

Patricia D. Cole · Shelley A. Adamo

Cuttlefish (*Sepia officinalis*: Cephalopoda) hunting behavior and associative learning

Received: 24 March 2004 / Accepted: 16 June 2004 / Published online: 11 August 2004
© Springer-Verlag 2004

Abstract Because most learning studies in cephalopods have been performed on octopods, it remains unclear whether such abilities are specific to octopus, or whether they correlate with having a larger and more centrally organized brain. To investigate associative learning in a different cephalopod, six sexually mature cuttlefish (*Sepia officinalis*) participated in a counterbalanced, within-subjects, appetitive, classical conditioning procedure. Two plastic spheres (conditioned stimuli, CSs), differing in brightness, were presented sequentially. Presentation of the CS+ was followed 5 s later by a live feeder fish (unconditioned stimulus, US). Cuttlefish began to attack the CS+ with the same type of food-acquisition seizures used to capture the feeder fish. After seven blocks of training (42 presentations of each CS) the difference in seizure probability between CS+ and CS− trials more than doubled; and was found to be significantly higher in late versus early blocks. These results indicate that cuttlefish exhibit autoshaping under some conditions. The possible ecological significance of this type of learning is briefly discussed.

Keywords Mollusca · Invertebrate · Foraging · Autoshaping · Associative conditioning

Introduction

Cephalopods (Phylum Mollusca) first appeared in the late Cambrian Era, about 60 million years before the earliest vertebrates (Teichert 1988). Their closest relatives include gastropods (e.g., snails) and bivalves (e.g., clams). Given

such a phylogeny, one might expect creatures with a small, distributed central nervous system, and hence limited learning abilities. However, coleoid cephalopods (octopus, squid and cuttlefish) have a large, coalesced central nervous system (Budelmann 1995). These large brains show a very different neural anatomy from those of vertebrates, reflecting their molluscan heritage (Budelmann 1995). Investigations of octopod species suggest extensive learning abilities (see reviews Mather 1995; Boal 1996a). However, because very few studies (e.g., Dickel et al. 2000) have tested the associative learning abilities of cephalopods outside the octopods, it remains unclear whether such abilities are specific to only some octopus species, or whether they correlate with having a larger and more centrally organized brain. For example, observational learning has been reported in octopus (Fiorito and Scotto 1992), but has not been found in cuttlefish (Boal et al. 2000). Cuttlefish also lack the ability to recognize individual conspecifics (Boal 1996b), but do exhibit some forms of spatial learning (Karson et al. 2003).

During one kind of associative learning procedure, appetitive classical conditioning, a neutral stimulus (the conditioned stimulus or CS), is presented to an animal just prior to the presentation of food (the unconditioned stimulus or US). After repeated presentations of the CS followed by the US, some animals begin to behave towards the CS as if it were the US. For example, pigeons will attempt to “eat” the CS. This phenomenon is called autoshaping (Brown and Jenkins 1968). Under some circumstances pigeons are so compelled to peck at the neutral stimulus (e.g., a lighted disc) that they ignore the food when it appears (Hearst and Jenkins 1974). However, some vertebrates, especially some mammals (Tomie et al. 1989; Moore 2004), behave in a more adaptive manner by treating the CS as if it were a sign indicating the imminent arrival of food, rather than treating it as if it were food. This behavior has been called sign-tracking (Hearst and Jenkins 1974; but see Moore 2004). The ability to modify reflexive behavior patterns represents a crucial step in the

Electronic Supplementary Material Supplementary material is available for this article at <http://dx.doi.org/10.1007/s10071-004-0228-9>

P. D. Cole · S. A. Adamo (✉)
Department of Psychology, Dalhousie University Halifax,
Nova Scotia, Canada
e-mail: sadamo@dal.ca
Tel.: +1-902-4948853
Fax: +1-902-4946585

evolution of learning and the adaptability of behavior (Moore 2004).

Purdy et al. (1999) found that cuttlefish exhibit sign-tracking. Purdy et al. (1999) presented cuttlefish (*Sepia officinalis*) with a small, flashing light, followed by the delivery of a feeder fish. The cuttlefish oriented and approached the light but did not direct consummatory behavior towards it. We test whether cuttlefish exhibit autoshaping under different experimental conditions.

