COMMUNICATIONAL ASPECTS OF NEURONAL CIRCUITRY IN THE CEREBELLAR CORTEX OF THE ALLIGATOR.

by

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PREFACE

The studies leading to this thesis were commenced in October 1965 in the Department of Communication at the University of Keele under the supervision of Professor D.M. MacKay. In September 1967, with the permission of the senate of the University and at the invitation of Sir John C. Eccles, I went to the Institute for Biomedical Research of the American Medical Association in Chicago to become acquainted with experimental techniques in the laboratory of Dr. R.R. Llinás.

Chapters I and V and Appendix B owe much of their content to ideas which I originated at Keele and which I subsequently developed and modified in the light of experimentation in Chicago. Chapters II, III and IV describe the experimental work carried out in Chicago as part of a programme of comparative studies

initiated by Dr. Llinás. The majority of the experiments were executed in collaboration with Dr. Llinas; during the course of the work I have carried out, however, at some time, all the experimental techniques described in this thesis.

A preliminary note describing the main findings reported in Chapters II and III has already appeared as:

> Dendritic spikes and their inhibition in alligator Purkinje cells. By R. Llinás, C. Nicholson, J.A. Freeman and D.E. Hillman. Science 160: 1132-1135 (1968).

A further note detailing the main findings of Chapter IV is in press under the title:

> Preferred centripetal conduction of dendritic spikes in alligator Purkinje cells. By R. Llinás, C. Nicholson and W. Precht. (To appear in Science)

During the period of the studies reported here I also visited the laboratory of Dr. R. Nieuwenhuys at the Netherlands Central Institute for Brain Research, Amsterdam, which increased

my appreciation of comparative studies and

resulted in the note:

> Cerebellum of Mormyrids. By R. Nieuwenhuys and C. Nicholson. Nature 215: 764-76 (1967).

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I wish to sincerely thank Professor D.M. MacKay for his guidance, inspiration and wholehearted encouragement throughout the course of these studies; Sir John C. Eccles for his generous invitation to the Institute for Biomedical Research in Chicago and Dr. R.R. Llinás for the constant benefit of his experience and instruction and above all for giving me the opportunity for fruitful and satisfying collaboration in a wide range of cerebellar research.

I am also indebted to Dr. D.E. Hillman for the use of his excellent histological material and to Dr. J.A. Freeman for initiation into many principles of neurosurgery and electronics, and much valuable discussion.

Finally the work would not have been completed without the thoughtful and efficient technical assistance of Miss S.A. Stopak, the typing of Mrs. J. Nakashima and the photographic

work of Mr. J. Obregon.

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CHAPTER I

CEREBELLAR RESEARCH, COMPARATIVE STUDIES AND THE SIGNIFICANCE OF THE ALLIGATOR.

The cerebellum has long been studied, but perhaps never so actively as at the present time. Despite this it is not known what the cerebellum does nor how it does it.

This chapter will begin with a brief review of the major landmarks in cerebellar studies followed by a discussion of some important problems in cerebellar physiology leading to an explanation of the reasons for the research presented in this thesis.

1.1 The Pattern of Discovery in Cerebellar Research

The current resurgence of interest in the cerebellum has led to several reviews covering all aspects of the literature. In particular, the anatomy of higher animals has been reviewed by Jansen and Brodal (1954) and the lower animals treated by Larsell (1967) and Nieuwenhuys (1967). Dow and Moruzzi (1958) covered all aspects of cerebellar physiology and clinical pathology up to that time, Brookhart (1960) again reviewed the physiology

and Eccles, Ito and Szentágothai (1967) dealt exhaustively with the more recent cerebellar histology and physiology to which they themselves had been major contributors. Fadiga and Pupilli (1964) reviewed the teleceptive aspect of cerebellar physiology and Oscarsson (1965) gave a comprehensive description of the spino-cerebellar system. Finally, Bell and Dow (1967) gave a short survey of our current knowledge of the whole cerebellar system. No attempt will be made to duplicate this work here but the main contributions to cerebellar research will be briefly mentioned below to give perspective to the discussion which follows. The anatomical literature relevant to the topic of this thesis is reviewed in detail in Appendix A.

In the anatomical field, the histological studies of Ramón y Cajal, published in the French translation in 1911, represent one of the greatest contributions to cerebellar studies and it is to Cajal that we owe the elucidation of the fundamental neuronal circuitry of the cerebellar cortex. No major addition to cerebellar histology was made until contemporary workers such as Fox and Szentágo-

thai used the electron microscope to confirm and amplify the details of synaptic connections.

Progress in systematisation of the gross anatomy of the cerebellum was slow and ambiguous so long as attention was devoted to the complex human form. The basic structure became clearer with the commencement of comparative studies by Herrick in 1891; this approach was quickly taken up by such workers as Johnston, Stroud, Smith, Bradley, Bolk and Ingvar. Finally, with the advent of Larsell, who first published in 1920 and subsequently devoted a lifetime to the comparative anatomy of the cerebellum, the underlying pattern of cerebellar morphology became clear and a generally acceptable cerebellar nomenclature evolved.

The afferent and efferent systems of the cerebellum became known as more knowledge accumulated about the brain, but this information improved in accuracy and usefulness with the introduction of degeneration and electrical stimulation techniques. Most notable in this field are the long continued studies of the Norwegian school under Jansen and Brodal and the contributions of Oscarsson and his

collaborators. In general, cerebellar connections revealed a rich and intricate relationship with neural centres closely related to motor control.

Progress in understanding the physiology of the cerebellum has become steadily more dependent on the introduction of new techniques. Speculation and dogmatism about the function of the cerebellum began with the scientific schools of ancient Greece and continued until the commencement of experimental studies using ablation techniques. Using such methods, Rolando showed in 1809 that the cerebellum influenced the motor system of the animal; in 1822 Flourens demonstrated a cerebellar role in the co-ordination of movement and showed that animals could, with time, recover from the effects of limited cerebellar ablation. Further results were obtained with this technique by such workers as Fodera and Magendie but the most important ablation studies were those of Luciani, published in 1891, reporting many careful experiments on chronic preparations. By the end of the nineteenth century, the technique of pure ablation was giving way to electrical stimulation, although Rademaker in 1931 published

important ablation studies on the cerebellar influence in reflex movements. The information gained by the techniques was supplemented considerably by clinical studies on patients with cerebellar disorders, but the value of such work was always limited by the lack of precision in the clinical tests and in the knowledge of the localisation of the lesion.

One of the first and most important discoveries using electrical stimulation was made simultaneously by Lowenthal and Horsley and by Sherrington in 1897. These workers discovered that decerebrate rigidity could be abolished by stimulating the cerebellum. Following electrical stimulation came recording with electrodes on the surface of the cerebellum. Using this method Adrian in 1935 found the now characteristic high frequency activity of the cerebellum and in 1944 a similar recording technique led Snider and Stowell to make the unexpected discovery that there were visual and auditory inputs to the cerebellum. With the advent of the microelectrode it became possible to record from either small collections of cerebellar neurones or single elements

and such techniques were first exploited by Brookhart, Moruzzi and Snider in 1950, followed by Buser and Rouguel in 1954 and an extensive study by Granit and Phillips, published in 1956 and 1957. The full potential of this technique, particularly the close correlation with Cajal's histology, were not fully exploited, however, until Eccles and his collaborators began their prodigious studies, first published in 1963 and continuing with increasing momentum ever since. These studies extended our knowledge of single neurones in general and identified each type of synapse within the cerebellar cortex as either excitatory or inhibitory. Finally, Ito demonstrated that the cortical efferent system, the Purkinje cell axons, formed inhibitory synapses on their target nuclei.

1.2 Current Status and Problems in Cerebellar Research. The great distinguishing feature of animals is that they move about. Movement requires that the limbs, and in particular the muscle groups associated with the limbs and body operate in certain sequences whilst those which are not operating maintain a degree of rigidity or tone so as to

provide a platform with which the active muscles can react. Several parts of the animal must be in motion at the same time so that muscle groups must be coordinated. Finally, if the animal is to move, it must create a reaction with some/ stationary medium so that muscles must exert varying amounts of force. All this results in setting the animal in motion, but motion is subject to the constraint that the animal maintains its equilibrium.

Movement is seen to be a complex operation and the components sequence, tone, coordination, force and maintenance of equilibrium are some of the elements into which it may be analyzed. The wealth of experiments with ablation and gross stimulation together with years of clinical experience have led to the conclusion that the cerebellum is intimately concerned with movement, but does not initiate action. Instead it modulates activity initiated elsewhere in the central nervous system.

The older researchers asked which elements of movements were mediated or modified by the cerebellum and they sought to describe cerebellar function

in a vocabulary related to the gross behaviour of the animal or its muscle groups. This may be called the macroscopic viewpoint. All the experimental methods which have sought to provide a description of cerebellar function in macroscopic terms have failed to provide any clear cut concept of what the cerebellum does. The reasons for this are probably twofold. Firstly, the methods themselves lack precision; for example, ablation, the principal technique merely enables one to say what the animal can do in the absence of the ablated region of its nervous system; it does not enable one to conclude that any missing function resided in the ablated region. A second reason for the lack of progress with macroscopic methods is that the cerebellum is functionally deep in the nervous system. It has a multiplicity of afferents and efferents almost all of which go to other structures of the central nervous system and not directly to the periphery. Thus, any activity which is observed at the periphery of the animal is, at best, likely to be represented in the cerebellum in a form con-

siderably modified by the interplay of other neural centres and in a way which may defy any simple form of decomposition.

