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FACTORS AFFECTING THE CONDUCTIVITY OF PATHWAYS IN THE CEREBRAL CORTEX

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SUMMARY

1. We have investigated the conductivity of neural pathways in slabs of unanaesthetized, isolated, cerebral cortex, cut from the isolated forebrains of twenty-five cats.

2. Neurones within the isolated area were indirectly excited, either by a small electrode thrust into the subcortical white matter, or by remote stimulation of the pial surface. Sometimes a small electrode was employed for intracortical stimulation.

3. The response of single neurones to these stimuli was recorded with extracellular micropipettes. Submaximal stimuli produced a stochastic response which was measured from the post-stimulus histogram (PSH) and provided an estimate of the probability of discharge at various times after the stimulus.

4. The PSH often displayed several discrete humps of different latencies, indicating several pathways between stimulated and recording point. Conductivity measurements were usually restricted to the pathway of shortest latency.

5. The conductivity of a pathway was defined as $C = \underline{xy}/x$, averaged over 1 or 2 min, where

x = frequency of afferent test volleys,

$\underline{xy}$ = frequency of response: i.e. of those action potentials contained within a well-defined hump of the PSH,

(y = frequency of all discharges of the recorded neurone).

6. The progress of conductivity was tested with some constant form of cortical stimulation, repeated at regular intervals of 1–5 sec. Reliable results were obtained for twenty-six pathways subjected to thirty-eight experiments.

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7. Temporary alteration of x, y or $\underline{xy}$ (conditioning with Δx, Δy or $\Delta \underline{xy}$), for a period of 6–25 min, often caused a subsequent change in the conductivity (ΔC) of a pathway which sometimes attenuated with a time constant of about 10 min, but which could persist without detectable attenuation for 20–30 min.

8. Conditioning periods less than 6 min rarely produced changes in conductivity; alterations of conductivity were more likely to be caused by conditioning periods longer than 17 min, than by periods of 6–16 min.

9. Changes in conductivity were usually correlated

negatively with temporary changes in $\underline{xy}$,
negatively with temporary changes in $\overline{x}$,
positively with temporary changes in y.

10. Nineteen of twenty-six pathways tested showed properties consistent with the formula

$$K.\Delta C = G_1 \frac{\Delta x}{x} + G_2 \frac{\Delta y}{y} + G_3 \frac{\Delta \underline{xy}}{\underline{xy}},$$

where

G_1 lies between $-1{\cdot}0$ and $-0{\cdot}16$, with mean value $-0{\cdot}50$,
G_2 lies between 0 and 0·42, with mean value $+0{\cdot}12$,
G_3 lies between $-0{\cdot}61$ and 0 with mean value $-0{\cdot}38$,
K is a coefficient which is usually different for each experiment.

Four out of twenty-six pathways so tested provided results which did not fit this formula; three out of twenty-six pathways did not give adequate information.

INTRODUCTION

Since the time of Pavlov, it has usually been assumed that the cerebral cortex plays an essential role in mammalian learning (Pavlov, 1927; Hebb, 1949; Burns, 1958). This view does not of course imply that other parts of the brain make no contribution to learning—the decorticate rat can learn to discriminate light from darkness (Lashley, 1950); nor does it imply that all parts of the cerebral cortex are equally important to the learning process (see, for instance, Milner, 1962). It merely suggests that examples of those neural mechanisms that are responsible for learning are likely to be found within the cerebral cortex.

It seems reasonable to assume that the changes of behaviour which follow learning are the consequence of persistent alteration in the conductivity of central synapses. In support of this assumption, it can be argued that after learning is complete, certain sensory inputs to the nervous system acquire a high probability of functional connexion to motor outputs with which they were not commonly associated before

learning occurred. This observation implies a re-routing of activity within the nervous system, which is most readily explained in terms of an enduring change in the conductivity of some central synapses.

The most convenient definition of synaptic conductivity in terms that allow measurement by extracellular recording is one involving the probability that activity will cross the junction between two neighbouring nerve cells. Suppose that a neurone, X, is the only one capable of transmitting excitation directly to another neurone, Y. Then if X fires 10 times within an arbitrarily chosen time and Y only fires 8 times within the same time interval, one would describe the conductivity of the functional junction XY as $C_{XY} = 0{\cdot}8$. ('Synaptic resistance' would be the reciprocal of conductivity, $R_{XY} = 1{\cdot}25$.) Where X is not the only neurone capable of exciting Y, as will usually be the case within the central nervous system, one must define C_{XY} as that fraction of discharges of X which *cause* excitation of Y within a few milliseconds of their occurrence.
Thus we may write

$$C_{XY} = \underline{xy}/x,$$

where

x = frequency of discharge of X,
$\underline{xy}$ = frequency of those discharges of Y, 'caused by' discharge of X,
($\overline{y}$ = frequency of all discharges of Y).

Unfortunately it is not easy to find and test monosynaptic pathways in the cerebral cortex. On the other hand, it is possible to measure the conductivity of neural pathways, through which conduction is so rapid that they cannot contain more than three or four synaptic junctions. Our first experiments of this sort, performed in collaboration with Dr G. K. Smith (unpublished experiments), depended upon excitation of the subcortical white matter immediately below a recorded cortical unit in the isolated, unanaesthetized cat's forebrain. We were able to measure conductivity from the stimulated point to the recorded neurone, and established that various conditioning procedures could induce long-lasting changes in conductivity of the test pathway. However, unambiguous interpretation of the results was made difficult by variations in spontaneous activity; moreover, since the subcortical stimulating electrode could excite both cortical afferents and cortical efferents, we were never sure that the observed changes of conductivity had a cortical origin. For these reasons, we did not continue with experiments in the intact brain.

The experiments described below have all been conducted in slabs of neurologically isolated cerebral cortex (Burns, 1951). Because the volume of nervous system involved is limited and the degree of ongoing activity can be controlled, interpretation of results is considerably easier than it is with similar experiments in whole brain. Our intention was to determine

whether long-lasting alterations in the resistance of cortical synapses could be induced by artificial stimulation. If this were so, we also hoped to find out which factors in the past history of a neural pathway determine its present conductivity.

A common assumption is that learning depends upon the presence of central synapses which display a long-lasting facilitation, or increase of conductivity, directly dependent upon previous repeated usage (Eccles, 1953, 1964; Hebb, 1949). Studies of post-tetanic potentiation in the peripheral nervous system (Brown & Euler, 1938; Larrabee & Bronk, 1938), and in the mammalian spinal cord (Eccles & McIntyre, 1953), seem to have supported this concept. On the other hand, elsewhere in the peripheral nervous system, the opposite is usually true (Sharpless, 1964). Moreover, on theoretical grounds, Uttley (1956) has pointed out that the formation and extinction of conditioned reflexes require that C_{XY} be determined by two opposing principles, involving the *relative* activities of the two neurones X and Y.

We have described below the first results from a set of experiments that were designed to:

(*a*) develop an experimental method for the measurement of conductivity along cortical pathways comprising small numbers of synapses;

(*b*) detect long-lasting changes in the conductivity (C_{XY}) of the pathway (X) between the stimulating electrode and the recorded cell (Y), that might be induced by transient alterations in the behaviour of X and Y;

(*c*) establish the sign of the influences of past x, y and $\underline{xy}$ upon present conductivity, C_{XY}.

METHODS

The preparation. Cats of either sex weighing between 1·8 and 2·7 kg were anaesthetized with ethyl chloride followed by ether. A tracheal tube was inserted and the forebrain was then neurologically isolated from the caudal nervous system by a cut close to the plane of the tentorium cerebelli. The decerebration cut was made with a bent wire passed through two holes in the skull just behind the tentorium, and caused no damage to the vertebral arteries supplying the forebrain (G. Mandl, in preparation). Anaesthetic was discontinued and the animal was put on artificial respiration. The left temporal muscle was ligated at its base and removed. The venous sinuses between the two tables of the skull on the left side were blocked by an injection of plasticine mixed with Vaseline (1 part of 'Vaseline': 5 parts of plasticine, by weight), made through two or three small holes drilled in the outer table near to the mid line. For this useful haemostatic technique we are indebted to Professor J. G. Robson (personal communication). The left parietal skull was then removed. Before the dura mater was cut away, a hypodermic needle was passed through the ectosylvian gyrus into the lateral ventricle in order to drain off any accumulated cerebrospinal fluid. The dura was then removed, care being taken to seal any communicating vessels with diathermy before they were severed. The drainage hole into the lateral ventricle was enlarged. The animal was given an injection of 10–20 mg/kg gallamine (Flaxedil) after which a slab of neurologically isolated cerebral cortex, together with underlying white matter, was cut from the suprasylvian gyrus (Burns, 1951). The animal's head was clamped firmly in a

Tschermak holder so that the surface of the isolated area was horizontal; the margins of the cranial skin incision were tied to a metal ring so as to produce the walls of a paraffin-oil bath covering the exposed brain. Body temperature was maintained at 37·5° C with a heating pad governed by a rectal thermostat. The animal was connected to an intravenous drip providing 2·5 ml./hr of physiological saline containing 5 % glucose and 0·6 % gallamine (100 ml. of solution contained 70 ml. of 5 % glucose in saline and 30 ml. of 20 mg/ml. gallamine).

