## Transverse Stimulation of a Single Node of Ranvier by Middle-Frequency Sinusoidal Current Bursts

The stimulating action of middle-frequency current bursts does not depend on variation of polarity, because with longitudinal stimulation of nerve, the latency of the responding action current remains the same if the leads to the stimulating electrodes are interchanged[1,2]. This fact provides evidence that a 'principle of convertibility' underlies the stimulating action of middle-frequency current bursts, and predicts the possibility of carrying out ambipolar as well as true transverse stimulation[1-4]. It is obvious, however, that in the case of transverse stimulation of an intact nerve trunk, symmetry of the electrode tips in relation to all the interlying nodes of Ranvier cannot be achieved. The present investigation was therefore devoted to the study of transverse stimulation applied to a single node of Ranvier.

Single motor fibres of the nervus ischiadicus of *Rana esculenta* were isolated under Ringer solution[5] using the STÄMPFLI technique[6], slightly modified. Excitation of the node was controlled by observing the twitch contraction of the muscle fibres of the corresponding motor unit.

The stimulating electrodes were two platinum wires insulated except at the cut end, the conducting face of which had a diameter of 30 μm. The electrodes were moved by means of micromanipulators and their position relative to the node was observed through a binocular microscope (×30). The middle-frequency (20 kHz) current bursts[7] had a duration of 0.5 msec and could be increased up to 300 V peak to peak. The threshold intensity was measured on an oscilloscope over a series resistance of 100 ohms intercalated in the stimulation circuit. The upper limit of the stimulus strength was controlled by observing gas bubbles arising from the electrodes.

The electrodes were placed in the Ringer bath, symmetrically on either side of the node. In a first series of experiments, they were moved away from the node in radial direction (along an axis perpendicular to the axon) and threshold strength of the stimulation current was determined at 5 different positions 20–250 μm distant from the node surface. In a second series of experiments, the electrodes were shifted in longitudinal direction (15 μm away from and parallel to the axon), and threshold strength was measured at the level of the node, and at 3 further positions along the axon distant 25–75 μm from the first. The relationship between threshold strength and distance in both series of experiments was established by subtracting the variance between the individual single nerve fibres from the overall variance. The results are presented in Figures 1 and 2 (points). Figure 1 shows that the threshold strength in the first series increases progressively with the distance from the node. Figure 2, on the other hand, shows that the threshold rise occurring in the second series is at first slow, and then rapid.

In order to analyze these results better, measurement was made of the electric field produced by the two stimulating electrodes. For this purpose a model was built of the array ×500. The stimulating electrodes were suspended in an electrolyte tank, their conducting surfaces face to face. A bipolar silver-wire electrode (distance between tips 2 mm) was suspended in the midplane between the stimulating surfaces in order to measure the electric field between and perpendicular to the latter. The 5 positions of the first series of single fibre experiments were simulated by displacing the 2 stimulating electrodes symmetrically away from to the midplane, the 4 positions of the second series by displacing the recording electrode along a horizontal axis (parallel to the plates). The tip of the bipolar electrode therefore represented the site of the node in the fibre experiments. Current strength for the various positions simulated was in relation to a given value representing the electric field at the tip of the recording electrode ('node'). The weighted means of the current strengths obtained were then equated with those obtained in the fibre experiments. Figures 1 and 2

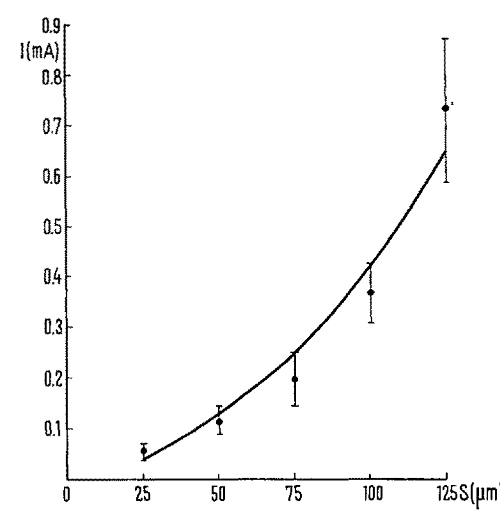


Fig. 1. Relation between threshold strength and distance of the electrodes from the node measured in radial direction. The points indicate average values from the single fibre experiments. Curve traced from experiments with the model.

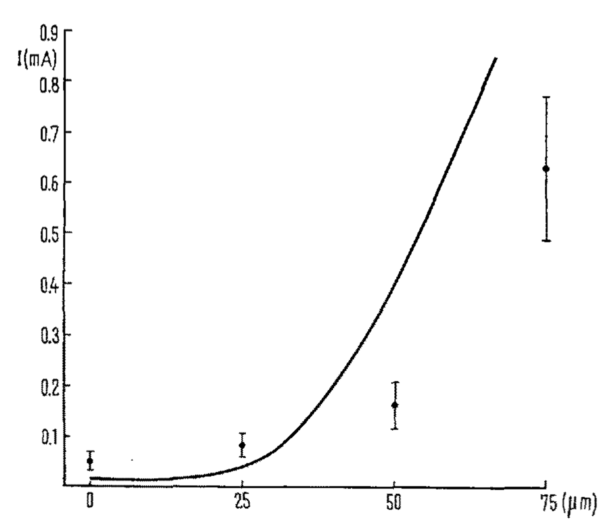


Fig. 2. Relation between threshold strength and distance of the electrodes from the node measured in longitudinal direction. The points indicate average values from the single fibre experiments. Curve traced from experiments with the model.

