Table 1. Mean intake of water by variously treated rats (see Fig. 1). The drug used was norepinephrine and the placebo was saline. Conditions of treatment (*A–D*) are defined in Fig. 1.

| Treatment | | Intake (ml) |
|---|---|---|
| Food | Injection | |
| As desired | Drug (*A*) | 6.4 |
| As desired | Placebo (*B*) | 8.3 |
| None | Drug (*C*) | 4.1 |
| None | Placebo (*D*) | 5.0 |

prived of food—half of them deprived of water and the other half satiated with water for any one trial. We could not replicate Grossman's finding of cholinergic depression of intake of food (*1*). The most plausible explanation of this failure is in the fact that we used solutions of carbachol as our stimuli, whereas Grossman employed crystals; thus he delivered a considerably larger quantity of chemical to the locus of stimulation. Grossman has demonstrated in the hypothalamus (*3*) that, when chemicals are used as blocking agents (as by us), relatively high dosages are needed, and even then the blocking effect may not necessarily be complete.

Functional checks of the accuracy of placements were made during the experiments. Appropriately deprived subjects were tested, by cholinergic or adrenergic stimulation, for the now well-established increases in drinking or eating, respectively (*1*). Of seven subjects checked with both carbachol and norepinephrine, four showed positive responses to both chemicals, two responded to norepinephrine only, and one responded to carbachol only. Differences in response possibly reflected minor variations in placement. The other four subjects all gave positive responses to carbachol. Ultimately all subjects were killed and the brains were removed, sectioned, and stained. The locus of stimulation was then determined with the de Groot atlas. Each subject had at least one cannula tip placed in the immediate vicinity of the amygdaloid cortical nucleus.

Thus Grossman's suggestion that the hunger-thirst interaction in the amygdala is mediated by activated circuits is not supported by the results. His alternative hypothesis of direct neurohumoral blocking seems more likely. The nature of such a mechanism is not yet known, but presumably it would be analogous, and perhaps similar, to the lateral inhibition demonstrated peripherally in receptors, as has been suggested (*2*).

An alternative explanation of our results may be that the adrenergic amygdaloid circuit can modulate ongoing, deprivation-induced, consummatory behavior, but not initiate such behavior in the satiated animal; but the circuit is still functional in the satiated animal and thus can stimulate inhibitory circuits. This explanation would preserve Grossman's interacting-circuit hypothesis, but evidence of such a generality of function for the amygdaloid circuits is not yet forthcoming.

On the other hand, the mechanism we posulate, whereby the synaptic release of a specific neurohumor from activated neurons would serve both to stimulate the next segment of that specific circuit and to inhibit adjacent, possibly antagonistic, circuits, would display the functional type of role postulated by Coury (*7*) for the overlapping of neurobehavioral systems; it could be an important contributor to changes in the "central set" of the organism (*8*) and to the integration of ongoing drive states (*9*). Of course, interaction by other activated circuits in the central nervous system (in the septal area, for example) also would not thus be precluded from contributing to the hunger-thirst interaction, although the amygdala is apparently not involved in such a mechanism; current thought in this field tends to support the notion of multiple mechanisms underlying the thirst and hunger drives (*9*).

G. SINGER
R. B. MONTGOMERY
*School of Behavioural Sciences, Macquarie University, Eastwood, New South Wales, Australia 2122*

