FLUCTUATION IN EXCITABILITY

A. A. VERVEEN

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RESEARCH REPORT ON SIGNAL TRANSMISSION IN NERVE FIBERS

BY

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"I returned, and saw under the sun, that the race is not to the swift, nor the battle to the strong, neither yet bread to the wise, nor yet riches to man of understanding, nor yet favour to men of skill; but time and chance happeneth to them all."

Ecclesiastes 9:11

"Wederom zag ik onder de zon, dat niet de snelsten den wedloop winnen, noch de sterksten den strijd, noch ook de wijzen het brood, noch ook de schranderen den rijkdom, nog ook de verstandigen de gunst, want tijd en toeval treffen hen allen."

Prediker 9:11

I. INTRODUCTION

1. Subject of the investigation

An electrical stimulus, applied to a living nerve fiber, may initiate an action potential in that fiber. The occurrence of a response 1) depends on, among many factors, the intensity of the stimulus.

When conditions are maintained as constant as possible, the relation between the intensity of the stimulus and the occurrence of the action potential shows a remarkable detail (Fig. 1). A stimulus whose intensity surpasses a specific value

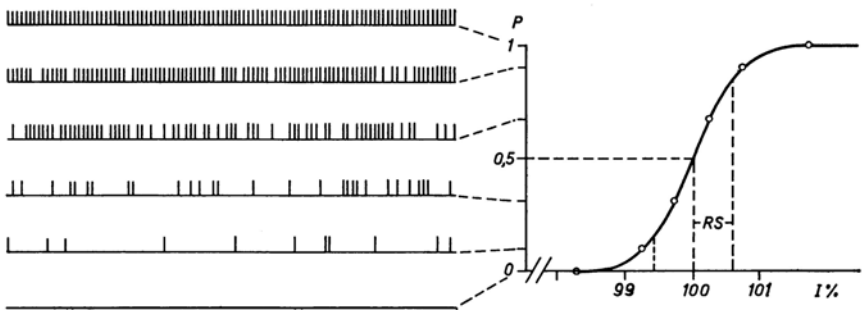

Fig. 1.
The relation between probability of response and stimulus intensity for a frog nerve fiber. Stimulus intensity in percentage of threshold. Stimulus given every 2 seconds.

nearly always produces an action potential in the nerve fiber; a stimulus with an intensity below another, lower, value practically never does so. The range between these intensity values may be called the threshold range. When a stimulus whose intensity falls within this range is given repeatedly with a sufficient long interval, an action potential will appear after some of the stimuli and will be absent following the others. The sequence of these responses is apparently a haphazard one. Within the threshold range the probability of response increases from almost zero to nearly one, when the intensity of the electrical stimulus is increased.

The existence of such a threshold range, viz., the existence of electrical

[1]) The appearance of an action potential following a stimulus is also called a 'positive response' or 'positive reaction'; this is mostly referred to simply as 'response' or 'reaction'; e.g., probability of response. In another context the action potential is also called 'output-signal' or 'output'. When an action potential does not occur after the application of a stimulus, this is always indicated by 'negative' or by 'no'; e.g., negative reaction; no output-signal.

stimuli having only a certain probability of producing an action potential in the nerve fiber studied, might indicate that the nerve fiber is subject to spontaneous and unpredictable changes in excitability the so called 'fluctuation in excitability' (Pecher, 1937).

The aim of the research described in this monograph is a further analysis of this uncertainty phenomenon.

2. Historical Outline

Blair and Erlanger (1932) observing the fluctuation in excitability of the frog nerve (fiber, noted that the succession of positive and negative reactions appears to be distributed at random, but only when a low frequency of stimulation is used (usually 1 every 2 seconds).

Together with the fluctuation in excitability, a variation is seen in the interval between the initiation of the stimulus and the passage of the action potential at the part of the nerve fiber below the recording electrodes (Blair and Erlanger, 1935, 1935/36b). This fluctuation in response-time occurs at the point of stimulation as a variation in latency. It is independent of the site of the recording electrodes on the nerve fiber (Blair and Erlanger, 1933).

It was also noted that a relatively weak stimulus occasionally produces an action potential; when the intensity is increased the number of positive responses is more frequent. To obtain a positive response to every stimulus, the stimulus intensity has to be increased by 2-10 % of its initial value (Blair and Erlanger, 1933, 1935/36b).

In preparations with two, approximately equally excitable fibers, Blair and Erlanger observed that the responses in one fiber are independent of those in the other fiber {Fig. 2). They concluded that the fluctuation in excitability is not due to external causes, but to a spontaneous and independent fluctuation in the excitability within each of the two fibers (1933).

In 1932 Monnier and Jasper also observed the fluctuation in the excitability of the frog nerve. They claim that the phenomenon is probably subject to the laws of chance.

In the period between 1936 and 1939 Charles Pecher made a detailed study of this subject in frog nerve fibers. He considered fluctuation in excitability a fundamental property of nervous tissue.

In his earlier work (1936) he attempted to determine whether the succession of positive and negative responses to identical stimuli is distributed randomly. As a criterion for randomness he used the linearity of the relationship between two variables: The first variable being the number of responses in a group of positive (or negative) responses, bordered by two negative (or positive) responses
a so-called 'run'. The second variable is formed by the logarithm of the number of 'runs' of this size. In an analysis of a series of 3.764 stimulations, he found a linear relationship between these variables both for the positive and negative runs. He concluded that there is a random distribution of positive and

negative responses to identical stimuli. He also reported that linearity of the relationship only exists when the successive stimuli are applied at an interval of one second or more.

In a later study, Pecher discussed the relationship between the fluctuation in excitability of two, almost equally excitable fibers (1937). Similar to the findings

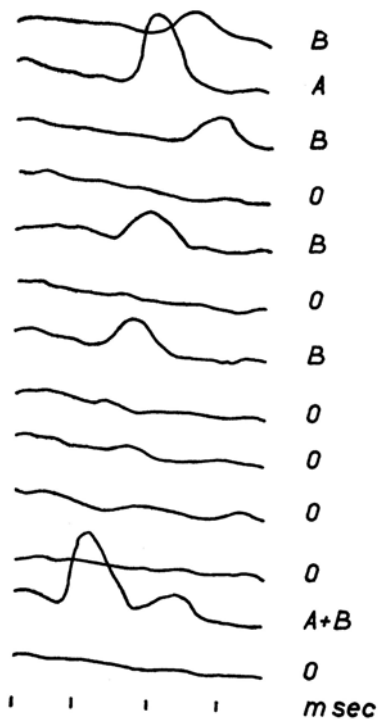

Fig. 2.
Responses of a two-fiber preparation.
Redrawn after Pecher (1939).

of Blair and Erlanger (1933), Pecher observed that repeated stimulations reveal all possible combinations of responses (Fig. 2). At times, both fibers show no responses, while at other times one, occasionally the other and sometimes both respond to one and the same stimulus. If a variation in external circumstances, e.g., stimulus intensity, was responsible for this phenomenon, it would be impossible for the fiber with the higher threshold to discharge alone. If the fluctuation in excitability in the one fiber is independent from that in the other fiber, then the probability for joint discharge must be equal to the product of the response probabilities for each fiber. External influences would then give a certain degree of correlation between the responses of the two fibers. This had not been revealed by the above-mentioned observations. Therefore, Pecher resorted to a numerical analysis. The results of his observations have been

reproduced in Table I. These data, corresponding to the hypothesis of 'no correlation' enabled Pecher to assume the mutual independency of the fibers analyzed (1937, 1939).

He then observed that the probability of a positive response (referred to as 'probability of response') is variable and depends on the intensity of the stimulus (1936, 1939). Pecher described the relationship between probability of response and stimulus intensity as having the shape of a symmetric sigmoid curve

TABLE I

Fluctuation in excitability
in two-fiber preparations of the frog
After Pecher (1939)

exp.	N	A	B	A+ B	AB/N
1	100	78	25	19	19.5
2	188	129	26	18	17.8
3	285	205	33	18	23.6
4	222	150	79	56	53.4
5	370	214	93	50	53.7
6	194	113	34	19	19.7
7	155	110	62	40	44.0
8	218	168	87	59	66.5
9	236	152	24	17	15.5

N number of stimulations
A number of responses of fiber A
B number of responses of fiber B
A + B number of simultaneous responses
AB/N expected number of simultaneous responses, calculated under the assumption of independency

(Fig. 3). The graphical representation of the first derivative resembles a bell-shaped curve, similar to that of the Gaussian or normal density function (Fig. 4) [2]).

Since Pecher's last publication, no systematic study has been presented dealing with the characteristics and nature of the fluctuation in excitability of nerve fibers following electrical stimulation. A few indirect studies on this subject appearing at a later date will be dealt with in the appropriate sections. Fluctuation in excitability upon stimulation of specific receptors has been studied more extensively:

Katz (1949, 1950a, 1950b), Buller, et al. (1953) and Hagiwara (1954) investigated the responses of muscle spindles to stretching. Frishkopf (1953, 1956) and Rosenblith (1954) studied probability phenomena in auditory nerve fibers following acoustic stimulation. FitzHugh (1957, 1958) and ten Doesschate *(1958)* studied response probabilities of single ganglion cells in the retina to illumination.

2) This function, usually ascribed to Gauss, was originally utilized by de Moivre and by Laplace Feller, 1957; Freudentahl, 1957).

Probability phenomena are also part of McCulloch's theories on the functional organization of the brain (1958, 1959a and b, 1960).

All these investigations support Pecher's concept, that the excitability of neural elements is subject to an endogenous fluctuation.

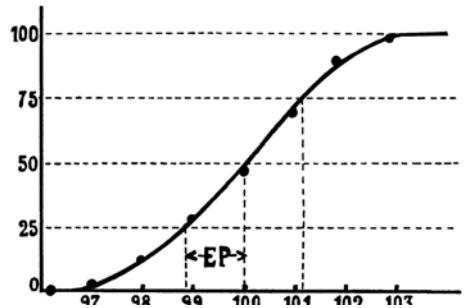

Fig. 2 - Relation entre l'intensité d'excitation (en abscisse) et la probabilité de réaction exprimée en nombre de réactions pour 100 excitations (en ordonnée).

Fig. 3.
The relation between probability of response and stimulus intensity for a frog nerve fiber.
Reproduction of the figure presented by Pecher in 1939.

3. Aim of the investigation

The aim of this investigation has been the further elucidation of fluctuation in excitability with regard to the function of the nerve fiber the transmission of signals.

The nerve !fiber is considered a signal-transmitting unit possessing the following properties:

Due to the arrival of a stimulus, also termed the 'input-signal' or `input', the stimulated part of the nerve fiber is activated. When the activation results in a discharge (the occurrence of which is determined by certain intrinsic properties of the nerve 'fiber), the resulting action potential, the 'output-signal' or `output', is conveyed along the nerve fiber and is registered. From the historical outline it becomes evident that an `uncertainty' enters the picture. With an input-signal of known parameters it is, in principle, impossible to predict, aside from the probability of response, whether or not an action potential will occur. The unit is said to exhibit a fluctuation in excitability.

The problem here is one concerning the characteristics of this process; an attempt will be made to elucidate this in a step-wise manner.

To this end the investigation has been formulated to find answers to the following questions:

1. Can the successive reactions of the nerve fiber to repeated, low-frequency application of a stimulus with fixed parameters, be considered to occur on each trial with an equal and independent probability of response? (Chapter III, la, b; Chapter IV).
2. Does Pecher's statement concerning the type of the relationship between

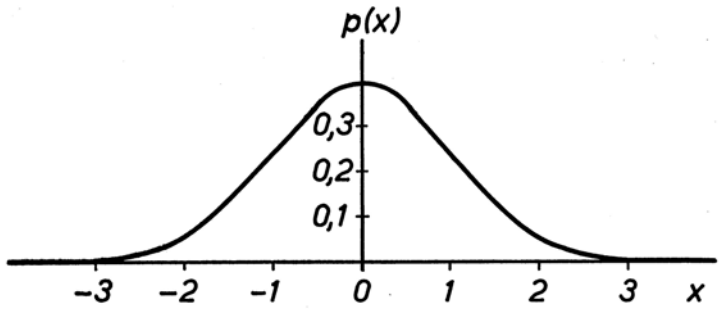

Fig. 4a.
The Gaussian or normal density function.

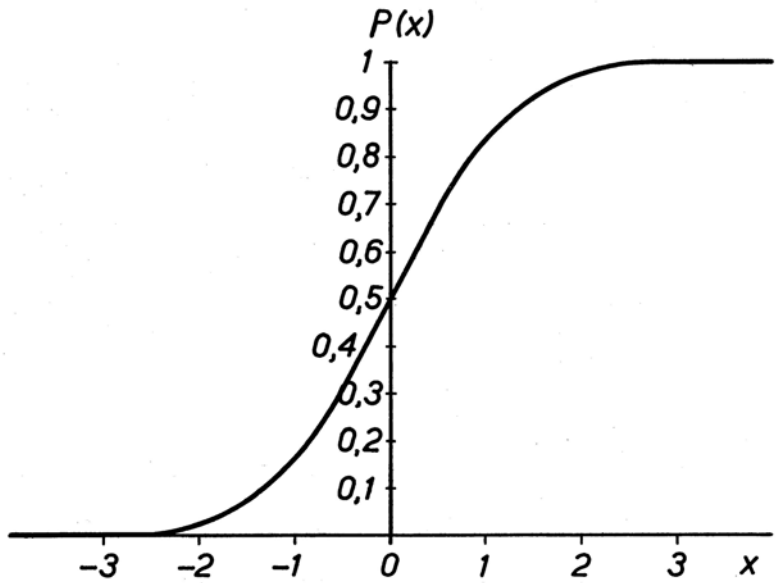

Fig. 4b.
The Gaussian or normal distribution function.

stimulus intensity and probability of response hold true here? This necessitated investigating whether the Gaussian or normal type of distribution function can be considered the working hypothesis for this relationship. (Chapter III, 1b; Chapter IV).

3. What type of relationship exists between stimulus duration and probability of response? Is it possible to describe the relationship between stimulus intensity, stimulus duration and probability of response? What information concerning the characteristics of the nerve fiber can be derived from it? (Chapter III, 1c; Chapter IV; Chapter V).
4. What can be determined concerning the function of the intrinsic characteristics of the nerve fiber with respect to the input-output relationships? This involved investigating the influence of conditioning stimuli, for both a subrheobasic current and for an above-threshold stimulus, as well as the effect of strychnine and urethane. (Chapter III, 2, 3; Chapter V).
5. Do the characteristics of fluctuation in excitability apply to nerve (fibers of species other than the frog (Chapter IV), and to neural structures other than peripheral nerve fibers? (Chapter VI).

A preliminary communication on some of the results has already been reported (Verveen, 1960).

II. MATERIALS AND METHODS

1. Nerve preparations

The observations were made on myelinated nerve fibers in the sciatic nerve of the frog (Rana esculenta), and on unmyelinated nerve fibers in the cheliped of the European cray-fish (Astacus leptodactylus).

a. Amphibian sciatic-phalangeal nerve preparation (Rana esculenta)

A modification of the sciatic-phalangeal preparation described by Blair and Erlanger (1933) was used throughout all experiments. After decapitation both sciatic nerves are prepared under Ringer's solution [1]). The sheaths of the two main lumbosacral rami are circumcised at their entrance to the vertebral column; by gently pulling the nerves the roots appear. The nerve and its branches are then prepared as far as the anterior phalangeal branches. A cotton thread is tied around the end of each branch. The preparation is stored in Ringer's solution at a temperature of 0.5 ° C.

Prepared in this manner, the nerve will remain in good condition for at least one week. Nevertheless, the experiments were made during the first four days; within this period no differences in electrical behavior are detectable.

Prior to performing the experiments the nerve preparation is mounted in the following manner: The cotton threads are spread over a plastic frame. Only the nerve preparation remains in the Ringer's solution. A similar frame is then screwed on the former, to fix the threads. Thereafter the frame with the nerve is placed in a tray containing a layer of mineral oil. The branches of the nerve are then spread by gently pulling the threads apart and fixing these in position by tightening the screws.

The thickness of the paraffin layer above the nerve preparation is approximately 4 mm. No specific aerating procedures were employed.

A plastic sheet on which the electrode-systems are mounted is screwed to the tray. When a suitable action potential is registered (cf. page 11), a cover is screwed over the electrode-systems. The whole set-up (water-tight) is then placed in a water-bath maintained at a temperature of 20° C.

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[1]) Composition of the Ringer's solution:

NaCl	6.5 gm	$NaH_2PO_4.2H_2O$	0.013 gm
KCl	0.14 gm	glucose	2.0 gm
$CaCl_2.6H_2O$	0.236 gm	aqua ad	1000 cc
$MgCl_2.6H_20$	0.011 gm	pH ± 7.2	
$NaHCO_3$	0.20 gm		

b. Crustacean nerve preparation (Astacus leptodactylus)

The observations on unmyelinated nerve fibers were made on the most excitable fibers in the nerve from the cheliped of the cray-fish.

The animal is forced to autotomise the cheliped. Thereafter, the nerve is prepared under a physiological Astacus solution [2]). After removing the shell with the accompanying muscles of the ventral part of the cheliped two nerves are seen: a thick one containing the axons to the muscles closing the claw and a thinner one innervating the muscle opening the claw (Segaar, 1929). The former is dissected out and split into its principal bundles. Cotton threads are tied around both ends of each bundle. The preparations are then stored in Astacus solution at a temperature of 0.5° C, where they remain in good condition for at least four days. The experiments were performed on the second day. A similar mounting procedure as used with the frog nerve is employed. The temperature is maintained at 15° C.

2. Stimulating and recording systems

a. Electrodes

Stimulating electrodes. A symmetrical tripolar electrode system is used, in order to stimulate exactly at the site of the central electrode: the cathode (Rashbass and Rushton, 1949). In preliminary experiments Ag-AgCl electrodes were used. Since no differences in results could be detected between these electrodes and electrodes made of tungsten wire (probably because of the small currents used, cf. Silver, 1958), the tungsten electrodes were preferred. They possess good mechanical properties, are easy to handle and clean and remain intact for long periods of time.

The electrodes are made of tungsten wire (diameter 0.5 mm) which has been wound to form a tube. The central electrode consists of a single winding, the peripheral electrodes six windings each. The diameter of the inner core is 0.7 mm. The three electrodes are then fixed in plastic, forming one cylindrical electrode system. The distance between the central electrode and each peripheral electrode is 3 mm.

Using a cotton thread, one of the two rami of the frog nerve is pulled through this cylindrical electrode system. The thread is then fixed in the plastic frame, and the electrode system can be moved along the ramus by means of a micromanipulator.

Recording electrodes. The recording system for the frog nerve consists of two

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[2]) Composition of the Astacus solution, prepared according to the ionic composition of Astacus blood, determined by Scholles (1933):

NaCl	9.7 gm	$NaH_2PO_4.2H_2O$	0.04 gm
KCl	0.39 gm	glucose	2.0 gm
$CaCl_2.6H_2O$	2.26 gm	aqua ad	1000 cc
$MgCl_2.6H_2O$	0.53 gm	pH ± 7.2	
$NaHCO_3$	1.20 gm		

pairs of silver electrodes. The distance between the two electrodes of one pair is 2 mm. Each pair is mounted in a metal husk, movable up and down; in a ball-and-socket joint. In this manner, each pair of electrodes can be moved independently along the phalangeal branches.

The distance between the stimulating electrode system and the pairs of recording electrodes varies from 10 to 15 cm, according to the length of the nerve preparation.

