Electromyographic analysis of goal-directed grasping behavior in the American lobster

Yusuke Tomina*‡ and Masakazu Takahata

ABSTRACT
Animals spontaneously initiate goal-directed behavior including foraging action based on their appetitive motivation. The American lobster *Homarus americanus* exhibits grasping behavior with its crusher claw as feeding behavior that can be initiated after appropriate operant conditioning. In order to quantitatively characterize the goal-directed grasping behavior with a time resolution fine enough for neurophysiological analysis of its initiation and control mechanisms, we made simultaneous electromyographic (EMG) recording from grasping- and reaching-related muscles of the crusher claw while animals initiated grasping behavior. We developed an *in vivo* extracellular recording chamber that allowed the animal under a semi-restrained condition to perform operant reward learning of claw grasping. Three muscles in the crusher claw (propodite-dactyl closer/opener and coxal protractor) were found to be closely associated with spontaneous grasping behavior. In spontaneous grasping, the activation of those muscles consistently preceded the grasping onset time and exhibited different activity patterns from the grasp induced by a mechanical stimulus. Furthermore, we found that the timing of coxal protractor activation was closer to the grasp onset and its activity was briefer for goal-directed grasping behavior in trained and hungry animals than for non-goal-directed spontaneous grasping behavior in naive or satiated animals. It is suggested that the goal-directed grasping behavior of lobster is characterized, at least partly, by experience-dependent briefer activity of specific muscles involved in reaching action.

KEY WORDS: Electromyogram, Goal-directed behavior, Manipulative behavior, Crustacean, Lobster

INTRODUCTION
Feeding behavior is, in general, initiated spontaneously as a searching phase or reflexively as an appetitive phase in response to distant chemoreception, directed toward the final goal of satiation. When food is located and secured, the organism initiates the consummatory phase of the feeding behavior that is specific to the animal species and the targeted food (Keen-Rheinhardt et al., 2013). Some animals show highly complex manipulative behavior at this phase of feeding behavior. This type of behavior is shared widely by a variety of animal species including vertebrates as well as invertebrates such as molluscs and insects (Shepherd, 1994). Manipulation is characterized by a defined purpose, i.e. satiation, and specialized appendages to accomplish it, for instance, as the lobster cheliped for crushing the shell to obtain meat food (Derby and Atema, 1982), and forepaws of raccoon for handling foodstuff (Iwaniuk and Whishaw, 1999). Behavior that is motivated by a specific purpose or goal is referred to as goal-directed behavior (Gazzaniga et al., 2009). Satiation-driven manipulation as the final phase of feeding behavior can be regarded as a component of goal-directed behavior.

The manipulative behavior consists of complex sequence of actions that can be adaptively modified depending on the environment, and involves higher levels of nervous control over initiation, maintenance and termination of the action sequence (Shepherd, 1994). Cellular and synaptic mechanisms underlying manipulative behavior have been studied chiefly by operant conditioning of the behavior (e.g. Kelley, 2004; Schultz, 2006; Gazzaniga et al., 2009; Redgrave et al., 2010) with the advantage that the behavior can be reliably repeated by experimenters and its sequence from initiation to termination can be analysed individually and totally. However, the neurophysiological mechanism of manipulative behavior remains largely unknown.

The feeding behavior of crustaceans has been well studied at behavioral (Derby and Atema, 1981; Derby and Atema, 1982; Devine and Atema, 1982), anatomical (Lavalli and Factor, 1995) and physiological levels (Laverack, 1962; Maynard and Sallee, 1970; Robertson and Laverack, 1979; Atema and Voigt, 1995). A pair of claws or chelipeds are often used to manipulate food during the consummatory phase (Derby and Atema, 1982). Clawed lobsters use the larger claw (the crusher) to grasp and break up hard prey such as bivalves and crustaceans (Childress and Jury, 2006). This final phase of feeding behavior in lobsters is subject to operant conditioning motivated by the goal of satiation (Tomina and Takahata, 2010; Tomina and Takahata, 2012). Thus the lobster can be trained to grasp a vertical bar for food reward and yet can perform operant discrimination learning with a light cue in restrained conditions. The conditioned grasping behavior of the lobster therefore provides a useful experimental framework for studying the highly complex mechanism of goal-directed manipulative behavior.

The present study was undertaken to characterize electromyographic (EMG) patterns of crusher claw muscles during the goal-directed grasping behavior in the lobster *Homarus americanus* H. Milne-Edwards 1837. Since the manipulative behavior has a definite action target, it is expected to be different in the motor program from other non-goal-oriented behaviors having no definite action target, both involving the same body parts such as claws and hands. Furthermore, if the manipulative behavior is to be adaptive, then it is expected that the motor program for the behavior should change during the course of behavioral consolidation in a given environment so that the behavioral pattern becomes more efficient than before. We addressed the following questions in the present study: how is the goal-directed grasping behavior characterized at the...
level of muscle activity pattern?; and how does the motor program change during the course of training? We applied chronic EMG recording to a tethered lobster to make quantitative analyses of multiple muscle activities and grasping action simultaneously. The results suggest that at least three claw muscles could be used as the indicator of the onset of spontaneous grasping. In comparison with non-goal-directed grasping behavior, the goal-directed one is characterized by briefer activity of the coxal protractor muscle activity as a consequence of training by operant conditioning.

