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Differential Reflex Activity Determines Claw and Closer Muscle Asymmetry in Developing Lobsters

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The paired claws and closer muscles of the lobster, *Homarus americanus*, are identical in the early juvenile stages, but subsequently differentiate into a stout crusher claw with only slow fibers and a slender cutter with largely fast fibers. Rearing with different substrates or exercise of the claws revealed that claw laterality is determined in the central nervous system by differential reflex activity in the paired claws; the side with greater activity becomes the crusher, while the contralateral side becomes the cutter.

In many animals, asymmetry in an otherwise bilaterally symmetrical body plan often results in a specialization of function on opposite sides. A striking example of such asymmetry is seen in the paired claws of the lobster, *Homarus americanus*, where one of the claws is a stout, slow-acting crusher and the other is a slender, fast-acting cutter (*1*), each with its role in food collection and territorial defense (*2*). The muscular basis for this functional asymmetry resides in the fiber composition of the paired closer muscle; the cutter has 60 to 80 percent fast fibers and a small ventral band of slow fibers, and the crusher has 100 percent slow fibers (*3*). The crusher claw appears with equal probability on the right or left side of the animal (*4*); this suggests that claw laterality is randomly determined. Such determination occurs during early juvenile development, in the fourth and fifth stages (*5*). Prior to this the paired claws and closer muscles are symmetric; both look like cutters and have a central band of fast fibers sandwiched between slow fibers (*6*). Once claw laterality is determined in the early juvenile stages, the central fast fiber band becomes slow fibers in the crusher, while in the cutter the slow fibers change to fast, except for a small ventral band. Because claw laterality can be experimentally altered by manipulating the environment or the claws in the early juvenile stages (*5*), it may be possible to uncover mechanisms that govern asymmetry. Here we report such experiments, which demonstrate that differential reflex activity in the paired claws converges in the central nervous system (CNS) and determines claw and closer muscle asymmetry in juvenile lobsters.

Experiments were performed at the Marine Biological Laboratory, Woods Hole, Massachusetts, where the rearing facilities enabled us to follow the development of individual lobsters from hatching to late juvenile stages when their claws were clearly asymmetric. After hatching, the first three larval stages were reared communally. After the molt to the fourth stage, which is the first juvenile stage, each animal was transferred to an individual tray so that we could monitor its molt history and to prevent internecine behavior (*7*). Experimental treatments were carried out in the fourth and fifth juvenile stages because, during these 2 to 3 weeks, claw laterality is determined (*5*) and, once determined, remains fixed for life. Juvenile lobsters were reared to at least the eighth stage, when claw asymmetry is well established. At this stage the cutter claw was long and slender, and had distinctive sensory bristles and a central incisor-like tooth on its pollex; the crusher claw was short and stout, lacked the sensory bristles, and had a central molar-like tooth (Fig. 1). The cutter closer muscle clearly had the adult fiber composition of predominantly fast muscle with a ventral slow band, while the crusher muscle had not acquired its final composition of 100 percent slow fibers and still retained a narrow central fast fiber band (Fig. 1). Because muscle composition is closely tied to external claw morphology (*8*), the external morphology was utilized to assess the internal muscle composition. The fiber composition of the closer muscle was evaluated histochemically in three representative lobsters from each experimental condition, each with a different configuration of its paired claws (Table 1). Fiber analysis was performed on frozen cross-sections of the claw muscle that were stained for myofibrillar adenosine triphosphatase (ATPase) activity (*9*).

