Exploration and Habituation in Intact Free Moving Octopus vulgaris

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Despite the huge numbers of studies published on the learning of cephalopod mollusks, studies on non-associative learning are scarce. We tested non-associative learning (habituation) and exploration in Octopus vulgaris in two different studies using a prey-shaped object (Study A) and inanimate objects and food objects (Study B). Study A consisted of the repeated presentation of a prey-like stimulus, which 23 subjects could only explore visually. In study B, 14 octopuses were presented two Lego blocks (one black and white with a smooth surface, one a blue "snowflake" with a rough surface) and two food items, one preferred (clams) and one non-preferred (mussels) inside their home tanks. As hunger is a motivational factor for exploratory behavior, different levels of food satiation (feeding 2h or 24 h prior to experiments) were tested. Within trial habituation was clearly documented in both experiments. In study A across trials habituation was found for all animals, whereas it was only significant in 5 animals in Study B.

The acquisition of knowledge about the environment and its changes is probably one of the principal tasks each organism has to face. The wealth of ethological, physiological and morphological data, and the lifestyle and learning capacities of Octopus vulgaris (Hanlon & Messenger, 1996; Nixon & Young, 2003; Wells, 1978) make this invertebrate species a perfect subject for studies to investigate the interplay between exploration and habituation. Exploration is the extraction of information from the surrounding environment (Hutt, 1966). During this process an animal can repeatedly encounter specific stimuli. This then is presumed to lead to habituation, the waning of response to a stimulus that is continuous or repeatedly present (Baldwin & Baldwin, 1997). The importance of exploration and habituation lies not only in the establishment of a familiar area but also in being able to monitor changes in it. For free moving animals in the wild the maintenance of familiarity with the environment necessitates regular inspections (Russell, 1983). On the other hand, captive animals face lack of changes and a rather stimulus-deprived setting. In both situations, novel, non-threatening stimuli evoke exploration in intact animals of all phyla (see Archer & Birke, 1983).

Exploration is hard to define; it has historically been determined, in effect, by what could be easily measured (Renner, 1990). This resulted in a conflict of definitions and a debate on motivational states and behaviors of exploration (see Archer & Birke, 1983; Power, 2000). Renner (1990) criticized the fact that exploration was often treated as the animal’s equivalent to the Brownian motion in mol-
ecules. He claimed that evidence shows the functional significance of exploration so more investigations have to be done on the interplay of learning and exploration.

The inner drive to search for conspecifics at times of reproduction and foraging due to hunger are probably the key motivational factors for exploration (Toates, 1983). Additionally, stimulus seeking to avoid boredom is an important factor modifying intrinsic as well as extrinsic exploration (Toates, 1983). This extrinsic exploration, response-obtaining information about a conventional reinforcer with its seemingly obvious motivational cause, has received little attention (Russell, 1983). Laboratory research on birds and mammals has focused on intrinsic exploration instead (e.g., Costall, Jones, Kelly, Naylor, & Tomkins, 1989; Heinrich, 1995; Huber, Rechberger & Taborsky, 2001; Karl, Burne, & Herzog, 2006), where intrinsic exploration is defined as a behavior directed towards a stimulus of little biological importance (Russell, 1983). Early studies on intrinsic exploration in rats (for a review see Toates, 1983) and later work on farm animals (Day, Kyriazakis, & Lawrence, 1994; Wood-Gush, Stolba & Miller, 1983) focused on the effect of hunger or punishment on exploration. Most of the time rats did not reduce or cease exploration when food deprived, even if food was offered, and they would also endure electrical shocks for an opportunity to explore a Dashiell maze filled with novel objects.