RESULTS
In this study, we carried out a series of EMG analyses of grasping behavior as a goal-directed behavior of lobsters in order to quantitatively characterize the motor programs at the level of muscle activity. We firstly examined which muscle group was reliably recruited in the grasping initiated spontaneously. We next analysed the goal-directed natures of grasping behavior associated with food reward. In this test, we conducted EMG recording from those muscles selected in the first part of this study to characterize the goal-directed grasping behavior.

Muscles recruited in spontaneous grasping behavior
In order to clarify which muscle was associated with spontaneous grasping, we made EMG recordings from four animals without learning experience while they were performing grasping behavior (Fig. 1). We adopted two criteria for examining the muscle activity: the robustness (or reliability) and the disparity in the activity pattern between spontaneous and reflexive actions. The robustness indicates that the muscle activity consistently precedes spontaneous grasp action independent of relative positions of the target (grasp sensor) and the claw. The disparity indicates that different activity patterns appear in spontaneous and reflexive (mechanical stimulus-induced) grasping. In this experiment, EMGs were recorded from five cheliped muscles: the propodite-dactyl closer (PDC) and propodite-dactyl opener (PDO) as grasping-related muscles, and the mero-carpodite flexor (MCF), ischio-meropodite levator (IML) and coxal protractor (CP) as reaching-related muscles.

We first tested which muscle activity consistently preceded the spontaneous grasping action, independent of the relative positions of the grasp sensor and the claw. Two set positions of the grasp sensor were tested: the inside condition where the sensor was set inside the cheliped at the midpoint level of carpus and propus (Fig. 2A), and the hold condition where the sensor was positioned within the claw between the dactyl and propus at the start of each experiment. The position of the sensor bar was switched manually between the inside and hold conditions. It was done under the dim red light during the experiment. Although we made every effort to place the bar quietly so as to avoid any disturbance to the animal, the placement action inadvertently stimulated the animal by

---

**List of abbreviations**

- CP: coxa protractor
- IML: ischio-meropodite levator
- MCF: mero-carpodite flexor
- PDC: propodite-dactyl closer
- PDO: propodite-dactyl opener

---

**Fig. 1. Experimental set-ups and the *Homarus americanus* lobster claw.** (A) Experimental chamber system for behavioral and electromyographic analysis used in the present study. The animal was restrained by an acrylic slab. Bar-grasping was detected by a load sensor whose output was fed into a PC. Since lobsters can be left-handed or right-handed with regard to which claw is differentiated as the crusher claw, the relative position of the sensor bar could be adjusted for either case. The EMG signal was also fed into the same PC. Circulation and cooling systems kept the animal in vigorous state throughout the experimental period. (B) Dorsal view of the crusher claw and its segments (partial modification of Herrick (Herrick, 1909)). EMG recording was made from the propodite-dactyl closer (PDC), propodite-dactyl opener (PDO), mero-carpodite flexor (MCF), ischio-meropodite levator (IML) and coxal protractor (CP). PDC and PDO are grasping-related muscles, and MCF, IML and CP are reaching-related muscles.
unidentified visual and/or mechanosensory stimulus so that the animal became active in some cases. In such cases, the animal was left undisturbed for a while until it became quiescent.

A typical EMG record from the inside condition is shown in Fig. 2A, while an EMG from the hold condition is shown in Fig. 2B. One bout of grasping behavior was initiated

Fig. 2. Crusher claw muscle activities at the initiation of spontaneous and tactile stimulus-induced grasping behavior. Typical EMGs recorded at the onset of grasping from the propodite-dactyl opener (PDO) and propodite-dactyl closer (PDC) (upper traces), and the mero-carpodite flexor (MCF), ischio-meropodite levator (IML) and coxa protractor (CP) (lower traces) in three conditions: inside (A), hold (B) and stimulation (C). In the inside condition the sensor was set inside of the cheliped at the midpoint level of carpus and propus (inset of A) while in the hold condition the sensor was positioned within the claw between dactyl and propus (inset of B) at the start of each experiment. In the inside condition, the claw had to be once retracted behind the sensor and then protracted to grasp it using its dactyl and propus. The traces of grasp force are shown above the EMG traces. The time difference between grasp onset and muscle activation was measured in each muscle. The negative value indicates that the muscle was active prior to the grasping action. The traces in each panel were obtained in two different recording sessions using different animals. The top three traces including EMGs from opener (PDO) and closer (PDC) muscles and the load sensor signal illustrate the detailed timing of grasp force development and underlying muscle activities. The next four traces showing simultaneous recordings from MCF, IML and CP muscles together with the sensor output illustrate muscle activities related to reaching movement of the cheliped prior to the grasping action.
spontaneously from the resting state that had lasted at least for 30 s. We measured the time difference between the onset of force change and that of muscle activation. When the EMG activation onset time preceded the force change onset, the time value was assigned a negative value. The latencies to muscle activation in the inside condition were −0.223±0.012 s for PDC, −1.435±0.045 s for PDO, −22.714±3.368 s for MCF, −60.480±10.867 s for IML and −62.109±11.184 s for CP. The latencies to muscle activation in the hold condition were −0.057±0.004 s for PDC, −0.753±0.022 s for PDO, 0.252±0.042 s for MCF, 0.355±0.045 s for IML and −0.311±0.616 s for CP.

