

Brief Report

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Early Exposure to Odors Changes Later Visual Prey Preferences in Cuttlefish

ABSTRACT: Developmental studies have shown that environmental stimulation received by a developing sensory system can alter the developmental outcome of both that sensory system and other aspects of the nervous system. We investigated the ecologically relevant question of whether prior exposure to prey early in development within one sensory modality could influence later prey choice within a different sensory modality. Cuttlefish are visual predators; they can detect prey odors but attacks on prey cannot be elicited without visual stimulation. Cuttlefish eggs were exposed to the odor of shrimp (preferred prey), crabs (non-preferred prey), mollusks (non-prey), or a seawater control (no prey). Seven days after hatching, prey preferences were tested with a visual choice test between crabs and shrimp. Hatchlings exposed to crabs odors and the seawater control were significantly more likely to attack shrimp. Hatchlings exposed to mollusk odors showed no visual prey preference, while those exposed to shrimp preferentially attacked crabs. These results demonstrate a complex relationship between an early sensory exposure and later prey preference. © 2010 Wiley Periodicals, Inc. *Dev Psychobiol* 52: 833–837, 2010.

Keywords: cross-modal effect; invertebrate; chemoreception; predation; sensory development

INTRODUCTION

Stability of the environment of an embryo is fundamental to normal development in all species. Embryonic stimulation of one of the sensory systems can affect the development of all the sensory systems and alter subsequent behavior; for example, embryonic visual stimulation affects auditory responsiveness in bobwhite quail (Foushée & Lickliter, 2002), suppresses auditory learning in ducklings (Gottlieb, Tomlinson, & Radell, 1989), and affects homing behavior in rat pups (Kenny & Turkewitz, 1986). The present study aimed to explore the

effect of embryonic olfactory stimulation on the visually controlled predatory behavior of an innovative and original model species, the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda).

Predators rely on keen sensory systems to detect prey and solitary predators must recognize prey from their first exposure. As cuttlefish do not receive any parental care (Richard, 1971), hatchlings are precocious predators that begin hunting 3–12 days after they hatch (Wells, 1958). Cuttlefish are highly visual predators (Messenger, 1968) and previous studies have demonstrated that embryonic exposure to prey influences later visual choice of prey. Young cuttlefish prefer small shrimp to crabs as prey (Darmaillacq, Chichery, Poirier, & Dickel, 2004; Wells, 1958), but if the hatchlings are exposed to the sight or the sight and odors of crabs in the first hours after hatching, or even in the hours just before hatching, this preference is reversed (Darmaillacq, Chichery, & Dickel, 2006; Darmaillacq, Chichery, Shashar, & Dickel, 2006).

A surprising result from an experiment on observational learning raised the possibility that odors are also important to the development of predatory behavior in

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cuttlefish. Cuttlefish exposed to the odors (only) of crabs later subdued crabs with fewer pinches than did naïve cuttlefish (Boal, Wittenberg, & Hanlon, 2000). Predatory behavior is thought to be visually guided. Although cuttlefish also detect odors, including odors of prey (Boal & Golden, 1999), they do not orient towards or approach particular prey odors (Boal, unpublished data). The investigators in the observational learning study suggested that some kind of priming could have been responsible for the improved predatory technique of the odor-exposed cuttlefish.

Cuttlefish, like other coleoid cephalopods, possess complex visual systems and multi-lobed brains. Visual information is processed first by a pair of optical lobes; information is then passed to integrative lobes such as the superior frontal lobes (Nixon & Young, 2003). Little is known about the neural processing of olfactory information. Olfactory information is perceived by the suckers and lips and then is passed to the brachial lobes, which are connected to the inferior frontal lobes, which are integrative centers. The inferior frontal lobes are linked to the superior frontal lobes (Nixon & Young, 2003). Potentially, information from olfactory and visual systems could be integrated in the superior frontal lobes; whether this actually happens is unknown.

In the current experiment, we hypothesized that odors play a significant, organizing role in predatory behavior of young cuttlefish. *Sepia officinalis* cuttlefish were exposed to the odors (only) of prey shortly before hatching. Prey preference was tested approximately 1 week after hatching using a visual (only) choice experiment. Cuttlefish were not fed before testing; they subsisted on any yolk remaining from the egg. We hypothesized that if the two sensory systems, olfaction and vision, were independent, then cuttlefish exposed to odors of prey should express the same prey preferences as control cuttlefish not exposed to prey odors. If, however, the visual and chemoreceptive systems of cuttlefish are interdependent, for example to prime predatory responses or support early recognition of suitable prey, exposure to the odors of prey could initiate an internal change leading to altered visual prey preferences relative to control cuttlefish.

METHODS

All experiments were conducted at the “Centre de Recherches en Environnement Côtier” (CREC) in Luc-sur-Mer, France.