Methods

Subjects

Laboratory-bred cuttlefish were obtained from the National Resource Center for Cephalopods, Galveston, Tex., USA. Cuttlefish (4 female and 2 male) were individually housed and tested at the Dalhousie University Aquatron facilities, N.S., Canada, in an open seawater system. Water temperature was maintained at 21°C. The light cycle was 12L:12D. Cuttlefish had mantle lengths ranging from 10.8 cm to 13.3 cm and were sexually mature. Sex was determined by the presence of testis or ovary during necropsy.

Stimuli used as signals for food presentation

Cuttlefish were presented with live goldfish as food targets (US), which they avidly hunted (personal observation). We used two CSs. Each CS consisted of a dense plastic sphere, either dark blue or light pink, and 3.8 cm in diameter. Spheres were attached to the end of a 25-cm piece of clear, rigid, plastic tubing filled with brown gravel.

Apparatus

Home-tanks were made of opaque molded fiberglass (59 cm wide × 89 cm long × 46 cm high). Water depth was maintained at 41 cm. Each home tank contained six rocks (5–8 cm in length), one piece of PVC tubing measuring 11.4 cm in diameter and 20 cm in length, and one round plastic floating bucket-lid (25 cm in diameter).

Conditioning sessions took place in an experimental tank, located in a room adjacent to the home tank room. The experimental tank had the same dimensions as the home tank, however its bottom was covered with brown gravel, to a depth of approximately 2 cm, and a 23 cm wide × 10 cm deep pile of rocks spanned the width of the tank along the centerline (individual rocks measured between 8 cm and 15 cm in length). A rectangular plate (30.5 cm × 58.5 cm) of dark Plexiglas was mounted over one end of the tank to produce a low-light area to which the cuttlefish could retreat. The plastic tubing assembly that supplied seawater to the experimental tank was outfitted with an in-line funnel, and served as the US

delivery system. The tubing assembly entered the water 30 cm away from the back wall. The end of the tube was submerged 6 cm below the water's surface.

The experimenter manually controlled all experimental contingencies from behind an opaque curtain, and remained behind this curtain for the duration of each session to prevent the possibility of cueing.

All conditioning trials were recorded on Hi8 video. Video output from the camera was sent to a color monitor, which allowed the experimenter to observe the sessions in realtime from behind the opaque curtain. Timers were used to mark the duration of trials.

Procedure

To familiarize the animals with the experimental tank and the process of being moved, they were individually placed in the experimental tank for a period of 35–40 min once a day for 5 consecutive days. During each stay six food deliveries were made at random time intervals, via the US delivery tube. After a 2-day break, conditioning trials began. Each subject's conditioning session occurred at approximately the same time each day (± 30 min), before the individual's daily feeding, and consisted of six trials.

A counterbalanced, within-subjects design was used. Each subject was exposed to both CSs; half the subjects were randomly assigned to the CS dark (blue) +/CS light (pink) – condition, and the other half to the CS dark –/CS light + condition. The stimulus presentation order (CS+ vs CS–) was randomized for each 2-day block (of 12 trials).

For conditioning sessions, animals were placed individually in the experimental tank, and allowed to rest for 5 min before any trials commenced, then for each trial a CS was lowered into the tank via a pulley assembly. On the first leg of its descent, the CS stopped momentarily 16 cm above the water's surface (to avoid a fear-inducing splash), and then descended to its final location 11 cm below the water's surface. On CS– trials, the CS was removed after 10 s; on CS+ trials, the presentation of the CS was followed 5 s later by the appearance of the US (fish). Actual delivery of the US took approximately 2 s. The CS+ was also removed 10 s after its initial presentation, which resulted in a 3-s overlap of CS+ and US. Trials were separated by a 5-min rest period.

After each session, the cuttlefish was returned to its home tank and, over the next hour, fed to satiation on previously frozen prawns. Food was not provided again until the next day's conditioning session.

Behavioral measures

Cuttlefish engage in a relatively fixed sequence of hunting behaviors, which include attending, orienting, positioning within striking distance, and finally striking with the tentacles (Messenger 1968). They are decapods, possessing eight arms and two tentacles. The tentacles are normally retracted and only deployed during hunting.

