

Heat- and Cold-sensitive Mammalian Nerve Fibres. Some Somatic Reflexes to Thermostimulation.

By

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All methods by means of which nerve fibres can be differentiated would seem to be of very great interest. Hence, when it was noted, in work on thermostimulation (BERNHARD and GRANIT, 1946), that the cat showed evidence of struggling when limb nerves were locally warmed but remained quiet when they were cooled, it was decided to devote some attention to this mode of stimulation. With an impulse integrator connected to the amplifier BERNHARD and GRANIT had already shown that both local cooling and moderate local warming initiated a discharge but this instrument could not analyze the nature of the discharge obtained. Their intention was to create an 'artificial sense organ', and they succeeded in demonstrating a local drop of potential relative to normal nerve when the temperature was raised or lowered from the level to which the nerve had been adapted. (For earlier work, see their paper, for a full review, C. v. EULER, 1947). GRANIT and SKOGLUND (1945) found that local cooling augmented the transmission through their artificial synapse, formed by the cut end of a mammalian nerve, but that warming, if it had any effect at all, rather tended to diminish the transmitted amount. This experiment too indicated some differentiation between these two modes of increasing the negativity at the treated region.

An extensive and systematic analysis of vegetative responses was now undertaken in this laboratory by C. VON EULER (1947)

whereas we ourselves decided to begin with an inspection of the impulse pattern elicited by local temperature changes to be combined with a preliminary survey of possible somatic reflexes obtainable by this mode of stimulation. Our results will be briefly reported in this paper.

Technique and Procedure.

Some 50 cats were used, nearly all decerebrated, some in addition made spinal by a section in the lower thoracic region. A few animals received chloralose (5 cc per kg of a 1 % solution). The standard technique for impulse recording with cathode ray, loudspeaker and necessary amplifiers was employed. The thermode was a small lacquered metal container with a 1 cm groove for the nerve. In its latest standardized pattern it was made of silver and several times coated with a special isolating material, each fresh coating dried at 300° C for several hours. This secured the perfect isolation necessary for the recording of thermopotentials. Our analysis of thermopotentials falls outside the scope of this paper.

The thermode contained a thermocouple, recording the temperature of the water circulating through it, and connected to a moving coil Multiflex mirror galvanometer, critically damped to a period of 1 sec. Its deflexion was projected vertically on the film. In order to preserve a reasonable sensitivity without increase of film width the galvanometer mirror was illuminated by two adjacent light points in such a manner that when a large deflexion removed the one light spot from the film the other light spot took over and continued the temperature record (see figs. 1, 3 and 4). The sensitivity was then 1.3—1.6 mm per degree C, depending upon the thermocouple used.

For reflex work the standardized denervated decerebrate preparation of the SHERRINGTON type (see CREED *et al.*, 1932) was used. For myographic recording *m. tib. ant.* or *m. gastroc.* (not isolated from *m. soleus*) was freed and connected to the isometric lever of the spring of a Brown-Schuster myograph, fitted with two crossing coils one of which was fed by a high frequency circuit. Since the one coil remained fixed and the other altered its position relative to the former with the deflexion of the myograph spring, capacitative changes were set up by the isometric muscle contraction, and these were transmitted to a cathode ray oscillograph by means of an amplifier. In this manner a vertical record of the reflex response was filmed together with the thermode temperature. The impulses in the nerve or in the muscle were recorded horizontally by a sweep circuit deflecting another cathode ray, generally every 1.2 sec. During the relatively slow warming and cooling of a nerve, samples of its impulse activity could thus be inspected at every desirable sweep speed without an inconvenient vertical expansion of the temperature and muscle records owing to high film speeds.

The cat was kept in an isolated box in steam at about 25—30° C.

Results.

