The authors claim that this learning could be adaptive because it allows speculative hunting response to recurring environmental features.[65] In terms of costs and benefits, speculative hunting is unlikely to occur in cuttlefish, particularly because cuttlefish are visual hunters.[7] Taken together, these studies show that cuttlefish are capable of associative learning in classical conditioning procedures.

Operant Conditioning

Operant conditioning is another type of associative learning in which an animal learns to associate a behavior with its consequences. Reinforcement and punishment are the core tools of operant conditioning. The former causes a behavior to occur with greater frequency, whereas the latter causes a behavior to occur with lower frequency, sometimes even until its complete inhibition (cf. the PT procedure). Darmaillacq et al.[66] designed a paradigm of aversion learning. In this experiment, cuttlefish were trained not to attack their preferred prey. This prey was rendered distasteful with a coating made of quinine (a substance that tastes bitter to humans) and presented to the cuttlefish every 15 min until they stopped attacking the prey twice in a row. In such condition, cuttlefish inhibit their predatory behavior within a few trials (<10), whereas control cuttlefish that tried to catch the prey but did not get it (removed before it is seized) kept on trying to get it. Indeed, after the cuttlefish inhibited their predatory behavior, a choice between the preferred prey and another prey was presented 24 or 72 hr later. For each retention delay, significantly more cuttlefish chose the other prey than their preferred one, although control cuttlefish still chose their preferred food. Beyond the demonstration that cuttlefish are capable of instrumental learning, these results showed for the first time 72-hr retention capabilities in cuttlefish. In an ecological perspective, this learning appears adaptive. Prey choice may be affected by prey defense mechanisms, such as the presence of toxins, escape strategies, and the availability of prey.[65] Quinine is a natural and widespread chemical. Thus, it makes sense that cuttlefish avoid prey that is distasteful, particularly when they experience it several times. Furthermore, cuttlefish are able to store for a long time information that is crucial to their survival and welfare. Unpublished data obtained using CO histochemistry[24] showed that taste aversion learning induced changes in CO staining with a pattern in the cuttlefish brain that depended on the delay after learning. Immediately after training, CO staining was decreased in the posterior superior frontal lobe; after 24 hr, it was increased in the inferior frontal lobe; and after 72 hr, significant changes in CO activity were observed in the VL and the superior frontal lobe (unpublished data). Like previous studies in cuttlefish and vertebrates,[24,67] these results suggest a differential temporal evolution

of post-training changes in regional brain activity supporting an evolution of the neural substrate of memory. They also confirm the involvement of the VL system in learning and memory processes. Unlike in the study by Agin et al.,[24] the superior frontal lobe is not the only brain region involved in these processes. This difference can be explained because the learning procedures in the two studies are different in terms of learning length, reinforcements, and control groups. However, the results presented here were obtained with a small number of animals and need further investigation.

DEVELOPMENTAL PERSPECTIVES

Behavior and Brain Development in Juveniles

Hatchlings appear to have similar behaviors as those of adults; a few days after hatching, they are active predators able to catch shrimp and shrimp-like prey, fish, and crabs that are large relative to their own size. They have the same prey-capture strategies as adults, but the tentacle strike strategy seems preferentially used, even to catch small crabs.[33,68] They assume the same nectobenthic life and the same defensive strategies (sand digging, changing of body patterns to conceal, inking, etc.) as adults. They do not benefit from parental care, so they have to cope on their own to avoid predators and find prey; thus, one can expect high plasticity of adaptive behavior in early juveniles. As a consequence, cuttlefish is a remarkable model to investigate early development of learning and memory, with the same learning paradigm being applicable from hatching to adulthood.