**References and Notes**

1. S. P. Grossman, *J. Comp. Physiol. Psychol.* **57**, 29 (1964).
2. ———, *Amer. J. Physiol.* **202**, 872 (1962).
3. ———, *ibid.*, p. 1230.
4. ———, *J. Comp. Physiol. Psychol.* **58**, 194 (1964).
5. The adrenergic dose level [$648 \times 10^{-4}M$, used by N. E. Miller, K. S. Gottesman, N. Emery, *Amer. J. Physiol.* **206**, 1384 (1964)] was the one showing maximum effect in the hypothalamus. The carbachol dose level ($3 \times 10^{-4}M$, used by R. W. Russell, G. Singer, F. V. Flanagan, M. Stone, J. Russell, in preparation) was the one showing maximum effect in the amygdala.
6. Q. McNemar, *Psychological Statistics* (Wiley, New York, 1962), pp. 101–2.
7. J. N. Coury, *Science* **156**, 1763 (1967).
8. E. Von Holst and U. Von St. Paul, *Sci. Amer.* **206**, 50 (1962).
9. S. P. Grossman, *A Textbook of Physiological Psychology* (Wiley, New York, 1967), pp. 610–11.
10. Supported by the Australian Research Grants Committee's grant 65/15607 to one of us (G.S.).

25 March 1968

## Junctional Physiology and Motor Nerve Distribution in the Fast Adductor Muscle of the Scallop

Abstract. *Electrical recordings from single muscle cells in the fast portion of the scallop adductor have revealed a multiterminal distribution of motor nerves. All motor junctions appear to be of the fast designation, and several nerve fibers supply each muscle cell. The muscle fibers, by virtue of common innervation, are grouped into functional motor units. The pattern of innervation in scallops thus shares functional similarities with the motor distribution to skeletal muscle fibers of both vertebrates and arthropods.*

The electrophysiology of molluscan muscle is not well known. Although there have been some extracellular recordings from cephalopods (*1*) and bivalves (*2*), intracellular recordings from single muscle fibers have, so far, been confined to only one, rather unusual, muscle type—the anterior byssal retractor of the mussel *Mytilus* (*3*). A growing number of neurophysiological investigations deal with molluscan material in an effort to elucidate the manner in which the central nervous system programs behavior in these diverse forms. It is thus important to establish comprehensive descriptions of molluscan neuromuscular mechanisms. I have studied the neuromuscular physiology of the fast portion of the adductor muscle in the scallops *Aequipecten gibbus* (from Panacea, Florida) and *Aequipecten irradians* (from Woods Hole, Massachusetts). Electrical responses from the two species appeared to be similar.

The fast portion of the scallop adductor receives up to four major nerve branches from tracts on each side of the parietovisceral ganglion. These radiate over the surface of the muscle and branch profusely. However, the individual adductor nerves are small and usually hard to identify in living preparations; therefore, muscle responses were

evoked by a stimulus cathode (a saline-filled capillary) placed directly on the muscle surface in the neighborhood of the recording site, so that a limited number of nerve branches were excited at any one time. This procedure elicited minimum response from the muscle as a whole, insuring that movement and dislodging of the recording electrode never constituted serious problems. All preparations were bathed in natural or artificial seawater. Intracellular records were obtained from fibers near the ventral surface of the adductor muscle by means of capillary microelectrodes filled with 2.5*M* KCl. Penetration of a fiber membrane was signaled by a stable, negative shift in the trace of an oscilloscope. The mean value of such resting potentials from 113 different fibers was 56 mv. All fibers examined in the fast portion of the scallop adductor receive a multiple innervation from the motor nerve branches of the parietovisceral ganglion (Fig. 1a). Increments in the strength of a single, brief (0.1 to 0.5 msec) electrical stimulation caused stepwise increases in the amplitudes of excitatory junctional potentials (EJP's) recorded from a single muscle fiber. These often had different latencies, were dissimilar in individual amplitudes, and had constant and reproducible thresholds. The data indicate that several separate excitatory pathways may impinge upon one region of a muscle fiber from the ganglionic motor centers. Indeed, the total number of separate excitatory axons which reach each fiber may be greater than indicated by individual records, since these were obtained at only a single recording locus on the cell in each case, and since the EJP's were evoked by stimulating only one site on the muscle.