Stimulating and recording electrodes. Miniature subcortical stimulating electrodes were constructed with shafts that were straight for about 15 mm. The first ones that we used were constructed from two parallel and straight lengths of varnished nichrome wire glued together with a film of Araldite. The tips were cut across at 45° to the axes, thus producing a sharp chisel-shaped end; these electrodes could be used for monopolar stimulation at two different but neighbouring sites, or for bipolar excitation. Later we used non-polarizable, monopolar stimulating electrodes made from glass-micropipettes of some 8 μ internal tip diameter; these were filled with 90 % saturated NaCl in water and a silver–silver–chloride lead was inserted in the wide butt-end. Direct stimulation of the grey matter was achieved either with a bipolar platinum electrode resting lightly upon the slab's surface or with a non-polarizable glass micropipette. The latter were sometimes used for stimulating the surface of the isolated area, but were also sometimes inserted through the pial surface so that their tips rested within the grey matter. There was no apparent difference between the results obtained with metal stimulating electrodes and those obtained with the non-polarizable glass pipettes. The stimuli used were 100–250 μsec in duration.

Extracellular micropipettes of 2–4 μ internal tip diameter were employed for recording discharges of the somata of individual neurones (Burns, 1961). These were suspended from a weak spring (Smith & Smith, 1965) and mechanical stability was often improved by covering the surface of the brain with a layer of 2 % agar in physiological saline, before insertion of the micropipette.

At the beginning of the experiment, a platinum electrode was applied lightly to the surface of the slab at a site convenient for the subsequent insertion of a micropipette. The tip of the subcortical stimulating electrode was then inserted into exposed white matter at the caudal end of the slab and the stimulating electrode was pushed horizontally forward under stereotaxic control, through the subcortical white matter. The provisional target of this operation was to place the stimulating electrode 2–3 mm immediately below the surface recording electrode. A final site was chosen for the stimulating electrode, such that evoked responses recorded at the surface were maximal. Sometimes, after a cell had been found by the recording micropipette it was necessary to adjust the position of the subcortical stimulating electrode in order to obtain a satisfactory unit response. The final position was rarely more than 0·5 mm from the original target.

The surface electrode was now moved aside (about 1 mm) and a micropipette was inserted at the chosen site. This extracellular recording electrode was driven slowly through the cortex with a hydraulic micromanipulator during test stimulation, until a satisfactory unit record was obtained. The pre-amplifier was connected so as to record voltage developed between the micropipette and the surface electrode.

Stimulating procedure. In most preparations, two pathways were potentially available for the excitation of cortical neurones—a subcortical or radial pathway, from the subcortical stimulating electrode to the recorded unit and a transcortical or tangential pathway originating at a remote surface or intracortical stimulating electrode. The timing of all stimuli was controlled by a battery of Tektronix 'wave form and pulse generators' (Types 161 and 162), which were arranged so that the stimulus parameters for the two pathways could be altered independently. Strong stimulation of either grey or white matter could cause burst responses (Burns, 1951); consequently, we usually kept stimulus strengths low so that for either stimulus there was not more than one discharge of the recorded unit. Subcortical stimuli were usually more effective when delivered in bursts of, say, 5 stimuli

at 32/sec repeated once every 2 sec. The conductivity of either tangential or radial pathways was monitored continuously by delivering some chosen form of test stimulus once every 1–5 sec. The test stimuli employed were always initially submaximal in the sense that, on average, they elicited less than one response per stimulus. Where possible, conditioning procedures were not begun until conductivity had settled to a constant value.

Recording procedure. Action potentials picked up by the extracellular micropipette were passed through a Grass P4 preamplifier fitted with a 10 msec coupling time constant. They were then fed to one channel of a Phillips 400 stereo tape recorder. A microswitch between the preamplifier and the taperecorder was used to interrupt the recorded signal by shorting it to ground for some 2·0 msec, bracketing any stimulus delivered to the animal. The duration of the block could be adjusted so that a watch could be kept for possible short-latency responses. This device eliminated stimulus artifact from the records. The recorded action potentials were monitored on a Tektronix 502A oscilloscope and were fed to both a loudspeaker and an averaging computer (Burns, Ferch & Mandl, 1965). Signals indicating the times and nature of stimuli were supplied to the remaining channel of the tape deck; they were also fed to the averaging computer.

Computation. A careful analysis of records was made after the experiment was over, by replaying the magnetic tape. On-line control of experimental procedure was effected by watching the post-stimulus histograms accumulated each minute by the computer, in response to test-stimulation. The computer provided a visual display of the number of responses 'caused by' the average stimulus (Fig. 1), and thus made possible a rapid assessment of conductivity along the test pathway.

Nomenclature and symbols used in the text. Many recorded neurones, Y, could be excited through either of two pathways—a transcortical or *tangential* pathway and a subcortical or *radial* pathway, one of which was chosen for the test pathway, X. (In those experiments where both pathways were used, the test pathway was labelled X_1 and the other pathway X_2.)

x = the number of stimuli per minute to an afferent pathway.

y = the discharges per minute of the recorded neurone, Y.

$\underline{xy}$ = the discharges of Y per minute 'caused by' the stimulus (i.e. those discharges of Y contained within a well-defined short-latency hump in the post-stimulus histogram).

C_{XY} = conductivity along the test pathway, X, between the point of stimulation and the recorded cell, Y.

Δx, Δy, $\Delta\underline{xy}$ = the transient changes in x, y and $\underline{xy}$ that might be used for or caused by periods of conditioning.

ΔC = the resultant alteration of C produced by a period of conditioning (i.e. the difference between conductivity before conditioning and that immediately after the end of the conditioning period (see Fig. 2)).

RESULTS

The measurement of conductivity

Single subcortical stimuli were often not successful in exciting cortical neurones. Many cortical units that were not excited by single stimuli would respond readily to bursts of subcortical stimuli. Moreover, it was easier to obtain responses that were stochastic in nature with bursts of this sort. Consequently, we often stimulated radial pathways with bursts of 2–6 stimuli at 20/50 sec repeated every 1 or 2 sec.

The response to excitation of a tangential or radial pathway appeared as a well-defined hump in the post-stimulus histogram (Fig. 1*a*), indicating those discharges of the recorded neurone *Y* which occurred soon after the stimulus. Figure 1*a* shows that the recorded neurone always responded with a latency greater than 2 msec and usually less than 6 msec. (Thus, it seems unlikely that this pathway involved more than two or three synaptic junctions.) The area under the hump of Fig. 1*a* indicates the number of discharges of *Y* 'caused by' the test stimuli and from a knowledge of the number of stimuli used in the formation of this post-stimulus

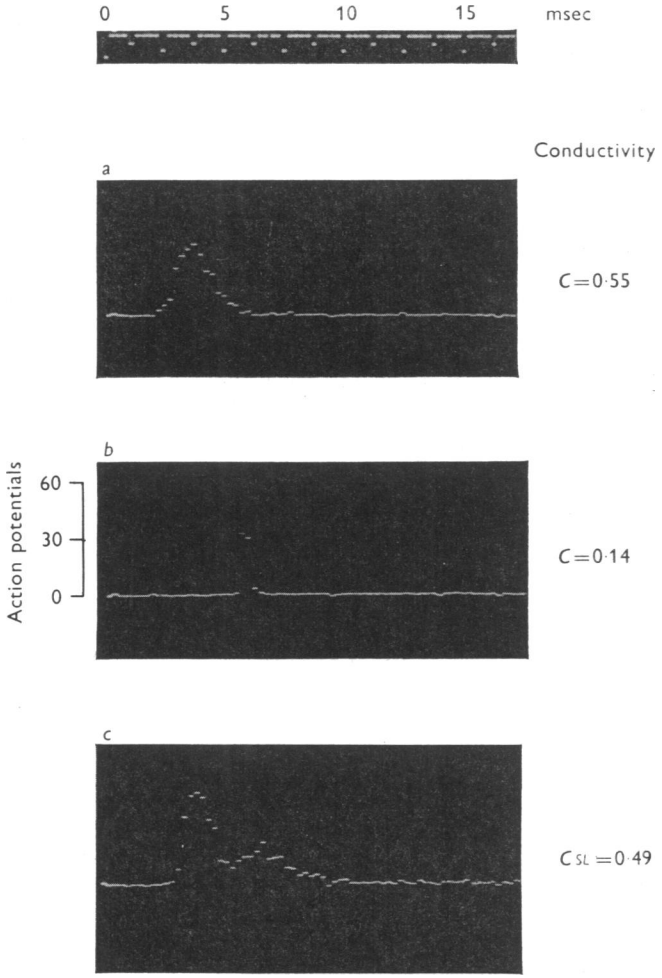


Fig. 1. Various post-stimulus histograms obtained from different cells in isolated cerebral cortex. (C_{SL} = conductivity calculated for short-latency hump.)

histogram, average conductivity for the integration period can be calculated as $C = \underline{xy}/x$. Pathways presumably involving a greater number of synaptic junctions were occasionally encountered (Fig. 1*b*).

