ALGEBRAICAL SUMMATION IN SYNAPTIC ACTIVATION OF MOTONEURONES FIRING WITHIN THE 'PRIMARY RANGE' TO INJECTED CURRENTS

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SUMMARY

1. In intracellular studies of cat lumbar motoneurones constant synaptic stimuli such as stretch, contraction or a high-frequency stimulation of a cut afferent nerve have been superimposed on firing in response to injected currents.

2. As long as the slope relating spike frequency to injected current remained constant, which by definition is the 'primary range' of firing, algebraical summation of superimposed synaptic stimulation prevailed. Added quantities in these experiments were then between 2·0 and 56·2 impulses/sec for excitation, between − 2·8 and − 21·8 impulses/sec for inhibition.

3. Data were obtained correlating firing rates with amount of synaptic potential and current respectively.

4. Theoretical implications are dealt with in the Discussion.

INTRODUCTION

A knowledge of how perfectly the firing motoneurone adds or subtracts a constant synaptic contribution to its level of excitability seems essential for the understanding of the properties of central exchange stations in which impulse frequencies are translated into synaptic currents and the latter again into impulse frequencies. Our first attack on this problem (Granit & Renkin, 1961) was made by recording from single ventral root fibres of the decerebrate cat. The motoneurones of ankle extensors were fired repetitively by high-frequency tetani from their muscular afferents and exposed to recurrent inhibition by similar tetani to the rest of the severed ventral root. Constant recurrent inhibition was found to subtract a constant number of impulses independent of the rate at which the motoneurone was fired. The present paper and the next one (Granit, Kernell &

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Lamarre, 1966) concern similar problems but the steady-state background of discharge at different rates is now being provided by trans-membrane currents injected from the tip of an intracellular micro-electrode. A preliminary report of some of the findings has been given by Granit (1966).

The linear relation between current strength and firing rate in such experiments (Granit, Kernell & Shortess, 1963a; Shapovalov, 1964) was found in recent work on cat motoneurones (Kernell, 1965a, 1966) to be restricted to a range corresponding to about 85% of the tension that such motoneurones could mobilize in their muscles. This was called the 'primary range' of firing. When stronger currents were applied there appeared a second branch of the curve, a straight line of steeper course so that less current was then needed for a given increase of frequency. This is the 'secondary range' of firing. It was not possible to demonstrate a 'secondary range' in all motoneurones but then technical reasons (some damage to the cell, leaks, electrode polarization) may have been responsible for the failures.

The previous work making use of recurrent inhibition had been restricted to what is now called the primary range of firing and to the addition of two synaptic stimulations. But if there be, as it were, two amplifiers in the motoneurone, one for its main range of operation, another for maximum performance, then the problem of algebraical summation of synaptic stimuli must be investigated for both ranges. This will be done below for the primary range and in our second paper (Granit et al. 1966) for the secondary range.

On the background discharge provided by injected current will be superimposed alternatively: (i) a constant stretch reflex, (ii) a constant high-frequency tetanus to a mixed nerve or (iii) a constant contraction of the gastrocnemius–soleus muscle as elicited from the peripheral stump of a cut ventral root. This limits the investigation to large motoneurones capable of giving steady firing rates to injected currents for not less than some 40 min.

The present work has also made it possible to compare the amount of post-synaptic potential for given steps in impulse frequency with that of current intensity required for the same steps. Further findings relate to comparisons of synaptic changes with induced trans-membrane effects.

METHODS

In most cases we have used the 'anaemically narcotized' cat (Granit, Kellerth & Williams, 1964) for experiments on excitation. One animal was decerebrated and some received pentobarbitone (Nembutal) 35–40 mg/kg. Two animals received 20 mg/kg of this drug and 15 mg/kg chloralose (see Table 1). The anaemically narcotized cat had the carotid artery and all its visible branches including the external and internal ones tied under ether. This done, ether

anaesthesia was discontinued and step by step replaced by Nembutal injected in small doses. Rarely, more than 20 mg/kg was needed in spite of which slight forelimb rigidity was generally left in an animal otherwise sound asleep. Artificial respiration was the rule. Unless contraction was studied the preparation often received 20 mg/kg gallamine triethiodide (Flaxedil).

The nerve supply to gastrocnemius–soleus was always left intact. The effect of stretch of the ankle extensors gastrocnemius and soleus was tested with most motoneurones. Stimulating electrodes were placed on several cut nerves, the ones used being collected in Table 1. The ventral roots L 7 and S 1 were severed for antidromic stimulation, sometimes so as to permit separate electrodes to be placed on the peripheral stump.

Essentially the technique described is that used in several earlier papers in this *Journal*, most fully in Granit *et al.* (1964), and, with regard to stimulation by trans-membrane current, in Granit, Kernell & Smith (1963*b*). In the majority of the experiments K citrate microelectrodes of conventional type were used.

Procedure. After ascertaining for the cell its rhythmic threshold, defined as the minimal firing rate to injected current, the most suitable of the three modes of synaptic stimulation (see Introduction) for that particular motoneurone was selected. The experiment was begun by firing the cell, adding the synaptic stimulus after a couple of seconds or as soon as a semistationary control value for one half or a full second of trans-membrane stimulation had been recorded. The duration of the synaptic test stimulus was determined by the same requirement for a semistationary state whose firing rate should be measurable for not less than 0·5 sec or 1 sec. With slowly rising stretch it was often possible to separate a phasic component, measurable over a shorter time, from the later semistationary static component. Records serving to illustrate the experimental procedure will be found in Fig. 9. The experiment proceeded in some 5 sec periods of stimulation between pauses lasting 0·5–2 min, depending on current strength, which was augmented stepwise from minimum to maximum. Occasional controls were taken of the test stimulus by itself, and the membrane potential was checked by inserting an antidromic shock between each current step to measure spike height or, in about half of the experiments, by recording it continuously at high sensitivity on a d.c. penwriter (Enograph-G, Type ZSG, Rhodhe and Schwartz).

Table 1 is a summary of the types of experiment analysed in this and the subsequent paper (Granit *et al.* 1966).

RESULTS

Primary range defined. Within this range, impulse frequency (F) is linearly related to strength of injected current (nA) by a straight line whose slope constant (k_A) is given in impulses sec^{-1} nA^{-1}. The lower limit is the minimal firing rate, the upper limit is at a definite rate of discharge in any one motoneurone but highly variable from cell to cell. In our material, values around 40–50 impulses/sec were common, low ones around 30 impulses/sec rare, and high ones such as the 110 impulses/sec (at least) of the motoneurone shown in Fig. 1 still rarer. The lowermost recording is below the rhythmic threshold, which in this case was very precise. Our experience agrees with that of Kernell (1965*a*, 1966) who found that, for the upper limit, firing rate as such and not current intensity is decisive. Early in the discharge most cells can be pushed into the secondary range of firing but the 'adaptation' (in the sense of Granit *et al.* 1963*a*) makes it necessary with maintained stimulation to use strong currents in order to

overcome the reduction in firing rate. Entry into the secondary range is characterized by a partial breakdown of the mechanism of after-hyperpolarization, which is normal throughout the primary range (summary by Kernell, 1966).