The cray-fish nerve preparation. The stimulating electrodes used are the same as for the frog nerve. The recording electrode system consists of two tungsten wire electrodes, with an inter-electrode distance of 3 mm. This system is mounted at a distance of about 4 mm from, the stimulating electrode system.

b. Stimulating and recording apparatus

A stimulator was built which delivers negative rectangular currents of variable duration. The current intensity can be varied in small steps. With each step the current stimulating the nerve fiber is changed by a certain percentage of the threshold current intensity. This is achieved in the following way.

The voltage of the screen grid of a current delivering pentode is changed in steps, by mounting a switch with a set of equal resistors in the potential divider. The value of the resistors is so chosen, that the switching in or out of each resistor produces a change in output current intensity of the tube of 0.25 μA. Only the linear part of the tube characteristic is used. With an initially fixed output current of 100 μA, this amounts to a change in intensity of 0.25 % per step. When the given current is 400 μA, each step brings about a change in intensity of 0.6 $^{0}/_{00}$. The drift of the current is at most 1 μA per hour.

With the output of the stimulator thus fixed, it is necessary to stimulate the nerve with a potentiometer placed in parallel to the nerve. The threshold of the chosen nerve fiber in the preparation is determined by adjusting the value of this shunt. These values are read in scale divisions.

In the case of combined stimulation, two stimulators of the same type are used. The interval between the onset of the two pulses can be varied.

The two leads of a pair of recording electrodes are fed into a differential input amplifier of the construction type developed by Bok (1950, 1955). This amplifier is used as a preamplifier for a Tektronic 502 dual-beam oscilloscope. The responses are recorded by counting from the screen.

Specific technical procedures will be described later.

c. Variability of the stimulus

Changes in intensity or duration of the stimulus will alter, accordingly, its stimulation value. Checking the stability of the stimulus is made possible by the phenomenon of mutual independency, in two-fiber preparations. Pecher's experiments on preparations with two, almost equally excitable fibers revealed that the fibers react independently to the application of one and the same

stimulus. Rapid changes in the stimulus will give a correlation between the response probabilities of two, approximately equally excitable fibers. Therefore, when such a preparation was obtained, this was used to check the reliability of the stimulating apparatus. No significant correlation was detectable between these response probabilities (cf. Table XII). Since the drift is also very small, variability of the stimulus is, therefore, eliminated as a significant factor.

3. Nerve fiber preparation

a. The functionally isolated nerve fiber

Since the phalangeal branches of frog and the nerves of crayfish contain a few axons of larger diameter (Young, 1936), it is possible to study the electrical behavior of single nerve fibers in detail, because they are the most excitable ones. These fibers will be the first to react to the application of a stimulus to the nerve. At the recording site the differentiation between fibers is possible by observing the differences in conduction time between the fibers, the differences in amplitude and the form of the action potentials.

In this way it is nearly always possible to obtain the response of a single nerve fiber in the preparation and to study its input-output relations with regard to the stimulus applied to the nerve. Therefore, when reference is made to the nerve fiber investigated (which is either the Ranvier node of the frog or the unmyelinated axon of the crag-fish) this applies to the nerve fiber preparation described here.

b. The functionally isolated node of Ranvier

Within the stimulated ramus of frog nerve a spatial difference in excitability of the chosen A-fiber is present, due to the existence of the nodes of Ranvier.

In preliminary experiments the stimulus was applied to one of the roots from nerves of large frogs. This permitted comparing the internodal distances on this root, with its much thinner connective tissue sheath, with the data obtained from the sheathed ramus. It was found that the internodal distances, determined by a modification of Lussier and Rushton's method (1952), are comparable (Fig. 5). The excitability between the adjacent nodes of Ranvier is very low. Though this is more marked for the 'sheathless' roots it still is considerable for the sheathed ramus. Owing to the fact that the excitability is low between the nodes, and because the most excitable nerve fiber in the ramus is used, stimulated at the most excitable place along the ramus, it is clear that in each case only one node of Ranvier of the chosen A-fiber is stimulated. That single nodes, indeed, exhibit a fluctuation in excitability follows from the experiments of del Castillo-Nicolau and Stark (1951) and from those of Huxley and Stämpfli (1951) on anatomically isolated single nodes of Ranvier of the frog; this has also been reported by Tasaki (1959).

It must also be pointed out that no gross functional difference with respect to the fluctuation in excitability is found between the portions of a nerve with or without a sheath. These aspects have not been analyzed quantitatively.

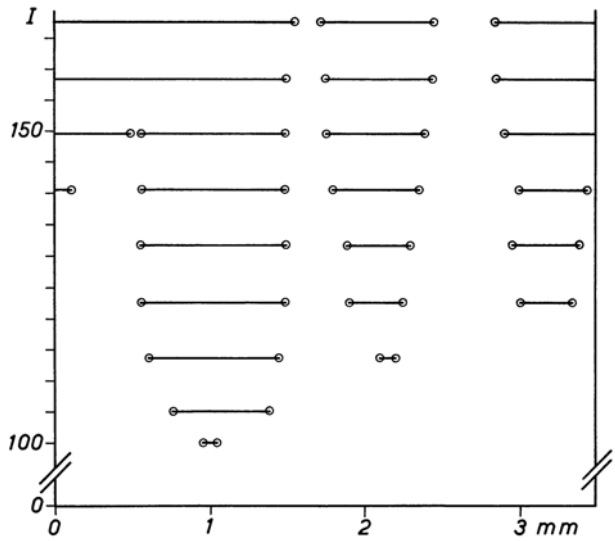

Fig. 5a.
The relation between the excitability of a functionally isolated single nerve fiber of the frog and the site of the cathode on the nerve:
a. determined for a sheathless root.

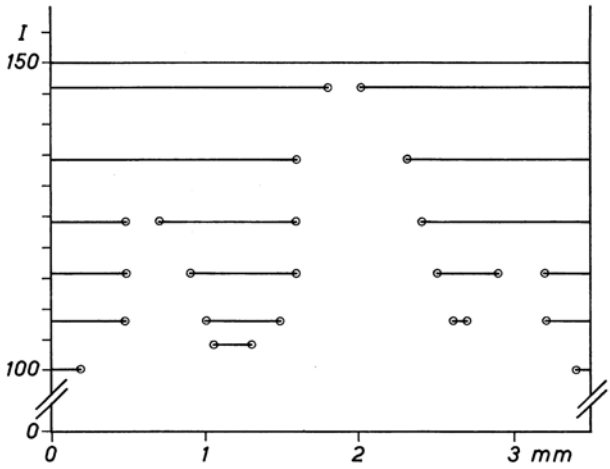

Fig. 5b
b. determined for a sheathed ramus.

4. Statistical procedures

a. Introduction

This study is based on the supposition that a random process model (cf. Siebert, 1959) is applicable to the input-output relations of nerve fibers.

The question is which model fits the relationship best, and what aspects and properties are to be ascribed to the model. This is answered in a step-wise manner; the next characteristic is investigated only after the applicability of the preceding one is made feasible, which one is called a working hypothesis.

To arrive at such a working hypothesis use is made of specific statistical techniques (cf. Bok, 1948; de Jonge, 1958, 1960), because the data obtained from each fiber are estimates of the supposed properties of the fiber investigated. In turn, the series of fibers investigated is a sample of the population of nerve fibers for which this property is supposed to apply. The specific statistical tests required to investigate the applicability of such a property were chosen and the data collected. The selection of the appropriate tests was made in conjunction with the Mathematical Centre at Amsterdam, were the calculations from the data were performed [3]). A level of significance of 5 % is used in all the tests. In this manner a hypothesis concerning a property is considered a 'working hypothesis' when no contradictory evidence is compiled.

In the text no distinction is made between the estimates of a property and the property itself. Confusion is, however, not likely, since the tables contain the estimates and because deductions are based on the working hypotheses.

b. Statistical procedures

Three statistical procedures were practiced for analysis of the data.

The first procedure used is *the run test* (cf. Swed and Eisenhart, 1943; de Jonge, 1958). This test is utilized to investigate whether the successive reactions to repeated stimulation with identical stimuli appear in such a way, that the probability of response has the same value for every trial, independent of the preceding reactions. This is made on series of exactly 100 responses, with complete runs at the beginning and end of each series. The test is then employed under the null-hypothesis of equal and independent probabilities for the occurrence of a positive response to every trial within that series.

The series investigated were obtained by prolonging the registrations for some time after the first 100 reactions. The first series of 100 responses with complete runs was then submitted to analysis. The number '100' was taken to simplify calculations.

The second procedure used is *the probit analysis* (Finney, 1947). This

3) Many thanks are due to Ir. A. R. Bloemena and T. Harkema for the statistical analysis and to K. J. Arwert for computations (Mathematical Centre at Amsterdam, Statistical Department; contract number 1961-38).

analysis is applied to each of the sets of data, covering the relations between probability of response and stimulus intensity.

The method is based on a transformation of the Gaussian distribution function in such a way that the function is made linear. The method is used to determine whether the Gaussian type of distribution function can be considered to be a working hypothesis for the relationship between probability of response and stimulus intensity. In this transformation the inverse of the standard deviation appears as the slope of the transformed function. Owing to the technique used, the reciprocal of the slope is not an estimate of the standard deviation, but of the coefficient of variation (cf. page 18).

Each set of data was obtained in the following way: The shunt is adjusted to such a value that upon stimulation the nerve fiber reacts with a probability of about 50 %. This shunt value is then an estimate of the threshold. Thereafter, the stimulus intensity is varied in steps, each step equal to a given percentage of the threshold value. The threshold range is scanned in this way, until, unless otherwise stated, 25 stimuli are given at each step. The total number of positive reactions per step is noted. The total number of steps is at least 7, which is achieved by choosing a percentage value for the steps of about 0.25 % for frog and $0.6\ ^{0}/_{00}$ for crag-:fish axons [4]).

The steps are small with respect to the value of the mean of the distribution function, the threshold. Therefore, the shunt value (or a direct reading in μA) is considered to be a sufficient estimate for the value of the threshold.

The third procedure used is *Wilcoxon's test for symmetry,* also called the 'signed rank test' (cf. Benard, van Eeden and Rümke, 1957; de Jonge, 1958). This test is applied to series of paired estimates, under the null-hypothesis that the differences between the members of each pair, taking their signs into account, are distributed symmetrically around zero, i.e., whether there is a detectable difference within the pairs or not. The test is used in those cases in which the influence of a certain agent on some parameter is investigated.

4) The initial position of the switch is always at step 10 (cf. Fig. 6). For frog nerve fibers the calculated values for the relative spreads (the coefficients of variation) are presented in the tables in double steps.

III. PROBABILITY PHENOMENA IN FROG MYELINATED NERVE FIBER

1. Stimulation with single pulses

a. Successive reactions to repeated stimulation with identical pulses

Firstly, it is necessary to verify Pecher's conclusion that the succession of positive and negative responses is distributed at random. This involves determining whether the probability for the occurrence of a positive response has the same and independent value for each trial.

Continuous series of responses upon stimulation with identical pulses of about threshold intensity and with a frequency of one stimulus per two seconds were registered from 10 nerve fibers. One series consists of 700 stimulations, the other 100 stimulations each. The results are presented in Table II.

TABLE II

Application of the run test
to continuous series of reactions for 10 frog nerve fibers

exp.	N	m	u	μ	T	k
1	700	409	341	341.1	0.00	1.00
2	100	59	46	49.4	0.60	0.55
3	100	76	39	37.5	0.28	0.78
4	100	46	58	50.7	1.38	0.17
5	100	37	38	47.6	1.97	0.05
6	100	72	37	41.3	0.95	0.34
7	100	36	50	47.1	0.53	0.60
8	100	50	47	51.0	0.70	0.48
9	100	49	45	51.0	1.10	0.27
10	100	73	38	40.4	0.49	0.62

N number of stimulations
m number of positive responses
u number of runs
μ expected number of runs
T test statistic
k probability of exceedance

The last three columns show the data from the application of the run test. This test was employed under the null-hypothesis of equal and independent probabilities for the occurrence of a positive response within each series. The results in the last column do not reveal any evidence disproving the null-hypothesis. The working hypothesis is, therefore, that following stimulation at

a low frequency and with identical stimuli the probability of response, i.e., the probability for the occurrence of an action potential, has the same value each time; independent of the preceding reactions. Hence, in all experiments on frog nerve a stimulus interval of 2 seconds is used.

b. Relationship between probability of response and stimulus intensity

Altering the intensity of the stimulus, the probability of response changes accordingly. The relation between the probability of response and the stimulus intensity with a fixed stimulus duration may possibly be described by the Gaussian type of distribution function, as was noted by Pecher. This hypothesis was tested on 18 nerve fibers. For each nerve fiber this relation was determined with both a stimulus of short (0.25 msec) as well as of a longer duration (2.5 msec). The reason for applying stimuli of two different durations will be given in the next section.

Each set of observations was made with a total of 50 stimulations per step. The value of each step was 0.45 % of the initially determined threshold (cf. page 14). The total number of 36 sets were subjected to the probit analysis in order to confirm the hypothesis of a Gaussian type of distribution function. These sets of observations, after the probit transformations, had to fit to straight lines. Within statistical limits this appeared to be the case for each set (cf. Fig. 6). Since no evidence is compiled to disprove the Gaussian type of distribution function, this type of function can now be accepted as a useful working hypothesis for the relation between probability of response *P (A = 1)* and stimulus intensity (c) for stimuli of a ;fixed duration (z), whether this duration is short or long.

Accepting this hypothesis it is possible to describe the threshold range more exactly (Fig. 1) by the use of the Gaussian distribution function (Fig. 4). The equation to be used here for the function is:

$$P(A=1|\iota,\tau) = \frac{1}{\sigma(\tau)\sqrt{2\pi}} \int_{-\infty}^{\iota} \exp\left\{-\tfrac{1}{2}\left(\frac{x-\mu(\tau)}{\sigma(\tau)}\right)^2\right\} dx$$

The function can be characterized by its mean $\mu(\tau)$ and its standard deviation $\sigma(\tau)$.

The mean and median $\mu(\tau)$ *is the 50 % stimulation threshold,* called in short *the threshold. This* is in accordance with the arbitrary definitions of threshold by Erlanger and Blair (1935/36a) and by Hodgkin and Rushton (1946).

In this study, 'threshold' has been defined as follows:

The threshold of a nerve fiber preparation for stimulation with negative electrical currents of a fixed duration is that intensity of the applied stimulus to which the nerve fiber reacts with a probability of 50 %, after stimulation at a frequency that the successive responses are not correlated.

The standard deviation $\sigma(\tau)$ is called *the spread.*

c. Strength-duration-probability relation

The probability of response does not depend on stimulus intensity alone,

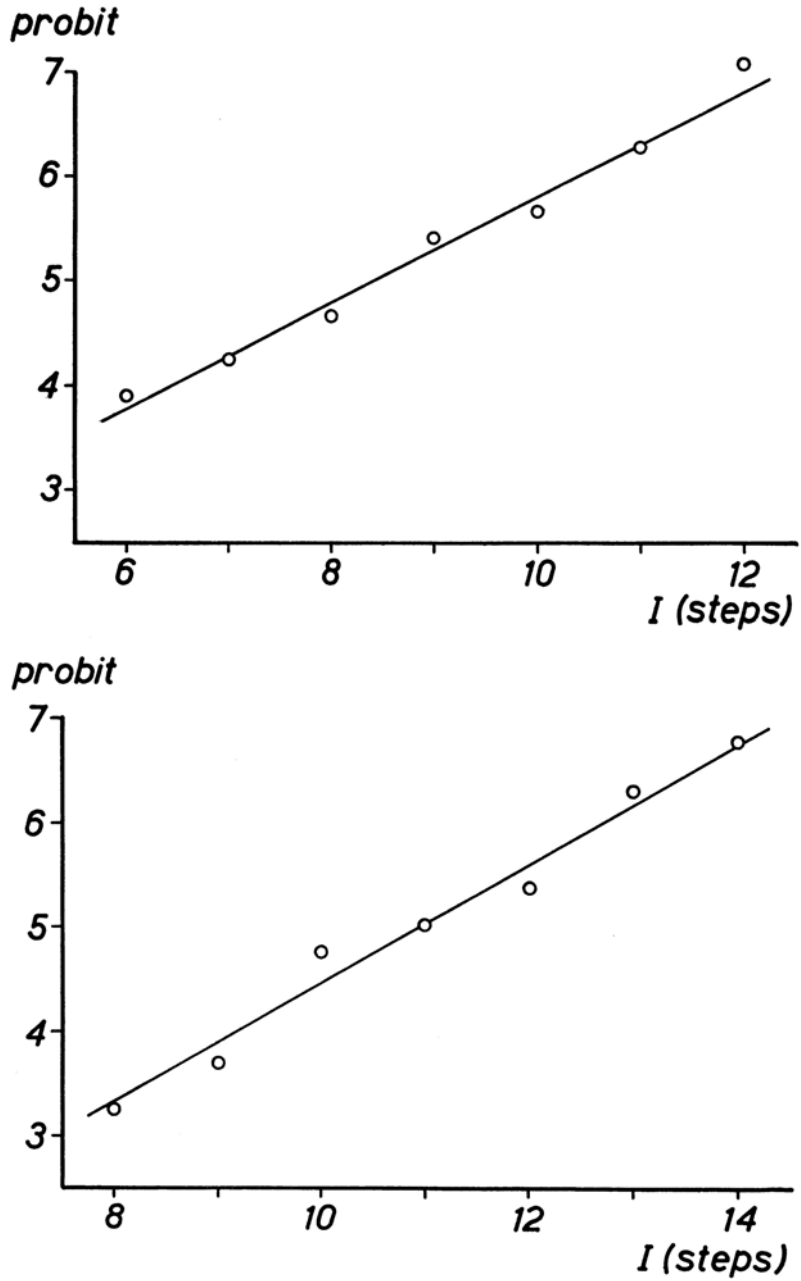

Fig. 6.
Two examples of the sets of probability-intensity relations after the probit transformation.

because factors such as stimulus duration must be taken into account. It is apparent from Fig. 9, that no simple expression can be derived from the experimentally derived relations between probability of response and stimulus duration. The Gaussian type of distribution function, however, describes the relation between probability of response and stimulus intensity, whether the stimulus is of short or long duration. It is therefore likely, that the characteristics of this function will be related to the duration of the stimulus. Such a relation is known for the threshold as the strength-duration relation. Here, it is presented in its simplest form (equation of Weiss and Hoorweg; cf. van Lier, 1955):

$$\mu(\tau) = \frac{a}{\tau} + b \qquad (2)$$

The value of the spread, determined with a short stimulus of high intensity and with a stimulus of long duration and lower intensity, appears to be proportional to the value of the threshold (Fig. 7). Therefore it is feasible to assume that the relationship between spread and threshold might be a linear one, and, moreover, that the quotient of spread and threshold might be a constant for a fiber, as was already noted by van Lier (1955). Stated in another manner, we can say that the relation between probability of reaction and stimulus intensity at different stimulus durations will be similar when the value of the threshold has been standardized.

To test this hypothesis, the sets of observations previously described were used. Since the steps in intensity are given as a percentage of the *initially determined* threshold, it is possible to calculate directly the estimate of the quotient of spread and threshold by means of the probit analysis. Thus, from each nerve fiber two quotients were calculated, one for the stimulus of short duration, the other for the stimulus of long duration. These 18 pairs of quotients were subjected to Wilcoxon's test for symmetry. From the data compiled in Table III it follows, that the symmetrypoint of the differences within the pairs of estimates is not found to differ significantly from zero. Therefore, the test does not offer evidence against the null-hypothesis. This is accepted to be the working hypothesis, so

$$\frac{\sigma(\tau)}{\mu(\tau)} = c \qquad (3)$$

This quotient, the coefficient of variation of the function, is called *the relative spread (RS).* The *RS* is considered to be the parameter of the fluctuation in excitability, because it is independent of the intensity and the duration of the stimulus.