Sometimes the test stimulus produced multiple humped post-stimulus histograms, such as those of Figs. 1*c* and 6*b*. Records of this sort indicate either that the neurone fired more than once following each stimulus or that two alternative pathways of different lengths were available for its excitation. In the great majority of such cases the latter explanation proved correct, since the recorded unit could be seen to give not more than one discharge per stimulus. Unfortunately, these multiple-humped records did not usually allow us to measure the effects of one conditioning procedure on two pathways from stimulated to recording point. The probabilities of response to excitation arriving by the two pathways were not independent; if the first response grew in size, the second would diminish, presumably because of refractoriness of the recorded unit. In this situation we only measured conductivity in the relatively short-latency pathway.

We therefore used post-stimulus histograms like those of Fig. 1, accumulated every 1 or 2 min, in order to monitor conductivity during test stimulation repeated every 1 or 2 sec. In most experiments, we had to continue with test stimulation for some 10–15 min before conductivity settled to a relatively constant value (see for instance, Fig. 5); we would then initiate a conditioning procedure and observe the subsequent change in C.

Conditioning procedures

Had we been able to find a cortical neurone connected with a suitable test pathway, it would have been desirable to change the three parameters x, y and $\underline{xy}$, one at a time in successive conditioning periods; we would then have observed the effects of these three changes on the same test pathway. In practice, this has not so far proved possible. We often lost contact with the recorded neurone after 1 hr or so, and a period of 1 hr was required to complete any one of these tests. Thus our usual procedure was to find with the recording electrode a neurone that promised a relatively stable record, and then spend some 10 min determining the conditioning procedure that seemed best suited to that particular unit's functional connexions. We would then begin a control period of test stimulation lasting some 20 min after which a period of conditioning was initiated. Figure 2 illustrates this sequence of manoeuvres. In this experiment one direct cortical stimulus per second was used to sample conductivity in a tangential pathway for a control period of 18 min. At the end of this period the rate of subcortical stimulation was increased to 7/sec

for 7 min. This produced an increase (Δx) in x and a consequent increase ($\Delta \underline{xy}$) in $\underline{xy}$, as indicated in Fig. 2. At the end of 7 min, the rate of subcortical stimulation was returned to the control value of 1/sec. It will be seen that the period of conditioning produced a fall in conductivity, ΔC. In this case the after effect of conditioning upon conductivity appeared to be temporary, and conductivity returned toward the control level with a time constant of roughly 20 min.

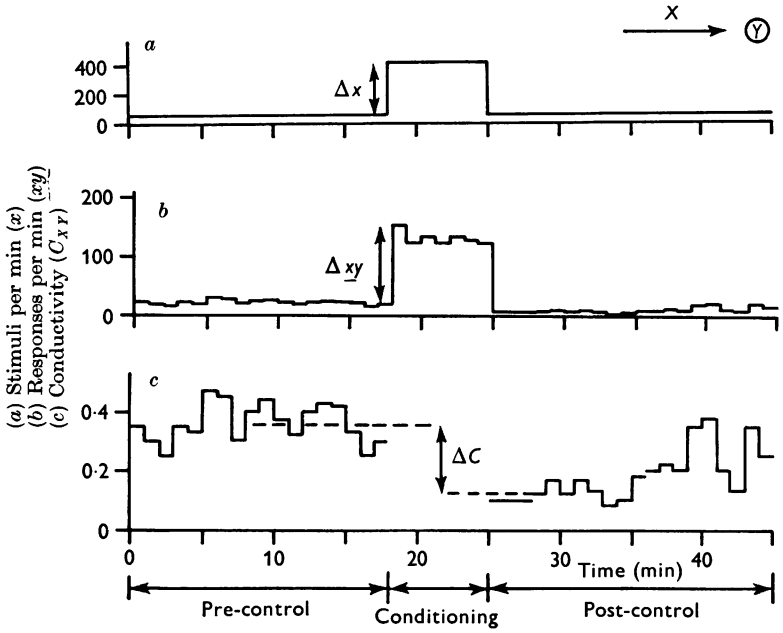


Fig. 2. The effect (ΔC) of a period of increased ($\Delta \underline{xy}$) upon the subsequent conductivity of a tangential pathway, X. Test stimulation of X during the pre- and post-conditioning control periods was at 1/sec. During conditioning the same stimuli were given at 7/sec. *a*: The frequency of stimulation of X. *b*: The frequency of discharge of cell Y; all discharges of this cell were caused by the stimuli. *c*: Calculated conductivity, C, between point of stimulation and Y (Expt. no. 6).

A similar procedure was adopted in all of the tests described below.

The effects of change in x alone. We made use of temporal summation in order to alter the input to the test pathway X, without causing an immediate change in the frequency of discharge, y, of the recorded neurone. Most radial pathways showed temporal summation; indeed, we have already pointed out that short bursts of subcortical stimulation were often more effective than were single stimuli. Thus, we were able to provide a period of conditioning, during which a number of stimuli at a lower frequency were added between the standard bursts of test stimuli. These conditioning stimuli did not excite extra responses from the recorded

neurone, but, since they were the same strength as the test stimuli, they were presumably exciting the origin of the test pathway.

Figure 3 illustrates the results of an experiment of this type. Conductivity was measured with single test stimuli repeated every 1·68 sec. During the control period of 8 min, six extra subcortical stimuli were given in each cycle at 10/sec. These additional stimuli were slightly weaker than

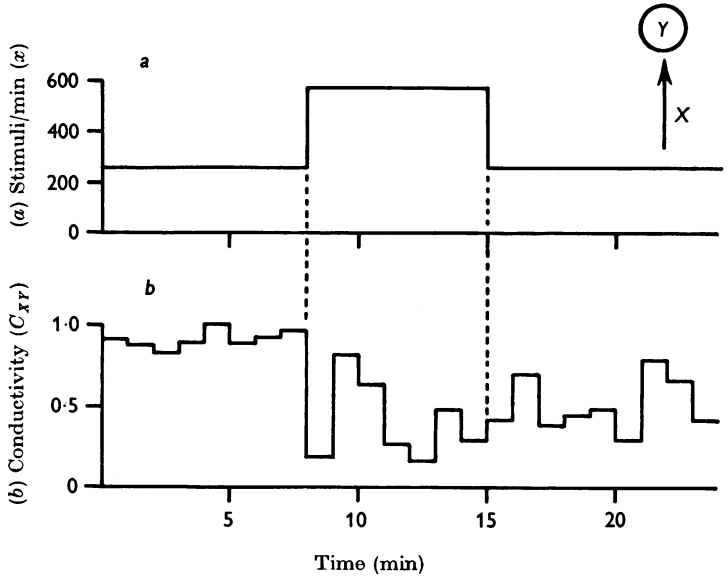


Fig. 3. The effects of increase in stimulation of an afferent radial pathway, X, without an increase in the rate of response of the recorded cell Y (i.e. increase of x alone). During the control periods preceding and following conditioning a single test shock was given to X every 1·68 sec, followed by a buzz of 6 weaker stimuli at 10/sec. These weaker stimuli did not drive the cell, but were capable of doing so when given at 50/sec. During conditioning, the buzz duration was increased to 14 stimuli at 10/sec. a: The frequency of all stimuli given to X. b: The conductivity between stimulated point and cell Y, calculated from the single test shock (Expt. no. 25).

the test stimuli and produced no response from the recorded neurone; nevertheless, when given at 50/sec, they produced responses. During the conditioning period of 7 min, the number of these just-subthreshold stimuli was temporarily increased to fourteen in each cycle. At the end of conditioning we reverted to the control sequence of one test stimulus followed by six subthreshold stimuli. It will be seen that conditioning produced a subsequent fall in conductivity which remained at its new level without significant change for 9 min until the cell was lost.

The effects of change in y alone. When two pathways were available for excitation of the recorded neurone, it was possible to vary the frequency of discharge, y, of the recorded unit, without changing the frequency of stimuli, x, given to the test pathway. Thus we would find a unit which took part in the burst response of isolated cortex (Burns & Grafstein, 1952) and begin sampling the conductivity of a radial pathway to this unit with a

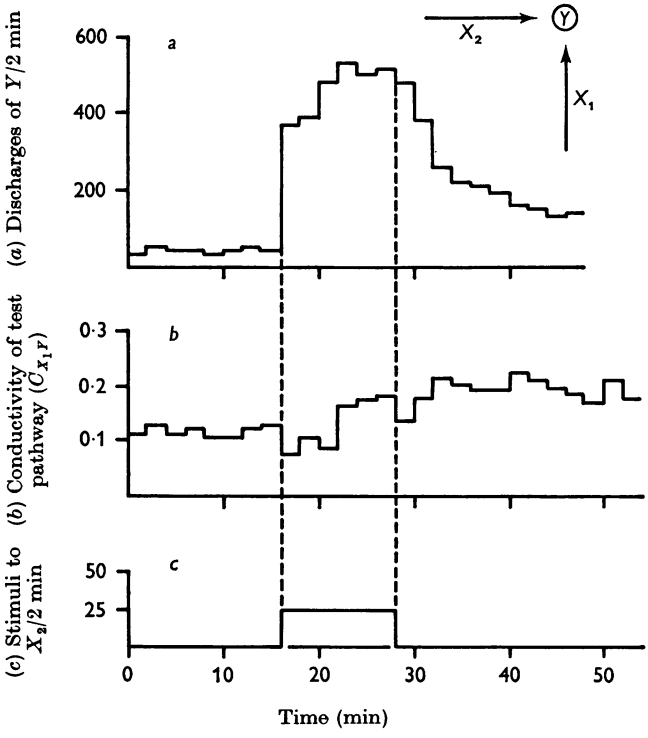


Fig. 4. The effect upon conductivity in a radial test pathway, X_1, of exciting the recorded cell Y by stimulation of a tangential pathway X_2. The test stimulus was a buzz of 6 stimuli at 50/sec repeated every 5 sec. *a*: Frequency of all discharges of Y. *b*: Calculated conductivity along the radial pathway, X_1. *c*: Frequency of conditioning stimuli to X_2. The conditioning stimuli set up after-bursts which largely account for the rise in y following the conditioning period (Expt. no. 12).

