Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 4232–4236, July 1984
Neurobiology

Functional history of two motor neurons and the morphometry of their neuromuscular junctions in the gill of *Aplysia*: Evidence for differential aging

(junctional transmission/facilitation)

B. PERETZ*†, A. ROMANENKO*†, AND W. MARKESBERY†‡

*Department of Physiology and Biophysics, ‡Department of Pathology and Neurology, and †Sanders–Brown Research Center on Aging, University of Kentucky Medical Center, Lexington, KY 40536

Communicated by Richard F. Thompson, March 26, 1984

ABSTRACT **Because the viability of the gill withdrawal reflex is dependent on age in *Aplysia*, we examined physiologic and morphometric properties of two motor neurons, L_7 and LDG_1, involved in the reflex in three postmetamorphic age groups: young, mature, and old. L_7's capability to elicit pinnule contraction, a major component of the reflex, was reduced markedly in old gills; facilitation at old L_7 terminals, upon which contraction is dependent, was significantly reduced. The morphology of the pinnule neuromuscular junctions changed with increased age. In contrast, LDG_1's capability to elicit efferent vessel contraction, a major component of respiratory movements, was not significantly altered by age; facilitation also was relatively unaffected; and morphologic changes at neuromuscular junctions were poorly correlated with age. Aging occurs differentially in two motor neurons innervating the gill. The dissimilarity in function and in frequency of activation of L_7 and LDG_1 may help explain the greater vulnerability of L_7 to the effects of aging. The possibility that disuse is involved in the aging process is discussed.**

Studies of the aging nervous system tend to describe putative degenerative changes, yet not all central nervous system neurons show age-dependent morphological and biochemical changes (1–4). These findings suggest that aging occurs differentially in the central nervous system and that functional differences exist between "aging" and "non-aging" neurons. The behavioral consequences of morphological and of functional alterations with age at the cellular level remain to be demonstrated.

Theories of cellular aging can be tested in *Aplysia* by investigating the neural substrates of well-defined behavior. *Aplysia* have a life span of 12–14 months (5, 6), with developmental stages being *ca.* 45 days (7), after which they metamorphose into the adult form. Approximately 80% of the life span then is spent in the adult form. The *Aplysia* nervous system is comprised of neurons of known function that can be identified in different aged animals (8–10). The neurons in the adult form and the behaviors they mediate have been most studied.

In *Aplysia*, underlying studies of behavioral plasticity and its neural substrates has been the assumption that they are unaffected by age during postmetamorphic life. Recent studies, however, showed that central nervous system control of the gill withdrawal reflex and habituation of the reflex changed with increasing age. In young animals, muscle contraction during the reflex was greater and the rate of habituation was slower than that in mature animals (8, 10). Also, the young central nervous system failed to regulate the rate of habituation in response to varying stimulus strength (8). With increased age the stimulus threshold to elicit the reflex was greater than in either young or mature animals, and the reflex amplitude was reduced and the rate of habituation was faster than that in mature animals (9). More recently, long-term habituation was shown to be impaired in older *Aplysia* (11). Neural substrates of the gill reflex then appear modifiable not only by training but by the effects of aging. The *Aplysia* nervous system appears to undergo change throughout postmetamorphic life.

We investigated properties of two motor neurons, L_7 and LDG_1, which are involved in the gill reflex in three age groups of *Aplysia*: (*i*) motor neuronal efficacy; (*ii*) facilitation of junctional transmission in the gill; (*iii*) morphometry of neuromuscular junctions (NMJs) innervated by L_7 and LDG_1, respectively. These properties of L_7 changed significantly in older animals in contrast to those of LDG_1, which were relatively unchanged. L_7 mediates the gill pinnule contraction (12, 13), which is a major component of the reflex (13), and LDG_1 mediates the efferent vessel contraction (12, 13), which is a component of respiratory movements and, to a lesser extent, the reflex (13). Our findings show that motor neurons innervating the gill are differentially affected by aging, with distinct effects at the NMJ.

METHODS

Aplysia californica of three different postmetamorphic ages (PMA) were used: young, PMA of 43 days; mature, PMA of 117 days; and old, PMA of 205 days. Age was determined by the size of the internalized shell located in the mantle (14). A total of 7 young, 13 mature, and 15 old animals was used; they were obtained from Alacrity Marine (Venice, CA) and Sea Life Supply (Sand City, CA).

