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Environmental enrichment affects the ontogeny of learning, memory, and depth perception of the pharaoh cuttlefish *Sepia pharaonis*

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ABSTRACT

We investigated the effects of environmental enrichment on the cognitive abilities of pharaoh cuttlefish, *Sepia pharaonis*, which were reared from day seven in four different environments: isolated, poor, standard, and enriched. First, we used "prawn-in-the-tube" to test whether environmental enrichment affects the ontogeny of learning and memory of *S. pharaonis*. The results showed that cuttlefish could usually learn the task regardless of their age and environment. At early age (74 – 81 d), cuttlefish from the isolated environment memorized the task for 24 h. However, at later age (104 – 171 d), the isolated cuttlefish were unable to remember the task. In addition, cuttlefish from the poor environment could not memorize the task at all ages examined. Cuttlefish from the standard environment could memorize the task in later ages (134 – 171 d). In contrast, cuttlefish from the enriched environment could memorize the task at all ages examined. Second, we examined the effect of environmental enrichment on the ontogeny of depth perception of *S. pharaonis* by observing their hunting behavior. Distance from the prey during hunting was always greater in isolated cuttlefish than those from the other three environments. In addition, hunting success and number of prey captured were always lowest in the isolated cuttlefish for all ages. In contrast, hunting success was always the highest in cuttlefish from the enriched environment. These variations in behavior among cuttlefish raised in different environments suggest that the visual/tactic input derived from social and physical factors of the surrounding environment could promote maturation of learning, memory, and depth perception in *S. pharaonis*.

1. Introduction

In phylogenetically advanced vertebrates with higher-level neural circuits, such as mammals, the raising environment influences the development of visual cognition. For example, in rat pups, continuous exposure to enriched environments (e.g., a combination of complex inanimate and social stimulation) for two months enhances learning and memory retention relative to standard housed controls (Leggio et al., 2005). Meanwhile, rats raised in light conditions showed improved depth perception after two months of age, whereas those raised in dark conditions did not (Tees, 1974). The impact of environmental influences on visual cognition also depends on the timing of exposure to the environment. Rats raised in dark conditions for a month after birth easily recovered depth perception when exposed to appropriate visual experiences, although those deprived of vision for two months after birth did not fully recover (Walk and Walters, 1973). Certain behaviors and their neural circuits do not develop and mature if surrounding stimulation is not appropriately received during a limited postnatal period, which is known as the critical or sensitive period (Hensch, 2004; Knudsen, 2004).

Coleoid cephalopods (octopuses, squids, and cuttlefishes) are one of the most advanced invertebrates since they possess camera-type lens eyes and the largest central nervous systems (i.e., brain) among invertebrates (Packard, 1972; Budelmann, 1995). Based on these anatomical characteristics, cephalopods can learn and memorize complex tasks and communicate both intraspecifically (e.g., through agonistic or sexual displays) and interspecifically (e.g., crypsis) using complicated neutrally controlled behavior, namely, body patterning consisting of chromatic, textural, postural, and locomotor components (Hanlon and Messenger, 1996; Hanlon et al., 2009). In all these behaviors, cephalopods percept, via the ambient environment, visual information, such as polarized light, edges, contrast, object area, and depth cues. Thus, it is reasonable to speculate that early exposure to ambient visual information would alter the ontogeny of behavior in cephalopods, similar

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to that of the mammals previously mentioned.

It has been proposed that early experience influences the ontogeny of behavior in cephalopods. California market squid (*Loligo opalescens*) fed on only slow-moving prey items (brine shrimp) during the first 40 days of life does not acquire normal hunting skills, whereas the cohorts fed on fast-moving prey (copepods) hunt well (Chen et al., 1996). Although hatchlings of European cuttlefish (*Sepia officinalis*) usually prefer shrimps to crabs, those exposed to crabs prefer crabs (Darmaillacq et al., 2006a). This phenomenon of food familiarization is thought to occur within the first day of life (Darmaillacq et al., 2006b). Dickel et al. (2000) and Poirier et al. (2004, 2005) reported the effect of environmental enrichment for *S. officinalis*; young individuals reared in tanks with conspecifics, sand, rocks, and artificial objects for 90 days after birth acquire more advanced learning, memory, and cryptic abilities than those reared in isolation in visually poor tanks.

In contrast to these studies, there have been no studies on the long-term impact of environmental exposure on the ontogeny of behavior in cephalopods. Under experimentally controlled conditions, it is generally difficult to monitor the long-term effects of environments on neural circuit formation and its related behaviors of phylogenetically and neutrally advanced animals because of their long lifespan, such as several years to decades. Regarding this, cephalopods are unique animals as they possess advanced neural and visual systems, but their life span is relatively short as they generally live for less than a year, which names they "live fast, die young" (Boyle and Rodhouse, 2005). This feature must be advantageous and can situate cephalopods as a suitable animal model to study environment-neural ontogeny relationships. Recent advancements in the culture techniques of cephalopods (Iglesias et al., 2014) can provide additional opportunities for long-term experiments on live specimens, by which the effect of environmental factors on the ontogeny of behavior among different species of cephalopods can be investigated. Besides neuroscientific interest, it is important for us to understand the longitudinal influence of environments on cephalopod behavior since they have been proposed for considerations of animal welfare similar to vertebrates (Fiorito et al., 2015).

