

FACILITATION IN SEA ANEMONES. IV. THE QUICK RESPONSE OF *CALLIACTIS PARASITICA* AT HIGH TEMPERATURES

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Preceding papers of this series have dealt with the effects of a number of treatments on the quick responses of the sea anemones *Calliactis parasitica* and *Metridium senile*. Pantin's (1935) analysis of the quick closing response in these animals showed that it is not normally elicited by single electrical stimuli, nor by stimuli following each other at intervals greater than about 3 sec. To get the closing response electrical stimuli must be given within a definite frequency range, at intervals of from about 0.2–2.5 sec. at room temperatures. Between these limits, the closer the stimuli the bigger the response, with responses occurring, of course, only to the second and subsequent stimuli. This remarkable example of a facilitated response in which the response can only occur when facilitation has been set up, and in which the amount of facilitation determines the size of the response, raised many interesting problems. My work has centred on the approach of trying to set up experimental conditions in which single stimuli are effective, as this seemed a promising way of finding out more about the nature of this kind of facilitation.

Few of the treatments employed so far have caused either *Metridium* or *Calliactis* to respond to single stimuli. Radical changes in ionic concentration do not do so, though they have big effects on the size of individual contractions (Ross & Pantin, 1940). A few drugs do so, e.g. tyramine, but only for brief periods after long exposure (Ross, 1945*a*). Some promising results with anemone extracts (Ross, 1945*b*) proved on further investigation to be unspecific effects shared with other substances (Ross, 1952) and were apparently due to a sensory rather than a direct facilitating action. Thus the aim of finding a chemical treatment which enables *Calliactis* or *Metridium* consistently to respond to single stimuli remains unfulfilled.

Recently I have found that if *Calliactis* is exposed to temperatures above 25° C., responses to single stimuli occur with great regularity. The responses obtained are generally small and might be regarded as insignificant were it not that so many other treatments have failed to produce this kind of effect. It seemed desirable, therefore, to study this effect as closely as possible and to report it briefly, since it reveals an unsuspected property of the quick response in these animals.

RESULTS

In these experiments the temperature of the sea water bathing the anemone was raised slowly by means of a small aquarium heater with thermostat attachment, the water being circulated by vigorous aeration. The animal was stimulated as required by condenser discharges delivered through fluid electrodes as in Pantin's (1935) original experiments. The experiments were carried out in London on animals obtained from Plymouth.

As the temperature rises the responses to stimuli become smaller and smaller at frequencies within the range effective at room temperatures. This is, of course, the effect of temperature on the rate of decay of facilitation described by Hall & Pantin (1937) in *Metridium*. Above 25° C., however, one begins to see tiny movements around the margin of the disk occurring in response to single stimuli. As the temperature rises still further these tiny movements become bigger and they have the effect of lifting all the tentacles quite sharply. When this happens it becomes clear that one is witnessing genuine withdrawal movements of the marginal sphincter that are similar in character and dimensions to the responses one gets normally to the second of a pair of stimuli as one approaches the end of the period of facilitation. At this point and beyond it is possible to record these movements on smoked paper.

At some point, usually between 30° and 34° C. in different animals, *Calliactis* becomes quite insensitive to electrical or tactile stimulation. It is possible, therefore, to follow the development of these responses to single stimuli only over a short temperature range of 5–9°.

Metridium does not behave like *Calliactis* in this respect. Hall & Pantin (1937) reported that temperatures above 25° C. were fatal to *Metridium*. In my experience, *Metridium* will recover temporarily from a temperature as high as 29° C., and death occurs some days later. Above 25° C., however, *Metridium* remains so tightly closed that its responses to stimulation can no longer be recorded or clearly observed. *Calliactis*, on the other hand, shows no ill effects from the treatment. Recovery of excitability occurs quickly as the temperature falls to a normal level, even when it has been exposed to temperatures as high as 35° C. It is noteworthy that as the temperature falls again the first responses to reappear are the small responses to single stimuli, usually in the neighbourhood of 30° C. Animals may be subjected to this treatment time and again without any apparent change in their normal behaviour or in their response to stimulation.