Evidence for an inhibitory innervation of the fast adductor muscle was not seen in the electrical data. Several hundred individual muscle fibers were examined with intracellular electrodes, and in no case were hyperpolarizing potentials evoked by apparent presynaptic stimulation, nor were any excitatory responses reduced in amplitude by increases in stimulus strength. Nonetheless, the possibility of inhibition in the fast muscle cannot be ruled out in the absence of confirmatory evidence involving tension measurements.

Very intense electrical stimulation of muscle preparations often evoked not only graded EJP's having the usual waveform and time course but much briefer activity as well (Fig. 1, c and f).

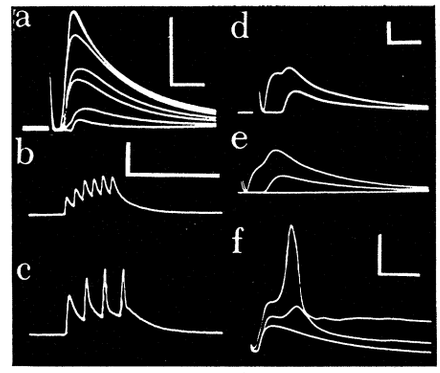


Fig. 1. In (a) superimposed records show the response of a single muscle fiber to brief shocks of increasing intensity. Repetitive stimuli at 20 per second (b) evoke nonfacilitating junctional potentials from a muscle fiber. In (e) another cell responds with graded responses—possibly involving regenerative activity—to four identical stimuli recurring at 10 per second. Records in (d) and (e) show the similarities in compound EJP's from two adjacent muscle fibers. Shown in (f) are EJP's and a muscle spike in response to presynaptic stimulation. Note the extra response to a stimulus of intermediate strength. Calibration marks: (a) 20 mv and 20 msec; (b) and (c) 30 mv and 500 msec; (d) and (e) 20 mv and 20 msec; (f) 25 mv and 50 msec.

These responses appear superimposed on the junction potentials and sometimes can be observed to overshoot the zero potential level. At 15°C, they have a duration at half-amplitude of approximately 20 msec. These transients will be referred to as muscle spikes. The thresholds for muscle spikes can be high (Fig. 1); moreover, in some cases (Fig. 1a) even large junctional potentials failed to evoke them. In other records, spikes may be seen which do not overshoot the zero potential level. These observations raise questions about the distribution of spike-supporting membrane along the fast muscle fibers. As with arthropods, the possibility that muscle spikes may be actively conducted remains problematical.

My observations indicate an absence of appreciable neuromuscular facilitation in the fast muscle (Fig. 1b). Liminal electrical stimuli were presented to the preparation, so that an apparently unitary EJP would be evoked in the postjunctional muscle fiber following each shock. Brief trains of identical stimuli at 10 and 20 per second were usually ineffective in altering the peak amplitude of the individual junction potentials. In some instances, later EJP's of the train did increase in amplitude somewhat. This could usually be attributed to a change in stimulating situation caused by movement of the preparation by muscle contraction. In other cases (Fig. 1c) the increased response amplitude was clearly due to the participation of regenerative phenomena, undoubtedly triggered by temporal summation of individual EJP's in the train. The stability of the junctional responses at frequencies of up to 20 per second is favorably correlated with the normal swimming frequencies of three to five strokes per second observed in intact animals at similar temperatures (15°C). At physiological frequencies, therefore, neither facilitation nor fatigue are characteristic features of junctional transmission in this muscle.