[1] O. A. M. WYSS, Helv. physiol. Acta *21*, 173 (1963).
[2] O. A. M. WYSS, Experientia *23*, 601 (1967).
[3] O. A. M. WYSS, Experientia *18*, 341 (1962).
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[5] I. TASAKI, Am. J. Physiol. *125*, 367 (1969).
[6] R. STÄMPFLI, Helv. physiol. Acta *4*, 411 (1946).
[7] O. A. M. WYSS, Helv. physiol. Acta *23*, 31 (1965).

show that the results obtained from the model (curves) give a satisfactory fit with those obtained from the fibre experiments (points). The slight deviation between model and fibre in the first series of experiments (Figure 1) can probably be attributed to distortion of the electric field caused by the presence of the nerve fibre in the live experiments. The relatively greater deviation between model and fibre in the second series of experiments (Figure 2) is probably due to the same factor, and to the fact that capacitative currents passing through the insulated electrode shafts modify the critical level (threshold) in an unknown manner.

Finally, using the data obtained from the model (and taking into account the conductivity of the electrolyte in the tank, etc.), the strength of the electric field at the node in the single nerve fibre experiments was established. Calculations, however, were only made for the first type of experiment (distance between node and stimulating electrodes in radial direction), since there was better agreement between live experiment and model in the first type than in the second (distance in longitudinal direction). The result gave a peak to peak amplitude of $50 \pm 16.5$ V/cm. Variance analysis of the value revealed that difference between the individual single nerve fibres could, however, not be established with certainty.

On the basis of the data presented, it can be concluded that middle-frequency current bursts evoke a true transverse stimulation of a node of Ranvier, and that the presence of longitudinal current components can be excluded.

The findings confirm previous observations on transverse stimulation of whole nerve trunk by means of middle-frequency current bursts[4]. They also show that a stronger electric field is required to attain threshold level when applying middle-frequency current than is the case when using conventional stimulation, namely, longitudinally applied direct current or low-frequency alternating current.

*Zusammenfassung*. Einzelne markhaltige Nervenfasern von *Rana esculenta* wurden mit Mittelfrequenz-Impulsen (20 kHz) quer durchströmt (Mittelfrequenz-Querreizung). Die Lage der Reizelektroden bezüglich eines Schnürrings wurde variiert, und die zugehörigen Reizschwellen wurden bestimmt. Die Simulierung der Experimente mit einem Modell zeigte, dass wirkliche Querreizung einer Nervenfaser im Bereich eines Schnürrings möglich ist, wobei für einen Schwellenreiz eine Feldstärke von $50 \pm 16.5$ V/cm (Scheitel zu Scheitel) notwendig ist.

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## Dietary Fat and Tissue Cholesterol in Female Rats

Coronary disease occurs in men significantly earlier in life than in women. Pathological evidence indicates that coronary atherosclerosis increases in occurrence, severity and extent to age 60 in men and 80 in women[1]. A search for a causal factor in the environment cast early suspicion on dietary fat[2], although Mayer[3] feels that the decreased physical activity of the American today, in comparison to that of 50 or even 25 years ago, may also play a significant part.

In earlier work we reported the effects of exercise on young, adult male rats fed high and low fat diets[4,5]. The effects of exercise on young, adult female rats fed high and low fat diets has not been adequately studied and this report is one attempt toward correcting this imbalance in emphasis between the sexes.

*Materials and methods*. The 35 rats used in this study were female Sprague-Dawley strain obtained at 100 days of age and they were held on a commercial stock diet for 1 week prior to the start of the study. 5 rats were then randomly selected and sacrificed in order for initial carcass and serum composition to be determined[5].

The 30 experimental animals were assigned in groups of 15 to one of 2 diets as described in Table I. The high fat diet contained around 60% of its calories as fat while the low fat diet contained only 20% fat calories. Approximately 24% of the added fat was provided by a polyunsaturated fatty acid source (corn oil), which is representative of U.S. 'market basket' diets. The source of carbohydrate in both diets was a mixture of carbohydrates simulating that found in U.S. 'market basket' diets[6]. Lactalbumin, mineral and vitamin content of the high fat diet was elevated so that equicaloric amounts of the 2 diets provided an equal intake of these nutrients.

One group of 5 rats on each diet were forced to swim for 30 min each day in 27 °C water with a weight equivalent to 2% of body weight attached to the tail of each rat. A second group on each diet were placed in wire restraining cages and immersed in 27 °C water up to their necks for 30 min daily. The third group on each diet were allowed to remain sedentary in their cages. After 8 weeks the 30 animals were sacrificed and changes in serum and carcass composition determined.

*Results*. Animals fed the high fat diet gained proportionately more fat (Table II) than those fed the low fat diet ($p < 0.01$). This despite the fact that the calorie intake for rats gaining similar ingesta-free weights on the 2 diets was nearly the same. This confirms earlier

Table I. Composition of high fat and low fat diets fed to forced-exercised, immersed and sedentary female rats

| | High fat (%) | Low fat (%) |
|---|---|---|
| Lactalbumin | 36.70 | 27.50 |
| Corn starch | 6.47 | 22.70 |
| Lactose | 3.23 | 11.35 |
| Dextrin | 1.62 | 5.67 |
| Sucrose | 1.62 | 5.67 |
| Glucose | 1.62 | 5.67 |
| Fructose | 1.62 | 5.67 |
| Corn oil | 9.30 | 2.30 |
| Beef tallow | 29.50 | 6.90 |
| Vitamin A and D concentrate | 0.07 | 0.05 |
| Cellulose | 2.00 | 2.00 |
| Salt mix, Jones and Foster[7] | 5.40 | 4.00 |
| Vitamin mix[5] | 0.85 | 0.52 |
| Total | 100.00 | 100.00 |
| Gross Calories (kcal/g) | 6.45 | 4.60 |