Superposition of synaptic excitation. The lower curve of Fig. 2 illustrates the 'control' which describes the effect of injected current by itself on the firing rate of a hamstring motoneurone of 100 mV spike height, the upper one the added effect of a gastrocnemius–soleus contraction elicited from the ventral root. The control values were measured for the 0·5 sec just

TABLE 1

Type of preparation	Motoneurones	Type of activation*		Range of firing†
Anaemically narcotized	Gastroc.–soleus	Stretch	+ + +	P, S
	Gastroc.–soleus	Stretch	+ + +	P, S
	Gastroc.–soleus	Stretch	+ +	S
	Gastroc.–soleus	Stretch	+ +	P
	Gastroc.–soleus	Stretch	+	P
	Gastroc.–soleus	Stretch	+ + +	P
	Gastroc.–soleus	Stretch	+ +	P
	Gastroc.–soleus	Tet. pop.	+	P, S
	Gastroc.–soleus	Stretch	+ +	P, S
	Hamstring	Tet. ham.	+ +	P, S
	Hamstring	Tet. ham.	+ + + +	P
	Unidentified	Tet. ham.	−	P, S
	Popliteal	Tet. pero.	−	P
	Popliteal	Tet. pero	− −	P, S
	Popliteal	Tet. pero.	−	P, S
Decerebrate	Hamstring	Tet. ham.	+ +	P
	Hamstring	Tet. pop.	+	P
Pentobarb.–chloralose	Hamstring	Contraction	+	P, S
	Hamstring	Contraction	+	P
Pentobarbitone alone	Popliteal	Tet. pero.	− −	P, S
	Unidentified	Tet. quadr.	−	P
	Popliteal	Tet. ham.	− −	P, S
	Unidentified	Tet. pero.	− −	P
	Hamstring	Tet. pero.	− − − −	P
	Hamstring	Tet. ham.	+ +	P
	Hamstring	Tet. pero.	− −	P, S

* + = Facilitation { of repetitive firing induced by trans-membrane current by stretch
− = inhibition { or contraction of triceps surae, or by tetanization of indicated
{ nerves, each sign indicating one experiment.

† P = 'primary range', S = 'secondary range'. Tet. = tetanization.

preceding onset of the root tetanus. The contraction slowly diminished and its average effect upon the firing rate was measured for a full second. By the method of least squares the two slope constants were found to be 3·6 and 3·5 impulses sec^{-1} nA^{-1} (see legend). The difference between the curves was 6·5 impulses/sec. This facilitation was largely if not wholly due to Golgi tendon organs. The difference of 0·1 impulses sec^{-1} nA^{-1} between the slope constants is negligible. (Below details of individual experiments will be restricted to the legends. For statistics to be used below, see Spiegel, 1961.)
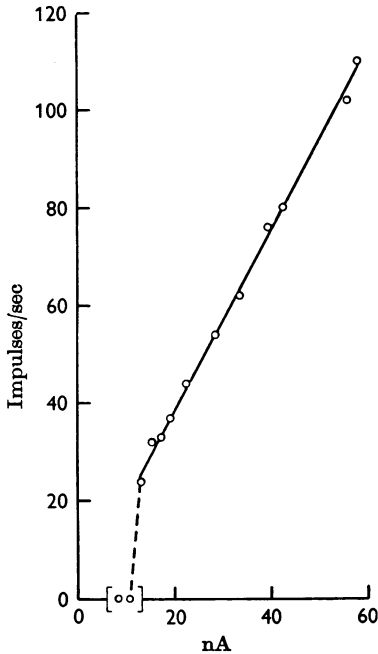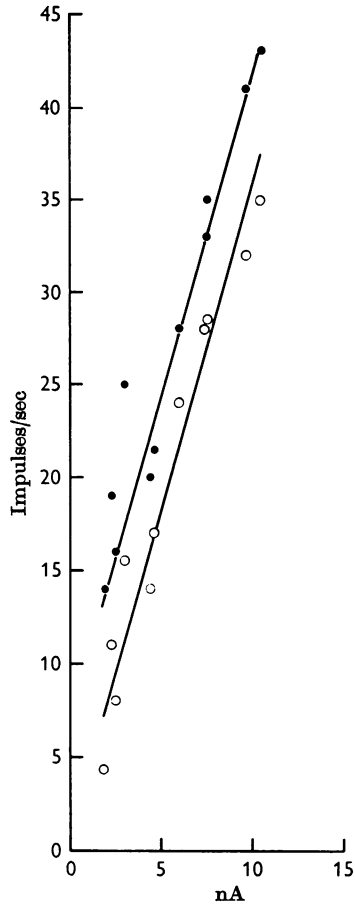
Fig. 1

Fig. 2

Fig. 1. Decerebrate cat. Hamstring motoneurone of spike height 85 mV, recorded by 2 M potassium citrate (intracellular) electrode. The curve shows relation between steady discharge frequency (measured during first 0·5 sec) and maintained trans-membrane stimulation from tip of micro-electrode. This cell fires repetitively in the 'primary range' from 22 impulses/sec (minimal firing rate) up to at least 110 impulses/sec. The slope of the straight line (by the method of least squares) is 1·84 impulses $sec^{-1} nA^{-1}$. The precision of the rhythmic threshold is indicated by the two readings on zero ordinate.

Fig. 2. Pentobarbitone–chloralose cat. Hamstring motoneurone of spike height 100 mV (2 M potassium citrate electrode) facilitated by steady tetanic contraction of gastrocnemius–soleus muscle elicited from peripheral stump of cut ventral root. Lower curve, relation between steady discharge frequency and maintained trans-membrane stimulation from tip of micro-electrode. Slope constant $k_A = 3·59$ impulses $sec^{-1} nA^{-1}$. Upper curve (filled circles), same facilitated, $k_A = 3·48$ impulses $sec^{-1} nA^{-1}$. The difference between the two curves is $6·5 \pm 2·03$ (s.d.) impulses/sec.