Electrophysiology. The motor neuron efficacy of L_7 and LDG_1 was examined in reduced preparations as described (15) (see Fig. 2*A Inset*). Single microelectrodes were used for both intracellular recording and stimulation via a bridge circuit. Junction potentials were recorded extracellularly with a suction electrode, which insured the recording of junction potentials during muscle contraction elicited by spike trains (15, 16). Extracellular junction potentials (CJPs) evoked by L_7 were recorded *only* on the medial surface of the gill pinnule, and those evoked by LDG_1 were recorded *only* on the upper surface of the efferent vessel (Fig. 1 *Insets*). Gill contraction elicited by spike trains was measured by a transducer connected to the gill by surgical thread and referenced to gill weight so that comparisons could be made between ages (15). The response to each spike train frequency was tested three times in each preparation with a 2-min interval be-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CJP, extracellularly recorded junction potential from a population of junctions; EJP, intracellularly recorded junction potential; EV, efferent vessel of the gill; PN, pinnule of the gill; PMA, average postmetamorphic age; NMJ, neuromuscular junction.

tween tests (15). Measurements of CJP amplitude and of muscle contraction were made from polygraph recordings.

Fig. 1 shows simultaneous recordings of CJPs and intracellular junction potentials (EJPs) from gill muscles evoked by spike trains; the increased CJP amplitudes during the train corresponded to the facilitation and summation of EJPs recorded in a single muscle fiber (15, 16). The suction electrode used had a diameter of *ca.* 400 μm and recorded an average CJP activity from a population of muscle fibers under it; the average fiber diameter from the three age groups was 6.6 ± 0.6 μm (unpublished result). CJPs evoked by spike trains or by single spikes were not contaminated by spikes in presynaptic fibers, by movement artifact, or by muscle spikes (15).

Electron Microscopy. Medial pinnule (PN) and efferent vessel (EV) longitudinal muscles, innervated by L_7 and LDG_1, respectively, were identified by CJPs evoked by intracellular stimulation (15) and then were processed. Simultaneous processing of paired muscles from the same gill and of pairs from different aged gills served to minimize, ascribing to fixation artifact, morphological differences between PN and EV NMJ and age-related changes in NMJ morphology, respectively. Muscle fiber morphology and connective tissue were not significantly different across age (unpublished data). The muscles were excised and then processed according to the methods of Orkand and Orkand (18). This method gave the optimal tissue penetration and fixation irrespective of animal age. Sections were viewed with a Philips 201 electron microscope. Junctional cleft width, at presumed active zones (Figs. 4 and 5), and the length of contact between the terminal and muscle fiber were measured at 30 junctions in both PN and EV muscles in each age group (Fig. 5 *Inset*); three gills per age group were used.

No selection of NMJ was made other than terminals being in direct contact with muscle. Measurements were made on one section per NMJ and the plane of section through the NMJ obviously was random. Morphometry was determined from photomicrographs, magnified no less that ×61,500, with a Zeiss image analyzer and an ocular micrometer. The measurements were made by using a blind procedure. An analysis of variance was applied to the data.

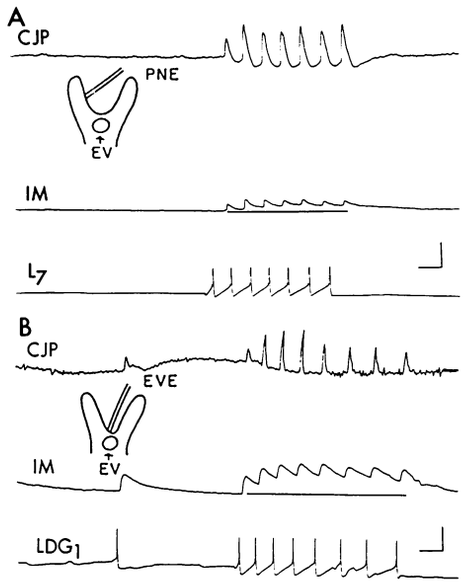

FIG. 1. Simultaneous recordings of CJPs and EJPs in response to a spike train. The line under EJPs denotes resting membrane potential of muscle fiber. Facilitation was determined as described (17), and a CJP and EJP derived facilitation parallel each other. (*A*) L_7-evoked junction potentials. PNE, extracellular electrode on pinnule; IM, intracellular electrode in a fiber at base of pinnule. (*Inset*) PNE placed on medial pinnule surface recorded CJPs only evoked by L_7. Scale: CJP, 25 μV; IM, 5 mV; L_7, 20 mV; 0.2 s. (*B*) LDG_1-evoked junction potentials in EV. EVE, extracellular electrode on EV; IM, intracellular electrode in a longitudinal muscle fiber in EV. (*Inset*) EVE placed on dorsal surface of EV recorded CJPs only evoked by LDG_1. Scale: CJP, 8 μV; IM, 5 mV; LDG_1, 20 mV; 0.5 s.

