

BILATERAL SYMMETRY IN CRAYFISH BEHAVIORAL REACTIONS*

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The crayfish, *Procambarus cubensis*, placed in the central area of a plus-maze preferred to go forward in about 50% of trials; they chose the right or left arm in 20.7 and 18.9% of the trials, respectively. In a T-maze, the difference between right and left directions was also insignificant. When exploring a plus-maze, the crayfish turned to 180° at the end of the arms, then turned to 90° going to the next arm. The mean difference between the right and left U-turns, and the right and left turns was insignificant though some animals demonstrated a left or right preference. There was a strong correlation between the direction of U-turns and following turns ensuring the clockwise or anti-clockwise movements of the crayfish. Also we examined a possible preference of the right or left claw in the feeding behavior of the crayfish. The crayfish caught a small bloodworm given from above equally with the right or left claw. The crayfish conditioned to take a bloodworm with a claw did not demonstrate any stable preference of left or right claw in the course of the experiments. The question of bilateral asymmetries within the decapod crustaceans is discussed.

Keywords: Crayfish – plus-maze – T-maze – directional preference – claw preference

INTRODUCTION

The main body plan of most animals is bilaterally symmetrical, an advantageous feature that evolved a long time ago. An availability of two sides each having symmetrical appendages is especially important for animals using their legs for walking (maintenance of equilibrium in rest, an ability to produce various different gaits, etc.). At the same time, “different kinds of asymmetries emerge in different time

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scales of evolution" [23]. There are numerous examples of asymmetries, not only in man but also in lower vertebrates [2] as well as in all the groups of invertebrates [13].

Decapod crustaceans are famous by the conspicuous asymmetries of the right and left sides of their body, first of all, of the right and left claws [11, 15]. The crayfish that also belongs to this group has no anatomical distinctions between the two sides. In this study we tested the crayfish for putative functional asymmetries at the behavioral level using the technique accepted in many studies on the most commonly used laboratory vertebrates, rats: turning behavior, choice of the direction in the plus-maze and T-maze, and usage of the right and left claws in prey capture.

MATERIAL AND METHODS

The experiments were carried out on specimens of the crayfish, *Procambarus cubensis* (Erichson) of both sex reared in the laboratory [3, 18]. Between the experiments, the crayfish lived in individual round tanks (diameter of 17 cm) whose floors were covered with sand and gravel (water level was about 4–5 cm). The animals were fed 1–2 times a week with live bloodworms or turbiform worms. They were maintained under room temperature (18–22 °C) and an artificial light cycle (dark from 20:00 to 8:00 hours). The experiments were conducted in a sound-proof room, during the day (13:00–20:00 h).

1. In order to test spontaneous choice of the direction, we used juvenile (2–5 month) crayfish (N = 18; 8–10 trials, separated by 24 h, on each crayfish; total number of trials, n = 164). The crayfish taken from its home tank was placed in the centre of the uniformly illuminated (50–60 Lx) plus-maze, with its anterior end looking at certain arm. The plus-maze consisted of a central square (65×65 mm²) and four arms (length 145 mm each). In the walls of each arm, two pairs of optical channels were situated; each approach of the crayfish to a sensor or leaving it was recorded with a computer program [see 20].

In all other experiments, we used adult (1–2-year old) crayfish, whose behavior was recorded with video camera (Panasonic VHS-C).

2. One arm of the same plus-maze was closed, and it was used as a T-maze. The crayfish (N = 7) was placed in the start arm, with its anterior end looking at the exit or the end of this arm. After it walked to the right or left arm, it was gently impelled to return to the start arm (some crayfish did this by themselves). An experiment consisted of only 5 trials, in order to avoid habituation; it was repeated 4–5 times with a 24 hour interval. After the crayfish demonstrated its spontaneous choice of the right or left directions, we did one (right or left) arm attractive for the crayfish: when it reached its end, it received a bloodworm. When the crayfish changed its initial preference, a worm was given in both the arms; thus the right and left directions became equally attractive for the crayfish. The choices made by each crayfish were observed on the monitor and averaged for 10 subsequent trials.