1. Impulse records from root filaments in response to thermostimulation of the sciatic.

Some 50 filaments, nearly all from the sensory roots L7 or S1 (since motor effects could be observed myographically), were isolated as thin as possible, listened in to, and the discharge photographed. For consistent results the following precautions have to be observed: (i) the thermode must not be placed too near a cut or crushed region of the nerve since this is hypersensitive, as is well known from work by ADRIAN (1930) and SKOGLUND (1942). (ii) It is necessary to make certain, by root sectioning and denervation, that the impulses recorded do not come from proprioceptors activated by reflex or direct muscle contractions elicited by thermostimulation. (iii) The cat must not be hyperventilated. Hyperventilation is well known to increase excitability. Recently KUGELBERG (1944) has measured the drop in rheobase and accommodation and studied the sensory paresthesias set up by hyperventilation.

If these precautions be observed, it is found that the typical response to warming (keeping below 45° so as not to damage the nerve) is difficult to obtain whereas in most preparations large spikes come bursting when the nerve is cooled. The response to heat is a slight increase of noise level, better heard than seen.

Three records to warming are shown in fig. 1 a, b and c. The level at which effects appear depends somewhat upon the level of previous thermal equilibration. The records are seen to consist of small fibre activity, undoubtedly a considerable proportion of unmyelinated C-fibres in addition to small myelinated ones. The responses to cold, fig. 1 d and e, the latter at lower amplification, are very different. In the former there is also a characteristic off-response when the temperature swings back to normal, unusually brisk in this particular case. Such off-effects should not be mistaken for heat responses which start at temperatures above 37°. This mistake is easily avoided if warming is preceded by a period of equilibration removing off-effects. Quite often the latter are missing.

Fig. 1 e shows that one often obtains filaments with a large number of clearly distinguishable spikes of different size. We expected that in such cases it sometimes would be possible to