RESULTS

Both L_7 and LDG_1 innervate smooth muscle fibers that do not spike (Fig. 1) (15). For nonspiking muscle fibers, contraction is graded and depends upon the level of depolarization evoked by summation and facilitation at the NMJs (18, 19). Consequently, spike trains in gill motor neurons were requisite to elicit muscle contractions (12, 15). Fig. 2 shows that muscles innervated by L_7 and by LDG_1 had thresholds to distinct spike rates. Trains of 3-s duration were used to insure eliciting maximal contractions in old gills (15).

L_7-Elicited PN Contractions. The antiflaring PN contractions of the pinnule are a component of the gill withdrawal reflex (Fig. 2*A Inset*). The spike rate in L_7, which initiated contractions, was 15–19 spikes per 3 s in young, mature, and old pinnules (Fig. 2*A*). Contractions of young and mature PN increased as spike rates increased, and they appeared to level off at *ca.* 600 mg/g of gill, in response to 40–44 spikes per 3 s. In contrast, contractions of old PN changed very little in response to increasing spike rates; they ranged from 10 mg/g of gill to a maximum of only 71 mg/g of gill (Fig. 2*A*). A

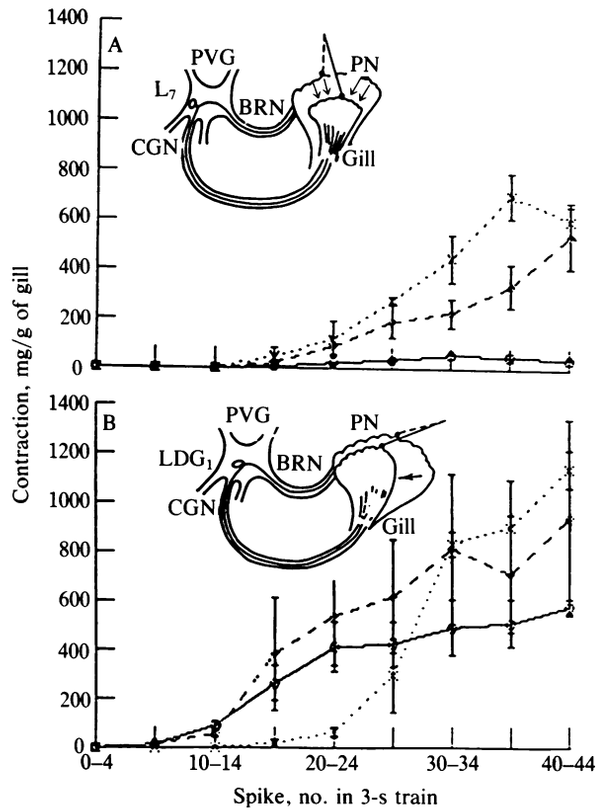

FIG. 2. Gill contraction as related to spike rate in the three age groups. ×····×, Young; ◇- - -◇, mature; ○——○, old. Note that each type of movement has a distinct threshold in mature and old animals; for PN it is 15–19 spikes per 3 s and for EV it is 5–9 spikes per 3 s. L_7 and LDG_1 have axons in both the branchial nerve (BRN) and ctenidio-genital nerve (CGN). The mean ± SEM is given for each spike rate. (*A*) L_7-elicited PN contraction. (*Inset*) Spike trains elicit an antiflaring movement of PN. Solid line, displacement of the thread connecting PN to tension transducer from rest position (dashed line). Arrows indicate direction of movement. (*B*) LDG_1-elicited EV contraction. (*Inset*) Spike trains cause movement of gill rostrally (arrow).

multiple comparison of PN contraction evoked by the spike rates tested (see Fig. 2*A*) showed that there was a significant age effect: F (2, 136) = 13.9; $P < 0.0001$. Contraction of old PNs evoked at spike rates of 25–29 spikes per 3 s and higher were significantly less than that of young and mature PNs, as determined by a Newman–Keuls test.