Hatchlings of cuttlefish probably possess sensory and motor skills equivalent to those of adults.[5,27,33,68,69] These behavioral assessments are in agreement with some preliminary observations of brain development in cuttlefish.[33] A developmental study of the cuttlefish brain from hatching to 30 days of age was performed using immunohistological labeling of 'heavy neurofilaments' (NF-H). The presence of these elements of neuronal cytoskeletons indicates that neuronal networks are structurally stabilized.[70] Low, intermediate, and high motor centers of the brain are well labeled from hatching. In contrast, NF-H progressively appear in the neuropil of associative lobes of the brain ('silent area')—that is, the VL, subvertical lobe, and superior frontal lobe. At 1 month of age, intensity and number of labeled fibers in the VL system are comparable to those observed in the adult VL system.[33] In the optic lobes, whereas the most central part of the medulla and the deep retina contain numerous stabilized fibers from hatching, NF-H labeling fibers in
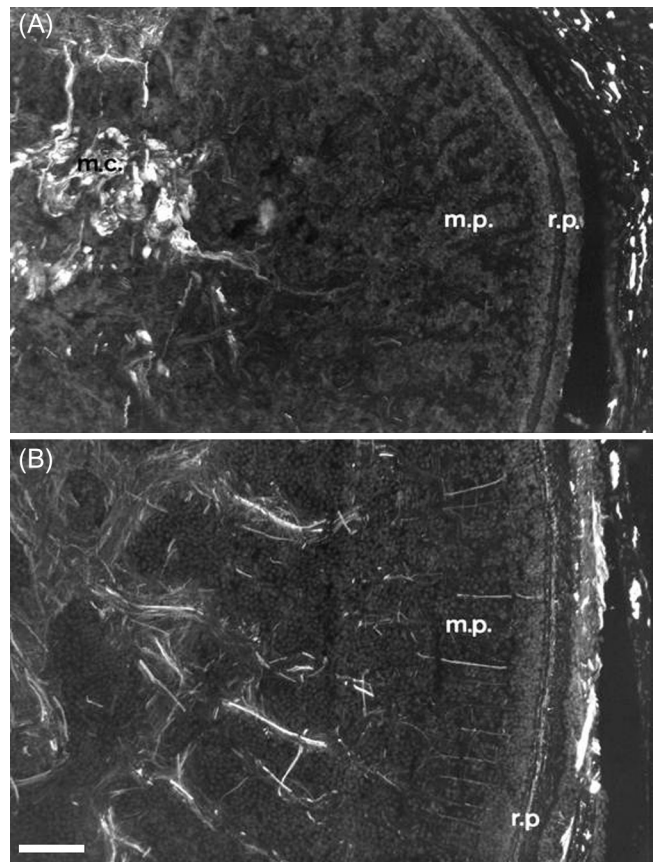
FIGURE 25.6 **Heavy neurofilaments immunoreactivity in optic lobes at hatching (A) and 9 days of age (B).** The microphotographs were taken from sagittal sections. Immunoreactive fibers are visible in the peripheral medulla of the optic lobe at 9 days, whereas they are abundant only in the central medulla and deep retina (well visible at higher magnification; not shown) from hatching. m.c., central medulla; m.p., peripheral medulla; r.p., deep retina. Scale bar = 100 μm.

the peripheral medulla of the optic lobes appear later, beginning at the end of the first week of life (Figure 25.6).

Development of Long-Term Memory and Brain Correlates

The PT procedure was used for the first time in early juveniles by Wells[6] and Messenger.[9] Using this paradigm with apparatuses of suitable size, Wells was the first to mention the poor learning capabilities of very young cuttlefish. In more detailed studies, Messenger[9] and Dickel et al.[35] showed a gradual improvement of training performance in the course of the first 3 months of life. Specifically, 8-day-old animals displayed memory capacities sufficient to inhibit their predatory behavior within a continuous presentation of PT, showing the existence of short-term memory capabilities from this age.[35,40] However, retention of the task at 60 min (corresponding to long-term memory storage[39])

