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Food imprinting, new evidence from the cuttlefish *Sepia officinalis*

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Imprinting provides precocial offspring with an efficient means to optimize their subsequent behaviours. We discovered food imprinting using a sophisticated invertebrate model: the cuttlefish. We showed that early juveniles preferred the prey to which they have been visually familiarized, when the amount of information was sufficient and only if such familiarization occurred during a short sensitive period. We also demonstrated that the effects of visual food imprinting overcame those of the first food ingested. Our study shows that visual imprinting is a critical process in animals, surpassing more direct reward experiences that occur outside the critical exposure period.

Keywords: food imprinting; prey selection; cuttlefish; learning; ontogenesis

1. INTRODUCTION

Imprinting was one of the first phenomena tackled by the field of ethology. Beyond a theoretical interest, it has considerable ecological and evolutionary significance (Immelmann 1975). Originally, it was studied in precocial birds and referred to the formation of social bonds between offspring and parent (Lorenz 1935). Imprinting meets particular criteria different from other kinds of learning. First, it occurs even without any obvious reinforcement (this point was widely debated). Second, it occurs during a sensitive period, typically shortly after birth or hatching. Third, imprinting is thought to be persistent into adulthood (Lorenz 1935; Immelmann 1975; Bolhuis 1991). Subsequently, the term ‘imprinting’ has been applied to a wide variety of behaviours (e.g. habitat preference, song learning or host imprinting) and investigations have also been carried out with insects (Jaisson 1975), fish (Russock 1991) and mammals (Horn 1985). Food imprinting was originally suggested to occur in the snapping turtle, *Chelydra serpentina*, which showed a preference for the food first experienced early in the post-hatching period (Burghardt & Hess 1966; Burghardt 1967). Whether food imprinting really occurred is still controversial, partly because in these and other studies (Punzo 2002), the establishment of food preference was

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based on food ingestion (i.e. on a possible association between the food item and positive reinforcement). Since imprinting could be distinct from associative learning, subsequent studies on other animals reported a primacy effect for food preference but did not mention whether imprinting had occurred (Allen & Littleford 1955; Capretta 1969; Rabinowitch 1969; Apfelbach 1973; Burghardt 1992).

Newly hatched cuttlefish, *Sepia officinalis*, innately recognize, prefer and capture shrimp-like prey throughout the first week of life (Wells 1958, 1962; Darmaillacq *et al.* 2004b). Nevertheless, visually exposing crabs—a naturally non-preferred prey—to juvenile cuttlefish for 5 h immediately after hatching is sufficient to change this innate preference. The switch in preference occurs without reinforcement (i.e. no consumption of the prey), and is detectable only three days later, when the cuttlefish first feed (Darmaillacq *et al.* 2006). This result is apparently paradoxical, since previous studies failed to show even a 24 h retention of an associative learning task in cuttlefish before the third month of life (Messenger 1973; Dickel *et al.* 2000, 2001). Taken together, these results suggest that the establishment of prey preference and the acquisition of associative learning are separate processes. Here, we examined food imprinting considering the original criteria for classical imprinting, using an invertebrate model—the cuttlefish. Since induction of prey preference without food reinforcement has already been demonstrated (Darmaillacq *et al.* 2006), we aimed to determine whether the other two criteria for imprinting could be met. The first two experiments assessed the sensitive period for food imprinting, and a third experiment investigated the persistence of the early experience on subsequent prey preference.

2. MATERIAL AND METHODS

Newborn cuttlefish hatch gradually throughout the night (Paulij *et al.* 1991). Consequently, hatchlings used in the following experiments were collected immediately at sunrise (*ca* 6.00; day 0) and individually isolated in opaque black plastic tanks (7 × 8 × 8 cm) provided with running sea water (20 ± 1 °C). They were then left to acclimatize for 2 h, before the start of the experiment, under day light conditions. Unless otherwise specified, cuttlefish were not fed until the end of the experiment. In each experiment, the eggs were obtained from a single egg-laying bout from one female.