3. The left-right preferences in turning behavior were examined in crayfish (N = 12, n = 39) placed in the plus-maze for 30 min. Then the crayfish explored the

maze walking mostly from one arm to another. At the end of each arm, it did a U-turn through its right or left side; this turning within an arm mostly followed by its turn between arms. Observing crayfish behavior on the monitor, we scored all its "movements" (as a single movement, we considered an exit from, or entry to an arm) directed forwards (F), backwards (B), to the right (R), or to the left (L). In addition, we marked the U-turns as [R] or [L] if they were performed through the right or left "shoulder", respectively.

4. The use of the left or right claw in the feeding behavior of the crayfish (N=4) was examined in their home tanks. The large (length from rostrum to telson, of 55–65 mm) crayfish with equally developed claws were chosen. The crayfish was accustomed to search for live bloodworms in the sand covering the bottom of the tank; usually, it caught them by a second walking leg. During the experiment, a small (about 10 mg) bloodworm was presented with a pair of forceps, and the crayfish could catch it only by using one of its claws. An experiment consisted of ten trials; it was repeated 7–8 times, with a 24 hour long interval.

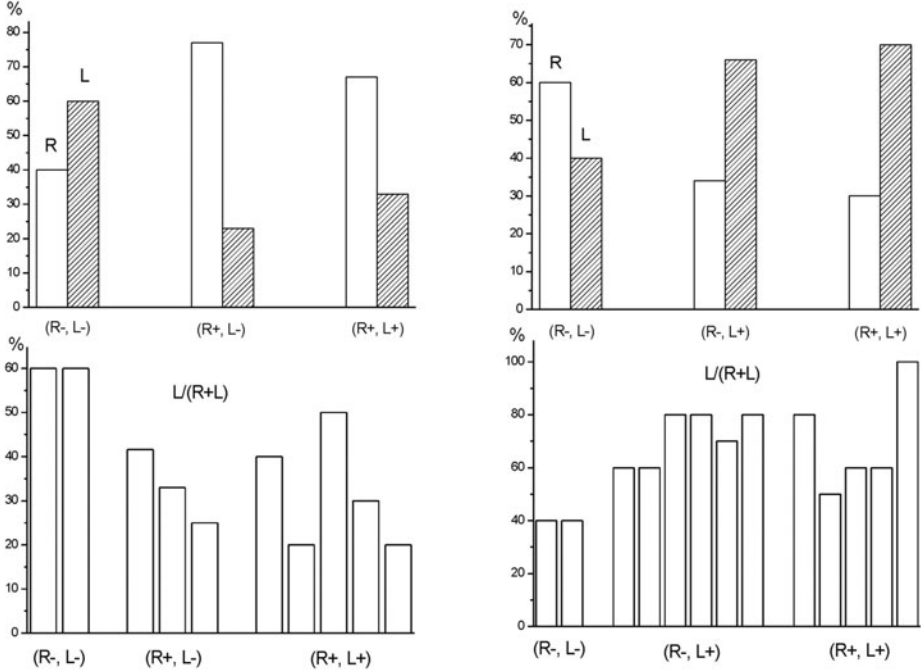
5. In the last experiment, the crayfish was conditioned to catch a bloodworm from a "feeder". The experimental chamber was made from the same plus-maze, with two arms closed completely, and with an arm closed with a door having an oval opening near its bottom. Behind the opening, there was a "feeder" where an end of a large worm (about 70 mg) was fastened. To take the bloodworm, the crayfish had to thrust one (right or left) claw into the opening. Six large crayfish were tested but only two performed the task (the others had very low motility and refused to eat in the experimental chamber). The experiments lasted 1–2 months, with one-two-day intervals. When the habit was elaborated, the crayfish usually caught and ate five bloodworms in each session.