The advent of the recording micro-electrode changed the whole perspective of cerebellar studies. It is true that the light microscopy of Cajal had provided a detailed picture of the neuronal elements of the cerebellum by the early part of the twentieth century, but apart from Cajal himself there was little enthusiasm for speculation on its significance before the electrical activity of these elements could be directly recorded. With the use of the micro-electrode, the structural regularity of the cerebellum could be exploited to the full and each neural element could be characterised as excitatory or inhibitory. Investigators quickly saw an analogy between electronic circuits and the "cerebellar circuit" consisting of a characteristic system of interconnected neurones which was duplicated throughout the cerebellum. The single neurone was taken as the basic descriptive element and interactions were described in the vocabulary

of electrical phenomena; this may be called the microscopic viewpoint. These developments led to an important question: whether or not an understanding of the nature of the individual cerebellar elements together with a knowledge of the connections of the elements and experience with possibly analogous man-made systems, particularly those designed for information processing, would enable the mode of operation of the cerebellar cortex to be deduced. The answer to this question would seem to be no for the following reasons. All information processing systems designed and analysed by man presuppose a knowledge of the information to be processed. With the cerebellum, we have no clue as to what information it is designed to operate on. This point should be amplified.

Our microscopic description of the cerebellum is in terms of the electrical interaction of neuronal elements. At this level, information would consist of a spatio-temporal pattern of electrical impulses travelling along the afferent and efferent pathways. The spatial elements would be the pat-

tern of fibres activated and the temporal element the sequence of nervous impulses in any particular fibre. The function of the cerebellum might then be described in terms of the transformation which it would perform on afferent patterns of electrical activity to convert them into the efferent patterns. Such a description of cerebellar function would admit of no immediate interpretation at the macroscopic level. If one wished, as one surely does, to relate the observed activity of the animal to the neural function of the cerebellum, it would be mandatory to understand the coding of macroscopic behaviour into patterns of microscopic electrical activity. Unfortunately, as previously pointed out, almost all information arising at the periphery of the animal passes through other centres of the central nervous system before reaching the cerebellum and each of the centres will doubtless transform the information in ways of which we have no knowledge at the present time.

One approach to this problem is to look for the receptor and effector systems which have a

monosynaptic connection with the cerebellum. In such a system, there is the possibility of correlating macroscopic and microscopic description. If one can then make the assumption that morphological similarity implies functional similarity within the cerebellar cortex, an understanding of cerebellar function gained with monosynaptic systems can be extended to other more complex afferent and efferent pathways. A second fact implicit in this approach is that adequate natural stimuli be used. The nervous system must be assumed to be capable of highly complex patterns of behaviour and this makes it impossible to predict how the system would react to adequate stimuli given only the results of physiological stimulation. Physiological stimulation is, however, of value in characterising different systems and combined with adequate stimuli becomes a powerful tool for analysis.

1.3 Comparative Cerebellar Studies. The cerebellum of the cat has received by far the most attention from both anatomists and physiologists, but, it is like the animal itself, a highly evolved struc-

ture and indeed there is little difference histologically between the cerebellum of a cat and the cerebellum of man. It can, therefore, be asked what one might gain from studying a sequence of more primitive animals which represent, to some extent at least, earlier phases in evolution.

1.3-1 Questions to Be Answered by Comparative Studies. One might find answers to the following questions: a) Are certain elements present throughout the evolution of the cerebellum whilst others appear only in the more advanced forms? If this were true, then one could concentrate initially on understanding the basic cerebellum. b) Does morphological similarity imply functional similarity? This question is particularly relevant at the neuronal level, together with the question of how much variation is necessary before a functional change takes place. c) At particular stages of evolution, did some animals acquire or lose certain functions which made special demands on the cerebellum? If so, then such animals might provide valuable preparations for cerebellar research.

Examining a variety of animals with the above questions in mind might also lead to the discovery of a preparation or group of preparations better suited to solving current cerebellar problems than the animals presently in use.

In attempting to retrace evolution by a study of living animals, some points should be kept in mind. Firstly, in the majority of cases, it is the least specialised representative of a group of animals which form the "main stem" of evolution but which almost always disappear when that group loses its dominance. The animals that survive are related forms which are highly adapted to some way of life which preserves them from the predators that have destroyed the more representative animals. This specialisation can lead to degeneration of features which are no longer required for the restricted way of life and the development of other features which aid survival. This raises the second point; that once a group of animals has branched off from the main stream of evolution, it may evolve independently and in parallel and by this process come to acquire features which resemble those found in other animals. There is reason to believe that

both specialisation and parallel evolution have played their part in producing the wide spectrum of cerebella available for study and this will be discussed in more detail in Chapter V.

1.3-2 The Phylogenetic Position of Different Animals and Their Cerebella with Special Reference to the Alligator. In order to explain why the alligator was chosen for study, it is necessary to recall how it and its cerebellum are related to other animal forms.

Although /the external appearance of the cerebellum undergoes considerable changes as one ascends the phylogenetic scale, the histology remains surprisingly constant with a number of basic features such as the three characteristic layers, molecular, Purkinje and granular, the mossy and climbing fibres, the basic morphology of the Purkinje cells and perhaps most important the characteristic geometrical relationship between the processes of cerebellar elements. On the other hand, the inhibitory interneurones seem to change with phylogeny. They may, however, play a secondary role in cerebellar func-

tion and this will be discussed in Chapter V. This provides a reason for supposing that the cerebellum may function in a similar way at the microscopic level in all vertebrates. On the other hand, the macroscopic role of the cerebellum may change substantially with phylogeny, although continuing to act predominantly in the motor sphere, since in lower aquatic animals it seems to have a substantial part to play in connection with the acoustico-lateral system whilst in higher animals expansion is related to the development of the cerebral cortices. As Eccles (1965) has pointed out it seems that as the brain has extended its range of functions, so the cerebellum has been utilised for an increasing range of purposes, whilst probably still retaining its basic mode of action.

Turning now to the animals themselves, and following the classifications described by Romer (1959), Figure 1 illustrates the phylogenetic relationship between those animals which have a significant cerebellum. Only the cyclostomes, the ancestors of the fish have been omitted since their

cerebellum is little more than a speck of tissue. The most ancient animals are the fish and amongst them the elasmobranchs or cartilaginous fish are the most primitive. Modern representatives fall into three classes, the sharks, skates and rays. After the cartilaginous fish come the bony fish which may be divided into the ray-fins (Actinopterygians) comprising the vast majority of all living fish and the lobe-fins (Sarcopterygians) represented now only by the lung fish and coelocanth. It is from this latter group that all higher animals developed. The variety amongst fish is astonishing and this variety is reflected in the shapes and sizes of the cerebella. In these animals, the cerebellum is linked with the acoustico-lateral system as well as the motor-system and in some fish which have very large cerebella (elasmobranchs, mormyrids, gymnotids and silurids) the cerebellum may be part of an electro-sensory system (Llinás, Nicholson and Nicholson, 1967). Bullock, unpublished observations; Nieuwenhuys and /

Following the fish come the amphibia. These have been relatively unsuccessful as a group of ani-

mals, being too constrained by the necessity of spending part of their life in the water and part on land, but lacking the specialisation necessary to fare well in both environments against increasingly efficient predators. Today only three groups remain, the anurans--frogs and toads--having a highly specialised mode of locomotion, the urodeles--newts and salamanders--probably the least specialised amphibians, and the apodians, rare and degenerate worm like forms. In all amphibians, the cerebellum is rather small and recently it has been shown that the frog cerebellum has virtually no inhibitory interneurones (Llinás and Bloedel, 1967), thus it is the simplest cerebellum yet found; apart from the cyclostomes.

After the amphibians came the reptiles and from the reptiles sprang three groups of animals, the dinosaurs, the birds and the mammals. The most primitive representatives of the reptiles proper are the turtles and related forms which were virtually "petrified" from an evolutionary point of view in their thick shells; they were as usual, a

rather specialised branch of the reptiles. Somewhat later the lizards developed and one archaic lizard, sphenodon, survives in geographical isolation in New Zealand, otherwise lizards have developed substantially from their prototypes, whilst still remaining fairly representative reptiles. From lizards arose the more specialised snakes which have proved very successful. Marine reptiles also existed at one time, but of these no survivors remain; nor can any representative now be found of the reptilian branch which eventually developed into mammals. The final branch of the reptilian radiation were the ruling reptiles, the animals which became the dinosaurs and dominated the earth, the reason for their demise still remaining a mystery. An early development amongst the ruling reptile branch were the crocodiles, somewhat degenerate in their four-footed gait (primitive dinosaurs were bipeds), but nevertheless not highly specialised. Today they are the sole survivors of the ruling reptiles. From this branch of the reptiles, however, there also developed the birds which will be considered later. The

the
cerebellum of/turtle is fairly simple whilst lizards and snakes show a considerable variation between species. The best developed cerebellum however is found amongst the crocodiles, where it is rather large and orderly (Its structure is fully treated in Appendix A).

The birds, although originating as relatives of the dinosaurs came to parallel mammals with their regulated blood temperature and efficient circulation. Birds are of course highly specialized for their peculiar mode of life but despite the enormous variety apparent in this group of animals, all birds share rather similar basic features. It is probable that the flightless birds, such as the ostrich and kiwi, are amongst the most primitive, but even these do not differ too greatly from the most advanced. The cerebellum is quite complex with many lobes and fissures, the number of lobules increasing with the size of the bird.