Sepia officinalis hatchlings do not benefit from any parental care. They hatch with yolk reserves that allow them not to eat and hence to survive until they can ingest their first meal (von Boletzky 1975, 2003; Dickel *et al.* 1997). This short period of time after hatching is crucial to their survival and is likely to be a sensitive period for food imprinting to occur. According to Bateson & Hinde (1987), the term sensitive period, ‘implies a sharply defined phase of susceptibility; if the relevant experience is provided before or after the period, no long-term effects are supposed detectable’. First, to determine the minimal exposure time required to change innate preference, five crabs, *Carcinus* sp. (carapace width 2–3 mm), were put into the tank of each cuttlefish ($n=95$, mean dorsal mantle length, $DML \pm s.e.m. = 8.6 \pm 0.4$ mm,) for 15, 30, 60, 90 or 120 min, starting at 8.00, after the 2 h period of acclimatization. We also tested the effect of the number of prey on the familiarization efficiency by presenting 5 or 20 crabs for 1 h to each juvenile ($n=35$). At the end of the exposure period, the crabs were counted to verify that no cuttlefish had eaten, and were gently removed. Control cuttlefish had no crab-exposure at hatching. The feeding preference of each cuttlefish was assessed on day 3 in a two-way choice test between crabs and shrimps, *Crangon crangon* (detailed in Darmaillacq *et al.* 2004b). Subsequently, to determine the period at which a visual crab exposure was the most effective, we tested 54 cuttlefish ($DML = 8.5 \pm 0.2$ mm,) in the following experiment. After the 2 h acclimatization period, they were assigned to one of four groups, successively familiarized with five crabs for a 2 h

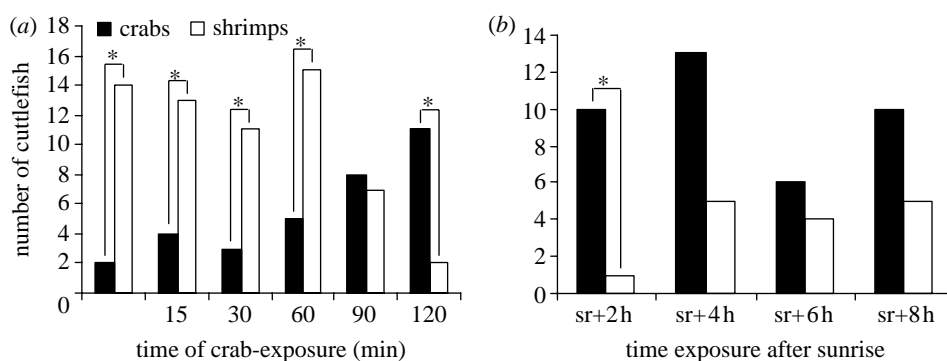


Figure 1. Preference for crabs and shrimps by 3-day-old cuttlefish. (a) After no crab exposure ($n=16$) and after different lengths of a five-crab exposure: 15 min ($n=17$), 30 min ($n=14$), 60 min ($n=20$), 90 min ($n=15$) or 120 min ($n=13$). (b) By period of familiarization, post-hatching. The first day of life was divided into 2 h periods, 2 h after sunrise (sr), during which cuttlefish were exposed to five crabs (sr+2 h, $n=11$; sr+4 h, $n=18$; sr+6 h, $n=10$; sr+8 h, $n=15$). χ^2 -exact test: * $p<0.05$.

period beginning on their first day of life, from 8.00 to 16.00. Their feeding preference was subsequently tested on day 3 in a two-way choice test (mentioned earlier).

Imprinting should be persistent (Immelmann 1975). To test this hypothesis for food imprinting, cuttlefish ($n=29$, DML= 9.0 ± 0.1 mm,) were familiarized with five crabs for 5 h on day 0 and then were given a single shrimp of suitable size (total length 8 mm), on day 3 ($n=15$). Control cuttlefish were only given a shrimp on day 3 ($n=14$). Their feeding preference was assessed on day 7.