Subjects

Adult cuttlefish, *Sepia officinalis*, were trawled in April and May from the English Channel near Normandy, France, as they moved inshore to spawn. Adults were placed in large housing tanks (1,000 L) with flow-through natural seawater (15–18°C). Any eggs laid in these tanks were collected and placed in a

similar but separate holding tank. Eggs were visually inspected weekly and those that appeared close to hatching (see Boletzky, 1983 for details) were collected for use as described below.

Odor Detection

Preliminary trials using ventilation rate as a bioassay (Boal & Golden, 1999) confirmed that hatching cuttlefish (approximately 4–6 weeks post-hatching) detect the odors of prey.

Prey Exposure

Cuttlefish eggs ($N = 120$) that appeared close to hatching (Boletzky, 1983) were placed in mesh baskets that floated within large tanks; a continuous supply of seawater passed first through a tank that contained the appropriate prey species and then to the tanks with the floating baskets. Each basket initially contained 30 eggs but some eggs failed to hatch and some cuttlefish died before being tested; thus, the total sample was reduced to 88. Each of the four treatment groups was exposed during daylight hours to odors from one of the following: shrimp (*Crangon crangon*; preferred prey; $n = 25$), crabs (*Carcinus maenas*; less-preferred prey; $n = 21$), mollusks (*Mytilus edulis*; non-prey; $n = 21$), or a seawater control (no prey; $n = 21$). Cuttlefish eggs typically hatch at night (Paulij, Herman, Roozen, & Denucé, 1991). To ensure that odor exposure was limited to the pre-hatching stage, the floating baskets of eggs were removed each evening and placed in similar tanks without any prey odors. At dawn, any cuttlefish that had hatched were removed from their hatching environment and housed in isolation until testing. The mean duration (\pm range) of odor exposure was 6.7 ± 4.3 days.

Visual Choice Test

Seven days after hatching, each individual cuttlefish was given a visual choice between live crabs and live shrimp; no odor cues were provided. Trials were conducted at dawn, when cuttlefish are still active (hatchlings are nocturnal; Boletzky, 1983), and were conducted as follows. The prey were introduced: five live crabs (carapace width 2–3 mm) and five live shrimp (body length ~ 1 cm) within separate seawater-filled glass beakers (3.5 cm diameter). Beakers were placed behind a black PVC slide within a small tank (10 cm \times 15 cm). The position of the crabs and shrimp was randomized (left/right). Next, individual cuttlefish were placed into a start box (4.5 cm diameter glass cylinder). After a one-min delay to allow the cuttlefish to settle, the PVC slide was removed. If the cuttlefish showed any attention behaviors (eye movement, color changes, arm extensions) towards the prey, the glass cylinder was raised and the cuttlefish could freely approach the prey. The cuttlefish typically showed one or more standard hunting behaviors of attention, positioning, and seizure (tentacle strike; Messenger, 1968) directed toward just one of the prey (crabs or shrimp). If the cuttlefish failed to respond to the prey within 5 min, it was returned to its home tank and tested again the following day (mean age at choice was 9.8 ± 0.3 days). The statistical significances of prey choices were analyzed using chi-squared tests using StatXact 7.0 (Cytel

Software ©) with a Bonferroni correction for multiple tests using the same control group.

RESULTS

Prior exposure to the odors of prey had a significant effect on later preference for the sight of prey. Naïve hatchlings (controls) preferentially attacked shrimp (15 shrimp: 6 crabs), as did hatchlings previously exposed to crab odors (16 shrimp: 5 crabs). Hatchlings previously exposed to mollusk odors (non-food) showed no visual prey preference (10 shrimp: 11 crabs) while hatchlings exposed to shrimp odors preferentially attacked crabs (7 shrimp: 18 crabs). The preferences of both the mollusk odor group and the shrimp odor group were significantly different from the preferences of the control group of naïve hatchlings (mollusks: $\chi^2(1, n = 42) = 5.83, p < 0.05$; shrimp: $\chi^2(1, n = 46) = 19.42, p < 0.001$).

Cuttlefish hatched after different numbers of days of odor exposure; all cuttlefish exposed to odors for no more than 3 days (value determined from post hoc observations) preferred to attack shrimp (Fig. 1). For cuttlefish that hatched after more than 3 days in the experimental apparatus, control cuttlefish exposed to no odors ($n = 4$; 17) and cuttlefish exposed to the odors of crabs ($n = 14$; 7) preferentially attacked shrimp. Cuttlefish exposed to mollusk odors ($n = 6$; 15) showed no prey attack preference while cuttlefish exposed to shrimp odors ($n = 5$; 20) preferentially attacked crabs. There was no

