A behavioral analysis of force-controlled operant tasks in American lobster

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Abstract

Operant conditioning is a common tool for studying cognitive aspects of brain functions. As the first step toward understanding those functions in simple invertebrate microbrains, we tested whether operant conditioning could be applied to train American lobster *Homarus americanus* that has been extensively adopted as an animal model for neurophysiological analyses of nervous system functions and behavioral control. The animal was trained by food rewarding for gripping of a sensor bar as the operant behavior. Lobsters were first reinforced when they acted on the bar with a stronger grip than a pre-set value. After this reinforcement, the animal learnt to grip the bar for food pellets. The yoked control experiment in which the animal received action-independent reinforcement excluded the possibility of pseudoconditioning that the food simply drove the animal to frequent gripping of the sensor bar. The association of the bar grip with food was extinguished by rewarding nothing to the operant behavior, and was restored by repeating the reinforcement process as before. In addition, lobsters successfully carried out differential reinforcement regarding the gripping force: their gripping force changed depending on the increased force threshold for food reward. These data demonstrate that lobsters can be trained by operant conditioning paradigms involving acquisition and extinction procedures with the precise claw gripping even under the force control.

Key words: operant conditioning, lever-press type task, differential reinforcement, gripping behavior, invertebrates, action force control, American lobster

1. Introduction

Operant conditioning is one of the most common tools for the studies of animal learning and cognition [1-3]. In cognitive neuroscience, mammals and avian species have been mainly used as experimental animals and various manipulative operant tasks (lever-press or key-peck) have been developed for them [4-6]. Recent research using operant paradigms, however, has shown that some invertebrates with a 'microbrain' [7] or a 'mini-brain' [8] that is characterized by not only its size but also its cytoarchitecture and neuronal organization also possess cognitive abilities [9, 10] including simple forms of rule learning or concept formation [11], count [12] and observational learning [13]. Physiological mechanisms underlying these higher-order functions of the microbrain, however, remain to be clarified because of experimental difficulties in most cases.

American lobster *Homarus americanus* has major three advantages for neurophysiological analysis of brain functions. First, the animal can perform a precise limb movement that is recommended as an operant target in most learning experiments [14, 15]. The lobster has a pair of asymmetrical claws as the first thoracic appendages: the crusher is a stout, molar-toothed, slow-acting claw while the cutter is a slender, incisor-toothed, fast-acting claw. The former type is usually used for breaking clamshell by gripping (defined by [16]) to eat shellfish meat so that its action can be precisely controlled regarding the direction of movements and the grip force [17]. Second, the lobster nervous system is easily accessible for neurophysiological analysis. Decapod crustaceans, such as lobsters, crayfish, and crabs, have been used for many researches on sensory and motor functions at the level of identifiable neurons and neuronal networks [18]. This characteristic of the nervous system can be also useful to analyze mechanisms underlying brain functions in crustaceans. Finally, crustaceans including lobsters and crayfish are phylogenetically close to insects that were demonstrated to show a variety of cognitive brain functions. Recent studies on phyletic evolution of arthropods have revealed that crustacean is quite likely an ancestral lineage of hexapoda [19-22], suggesting that some primitive forms of cognitive brain function can be found in crustaceans. In particular, it is noteworthy that anatomical similarity of brain structure between crustaceans and insects also indicates their phyletic relationship [23].

As the first step toward understanding the higher-order brain function in lobsters, basic operant procedures were conducted in the present study to associate manipulative task with food as the reward. Since lobsters can perform precise gripping actions by chelipeds [15, 24, 25], we utilized them as an operant target. For this purpose, we developed an operant chamber for lever-press type conditioning. This system allowed the animals to perform free operant reward learning. The pressure sensor system in the chamber quantitatively measured the gripping force of lobsters. Using this quantitative measurement system, we also analyzed whether lobster could carry out differential reinforcement procedures on the grip force. The results demonstrated the applicability of the bar-grip paradigm in lobsters for studying their learning ability and higher-order brain functions.

2. Materials and Methods

2.1. Animals

Adult lobsters, *Homarus americanus*, of both sexes were purchased at a commercial retail market (Sato-Suisan, Sapporo, Japan). Lobsters were imported from Canada and the United States, and kept in cooled aquariums for sale in the shop. In our laboratory, they were kept individually in separate aquariums filled with artificial or natural seawater at 15-18 °C under continuous filtration. Animals were fed every four days with small pellets of dried fish sausage. Acclimation was carried out at least 2 weeks prior to training under a day/night rhythm of 12L/12D: the light period started at 6 o'clock in the morning while the dark period at 6 o'clock in the evening. For one week prior to experimental use they were kept off feeding to increase their motivation toward the food pellets. It is noted here that decapoda crustaceans can survive for a long period without food [14, 26]. They were habituated to the operant chamber for one or more days before experiment. During the experimental period, animals were fed only in the operant conditioning procedure as reinforcements. Animals ranged between 10.5 – 13.5 cm in carapace length and 482 - 547 g in weight.