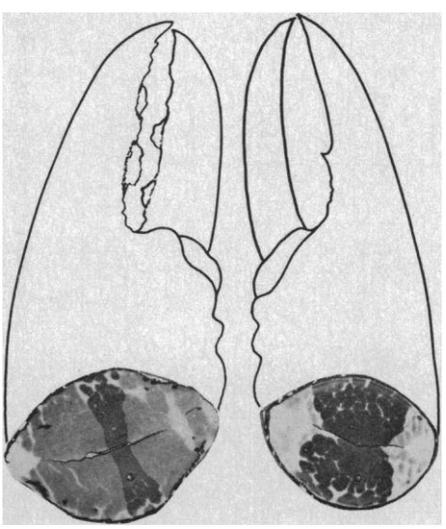

Fig. 1. Closer muscles of a left crusher claw and right cutter claw from an eighth stage juvenile lobster in which the left claw was exercised (magnified ×24).

When juvenile lobsters are reared with various substrates (such as oyster chips, plastic chips, gravel, or mud), as adults they have asymmetric claws and closer muscles in approximately equal numbers of right- and left-handedness (*8*). Such a ratio is to be expected because claw laterality is randomly determined (*4*). When they are reared without a substrate, however, they have symmetrical claws with closer muscles of the cutter type. In contrast to the substrates in the

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trays, the smooth molded plastic of the rearing trays provided little opportunity for gripping by the claws; this suggested that a substrate that can be manipulated by the claws was necessary for the development of asymmetry. In fact, juvenile lobsters were observed handling the substrate with their claws. To test this proposal, we reared juvenile lobsters with no substrate, but with spots resembling chips painted on parts of the bottom and sides of the plastic trays, and compared these with lobsters reared with oyster chips as substrate and with no substrate (Table 1). When they were reared with oyster chips a majority of the lobsters had asymmetric claws, but both the painted chip and no substrate conditions gave rise to lobsters with symmetric claws. Thus, the presence of a physical substrate seemed to be required for claw asymmetry to develop. Even one oyster chip was adequate to result in a significant majority of lobsters with asymmetric claws. Thus any substrate that allowed the claws to be exercised could cause the development of bilateral asymmetry.

Table 1. Configurations of paired claws in juvenile lobsters reared under different environmental conditions and with exercise of the claws. Lobsters were reared individually in smooth plastic trays from the fourth to the eighth stage. Claw configuration was then assessed visually and a representative example of each of the three claw configurations was histochemically treated to determine fiber composition of the paired closer muscles. The different environmental conditions prevailed throughout the entire rearing period and the exercise conditions were applied only in the fourth and fifth stages. The contingency χ^2 test (with Bonferroni correction to probabilities) was used to determine the significance (P) between oyster chips (control) and each of the environmental conditions, and between handling animals (control) and exercising of the claw. Abbreviations: R., right; L., left; and NS, not significant. Under environmental conditions promoting asymmetry, 11 out of 64 lobsters developed paired cutter claws; this is a value much higher than in the wild and due largely to the paired cutter claws that developed in the one chip condition.

Treatment	Paired claw configurations			Total number reared	P
	R. crusher L. cutter	L. crusher R. cutter	R. cutter L. cutter		
Environmental condition					
Oyster chips (control)	8	9	1	18	
One oyster chip	7	9	7	23	NS
Painted chips	4	0	16	20	<0.005
Two animals	9	11	3	23	NS
Mirror	7	3	19	29	<0.005
No substrate	2	4	20	26	<0.005
Exercise					
Handled animal (control)	2	2	18	22	
Left claw	3	13	1	17	<0.002
Both claws	4	3	11	18	NS

To provide further support for this conclusion, we reared two lobsters together in a single tray and allowed them to interact during the fourth and fifth stages. Lobsters are by nature aggressive animals and, when reared together, fight with their claws. Usually one animal lost either one or both claws during the period they were together. However, when claw laterality was assessed in lobsters with both claws, the majority developed asymmetric claws and closer muscles (Table 1). To test whether it was the use of the claws in antagonistic encounters with another lobster that promoted asymmetry, and not simply the visual presence of another animal, we reared juvenile lobsters without a substrate but with a mirror along one of the long sides of the tray. The lobsters occasionally approached their reflections but did not attempt to interact with them further. A significant number of these lobsters developed paired cutter claws similar to their counterparts raised without a substrate but unlike those raised with oyster chips (Table 1). Clearly the lack of a substrate and the resulting lack of claw activity prevented one of the paired claws from differentiating into a crusher.