Finally, we come to mammals,and it is impossible to do justice to this vast collection of animals in a few sentences, but the main groups may be mentioned. Most primitive are the monotremes (duck bill and

spiny anteater) and the marsupials. The majority of these animals have been preserved solely by geographical isolation. From these one passes via the early insectivors (of which the shrew is a living, if developed, representative) to the rodents, carnivores, subungulates, ungulates, edentates, cetacea and primates to mention the more numerous groups.

In all mammals the cerebellum is a complex folded structure reaching its greatest complexity in the primates, but having a considerable similarity from mammal to mammal.

It is apparent from this brief survey that the crocodiles occupy a very interesting place in evolution at a point linking together amphibia, reptiles, dinosaurs, birds and mammals. The cerebellum is in gross morphology very much simpler than that found in any mammals but histologically it is not far enough removed to make comparisons difficult. On the other hand, there is sufficient difference in the cyto-architecture, notably in the morphology of the Purkinje cell dendrites and the

absence of basket cells,to make the crocodiles a worthwhile preparation for seeking functional variation. The present work has been limited to the use of physiological stimuli similar to those used by Eccles and co-workers in the cat. The aim has been to use these techniques to characterise the responses of the neural elements in a representative of the crocodilians (the South American alligator, Caiman Sklerops) so that by comparison and contrast with those of the cat and other animals, where possible some insight may be gained into modes of interneuronal communication in the cerebellar cortex in general. In particular, the findings and conclusions presented here provide new information on the way in which the Purkinje cell can function. Chapters II, III and IV present the results of the physiological experiments. Chapter V discusses these results from the point of view of neurocommunications. In particular the role of Purkinje cell dendrites is explored in the light of the experimental findings and a mathematical analysis of branched dendritic systems developed in detail

in Appendix B. Chapter V also discusses the roles of inhibition in the cerebellar cortex and compares the mossy and climbing inputs. In order to facilitate comparison of the cerebellar physiology of the cat and alligator, Appendix A contains a comprehensive survey of the literature on the cerebellar anatomy of the alligator and relates it to the cat.

CHAPTER II

PARALLEL FIBRE FIELDS AND SPIKE ACTIVITY IN THE DENDRITES OF PURKINJE CELLS

This chapter and the next two present the results of physiological experiments on the cerebellar cortex of the South American alligator Caiman sclerops using micropipette recording techniques. The results are representative of experiments on some 30 animals. The anatomy of the cerebellum is reviewed in Appendix A and Figures 22, 27-33 summarise the gross morphology and histology.

Methods

The animals weighed between 150 g and 2000 g and were anaesthetized with an intraperitoneal injection of between 10 and 20 mg/kg of sodium thiopental (Pentothal, Abbott). In some experiments the animals were lightly anaesthetized, artificially ventilated via a tracheal cannula, using compressed air regulated by a respirator, and then paralysed with gallamine triethoiodide (Flax-

edil, Davis and Geck) administered intraperitoneally with a dosage of between 25 and 50 mg/kg.

The head of the animal was clamped in a stereotaxic head holder fitted with a rabbit adapter (David Kopf), the whole assembly being mounted on a Canberra type spinal frame with the body of the animal supported on foam plastic blocks.

The dura mater covering the cerebellum was exposed by a craniotomy. Embedded within the dura were sizable medial and lateral venous sinuses which often had to be removed. In such cases, the sinus was injected with Vaseline before cutting it; electric coagulation was also used on some occasions. Any subsequent bleeding, particularly from small vessels associated with the lateral vascular system, was stopped by the application of oxidised cellulose (Oxycell, Parke-Davis) or thrombin (Parke-Davis). After opening the dura with fine forceps, the arachnoid was teased away and the dorsal aspect of the medial and often the posterior lobule revealed. Occasionally after surgery the circulation appeared sluggish and in such cases the animal

was given an injection of Metaraminol bitartrate (Armine, Merck, Short and Dohme). If there was any prospect of the cerebellar surface drying, it was covered with either Ringer solution, balanced as for the frog, or mineral oil.

Stimulating electrodes were of the bipolar type consisting of two strands of twisted steel wire, diameter 120μ, insulated to the tip and shielded to within 1 cm of the ends. These electrodes were held in miniature micromanipulators (Narishige type MM3) attached to the Canberra frame. For surface stimulation (Loc stimulation) the tips of the electrodes were placed on the pia mater of the cerebellum or more usually on the surface of the cortex itself, after a small hole had been made in the pia. This latter procedure substantially reduced the stimulus artifact. For stimulation of the cerebellar white matter (WM stimulation) a bipolar electrode was inserted into the rostral-most part of the cerebellum and pushed down until characteristic antidromic re-

sponses were obtained with the recording electrode (See Chapter IV). The arrangement of the stimulating electrodes is shown in Figure 2. Stimuli were delivered to the bipolar electrodes via isolation units (Grass SIU 478A or Bioelectric ISA) fed from either a Grass S8 stimulator or from a set of Tektronix 161 pulse generators controlled by a programmable digital delay and sequencing device designed and built by Dr. J.A. Freeman.

For recording extracellular field potentials, micropipettes filled with 4M NaCl were used with a tip resistance in the range 1-5 Megohms. For intracellular recording, the micropipettes were filled with 3M KCl and had a tip resistance of 8-15 Megohms. The recording electrodes were mounted on single, double or triple electrode holders (Narishige HMD-2, HMT-3).

In some experiments, a fourth microelectrode was cemented to one of the lateral electrodes in the triple electrode carrier and such a configuration is shown, diagrammatically, in Figure 2. The electrode / carrier was held in a Canberra-type micro drive (Narishige

ME7) and the recording electrodes fed either a negative capacity bridge amplifier (Rockefeller Model 65 NACF 5, SER) or, for multiple electrode recording, up to four separate negative capacity field effect transistor headstages (Bioelectric NF1). The signal subsequently underwent two more stages of amplification using either a Tektronix 2A63 or 3A3 amplifier followed by a 3A74 four channel amplifier, the output of which was displayed on a Tektronix RM 565 dual beam oscilloscope. Records were photographed with a Grass Kymograph camera coupled to the oscilloscope via a reflexor housing (Bioelectric). In addition, signals were monitored on a large diplay oscilloscope and an audio monitor.

When required, waveforms were averaged on a Fabritek 1064 instrument computer having a 4096 word memory and limited arithmetic capabilities; the output of this instrument could also be displayed on the RM565 for photographic recording.

Calibration signals were generated either internally in the Rockefeller headstage or with a Bioelectric calibrator (Type CA5) inserted in the

ground lead to the animal when several micropipettes were in use. In addition, time marks were produced with a Tektronix time mark generator (Type 184). The animal was grounded with a large hypodermic needle inserted under the skin in the abdominal region.

Extracellular records were taken at various depths in the cerebellar cortex by firstly obtaining a control surface record, then driving the micropipette below the greatest depth required and then taking records as the electrode was withdrawn towards the surface, finally checking to ensure that the surface record did not differ substantially from the control. Intracellular records were usually obtained as the electrode descended into undamaged tissue.

In some instances, the pia mater of the cerebellar cortex was almost impenetrable to the microelectrode. In these cases, a solution of trypsin (Sigma) was applied locally for a short period to soften the membrane.

2.1 The Fields Evoked within the Cortex by Local Stimulation. A bipolar electrode was used to stimulate the surface of the cerebellum; this is termed local (Loc) stimulation. The resulting fields were recorded with a micropipette positioned so that it passed through the bundle of parallel fibres which were contacted by the stimulating electrode (Figure 2, recording electrode 1).

Characteristic fields are shown in Figures 3A, 3B and 4A. In Figure 3A several oscilloscope traces have been superimposed to show the almost total lack of variation of the field potentials evoked in this manner. Figures 3B and 4A show a record of 16 responses averaged with the instrument computer. The responses have the following characteristics. Superficially, following the stimulus, a fast positive-negative-positive wave is seen, the negative component of which is very pronounced. The final positivity of this wave is obscured in Figure 3, but clearly visible in Figure 4A. Figure 3A shows that by 300μ this triphasic component is wholly gone, whilst in

Figures 3B and 4A it is seen to be absent by 200μ. Following the triphasic wave there is a slower negativity which has a maximum value between 200μ and 300μ, but is still clearly visible at 500μ and it is even seen at a depth of 700μ in Figure 3A. It is convenient for the purposes of further analysis to call the first triphasic wave the A-component and the second, slower negative wave the B-component. It will be shown that these two components arise from different populations of neural elements. For the present, however, it may be noted that there is a very clear-cut way of operationally distinguishing between the two components. This is illustrated in Figure 4B where the Loc evoked field of Figure 4A has been preceded, at an interval of 20 msec, by a similar Loc stimulus applied through the same electrode. The resulting interaction leaves the A-component unchanged, but drastically reduces the B-component. An investigation of paired Loc stimuli will be presented in Chapter III.

2.1-1 The A-Component and its Identification with Direct Activation of Parallel Fibres.

Figure 5A shows a series of potentials recorded close to the surface of the cerebellum as the recording electrode was moved further and further away from the Loc electrode in the same direction as the parallel fibres. It is seen that the latency of the A-component becomes progressively longer as the distance between stimulating and recording electrodes increases. In Figure 5B the distance of the recording electrode from the periphery of the stimulating electrode is plotted as a function of the latency of the peak of the negativity for the records of Figure 5A and two other series of similar measurements. From this graph it is calculated that the peak propagates with a velocity of 0.18m/sec. It is also seen that the A-component diminishes and broadens with distance. From the evidence presented above, it is safe to conclude that the A-component is the field generated by a superficial bundle of parallel fibres activated by Loc stimulation.