Figs. 1–3 show records of the responses of *Calliactis* to stimuli at different frequencies at temperatures ranging from room temperatures up to 33·5° C. In Fig. 1 we see responses at a frequency of 1 stimulus per second. At a temperature of 19·5° C. the steps of the staircase are big and the response begins, of course, only on the second stimulus, so that the five stimuli give a staircase consisting of four big steps. At 25° C. the response still begins on the second stimulus, but the four steps of the staircase are now quite tiny, as Hall & Pantin (1937) showed with *Metridium*. With a further rise in temperature, however, the response takes on a

different character. A small contraction can be seen on the first stimulus, and usually on all subsequent stimuli, so that the five stimuli now give a staircase with five small steps instead of four, and the response is an example of simple summation. At these temperatures the quick response of *Calliactis* clearly loses its normal facilitated character, and responses occur to each stimulus without requiring a preceding facilitating stimulus.

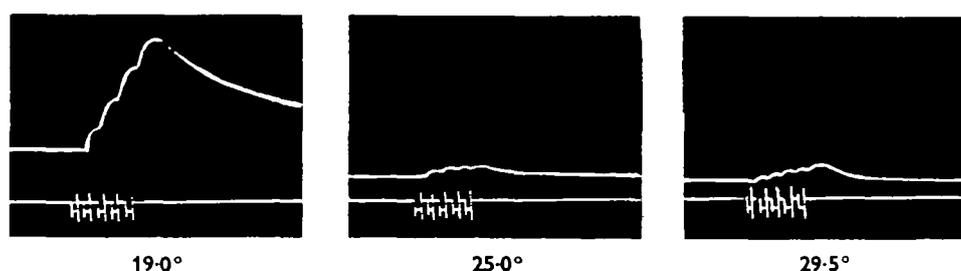


Fig. 1. Responses of *Calliactis parasitica* to 5 stimuli at 1 per second at the temperatures indicated.

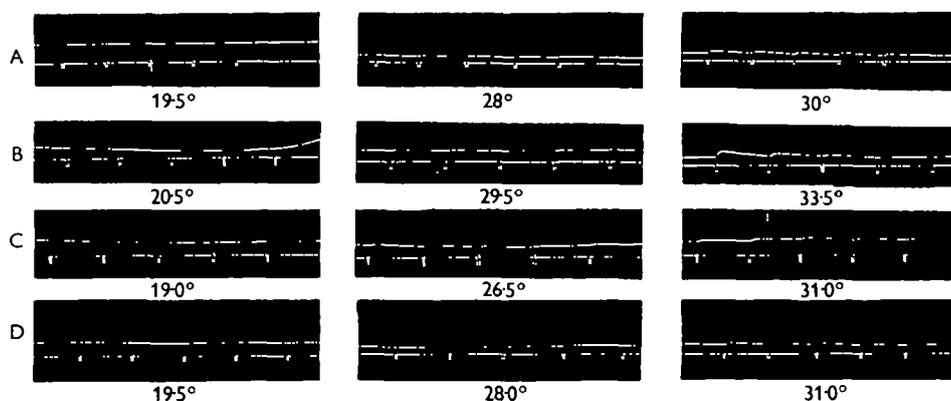


Fig. 2. Responses of *Calliactis parasitica* to 5 stimuli at 1 per 10 seconds at the temperatures indicated. A, B, C & D are four different experiments.

Fig. 2 shows the same effect in another way. The stimuli here are delivered at intervals of 10 sec. This is well outside the normal period of decay of facilitation, so that each stimulus is in effect a single stimulus. These stimuli have no effect at room temperatures, but as the temperature rises to 30° C. and beyond, each stimulus elicits a distinct movement of the animal. These responses are never large, and their size varies considerably from one animal to another, as the records taken from four different experiments show. In my experience the responses given by any one animal to the separate stimuli in a series are usually all approximately the same size at a given temperature, as in Figs. 2a, c and d, but occasionally bigger responses occur on certain stimuli as in Fig. 2b.

It is evident from these records that these movements in response to single stimuli get bigger as the temperature rises. This feature of the effect is sometimes

easier to see by eye, as a lifting of the tentacles which becomes more and more pronounced, than it is to record on a kymograph. This heightening and sharpening of the response continues until the temperature is reached at which the animal becomes inexcitable. One gets the impression that the response would get still bigger and would eventually approach the size of the normal quick closing movements if *Calliactis* could tolerate still higher temperatures.