By the use of the *RS* and the strength-duration relation (the equation for the threshold, the other parameter), the strength-duration-probability relation may now be expressed by equations (1), (2) and (3).

Given a strength-duration relation and the *RS,* the relations between probability of response and stimulus duration can be derived for given stimulus intensities. The mathematical formulas are somewhat difficult to handle for

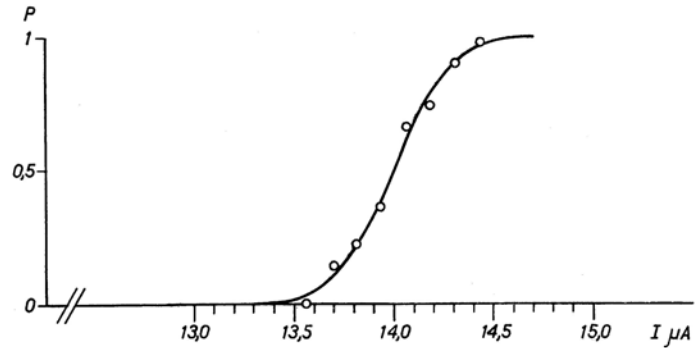

Fig. 7a.
The relation between probability of response and stimulus intensity:
a. for a stimulus of 2.5 msec duration

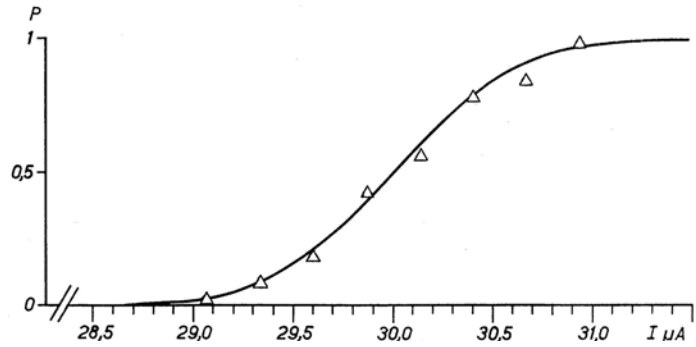

Fig. 7b.
b. for a stimulus of 0.25 msec duration

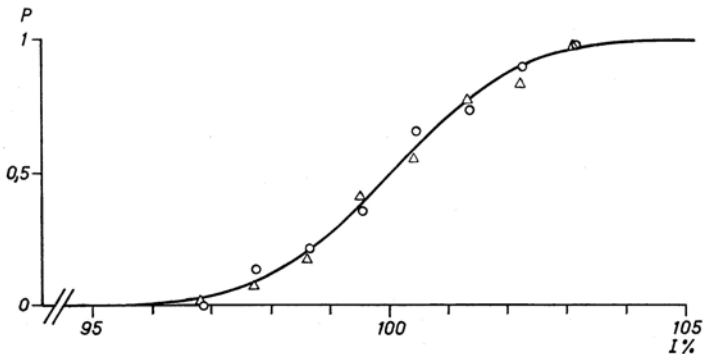

Fig. 7c.
c. same relations after standardization of the threshold

TABLE III

Influence of stimulus duration on relative spread
Frog nerve fibers

exp.	relative spread for durations of		difference * 100		rank	
	0.25	2.5 msec	+	–	+	–
1	1.39	1.42	3		1	
2	1.34	1.14		20		6
3	2.51	3.10	59		11	
4	2.22	2.60	38		9	
5	3.85	3.98	13		4	
6	1.79	2.14	35		7.5	
7	3.29	3.77	48		10	
8	4.74	6.02	128		15	
9	5.35	4.74		61		12
10	2.74	1.82		92		14
11	2.84	2.07		77		13
12	1.82	2.17	35		7.5	
13	2.88	4.37	149		16	
14	2.95	3.11	16		5	
15	1.15	1.03		12		3
16	2.28	3.94	166		18	
17	2.70	2.63		7		2
18	6.37	4.78		159		17
					+104	- 67
					T = + 37	
					k = 0.44	

The relative spread is presented in steps. The value of each step is 0.45 % of the value of the threshold.

direct comparison with the experimentally derived relations. For this reason, and because a certain inexactitude exists in the experimentally derived relations, due to a longterm instability of both the stimulus and the nerve fiber, because of the unavoidably long observation times necessitated, the properties of the relations have been derived in a graphical way and are compared with those found for the nerve fiber. With the aid of a strength-duration relation and the *RS*, a graph has been constructed (Fig. 8a). From this graph, and from its projection to the horizontal plane (Fig. 8b), the following properties for the duration-probability relations are found:

1. The curves are asymmetrical and the first derivative shows a skewness to the right.
2. The higher the stimulus intensity, the steeper the curve.
3. At stimulus intensities in the neighborhood of the rheobase, the curves never reach the 100 % probability.

When these relations are investigated for the frog nerve fiber (Fig. 9), it is evident, that the characteristics which were expected are present.

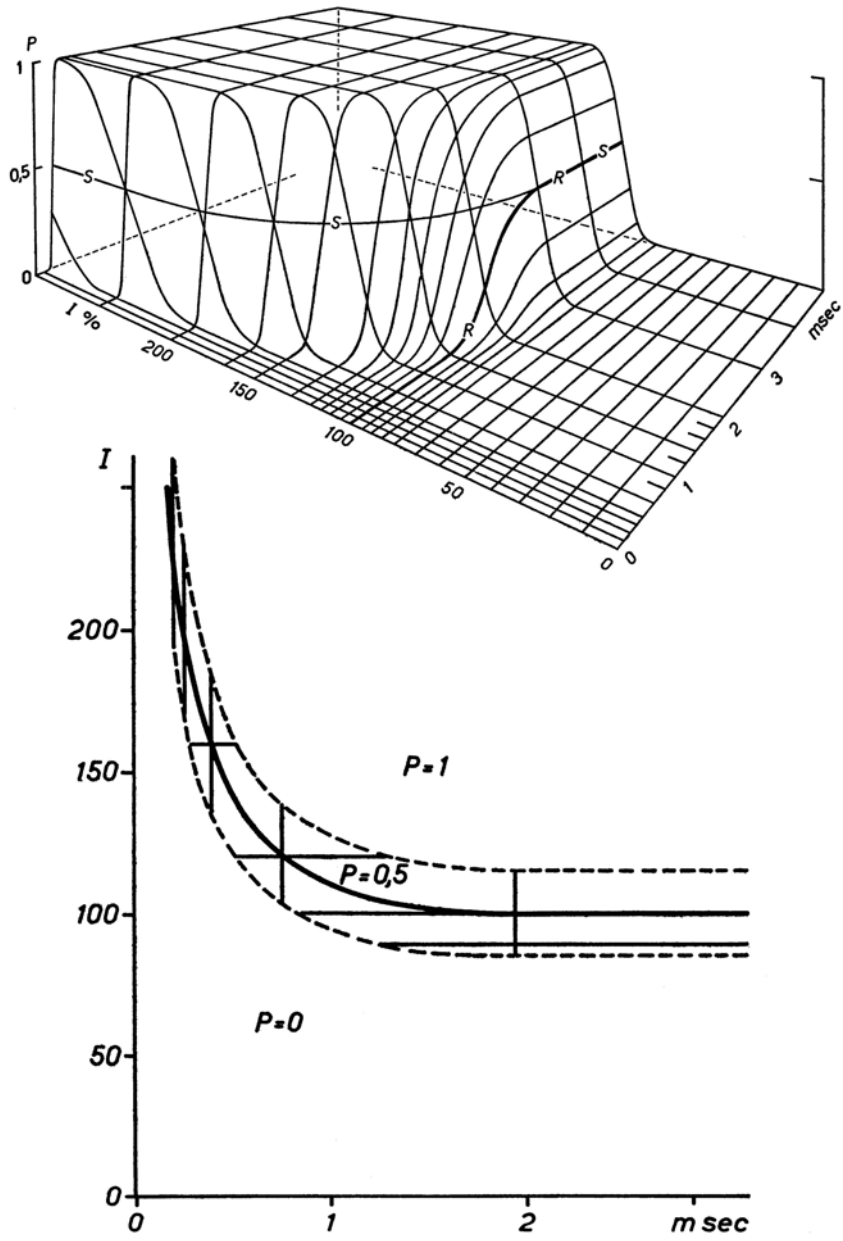

Fig. 8.
Strength-duration-probability graph and its projection to the horizontal plane. The value of the relative spread has been chosen somewhat larger than encountered in the frog nerve fibers studied. S, strength-duration curve; R, rheobase.

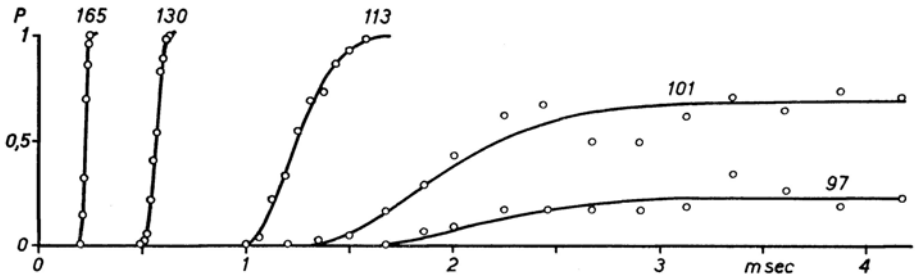

Fig. 9.
Duration-probability curves for a frog nerve fiber. [1])
Stimulus intensity in percentage of rheobase.

Conclusion: The input-output relations of the nerve fiber preparation can be described by the Gaussian type of distribution function. For a given nerve fiber, viz., node of Ranvier, this relation is denoted by the characteristics of the function, presented here as parameters of the nerve fiber:

a. The threshold (the mean of the function), given by the strength-duration relation.
b. The relative spread (the coefficient of variation of the function), a dimensionless number, independent of intensity and duration of the stimulus.

It should be noted that these parameters have different values for different nerve fibers.

2. The influence of conditioning stimuli

a. Introduction

After increasing the stimulus frequency the observations suggest that the independency between the successive reactions disappears. Complicated patterns of positive and negative reactions are seen (Fig. 10), and these are not easy to interpret (Blair and Erlanger, 1935/36a; Pecher, 1936).

When the stimulus frequency is increased to about one per second, random series may be encountered, but occasionally an alternation of the positive and negative reactions and, sometimes, a grouping of the positive and negative reactions is seen. At higher frequencies a succession of large groups of positive and negative reactions is observed. Such a group may vary from one complete run of positive (or negative) reactions to series of which each sequence resembles the response pattern of a slowly accommodating nerve fiber upon direct current stimulation (Fig. 10). This gives the impression that after a more or less abrupt increase of the probability of response, a gradual decrease of the response probability is present.

It is possible that these reaction patterns are the result of a complicated

[1]) Fig. 1, 8 and 9 have been redrawn after A. A. Verveen (1960) in: Tower, D. B. and Schadé, J. P., Structure and Function of the Cerebral Cortex. Amsterdam, Elsevier.

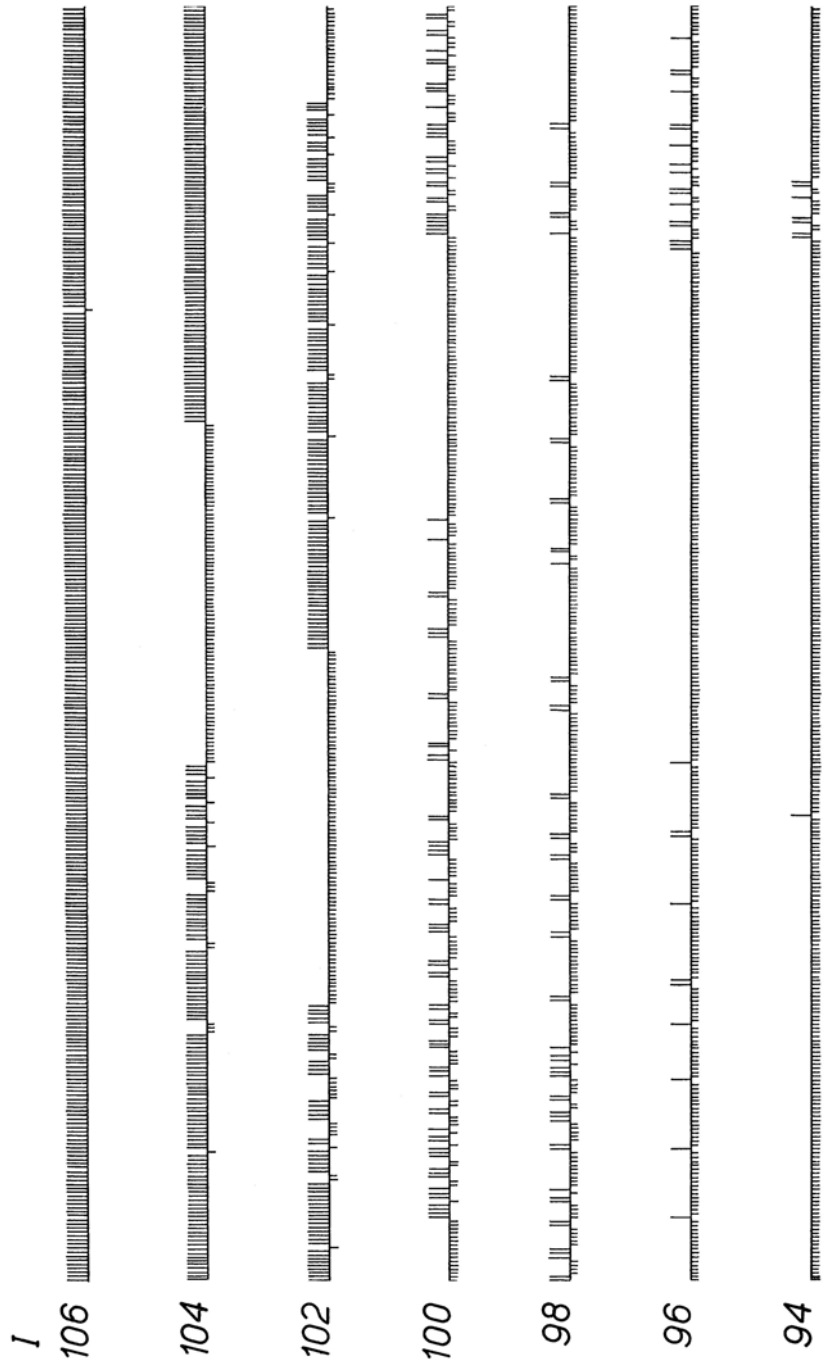

Fig. 10.
Response patterns of a single frog nerve fiber to stimulation with a frequency of 16 stimuli per second (redrawn)
Stimulus intensity in percentage of threshold. Long bars indicate action potentials, smaller bars indicate negative reactions.

interplay of excitability changes due to a number of factors, such as one recovery period following the action potentials, the stimuli themselves, together with the fluctuation in excitability.

With respect to the fluctuation in excitability it was therefore decided to investigate the following aspects:

1. The recovery of excitability following the discharge of the nerve fiber.
2. The change in excitability due to an ineffective stimulus.

b. Recovery of excitability following a discharge

The three phases in the recovery of excitability are found to be present in the

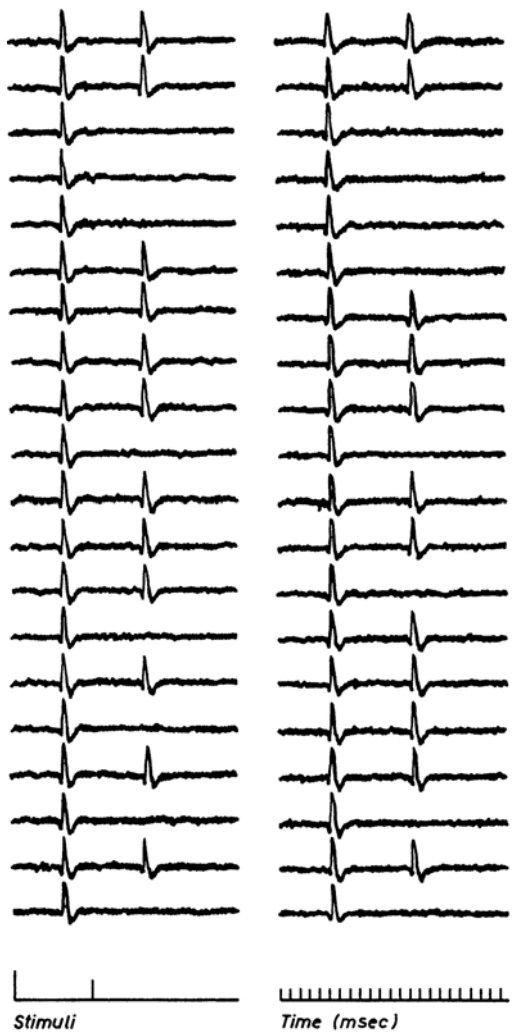

Fig. 11.
Fluctuation in excitability in the recovery period (redrawn).

frog nerve fibers studied (the absolute and relative refractory period, the supra-normal period and the sub-normal period). The entire recovery period lasts up to about 1½ seconds. This explains the necessity of using an interval of two seconds between consecutive stimuli to avoid cumulative effects.

When the test-stimulus is applied in the recovery period it is found that the threshold range is displaced. With a test-stimulus of about, threshold intensity the second action potential appears after a number of the given stimuli (Fig. 11), whether it is a full size action potential or not. The succession of positive and negative reactions appears to be unpredictable.

To test this, continuous series of reactions were registered from 10 nerve fibers. Two short stimuli were given: one, 0.2 msec (a conditioning stimulus of above-threshold intensity) and the second, 0.12 msec (the test-stimulus), 8 msec after the first one. The intensity of the test-stimulus was so adjusted to fall in the threshold range. Each of the 10 series consists of 100 stimulations, with an interval of 2 seconds between the successive stimulus-complexes. The results are compiled in Table IV. The data obtained from the application of the run

TABLE IV

Application of the run test
to continuous series of reactions for 10 frog nerve fibers

exp.	N	m	u	μ	T	k
1	100	48	53	50.9	0.32	0.75
2	100	39	46	48.6	0.44	0.66
3	100	43	54	50.0	0.71	0.47
4	100	50	56	51.0	0.91	0.36
5	100	36	55	47.0	1.62	0.11
6	100	75	37	38.5	0.27	0.79
7	100	37	47	47.6	0.03	0.98
8	100	32	47	44.5	0.46	0.65
9	100	39	47	48.6	0.23	0.82
10	100	16	26	27.9	0.52	0.60

The stimulus was applied during the supra-normal period.
For explanation of symbols see Table II.

test are presented in the last three columns. From this it is seen that the test does not provide evidence against the null-hypothesis of equal and independent probabilities for the occurrence of a positive response within each series.

It is clear from this that a fluctuation in excitability also exists during the recovery period. This necessitates studying the relationship between probability of response and stimulus intensity for the recovery period.

Two questions must be answered: What is the form of the relationship between stimulus intensity and probability of response, and, if it is Gaussian, what happens with the parameters, the threshold and the *RS*?

To this end, 15 axons were investigated. The interval between the condi-

tioning stimulus and the test-stimulus was fixed at 8 msec; the test-stimulus arriving about the moment of maximal excitability in the supra-normal period. An interval of 2 seconds between the successive stimulus-complexes was used.

The relative refractory period was not investigated in detail, because of the large gradient of the threshold during this period.