With regard to MCF and IML muscles, there was no preceding activity when the spontaneous grasping behavior was initiated in the hold condition (Fig. 3, no activity: n=12/26 trials; delayed activity: n=14/26). Therefore, these two muscles were judged not to be involved in spontaneously initiated grasping behavior. In the other muscles, i.e. PDC, PDO and CP, preceding activity was consistently observed in both conditions (Fig. 3), suggesting that these muscles are consistently recruited in grasping behavior, although there was a significant difference in the timing between the two conditions (likelihood ratio test: P=2.2×10−16 for PDC, P=2.2×10−16 for PDO, P=5.043×10−2 for CP). In the inside condition, the animal had to retract the claw and protract it in a different direction so that it could reach the sensor bar with its dactyl. The CP activity in the EMG record shown in Fig. 2A corresponded to the protracting action of the coxa. Such activity was not observed in the hold condition (Fig. 2B).

We then studied the disparity in the muscle activity pattern between spontaneous and reflexive behavior by testing whether there was any difference in the EMG. First, we analysed the activation timing. In the PDO muscle, no consistent preceding activity was observed in the stimulus-evoked grasping, whereas in spontaneous grasping in the hold condition it was activated reliably (no activity: n=28/36 trials, Fig. 4A). This muscle thus appeared to be activated characteristically in spontaneous grasping. The mean value for the activation period in the hold condition was −0.057±0.004 s for PDC, −0.753±0.022 s for PDO and −0.311±0.616 s for CP. Those values in the stimulation condition were −0.053±0.005 s for PDC, −0.430±0.073 s for PDO and −3.288±0.259 s for CP. In the CP muscle, there was a significant difference in the activation period between these conditions (Fig. 4A, likelihood ratio test: P=2.2×10−16, <0.05), suggesting that this muscle is also characteristically activated in the spontaneous action. However, the PDC muscle showed no significant difference between the two conditions (Fig. 4A, likelihood ratio test: P=0.476, >0.05).

For further analysis, we focused on the burst latency that was defined as the time difference between the onset of sustained muscle activation and the onset of burst activity (Fig. 4B). The EMG activity pattern during spontaneous grasping was found to be characterized by a leading sustained activity of small units and a following burst activity of large units (e.g. Fig. 2A, PDC). The former was assumed in this study to be due to activities of tonic motoneurons while the latter was due to phasic motoneurons, both of which are known to innervate the closer muscle (Costello et al., 1981). We analysed the burst latency in both conditions. The mean value of burst latency for the hold condition was 0.54±0.05 s for PDC, 0.12±0.01 s for PDO and 0.53±0.05 s for CP. The mean value for the stimulation condition was 0.27±0.03 s for PDC, 0.13±0.02 s for PDO and 0.29±0.04 s for CP. Regarding PDC and CP, there was a significant difference in the latency between the two conditions (Fig. 4C, likelihood ratio test: P=2.2×10−16 for PDC, P=2.2×10−16 for CP). In these muscles, the burst latency in spontaneous grasping was longer than that in reflexive grasping. Thus the PDC muscle was concluded to be involved in the grasping behavior initiated spontaneously. Taken together, these three cheliped muscles (the PDC, PDO and CP) are characteristically involved in spontaneous grasping.

Fig. 3. Time differences between the onset time of grasping behavior and that of muscle activation in different target conditions. (A) Comparison of the timing data between the inside and hold conditions. (B) Comparison of the timing data among the five muscles. The EMG recordings were made from the PDC, PDO, MCF, IML and CP (N=4 individuals, n=48 trials in each muscle in the inside condition; N=4, n=28 in PDO muscle and n=26 in the other muscles in the hold condition). MCF and IMF muscles showed no preceding activity in the hold condition (no activity: n=12/26 trials; delayed activity: n=14/26). PDC, PDO and CP muscles consistently showed preceding activity in both conditions. The box plots show the median, and first and third quartiles of the data distribution. Whiskers denote the minimum–maximum range of the data within 1.5 times the length of the box. Outliers are denoted by open circles.
Goal-directedness of trained grasping action