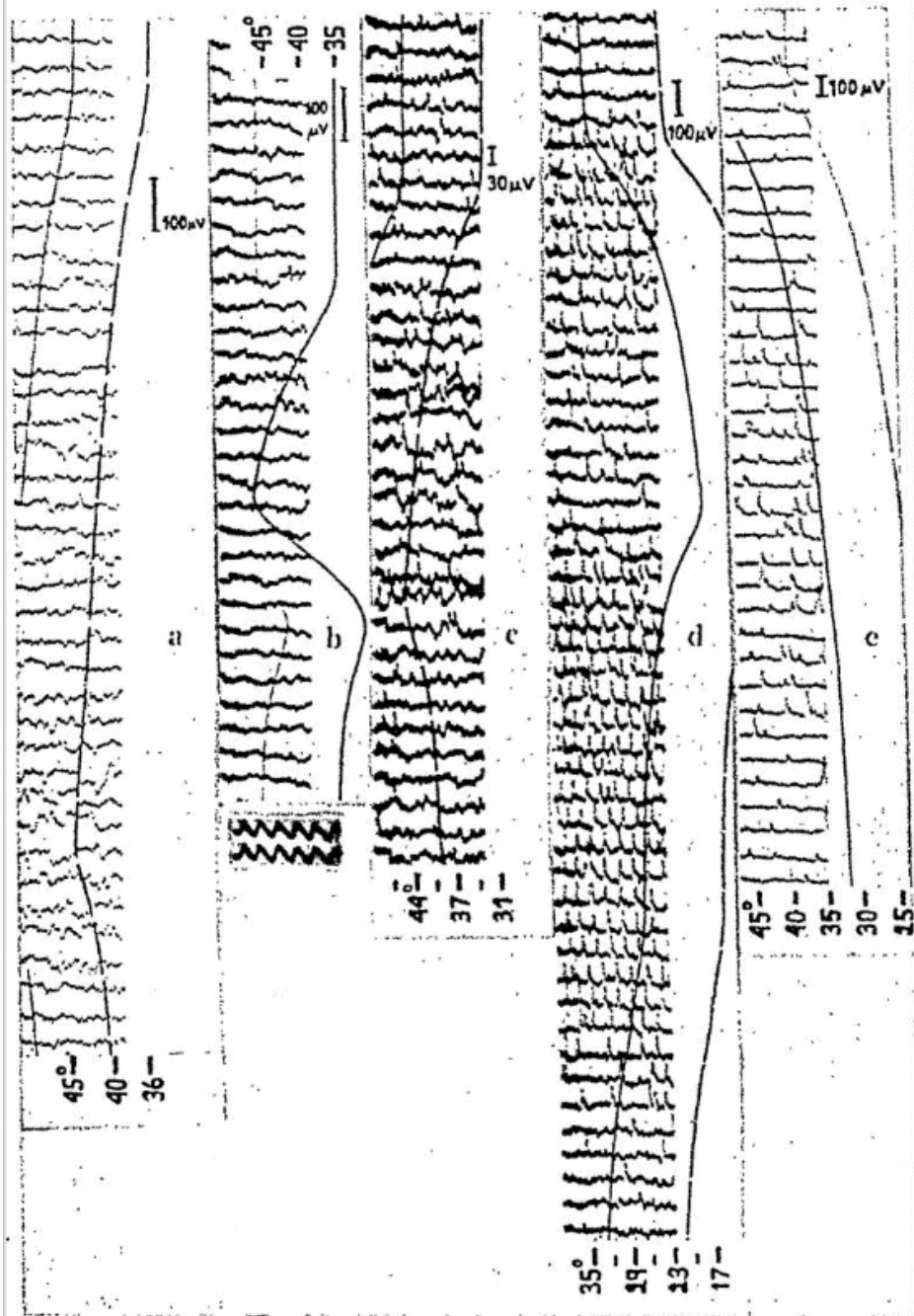


Fig. 1. To be read downwards. Records from thin filament strands of sensory roots. Stimulation by thermode on the sciatic nerve. a, b and c in response to warming. Thermode temperature recorded by parallel black lines one of which interrupted every second. Temperature scale refers to that particular line which embraces the significant range. d and e, same in response to cooling. Calibration of cathode ray marked on each film, sweep speed by separate record of period 100 Hz.

find spikes which were activated both by cooling and warming. But we have not succeeded in demonstrating the expected overlapping of heat- and cold-sensitivity though other experiments suggest that it might exist. There must, at any rate, be a relatively insensitive zone between the smallest heat-sensitive and the largest cold-sensitive fibres.

In good agreement with these results is the absence of a motor effect to heat. Occasionally a just perceptible response is seen, but the effect is very rare. Heat might nevertheless stimulate the small fibres of the motor roots. LEKSELL's (1945) careful analysis in this laboratory of the motor gamma and delta fibres has shown very definitely that these fibres do not cause myographically measurable contractions. Their effect can only be observed indirectly as an increase of activity in the proprioceptive fibres, picked up in the sensory roots. For the time being the possibility that heat might stimulate these fibres must be left open.

Cold, however, does stimulate the motor fibres but the effects are generally much smaller than a maximal tetanus, unless there is evidence of hyperexcitability due to forced breathing. On the whole it seems as if the afferent fibres were a great deal more sensitive to thermostimulation than the efferent ones. Accommodation, which is greater in the motor than in the sensory fibres of the cat (SKOGLUND, 1942), may play a rôle in determining the magnitude of the effect.

C. v. EULER's extensive work (1947) with the same type of apparatus and mode of stimulation has demonstrated that warming elicits a very strong blood pressure reaction combined with the other components of the general reflex pain response: panting, struggling, pupil dilatation etc. Cold does not elicit this characteristic response pattern. Occasionally he noted a blood pressure increase to cooling but these cases were very rare. Within the efferent vegetative system responses to thermostimulation are practically non-existent. Some idea about the overlap may be obtained from C. v. EULER's experiments on the rabbit's depressor (fibres below about 5μ) in which a strong depressor reaction is elicited by warming and a small one by cooling. Size, on the other hand, need not be the sole determinant of the effects found in different types of nerve in different animals, though it gives a good first approximation. More experience is needed for detailed differentiation.

