Discrimination learning with light stimuli in restrained American lobster

Yusuke Tomina* and Masakazu Takahata

Animal Behavior and Intelligence, Graduate School of Life Science,

Hokkaido University, Sapporo 060-0810, Japan.

*Address correspondence to

Yusuke Tomina

Animal behavior and Intelligence

Graduate School of Life Science

Hokkaido University

Sapporo 060-0810

Tel: +81 011 706 2753

Fax: +81 011 706 4923

Email: tomina@mail.sci.hokudai.ac.jp

Abstract

Operant discrimination learning has been extensively utilized in the study on the perceptual ability of animals and their higher-order brain functions. We tested in this study whether American lobster *Homarus americanus*, which was previously found to possess ability of operant learning with claw gripping, could be trained to discriminate light stimuli of different intensities. For the current purpose, we newly developed a PC-controlled operant chamber that allowed the animal under a body-fixed condition to perform operant reward learning with claw gripping. Lobsters were first reinforced when they gripped the sensor bar upon presentation of a light cue. Then they were trained to grip the bar only when the light stimulus of a specific intensity was presented to obtain food reward while the stimuli of three different intensities including the reinforced one were presented in a random order. Finally, they were re-trained to grip the bar only when the light stimulus of another intensity that was not rewarded in the preceding training to obtain food while other intensities including the one that was rewarded previously were not rewarded any more. In these training procedures, the operant behavior occurred more frequently in response to the rewarded cue than to the non-rewarded one. The action latency for the reinforced stimuli showed a significant decrease in the course of training. These data demonstrate that lobsters can be trained with the light cues of different intensity as discriminative stimuli under a restrained condition that would allow application of electrophysiological techniques to the behaving subjects.

Key words: operant conditioning, discrimination learning, light cue, invertebrate, crustacean, American lobster

Highlights

>We newly developed an operant chamber for discrimination learning in the restrained lobsters.

>Restrained lobsters could be trained by a simple light(+)/dark(-) discrimination task.

>Light stimuli of three different intensities could be used as the discriminative cues.

>Restrained lobsters could learn to discriminate the light of a specific intensity among the three to obtain food reward.

1. Introduction

Animals learn to discriminate signals for reward from those for nonreward [1]. Operant discrimination learning has been a powerful tool for studying not only the sensory or perceptual ability of animals but also their ability of higher-order learning and cognitive behavior mainly in mammals and birds [1, 2]. Especially, in their investigation into neural mechanisms underlying cognitive brain functions including decision-making and category/concept formation, many neuroscientists have used discrimination tasks with a restrained monkey that was trained to manipulate a lever when an appropriate light cue was presented [3-5]. Recent studies utilizing the operant discrimination learning have demonstrated that some invertebrates also possess the ability of higher-order, non-elemental learning [6, 7]. Their brains, called "microbrains" [8] or "minibrains" [9], are characterized by not only their size but also the cytoarchitecture and organization of neurons that are small in their population and large in their individual cell size. Intensive behavioral and molecular biological studies have been done on the learning ability in many invertebrates including insects [6,7,10-32] and crustaceans [15, 33-38]. Electrophysiological techniques have been applied to the study of learning mechanisms in some invertebrates [39-47]. However, neurophysiological mechanisms responsible for their brain functions remain to be clarified at the level of identifiable nerve cells in the future chiefly because of experimental difficulties in recording their activities from freely behaving animals.

The American lobster *Homarus americanus* has a nervous system that is easily accessible with a variety of neurophysiological techniques [48-52] and yet can perform a

precise limb movement that is recommended as an operant response in most learning experiments [53, 54]. The lobster has a pair of bilaterally asymmetrical claws as the first thoracic appendage: the crusher is a stout, molar-toothed, slow-acting claw while the cutter is a slender, incisor-toothed, fast-acting claw. Which type grows on which side depends on the life history of individual animals [55]. The former type is usually used for breaking clamshells by gripping (defined by [56]) to eat shellfish meat so that its action can be precisely controlled regarding the direction of movements and the grip force [54, 57]. Thus lobsters can perform manipulative behavior that, as in the case of octopus tentacles and mammalian hands, requires nervous control by more complex mechanisms than those mediating locomotion and posture [5]. If we can train lobsters to perform operant discrimination learning, then it would pave the way for intensive neurophysiological analysis of higher-order brain functions, including rule learning and working memory [6, 7], at the cellular level taking advantage of the microbrain system consisting of identifiable neurons.

The present study was undertaken to test whether lobsters can achieve operant discrimination learning using visual stimuli of different intensity. Although lobsters have been known to be monochromatic in their vision [58], many physiological studies have demonstrated that they can discriminate between light intensities in the neural activities of receptor (or sensory) neurons [59-62]. We demonstrated in a previous study that lobsters could be trained by operant reward learning involving acquisition and extinction procedures with the gripping behavior even under the force control [54]. It remains unknown, however, if lobsters can perform more advanced form of learning. Since the

behavior of lobsters, having a relatively long life [63, 64] in invertebrates, is more likely to be affected by past experiences than that of less long-lived animals, we could expect that they have potential ability of higher-order learning including operant discrimination learning.

In the current experiment, tethered lobsters were first trained in an aquarium to grip a vertical bar to obtain food reward when a light stimulus was applied in a dark condition. Then they were trained to grip the bar to obtain food in response to the light stimulus of a specific intensity while light stimuli of three different intensities were presented serially at random. We conducted rigorous control experiments including reversal discrimination test to make sure that lobsters really responded to the light stimulus specifically designated in each test by food reward. Every animal thus went through the same single line of experiments consisting of seven procedures. The results obtained in ten animals demonstrated their ability of operant discrimination learning with light stimuli in the body-fixed condition, suggesting the applicability of this type of training for the neurophysiological study of their learning ability and higher-order brain functions at the level of identifiable neurons.

2. Materials and Methods

2.1. Animals

Adult lobsters, *Homarus americanus*, of both sexes were purchased at a commercial retail market (Daisan-Nishizawa, Sapporo, Japan). They 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 10-15 °C under the condition of continuous filtration. Animals were fed every four or five days with small pellets of dried fish sausage. The food was chosen because of its low-cost availability, easy preservability and handy processability. The same type of food was consistently used in the keeping aquarium as food and in the experimental aquarium as reward because lobsters show a preference for odor of familiar food [65]. Acclimation was carried out at least two weeks prior to the training under a day/night rhythm of 12L/12D: the light period started at 6 o'clock in the evening while the dark period at 6 o'clock in the morning. All experiments were done in the subjective dark period for the animal because they are generally nocturnal [66].

When a naïve lobster was placed in the experimental aquarium for the first time, it was in a highly alert and vigilant state with restless movements and unresponsive rigidity. Such an animal did not eat diet nor show spontaneous gripping behavior in many cases. Therefore, we made the animal habituated to the experimental environment prior to the experimental procedures starting with the pre-shaping process. The animal was left undisturbed in the experimental aquarium under the body-fixed condition for at least 3 hours per day for two or more days before experiment. The animal became calm in the

restrained state and responsive after the habituation. Once the pre-shaping and experimental procedures were started, the habituation process was never resumed throughout all the experiment with the animal. Thus, the habituation was not targeted to the visual stimulus but to the general environment before experiment. We tested twenty-eight animals in all. Four of them died before experiment and fourteen were judged to be unfit for the current experiment chiefly because they did not positively feed on the pellets of dried fish sausage nor spontaneously act on the sensor bar. The judgment was made during the habituation period. As a result, only those ten lobsters that could grip the sensor bar and get food directly from the feeder pipe in this habituation period were used as experimental animals. During the experimental period, animals were fed only in the operant training procedure as the reinforcement. Animals ranged between 10.9 – 14.2 cm in carapace length and 477 - 565 g in weight.

2.2. Apparatus

In a glass aquarium (90 x 45 x 45 cm) the animal was physically fixed to an acrylic tether bar and a bolted-down plate glued to its carapace using a quick-drying adhesive (Aron Alpha, TOAGOSEI, Tokyo). The animal could not move around but could freely move its appendages (Fig. 1A, B) and could be released by loosening bolts after experiment. The experimental animal was held in a position that it could grip the sensor bar with the crusher claw. The seawater was continuously filtered at 15 ± 1℃, and maintained at the depth of about 3 cm above the lobster's eyes. The whole apparatus was placed in a Faraday cage covered with lighttight curtains that completely shielded the

animal from the outside electromagnetic waves and 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 20 - 30 lx during the L period (night) and 0 lx during the D period (day) to avoid light adaptation of the animal. We carried out experiments during the day period under low-intensity red light to which lobsters are scarcely sensible (the sensitivity of the lobster visual pigment is the greatest near 525 nm, a wavelength corresponding to blue-green light [57, 67, 68]). A light cue for discrimination learning was presented by a white LED covered with a plastic hemisphere that was located immediately above the animals' head (Fig. 1A, B). The illuminance of the light stimuli was 20, 40 or 60 lx, depending on the experimental session, which can be discriminated by a closely related Astacidea crayfish species in the neural activity of its oculomotor fibers [69].

The animals obtained food, one pellet at one time, which was dropped into a water stream spouting out from a small pipe at its mouthpart. This feeder system provided the animal with food reward that was associated with the gripping behavior (see below). The detailed information of gripping sensor bar is described in [54]. The sensor was functionally coupled with the feeder motor (Oriental motor CPL28), controlled by a personal computer (CPU1 in Fig. 1A): We measured the grip force of the lobster's claw with the sensor and digitized it every 15-ms by a 16-bit A/D converter (National Instruments USB-6009) connected to CPU1 which controlled the feeder and LED by a home-made program 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. 1A).

The training program was written in Objective-C using Cocoa framework. 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 the grip force exceeded the threshold (Fig. 1C). The reinforcement delay, i.e., the latency from the time of threshold attainment to the time of pellet release, was within a second in every experimental session. One training session was finished when the sensor bar was manually removed from the set position at a scheduled time. We also observed the lobster behavior during experiment under a low-intensity red light.

2.3. Experimental design

All ten animals went through the same seven procedures including (1) pre-shaping, (2) shaping, (3) post-shaping, (4) light(+)/dark(-) discrimination, (5) dim(+)/middle(+)/bright(+) training, (6) dim(-)/middle(-)/bright(+) or dim(+)/middle(-)/bright(-) discrimination, and (7) reversal discrimination. We trained two animals per day rotationally in this study. The values of experimental parameters such as intensity and duration of light stimulation were determined by repeated pilot studies conducted before the reported experiment and were rigorously controlled throughout this study.

2.3.1. Pre-shaping procedure

In order to acclimate the experimental animals to the light stimulus, naïve lobsters were exposed to 5-min dark-situation and 5-min light-presentation (40 lx) alternately in a 30-min session under the body-fixed condition. Lobsters obtained no reward for their gripping actions. The procedure was performed for 2 day, three 30-min sessions per day.