Behavioral analysis

In order to examine whether or not the grasping behavior that had been reinforced with the operant conditioning procedures was initiated by appetitive motivation, we next carried out behavioral tests in four animals. We first trained them to associate their grasping action on a load sensor bar with a food reward by an acquisition procedure (Fig. 5A). The bar-grasp action of the lobsters (N=4) showed a significant increase in frequency during the acquisition procedure (Fig. 5B, likelihood ratio χ² test; P=9.317×10⁻⁶, <0.05). In the test 1 phase after this training, the bar-grasp frequency was kept at an increased level above baseline in the preconditioning procedure (Fig. 5B, likelihood ratio χ² test; P=4.993×10⁻¹², <0.05). By contrast, in the phase of test 2 in the satiety state, the frequency of bar-grasp action tended to decrease near to the baseline, i.e. the average count during the preconditioning procedure, and there was no significant difference between preconditioning and test 2 phases (the baseline was 1.44, and the mean value in test 2 was 2.5; Fig. 5B, likelihood ratio χ² test; P=0.153, >0.05). In the test 3 phase where the animal was kept in the starvation state that followed the test in the satiety state, the bar-grasp frequency was kept at an increased level above baseline (the mean value in test 3 was 9.38; Fig. 5B, likelihood ratio χ² test; P=7.617×10⁻¹⁴, <0.05). It is noted here that the animal recovered from satiation within the time between the second session (test 2) on day 4 and the first session on day 5, i.e. about 24 h. This is consistent with our preliminary study using the antennal flicking test showing that the animal recovered the normal flicking frequency 24 h after satiation (data not shown).

In order to exclude the possibility that the decrease in grasping frequency during test 2 was simply caused by an extinction effect, we prepared a control group (N=3) that experienced the same operant procedures, but no satiation manipulation in the phase between tests 1 and 2. The bar-grasp action of the control animals showed a significant increase in frequency during the acquisition procedure in the same way as the satiation group (Fig. 5C, likelihood ratio χ² test; P=4.332×10⁻⁶, <0.05). In the test 1 phase after this training, the bar-grasp frequency was kept at an increased level above baseline in the preconditioning procedure (Fig. 5C, likelihood ratio χ² test; P=3.099×10⁻³, <0.05). In the phase of test 2 without
satiation, the frequency of bar-grasp action was also kept at an increased level above baseline in the preconditioning procedure (Fig. 5C, likelihood ratio χ² test; \( P=1.107 \times 10^{-8} \), <0.05). In the test 3 phase where the animal was kept in the starvation state that followed test 2, the bar-grasp frequency was kept at an increased level above baseline (Fig. 5C, likelihood ratio χ² test; \( P=3.879 \times 10^{-8} \), <0.05). Taken together, these data demonstrate that the reinforced grasping action possessed goal-directedness for food reward.

**EMG analysis**

We conducted a chronic EMG recording experiment (Fig. 6A) through a series of behavioral tasks to characterize the motor activity pattern of the goal-directed grasping behavior based on appetitive motivation. Four animals were passed through eight successive procedures: (1) test 1 (untrained spontaneous grasping), (2) shaping, (3) acquisition, (4) test 2 (test after training), (5) satiation, (6) test 3 (test in satiety state), (7) light-cue training, and (8) test 4 (light-cue test). These procedures were carried out successively over a period of 20 days (Fig. 6A). The bar-grasp action showed a significant increase in frequency during the acquisition procedure (Fig. 6B, likelihood ratio χ² test; \( P=0.007 \), <0.05). During the light (+)/dark (−) training procedure, the bar-grasp action showed a significant increase in frequency during the light presentation (Fig. 6C, likelihood ratio χ² test; \( P=0.0002 \), <0.001) compared with the dark situation (Fig. 6D, \( P=0.690 \), >0.05). In the light-cue test, the latency with which the animal initiated the grasping behavior after the light cue was turned on was comparable to that previously reported in the light/dark discrimination training (Tomina and Takahata, 2012), indicating that the grasping behavior in this test was induced by the light cue associated with food reward (Fig. 6E, mean value: 27.423±4.336 s).

EMG recordings from the PDC, PDO and CP muscles were conducted during spontaneous grasping behavior during the procedures: (1) test 1 (untrained spontaneous grasping), (4) test 2 (test after training), (6) test 3 (test in satiety state) and (8) test 4 (light-cue test). We recorded the EMG on a total of 10 behavioral bouts for every individual in each procedure. Grasping behavior initiated in the test after training (test 2) and the light-cue training (test 4) showed goal-directedness as described above (Fig. 5B,C). The question here was whether there was any common characteristic in the EMG pattern between goal-directed grasping and non-goal-directed spontaneous grasping? We first compared the burst latency of three
muscles among the four tests (N=4, n=40 in each muscle). In any muscle, there was no significant difference in the burst latency among the four procedures (Fig. 7A; ANOVA: P=0.338 for PDC, P=0.730 for PDO and P=0.062 for CP), indicating that the motor activity pattern or the command input from the central nervous system for initiating grasping spontaneously was not fundamentally different among those procedures. We next examined the time difference between EMG activation onset time and the grasp force change onset time in the three muscles (N=4, n=40 in each muscle). In the results, there was a significant difference only in the CP activity (Fig. 7B; ANOVA: P=9.38×10^{-12}, <0.05). In this muscle, the time difference in the test after training and the light-cue test was shorter than that in the untrained grasping behavior and the test in satiety (Fig. 7B; Tukey’s test: test 1–2: P=6.519×10^{-9}; test 1–3: P=0.422; test 1–4: P=3.125×10^{-18}; test 2–3: P=9.339×10^{-16}; test 2–4: P=0.990; test 3–4: P=3.509×10^{-5}). Regarding the PDC and PDO muscles, there was no significant difference in any of the four tests (Fig. 7B; ANOVA: P=0.852 for PDC, P=0.153 for PDO). In order to clarify whether or not the shortening of activation time in the CP muscle reflects any reduction in the total duration of muscle activation for reaching action, we examined how long the protractor muscle activity took during one behavioral bout in the four procedures. Fig. 7C shows a typical example of EMG records from the CP in the same lobster. The mean value of the total duration was 54.800±6.512 s for test 1, 28.878±3.058 s for test 2, 61.471±7.968 s for test 3 and 27.276±3.878 s for test 4. The total duration of muscle activation in the post-training and the light-cue tests (tests 2 and 4) was shorter than that in the untrained grasping behavior and the
satiety test (tests 1 and 3) (Fig. 7D; ANOVA: $P=8.55 \times 10^{-6}$, <0.05; Tukey’s test: test 1–2: $P=0.009$; test 1–3: $P=0.842$; test 1–4: $P=0.005$; test 2–3: $P=0.0005$; test 2–4: $P=0.997$; test 3–4: $P=0.0002$). These data suggest that the reaching action in the goal-directed grasping behavior of lobster can be characterized by use of a specific muscle, i.e. CP as distinct from non-goal-directed spontaneous grasping behavior in which the muscle showed longer burst latency independent of the light cue.