increased progressively between 15 and 60 days of age. In pioneering work, Messenger[9] used eight 3-min presentations of the PT (hereafter referred to as 'trials') with intertrial intervals of 30 min during which the juveniles were left undisturbed. All juveniles were tested 24 hr later. The author showed an increase in both learning (i.e., waning in number of strikes between the first and the eighth trial) and 24-hr retention performance (i.e., difference between the number of strikes during the first trial and the retention test) between 7 and 112 days of age. Messenger hypothesized that the poor memory capabilities of juveniles were related to the immaturity of their vertical and superior frontal lobes. This assessment was further investigated by Dickel et al..[35] By using morphometric measurements of different lobes of the brain and PT learning experiments in juveniles aged 8−90 days, these authors showed that only the growth of the superior frontal lobe and the VL was significantly correlated with the improvement of learning and long-term retention performances with age. This developmental approach confirms Messenger's hypothesis and appears to be in agreement with results obtained from adults in lesion experiments: The VL system seems to be involved in training of an associative learning[71−73] as well as in retention processes in *Octopus*.[20,73] In this species, Young[74] emphasized the likely role played by these structures in building associations among various stimuli. This strong hypothesis was recently supported by both physiological[24] and electrophysiological studies (see Chapter 24).

Influence of Early Experience on Memory and Brain

There is a substantial body of literature on the influence of rearing environment on behavioral and brain maturation in mammalian and avian species (for review, see Renner and Rosenzweig[75]). This factor is crucial on a fundamental level to further investigate the mechanisms of 'phenotypic plasticity'[76] and from an animal husbandry standpoint. The European regulation about the use of animals in laboratories now integrates cephalopods. It requires environmental enrichment in the animal enclosure. As such, one can ask whether enrichment of rearing tanks can have consequences on both brain and behavioral plasticity in cuttlefish.

In a series of experiments, Dickel et al.[34] tested three groups of cuttlefish reared in different conditions: standard conditions, SC (large and bare tanks, reared in groups); impoverished conditions, IC (small and bare tanks, solitary condition); and enriched conditions, EC (large tanks with sand, rock shelters, shells, and artificial seaweed). Some animals were maintained in impoverished conditions until 1 month of age and then transferred in enriched conditions (I/EC), and others were kept in enriched conditions until 1 month of age and then transferred to impoverished conditions (E/IC). Using the PT paradigm, retention performance of the juveniles from the SC, IC, and EC groups was assessed at 1 month, and that of juveniles from all groups (SC, IC, EC, I/EC, and EC/IC) was assessed at 3 months.[34] For an easier comparison of 24-hr retention performance between the different groups, the level of learning acquisition was standardized for all animals. Learning and 24-hr retention performance developed faster in EC than in IC cuttlefish at the same age, with learning and retention performance of SC cuttlefish intermediate between EC and IC. Interestingly, at 3 months, cuttlefish from the E/IC group showed the same learning and retention performance as the IC group at the same age, whereas the I/EC group did not differ in its performance from that of the EC group. These results indicate that an enriched environment facilitates development of learning and memory capabilities in young cuttlefish.

Preliminary morphometric studies of brain structures have shown that the VL and medulla of the optics lobes develop faster in cuttlefish reared for 3 months in EC than in cuttlefish from IC.[33,77] There is no significant difference between IC and EC cuttlefish at 2 months. In cuttlefish from the IC and EC groups, cell proliferation was quantified by bromodeoxyuridine (BrdU) labeling of dividing cells of the brain in hatchlings and at 1, 15, 30, and 60 days of age. Brains were removed 4 hr after injection of BrdU, and labeled cells were analyzed by immunohistochemistry.[78] This study focused on the VL system (vertical, subvertical, and superior frontal lobes) and the optic lobes. This experiment showed that cell proliferation, which is intense in all brain structures on day 1, strongly decreases during the first 2 months of life. However, the rate of decrease is lower in the EC group than in the IC group in both neuropil and deep retina of the optic lobes. We did not find any significant differences in the lobes of the VL system. Although preliminary, these data indicate that the maturation of the most associative structures of the brain is sensitive to early experience of juveniles. Enriched environment of rearing would facilitate neurite growth in the VL and in the superior frontal lobe, whereas at least cell proliferation intensity (i.e., the density of labeled cells) is affected by an impoverished environment in structures that are involved in the primary integration of visual information (i.e., deep retina of the optic lobes). However, the latter result has to be considered with caution because the sequence of neuronal development is still unknown. Further investigations also have to be conducted to study the effect of enriched conditions of rearing on the maturation of