An advantage of using stretch rather than contraction in such experiments is that two facilitated values can be obtained, one referring to the initial phasic rise of impulse frequency from the primary spindle organs responsible for the stretch reflex, another to the subsequent semistationary state of firing of the same organs. Two such experiments are
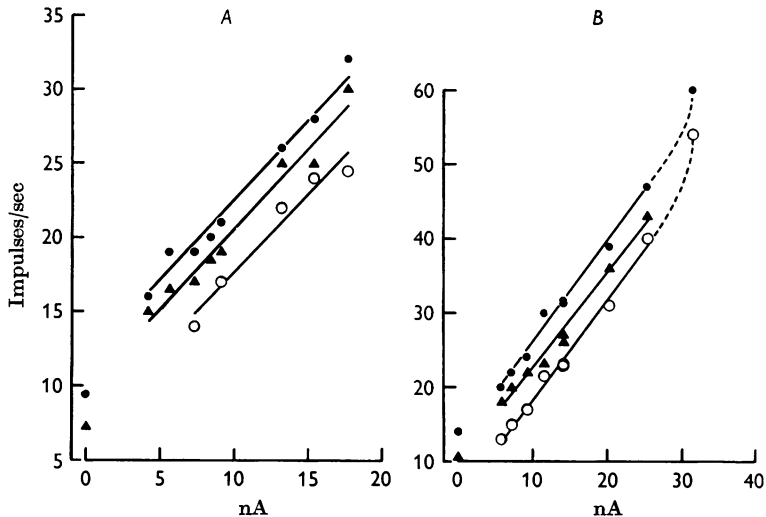

Fig. 3. Anaemically narcotized cat. *A* and *B* from two different ankle extensor motoneurones (3 M potassium chloride electrode). *A*, gastrocnemius–soleus motoneurone of spike height 65 mV. Lower curve (open circles) is the 'control' showing the relation between steady discharge frequency (measured for 1·0 sec before onset of stretch) and trans-membrane stimulation alone; $k_A = 1·05$ impulses sec^{-1} nA^{-1}. The two upper curves (filled symbols), same with facilitation by 10 mm stretch of the gastrocnemius–soleus muscle. Stretch rose in 1·5 sec (phasic) and was maintained for 2·5 sec (tonic). *Filled circles* represent discharge frequencies measured for 0·5 sec during the phasic rise for which $k_A = 1·07$ impulses sec^{-1} nA^{-1}; *filled triangles* those measured during the first 1·0 sec of steady pull, $k_A = 1·08$ impulses sec^{-1} nA^{-1}. The difference is $4·9 \pm 1·35$ (s.d.) impulses/sec between the phasic curve and the control and $2·9 \pm 1·49$ (s.d.) impulses/sec between the tonic and the control. Note that stretch by itself produces steady discharge rates at 9·5 and 7·0 impulses/sec respectively for phasic and tonic part of the reflex (see values at zero current) while the minimal firing rate with the injected current alone was 14 impulses/sec. *B*, experiment identical with that of *A* but with another gastrocnemius–soleus motoneurone of spike height 70 mV. Control curve measured for 0·5 sec before onset of stretch. Phasic curve (filled circles) represents the discharge frequency during half a second on top of rising phase; tonic curve (filled triangles) obtained during the first second of steady pull. Control $k_A = 1·32$ impulses sec^{-1} nA^{-1}, 'phasic' $k_A = 1·33$, 'tonic' $k_A = 1·25$ impulses sec^{-1} nA^{-1}. The difference between 'phasic' curve and 'control' is $7·6 \pm 0·67$ (s.d.) impulses/sec; between 'tonic' curve and 'control' $3·9 \pm 1·22$ (s.d.) impulses/sec. The two upper points represent values within the 'secondary range' of firing indicated by dotted lines. Values at zero current show that stretch by itself fired the cell at 14 impulses/sec (phasic) and 10 impulses/sec (tonic).

shown in Fig. 3*A* and *B*. The difference between the phasic (filled circles) and the tonic values (filled triangles) is a consequence of the spindle properties just referred to, as reproduced by the motoneurone in terms of impulse frequencies (see Fig. 9). Spindle secondaries and Golgi tendon organs also contribute to the input, but both oppose the primaries (in the ankle extensors used) so that the motoneurone records a net depolarization of excitation minus inhibition. In spite of this complexity of synaptic action, algebraical summation was maintained within the whole primary range.

Figure 3*A* raises another point of some interest. By injected currents alone it proved impossible in this case to elicit a steady discharge below 14 impulses/sec while a tonic stretch reflex (see values at zero current in Fig. 3) could be adjusted to a steady minimum firing rate of as little as 7 impulses/sec. Similar observations have been reported by Kernell (1966). Later examples of the greater efficacy of the synaptic approach compared with trans-membrane stimulation refer, as a rule, to the upper portion of the curve in the region where it changes slope (see Figs. 3*B*, 4*B* and subsequent paper, Granit *et al.* 1966).

For very large facilitatory effects it is convenient to use high-frequency tetani to a cut nerve (see Fig. 9). Two such cases are illustrated in Fig. 4*A* and *B*. It was a little surprising to find several instances of satisfactory additive behaviour in motoneurones in spite of the mixed nature of the nerve, the polysynaptic character of the circuits involved and their powerful effect on the cell. Owing to the high rate of firing induced under facilitation, the motoneurone of Fig. 4*B* has reached the secondary range, as is indicated by the increase in the slope constant (to be discussed in the subsequent paper, Granit *et al.* 1966).

Errors illustrated. In order to present an example of a source of error, which is easily detected when it is systematic, Fig. 5*A* and *B* has been chosen. This is our standard type of experiment, but in Fig. 5*A* neither of the two curves became linear. During this experiment—the first with this long-lasting motoneurone—the membrane potential slowly rose by − 7 mV while the readings were taken in the usual order corresponding to increasing current strengths on the abscissa. This means that the threshold of excitation rose in the same way so that increasingly greater steps of current would have been required in order to maintain linearity of the curve. For the comparison of the difference between the two curves it was now less convenient to calculate with proportionality constants as above. Therefore, in Fig. 5*B*, the plot of Granit & Renkin (1961) was used, in which facilitated values occur as ordinates, the corresponding controls as abscissae. If there had been no facilitation the plot would have followed the 45° line drawn through the origin. A curve of perfect algebraical

summation should take the same course above it. The regression co-efficient of the experimental curve is 0·95, or near enough to the theoretical 1·0 to justify the conclusion that there was algebraical summation. Figure 4A was an experiment with the same cell later in the day but in this case the membrane potential of 80 mV was perfectly stable. Now a facilitated difference of as much as 32·6 impulses/sec could be held constant within the whole primary range and the curve stayed linear.

The effect of a gradually diminishing test response is illustrated in Fig. 6 with a spike which maintained its size of 85 mV during the whole experiment at a steady membrane potential. This led to a diminution of
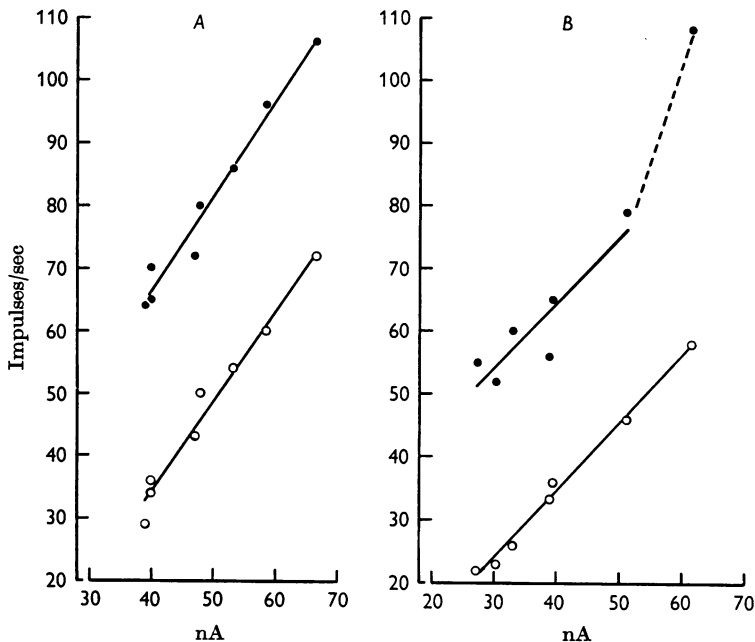