In electromyograms obtained by extracellular recording from the fast muscle during swimming sequences, only single compound action potentials are observed during each power stroke. These are typically of the same duration as muscle spikes recorded intracellularly from individual muscle fibers in response to presynaptic stimulation. This implies that, during swimming, the muscle responds to single nerve volleys at each power stroke and that excitatory electrical activity may occur synchronously in all fibers. One explanation of these findings would be simultaneous central excitation of all relevant nerve fibers. Peripheral mechanisms—perhaps electrical coupling between muscle fibers—might also occur, however. The latter possibility was examined. As a micropipette is advanced vertically into the fast muscle from its surface, successive penetrations indicate functional groupings among the muscle fibers themselves. Each fiber of one group exhibits an identical program of excitatory junctional potentials (Fig. 1, d–e). Waveforms of these compound EJP's are not only similar in the temporal relationships of their individual components, but respective component thresholds are identical as well (Fig. 2A). In each frame the upper and lower oscilloscope traces are electrical records obtained simultaneously from two neighboring muscle fibers. Superimposed sweeps show, in sequence, the response to a subthreshold shock, some responses of constant amplitude evoked by several shocks of gradually increasing intensity, and finally, a postjunctional response of a compound nature following a stimulus of still higher intensity. In this particular intensity series, the programs of response increment in the two neighboring muscle fibers are not only similar in waveform, but the increment steps

have identical thresholds in the two cells. Thus, neighboring fibers in this muscle can have identical junctional response characteristics. What is the basis for this identity? The possibility that neighboring fibers are electrically coupled, as in some other (*4*) fast-fibered invertebrate muscles, appears to be remote. In some cases, where simultaneous recordings were obtained from two neighboring muscle fibers and where it was established that similar response programs appeared in both fibers at identical stimulus thresholds, the recording pipette in one cell was connected to the output of an isolating transformer, and current was passed across the cell membrane. During this procedure, no resulting potential shift was ever observed in the other fiber (Fig. 2C).

Examination of the component latencies in different fibers which show identical EJP programs indicates that the latencies can be quite different from cell to cell (Fig. 2, E–I)—evidence which militates against any low-resistance pathways between individual muscle fibers. These observations appear to exclude direct electrical coupling between muscle cells as the basis for similar response programs. Thus, it seems probable that the similarities result from parallel excitatory innervation, undoubtedly involving multiple branching of an individual motor axon. The evidence further indicates that all muscle fibers included in such a functional group, or motor unit, receive branches from all the same motoneurons.

The pattern of innervation of the fast adductor in this animal shares similarities with both arthropods and vertebrates. In the latter, skeletal muscles are functionally divided into large numbers of separate motor units, each unit receiving the output of a single motor neuron which distributes its terminal branches to as many as 100 individual muscle fibers. A similar division of output occurs in the fast adductor of the scallop, and it is likewise possible to assume that the rapidity and strength of contraction in this animal may be programmed by fractional or total recruitment of a motoneuron pool. In addition, muscle fibers in the fast adductor receive a multiple innervation, the standard trademark of many other invertebrate preparations. In the scallop, however, all of the motor pathways which innervate a fast muscle fiber are functionally similar, possibly because selected recruitment of motor units obviates some of the necessity for a typical frequency-controlled slow innervation, as does the existence of an entirely separate muscle which controls long-term maintenance of valve position. I conclude that multiple innervation is necessary in evoking maximum electrical response in fast fibers, and in this animal it may be the only mechanism which will insure the generation of muscle spikes in all competent regions of the fiber membrane.

