duce functional deficits. Our findings indicate, however, that the fetal organism is particularly sensitive to chronic CO exposure and may be impaired at levels of exposure similar to those found among cigarette smokers. Cigarette smoke contains roughly 3 to 5 percent CO (*20*) and inhalation of tobacco smoke is the major source of HbCO in the general population. Cigarette smokers have an average HbCO concentration of approximately 5 percent (*15*), but the level ranges from about 1 to 16 percent in individuals, depending on occupation, smoking habits (frequency and inhalation patterns), and ambient CO concentrations (*15*). In nonsmokers HbCO levels average approximately 0.5 percent and rarely exceed 2 percent (*15*). Carboxyhemoglobin levels also tend to be somewhat elevated during pregnancy, reflecting enhanced endogenous CO production (*4*).

Our results suggest that indices of maternal cigarette smoking such as enhanced neonatal mortality and reduced birth weight reflect only the most readily measurable effects of this toxin; the potentially more serious consequences of altered central nervous system function and biochemistry early in life or, perhaps, permanently are only now beginning to be discovered by use of animal models.

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9 February 1977; revised 29 March 1977

Developmental Neuroethology: Changes in Escape and Defensive Behavior During Growth of the Lobster

Abstract. *The changes in relative efficacy of two incompatible behaviors was investigated during growth of the lobster,* Homarus americanus. *In larval and early juvenile stages, physiological and morphological factors favor use of the escape response over defensive behavior. In large animals, defensive behavior is preferred almost exclusively to escape behavior unless the claws are lost. The interaction of escape and defensive behavior is modified by neural and morphological factors, which are dependent on the stage in the life cycle of the organism.*

One of the goals of neuroethology (*1*) is to determine the physiological substrates for behavior. Within the past decade, a number of studies have provided an excellent foundation for this emerging field (*1–4*). Most of these studies have been of invertebrates, primarily mollusks and arthropods because these animals have relatively simple nervous systems and relatively simple behaviors. In general, the approach has been to take one or a few simple behavioral acts, determine the neural structures controlling the behaviors, and then determine the interactions among the elements involved. However, these studies have generally focused on the substrates of a simple behavior (*3*) or the interaction of several behaviors (*4*) in adult animals. Other investigators have pursued the changes in a single behavior during growth and development (*2*). However, these studies have rarely been concerned with correlating both neural and morphological substrates with changes in behavior. We have examined both physiological characteristics and allometric relationships among various components of two incompatible behaviors of the lobster—escape and defense—from the larval stages to sexual maturity. During this period of growth, the components of these behaviors develop and differentiate considerably: the animal clearly favors escape early in its life cycle but defensive behavior in the later stages. Furthermore, certain physical characteristics, which determine the efficacy of these behaviors, exhibit transitional stages that occur during the same stage of development.

The primary flight response of the lobster is the well-known tail flip escape response common to the reptantian crustaceans (*5, 6*). The tail flip results from the contraction of the large abdominal flexor muscles and serves to propel the animal backwards. The behavior is mediated by two pairs of giant interneurons, the medial giants (MG) and the lateral giants (LG), as well as by nongiant interneurons (*7–11*). We have studied two aspects of escape behavior during growth of lobsters. We calculated (i) the time for the action potential in the medial giant axon to propagate the length of the animal and (ii) the relative size of the abdomen as a function of the total size of the animal.