2.3.2. Shaping procedure

Since naïve animals did not know that gripping the sensor bar would bring them food reward, we let them know it by this procedure. On the day following the pre-shaping procedure, we forced the animal to grip the sensor bar reflexively by holding the meropodite part of the claw close to it with a waterproof wire wound around them. In this situation, small amounts of food were dispensed when the lobster gripped the bar. The animals were exposed to 5-min dark-situation and 5-min light-presentation (40 lx) alternately in a 30-min session. These exercises were conducted repeatedly 5 times in each light and dark condition per one session and all 3 sessions were carried out. After this training, we observed that the animal showed gripping behavior more spontaneously than before (Fig. 2). The animal was regarded to have been "shaped" for the bar-gripping task. Those animals that did not present gripping behavior were excluded from the present study.

2.3.3. Post-shaping procedure

This procedure was carried out to test the possibility that the animal has developed a preference for either the light or dark condition during the shaping procedure. In this

procedure, lobsters obtained no reward even when they gripped the bar spontaneously. The procedure was performed on the day following the shaping procedure in three 30-min sessions. Lobsters were exposed to 5-min dark-situation and 5-min light-presentation (40 lx) alternately in a session. 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.

2.3.4. Light(+)/dark(-) discrimination

This procedure was undertaken to let the animal learn that gripping the bar in the light condition is rewarded while that in the dark condition is not on the days next to the post-shaping. In the light(+)/dark(-) discrimination schedule, the animal obtained food reward for gripping action in the presence of light stimulus (40 lx) and not in the dark condition. Dark situation (5-min) and light presentation (5-min) were switched around in a 30-min session. The procedure was performed over 4 successive days, three 30-min sessions per day.

2.3.5. Dim(+)/middle(+)/bright(+) training

On the day following the light(+)/dark(-) discrimination procedure, we provided the animal with three light conditions with different intensities, but the animal was rewarded with food when it gripped the sensor bar in the light condition regardless of the light intensity. This procedure was undertaken to test whether animals could be trained to grip the bar to obtain food reward when the light stimuli that were different from the

preceding one in illuminance were provided. The dim(+)/middle(+)/bright(+) training was carried out for total 12 sessions. In this procedure, 1-min presentations of bright light (60 lx), middle light (40 lx) and dim light (20 lx) were pseudorandomly switched around with 30-sec dark intervals in a 45-min session. The pseudorandomness was generated by an online statistical utility (the website WebCalculator [http:// www.webcalculator.co.uk]). Each light cue was presented 10 times in total respectively and the dark interval was inserted 30 times in a session. The animal was reinforced for gripping action during the bright/middle/dim light presentations with food reward. The procedure was performed for 4 days, two to four sessions per day.

2.3.6. Light intensity discrimination

This was the main experiment in the present study. All preceding procedures were carried out to standardize the animal for the discrimination training. After the dim(+)/middle(+)/bright(+) training, animals learnt to grip the bar when the light was turned on in any intensity to obtain the food. On the day next to the dim(+)/middle(+)/bright(+) training that lasted for 4 days, we randomize subjects into two groups: dim(-)/middle(-)/bright(+) group and dim(+)/middle(-)/bright(-) group. In both discrimination schedules, bright/middle/dim lights were presented in the same manner as in the dim(+)/middle(+)/bright(+) schedule. In the dim(-)/middle(-)/bright(+) group, lobster was reinforced for gripping action only in the bright light (60 lx) presentation, while in the dim(+)/middle(-)/bright(-) group gripping behavior was reinforced only in dim light (20 lx) presentation. The gripping in other light conditions

was never reinforced. These procedures were conducted for total 12 sessions in each group. Both procedures were performed for 4 successive days, two to four sessions per day. What was expected in this experiment was that if the animal could discriminate the light conditions of different intensities then it would continue, following the dim(+)/middle(+)/bright(+) training, to grip the bar only when the light was turned on at the specified intensity and became more and more reluctant to grip it at different light intensities.

2.3.7. Reversal discrimination

In order to test the possibility that the animal simply continued to grip the bar and did not positively discriminate the light of specific intensity in the preceding discrimination experiment, we carried out the reversal discrimination experiment in which the animal was retrained to learn in the opposite way to obtain food reward. In this procedure, the stimulus-reinforcement conditions were reversed in each group: the dim(-)/middle(-)/bright(+) group in the preceding procedure was switched over to the dim(+)/middle(-)/bright(-) procedure and vice versa. These procedures were carried out for total 12 sessions in each group. Both procedures were performed for 4 successive days, two to four sessions per day.

2.4. Statistical Analysis

Statistical analysis was performed by generalized linear mixed models (GLMMs) [70] using the R programming software (2.12.1 version) and lme4 package (0.999375-39

version) in R (R Development Core Team). In each procedure to which the statistical analysis was applied, we constructed two models to explain the behavioral data: the alternative model and the null model, and tested these models by a likelihood ratio test, asymptotically applying the chi-square distribution with degrees of freedom equal to the difference in the number of identifiable parameters in the two models as described by [54, 71]. The difference was considered to be significant when p-value < 0.05.

3. Results

We carried out a series of training procedures to confirm that the lobsters could learn light discrimination tasks in the restrained condition. In this experiment, ten animals that were screened by the criteria (described in Materials and Methods) were passed through 7 successive procedures: Pre-shaping, shaping, post-shaping, light(+)/dark(-) discrimination, dim(+)/middle(+)/bright(+) training, light intensity discrimination and reversal discrimination. These procedures were carried out on successive 20 days (Fig. 1D). Each procedure before the discrimination training was not independent, but was intentionally related to its preceding and following ones to standardize animals' internal motivation toward bar gripping as practically as possible.

Pre-shaping, shaping and post-shaping procedures

Naïve animals did spontaneously grip the sensor bar, but the frequency of this behavior was so low as to take a very long time to practically complete the whole series of experiments. We therefore had to let them know that bar gripping is rewarded with food by a shaping procedure. Shaping means creation of a behavior that is not originally in the behavioral repertoire of an animal by forcing it to do that behavior and obtain reward for the behavior [15]. Statistical comparison of bar gripping frequency before and after the shaping procedure revealed that the frequency increased significantly (likelihood ratio test, $P < 0.05$).

We then confirmed whether lobsters had any preference for bar gripping in the light or dark condition before and after the shaping procedure. In the pre-shaping and

post-shaping procedures, where the animals (N = 10) were exposed to the light and dark situation alternately in a session but obtained no reward upon bar gripping, there was no significant difference in the grip counts between the light and dark conditions (Fig. 2, pre-shaping: $P = 0.9986 > 0.05$, post-shaping $P = 0.8314 > 0.05$). The data suggests that lobsters potentially have no definite preference to grip the bar in either the light or dark condition. At the beginning of the post-shaping procedure, the experimental animals tended to grip the bar regardless of the light condition as a consequence of the shaping procedure in which gripping was rewarded with food regardless of the light condition. At the end of the post-shaping procedure, however, they became unresponsive to the sensor bar in either light condition (Fig. 2).

Light/dark discrimination procedure

In order to test if lobsters can discriminate between light and dark condition, we next carried out the light(+)/dark(-) training procedure using the same ten animals. The dark (5-min at 0 lx) and light (5-min at 40 lx) conditions were switched around in a 30 min session. The lobsters obtained food reward for the gripping action in the light condition but not in the dark condition. During this discrimination procedure, the bar-grip action showed a significant increase in frequency at the light presentation ($P = 2.200 \times 10^{-16} < 0.001$) compared with the dark situation ($P = 0.7579 > 0.05$) (Fig. 3A). The cumulative action profile clearly shows that the gripping count increased steadily in a whole one session at the end of the training (Fig. 3B). The results indicate that the light presentation at the illuminance of 40 lx can be utilized as a discriminative cue for lobsters

in this paradigm. It is noted here that the lobster gripped the bar to obtain food more than one time in a single light stimulus (Fig. 3C). The grip action record in Fig. 3D shows that the animal can grip the bar to obtain food at least three times in 5 minutes of the light condition. This observation implied a possibility that the food reward itself actively drove the animal to grip the bar: the action frequency (grips/stimulus) during the light presentation of 5 minutes rose above the value of 1.0 through sessions. This possibility, however, turned out to be excluded in the later experiment in which the bar gripping in a short-duration (1 minute) light condition was selectively reinforced with food reward among other two light conditions that were presented to the animal in a random order in the light intensity discrimination procedure.

Multi-level light reinforcement procedure

We next tested whether the lobsters could be trained by multi-level light cues including bright (60 lx), middle (40 lx) and dim (20 lx) light stimuli. This training was intended to confirm that the animal could learn the association of gripping with reward in the period of 12 sessions over 4 days, not to test the discrimination ability of the animal that was to be tested in the following procedures using the same period of 12 sessions over 4 days. The training was characterized by equal reinforcement of the bar-gripping action in the conditions of three different light intensities, i.e., dim(+)/middle(+)/bright(+) training. The bar gripping in the dark condition was not reinforced. 1-min presentations of bright light, middle light and the dim light were switched around with 30 sec dark intervals in a 45-min session. Each light cue was totally

presented 10 times and a dark interval was inserted 30 times in a session. The gradual increase in the frequency count of gripping behavior along with the session number under presentation of all three-level light stimuli (Fig. 4A; dim(+): $P = 2.843 \times 10^{-11} < 0.001$, middle(+): $P = 1.571 \times 10^{-7} < 0.001$, bright(+): $P = 1.545 \times 10^{-11} < 0.001$) demonstrated that lobsters could be trained to grip the bar to obtain food when any of the light stimuli was present, although it remained unknown in this experiment whether they could discriminate different light intensities. There was no remarkable difference among the action frequency in three different light conditions ($P = 0.7398 > 0.05$). The gripping frequency under the dark condition remained at a low level (Fig. 4A; $P = 0.084 > 0.05$). The cumulative action profile shows that the animals, reluctant to grip the bar at the beginning of training, became positively grip the bar to obtain food at the end of the training in any of the different light conditions (Fig. 4B). In this procedure, the animal seldom gripped the bar more than one time during a single light stimulus (Fig. 4C). It can be seen in the action record illustrated in Fig. 4C that the animal came to grip the bar almost whenever the light was turned on at any of the three different intensities when the training was completed.

Selective discrimination procedures

To examine whether the lobsters could discriminate those light stimuli of different intensities, we next carried out the light intensity discrimination procedures. After the dim(+)/middle(+)/bright(+) training, we divided the ten animals into two groups: Dim(-)/middle(-)/bright(+) (N = 5) and dim(+)/middle(-)/bright(-) (N = 5) groups. In

both the dim(-)/middle(-)/bright(+) and dim(+)/middle(-)/bright(-) procedures conducted for four successive days just following the dim(+)/middle(+)/bright(+) procedure, light stimuli of three different intensities were presented in the same manner as in the preceding dim(+)/middle(+)/bright(+) procedure. Both procedures were performed for 12 sessions, two to four sessions per day for 4 successive days. In the dim(-)/middle(-)/bright(+) discrimination procedure, lobsters were reinforced for gripping action only in the bright light (60 lx) presentation whereas in the dim(+)/middle(-)/bright(-) discrimination procedure, other lobsters were reinforced for gripping action only in the dim light (20 lx) presentation (Fig. 5A).