The choice tests were performed by an experimenter blind to the experimental status of the animals. Cuttlefish were tested individually and only once. All data were analysed with non-parametric tests (Siegel & Castellan 1988) and computed using STATXACT-6. Differences between the values were considered significant at $p<0.05$. All tests were two-tailed.

3. RESULTS

When exposure time was 60 min or less, juveniles significantly preferred shrimps to crabs (15 min: $\chi^2=4.76$, $p<0.05$; 30 min: $\chi^2=4.57$, $p<0.05$; 60 min: $\chi^2=5$, $p<0.05$; figure 1a), just as did cuttlefish with no crab exposure ($\chi^2=9$, $p<0.01$; figure 1a). When exposure time was 90 min, juveniles showed no significant preference ($\chi^2=0.07$, $p=0.8$); however, at 120 min exposure, they significantly preferred crabs to shrimps ($\chi^2=6.23$, $p<0.05$; figure 1a). Furthermore, cuttlefish tended to prefer crabs to shrimps when the exposure time was 60 min, if the number of crabs was increased from 5 to 20 (Fisher's exact test, $p<0.05$).

A significant preference for crabs was demonstrated only by cuttlefish familiarized with crabs within 2 h after sunrise ($\chi^2=7.36$, $p<0.01$; figure 1b). The familiarization efficiency appeared lower in the 4–6 h window after sunrise ($\chi^2=3.56$, $p=0.059$).

Cuttlefish familiarized with five crabs for 5 h and then given a shrimp on day 3 significantly preferred crabs over shrimps on day 7 ($\chi^2=5.4$, $p<0.05$; figure 2), while control cuttlefish that only ate one shrimp on day 3 significantly preferred shrimps to crabs ($\chi^2=4.57$, $p<0.05$).

4. DISCUSSION

First, our results show that the efficiency of the familiarization depends on the length of the exposure as well as the quantity of prey exposed, i.e. the information flow perceived during the familiarization. Then, we have shown that cuttlefish significantly preferred crabs only when such familiarization

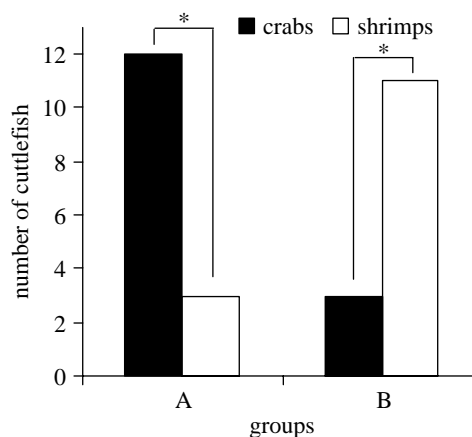


Figure 2. Preference for crabs and shrimps by 7-day-old cuttlefish. In group A, cuttlefish were familiarized with crabs for 5 h post-hatching and then first fed one shrimp, on day 3 ($n=15$); in group B, cuttlefish without crab exposure were fed one shrimp on day 3 ($n=14$). χ^2 -exact test: * $p<0.05$.

occurred during a 2–4 h window, which gradually terminates within 6 h after sunrise. These results suggest the existence of a sensitive period that closes early within the first day of life, whatever the hour of the night the cuttlefish have hatched. Second, in this study, we pointed out that the primacy of the early familiarization clearly outweighed untrained preferences. This effect could account for lasting feeding preferences observed in adult cuttlefish (Darmaillacq *et al.* 2004a).

On the basis of three main criteria—absence of reinforcement, sensitive period and primacy effect of early familiarization—we can state that food imprinting occurs in *S. officinalis*. Cuttlefish will eat prey other than the imprinted one, but preferentially select the imprinted prey, as for mate preference established through sexual imprinting (Immelmann 1975). Since there are possible changes in food availability, rigid invariability in prey selection is likely to be disadvantageous. Accordingly, preferring the prey to which they have been visually familiarized could allow early juveniles to maximize their chances of survival immediately after hatching. Food imprinting in animals that do not benefit from parental care constitutes an advantageous compromise between selecting food by trial/error learning—which can be long and

risky—and by innate processes that restrict the young to feed on only one kind of prey.

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