2.2. Apparatus

A glass aquarium (90 × 45 × 45 cm) was divided into two compartments for feeding and resting by a computer-controlled gate (Fig.1A). The seawater filling both compartments was continuously filtered at 15 ± 1℃. The whole apparatus was placed in a wooden box (130 × 60 × 80 cm) that was completely shielded from the outside to avoid any visual disturbance. Lobsters were subjected to 12L/12D photoperiod as in the same manner during the acclimation period. The illuminance of white fluorescent lamp was maintained at 10-40 lx during the L period (day) and 0 lx during the D period (night). We carried out experiments during the day period. Animals could rest in the shelter or move freely in the resting compartment and were allowed to enter the feeding compartment only when the gate was open up. Between experimental sessions, the animals were housed in the shelter. The animals obtained food, one pellet at one time, which was dropped to a water stream spouted out from a small pipe at the feeding place. This feeder system provided the animal with food reward that was associated with the gripping behavior (see below). The gripping sensor bar (manufactured by Keisoku Support, Hiroshima, Japan) was comprised of a brass bar as the core, a sheet-type load sensor wrapping around the bar, and an outermost waterproof tube shielding the entire sensor bar. The diameter of the grip bar was 2 cm so that lobsters can grip it with no difficulty. The bar was fixed vertically in front of the feeding place. The sensor was functionally coupled with the feeder, mediated by a personal computer (CPU1 in Fig.1A): we measured the grip force of lobster's claw with the sensor and digitized it every 15 msec by a 16-bit A/D converter (National Instruments USB-6009) connected to CPU1 which controlled the feeder through an electronic relay system and to CPU2 which stored the original sensor data at the sampling rate of 1 kHz using a PowerLab 8RSP (ADInstruments, Tokyo, Japan). The data stored in CPU2 was analyzed with Chart software version 5.3 (ADInstruments, Tokyo, Japan) and R programming software. (Fig.2B). The training program schematically shown with a flow chart in

Fig.1B was written in BASIC using Microsoft Visual Studio 2008. A grip force threshold, called reinforcement threshold in this study, was set in CPU1 for providing food rewards from the feeder according to the experimental procedure when its grip force exceeded the threshold. The reinforcement delay, i.e., the latency from the time of threshold attainment to the time of pellet release, was about 3 seconds in every experimental session (Fig.2B). One training session was finished when the gate was closed down automatically at a scheduled time of 30 minutes. If the animal was under the closing gate at this time, the gate moved up and down repeatedly with small movements until the animal escaped from there to the shelter. The animal position in the aquarium was monitored with two pairs of optical sensors (850 nm in peak wavelength) to control the gating system. We observed the lobster behavior during experiment using a video camera with an infrared illumination device (Victor TK-N1100).

Figure 1.

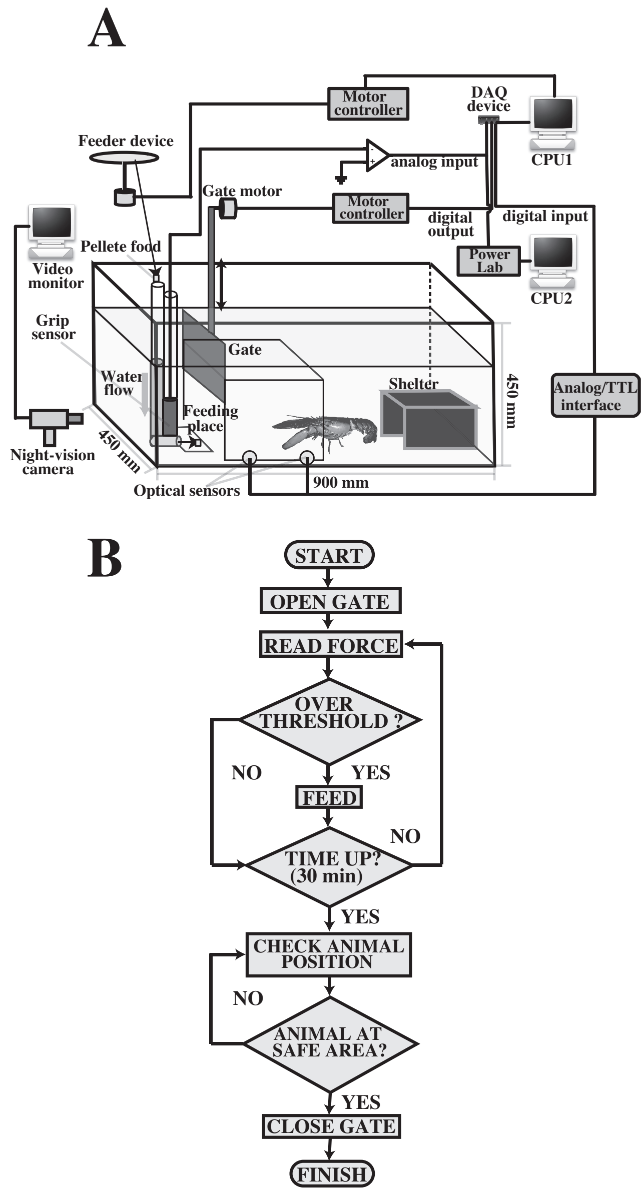

Figure 1.

Experimental set-ups. A: Operant chamber system adopted in the present study. Bar gripping was detected by a load sensor whose out put was fed into CPU1 that controlled the feeder system and the gate for feeding place depending on the griping force. The sensor signal was also fed into CPU2 for continuous recording throughout the experiment. B: Flowchart of the training program. All of the possible actions and responses by the subject during training were presupposed and appropriately dealt with in the program by continuously monitoring the animal behavior throughout two pairs of optical sensors as well as a grip sensor.

Figure 2.