The gripping frequency during the reinforced light stimulus gradually increased along with the session number from the initial level that was attained by the previous dim(+)/middle(+)/bright(+) training (Fig. 5B; $P = 3.118 \times 10^{-6} < 0.001$ for the bright(+) condition in the dim(-)/middle(-)/bright(+) group, $P = 0.0032 < 0.01$ for the dim(+) condition in the dim(+)/middle(-)/bright(-) group) while the gripping during the unreinforced light stimulus showed a gradual decrease in its frequency from the initial level (Fig. 5B $P = 1.102 \times 10^{-10} < 0.001$ and $P = 0.0411 < 0.05$ for the dim(-) and middle(-) conditions respectively in the bright(+)/(middle(-)/dim(-) group; $P = 1.573 \times 10^{-7} < 0.001$ and $P = 0.0103 < 0.05$ for the bright(-) and middle(-) conditions respectively in the bright(-)/(middle(-)/dim(+) group). The cumulative action profile shows that the bar gripping during unreinforced light stimuli clearly decreased at the end of the training (Fig. 5C Middle and Bright, Fig. 5D Dim and Middle) while the gripping during reinforced light stimuli showed an increase when the training ended (Fig. 5C Dim, Fig. 5D Bright).

It should be noted here that the slope of decrease in the gripping frequency for the middle light stimulus tended to be less steep than that for another non-reinforcement light stimulus, i.e. dim(-) or bright(-). The estimated value of the negative slope for the dim(-) and middle(-) stimuli was -0.35 and -0.11 respectively in the dim(-)/middle(-)/bright(+) procedure, and that for the bright(-) and middle(-) stimuli was -0.33 and -0.19 respectively in the dim(+)/middle(-)/bright(-) procedure. The differences were statistically significant ($P = 0.0014 < 0.01$ for dim(-)/middle(-) in the dim(-)/middle(-)/bright(+) group and $P = 0.0119 < 0.05$ for the bright(-)/middle(-) in dim(+)/middle(-)/bright(-) group).

The GLMM analysis adopting the group category as the factorial explanatory variable revealed a statistically significant difference between the bright(+) and bright(-) groups (Fig. 5B Bright) and between the dim(+) and dim(-) groups (Fig. 5B Dim) ($P = 2.481 \times 10^{-7} < 0.001$ for bright(+)/bright(-) groups and $P = 0.0012 < 0.01$ for dim(+)/dim(-) groups). As to the middle(-) groups, there was no significant difference between them (Fig. 5B Middle) ($P = 0.4334 > 0.05$). The mean action count during each reinforced light presentation never rose above 1.0 throughout all 12 sessions over 4 days.

In order to exclude the possibility that the lobsters just kept gripping simply because food was coming and to confirm that they positively discriminated the reinforced light intensity, we re-trained the same animals to grip the bar in the reverse condition: the training conditions for the dim(-)/middle(-)/bright(+) group was changed to the dim(+)/middle(-)/bright(-)condition and vice versa. Bright/middle/dim lights were presented in the same manner as before. Both procedures were performed for 4 days, two

to four sessions per day (Fig. 6A). The gripping frequency during the newly reinforced light stimulus increased gradually along with the session number from the initial low level that was attained by the extinction process provided by the previous training in which the dim or bright light was not reinforced together with the middle light. The increase in the gripping frequency was statistically significant (Fig. 6B Dim and Bright. $P = 6.556 \times 10^{-7} < 0.001$ for the dim(+) condition in the bright(-)/(middle(-)/dim(+) group, and $P = 5.304 \times 10^{-9} < 0.001$ for the bright(+) condition in the bright(+)/(middle(-)/dim(-) group). On the other hand, the gripping frequency during the unreinforced light stimulus showed a decrease along with the session number from the initial high level that was attained by the preceding reinforcement training. The decrease in the gripping frequency was statistically significant ($P = 1.620 \times 10^{-6} < 0.001$ for the bright(-) condition in the bright(-)/(middle(-)/dim(+) group, and $P = 3.011 \times 10^{-8} < 0.001$ for the dim(-) condition in the bright(+)/(middle(-)/dim(-) group). The grip count in the unreinforced middle light showed no significant difference between the two procedures (Fig. 6B Middle. $P = 0.4189 > 0.05$ for the middle(-) condition in the bright(-)/(middle(-)/dim(+) group, and $P = 0.2592 > 0.05$ for the middle(-) condition in the bright(+)/(middle(-)/dim(-) group). The cumulative action profile (Fig. 6C, D) shows that the light intensity for the gripping behavior was clearly reversed: thus, two representative animals that first tended to grip the bar when the bright light was presented ceased to do so (Fig. 6C Bright) and instead tended to grip it when the dim light was presented at the end of this reversal training (Fig. 6C Dim). Other animals that first tended to grip the bar when the dim light was presented ceased to do so (Fig. 6D Dim) and instead came to grip it when the bright light was

presented as the training proceeded (Fig. 6D Bright).

There was a statistical difference between the bright(-) and bright(+) groups (Fig. 6B Bright), and between dim(+) and dim(-) groups (Fig.6B Dim) ($P = 0.0053 < 0.01$ for bright(+)/bright(-) groups and $P = 0.0298 < 0.05$ for dim(+)/dim(-) groups). As to the middle(-) groups, there was no significant difference between them ($P = 0.2634 > 0.05$) (Fig. 6B Middle). The mean action count during each newly reinforced light presentation never rose above 1.0 throughout all 12 sessions over 4 days.

Latency analysis

We further analyzed the action latency, i.e., the time between the stimulus onset and the beginning of the first gripping action in the procedures of light(+)/dark(-) discrimination, dim(+)/middle(+)/bright(+) training, light intensity discrimination and reversal learning all of which were carried out in the training period of 1 minute except the first one that lasted for 5 minutes. The gripping action was observed less than once in average during a single light stimulus of 1 minute duration while it occurred more than once when the stimulus duration was 5 minutes in the light(+)/dark(-) discrimination procedure. Quantitative analyses revealed that the latency of the gripping action in response to light stimulation showed a statistically significant decrease as the session number increased in the 5-min light(+)/dark(-) discrimination procedure (Fig. 7 A, B, $P = 0.0006 < 0.05$). In the dark situation, the latency from the time when the light was switched off to the time when the animal first gripped the bar showed a statistically significant increase as the training proceeded (Fig. 7C, D, $P = 0.0021 < 0.05$).

In the dim(+)/middle(+)/bright(+) training procedure in which the light stimulus lasted for 1 minute, the action latency in each light presentation generally decreased as the twelve sessions proceeded over four days (Fig. 8) The decrease was statistically significant in either light intensity ($P = 1.733 \times 10^{-8} < 0.001$ for the bright light (Fig. 8A, B), $P = 7.372 \times 10^{-11} < 0.001$ for the middle light (Fig. 8C, D), and $P = 4.152 \times 10^{-8} < 0.001$ for the dim light).

In the light intensity discrimination procedure, the latency for the reinforced light presentation was maintained at a short value that was attained by the preceding dim(+)/middle(+)/bright(+) training throughout the twelve sessions (Fig. 9A. $P = 0.9296 > 0.05$ for the bright(+) condition in the dim(-)/middle(-)/bright(+) training, $P = 0.3982 > 0.05$ for the dim(+) condition in the dim(+)/middle(-)/bright(-) training). The latency for the unreinforced light presentation showed a significant increase in the course of the training (Fig. 9A. $P = 0.0483 < 0.05$ for the dim(-) condition and $P = 6.016 \times 10^{-4} < 0.001$ for the middle(-) condition in the dim(-)/middle(-)/bright(+) training, $P = 0.0020 < 0.01$ for the bright(-) condition and $P = 0.0016 < 0.01$ for the middle(-) condition in the dim(+)/middle(-)/bright(-) training). In the reversal learning procedure, the prolonged latency attained by the preceding training showed a statistically significant decrease when the light intensity for reinforcement was switched over (Fig. 9B. $P = 0.0016 < 0.01$ for the dim(+) condition in the dim(+)/middle(-)/bright(-) training and $P = 0.0153 < 0.05$ for the bright(+) condition in the dim(-)/middle(-)/bright(+) training). For the unreinforced light stimuli, the action latency that was shortened in the preceding training showed a statistically significant increase (Fig. 9B. $P = 0.0253 < 0.05$ for the bright(-) condition in

the dim(+)/middle(-)/bright(-) training and $P = 0.0238 < 0.05$ for the dim(-) condition in the dim(-)/middle(-)/bright(+) training). The action latency for the middle-intensity light stimulation that was never reinforced in both dim(+)/middle(-)/bright(-) and dim(-)/middle(-)/bright(+) groups was maintained at a prolonged level as attained in the preceding training (Fig. 9B. $P = 0.6543 > 0.05$ for the middle(-) condition in the dim(+)/middle(-)/bright(-) training and $P = 0.8072 > 0.05$ for the middle(-) condition in the dim(-)/middle(-)/bright(+) training).

4. Discussion

Operant discrimination learning has been extensively utilized in the neurophysiological analysis of brain mechanisms underlying higher-order or cognitive aspects of voluntary behavior using primates [3-5]. Now that evidence has been accumulating that invertebrate animals also show voluntary behavior [72-74] and display highly complex behaviors that suggest cognitive processes including rule learning and concept/category formation [6, 7, 30], it is an urgent problem to develop an experimental system that will enable neurophysiological analysis of the cellular mechanisms underlying these processes at the level of identifiable neurons although criticisms do exist regarding the "cognitive" nature of invertebrate behavior [75, 76].

Discrimination learning is displayed by nearly all animals including unicellular organisms [1]. Various types of discriminative operant learning have been reported in many arthropods such as honeybees [6, 7, 30], bumblebees [7], and fruit flies [6, 7, 23] as well as in other invertebrates [7, 14, 23, 77]. But since the operant behavior in these studies involves almost all of the body parts with the exception of a few [e.g. 77], they cannot be used in neurophysiological investigation that requires extracellular or intracellular recording from the central neurons. Almost no operant discrimination paradigm targeted for any spontaneous manipulative action has been reported in invertebrate animals except honeybees for which the operant reward learning of antennal movements was developed in a body-fixed condition [20, 41] and the solitary bee *Meloipona anthidiodes* in which the lever-pressing by front leg movement was targeted as the operant behavior [12, 15]. In this study, we newly developed an operant chamber

system to make lobsters perform a discrimination task under the body-fixed condition illustrated in Fig. 2A. We could demonstrate that American lobster *Homarus americanus*, having a nervous system that is easily accessible with a variety of neurophysiological techniques, could be trained by discrimination learning using light cues under a restrained condition.