RESULTS

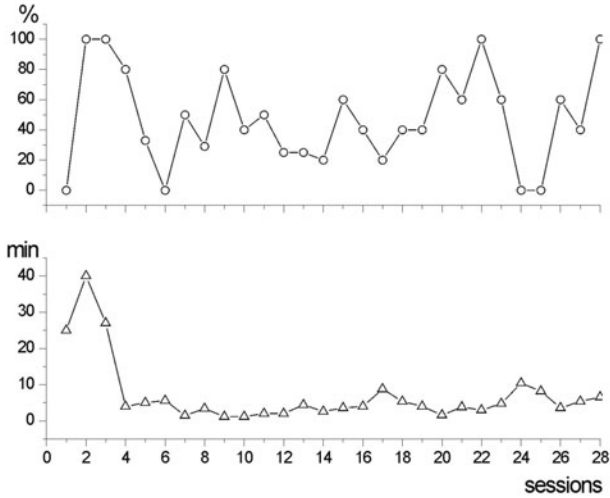
1. The crayfish placed in the central area of the plus-maze walked, sooner or later, into one of its arms. Mostly (about 50% of observations) they walked forward, sometimes (11%) they walked or jumped backward. They walked to the right or to the left in about 20% of observations; mean frequency of choosing right and left arm for an animal was 1.94 ± 0.23 , and 1.61 ± 0.34 , respectively; $p=0.429$).

2. In a T-maze, individual crayfish walked more to the right or to the left, but the difference was small. Pooled data for six crayfish demonstrate that difference between right and left choices was insignificant: 11.83 ± 1.27 and 10.83 ± 1.32 , respectively; $p=0.6$. One (the 7th) crayfish showed an obvious directional preference: it walked almost always to the left. This was caused, however, by deterioration of its right eye after molting; therefore, we excluded its data from pooling. Thus, intact crayfish did not demonstrate any directional preference when both arms had nothing attractive for the animal (Fig. 1, A: (R-, L-)). However, they changed this attitude when the right and left arms differed: then they began to prefer the "rewarded" arm. This happened quickly, in the first-second sessions, though the preference

A



B



of the “rewarded” arm was not absolute (Fig. 1, A: (R+, L-) or (R-, L+)). After this training, we made both arms equally attractive: the crayfish received a bloodworm when it walked either to the right or to the left. It appeared, however, that the crayfish rather slowly changed its previously elaborated directional preference: it could maintain after five sessions (50 trials) (Fig. 1, A: (R+, L+)).

3. When the crayfish was placed in the plus-maze for a long time, it appeared to explore it by walking from one arm to another. The total number of movements recorded in these experiments was 730 (forwards, 9.9%; backwards, 7.4%; to the right, 36.8%; to the left, 45.6%). If the crayfish entered an arm, it used to go up to its end where it spent time mostly exploring the angles. Then it did a U-turn and went to the exit of the arm. From 627 U-turns recorded, 327 were made through the right, and 300 – through the left “shoulder”, on the average, 8.58 ± 0.89 and 7.84 ± 0.76 , respectively; $p=0.6$. After a U-turn, the crayfish usually (80% of observations) turned to the next, right or left, arm. The mean number of right and left turns also differed insignificantly: 6.84 ± 0.66 and 8.48 ± 0.92 , respectively; $p=0.2$. It is remarkable that after the right U-turn it turned mostly to the left, and after the left turn, to the right (about 87% of observations). More rarely (about 13% of observations), the direction of the turn coincided with the direction of the preceding U-turn.

Though the mean data for the right and left U-turns as well as for the right and left turns differed insignificantly, individual crayfish could demonstrate rather stable preference in their U-turns and turns. The asymmetry Index, AI = ratio of turns to the left over total number of turns, counted for all (2–5) experiments on each crayfish, mostly was near 50% but in one animal it was close to 0, whereas in two animals, it was close to 100%. Interestingly, this preference was maintained in subsequent experiments separated by 24 h.

It also was noted that usually the crayfish made several (2–8) U-turns (and turns) in the same direction, and then it suddenly changed the direction, again for a rather long time.