Fig. 4. A, anaemically narcotized cat. Hamstring motoneurone of spike height 84 mV and steady resting membrane potential − 80 mV (2 M potassium citrate electrode) facilitated by high-frequency stimulation of cut hamstring nerve. Lower curve, 'control', measured for 0·5 sec before onset of nerve stimulation. Upper curve, same facilitated during the first 0·5 sec of hamstring nerve stimulation. 'Control' $k_A = 1·44$, facilitated curve $k_A = 1·51$ impulses sec^{-1} nA^{-1}. The difference between the two curves is $32·6 \pm 2·49$ (S.D.) impulses/sec. B, anaemically narcotized cat. Hamstring motoneurone of spike height 87 mV (3 M potassium chloride electrode). In this case the 'control' was measured during 0·5 sec after cessation of high-frequency hamstring tetanus while the facilitated curve (filled circles) was measured during the last 0·5 sec of nerve stimulation. Control $k_A = 1·07$ impulses sec^{-1} nA^{-1}; for the facilitated curve $k_A = 1·03$ impulses sec^{-1} nA^{-1}. The difference between the two curves is $30 \pm 3·9$ (S.D.) impulses/sec. As in Fig. 3B, the last point of the facilitated curve has reached the 'secondary range' of firing.

the slope constant of the facilitated curve to 1·4 from 1·6 impulses sec^{-1} nA^{-1} in the control. The test response by itself gave 26 impulses/sec in the beginning, 22 in the middle and 16 impulses/sec at the end.

Clearly the sources of error, which in these two instances were systematic and hence detectable, may also occur as unsystematic deviations during the measurements and then escape detection. Progressive deterioration of a cell is less serious an obstacle in this type of work because it is heralded by very obvious signs and easily checked. Such cells had to be rejected.

Statistical considerations. Rather than to present all of the individual experiments we have chosen to review the whole material on excitation in some graphs based on statistical points of view. Since linearity of the
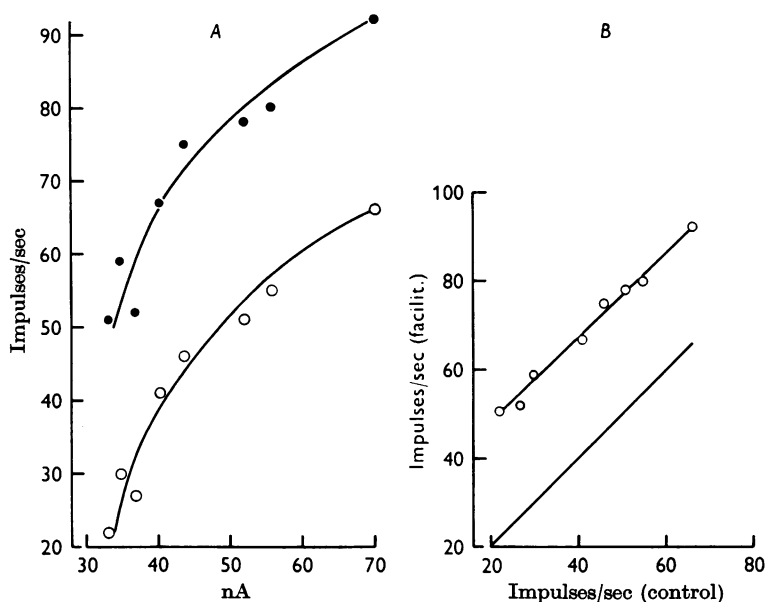

Fig. 5. *A*, same hamstring motoneurone as in Fig. 4*A* but recorded earlier in the day and facilitated by weaker stimulation of the hamstring nerve. The two curves represent values measured in the same way as in Fig. 4*A* but, in this case, neither of the two curves became linear. The resting membrane potential was − 73 mV at the beginning of this experiment and slowly rose by − 7 mV while the readings were taken in the usual order corresponding to increasing current strengths on the abscissa. Then, the membrane potential remained steady at − 80 mV during which readings were taken for the second experiment illustrated in Fig. 4*A* in which the curves became linear and parallel. *B*, same experiment as in *A*. Here, facilitated values (ordinates) plotted against coresponding controls (abscissae). A 45° line is drawn through the origin. If there had been no facilitation, the plot would have followed this line. The experimental curve shows an increase of frequency amounting to 27 ± 1·7 (s.d.) impulses/sec with a regression coefficient of 0·95, thus near enough to the theoretical 1·0 to justify the conclusion that there was algebraical summation in spite of the non-linear characteristics shown above in *A*.

control curves is so well demonstrated above and in the papers referred to (cf. Introduction), the present analysis will be restricted to the problem of how well algebraical summation within the primary range can be substantiated with a greater number of motoneurones.
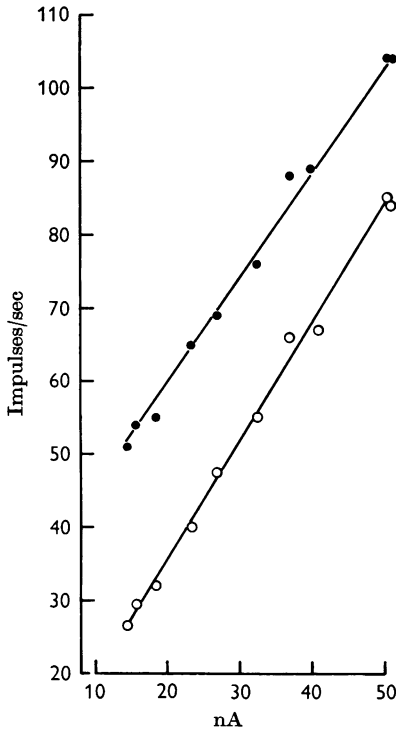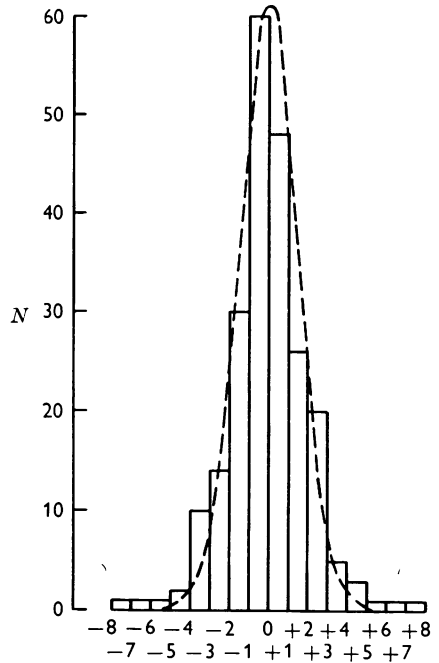