Discrimination learning in the lobster

The lobster could be trained to grip the sensor bar to obtain food reward only when the light stimulus was turned on in the light(+)/dark(-) procedure (Fig. 3) at the end of which the animal tended to discriminate the light cue (40 lx) that lasted for 5 minutes for reward from the dark state for nonreward, although there was no such a tendency in the pre-shaping and post-shaping procedures (Fig. 2). In the current study, we did not confirm if the animal could be trained by the reversed light(-)/dark(+) procedure so that it shows bar gripping action only in the dark condition. However, lobsters were found to grip the bar more frequently during light presentation than before (Fig. 3), suggesting that they could behave in response to a discriminative cue for reward.

After the simple light(+)/dark(-) discrimination training, the same animal was subjected to the dim(+)/middle(+)/bright(+) training using the light stimuli of 60 lx, 40 lx and 20 lx in illuminance. The bar-gripping action under the presence of these light stimuli showed a gradual increase in its frequency regardless of the intensity (Fig. 4). These results demonstrated that the lobster could respond to any of the three light intensities to grip the bar for food reward, suggesting the possibility that multilevel light stimulation

could be used as the discriminative stimuli. We thus tested if the animal could discriminate these light intensities or not by selective reinforcement procedures. Those procedures from the pre-shaping to the dim(+)/middle(+)/bright(+) training were applied to ten animals individually. They were divided into two groups: the dim(-)/middle(-)/bright(+) and dim(+)/middle(-)/bright(-) discrimination test sets. The grip count during the reinforced light stimuli tended to be kept high and even show an increase from the level attained by the preceding dim(+)/middle(+)/bright(+) training while that during the no-reward light showed a gradual decrease from the high level attained at the end of the preceding training (Fig. 5). These results suggested that lobsters could discriminate different light intensities of 20, 40 and 60 lux in illuminance to behave differently in response to them.

There was an alternative interpretation of the obtained results: the lobsters might just have kept gripping if food was coming and stopped gripping if gripping was not followed by food regardless of which light intensity was being presented. This interpretation, however, is unlikely by threefold reasons. First, the reversal discrimination training that followed the selective discrimination procedure demonstrated that the lobsters positively learnt to grip the bar to obtain food when the light stimulus that was not rewarded in the preceding training but switched to be rewarded in the reversal training was presented (bright(+) or dim(+) in Fig. 6). They also positively learnt not to grip the bar when the light stimulus that was rewarded before but not in the reversal training for extinction (bright(-) or dim(-) in Fig. 6). Second, the light stimuli of different intensities were presented to the animal in a random order so that, for instance in the

dim(-)/middle(-)/bright(+) experiment, the bright light stimulus that was rewarded by food was randomly followed, with a dark interval of 30 seconds, by one of other stimuli including not only the same rewarded one but also other two that were not rewarded (Fig. 5A). Finally, the gripping count during the rewarded light stimulus showed a statistically significant increase from the level attained in the preceding dim(+)/middle(+)/bright(+) training ($P < 0.05$; Fig. 5B). Taken together, the experimental data obtained in this study unambiguously demonstrate the capability of lobsters to perform discriminative tasks on different light intensities ranging 20 - 60 lx. In addition, the shortening of the action latency upon light stimulation (Figs.7-9) also support our conclusion on the analogy of psychological investigations into discriminative operant tasks using mammals reporting considerable shortening of latencies for the reinforced action [78-81].

Physiological implications

The lobster *H. americanus*, which is a nocturnal animal distributing from intertidal area to continental shelf at a depth of 700 m [51] in their habitat, have been assumed to possess a visual system adapted for use in low-intensity light environments, defensively responding to shadows of potential predators looming above them and capturing live preys [50] presumably by detecting small changes in the reflecting light intensity. The discrimination threshold for the visual sense in the lobster is unknown, but in a crustacean *Daphnia* it has been reported to be about 1/10 [82]. The intensity differences in the current discrimination training ranged from 0.3 to 2.0, much larger than the widely accepted general value for the discrimination threshold in visual sense [83]

and the reported value for *Daphnia*. The behavioral discrimination of different light intensities observed in this study is thus consistent with the physiological characteristics of the lobster visual system.

Nonetheless, the exact behavioral significance of light intensity discrimination at the range used in this experiment is not satisfactorily understood for lobsters in the natural marine environment. Electrophysiological analyses for visual properties of lobster using electroretinogram (ERG) also indicated its ability to discriminate between different light intensities [58-61]. The relationship between ERG and the light intensity (response-energy or V-log *I* relationship) followed Weber-Fechner's low approximately in a range of 3 log units in the dark-adopted lobster, but the absolute value of light illumination in their experiments is not described explicitly. Furthermore, little is understood in lobster about to what extent the ERG responses must differ in order to cause the animal to behave differently in nocturnal environment. In the crayfish *Procambarus clarkii*, it has been reported that the illuminance of 20, 40 or 60 lx can be discriminated in the neural activity of oculomotor fibers that control the movements of eyestalks [57]. In the lobster, such data on the visual responses in the motor system is critically lacking.

It would be interesting note here that in the selective discrimination procedures, the grip count in the middle(-) light cue tended to decrease less than that for other bright(-) or dim(-) light ($P < 0.05$; Fig. 5B). It means that while the gripping during bright (dim) light stimulation was reinforced, the action during dim (bright) light stimulation was extinguished rapidly but that during middle light stimulation less rapidly. This

tendency suggests the presence of generalization effect based on the light intensity, meaning that lobster could make discrimination between different light illuminances.

Conclusions

In this study, we demonstrated by using a specific operant paradigm that the lobsters are apparently able to discriminate between light stimuli of different intensities under the dark condition. A line of neurophysiological evidence has been reported for the chemically discriminative ability of the lobster on amino acids with its lateral antennule [54]. The physiological basis of visually discriminative ability of the lobsters, however, remains to be analyzed in the future study as well as the ecological meaning of such ability. But such an ability of the animal that is highly social [57, 66, 84, 85], has a simple nervous system with identifiable neurons, and yet shows clever behavior [66, 84-86] would make the lobsters a useful experimental system for the neurophysiological analysis of highly complex functions of their brain such as rule learning and concept/category formation reported in some insects that are thought to have arisen from within the crustaceans to which lobsters belong [87].

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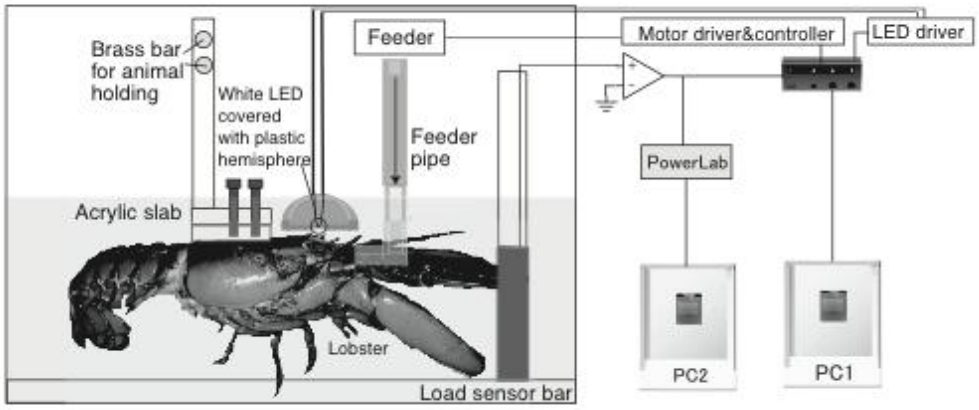
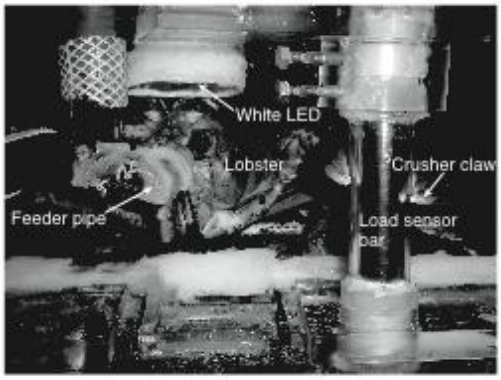
Figure 1

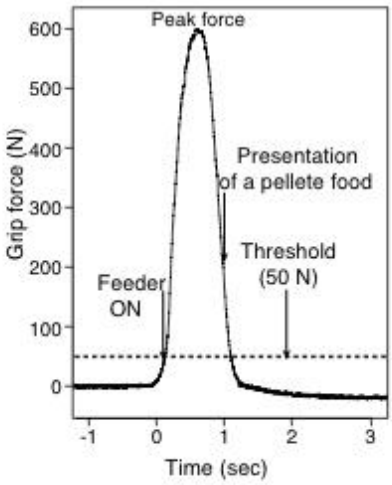
A



B



C



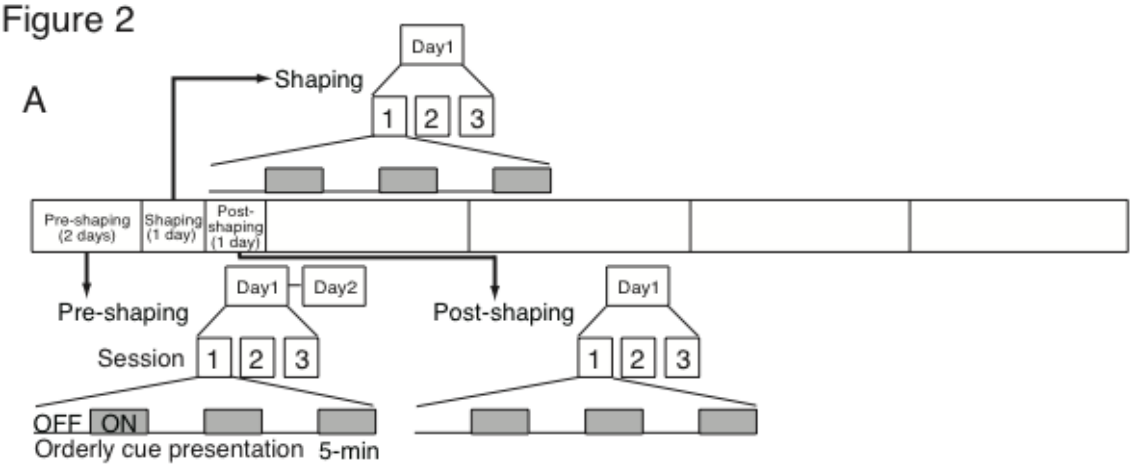
D

Pre-shaping (2 days)	Shaping (1 day)	Post-shaping (1 day)	Light(+)/dark(-) (4 days)	Dim(+)/middle(+)/brgiht(+) (4 days)	Light intensity discrimination (4 days)	Reversal learning (4 days)

Figure 1.

