

NEURAL REDUNDANCY AND REGULARITY OF SWIMMING BEATS IN SCYPHOZOAN MEDUSAE

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INTRODUCTION

The swimming beat of scyphozoan medusae can be generated by any one of the marginal ganglia present in the intact animal (Bullock & Horridge, 1965, summarize the data used in this Introduction). This finding of multiple neural units which all perform similar or perhaps even identical functions is relatively common. It raises the question, 'What function is served by the apparent neural redundancy?' Several possible functions can be suggested for the multiplicity of pacemakers for the jellyfish swimming rhythm.

First, the multiplicity of ganglia which can trigger a beat may facilitate responses of beat frequency to local sensory stimuli. This speculation is suggested by the following two observations: (1) each ganglion serves as an integration centre at which the swimming beat frequency can be influenced by excitation induced in the diffuse nerve net by local stimulation; (2) a ganglion is found near each major sensory area (rhopalium) with the number of ganglia varying in different species according to the number of rhopalia. Indeed, the marginal ganglia represent considerable centralization of neural tissue and of function as compared to the kind of neural organization believed to be phylogenetically oldest, namely, local neural co-ordination of local responses to local stimuli. We have made no attempt to test this possible function of redundancy, but have concentrated instead on ways in which the redundancy might contribute to the regularity of jellyfish beating.

Several jellyfish have arrived in our laboratory with more or less normal swimming beats but with one or more marginal bodies that appeared damaged. Activity of an individual ganglion was tested by cutting out a piece of jellyfish that contained no other ganglia. In such tests the apparently damaged ganglia often proved incapable of initiating swimming beats. In these cases the redundancy of pacemakers permitted swimming to continue despite serious damage to part of the nervous system.

Horridge (1959) has suggested that even in intact animals the multiplicity of ganglia may play an important role in increasing the frequency and regularity of the swimming beat. (Note that this is an adaptive function of the redundancy only if fast and regular swimming beats are more useful to the animal than slow and irregular swimming beats. Ecological data to test this assumption are not available to us.) Horridge's suggestion that redundancy of pacemakers contributes to regularity of beating was based initially on the observation that pieces of jellyfish containing only

one ganglion generally beat more slowly and irregularly than the whole jellyfish. more specific argument was developed from two observations: (1) when one ganglion generates a beat, electrical excitation sweeps through the giant-fibre nerve net; (2) activity in this nerve net can trigger activity in the other ganglia (Passano, 1965), and re-sets the pacemaker of an isolated ganglion. (Re-set is observed as a pause before the next spontaneous beat; this pause averages at least as long as the intervals between spontaneous beats generated by that ganglion (Horridge, 1959).) Specifically, Horridge proposed that, after each beat in the intact animal, each ganglion was re-set and proceeded to generate a beat as it would when isolated; the ganglion which generated a beat first would trigger all the others and re-set them.

On this basis Horridge proposed an elegantly simple method for predicting the distribution of intervals between beats of the intact jellyfish from the interval distributions of the isolated ganglia. To characterize the intervals between beats generated by an isolated ganglion, let $f(t)$ be the proportion of intervals longer than $t - 1$ seconds but less than or equal to t seconds. Then $F(x) = 1 - \sum_{t=1}^x f(t)$, is the probability that a ganglion will not have fired again at a time x after the last beat. The probability that no ganglion in a whole jellyfish containing eight ganglia will have fired by time x is $[F(x)]^8$. Therefore, in the whole jellyfish, the proportion of intervals of duration between $x - 1$ and x is $[1 - F(x)]^8 - [1 - F(x - 1)]^8 = F(x - 1)^8 - F(x)^8$.

Horridge mentioned two complications that must be taken into account. The rhythms produced by different isolated ganglia from the same jellyfish are not the same. This observation is easily incorporated into the model by substituting $F_1(x) \cdot F_2(x) \cdot F_3(x) \cdot F_4(x) \cdot F_5(x) \cdot F_6(x) \cdot F_7(x) \cdot F_8(x)$ for $[F(x)]^8$. The second complication is that when a ganglion beats it is not entirely re-set, i.e. each interval is not independent of the preceding interval. Since the average interval for the intact jellyfish is shorter than for the isolated ganglion, it might be assumed that the average state of each ganglion when it starts to generate a beat is different in the two cases. Thus the interval distribution of the isolated ganglion would not be exactly the interval distribution that should be used in the prediction for the whole. Horridge considered this effect to be quantitatively unimportant. We have tried to evaluate this point by making quantitative tests of the model. These tests proved inconclusive for reasons described below. We have also explored the reasons for the differences in activity among apparently similar ganglia from the same jellyfish. Lastly, our data suggest another mechanism which may contribute as much as redundancy to the regularity of the swimming rhythm of medusae.