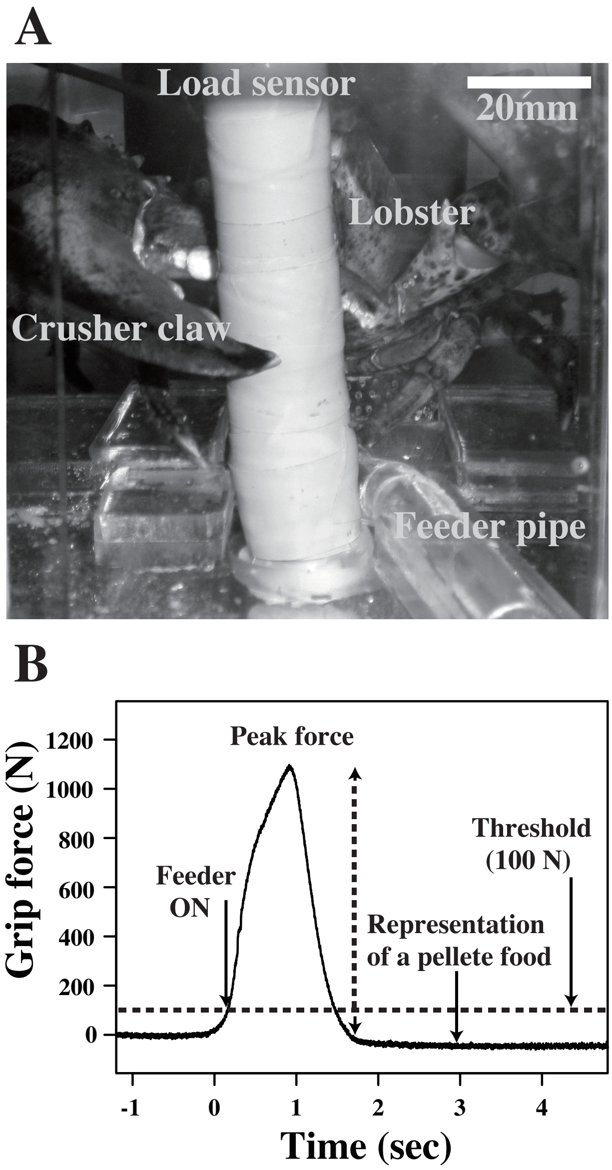

Figure 2.

Gripping behavior of lobster. A: A trained lobster gripping the sensor bar with crusher (left) claw. B: Temporal profile of the grip force development. The gripping behavior was maintained for more than 1 second in most cases. Reinforcement threshold, which was the criterion value of reinforcement for the operant target, was 100 N in this case. The latent time for the reward, i.e., the time between threshold passing and pellet food falling, was about 3 seconds in every experiment.

2.3. Experimental Design

2.3.1. Experiment 1

2.3.1.1. Basic operant procedures

The experiment consisted of five procedures: preconditioning, acquisition, maintenance, extinction, and reacquisition. These procedures were performed over consecutive days. Five lobsters were used in these successive procedures and their gripping actions were counted through sessions. Before the preconditioning, we carried out shaping of the lobster's behavior.

2.3.1.2. Shaping

Naïve lobsters did not show any tendency to approach the feeder place nor to grip the sensor. One day before the preconditioning procedure, a small amount of scallop extract was presented from the feeder pipe for one minute so that lobster was chemically attracted to the feeding place. In this situation, small amounts of food were dispensed when the lobster approached there. These exercises were conducted repeatedly until the animal spontaneously approached to the place sufficiently frequent times in one day. After this training, we observed that the animal spontaneously showed searching behavior around the feeding place, touched and held the sensor with pereiopods, and continuously gripped it with the crusher claw (Fig. 2A). The animal was regarded to have been "shaped" for the lever-press task. Those animals that did not approach the feeding place spontaneously or did not present gripping behavior were excluded from the present study.

2.3.1.3. Preconditioning

In this procedure, lobsters obtained no reward for their gripping actions. The procedure was performed for 2 days, two 30-min sessions per day. We counted gripping actions in each session and determined the operant level of the spontaneous activity as an average value of the gripping count through the sessions.

2.3.1.4. Acquisition, Maintenance, Extinction, and Reacquisition

After the preconditioning, four types of basic operant procedure, i.e., acquisition, maintenance, extinction, and reacquisition, were performed successively. Each procedure was conducted for 3 consecutive days, two 30-min sessions per day. In the acquisition procedure, lobsters obtained food reward for gripping actions. Abortive or unfinished actions in which the force was less than 100 N were not reinforced. In the maintenance procedure, animals were reinforced in the same manner. These two procedures can be regarded as a fixed-ratio training. After the maintenance, we ran the extinction procedure where no reinforcement was provided to the operant behavior irrespective of the gripping force. The reacquisition procedure where lobsters were reinforced again was carried out after the extinction was completed.

2.3.1.5. Controls for experiment 1

Two types of control procedure were performed for 3 or 2 consecutive days, two or three 30-min sessions per day, respectively: one group was the yoked control (N=4) and another group was non-reinforcement control (N=4). The animals used in these control experiments first experienced the shaping and preconditioning procedures in the same way during the basic operant experiment. Yoked control animals then received response-independent reinforcement. They were provided food reward when they stayed at the feeding place for 1 min long. The feeding interval was at least 3 min. Non-reinforcement control animals obtained no food reward for gripping behavior during the sessions. This situation was the same as the preconditioning procedure.