Non-associative learning (habituation and sensitization) is an important process to modify exploratory behavior (e.g., Balwin & Baldwin, 1997). Habituation, one of the most basic manifestations of behavioral plasticity, is defined as a decrease of response to a repeated stimulus (Thompson & Spencer, 1966). It has been studied less in whole animals, than in semi-intact ones or even isolated nerve cells (e.g., Abramson, 1994; Brown, 1998, 1997; Chase, 2002; Hawkins, Cohen, Greene & Kandel, 1998; Mongeluzi & Frost, 2000; Zaccardi, Traina, Cataldo & Brunelli, 2001). In contrast to habituation, sensitization is normally seen as an aversive or positive reaction towards a noxious or worthwhile stimulus (Hutt, 1983; Zaccardi & colleagues, 2001). Sensitization can lead to dishabituation and override the initial habituation process. This interplay between habituation and sensitization is a manifestation of behavioral flexibility found in all of the animal kingdom. Studies on these behaviors have provided some of the most important and fundamental insights into the process of learning and showed that non-associative learning and associative learning share several basic principles.

Octopuses are generalist predators and detect their prey both visually and by touch (Hanlon & Messenger, 1996). The loss of the external molluscan shell is linked to their more complex system for predator avoidance, an increase in brain size, the evolution of more effective sense organs, all of which result in higher cognitive abilities (Hanlon & Messenger, 1996). Octopuses were always believed to be curious animals. In the first reports on the octopus’ natural history, Aristotle claimed that the octopus is one of the most stupid animals, as it would always come out to examine a novel stimulus provided by fisherman. Yet as he also pointed out, this exploration ended with the entry of the animal into the human food chain. More recent studies (Kuba, Byrne, Meisel, & Mather, 2006; Kuba, Meisel, Byrne, Griebel & Mather, 2003; Mather & Anderson, 1999) focused on behavioral processes during object exploration in octopuses and initiated research
of the interplay of exploration, habituation and exploratory play in these animals. For the first time, an invertebrate was shown to engage in playful interactions with objects.

Choosing *Octopus vulgaris* for research on exploration and habituation gives us the opportunity to fill a gap in our knowledge of this otherwise behaviorally and neurophysiologically well studied animal (Hanlon and Messenger, 1996; Wells, 1978) to inquire into the simplest aspect of behavioral plasticity. There have been numerous studies on learning and other cognitive abilities in the genus *Octopus* (e.g., Boal, 1996; Fiorito & Scotto, 1992; Hanlon & Messenger, 1996; Nixon & Young, 2003; Wells, 1978). Nevertheless, studies on non-associative learning are scarce (Angermeier & Dassler, 1992; Kuba, Zullo, Byrne & Hochner, in press; Kuba, Zullo, & Hochner, 2005; Mather & Anderson, 1999; Wells & Wells, 1957). In contrast to Wells and Wells’ (1956) claim that octopuses do not habituate to stimuli visually, two recent studies (Kuba et al., 2005; Kuba et al., in submission) showed that octopuses can habituate to a visual stimulus. Interestingly, the subjects in one study (Kuba et al., in submission) showed no significant long-term effects in habituation. The experimental session 24 h after the initial presentation was not significantly different from the first session. This raises the question how octopuses habituate to visual prey stimuli presented over a longer period of time.

One objective of the present study is to learn more about the mechanisms underlying directed exploration in octopuses. In order to do so different levels of feeding motivational state (hungry and satiated) will be used as influences on the octopuses’ response to an extrinsically-linked conventional reinforcer (food) and intrinsically-linked innate objects (non-food objects). Our hypothesis is that there is a clear difference in the exploration and habituation of the animals to food and non-food objects, and this should change according to the different levels of motivation. This will provide information about the difference of intrinsic and extrinsic exploration in octopus.

**Method**

**Subjects**

Subjects were 37 *Octopus vulgaris* (20 females, 17 males) obtained from Naples, Italy (Stazione Zoologica di Napoli), with mantle lengths ranging from 5 - 18 cm. Study A used 23 subjects and study B used 14 subjects. After the experiments 15 subjects died of old age, 5 were given to public aquariums and 17 were set free about 10 km north of the Bay of Naples.

