J. exp. Biol. 108, 163–177 (1984)
Printed in Great Britain © The Company of Biologists Limited 1984

AXON SURFACE INFOLDING AND AXON SIZE CAN BE QUANTITATIVELY RELATED IN GASTROPOD MOLLUSCS

BY ROGER D. LONGLEY*

Department of Biology, University of Oregon, Eugene, Oregon 97403, U.S.A. and *Department of Biology, University of California, Los Angeles, California 90024, U.S.A.*

Accepted 4 May 1983

SUMMARY

A quantitative estimate of infolding and of its effect on electrical characteristics in axons of different sizes is given by expressions based on the allometric equation, $y = \alpha x^{\beta}$. These expressions can be used to describe the effect of stretching the axon and the size dependence of the axo-somatic conductance ratio.

The parameters in the allometric equation are determined for a range of axon sizes in three species of opisthobranchs from original and from published data. Analysis of variance for these data suggests that interaxonal variability of infolding is less for axons of an identifiable neurone from different specimens of the same species than for axons of different neurones from the same specimen. With the exception of the *Aplysia* R2 axon where the infolding size dependence may be less, the ratio of axon volume to surface in these neurones is given approximately by the equivalent axon diameter to the one-half power. For this relation of infolding to size (and if it is assumed that infolding in the soma varies with size in the same way as in the axon), the axo-somatic conductance ratio is inversely proportional to soma diameter to the three-fourths power.

These results suggest that conduction velocity increase with size is small, and because of the penalty in the concomitant increase in axon surface membrane with size, selection for larger axons probably does not occur to satisfy a requirement of decreased response time.

INTRODUCTION

In certain species of gastropod molluscs, neurone DNA content (Bezruchko *et al.* 1969; Coggeshall, Yaksta & Swartz, 1970; Lasek & Dower, 1971) and size (Arvanitaki & Tchou, 1942) continue to increase during adult life. The axon membranes of those neurones in which growth persists develop a complex morphology with elaborate invaginations. These surface indentations, which vary in amount for different neurones, affect synaptic integration and axon electrical properties (Mirolli & Talbott, 1972; Gorman & Mirolli, 1972).

* Present address: Pacific Sciences Institute, P.O. Box 835, Friday Harbor, WA 98250, U.S.A.
Key words: Axon infolding, axon size, gastropod, axon conduction velocity.

For axons of irregular shape, the ratio of volume to surface area is the geometric variable of interest; it has been suggested that this ratio should be independent of changes in axon length if the axolemma is not deformable at the ultrastructural level (Hodgkin, 1954). This analysis has been used to explain the independence of conduction velocity (and, more generally, of length constant) on stretching in infolded axons. Mirolli & Talbott (1972) measured volume and surface area for G-cell axons in the nudibranch *Anisodoris nobilis* and developed equations to relate these measurements to the axon passive electrical characteristics. They estimated axon volume and surface area from cross-sectional area (A) and perimeter (P), respectively. Area and perimeter were combined to give a geometric factor for the length constant, $H = (A/P)^{1/2}$, and for the input conductance, $M = (AP)^{1/2}$. They concluded that, although axon area and perimeter varied widely in different nerve cross-sections, both H and M could be approximated by values averaged along the axon length, and that H remains constant when the axon is stretched, while M decreases.

Although infolding has been described in some detail for specific gastropod neurones (Mirolli & Talbott, 1972; Pinsker, Feinstein, Sawada & Coggeshall, 1976) and a correlation with size has been shown in *Aplysia* (Graubard, 1975), volume and surface have not been analytically related for axons of different sizes. This report shows that infolding can be predicted by an *empirical* formula which gives the ratio of axon volume to surface area as a function of equivalent axon diameter in the form of the allometric equation, $y = \alpha x^{\beta}$ (Huxley, 1932). Such a relation can be used to describe the size-dependent effect of infolding on the passive electrical properties of axons; infolding can be quantitatively related to axon size through the parameters α and β; but, because of the empirical nature of this relation, α and β must be determined for each axon type or group of axons considered. These parameters are estimated from measurements given here for *Tritonia* axons and from the available data for *Aplysia* and *Anisodoris* fibres (Mirolli & Talbott, 1972; Graubard, 1975; Pinsker *et al.* 1976).

MATERIALS AND METHODS

Tritonia *axon measurements*

The lateral buccal nerve (Longley, 1976) from a 40 g *Tritonia diomedia* (Bergh) obtained from East Sound in the San Juan Islands of northwest Washington State was prepared by immersing the buccal mass, with ganglia and nerves exposed, for 12 h in fixative (4% glutaraldehyde, 3% formaldehyde, and 0·2 M-NaH_2PO_4 in distilled water; pH adjusted to 7·3 with NaOH). The nerve was then dissected, postfixed for 2 h in 2% OsO_4, dehydrated in ethanol, and embedded in Epon. A silver section of the whole nerve cross-section was placed on a support film, stained with uranyl acetate and lead citrate, and photographed at a magnification of 1800 using a Philips 300 electron microscope. In a montage of the nerve enlarged 4800 times, axon area, A, was measured with a planimeter and by weighing axon tracing cutouts, while the perimeter, P, of each axon was measured with a measuring wheel. These measurements were repeated at least two times. Uncertainty in measurements of axon cross-sections did not seem to be an important source of variation, since changes in area and perimeter with repeated measurements of axon tracings were small compared to the