2.3.2. Experiment 2

2.3.2.1. Differential reinforcement

This experiment was consisted of four successive procedures: pre-reinforcement, low-threshold reinforcement, middle-threshold reinforcement, and high-threshold reinforcement. To assess the performance, we obtained the ratio of food-rewarded grips to total grips in sessions of the middle- and high-threshold reinforcement, in addition to the analysis of the grip force. We used those animals that had been trained by the acquisition procedure within 3 weeks. In the pre-reinforcement procedure, lobsters were tested regarding whether or not they spontaneously gripped the sensor bar more than 10 times in total within two 30 min-sessions in a day. We finally screened out 5 animals for the next step. One day after the pre-reinforcement, the selected lobsters were trained by low-threshold reinforcement in which the reward threshold was constant at 100 N. This training procedure was conducted for 6 consecutive days, two 30 min-sessions in a day (12 sessions). These trained animals were further conditioned by the middle-threshold and the high-threshold reinforcement procedures successively, both carried out over 3 consecutive days, two 30-min sessions in a day (both procedures were consisted of 6 sessions respectively). We determined individually the threshold elevation value as the proximal 75 percentile of the force distribution in the preceding procedure.

2.3.2.2. Controls for experiment 2

As control groups, four lobsters were used in the all-low-threshold reinforcement control and three in the low/middle threshold control. Both control procedures were conducted for 12 or 8 consecutive days, two or three 30-min sessions in a day respectively. The all-low-threshold control was consisted of 4 successive procedures with the same low-threshold reinforcement (24 sessions). The low/middle threshold control was divided into the low-threshold part (12 sessions) and the middle threshold part (12 sessions). In these control groups, the low-threshold value and the threshold elevation value in the middle-threshold experiment were the same as those in the experimental group.

2.3.3. Statistical Analysis

Statistical analysis was performed by generalized linear mixed models (GLMMs) [27,28] using R programming software (2.9.2 version) and lme4 package (0.999375-32 version) in R (R Development Core Team). In experiment 1, we focused on the gripping count as the response variable. To assess the animal's performance in the acquisition and extinction procedures, we analyzed the gripping counts through 6 sessions in each procedure. We constructed two models to explain the behavioral data: the alternative model and the null model. The former model assumed that the value changed according to session numbers, whereas the latter model assumed that the value was constant through sessions. We assumed that the mean gripping count (g) followed a normal distribution for the error structure, which was approximated by an identity link function as

$$g = b_0 + b_1 \times (\text{session number}) + r_i$$

where session number denotes the number of present sessions (an integer ranging from 1 to 6) as a numeric explanatory variable. The r_i term denotes random effects due to individual disposition or noise that could not be experimentally controlled (page 153-154 of [27], a real value). b_0 and b_1 are parameters of linear predictors to be estimated together with r_i. In the null model, session number was disregarded and assigned 0. We tested these models by a likelihood ratio chi-square test. In the same way, the statistical analysis was conducted in the yoked and the non-reward control groups. The difference was considered to be significant in case of $P < 0.05$.

To assess the animal's performance in the maintenance and reacquisition procedures, we compared the gripping counts among the preconditioning, maintenance and reacquisition procedures within the basic operant group. We constructed two models to compare the effect of schedule this time, not the effect of session numbers as before. Thus the mean gripping count (g) was assumed to follow a normal distribution for the error structure, which was approximated by an identity link function as

$$g = b_0 + b_1 \times (\text{procedure}) + r_i$$

where procedure denotes the type of training (preconditioning, maintenance, and reacquisition) as a factorial explanatory variable. Other terms follow the same convention as before. In the null model, the schedule variable was disregarded and assigned 0. We tested these models by a likelihood ratio chi-square test. The difference was considered to be significant when $P < 0.05$.

In experiment 2, we focused on two observation values as the response variables: the percentage of successful gripping and the gripping force. To assess the animal's performance in the differential reinforcement procedure, we first analyzed the percentage of successful gripping in which the force exceeded the reinforcement threshold in all gripping counts through 6 sessions in elevated threshold procedures. We constructed two models to explain the behavioral data. The alternative model assumed that the value changed according to session numbers, whereas the null model assumed that the value was constant through sessions. We assumed that the mean successful gripping count (s) followed a Poisson distribution for the error structure, which was approximated by a log link function as

$$\log s = b_0 + b_1 \times (\text{session number}) + r_i + \log (\text{total gripping})$$

or

$$s = \exp (b_0 + b_1 \times (\text{session number}) + r_i) \times (\text{total gripping})$$

where session number is an integer ranging from 1 to 6 functioning as a numeric explanatory variable. The logarithm of the total gripping, log (total gripping), enters the linear predictor as an offset term that serves to normalize the successful gripping count to its proportion. In the null model, session number was disregarded and assigned 0. We tested these models by a likelihood ratio test, referred to as the deviance adjustment test. The P-value of this test was obtained by parametric bootstrapping with 10,000 bootstrap replicates [28-30] and the difference was considered to be significant when P-value < 0.05.

To assess the effects of threshold elevation on the gripping force, we adopted model selection method using GLMMs for gripping force data in group1, 2 and 3. We assumed that the mean logarithmic gripping force (f) followed a normal distribution for the error structure, which was approximated by an identity link function as

$$f = b_0 + b_1 \times (\text{procedure}) + r_i$$

where procedure denotes the difference of threshold condition as a factorial explanatory variable. In order to represent changes in the gripping force in the course of training, a total of 8 models were constructed each of which consisted of 4 consecutive variables for the force strength corresponding to 4 consecutive procedures (Table 1). For example, the model 1 describes that lobster yielded gripping force with different mean values in different procedures. In contrast, the model 8 describes that the animals yielded the same mean value through all procedures. The model 1 was symbolized as ABCD whereas the model 8 as AAAA in Table 1, different alphabetical characters representing different force strengths. It should be noted here that the alphabetical order of these characters indicates no specific order in the gripping force strength. They simply indicate that the force strength during a procedure was the same as or different from that during another procedure.