subcortical stimulating electrode. Direct excitation of the grey matter with a remote electrode could be used to produce burst responses and provided an independent pathway for the control of mean unit activity. This type of experiment is illustrated by Fig. 4. In this experiment, test stimulation consisted of bursts of six stimuli at 50/sec repeated every 5 sec, and was given to a radial pathway, X_1, through a subcortical electrode. After a control period of 17 min, the unit was also excited by a

single stimulus given to the surface of the slab, 2·5 sec after each test stimulus (pathway X_2). These conditioning surface stimuli produced burst responses and therefore caused a dramatic increase in y. It will be seen that the conditioning stimulus produced an increase of conductivity in the test pathway that persisted without measurable change for 26 min. Unfortunately, in this experiment the surface stimuli used for conditioning set up a remote focus for spontaneous activity in the form of after-bursts

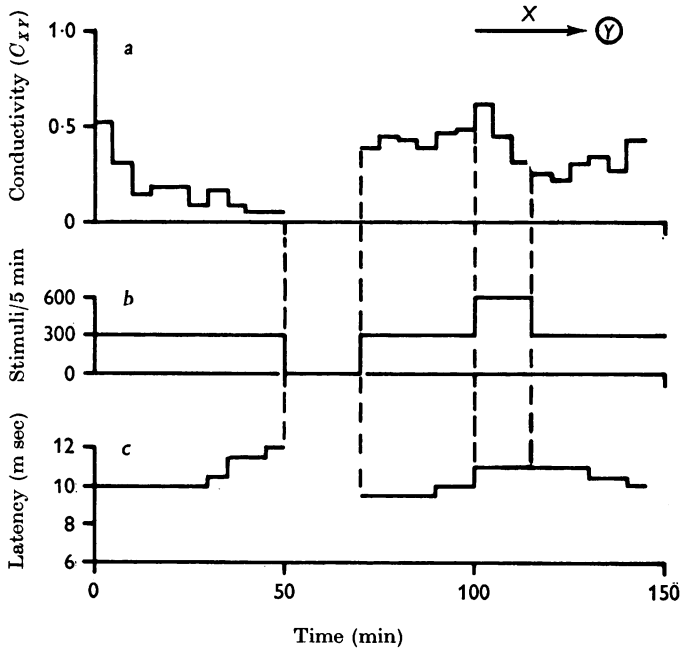


Fig. 5. The effects of rest and increased use upon the conductivity of a tangential pathway, X. Single test stimuli were given at 1/sec. During the first conditioning period, this stimulus was turned off. During the second period of conditioning the same stimulus was given at 2/sec. a: Conductivity between the stimulated point and the recorded cell, Y. b: Frequency of stimuli given to X. c: Latency of response, measured to peak of first hump in post-stimulus histograms illustrated in Fig. 7 (Expts. 19 and 20).

(Burns, 1954), which caused values of y to remain high for some time after the conditioning stimulus was withdrawn. This is one of the many factors that can complicate interpretation of results from a relatively simple preparation.

We have also made some use in similar experiments of the fact that the duration of a burst of activity set up by the surface stimulus can be curtailed by the administration of a second appropriately timed surface or

deep stimulus (Burns, 1951). This fact enables one to control y without involving a risk of unwanted after-bursts (Expt. 10).

The effects of simultaneous change in x and xy. Simultaneous changes of like sign in x and xy were easy to arrange. A temporary alteration in the usage of any test pathway could be produced by altering the frequency of the stimulus employed for testing. Figure 2 shows the results of an experiment in which synaptic usage along a radial pathway was increased during a conditioning period of 7 min. This manoeuvre produced a subsequent

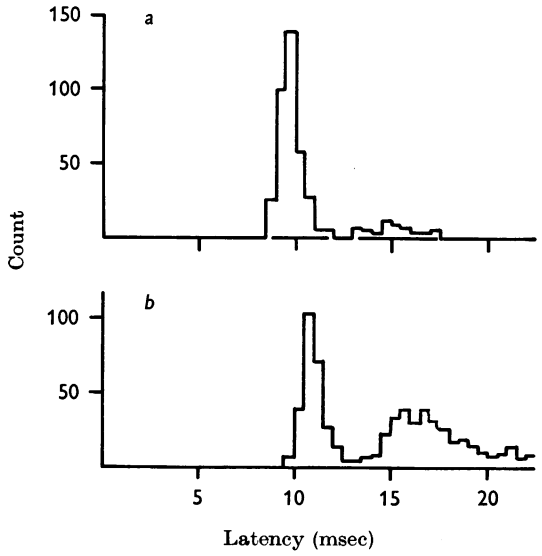


Fig. 6. The changes of latency produced by a period of increased usage. Same experiment as Fig. 5. Ordinate = number of discharges of Y per 0·5 msec time-bin, per 10 min record. Abscissa = time after stimulation of X. *a*: Response before conditioning, averaged from the 91st to 100th min of Fig. 5. *b*: Response after conditioning, averaged from the 121st to 130th min of Fig. 5 (Expt. no. 20).

reduction in conductivity, which slowly drifted back towards control levels. Figure 5 shows the results of a similar experiment, in another cat, upon a tangential or transcortical pathway. After a control period of 50 min, the test stimulus was stopped and the preparation was rested for 20 min. After this rest, conductivity was almost double the average value before conditioning, and remained at this level for 30 min until the next test. For this test, the rate of test stimulation was doubled for 16 min, after which conductivity showed a decline which appears to drift back towards the preconditioning level with a time constant of some 30 min.

Latency changes. Conditioning produced measurable alterations in the latency of response in six experiments (Nos. 19, 20, 22, 44, 45 and 48). In

every case the observed change was that expected from the calculated change of conductivity. When conductivity increased, the latency of response became less—and vice versa. This negative correlation between conductivity and latency is illustrated in Fig. 5*c*. Figure 6 shows the sort of post-stimulus histogram from which the data for Fig. 5*c* were derived. It illustrates both the fall in conductivity and the increased conduction time of the short-latency pathway, caused by a period of increased usage. (The consequent increase in conductivity of a dependent, longer latency pathway is also visible.)

Summary of all experiments

The Figures of the preceding sections provide examples of our experimental results. We have performed tests of this sort on preparations from twenty-five cats. Some of these experiments were unsatisfactory and their results were discarded. The two most frequent reasons for discarding result, were drift or instability of conductivity and early loss of the recorded neurone. However, we managed to collect apparently reliable results from thirty-eight experiments performed on twenty-six different pathways in twenty cats. The results of these experiments are summarized in Table 1.

The Table shows that we have used a variety of conditioning periods ranging from 3 to 26 min with one of 60 min. This is partly because we began this work with no idea of the relation between conditioning period and the probability of producing an after-effect. Moreover, we sometimes chose relatively short periods for conditioning because we had the impression that we might soon lose contact with the recorded neurone. Table 2 is derived from Table 1 and provides an attempt to assess the relationship between duration of conditioning and its effect upon subsequent conductivity.

The first row of entries in this Table was derived from column *n* of Table 1, and shows that changes in conductivity are more likely to occur, the longer the duration of conditioning. The second row summarizes column *o* of Table 1, and provides no evidence that longer conditioning periods are more likely than short ones to produce persistent changes in conductivity.

Examination of Table 1 provides no reason for believing that any one of the conditioning procedures we have used is more likely than the others to produce a change in conductivity. Moreover, it appears that the probabilities of producing alterations of conductivity in radial and tangential pathways are similar. Table 3 was derived from Table 1 and illustrates this point.

Analysis of results

We have attempted to summarize the results of all our separate experiments by searching for an approximately linear relationship between some function of Δx, Δy, and $\Delta \underline{xy}$, the changes in frequency produced by conditioning, and the consequent change in conductivity ΔC. Such relationships were not obvious from the data, because more than one of these frequencies usually changed during a conditioning period. Moreover, in each experiment the number and initial state of the neural pathways involved were unknown, and such factors might be expected to alter the effectiveness of conditioning. So, for the equation

$$K.\Delta C = F_1\Delta x + F_2\Delta y + F_3\Delta \underline{xy} \qquad (1)$$

although K could not be determined, it seemed reasonable to consider the ratios $F_1:F_2:F_3$ and to seek values consistent with all the experiments.

We found that for nineteen out of the twenty-six pathways listed in Table 1, the experimental results were consistent with the above equation, where K is unknown and

F_1 lies between $-1{\cdot}0$ and $-0{\cdot}15$, with a mean value $-0{\cdot}45$,

F_2 lies between 0 and 0·39, with a mean value $+0{\cdot}13$,

F_3 lies between $-0{\cdot}81$ and 0, with a mean value $-0{\cdot}42$.