Although these experiments suggested that activity influences the development of a crusher, convincing proof was needed from experiments that promote the differentiation of a crusher claw rather than suppress it. To this end we reared lobsters without a substrate but with exercise of one of the paired claws during the critical juvenile stages. The exercise regime consisted of holding the lobster and stroking its claw with a small paint brush so that the claw gripped the bristles six to eight times during a 40 to 60 second session. Each lobster was exercised in three sessions per day separated by approximately 5-hour intervals over the entire period of the fourth and fifth stages. Usually the exercised claw became the crusher (Table 1) even though the lobsters were raised under conditions that would tend to suppress crusher development, that is, with no substrate. As a control, another group of juvenile lobsters was raised under identical conditions but not exercised, that is, they were picked up and held for 1 minute three times daily. The distribution of claw laterality was significantly different between these two groups (Table 1). Moreover, the probability of the treated claw becoming a cutter or crusher was tested between control and experimental conditions, and the number becoming crushers was significantly greater in the exercised group than in the control group ($P < .001$, contingency χ^2 test). Thus, some minimal level of reflex activity in the claws is necessary to differentiate a crusher claw and to transform fast fibers to slow.

If the only requirement for producing a crusher is a minimal (threshold) level of reflex activity, then why are lobsters with paired crusher claws so rare? In more than a decade of rearing lobsters we have not seen any with paired crusher claws and, in the one wild animal that had paired claws, one of the closer muscles had a substantial proportion of fast fibers (*10*). Apparently, reflex activity by itself may not directly induce crusher development, but may act at a more distant site, such as the CNS, where inputs from reflex activation of the paired claws converge. Support for this view comes from our final experiment in which both claws were exercised. The majority failed to develop asymmetric claws but developed paired cutter claws (Table 1). Thus, the distribution of claw laterality when both claws were exercised was not significantly different from the control condition when the lobsters were handled but not exercised. Yet these juvenile lobsters were subjected to an exercise regime that, when applied to one of a pair of claws, induced crusher development. However, because both claws were exercised, bilateral differences were presumably reduced or nonexistent, resulting in symmetrical claws and closer muscles. Development of claw asymmetry therefore depends on the differential reflex activation of the paired claws, the side with the relatively higher level becoming the crusher while the opposite side becomes the cutter.

A simple and possible explanation of these results is that the neural input (sensory or proprioceptive) due to reflex activity from both claws converges in the CNS—in this case, the first thoracic ganglion serving the claws. Here, a comparison of the neural input from both sides occurs, resulting in the determination of claw asymmetry. Once laterality is determined in the CNS in the early juvenile stages, it is expressed at the periphery during subsequent development in terms of external claw morphology and fiber composition of the closer muscle. A

more complex explanation of our results is that, although reflex activity promotes crusher development, another signal to the CNS inhibits its induction in the contralateral claw. Consequently, if inhibition took priority over induction, then exercise of both claws would result in paired cutter claws even though reflex activity induces crusher formation. In either case, our results demonstrate that control of claw asymmetry resides in the CNS. Although asymmetry of the vertebrate brain with respect to vocalization in song birds (*11*) and language processing in humans (*12*) may be experimentally altered by ablation and deprivation, we do not know the degree to which use and disuse determines the original asymmetry. Our experiments with lobster claws show how use promotes asymmetry. Moreover, the neuronal changes involved in determining such asymmetry may be more easily understood in lobsters because of the relative simplicity of their nervous system as compared to that of vertebrates. Insights can be gained here that will be of help in understanding neural asymmetry in vertebrates—for example, cerebral lateralization in humans (*13*).