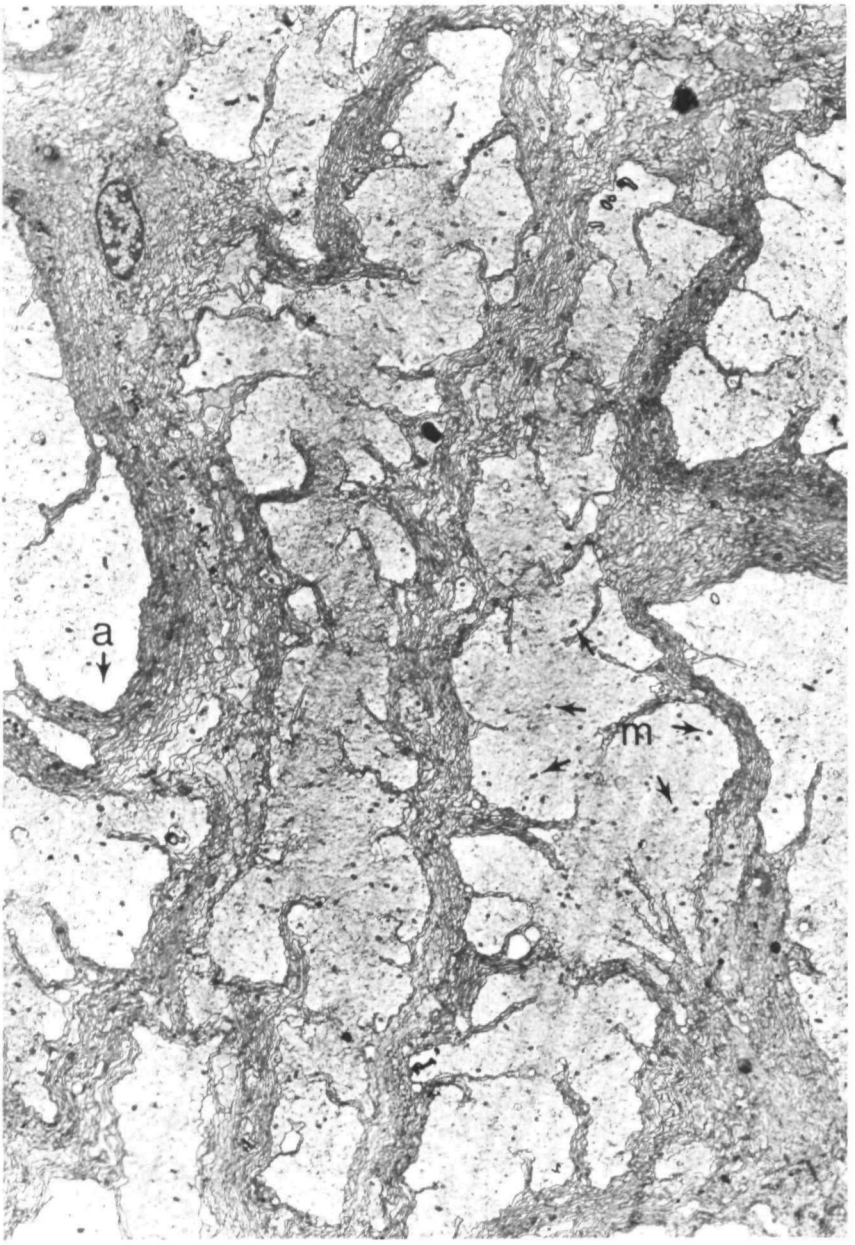

Fig. 1. Electron microphotograph of a cross-section of the lateral buccal nerve of *Tritonia*. In some of the larger axons a fixation artifact (a) can be recognized as a low density region of the axoplasm near the axolemma, but this involved only a small percentage of axon area. Mitochondria (m), ranging in number from 6–177 in the 23 largest axons in the cross-section of this nerve (see Fig. 2), were present at a density of $0{\cdot}4\,\mu m^{-2}$. The number of mitochondria was correlated (after log-transformation of variables) against axon area ($r = 0{\cdot}91$, $N = 23$) and perimeter ($r = 0{\cdot}90$, $N = 23$). The slope of a line fitted to the transformed data by the least squares method estimates the exponent in a parametric equation of the form $y = \alpha x^{\beta}$ relating the number of mitochondria to either axon area or perimeter. This exponent would be expected to be one if the number of mitochondria was directly proportional to either of these variables. For the number of mitochondria correlated to axon perimeter, the exponent is 0·916 and is not significantly different from 1·0 ($t = 0{\cdot}90$, $P > 0{\cdot}2$). When correlated to axon area, the exponent (= 0·806) is significantly less than 1·0 ($t = 2{\cdot}55$, $P < 0{\cdot}02$). (a) fixation artifact; (m) mitochondria; magnification: ×3000.

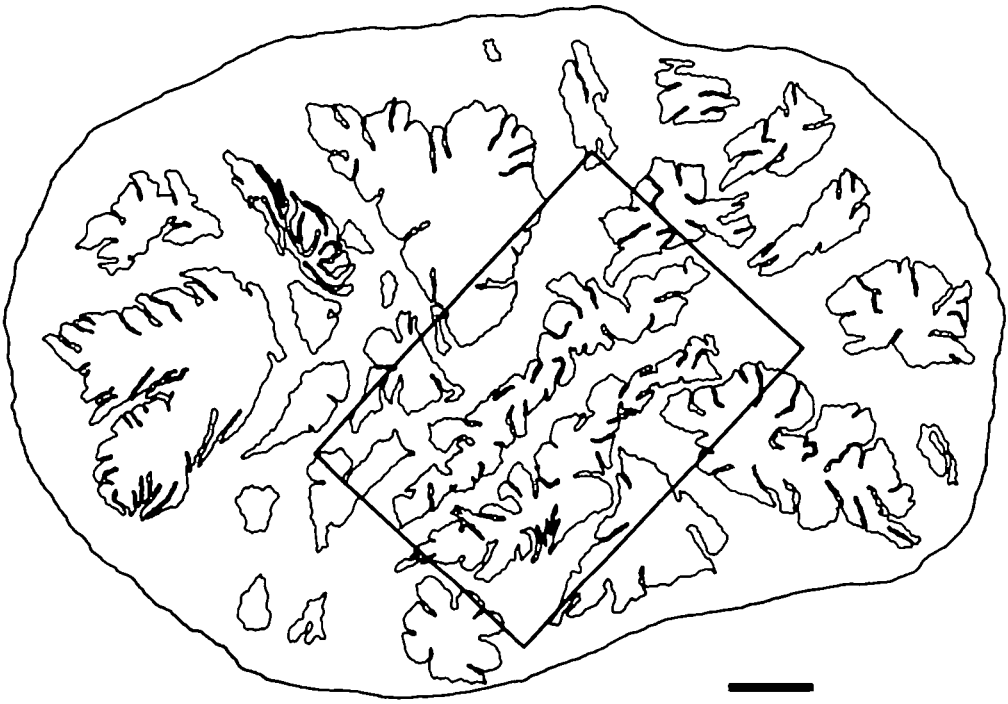

Fig. 2. Tracing of axonal profiles from a cross-section of a lateral buccal nerve of *Tritonia*. The 26 largest axons in a single cross-section of this nerve are outlined from a montage of electron microphotographs. The central plate of this montage is shown in Fig. 1. Scale bar: 10 μm.

interaxonal scatter of the data. No correction was made for artifacts introduced by fixation and handling of the tissue (see Fig. 1).