Fig. 6   Fig. 7

Fig. 6. Decerebrate cat. Same hamstring neurone as in Fig. 1. Spike size 85 mV (2 M potassium citrate electrode). The lower curve is the control as measured for 0·5 sec before onset of facilitation. The upper curve (*filled circles*) shows facilitated values obtained during the first 0·5 sec of high-frequency stimulation of the popliteal nerve. The difference between the two curves is $22·3 \pm 1·9$ (s.d.) impulses/sec. 'Control' $k_A = 1·60$ impulses $sec^{-1} nA^{-1}$, 'facilitated' $k_A = 1·42$ impulses $sec^{-1} nA^{-1}$. In this case, the lower slope constant of the facilitated curve can be satisfactorily explained by a gradually diminishing test response of the popliteal nerve stimulation. In the beginning of the experiment, the test response was 26 impulses/sec; in the middle 22 impulses/sec and at the end only 16 impulses/sec. During the whole experiment the spike maintained its size of 85 mV at a steady resting membrane potential.

Fig. 7. Histogram of 224 deviations from the mean difference between control and facilitated discharge frequency for each of thirty experiments. Unit variate for the classes is 1·0 impulse/sec. The curve computed from the experimental data and drawn over the histogram (interrupted line) shows how well a theoretical normal distribution of variates fits the data.

Looking at this problem, first, from the point of view of the proportionality constant k_A, perfect algebraical addition would mean that, if all the thirty experiments (twenty-eight with linear controls) on facilitation within the primary range were to be regarded as one single experiment, plotted as in Fig. 5B, the regression coefficient b in this plot should be 1·0. For this calculation it is necessary to use weighted means because of the varying number of observations N in each case. When this was done, it was found that

$$\Sigma(bN)/\Sigma(N) = 0{\cdot}985 \pm 0{\cdot}157.$$

The corresponding figure of Granit & Renkin (1961) was 0·996. These authors studied the addition of two static synaptic effects with an average difference of $-5{\cdot}5$ impulses/sec. In the present experiments a much wider range of differences was used, the weighted mean being $13{\cdot}6 \pm 1{\cdot}95$ (S.D.) impulses/sec, the range being from 2·0 to 56·2 impulses/sec.

Since the difference between the control and the facilitated curve should be constant, it is of interest to investigate by how many impulses/sec this difference in each pair of measurements deviated from the mean. This involves an analysis of 224 observations. Their deviation from a theoretically constant difference $(= 0)$ is plotted in the histogram of Fig. 7, taking 1·0 impulse/sec as unit variate. Figure 7 demonstrates a perfectly normal distribution of variates (see legend). Some 73 % of the differences measured have been constant within ± 2 impulses/sec, which (we believe) is as well as one can do without imposing far-reaching restrictions on the quality of the individual experiments. It may well in the end be more difficult to defend such restrictions than to accept a greater latitude for variability and try to find out whether its distribution is reasonably normal.

The range of slope constants in the controls of this material is also of interest, both as such, as well as compared with the corresponding slope constants of the facilitated curve. These two slope constants have been plotted against each other in Fig. 8. The regression coefficient obtained is $0{\cdot}96 \pm 0{\cdot}11$ ($S_{y.x}$) and thus very close to the theoretical value of 1·0. The mean slope constant of the controls is $1{\cdot}33 \pm 0{\cdot}54$, that of the facilitated ones $1{\cdot}31 \pm 0{\cdot}51$ impulses $\text{sec}^{-1}\,\text{nA}^{-1}$. (The coefficients are given with standard error of estimate, $S_{y.x}$).

Resistance of firing motoneurones. In several experiments the strength of the superimposed test stimulus could be adjusted to a value below the firing threshold of the motoneurone and then it gave rise to a post-synaptic potential, samples of which are given in Fig. 9. When this was added to the effect of injected current it produced the constant difference in impulse frequencies that has been analysed in Fig. 7. Such experiments gave a

number of measurements relating the constant increase of frequency to the amount of post-synaptic potential required for it. If the latter was of the gently sloping type several measurements from the counting-period were averaged. (It was decided to measure from base line to the peaks of the synaptic activation noise, when noise was present.)
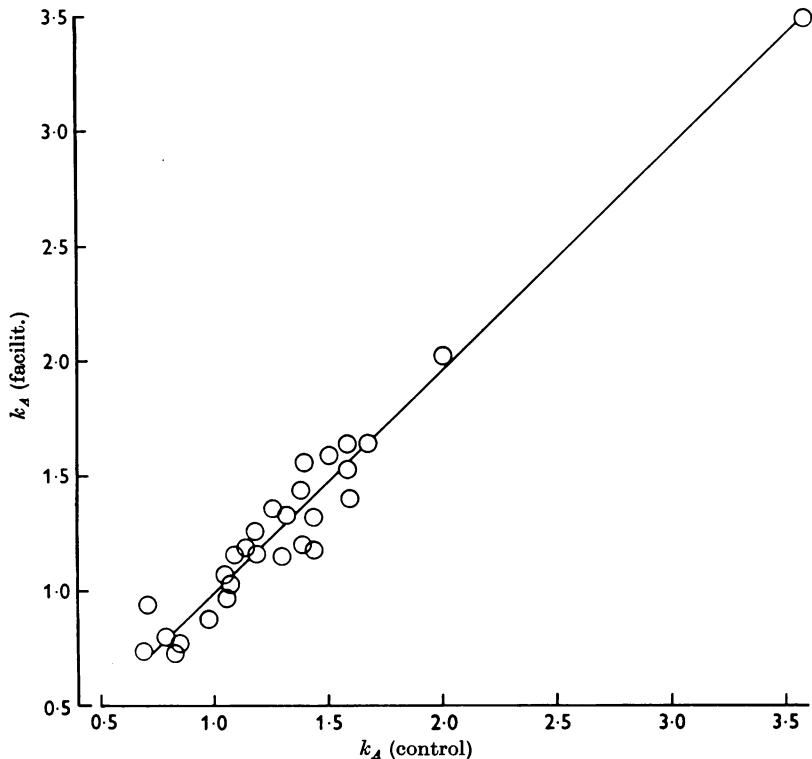

Fig. 8. Plot of slope constants of facilitated curves (ordinates) against slope constants of control curves (abscissae), for the twenty-eight experiments in which linear curves were obtained. The regression coefficient is 0·96 ($S_{y \cdot x} = \pm 0·11$) and the linear correlation coefficient $r = 0·974$.