The model selection was based on Akaike's information criterion (AIC) that can be expressed as:

$$AIC = -2 \log L + 2k$$

where L is the maximum log likelihood and k the number of parameters involved in the model [31]. A lower AIC value implied a better fit to the model. AICs for these 8 statistical models were compared in each group.

3. Results

3.1. Basic operant procedures

We analyzed first the ability of lobsters to associate their gripping action on a vertical bar with food reward by basic operant procedures. The bar-grip action of experimental group animals (N =5) showed a significant increase in frequency during the acquisition procedure compared with the preconditioning procedure (Fig. 3, likelihood ratio chi-square test; $p < 0.01$). By contrast, in the extinction procedure, the frequency of bar-grip action tended to decrease gradually near to the baseline, i.e., the average count during the preconditioning procedure (Fig.3, likelihood ratio chi-square test; $p < 0.01$). In the maintenance and reacquisition procedures, the bar grip frequency was kept above their baseline at an increased level (Fig. 3, likelihood ratio chi-square test; $p < 0.01$).

In order to exclude the possibility that the association between bar grip action and food reward was simply caused by their contingent occurrences, we prepared two control groups: the yoked control (N = 4) and the non-reward control (N = 4). Both control groups had access to the grip-bar but it was not coupled to the feeder. In the yoked control procedure, where the lobsters received a variable amount of food depending on the stay time at the feeding place, the bar-gripping action did not significantly increase in frequency over the baseline (Fig. 4a, likelihood ratio chi-square test; $p > 0.01$). Similarly, the gripping frequency was not significantly elevated in the non-reward control procedure, in which the lobsters obtained no reward upon gripping (Fig. 4b, likelihood ratio chi-square test; $p > 0.01$). Both control animals successfully performed the following acquisition procedure (Fig. 4a, Fig. 4b, likelihood ratio chi-square test; $p < 0.01$). Taken together, these data demonstrate that lobster can be trained to associate bar-gripping action with food reward by basic operant procedures.

Figure 3.

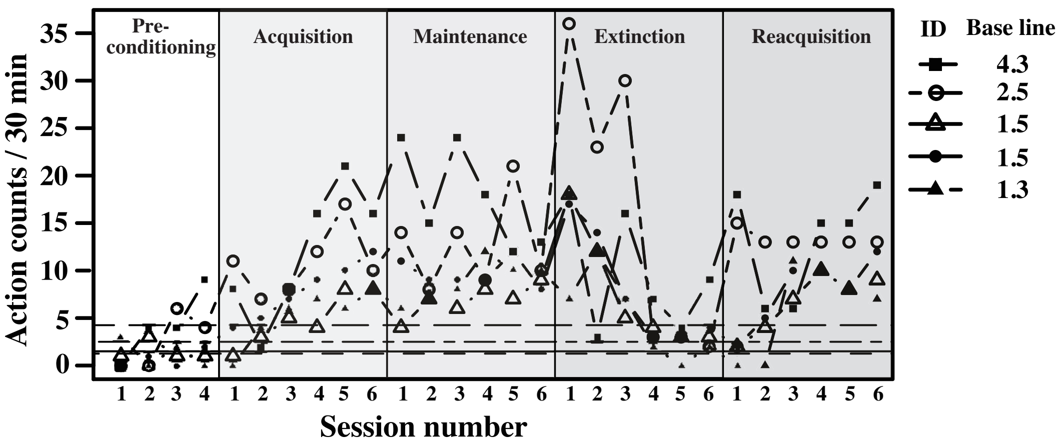

Figure 3.

Bar-grip actions of five lobsters basic operant procedures including preconditioning, acquisition, maintenance, extinction and reacquisition. Each session was 30 minutes in length, and 2 sessions were carried out in a day. After 4 sessions of preconditioning, 6 sessions were carried out for each of the procedures of acquisition, maintenance, extinction, and reacquisition. Horizontal lines represent the average of action counts in the four 30-minutes sessions of preconditioning for five subjects. The lines are called baselines in this study. The average value is shown on the right for each subject that is represented by a unique symbol.

Figure 4.