As shown previously (15), the significant reduction of contraction of old PNs was not the result of conduction failure in L_7 axons nor failure at the NMJs. Also, PN muscle fiber diameter and fiber density in the three age groups were not significantly different and were unlikely to account for the significant differences in contraction (unpublished data).

Facilitation at PN NMJs. Facilitation was calculated as described (15, 17, 20) from CJP amplitudes measured in preparations whose PN contractions are shown in Fig. 2*A*. Fig. 3*A* shows the average value of facilitation per spike during a train and that facilitation occurred at NMJs in old pinnules. A maximal value was reached at 5–9 spikes per 3 s, which was below the rate initiating contraction, and it then gradually decreased in response to increased spike rates. In young and mature PN NMJs, facilitation was increased with increased spike rates and was maximized at 25–29 spikes per 3 s, a rate well above threshold.

An analysis of variance showed that the age by spike rate effect was significant: F (2, 39) = 2.94; $P < 0.018$. The post-hoc test established that facilitation at old NMJs was significantly less at 25–29 spikes per 3 s and higher rates than that at younger NMJs. Facilitation to lower rates was not significantly different among the three groups. The results in Figs. 2*A* and 3*A* are consistent with each other, in that the spike rates at which contraction and facilitation are significantly reduced in old pinnules are the same for both.

LDG_1-Elicited EV Contractions. EV contractions are components of respiratory movements and the reflex (Fig. 2*B Inset*). The threshold spike rate eliciting contraction of mature and old EVs was 5–9 spikes per 3 s. With increasing spike rates, contraction increased and continued to do so up to the maximal spike rate tested, 35–39 spikes per 3 s (Fig. 2*B*). The threshold for EV contraction in young gills was 15–19 spikes per 3 s; nevertheless higher spike rates resulted in contractions equaling those in the gills of the two older groups. Analysis of age by spike rate effect was significant: F (2, 72) = 3.44; $P < 0.042$. This was due to contraction of mature and old EVs being significantly greater than young EV contraction to spike rates from 5–9 to 20–24 spikes per 3 s, determined by the Newman–Keuls test. In response to rates from 25–29 to 35–39 spikes per 3 s, there were no significant differences in contraction among the three groups. No differences in EV contraction were demonstrated between mature and old EV at any spike rate.

Facilitation at EV NMJs. Facilitation at mature and old NMJs was maximum at 5–9 spikes per 3 s, the same rate that initiated vessel contraction. In response to higher spike rates the average facilitation per spike decreased in both groups. It is apparent that the differences between the two groups were not significant (Fig. 3*B*). At young NMJs, the pattern of facilitation to increased spike rate was markedly different from that at mature and old NMJs. Facilitation progressively increased and reached a maximum at 25–29 spikes per 3 s. Multiple comparison showed that an age by spike rate effect

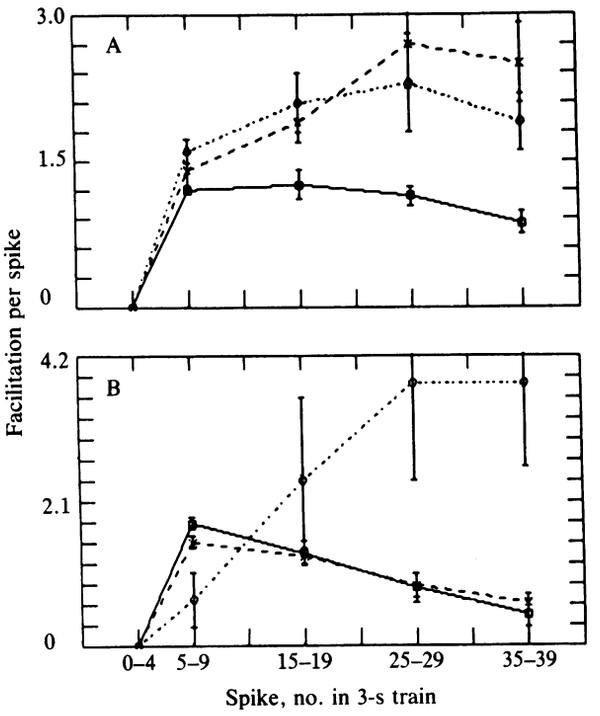

FIG. 3. A plot of facilitation per spike is obtained from averaging facilitation for each spike in a train, for each age group. An average value of 1 denotes CJP amplitude was unchanged; CJP increasing during a train resulted in values >1; values <1 denote CJP amplitudes were depressed. ○···○, Young; ×----×, mature; □——□, old. Values are mean ± SEM. (*A*) Facilitation at L_7 NMJs in the PN. At young and mature NMJs, facilitation is maintained at values of 2 and greater to spike rates of 15–19 spikes per 3 s and higher. At old NMJs, it does not exceed levels much above 1 and is depressed at higher rates. (*B*) Facilitation at LDG_1 NMJs in the longitudinal muscle of the EV; at mature and old NMJs, it does not quite reach 2 and is depressed in response to spike rates >15–19 spikes per s.