METHODS

We present data on *Aurelia aurita* from the Woods Hole area and *Dactylometra quinquecirrha* from the Gulf of Mexico (both from Order Semaestomeae). Sketchy but similar results were obtained for two other species: *Cyanea capillata* (also Order Semaestomeae) and *Cassiopea frondosa* (Order Rhizostomeae).

Our primary data are the sequences of interval durations for swimming beats of whole jellyfish and for pieces containing only one marginal body. Pieces were generally obtained by radial cuts at a 45° angle. Beats were recorded by an observer who, in synchrony with each contraction, pushed a switch that activated a pen on an Esterline

mus recorder. Activity of pieces was not recorded until at least half an hour after cutting, when sporadic bursts of beating had ceased and the activity seemed stable. This procedure is compatible with the more systematic observations of Romanes (1877) who showed that rate of beating became constant about half an hour after cutting and remained so for several hours.

The records of muscle contractions were meant to indicate the time of the activity induced in the giant-fibre nerve net by a burst of activity in the ganglion. It would seem possible, however, that sometimes a spike passed through the giant-fibre nerve net while the muscle was still refractory due to previous activity. For *Aurelia* we observed intervals as short as a quarter of a second in the smallest jellyfish ($2\frac{1}{2}$ " diameter), and beats as short as three quarters of a second for the larger jellyfish ($3\frac{1}{2}$ " diameter); Bullock (1943) reports a 0.7 s absolute refractory period for the muscle of *Aurelia* of unspecified size (temperature 16–18 °C for all observations). For *Dactylometra* we commonly observed intervals as short as 0.4 s (temperature 12–13 °C, corresponding to the winter temperature in their natural habitat). If beats were obscured by muscle refractoriness, then, when the interval between ganglion bursts was approximately equal to the absolute refractory period, the observed beat interval distribution should be bimodal, with modes equal to the refractory period and an interval twice as long. We never observed this, and therefore hope that muscle refractoriness was not a serious source of error.

The sequence of intervals was partially characterized by serial rank-correlation coefficients up to order 15. An n th order rank correlation coefficient is a conventional Spearman rank order correlation in which each interval length is compared to the length of the n th subsequent interval in the sequence. Significance was accepted for $P < 0.05$.

RESULTS AND DISCUSSION

Ganglia differ

Our attempts to test the model by comparing actual interval distributions for the whole jellyfish with interval distributions predicted on the basis of activity of a one-ganglion piece gave inconsistent results. This inconsistency was due to the differences between isolated ganglia (see Figs. 1, 2). Given the apparent symmetry of the animals, it seemed possible that the differences between ganglia might be artifacts caused either by inequalities in the cutting procedure or by inadequate samples of varying activity.

Several of our results suggest, however, that these differences between ganglia are not artifacts. In a few intact *Dactylometra* it was possible to see where the contraction wave started and thus to determine which ganglion had initiated the beat. Fig. 1 shows that certain ganglia consistently initiated a high proportion of the beats. Thus some differences in ganglionic activity were present before the cutting and could not have been caused by it. We were unable to make an adequate test of whether those ganglia which initiated more beats before cutting beat more rapidly after cutting, but our very sketchy data did not support this notion. We did, however, observe that the differences between isolated ganglia tended to persist through several observation periods (Fig. 1). This suggests that differences were not due to inadequate statistical samples.

Despite occasional unexplained large changes in beating rate of individual ganglia, the general consistency of differences suggests that some intrinsic property of the