The relations between stimulus intensity and probability of response were determined both for the test-stimulus applied in the supra-normal period and for the unconditioned test-stimulus. This was done to compare the *RS* of the conditioned test-stimulus with the *RS* of the unconditioned test-stimulus.

These sets of observations, 15 pairs, were subjected to the probit analysis. No evidence could be produced to disprove the Gaussian type of distribution function for both the conditioned and the unconditioned stimuli.

TABLE V

Influence of supra-normal period on threshold and relative spread
Frog nerve fibers

exp	conditioned threshold	relative spread unconditioned	relative spread conditioned	difference x 100 +	difference x 100 -	rank +	rank -
1	88	1.56	1.40		16		7½
2	87	1.36	1.46	10		6	
3	76	3.02	1.66		136		15
4	91	1.92	1.94	2		2	
5	83	1.51	1.96	45		14	
6	84	1.47	1.29		18		9
7	82	1.72	1.34		38		12
8	76	0.92	1.08	16		7½	
9	87	1.28	1.29	1		1	
10	85	0.78	0.75		3		3
11	96	1.54	1.60	6		5	
12	89	1.57	1.84	27		10	
13	92	1.54	1.59	5		4	
14	92	1.95	1.67		28		11
15	89	1.91	2.30	39		13	
						+ 62½	- 57½
						T = +5	
						k = 0.91	

The conditioned threshold is presented in percentage of the unconditioned threshold. The relative spread is presented in steps. The value of each step is 0.55 % of the value of the threshold.

The estimates of the *RS* were calculated, and these 15 pairs of relative spreads subjected to Wilcoxon's test for symmetry. The data are presented in Table V. From these data it is concluded that no systematic difference can be

detected within the pairs of relative spreads. The actual thresholds differ systematically as is evident from the data in column two, where the threshold values of the conditioned stimuli are given in percentages of the threshold values of the unconditioned stimuli. The threshold values of the conditioned stimuli are lower, because they were applied in the supra-normal period.

As was mentioned previously, with the exception of some orientating investigations no systematic series of experiments was performed in the relative refractory period. From these experiments the impression was gained that, although the threshold is raised considerably, the *RS* is not changed during this period.

Conclusion: From the data presented it can be concluded that in the recovery period:

a. A fluctuation in excitability exists.
b. The successive reactions occur with the same and independent probability of response, when the stimuli are applied at the same moment during the recovery period.
c. The relation between probability of response and stimulus intensity can be described by the Gaussian type of distribution function.
d. The first parameter of this function is the threshold, which is known to be also a function of the time following the initiation of the preceding action potential.
e. No significant influence of the preceding action potential on the relative spread is found.

c. Influence of a sub-rheobasic direct current

The influences of sub-rheobasic stimuli of longer duration on the fluctuation in excitability were determined. The test-stimulus (0.12 msec duration) was applied 5 msec after the onset of a sub-threshold direct current, which was switched off 4 msec after the application of the test-stimulus. The period of 5 msec covers the maximum of the induced change in excitability. The intensity of the direct current was of an order that the test-stimulus, with an intensity of 50 % of the threshold of the unconditioned test-stimulus, was just at threshold.

An interval of 2 seconds between successive stimulus-complexes was used as in all the experiments.

With identical stimulus-complexes the succession of positive and negative reactions appears to be unpredictable again. This is now taken for granted.

The intensity-probability relations were determined for the conditioned and unconditioned test-stimulus. These experiments were carried out on 12 nerve fibers. From the probit analysis it followed again, that the Gaussian type of distribution function can be considered to be the working hypothesis for the relationship between probability of response and stimulus intensity.

The values of the relative spread of the conditioned test-stimulus (RS_c) and of the unconditioned test-stimulus (*RS*) were calculated. The results are listed

in Table VI-A, together with the data obtained from the application of Wilcoxon's test for symmetry, applied on the differences between the 12 pairs of relative spreads. From these data it can be concluded that the difference

TABLE VI-A

Influence of sub-rheobasic current on relative spread
Frog nerve fibers

exp.	relative spread unconditioned	relative spread conditioned	difference x 100 +	difference x 100 –
1	2.55	3.98	143	
2	1.14	2.08	94	
3	1.26	2.43	117	
4	1.32	3.92	260	
5	1.67	2.92	125	
6	0.60	1.95	135	
7	1.41	4.27	286	
8	1.35	2.87	152	
9	1.86	3.58	172	
10	1.26	4.02	276	
11	1.26	2.25	99	
12	0.80	2.10	130	

Rank: all values are positive:
T = +78
k = 0.0005

The relative spread is presented in steps. The value of each step is 0.5 % of the value of the threshold. The value of the conditioned threshold is 50 % of the value of the unconditioned threshold, except for experiment 2, where it is 55.5 %.

between RS_c and RS is significant, the RS_c being larger than the RS. Since the conditioned threshold is lower than the unconditioned threshold, the question is raised as to what information can be obtained from the products of the threshold and the relative spread for both conditions.

It is noted from Table VI-B that no difference can be detected within the pairs of factors consisting of $(RS.100)$ and (RS_c.percentage intensity of test-stimulus). In other words, the RS, can be assumed to be inversely proportional to the intensity of the test-stimulus. Evidently the change in threshold of the test-stimulus, induced by the direct current, produces an opposite change of the relative spread. The 'stimulation value' of the direct current at the moment the test-stimulus is applied is equal to a current pulse of test-stimulus duration and with an intensity equal to the difference between the unconditioned stimulus and the test-stimulus. Thus, with regard to the stimulation value of the whole stimulus-complex at the moment of summation the RS is not found to be altered.

TABLE VI-B

Influence of sub-rheobasic current on product of relative spread and threshold
Frog nerve fibers

exp.	*RS x* threshold *x* 100 difference uncon- ditioned	con	+	–	rank +	–
1	255	199		56		9
2	114	115	1		1	
3	126	122		4		2
4	132	196	64		10	
5	167	146		21		6
6	60	98	38		8	
7	141	214	73		11	
8	135	144	9		4	
9	186	179		7		3
10	126	201	75		12	
11	126	113		13		5
12	80	105	25		7	
					+53	-25

T = + 28
k = 0.30

Conclusion: Currents that do not initiate an action potential, alter the threshold, and produce an exactly opposite change of the relative spread.

With respect to the stimulation value of the whole stimulus-complex at the moment of summation, no change in the relative spread is detectable.

3. Influence of strychnine and urethane on fluctuation in excitability

In 1941 a study was published by Erlanger, Blair and Schoepfle, describing the effects of a few agents on the fluctuation in excitability. As a measure for the fluctuation these investigators had chosen the difference between the stimulus intensity which, gave about 5 responses to 6 stimulations on the one hand, and the smaller intensity which elicited about 1 response to 6 stimulations on the other. This interval in stimulus intensity was called the 'amplitude' and the influence of a few agents on the amplitude was investigated. The most striking observation by Erlanger et al. was the effect of strychnine. Very low concentrations of strychnine ($1{:}10^4$—10^6) appeared to have a profound influence, causing an increase of the 'amplitude', while the threshold hardly increased. Other effects on the nerve fiber were not seen with these doses. Strychnine has otherwise hardly any effect on the peripheral nerve (cf. von Muralt, 1954); tot he contrary it is known to be a powerful excitant of the central nervous system.

The above mentioned interesting observation, which was made on four nerve fiber preparations, led us to study the effect of strychnine on the *RS*. It was also

desirable to look for a substance which could possibly have a reverse influence on the *RS*. Urethane, an anesthetic, was chosen, because of the possibility of grading the `degree of narcosis' of the nerve fiber. This had been found to be proportional to the concentration of this substance (Tasaki, 1953).

The doses used in these experiments were 5 mg of strychnine nitrate per l00 cc of Ringer's solution (1 : 20 000), and 1 gm of urethane per 100 cc of Ringer's solution (1 : 100) . Owing to the fact that the solubility of free strychnine is 1 : 6400 at 20° C (cf. Fullerton Cook and Martin, 1948), the nitrate could be used in the Ringer's solution worked with here.

Preliminary experiments had already confirmed that this strychnine solution might increase the *RS* and had suggested that the urethane solution might decrease the *RS*. This enabled the formulation of specific hypotheses for the statistical procedures to be used: an increase in the *RS* by strychnine, and a decrease in the *RS* by urethane.

It was decided to test the influence of both substances and the influence of two control solutions which contained only Ringer in a blind test. The four solutions were contained in coded identical ampullae.

In each of 14 experiments, 4 fibers from one frog were used (the two rami of each sciatic nerve were stimulated separately). At the beginning of each experiment 4 control solutions (Ringer) from the same stock the test solutions were made of, were applied to each of the four rami, with the electrodes in situ. This was done to equilibrate the rami with the control solution. This procedure was adopted to eliminate possible influences of different Ringer's solutions. After the equilibration the solutions were sucked away and the probability-intensity relations determined. The stimulus duration was 0.12 msec; the interval between successive stimuli was 2 seconds. The thresholds, in scale units of the shunt, were also noted. The same procedure was repeated after the application of each of the four test solutions. Since the rami are thick and possess sheaths, each solution had to be applied for a period of 3 hours.

In this manner one pair of intensity-probability sets and one pair of threshold values were obtained for each of the nerve preparations. A total of 33 fibers was successfully investigated. After the series of experiments was completed, the sets were decoded, the relative spreads calculated by means of the probit analysis (all sets again appeared to fit to the Gaussian type of distribution function) and for each substance investigated, the pairs of relative spreads and also of threshold values were subjected to Wilcoxon's test for symmetry. The data are presented in Tables VII, VIII and IX, together with the results of the application of the symmetry-test. Numerical exclusions in the tables represent failures.

From these data the following conclusions are drawn with respect to the substances applied:

With regard to the influence of *strychnine:*

a. The null hypothesis is rejected against the contra hypothesis of a larger *RS* after the application of strychnine (Table VII-A).

TABLE VII-A

Influence of strychnine on relative spread
Frog nerve fibers

exp.	relative spread before application	relative spread after application	difference * 100 +	difference * 100 –	rank +	rank -
3	3.55	2.70		85		5
4	1.76	2.36	60		3	
5	3.66	5.74	208		9	
8	1.83	2.17	34		2	
9	2.15	3.97	182		7	
11	2.91	2.78		13		1
12	1.12	1.78	66		4	
13	*1.56*	2.99	143		6	
14	1.26	3.27	201		8	
					+39	- 6

T = + 33
k = 0.027
(one-sided)

The relative spread is presented in steps. The value of each step is 0.5 % of the value of the threshold.

TABLE VII-B

Influence of strychnine on threshold
Frog nerve fibers

exp.	threshold before application	threshold after application	difference +	difference –	rank +	rank –
3	47	43		4		4
4	27	25		2		1 ½
5	31	28		3		3
8	60	55		5		5
9	29	31	2		1½	
11	38	46	8		6	
12	65	83	18		8	
14	57	43		14		7
					+ 15½	-20½

T = –5
k = 0.74

The threshold is presented in scale units of the shunt.

b. With respect to the threshold no evidence is found against systematic difference within the pairs (Table VII-B).
These results are also in accordance with the observations of Erlanger, et al.

Concerning the influence of *urethane:*

TABLE VIII-A

Influence of urethane on relative spread
Frog nerve fibers

exp.	relative spread before application	relative spread after application	difference x 100 +	difference x 100 –	rank +	rank –
1	1.80	2.24	44		1	
2	2.80	1.40		140		5
3	2.34	1.38		96		2
4	2.70	1.38		132		4
5	5.46	1.86		360		8
8	1.41	2.41	100		3	
9	3.31	1.31		200		6
11	4.22	1.32		290		7
					+ 4	- 32

T = - 28
k = 0.027
(one-sided)

The relative spread is presented in steps. The value of each step is 0.5 % of the value of the threshold.

TABLE VIII-B

Influence of urethane on threshold
Frog nerve fibers

exp.	threshold before application	threshold after application	difference +	difference –	rank +	rank –
1	24	19		5		1
2	28	44	16		4	
3	19	25	6		2½	
4	43	49	6		2½	
5	35	52	17		5	
8	58	58				
9	33	52	19		6	
11	75	152	77		7	
					+27	- 1

T = + 26
k = 0.03

The threshold is presented in scale units of the shunt.

a. The null-hypothesis of no systematic difference is rejected against the contra-hypothesis of a smaller *RS* after the application of the substance (Table VIII-A).
b. With respect to the values of the threshold the null-hypothesis is rejected (Table VIII-B). The threshold is increased by the application of urethane.
c. The changes mentioned in a and b appear to be in an opposite direction. Therefore, from these data the products of *RS* and threshold (in scale units) were calculated. These pairs of products are also compared with the symmetry test (Table VIII-C). After application of this additional test it appears

TABLE VIII-C

Influence of urethane on product of relative spread and threshold

exp	*RS* x threshold x 100 before application	after application	difference +	difference –	rank +	rank –
1	43	43				
2	77	62		15		2
3	44	35		9		1
4	116	68		48		4
5	191	97		94		6
8	82	140	58		5	
9	109	68		41		3
11	317	201		116		7
					+5	- 23

T = -18
k = 0.16

that no evidence can be compiled to reject the null-hypothesis of no systematic difference within the pairs of products.

With regard to *the control solutions:*

a. Tables IX-A and IX-B show that no arguments are found against the null-hypothesis of no differences within the pairs both for the *RS* and for the threshold.
b. The dispersion in the obtained value of the *RS* appears, however, to be great (Table IX-A). This is disappointing, because no numerical values can now be obtained for the degree of change in *RS* caused by strychnine and urethane. This may possibly be caused by the experimental set-up, in which very long waiting times had to be taken into account. This is a great drawback for the investigation of the chemicals applied. Therefore, no attempts were made to wash them out or to test the influence of other chemicals and of changes in the ionic composition of the applied fluid.

The *conclusions* drawn from these experiments are:

TABLE IX-A

Influence of control-solution on relative spread
Frog nerve fibers

exp.	relative spread before application	relative spread after application	difference x 100 +	difference x 100 –	rank +	rank –
1	2.67	1.81		86		12
	2.31	1.77		54		8
2	1.94	2.43	49		7	
	3.24	2.22		102		13
3	3.37	11.24	787		16	
4	2.61	3.34	73		11	
	1.88	7.52	564		15	
5	1.61	1.75	14		1	
	2.65	2.36		29		3
8	1.58	1.22		36		5
9	4.93	4.59		34		4
11	2.19	1.64		55		9
	2.23	3.36	113		14	
12	2.45	1.77		68		10
13	2.67	2.23		44		6
14	1.56	1.28		28		2
					+64	- 72

T = -8
k = 0.86

The relative spread is presented in steps. The value of each step is 0.5 % of the value of the threshold.

1. Strychnine increases the relative spread, while there is no influence on the threshold.
2. Urethane raises the threshold and decreases the value of the relative spread, probably in such a way that their product is not altered.

4. The value of the relative spread

The *RS* was determined in all the 80 nerve fibers investigated. The distribution of the obtained values is shown in Fig. 12. A skewness to the right is present. The mean value of the *RS* is 0.011 (80 values; S.D. 0.005).

Pecher (1939) and Erlanger, et al. (1941) determined the width of the threshold range with a similar type preparation, viz., the functionally isolated A-fiber from the sciatic nerve of the frog. The relative spreads were also calculated from these data. This resulted in 11 values for the *RS*, with a mean value of 0.016 (S.D. 0.006), calculated from Pecher's data. From Erlanger's data, 20 values were calculated. The mean value is 0.015 (S.D. 0.004). These authors did not try to stimulate single nodes of Ranvier, but, nevertheless, the

TABLE IX-B

Influence of control-solution on threshold

exp.	threshold before application	threshold after application	difference +	difference –	rank +	rank –
1	26	44	18		12	
		45	37	8		9½
2	41	44	3		2½	
	23	23				
3	39	47	8	9½		
4	56	92	36		15	
		80	86	6	8	
5	31	26		5		6½
		28	25	3		2½
8	62	88	26		14	
9	42	41		1		1
11	61	65	4		4½	
	53	49		4		4½
12	67	62		5		6½
13	85	62		23		13
14	68	56		12		11
					+65½	-54½

T = +11 k = 0.78

The threshold is presented in scale units of the shunt.

mean values calculated from their observations and that obtained here are of the same order.

5. Summary and conclusions

The aim of the investigation described in this chapter is the study of fluctuation in excitability of frog nerve fibers. This was done by studying the input-output relations for functionally isolated single nodes of Ranvier. The probability of response was examined with respect to the parameters of a negative rectangular current, repeatedly applied and with an interval of two seconds.

Results:

1. The successive reactions to identical stimuli or stimulus-complexes are distributed in accordance with the hypothesis that the probability of response has, each time, the same value, independent of the preceding reactions.
2. With a given stimulus duration, the relationship between probability of response and stimulus intensity can be described by the Gaussian type of

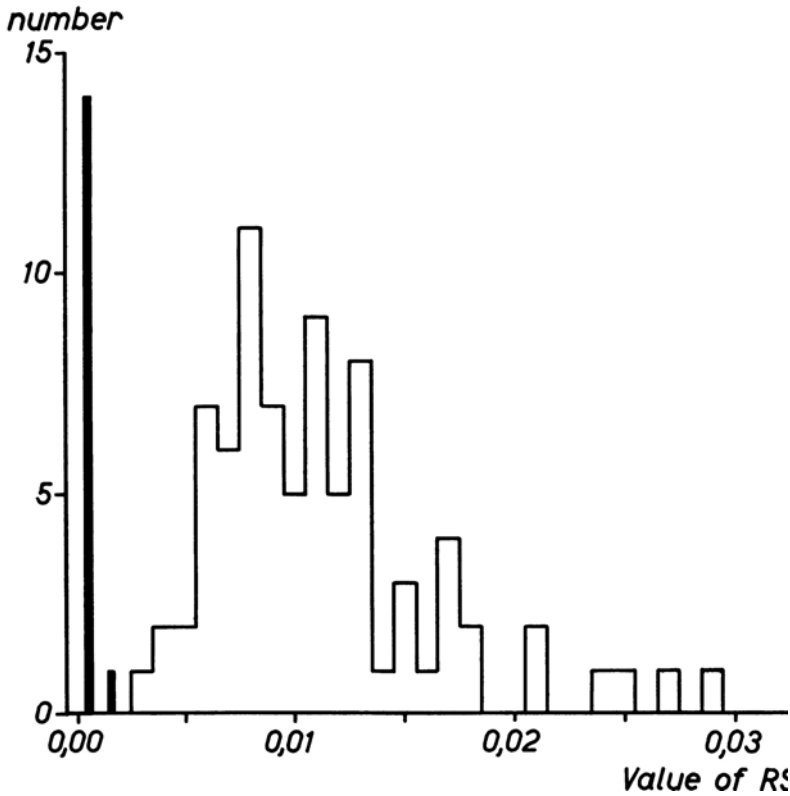

Fig. 12.
The values of the relative spreads obtained from the 80 frog nerve fibers investigated. (The black columns at the left indicate the relative spreads obtained from 15 cray-fish axons. Cf. Fig. 15).

distribution function for all the 80 nerve fibers investigated.
This function is characterised by:

a. the 50 % stimulation threshold, in short called the `threshold', being the mean and median of the function, and by
b. the spread, being the standard deviation of the function.

Both parameters are dependent on the stimulus duration.

3. The strength-duration-probability relation can also be described by this function. It appears that the relation can then be characterized easiest by:

a. the threshold, the value of which depends on the stimulus duration and is given by the strength-duration relation; and
b. the coefficient of variation, the quotient of spread an threshold, called the relative spread (*RS*). The *RS*, a number, is independent of the intensity and duration of the applied stimulus.