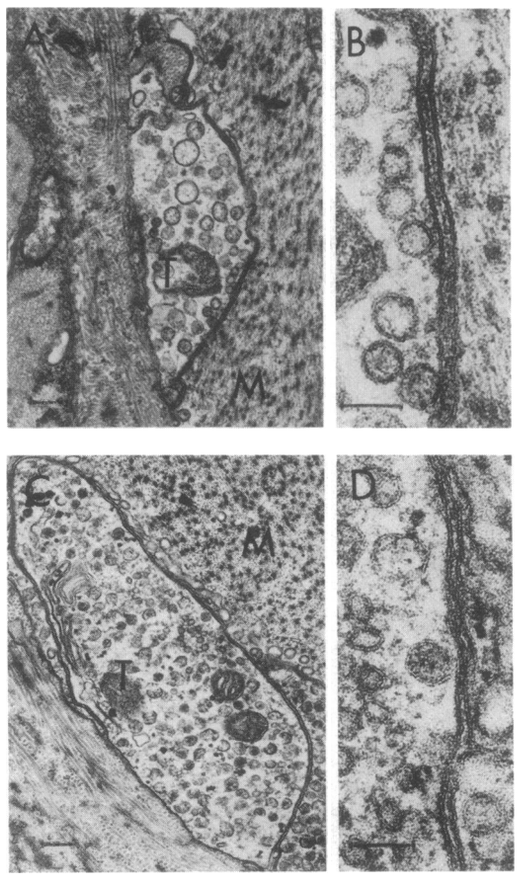

FIG. 4. Electron micrographs of PN NMJs. Young NMJ (*A*) and old NMJ (*C*) with nerve terminals (T) situated in a groove in the smooth muscle fiber (M). Both clear and electron-dense vesicles are present in a terminal. There were no obvious signs of degeneration at old NMJs. (*B*) Young NMJ with a cleft width of 13.0 nm; (*D*) Old NMJ with a cleft width of 6.3 nm. (*A* and *C*, bars = 0.25 μm; *B* and *D*, bars = 0.1 μm.)

was significant: F (2, 33) = 3.05; $P < 0.045$. Facilitation to spike rates of 25–29 spikes per 3 s and higher were significantly larger at young NMJs than at the two older NMJs, as determined by the post-hoc test.

In young EV, the fiber density of the longitudinal muscle is significantly less than in either mature or old EVs (unpublished data). For contraction of a young EV to attain amplitudes equal to or greater than those produced in the older EVs, it is likely that individual fibers contract to a greater extent to compensate for the lower fiber density. Because contraction of nonspiking *Aplysia* muscle appears dependent upon the level of fiber depolarization (18, 19), increased levels of facilitation would increase depolarization and result in strengthened contraction. The necessity for higher spike rates to elicit EV contraction (Fig. 2*B*) and the increased facilitation at NMJs (Fig. 3*B*) in young EVs is consistent with the means proposed to compensate for the low fiber density.

Morphometry of NMJs. Fig. 4 shows that the nerve terminal sits in a groove in close proximity to the small muscle fiber. There were no obvious signs of degeneration at old NMJs.