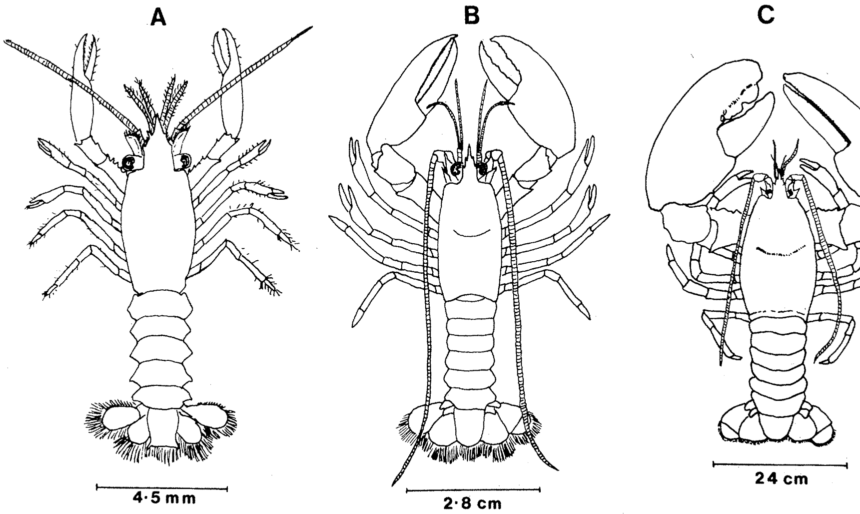

Fig. 1. Lobsters at three stages of growth. The positive allometric growth rate for the claws and the negative allometric growth rate for the abdomen are clearly demonstrated. Approximate weight, total length, and age are: (A) 0.2 g, 14 mm, 14 days (fourth stage); (B) 15 g, 65 mm, 14 months; (C) 8.8 kg, 49.5 cm; the age of this animal was estimated to be between 20 and 30 years. Claws of the postlarval animal (A) are identical; the crusher claw is on the right in the 15-g animal and the left on the 8.8-kg animal.

The primary fighting response of the lobster employs the chelipeds (claws). The adult animal has dimorphic claws, a cutter and a crusher (*12*) (Fig. 1). The former has a closer (adductor) muscle, which contains about 60 to 70 percent fast muscle fibers in the adult (*13*), and is capable of closing very rapidly (*14, 15*), fast enough, in fact, to capture fish (*Fundulus*) (*16*). The crusher claw is composed of slow muscle fibers only (*13*), but it is able to close with sufficient force to break the shells of large mussels (*Mytilus*). Thus, these claws serve different functions in both feeding and fighting (*17*) behaviors. However, in larval and early juvenile lobsters, the claws are symmetrical and relatively small. We have studied two aspects of the use of the claws during the animals' growth: (i) the relative size of the claw as compared to the total size of the animal and (ii) the rate of differentiation of the claw from the symmetrical to the asymmetrical condition.

Larval lobsters were obtained from the State Lobster Hatchery, Martha's Vineyard, Massachusetts, and were reared as previously described (*18*). Conduction velocities were measured in the MG and LG axons in the anterior abdominal region (*19, 20*). During growth of the animal, the diameter of the MG axon increased from about 20 μm in the fourth stage (11 mm total length) to about 100 to 120 μm in a 16- to 18-cm animal. The LG axon diameter is less than 10 μm in the fourth stage (*20*). Although it is not "giant," it also grows substantially during the same period, reaching a diameter of 90 to 110 μm in a 16- to 18-cm animal. Most of the growth of the MG and LG has been achieved when the animal is 6 to 7 cm in length. At this time, the MG is 80 to 90 μm in diameter. Since conduction velocity increases as the 0.5 power of the diameter, the further increase in the MG diameter does not substantially increase the conduction velocity.

The early hypertrophy of the giant axons takes place rapidly enough to compensate for increases in the length of the animal (Fig. 2A). Initially, the conduction time from the brain to the telson is about 4 msec; it remains constant until the animal reaches about 5 cm in length. At this point, conduction time gradually increases, in part because the subsequent increase in MG axon diameter is only a small percentage of the total diameter and thus produces only a small increase in conduction velocity. In addition, this small increase in velocity is offset by the increase in the length of the animal, which results in a striking increase in conduction time.

Another observation that reflects the efficacy of the tail flip as an escape mechanism is the allometric relationship of abdomen size to animal size. In larval and early postlarval animals, the abdomen forms a substantial portion of total animal weight (Fig. 2B). This ratio remains nearly constant until the animal becomes 40 to 60 mm long; thereafter, the tail increases in size more slowly than the rest of the animal. In large animals it is less than 15 percent of the total weight. A similar result obtains when abdominal length is compared to carapace length (Fig. 2C) (*21*).

When similar data are collected for the growth of the claws, the opposite picture emerges. In larval and early postlarval animals, claw weight and length are a small fraction of total weight and carapace length, respectively (Fig. 2, B and C). During subsequent growth, claw length increases disproportionately faster than carapace length until the animal reaches about 5 cm in total length. At this time the length of the propodite reaches a ratio of just under 1.4 times the carapace length. The maximum ratio of just more than 1.4 is achieved during the next 50 mm of growth. Similarly, claw weight as a percentage of total weight increases rapidly from the larval stages until the animal is about 50 mm in total length. Thereafter, claw weight continues to exhibit a positive allometric relationship to total weight (*22*).