The triphasic configuration agrees with the field for a synchronised volley of action potentials propagating in a fibre bundle immersed in a volume conductor (Lorente de Nó, 1947a; Clark and Plonsey, 1968). The disappearance of the wave in the lower part of the molecular layer indicates that only the superficial parallel fibres are activated. The slow conduction velocity of the compound action potential is entirely consistent with the very small diameter of these fibres. In the cat, a maximum velocity of 0.5m/sec was reported by Dow (1949) using a gross recording electrode and Eccles, Llinás and Sasaki (1966a), using techniques similar to those employed in the present experiments, found a conduction velocity of 0.3 to 0.39m/sec. The slower conduction found in the alligator may be accounted for by the fact that the alligator is a poikiotherm and thus is at the temperature of the laboratory (about 24°C), whilst the cat actively maintains a constant, higher temperature. The different conduction velocities found in the cat and alligator cannot be attributed to a difference in parallel fibre

diameter since the diameters of the superficial parallel fibres in the alligator lie between 0.1 and 0.25μ (Hillman, personal communication), and thus do not differ greatly from those of the cat which range from 0.2 to 0.3μ (Fox, Siegesmund and Dutta, 1964; Hámori and Szentágothai, 1964). On the other hand, in the frog, which is also a poikiotherm, the conduction velocity is about 0.2 m/sec (Llinás, Bloedel and Hillman, personal communication) but it is not known how the parallel fibre diameters compare with those found in the alligator.

The reduction in size of the A-component with distance is explicable if the parallel fibres do not extend from end to end of the cerebellum in the alligator and if there is velocity dispersion in fibres of different diameters. The extent of the parallel fibres has not been confirmed histologically in this animal so the diminution may be evidence that at least a substantial proportion of the fibres are considerably shorter than the maximum span of the lobule. A similar

situation holds for the cat where the parallel fibres are considerably shorter than the length of a typical folium. The velocity dispersion will also account for the broadening of the negativity, with distance from the stimulating electrode. No attempt has been made to measure the conduction velocity of the deeper parallel fibres, but since these appear to be of larger diameter than the superficial ones (as in the cat), the conduction velocity should be greater.

Finally, Figure 4B shows that the Loc evoked A-component is unaffected by a similar stimulus 20 msec beforehand. This is in complete agreement with the identification of the A-component with directly induced parallel fibre excitation.

2.1-2 The B-component and a Hypothesis for its Mode of Production. Since the B-component follows the A-component at a fixed latency (Figure 5A) it is reasonable to conclude that it is related to the A-component. Parallel fibres make synaptic contact with stellate and Golgi cell dendrites, but the overwhelmingly numerous synaptic contacts

are with Purkinje cells (Appendix A). It may thus be assumed that the B-component is related to post-synaptic events taking place predominantly in Purkinje cells. Since the B-component is clearly visible even in the most superficial regions of the molecular layer, it is safe to conclude also that it is mainly a dendritic phenomenon. Further consideration of the B-component demonstrates an interesting feature: Figures 3A and 3B show that the B-component becomes progressively later as it is measured at ever increasing depths. In Figure 3C, the depth of the recording electrode is plotted as a function of the latency of commencement of the negativity (leftmost points) and as a function of the latency of the peak of the negativity (rightmost points). From this it is clear that the negative wave may be regarded as propagating downward with a velocity of about 0.1m/sec in the first 300μ and 0.4m/sec thereafter. Downward propagation of the wave would seem to be the only mechanism possible since it has been shown that the activated beam of parallel fibres

is very superficial.

It is seen that nowhere within the soma-dendritic complex of the Purkinje cell does the B-component reverse to a positivity, suggesting that there is excitation of the Purkinje cell throughout its length. This latter point is especially noteworthy as it contrasts markedly with the situation found in the cat. In that animal a negativity is recorded in the superficial regions of the molecular layer at the same time as the B-component found in the alligator, but in the cat there is a clear-cut reversal of the negativity to a positivity below 100μ (Eccles, Llinás and Sasaki, 1966a; Eccles, Sasaki and Strata, 1966a). The explanation put forward by Eccles, Llinás and Sasaki for the superficial negativity found in the cat is that it is an extracellular manifestation of the EPSP evoked in the superficial dendritic regions of the Purkinje cell by the synaptic action of the parallel fibre volley. Lower regions of the Purkinje cell are regarded as acting in the role of passive current sources to the superficial sink of the synaptic activation, thus accounting for the

positivity. There would be no a priori reason to suppose that the alligator Purkinje cells should behave in a substantially different manner from those in the cat, especially as it has been shown by Llinás and Bloedel (1967) that the negativity and its reversal are present in the frog. Since, however, the response in the alligator is very different from that in the cat and frog, it is concluded that the B-component is not mainly an extracellularly recorded EPSP and the hypothesis is advanced that it is the result of action potentials within Purkinje cell dendrites induced by the parallel fibre activation. Such an explanation would account for both the propagation of the B-component from dendrite to soma and the failure to reverse at any point. This hypothesis requires careful testing and discussion, especially because dendrites can cause purely passive slowing of the EPSP (Appendix B, Rall, 1964, 1967; MacGregor, 1968).

These findings, and the results of other workers on dendritic spike potentials, will be discussed after more evidence has been presented in favour of the present hypothesis. All the records so far

presented were, of course, taken in animals in good physiological condition. Sometimes, however, when there was reason to believe that the condition of the cerebellum had deteriorated, the typical B-component was absent and fields resembling those of the cat, that is with a reversal of the superficial negativity, were seen. This again helps to distinguish the phenomenon in the alligator from that in the cat.

2.2 Extracellularly Recorded Dendritic Spike Potentials. Following Loc stimulation, sharp, negative all-or-none potentials were often recorded in the molecular layer. Examples of such potentials, recorded at a depth of 150μ, are shown in Figure 6B-E. In Figure 6A a weak Loc stimulus gives rise to the usual A and B components of the field, but, when the intensity is increased slightly (6B) a large sharp negativity sometimes arises from the B-component. This negativity is preceded by a small positive deflection, rises smoothly and rapidly to a peak and then falls to the baseline with a markedly notched profile. Figure 6C shows several records superimposed and both the all-or-none character and the reproduc-

ibility of the phenomenon are seen. In Figure 6E, the first Loc is followed 25 msec later by a second (control for second Loc is shown in Figure 6D). It is observed that in every case, except one, the second Loc fails to evoke/negativity. From this result it is concluded that the negativity is inhibited by a preceding Loc. These observations, taken in conjunction with those of Section 2.1-2 suggest that the negativity is caused by dendritic spikes generated closed to the tip of the micropipette. There are two reasons for postulating this mechanism. Firstly, the all-or-none nature of the negativity is indicative of an action potential. The positivity preceding the negativity indicates that the potential was generated at a small distance away from the recording position. Secondly, the demonstration of inhibition eliminates the possibility that the negativity was produced passively and postsynaptically, since under such circumstances the negativity would be enhanced by a preceding Loc stimulus, as shown in the cat (Eccles, Llinás and Sasaki, 1966a) and frog (Llinás and Bloedel, 1967). Presynaptic inhibition in the molecular layer can be excluded since there is no

morphological basis for such a mechanism, and much physiological evidence against one. It may be asked if the all-or-none negativity could arise in any element apart from the Purkinje cell. The only other source of such potentials in the molecular layer would be the stellate cells but these cells would not show inhibition following a preceding Loc; on the contrary, they should be facilitated (Eccles, Llinás and Sasaki, 1966d).

An interesting observation is the notched falling phase of the dendritic spike. This suggests that not just one, but several spikes are being recorded by the micropipette. These spikes could either be all recorded from the same locus, in which case they must follow each other in quick succession and the absolute refractory period of the site must be very short, or they are recorded from different sites in the same or even different dendrites. Since the spike waveform has a rather constant shape, it is likely that the generation of these spikes is not completely independent but that the production of one spike leads to the production of another. If the components of the spike waveform are not independent, then it is likely that the spikes arise in the same dendritic

tree and this hypothesis is strengthened by the all-or-none nature of the spike waveform complex.

Dendritic spikes were further investigated by using a pair of micrcpipettes, mounted in a double Narishige electrode holder (See Methods) and so adjusted that the tips of the electrodes were 200μ apart vertically with one electrode tip above the other. As the microelectrodes are moved through the molecular layer it is occasionally possible to record spike potentials on both electrodes simultaneously and sometimes these spikes have similar characeristics and are both evoked in an all-or-none manner by a Loc stimulus (Figures 7 and 8). On these occasions it is concluded that the spikes recorded on each microelectrode are related and belong to the same dendritic tree; for if not, the spikes should arise independently with different thresholds and varying latencies. It will be shown below, using the data in Figure 8, that the latency between the two spikes is fairly constant, although the instant of initiation is somewhat labile for a given stimulus strength.