motor and sensory structures. Taken together, however, these studies indicate that exposure to an enriched environment induces both structural and functional changes in the cuttlefish brain. There is further evidence that early experience also affects the maturation of defensive behavior such as body patterning[78,79] and sand digging.[80] Enrichment is a complex combination of physical activities, learning, and inanimate and social stimulation. It is still unknown which of these factors is critical for brain and behavioral plasticity in cuttlefish juveniles.

Imprinting and Early Cognition

Imprinting, originally described by Lorenz,[81] refers to a specific form of persistent learning that occurs during a sensitive period without any obvious conventional reinforcement.[82] In chicks, the social preference induced in an individual by imprinting is then generalized to all the individuals of the same species.[81] Other imprinting-like behaviors have since been reported, including habitat imprinting[83] and food imprinting.[84] Cuttlefish hatch with internal nutritive reserves. Even if active predation can begin before the inner yolk is entirely used up,[27,68] these reserves allow young cuttlefish not to feed for a number of days. This short period is of critical importance in the life of young cuttlefish because ambushed hatchlings can collect information about their environment (available prey, predation risk, etc.) before they start foraging.[85] When naive cuttlefish start feeding, they spontaneously prefer shrimp-like prey to any other type of prey, such as as crustacean or fish.[6,86] However, it has been shown that a preference for crabs (a nonpreferred prey) could be induced after a mere visual familiarization with crabs during a sensitive period within the first hours of life after hatching.[87,88] Interestingly, the efficiency of this familiarization depends on the length of the exposure as well as the density of prey exposed—that is, the flow of information perceived during the sensitive period. Finally, it appears that the primacy of the early familiarization outweighs untrained preference for shrimp.[88] It has also been shown that if naive juvenile cuttlefish are presented a choice between black and white crabs, they prefer the dark phenotype.[89] However, if they are exposed to white crabs at hatching during the sensitive period, they subsequently prefer white rather than black crabs (two-way choice: white vs. black crabs) and black crabs over shrimp (two-way choice: black crabs vs. shrimp). This result suggests that cuttlefish can make intracategorical discrimination (white vs. black phenotype) and that they can generalize the learning of the characteristics of the prey to which they were familiarized to a

novel prey that shares the same morphological features.[89] Early diet selection may utilize food-item generalization to reduce energy and time devoted to information processing.[90] Generalizing among initial prey preferences would allow a young cuttlefish to diversify its diet while dispersing away from the laying site without the increased cost associated with trial-and-error learning.[85]