Aplysia *and* Anisodoris *data conversion*

Data on infolding analysed here from other papers were obtained from graphs (Mirolli & Talbott, 1972; Graubard, 1975) and from tables and figures of axon cross-sections (Pinsker *et al.* 1976). When necessary, these materials were enlarged photographically to increase accuracy in translating the results of these authors to axon area and perimeter for comparison to results obtained from *Tritonia* (Fig. 3). Data for axons of the *Aplysia* right abdominal connective (Fig. 4A and filled squares in Fig. 4B) are from Graubard (1975). For the *Aplysia* R1 and R2 axons (Fig. 4B, open and filled inverted triangles), the data are from Pinsker *et al.* (1976). Area and perimeter values of the G-cell axon of *Anisodoris* used in plotting Figs 5 and 6 are taken from Mirolli & Talbott (1972).

Statistical methods

The constants a and b (in equation 1) required to solve the allometric equation were estimated by fitting a straight line with the least squares method to logarithmic transformations of the data. The validity of this straight line model was tested using linear regression statistics.

Biological variability in interaxonal results is estimated from the residual mean

squares of the infolding variable about the regression line. By using logarithmic transformations of the data, the size dependence of the variance is removed (Lewontin, 1966). In Figs 3A and 4A, values of log$U$ are symmetrically located about the regression lines and tend to form a normal distribution (D'Agostino's test, $P > 0{\cdot}2$). On this basis, the F-test is used in comparing the residual mean squares of log$U$ in different axon groups.

Different techniques and procedures used by the different authors also affect the variability of the data. Variations may occur in shrinkage and distortion because of different histological techniques. If swelling or shrinkage occurs uniformly throughout the tissue without changing axon perimeter, the exponent b in equation (1) will not be affected, but the constant a will be changed by the factor f^{2-b}, where f is the fractional change in the dimensions of the tissue. Such a uniform change in size would not affect the variability of the results for axons in a single nerve, but where axons in several nerves are compared, differences in f may increase the variance of the data. The extent of non-linear distortion, which could change both b and a and also increase the variance of the data, cannot be estimated except from the overall consistency of the results.

Where an identified axon is measured in more than one nerve, differences in the amount of stretching of the nerves before fixation will cause variations in the size of the axons (see Appendix). For the G-cell axons stretched over a range of 1·5–1·8 times, as reported by Mirolli & Talbott (1972), possible effects on the variance of the data are negligible. In R1 and R2 of *Aplysia*, where stretching of the nerve was based on an electrophysiological criterion (Pinsker *et al.* 1976), the apparent interaxonal variability of infolding (in different nerves) may have been increased by different amounts of stretching.

RESULTS

Analytical relation of axon size to infolding

In describing the size of invaginated axons, it is convenient to use an equivalent diameter, $D = (4A/\pi)^{1/2}$, previously defined by Mirolli & Talbott (1972), where A is the axon cross-sectional area. A ratio, U, of volume to surface area is estimated from cross-sections as $U = A/P$, where P is the axon perimeter. U is related to D in the allometric equation by

$$U = aD^b. \tag{1}$$

This equation is limited in range to values of $D \geqslant 4U$, since U cannot exceed the ratio of volume to surface area of a cylindrical axon where $a = 1/4$ and $b = 1$.

A plot of the equivalent axon diameter and ratio of volume to surface area used in the above equation shows the effect of infolding, but the importance of this relation is in defining the parameters a and b. When a and b are known, H, M, and other infolding variables (see Appendix) can also be expressed as a power function of D. If the least squares method is used in the calculation of a and b in this power function, the choice of variable is arbitrary. However, U, the variable used here, retains the magnitude of the biological variability in measurements of surface area and perimeter to a greater extent than other possible variables and is less misleading in its relation

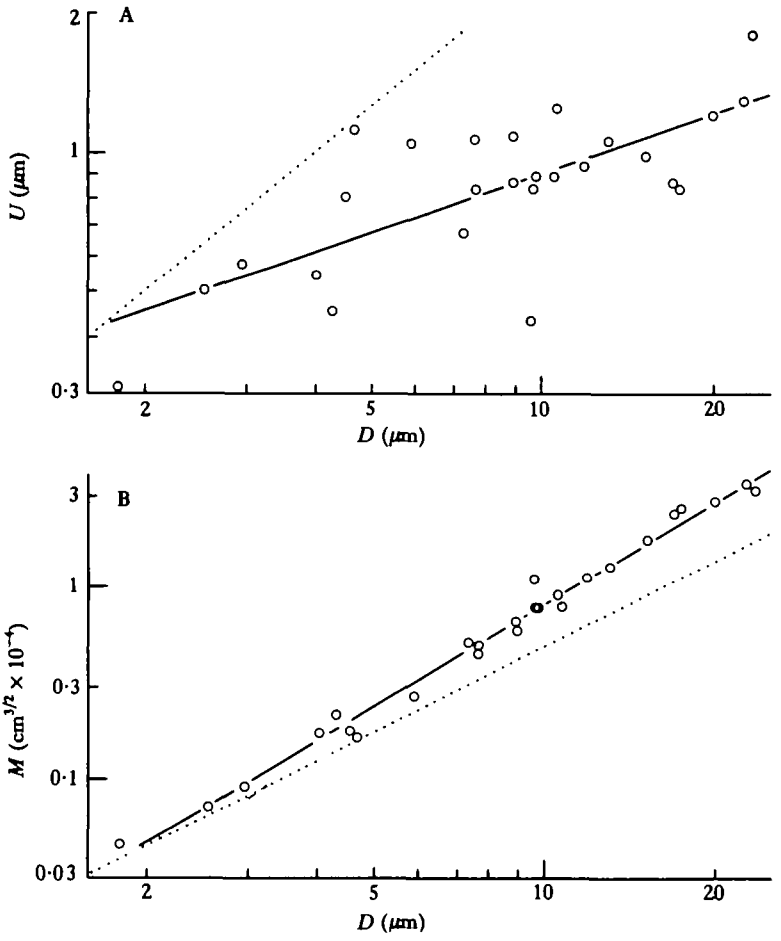

Fig. 3. Infolding variables for *Tritonia* axons outlined in Fig. 2. (A) Correlation of U, the ratio of axon cross-sectional area to axon perimeter, *vs* the equivalent axon diameter, D. (B) M, the geometric axon conductance factor, *vs* D for the same axons as in (A). Lines through data points are fitted by least squares. Cylindrical axons with no infolding have the maximum possible values of U and the minimum values of M; these limiting values are indicated by dotted lines in (A) and (B). The extent of infolding is shown by the departure of the data from these dotted lines.

to D when presented graphically (e.g., compare the scatter of *Tritonia* data for U in Fig. 3A and M in Fig. 3B).