Hence threshold and relative spread characterize the input-output relations of a nerve fiber.

4. The relations between probability of response and stimulus duration (with given stimulus intensities) were derived from the strength-duration-probability relation. They appeared to be comparable to those found on the nerve fiber.
5. The influences on fluctuation in excitability were investigated for:
   1e. the recovery period;
   2e. a sub-rheobasic direct current;
   3e. the application of strychnine;
   4e. the application of urethane.

   The following *conclusions* were drawn:

   a. Fluctuation in excitability is always present.

   b. The relation between probability of response and stimulus intensity can always be described by the Gaussian type of distribution function.

   c. The influences are exerted on the parameters characterizing the input output relations, i.e., the threshold and the relative spread:

      1e. In the supra-normal phase (and probably also in the other phases) of the recovery period the threshold is decreased (changed), while no change in the relative spread is detected.

      2e. A sub-rheobasic current decreases the threshold and increases the relative spread. No changes are found when the stimulation value of the sub-rheobasic current and the test-stimulus at the moment of summation is taken into consideration.

      3e. Strychnine increases the relative spread; no influence on the threshold is detected.

      4e. Urethane increases the threshold and decreases the relative spread; no influence on the product of threshold and relative spread, the spread, is found.
6. The mean value of the relative spread is found to be 0.011 (80 determinations, S.D. 0.005) for functionally isolated nodes of Ranvier of frog A-fibers.

IV. PROBABILITY PHENOMENA IN UNMYELINATED CRAY-FISH AXON

1. Introduction

The question arose, whether probability phenomena are present in species other than frog and in structures other than the myelinated nerve fiber and, if so, what could be stated about their character. To this end, the unmyelinated axon of the cray-fish *Astacus leptodactylus* was chosen. The method of preparation has been described in chapter II, 1b. From each bundle of fibers obtained, the most excitable axon was used.

The study of this problem has led to the following successive questions:

1. Is there a threshold range, in which the reaction of the axon is unpredictable?
2. If so, do the successive reactions have the same and independent probability of response to stimulation with identical stimuli?
3. Do the axons react independently of one another to one and the same stimulus?
4. What type of relationship exists between probability of response and stimulus intensity; what is the influence of the stimulus duration and what is the general kind of the input-output relationship with regard to negative rectangular currents?
5. In what respect does the cray-fish axon differ from the frog nerve fiber, with regard to these probability phenomena?

2. Results

Before these questions could be answered, the appropriate interval between successive stimuli had to be determined. Since the recovery period is about 500 msec, a one second interval was chosen. The procedures used in the investigations are the same as for the frog nerve fiber.

Regarding *the first question*, it was immediately apparent that a threshold range also exists in the cray-fish axon. The width of this range, however, is much smaller than for the frog nerve fiber.

With respect to *question two*, a continuous series of responses to stimulation with identical, stimuli of about threshold intensity was obtained from each of 10 axons, both for a single stimulus and for a stimulus falling in the relative refractory period. These series are presented in Tables X and XI. In the last three columns of each table the data obtained from the run test are given. These show that no evidence is produced to disprove the null-hypothesis of the same

TABLE X

Application of the run test
to continuous series of reactions for 10 cray-fish axons

exp.	N	m	u	μ	T	k
1	100	44	48	50.3	0.37	0.71
2	100	24	35	37.5	0.55	0.58
3	100	35	46	46.5	0.00	1.00
4	100	34	43	45.9	0.54	0.59
5	100	26	44	39.5	1.05	0.29
6	100	77	35	36.4	0.26	0.79
7	100	69	34	43.8	2.18	0.03
8	100	38	46	48.1	0.34	0.73
9	100	35	39	46.5	1.55	0.12
10	100	67	46	45.2	0.07	0.94

A single stimulus was used.
For explanation of symbols see Table II.

TABLE XI

Application of the run test
to continuous series of reactions for 10 cray-fish axons

exp.	N	m	u	μ	T	k
1	100	51	57	51.0	1.11	0.27
2	100	49	61	51.0	1.92	0.06
3	100	49	40	51.0	2.11	0.04
4	100	46	54	50.7	0.57	0.57
5	100	41	43	49.4	1.22	0.22
6	100	40	50	49.0	0.10	0.92
7	100	50	59	51.0	1.51	0.13
8	100	43	50	50.0	0.00	1.00
9	100	47	50	50.8	0.06	0.95
10	100	3 6	50	47.1	0.52	0.60

The stimulus was applied during the relative refractory period.
For explanation of symbols see Table II.

and independent probability of response, both for a single stimulus and for the conditioned stimulus in the relative refractory period. Experiments number 7 (Table X) and 3 (Table XI) with a probability of exceedance below the level of
significance do not form an objection against the null-hypothesis in the whole set of tests, covering the total number of observations. It is clear that with the null-hypothesis given, some series will be found in the tails of the distribution.

With regard to *the third question,* the following procedure was employed. Almost all the nerve bundles investigated present a small number of axons with low thresholds. In most cases the strength-duration relations of these axons

show intersections (Fig. 13). By gradually increasing or decreasing the stimulus duration, the intersection point for two axons can be detected. Carefully adjusting the stimulus duration, a discharge of these two axons is obtained with about the same probability of response for each axon. Such two-fiber preparations were investigated on mutual independency. From each of 8 preparations

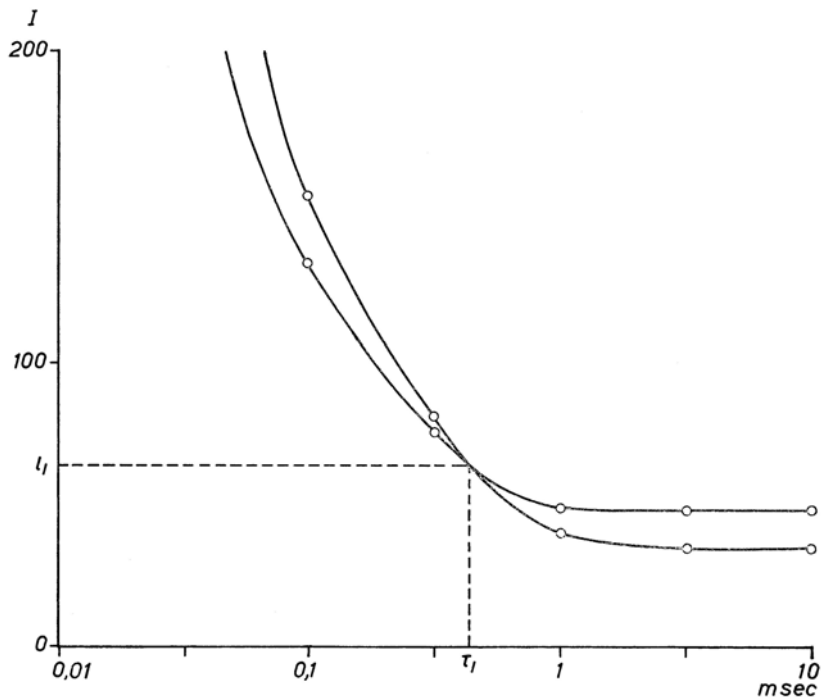

Fig. 13.
Strength-duration relations of two cray-fish axons in one nerve. When the applied stimulus has a duration i, both axons have the same threshold cl.

a series of 100 stimulations was registered. The results are presented in Table XII. It can be seen that the registered number of double responses is about equal to the number expected under the hypothesis of no correlation between the responses of the two axons. This leads to the conclusion that the fluctuation in excitability occurs in both axons independently of each other.

With respect to *question four*, the relations between probability of response were investigated in 15 axons, both for a short pulse (0.12 msec) of high intensity and for a longer one (1.2 msec) of lower intensity. These 15 pairs of sets were subjected to the probit analysis. Since they then appeared to fit to straight lines (cf. Fig. 14), the Gaussian type of distribution function can be considered to be

TABLE XII

Fluctuation in excitability
in two-fiber preparations of the cray-fish

exp.	N	A	B	A+ B	AB/N
1	100	47	47	20	22.1
2	100	36	19	7	6.8
3	100	68	41	27	27.9
4	100	65	50	30	32.5
5	100	56	44	24	24.6
6	100	72	25	20	18.0
7	100	61	44	24	26.8
8	100	59	50	32	29.5

N number of stimulations
A number of responses of fiber A
B number of responses of fiber B
A + B number of simultaneous responses
AB/N expected number of simultaneous responses, calculated under the assumption of independency.

the working hypothesis for these unmyelinated axons as well. The values of the relative spreads were calculated and the resulting 15 pairs of estimates subjected to the symmetry-test (Table XIII). Since no evidence is produced against the

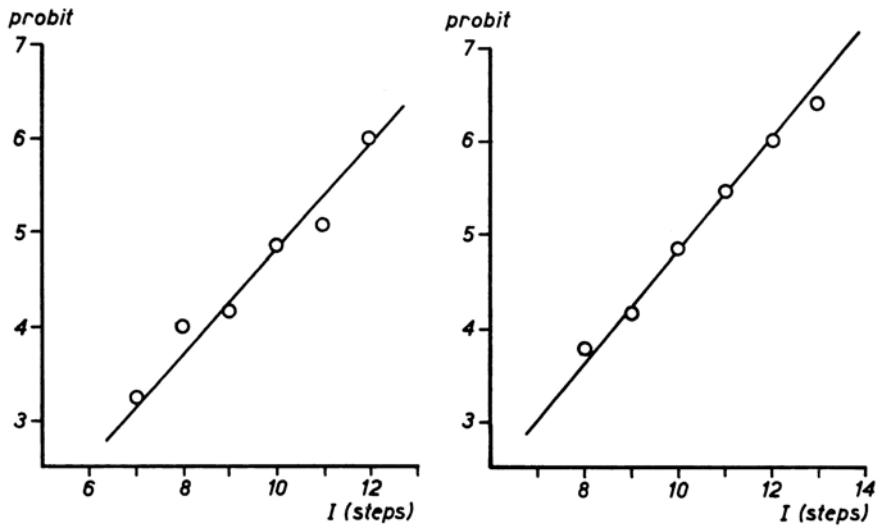

Fig. 14.
Two examples of the sets of probability-intensity relations for cray-fish axons after the probit transformation.

TABLE XIII

Influence of stimulus duration on relative spread Cray-fish axons

exp.	relative spread for durations of		difference x 100		rank	
	0.12	1.2 msec	+	–	+	–
1	1.53	1.68	15		7	
2	1.69	1.61		8		22
3	1.70	2.25	55		14	
4	1.81	1.73		8		22
5	2.14	2.28	14		6	
6	1.86	2.09	23		9	
7	3.40	3.16		24		10
8	1.54	2.28	74		15	
9	1.56	2.06	50		13	
10	1.80	1.81	1		1	
11	1.30	1.09		21		8
12	2.30	2.75	45		12	
13	1.75	1.62		13		5
14	1.67	1.78	11		4	
15	1.57	1.30		27		11
					+81	- 39
						T = + 42
						k = 0.25

The relative spread is presented in steps. The value of each step is 0.6 ‰ of the value of the threshold.

the null-hypothesis of no difference within the pairs, the *RS* is considered to be independent of the intensity and duration of the applied stimulus. It follows that the relationship between probability of response and the parameters of the applied stimulus can also be described by the equations (1), (2) and (3), presented in Chapter III.

With regard to *question five,* the estimates of the relative spreads were calculated only for the 15 aforementioned axons. These values are presented in Fig. 15. The mean value of the *RS* is 0.0012 (15 values, S.D. 0.0003). It is clear that the obtained values are much smaller than those recorded in frog A-fibers (mean 0.011).

All the features of the input-output relations known for the frog nerve fiber (i.e., node of Ranvier) are present in the unmyelinated cray-fish axon. The resemblance in general features is striking. Aside from this nearly perfect similarity in general behavior, with regard to the input-output relationships, the most remarkable observation is the small value of the relative spread (Fig. 12 and Fig. 15).

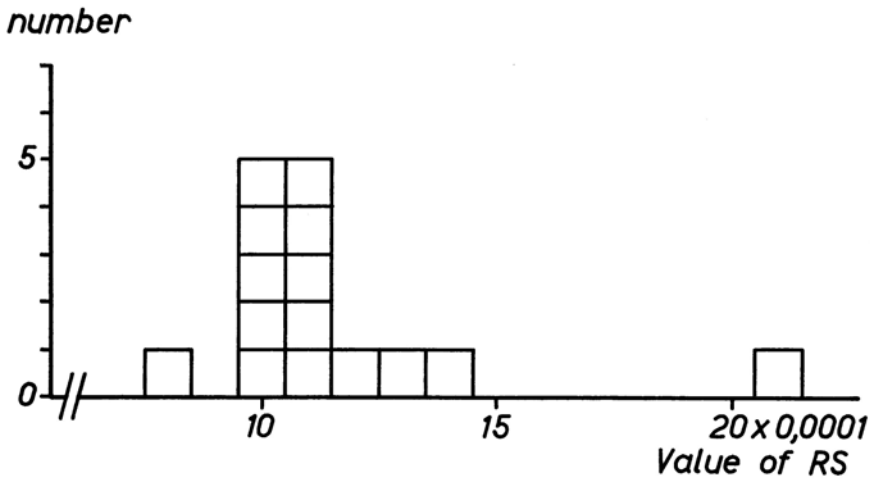

Fig. 15.
The values of the relative spreads obtained from the 15 cray-fish axons investigated (cf. Fig. 12).

3. Summary

The most excitable axons in the cheliped of the cray-fish were investigated with respect to fluctuation in excitability.

Results:

1. A threshold range exists, in which the fiber will respond to the applied stimulus with a certain probability.
2. The probability of response has the same and independent value each time, if stimulated with identical stimuli at one second intervals.
3. The different axons in the same bundle react to the same stimulus independently of each other.
4. The relationship between probability of response and stimulus intensity can be described by the Gaussian type of distribution function. The coefficient of variation of this function, the relative spread, is also independent of the intensity and duration of the applied stimulus. The general behavior of the cray fish axon with respect to the input-output relationship is similar to that of the frog nerve fiber.
5. The mean value of the relative spread of the cray-fish axons investigated is about one tenth of the mean value of the relative spread of frog A-fibers.

V. DISCUSSION

1. Probability of response

The input-output relationship of the stimulated part of the nerve fiber, seen as a signal-transmitting unit, can be approximated as a step function. This unit, or part of it, acts as a trigger (Bullock, 1956). If an input-signal is not presented, the unit is in 'resting' state. Upon the introduction of a signal, i.e., an electrical stimulus, the unit can adopt one of two possible states, dependent on the 'stimulation value' of the input signal: an action potential is or is not produced.

In former chapters it was made clear that, instead of a discontinuous 'threshold', a continuous transitional range exists, which is called the 'threshold range', both for the frog node of Ranvier and the cray-fish axon. When the stimulation value of the input signal is above the threshold range, an action potential will nearly always be produced. A stimulation value below the threshold range is practically never followed by an action potential, when the value falls within the threshold range, the state of the unit is uncertain; there is only a probability of response (Fig. 1). At the upper limit of the threshold range the probability of response approaches one, at the lower limit it approaches zero.

For a careful investigation of the threshold range, i.e., the relation between probability of response and the stimulation value of the input signal, one condition must be fulfilled. At the repeated presentation of the input signal, there should be no correlation between the successive responses.

This primary condition was also the first problem on which Charles Pecher (1936) focussed his attention. There is some ambiguity in his conclusions (Chapter I, 2), because the criterion he used does not imply that the successive reactions are unrelated, as was also noted by Frishkopf and Rosenblith (1958).

To this end, the primary condition was investigated again, on a number of nerve fibers both for single stimuli and for stimulus complexes applied with an interval of two seconds between successive stimulus complexes. It was found (Tables II, IV, X and XI) that no evidence could be compiled against the hypothesis that at the low-frequency input of identical signals the probability of response each time has the same value and is independent of the preceding reactions, both for the frog node of Ranvier and the cray-fish axon.

2. Input-output relationship

Since the primary condition was fulfilled, the relationship between probability of response and stimulus intensity was investigated. In these experiments, 80 nerve fibers of the frog and 15 of the cray-fish were studied. For each axon two sets of observations were obtained, one set for a short stimulus without

specific conditions, the other for a long stimulus, or conditioned stimulus, or for the application of strychnine or urethane. All 190 relations of all 95 axons studied appeared to fit the Gaussian type of distribution function within statistical limits [1]) . This type of function is, therefore, accepted to be the model of choice for this relationship, both for the frog peripheral nerve fiber (viz., the node of Ranvier) and for the cray-fish axon. It appeared that the relationship between probability of response, stimulus intensity and stimulus duration could be characterized by the relative spread (*RS*) and the threshold (being a function of the stimulus duration, a relation known as strength-duration relation). The *RS* is obtained by the quotient of spread and threshold of the Gaussian distribution function. It is a dimensionless number, independent of the intensity and duration of the applied stimulus. With the *RS* and the threshold, the input output relation of the nerve fiber preparation is described in a useful approximation by equations (1), (2) and (3). The characteristics of the duration-probability relations obtained with the use of these equations were also found to apply to the nerve fiber. The *RS* and the threshold (strength-duration relation) are, therefore, the parameters characterizing its input-output relations.

In these experiments the rectangular current is applied to the whole part of the nerve surrounded by the cathode (cf. Chapter II). The effects studied, however, apply to the frog node of Ranvier or the cray-fish axon, situated somewhere within the stimulated part of the nerve. These excitable units can be looked upon as containing a trigger mechanism, which induces the unit to generate an action potential or not, depending on the value of the change induced in the unit by the stimulating current applied to the preparation and also on the fluctuation in excitability of the unit. The change induced in the unit by the application of the stimulus is termed *the local activation process.* It is not relevant here to go into further detail about the mechanism and the nature of the processes that form the local activation process. It will be considered as a transformation of the electrical stimulus, its (maximal) value being given by a correction of the stimulus intensity with the use of *a transformation factor,* symbolized by φ. The supposition is, thereby, that this factor does not change when the intensity of the stimulus is altered.

Thus, measures based on the use of stimulus intensity must be corrected by this factor when they are applied to the intrinsic processes supposedly occurring at the trigger-part of the unit. Therefore, the measures to be corrected are the threshold and the spread, because they are expressed in stimulus intensity. This gives rise to the notion of two intrinsic parameters:

a. *The intrinsic threshold,* symbolized by *h,* and obtained by the correction of the threshold $\mu(\tau)$ by the transformation factor φ, which gives

$$h = \mu(\tau) * \varphi \qquad (4)$$

[1]) It is superfluous to present all the transformations of the 190 sets of relations obtained, or the data on their closeness of fit. The hypothesis can be considered proved by sheer evidence.

b. *The intrinsic spread,* symbolized by σ, obtained by the correction of the spread σ(τ) by the transformation factor which gives

$$\sigma = \sigma(\tau) * \varphi \ . \tag{5}$$

The intrinsic threshold is considered to be the value which must be reached by the local activation process initiated by the applied stimulus, if the unit is to generate an action potential. The intrinsic spread is the parameter of the fluctuation in excitability; a process that introduces an uncertainty for the local activation process to reach the intrinsic threshold, a property of the unit itself as was clarified previously by the mutual independency from fibers in the same preparation.

Both spread and threshold are functions of the stimulus duration τ. Their quotient, the relative spread, is found to be independent of the stimulus duration. From equations (4) and (5) follows for the relative spread (*c*):

$$c = \frac{\sigma(\tau)}{\mu(\tau)} = \frac{\sigma/\varphi}{h/\varphi} = \frac{\sigma}{h} . \tag{6}$$

The duration-dependent factor disappears from the quotient. This factor is, therefore, ascribed to the transformation factor. Hence, the transformation factor is a function of the stimulus duration, and represented by (z), while the intrinsic spread and the intrinsic threshold are independent of the applied stimulus.