The implications of these findings in terms of escape and defensive behavior are clear and lead to an obvious hypothesis regarding use of these incompatible behaviors. In order to survive, the animal must either be able to escape from a potential predator or be able to defend itself. In the early growth stages, the lobster is small and defenseless. At this period of its life, the primary strategy is one of escape. This necessity is well served by the low threshold and short latency for the tail flip as well as by the large size of the abdominal musculature relative to the size of the animal. Other studies have reported that small crayfish have a much lower threshold for escape behavior (tail flips) than larger ones (*8, 11*). We have observed similar behavior in the lobster. When a rapidly moving visual stimulus is presented to a juvenile lobster, it invariably retreats. Juvenile animals (31 to 52 mm long) respond with a tail flip 98 percent of the time, but adult animals (17 to 25 cm long) respond with a tail flip only 18 percent of the time. Clawless adults respond with a tail flip 84 percent of the time (*23*).

As the animal grows, the escape behavior becomes replaced by a defensive strategy. At this time the tail flip becomes less effective as a means of escape for two major reasons. (i) Conduction time for an MG impulse to travel from the brain to the sixth abdominal ganglion increases dramatically and results, in turn, in an increased latency for the escape response. (ii) The relative length of the abdomen decreases from about twice the carapace length in the first larval stage to about 1.4 times the carapace length in the adult. Thus, the absolute

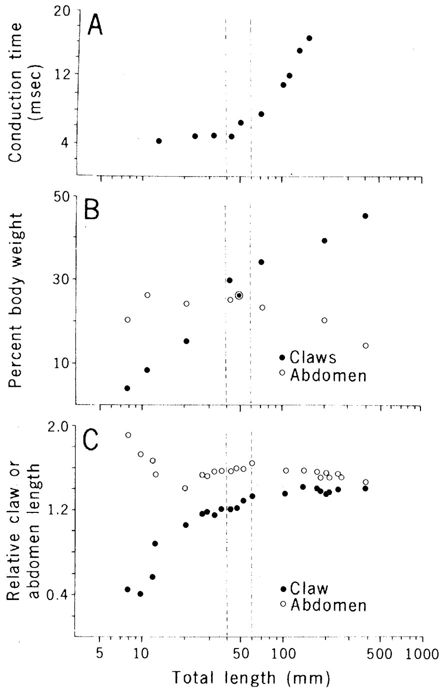

Fig. 2. Changes during growth of the lobster. (A) Conduction time (in milliseconds) for a spike in the medial giant axon traveling from the brain to the sixth abdominal ganglion. The conduction velocity was measured in the anterior abdominal segments (*20*). (B) Relative change in weight is given for the abdomen and the claws. The latter include both claws, severed at the autotomy plane; body weight is the total weight of the intact animal. (C) Relative change in claw (propodite) length or abdomen length as compared to carapace length (from the eye to the posterior end of the thorax) during growth of the lobster. The data given for the claw is for the cutter. Data in (A) are from animals in the first postlarval (fourth) stage and larger; (B) and (C) include the three larval stages. The semilog plot was used to compress the abscissa to accommodate the large range in animal size. Each point is an average from at least 5 animals.

force developed by the flexing abdomen may increase during growth, but the ratio of force to animal mass decreases, and the abdomen is less able to impel the animal away from danger.

Conversely, the claw increases its size in a positive allometric fashion while differentiating into the specialized cutter and crusher claws. With larger, differentiated claws, the animal is in a position to readily defend itself. Now when the animal is attacked with a probe, it seldom responds with a tail flip regardless of the viciousness of the attack. Rather, threatening evokes the defensive behavior of raised, open claws. Indeed, if the animal is actually jabbed, it will often attempt to grab the probe with both claws.

This preference for defensive behavior in older animals is not simply due to a slow disappearance of the tail flip with age. We have observed that when the claws of a 3600-g animal are removed, the animal immediately escapes from a net by using the tail flip several times in quick succession. Thus, while the neural circuit for the reflex is intact and functional, it is simply not an efficacious response for a large, clawed animal. Similar results have been reported in crayfish (*24*).

It would be of interest to compare some of the same measurements in other reptantians that either lack claws (for example, *Panulirus* sp.) or have relatively smaller claws (for example, crayfish). Preliminary experiments on the latter suggest that crayfish (*Procambarus acutus*) differ from lobsters in their behavioral strategy. Their claw weight is about 10 percent of total weight even in sexually mature males (50 mm long). (In lobsters of comparable size, which are not sexually mature, the claws are 27 percent of the total weight.) Another interesting comparison between these animals is the relative size of the giant axons. Crayfish giant axons are 16 percent of the total cross-sectional area of the nerve cord in a 7-cm animal. In a smaller lobster, 23 mm in length, the comparable figure is 11 percent, and in a 7-cm lobster it is only 8 percent (*25*).