**Maintenance**

The octopuses were held in tanks which were part of a closed circulation system of approximately 4,500 l of artificial sea water with a turnover rate of 24 times per day. Within this system up to 8 subjects were kept individually in 1.0 x 0.6 x 0.5 m and 1.0 x 0.7 x 0.5 glass tanks used for the experiments. Water was filtered with protein-skimmers and biological filters. Air stones produced a weak current and additional aeration in each tank. Illumination was provided by artificial light with a daylight emission spectrum from 0800 to 2000 h. Water temperature was kept at 16 °C in winter and 22 °C in summer, and an escape-proof Plexiglas lid was used to cover the tanks. The octopuses were provided with an enriched environment in their tanks, consisting of sand, gravel, rocks with epigrowth and *Caulerpa sp.* (Dickel, Boal, & Budelman, 2000, have shown in cuttlefish that an enriched environment positively affects not only growth rate but also the acquisition and retention of learning tasks).
Study A

Observations were conducted between Aug. 1999 and Sept. 2002. All sessions took place between 1500 and 2100 hours, were recorded with a digital video camera (Sony DVX 1000 or 2000), and were filmed for 1 h each on 5 days that were at least 4 days apart ($M \pm SD = 10 \pm 6$ days). The video camera was positioned in front of the tank with an operator who followed the movement of the octopus. A second person sat on the floor between the camera and the tank, and attracted the octopuses to the front glass of the tank by moving a life-sized plastic crab model (resembling *Pilumnus* sp., ca. 7.5 cm diameter) mounted on a transparent Plexiglas stick. The model was presented at five positions equidistant to each other (17 cm) along the length of the tank and was held at each position for 3 min according to a restricted random sequence, so that the time at each of the five positions was the same over one hour, to avoid directional bias.

We measured the time the octopus was holding on to the front glass of its tank, actively moving around and looking out using at least one eye. Byrne, Kuba and Griebel (2002) called this behavior ‘curiosity,’ but as no responses to novelty have been defined in this former study, it was called ‘inspection behavior’ in Byrne, Kuba & Meisel (2004). The amount of time spent in each of the following behaviors was recorded: rubbing its mantle on the front glass; resting (which was defined as when it was not moving for more than 20 s); not reacting to the stimulus of the crab-model (the pigmentation on the ventral side of the arms changed into a patchy red and white pattern, the tips of the arms curled up and the pupils were smaller than when it was active, Byrne et al., 2002); being located somewhere else in the tank. These data were recorded for later analysis as bouts of non-inspection behaviour. We checked for habituation within trials by dividing each experimental session into ten min intervals, resulting in 6 intervals of 10 min each. Those were ranked according to the percentage of inspection behavior (Byrne et al. 2002, 2004) and were compared using a Kruskal-Wallis test. Habituation across trials was investigated similarly by comparing the amount of inspection behavior across the five sessions using a Kruskal-Wallis test.

Study B

Observations were conducted between Dec. 2002 and Sept. 2003. All sessions took place between 0900 and 2100 hours and were recorded with a digital video camera (Sony DVX 1000 or 2000). The video camera was positioned in front of the tank and the animals were visually shielded for 30 min prior the experiments with opaque curtains (Figure 1). Subjects were presented two food items (Figure 2), clams and mussels. Personal observation suggested that clams were a favored food item whereas mussels were non-preferred food. Octopuses also received two different inanimate objects made of Lego® blocks (Figure 3). One was a smooth-surfaced cubic block made of black and white Lego® blocks (smooth surfaced object, 7.7 x 4.3 x 5.7 cm). As octopuses were shown to have deficits discriminating colors (Kühn 1950; Messenger, 2001) the black and white Lego® pieces provide maximal contrast. The other object was a single-colored (blue) snowflake-like Lego® construct (8.2 cm axis length), which maximized rough surface areas. In order to position the objects in an equal distance from the octopus at the beginning of each session, three equidistant square openings were cut into the Plexiglas® lid (Figure 1). Using these three openings we were able to compensate for variable octopus locations in the tank and equalize the distance of the animal to the object at the beginning of each experimental session. Each series of sessions lasted for seven consecutive days for each of the two food and non-food objects and all experimental sessions lasted for 30 min. Experiments were carried out either 2 hours after feeding (satiated) or 24h after feeding (hungry). A total of 8 weeks of sessions per animal were filmed. After a week of testing there was a two to three days break before the next session started.
Figure 1. Experimental set-up. Circular marks indicate the three openings in the Plexiglas® lid.