Fig. 3 shows the responses of *Calliactis* to pairs of stimuli at intervals of 0.5, 0.3 and 0.2 sec. We see that at the highest temperatures employed, 31–32° C., the decay of facilitation is so speeded up that only the small responses to single stimuli are apparent at 0.5 sec. The existence of these responses makes it impossible to determine the period of decay of facilitation with any precision because it is no longer clear at what frequency the response to the second stimulus of a pair fades

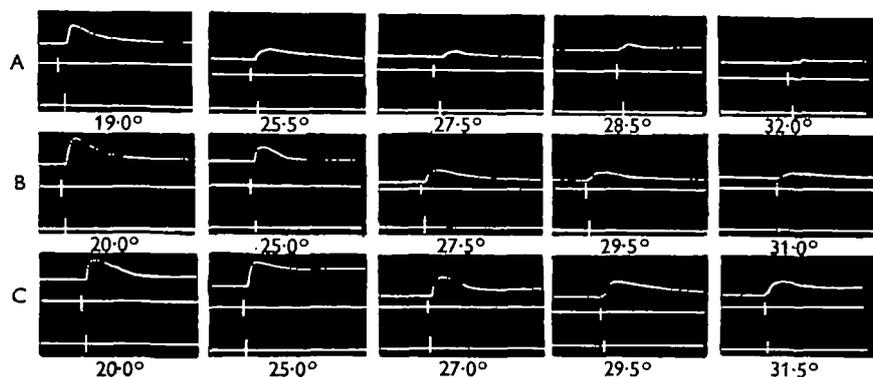


Fig. 3. Responses of *Calliactis parasitica* to pairs of stimuli at frequencies of: (A) 0.5 sec.; (B) 0.3 sec.; (C) 0.2 sec. at the temperatures indicated.

out when every stimulus gives a small response. At higher frequencies, however, the normal type of facilitated response to the second stimulus is superimposed on, and at 0.2 sec. quite overshadows, the response to the first stimulus (Fig. 3c, 31.5° C.).

DISCUSSION

Earlier work has produced abundant evidence that facilitation and excitation are separate steps in transmission of the quick response in *Calliactis* (Pantin, 1937; Ross & Pantin, 1940; Ross, 1945). Within the framework of this general conception it is possible to put forward a number of possible explanations for the temperature effect here described. It is conceivable that at high temperatures a slight state of permanent facilitation might be set up perhaps as a result of the metabolic effects of the treatment. This is not, however, an example of the residual facilitation described by Ross & Pantin (1940) in *Calliactis* and by Pantin & Vianna Dias (1952) in a Brazilian species of *Bunodactis*. That effect occurs only after repeated stimulation, whereas the responses to single stimuli described here occur in the absence of previous stimulation. It is also possible that this treatment so affects the electrical conditions at the membrane of the muscle cells or at the junctions that the normally

ineffective single impulse is just able to initiate contractions without prior facilitation. Or perhaps at these temperatures there are enough random impulses passing in the nerve net to keep all the junctions slightly facilitated. Yet if the treatment really had effects on the electrical state of the muscle or the junctions, or if it set up numbers of impulses, one would expect a conspicuous increase in the number of spontaneous quick contractions. This does not happen.

We are in no position to discuss these possibilities further, since they are concerned with aspects of neuromuscular activity on which we have little or no information at present in these animals. I propose, however, to discuss one other possibility in more detail, partly because it arises from the idea which led me to undertake these experiments. This is that the puzzling failure of the single stimulus in *Calliactis* and other anemones may simply be a matter of timing between the facilitatory and excitatory steps in neuromuscular transmission in these animals.

Suppose that in this response, where facilitatory and excitatory processes are both essential for transmission, facilitation is a slower process than excitation. In that case a single stimulus might fail simply because the excitatory state which starts off the quick contraction has subsided before any facilitation has been set up. Now if facilitation could be speeded up more than excitation, say by raising the temperature, so that some facilitation is set up before the excitatory state has died away, one should get responses to single stimuli as the two processes begin to overlap in time.