The parameters characterizing the input-output relations can now be expressed in the factors derived here. This gives for the threshold:

$$\mu(\tau) = \frac{h}{\varphi(\tau)} , \tag{7}$$

the strength-duration relation.

The spread becomes:

$$\sigma(\tau) = \frac{\sigma}{\varphi(\tau)} , \tag{8}$$

The relative spread is:

$$c = \frac{\sigma}{h} . \tag{9}$$

In the appendix a descriptive mathematical model is presented, based on Rashevsky's theories on excitation and fluctuation (1948), but modified by some simplifications. The input-output relations for this model are calculated under certain restrictions. It follows that they are equal to the equations (1), (7), (8) and (9).

We might, therefore, consider intrinsic threshold, intrinsic spread and the transformation factor to be the parameters determining the input-output relations of the units studied. Additional evidence in support of this conclusion is presented in the next section.

In the experiments the relative spread is the only parameter which can be measured directly, as is done here, because of its independence of the stimulus. Threshold and spread can only be measured indirectly, since the value of the transformation factor is not known. The investigations were restricted to studying the changes in the threshold, and direct measures of the relative spread; the behaviour of the spread being known from its relation to threshold and relative spread. The relative spread has, however, still another important value. Aside from being measured without much difficulty, it might be considered upon as a measure for the indeterminateness of the unit (cf. Chapter VI, 4).

3. The influence of conditioning stimuli and of chemical substances

a. Introduction

Further investigations of fluctuation in excitability were now faced with the question what would happen during the recovery period and during the application of an ineffective stimulus. It was also interesting to learn what would happen with the input-output relations after the application of strychnine and urethane.

From all experiments reported in Chapter III, 2 and 3, the following general conclusions have been drawn:

a. A fluctuation in excitability is always present, even during the recovery period.
b. The general form of the relationship between probability of response and stimulus intensity remains the same. It is always possible to describe these relations with the Gaussian type of distribution function.
c. The influences exerted on the relations are, therefore, restricted to changes in the parameters of this type of function, viz., the threshold and the relative spread 2).

The following influences on the parameters of the input-output relations were found:

1. In the supranormal phase of the recovery period the threshold is decreased; no change in the relative spread was detected.
2. A sub-rheobasic current decreases the threshold and increases the relative spread. Their product, the spread, is not altered.
3. Strychnine increases the relative spread; no influence on the threshold was detected.
4. Urethane increases the threshold and decreases the relative spread; no influence on their product, the spread, was found.

What is revealed by these results concerning the properties of the nerve fiber, viz., the node of Ranvier?

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[2]) When the induced change in these two parameters appeared to be in an opposite direction, an attempt was then made to determine what had happened to the product of these parameters, the spread. In the other cases, this product was also investigated.
However, since the observed change was always equal to the alteration expected from the change observed in threshold and relative spread, these secondary conclusions were not explicitly presented.

For the sub-rheobasic current it became apparent that no changes in the parameters were detectable when the stimulation value of the whole stimulus-complex, at the moment of summation (sub-rheobasic stimulus plus test-stimulus), was considered.

Such an explanation, however, does not apply to the effects mentioned in 2, 3 and 4. Moreover in these cases the pattern of the induced changes differs. During the recovery period the threshold is changed, but not the relative spread. Strychnine increases the relative spread, but not the threshold. Urethane, on the other hand, changes both the threshold and the relative spread, but in opposite directions and in such a manner, that their product, the spread, remains unaltered. It seems, therefore, feasible that these two parameters are functions of other, intrinsic, parameters of the nerve fiber preparation.

In view of the type of the changes mentioned above, it can be assumed that at least three intrinsic properties are present:
The first one contributes to, let us say, the threshold.

A change in this property would alter the threshold, leaving the relative spread unchanged.

A second property should contribute to the relative spread. Any change in this property would alter the relative spread, leaving the threshold unchanged.

The third property would contribute to both threshold and relative spread; a change in this factor inducing exactly opposite changes in threshold and relative spread, leaving their product, the spread, unchanged.

From the properties previously discussed, and the equations derived for the threshold and the relative spread it follows that, with the use of these attributes, the observed findings can be explained. Equations (7) and (9) show that threshold and relative spread have one common factor (the intrinsic threshold h), the alteration of which induces exactly opposite changes, and that each one possesses a factor not present in the other parameter. Hence, based on the use of these properties, derived before, the results of the experiments can be discussed in more detail.

b. Sub-rheobasic direct current

In order to investigate this influence, the threshold was decreased to 50 % of its original value by the introduction of a sub-rheobasic direct current. Because the probability-intensity relations were determined with respect to the intensity of the conditioned test-stimulus, and not to the stimulating value of the whole stimulus-complex at the moment of summation, the situation is comparable to a reduction of the intrinsic threshold h by 50 % of its original value. From equations (7) and (9) it is therefore expected that this would result in a decrease of the threshold $\mu(\tau)$, an increase of the relative spread (c), while their product, the spread $\sigma(\tau)$ remains unaltered. This is in accordance with the experimental observations. If the direct current had caused a change in the intrinsic spread σ, the resulting change in the *RS* (c) would have been found to be disproportional to the change in the threshold $\mu(\tau)$ and their product would have been altered. The same type of reasoning applies to a change induced in the intrinsic threshold

h or in the transformation factor $\varphi(\tau)$. No disproportionality has been detected. Therefore, no evidence has been brought to light that a conditioning current influences the intrinsic properties of the nerve fiber; the influence of the current being comparable to a reduction of the intrinsic threshold.

c. Recovery period

'The recovery period is an additional factor, the change in excitability following the production of an action potential. This is not accounted for in the derivations presented before. The results of the experiments indicate that the *RS* may not be influenced by it, while the threshold is altered, being decreased because of the supra-normal period. This decrease, however, was small. The interpretation of these results, therefore, must be made with reserve. These results may indicate, that no influence is exerted on the intrinsic spread σ and on the intrinsic threshold *h*, but, possibly, that only the transformation factor $\varphi(\tau)$ has changed.

d. Application of strychnine and of urethane

Following the application of strychnine it was noted that the threshold was not found to be altered, while the *RS* was significantly increased. This can be explained by an increase in the intrinsic spread σ, while the intrinsic threshold *h* and the transformation factor $\varphi(\tau)$ remain unchanged. This is exceptionally interesting, since strychnine in these low concentrations has practically no other effect on peripheral nerve fibers, though it is used in concentrations that have a definite influence on the central nervous system.

Urethane was found to increase the threshold and decrease the *RS*, while their product was not found to be altered. The observed increase in threshold is in accordance with the observations of Tasaki (1942, 1953). The results of our experiments may be interpreted as an effect exerted only on *h*, the intrinsic threshold. Although urethane was chosen with the idea that it might possibly cause a depression of fluctuation in excitability, a fortunate fortuity of the result of this experiment is that it influences, especially, the other parameter determining the relative spread, the intrinsic threshold *h,* while strychnine affects σ, the intrinsic spread.

e. Conclusion

The input-output relations of the nerve fibers to stimulation with rectangular electrical currents are described by the Gaussian type of distribution function and characterized by the threshold and the relative spread. The relations are, however, determined by the parameters of the properties of the preparation: the local activation process (parameter: transformation factor), the fluctuation in excitability (parameter: intrinsic spread) and the intrinsic threshold.

The results of the investigations on the influence of conditioning stimuli and of the application of strychnine and of urethane support the employment of the

derived properties of the nerve fiber preparation. They are concluded to be independent, because they behave independently of each other.

The intrinsic parameters of the nerve fiber are not influenced by changes in stimulus duration (page 46), nor by summation of currents (page 48) and perhaps also not in the recovery period (page 49). It is by chemical influences only that they are found to alter. Strychnine increases the intrinsic spread and has no influence on the intrinsic threshold. Urethane increases the intrinsic threshold and has no influence on the intrinsic spread.

VI. GENERAL ASPECTS

1. Pecher's fluctuation in excitability

In 1932 Adrian suggested that irregular fluctuations in the threshold [1]) of the nerve could account for the irregular injury discharge. In the same year Blair and Erlanger and also Monnier and Jasper made their observations on the occasional responses of the nerve fiber to identical stimuli. Pecher called this phenomenon 'la fluctuation d'excitabilité' and after describing painstakingly his observations on the frog nerve fiber (1939) he posed the question: 'Des fluctuations analogues existent elles lors de l'excitation du neurone par des processes physiologiques?' His untimely death never permitted him to find the answer to this question. At present, this may be given in the affirmative.

Before going into details, it is necessary to define fluctuation in excitability:

Fluctuation [2]) in excitability is the property of that biological unit, which, upon the presentation of a non-random input, may produce an output according to a certain probability.

The input may be a signal described by a step-function (e.g., the sudden stretch of a muscle spindle (Ratz, 1949, 1950 a and b; Buller, et al., 1953; Hagiwara, 1954), or by a more complex function with (fixed parameters (e.g., the repeated application of a rectangular current as used in our investigations), or the input may even be 'zero', the random output if present is then a form of the so-called 'spontaneous activity' (cf. Bullock, 1953).

The output may be a series of action potentials with a random fluctuation of the intervals around a mean (e.g., the muscle spindle cited above) or the presence or absence of an impulse, the succession of which on repeated application of the stimulus appears to be distributed at random.

Given such a unit, or a population of units (a system) each of which exhibits a fluctuation in excitability, two problems arise:

1. In what way is the output related to the input of the unit, i.e., what are the

[1]) The term 'fluctuation of threshold' is not upheld, because a parameter-free term is to be preferred. The term 'fluctuation of threshold' might, for example, imply that the degree of fluctuation is a function of the value of the threshold. This appears to be so, if the intrinsic properties are not considered (Fig. 8b), because both threshold and spread are functions of the intrinsic properties of the nerve fiber. The intrinsic spread is independent of the value of the intrinsic threshold, as shown in this study. The term 'fluctuation of threshold' would be justified, if these intrinsic parameters are not independent, but whether such a situation applies to neuronal units has to be doubted for the reasons already elaborated.

[2]) Thee frequently used term 'oscillation' should be avoided when fluctuation in excitability is meant. 'Fluctuation' is the term for a random variation of some parameter occurring in time, but essentially independent of it. The term 'oscillation' should be reserved for a cyclic time-dependent variation of some parameter.

characteristics of the unit? This problem can be dealt with by investigating the input-output relations of the unit and, secondly, by interfering with its internal structure.

2. The second problem concerns the nature and source of this phenomenon.

This study is limited to the first problem, the object being a simple unit, the single nerve fiber of which the yes-or-no output was related to a simple input, namely to the rectangular stimulus with its two parameters, intensity and duration.

The primary condition mentioned in Chapter V, 1 led to investigate whether the probability of response has, each time, the same and independent value following the repeated presentation of the same input signal. This was shown to be true for the units investigated, viz., the peripheral nerve fiber of the frog and cray-fish.

Frishkopf (1956) reports the same results for the population of auditory neurons upon the presentation of clicks. Krnjevic and Miledi (1959) describe a partial block in the most distal branches of the motor nerve fiber after tetanic stimulation. During the presence of this block the positive and negative responses are grouped at random to repeated stimulation with frequencies below 20/second, which they could demonstrate by means of the run-test.

From these investigations it can be concluded that, whether there is a natural (clicks, action potentials) or artificial input (negative rectangular currents), a random output exists in the presence of a non-random input, viz., a fluctuation in excitability is present in the systems investigated.

Other reports on the existence of a fluctuation in excitability have been published, as mentioned in the introductory chapter. Rather complex systems have also been investigated, but if only the input-output relations are considered they are comparable to the system described above:

Reports were published by Amassian and co-workers (1958, 1959) on single cortical units in monkeys; Rosenblith (1954) on single auditory neurons; Granit and Strom (1951) ; Lloyd, et al. (1955, 1957, 1958) and Hunt (1955 a and b) on the motoneurons in the spinal cord; and by Darian-Smith (1960) on the neurons in the main trigeminal nucleus.

A similar phenomenon was also found in the neuro-muscular junction by Fatt and Katz and co-workers (1950, 1951, 1952; del Castillo and Katz, 1953 a and b, 1954 a en b) and in myocardial tissue (van Dam, 1960).

It can safely be assumed that general agreement exists regarding fluctuation in excitability as being a general property of excitable tissue.

Pecher's pioneering investigations can best be honored by naming this phenomenon after him, viz., Pecher's fluctuation in excitability.

2. The input-output relationship

We could now ask whether the Gaussian type of distribution function applies to the intensity-probability relations of neuronal units. For this purpose, a search was made in the pertinent literature for intensity-probability relations in relatively simple systems.

Lloyd and McIntyre (1955; Lloyd, 1957, 1958) and Hunt (1955 a and b) investigated the mono-synaptic reflex responses of individual motoneurons. In these studies the relationship between probability of response (the `firing index')

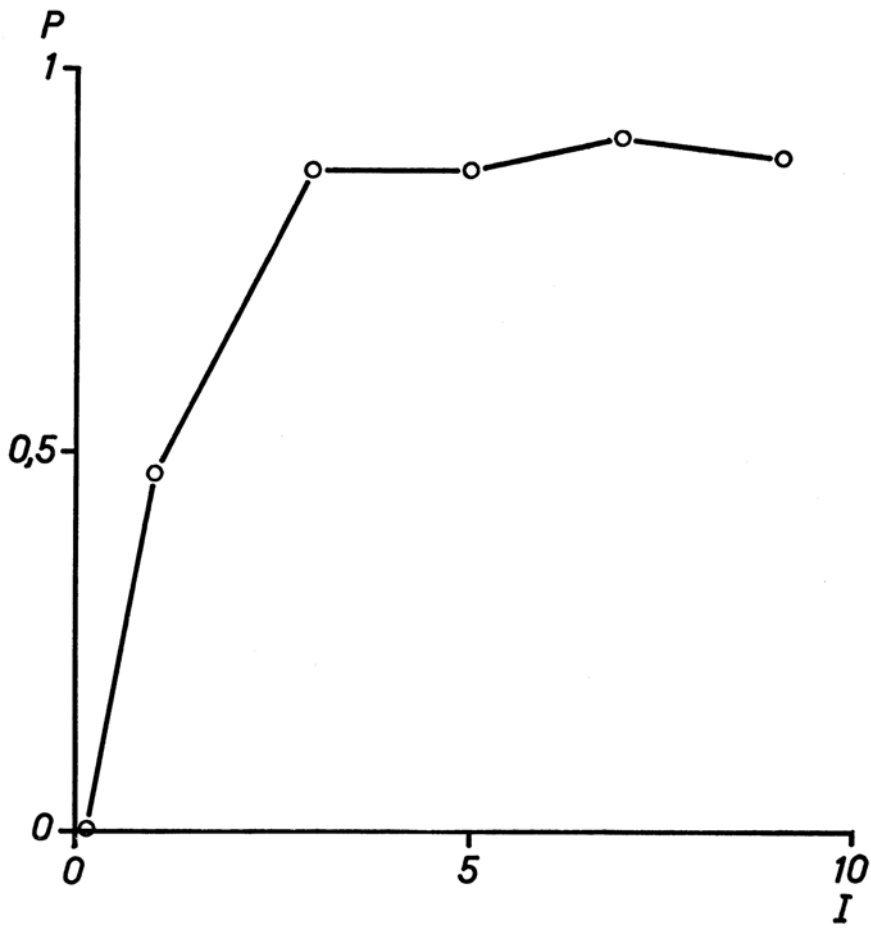

Fig. 16.
The relation between probability of response of a cortical unit and the intensity of a stimulus applied to the digits (monkey).
Redrawn after Towé and Amassian (1958).

and the intensity of the stimulus at its arrival at the neuron (the 'synaptic drive' or 'transmitter potentially') was evidently Gaussian (Lloyd, 1957). The observed relationship is in part due to a random input, the so-called 'background fluctuation' (Lloyd and McIntyre, 1955), but a fluctuation in excitability is also present (Hunt, 1955 a; Rail and Hunt, 1956).

The most explicit study has appeared on the input-output relations of trigeminal neurons, i.e., Darian-Smith's investigation of the neurons in the main

trigeminal nucleus of the cat (1960). He first investigated the relationship between the voltage applied to the skin and the peak-to-peak amplitude of the pre-synaptic potential. This relation appeared to be linear. Therefore, he could

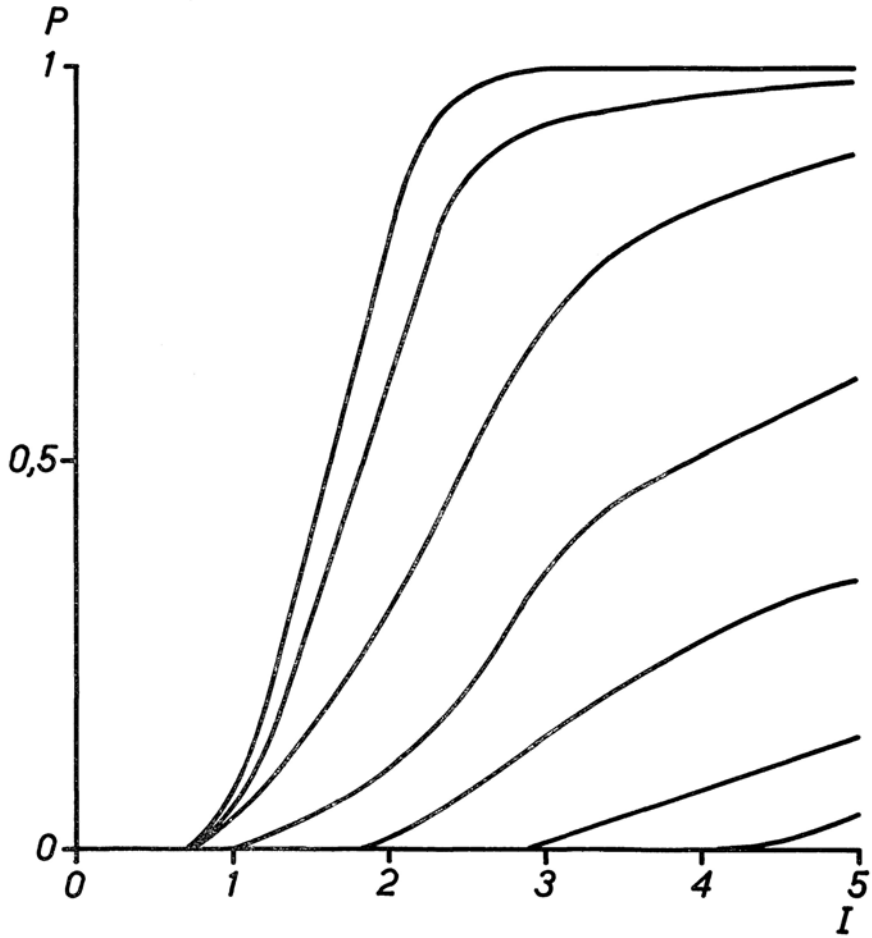

Fig. 17.
The relation between probability of response of a motoneuron and the intensity of a stimulus applied on the dorsal root (cat).
Redrawn after Hunt (1955b).

relate the probability of response directly to the intensity of the applied stimulus. Studying 120 units he found a Gaussian type of distribution function applicable to the relation between probability of response and the stimulus intensity. For 80 of the 120 neurons investigated the relationship is due to a fluctuation in excitability. This is due to the fact that these neurons are monosynaptically activated, and because the convergence on these neurons is very limited. This severely restricts the possible influence of background fluctuation.