Comparison of infolded axons in different species

Tritonia *buccal nerve*

The buccal nerve axons outlined in Fig. 2 are primarily from monopolar neurones and one pair of bipolar neurones whose somata are found in the buccal ganglion of *Tritonia*, but one or two branches of the giant cerebral neurones are also present (Longley, 1976). The number of mitochondria in these infolded axons tends to be proportional to axon perimeter but not to axon area (see Fig. 1). Values of U for these axons are plotted in Fig. 3A against their equivalent diameters, and estimates of the

parameters of the regression line through the log-transform of these data are given in Table 1. For the value $b = 0{\cdot}42$ of the exponent in equation (1), from the slope of this line, the geometric factor for the axon length constant H ($= U^{1/2}$) would vary as $D^{0{\cdot}21}$ instead of as $D^{0{\cdot}5}$ in cylindrical axons with no infolding. The fit achieved with a power function of D to the geometric factor for axon conductance (M) is shown in Fig. 3B.

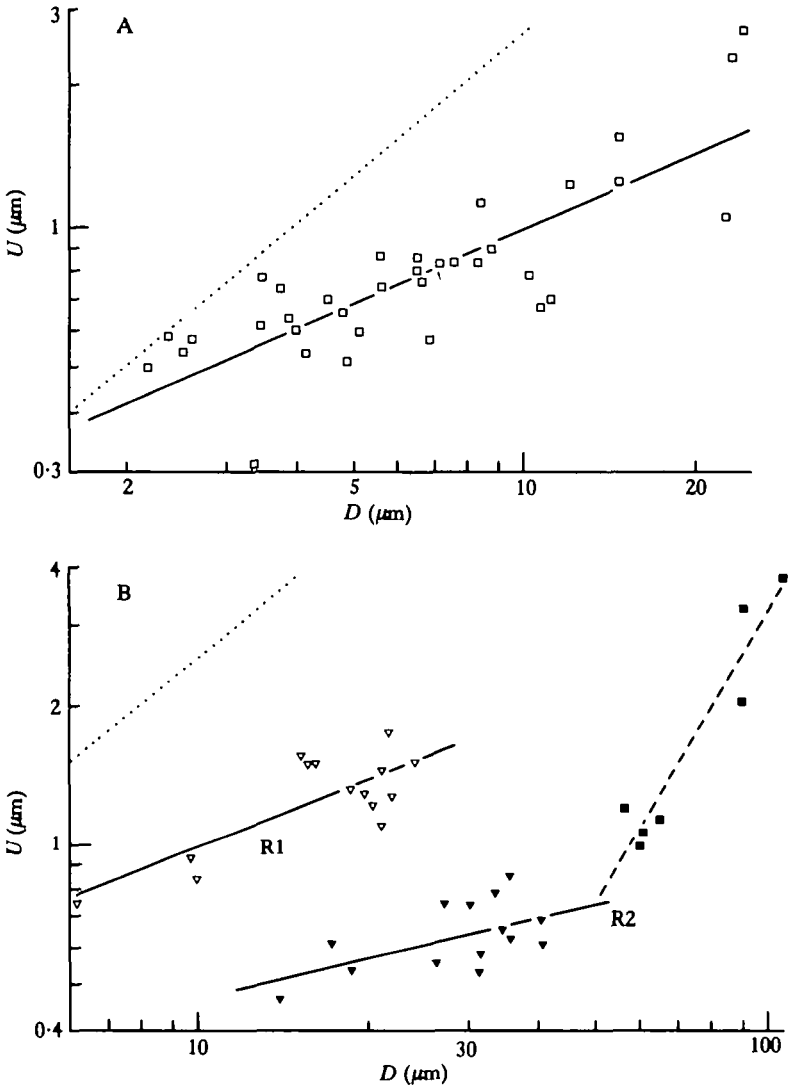

Fig. 4. Infolding in axons of *Aplysia californica* abdominal ganglion right connective. (A) Correlation of U *vs* D from light microscope data of Graubard (1975). Values of area (A) and perimeter (P) were calculated from F (relative infolding factor, see Appendix) and D in Graubard's Fig. 1D using $A = \pi D^2/4$ and $P = \pi DF$; U is given by the ratio A/P. (B) Correlation of U *vs* D for R1 (▽) and R2 (▼) from Pinsker, Feinstein, Sawada & Coggeshall (1976) using their values of A and P averaged from electron microphotographs of three cross-sections along the connective. Relative values of centimetres and grams for P and A in their Table 2 have been converted to length and area by using measurements from the representative axons in their Fig. 5. Conversion factors used were $1{\cdot}17\ \mu m\ cm^{-1}$ and $58{\cdot}2\ \mu m^2\ g^{-1}$. Values of U in (B) for larger R2 axons (■, dashed line) are from Graubard (1975). Dotted lines represent cylindrical axons.

M was calculated from area and perimeter of the axons outlined in Fig. 2 using $M = (AP)^{1/2}$. The line through these points (Fig. 3B) can either be drawn by using the values of a and b in Table 1 for these axons and the expression for M in the Appendix or calculated directly from log$M$ and log$D$ using least squares.

Aplysia *abdominal ganglion connective*

Axon infolding in the right abdominal ganglion connective of *Aplysia californica* has been examined by Graubard (1975). Values of U for axons with $D < 25\ \mu m$ (a size range comparable to the *Tritonia* buccal nerve axons) are shown in Fig. 4A. In these axons, neither of the estimated parameters a and b (Table 1) differ significantly ($P > 0{\cdot}2$) from those found for *Tritonia* axons. Infolding in the two largest axons in this connective from neurones R1 and R2 has been described by Pinsker *et al.* (1976). Measurements were made by these authors on both R1 and R2 axons, in sections cut from 14 nerves taken from animals ranging in size from 5 to 300 g. The regression line for their R1 axon data (Fig. 4B, open inverted triangles) overlies the results for the above groups of *Tritonia* and *Aplysia* axons (see estimated parameters in Table 1).