Experimental setups for the present study. A: An operant chamber. The animal was fixed to a holder by acrylic implements. Bar gripping was detected by a load sensor whose output was fed into PC1 that controlled the feeder system and the LED driver for presentation of light cues. The sensor signal was also fed into PC2 for continuous recording of the sensor signal throughout the experiment. B: A trained lobster gripping the sensor bar with its crusher (left) claw. C: Temporal profile of the grip force development. The gripping behavior was maintained for more than 1 second in most cases. The reinforcement threshold, which was the critical value for reinforcement of the operant response, was 50 N in this case. The latent time for the reward, i.e., the time between threshold passing and pellet food falling, was about a second in every experiment. D: Schematic drawing of the experimental schedule. Every animal in this study passed through this schedule.

Figure 2


A
Shaping
Day1
1
2
3
Pre-shaping (2 days)
Shaping (1 day)
Post-shaping (1 day)
Pre-shaping
Day1
Day2
Session
1
2
3
Post-shaping
Day1
1
2
3
OFF
ON
Orderly cue presentation
5-min

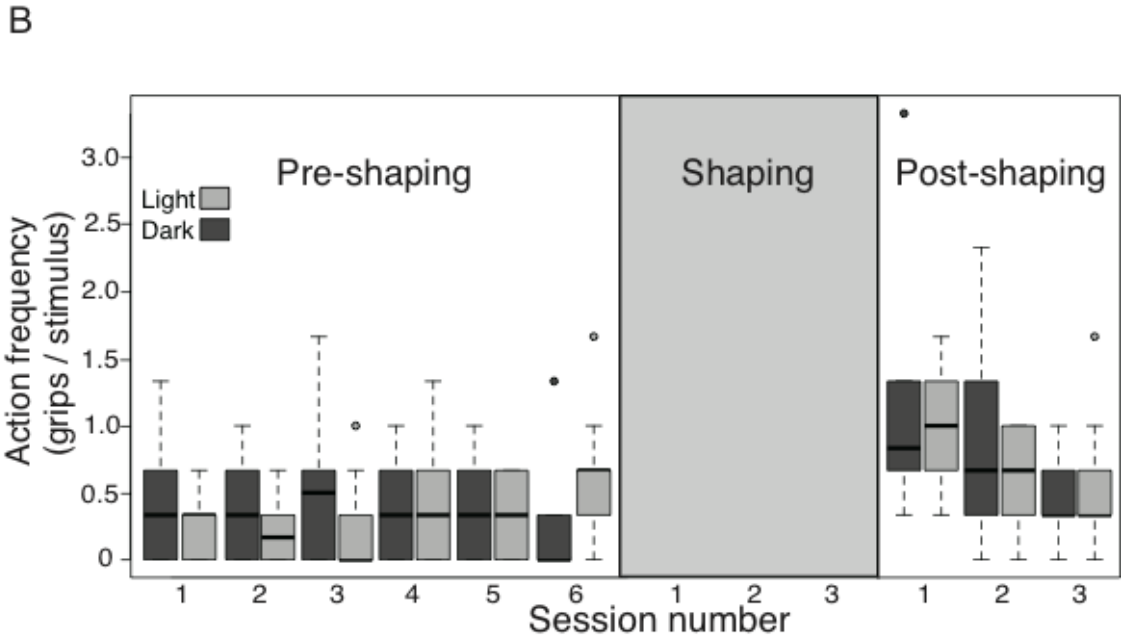

B
Pre-shaping
Shaping
Post-shaping
Light
Dark
Action frequency (grips / stimulus)
3.0
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
Session number

Figure 2.

Bar-gripping actions (grips/stimulus) in the pre-shaping, shaping, and post-shaping procedures (N = 10). A: Timing of these procedures in the whole experimental schedule. In these procedures, the dark (5-min) and light (5-min) conditions were switched around in a 30 min session. B: Gripping actions before and after the shaping procedure. The ordinate shows the number of gripping action during each dark and light condition in the training session represented by the abscissa. Box plots show the median, first and third quartiles of the data distribution. Whiskers denote the minimum-max range of the data within 1.5 times the length of box. Outliers are shown with closed circles. The black box on the left side and the gray box on the right side represent the dark and light condition, respectively for each session. The horizontal line represents the value of 1.0 where the number of gripping is equal to the number of stimulus. In the pre-shaping and post-shaping procedures, there was no significant difference in the action frequency between the light and dark conditions ($P > 0.05$).

Figure 3

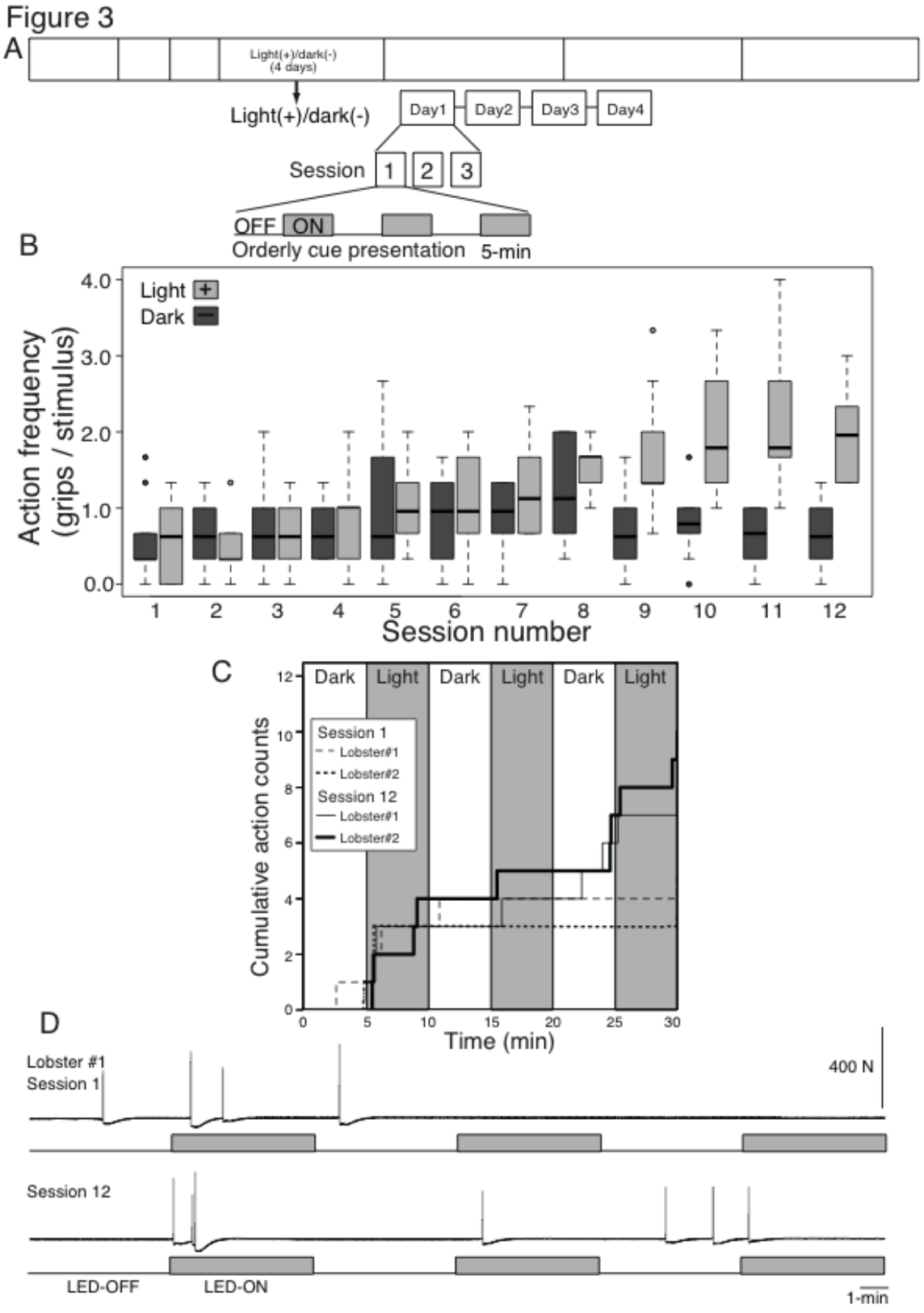

A
Light(+)/dark(-)
(4 days)
Light(+)/dark(-)
Day1
Day2
Day3
Day4
Session
1
2
3
OFF
ON
Orderly cue presentation
5-min
B
Light
Dark
Action frequency
(grips / stimulus)
4.0
3.0
2.0
1.0
0.0
1
2
3
4
5
6
7
8
9
10
11
12
Session number
C
Dark
Light
Dark
Light
Dark
Light
Session 1
Lobster#1
Lobster#2
Session 12
Lobster#1
Lobster#2
Cumulative action counts
12
10
8
6
4
2
0
0
5
10
15
20
25
30
Time (min)
D
Lobster #1
Session 1
400 N
Session 12
LED-OFF
LED-ON
1-min

Figure 3.

Light(+)/dark(-) discrimination procedure. A: Timing of the procedure in the whole experimental schedule. The dark (5-min, unreinforced) and light (5-min, reinforced) conditions were switched around in a 30 min session. B: Box plot of the action frequency (grips/stimulus) for the dark (black) and light (gray) conditions through 12 sessions (N = 10). The black box on the left side and the gray box on the right side represent the dark condition and light condition respectively for each session. The horizontal line represents the value of 1.0 where the number of gripping is equal to the number of stimulus. The bar-gripping frequency showed a significant increase in the light condition ($P < 0.05$) but not in the dark condition ($P > 0.05$). C: Cumulative records of the gripping action in the first and twelfth sessions in two representative subjects (Lobster #1 and #2). Broken lines indicate their performance in the first session and solid lines their performance in the twelfth session. The gripping behavior in the light condition occurred more frequently in the last session than in the first one. D: Action record examples for the first (the upper set of traces) and twelfth (the lower set) sessions in the training of Lobster #1. Spikes in the upper trace represent gripping actions while the lower trace schematically shows the time course of stimulus presentation. The animal gripped the bar more frequently in the last session than in the first one.