We also considered the relation between ΔC and relative changes in frequency; the corresponding equation is:

$$K.\Delta C = G_1\frac{\Delta x}{x} + G_2\frac{\Delta y}{y} + G_3\frac{\Delta \underline{xy}}{\underline{xy}}. \qquad (2)$$

We found that for the same nineteen pathways, the results were consistent with the above equation with

K unknown and

G_1 between $-1{\cdot}0$ and $-0{\cdot}16$, with mean value $-0{\cdot}50$,

G_2 between 0 and 0·42, with mean value $+0{\cdot}12$,

G_3 between $-0{\cdot}61$ and 0, with mean value $-0{\cdot}38$.

These conclusions were derived in the following way, which is explained in detail in the Appendix. The ratios between three numbers can be displayed by means of areal co-ordinates (Milne, 1924), as in a colour triangle. In triangle XYZ of Appendix Fig. 1, any point P uniquely describes the ratios of the three triangular areas YZP, ZXP and XYP. These areas may be used to represent the constants F_1, F_2 and F_3 of equation (1) (or

TABLE 1. SUMMARY OF RESULTS

Expt. no. *a*	Pathway no. *b*	Tangential or radial *c*	Mean latency response (msec) *d*	Conditioning: With *e*	Conditioning: For (min) *f*	Pre-conditioning values (min^{-1}): *x* *g*	*y* *h*	*xy* *i*	*C* *j*	Changes during conditioning: Δx *k*	Δy *l*	Δxy *m*	After effect ΔC *n*	Time constant of ΔC (min) *o*	Observed for (min) *p*	Fits equation: *F* *q*	*G* *r*
1	1	R	2–7	$-x, -\underline{xy}$	8	15	125	15	0·42*	−15	−25	−15	−0·29	INF	42	No	No
2	1	R	2–7	$+x, +\underline{xy}$	6	15	110	4	0·18*	90	61	79	0·58	INF	6	No	No
3	2	R	2–5	$+\underline{xy}$	6	90	30	30	0·33	0	90	90	0	—	—	Yes	Yes
5	3	T	7–8	$+x$	13	60	30	30	0·50	360	0	0	0	—	—	No	—
6	3	T	7–8	$+x, +\underline{xy}$	7	60	20	20	0·33	360	240	240	−0·21	20	—	Yes	Yes
6*a*	4	T	4–5	$+y$	5	46	5	5	0·11	0	38	0	0	—	—	Yes	Yes
7	5	R	6–7	$+y$	5	226	55	55	0·24	0	75	−50	0	—	—	Yes	Yes
10	6	R	11–13	$-y$	12	180	395	180	1·0	0	−67	0	−0·23	INF	10	Yes	Yes
11	7	R	6–12	$+x, +\underline{xy}$	6	37	600	105	1·0*	188	−144	145	−0·22	8	—	Yes	Yes
12	8	R	6–8	$+y$	12	72	12	9	0·13	0	228	0	0·07	INF	26	Yes	Yes
15	9	T	10–11	$-x, -\underline{xy}$	6	231	79	79	0·34	−231	−79	−79	0·18	8	—	Yes	Yes
16	9	T	10–11	$+x, +\underline{xy}$	6	231	79	79	0·28	231	46	46	−0·12	INF	14	Yes	Yes
18	10	T	10–11	$-x, -\underline{xy}$	25	60	15	15	0·22*	−60	−15	−15	0·31	10	—	Yes	Yes
19	10	T	10–11	$-x, -\underline{xy}$	20	60	12	12	0·06*	−60	−12	−12	0·38	INF	30	Yes	Yes
20	10	T	10–11	$+x, -\underline{xy}$	16	60	32	32	0·42*	60	42	42	−0·16	20	—	Yes	Yes
21	10	T	10–11	$+x, -\underline{xy}$	5	60	48	48	0·34*	180	−43	−43	−0·03	INF	30	Yes	Yes
22	11	R	23–26	$-x, -\underline{xy}$	17	60	60	6	0·05	−60	−60	−6	0·85	6	—	Yes	Yes
23	12	T	10–17	$-x, -\underline{xy}$	17	60	60	54	0·90	−60	−60	−18	0·55	INF	12	Yes	Yes
24	13	T	20–25	$-x, -\underline{xy}$	20	60	3	3	0·05	−60	−3	−3	0·10	INF	10	Yes	Yes
25	14	R	8–11	$+x, -\underline{xy}$	7	270	34	34	0·88†	318	−18	−18	−0·41	INF	10	Yes	Yes
29	14	R	6–8	$+\underline{xy}$	6	270	42	42	1·1†	0	168	168	0	—	—	Yes	Yes
30	14	R	6–8	$+\underline{xy}$	10	270	42	42	0·67*†	0	109	109	−0·48	INF	17	Yes	Yes

TABLE 1 (*cont.*)

Expt. no.	Path-way no.	Tan-gential or radial	Mean latency response (msec)	Conditioning		Pre-conditioning values (min^{-1})				Changes during conditioning			After effect	Time con-stant of ΔC	Observed for	Fits equation	
				With	For (min)	x	y	$\underline{xy}$	C	Δx	Δy	$\Delta \underline{xy}$	ΔC	(min)	(min)	F	G
a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*	*k*	*l*	*m*	*n*	*o*	*p*	*q*	*r*
26	15	R	4–5	$+x$, $-\underline{xy}$	8	57	80	14	0·25	95	0	−8	−0·25	INF	6	Yes	Yes
31	16	R	8–10	$+x$	10	192	25	25	0·13	121	0	0	0	—	—	No	—
34	17	R	2–3	$+x$, $-\underline{xy}$	5	190	50	50	0·26	228	−10	−10	0	—	—	No	—
35	17	R	2–3	$+x$, $-\underline{xy}$	3	190	40	40	0·21	228	−30	−30	0	—	—	No	Yes
38	18	T	3–5	$-\underline{xy}$	16	90	330	82	0·10*†	0	0	−7	0	—	—	Yes	Yes
40	19	T	2–3	$-x$, $-\underline{xy}$	60	60	540	55	0·92	−60	−22	−55	0	—	—	No	No
41	19	T	2–3	$+x$, $+\underline{xy}$	13	60	325	51	0·85	90	75	91	−0·12	4	—	No	No
44	20	R	1–5	$-\underline{xy}$	10	90	59	59	0·65	0	−54	−54	−0·31	INF	10	No	No
47	21	T	4–5	$+y$, $-\underline{xy}$	5	90	15	15	0·17	0	81	−14	0·65	4	—	Yes	Yes
48	22	T	2–4	$+y$, $-\underline{xy}$	13	90	61	61	0·67	0	119	−12	−0·39	INF	9	No	No
49	23	T	2–3	$-\underline{xy}$	11	72	48	48	0·67	0	0	−44	0	—	—	Yes	Yes
51	23	T	2–3	$+y$	9	72	36	36	0·50	0	0	47	0	—	—	Yes	Yes
54	24	T	1–3	$+y$	5	90	43	8	—	0	35	0	0	—	—	Yes	Yes
55	25	R	4–15	$+y$	1·5	180	2	2	0·01	0	170	0	0	—	—	Yes	Yes
100	26	T	1–20	$+\underline{xy}$	3·0	92	252	32	0·35	0	−62	18	0·46	16	—	Yes	Yes
101	26	T	1–15	$-\underline{xy}$	3·0	92	162	7	0·08	0	0	−2	0·06	INF	20	Yes	Yes

Notes

Column *e*. The direction of the change in x, y or $\underline{xy}$ during conditioning is listed as '+' (increase) or '−' (decrease).

Columns *g–j*. Average values for last 6 min before conditioning.

Columns *k–n*. Average values for first 6 min after conditioning.

Column *o*. *Time constant* is defined as the estimated time for ΔC to fall to one-third of its initial value. INF implies no detectable drift of C towards pre-conditioning level during period of observation listed in column *p*.

Columns *q–r*. Yes, fits equation; No, does not fit; — = indeterminate.

Column *j*. *, † indicates that $C = \underline{xy}/x$, either because C was measured from the first hump of a multimodal PSH (*), or from the first of a burst of test stimuli (†).