The R2 axons of smaller diameter in Fig. 4B (filled inverted triangles) were shown by Pinsker *et al.* (1976) to have relatively greater infolding than R1 axons from the same connectives. This finding is illustrated quantitatively in Fig. 4B and Table 1 as a displacement to a lower elevation and slope of the regression line for R2 data relative to results obtained for R1. Measurements on larger R2 axons in *Aplysia* (Fig. 4B, filled squares) from Graubard (1975) have an entirely different slope, with a value of b near 2. If $b = 2$, the perimeter of the axon remains constant as its equivalent diameter, D, increases and the relative infolding factor, F, varies inversely with D (see equations for P and F in Appendix). This is not consistent with any of the other results presented here and indicates in this axon a marked change in growth relations for infolding in larger animals. Changes in growth of *Aplysia* abdominal ganglion neurones with animal size have been reported previously (Arvanitaki & Tchou, 1942), and a decrease in efficacy with age has also been reported in an *Aplysia* abdominal ganglion neurone (Peretz, Ringham & Wilson, 1982). The above results suggest an age-related infolding change in R2 axons.

Table 1. *Estimated infolding parameters of different axon groups for the axon volume to surface ratio given by* $U = aD^b$

Data source	a*	$b \pm$ S.E. (N)	r**
Tritonia buccal nerve, Fig. 3A[a]	0·34	0·42 ± 0·08 (26)	0·74
Aplysia connective, Fig. 4A[b]	0·29	0·53 ± 0·07 (35)	0·81
R1 axon, Fig. 4B[c]	0·31	0·50 ± 0·11 (14)	0·79
R2 axon, Fig. 4B[c]	0·24	0·30 ± 0·12 (14)	0·57
G-cell axon, unstretched, Fig. 6A[d]	0·10	0·59 ± 0·18 (8)	0·80
G-cell axon, stretched, Fig. 6A[d]	0·16	0·46 ± 0·14 (8)	0·80

* Values of a are for the equivalent axon diameter D in microns, i.e. a is the value of U for an equivalent axon diameter of 1 μm.

** The correlation coefficient r is significant in each case.

[a] Present study; [b] Graubard, 1975; [c] Pinsker, Feinstein, Sawada & Coggeshall, 1976; [d] Mirolli & Talbott, 72.

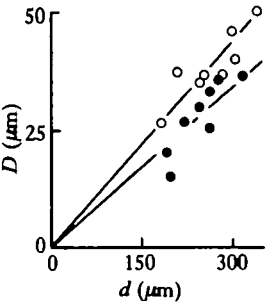

Fig. 5. Correlation of axon (D) and soma (d) equivalent diameters of *Anisodoris nobilis* G-cell axons, from light microscope data of Mirolli & Talbott (1972). In their Figs 5 and 6, these authors correlate averaged values of H and M with d, a measure of soma size. Using the relations $A = HM$ and $P = M/H$, A and P can be calculated for an axon corresponding to a particular soma of size d. The equivalent axon diameter, D, is obtained from the area, A, and plotted against d. The regression lines through the origin have for unstretched axons (○) a slope of $k = 0{\cdot}146$, ±S.E. 0·005 ($N = 8$) and for stretched axons (●) a slope of $k = 0{\cdot}116$, ±S.E. 0·006 ($N = 8$).

G-cell axon in Anisodoris

The data presented on infolding in the *Anisodoris* G-cell axon by Mirolli & Talbott (1972) are unique in that these authors examined histologically the effect of stretching on an identifiable fibre. It is possible from these data to show a proportionality between axon size (D) and their estimate of G-cell soma size (d), so that $D = kd$. In Fig. 5, this proportionality constant, k, is calculated for both the unstretched and stretched axons by regression through the origin. Stretching of the axons leads to a reduction in k.

The data of Mirolli & Talbott (1972) on the G-cell for both unstretched and stretched axons (Fig. 6A, open and filled circles, respectively) give values of the exponent in equation (1) which are similar to results from other axon groups shown in Table 1. For the regression line through values of $\log U$, stretching should produce a shift with constant slope towards smaller D and an increase in the elevation, a, consistent with that shown in Fig. 6A, although the value of U for a particular axon should not change with stretch (see Appendix). Even though the exponent b of the G-cell axons is similar to that for other axons in the *Tritonia* buccal nerve and the *Aplysia* connective, the elevation of the regression line is lower, placing this line near the R2 results from Pinsker *et al.* (1976). For the other four groups of *Aplysia* and *Tritonia* axons in Table 1, the mean value of a is 0·30 with a range of 0·24–0·34, but for the unstretched G-cell axons, $a = 0{\cdot}10$. This difference may indicate relatively greater shrinkage of this axon in histological procedures, which could reduce a and leave b unchanged, or it may represent a real difference in the G-cell in this size range.

Mirolli & Talbott (1972) also give measurements on infolding along the length of a single G-cell axon. A correlation between U and D based on their results is shown in Fig. 6B. Although equation (1) is used to relate U and D for these data where changes in cross-sectional area and perimeter are occurring along the length of a single axon, the parameters a and b in this case are not comparable to results from other axons in Table 1, where a and b are derived from an interaxonal correlation (i.e. the average of the values of $\log U$ and $\log D$ in Fig. 6B would appear as one data point in Fig. 6A). A strong correlation is present between U and D measured at different

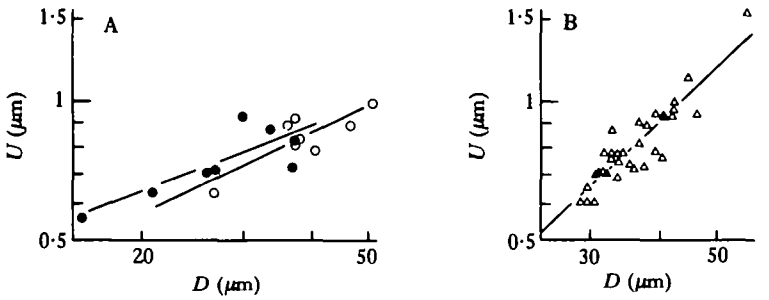

Fig. 6. *U vs D* in the G-cell axon of *Anisodoris nobilis*. In (A) *U* and *D* are correlated for eight unstretched (○) and eight stretched (●) axons. Each data point is the average of 10 cross-sections measured using the light microscope. Data are from Figs 5 and 6 of Mirolli & Talbott (1972); see Fig. 5, this paper, for method of conversion of data to *A* and *P*. (B) Intra-axonal correlation of *U vs D* ($r = 0{\cdot}90$, $N = 33$) along a 5 mm segment of a single G-cell axon at a distance 6·5–11 mm from the G-cell soma (from light microscope data in Figs 3 and 4 of Mirolli & Talbott, 1972). Measurements of *D*, *H* and *M* at each axon cross-section were used to calculate *U* and *D*. If the outlying value of log*U* (more than three standard deviations greater than the mean) is omitted, $r = 0{\cdot}85$.