In Figure 7, pairs of records are shown at various sweep speeds. In each case, the smaller record was obtained from the lower electrode but relative size is of no importance since it is not feasible to move each micropipette independently to maximise the potentials. In Fig. 7, the waveform pairs are all seen to be rather similar in shape and in some cases a second spike is visible on both records (Figure 7I, K and L). Similarly shaped inflections are seen on both the rising and falling edges of the potentials recorded from both pipettes in many cases (Figure 7D-J). This is further evidence that a spike complex is propagating from one recording site to the other. Thus both sites must be in the same dendritic tree.

Figure 8 shows another series of records taken in a similar manner to those above. The spikes recorded by the more superficial micropipette are particularly large and clear in this case. Records 8J, K, F and L show superimposed successive sweeps and a slight degree of lability in spike generation can be seen, but it is to be noted that the spike recorded on the lower electrode shows the same

lability characteristics as the upper one. At the fastest sweep speed (Figure 8, F-L) the negativity appears to have a smooth contour, without pronounced notches, suggesting that in this case it represents only a single spike. If this is so, then the width of the spike is large compared with those seen in soma-axonal recordings. Another unusual feature is the very small, and sometimes nonexistent, positivity preceding the negative spike. When recording from a position adjacent to the soma or axon, the initial positivity is almost always large (See Figure 17) because the spike usually does not originate directly beneath the microelectrode tip (since the tip will probably have raised the threshold of the adjacent membrane through contact) and therefore the spike propagates into the recording area. The present records indicate that the spike originates directly under the electrode and since this is found repeatedly it might imply that a fairly large region of membrane is simultaneously active. The width of the spike also suggests that a large area is active. It may even be that the membrane is composed of a mosaic

of active and passive regions which couple passively so that spikes do not move continuously.

As would be expected, there is a latency between the spikes recorded on the two electrodes. Figures 8M, N and O show histograms of the number of pairs of spikes at a given latency for a series of decreasing stimulus strengths. In M, the stimulus strength was such that the lower spike sometimes arose at or before the time of the upper one, suggesting that a large part of the dendritic tree was being activated at the same time. As the stimulus strength decreased (Figures 8N and O) the upper spikes tended to arise almost always before the lower ones, suggesting that only the more distal dendrites were synaptically activated. Figure 8P shows the composite histogram and the pronounced peak indicates that there is preferred latency, that is, the time for a spike to propagate in a dendrite from one recording site to the other has a rather constant value.

2.3 Discussion

The application of Loc stimulation to the surface of the cerebellum in the alligator produces a synchronised volley of action potentials in the parallel fibres which closely resemble those seen in the cat, under similar conditions. In one aspect the fields do differ: The lower body temperature of the alligator, compared to the cat, causes the conduction velocity of the parallel fibres in the former animal to be half that in the latter.

The postsynaptic events induced in Purkinje cells by the parallel fibre volley show a very significant departure from those seen in higher animals. The present findings are a) a large negativity follows the presynaptic volley and does not reverse to a positivity in depth, b) the negativity has a progressively increasing latency with depth, the velocity of propagation of the peak ranging from 0.1m/sec to 0.4m/sec, c) the negativity is abolished by a preceding Loc stimulation at an interval of about 20 msec, and d) a micropipette within the molecular layer often records sharp negative-going spikes which arise out of the

negativity. With just threshold stimulation, these spikes exhibit all-or-nothing behaviour and are often quite labile. A second Loc stimulus usually abolishes such spike activity.

From this evidence it is concluded that a Loc stimulus generates action potentials within the superficial dendrites of Purkinje cells. The abolition of Loc evoked spikes by a preceding Loc is entirely consistent with the action of the inhibitory stellate cells (See Chapter III). It remains to dispose of the argument that these potentials are purely passive phenomena; the arguments for their being passive are a) if a synaptic current is applied to the distal region of a "leaky cable", such as dendrite, it will produce a PSP whose peak value will occur at progressively later times as it is measured further and further from the point of initiation (Appendix B, Davis and Lorente de Nó, 1947; Rall, 1964, 1967; MacGregor, 1968; Harmon, Kado and Lewis, 1968; Calvin and Hellerstein, 1968). It has been claimed that this will cause the extracellular fields also to show a delayed peak (Calvin and Hellerstein, 1968) for a

purely passive spread of potential. b) The size of the PSP is a function of the impedance of the nerve process which in turn is inversely related to diameter (Katz and Thesleff, 1957; MacGregor, 1968). In fine nerve processes, such as dendrites, very large PSP's will therefore be observed in response to synaptic activation and these PSP's may be mistaken for spikes (MacGregor, 1968).

In reply to criticism a) it must first be pointed out that the PSP is the transmembrane potential, that is, the potential which would be measured by a micropipette within the cell and an indifferent electrode outside. The extracellular potential is measured between two points in the outside medium and is a function of the resistivity of the external medium and the current leaving the membrane which is proportional to the second spatial derivative of the PSP in the one-dimensional cable equation approximation. The extracellular field is, however, a rather complex function of the transmembrane current. To see this it may be noted that the current attenuates rapidly with distance and the micropipette measures an average

of the current derived from a relatively large area of membrane and hence from currents having a spectrum of delays and magnitudes. One may also have to take into account that not all current flows parallel to the vertical axis of the cell (Clark and Plonsey, 1967), although the laminar structure of the cerebellum gives some reason to believe that most current flows vertically in sizeable populations of simultaneously active Purkinje cells. These considerations mean that the peak of the extracellularly recorded field is not simply related to the intracellular PSP. One point is clear, however: at all regions of the cell, other than the synaptically active region, currents will be flowing out across the membrane. These regions will be sources of current and a micropipette tip in the vicinity of these areas should record a positivity. The active region will appear as a sink, so that, given a purely passive spread of potential, the sign of the field will change when the electrode is no longer recording predominantly from the synaptic region. In the present case, the sign of the potential does not change at any point (Section 2.1-2).

It has been argued that in the case of complex neuronal geometries the fields may remain negative in regions where only passive processes are taking place (Rall, 1962b). In the present case it is impossible to comment on this since it would require detailed calculations involving data which are not available at the present time. Finally, given a passive hypothesis, it is not possible to account for the removal of the Loc evoked B-component by a preceding Loc stimulus. It will be shown (Chapter III) that this effect is caused by the inhibitory stellate cells. These cells would not block an EPSP, in fact, the hyperpolarization would increase its size. The inhibition would, however, prevent the dendrite from attaining the threshold at which spikes could be produced. Argument b) would, if true, account for the spikes recorded in the molecular layer. Such large potentials would, however, only be visible with intracellular recording because the synaptic current would not be increased; but there was never any evidence that these spikes were recorded intracellularly (intracellular records are described in Chapter IV). Thus the spikes seen become

further evidence against argument a). In conclusion, it may be said that extracellular field potentials are dependent on a number of complex factors, which makes them difficult to predict quantitatively, even in the cerebellum where the laminar structure is particularly favourable to such analysis. Field potentials are best regarded as useful empirical evidence. In the present case, they have been accompanied by other evidence which gives a very strong case for believing that the present interpretation is correct.

There have been several previous reports of dendritic spikes. They have been found in the pyramidal cells of the hippocampus. Here the apical dendrites are some 500-600μ from the soma of the cell and thus active dendritic processes might enable the remote dendrites to exert an appreciable influence on the soma. Cragg and Hamlyn (1955) reported apical dendritic spikes initiated orthodromically with a conduction velocity of 0.3 to 0.5m/sec. Andersen and co-workers confirmed this finding in the CA1 and CA3 neurones (Andersen, 1960; Andersen, Holmqvist and Voorhoeve,

1966a; Andersen and Lømo, 1966) finding a range of conduction velocities between 1.4 and 0.25m/sec with a mean of 0.4m/sec. Andersen, Holmqvist and Voorhoeve (1966b) also found that spikes could be initiated orthodromically within the dendrites of the dentate granule cells of the hippocampus after which the spike invaded both somatofugally and somatopetally with a velocity of 0.5 m/sec. Spencer and Kandel (1961) reported fast prepotentials in the apical dendritic shafts of hippocampal pyramidal cells which were interpreted as evidence of local spikes. These authors believed that such spikes did not propagate all the way to the soma, but influenced this region by passive spread. Fujita and Sakata (1962) found spikes in the apical dendrites of CA1 and CA3 neurones, originating in the apical dendritic shafts and propagating to the soma with a velocity of between 0.5 and 0.15m/sec. Purpura and co-workers have also reported dendritic spikes in hippocampal neurones following trans-hippocampal polarization and during seizure (Purpura and Malliani, 1966; Purpura, McMurtry, Leonard

and Malliani, 1966). On the other hand, in the neocortex, spikes have rarely been observed and Purpura and his co-workers concluded that dendrites of the pyramidal cells of this region usually supported spikes only when they were immature (Purpura, 1967). Evidence has been produced for dendritic spikes in the motoneurone. Lorente de Nó (1947) reported antidromic spike invasion of motoneurone dendrites with a velocity of 2 m/sec suggesting that the dendrites could support active processes; antidromic invasion was confirmed by Fatt (1957a) and centrifugal invasion of motoneurone dendrites demonstrated following a somatic spike (Fatt, 1957b). These results were questioned by Nelson and Frank (1964a) on the grounds that passive electrotonic spread could account for the apparent active dendritic invasion. These same authors (Nelson and Frank, 1964b) showed that orthodromically induced dendritic depolarization might facilitate antidromic spike invasion of the dendrites. Eccles, Libet and Young (1958) showed that chromatolysed motoneurones aquired highly excitable patches of dendritic membrane where par-

tial responses (spikes) could be elicited with orthodromic stimulation.