Figure 4

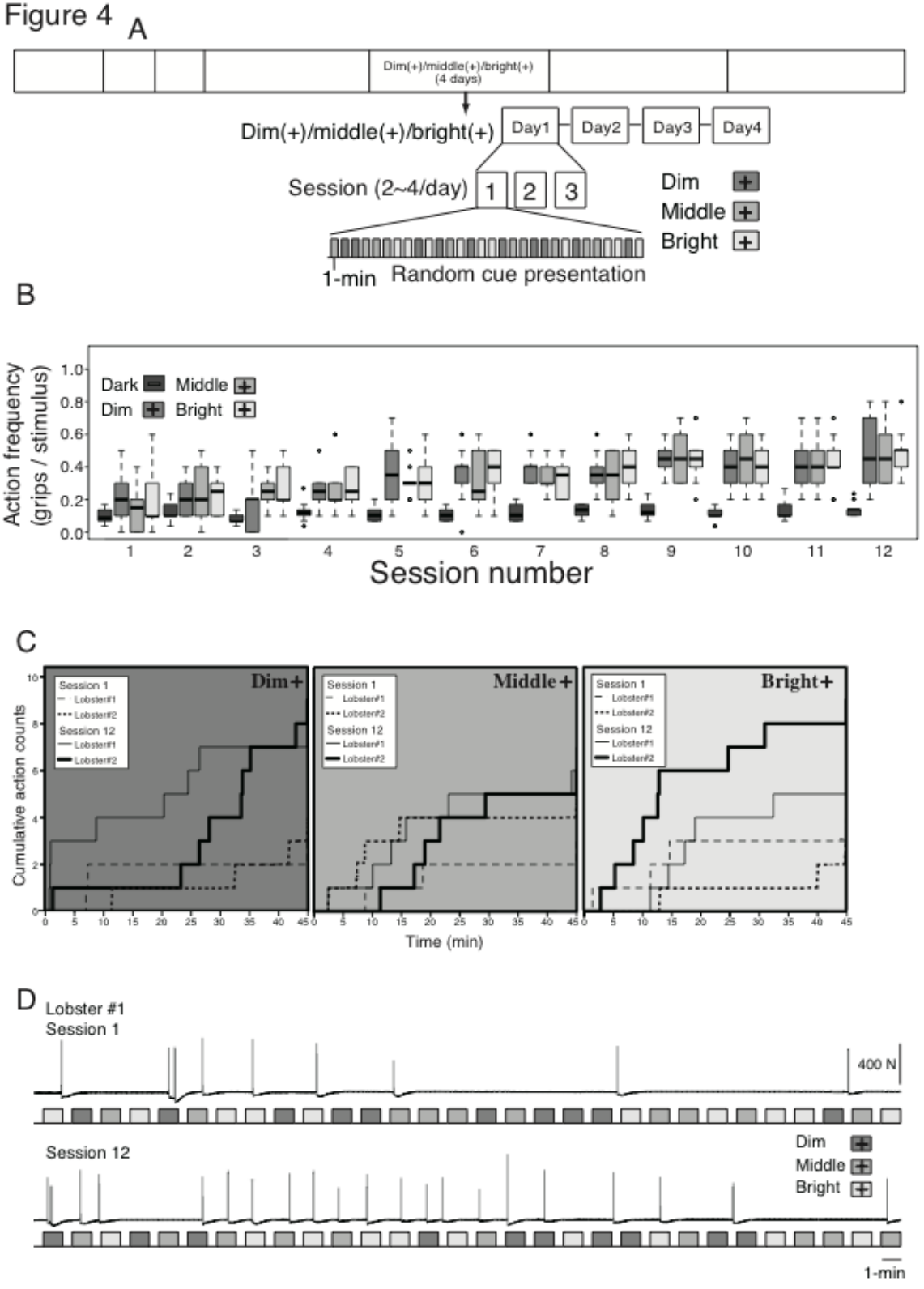

A
Dim(+)/middle(+)/bright(+)
(4 days)
Dim(+)/middle(+)/bright(+)
Day1
Day2
Day3
Day4
Session (2~4/day)
1
2
3
Dim
Middle
Bright
1-min
Random cue presentation
B
Action frequency
(grips / stimulus)
Dark
Middle
Dim
Bright
Session number
C
Cumulative action counts
Session 1
Lobster#1
Lobster#2
Session 12
Lobster#1
Lobster#2
Dim+
Middle+
Bright+
Time (min)
D
Lobster #1
Session 1
400 N
Session 12
Dim
Middle
Bright
1-min

Figure 4.

Dim(+)/middle(+)/bright(+) procedure. A: Timing of the procedure in the whole experimental schedule. The bright (60 lx, reinforced), middle (40 lx, reinforced) and dim (20 lx, reinforced) light stimuli of 1-minute duration were pseudorandomly switched around with 30-sec dark intervals in a 45-min session. B: Box plot of the gripping action frequency (grips/stimulus) for the dark condition (black) and for the dim (dark gray), middle (gray) and bright (light gray) light conditions through 12 sessions ($N = 10$). The ordinate indicates the mean count of gripping action during the dark and light conditions in each session. The count gradually increased with the session number in any of the light condition ($P < 0.05$) but not in the dark condition ($P > 0.05$). There was no statistically significant difference in the grip frequency among the three light levels ($P > 0.05$). C: Cumulative record of the gripping action in the first and twelfth sessions in two representative subjects (Lobster #1 and #2). The left graph shows the gripping record in the dim light condition, the central in the middle light, and the right in the bright light condition. Broken lines indicate their performance in the first session and solid lines their performance in the twelfth session. The gripping behavior in all three light conditions occurred more frequently in the last session than in the first one. D: Action record examples for the first (the upper set of traces) and twelfth (the lower set) sessions in the training of Lobster #1. During these light presentation, the animal became to more frequently grip the bar in the last session than the first one.

Figure 5

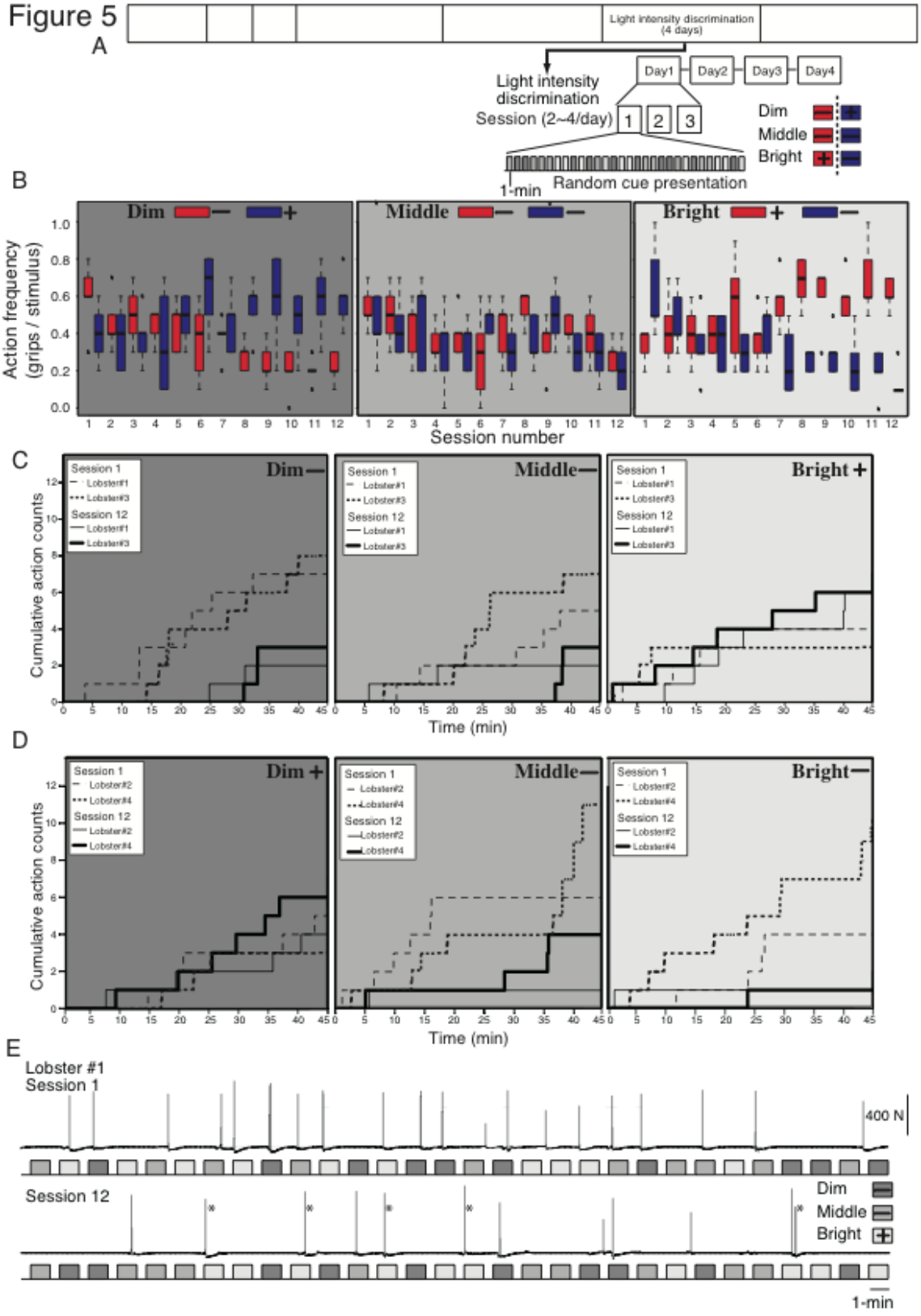

A
Light intensity discrimination (4 days)
Day1
Day2
Day3
Day4
Light intensity discrimination
Session (2~4/day)
1
2
3
Dim
Middle
Bright
1-min
Random cue presentation
B
Dim
Middle
Bright
Action frequency (grips / stimulus)
Session number
C
Cumulative action counts
Time (min)
D
Cumulative action counts
Time (min)
E
Lobster #1
Session 1
400 N
Session 12
Dim
Middle
Bright
1-min

Figure 5.

Light intensity discrimination procedure. A: Timing of the procedure in the whole experimental schedule. Experimental animals were divided into two groups. For one group, the bright (reinforced), middle (unreinforced) and dim (unreinforced) light stimuli were pseudorandomly switched around with 30-sec dark intervals in a 45-min session. For another group, the bright (unreinforced), middle (unreinforced) and dim (reinforced) light presentations were pseudorandomly switched around in the same was as in the other group. B: Box plot of the gripping action frequency (grips/stimulus) for the dim(-)/middle(-)/bright(+) group (N = 5, red) and dim(+)/middle(-)/bright(-) group (N = 5, blue). The left graph shows the gripping count data in the dim light presentation, the central in the middle light, and the right in the bright light presentation. The gripping action frequency during the dim (left graph) or bright (right graph) light presentation for reinforcement gradually increased along with the session number ($P < 0.05$) while the frequency during the dim (left) or bright (right) light presentation for non-reinforcement gradually decreased ($P < 0.05$). The gripping action frequency during the unreinforced middle light did not change significantly in both the dim(-)/middle(-)/bright(+) and dim(+)/middle(-)/bright(-) procedures ($P > 0.05$). C: Cumulative record of the gripping action in the first and twelfth sessions in two representative subjects belonging to the dim(-)/middle(-)/bright(+) group (Lobster #1 and #3). Broken lines indicate their performance in the first session and solid lines their performance in the twelfth session. The gripping behavior in the bright light condition occurred more frequently in the last session than in the first one, while in middle and dim light conditions it occurred less

frequently. D: Cumulative record of the gripping action in the first and twelfth sessions in two representative subjects belonging to the dim(+)/middle(-)/bright(-) group (Lobster #2 and #4). The gripping behavior in the dim light condition occurred more frequently in the last session than in the first one, while in the middle and bright conditions it occurred less frequently. E: Action record examples for the first (the upper set of traces) and twelfth (the lower set) sessions in the training of Lobster #1. At the beginning of this training, the animal tended to grip the bar regardless of the light intensity, but at the end, it tended to grip the bar when the reinforced bright light stimulus was provided as indicated by asterisks.

