Functional subdivision within a lobster motor unit

R.H. Hill[1] and C.K. Govind[2]

Boston University Marine Program, Marine Biological Laboratory, Woods Hole (Massachusetts 02543, USA), and Scarborough College, University of Toronto, West Hill (Ontario M1C 1A4, Canada), 13 July 1981

Summary. A discrete middle band of tonic fibers in the claw closer muscles of lobsters functions as a sub-unit of the slow motor unit in maintaining dactyl posture at a low frequency of firing due to its synaptic properties and strategic location.

Crustacean limb muscles are particularly suited for the analysis of motor activity because they are innervated by relatively few motoneurons[3] and yet display a wide range of contractile responses[4]. For example, the dimorphic cutter and crusher claws in the lobster *(Homarus americanus)* can close their dactyls at different speeds and strengths[5,6], yet their closer muscles are innervated by only a fast closer excitor (FCE), a slow closer excitor (SCE) and a closer inhibitor (CI) axon[3]. One way to achieve the full range of closing responses typical of the claws with only 2 (FCE and SCE) motor units is to divide the motor unit into functional sub-units. We find such a sub-unit in the form of a small, discrete bundle of tonic fibers responding to low frequency firing of the SCE and strategically located within the muscle for postural control of the dactyl.

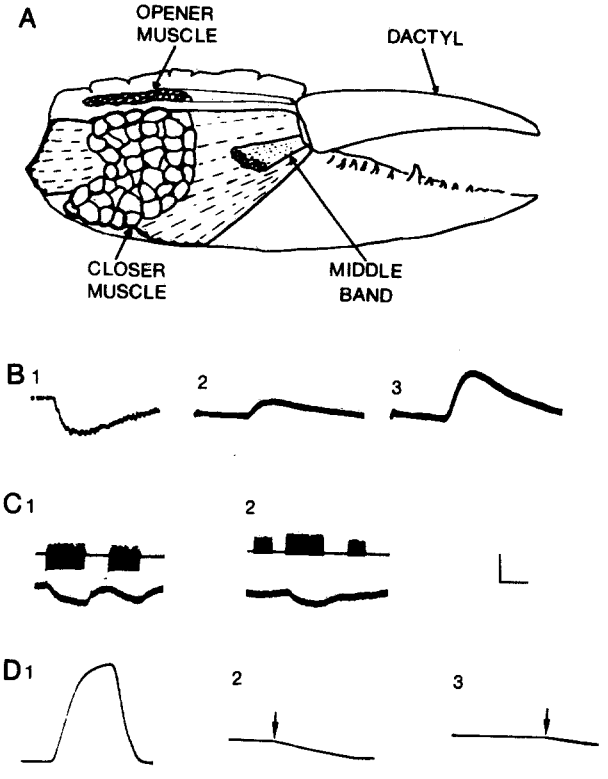
The claw closer muscle in the lobster is a large pinnately arranged muscle which when fixed with alcoholic Bouins fluid separates into 4 groups of fibers[7] viz. a large dorsal and ventral group and a smaller more proximally located dorsal and ventral group. Though the distribution of FCE, SCE and CI axons within each group is complex[7,8], there is a tendency for small bands of fibers to receive only one of the two excitor axons. We examined one such band in the distal region of the muscle reported to be innervated by SCE only[8]. The fibers within this middle band (fig. A) were shorter in length and inserted on the tendon at a greater angle than the remainder of the distal fibers. They were approximately 25μm in diameter and had a thin to thick filament ratio of 5:1[9]. Both features are characteristic of crustacean tonic muscle[4] as is the fact that these fibers have low levels of myofibrillar adenosine triphosphatase activity and a high oxidative capacity[10].