Figure 2. Octopus feeding on a clam
Figure 3. Octopus exploring the smooth surfaced Lego® block

After a two-week period of accommodation to captivity, each animal was first given a session with one food object. Thereafter the presentation of sessions with food and non-food objects alternated. This sequence of the experiments was arranged in order to provide a time interval of at least 21 days before the animal received the same object again.

One person analyzed the film material using prearranged criteria (Bakeman & Gottman, 1997). We designated two levels of exploration: Level 1 was exploratory interaction with an object using one or several arms. Level 2 interactions occurred when the animal had the object within the interbrachial web. Both levels of interaction can be seen as chemotactile exploration, with Level 2 being analogous to the treatment of prey while feeding. We recorded time of initiation and the duration of every contact of octopus to item. Using this information we could analyze by day, object and feeding regime how long each contact lasted, when it took place, and how many other contacts occurred under the same circumstances. We used Thompson and Spencer’s (1966) operational definition for habituation.

A Mann-Whitney U test was used to compare the frequencies of sessions with no contact in food and non-food items. This non-parametric test was also used to compare the duration of contacts for the different objects and conditions. A Kendall’s W test was used to compare the number of contacts across each third (10 min each) of the experimental sessions. To correlate the time of the start of a contact with its duration a linear regression was used. A two-tailed ANOVA and a post hoc (Scheflé) test were used to test for significant differences in the changes in contact duration in dependence to object and day of the experiment for each subject.

Results

Study A

We checked for habituation within trials by dividing each experimental session into ten minutes intervals, resulting in 6 intervals of 10 min each. Those were ranked according to the percentage of inspection behavior. Within each experimental session a highly significantly decrease of attentiveness was shown by all 23 subjects (Kruskal-Wallis test: $H_5 = 52.32, P < .001$). Habituation across tri-
als was investigated similarly by comparing the amount of inspection behavior across the five sessions all 23 subjects habituated significantly to the stimulus presented across the observational sessions (Kruskal-Wallis test: $H_4 = 17.99, P < .01$).

**Study B**

**Exploration.** Healthy, non-senescent octopuses in captivity immediately react to any stimulus dropped into their tanks by approaching it within a few minutes (food objects $M \pm SD = 124 s \pm SD 35 s$, inanimate objects $M \pm SD = 406 s \pm SD = 67 s$). Latency to approach the stimulus was significantly different for food and non-food objects (Wilcoxon $W = 105, Z = -4.503, p < .001$), but there was no significant change in latency over days for any of the objects across the two feeding conditions (Mann-Whitney U test: food: $Z = -1.549, ns$; non-food: $Z < 1.0, ns$). Yet, there was a significant difference in the number of trials in which octopuses did not tactiley explore the stimulus at all (food $M \pm SD = 8.1 \pm 6.5$; non-food $M \pm SD = 1.5 \pm .5$; Mann-Whitney U test: $Z = -2.323, p = .02$). When the animals were fed 2 hours before the experiment, the number of sessions without contact of subject and object doubled for mussels (24h - 7.4% of contacts, 2h - 13.2%) and clams (24h - 11.1% of contacts, 2h - 20.6%) compared to the 24 h feeding regime. For the two non-food objects ($M \pm SD = 3.1 \% s \pm .1 \%$) the number of sessions without contact was not significantly different.

