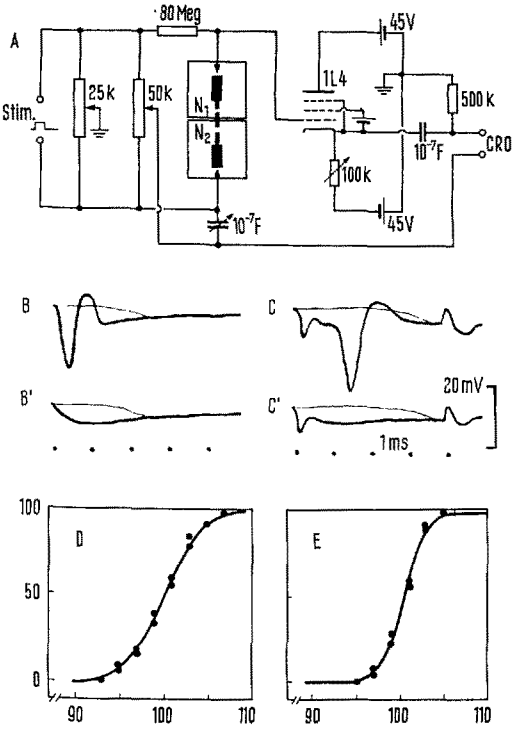
Fluctuations in Excitability of Single Myelinated Nerve Fibres

An electrical stimulus applied to an excitable tissue evokes an action potential if its strength exceeds a certain amount. At near-threshold intensities the excitatory effect is inconstant and successive identical pulses will either elicit or fail to elicit a spike[1]. When the trials are separated by intervals larger than the recovery cycle, the likelihood of triggering a spike in any given trial is not influenced by the previous stimulations. The successes and the failures are then distributed at random in the trials and the probability of response can be defined by the percentage of successes in a large number of trials[2-4]. The whole range of uncertainty considered can be explored by making trials with stimuli of various near-threshold intensities and by estimating the probability of response for each intensity. If such probability is plotted versus intensity, an S-shaped curve is obtained[2,5]. The steepness of this curve is related to the amplitude of the excitability fluctuation which can thus be studied and compared under various experimental conditions. The present paper deals with experiments in which amplitudes of excitability fluctuations have been compared for long and short current pulses. As suggested to me by Professor RIJLANT, some information could be obtained about the still unanswered question of the frequency spectrum of the excitability fluctuation. Indeed, if the mean period of the fluctuation and the effective duration of the stimulus are of the same order, the fluctuation amplitude must be reduced for the longer current pulses.

Single myelinated fibres were dissected from the sciatic nerve of *Rana temporaria*, following the technique of HUXLEY and STÄMPFLI[6] with some modifications. The nerve sheath is cut open with a microscalpel over a length of about 3 cm and all nerve fibres, except a large-sized one, are cut. The tissue surrounding one internode and the adjacent nodes of Ranvier is cleaned away. The internode is laid across a 0.5 mm air gap, between two pools of Ringer solution. Two Ag/AgCl electrodes connect the pools to a bridge circuit[7,8], designed to pass current pulses and to record the spike with adequate compensation for the artifact (Figure A). The experiments lasted up to six hours and by that time the spike remained almost unchanged, while the threshold had almost doubled. The cathode follower picks up action potentials of about 20 mV. The responses are diphasic, the downward wave originates in the node of Ranvier N_2. The second upward wave originates in the node N_1 and is triggered by the spike of N_2 (Figure A). Both nodes are immediately silenced by substituting isotonic KCl for the Ringer solution in the corresponding pool.

It was important to use a single nerve fibre preparation for the present purpose because, as was pointed out by several authors[9-11], the spikes of the neighbouring fibres affect the threshold of a given fibre to a far from negligible extent. This mutual disturbance varies at random in height and shape if the stimulus is within threshold range for a bundle of fibres and is bound to broaden the range of uncertainty of response. Indeed, some difficulties encountered in our first experiments and in those of VERVEEN[4], carried out on a whole nerve preparation, seem to have been solved by using single nerve fibre preparations.