Wall (1965) claimed that spikes could be induced orthodromically in the dendrites of neurones of the substantia gelatinosa.

Segundo, Takenaka and Encabo (1967) reported the possibility of dendritic spikes in the bulbar reticular formation.

In invertebrates, Mellon and Kennedy (1966) showed that spikes arose and propagated in the dendritic region of the crayfish tactile receptors and Grampp (1966a, 1966b) showed that reverberating dendritic spikes could be set up in the slowly adapting stretch receptor neurone of the lobster.

In the cerebellum, Hild and Tasaki (1962) demonstrated spikes in the dendrites of Purkinje cells, grown in tissue culture taken from the cat and rat. These spikes were not only capable of invading the soma, but could also propagate back into other dendritic regions. Eccles, Llinás and Sasaki (1966c) showed that antidromic impulses

could invade Purkinje dendrites as far as 200μ from the surface in the cat and that all the dendrites below this region tended to fire at the same time. These authors suggested that this was because of "trigger zones", possibly located at major dendritic branch points. Eccles, Llinás and Sasaki (1966f) also produced evidence that climbing fibres could initiate spikes in Purkinje dendrites at about 150-200μ below the surface. Fujita (1968) confirmed these results in the rabbit by recording extracellular dendritic spikes in the molecular layer following stimulation of the white matter. Fujita hypothesised that dendritic spikes were the basis of the "inactivation response" of Granit and Phillips (1956); this response had already been attributed to a climbing fibre mechanism by Eccles, Llinás and Sasaki (1966f). Finally, Eccles, Llinás and Sasaki (1966a, Figure 11A) recorded a Loc evoked spike at 250μ depth in the cat which they attributed to a Purkinje cell; in general, however, the field potentials in the cat never resembled the B-component seen in the alligator.

In summary, there is good evidence for dendritic spikes in the hippocampus and in invertebrate nervous systems. In other regions of the vertebrate nervous system the evidence is more equivocal. The motoneurone evidence is still disputed and whilst Hild and Tasaki's results were unquestionable, they were made in tissue culture where the neurones were not in normal condition and may have demonstrated hyperexcitability as in the chromatolysed motoneurones of Eccles, Libet and Young. Alternatively, the neurones of Hild and Tasaki may have been immature and the observation of Purpura (1967) could be relevant: that immature neocortical cells are capable of spike generation but that this capacity is mostly lost as the cells mature. The demonstration that climbing fibres can produce dendritic spikes in higher animals is interesting because it would be expected that this very powerful synaptic system should produce spikes if the dendritic membrane were excitable. These spikes have not been seen, however, with parallel fibre bombardment (with the exception of the spike seen by Eccles, et al, and described

above) in any animal except the alligator. This raises the question of whether the spikes seen in the alligator might somehow have a climbing fibre origin. In order to test this, one alligator was deafferented 36 hours prior to the experiment. The dendritic spikes appeared with Loc stimulation and subsequent histological examination showed the deafferentation to be almost complete, so that, providing climbing fibres have an extra-cerebellar origin in the alligator, it would seem unlikely that they could have been activated in this experiment. It is also difficult to account for any mechanism which would give such consistent Loc evoked spikes with a climbing fibre origin as are seen in the present experiments. Finally, Eccles, Llinás and Sasaki (1966f) showed that when climbing fibres are activated, the negativity had its minimum latency at the soma of the Purkinje cell, not at the periphery of the molecular layer, so that climbing fibre evoked spikes should be initiated in the lower region of the cell. This is not the case in the present experiments.

CHAPTER III

STELLATE CELL INHIBITION AND THE RELATIONSHIP BETWEEN THE IN-LINE AND OUT-OF-LINE FIELDS.

This chapter describes the inhibition of the B-component of Loc evoked fieldsand the reasons for believing it to be caused by the activation of stellate cells. The relationship between the in-line fields, that is fields directly below the excited beam of parallel fibres and the out-of-line fields, is described and more data presented on the behaviour of dendritic spikes.

3.1 Double Loc Stimuli at Short Intervals and the Effect on the B-component. A very useful experimental procedure in studying the electrophysiology of the cerebellum is to apply two Loc stimuli through the same bipolar electrode with a short time interval between them. The first stimulus is designated the conditioning Loc, the second the testing Loc. The purpose of this procedure is to utilise the modification which the test field undergoes, when preceded by the condi-

tioning volley, as an analytical tool to investigate the way in which the extracellular potential fields are generated.

If the two stimuli are separated by only one or two msec, the second stimulus will find the parallel fibres in a refractory state and thus no fields will be seen following the testing stimulus. As the interval between stimuli is increased, the effects shown in Figure 9 are observed. These records were taken at 200μ depth and the upper record in 9A shows the averaged testing response alone. It is seen that the A-component is very small and that the B-component is preceded by a marked positivity; this is almost certainly caused mainly by the dendrite in the vicinity of the recording electrode acting as a current source to superficial current sinks caused by the spikes in the distal dendrites. The B-component is very pronounced and thus ideal for testing the influence of a preceding Loc stimulus. Subsequent records of Figure 9A show the response to a testing Loc at various intervals after a conditioning Loc. The residual field of the conditioning Loc has

been removed from these responses by averaging an equal number of conditioning Loc stimuli alone and then subtracting the result from the combined conditioned plus test response using the arithmetic capability of the instrument computer. Figure 9B shows single traces of the double stimulus evoked records for comparison. It is seen that at short intervals only the parallel fibre component and positivity preceding the B-component remains and that the negativity is wholly absent, returning only at 33 msec and thereafter increasing rapidly. Figure 9C shows a graph of the percentage height of the average test response after conditioning as a function of the average conditioned negativity (the conditioning and test stimulus waveforms were identical). In each case the quantity measured was the height of the waveform at the position where the peak would have occurred in the absence of a conditioning stimulus: thus the negative percentage indicates that a positivity existed at this point. It is seen that the inhibitory depression reaches

a maximum at about 16 msec, then the depression decreases rapidly reaching its former value after an interval of about 75 msec and thereafter showing an increase above its former value. This facilitation at long intervals will be discussed later, but at present attention will be focused on the reduction of the negativity of the B-component. The duration of the effect eliminates refractoriness in dendrites as a mechanism, assuming that dendritic membranes have properties similar to those of other active membranes. It is therefore concluded that this effect is caused by inhibition and the anatomical evidence points to the stellate cells as the only element which could be involved (Appendix A). This is further strengthened by the results in the cat (Eccles, Llinás and Sasaki, 1965) where stellate cells were inferred to be inhibitors, although this could not be conclusively demonstrated because of the dominant basket cell inhibition, and in the frog where there are no stellate or basket cells and no inhibition is found (Llinás and Bloedel, 1967).

In the cat of course the negativity following the parallel fibre component is not inhibited, since it is evoked by the current which produces the EPSP, but the firing of the Purkinje cell is reduced or prevented. The present elimination of the B-component at short testing intervals is further evidence in favour of this being caused by an active process. Figure 4 illustrates other features of the inhibition. Here the testing volley along is shown in 4A and the conditioned testing volley in 4B at various depths below the surface of the cortex. It is seen that at this testing interval (20 msec) the B-component is absent at all depths but that a smaller, longer lasting negativity is seen in the most superficial responses. This negativity should be caused by the EPSP currents which in the uninhibited situation would lead to spike production. It is seen that in depth the positivity is a mirror image of the superficial negativity, that is, it reverses, as would be expected. Some of the positivity at intermediate depths may be caused by the IPSP current produced in the Purkinje cells by stellate cell action.

Thus, it may be concluded that there is active inhibition in the molecular layer of the alligator cerebellum, mediated by the stellate cells. The inhibition has its peak effect at about 16 msec judging by the maximum positivity in Figure 9A. In two other series of experiments, similar to that illustrated in Figure 9, the maximum positivity was found at 16.5 msec and 13 msec. The inhibition passes off at about 75 msec.

A further method of investigating the inhibition on the dendrite is to use local stimulation combined with antidromic activation. The results of such experiments will be presented in Chapter IV. For the present, the effects of two Loc stimuli can be summarised in Figure 10. In 10B there is a superposition of the conditioning and testing response at a sequence of different intervals. In this picture the testing responses ride on the field produced by the conditioning stimulus. It can be seen, however, that the inhibition of the B-component at short intervals is clearly visible. Figure 10C summarises in diagrammatic form the

relationship between the stellate cells and the Purkinje cells.

Figure 9C illustrates another phenomenon. When the two Loc stimuli are separated by a long interval, the Purkinje cells will have recovered from the stellate inhibition, induced by the first stimulus, by the time the second stimulus is applied. Under these conditions, the response to the second Loc is potentiated. Figure 9C shows that there is some variability but the predominant effect is that the conditioned testing response is about 120% of the unconditioned response. The effect could still be observed at 500 msec intervals in this experiment and it has also been observed on other occasions.

3.2 Fields Evoked by Loc Stimulation Perpendicular to the Parallel Fibres. Loc stimulation activates a narrow beam of superficially situated parallel fibres. The records so far presented have been obtained from a micropipette within, or directly below the centre of this beam. The results, to be presented now, relate to fields recorded at various distances from the centre of the beam and perpendicular to it. For these studies, an array of three

or four micropipettes were used (See Methods, Chapter II) with their tips arranged so that they entered into contact with the cerebellum at the same time and aligned in a direction strictly perpendicular to the parallel fibres. The distance between the tips of the electrodes was between 200μ and 250μ; but it is unlikely that this spacing always remained constant as the electrodes penetrated the cortex, the general tendency being for the electrodes to converge slightly.