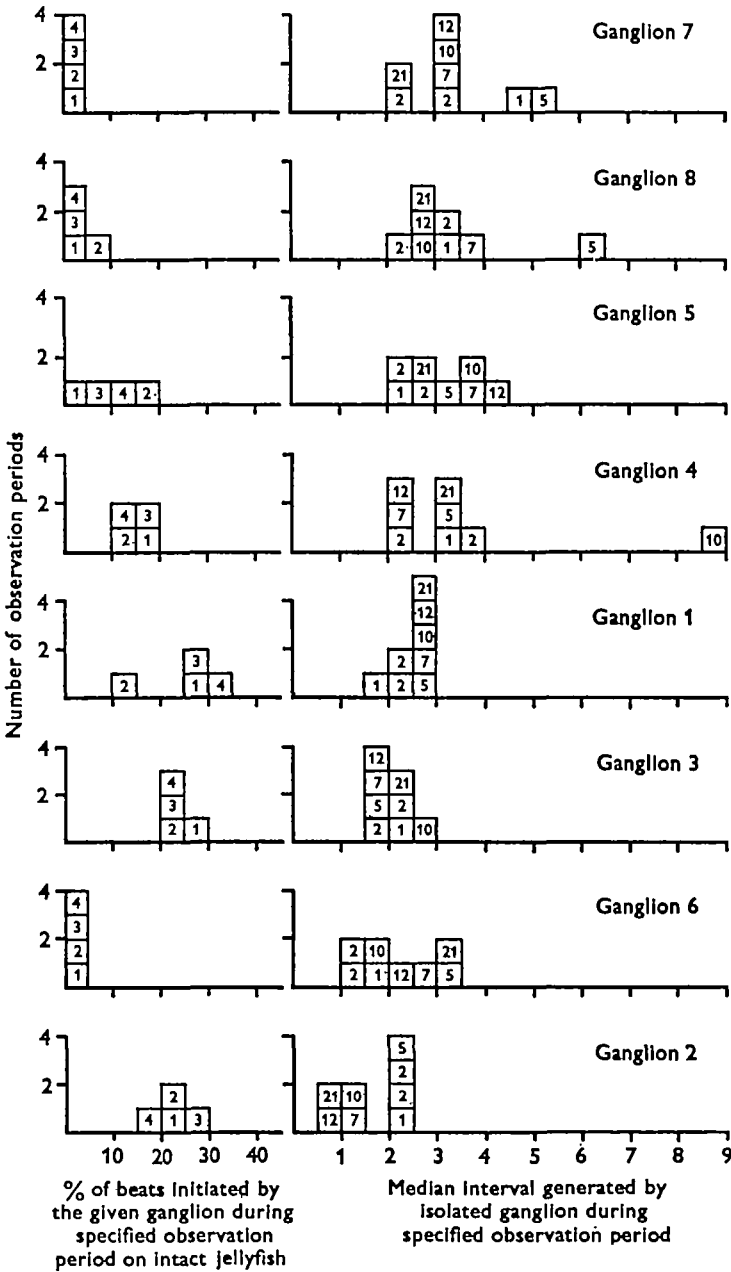


Fig. 1. Activity levels of the ganglia of a *Dactylometra*. The histograms on the left indicate activity of a given ganglion in initiating beats in the intact animal. The histograms on the right indicate activity levels of the same ganglion, after the animal has been dissected into eight pieces each containing only one ganglion. The number in each box indicates the number of hours by which the reading preceded (or followed) the cutting. The ganglion number indicates position around the margin of the medusa; no. 1 position is arbitrary and next to no. 8. Graphs for the slowest ganglia (after cutting) have been put at the top. Differences in level of activity persisted over several hours both in the intact animal and the isolated ganglia.

Isolated ganglion plays an important role in producing different beating patterns. A reasonable hypothesis is that the difference for isolated pieces might be due to differences in the amount of tissue. However, our data suggest that this is not so. In the most extreme case, comparisons were made between the interval distribution of 1/8 pieces cut from *Aurelia* as a piece of pie is cut and the interval distributions for jellyfish from which only enough tissue had been removed confidently to eliminate 7 of the 8 ganglia. Despite the considerable difference in mass of tissue attached to these two kinds of single-ganglion pieces, they both produced interval distributions in the same range.