In this manner, then, the points in the graph of Fig. 10A were assembled, all of which were from experiments on anaemically narcotized animals. Some inhibitory post-synaptic potentials obtained with the same type of preparation were also included. (In the Nembutal-dosed animals used in the experiments on inhibition the membrane potentials varied between -85 and -54 mV, whereas in the inhibited cells from the anaemically narcotized animals they only varied between -64 and -62 mV.) The graph of Fig. 10A shows impulse frequency (ordinates) against the amount of post-synaptic potential (abscissae). There is considerable spread around the regression line whose slope $K_V = 2·28$ impulses $sec^{-1} mV^{-1}$. Some

homogeneity of the properties of the motoneurones of Fig. 10 A is required to justify the correlation. There is, to begin with, the fairly uniform cell size forced upon us by the need for long-lasting penetrations. This deduction is supported by an analysis of the duration of the after-hyperpolarizations, which averaged 63 msec, thus indicating large cells (Eccles, Eccles & Lundberg, 1958; Kernell, 1965 b). There is further the similarity in the treatment of the animals (which, as stated, implied slight rigidity).

From this curve a fictitious value can be derived by extrapolation for the amount of post-synaptic potential needed for the upper limit of the
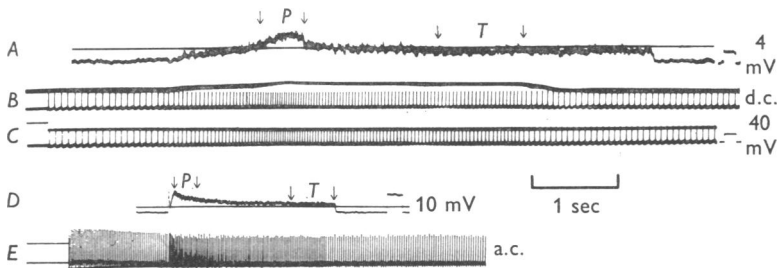

Fig. 9. Records of post-synaptic potentials and of repetitive discharges set up in two different motoneurones by injected currents (2 M potassium citrate electrode) in two anaemically narcotized cats. A–C from a gastrocnemius–soleus motoneurone of spike height 78 mV. In A, excitatory post-synaptic potential produced by a 10 mm stretch of the gastrocnemius–soleus muscle. B and C recorded simultaneously. Repetitive discharge initiated by depolarizing trans-membrane current of 14 nA (indicated by the downward deflexion of the upper trace in C). Stretch of the gastrocnemius–soleus muscle indicated by myograph record in upper trace of B beginning about 1·5 sec after onset of trans-membrane stimulation. A illustrates the measured phasic and tonic component potentials of the synaptic test stimulus (labelled P and T respectively). Hence, two facilitated values were obtained, one referring to the phasic rise of impulse frequency, another to the semi-stationary state of firing, as measured between arrows (cf. Fig. 3 A and B). D and E are from a hamstring motoneurone of spike height 84 mV. In this case, the synaptic effect elicited by high-frequency stimulation of cut hamstring nerve. D, excitatory post-synaptic potential produced by nerve stimulation alone. In E, the cell is fired repetitively by trans-membrane current of 43 nA (downward deflexion of upper trace) and, after about 1 sec, the synaptic test stimulus is added. Here again, two facilitated values of spike frequency were obtained; one phasic, the other tonic (P and T between arrows).

primary range, assuming the latter to be 40–50 impulses/sec and the minimum firing rate about 15 impulses/sec. This would be 11–15 mV, to which should be added 6–10 mV for the rhythmic threshold, in all therefore 17–25 mV.

In the primary range the addition of a constant synaptic excitation obviously has the same effect as the addition of a constant amount of injected current. It is also of interest to plot the ordinates of Fig. 10 A

against the equivalent amount of injected current on the abscissa (instead of post-synaptic potential). This has been done in Fig. 10B, which shows a regression coefficient $K_A = 1\cdot06$ impulses sec^{-1} nA^{-1}. From the constants K_V and K_A can be computed the values for voltage and current required for any given spike frequency. The ratio of these values is proportional to K_A/K_V and has the dimension of resistance; if accepted as such, it corresponds to a membrane resistance of 0·47 MΩ. If the experiments are treated individually and then averaged, a value of 0·55 MΩ is
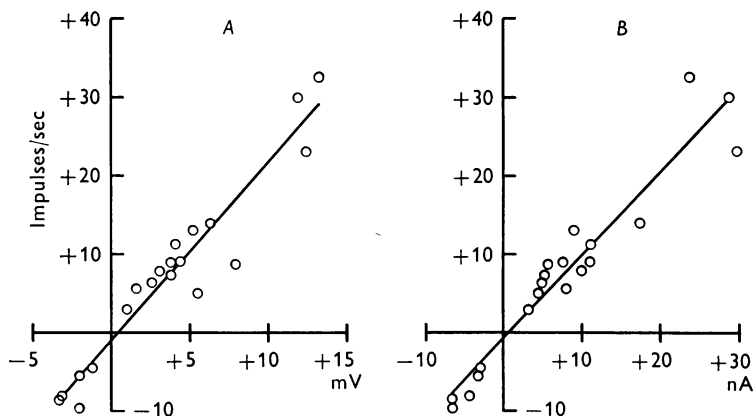

Fig. 10. A, plot of constant increase or decrease in impulse frequency (ordinate) against the corresponding amount of post-synaptic potential required for it (abscissa). The slope constant $K_V = 2\cdot28$ impulses sec^{-1} mV^{-1}. B, same ordinates as in A but plotted against current on the abscissa. The slope constant $K_A = 1\cdot06$ impulses sec^{-1} nA^{-1}. Five readings refer to experiments with inhibition, as in Fig. 11.

obtained. The range is from 0·3 to 1·3 MΩ. We shall return in the Discussion to the question of whether or not these figures actually measure 'resistance'. Direct methods in heavily anaesthetized cats (Coombs, Curtis & Eccles, 1959) as well as an indirect method (Frank & Fuortes, 1956) have given higher values for silent cells. Thus, in Table 1 of Coombs *et al.* (1959) the recorded membrane resistances fell between 0·5 and 2·5 MΩ, the average being 1·2 MΩ. The sample was stated to consist of large motoneurones. These methods are without demur.

Superposition of synaptic inhibition. For the corresponding experiments on inhibition it was necessary to use firing rates a good deal above the minimal firing rate because the latter sets an absolute limit of complete silence to the difference in spike frequencies that is to be measured. For this reason a number of particularly instructive observations could be made on inhibition at the high firing rates within the secondary range which will be presented in the subsequent paper (Granit *et al.* 1966). The

results in seventeen experiments within the primary range fell in line with those obtained by adding synaptic excitation and can therefore be discussed in an abbreviated form.