These results demonstrate that physical factors place constraints on particular behaviors. Since many structures of animals grow at different rates, their allometric relationships can provide insight into their relative importance in the behavioral strategies of an animal. With parallel physiological studies, one can further appreciate the particular function and importance of these structures.

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5. Practically speaking, the only means of locomotion available to adults is walking, as the swimmerets (paired abdominal appendages) are much too small to propel the animal and the tail flip is only rarely used. Larval lobsters (stages 1 to 3) are pelagic and swim by means of exopodites, extensions of the thoracic appendages, which beat rhythmically [D. M. Neil, D. L. Macmillan, R. M. Robertson, *Phil. Trans. R. Soc. London Ser. B.* **274**, 53 (19 D. L. Macmillan, D. M. Neil, M. S. Laverack, *ibid.*, p. 69; M. S. Laverack, D. L. Macmillan, D. M. Neil, *ibid.*, p. 87.] Early postlarval animals (stages 4 to 5) are often pelagic but, having lost the exopodites at the molt to stage 4, move about by using the swimmerets. Sometime during stages 4 to 6, the animals settle to the bottom and assume a benthic existence and thereafter are only rarely observed to use the swimmerets.
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19. The methods have been described in detail (*20*). Briefly the ventral nerve cord was exposed in the abdominal region (using a ventral approach in postlarval animals and a dorsal approach in larger animals). Suction recording electrodes [F. Lang, in *Experiments in Physiology and Biochemistry*, G. A. Kerkut, Ed. (Academic Press, New York, 1972), vol. 5, p. 127] were placed anterior to each of the first two abdominal ganglia. Stimulation was through fine platinum wires placed between abdominal ganglia 5 and 6. These nerve cords were fixed for microscopy and sectioned in the connectives anterior and posterior to the first abdominal ganglion to obtain diameters for the giant axons.
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21. Carapace length is taken as the distance from the eye socket to the posterior end of the thorax.
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23. Lobsters were placed unrestrained in a large aquarium. The stimulus was given by suddenly introducing a wooden rod into the visual field. Each of the three groups (juvenile, adult, and clawless adult) consisted of ten animals, with five trials each; there was a minimum of 5 minutes between trials.
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3 February 1977; revised 5 April 1977

Return of Myosin Heads to Thick Filaments After Muscle Contraction

Abstract. *The heads of myosin molecules, which move to the vicinity of the thin filaments to react with actin during muscle contraction, return to the thick filaments after contraction. The return occurs in two stages; a rapid return of the majority of the myosin heads is followed by a slow return of the rest.*

The heads of myosin molecules play a major role in producing the contractile force of muscle. They are arranged in a regular manner in the vicinity of the thick filaments in living resting muscle (*1*), and, when the muscle is activated, move promptly to the vicinity of the thin filaments (*2*) to undergo tension-generating reaction with actin. After contraction, the myosin heads return to their resting positions, but the time course of the return has not been well defined; Huxley (*3*) has concluded, from his observation on the intensities of the axial x-ray reflections of muscle after a short tetanus, that the return takes at least several seconds; whereas Podolsky *et al.* (*4*) have concluded from their observation on the intensities of the equatorial x-ray reflections that the return is almost completed within 100 msec after the fall of contractile tension. In a further study on the time-dependent intensity changes of the equatorial reflections, we obtained a result suggesting that the return occurs in two stages; a prompt change in the equatorial intensities on cessation of tetanic stimuli is followed by a gradual change lasting several seconds.

A sartorius muscle, together with the pubic bone, was isolated from the bullfrog *Rana catesbeiana*. The muscle was held isometrically in a specimen chamber by clamping the pubic bone at one end and connecting the tendon to a force transducer (Shinkoh, type UL) at the other end. The chamber had Mylar windows for passing x-rays through the middle of the muscle and was filled with oxygenated Ringer solution (4°C), which was continuously renewed with a perfusion pump. At the beginning of each experiment the sarcomere length of the muscle was adjusted to 2.2 μm by moving the force transducer; the sarcomere length was measured by passing a helium gas laser ($\lambda = 0.6328$ μm) through the same part of the muscle as to be exposed to x-rays, and observing the optical diffraction pattern. The muscle was stimulated tetanically, for 1 second at a time, with supramaximal electrical pulses (20 hertz) given through a pair of electrodes placed parallel to the muscle axis.