The rationale for measuring length of contact at the junction was suggested by the findings at crustacean NMJs, that highly facilitating NMJs tend to have a smaller region of contact between the terminal and muscle than at poorly facilitating NMJs (21, 22). Possibly, decreased facilitation at old L_7 terminals resulted from a change of contact length, and the difference between facilitation patterns at L_7 and LDG_1 NMJs resulted from differences in contact length. The rationale for measuring the junctional cleft was the suggestion that old rat skeletal muscle NMJ terminals swell with age (23); possibly old L_7 terminals became swollen and consequently the cleft width decreased with age. With random sections taken through NMJs, it would be reasonable to expect no differences in morphometry at both PN and EV

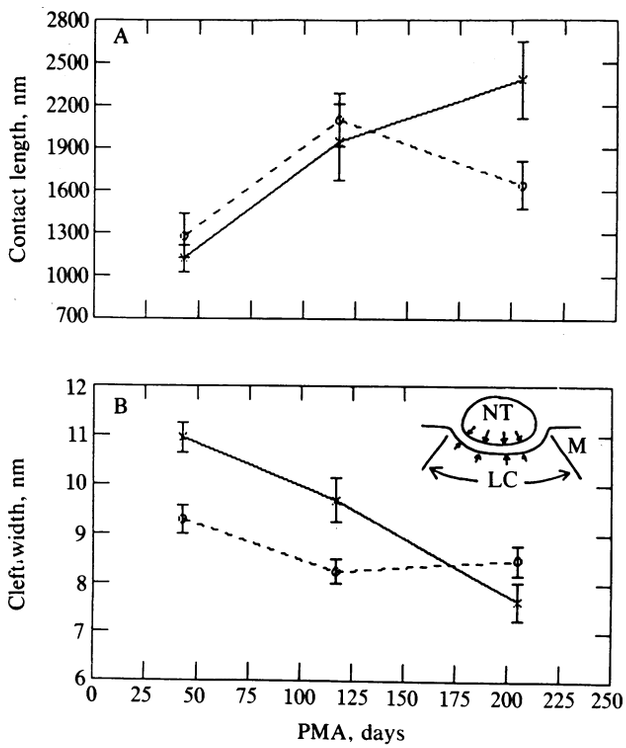

FIG. 5. Morphometry of NMJs as a function of age. ×——×, PN; o- - - -o, EV. Values are mean ± SEM plotted for each age group. Significant differences between groups are shown in Table 1. (*A*) LC of PN and EV NMJs. (*B*) Cleft width at PN and EV NMJs. (*Inset*) Length of contact, LC, and cleft width; arrows show sites of measurements. NT, nerve terminal; M, muscle fiber.

Table 1. Comparisons of NMJs with resulting probabilities, P, from the Newman–Keuls test

Comparison	P LC	P CW
Across age		
Y_{PN} vs. M_{PN}	<0.01 (+74%)	>0.1 (−12%) NS
Y_{PN} vs. O_{PN}	<0.001 (+114%)	>0.005 (−31%)
M_{PN} vs. O_{PN}	>0.1 (+22%) NS	<0.05 (−21%)
Y_{EV} vs. M_{EV}	<0.005 (+64%)	<0.01 (−11%)
Y_{EV} vs. O_{EV}	>0.1 (+12%) NS	<0.025 (−8%)
M_{EV} vs. O_{EV}	<0.05 (−22%)	>0.3 (+3%) NS
Within age		
Y_{PN} vs. Y_{EV}	>0.3 (+14%) NS	<0.05 (−16%)*
M_{PN} vs. M_{EV}	>0.3 (+8%) NS	>0.1 (−15%)*
O_{PN} vs. O_{EV}	<0.005 (−31%)	>0.2 (+11%)*

LC, length of contact; CW, cleft width. Values in parentheses indicate percent change of second value with respect to first from the sites designated in the left-hand column—e.g., LC in M_{PN} was 74% longer than that in Y_{PN}. NS, not significant. Y, young; M, mature; O, old.

*This value of P for F (2, 6) = 4.47; P = 0.065 (see text).

NMJs among the age groups. However, the length of contact between nerve terminals and muscle fibers at PN NMJs did increase linearly with age: r = 0.97 (Fig. 5*A*). Analysis of variance showed that the increase was significant: F (2, 6) = 10.00; $P < 0.04$; a Newman–Keuls test showed that the significant lengthening occurred between young and mature and between young and old (Table 1). Contact length was greater at old than at mature NMJs but not significantly so: $P < 0.1$. The cleft widths, from the same PN NMJs, decreased linearly with increasing age (r = −0.997) (Fig. 5*B*) and were significantly narrower: F (2, 6) = 6.04; $P < 0.037$; the Newman–Keuls test showed that the narrowing was significant between young and old clefts and between mature and old clefts and not so between young and mature clefts (Table 1). These two morphological properties of PN NMJs are progressively altered during postmetamorphic life.