One merit of this approach is that, unlike the suggestions made above, it postulates only that the effect of raising the temperature is to speed up reactions. There is, of course, no direct evidence that facilitation is established more quickly at higher temperatures. But it decays more quickly and the whole frequency/response curve is shifted, so it would be surprising if the build-up of facilitation were not affected in the same way.

The notion that facilitation might be speeded up more than the process of excitation is less plausible and would require some experimental support. Although the junctional and muscle excitation time cannot be measured directly, there are two quantitative features of the quick response that might give some indication about the effect of higher temperatures on excitatory processes in general. The latent period, the time between the application of a successful stimulus and the beginning of the contraction comprises the conduction time of the impulse in the nerve net and the excitation time at the muscle, the latter being the property which one would like to measure. But both of these components no doubt are determined by processes involving movements of ions at membranes, and the effects of temperature on them might reasonably be expected to be of the same order of magnitude. The absolute refractory period is the shortest interval between a pair of stimuli that still permits a response to occur on the second. It is thus a measure of the restoration of excitability and should also indicate how higher temperatures affect the excitatory process from which the system is recovering during this period.

With this in mind I compared the effects of higher temperatures on the three quantities, rate of decay of facilitation, absolute refractory period, and latent period

in several animals. The results are shown in Fig. 4. There are certain difficulties in measuring these quantities exactly at temperatures above 28° C. (especially the decay of facilitation, owing to the appearance of responses to single stimuli), but the trend is decidedly in favour of a much bigger temperature effect on facilitation than on the other two quantities. This is true particularly above 23° C., where latent and refractory periods show little change. This result is consistent with the idea advanced above, that at higher temperatures facilitation might begin to overtake the excitatory process. Certainly if this were happening one would expect the effect to begin with tiny responses which gradually get bigger as the two processes overlap more and more. This would be more convincing, of course, if the responses obtained at the highest temperatures were not so small. In this connexion it would be interesting to repeat these tests on *Calliactis* from warmer waters, since they might tolerate still higher temperatures and so allow the effect to be studied over a wider range.

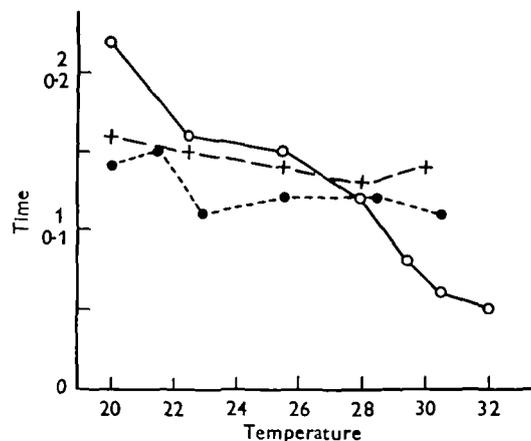


Fig. 4. Effect of temperature on Facilitation, Refractory Period and Latent Period in the quick response of *Calliactis parasitica*. —○— Duration of facilitation, (seconds). - - + - - Absolute refractory period (tenths of seconds). - - ● - - Latent period (tenths of seconds).

A comment might be added about the bearing these observations have on comparative aspects of neuromuscular transmission, and especially on the relationship between facilitated and direct modes of transmission. The records in Fig. 1 show that at high temperatures *Calliactis* goes over from a facilitated to a direct mode of transmission with simple summation, though it is direct transmission of a very imperfect and inefficient kind. Whether this happens by speeding up the facilitation process so that it begins to overtake the more rapid excitation process as I have suggested, or in some other way, it does show that the distinction between the two modes of transmission is not absolute. This encourages the belief that it will yet be possible to solve the elusive problem of fitting the facilitation process in anemones into more general concepts of neuromuscular transmission which have been found to apply in most other animals.

SUMMARY

1. At temperatures above 25° C. small quick contractions of the sphincter of the sea anemone, *Calliactis parasitica*, occur in response to single electrical stimuli which are normally ineffective.
2. These contractions, though they never become large, increase in size as the temperature rises until they vanish, along with all other responses, between 30 and 35° C.
3. The effect is discussed in relation to the timing of the two processes, facilitation and excitation, involved in the quick response in this animal.

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