At near-threshold intensity a 'short' square current pulse (0.1 msec) may elicit a spike in some trials (Figure B) while being ineffective in others (B'). In the same way the 'long' square current pulse (4 msec) of near-threshold intensity may either evoke (C) or fail to evoke (C') a spike. The random distribution of presences and absences of the spike was checked by applying the 'run test' of SWED and EISENHART[12], which indicates the number of runs of consecutive successes and of consecutive failures compatible (at the level of 0.05) with a random distribution, given the total number of trials and the proportion of successes. Under the conditions of the present experiments, this test was satisfied for stimulation frequencies of 3–4/sec or less, but a definite clustering of responses and of absences of response was observed at higher stimulation frequencies. Using square current pulses of a given duration, several near-threshold intensities were selected covering the whole range of uncertainty of response. A series of 100 identical stimuli of each intensity was applied to the fibre, from the lowest intensity to the highest and back to the lowest, as a check for reversibility. The responses occurring during each series were counted, their number



A, sketch of the experimental set-up for stimulation and recording in a single nerve fibre. B and C, tracings of cathode-ray oscillograms of responses evoked by near-threshold square pulses of 0.1 msec (B) and of 4 msec (C). Identical stimuli failed to elicit a spike in B' and C'. D and E, probability of response for the shorter (0.1 msec) stimuli in D and for the longer (10 msec) stimuli in E. Abscissa, stimulus voltage in % of threshold; ordinate, probability of response estimated on the basis of 100 trials for each point.

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giving in % the probability of response. This was plotted versus intensity of stimulus given in % of threshold. The points fit an S-shaped curve (Figure D and E). When the ordinates are subjected to the Probit[13] transformation, a straight line is obtained which shows the S-shaped curve to be the integral of a Gaussian distribution[2]. The sigmoid obtained for the longer current pulses (Figure E) is steeper than the one for the shorter pulses (D) in the same fibre. The amplitude of the excitability fluctuation is thus smaller when longer pulses are used. This result has been consistently observed in 11 nerve fibres in which the effect of square pulses of 0.1 and 10 msec has been systematically compared. Indeed, by applying Student's test by pairs to the experimental results, the difference proved significant at the level of 0.001.

Whatever the intrinsic processes governing the uncertainty of response near threshold, some conclusions about the frequency spectrum of the resultant fluctuation might be drawn from the experimental data. Of course, the really effective duration as a stimulus of the longer current pulses will be limited by accommodation. If the random changes in excitability were rather slow compared to the time scale of the electrical stimuli used, the duration of the latter would not affect their ability to trigger a spike and the probability of response would depend only on the level of depolarization produced at the membrane of the node (within the limits allowed by 'accommodation'). On the contrary, if the mean period of the random fluctuation in excitability was of the same order as the duration of the current pulses used, the probability for a stimulus inducing a given depolarization to trigger a spike would increase with its duration, because the longer depolarization has more chance to benefit from any random intermittent change in the membrane which facilitates the firing off. In other words, when the voltage of the stimuli is increased close to the region of uncertainty, a point is reached where the depolarization produced by either the longer or the shorter pulses triggers a response in only a few of the trials; further increase in depolarization will augment the probability of response more rapidly in the case of the longer pulse, hence the steeper S-shaped relation seen in E. Along this line, it can be suggested that the duration of the current pulses used is of the same order as the mean period of the fluctuation process.

No specific hypothesis can be elaborated from the data but it can be pointed out that such rather rapid random changes in excitability might be related to thermal agitation at molecular level[14].

Résumé. Il est possible de montrer des fluctuations aléatoires de l'excitabilité électrique de fibres nerveuses myélinisées isolées excitées au moyen de chocs proches du seuil. L'amplitude de cette fluctuation a été comparée pour des excitants rectangulaires de deux durées (0,1 et 10 msec); elle est plus grande quand on utilise des chocs brefs. Ces résultats suggèrent que les composantes de relativement haute fréquence du spectre de la fluctuation sont importantes.

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Reduction of Dynamic Sensitivity of Primary Muscle Spindle Endings in Experimental Tremor

The main sensing elements of the proprioceptive feedback system, primary muscle spindle endings, respond statically to amount, as well as dynamically to rate, of muscle length changes[1–3]. In a servomechanism of this type, the derivative component improves the stability of the whole system and counteracts inherent oscillatory tendencies[4–7]. Supraspinal muscle spindle activation modulates the dynamic properties of the spindle endings[8]. Furthermore, relatively independent fusimotor controls of their static and dynamic responses, respectively, seem to exist[7,9]. Hence, if some differential fusimotor action would predominantly reduce the dynamic sensitivity of primary spindle endings, this would, by analogy with technical feedback systems, favour oscillations in the proprioceptive feedback loop and could thus be one possible cause for tremor motor phenomena.

We have induced, in cats under steroid anaesthesia with 'Presuren'[10], a state of experimental tremor by perfusing the cerebral ventricular system with a solution of *d*-tubocurarine chloride[10] in artificial cerebrospinal fluid (5–100 μg/min)[11,12]. Methods and results of a detailed analysis of the mechanical and electrophysiological aspects of this type of tremor will be published elsewhere[13].

Here we wish to report on the responses of innervated muscle spindles from ankle extensors and flexors, recorded from filaments of centrally cut dorsal roots. Impulses conducted at velocities exceeding 72 m/sec were considered to originate from primary spindle endings. A

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