**DISCUSSION**

Manipulation is a key element of the feeding behavior of most invertebrate and vertebrate species. Although the central nervous mechanism underlying such manipulative behavior has been intensively studied in vertebrates, it remains largely undescribed in invertebrates, although behavioral observation and analyses have been in made many species (Flash and Hochner, 2005).

We focused on muscle activation as an indicator of the behavioral onset, because EMGs could be recorded in high-time resolution. In addition, EMG recording allowed us to detect isometric contraction that could not be detected by video recording. Muscle activity has been used as an indicator of behavioral onset in previous studies (Kagaya and Takahata, 2010; Kagaya and Takahata, 2011). Electromyographic studies (Chikamoto et al., 2008; Tomina et al., 2013) revealed that the temporal pattern of leg muscle (merocarpodite flexor) activation is statistically different between spontaneously evoked and stimulus-evoked walking. In the current study, we made EMG recordings from the crusher claw muscles of lobster to characterize the motor program subserving spontaneous initiation of the grasping action by cheliped for manipulating foodstuff. The animal was trained to grasp a vertical load sensor when a light cue was turned on. The chronic EMG analysis combined with learning experiments demonstrated that goal-directed
grasping behavior is characterized by changes in the discharge of a reaching-related muscle relative to non-goal-directed spontaneous grasping behavior that was independent of the light cue.

**Muscle activity patterns in spontaneous grasping behavior**

In this study, we found that at least two grasping-related muscles (PDC and PDO) and one reaching-related muscle (CP) were consistently activated prior to the initiation of grasping action, defined as a force change in the load sensor independent of positional relationship between the sensor bar and the claw (Fig. 3). Our finding that a specific muscle (CP) is consistently activated prior to the grasping action while others (MCF and IML) are not appears to illustrate the defined control of the motor command proceeding to grasping behavior. In addition, spontaneous grasping was found to differ from mechanically induced reflexive grasping in activity patterns of those three muscles. In particular, the burst latency in the EMG from PDC and CP was significantly longer in spontaneous grasping than in reflexive grasping (Fig. 4A,C). In the EMG studies using human subjects, it has been shown that EMG activity patterns are different in voluntary and stimulation-evoked muscle contraction (Hoffer et al., 1996). Previous studies using crayfish also successfully characterized EMG patterns in spontaneously initiated walking as different from those in mechanical stimulus-evoked walking (Chikamoto et al., 2008; Tomina et al., 2013). Those investigations revealed that a specific muscle of the walking leg was tonically activated prior to the onset of rhythmical stepping movements or bursting activity when the animal initiated walking spontaneously, and this pre-activation time was relatively shorter in the reflexive walking (Chikamoto et al., 2008; Tomina et al., 2013). The preceding activation of leg muscles prior to behavioral initiation of spontaneous walking can be interpreted as a revelation of central nervous mechanisms underlying initiation and control of stepping action. The results of our current study focusing on manipulative behavior of the lobster were consistent with those of the previous study in the crayfish walking study focusing on manipulative behavior of the lobster were mainly due to the accessibility with wire electrodes and ease of chronic recording, it remains possible that some other muscles of the crusher claw are also activated in association with the spontaneous initiation of grasping behavior. However, since we did not intend to make an exhaustive survey of the muscles involved in spontaneous grasping but to characterize how the motor program changes in the course of conditioning, we focused our present analysis to those three muscles (PDC, PDO and CP).