Figure 6

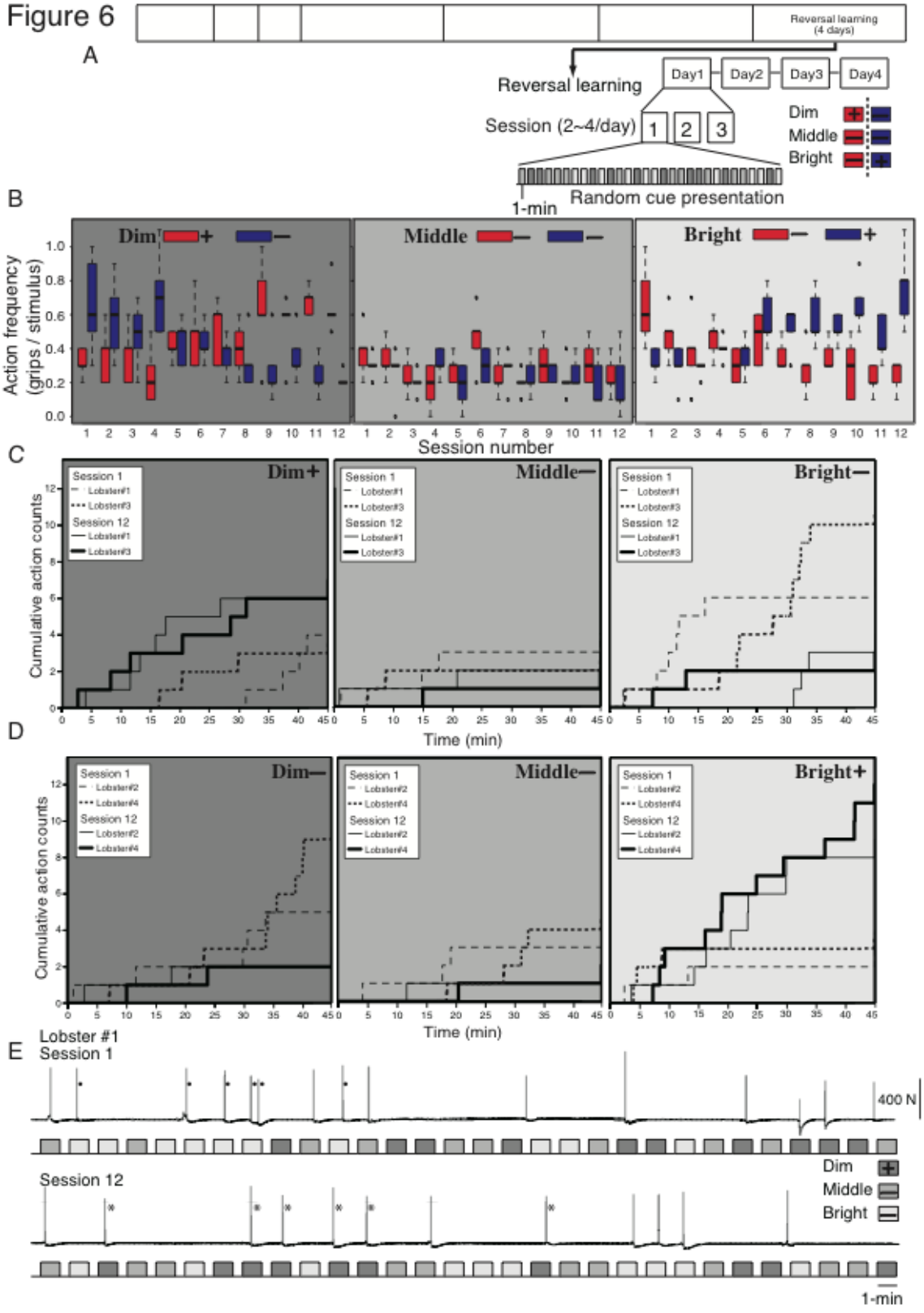

A
Reversal learning
(4 days)
Reversal learning
Day1
Day2
Day3
Day4
Session (2~4/day)
1
2
3
Dim
Middle
Bright
1-min
Random cue presentation
B
Dim
Middle
Bright
Action frequency
(grips / stimulus)
Session number
C
Dim+
Middle—
Bright—
Session 1
Lobster#1
Lobster#3
Session 12
Lobster#1
Lobster#3
Cumulative action counts
Time (min)
D
Dim—
Middle—
Bright+
Session 1
Lobster#2
Lobster#4
Session 12
Lobster#2
Lobster#4
Cumulative action counts
Time (min)
E
Lobster #1
Session 1
400 N
Session 12
Dim
Middle
Bright
1-min

Figure 6.

Reversal learning procedure. A: Timing of the procedure in the whole experimental schedule. The group that was subjected to the dim(-)/middle(-)/bright(+) training in the preceding step was re-trained by the reversed dim(+)/middle(-)/bright(-) procedure, and vice versa. For the previous dim(-)/middle(-)/bright(+) training group, the bright (unreinforced), middle (unreinforced) and dim (reinforced) light stimuli were pseudorandomly switched around with 30-sec dark intervals in a 45-min session. For another group, the bright (reinforced), middle (unreinforced) and dim (unreinforced) light presentations were pseudorandomly switched around in the same was as in the other group. B: Box plot of the gripping action frequency (grips/stimulus) for the dim(+)/middle(-)/bright(-) group (N = 5, red) and dim(-)/middle(-)/bright(+) group (N = 5, blue). The left graph shows the gripping count data in the dim light presentation, the central in the middle light, and the right in the bright light presentation. The gripping action frequency during the dim (left graph) or bright (right graph) light presentation for reinforcement gradually increased along with the session number ($P < 0.05$) while the frequency during the dim (left) or bright (right) light presentation for non-reinforcement gradually decreased ($P < 0.05$). The gripping action frequency during the unreinforced middle light did not change significantly in both the dim(-)/middle(-)/bright(+) and dim(+)/middle(-)/bright(-) procedures as the reversed learning ($P > 0.05$). C: Cumulative record of the gripping action in the first and twelfth sessions in two representative subjects belonging to the dim(+)/middle(-)/bright(-) group (Lobster #1 and #3). After the dim(-)/middle(-)/bright(+) procedure, they were reinforced for the gripping action only in

the dim light presentation. Broken lines indicate their performance in the first session and solid lines their performance in the twelfth session. The gripping behavior in the dim light condition occurred more frequently in the last session than in the first one, while it occurred less frequently in the bright light condition. D: Cumulative record of the gripping action in the first and twelfth sessions in two representative subjects belonging to the dim(-)/middle(-)/bright(+) group (Lobster #2 and #4). After the dim(+)/middle(-)/bright(-) procedure, they were reinforced for the gripping action only in the bright light (60 lx) presentation. The gripping behavior in the dim light condition occurred more frequently in the last session than in the first one, while it occurred less frequently in the middle and bright light conditions. E: Action record examples for the first (the upper set of traces) and twelfth (the lower set) sessions in the reversed training of Lobster #1. At the beginning of this training, the animal tended to grip the bar when the bright light was presented as indicated by dots, but at the end, it tended to grip the bar when the reinforced dim light stimulus was provided as indicated by asterisks.

Figure 7

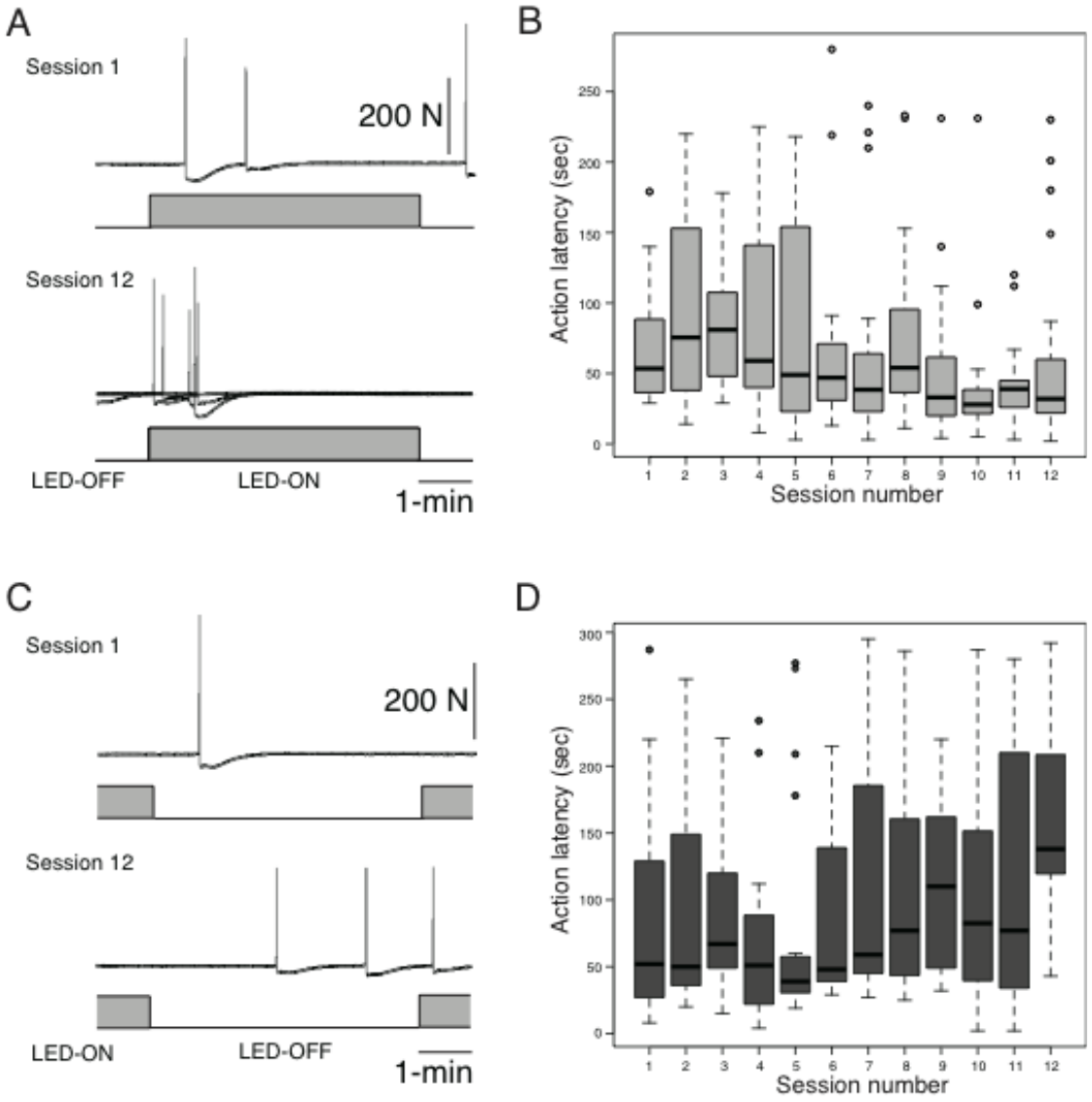

A
Session 1
200 N
Session 12
LED-OFF
LED-ON
1-min
B
Action latency (sec)
Session number
C
Session 1
200 N
Session 12
LED-ON
LED-OFF
1-min
D
Action latency (sec)
Session number

Figure 7.