Nevertheless, it is desirable to summarize our present state of knowledge of this interesting property of nerve fibres in a tentative diagram such as that of fig. 2, to be modified and improved as experiences multiply. The region of overlap is placed around 5μ . The electrical records and the very strong blood pressure responses suggest a large component of C-fibre activity (RANSON and BILLINGSLEY, 1916; ZOTTERMAN, 1933; CLARK, HUGHES and

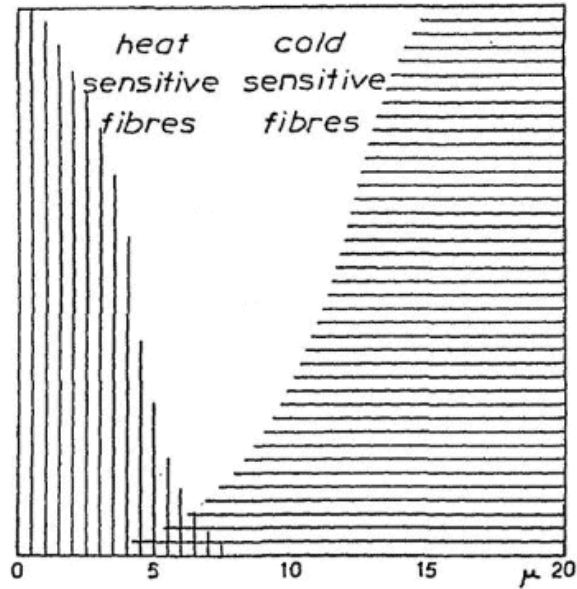


Fig. 2. Diagram illustrating heat- and cold-sensitive nerve fibres. Abscissae: fibre diameter in μ .

GASSER, 1935) but it will be remembered that blood pressure responses, respiratory reactions etc. also are obtained by stimulation of δ -fibres (HEINBECKER, BISHOP and O'LEARY, 1933).

We have reasoned as if the difference between the excitatory effects to cold and heat were altogether due to differences of excitation without mentioning the possibility that cold may inhibit impulse generation in small fibres and heat, *vice versa*, in large fibres. For the former view there is no evidence but from time to time we have seen a spontaneous discharge in large fibres be inhibited by warming. Occasionally one also finds a half maximum alpha spike sent through a warmed region to diminish in size. A record of inhibition of a spontaneous discharge is shown in fig. 3. Such records suggest that the mechanism of

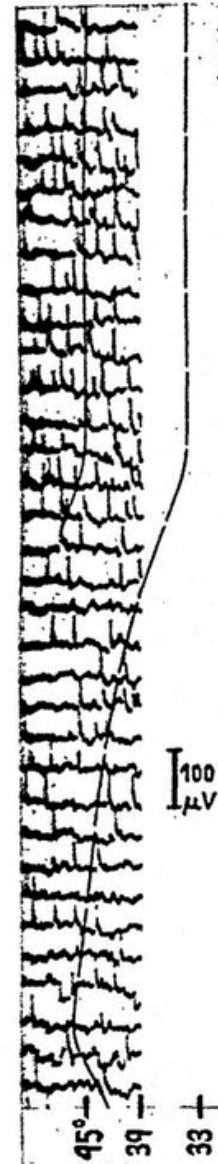


Fig. 3. Spontaneous discharge, picked up in thin strand of root filament, is diminished by warming thermode on sciatic. To be read downwards. Marked as fig. 1.

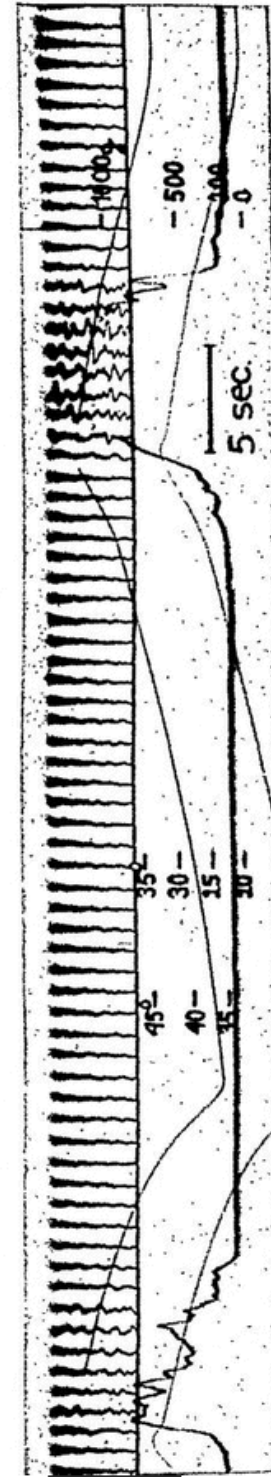


Fig. 4. The tibial muscle responding reflexly to thermostimulation of the ipsilateral popliteal nerve. Silver pins in the muscle record spike activity on vertical sweep (upper half). Temperature as in fig. 1. Note, separate scales for the two lines. Remaining record, myograph.