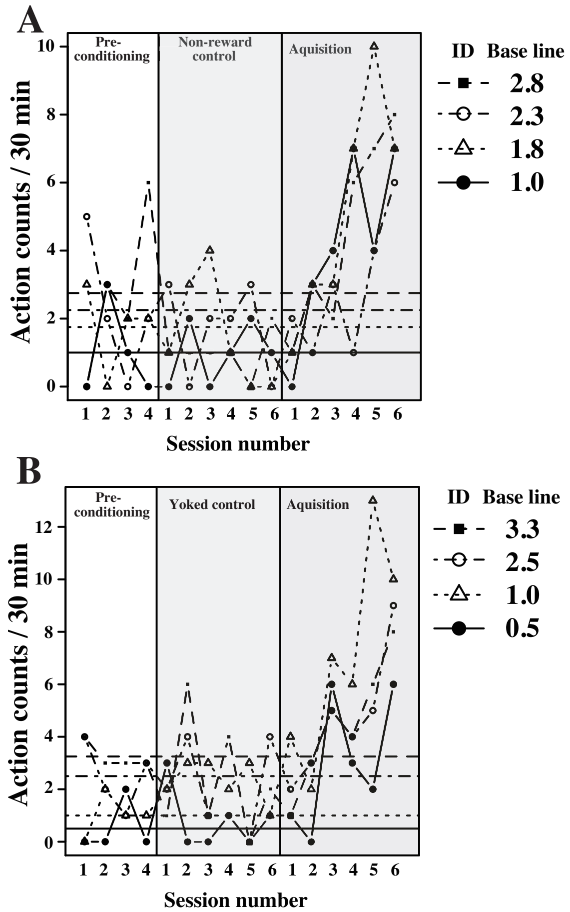

Figure 4.

Bar-grip actions of lobsters in the control experiments. A: Non-reward control group consisting of four animals. After 4 sessions of preconditioning, 6 sessions of non-reward procedure were imposed on them, followed by 6 sessions of acquisition procedure in each schedule. B: Yoked control group consisting of four animals that were different from those used in the non-reward control experiment. After 4 sessions of preconditioning, 6 sessions of yoked control procedure were imposed on them, followed by 6 sessions of acquisition procedure in each schedule. Each session was 30 minutes in length, 2 or 3 sessions carried out in a day. Horizontal lines represent the baselines for the experimental animals.

3.2. Differential reinforcement

We next tested whether lobsters could be trained by differential reinforcement on the grip force. In this experiment, the animals were passed through 4 successive procedures: pre-conditioning, low-threshold reinforcement, middle- and high-threshold reinforcement with increasing grip-force threshold for food reward. To assess the performance, we first analyzed the percentage of the number of successful gripping that yielded food reward in the total number of gripping bouts under the elevated threshold condition (Fig. 5). Each symbol in Fig. 5 represents an individual lobster. Gray-colored bold lines show exponential regression based on the alternative model in which the successful grip ratio was regarded to have changed through sessions, whereas black-colored dashed lines represent linear regression based on the null model where the successful grip ratio was regarded to have been constant through sessions. In the middle-threshold condition, the successful gripping ratio showed a significant increase through 6 sessions (Fig. 5a, $N = 8$, $p < 0.01$). Similarly, it also increased significantly through 6 sessions in the high-threshold condition (Fig. 5b, $N = 5$, $p < 0.05$). In both elevated-threshold conditions, the alternative model was adopted by parametric bootstrap approach, suggesting that the lobsters could be trained to associate gripping action with food reward by differential reinforcement regarding the grip force.

Figure 5.

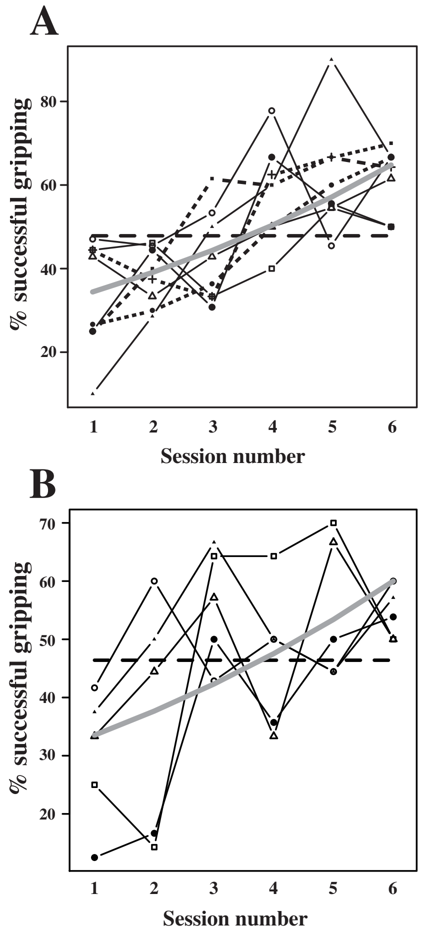

Figure 5.

Ratio of successful gripping for reinforcement through 6 sessions of two types of differential reinforcement procedure. The group 1 subjects (N =5) were imposed 4 successive conditions: low-threshold, low-threshold, middle threshold, high-threshold. The group 2 subjects (N =3) were imposed 4 successive conditions: low-threshold, low-threshold, middle threshold, middle-threshold. A: Successful gripping ratio during the third condition, i.e., middle-threshold condition after the second low-threshold condition for group 1 and group 2 subjects. Each of the 4 conditions consisted of 6 sessions, but only the successful ratios for the 6 sessions during the third condition are shown here. B: Successful gripping ratio during the forth condition, i.e., high-threshold condition after the third middle-threshold condition for group 1 subjects. Each symbol represents individual subject. Solid lines indicate group 1 subjects and dashed ones group 2. Bold curve lines show the mean value yielded by the alternative model. Dashed horizontal lines show the mean value yielded by the null model.