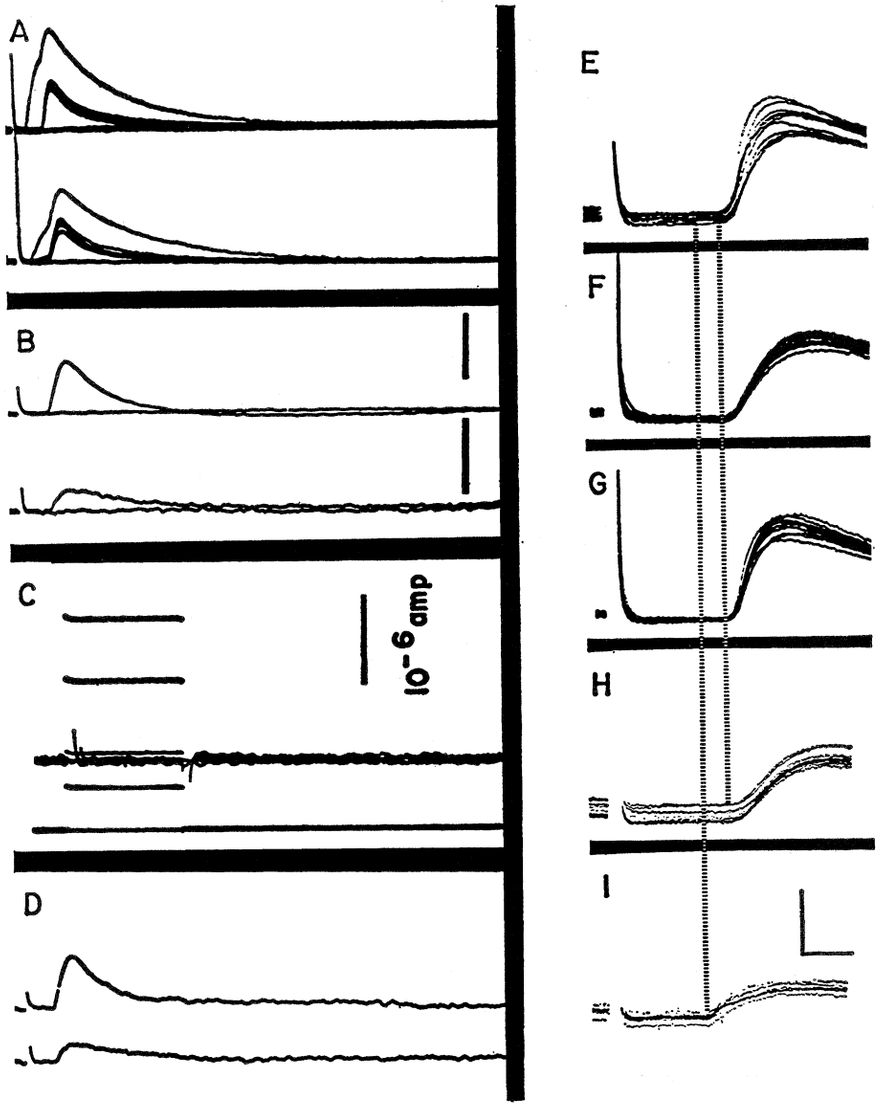


Fig. 2. (A–D) Records were obtained simultaneously from pairs of neighboring muscle fibers. In (A) the stimulus intensity series delivered to the motor nerves evokes similar response waveforms at identical stimulus strengths in two fibers. In another pair of fibers, records show unitary responses to threshold stimuli before (B), and after (D) passing current pulses through one of the recording electrodes. (E–I) Records are shown from five muscle cells in the same motor unit, obtained in successively deeper penetrations by advancing the recording electrode. A unitary response was evoked in each fiber by the stimulus. (E–I) The latencies for are significantly different from each other. Each trace represents ten superimposed sweeps recurring at one per second. Calibration: (A–D) vertical bars for upper and lower traces are both 10 mv. Duration of the current pulses in (C) is 110 msec; sweep speed is the same in all four records; (E–I) 5 mv and 10 msec.

DEFOREST MELLON, JR.
*Department of Biology, University of Virginia, Charlottesville,* and *Marine Biological Laboratory, Woods Hole, Massachusetts*

**References and Notes**

1. D. M. Wilson, *J. Exp. Biol.* **37**, 57 (1960).
2. J. Lowy, *J. Physiol.* **124**, 100 (1954); C. M. Fletcher, *ibid.* **90**, 415 (1937).
3. B. M. Twarog, *ibid.* **192**, 847 (1967).
4. J. P. Reuben, *Biol. Bull.* **119**, 334 (1960); I. Parnas and H. L. Atwood, *Comp. Biochem. Physiol.* **18**, 701 (1966).
5. Supported by PHS grant NB-04989 and NSF grant GB-3625.

10 April 1968