Taken together, these studies show that the learning of the visual characteristics of a potential prey meets all the criteria of imprinting as described previously: no reinforcement, existence of a sensitive period, persistence,[88] and generalization of the prey preference.[89] It was proposed that food imprinting could account for the prey preference observed in week-old cuttlefish. Food imprinting could be a good compromise between a certain degree of flexibility in response, useful for learning information for which the timing is likely to be predictable—food seen in the first few hours of life—but in which specifying the exact details of the experience is not useful. Such early learning capabilities would allow juveniles to take advantage of a changing environment and to deal with a world in which shrimp (i.e., their 'innate' food preference) would be unavailable. Contrary to filial or sexual imprinting, food imprinting would seem costly and even disadvantageous in the long term,[91] particularly for long-lived individuals living in somewhat changeable environments. As such, it may be helpful for juveniles to become highly attracted by available prey in the weeks following hatching before they starts foraging around. This preference for a prey that was seen for a few hours soon after hatching and expressed 7 days later suggests a rapid acquisition and long-term memory capabilities. This appears to contradict previously mentioned results obtained from studies using the PT procedure in juveniles, in which the authors showed that 8-day-old juveniles had a poor acquisition of the associative task[35,40] and that long-term memory only appeared from the age of 1 month.[9,34,35,40] One possible explanation is that the acquisition and retention processes involved in the establishment of prey preference in juvenile cuttlefish may depend on different rules than those in avoidance learning.[85] In the chick, it has been shown that memories supporting imprinting preferences and those consecutive to the acquisition of a heat-reinforced discrimination using the imprinted objects are functionally different and are located in different areas of the brain.[92] In the cuttlefish, one could argue that the two kinds of memories may involve separate brain structures. Because the VL is very immature in the first weeks of life, good candidates would be the optic lobes. These brain structures process visual information coming directly from the eyes,[93] and they may also be involved in learning and

memory.[94] Dickel[33] showed that neural maturation occurs earlier in the optic lobes than in the vertical system (Figure 25.6). This finding is also supported by a recent study in which crabs were exposed to the cuttlefish's right or left eye (unpublished data). Only cuttlefish exposed to crabs with their right eye were significantly more likely to choose crabs than were control cuttlefish that did not see crabs. This study indicates that the right eye pathway involving the optic lobes is sufficient for food imprinting and that a kind of hemispheric specialization may exist in the cuttlefish. Thus, the right visual field would be more generally specialized in foraging and feeding behavior, as in many vertebrate species[95] and in honeybees.[96]

One of the characteristics of cuttlefish is that they are semelparous, which means that adult cuttlefish only mate once in their life and females die after having laid hundreds of eggs. A direct consequence is that embryos and juveniles develop without parental care. Unlike other cuttlefish species, in *S. officinalis* the egg capsule is stained in black by the ink of the female. Immediately after spawning, the capsule is completely opaque; as the embryo grows and the osmotic pressure of the perivitelline fluid increases,[69] it is dilated and peels off. Consequently, the capsule becomes more transparent and, hence, it is likely that several types of information can reach the embryo. It is known that in birds and mammals, the onset of the sensory systems follows a fixed sequence in which the tactile system appears first and then the vestibular, chemical, auditory, and visual systems.[97] In the cuttlefish, the tactile, chemical, and visual systems are functional before hatching, although the precise onset for the tactile and chemical systems is still not known (unpublished data). However, there is some behavioral evidence that the visual system is functional long before hatching. Indeed, Darmaillacq *et al*.[98] showed that embryos exposed to crabs (unpreferred prey) between 1 and 2 weeks before hatching subsequently preferred crabs to shrimp 7 days after hatching. This first demonstration of embryonic visual learning also highlights the extraordinary long-term memory capabilities of this marine invertebrate. In addition, the learning of the characteristics of prey is not limited to the general shape and motion but also includes the phenotype of the prey. Indeed, when offered a choice between white and black crabs, young cuttlefish visually familiarized *in ovo* to white crabs prefer white crabs for their first meal compared to the control cuttlefish that prefer black crabs; they also prefer black crabs to shrimp.[89] This result confirms that juvenile cuttlefish have the capability to categorize and generalize prey, but interestingly, it also shows that embryos are capable of fine perception of the features of prey, notably its brightness. The ability of cuttlefish embryos to perceive