4. When a small bloodworm approached the crayfish staying in its home tank, it raised both claws simultaneously. It caught the worm either with the right or the left claw, and sometimes it took the same worm with one, and then with another claw. The mean number of captures with the right or the left claw differed insignificantly (33.25 ± 3.6 and 36.5 ± 4.0 , $p=0.57$). No individual differences in using the right or

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Fig. 1. A. Choice of the right or left arm in T-maze by two crayfish that were tested in three situations: initial preference, when both arms were similar (R-, L-); learned preference when visiting one of the arms was reinforced with food (R+, L-, for the left animal, or R-, L+ for the right one); and re-learned preference when visiting both arms was equally reinforced (R+, L+). Top, data pooled for the whole situation; bottom, Asymmetry Index, L/(L+R)%, in the course of the experiments (each column corresponds to 10 choices). B. Time course of the experiments with learning the crayfish to take a bloodworm by a claw. Upper graph, Asymmetry Index, L/(L+R)% for using the left claw; lower graph, mean time spent for taking a bloodworm; abscissa, number of session. After regular (1–21) sessions, the experiments were performed with a one-week interval

left claw were present. Some changes in general behavior, which could be indicative of the crayfish learning at the course of the experiments, also were not observed.

5. To test using a single claw in feeding behavior of the crayfish, a special technique was employed (see above). After habituating to the experimental conditions, the crayfish learned to catch a large bloodworm oscillating in the water. To do this, it thrust a claw through an opening in the door. When the habit was elaborated, the crayfish caught the worm with a quick, strong and accurate movement of a claw. The habit remained stable well after an interruption in experiments for a week. It did not vanish during a month when the experiments were conducted with one-week intervals. In different experiments, the number of captures with the right or left claw was variable but close to 1 : 1. In single experiments, one (right or left) claw could be dominating but any persistent tendency in using this or that claw was not found (Fig. 1B).

DISCUSSION

In the literature, there is little evidence on the preference of crayfish for the right or left side. In the first study devoted to an ability of the crayfish (*Cambarus affinis*) to learn simple habits, Yerkes and Huggins [24] did not find their spontaneous preference in using the right or left exit from the experimental chamber. The chamber used in this study is similar to the T-maze used in our work. The above-described results also indicate an absence of initial directional preference of the crayfish when both arms of the T-maze were similar. An exception was only one crayfish, with a defective eye. This may be considered a kind of “natural” control: if the crayfish has a defect, it can demonstrate some asymmetric behaviors. It should be noted, however, that even this individual, and all the intact animals quickly changed their initial preference when one of the arms became positively reinforced.

There is, however, a paper where the right-side preference in the exploratory behavior of the crayfish, *Astacus leptodactylus*, is described [1]. Regretfully, the authors did not give any explanation for their results. In addition, the conditions of their experiments are not described in sufficient detail, therefore we cannot comment on the discrepancy of our results with those of Baranyuk et al. [1].

The construction of the plus-maze was favorable for studying both U-turns (180°) within arms, and subsequent turns (90°) between arms. On the average, there was no preference in U-turns through the right or left “shoulder”, though the number of right U-turns was slightly more, than the left ones. Curiously, the number of turns between arms was slightly more after U-turns through the left “shoulder”. In both cases, however, when turning between arms, the crayfish continued to move in the same circular direction (clockwise or anti-clockwise), as during previous U-turn. Hence, after the right U-turns it went mostly to the left arm (i.e., clockwise), and after the left U-turns it went mostly to the right arm (i.e. anti-clockwise).

Though there was no significant mean difference between the right and left U-turns and turns, three crayfish out of twelve showed a preference for the right or left

direction that was maintained in several experiments performed on the same animal. Such preference within any one session could be explainable by some inertness of nerve processes noted above and caused probably by an inability of quick changes of motor programs in the crayfish. However, it is unclear why it can conserve the same circular movements after a long (24 h) delay between the experiments. This question needs to be investigated further.

The data concerning spatial preference are, however, rather ambiguous even for laboratory mammals such as rats or hamsters [17, 22]. Most evidence concerning motor bilateral asymmetries in laboratory mammals is from the study of paw preference in “reaching” reactions [8]. Kelly and Chapple [9] assumed that the defense reaction of the crayfish (raising both claws in response to moving shadow) is similar to reaching in primates. Thus we tried to investigate a putative preference of a claw in prey capture. In preliminary experiments, we fixed a bloodworm on the thread. Then the crayfish tried to catch it always by both claws. In order to make his task easier for the crayfish, we slightly fixed a bloodworm by a pair of forceps. If the crayfish touched the worm by a claw, the forceps opened. However, even then the capture of the worm by the right or left claw was random. Each case the crayfish raised both claws; moreover, sometimes it took the worm and threw it aside. Thus, we suggest that this reaction does not have much in common with feeding motivation; in addition, it differs strongly from mammalian reaching because it is accomplished by two symmetrical appendages.