This last study, especially, provides strong evidence in support of the hypothesis, that the Gaussian type of distribution function applies to the relationship between probability of response and stimulus intensity of neurons. It is, therefore, feasible that in a neuron discharging with a certain probability upon the arrival of a stimulus, the relationship between probability of response and intensity of the *locally arriving* stimulus is defined by a Gaussian type of distribution function.

Possible deviations of this hypothesis must be examined first, as the possibility exists that the observed distribution may be due to the fact that a complex

system of such units is investigated. Two possibilities can be distinguished:

a. The output recorded is the output of a whole population of units. This problem is discussed in detail by Frishkopf (1956).

b. The output is recorded from a single unit forming the terminal point of a more or less complicated chain, at the beginning of which the (peripheral) stimulus is applied. Though the input-output relation of the unit might be Gaussian, in general no Gaussian type of intensity-probability relation will be obtained from the system, because the relation between the intensity of the signal arriving at the unit and the intensity of the peripherally applied stimulus will, in general, not be linear.

In the monosynaptic system investigated by Darian-Smith this relation is linear, but in the aforementioned monosynaptic motoneuron system this relation is not linear, or at most only in some specific cases.

Amassian and co-workers *(1958,* 1959) worked on the relation between the response-probability of single cortical neurons and the intensity of the stimulus applied to the digits. The relation is clearly not Gaussian, but if this graph (Fig. 16) and also those given by Hunt (1955 b) (cf. Fig. 17) are compared with the duration-probability curves (Fig. 9), the similarity is striking. This gives the impression that, given a Gaussian type of intensity-probability relation for the neuron, it is the *duration* of the stimulus locally activating the neuron, that is related to the *intensity* of the peripherally applied stimulus.

Conclusion: The input-output relationships reported for complete and for higher-order neurons do not provide evidence against the hypothesis that the intensity-probability relations of these units themselves can be described by a Gaussian type of distribution function.

3. The Relative Spread

The relative spread was experimentally found to be the parameter for the fluctuation in excitability. This value characterizes, together with the threshold (the strength-duration relation), the input-output relations of the nerve fiber (equations (1), (2) and (3)). The relative spread is independent of intensity and duration of the applied stimulus.

It was concluded that three independent properties exist in the nerve fiber preparation. The input-output relations calculated on these propositions (Appendix), also showed that the relative spread is a parameter, independent

of the intensity and duration of the applied stimulus. It followed that the relative spread is a function of the intrinsic spread and the intrinsic threshold, being their quotient. Both intrinsic parameters will display their existence, however, only after a, rigorous, interference with the structure of the system to which they apply. This was achieved by the application of strychnine and of urethane. The results were indicative of an increase in the intrinsic spread due to strychnine, while the intrinsic threshold was left unaltered. Urethane application resulted in an increase in the intrinsic threshold, while the intrinsic spread remained unaltered (Chapter III, 3 and Chapter V, 3).

The question may now be asked as to whether there is evidence that the concept of the relative spread is applicable to other units in the nervous system.

Again, the study of Darian-Smith provides some illustrative data. In one graph (1960, Fig. *5a)* he plotted the probability of response against the stimulus intensity of a repetitively discharging neuron and in another (1960, Fig. 5b) he presented the value of the threshold and the variance for each successive spike. We attempted to reconstruct the original values from this graph and to calculate the relative spreads for each successive spike (Table XIV). It is apparent that

TABLE XIV

Relative spreads,
calculated for successive spikes of a repetitively discharging trigeminal neuron

spike no.	threshold (Volts)	variance $(Volts)^2$	spread (Volts)	relative spread
1	1.4	0.22	0.47	0.34
2	4	1.0	1.0	0.25
3	10	2.0	1.4	0.14
4	30	6.3	2.5	0.08
5	54	140	11.8	0.22
6	80	400	20	0.25

The values for threshold and variance were estimated from the graphs published by Darian-Smith (1960, Fig. 5b).

the relative spreads for the second and the sixth spikes are alike, despite the great difference in threshold. This finding for a trigeminal neuron is, therefore, an argument, that the concept of the relative spread is also applicable to units other than the peripheral nerve fiber.

It should also be noted, that rather high values for the relative spreads were obtained from the data of Darian-Smith (Table XIV). He distinguishes two kinds of trigeminal neurons. The first is monosynaptically activated. For these neurons he gives a mean value of the spread of 0.79 V and states that the threshold for most units is below 30 V. The second type of neuron is a type which is polysynaptically activated. He reports that their variances might be about 400 times as large as those found for the monosynaptically activated neurons, but that the mean threshold is of the same order. This implies that the

spreads might be 20 times larger than those of the monosynaptically activated neurons and consequently the relative spreads as well. This may be partly due to a background fluctuation, but these values might also indicate that the relative spreads themselves are large.

It is interesting to compare these findings with our investigations on frog and crayfish peripheral nerve fibers. In frog A -fibers, a mean relative spread of 0.011 was registered and for the most excitable cray-!fish axons the mean value was 0.0012. These values are smaller than those calculated from Darian-Smith's data on trigeminal neurons.

Upon considering these values for the relative spreads, some additional questions may arise: Is the value of the relative spread a species-characteristic? Does the relative spread increase with increasing order of the neurons? We cannot answer these questions at this time, but we might ask ourselves: can a specific function be assigned to Pecher's phenomenon?

4. On the possible meaning of Pecher's fluctuation in excitability on the function of the nervous system

Is there any significance of the fluctuation in excitability for the function of the nervous system? One possibility is apparent and this was already mentioned by Hunt (1955 a): The fluctuation in excitability may be useful for finer gradation of response, because the probability of response is graded as the stimulus varies.

We may, however, add that it is the width of the threshold range, relative to the value of the threshold, that is important (Fig. 1)

If the width of the threshold range is small, the output will be determined for most input-values, the probability of response being generally zero or one. If the width of the range is large, the response to a single input-signal is generally undetermined; the reaction of the unit to such a signal gives the impression that the unit 'chooses the state it will assume', i.e., whether it 'will' produce an output-signal or not. The unit is thus provided with a degree of undeterminateness, a 'degree of freedom'. This might enable a complex system, composed of these units, to achieve a determinateness which is not strict and therefore allows other means of behavior.

Let us, therefore, repeat in plain words what occurs in such a unit upon the arrival of a given signal: The input-signal initiates a 'local activation process', considered as a transformation of the input-signal, depending therefore, on the properties of the preparation and on the form of the input-signal. For an input-signal of a given form, the amplitude of the transformed signal is (approximately) linear to the intensity of the input-signal. (This process may also be influenced by preceding input-signals and discharges and by signals arriving from other inputs). An independent variable, the fluctuation in excitability, is present, characterized by its parameter, the 'intrinsic spread'. The sum of the transformed signal and of the momentaneous value of fluctuation in excitability initiates a discharge at the moment that a critical value, the 'intrinsic threshold', is exceeded. The intrinsic threshold is also independent in itself.

These postulated intrinsic factors and processes *determine* the input-output relations of the unit (equations (1), (7), (8) and (9)). These relations are, however, *characterized* by the threshold (equations (2) and (7)) and the relative spread (equations (3) and (9)).

What do these two parameters indicate with respect to the function of the unit?

The threshold is generally considered as a measure for the excitability of the unit, which, when used in this connotation, is expressed as the reciprocal of the threshold. It is known, therefore, that: *It the threshold is high, the excitability of the unit is low; if the threshold is low, the unit is highly excitable.*

The relative spread is a measure for the width of the threshold range, relative to the threshold (Fig. 1). It is clear from the preceding that this parameter also specifies a property of the unit its 'undeterminateness'. *I f the relative spread is small, the unit is more determinate, if the relative spread is large, the unit is less determinate.* It is the expectation that *if the relative spread is very large, the unit will discharge spontaneously.* (This spontaneous activity (cf. this Chapter, 1) will be present if the relative spread exceeds 0.3). All possible combinations of excitability and undeterminateness may occur, according to the specific values of the parameters of the intrinsic properties (equations (7) and (9)). These intrinsic parameters may be variable too, as the experiments with strychnine and urethane have shown:

Strychnine, increasing the intrinsic spread, renders the unit less determinate, while the excitability remains the same.

The unit is less determinate, because the relative spread is increased:

Sometimes signals of smaller intensity initiate a discharge, while before the application of strychnine they had a response probability of zero; sometimes signals of high intensity, which, before, had a response probability of one are 'blocked'. The mean level, the threshold, is, however, not altered. It is feasible that the stability of a complex system, composed of these units, is now greatly decreased; a well-known situation encountered in strychnine intoxication.

Urethane on the other hand increases the threshold, and, therefore, decreases the excitability of the unit. The secondary decrease of the relative spread, which tends to make the unit more determinate, is unimportant with respect to the depression of excitability.

Other questions emerge now. Is there any significance to the difference between the relative spread of frog and cray-fish axons? Is the one less determinate and the other more 'fixed' in its reaction-pattern?

Annexed to these questions is the problem concerning the nature of Pecher's fluctuation. A more detailed discussion, however, falls outside the scope of this work, which is primarily concerned with the properties of Pecher's fluctuation with regard to the function of the nerve fiber, the transmission of signals, as it is stated in the introduction. Since the material concerning the problem of the nature of Pecher's phenomenon is rather conflicting, it is also useless to present a brief outline. (For further orientation see: Pecher, 1939; Landahl, 1945; Fatt and Katz, 1952; Buller, et al., 1953; van Lier, *1955.)* Even the equations of

Hodgkin and Huxley are of no help in explaining the factors responsible for it (cf. Cole, Antosiewicz and Rabinowitz, 1955). The problem of the nature and source of Pecher's fluctuation is very intricate and further research is necessary if more light is to be shed on it.

In conclusion, we may say, that Pecher's fluctuation in excitability is the manifestation of an uncertainty, being an inherent property of nervous tissue. This uncertainty might be considered to be of functional importance for biological systems.

5. Summary

In this chapter, Pecher's question whether a fluctuation in excitability exists in other neural elements aside from the peripheral nerve fiber, is discussed:

1. A more exact description of the term 'fluctuation in excitability' is given.
2. From a survey of the literature, it was concluded that there is a general agreement concerning the existence of this phenomenon as a property of excitable tissue.
3. Evidence was presented that the Gaussian type of distribution function applies to other neuronal elements aside from peripheral nerve fibers.
4. Some remarks are made concerning the function ascribed to the two parameters (the threshold and the relative spread) characterizing the input-output relations:

   The threshold is generally used as a measure for the excitability of the unit. A hypothesis is discussed regarding the 'undeterminateness' of the unit, for which the relative spread can be considered to be the measure. It is concluded that Pecher's phenomenon is the manifestation of an uncertainty inherent to signal transmission in biological systems.

VII. APPENDIX

A mathematical model

Introduction

The problem to be investigated is the general form of the input-output relationship with respect to the intrinsic properties of the unit.

These aspects are given only in a descriptive way and are presented in the simplest equations possible, based on Rashevsky's theories of excitation and fluctuation (1948; Landahl, 1945) [1]). More 'realistic' concepts, such as that of membrane potential are, therefore, neglected.

Since the nerve (fiber preparation in our experiments made is stimulated with electric current, the intensity of the stimulus is expressed in terms of this dimension [2]).

General aspects of the unit

The input-signal S (ι, τ), a negative rectangular current, is transformed by the unit (and its surroundings) according to its specific characteristics. It is assumed that the unit is linear.

A fluctuation in excitability, described by a Gaussian random variable ξ, is present in the unit.

When the sum of these two processes reaches a critical value h, called the 'intrinsic threshold', the unit produces an output-signal, the action potential ($A = 1$). When this value is not reached, no action potential is produced ($A = 0$).

The problem to be solved is the equation for the probability of response P ($A = 1$), with regard to a rectangular input-signal S (ι, τ), with an intensity ι and a duration τ: $P(A = 1 \mid \iota, \tau)$.

The input-signal

When τ is given as

$$\tau = t_{off} - t_{on},$$

the input-signal can be described as

$$S(\iota, \tau) = \begin{cases} 0, & t < t_{on} \ \ and \ \ t > t_{off} \\ \iota, & t_{on} \leq t \leq t_{off} \end{cases}$$

---

[1]) Additional problems such as the fluctuation in latency and the influence of the recovery period are not covered here. For reference to these aspects of the problem see: Rashevsky (1948), Hagiwara (1954) and Viernstein and Grossman (1960).

[2]) For explanation of symbols see page 65.

Because of the linearity of the unit it can also be expressed as the difference of two signals of infinite duration with equal intensities t, but with different times of arrival:

$$S(\iota, \tau) = S(\iota, t - t_{on}) - S(\iota, t - t_{off}),$$

with

$$S(\iota, t - t_x) = \begin{cases} 0, & t < t_x \\ \iota, & t > t_x \end{cases},$$

when x stands for 'on' or 'off'.

The characteristics of the unit

a. The transformed signal

The 'local activation process' is treated as a transformation of the input-signal. The transformed signal is presented with respect to an input-signal of infinite duration. It is determined by the difference between two intrinsic processes [3]):

1. An excitatory process e, approximated by

$$\varepsilon = \iota\,[1 - \exp\{-k(t - t_x)\}],$$

in which k is a time constant.

2. An inhibitory process j, approximated by

$$j = \iota\,[1 - \exp\{-m(t - t_x)\}],$$

in which m is a time constant.

The condition is that

$$0 \leq m < k.$$

The transformed signal, $E(\iota, t - t_{on})$, is now given by

$$E(\iota, t - t_{on}) = \varepsilon - j = \iota\,[\exp\{-m(t - t_{on})\} - \exp\{-k(t - t_{on})\}].$$

This function reaches its maximum at t_u:

$$t_u = t_{on} + \frac{1}{k - m} \ln \frac{k}{m}.$$

With respect to the input-signal $S(\iota, \tau)$ the transformed signal is

$$E(\iota, \tau, t) = \begin{cases} 0 & , t < t_{on} \\ E(\iota, t - t_{on}) & , t_{on} \leq t \leq t_{off} \\ E(\iota, t - t_{on}) - E(\iota, t - t_o) & , t > t_{off} \end{cases}$$

---

3) The following simplifications are made in Rashevsky's equations:

$$\frac{K}{k} = \frac{M}{m} = 1 \quad \text{and} \quad j_0 - \varepsilon_0 = h.$$

$E(\iota, \tau, t)$ reaches its maximal value at t_{max}:

$$t_{max} = \begin{cases} t_u\ ,\ t_{off} > t_u \\ t_{off},\ t_{off} < t_u \end{cases}$$

$E(\iota, \tau, t_{max})$, the function of the maxima of $E(\iota, \tau, t)$, *is*

$$E(\iota, \tau, t_{max}) = \_\iota \bullet \varphi(\tau) \tag{1}$$

With

$$\varphi(\tau) = \exp\ \{ - m(t_{max} - t_{ton})\} - \exp\ \{ - k(t_{max} - t_{on})\}.$$

$\varphi(\tau)$ is a dimensionless factor, *the transformation factor.*

b. The fluctuation in excitability

The fluctuation in excitability is represented by a Gaussian random variable ζ, of the same dimension as $E(\iota, \tau, t)$. During each time interval Δt, ζ has some constant value ξ. This fluctuation therefore applies to the model unit under the following condition [4]):

$$\tau \ll \Delta t \ll T\,. \tag{2a}$$

The value of τ is small, with respect to Δt. This implies that the specific value ξ does not change during the presence of the signal. The time interval Δt is small with regard to the interval between successive stimuli, T. The successive reactions are, therefore, independent.

The probability of ζ to have at least a certain value ξ upon the application of an input-signal is now given by the Gaussian distribution function:

$$P(\boldsymbol{\xi} \geqslant \xi) = \frac{1}{\sigma\sqrt{2\pi}} \int_{\xi}^{+\infty} \exp\left\{ -\tfrac{1}{2}\left(\frac{x}{\sigma}\right)^2 \right\} dx. \tag{3}$$

The value of the mean of this distribution is zero. Its standard deviation σ is called *the intrinsic spread.*

c. The condition for excitation

A certain critical value *h,* called *intrinsic threshold,* exists in the unit. The condition for excitation is then represented by

$$E(\iota, \tau, t) + \zeta \geq h. \tag{4a}$$

When condition (2) is taken into account, this condition (4a) becomes

$$E(\iota, \tau, t_{max}) + \geq h. \tag{4b}$$

---

[4]) When $\sigma \ll h$, the probability of response is still approximately zero for the already large values of $E(\iota, \tau, t)$. The influence of the fluctuation in excitability is then only present when $E(\iota, \tau, t)$ already has a value very close to that of the intrinsic threshold. In this case, τ might be as great as (or even exceed) Δt. This process is, therefore, applicable with the following condition: instead of (2a)

$$\sigma \ll h \text{ and } \tau \leq \Delta t \ll \mathrm{T}. \tag{2b}$$

The input-output relation

The problem to be solved is the input-output relation of the system, i.e., the equation of the probability of response, with respect to the input-signal $S(\iota, \tau)$. From equations (3) and (4b) follows

$$\left.\begin{aligned} &P[\xi \geqslant h - E(\iota,\tau,t_{\max})] = \\ &= P(A=1|\iota,\tau) = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{E(\iota,\tau,t_{\max})-h} \exp\left\{-\tfrac{1}{2}\left(\frac{x}{\sigma}\right)^2\right\} dx. \end{aligned}\right\} \quad (5)$$

The strength-duration relation $\mu(\tau)$ should now be calculated:

By definition of the threshold, $\mu(\tau)$ is the value for ι, for which

$$P(A = 1|\, \iota,\, \tau) = \tfrac{1}{2},$$

which gives(6a)

$$E(\iota,\, \tau,\, t_{\max}) - h = 0, \quad (6a)$$

With

$$\iota = \mu(\tau) \quad (6b)$$

From equations (1) and (6) it follows that

$$\mu(\tau) = \frac{h}{\varphi(\tau)} \quad (7)$$

(cf. equation V (7)).

$E(\iota, \tau, t_{\max}) - h$ can now be expressed in ι and $\mu(\tau)$, with the use of equations (1) and (7)

$$E(\iota,\, \tau,\, t_{\max}) - h = \frac{h}{\mu(\tau)}\{\, \iota - \mu(\tau)\}. \quad (8)$$

Substitution in equation (5) gives:

$$P(A=1|\iota,\tau) = \frac{1}{\frac{\sigma\,\mu(\tau)}{h}\sqrt{2\pi}} \int_{-\infty}^{\iota} \exp\left\{-\tfrac{1}{2}\left(\frac{x-\mu(\tau)}{\frac{\sigma\mu(\tau)}{h}}\right)^2\right\} dx. \quad (9)$$

This equation describes the relation between probability of response and the parameters of the input-signal $S\,(\iota, \tau)$ for the model of the unit described above.

The standard deviation of this function (9):

$$\sigma(\tau) = \frac{\sigma\,\mu(\tau)}{h} = \frac{\sigma}{\varphi(\tau)} \quad (10)$$

is comparable to the spread (equation V–(8)).

The coefficient of variation is

$$c = \frac{\sigma}{h} \quad (10)$$

and is comparable to the relative spread (equation V– (9)). This coefficient is

independent of the intensity and duration of the input-signal and is a dimensionless number; this is in accordance with the experimental results.

Comment

The calculations which led to these equations are based on two restrictions.

The first restriction regards the linearity of the unit. The nerve fiber actually does not behave in this way (cf. Tasaki, 1956; Monnier and Lavigne, 1952). How non-linearity will interfere with the assigned properties of the unit is not clear at this time. This implies, for instance, that a positive rectangular current will behave solely as described here. A restriction which also applies to equation III – (1). Because of the close agreement with the experiments this point is regarded to be no problem here.