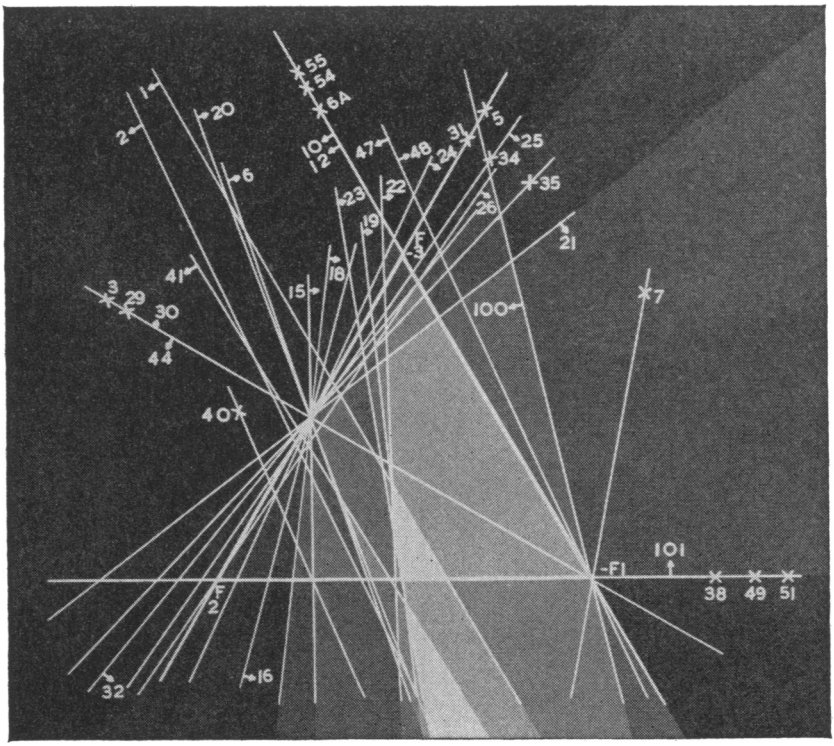


Fig. 7. The results of Table 1, displayed in areal co-ordinates appropriate to equation (1). The lighter any district of this diagram, the more likely is this district to contain the true values of F_1, F_2 and F_3. See text.

TABLE 2. The relationship between duration of conditioning and subsequent changes in conductivity

	Duration of conditioning (min)		
	0–5	6–16	17–60
% of experiments showing changes of conductivity	40 (10)	68 (22)	83 (6)
% changes in conductivity that were persistent (time const. = INF)	50 (4)	67 (15)	60 (5)

Note: the numerals in parentheses indicate the totals from which entries were derived.

TABLE 3. The distribution of various changes in conductivity with type of pathway

	Type of pathway	
Change of conductivity	Radial	Tangential
Number showing no change	7	7
Number showing transient change	2	7
Number showing permanent change	8	7
Showing some change (%)	59	67

G_1, G_2 and G_3 of equation (2)). The results of each experiment can then be uniquely described by a straight line, such as QR, where:

$$\frac{RX}{ZX} = \frac{\Delta x}{\Delta x - \Delta xy}; \quad \frac{QY}{ZY} = \frac{\Delta y}{\Delta y - \Delta xy}.$$

The sign of ΔC determines on which side of QR lies the unknown point P which we seek.

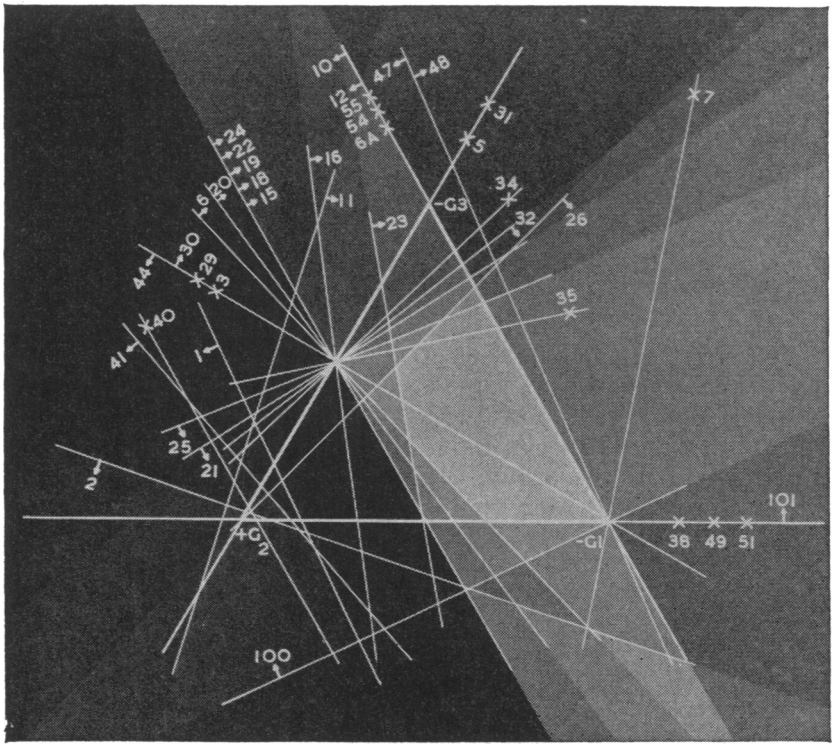


Fig. 8. The results of Table 1, displayed in areal co-ordinates appropriate to equation (2). The lighter any district of this diagram, the more likely is this district to contain the true values of G_1, G_2 and G_3. See text.

Figure 7 shows, in this graphical form, the results of all experiments using equation (1). For each experimental line (equivalent to QR in Appendix Fig. 1) an arrow indicates on which side the point P must lie; note the negative signs of F_1 and F_3. Figure 8 shows the results for equation (2) displayed in a similar manner. In order to indicate the extent to which equations (1) and (2) provided satisfactory descriptions of the experimental results, the figures were obtained by multiple exposure photo-

graphy. The same short exposure was made on one side only of the line describing each experiment—the side opposite to the arrow. Thus, the over-all brightness of any area is directly related to the number of experiments whose results would be satisfactorily described if P fell within that area. Put another way, the brightest areas of Figs. 7 and 8 indicate the approximate ratios of $F_1:F_2:F_3$ or $G_1:G_2:G_3$ that are consistent with the majority of our experimental results.

It would appear reasonable, at this stage, that we should not be able to determine whether C is a logarithmic or a linear function of, say, y. The important general conclusions are that, for a majority of experiments:

(*a*) ΔC is negatively correlated with Δx and Δxy;

(*b*) ΔC is positively correlated with Δy;

(*c*) the effects upon C of Δx and Δxy are approximately equal and about three times larger than the effect of Δy.

DISCUSSION

In all of the experiments described above, we have used constant submaximal stimulation of a pathway running through the grey matter of the cerebral cortex in order to test conductivity of this pathway between the stimulating electrode and recorded cortical neurone. Responses of the latter were stochastic in nature; although the average number of responses per minute remained fairly constant under unchanging experimental conditions, the presence or absence of response to any individual stimulus could not be predicted with certainty. This unpredictability of unit response may be due to synaptic noise (Li, 1961) akin to end-plate noise in skeletal muscle (Fatt & Katz, 1952). Alternatively, it could be due to minor variations in the thresholds of structures accessible to excitation by the stimulating electrodes. Again, it could be due to minute movements of the stimulating electrodes that we used, relative to the surrounding neural tissue. We have no way of deciding between these alternative explanations; but fortunately the cause of the stochastic behaviour of cortical units is not immediately relevant to the interpretation of the experimental results given below.

The experiments have established that relatively long-lasting changes in the conductivity of cortical pathways can be set up by forced transient alterations in the relative activity of input and output of these pathways. There is no reason to believe that tangential or transcortical pathways differ in this respect from radial pathways, originating in the subcortical white matter; similar evidence of plasticity has been obtained in both

classes of pathway. However, alteration of conductivity is more likely to follow long conditioning procedures than short ones. The changes of conductivity that were recorded were not always 'permanent'; in about half the trials, conditioning set the conductivity to a new level, which was not maintained and conductivity drifted slowly back to control levels. In the remaining half of these trials, the new level of conductivity was maintained without significant drift for the period of observation—some 10–30 min. It would be of great interest to know how long these alterations of conductivity that we have called persistent can last. Unfortunately, changes persisting for 40 min are the longest that we have recorded; longer periods of observation have usually been prevented by loss of contact with the recorded unit. On those occasions when the preparation was very stable we have felt it more important to expose the pathway to several tests, than to extend observation of the consequences of one experiment. There is no evidence from our experiments to suggest that comparatively long periods of conditioning are more likely to produce enduring changes of conductivity. It therefore seems possible that some of the pathways we have tested contained synaptic junctions that were incapable of persistent change.

Having developed a method for the measurement of conductivity through cortical pathways and having established that long-lasting changes of conductivity can be induced, we have tried to find out which aspects of the past history of a pathway determine its present conductivity. We have attempted to derive an equation relating

$$\Delta C \quad \text{to} \quad \Delta x, \quad \Delta y \quad \text{and} \quad \Delta \underline{xy},$$

that would fit the greatest number of our experimental results. It would have been convenient, had we been able, to expose every pathway sequentially to changes in each of the three independent variables, one at a time. This has not so far proved possible, because we were usually unable to record from one pathway for long enough. Moreover, it was not usually possible to arrange that only one of these variables changed during a conditioning period. For instance, we have not managed to alter $\underline{xy}$ without simultaneous alteration of y; furthermore, in slabs of isolated cortex that gave burst responses of uncontrolled duration, it was often difficult to control the value of y. Consequently, we have sought a formula for description of the results, that assumed the ratios of contributions to final conductivity from alterations in x, y and $\underline{xy}$ to be the same for all pathways tested, although the absolute effect upon conductivity might vary from test to test. In this way we found that the formula

$$K.\Delta C = G_1 \frac{\Delta x}{x} + G_2 \frac{\Delta y}{y} + G_3 \frac{\Delta xy}{\underline{xy}}$$

fitted the results from 29/38 tests and from 19/26 pathways provided that:

G_1 was negative, and lay between $-1{\cdot}0$ and $-1{\cdot}6$,
G_2 was positive and lay between 0 and $+4{\cdot}2$,
G_3 was negative, and lay between $-0{\cdot}61$ and 0,
while K might vary from experiment to experiment.