Fast-moving prey is seized with the tentacles, and tentacles and prey are then drawn back towards the buccal cavity and enveloped in the ring of eight arms (Messenger 1968). We recorded whether cuttlefish seized the CS with their tentacles.

Results

Cuttlefish always seized and ate the fish (US). On the very first presentation of the stimuli (trial 1 of the 12 trials in total that make up block 1), four out of the six cuttlefish seized a CS (two seized the CS+, and two seized the CS−). One cuttlefish continued to attack both CSs on nearly all the remaining trials of block 1. However, the other five cuttlefish did not seize a CS on the second trial or any of the following 11 trials during block 1. Attacks culminating in tentacle seizures directed towards the CS+ began to gradually increase in frequency relative to seizures directed at the CS− during block 3 (after 36 trials, half paired, half unpaired). Figure 1 represents a comparison of seizure probabilities across all training blocks (each block represents a total of 12 randomized trials, 6 CS+ and 6 CS−). These learning curves indicate a more than threefold increase in the probability of seizures directed to the CS+, between the first and last training blocks. They also indicate that by the last training block, seizures were more than twice as likely to be directed to the CS+, rather than to the CS−. For the purpose of statistical analysis, mean seizure probability difference-scores [(probability of seizure to CS+) − (probability of seizure to CS−)] for early (combined blocks 1 and 2, $M=-0.04$, $SD=0.14$) versus late (combined blocks 6 and 7, $M=0.26$, $SD=0.19$) conditioning blocks were compared using a simple repeated-measures t -test. The result was significant, $t_{(5)}=3.70$,

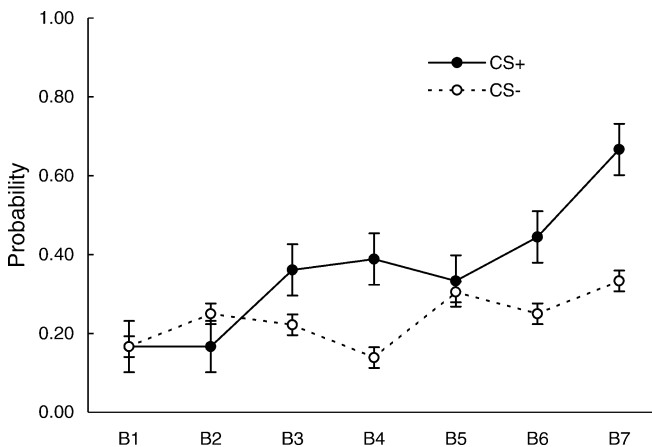


Fig. 1 The probability (\pm SE) of tentacle seizures across seven conditioning blocks of 12 trials each for $n=6$ cuttlefish (*Sepia officinalis*) exposed to a light or dark CS. The appearance of one stimulus was followed by the presentation of a live feeder goldfish (CS+), while the other was not (CS−). This video shows a CS− trial followed by a CS+ trial from block 7 for cuttlefish no. 7. During the CS− trial the cuttlefish initially moves away, eventually orients to, but does not approach the CS. During the CS+ trial, the cuttlefish's first strike is directed towards the CS, and the second strike captures the fish (US).

$P=0.01$, indicating that seizures were significantly more likely to be directed towards the CS+, rather than the CS−, as a result of conditioning.

Qualitatively, the seizures directed towards the CS+ resembled the seizures elicited by the fish (US) in that cuttlefish seized the CS+ with their tentacles. Typically they released the CS+ after the appearance of the fish (US) and directed their hunting behavior towards the live food. However, there were instances of cuttlefish seizing the CS+, and/or continuing to maintain their hold on it, even after the appearance the fish (US). A CS− and CS+ trial can be seen in Fig. 1 of the Electronic Supplementary Material.

Discussion

Cuttlefish were not required to make any response in order to receive a fish. Nevertheless, their tendency to attack the CS+ increased significantly. Qualitatively, the CS seizures appeared identical to the seizures elicited by fish. Therefore, the cuttlefish exhibited autoshaping.

In contrast to our study, Purdy et al. (1999) reported that only the first two components of cuttlefish hunting behavior, attention and orientation, were directed significantly more often to the CS+ (a flashing light). However, they did note that one cuttlefish repeatedly attempted to seize the CS+ (Purdy et al. 1999).