3.3. Effects of threshold elevation on lobster's grip force

To examine whether the changes in grip force observed during differential reinforcement were due to threshold elevation and attributable to no other factors, we next analyzed distributions of grip force in three groups that were passed through different combinations of reinforcement procedures (Fig. 6). The experimental procedure of group 1 consisted of four consecutive trainings: 1) low-threshold condition (6 sessions), 2) low-threshold condition (6 sessions), 3) middle-threshold condition (6 sessions), and 4) high-threshold condition (6 sessions). The experimental procedure of group 2 consisted of four consecutive trainings: 1) low-threshold condition (6 sessions), 2) low-threshold condition (6 sessions), 3) middle-threshold condition (6 sessions), and 4) middle-threshold condition (6 sessions). The experimental procedure of group 3 consisted of the same threshold condition tasks: 1) low-threshold condition (6 sessions), 2) low threshold condition (6 sessions), 3) low-threshold condition (6 sessions), and 4) low-threshold condition (6 sessions). The group 2 and 3 served as a middle-threshold control and a low-threshold control respectively. The grip force values obtained from the same group of individuals were box-plotted against the session number in each procedure (Fig.6). Bold lines in different gray shadings show the estimated mean value for different threshold types calculated by model selection method with AICs. Calculated AIC values of each model are summarized in Table 1. In group 1, the AIC value (2256) calculated for model 2, i.e., AABC model, was smaller than those for any other models (Fig. 6 A, Table 1). Model 2 represents the situation where the first and the second procedures yielded the same mean values of grip force~~,~~ while the third and the fourth procedures yielded the values that were different from each other as well as from that for the first and the second procedures. This conclusion was consistent with the threshold elevation schedule, supporting our conclusion on the grip force increase induced by differential reinforcement (Fig.5). In contrast, in groups 2 and 3, the AIC values calculated for model 6, i.e., AABB model (651.5), and model 8, i.e., AAAA model (550.0), were the smallest respectively. (Fig. 6 B, C, Table 1). Model 6 represents the situation that the first and the second procedures had the same mean value of grip force, while the third and the fourth had the same and greater than the first and second procedures. Model 8 represents the situation that all four procedures had the same mean value of grip force, corresponding to all low-threshold procedures. As shown in Fig. 6 and Table 1, no significant change in the estimated mean value was observed between the same threshold conditions. These results demonstrate that the increase in grip force under middle-threshold (groups 1 and 2) and high-threshold (group 1) conditions were caused by the threshold elevation.

Figure 6.

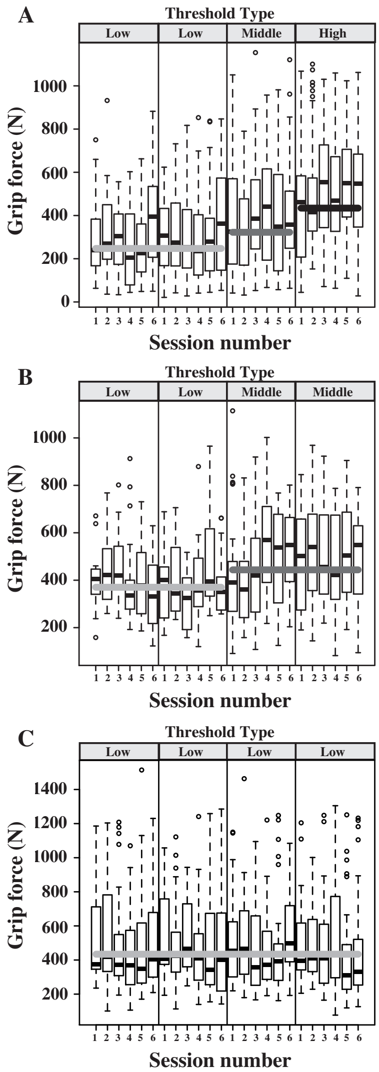

Figure 6.

Distribution of grip force in differential reinforcement experiment. Box plots show the median, first and third quartiles as well as extreme values (horizontal bars) and outliers (open circles). Three types of experimental procedure were adopted. Each procedure consisted of consecutive four schedules which included 6 sessions respectively. Each session was 30 minutes in length, 2 or 3 sessions carried out in a day. A: Grip force changes in group 1 (N =5) for the threshold raise sequence of low-, low-, middle-, and high-threshold procedures. B: Grip force changes in group 2 (N =3) for the threshold raise sequence of low-, low-, middle-, and middle-threshold procedures. C: Grip force changes in group 3 (N =4) for the threshold sequence of low-, low-, low-, and low- threshold procedures. Bold lines in gray colors represent the mean values estimated for each schedule by model selection method with AICs.

Table 1.

model	procedures 1	2	3	4	AIC group A	group B	group C
1 ABCD					2261	659.7	563.7
2 AABC					2256	654.8	557.3
3 ABBC					2277	667.2	557.2
4 ABCC					2288	656.4	561.9
5 AAAB					2282	662.5	550.5
6 AABB					2282	651.5	555.5
7 ABBB					2347	672.6	556.6
8 AAAA					2379	671.5	550.0

Table 1.

Eight statistical models designed for the differential reinforcement experiment and their AIC values in three groups. Model 1 represents a specific pattern of gripping force transition in the course of 4 successive procedures that comprised the experiment: mean values obtained in 4 procedures were different from each other, increasing with the threshold. The model is therefore referred to as an ABCD model in the text. The shading in the 4 successive box scheme in the table indicates assumed strength of the grip force: darker shading indicates stronger force. AIC values for group 1 (N =5), group 2 (N = 3) and group 3 subjects (N = 4) are shown for this model. Other seven models are also schematized in the same way and shown with AIC values for the three groups.