We have pointed out in the introduction that studies of animal learning are commonly taken to imply that the brain must contain synaptic junctions, the resistance of which falls, the more often they have transmitted activity (Hebb, 1949; Eccles, 1953, 1964). The results of our experiments lend little support to this belief. In fact the negative sign of G_3 implies that synaptic junctions with a precisely opposite mode of action are more common. The great majority of pathways we examined must have contained synaptic junctions that were less likely to transmit excitation the more often the pathway was used. Nevertheless, Expts. 1, 2, 40, 41, 44 and 48, suggest that at least four of the pathways tested (1, 19, 20 and 23 of Table 1) contained junctions of a different sort. Thus, it appears that the cerebral cortex contains plastic synapses of more than one type.

Most of the pathways we have tested almost certainly contained more than one synaptic junction. It is therefore reasonable to enquire to what extent behaviour of a pathway revealed by the various tests we have used could indicate the properties of individual junctions. The problem may be made clearer by reference to Fig. 9. In experiments involving conditioning with transient changes in x alone, or both x and $\underline{xy}$, only one pathway (X_1 or X_2) was used for excitation of the recorded cell Y. Thus, the observed changes in conductivity produced in a pathway by such tests must have resulted from similar changes in conductivity in at least one functional unit of the pathway, however many such units there were in the chain that conducted excitation from S_1 (or S_2) to Y. It is a reasonable guess that synaptic junctions are the units exhibiting variable conductance.

In those tests where variation in y alone was used as a conditioning stimulus, two pathways were used for excitation of the recorded neurone—pathways illustrated by X_1 and X_2 of Fig. 9. If one could be sure that these pathways were anatomically independent as shown in Fig. 9*a*, then it would be safe to ascribe the properties of a test pathway as revealed by this test, to similar properties of at least one synapse in the chain of neurones constituting the pathway. But in the majority of such experiments, the

pathways may not have been independent; the test pathway X_1 of Fig. 9*b* may have shared units with the pathway X_2, used for alteration of y without change in x or $\underline{xy}$. Thus, the conditioning excitation, travelling along the X_2 pathway, might have facilitated either or both of the junctions *cb* and *bY*; an increased conductivity of either junction would appear as increased conductivity in the X_1 pathway (see, for instance, Expts. 8 and

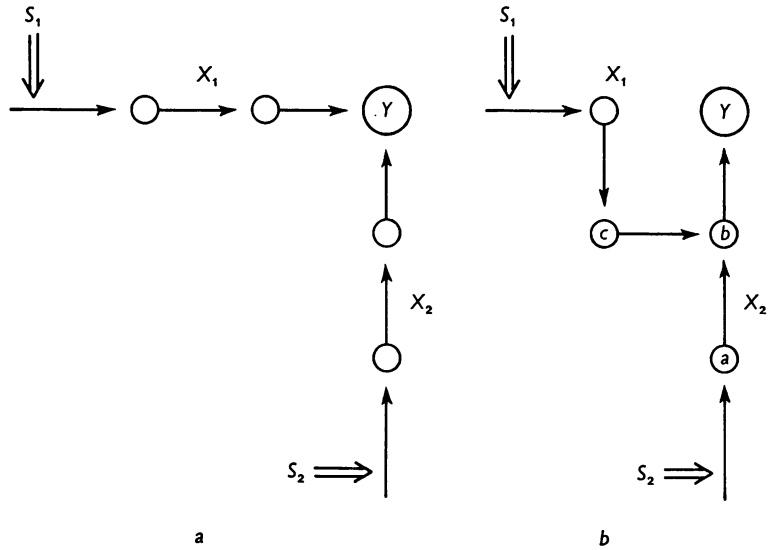


Fig. 9. Diagram illustrating the tangential and radial pathways from stimulating electrodes to recorded cell, discussed in the text.

12 of Table 1). Increased conductivity at the *cb* junction would then have been due to cell *b* firing without preceding discharge of cell *c*, during the conditioning period; increased conductivity of the junction between cells *b* and *Y* would have been due to an increased usage of this junction (an effect of increasing both x and $\underline{xy}$). Fortunately, the majority of experiments shows that conductivity is negatively correlated with simultaneous changes in x and $\underline{xy}$. Since conductivity of the pathways examined in Expts. 10 and 12 was positively correlated with alterations in y alone, it is probable that at least one synapse in the test pathway exhibited the same positive correlation.

For these reasons the estimates given for the signs of G_1, G_2 and G_3 may be accepted as the signs of changes at one or more individual synapses along the pathways tested. On the other hand, the estimates we have given of the magnitudes of G_1, G_2 and G_3, cannot safely be assumed to describe the properties of individual synapses. The true values of G_1 and G_3 for pathways can only provide upper limits for the values appropriate to a

single synapse, while the magnitude of G_2 for a test pathway cannot offer any indication of the equivalent value for a single synapse, unless the pathways used for testing and conditioning are known to be anatomically independent. Nevertheless, one can safely make one useful generalization about the properties of cortical synapses that display plasticity: *the majority of such junctions between two cells X and Y (so arranged that X can excite Y), show long-lasting changes of conductivity which are negatively correlated with both the firing rate of X and usage of the XY junction, but are positively correlated with the discharge frequency of Y.*

Milner pointed out (1957) that a nervous system containing only synapses whose conductivity was increased by usage ($\underline{xy}$) would be highly unstable. The mean frequency of discharge of cortical neurones remains constant for long periods of time and is not readily altered by afferent excitation (Burns, Heron & Pritchard, 1962; Burns & Smith, 1962; Bindman, Lippold & Redfearn, 1964); ideally, postulated properties for central synaptic junctions should account for this stability of behaviour (Burns, 1968). Those properties of synapses that would be satisfactory in this respect will be discussed by A. M. Uttley (in preparation). From this point of view, the properties described by the constants we have fitted to equations (1) or (2) are attractive; universal properties of this sort would lead to a stable nervous system.

Many different synaptic mechanisms have been postulated as possibly responsible for the changes of conductivity that must underly learning. For instance, Eccles & MacIntyre (1953) suggested that swelling of afferent terminals might account for the long-lasting post-tetanic potentiation that they had recorded in spinal synapses and which they thought might provide an example of those changes in the upper nervous system that are responsible for learning. But the assumption that learning is the consequence of facilitation through repeated usage depends upon little more than the observation that repeated exposure to a conditioning stimulus is required for establishment of a conditioned reflex. It does not follow that individual synapses obey the rule that 'practice makes perfect'. Indeed, Young (1967) has suggested that during the repeated stimulation which one has come to associate with learning, there is a progressive increase in the number of conducting pathways, rather than an increase in the conductivity of individual synapses. In either case, it seems reasonable to find out what happens before speculating upon how it comes about.

The preliminary experiments we have described are too few in number to permit exact quantitative statements about the recorded plasticity of cortical synapses. This work is being continued and a more exhaustive enquiry along the same lines will be reported at a later date. Meanwhile, it should be pointed out that the changes of conductivity we are recording in

this sort of experiment may have little to do with learning by the intact nervous system. In this connexion, one would like to know whether they are vulnerable to the effects of light general anaesthesia (Summerfeld & Steinberg, 1957; Robson, Burns & Welt, 1960) and of spreading cortical depression (Bures & Buresova, 1956). One would also like to know whether a network of neurones with these junction properties, simulated upon a computer, would exhibit learning behaviour similar to that of animals.

This work was partly supported by a grant to B.D.B. from the Canadian Medical Research Council, to whom we wish to express our thanks. Many of the experiments were carried out while T.V.P.B. and B.D.B. were working at the National Physical Laboratory; they would like to thank the Director for his hospitality. Thanks are also due to the Carnegie Institute of Washington which provided B.D.B. with a travelling fellowship and thereby made our collaboration possible.

APPENDIX

By A. M. Uttley

Analysis of results

One purpose of the experiments described above was to test the proposition that present conductivity, C, of a cortical pathway is some function of the past history of x (the input frequency to the pathway), of y (the output frequency of the recorded cell) and $\underline{xy}$ (the frequency of output signals 'caused by' the input); i.e. in the steady state, with x, y and $\underline{xy}$ constant, C settles at a value given by a relation of the form

$$C = f(x, y, \underline{xy}).$$

Seeking the simplest possible equation, we assume that the effects upon C of the three independent variables are independent of one another, and write a simpler equation

$$C = f_1(x) + f_2(y) + f_3(\underline{xy}).$$

It was not possible, however, to make a change in, say, x, and wait until C had reached a new steady value. This would have taken a long time during which experimental conditions might have changed. Instead, a change in x was made for a short period t_0 and a change in C was observed. We therefore differentiate the above equation with respect to time and seek a relation between increments of the form

$$\frac{\Delta C}{t_0} = f_1'(x)\,\Delta x + f_2'(y)\,\Delta y + f_3'(\underline{xy})\,\underline{\Delta xy} \qquad (1)$$

One simple solution would be to find that the three functions of the variables in this equation could be replaced by three constants F_1, F_2 and F_3, which were consistent with all our experimental results; a satis-

factorily descriptive equation would then be of the form

$$\frac{\Delta C}{t_0} = F_1\Delta x + F_2\Delta y + F_3\Delta \underline{xy}. \tag{2}$$