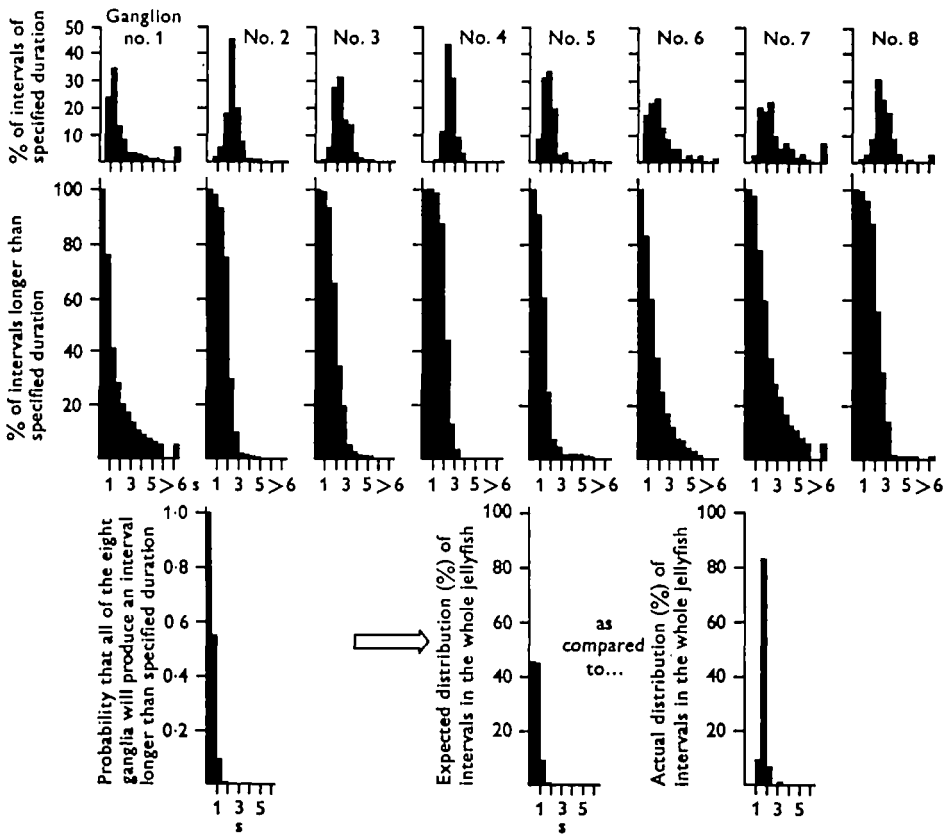


Fig. 2. A test of Horridge's model. The model is based on the idea that, in the whole jellyfish, long intervals will occur only when all eight ganglia independently fail to initiate a beat. A prediction of the interval distribution for the whole *Aurelia* has been made using the interval distributions for individual ganglia and the formulae developed by Horridge and specified in the Introduction. The prediction is significantly different from the observed interval distribution. However, for reasons explained in the text, we do not think this an adequate basis for rejecting Horridge's model.

We thus conclude that the differences between ganglia are not artifacts of the cutting procedure or of statistically inadequate samples. Although we do not know what caused the differences, our data suggest that incorporation of the differences between ganglia into the model represents incorporation of a biological reality rather than a statistical manoeuvre to improve predictions.

Test of the model

Predictions of the interval distribution of the whole jellyfish were made in accordance with Horridge's model, using the interval distributions for each of the eight ganglia isolated from that jellyfish. In almost all cases these predicted distributions differed from the distribution observed for the intact jellyfish. For each of the three *Dactylometra* tested the predicted distribution was slower and less regular than the actual ($P < 0.01$ in each case, using the Komolgorov-Smirnov non-parametric test for homogeneity of distributions). For each of the seven *Aurelia* tested the predicted distribution was faster than the actual ($P < 0.01$ in all but one case). Fig. 2 shows an example. This same result was observed for two other *Aurelia*, one of which had only five marginal bodies and the other of which had only three ($P < 0.01$ in both cases). For three other *Aurelia* the interval distribution for the whole was predicted from observations on two pieces, one containing only one ganglion and the other containing the other seven ganglia of that jellyfish. In all three cases, the predicted interval distribution was more irregular than the observed ($P < 0.01$ in each case).

We do not think, however, that the discrepancies between predicted and observed interval distributions constitute a sufficient reason for rejecting Horridge's model. These discrepancies could be due to several differences in external stimuli for isolated ganglia as compared to ganglia in the intact jellyfish. For example, ganglia in the intact animal received more stimulation from motion through the water but less stimulation from contact with gravel on the bottom, where many of the pieces tended to rest. The whole jellyfish slowed its beating whenever it reached the surface, and only resumed rapid beating after it had drifted somewhat deeper. Since the pieces never reached the surface they would not have been subject to this apparently inhibitory stimulation. Whenever a jellyfish or piece of jellyfish is strongly stimulated mechanically, it beats more rapidly, presumably as an escape response; the injury involved in cutting the pieces is an extreme form of this and causes very rapid beating. On the other hand, 1/8 pieces die after a day or two, and ill health is correlated with slower beating. We tried to record the beating pattern at a time when it was stable for several hours, but did not rigorously test for stability. For the *Dactylometra* only, the 1/8 pieces were kept in Syracuse dishes, which reduced their exposure to water currents and to oxygen-rich water; this may explain why the predictions based on these pieces were slower and less regular than the observed interval distributions for the whole jellyfish. In general, it would seem possible to explain away any difference between predicted and observed interval distributions. It does not seem possible to establish rigorously similar conditions for ganglia before and after isolation. We conclude that the sensitivity of ganglion activity to external stimuli makes it impossible to test Horridge's model satisfactorily by the method we have used.