visually and to process and learn particular characteristics of prey present in the vicinity of the eggs may confer important adaptive advantages. In mammals, prenatal olfactory learning of the maternal diet may provide the opportunity to learn about a safe and natural diet.[99] In the absence of parental care, neonates have to avoid harmful food on their own. Female cuttlefish generally lay their eggs in shallow water[69] and may choose places where newly hatched juveniles can easily find potential prey. The ability to learn the visual characteristics of the prey *in ovo* would facilitate postnatal imprinting on this kind of prey. Finally, these results imply that it is also very likely that the sensitive window that is active soon after hatching[88] may be open in the few weeks before hatching. Another series of studies showed that chemical information can also reach the embryo. In embryos, the chemical system is functional before the visual system (unpublished data). In a study addressing the effect of a chemical exposure on visual preference after hatching, embryos were exposed to odors from shrimp (*Crangon crangon*; preferred prey), crabs (*Carcinus maenas*; unpreferred prey), mollusks (*Mytilusedulis*; nonprey), or a seawater control (no prey).[100] They were then tested for their visual preference between crabs and shrimp. Cuttlefish that had previous experience with shrimp odor had a visual preference for crabs, whereas cuttlefish that had previously smelled crabs preferred shrimp and cuttlefish that had previously smelled bivalves had no preference. To explain these puzzling results, the authors noted a cross-modal effect between the chemical and the visual systems. Moreover, they hypothesized that an overstimulation of the chemical system during embryonic development could have disturbed the onset of the visual system and hence the visual perception in juveniles.

CONCLUSION

The studies reported in this chapter demonstrate the increasing interest in examining the biological bases of behavioral plasticity in *S. officinalis*. There is a significant effort at an international level to develop efficient and well-characterized behavioral tools to investigate the neural substrates of learning and memory in this species. Based on these tools and on the extraordinary amount of knowledge already collected in *Octopus* (for reviews, see[4,101]), studies on cuttlefish could allow a better understanding of the neural mechanisms of complex learning (imprinting, classical and instrumental conditioning, spatial learning, etc.). In this species, promising perspectives are still open to investigate high-order cognitive processes such as concept formation, representation building, and executive functions.

From a neurobiological approach, cuttlefish brain anatomy is well-known, and ablation or pharmacological lesions as well as more modern methods (neuropharmacology, pathway tracing, *ex vivo* electrophysiology, etc.) have provided precious knowledge on the circuitry and functioning of relevant brain regions (i.e., VL system[26] and optic lobes[93]). However, it is still difficult to apply techniques that would allow the determination of neural activity *in vivo* (calcium imaging, positron emission tomography, and magnetic resonance imaging) or during the different steps of memorization of complex tasks (markers of neuronal plasticity as immediate-early gene expression). Another promising perspective would be to apply neuropharmacological blockers or facilitators of selective neural pathways during learning, based on the pioneering works of Chichery,[102] Chrachri et al.,[103] and Messenger.[47] Some of these tracks would have to be conducted on a comparative basis between different species of cephalopods that show considerable differences (morphology, ecology, behavior, etc.), but all share impressive behavioral plasticity and an overall identical brain organization.

In parallel, the cuttlefish provides a unique opportunity to explore the development of brain and cognition in an invertebrate species. Data about learning and memory skills in embryos,[89,98] in hatchlings and juveniles (for review, see Dickel *et al*.[85]), during sexual maturation,[59] and during senescence[32,104,105] show several analogies with higher vertebrates such as birds and mammals. This is of major interest from a fundamental standpoint to better understand mechanisms of developmental plasticity of brain and behavior. It may also produce interesting outcomes for applied studies—to investigate, for example, some developmental disorders as long-lasting effects of early stress on brain and behavior or mechanisms of brain and cognitive aging.

However, behavioral biology research of *S. officinalis* suffers from a lack of knowledge about the behavior of cuttlefish in the field. The behavioral rules and constraints of cuttlefish in its natural environment remain largely unknown. Research in behavioral ecology in this species will allow the refinement and diversification of learning paradigms available for use in the laboratory. As already reported in the literature,[106] these kinds of investigations will be a challenging key to answering questions about the neurobiology of cognition in cuttlefish.

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