The second restriction regards the condition for the Gaussian random variable to be applicable as a model:

$$\tau \ll \Delta t \ll T$$

or, as was given in foot-note 4:

$$\sigma \ll h \text{ and } \tau \leq \Delta t \ll \mathrm{T}.$$

The greatest value of the relative spread encountered in our experiments was 0.05, which implies indeed, that $\sigma \ll h$ (cf. equation 11).

Two studies have been published regarding the value of Δt. Frishkopf (1956) in his experiments with the use of externally added noise, arrives at a minimal number of 2000 of these time intervals per second. This gives a maximal interval duration Δt of 0.5 msec. Viernstein and Grossman (1960) used a number of 1000 time intervals per second in their calculations; with an error of 50 %. This implies that the duration, of such a time interval is somewhere between 0.7 and 2 msec.

In our experiments the stimulus durations used were: 0.25 and 2.5 msec in the investigations concerning the strength-duration-probability relations of the frog nerve fibers; 0.12 and 1.2 msec in the same investigations on the cray-fish axons and 0.12 msec in the investigations of the influences of conditioning stimuli and of the application of strychnine and urethane on frog nerve fibers.

The short duration stimuli (τ) fall short of the values for Δt given by these authors. The longer duration stimuli are comparable with those given by Viernstein and Grossman. The time interval between successive stimuli (T) was always one or two seconds, hence Δt is small with respect to T.

Therefore, the condition given in equation (2) can be considered to be fulfilled for the investigations reported in this work.

SYMBOLS

P	probability
$A = 1$	occurrence of an action potential
$A = 0$	absence of an action potential
I	intensity (negative)
ι	intensity of the input-signal
t	time
t_{on}	arrival time of the input-signal
t_{off}	time at which the input-signal is switched off
τ	duration of the input-signal
$t_{\max}$	time at which the transformed input-signal has its maximal value
t	time-interval
T	interval between successive stimuli
$S(\iota, \tau)$	rectangular input-signal with intensity ι and duration τ
a, b	constants from Weiss formula
c	relative spread
$E(\iota, \tau, t)$	transformed input-signal
ε	excitatory process inhibitory
J	process
k	time-constant of excitatory process
m	time-constant of inhibitory process
$\varphi(\tau)$	transformation factor
$\mu(\tau)$	threshold
$\sigma(\tau)$	spread
h	intrinsic threshold
σ	intrinsic spread
ξ	Gaussian random variable

VIII. SUMMARY AND CONCLUSIONS

The aim of the study presented here has been the elucidation of fluctuation in excitability with regard to the function of the nerve fiber the transmission of signals.

When a nerve fiber is stimulated with negative rectangular currents with a large interval between successive stimuli, it will nearly always respond with an action potential when the stimulus intensity is above a certain value, and practically never when the intensity is below another, lower, value. When the intensity of the stimulus is within this *threshold range* it is impossible to predict, aside from the probability of response, whether or not an action potential will occur. The nerve fiber is said to exhibit a fluctuation in excitability (Pecher, 1937).

Fluctuation in excitability is considered to be a property of a biological unit, when, upon the presentation of a non-random input the unit may respond with a certain probability.

The investigations on fluctuation in excitability were made on functionally isolated single nodes of Ranvier of amphibian myelinated axons (A-fibers in the sciatic nerve of the frog), and on unmyelinated crustacean axons (the most excitable fibers in the cray-.fish cheliped).

The probability of response (i.e., the production of an action potential) was examined with respect to the parameters of a negative rectangular current, repeatedly applied with an interval of 2 seconds when investigating the frog nerve fiber, and 1 second for the cray-fish axon.

A. The investigation of the input-output relations for both frog and crayfish nerve fiber preparations led to the following results:

1. A threshold range exists, in which the fiber will respond to the applied stimulus with a certain probability of response.
2. The successive reactions upon identical stimuli are distributed in accordance with the hypothesis that the probability of response has each time the same value, independent of preceding reactions.
3. Different axons in the same nerve react independently of each other on the application of one and the same stimulus to the preparation.
4. With given stimulus durations, the relations between probability of response and stimulus intensity can be described by the Gaussian type of distribution function.

The parameters characterizing the relations are:

a. *The threshold,* the mean and median of the function, the value of which depends on the stimulus duration. A relation known as the strength-duration relation.

b. *The relative spread,* the coefficient of variation of the function, a constant for a fiber, a number, independent of intensity and duration of the stimulus.

With these parameters the strength-duration-probability relation is described by the function given above.

5. Three properties are deduced to be present in the nerve fiber preparation investigated:
   a. *A `local activation process',* initiated in the nerve fiber by the electrical stimulus applied to the preparation. This process is considered as a transformation of the stimulus applied. *A transformation factor is* introduced to account for the transformation.
   b. *An intrinsic threshold,* a value which has to be reached by the `activation process' for the fiber to produce an action potential.
   c. *The fluctuation in excitability,* being the manifestation of processes in the fiber introducing an uncertainty for the `activation process' to reach the intrinsic threshold; Gaussian, and therefore characterized by the standard deviation, called *the intrinsic spread.*

   The parameters of the input-output relations of a fiber can now be expressed in the parameters of these postulated properties.
6. A descriptive mathematical model, based on Rashevsky's theories on excita-tion and fluctuation (1948) is developed with regard to the postulated properties of the unit (Appendix). The equations calculated for the input output relations and for the parameters characterizing them are identical to those derived experimentally.
7. It appears that the relative spread, independent of the parameters of the applied stimulus, is also equal to the quotient of intrinsic spread and intrinsic threshold. The relative spread is, thus, a direct and highly convenient measure for the influence of fluctuation in excitability; it is a measure for the width of the threshold region, relative to the value of the threshold.

For the frog A-fibers (viz., nodes of Ranvier) the mean value of the relative spread was found to be 0.011 (80 determinations, S.D. 0.005). For the un-myelinated cray-fish axons investigated this value is 0.0012 (15 determinations, S.D. 0.0003); about ten times smaller than that for the frog.

B. The influences of certain conditions on fluctuation in excitability were investigated for the functionally isolated frog node of Ranvier. This was made by examination of the input-output relations for a sub-rheobasic current; the recovery period; the application of strychnine and of urethane.

Results:

1. A fluctuation in excitability is always present.
2. The relation between probability of response and stimulus intensity can always be described by the Gaussian type of distribution function.
3. The influences are found by changes in the parameters of this function, threshold and relative spread:

a. A sub-rheobasic current decreases the threshold and increases the relative spread; their product, the spread, is not changed. Upon consideration of the 'stimulation value' of the whole stimulus complex no change in the parameters is detected.
b. In the recovery period (supra-normal phase) the threshold is changed (decreased). The relative spread is not changed.
c. Strychnine increases the relative spread and does not change the threshold.
d. Urethane increases the threshold and decreases the relative spread; their product, the spread, is not changed.

4. An analysis of the observed changes learns that these can be explained by the assumption of the existence of three independent properties in the preparation. It follows that the previously deduced properties may also explain these changes:
   a. The influence of the recovery period is comparable with a change in the transformation factor.
   b. The effect of a sub-rheobasic current is comparable with a reduction of the intrinsic threshold.
   c. strychnine increases the intrinsic spread.
   d. Urethane increases the intrinsic threshold.

It is concluded that the employment of the postulated properties is a useful approach.

C. A survey of the literature reveals that there is a general agreement with regard to the existence of fluctuation in excitability as a property of excitable tissue.

Evidence is presented that the Gaussian type of distribution function applies also to the input-output relations of other neural elements aside from the peripheral nerve fiber.

Some remarks are made concerning the meaning of Pecher's fluctuation in excitability with reference to the function of the unit:

The reciprocal of the threshold generally is used as a measure for the excitability (in this special meaning) of the unit.

The relative spread is a measure of the `width' of the threshold range. If the width of this range is small the output will be determined for the largest fraction of input-values, the probability of response being mostly zero or one. If the `width' of this range is large (the relative spread thus being large), the response to a single input-signal is generally undetermined, there is only a probability of response. The relative spread can, therefore, be considered a measure for the degree of `undeterminateness' of the unit.

Pecher's phenomenon, the fluctuation in excitability, is the manifestation of an uncertainty inherent to the transmission of signals in biological systems.

IX. SAMENVATTING EN CONCLUSIES

Dit proefschrift handelt over een onderzoek naar de *fluctuatie in de prikkelbaarheid* van de zenuwvezel, zulks ten aanzien van diens functie, het overbrengen van signalen.

Het onderzoek is verricht op gemyeliniseerde zenuwen van de groene kikker en op ongemyeliniseerde zenuwen van de zoetwaterkreeft.

In elk van de onderzochte zenuwen is de reactie van een enkel axon bestudeerd, terwijl voor ieder kikkeraxon de reactie op prikkeling van één knoop van Ranvier is onderzocht. HFDST. II

Wanneer een bepaalde zenuwvezel met een negatieve, rechthoekige, elektrische stroomstoot wordt geprikkeld, is het in beginsel niet mogelijk om aan te geven of er een actiepotentiaal zal optreden of niet. Wel is het mogelijk om de kans op het ontstaan van een actiepotentiaal te formuleren, waarbij de grootte van die kans afhankelijk is van de parameters van deze prikkel. Ligt voor prikkels van gegeven duur de intensiteit beneden een bepaalde waarde, dan is deze kans praktisch gelijk aan nul; ligt zij boven een andere, grotere waarde, dan is deze kans praktisch gelijk aan één. In het geval dat de intensiteit tussen deze twee waarden in ligt, is het onzeker of er een actiepotentiaal zal ontstaan. Wordt een prikkel van zulk een intensiteit vaker gegeven, dan ontstaat er nu eens wel een actiepotentiaal en dan weer niet. Is zij sterker, dan is de kans op een actiepotentiaal groter. Dit wekt de indruk, dat de prikkelbaarheid van de vezel aan onregelmatige wisselingen onderhevig is. Pecher, die enkele aspecten van dit verschijnsel vrij nauwkeurig heeft onderzocht, sprak daarom van een `fluctuatie in de prikkelbaarheid'. Hij maakte voor de kikkeraxonen reeds duidelijk dat deze onzekerheid in de zenuwvezels zelf besloten ligt. Uit ons onderzoek blijkt, dat dit ook geldt voor de onderzochte axonen van de kreeft. HFDST. I FIG. 1 TABEL I TABEL XII

Resultaten van het onderzoek

Dient men opeenvolgende, constante, prikkels toe met een interval van enkele seconden, dan is *de kans op een actiepotentiaal* ieder keer gelijk en onafhankelijk van de tevoren opgetreden reacties. Bij kortere intervallen gaat de verandering in de prikkelbaarheid ten gevolge van de tevoren gegeven prikkels en de tevoren opgetreden actiepotentialen een rol spelen. TABEL II, X FIG. 10 FIG. 11

Valt de prikkel iedere keer op hetzelfde ogenblik in de herstelperiode, dan zijn de reacties weer onderling onafhankelijk. TABEL IV, XI

Uit het verdere onderzoek, verricht aan 80 axonen van de kikker en 15 van de kreeft, volgt, dat de relatie tussen de kans op een actiepotentiaal en de intensiteit van de toegediende prikkel voor elke vezel afzonderlijk is te be- HFDST. III, IV

schrijven met *de verdelingsfunctie van Gauss* (de 'normale' verdelingsfunctie). Binnen de nauwkeurigheid van de proeven kenmerken de mediaan en de standaarddeviatie daarvan de kans op actiepotentialen na het toedienen van elektrische prikkels van uiteenlopende sterkte maar gelijke duur.

De mediaan van deze functie, *de 50 % drempelwaarde*, genoemd 'drempelwaarde', is een functie van de duur van de prikkel (sterkte-duur relatie). De standaard deviatie, kortweg *spreiding* genoemd, blijkt eveneens een functie van de prikkelduur te zijn.

FIG. 7
TABEL III, XIII

Het blijkt echter, dat het quotiënt van spreiding en drempelwaarde ten aanzien van een gegeven zenuwvezel onafhankelijk is van de parameters van de prikkel. Dit getal werd *de relatieve spreiding (RS)* genoemd.

VGL.III-(1) (2) EN (3)

Met drempelwaarde (sterkte-duur relatie) en relatieve spreiding is *de sterkte-duur-waarschijnlijkheid relatie* te beschrijven met de bovengenoemde functie.

FIG. 8, 9

De hieruit theoretisch af te leiden relaties tussen prikkelduur en kans op een actiepotentiaal blijken ook op de zenuwvezel van toepassing te zijn. Drempelwaarde en relatieve spreiding zijn dus te beschouwen als de parameters, die de relatie tussen de prikkelsterkte, prikkelduur en kans op een actiepotentiaal kenmerken.

HFDST. V

Beschouwingen over het gebeuren in het preparaat leiden tot de conclusie, dat daarin drie eigenschappen aanwezig zijn:

a. Een activeringsproces, in de zenuwvezel opgewekt door de prikkel die aan het preparaat is toegediend. Het verband tussen de aan het preparaat toegediende stroomstoot en dit activeringsproces is benaderd door het te beschouwen als een transformatie van de prikkel, waarmee een transformatiefactor wordt geïntroduceerd. De parameters die de prikkelsterkte-waarschijnlijkheid relatie beschrijven, moeten hiermee worden gecorrigeerd, om toepasbaar te zijn op het locale gebeuren. Dit voert tot de begrippen intrinsieke drempelwaarde en intrinsieke spreiding.

b. De intrinsieke drempelwaarde is op te vatten als het niveau dat door het in de zenuwvezel opgewekte activeringsproces moet worden bereikt, om een actiepotentiaal te doen ontstaan.

c. De intrinsieke spreiding is te beschouwen als de parameter van de, Gausse, fluctuatie in de prikkelbaarheid. Een in de zenuwvezel aanwezig fenomeen, dat aan het activeringsproces een onzekerheid toevoegt.

APPENDIX

Gebruik makend van de hier veronderstelde eigenschappen is, met zekere restricties, een descriptief wiskundig model ontwikkeld, gebaseerd op Rashevsky's theorieën over prikkelbaarheid en fluctuatie. Het blijkt dat de vergelijkingen, die de relatie tussen prikkelsterkte, prikkelduur en kans op een actiepotentiaal beschrijven identiek zijn met de experimenteel gevonden relaties. Ook nu is de relatieve spreiding onafhankelijk van de prikkel. Deze is dus te beschouwen als een bruikbare maat voor de onzekerheid, die door de fluctuatie in de prikkelbaarheid wordt geïntroduceerd.

HFDST. III, IV

Voor de 80 axonen van de kikker (knopen van Ranvier) is voor de relatieve spreiding een gemiddelde grootte van 0.011 ± 0.005 gevonden. Voor de

15 onderzochte axonen van de kreeft is een gemiddelde waarde van 0.0012 ± 0.0003 gevonden; een waarde die 10 keer zo klein is als voor de kikkervezels.

Voor de zenuwvezels van de kikker is voorts nagegaan welke invloed op de relatie tussen prikkelsterkte en kans op een actiepotentiaal wordt uitgeoefend door de herstelperiode volgende op een actiepotentiaal, door prikkeling met een sub-rheobasische stroom, door de applicatie van strychnine en door die van urethaan. HFDST. III

Het blijkt dat de fluctuatie in de prikkelbaarheid steeds aanwezig is en dat de relatie tussen prikkelsterkte en kans op een actiepotentiaal steeds is te beschrijven met de Gausse verdelingsfunctie. De veranderingen treden op in de parameters van deze relatie. TABEL V

In de herstelperiode (supra-normale fase) is de drempelwaarde veranderd (verlaagd), terwijl de relatieve spreiding gelijk blijft. TABEL VI

Een sub-rheobasische prikkel verlaagt de drempelwaarde en vergroot de relatieve spreiding, terwijl de spreiding, het product van deze twee, niet verandert. Wanneer de prikkelende waarde van het hele prikkelcomplex sub-rheobasische prikkel plus testprikkel) wordt beschouwd, zijn er geen, veranderingen aantoonbaar.

Strychnine vergroot de relatieve spreiding en heeft geen invloed op de drempelwaarde. TABEL VII

Urethaan verhoogt de drempelwaarde en verkleint de relatieve spreiding, terwijl hun product, de spreiding, gelijk blijft. TABEL VIII HFDST. V

De reeds eerder veronderstelde eigenschappen van het preparaat leveren de factoren, die deze veranderingen verklaren. Met name valt op te merken, dat strychnine de intrinsieke spreiding vergroot en dat urethaan de intrinsieke drempel verhoogt. Het effect van een sub-rheobasische prikkel komt overeen met een verlaging van de intrinsieke drempel, terwijl de invloed van de herstelperiode overeen komt met een verandering van de transformatiefactor.

Deze experimenten wijzen op het belang van de veronderstelde drie eigenschappen. Zij kunnen onafhankelijk van elkaar van waarde veranderen. Zij *bepalen* dus de relatie tussen prikkelsterkte, prikkelduur en de kans op een actiepotentiaal. Een relatie, die *gekenmerkt* wordt door de drempelwaarde en de relatieve spreiding. HFDST. VI

Zoals de drempelwaarde is op te vatten als een maat voor de prikkelbaarheid (in engere zin) van de zenuwvezel, zo is de relatieve spreiding te beschouwen als een maat voor de onbepaaldheid van de reactie van de zenuwvezel. Bij een kleine relatieve spreiding is de reactie voor het grootste gedeelte van het prikkelbereik bepaald. Is de relatieve spreiding groot, dan is de reactie voor een groot deel van het prikkelbereik onbepaald en alleen maar in kansen uit te drukken. De verwachting wordt uitgesproken, dat bij zeer grote waarden van de relatieve spreiding (groter dan 0,3) spontane (onregelmatige) activiteit op zal treden, terwijl een geringe vergroting van de gegeven relatieve spreidingen de stabiliteit van het uit prikkelbare eenheden opgebouwde biologische systeem zal kunnen FIG. 1

verstoren; een werking die binnen deze redenering aan strychnine kan worden toegeschreven.

Pecher opperde reeds de gedachte, dat de fluctuatie in de prikkelbaarheid ook aanwezig zou zijn in de andere neurale elementen. Uit de literatuur blijkt dat dit inderdaad het geval is.

Het is zelfs waarschijnlijk dat hier de beschreven relatie tussen de aankomende prikkel en de kans op een reactie geldt voor alle neurale elementen. Men moet daarbij echter wel terdege rekening houden met de gecompliceerdheid van de structuur van het gedeelte dat gelegen is tussen de plaats van de toegediende prikkel en het element, waarvan de reactiekans wordt onderzocht.

De slotconclusie schijnt gerechtvaardigd, dat Pecher's fenomeen de uiting is van een onbepaaldheid, een onzekerheid, die inherent is aan prikkelbare biologische elementen. Een onbepaaldheid die de indruk geeft van een 'vrijheidsgraad' voor het beschouwde element en waaraan, opgenomen in het geheel van het organisme, vermoedelijk ook een functie is toe te schrijven.

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ESSENTIAL ERRATA

(corrected in the digital version)

Page	**position**	**was**	**changed to**
31	Table VBII-A	difference	difference * 100
31	Table VII-B	difference * 100	difference
33	Table VIII-C	RS * difference * 100	RS * difference
34	Table IX-A	k = 0.43	k = 0.86
35	4th line from below	value, and is	value,
62	3d line from below	exceed) t	exceed) Δt
63	equation (9)	between integral and brackets insert: exp	

Note:
Simple errors in spelling and printing were corrected during the OCR session.