In order to create experimental conditions similar to those in “reaching” responses of the laboratory mammals (rats) we changed the technique in such a way that the crayfish could take a bloodworm only by one claw. In fact, it could pass two claws into the opening but this was possible only when they were closed. The results of this study are preliminary because we tested fully only two animals (the other four animals were refused from this experiment because of their very low food motivation). They are presented because [1] the technique allows to elaborate a novel feeding habit in the crayfish, with a claw used as a true manipulator organ; [2] they demonstrate an absence of any initial preference of the right or left claw in feeding behavior of the crayfish, and inability to develop this or that preference in the course of long experiments. This differentiates the behavior of the crayfish from that of the rat whose dominance of a paw is seen often in the first ten trials, and it becomes more and more evident in the course of the experiments [8].

Thus the main conclusion from our experiment is that the crayfish has no functional asymmetry not only in their turning and choice behavior, but also in using their claws in feeding. This may seem trivial but in our opinion this needs to be commented. First, the crayfish belongs to decapod crustaceans, and this group is famous for the conspicuous asymmetry of the claws [11]. It is obvious from the Table 2 of [1] that most of the species having the asymmetric claws are marine animals (with an exception of the shrimps *Macrobrachium* living in the estuaries) and belong to Brachyura. Thus one may suggest that the less complex freshwater environment can be one of the reasons for the non-development of bilateral asymmetry of the claws in the crayfish. Additional factors acting in the same direction can be the lower position

of the crayfish and other Macrura, in comparison to Brachyura, and low level of mobility of the crayfish comparing with the shrimps that also belong to Macrura. However, the lobsters which are a “sister” group of the crayfish [5, 14, 16] and whose anatomical design is extremely close to that of the crayfish, are known to have a conspicuous asymmetry of their claws (this was noted even by Aristotle, in his “The History of Animals”). Thus the question is, did the common ancestor of the lobsters and crayfish have asymmetric or symmetric claws? In the first case, the crayfish probably lost the asymmetry after they left the sea and adapted to freshwater environment. In the second case, the lobsters developed claw asymmetry later, in a more recent stage of their evolution.

The clawed lobsters (Nephropidae) have strong morphological and functional distinctions between the claws: one of them (major claw, or crusher) is much bigger and more powerful than the other (minor claw, or cutter). Both claws also differ in their nerve-muscular physiology. The role of genetic and environmental factors in development of claw asymmetry in lobsters has been studied extensively [6]. Both factors appear to be important: the critical period for crusher determination (the 4th–5th juvenile stage) is genetically determined whereas the actual trigger is influenced by experience. The critical period defines a time when the central nervous system is susceptible to being lateralized into a crusher and cutter side. The lateralization is dependent, however, on the environment: juvenile lobsters reared during the critical period with a substratum that could not be grasped, or with reduced input from proprioceptors, failed to develop a crusher claw [7]. If the juvenile lobster has an appropriate substratum (sand, gravel, etc.), the side experiencing the greater activity becomes the crusher side while the contralateral side becomes the cutter. Thus the role of the environment in developing of claw asymmetry is obvious but the environment is acting *via* not less important abilities determined genetically. At least two of them seem to be significant: an ability of each claw to work independently of the other, and the great plasticity in the nerve-muscular system of the claws typical for the lobsters.

The high level of plasticity in the nerve-muscular system of crayfish claw and the walking leg is shown experimentally: long *in vivo* stimulation of a phasic motoneuron supplying the closer muscle of the crayfish, or the limb stretcher muscle can change the ultrastructure of its synaptic terminals to a more tonic phenotype [10, 12]. It seems, however, that the claws in the crayfish, as distinct of some other appendages [4, 19], fail to work independently. Thus, we suggest that the evolution of the crayfish went another way compared to the lobster. Instead of developing the diversity of both claws, the crayfish developed the common usage of both claws in all the behaviors where they are involved. This allowed it to remain, at least during three hundred millions of years, an ideal bilaterally symmetric animal.

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