In Figure 11, two sets of fields are shown, each recorded with the micropipettes at 100μ depth. The stimulus strength has been increased from the uppermost to the lowermost set of records. The lower records in each case are from the micropipettes closest to the centre of the parallel fibre beam and in these the A and B components are visible. The latency of the parallel fibre beam decreases very slightly with increasing stimulus strength, but the latency of the B-component noticeably decreases, owing to synaptic bombardment by increasingly deep parallel fibres and the activation

of parallel fibres nearer to the recording position as the Loc stimulus increases. In the central trace of each group of records a negativity is seen which has a rising phase synchronous with the parallel fibre volley of the lower trace but of longer duration. It would seem that not all this field is evoked by parallel fibre activity, but that the later part is related to postsynaptic events. This later part of the negativity cannot be a spike potential, however, since it is earlier than the spike potential in the centre of the beam whilst the parallel fibre activation of the Purkinje cell cannot be as great as that in the centre of the beam. This element of the wave can therefore be identified as an EPSP. The positivity separating the pre- and postsynaptic elements on beam must thus be related to the propagation of the parallel fibre volley and be an effect which is diminished when the fields have an asymmetry, as they must out of the centre of beam. Following this EPSP, there is a marked positivity which is seen to correspond to the on-beam negativity of the B-component. This can be ascribed to the off-beam regions of Purkinje cells acting as

passive current sources to on-beam regions. Finally, the upper trace of each group of records, about 400μ from the centre of the beam, closely resembles the middle trace, but has diminished amplitude. It is either the result of the weak action of the outermost regions of the parallel fibre beam or simply a field effect.

Spikes can be produced somewhat off-beam as can be seen in Figure 12, where four laterally spaced (200μ) recording electrodes were used. All the information acquired from such experiments is difficult to display in a quickly intelligible manner and here the same records have been presented in two different ways to illustrate two different points. In Figure 12A, blocks of four traces are presented, each block of traces representing the simultaneously recorded fields at a particular depth. In this way the temporal relationship between the fields is seen. In Figure 12B the same fields have been presented with the four simultaneous records arranged horizontally so as to give a more realistic picture of the whole field. It is

seen that the on-beam component again exhibits the typical configuration. At depths of 350μ to 450μ many small spikes are seen on the negativity, indeed they are so numerous in these superimposed records that they provide further evidence that the negativity largely represents the envelope of such spikes. Electrode 2, that is the electrode at 250μ from the centre of the beam, shows a sharp positivity following the parallel fibre component in the most superficial record. This is seen to correspond to a negativity at 100μ and below, so the positivity may be regarded as a reversal of this negativity. From Figure 12A it is seen that the negativity recorded at 100μ with the second electrode is markedly later than that recorded with the first electrode. However, whilst the latency of the spike in the first sequence increases with depth, that of the second sequence decreases with depth, so that a point is reached around 200μ where both negativities have the same latency. An explanation may be that the first negativity propagates down in the usual manner, but at 200μ it initiates an

invasion of more lateral dendrites which propagates up towards the surface but does not reach it, as the reversal at 100μ shows. Alternatively the EPSP in the second electrode track may initiate the spike at 200μ owing to a reduced threshold in this region. It is also seen in the records from Electrode 2 that spike potentials occur at 300μ. The two lateral most recordings (Electrodes 3 and 4) are very similar to each other and resemble the pattern of the second sequence. These are probably merely a field effect, although the presence of some spikes at 350μ, in the vicinity of the negativity in the third set of records, may indicate that there is some postsynaptic electrical activity at this point. The few spike potentials that occur in the fourth track are probably purely spontaneous, that is to say bearing no explicable relationship to the stimulus.

3.3 Partially Propagating Dendritic Spike Responses Evoked by Weak Loc Stimulation. In Figure 13A are shown the results of applying a very weak Loc and

averaging the results of 8 such stimuli to produce recognisable fields. A negativity is seen at the surface and at 50μ. It has a two peaks the first of which may be attributable to the parallel fibre volley as it is preceded by a small positivity. Below 50μ the negativity is absent and there is a positivity corresponding to the rising phase of the second peak of the superficial negativity. The peak of the second summit of the superficial negativity is markedly later at 50μ than at 0μ, and so it is suggested that these records show a very superficial action potential which failed to propagate to the soma. It is seen, however, that a negativity does exist at a level of 200μ. Two mechanisms might have produced this lower negativity. Either the EPSP which was the forerunner of the superficial action potentials was able to fire the soma-axon-hillock region, which then caused a spike to invade the dendrite so accounting for the negativity in the lower regions of the dendrite; or a few action potentials did succeed in invading the lower regions and initiating a larger spike.

The former mechanism seems the more probable because the initiation of the lower negativity is almost simultaneous with that of the second summit of the superficial wave, although the peak is late in depth. This is further supported by Figure 13B where the same experiment has been repeated with a stronger stimulus. There is a clear-cut case of two sets of spike potentials, simultaneously activated, one at the surface, the other in depth. Figure 13B also illustrates another remarkable feature; the velocity of propagation of the peak of the B-component in the first 100μ is 0.025m/sec, a very slow rate indeed. It is also apparent that in these two records the superficial spike potential is produced within a few tens of microns of the surface since there is a marked latency in the peaks at only 50μ.

3.4 Discussion. Evidence has been presented in this chapter for an inhibitory mechanism which acts on Purkinje cells and it has been suggested that the mediator of such an effect is the stellate cell. Since basket cells are not found in

the alligator cerebellum (Appendix A, Section A.3) the only other inhibitory mechanism which could act on the Purkinje cells would be the axon collaterals of Purkinje cells. These collateral synapses have not been identified on Purkinje cells, whilst stellate synapses have been found (Hillman, 1968). Furthermore, the evidence is that Purkinje collaterals in the cat do not inhibit Purkinje cells very effectively (Eccles, Llinás and Sasaki, 1966c). In the cat, basket cells were identified as producing a powerful inhibitory effect on Purkinje cells (Andersen, Eccles and Voorhoeve, 1963, 1964; Eccles, Llinás and Sasaki, 1966b; Eccles, Sasaki and Strata, 1967a) and it was hypothesised that the stellate cells of the cat were also inhibitory, but these cells could never be activated without simultaneous activation of the basket cells (Eccles, Llinás and Sasaki, 1965, 1966a, 1966d; Eccles, Sasaki and Strata, 1966a). In the alligator, where the stellate cells can be excited by themselves, it is possible to compare the time course of the inhibition with that found

in the cat. In the alligator, the inhibition seems to be a maximum at about 16 msec and to pass off at 75 msec. In the cat, the maximum is at 20-50 msec and the inhibition passes off at 120 msec (Eccles, Llinás and Sasaki, 1966c). It has been suggested that the slow rise time and long persistence of the inhibition in the cat is caused by the peculiar glial encapsulation of the basket cell synapses on the soma-axonal region of the Purkinje cell (Appendix A, Hámori and Szentágothai, 1965; Eccles, Llinás and Sasaki, 1966b; Eccles, Sasaki and Strata, 1967a).

The potentiation of second Loc evoked response after the inhibition has passed off could be explained by potentiation of transmitter release in the presynaptic terminals of the parallel fibre; a phenomenon which is known to occur at the neuromuscular junction (Eccles, 1964: Ch. VI). It may also be caused by the first Loc exciting Golgi cells which in turn inhibit the granule cells and in particular bring all the spontaneous activity to an end. The second Loc will then encounter

a population of parallel fibres which are free of the refractory effects following the passage of random spontaneous spikes and so a greater number of parallel fibres will be activated by the second stimulus than by the first stimulus.

The laterally evoked fields are consistent with the spike production hypothesis; a lateral positivity is seen which should be a sign of a passive current source to the dendritic sink. There may also be an active component caused by the hyperpolarization of dendrites outside the main beam by laterally spreading stellate axons. There is also evidence that on some occasions lateral regions of the dendrite are invaded by spikes evoked by intense Loc stimulation in the medial regions.

The partially propagating dendritic activity described in Section 3.3 illustrates some of the complicated patterns of activity which may be caused by dendritic spikes.

CHAPTER IV

UNIDIRECTIONAL TENDENCY FOR DENDRITIC SPIKES IN PURKINJE CELLS; AXONAL SPIKES AND INTRACELLULAR RECORDS FROM PURKINJE CELLS.

This chapter describes results obtained with white matter (WM) stimulation (See Methods Section, Chapter II) used to evoke antidromic fields in the cerebellar cortex and shows that action potentials prefer to move in a centripetal rather than a centrifugal direction in the dendrites of Purkinje cells. The spike output of Purkinje cells following Loc and WM stimulation is presented, and it demonstrates a possible relationship between dendritic spike activity and the pattern of output spikes of the cell. Finally, the results of intracellular recording are presented and are found to confirm the conclusions deduced from extracellular records.

4.1 Identification of Field Components Following WM Stimulation. Typical fields evoked by WM stimulation are shown in Figure 14A. The deepest

records (450μ) show a sharp, very early negative peak, followed by a second longer negativity which appears to have two components. This pattern persists to about 300μ where the second negativity loses its first component. At 200μ and above, the first very early negativity is replaced by a positivity. This superficial positivity is followed by a second, larger, positive wave from 100μ upwards. The second negativity increases steadily in latency as it is recorded more superficially.