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Interferon Inhibits the Establishment of Competence in G_0/S–Phase Transition

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Addition of mouse interferon-α/β (IFN) to confluent, quiescent BALB/c 3T3 (clone A31) mouse fibroblasts resulted in a block or delay in serum-induced activation of the cell cycle. It was necessary to add IFN within 6 hours after serum stimulation to inhibit nuclear labeling with [^{3}H]thymidine. This is consistent with the time required for platelet-derived growth factor (PDGF) to induce cells to become competent to respond to additional growth factors present in platelet-poor plasma. Simultaneous addition of IFN with PDGF inhibited the PDGF-induced synthesis of a 29-kilodalton and a 35-kilodalton protein that normally occurs within 1 hour after PDGF addition. IFN also suppressed the general increase in protein synthesis that occurs by the fifth hour after PDGF addition. These results show that IFN antagonizes the action of PDGF, thereby interfering with the activation of G_0 cells for G_1 traverse and S-phase entry.

INTERFERONS (IFN's) CAN INHIBIT cell proliferation by blocking cells in the G_0/G_1 phase of the cell cycle (*1–5*). Serum factors control the traverse of cells from G_0/G_1 to S phase. Platelet-derived growth factor (PDGF) initiates proliferation by rendering quiescent fibroblasts "competent" to respond to the progression activity contained in platelet-poor plasma-derived serum (PPP) (*6–8*). Plasma factors, which include epidermal growth factor (EGF) and somatomedin C (SmC), govern cellular progression through early G_1 phase to a mid-G_1 point (*8*). Only SmC is required for progression from mid-G_1 into S phase (*8*). We have investigated the antagonism between interferons and growth factors and report that mouse IFN-α/β inhibits the traverse of serum-stimulated quiescent mouse BALB/c 3T3 cells from G_0 to S phase by acting on processes that take place within the first 6 hours. IFN both selectively inhibits the early synthesis of at least two PDGF-induced proteins and suppresses the PDGF-induced increase in the overall level of protein synthesis.

We first defined the conditions under which mouse IFN-α/β (2.6×10^7 U/mg) inhibited the proliferation of a subline (*7*) of BALB/c 3T3 cells (clone A31). An exponential relationship was observed between IFN concentration and reduction in growth rate of logarithmically growing cells as determined over a 3-day interval (Fig. 1). A 20% reduction in growth rate was evident at 100 U/ml and a 46 and 51% reduction occurred at 5000 and 10,000 U/ml, respectively. The BALB/c 3T3 subline used in the present studies possessed an IFN sensitivity intermediate between the lower sensitivity of BALB/c 3T3 cells used by Sokawa *et al.* (*1*) and the higher sensitivity of cells used by Balkwill and Taylor-Papadimitriou (*2*).

The ability of IFN to delay or block serum-stimulated entry into S phase was evaluated by determining the percent of nuclei that incorporated [^{3}H]thymidine at various intervals after addition of serum to density-arrested BALB/c 3T3 cells (Fig. 2a). After an initial lag of 10 hours, the percent of labeled nuclei increased with time over the ensuing 14 hours in cultures that received serum without IFN. Treatment with IFN-α/β at either 1500 or 5000 U/ml delayed entry into S phase in a concentration-dependent manner. In addition, the rate of increase in DNA-synthesizing cells was clearly reduced in the presence of 5000 U/ml. These results are consistent with previous observations (*1, 2*).

The establishment of competence by PDGF is both concentration- and time-dependent. With an estimated PDGF concentration of the order of 1 ng or 1 U/ml in the medium containing 10% calf serum, the great majority of the cells that achieve competence do so within 6 hours (*7*). The inhibition of S-phase entry as a function of the time of IFN addition was measured in order to determine the time in the cell cycle at which the IFN-mediated block occurred. 3T3 cells were stimulated by the addition of fresh medium supplemented with 10% calf serum, and IFN (final concentration 5000 U/ml) was added simultaneously or at various intervals after serum addition. Cultures were allowed to incorporate [^{3}H]thymidine and the percent of cells that entered S phase

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