Two individual experiments are shown in Figs. 11 and 12. The former is a plot of the type used above, e.g. in Fig. 2, for excitation. The outcome is the same. Summation is algebraical with opposite sign for inhibition.
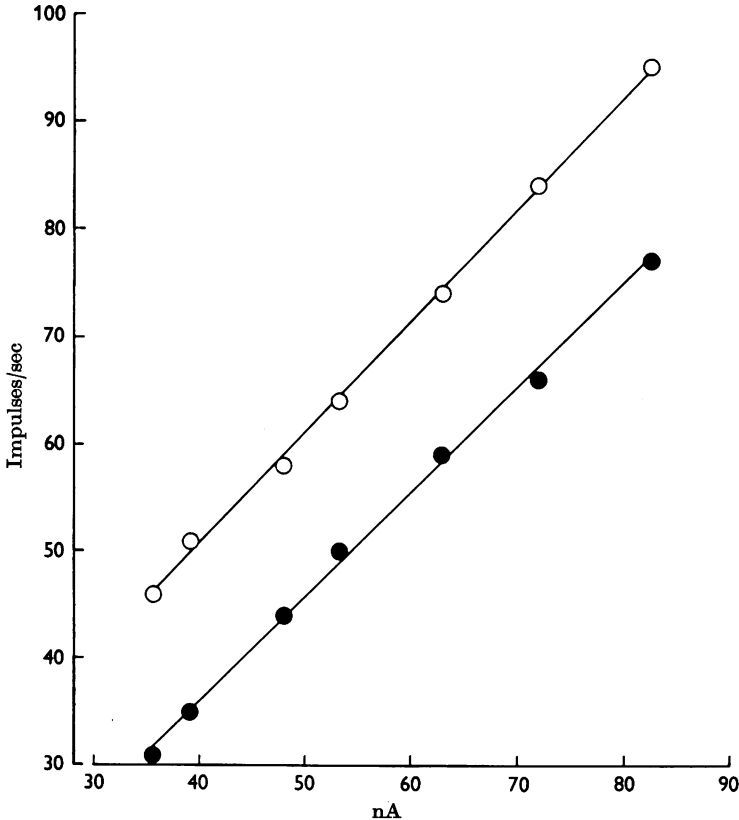

Fig. 11. Pentobarbitone cat (35 mg/kg). Hamstring motoneurone of spike height 67 mV and resting membrane potential − 63 mV (2 M potassium citrate electrode). Firing inhibited by high-frequency stimulation of deep peroneal nerve. Upper curve, 'control', as measured for 0·5 sec before onset of nerve stimulation. Lower curve (*filled circles*), same inhibited during the first 0·5 sec of peroneal nerve stimulation. 'Control' $k_A = 1·03$ impulses $sec^{-1} nA^{-1}$, 'inhibited' $k_A = 0·96$ impulses $sec^{-1} nA^{-1}$. The difference between the two curves is $15·7 \pm 1·6$ (S.D.) impulses/sec.

Figure 12 is from the only experiment in which the same motoneurone (steady for 3 hr) was used for both excitation and inhibition. In this case it is plotted in the manner of Granit & Renkin (1961): 'inhibited frequencies' against their 'controls', 'facilitated frequencies' against the

controls taken in the subsequent experiment on added synaptic excitation. The 45° line through the origin (for $E = 0$; $I = 0$) is inserted for comparison. The regression coefficient for the curve illustrating inhibition is 1·01, that of the corresponding curve for excitation 1·02. If the data of Fig. 12 were to be plotted in the manner of Fig. 11 the slope constants (k_A) would be obtained. These are 1·8 impulses sec^{-1} nA^{-1} for inhibition, 1·7 impulses sec^{-1} nA^{-1} for excitation, thus virtually identical.
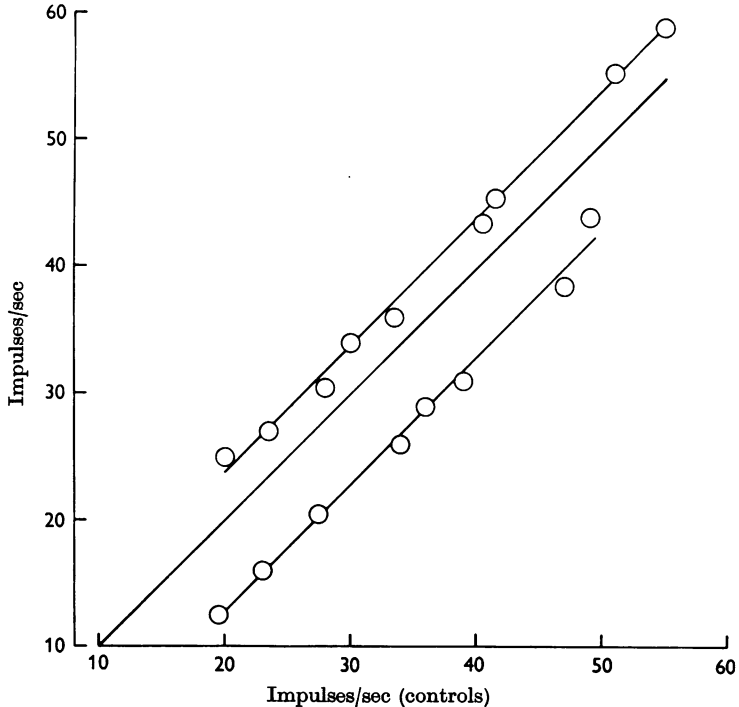

Fig. 12. Pentobarbitone cat (35 mg/kg). Hamstring motoneurone of spike height 75 mV (2 M potassium citrate electrode). This neurone studied for more than 3 hr and values could be obtained for both excitation (tetanus of cut hamstring nerve) and inhibition (tetanus of deep peroneal nerve). In this graph, inhibited and facilitated values are plotted against their own corresponding control values. Thus the 'inhibited' curve is found below and the 'facilitated' one above the 45° line (for $E = 0$ and $I = 0$) through origin, inserted for comparison. The perfect algebraical summation for both excitation and inhibition by the same motoneurone is shown by the regression coefficients of the two curves, which are 1·02 (excitation) and 1·01 (inhibition). The difference in impulse frequency is $+3·7$ impulses/sec for excitation and $-7·3$ impulses/sec for inhibition.

Figure 13 summarizes the results of the seventeen experiments on inhibition within the primary range in a plot of slope constants (k_A) of inhibited cells against those of the controls (as in Fig. 8 for excitation).

The regression coefficient is 1.06 ± 0.09 as against 0.96 with excitation. It is interesting to note that the average slope constant $k_A = 1.30 \pm 0.46$ impulses $sec^{-1} nA^{-1}$ for inhibition was identical with that obtained for excitation (1.31), although in the former case thirteen experiments were performed with animals which had received the full dose of pentobarbitone necessary for anaesthesia (carotids open) and only one experiment (Fig. 12) made use of the same motoneurone for both inhibition and excitation. The mean difference between inhibited values and controls was -8.4 ± 1.20 (S.D.) impulses/sec, the range from -2.8 to -21.8 impulses/sec.
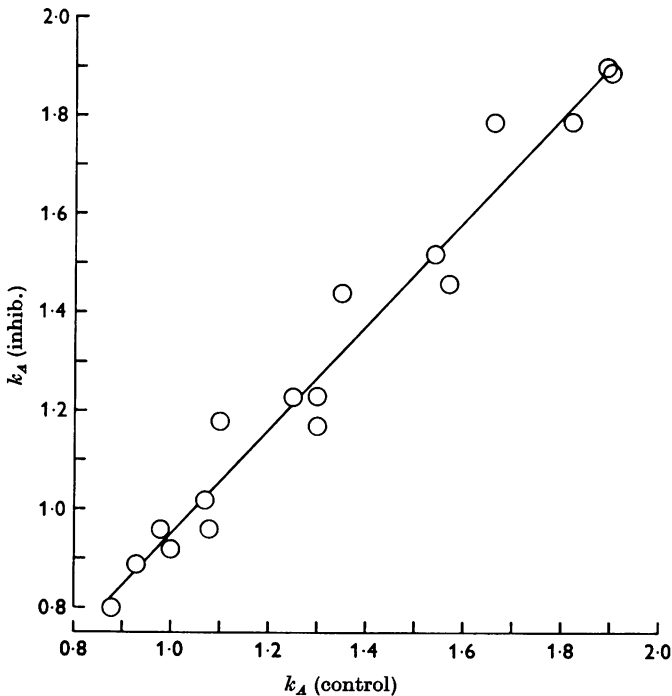