The equatorial x-ray diffraction pattern of the muscle was recorded by a position-sensitive counter of the type developed by Allemand and Thomas (*5*). The outputs of the counter were fed into a data collection system that was synchronized with the tetanus (see below). The recorded pattern (that is, the intensity distribution of the x-rays scattered along the equator) showed the 1,0 and the 1,1 reflections arising from the hexagonal array of the myofilaments. The intensities of these reflections were obtained by measuring the area under the peaks on the intensity distribution; the background level under each peak was drawn in by eye. This background level, relative to the peak height of each reflection, was of approximately the same magnitude as that of the densitometer traces of the equatorial patterns recorded on x-ray films.

Time-dependent changes in the in-

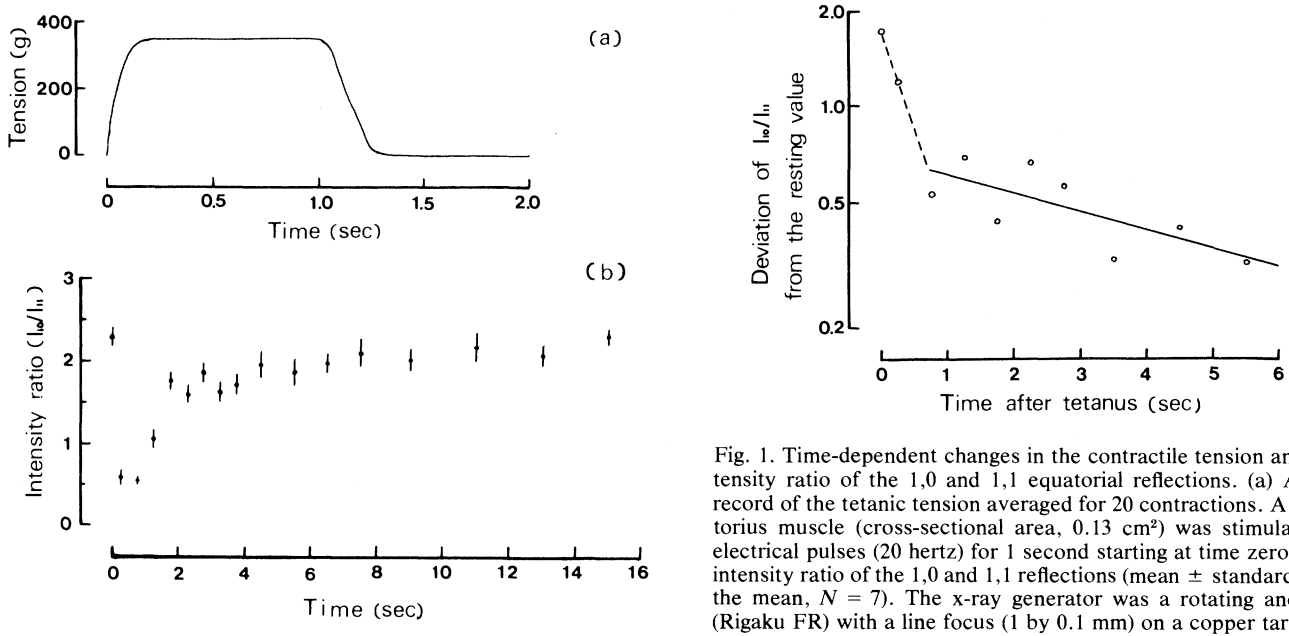

Fig. 1. Time-dependent changes in the contractile tension and the intensity ratio of the 1,0 and 1,1 equatorial reflections. (a) A typical record of the tetanic tension averaged for 20 contractions. A frog sartorius muscle (cross-sectional area, 0.13 cm^2) was stimulated with electrical pulses (20 hertz) for 1 second starting at time zero. (b) The intensity ratio of the 1,0 and 1,1 reflections (mean ± standard error of the mean, $N = 7$). The x-ray generator was a rotating anode type (Rigaku FR) with a line focus (1 by 0.1 mm) on a copper target. This was operated at 40 kv with a tube current of 80 ma. A low-angle camera of Huxley-Holmes type (*1*) was used with a specimen-to-counter distance of 40 cm. (c) A semilogarithmic plot of the deviation of the intensity ratio from its resting value against time after the cessation of stimuli. The solid line is the regression line for the points after the fall of tension.