5. Discussion

We newly developed a composite hardware/software system (Fig.1) to make lobsters perform a manipulative task for food reward and further to train them to increase the action intensity in that task by operant conditioning. Measurement of the action intensity changes associated with the threshold setting for reinforcement is useful for quantitative behavioral analysis of learning capability in animals [32-35]. An analog system for measuring action intensity was firstly applied by Skinner [32]. A digital measurement system for lever-press paradigm was firstly developed by Notterman et al. [44]. Fowler and his colleagues have developed further measurement techniques for action intensity of operant behavior [33, 35, 36]. Several other quantitative methods for measuring the intensity of operant behavior have been since then developed especially in rats [37] and mice [38]. Although various types of operant paradigm have been established in many invertebrates [1] and [39], almost no quantitative behavioral analysis on response intensity has been reported in any invertebrate species. In this study, we applied such a quantitative measurement system for the gripping force of the lobster crusher claw to demonstrate the trainability of the animal by operant conditioning paradigms.

5.1. Basic operant procedures

We demonstrated in the present study that American lobster *Homarus americanus* could be trained by operant conditioning targeted on gripping action by the crusher claw. The animal could be reinforced with food reward for bar gripping action in the procedures of acquisition and maintenance (Fig.3), but not in yoked (Fig.4 A) and non-reward (Fig.4 B) control procedures. In addition, the bar gripping action showed a gradual decrease in frequency throughout the extinction procedure, while it showed an increase again in the reacquisition procedure (Fig.3).

A possibility we had to consider in the basic operant procedures was that the increase in the frequency of bar gripping was due to the effect of food itself to vitalize the subject so that it showed bar gripping more frequently after obtaining more food reward. We excluded the possibility of this type of pseudoconditioning by a yoked control experiment in which lobsters obtained food depending on the stay time at the feeder place, but not on the timing of gripping action (Fig.4 A). The result showed that the gripping action could not be reinforced by food rewards that were not associated with the action. Another possibility we had to consider was that the animal came to grip the bar as the stay time at the feeding place simply accumulated. The result of non-reward control (Fig.4 B) excluded this possibility, even confirming that the animals with high feeding motivation due to starvation did not grip the bar spontaneously without gripping-associated food rewards.

The current work expanded the range of invertebrate species that are capable of learning manipulative tasks in operant chambers by adding lobster *Homarus americanus* to the previous list of cockroach [40], bee [41], *Aplysia* [42], crab [14] and snail [43]. It is noted here that green crab *Carcinus meanas*, a decapod crustacean like lobsters, has been reported to successfully perform acquisition (continuous reinforcement), extinction and reacquisition procedures for pressing a lever by extending the claw or whole body [14]. This result is consistent with our data in lobster, suggesting that decapoda crustaceans have a general ability of manipulative operant conditioning.

5.2. Differential reinforcement on behavioral intensity

In the differential reinforcement paradigm, we can evaluate the capability of the animal to adapt a particular aspect of operant target to changing environment. Rats can be reinforced with food for pressing lever with forces greater than a criterion [45, 46]. Budgerigars *Melopsittacus undulates* can be reinforced with food for producing calls that were above or below a criterion level of intensity [45]. The differential reinforcement paradigm used in these studies has demonstrated whether the animal can 'voluntarily' control the response intensity [45]. In the present study, we could not confirm if the animal would decrease the gripping force to lowered threshold for food reward since the current feeder system, with a time lag between the threshold attainment and the food pellet release, was not adequate for such exploration. However, lobsters were found to grip the bar more strongly in response to at least two elevation steps depending on the reinforcement threshold (Fig. 5,6), suggesting that lobsters possess the capability to 'voluntarily' control its gripping force.

Since the gripping action of crustaceans is controlled reflectively mediated by a proprioceptive sense organ [46-50], our results suggest that some neural mechanism should exist for the descending 'voluntary' motor pathway to override that reflective pathway. In crayfish, the reflex pathway from the propodite-dactyl (PD) organ afferents to claw motor neurons has been reported to be organized monosynaptically [48,49]. The gripping force is thus regulated by proprioceptive feedback from chordotonal organ embedded in the claw [48, 49, 51]. American lobster has been thought to retain a similar proprioceptive system in its gripping control [17, 24]. It remains unknown for this moment how the ‘voluntary’ motor pathway overrides the reflective motor pathway. If the reflex from the PD organ afferents to claw motor neurons in lobster is mediated monosynaptically as in crayfish, then the site of override should be sought on the motor neuron since no direct efferent control is found in this type of arthropod proprioceptors [52]. Some evidence, however, supporting the existence of indirect efferent control and modulation of sensory organ activity has been reported in the coxo-basipodite chordotonal organ (CBCO) of crayfish [53-55]. Further study is needed to clear the central mechanism of the grip force control based on the learnt information stored in the brain of lobster.

The bar-gripping behavior of lobster is considered to correspond to shell crushing behavior in nature because the behavioral sequence is similar with each other. In both behavioral sequences observed in our laboratory, the animal first touches and grabs the target object with mouthparts and legs, subsequently pinches it with the cutter claw, and finally grips it with the crusher claw [15]. Lobsters in the natural environment may also recognize the hardness of prey clams by trial and error, and may learn to grip the clams of the same type with adaptive force without executing unnecessarily stronger force. This possibility is also open to future research both in laboratory and in natural environment.

Acknowledgement

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (18657025, 20370028). We are grateful to Dr. K. Kagaya for his valuable suggestions and criticisms in the course of this study, Dr. T. Kubo for his tutorial on statistical computation, and Dr. Y. Fujimoto for his newly developing the grip sensor.

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