Fig. 13. Plot of slope constants of 'inhibited' curves (ordinates) against slope constants of control curves (abscissae) for seventeen experiments. The regression coefficient is 1.06 ($S_{y \cdot x} = 0.09$) and the linear correlation coefficient $r = 0.982$. The slope constant of one experiment on inhibition was left out as being based on two readings only.

A distribution curve for deviations from a constant difference corresponding to that of Fig. 7 for excitation was plotted also for inhibition. The outcome was the same and it was deemed unnecessary to reproduce the graph. The fraction $\Sigma(bN)/\Sigma N$, which for excitation was 0.985, was likewise computed for inhibition and found to be 0.973 ± 0.073. The mean amount of hyperpolarization by inhibition proved to be 1.7 ± 1.00 mV

(s.d.), the range from 0·1 to 3·8 mV. We suspect that in the pentobarbitone animals with high membrane potentials these values were too low owing to being cut off by the ceiling set by the equilibrium potential for post-synaptic inhibition. This would explain why it proved impossible to calculate K_V for inhibited motoneurones other than those belonging to anaemically narcotized cats (Fig. 10A). The variability was too large.

DISCUSSION

It is concluded from the findings presented that the primary range of regular rhythmic firing of motoneurones, which can be approximated so satisfactorily by a linear relation between impulse frequency and the strength of the injected current, is a range within which algebraical summation of superimposed synaptic currents is the rule. This most likely signifies that the conductance changes induced by peripheral impulses have released currents from synaptic batteries lying in parallel along the cell membrane. All of them contribute their own share to the activation of the common spike generator engaged in summing up their net value. Our type of data does not provide direct information as to the site of this pace-maker. In view of the difficulties encountered in such measurements, it is likely that within the primary range algebraical summation is as strictly realized in the motoneurones as it is in our very best experiments. The slope constant k_A appears to be independent of membrane potential.

The observations have been restricted to large motoneurones but Granit & Renkin (1961), working with recurrent inhibition, obtained the same result extracellularly in decerebrated animals. It is known that recurrent inhibition is particularly potent on the small lumbar motoneurones (Granit, Pascoe & Steg, 1957; Kuno, 1959; Eccles, Eccles, Iggo & Ito, 1961), which in decerebrate animals have low reflex thresholds and easily fire repetitively (Granit, Phillips, Skoglund & Steg, 1957). We have therefore every reason to assume our conclusion to be valid for them too. A more important limitation may be the restriction of this work to segmental reflexes. Changes of the slope constant (k_A) have actually been obtained by supraspinal stimulation (Kernell, 1966).

There have been strong polysynaptic components in the reflexes studied above, and therefore the notion that the synapses engaged have been restricted to the soma seems highly unlikely (see e.g. discussion by Terzuolo & Llinás, 1966). Therefore, algebraical summation is most probably not an exclusive property of purely somatic synapses. The area of membrane which integrates current for the common pacemaker must be a great deal larger. Membrane areas outside the soma are also likely to take an active part in the repetitive discharge itself and in the production

of the after-potentials, so essential for determining the properties of the firing cell. Granit *et al.* (1963*a*) found firing with after-hyperpolarization to indicate a considerable contribution from the dendrites, a true SD-activity in the terminology of Eccles (1957). We shall return to these problems in the Discussion of our second paper (Granit *et al.* 1966). The relation between after-hyperpolarization and firing rates has been subject to a recent quantitative study by Kernell (1965*b*).

The calculated value of some 17–25 mV depolarization for bringing the firing rate of the cell from its minimal to its maximal value within the primary range of firing may be fictitious but is not therefore devoid of interest. It shows how much the after-hyperpolarization would have to make up for, in order to hold down depolarization to near the level required for the minimal firing rate. According to Eccles (1957) compensation by after-hyperpolarization involves maximally 30–40 % of the membrane potential and so, at the upper limit of the primary range, a deficit of residual depolarization may begin to appear in some cells and cause partial inactivation (see e.g. Frankenhaeuser & Vallbo, 1965).

The events within the primary range are on the whole better understood in terms of current. The basic linear relation implies that the greater the driving force (current), the sooner will the next spike occur. Thus, as long as inactivation is absent or modest, the firing cell is held to a narrow range of deviation from the properties it has at the minimal firing rate as determined by the properties of the common pace-maker of its firing zone.

Our results have shown that within the primary range a constant synaptic inhibition or excitation has the same effect as the addition or subtraction of a constant amount of injected current; i.e. these synaptic stimuli seemed to affect neither the firing mechanism of the cell nor the efficiency of the injected currents. This is somewhat surprising, because at least the more powerful synaptic stimuli would be expected to lead to a definite increase of the input conductance of the cell (Eccles, 1964). The increased conductance should function as a shunt for the injected current, and the slope constants referring to facilitation and inhibition would therefore tend to be smaller than those obtained with injected currents alone. This was actually the case in similar experiments performed on the *Limulus* excentric cell (Fuortes, 1959; Rushton, 1959) and the crustacean stretch receptor (Terzuolo & Washizu, 1962). Light in the one case, stretch in the other, was added to varying amounts of injected current. In both cases the slope constant was smaller with added 'natural' stimulation than with injected current alone, and in both cases 'natural' stimulation appeared to increase the input conductance of the neurone. In many of our own cases, the synaptic stimuli may have been too weak for the shunting effect of synaptic action to be reflected in a change of slope constants.

However, even with the very strongest synaptic stimuli no tendency for the slope constant to decrease was seen. As we have not performed any conductance measurements, it is for the time being not very profitable to speculate on whether or not the preservation of the value of the slope constant is due to some compensating mechanism in the neurone other than the after-hyperpolarization.

With regard to the low average membrane resistance of some 0.5 MΩ, as calculated from K_V and K_A in the anaemically narcotized animals, the physical interpretation is somewhat equivocal. The value should predominantly reflect the input resistance, which should be low owing to the light anaesthesia providing a background of lively synaptic activity. On the other hand, the method of measurement is complicated by factors relating to the relative efficiency of synaptic and injected currents on the membrane potential of the soma and the firing rate of the cell. For a further discussion of these factors, the input resistance of the cells should also be measured in the conventional manner.

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