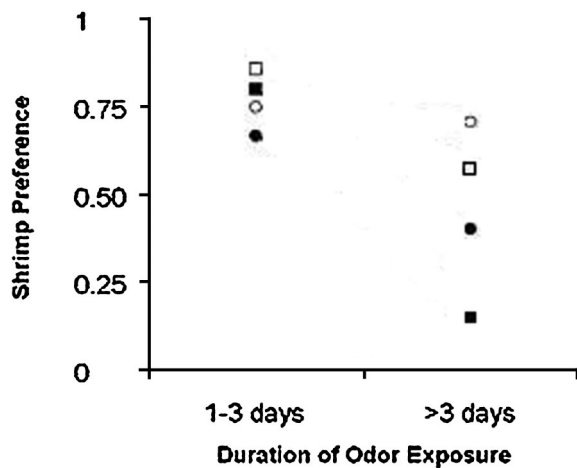


FIGURE 1 Ratio of shrimp preference in function of the odor exposure duration. Control cuttlefish exposed to no odors (open circles, $N = 4, 17$) and cuttlefish exposed to the odors of crabs (open squares, $N = 14, 7$) preferentially attacked shrimp. Cuttlefish exposed to mollusk odors (filled circles, $N = 6, 15$) showed no prey attack preference while cuttlefish exposed to shrimp odors (filled squares, $N = 5, 20$) preferentially attacked crabs.

correlation between exposure time and feeding motivation (age of the first attack with a daily presentation of prey since the age of 7 days) (coeff. = 0.19; $p = 0.006$).

DISCUSSION

Exposure to the odors of prey influences later prey choice by sight in cuttlefish. In these vision-only prey choice tests, naïve cuttlefish housed in natural seawater but not exposed to any prey odors preferentially attacked shrimp rather than crabs. An initial preference for shrimp has been documented previously (Darmaillacq et al., 2004). This preference remained intact after exposure to the odors of crabs, a less-preferred prey of young cuttlefish. Surprisingly, this preference disappeared after exposure to the odors of mollusks, which are not prey of young cuttlefish, and was reversed after exposure to the odors of shrimp, the preferred prey.

Several different hypotheses could explain these surprising findings. First, stress during embryonic development could have affected the cuttlefish's food preferences. In natural conditions, eggs are moved by water flux, but in the current experiment, to avoid the possibility of odor exposure after hatching, eggs were removed each night. Thus, eggs were handled twice daily and the last cuttlefish to hatch were handled more than the first ones to hatch. This stress could have been associated aversively with odors. Prenatal stress is known to affect, for example, mate selection in rodents (Meek, Schulz, & Keith, 2006), but in the current experiment, stress alone did not modify the food preferences of all the cuttlefish, since the control cuttlefish and those exposed to crabs maintained their natural preference for shrimp. This hypothesis also fails to address the observed interaction between sensory systems: odor exposure and sight preference.

Second, it is known that information from one sensory modality can influence behavior in another sensory modality ("cross-modal effects"; VanderSal, & Hebets, 2007). In naïve cuttlefish, prior exposure to the odors of crabs resulted in fewer pinches and quicker subduing of crabs during later predation events (Boal et al., 2000), and in predatory jumping spiders, the presence of chemical cues from ants increased motor activity and visual attentiveness (VanderSal & Hebets, 2007). In both of these examples, information from one sensory modality appeared to enhance readiness for predatory behavior involving other sensory modalities. In chicks, the presence of odors from insect warning displays induced color aversions that were otherwise hidden (Rowe & Guilford, 1996), in jumping spiders, the presence of a seismic stimulus in the environment improved color discrimination learning (Clark, Jackson, & Cutler, 2000),

and in avian predators, sound improved visual discrimination learning (Rowe, 2002). In these three examples, stimuli presented within one sensory modality influenced learning using a different sensory modality. Clearly, salient information from different sensory modalities are integrated within the central nervous system. All brains engage this multisensory integration strategy at multiple levels of the neuraxis (Calver, Spence, & Stein, 2004). In cuttlefish brain, both visual and olfactory information converge in the superior frontal lobes (from optical lobes for visual information and from brachial lobes for chemical information; Nixon and Young, 2003), so each type of sensory information could influence the other in these areas of integration. The pattern of influence observed here seems surprising, however: exposure to preferred prey induced an aversion to that same prey type. This aversion was not induced with visual exposure during embryonic development (Darmaillacq, Chichery, Dickel, et al., 2006; Darmaillacq, Chichery, Shashar, et al., 2006).

Third, external stimulation can affect the course of embryonic development and lead to novel behavior (Lickliter & Harshaw, 2010). Rearing eggs in artificial conditions is already a massive environmental change for a developmental system, and in this experiment, we also introduced chemical stimuli during the late phase of embryonic development. Sensory stimulation early in development can interact with specific organismic factors, such as the stage of organization of the sensory systems, to guide and constrain the developmental course of a species' perceptual preferences. Any changes in these basic processes can lead to modifications in typical behavior of the species, such as diet preference (Lickliter, 1995, 2005). This hypothesis appears to be the most consistent with our results.

We conclude that changes occurring during development can act on phenotypic plasticity, that these effects can extend across sensory modalities, and that they could lead to ecologically relevant, potentially adaptive, novel behavior. We believe our findings merit closer scrutiny in a wide range of species.

NOTES

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