points along the length of this axon, even though H ($= U^{1/2}$) was previously thought to be constant for these data (Mirolli & Talbott, 1972). Estimates of the parameters are $a = 0{\cdot}013$ and $b = 1{\cdot}15$, ±S.E. 0·10 (33). If b is close to one, as in this case, the axon perimeter tends to be proportional to the equivalent diameter, but the relative amount of infolding along the axon is independent of size variations (see expressions for P and F in Appendix). The exponent b for this intra-axonal correlation is significantly greater than that for other axons analysed as a group ($P < 0{\cdot}001$). This result for the size dependence of infolding along this axon suggests that local control of axon surface differs from regulation of the average volume to surface ratio in the axon during growth.

Biological variability of infolding

The principal variation of *U* in different axons results from its dependence on axon size, as shown in Figs 3A, 4 and 6A, but differences may also exist in the variance of *U* in neurones of the same size. This latter possibility, which can be examined with data assembled here, would suggest an intrinsic difference in the infolding mechanism in these neurones. Results for the interaxonal variation of log*U* of axon groups whose size dependence has been shown above are given in Table 2 as the residual mean squares.

The residual mean squares also contains the intra-axonal variance, which is a major source of uncertainty in estimating the variance of log*U* between axons. In the case of the G-cell axon (Fig. 6B), the intra-axonal variance of log*U* can be calculated and is shown in Table 2 for comparison to the residual mean squares of axon groups. However, this intra-axonal variance would not add directly to the variance of log*U* between axons because of the intra-axonal correlation of log*U* to log*D*. The values of log*U* from intra-axonal sampling in this axon would lie in an ellipse with a major axis slope of about 1·0. When the variance of these values is projected on the residual mean squares of a regression line through interaxonal data with a slope of 0·5, the contribution of this intra-axonal variance will be reduced eight times over that which

Table 2. *Size independent component of variance for the ratio of axon volume to axon surface,* U

Data source	Number of axons	Sections measured per axon	Residual mean squares*
Single G-cell axon, Fig. 6B[a]	1	33	0·00707**
G-cell axon, unstretched, Fig. 6A[a]	8	10	0·00153
G-cell axon, stretched, Fig. 6A[a]	8	10	0·00225
R2 axon, Fig. 4B[b]	14	3	0·00403
R1 axon, Fig. 4B[b]	14	3	0·00479
Aplysia connective, Fig. 4A[c]	35	1	0·01193
Tritonia buccal nerve, Fig. 3A[d]	26	1	0·01373

* The residual mean squares of axon groups was calculated from the regression line of log$U$ *vs* log$D$.

** The intra-axonal variance of log$U$ was calculated directly from measurements on a single axon. This variance decreases to 0·00464 ($N = 32$) if the outlying value of log$U$ (3·4 standard deviations greater than the mean) is dropped.

[a] Mirolli & Talbott, 1972; [b] Pinsker, Feinstein, Sawada & Coggeshall, 1976; [c] Graubard, 1975; [d] present study.

would be present if intra-axonal values of log$U$ were independent of axon size. For the two groups of G-cell axons, the intra-axonal variance present in the residual mean squares is further reduced an additional tenfold by averaging, and the residual mean squares shown in Table 2 for these axons should be almost entirely free of intra-axonal sampling effects. In other neurones, if the magnitude of the intra-axonal variation of log$U$ and correlation of log$U$ to log$D$ is similar to that shown for the G-cell in Fig. 6B, then intra-axonal variability will add less than 8% to the residual mean squares of other groups of axons shown in Table 2, even in those cases where there is no averaging.

Although the residual mean squares of the stretched G-cell axon group and the R1 and R2 axon groups are larger than that of the unstretched G-cell axons, these differences are not significant [for the unstretched G-cell axons and R1 axons, $F(13,7) = 3{\cdot}13$; $0{\cdot}1 > P > 0{\cdot}05$, one tailed test]. The residual mean squares from these identifiable neurone groups are lower than those for the axon groups from different neurones in the *Aplysia* connective and the *Tritonia* buccal nerve. When tested between the R1 and *Aplysia* connective groups where the values are closest, this difference is significant [$F(34,13) = 2{\cdot}49$; $P < 0{\cdot}05$, one tailed test]. Overall, these results suggest that the observed variability in U, after removal of size dependence, does not arise from random sampling along the axon, but is due to differences between individual neurones. This variability is greater in a group of axons from different neurones than in axons from an identifiable neurone.

Size dependence of axo-somatic conductance ratio

With empirical expressions for axon infolding, a quantitative estimate of the size dependence of the axo-somatic conductance ratio, ϱ, can be made. This passive electrical parameter is also affected by infolding changes with size in the soma surface, and, although soma infolding has only been reported for the G-cell in *Anisodoris* (Mirolli & Talbott, 1972), it is possible to make reasonable estimates for soma infolding which place limits on ϱ. From Mirolli & Talbott (1972), the axo-somatic

conductance ratio can be expressed analytically as

$$\varrho = (M/S)(R_m/R_i)^{1/2} \tag{2}$$

where M is the geometric factor for axon conductance, S is the unfolded soma surface area, R_m is the specific membrane resistance, assumed to be the same in both axon and soma, and R_i is the axoplasmic volume resistivity. If the axon does not taper and is much longer than its length constant, the size dependence of M is given by its relation to D (see Appendix). It should be noted that, unlike U, M is affected by stretching the axon, and ϱ can be expected to vary inversely with stretch (Mirolli, 1976). Infolding in the soma membrane can be approximated by using the same fractional amount of infolding as predicted for the axon (although other assumptions are possible). Soma surface is then

$$S = F\pi d^2 \tag{3}$$

where F is the axon infolding factor (expressed in terms of D in the Appendix) and d is a measure of soma size. A simple proportionality over a limited size range is assumed ($D = kd$) between axonal equivalent diameter D and soma size d, similar to that found for unstretched axons of the G-cell in *Anisodoris* (Fig. 5). Using this expression for D and substituting in (2) with S from (3) and M from the Appendix, one obtains:

$$\varrho = \mathrm{k}^{1+b/2}\mathrm{a}^{1/2}\mathrm{d}^{-1+b/2}(R_m/R_i)^{1/2}. \tag{4}$$