In contrast, measurements from NMJs in EV muscle show no consistent change of the two morphological features with increasing age (Fig. 5), for which a correlation was sought: r = 0.39 for contact length and r = −0.69 for cleft width. An age effect was significant for contact length: F (2, 6) = 6.75; $P < 0.029$; the basis for it was a decreasing length between mature and old NMJs and an increasing one between young and mature NMJs (Table 1). The age effect for cleft width was also significant: F (2, 6) = 6.05; $P < 0.036$; clefts in young NMJs were wider than those at mature and old NMJs, with no difference between the latter two (Table 1).

If change in NMJ morphometry in the three groups (Figs. 4 and 5 and Table 1) simply resulted from differential processing because of variant tissue properties, both EV and PN NMJs should have shown progressive change with increased age. EV NMJs did not meet this condition. In fact, comparison of length within age groups was significant: F (2, 6) = 6.81; $P < 0.029$; old PN NMJs were longer than old EV NMJs (Table 1). The multiple comparisons within age groups of cleft width exceeded the accepted level of significance, F (2, 6) = 4.47 and $P < 0.065$, yet reveal a difference between young PN and EV NMJs. Although the morphology of both PN and EV NMJs changed between young and mature animals, only in PN NMJs did it change progressively with increased age.

DISCUSSION

The results described here show that two motor neurons that innervate the gill are affected dissimilarly by increased age during postmetamorphic life. The differences were expressed as measurable characteristics: amplitude of muscle

contraction each neuron elicited by spike trains, facilitation at their respective terminals evoked by the trains, and morphological properties of NMJs innervated by each neuron. Increased age appears to affect discrete mechanisms and sites in the aging neuron.

PN contraction was significantly decreased in old gills as compared to that in young and mature gills. As shown here and previously (15), decreased contraction resulted from reduced facilitation at L_7 NMJs. The morphometry of L_7 NMJs changed progressively and was highly correlated with age. In old PN NMJs, decreased cleft width and increased contact length appeared to coincide with decreased facilitation. In contrast, EV contraction was not significantly different among the three age groups. Facilitation evoked by LDG_1 spike trains was the same in mature and old gills. The significantly greater facilitation in young EV NMJs appears to compensate for the low density of muscle fibers; this would account for higher spike rates being necessary to elicit contraction amplitudes equivalent to those elicited in the two older groups. Unlike the morphological alterations at L_7 NMJs, those at LDG_1 NMJs appeared unrelated to increased age.

Our findings do not show a simple relationship between length of contact, cleft width, and facilitation. They do suggest that in addition to size of the NMJ, as shown in crustacean NMJs (21, 22), cleft width should be considered when investigating characteristics bearing on facilitation—that is, if either contact length or cleft width changed significantly when compared across age groups, no marked change in facilitation was measured; if both changed across age groups, facilitation was significantly different. Possibly both length of contact and cleft width must be altered beyond certain levels before facilitation is noticeably affected. Further study of the relationship between morphology and junctional transmission is necessary, including serial sections of PN and EV NMJs to yield a more complete description of the morphological changes as related to age. Nevertheless, the morphologic alterations at PN NMJs were highly correlated with age in clear distinction to those at EV NMJs, which were poorly correlated with age. These results offer a means to relate further junctional morphology and transmission.

L_7 is more vulnerable to increased aging than LDG_1. Functional differences between the two neurons may provide insight into differential aging. L_7's contribution to the gill withdrawal reflex is greater than that of LDG_1 (13). In addition, LDG_1 is involved in respiratory movements of the gill, but L_7 is inhibited during these movements (12, 24). LDG_1 as a motor neuron is an active constituent in two pathways, both involving the gill, respiratory movement, and the withdrawal reflex, whereas L_7 appears to be an active constituent only in one of the pathways, the reflex.

Along with the defensive gill withdrawal reflex decreasing with age (9), L_7's motor neuronal efficacy also appears to decrease. Because young *Aplysia* are subject to predatory attacks (25, 26), they depend upon L_7's ability to elicit pinnule contraction to protect the gill from possible injury. As the animals grow larger and hence older (14) and predation appears less likely (25), pinnule contraction elicited by L_7 may be evoked less frequently and thus results in decreased motor neuronal efficacy. The reduced efficacy may result from naturally occurring disuse. A recent report, which showed ultrastructural changes at a central synapse to long-term habituation in *Aplysia* (27), lends support to this proposal and to our results—that is, long-term change in synaptic activity is related to synaptic morphology. LDG_1's effectiveness may not suffer the same fate because the viability of its junctions is maintained by continual activation of respiratory movements, which remain relatively unchanged with increased age (15). Possibly there is a hierarchy of neurons with respect to their vulnerability to aging: neurons such as LDG_1 primarily mediating an invariant behavior, as are respiratory movements, are not as vulnerable to aging as are neurons such as L_7, primarily involved in the modifiable gill withdrawal reflex.