We determined the innervation to this band by stimulating each of the FCE, SCE, and CI axons and recording the resulting excitatory (EJP) and inhibitory (IJP) junctional potentials[6] (fig. B). Almost all, i.e. 72 out of 77, fibers sampled in this bundle in 7 cutter and 5 crusher claws received the SCE axon only. Invariably these fibers also had CI innervation. 6 out of 77 fibers had both SCE and FCE but no CI. The synaptic properies of these SCE-innervated fibers were similar in both cutter and crusher claws (table). In both claws, the SCE axon evokes relatively large EJPs at a low frequency of stimulation and these facilitate to less than twice their size with 10 Hz stimulation (fig. B, table). Compared to EJPs in other regions of the closer muscle[8], these are large responses for the SCE axon. Usually fibers in the center of this bundle had 10 mV EJPs at 1 Hz and these did not facilitate and would often defacilitate at 10 Hz stimulation. Fibers towards the periphery had smaller EJPs. The variability in EJP size amongst fibers of this band is reflected by the large standard deviation in the table.

The discrete nature of the middle band, its homogenous innervation and the comparatively large EJPs prompted us to examine its possible role. We did this by recording the movement of the dactyl with a photo-electric transducer[9] when the SCE is stimulated in isolated claws. A stimulating

Amplitude of EJPs at 1 and 10 Hz stimulation of SCE axon in the middle band of fibers in the dimorphic claw closer muscles of lobsters

		EJP (mV) 1 Hz		10 Hz	
	N	*x*	*SD*	*x*	*SD*
Cutter	41	5.1	5.9	8.4	5.5
Crusher	27	5.0	4.2	9.3	3.6



A Diagram of a lobster cutter claw showing the position of the middle band of fibers in the closer muscle. *B* Intracellular recordings from the middle band of muscle fibers showing an IJP (1) in response to 1 Hz stimulation of the CI axon and an EJP at 1 Hz (2) and 10 Hz (3) stimulation of the SCE axon. *C* Closing of the dactyl (downward deflection in lower trace) during 2 periods of 5 Hz stimulation of the SCE axon (wide bursts in upper trace) in an intact cutter claw (1). For the same claw, when the middle band is cut, the dactyl closes only in response to 10 Hz stimulation of SCE axon (middle burst in upper trace) but not to 5 Hz stimulation (1st and 3rd bursts) (2). *D* Opening movement of the dactyl (upward deflection) from a fully closed position to fullly opened position in response to 20 Hz stimultion of the OE axon and its return to the closed position (downward deflection) upon cessation of the stimulation (1). With the dactyl held slightly open with 8 Hz stimulation of the OE, concurrent 5 Hz stimulation of the SCE (arrow) causes closure (downward deflection) (2). When the middle band is cut, the dactyl closes only in response to 20 Hz stimulation of SCE (arrow) but not to 5, 10 and 15 Hz stimulation (3). Calibration; horizontal 10 msec in B; 5 sec in C, D; vertical 0.2 mV in B1; 20 mV in B2, B3.

frequency of 5 Hz is sufficient to cause closing of the dactyl when the middle band is intact (fig. C1) but 10 Hz is required after it is cut (fig. C2). In other experiments the dactyl was held partially open by stimulating the excitor motoneuron to the opener muscle (fig. D). Under these conditions firing the SCE at 5 Hz caused the dactyl to close (fig. D2). But when the middle bundle was cut, closing only occurred at 20 Hz (fig. D3). These experiments show that the middle band of fibers close the dactyl at low (5 Hz) frequencies of firing of the SCE axon. Consequently the middle band may be regarded as a distinct functional sub-unit within the SCE motor unit.

The discovery of such sub-units in the isolated claw closer muscle leads to the equally interesting question of their role in the intact animal. Because of the location of the middle band, it was possible to obtain myograms from it via paired extracellular wire electrodes in intact lobsters. There was a background activity of muscle EJPs at an average frequency of 1-5 Hz in a quiescent state with the claw held in a partially closed position. During closing movement, firing frequencies were much higher. The middle band therefore appears to maintain the posture of the dactyl with a low firing frequency. Furthermore it is strategically located within the muscle to maintain posture with a minimal amount of tension since it inserts on the tendon at the pivotal point of the dactyl (fig.). Functional subdivision of the SCE motor unit in the lobster closer muscle therefore extends the contractile capabilities of this muscle and may have evolved for this reason in a large muscle with few motor units.