The use of a power function for the size dependence of infolding yields a simpler equation for ϱ than would result if a linear relation between size and M were assumed, as in Mirolli & Talbott (1972). This equation contains the spherical-soma-cylindrical-axon model ($a = 1/4$, $b = 1$) where ϱ varies as $d^{-1/2}$. When b is less than one, because of axon infolding, ϱ will decrease more rapidly as neurone size increases, i.e. typically about as $d^{-3/4}$ for $b = 1/2$. If soma infolding were not to change with size, F would be replaced with a constant in (3), and the axo-somatic conductance ratio would be more strongly dependent on k than shown in equation (4), but its size dependence would be decreased to $d^{-b/2}$.

Values for the calculation of the axo-somatic conductance ratio using equation (4) are available for the G-cell. For the unstretched axon, using μm as the length unit, $k = 0{\cdot}15$, $a = 0{\cdot}10$ and $b = 0{\cdot}59$. From Gorman & Mirolli (1972), R_m and R_i are $10^6\,\Omega\mathrm{cm}^2$ and $10^2\,\Omega\mathrm{cm}$, respectively. For soma diameters varying from 133–333 μm (corresponding to axon diameters from 20–50 μm), ϱ from equation (4) decreases from 8·6 to 4·5. If infolding in the G-cell soma has a constant value of 7·5, independent of size (Gorman & Mirolli, 1972), ϱ over the above size range would vary from 9·8 to 7·5. These values are in the range given by Gorman & Mirolli (1972) for their anatomical estimates of the axo-somatic conductance ratio in the G-cell.

DISCUSSION

Where infolding can be described by the allometric equation, there must be a mechanism which maintains the ratio of increments in volume and surface in proportion to the axon volume/surface ratio as the axons increase in size, even though the axons may be growing at different rates to reach their various sizes (Rosen, 1967). The

variability of the data suggests that there are differences in this process and that a range of parameter sets may be required to describe infolding in different neurone types. In the neurones considered here, infolding variability is less for axons from an identifiable neurone in different animals than it is for axons from neurone groups in the same animal. This suggests that the developmental history of the neurones is a more important factor in regulating infolding during growth than the identical genetic endowment of neurones from one individual. However, with the observed variability, it is still feasible to specify the parameters a and b in equation (1) both for axons from identified neurones at different growth stages and for axons of different sizes from the same animal. This demonstrates that the allometric equation, with its interpretative significance (Savageau, 1979), can be used to describe axon infolding, and the parameters of this equation, when determined empirically for a group of axons, can be used to predict the size dependence of geometric factors of axon passive electrical characteristics. The range of these parameters for other infolded axon groups in opisthobranch and pulmonate species remains to be investigated.

Increasing axon size is generally correlated with an increase in axon spike velocity, but the constants associated with this relation vary widely in different species (Bullock & Horridge, 1965). In the squid giant axon, spike velocity was found to be proportional to the square root of axon diameter (Pumphrey & Young, 1938), although a linear relation has also been reported (Hodes, 1953). The square root relation is predicted analytically (Hodgkin, 1954) and is widely accepted for spike velocity in unmyelinated cylindrical axons. In axons with infolding, the geometric factor for spike velocity is not the diameter but the square root of the axon volume to surface ratio, or H, as developed by Mirolli & Talbott (1972). In spike velocity comparisons in cases where there is grossly different infolding (axons of R1 and R2 in the *Aplysia* abdominal ganglion), the ability of H to predict relative spike velocity was verified, although other factors may be present since agreement between morphological and electrophysiological measurements was not exact (Pinsker *et al.* 1976). Using H as a correlate, spike velocity for the infolded axons described here can be expected to increase about as the fourth root of their equivalent diameter, although there may be exceptions, such as the R2 axon, where the size dependence is much less. In order to double spike velocity, it would be necessary to increase the perimeter 64 times and the cross-sectional area 256 times in these axons. This enormous increase in membrane required for infolding would seem to rule out selection of larger axons solely to obtain an increased spike velocity.

Infolded membrane may have a role in facilitating stretching of the larger axons in these gastropod species, but it may also offer an advantage of increased oxygen supply, since the number of mitochondria in the *Tritonia* buccal nerve axons is correlated with this disproportionate axon surface increase with size. Such a nutritive role for infolding has been repeatedly proposed (Pinsker *et al.* 1976). The increasing size of these axons during the animal's growth may be associated with an increased transport of proteins and smaller molecules from the neurone soma to the periphery. This possibility is supported by continuing DNA synthesis, which may increase up to 75 000 times the haploid amount of DNA in the *Aplysia* R2 neurone (Coggeshall *et al.* 1970).

It is striking that axons from three different species evolving in different environments have parameters for infolding which are very similar. Membrane infolding may

occur in an axon in a manner analogous to that proposed for formation of convolutions in the vertebrate brain where growth is limited by the skull (Clark, 1945). In such a model axon volume increases would be constrained by the nerve sheath, and excess axolemma production would result in infolding. Thus it is possible that determining the parameters for infolding, in addition to being useful in predicting size related electrical characteristics of these gastropod axons, will also allow separation of neurones having different metabolic activity.