selectivation might be quite complex. BERNHARD and GRANIT (1946) also noted that warming sometimes inhibited a spontaneous discharge from the cut end. They did not at that time realize that heat and cold excite different fibres. BREMER and TITECA (1946) report that in frog nerves, uniformly heated in a paraffin bath, the large fibre component of a response to a shock disappears.

2. Some somatic reflexes in response to thermostimulation.

The flexor reflex. In fig. 4 muscle contraction and thermode temperature are recorded vertically, the electrical activity in the *tib. ant.* muscle (with large silver pins) horizontally. The thermode was applied to various nerves, generally to the popliteal branch of the sciatic. It is seen that warming elicits a strong reflex contraction throwing the myograph out of focus and out of its proportionality range whereas cooling has no effect whatsoever. The record is typical. Cold responses are, however, sometimes obtained. We conclude from our observations on the spinal cat that an ipsilateral flexor reflex is easily elicited by heat but difficult to excite, or at least less regularly excited by cold.

If some other nerve twig be used for simultaneous stimulation with electrical shocks, cooling may facilitate the synchronized reflex responses set up in this manner. The present technique, planned for a general survey only, is not suitable for following up such observations. The result is mentioned in order to draw attention to the significance of reflex background. Assuming cold to stimulate a few fibres of medium size, capable of setting up a flexor reflex, it will depend upon the background whether the effect becomes visible or not.

SHERRINGTON (1910) distinguished two types of flexor reflexes, one that was a component in locomotion, another of nociceptive character requiring stronger stimulation, probably therefore smaller fibres. Our impulse records combined with v. EULER's results suggest that local warming of a nerve is a fairly selective stimulus for the nociceptive flexor reflex, a fact that would seem to be of methodical interest from various points of view.

A crossed extensor. This reflex was studied in decerebrate cats, de-afferented on the side of the muscle tested so as to remove the myotatic appendage. The results were very uniform. Cold did not elicit any effects, although it sometimes facilitated a simultaneous electrically initiated crossed extensor reflex. But heat applied to the opposite sciatic nearly always excited a good