The European common cuttlefish, *S. officinalis*, which is mostly benthic but also moves up in the water column similar to other cuttlefishes, can perceive and learn spatial information (Scatà et al., 2016, 2017). In contrast to *S. officinalis*, which inhabits turbid temperate waters, the pharaoh cuttlefish *S. pharaonis* inhabits clear tropical waters with visually complicated environments comprising corals, sponges, rocks and/or sea grasses (Reid et al., 2005). Additionally, *S. pharaonis* shows a larger repertory for body patterning than that of *S. officinalis*' (Nakajima and Ikeda, 2017). Under these circumstances, it can be assumed that *S. pharaonis* would be visually affected by the complexity of the habitats, that is environmental enrichment, especially during its young phase in which the neural circuit of the brain and vision would be drastically structured, as in other cephalopods (Kobayashi et al., 2013). Therefore, we investigated the effect of environmental enrichment on the ontogeny of visual cognitive abilities in *S. pharaonis*, with special reference to their learning, memory, and depth perception.

Regarding neural plasticity, it is necessary to focus on the sensitive period when the nervous system is highly responsive to environmental stimuli (Hensch, 2004; Knudsen, 2004). Based on this, if a sensitive period exists for the formation of cognitive functions in *S. pharaonis*, the state of their cognitive ontogeny would differ in different rearing environments. Therefore, the present study was designed to test the hypothesis that *S. pharaonis* raised in an enriched environment would acquire normal cognitive abilities for visual learning, memory, and depth perception, whereas the counterparts raised in impoverished environments would be inhibited, with the effects appearing in particular ages and being retained thereafter.

2. Materials and methods

2.1. Subjects and rearing environments

Egg masses of *S. pharaonis* were collected in April and May 2013 by SCUBA from the coastal waters of Okinawajima Island of the Ryukyu Archipelago, Japan. These egg masses were transported in buckets of seawater to the laboratory of the University of the Ryukyus Nishihara campus, then transferred into six circular plastic arenas (240 mm diameter × 130 mm height, bottom covered with a net-sheet) soaked in cylindrical tanks (300 mm diameter × 330 mm height) of a closed seawater system equipped with biological filters, a cooler/heater unit, and UV sterilization (Multihydense®, Aqua Inc., Shinagawa-ku, Tokyo, Japan). Walls of these cylindrical tanks, in which there were no objects, were covered with blackout cloths to avoid disturbance. Since *S. pharaonis* hatched over several days, we defined d 0 (i.e., hatching date) as the day when the highest hatchling proportion was observed. To examine the effects of environmental enrichment, 44 – 48 juveniles were raised in each of the following four rearing tanks connected to the Multihydense® system: "isolated" (solitude with no objects), "poor" (no objects but included conspecifics), "standard" (fine beige sandy bottom and conspecifics), and "enriched" (artificial sea grass and coral reef on fine beige sandy bottom, and conspecifics). Details of the methods for keeping cuttlefish have been described elsewhere (Yasumuro and Ikeda, 2016). After confirmation of normal and healthy behaviors, 7-d old juveniles were introduced to the above four tanks, and were raised for 171 d. The size of the tanks for the isolated groups were identical throughout the experimental period (a black plastic enclosure [170 mm diameter × 170 mm height]), whereas those for other three tanks were sequentially changed as the cuttlefish grew (7 – 27 d, an opaque gray-white acrylic enclosure [290 mm diameter × 300 mm height]; 27 – 171 d, an opaque gray-white plastic square tank [600 mm length × 435 mm width × 400 mm height]). We placed an air stone into each tank to providing oxygen for *S. pharaonis*. Cuttlefishes are benthic, and this is especially the case during young period (Hanlon and Messenger, 1996). Therefore, the space that single cuttlefish could use was almost similar between isolated and grouped condition regardless of tank size in this study; they actually did not actively swim around the tank. Similar situation was also conducted to the previous study on *S. officinalis* (Dickel et al., 2000). Water conditions of these four rearing tanks throughout the experiment were kept as follows: water temperature 25 – 26.5 °C, salinity 33 – 35 ppt, and pH over 7.8. Fluorescent lights were set above the tanks, with a 12L:12D cycle controlled by a timer.

Sepia pharaonis up to 30 d of age in the four rearing environments were fed on live adult mysid shrimp (*Neomysis japonica*) and frozen larval Japanese anchovy (*Engraulis japonicus*). After the age of 30 d, they were fed on frozen adult Sakura shrimp (*Sergia lucens*) and adult tiger prawns (*Penaeus monodon*). *S. pharaonis* were fed three times per day ($<$ 7-d old) or twice per day ($>$ 7-d old). After feeding, excrement and waste were immediately removed from the tanks.

To evaluate the effect of environmental enrichment, we randomly selected individuals from each of the four rearing tanks to conduct the behavioral experiments described below. After the experiments, we anesthetized the cuttlefish in 5% ethanol in seawater to measure their dorsal mantle length (DML), then the animals were killed by decapitation. Age and number of *S. pharaonis* examined were as follows: 74 – 81 d (Age 74 – 81: $N = 7$ standard, $N = 8$ remaining three environments), 104 – 110 d (Age 104 – 110: $N = 9$ isolated, $N = 7$ poor, $N = 8$ standard and enriched environments), 134 – 141 d (Age 134 – 141: $N = 9$ isolated, $N = 8$ remaining three environments), and 164 – 171 d old (Age 164 – 171: $N = 5$ isolated, $N = 4$ poor, $N = 6$ standard and enriched environments). In *Sepia officinalis*, the effect of the enriched or poor raised condition to the learning and memory of juveniles was documented up to 90 d old (Dickel et al., 2000). However, much longer-term effect of environmental enrichment has not been examined in any species of cephalopods. This is why we dealt with *S. pharaonis* aged 74 –