**Goal-directedness in the grasping behavior of lobster**

Goal-directed behavior could be defined as a behavior that depends on the representations of the operant contingency between an action and a particular outcome, and a representation of the outcome as a goal (Tolman, 1932; Dickinson and Balleine, 1994). Lobsters show one form of manipulative behavior (Shepherd, 1994), which is the grasping behavior with its crusher claw to break clamshells so as to eat shellfish meat (Derby and Atema, 1982), suggesting that the motor control system of the grasping behavior in lobster can be inherently connected to internal appetitive motivation for food. In our previous studies, we found that the grasping behavior could be reinforced by an operant conditioning paradigm in both the freely behaving and restrained conditions (Tomina and Takahata, 2010; Tomina and Takahata, 2012). In particular, the behavioral performance in reversal learning with different intensities of light cues indicated that their trained behavior was controlled by the operant contingency (grasping–food reward relationship) (Tomina and Takahata, 2012). However, it has not been experimentally tested so far whether the reinforced grasping behavior of lobster is affected by some motivational state and thus is goal directed or not. In this study, we confirmed the satiation effect on behavioral performance by feeding the animal with excessive food immediately before the behavioral test (Fig. 5B). As we expected, the grasping frequency in this test under the satiated condition decreased significantly compared with that in the test under starving conditions (Fig. 5B,C). Although further systematic study using devaluation manipulation is needed to strictly confirm representation of the outcome as a goal (Adams and Dickinson, 1981), we could show that the occurrence of trained grasping behavior could be affected by the appetitive motivational state of the lobster.

**EMG characterization of goal-directed grasping behavior**

In the current study using lobsters as the experimental subject, we performed chronic EMG analysis of three muscles (PDC, PDO and CP) under consecutive operant conditioning procedures. We regarded spontaneous grasping behavior that was initiated in the tests 2 (trained condition) and 4 (light-cue induction) as goal-directed action, and regarded the behavior initiated in tests 1 (untrained condition) and 3 (satiated condition) as non-goal-directed action. In test 1, there was no memory guidance with regard to the bar-grasp for food. In test 3, lobsters were in a satiated state, so that there was low appetitive motivation for food. Unlike the relationship between spontaneous and reflexive grasping, no discrepancy in the burst latency was observed between goal-directed (tests 2 and 4) and non-goal-directed grasping behavior (tests 1 and 3) in any of three muscles (Fig. 7A). Judging from the burst latency, command pathways for initiation of spontaneous grasping appear to be common among those four types of spontaneous grasping, independent of goal-directedness and light-cue induction.

Meanwhile, there was a significant discrepancy in the reaching pattern of CP muscle activity between goal-directed and non-goal-directed grasping behavior. The behavioral execution time of spontaneous grasping (the time difference between the CP muscle activation onset and the grasping initiation) under the trained condition (test 2) or light-cue induction (test 4) was shorter than that of spontaneous grasping actions initiated under the untrained (test 1) or satiated conditions (test 4) (Fig. 7B). In addition, the total duration time of CP muscle activity for the execution of spontaneous grasping under the trained or light-cue induction was shorter than that of spontaneous grasping actions initiated under the untrained or satiated conditions (Fig. 7D). These data suggest that the motor program for reaching and holding in the goal-directed grasping behavior, in contrast with that for non-goal-directed grasping behavior, can be characterized as an efficient use of the reaching-related muscle, i.e. CP, as in the ‘optimized limb movement’ (Kambara et al., 2009). Several studies have suggested that the central nervous system is optimizing arm movements so as to minimize some kind of cost function that specifies movement-related variables (Flash and Hogan, 1985; Uno et al., 1989; Harris and Wolpert, 1998). This idea could possibly be applied even in the case of the goal-directed grasping behavior in the lobster. Further neurophysiological study combined with integrated motor output analyses including anatomy, kinematics and electromyography is needed for testing this possibility, and for understanding central nervous mechanisms underlying goal-directed grasping behavior in the lobster with a ‘microbrain’ (Mizunami et al., 1999) system.
**MATERIALS AND METHODS**

**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 8–15°C under continuous filtration. Animals were fed every 4 or 5 days with small pellets of dried squid. 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 raising aquarium as food and in the experimental aquarium as reward because lobsters show a preference for odor of familiar food (Derby and Atema, 1981). Acclimation was carried out at least 2 weeks prior to the training (see below). All experiments were done in the subjective dark period for the animal in the same manner as our previous study (Tomina and Takahata, 2012). During the experimental learning period without satiety operation, animals were fed only in the operant training procedure as the reinforcement. The habitation process and the criteria for subject selection prior to the experiments also followed our previous study (Tomina and Takahata, 2012). We tested 32 animals in all. Eleven of them died before experimentation and six animals were judged to be unfit for the current experiment chiefly because they did not positively feed on the pellets of dried squid, nor did they spontaneously act on the sensor bar. The judgement was made during the habitation period. As a result, only those 15 lobsters that could grasp the sensor bar and get food in this habitation period were used as experimental animals. Four animals were used for preliminary survey of muscle activities associated with spontaneous grasping. Seven animals were used for analysis of goal-directedness in trained grasping behavior (N=4 for experimental group; N=3 for control group). Four animals were used for chronic EMG study of goal-directed grasping behavior. Animals used in our experiments ranged between 11.7 and 13.1 cm in carapace length and were 490–515 g in weight.