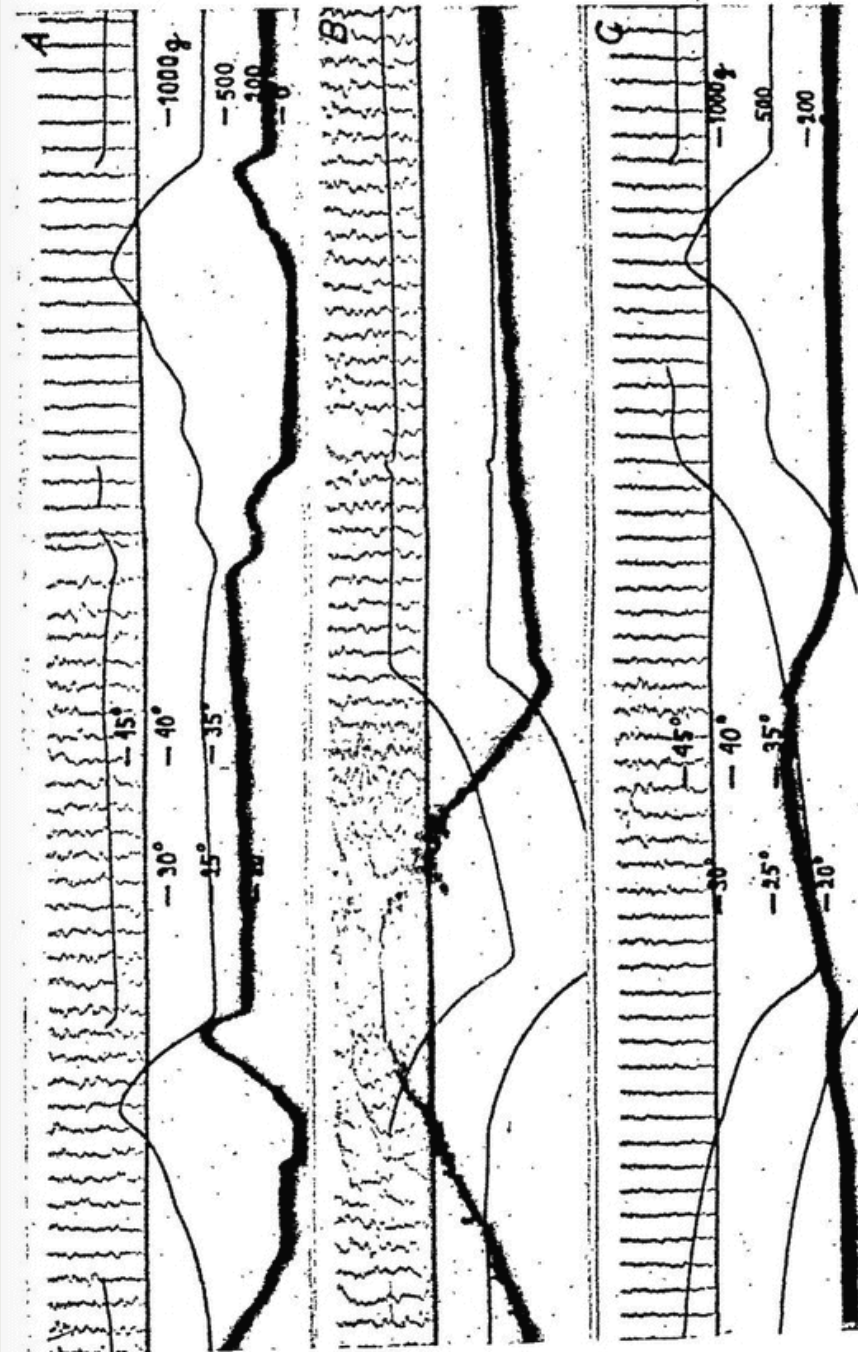


Fig. 5. Reflex and direct response of the gastrocnemius muscle to thermal stimulation of its own nerve. Marked as fig. 4. A. Warming of nerve, twice in succession. B. Directly continued from A, but this time response to cooling. C. Response to cooling of nerve to opposite gastroc. after deafferentation.

crossed reflex in the gastrocnemius muscle, thereby placing it in the small fibre range. Our results do not, of course, exclude the possibility that electrical synchronized volleys in large fibres could elicit crossed extensor reflexes but stimuli of this type are always more artificial than the slowly rising discharges set up by thermostimulation.

Reflexes with afferent and efferent path in the same muscle nerve.

This case is particularly well suited for a method that picks out the afferent small fibres without concomitant effects on the motor supply. Responses to heat will thus practically always be pure reflex in character whereas responses to cold will be combined, reflex *plus* direct motor. Theoretically it is simple enough in such cases to cut the motor nerve high up in the thigh after the test with cold, repeat the experiment and subtract the direct motor component. In actual practice this method is not always reliable since the response to thermostimulation rapidly deteriorates, particularly that to cooling, and so errors arise in estimating the reflex fraction of a contraction to cold. A more satisfactory way is to use the other side de-afferented and try the symmetrical muscle on that side as control. Both procedures have been employed.