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Trigeminal stimulation modulates vestibular unitary activity[1]

L. Petrosini, D. Troiani and B. Zannoni

Istituto di Fisiologia Umana, Università Cattolica S. Cuore, 644, Via della Pineta Sacchetti, I-00168 Roma (Italy), 21 May 1981

Summary. 139 vestibular neurons were analyzed in 38 guinea-pigs after the stimulation of trigeminal fibers: increase or decrease of discharge rate and also rhythmic modulation of vestibular unitary activity were observed.

Previous reports have provided indications of the involvement of trigeminal sensory afferent fibers in the vestibular system. Unilateral neurotomy of the trigeminal nerve in the compensated guinea-pig after hemilabyrinthectomy induced reappearance of labyrinthine postural asymmetry; curvature of the trunk, head torsion, eye nystagmus, extension of the forelimb and impairment of righting reflexes[2]. Other research[3] on vestibular compensation demonstrated, after trigeminal neurotomy, an increment in amplitude of N_1- and N_2-waves of vestibular field potentials recorded from the non-deafferented vestibular nuclei. These findings showed that the trigeminal system contributes to reestablishing a condition of balance between the 2 vestibular nuclear groups through a mechanism of inhibition of the hyperactive vestibular nuclei of the intact side. Furthermore a role for the trigeminal system in the control of posture and orientation has been documented in animals with poor vision such as rats, guinea-pigs, nocturnal and burrowing animals[4]. In fact in recent research[5] a trigeminal reflex leading to the righting of the head was described both in normal and bilateral labyrinthectomized animals when placed on their sides on the ground: the involvement of the vestibular neuronal pool for the actuation of this reflex was suggested. Thus, on the basis of the above reported data, an analysis of trigeminal influence on vestibular unitary discharge has been undertaken.

38 guinea-pigs were anesthetized (ketamine hydrochloride: 24 mg/kg), tracheotomized, curarized and artificially ventilated. Then the animals were surgically prepared to expose bilaterally the ampulla of the lateral semicircular canals and the trunk of the trigeminal nerves immediately distal to the semilunar ganglion. Labyrinthine stimulation was carried out by introducing into each ampulla bipolar copper electrodes insulated except at the tip (0.05 msec, 1-100 Hz, 0.2-2.0 mA). The left and the right trigeminal nerves were stimulated through bipolar tungsten microelectrodes (0.05 msec, 10-100 Hz, 50-200 μA). The stimulation period lasted 200-1000 msec. Non-nociceptive mechanical cutaneous stimulations of the areas innervated by the ophthalmic, maxillary and mandibular branches of the trigeminal nerves were separately performed with a hand-held plastic probe and included light touch with wisps of cotton and gentle pressure with small wooden dowels. Mechanical displacement of the vibrissae was also carried out. A tungsten insulated microelectrode (tip diameter 1-8 μm; resistance 900-1500 kΩ) was advanced by means of a Transvertex micromanipulator towards the region of the vestibular nuclear complex to record evoked potentials and single-unit discharges. The signals from the microelectrode were amplified by a conventional AC preamplifier then recorded on tape (HP 3960) and displayed on an oscilloscope (Tektronix 565). The signals were also led to a signal analyzer (HP 5480 A) for field potential analysis (average of potentials 64 times). The extracellular signals from the tape recorder were directed into a window discriminator for spike amplitude selection. Each selected spike was displayed on an oscilloscope channel to verify the constancy of its morphology. Then, the discriminated spike was used to trigger a rectangular pulse of constant amplitude and width (0.1 msec) which was directed to an EEG channel. Spike frequency was determined by counting at intervals of 250-500 msec. In each experiment the stereotaxic coordinates of each recorded unit were noted and, at the end of the experiment, at least 1 site of recording was marked by an electrolytic lesion. The animal was given a lethal dose of anesthesia and the brain was removed, fixed in Carnoy