Subjects explored objects either with one or several arms (Level 1 exploration, Figure 4a) or by bringing the objects under their interbrachial web (Level 2 exploration, Figure 4b). For the non-food objects, the number of contacts at two levels of exploration was not significantly different (Mann-Whitney U test: $Z = -1.549, p = ns$). However, there was a significant difference in number of level 2 contacts between food and non-food objects (Mann-Whitney U test: $Z = -2.323, p = .02$). Food items were approached and eaten or otherwise ignored and then were not subject to further exploration. Although there were no significant differences in duration of contact between the two feeding regimes, we found differences between food and non-food objects. Contacts with food objects lasted longer whereas contacts with non-food objects were more numerous; the number of contacts was three to four times higher with the non-food objects (Table 1). Difference in duration of contacts was significantly different for food and non-food objects (food $M \pm SD = 240.5 s \pm 165.5 s$; non-food $M \pm SD = 1.5 s \pm .5 s$; Mann-Whitney U test: $Z = -2.308, p = .021$).

**Within trial habituation.** Each of the 14 octopuses showed within-trial habituation as the number of contacts decreased over time. There is a highly significant decrease of the number of contacts in the second and third section of a thirty-minute trial (Kendall’s W test, Kendall’s $W = .796, df 2, \chi^2 = 22.86, P < .001$). Across all animals 58% of the contacts ($M \pm SD = 58\% \pm 11\%$) took place within the first 10 minutes ($M \pm SD = 27\% \pm 4.7 \%$ in the second and $M \pm SD = 15 \% \pm 2.6$ in the last 10 minutes).
Figure 4. (A): Level 1 exploratory behavior; the animal uses one or several arms to explore the object; (B): Level 2 exploratory behavior; the animal holds the objects in its interbrachial web

Table 1
Difference in duration of contacts for food and non-food items

<table>
<thead>
<tr>
<th>Objects</th>
<th>Contacts</th>
<th>25% (s)</th>
<th>Median (s)</th>
<th>75% (s)</th>
<th>min. (s)</th>
<th>max. (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mussel -24h</td>
<td>100</td>
<td>26</td>
<td>444</td>
<td>1552</td>
<td>1</td>
<td>1799</td>
</tr>
<tr>
<td>Mussel -2h</td>
<td>92</td>
<td>20</td>
<td>899</td>
<td>1790</td>
<td>1</td>
<td>1798</td>
</tr>
<tr>
<td>Clam -24h</td>
<td>88</td>
<td>359</td>
<td>647</td>
<td>904</td>
<td>1</td>
<td>1795</td>
</tr>
<tr>
<td>Clam -2h</td>
<td>81</td>
<td>5</td>
<td>601</td>
<td>1031</td>
<td>1</td>
<td>1798</td>
</tr>
<tr>
<td>Snowflake -24h</td>
<td>321</td>
<td>5</td>
<td>13</td>
<td>29</td>
<td>1</td>
<td>393</td>
</tr>
<tr>
<td>Snowflake -2h</td>
<td>431</td>
<td>4</td>
<td>11</td>
<td>21</td>
<td>1</td>
<td>1797</td>
</tr>
<tr>
<td>Smooth -24h</td>
<td>450</td>
<td>4</td>
<td>9</td>
<td>31</td>
<td>1</td>
<td>1800</td>
</tr>
<tr>
<td>Smooth -2h</td>
<td>361</td>
<td>3</td>
<td>10</td>
<td>35</td>
<td>1</td>
<td>1797</td>
</tr>
</tbody>
</table>

Across trial habituation. There was a decrease in response following an initial period of high exploratory activity on days one and two, but after this period of habituation the amount of contacts and their duration increased again. This was observed on the forth to sixth day out of seven days in the experiments. This stable, predictable decrease of the total time in contact with the objects was significant for the non-food items in 5 of 14 animals ($F(1, 97) = 4.150, p < .001$).

Figure 5a shows one week’s experimental sessions of animal 7. Differences in total contact time per experimental day were significant ($F(55, 83) = 1.808, p = .007$) for the 7 days of the experiment with the non-food objects. The other sessions resulted in a fluctuating total time of contacts over the various days. A graph of the total time in contact for a food object is shown in Figure 5b.
Figure 5. (A) Exemplary graph of one experimental week for animal 5. The graph shows the decrease of duration of all contacts (total time per day) with the tactile object over time; (B) Exemplary graph of one experimental week for animal 7. The graph shows the duration of all contacts (total time per day) with a food object over time.