**Experimental apparatus**

In an acrylic chamber (500 mm long×150 mm wide×130 mm high, Fig. 1A), 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, Japan). The animal could not move around but could freely move its appendages and could be released by loosening bolts after the experiment. The grasping sensor bar (manufactured by Keisoku Support, Hiroshima, Japan) comprised a brass bar as the core, a sheet-type load sensor wrapped around the bar, and an outermost waterproof tube shielding the entire sensor bar. The diameter of the grasp bar was 2 cm so that the lobsters could grasp it without difficulty. The bar was fixed vertically in front of the feeding place. The experimental animal was held in a position so that it could grasp the sensor bar with the crusher claw. As lobsters can be left-handed or right-handed with regard to which claw is differentiated as the first thoracic appendage: the crusher is a stout, molar-toothed, slow-moving appendage used for breaking clamshells by grasping (see Dollar, 2001) to eat shellfish meat. Extracellular recording from an individual muscle within the crusher claw segment was made through a pair of silver wires (225 μm in diameter) coated with Teflon except at the cut tip. A pair of fine holes was drilled through the cuticle by a dental drill, and the electrode tips were inserted into a relatively immobile region of the muscle. The holes were sealed and the wires were fixed to the cuticle with glue (Aron Alpha). EMG signals were differentially amplified (model 1700; A-M Systems, Sequim, WA, USA), passed through a band-pass filter (100 Hz and 20 kHz cut-off frequencies), displayed on an oscilloscope (Tektronix 5115; Tektronix, Pikachu, TO, USA), and digitized at 1 kHz using an A/D converter (Power 1401 mk II; Cambridge Electronic Design, Cambridge, UK) and the associated software (Spike2 versions 6 and 7, Cambridge Electronic Design).

A typical crusher claw is depicted in Fig. 1B (partial modification of Herrick (Herrick, 1909)). The claw consists of seven segments: dactyl, propus, carpus, merus, ischium, basis and coxa. In this study, EMGs were recorded from five claw muscles: the PDC, PDO, MCF, IML and CP (Ayers and Davis, 1977; Govind, 1995). We selected these muscles as candidate muscles because of ease of chronic recording. Since the musculature arrangement of the meropodite is highly complicated (Bush et al., 1978), we always had to be careful about recording contamination from muscles other than the targeted one. The grasping behavior was initiated in any one of three situations with regard to the set position of the load sensor bar: the inside condition, hold condition and stimulation condition. In the inside condition where the sensor was set inside the crusher claw (Fig. 2A), we observed spontaneously initiated grasping behavior consisting of reaching and grasping movement. In the hold condition where the grasp sensor was positioned within the claw between dactyl and propus, we observed spontaneous grasping behavior without reaching movement, but requiring only grasping movement to produce force on the bar (Fig. 2B). The sensor bar was quietly placed between the dactyl and propus by hand when the claw was open. The bar was once firmly grasped by the crusher claw probably due to mechanosensory or visual stimulation caused by the bar placement, but the animal finally released it when left undisturbed for a while. We then waited to observe the animal spontaneously initiating the grasping action. In the stimulation condition where the set position of the bar was the same as the hold condition, we induced reflexive grasping behavior by manually inserting a disposable pipette into the space between the dactyl and propus of the claw (Fig. 2C). When it was inserted into the space by one hand, an electrical pulse signal was sent to the EMG recording system by hitting a specific key on the PC keyboard by another hand so that the timing of the pipette insertion could be determined on the EMG record.

The minimum Akaike information criterion (AIC) procedure was applied to the EMG data in order to find out the time onset of muscle activation objectively, as described previously (Chikamoto et al., 2008; Kagaya and Takahata, 2010; Tomina et al., 2013). We performed the procedure using R programming software (versions 2.15.1 and 3.0.2, R Development Core Team) and the TIMSAC package (version 1.2.7, Institute of Statistical Mathematics).

**Electromyographic recording**

Unlike the crayfish, 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. The crusher is usually used for breaking clamshells by grasping (see Dollar, 2001) to eat shellfish meat. Extracellular recording from an individual muscle within the crusher claw segment was made through a pair of silver wires (225 μm in diameter) coated with Teflon except at the cut tip. A pair of fine holes was drilled through the cuticle by a dental drill, and the electrode tips were inserted into a relatively immobile region of the muscle. The holes were sealed and the wires were fixed to the cuticle with glue (Aron Alpha). EMG signals were differentially amplified (model 1700; A-M Systems, Sequim, WA, USA), passed through a band-pass filter (100 Hz and 20 kHz cut-off frequencies), displayed on an oscilloscope (Tektronix 5115; Tektronix, Pikachu, TO, USA), and digitized at 1 kHz using an A/D converter (Power 1401 mk II; Cambridge Electronic Design, Cambridge, UK) and the associated software (Spike2 versions 6 and 7, Cambridge Electronic Design).