Another simple hypothesis worth testing would be that the effects of the three variables upon conductivity depended upon the proportional change in these variables, in which case $f_1'(x)$ of equation (1) would be equal to G_1/x where G_1 is a constant, and similarly for y and $\underline{xy}$. We should then have an equation of the form

$$\frac{\Delta C}{t_0} = G_1\frac{\Delta x}{x} + G_2\frac{\Delta y}{y} + G_3\frac{\Delta \underline{xy}}{\underline{xy}}. \tag{3}$$

For these reasons we attempted to find parameters for a single equation of the form of equation (2) or of equation (3), which fitted all the experimental results summarized in Table 1. But, since the quantity C, as measured in our experiments, was dependent upon a number of unknown factors such as the number of synapses involved, and their state at the beginning of an experiment, we introduced into the left hand side of equations (2) and (3) a factor K which might vary from experiment to experiment and which included t_0, the duration of conditioning. These two equations may then be written

$$K\Delta C = F_1\Delta x + F_2\Delta y + F_3\Delta \underline{xy}, \tag{2a}$$

$$K\Delta C = G_1\frac{\Delta x}{x} + G_2\frac{\Delta y}{y} + G_3\frac{\Delta \underline{xy}}{\underline{xy}}. \tag{3a}$$

We then went on to test whether our results could be described by either of these equations ($2a$ or $3a$), in which the coefficients on the right-hand side remained the same in all experiments, while K might vary from experiment to experiment. This device permitted us to consider only the ratios between the three constants on the right-hand side of the equations and these could then be represented graphically (as in a colour triangle) by means of three areal co-ordinates, whose sum is unity (Milne, 1924).

The areal co-ordinate (x, y, z) of a point P in relation to a reference triangle XYZ are the areas of triangles x, y and z in Appendix Fig. 1, expressed as fractions of the area of triangle XYZ. The co-ordinate x is taken as positive if P is on the same side of the side YZ as is the point X. It can be shown that the equation

$$lx + my + nz = 0 \tag{4a}$$

represents a straight line: and

$$lx + my + nz = p, \tag{4b}$$

where p is a positive constant, represents the statement that the point (x, y, z) is on the positive side of that line.

The line $(4a)$ can be drawn from a knowledge of its intercepts with the sides of the triangle; for example, its intercept Q with the line YZ is found by making $x = 0$, whence

$$my + nz = 0$$

so

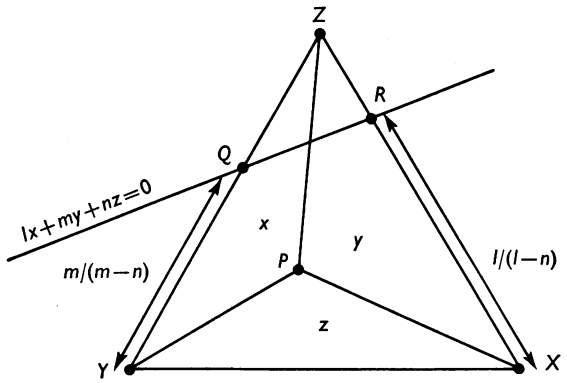
$$\frac{ZQ}{QY} = \frac{n}{m},$$

whence

$$\frac{QY}{ZY} = \frac{m}{m-n},$$

similarly

$$\frac{RX}{ZX} = \frac{l}{l-n}.$$



Appendix Fig. 1. The use of areal co-ordinates. See text.

Consider now the representation of equation $(2a)$. The quantities F_1, F_2 and F_3 are unknown so that they replace the quantities x, y, and z of equation $(4b)$. The quantities Δx, Δy, Δxy and ΔC are measured in the experiment so they replace the coefficients l, m, n and p, of equation $(4b)$.

The measurements of Δx, Δy, Δxy, of each experiment will be completely represented by a straight line of the form

$$lF_1 + mF_2 + nF_3 = 0,$$

while the sign of ΔC will indicate on which side of this line the unknown point (F_1, F_2, F_3) lies. Only the absolute value of ΔC is not used.

If an area can be found which is on the correct side of every line, then any point (F_1, F_2, F_3) within that area will satisfy the theory that the ratios F_1, F_2, F_3, remain constant from experiment to experiment. Furthermore, these ratios will have been found. If such an area cannot be found, the assumptions of equation $(2a)$ will have been disproved.

From preliminary calculations for equation ($2a$) it was found that F_1 was negative, F_2 was positive, and F_3 was negative, so equation ($2a$) was rewritten as

$$-l(-F_1)+mF_2-n(-F_3) = 0.$$

This ensured that the point ($-F_1$, F_2, $-F_3$) came within the triangle of Fig. 7 which shows the results of thirty-eight experiments. Figure 8 was derived in a similar manner.

REFERENCES

BINDMAN, L. J., LIPPOLD, O. C. J. & REDFEARN, J. W. T. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *J. Physiol.* **172**, 369–382.

BROWN, G. L. & EULER, U. S. v. (1938). The after effects of a tetanus on mammalian muscle. *J. Physiol.* **93**, 39–60.

BURES, J. & BURESOVA, D. (1956). A study on the metabolic nature and physiological manifestations of the spreading EEG depression of Leao. *Physiologia bohemoslov.* **5**, 4–6.

BURNS, B. DELISLE (1951). Some properties of isolated cerebral cortex in the unanaesthetized cat. *J. Physiol.* **112**, 156–175.

BURNS, B. DELISLE (1954). The production of afterbursts in isolated unanaesthetized cerebral cortex. *J. Physiol.* **125**, 427–446.

BURNS, B. DELISLE (1958). *The Mammalian Cerebral Cortex*, pp. 119. London: Edward Arnold.

BURNS, B. DELISLE (1961). Use of extracellular microelectrodes. *Meth. med. Res.* **9**, 354–380.

BURNS, B. DELISLE (1968). *The Uncertain Nervous System*. London: Edward Arnold. (In the Press.)

BURNS, B. DELISLE, FERCH, W. & MANDL, G. (1965). A neurophysiological computer. *Electron. Engng* **37**, 20–24.

BURNS, B. DELISLE & GRAFSTEIN, B. (1952). The function and structure of some neurones in the cat's cerebral cortex. *J. Physiol.* **118**, 412–433.

BURNS, B. DELISLE, HERON, W. & PRITCHARD, R. (1962). Physiological excitation of visual cortex in cat's unanaesthetized isolated forebrain. *J. Neurophysiol.* **25**, 165–181.

BURNS, B. DELISLE & SMITH, G. K. (1962). Transmission of information in the unanaesthetized cat's isolated forebrain. *J. Physiol.* **164**, 238–251.

ECCLES, J. C. (1953). *The Neurophysiological Basis of Mind*, pp. 314. Oxford: Clarendon Press.

ECCLES, J. C. (1964). *The Physiology of Synapses*, pp. 316. Germany: Springer-Verlag.

ECCLES, J. C. & MCINTYRE, A. K. (1953). The effects of disuse and of activity on mammalian spinal reflexes. *J. Physiol.* **121**, 492–516.

FATT, P. & KATZ, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117**, 109–128.

HEBB, D. O. (1949). *The Organization of Behaviour*, pp. 335. New York: John Wiley.

LARRABEE, M. G. & BRONK, D. W. (1938). Persistent discharge from sympathetic ganglion cells following preganglionic stimulation. *Proc. Soc. exp. Biol. Med.* **38**, 921–922.

LASHLEY, K. S. (1950). Physiological mechanisms in animal behaviour. In search of the engram. *Symp. Soc. exp. Biol.* **4**, 454–483.

LI, CHOH-LUH (1961). Cortical intracellular synaptic potentials. *J. cell. comp. Physiol.* **58**, 153–167.

MILNE, W. P. (1924). *Homogenous Coordinates*, pp. 164. London: Edward Arnold.

MILNER, BRENDA (1962). Les troubles de la memoire accompagnant des lesions hippocampes bilaterales. *Physiologie de l'hippocampe*, pp. 257–272. Paris: C.N.R.S.

MILNER, P. (1957). The cell assembly: Mark II. *Psychol. Rev.* **64**, 242–252.

PAVLOV, I. (1927). *Conditioned Reflexes*, pp. 430. London: Milford.

ROBSON, J. G., BURNS, B. DELISLE & WELT, P. J. L. (1960). The effect of inhaling dilute nitrous oxide upon recent memory and time estimation. *Can. Anaesth. Soc. J.* **7**, 399–410.

SHARPLESS, S. K. (1964). Reorganization of function in the nervous system—use and disuse. *A. Rev. Physiol.* **26**, 357–388.

SMITH, G. K. & SMITH, D. R. (1965). A statistical analysis of the continual activity of single cortical neurones in the cat unanaesthetized isolated forebrain. *Biophys. J.* **5**, 47–74.

SUMMERFELD, A. & STEINBERG, H. (1957). Reducing interference in forgetting. *Q. Jl exp. Psychol.* **9**, 146–154.

UTTLEY, A. M. (1956). A theory of the mechanism of learning based on the computation of conditional probabilities. *Proc. 1st Int. Congr. on Cybernetics. Namur.* Paris: Gauthier-Villars.

YOUNG, J. Z. (1967). *The Memory System of the Brain*, pp. 128. Oxford: University Press.