Pattern of beating produced by single ganglia

Horridge reports that the intervals produced by a single *Aurelia* ganglion tend to be alternately long and short. He measures this as significant negative first-order serial correlation coefficients. In analysing records from 40 *Aurelia* ganglia we found no significantly negative first-order serial correlation coefficients. In 13 cases the coefficients were positive, indicating that there were cyclic changes in frequency.

Higher-order coefficients indicated that cycle length varied from about two beats to more than 15 beats. Horridge has suggested that these cyclic changes in rhythm occur in 'over-stimulated or damaged preparations'. We do not think that the cyclic changes we observed were due to overstimulation or to damage beyond that necessarily involved in isolating a ganglion. The main basis for this opinion is the relative stability of beating pattern of these ganglia over several hours (Fig. 1). We have not, however, made systematic measurements of stability and variation in beating pattern over periods of hours to argue this point strongly.

Our records do, however, illustrate a point of considerable importance and relevance: individual ganglia often beat as fast and as regularly as does the whole jellyfish. The peaks in the interval distribution for ganglia 1 and 5 in Fig. 2 derive from just such periods of rapid and regular beating. This demonstrates that, under some circumstances, individual ganglia can produce a pattern like that produced by eight ganglia in the whole jellyfish. Apparently a ganglion can beat regularly without rhythmic input from seven other ganglia provided it receives enough excitatory input. Further evidence for this point of view comes from two experiments reported by Horridge in which he showed that stimulation of an individual *Aurelia* ganglion can cause it to produce rapid and regular beats. This is illustrated for stimuli due to prodding and orientation relative to gravity in his fig. 6 and for electrical stimulation of the diffuse nerve net (12 shocks in 3 s) in his fig. 2 (Horridge, 1959).

These observations suggest an alternative hypothesis to explain why the whole jellyfish beats more rapidly and regularly than the average piece containing only one ganglion. Perhaps the redundancy of pacemakers operating according to Horridge's model is not the crucial factor; perhaps greater excitatory input to each ganglion makes each independently a more regular pacemaker.

How large a role does redundancy play in the regularity of swimming beats?

Two basic explanations have been offered for why the whole jellyfish beats more rapidly and regularly than the average piece with only one ganglion. We have just proposed that in the whole jellyfish the pacemaker in each ganglion may be responding to greater tonic excitatory input by producing more rapid and regular beats. Horridge (1959) has previously proposed that long intervals are rarer when many ganglia are present because such long intervals occur only when all ganglia independently fail to initiate an earlier beat. Both proposals seem reasonable on the basis of the available data.

Perhaps the most interesting question to ask now concerns the quantitative importance of the two mechanisms. Is the greater regularity of beating in the whole jellyfish due *primarily* to redundancy of pacemakers or to greater excitation of individual pacemakers? One way to test the importance of redundancy would be to use Horridge's model to make quantitative predictions of the effect of redundancy and compare these predictions with actual observations. We have tried this technique, and find that it leads to no firm conclusions because sensory input cannot be equated for the pieces and the whole. Another approach to evaluate the relative importance of redundancy versus diffuse excitation would be to attempt to isolate the effects of diffuse excitation and evaluate these effects alone. Possibly this could be done by removing seven marginal ganglia from an otherwise undisturbed jellyfish and then looking at the beating rhythm

produced by the remaining ganglion. We would expect such an experiment to be inconclusive, however, primarily because of the probable importance of input from the statocyst and other marginal body sensory structures which apparently make contact with the diffuse nerve net only via the ganglion. Perhaps when more is known about the activity of the diffuse nerve net in a free-swimming jellyfish, the importance of this activity could be tested by comparing the rhythm produced by an unstimulated isolated ganglion with the rhythm produced by the same ganglion during electrical stimulation of the diffuse nerve net to simulate natural input. The magnitude of the effect of this input could help to distinguish whether redundancy of pacemakers makes a major contribution to regularity of the swimming beat, or whether individual pacemakers, under conditions of normal input, produce such regular beats as to make the contribution of redundancy minor.

SUMMARY

1. Despite the apparent symmetry of jellyfish the eight ganglia do not all have equal levels of activity in initiating swimming beats. Differences in activity level persist over several hours, both in the intact jellyfish and after isolation of the individual ganglia.
2. Horridge has proposed a specific model of how the regularity of the swimming beat should be increased by the redundancy of pacemakers (one in each ganglion). Our data are quantitatively incompatible with this model. However, this incompatibility may be due more to the impossibility of maintaining comparable levels of sensory input at different stages of the test than to deficiencies in the model.
3. Regular beating in the whole jellyfish is probably due to both redundancy of pacemakers *and* more regular functioning of individual pacemakers when they receive greater excitatory input.

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