**Operant conditioning and behavioral tests for goal-directedness**

In the operant learning experiments, naïve lobsters in the restrained condition commonly went through procedures including: (1) shaping, (2) pre-conditioning, (3) acquisition, (4) test 1 (test after training), (5) satiation, (6) test 2 (test in satiety state) and (7) test 3 (test in starvation state). The protocol of the shaping procedure has been described previously (Tomina and Takahata, 2012). After behavioral shaping, the pre-conditioning procedure was carried out, where the lobsters obtained no food reward for grasping action. The procedure was performed for 1 day with three, 30 min sessions. In the acquisition procedure, the animals were trained to grasp the bar to obtain a small piece of dried squid (3 mm×3 mm) as food reward that was presented manually to the mouthpart when the grasping force exceeded a certain strength as described below. The food was held by a pair of fine...
forces until the animal took it by maxillipeds. In the present study, we did not identify sensory organs responsible for this feeding, but the food reward was presented in the same way throughout the training and experiment. It took about 1–2 s from grasping the bar to obtaining the food, measured by a stopwatch (Fig. 5A). The reinforcement threshold of grasping force was constant at 50 N for every acquisition procedure in this study. The procedure was performed for 2 days, for four, 30 min sessions per day. The first test procedure (test after training) was performed ~24 h after the acquisition procedure, where the lobsters obtained no food reward for grasping behavior. The procedure was performed for 1 day with two, 30 min sessions. The satiation procedure was conducted immediately after the test sessions, where the lobsters were fully fed. Because starving lobsters generally exhibit antennal flicking for food odor (Derby and Atema, 1982), we observed the animal’s antennal movement during food presentation in order to confirm whether they had become satiated or not. The second behavioral test (test in satiety state) was carried out ~30 min after the satiation procedure. The procedure was performed for 1 day with two 30 min sessions. Finally, the third behavioral test (test in starvation state) was carried out ~24 h after the satiation procedure. The procedure was performed for 1 day with two 30 min sessions. Through all procedures, the interval between sessions was ~30 min. A control procedure was performed using three animals. They experienced the preconditioning, acquisition and test 1–3 procedures in the same way during the operant experiment without satiation procedure. Control animals underwent no satiation process between the first and second tests.

Chronic electromyographic recording in the trained behavior

Naïve lobsters were equipped with a pair of silver wires for EMG recording on their crusher claw and restrained in the chamber a day before experimental use. The animals went through successive procedures including: (1) test 1 (untrained spontaneous grasping), (2) shaping, (3) acquisition, (4) test 2 (test after training), (5) satiation, (6) test 3 (test in satiety state), (7) light-cue training and (8) test 4 (light-cue test). They are diagrammatically shown in Fig. 6A. We carried out EMG recordings in the test 1, 2, 3 and 4 procedures. Spontaneous grasping behavior was initiated in the inside condition, and was observed in 10 trials in each procedure. The sensor bar was manually presented to the animals in the rest state and was removed when the grasping behavior was terminated or when 5 min had elapsed. The protocols for procedures (3) to (6) were equal to those used in the former experiments (3) to (6). The acquisition experiment was carried out for 2 days, for four 30 min sessions per day. In order to rest the animals, all EMG wires were removed by cutting the wires close to the cuticle, and the lobsters were kept in aquarium tanks for 2–4 days after test 6 in satiety state. In the light-cue training following the rest period, a 40 lx light cue for light/dark discrimination was presented by a white LED that was located immediately above the animal’s head (Tomina and Takahata, 2012). This procedure was undertaken so that the animal could learn that bar grasping in the light condition was rewarded while that in the dark condition was not. In the light (+)/dark (−) discrimination schedule, the animal obtained a food reward for grasping 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 repeatedly in a 30 min session. The procedure was performed over four or five successive days, for four 30 min sessions per day. After the light-cue training, the animal was equipped with silver wires for EMG recording again on the same target muscles for the preceding recording experiment.

Statistical analysis

Statistical analysis was performed by a generalized linear model using the R programming software (version 2.12.1, 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. These models were examined by a likelihood ratio test based on asymptotical application of the $\chi^2$ distribution with degrees of freedom equal to the difference in the number of identifiable parameters in the two models as described previously (Faraway, 2006; Tomina and Takahata, 2010). For statistical analysis of differences in the burst latency and in the EMG onset time among four different conditions, one-way ANOVA was employed to determine the significance in the conditional effect. The Tukey–Kramer method was performed for post hoc tests. In all statistical tests, the difference was considered to be significant when the P-value was < 0.05. With regard to the sample number, N indicates the number of individuals, while n indicates the number of trials. If not otherwise specified, results are expressed as means ± standard error of the mean (s.e.m.) of the indicated number of trials.

Acknowledgements

We are grateful to Dr Y. Fujimoto for developing the grasp sensor, Dr K. Kagaya for his tutorial on statistical computation of the time series analysis, and technical staff at the workshop at the Faculty of Science in Hokkaido University for their help in constructing the present apparatus.

Competing interests

The authors declare no competing financial interests.

Author contributions

Y.T. and M.T. conceived the study and drafted the manuscript. Y.T. designed and carried out all experiments and data analyses. Both authors read and approved the final manuscript.

Funding

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (23370031). Y.T. was supported by a Japan Society for the Promotion of Science Research Fellowship for Young Scientists (23005250).

References


Iwaniuk, A. N. and Whishaw, I. Q. (1999). How skilled are the skilled limb movements of the raccoon (Procyon lotor)? Behav. Brain Res. 99, 35-44.