Gripping action latency changes in the light(+)/dark(-) discrimination procedure. The action latency was defined as the time difference between the light onset time and the peak time of the gripping force. A: Typical examples of gripping action records during the light presentation in the first (single record) and twelfth (three superimposed records) sessions (Lobster #1). Spikes in the upper trace represent gripping actions while the lower trace schematically shows the time course of light presentation. B: Changes in the gripping action latency during training (N = 10). Box plots show the median, first and third quartiles of the data distribution. Whiskers denote the minimum-max range of the data within 1.5 times the length of box. Outliers are shown with closed circles. The action latency decreased gradually as the training proceeded ($P < 0.05$). C: Typical examples of gripping action records during the dark interval in the first (single record) and twelfth (single record) sessions (Lobster #1). In this case, the time difference between the light offset and the peak time of gripping force was measured as the gripping action latency. D: Changes in the gripping action latency during training (N = 10). The latency gradually increased as the training proceeded ($P < 0.05$).

Figure 8

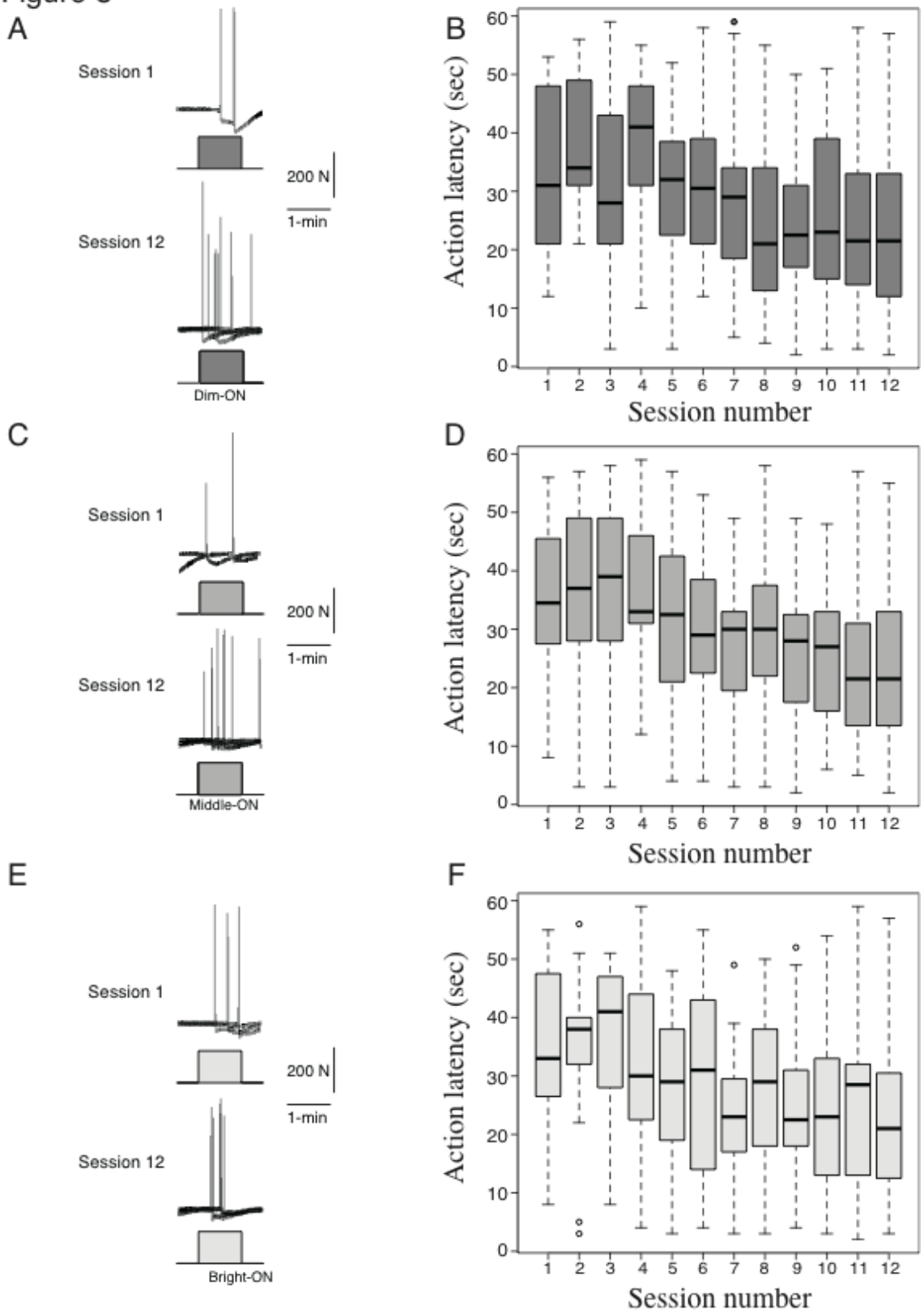

A
Session 1
200 N
1-min
Session 12
Dim-ON
B
Action latency (sec)
Session number
C
Session 1
200 N
1-min
Session 12
Middle-ON
D
Action latency (sec)
Session number
E
Session 1
200 N
1-min
Session 12
Bright-ON
F
Action latency (sec)
Session number

Figure 8.

Gripping action latency changes in the dim(+)/middle(+)/bright(+) procedure. A: Typical examples of gripping action records during the dim light presentation in the first (single record) and twelfth (five superimposed records) sessions (Lobster #1). Spikes in the upper trace represent gripping actions while the lower trace schematically shows the time course of light presentation. B: Changes in the gripping action latency during training (N = 10). Box plots show the median, first and third quartiles of the data distribution. Whiskers denote the minimum-max range of the data within 1.5 times the length of box. Outliers are shown with closed circles. The latency gradually decreased as the training proceeded ($P < 0.05$). C: Typical examples of gripping action records during the middle light presentation in the first (two superimposed record) and twelfth (six superimposed records) sessions (Lobster #1). Spikes in the upper trace represent gripping actions while the lower trace schematically shows the time course of light presentation. D: Changes in the gripping action latency during training (N = 10). The latency gradually decreased as the training proceeded ($P < 0.05$). E: Typical examples of gripping action records during the bright light presentation in the first (three superimposed records) and twelfth (five superimposed records) sessions (Lobster #1). Spikes in the upper trace represent gripping actions while the lower trace schematically shows the time course of light presentation. F: Changes in the gripping action latency during training (N = 10). The action latency generally decreased through 12 sessions ($P < 0.05$).

Figure 9

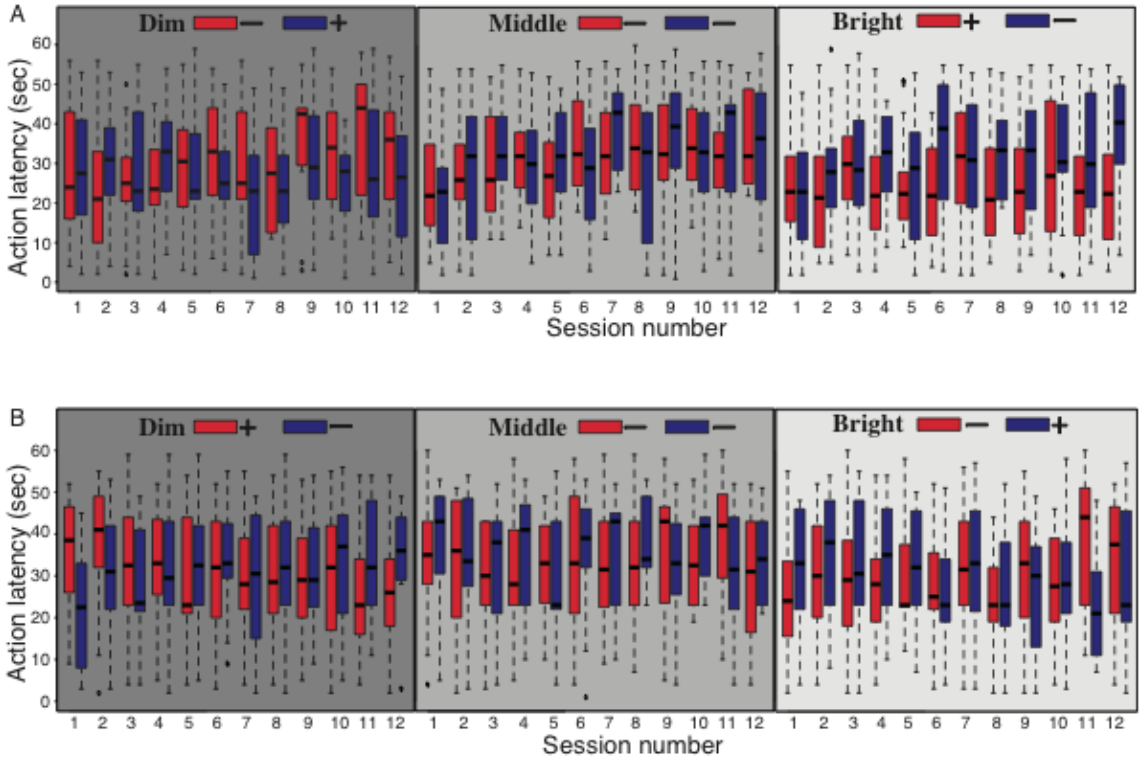

A
Dim
Middle
Bright
Action latency (sec)
Session number
B
Dim
Middle
Bright
Action latency (sec)
Session number

Figure 9.

Gripping action latency changes in the light intensity discrimination and reversal learning procedures. A: Changes in the gripping action latency in the course of the light intensity discrimination procedure. Box plots show the median, first and third quartiles of the data distribution. Whiskers denote the minimum-max range of the data within the 1.5 times the length of box. The box color is red for the dim(-)/middle(-)/bright(+) group (N = 5) and blue for the dim(+)/middle(-)/bright(-) group (N = 5). The left graph shows the gripping latency data in the dim light presentation, the central in the middle light, and the right in the bright light presentation. The gripping action latency in response to the reinforced light cue was maintained at the initial short level through 12 sessions while that in response to the unreinforced light cue increased gradually as the training proceeded ($P < 0.05$). B: Changes in the gripping action latency in the course of the reversal learning procedure. Graphic conventions are the same as those mentioned above. A statistically significant decrease in the action latency in response to the switched reinforced light cue ($P < 0.05$) and an increase in the action latency in response to the switched unreinforced light cue ($P < 0.05$) were observed as the reversal training proceeded. The action latency in response to the middle(-) light cue in both groups was maintained at a prolonged level ($P > 0.05$).