Note Added in Proof. We tested the possibility that L_7's function is decreased in old *Aplysia* because of disuse. Long-term (3 week) stimulation of the siphon/gill reflex in freely moving animals resulted in partial recovery of L_7's motor neuronal function (28).

We thank Drs. S. Feldman and J. Zolman for reading a draft of this paper and P. Campbell and T. Skinner for technical assistance. This research was supported by National Institute of Mental Health Grant 18611 (B.P.) and National Institute on Aging Grant AG 02759 (W.M. and B.P.).

1. Brizzee, K. R., Kaack, B. & Klara, P. (1975) in *Neurobiology of Aging*, eds. Ordy, J. M. & Brizzee, K. R. (Plenum, New York), pp. 463–484.
2. McGeer, E. G. & McGeer, P. L. (1975) in *Neurobiology of Aging*, eds. Ordy, J. M. & Brizzee, K. R. (Plenum, New York), pp. 287–305.
3. Tomlinson, B. E. (1977) in *Aging and Dementia*, eds. Smith, W. L. & Kinsbourne, M. (Spectrum, Jamaica, NY), pp. 25–56.
4. Scheibel, M. E. & Scheibel, A. B. (1975) in *Aging*, eds. Brody, H., Harmi, D. & Ordy, J. M. (Raven, New York), pp. 11–37.
5. Eales, N. B. (1921) *Proc. Trans. Liverpool Biol. Soc.* **35,** 183–266.
6. Kandel, E. R. (1976) in *Cellular Basis of Behavior* (Freeman, San Francisco), p. 74.
7. Kriegstein, A. R., Castellucci, V. & Kandel, E. R. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3654–3658.
8. Peretz, B. & Lukowiak, K. (1975) *J. Comp. Physiol.* **103,** 1–17.
9. Rattan, K. & Peretz, B. (1981) *J. Neurobiol.* **12,** 469–478.
10. Lukowiak, K. (1979) *Can J. Physiol. Pharmacol.* **57,** 987–997.
11. Bailey, C., Castellucci, V., Koester, J., Chen, M. & Koch, V. T. (1980) *Soc. Neurosci. Abstr.* **6,** 77.
12. Peretz, B. (1969) *Science* **166,** 1167–1172.
13. Kupfermann, I., Carew, T. & Kandel, E. (1974) *J. Neurophysiol.* **37,** 996–1019.
14. Peretz, B. & Adkins, L. (1982) *Biol. Bull.* **162,** 333–344.
15. Peretz, B., Ringham, G. & Wilson, R. (1982) *J. Neurobiol.* **13,** 141–151.
16. Jacklet, J. & Rine, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1267–1271.
17. Mallart, A. & Martin, A. R. (1967) *J. Physiol. (London)* **193,** 679–694.
18. Orkand, P. & Orkand, R. (1975) *J. Neurobiol.* **6,** 531–548.
19. Weiss, K. R., Cohen, J. & Kupfermann, I. (1975) *Brain Res.* **99,** 381–386.
20. Magleby, K. L. (1973) *J. Physiol. (London)* **234,** 327–352.
21. Atwood, H. L. (1976) *Prog. Neurobiol. (Oxford)* **7,** 291–391.
22. Lang, F. & Atwood, H. L. (1973) *Am. Zool.* **13,** 337–356.
23. Gutman, E. (1977) in *Handbook of the Biology of Aging*, eds. Finch, C. & Hayflick, L. (Van Nostrand, New York), pp. 445–469.
24. Byrne, J. & Koester, J. (1978) *Brain Res.* **143,** 87–105.
25. Winkler, L. R. & Tilton, B. E. (1962) *Pac. Sci.* **16,** 286–290.
26. Kandel, E. R. (1979) in *Behavioral Biology of Aplysia* (Freeman, San Francisco), pp. 272–274.
27. Bailey, C. H. & Chen, M. (1983) *Science* **220,** 91–93.
28. Zolman, J. F. & Peretz, B. (1984) *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, in press (abstr.).