In fig. 5 we can inspect the course of an experiment in which the thermode was on the medial and lateral nerves to the gastroc. muscle. It begins with record A, directly continued in B. It is first seen that heat elicits a small contraction followed by complete inhibition to the myograph zero. A rebound (hardly started as an off-effect in the nerve, since off-effects have only been seen after cooling as in fig. 1) follows the return of the temperature to the level of equilibration after which heating is repeated with the same outcome as before. Continuing the experiment in B we find cooling to begin at the moment when the rebound contraction has diminished so that the muscle again has approached its initial level of moderate tension, though the electromyogram indicates greater activity. The effect of cooling, both in the myograph and the electrical record, is so strong as to upset the whole system of recording. Record C shows the control of the pure motor component obtained with the de-afferented corresponding pair of gastroc. nerves on the other side of the animal. Heating causes no effect whatsoever, cooling a contraction of some 400 grams only.

The very characteristic inhibition to warming has only been seen in decerebrate cats, not in spinal ones. Hence it can hardly have been a peripheral inhibition in the nerve itself. Otherwise the result is typical in illustrating a large-fibre reflex to cold, probably coming from the large proprioceptives of the muscle spindles (SHERRINGTON, 1894) and a mixed response to heat initiated by small muscle afferents. Among known inhibitory effects in extensor reflexes upon the acting muscles themselves should be mentioned SHERRINGTON's well-known 'lengthening reaction' as well as the general effect noted by LEWIS (1942) upon injection of NaCl-solution in a muscle (see also, GELLHORN and THOMPSON, 1945).

M. tib. ant. responds reflexly to warming of its own nerve by contracting. The inhibitory component, which we found above in gastroc.-gastroc. stimulation with heat, does not appear, so that excitation must dominate for the flexors. The response to cooling is less regular, nevertheless often seen, and partly reflex in character. There are, of course, large afferent fibres in the flexors too (REXED, 1947).

In general the reflex thermoeffects are less regular than reflexes set up electrically and hence by synchronized impulses. The asynchronous thermal discharge has to withstand the full influence of slow accommodative counterprocesses and, besides, the thermally initiated impulses do not support each other by electrical forces in the same manner as those set up simultaneously.

Discussion.

As a complement to and in combination with the purely electrical methods of analysis thermostimulation may well prove to be of value in reflex work. A disadvantage in the methods utilizing electrical shocks is that one always is forced to pass from large to small fibres, when increasing stimulus intensity, and that it therefore is impossible to stimulate small fibres selectively without recourse to complicated and tedious methods of blocking of the larger ones. A reflex background of small fibre activity can easily be introduced by moderate local warming of a suitable nerve. We hope to return to this aspect of our work later.

Very intriguing is the question as to what constitutes this rather striking difference in sensitivity to heat and cold. BERN-

HARD and GRANIT (1946) thought that both heat and cold stimulate by means of the generator potential, set up by the thermic stimulation. In fact, they were led to their work by this particular and so far profitable theory. If this view is right it means that the heat and cold potentials, though of the same electrical sign, are set up in different fibres. C. v. EULER (1947) has made the valuable discovery that among the nerves studied by him some responded with a local drop of potential to heat, others to cold, others again to heat and cold. This will no doubt prove significant. We shall return to this aspect of the problem in another paper. The irregular inhibition of large fibres by heat suggests that selective stimulation of the small fibres by warming may be supported by a simultaneous depression in the large fibres. ZOTTERMAN (1936) has reported that *adequate* stimulation of the small fibres mediating temperature sensations may inhibit simultaneous excitation of the touch fibres, as when, for instance, a drop of cold or hot water is dropped onto the tongue (cat). The touch response may then be suppressed so that the temperature response appears alone.

Summary.

Records from thin strands of root filaments show a selective small fibre response to local warming and a selective large fibre response to local cooling of a nerve (cat) with a thermode.

The response to cooling is often accelerated into an off-effect when the thermode temperature suddenly swings back to normal.

The heat response is only seen in afferent fibres, the response to cooling is found in both efferent and afferent nerves, but better developed in the latter.

This being the case heat can be used for stimulation of the small-fibre component of a muscle nerve in order to study the reflex effect on its own muscle. This method has been applied for an analysis of such reflex responses upon *m. tib. ant.* and *m. gastroc.*

It has further been found that a flexor and a crossed extensor reflex are easily elicitable by heat but less regularly by cold.

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