APPENDIX

Other infolding variables

In addition to U given by equation (1), other ratios and products of area and perimeter (A and P) which are useful in describing the passive electrical properties of the invaginated axon may be derived as a power function of the equivalent axon diameter, D. From $U = A/P = aD^b$ and $D = (4A/\pi)^{1/2}$, the expression for the axon perimeter is:

$$P = (\pi/4)a^{-1}D^{2-b}.$$

Using the nomenclature of Mirolli & Talbott (1972), the geometric factor for the length constant, or spike velocity, of the infolded axon is:

$$H = U^{1/2} = a^{1/2}D^{b/2},$$

and the geometric factor for axon conductance is

$$M = PU^{1/2} = (\pi/4)a^{-1/2}D^{2-b/2}.$$

In an invaginated axon where $P > \pi D$, a dimensionless infolding factor (Graubard, 1975) can be defined as:

$$F = P/(\pi D) = (1/4)a^{-1}D^{1-b}.$$

Stretching of infolded axons

The effect of stretching on an idealized infolded axon can be determined from geometric considerations. For an axon with constant volume and surface which undergoes uniform unfolding throughout its invaginated membrane when stretched, both the cross-sectional area and perimeter will be reduced by s, where s is the factor by which axon length is increased. This results in a U independent of s (Hodgkin, 1954), but D, from its dependence on area, will be divided by $s^{1/2}$. In this model for axons after stretching, U would remain the same for each axon in the nerve, but U as a function of D would be shifted to smaller D. If the same stretch factor s applies to all axons in the nerve, the exponent b in equation (1) will not change, but if the stretched axons are to be described by this equation with U constant and D divided by $s^{1/2}$, a must be replaced by

$$a' = as^{b/2},$$

where $a' = a$ when the axon is at reference length.

The expected displacement of the regression line of U for the stretched *Anisodoris*

G-cell axons (Fig. 6A) can be calculated from the effect of axon stretching on D. There is a proportionality between axon and soma size in this neurone (Fig. 5) and since soma size should be unaffected by stretching the axon, the amount of stretching can be estimated from the change in the proportionality constant k (from $D = kd$) through the inverse relation of D to $s^{1/2}$. This leads to $s = (k/k')^2$, where k' is the constant for the stretched axons. For these data in Fig. 5, $s = 1{\cdot}58$, in agreement with the range of stretch values of 1·5–1·8 times indicated by Mirolli & Talbott (1972). From this result the values of U of the stretched axons in Fig. 6A would be expected to be shifted to a smaller D by the factor $s^{1/2}$, or 1·26. The expected increase in the elevation of the regression line for these stretched axons is 13 %, calculated from the expression for a' above.

The effect of axon stretch on other infolding variables above can be determined directly from the stretch related change expected in area and perimeter or by substituting into these equations modified values of a and D as indicated above. The length constant H should be independent of axon stretching, as is U, but P and M should be inversely proportional to s, and F inversely proportional to $s^{1/2}$.

I thank Drs Douglas Junge, Bill Moody, and Kathy Graubard for helpful criticisms of earlier versions of this manuscript and the anonymous referee for numerous helpful comments, many of which were included in the manuscript. I also thank Dr Alan Grinnell, director of the Jerry Lewis Center for Neuromuscular Research, and Dr Dennis Willows, director of Friday Harbor Laboratories, for use of facilities during the preparation of this paper. This work was supported in part by NIH Special Research Fellowship 5 F03 GM45283.

REFERENCES

ARVANITAKI, A. & TCHOU, S. H. (1942). Les lois de la croissance relative individuelle des cellules nerveuses chez l'Aplysie. *Bull. Histol. appl. Physiol. Path.* **19**, 244–256.

BEZRUCHKO, S. M., VOZHENINA, N. I., GAZARYAN, K. G., KULMINSKAYA, A. S. & KUKHTIN, V. A. (1969). Autoradiographic investigation of the synthesis of DNA in giant neurones of *Tritonia diomedia*. *Biophysics* **14**, 1107–1111.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous Systems of Invertebrates*, Vol. I. San Francisco and London: W. H. Freeman.

CLARK, W. E. LE G. (1945). Deformation patterns in the cerebral cortex. In *Essays on Growth and Form*, (eds W. E. le Gros Clark & P. B. Medawar), pp. 1–22. Oxford: Clarendon Press.

COGGESHALL, R. E., YAKSTA, B. A. & SWARTZ, F. J. (1970). A cytophotometric analysis of the DNA in the nucleus of the giant cell, R-2, in *Aplysia*. *Chromosoma* **32**, 205–212.

GORMAN, A. L. F. & MIROLLI, M. (1972). The passive electrical properties of the membrane of a molluscan neurone. *J. Physiol., Lond.* **227**, 35–49.

GRAUBARD, K. (1975). Voltage attentuation within *Aplysia* neurons: the effect of branching pattern. *Brain Res.* **88**, 325–332.

HODES, R. (1953). Linear relationship between fiber diameter and velocity of conduction in giant axon of squid. *J. Neurophysiol.* **16**, 145–154.

HODGKIN, A. L. (1954). A note on conduction velocity. *J. Physiol., Lond.* **125**, 221–224.

HUXLEY, J. (1932). *Problems of Relative Growth*. London: MacVeagh.

LASEK, R. J. & DOWER, W. J. (1971). *Aplysia californica*: analysis of nuclear DNA in individual nuclei of giant neurons. *Science, N.Y.* **172**, 278–280.

LEWONTIN, R. C. (1966). On the measurement of relative variability. *Syst. Zool.* **15**, 141–142.

LONGLEY, R. D. (1976). Analysis of the feeding mechanism in the mollusc *Tritonia*. Ph.D. thesis, University of Oregon, Eugene.

MIROLLI, M. (1976). Membrane resistance of molluscan neurons. In *Neurobiology of Invertebrates. Gastropod Brain*, (ed. J. Salanki), pp. 457–469. Budapest: Akademiai Kiado.

MIROLLI, M. & TALBOTT, S. R. (1972). The geometrical factors determining the electrotonic properties of a molluscan neurone. *J. Physiol., Lond.* **227**, 19–34.

PERETZ, B., RINGHAM, G. & WILSON, R. (1982). Age-diminished motor neuronal function of central neuron L_7 in *Aplysia*. *J. Neurobiol.* **13**, 141–151.

PINSKER, H., FEINSTEIN, R., SAWADA, M. & COGGESHALL, R. (1976). Anatomical basis for an apparent paradox concerning conduction velocities of two identified axons in *Aplysia*. *J. Neurobiol.* **7**, 241–253.

PUMPHREY, R. J. & YOUNG, J. Z. (1938). The rates of conduction of nerve fibres of various diameters in cephalopods. *J. exp. Biol.* **15**, 453–466.

ROSEN, R. (1967). *Optimality Principles in Biology*. London: Butterworths.

SAVAGEAU, M. A. (1979). Growth of complex systems can be related to the properties of their underlying determinants. *Proc. natl Acad. Sci. U.S.A.* **76**, 5413–5417.