There are two possible reasons for the difference between our study and Purdy et al. (1999). First, our CS, being a solid object in the water, may have had more features in common with prey than does a flashing light. In vertebrates, the degree of similarity between the CS and US influences how the animal responds to the CS (Tomie et al. 1989). Second, our CS–US interval was shorter, 5 s versus 30 s. In pigeons, shorter CS–US intervals are more likely to produce autoshaping (Terrace et al. 1975). It would be interesting to test whether a shorter CS–US interval would elicit autoshaping in the experimental paradigm of Purdy et al. (1999).

By seizing the CS, cuttlefish delayed their hunting of the US (fish). Autoshaping behavior reduces feeding efficiency in other animals too (Tomie et al. 1989). Purdy et al. (1999) suggest that in the natural environment sign-tracking could be adaptive by allowing cuttlefish to associate certain stimuli (e.g., specific rock crevices) with the presence of prey. Autoshaping may also be adaptive in this context. For example, attacking a hidden shrimp by striking into a rock crevice, may be an adaptive strategy if shrimp often hide in a particular crevice. The timing between the appearance of a recurring environmental feature (e.g., specific rock crevices) and capturing food may be important in determining whether the response of the cuttlefish to these features (the CS) is a ready-to-hunt posture (sign-tracking) or a sometimes successful speculative hunting response (autoshaping). Moreover, cuttlefish can also learn not to strike at prey under some conditions (e.g., Messenger 1973) and this may also shape the response in the field. More studies are needed to

determine the role of associative learning in cuttlefish behavior.

Acknowledgements This research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to S.A. Adamo. This study was approved by the Dalhousie University Animal Care Committee.

References

- Boal J (1996a) A review of simultaneous visual discrimination as a method of training octopuses. *Biol Rev* 71:157–190
- Boal J (1996b) Absence of social recognition in laboratory-reared cuttlefish (*Sepia officinalis*) L. (Mollusca: Cephalopoda). *Anim Behav* 52:529–537
- Boal J, Wittenberg K, Hanlon R (2000) Observational learning does not explain improvement in predation tactics by cuttlefish (Mollusca: Cephalopoda). *Behav Process* 52:141–153
- Brown P, Jenkins H (1968) Auto-shaping the pigeon's key peck. *J Exp Anal Behav* 11:1–8
- Budelmann B (1995) The cephalopod nervous system: what evolution has made of the molluscan design. In: Breidbach O, Kutsch W (eds) *The nervous system of invertebrates: an evolutionary and comparative approach*. Birkhäuser Verlag, Basel, pp 115–136
- Dickel L, Boal J, Budelmann B (2000) The effect of early experience on learning and memory in cuttlefish. *Dev Psychobiol* 36:101–110
- Fiorito G, Scotto P (1992) Observational learning in *Octopus vulgaris*. *Science* 256:545–547
- Hearst E, Jenkins HM (1974) Sign-tracking: the stimulus-reinforcer relation and directed action. Psychonomic Society, Austin
- Karson M, Boal J, Hanlon R (2003) Experimental evidence for spatial learning in cuttlefish (*Sepia officinalis*). *J Comp Psychol* 117:149–155
- Mather J (1995) Cognition in cephalopods. *Adv Study Behav* 24:317–353
- Messenger J (1968) The visual attack of the cuttlefish, *Sepia officinalis*. *Anim Behav* 16:342–357
- Messenger J (1973) Learning in the cuttlefish, *Sepia*. *Anim Behav* 21:801–826
- Moore BR (2004) The evolution of learning. *Biol Rev* 79:301–335
- Purdy J, Roberts A, Garcia C (1999) Sign tracking in cuttlefish (*Sepia officinalis*). *J Comp Psychol* 113:443–449
- Teichert C (1988) Main features of cephalopod evolution. In: Clark M, Trueman E (eds) *The Mollusca: paleontology and neontology of cephalopods*. Academic, San Diego, pp 215–288
- Terrace H, Gibbon J, Farrell L, Baldock M (1975) Temporal factors influencing the acquisition of an autoshaped keypeck. *Anim Learn Behav* 3:53–62
- Tomie A, Brooks W, Zito B (1989) Sign-tracking: the search for reward. In: Klein S, Mowrer R (eds) *Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory*. Erlbaum, Hillsdale, pp 191–223