Discussion

All 37 subjects showed behaviors that can be classified as directed exploration (Berlyne, 1960). We demonstrated that *Octopus vulgaris* does habituate to a stimulus that can only be visually explored. This is consistent with recent findings on habituation towards a visually presented prey stimulus (Kuba et al., 2005; Kuba et al., in submission). The clear difference in how the octopuses treated food (ex-
trinsic exploration) and non-food objects (intrinsic exploration) is important as this gives further experimental evidence for curiosity behavior in octopuses. Contacts with food objects lasted longer whereas contacts with non-food objects were shorter but more numerous. Such results make clear that the behavior with the Lego objects was not misplaced predation by an animal unable to discriminate prey from non-prey. Intrinsic exploration did not significantly change under different feeding regimes, which is puzzling. We expected hunger to be a strong motivational factor, as octopuses have a short lifespan and high growth rates resulting in high metabolic requirements (see Lee, 1994). Therefore, we anticipated octopuses would pay less attention to non-food objects when hungry. Still, the present results are consistent with those found in studies on intrinsic exploration in rats (see Toates, 1983, for reviews) and farm animals (Day et al., 1994; Wood-Gush et al., 1983). The octopuses' tendency to show directed investigation in the form of tactile discrimination as also described for vertebrates (Archer & Birke, 1983; Berlyne, 1960; Renner, 1990) is a striking convergence between cephalopods and vertebrates. This is one of a long line of experiments revealing general principles in animal neurophysiology and cognition based on the octopus as a model (Fiorito & Scotto, 1992; Hanlon & Messenger, 1996; Hochner, Brown, Langella, Shomrat & Fiorito, 2003; Mather, 1995). The fact that latency did not change over time is consistent with findings by Fiorito and Gherardi (1999) on duration of prey handling and jar opening experiments in *Octopus vulgaris* (Fiorito, Biedermann, Davey & Gherardi, 1998).

The number of exploratory contacts increased for both objects (snowflake & smooth) when the octopuses were hungry, yet the duration of contacts increased only for one object (snowflake). This slight difference in behavior is interesting and might be explained by earlier studies on tactile discrimination in octopuses (Wells, 1962) that showed the animals’ tactile discrimination of objects depends on the proportion of the sense organs (in the rims of the suckers) in contact with the object. If a sucker bridges the gap of a groove, the signal sent to the brain cannot be discriminated from a flat surface. Wells (1962) suggested that the proportion of sense organs exited determines the quality and quantity of the signal sent to the brain. The snowflake-like object, with its rough and irregular surface, should have been more complex and ‘interesting’ to the octopuses as there were more “grooves” into which the suckers can penetrate. This trend remains to be investigated further.

In contrast to the study by Kuba et al. (in submission), we were able to show that octopuses can show long-term effects when habituating towards a visual prey stimulus. The difference between these two studies might be due to different hierarchies in the octopus central nervous system. Habituation might first be taking place locally at the level of the optical lobes and only after a sufficient amount of repetitions it might also be manifested at the level of the vertical lobe which is involved in higher order learning (Hochner et al., 2003; Nixon & Young, 2003). Neurophysiological experiments are needed to tackle this question.

Our findings that habituation was stronger within days than across days are consisted with the results found by Mather and Anderson (1999). In their study, *Octopus dofleini* exhibited clear habituation towards the stimuli on the first day of the experiments. But on successive days, the patterns of response were more com-
plex and led sometimes to exploratory play. The less pronounced habituation across days in the present study might also be a result of the life history of octopuses. In a study on use of space, Mather (1991) reported that octopuses changed their small home ranges after periods of about 10 days. Although octopuses have the potential for long-term memory (Hochner et al., 2003; Wells, 1978) short intervals for habituation to stimuli might be more important to the animals in real-life situations. The difference found in habituation across days supports the idea that octopuses habituate more readily to a visual stimulus they could not interact with tactiley. Now that we have shown intrinsic and extrinsic exploration and habituation in Octopus vulgaris a more detailed investigation of a possible play-like behavior while interacting with these objects needs to be done.

References