The early negativity visible from 450μ to 250μ can be identified with the antidromic activation of the Purkinje cells. The reasons for this identification are a) its very short latency, since the Purkinje cells are the only elements which send their axons through the white matter, and b) the fact that the field reverses to a positivity at the surface, implying that the negativity arises in a vertically orientated core conductor and so other types of element are excluded as the source of this wave. The negativity may still have mossy and climbing fibre components at levels below 350μ,

the point at which mossy fibres terminate. Climbing fibres would extend up to 100μ below the surface but, in the cat, they conduct slowly and contribute to the N_2 wave, discussed below (Eccles, Llinás and Sasaki, 1966c). c) A preceding Loc stimulus (Figure 14B) does not abolish the early negativity, although there is some reduction in amplitude in the deepest records. This reduction caused by repetitive firing in some Purkinje cells following the first stimulus and so making these axons inexcitable to the WM stimulus. These considerations enable the early negativity to be identified with the N_1 component defined by Eccles et al (Eccles, Llinás and Sasaki, 1966e). The later negativity between the depths of 350 and 450μ has most likely a dual origin. In the first place mossy fibres excited by the WM electrode will excite granule cells and probably some Golgi cells at these depths. Thus, the dendrites and somas of these elements will act as current sinks whilst the axons of granule cells and the dendrites of Golgi cells within the molecular layer will act as sources, so that a negativity will be seen in depth.

This would account for the first component of the late negativity seen in the records between 350 and 450μ. It is not altered by a preceding Loc stimulus, which is consistent with its being caused by synaptic current. On these grounds, this component may be identified with the N_2 wave of Eccles et al. (Eccles Llinás and Sasaki, 1966e). The second component of the late negativity seen in Figure 14A, between 350 and 400μ, is related to the negativity which persists up to the surface. This wave has its minimum latency at 150-300μ and appears to originate, at least in part, in this region. It is most plausibly identified with the orthodromic Purkinje cell response. The component below 300μ has the characteristics of a field potential caused by spikes originating in the soma and propagating down axons. As such it may be homologised with the N_4 wave of Eccles and his collaborators (Eccles, Sasaki and Strata, 1967b). The records of Figure 14B show that a preceding Loc reduces this component, but does not abolish it. Between 300μ and 150μ the latency of the late negativity is a minimum; this is also reduced but not abolished by a preceding Loc. This

fact suggests that it is caused by very powerful synaptic activity and it may well represent the action of climbing fibres. Above 150μ the latency of this wave increases; moreover, it is removed by a preceding Loc above 100μ. Two possibilities arise. Either it is the local response of Purkinje dendrites to parallel fibre activation, the activation being successively later the higher it is recorded in the molecular layer owing to the increased length of granule cell axon that the afferent activity must traverse; or the dendrites are actively invaded in a centrifugal direction from the region of the initial activation (150-300μ). This latter process could be facilitated by parallel fibre bombardment of the dendrites, and this may be a necessary condition, since the antidromic activation does not invade beyond 200μ (See Sections 4.2 and 4.5). On these grounds it is difficult to identify the N_3 component of Eccles et al. (Eccles, Llinás and Sasaki, 1966e) since this is attributed to the parallel fibre-induced synaptic current flowing into Purkinje cells, whilst in the present situation

this process cannot be clearly identified. Finally, the positivities which are seen superficially are in the first case a manifestation of the superficial sink for the lower antidromic invasion and in the second a superficial sink for the intense depolarization between 150 and 300μ.

4.2 The Antidromic Component. The antidromic (N_1) component was examined in detail using a fast sweep speed on the oscilloscope and the results are shown in Figure 15A. It is seen that the antidromic wave slows down rapidly above 300μ and fails altogether at 200μ. The rate of penetration is illustrated in Figure 15B where it is seen that between 300μ and 200μ the rate of propagation is about 0.14 m/sec. This situation contrasts with the cat where there is almost instantaneous invasion to a depth of 250μ and thereafter slow penetration to a depth of 150μ (Eccles, Llinás and Sasaki, 1966c).

It is of interest to examine the fields evoked by WM stimulation and recorded perpendicular to the parallel fibres. Figure 16A shows records taken with three micropipettes 200μ apart, with their tips at the same level. In general these fields demonstrate the

same characteristics as those of Figure 15A and show that the antidromic invasion takes place over a wide area with this type of stimulation. A preceding Loc stimulus (Figure 16B), with its maximum fields in the lower record of each set has its greatest effect on the second negativity of the WM evoked response at a depth of between 0μ and 200μ and distance of 200μ out of line. This suggests that there is a definite lateral spread of inhibition extending beyond the confines of excitatory effects, thus providing confirmation of the lateral spread of stellate axons.

4.3 The Purkinje Cell Output. The presence of dendritic spikes in Purkinje cells raises the question of whether the firing pattern of the soma-axon hillock region of the cell shows any relationship to these spikes. In Figures 17A-C a micropipette recorded all-or-none (Figure 17B) spike activity, in the region of Purkinje cell bodies, elicited by Loc stimulation. It will be shown later that this activity could be inhibited by a preceding Loc and so it was identified as a Purkinje cell

response. It is seen that a sequence of spikes of diminishing size occurs, having a positive-negative configuration. The most interesting features are that the activity is repetitive and that the falling phases of the spikes are long and show several components. A preceding WM stimulus (Figure 17D-arrow) prevents any spikes from occurring. On the other hand, a strong WM stimulus (17E to G) produces a single sharper spike (in E three traces are superimposed). In 17 G a spontaneous spike occurs just prior to the WM stimulus on one occasion, also on the majority of trials the cell responded at rather short latencies with a spike followed by a very long lasting negativity, and on only one occasion was the spike similar to those shown in 17E and F. In Figure 17H, taken at a slower sweep speed, WM stimulation produces a single spike, but it is followed by a sequence of small oscillations which continue for some 12 msec. In the sequence shown in Figures 17I to K, a WM stimulus is given (arrow) and in 17J and K it is preceded by a Loc. It is

seen that the WM elicited spike of Figure 17I is extinguished by the preceding Loc in 17J. In Figure 17K an increase in WM stimulus strength produces a spike at very short latency which may be purely antidromic. In all cases, the Loc evokes a sequence of spikes as in 17A to C. Finally, Figure 17L shows some spontaneous activity at a slow sweep speed.

From these records it can be concluded that repetitive Purkinje cell firing occurs following Loc and WM stimuli. When WM stimuli are used the Purkinje cells may be activated by climbing fibres. The latency of the response is the same (about 10 msec) for both WM and Loc stimuli in all cases, except those shown in Figures 17E and K (second response); in the latter case a strong stimulus was used and the latency shortened. In Figure 17G, the response was all-or-none and some evidence will be presented below that the prolonged negativity following the spike in three cases could

be a sign of climbing fibre activity. When Loc stimuli are used, it is conceivable that climbing fibres could be excited by a) direct stimulation, b) excitation of climbing fibre collaterals, and c) extracerebellar reflex; in general, however, a climbing fibre component cannot be identified in studies of fields following a Loc stimulus (See Chapter II). It is thus entirely possible that the repetitive firing following a Loc stimulus is produced by a parallel fibre volley. The long duration and notched appearance of the falling phase of the spike might then be accounted for by a barrage of dendritic spikes arriving at the soma. On the other hand, if one compares the above spike sequences with those seen in the cat following stimulation of the olive or white matter (Eccles, Llinás and Sasaki, 1966f - Figures 3A to C), one sees a great similarity between the sequences of spikes elicited by climbing fibres in the cat and those produced by Loc stimulation in the alligator, except that the falling phases of the alligator spikes are a great deal longer than those in the

cat. These results raise the question of whether, by observation of the output of the Purkinje cell alone, it is possible to distinguish between the type of input, mossy or climbing fibre, which initiated it.

Some further responses of Purkinje cells are shown in Figure 18. These responses were recorded from about 400μ depth in the posterior lobule of the cerebellum. This should be the region of the Purkinje cell somas, but rather acute curvature of the posterior lobule may lead to some error in position (the position of the pipette tip was not checked histologically because the animal moved shortly after the records were taken and terminated the experiment). Figure 18A and B show spikes caused by Loc stimulation. In this case the spikes are of shorter duration than those of Figures 17A to C and closely resemble the climbing fibre response in the cat. Records 18C to F show a set of responses from a slightly more superficial position in response to WM stimulation, the stimulating electrode being pushed

through the caudal part of the medial lobe in this case. The responses consist of long lasting negativities with a sudden rise and sudden cessation. In the longest case the phenomenon lasted 25 msec and Figures 18C and E illustrate that it was all-or-none in nature. In Figure 18E three negativities have been superimposed to illustrate the repeatability of the effect. During this experiment and in this region the sudden and prolonged negativity was seen frequently; the animal was in excellent condition and responses from other regions of the cerebellum were very good. In no other alligator has such an effect been seen, but a similar potential has been observed occasionally in the cat cerebellum (Llinás, personal communication). A preceding Loc stimulation (Figures 18G to I) only inhibits the negativity at the shortest interval used, 12 msec. The origin of this effect cannot be deduced from these experiments, but the sharp rise and fall of the negativity together with its all-or-none nature suggest that it may be a very long lasting action potential.

4.4 Intracellular Records from Purkinje Cells. Purkinje cells were identified by a) the depth at which the cell was penetrated, b) size of action potentials, and c) IPSP's. All cells penetrated had similar characteristics and indeed there are no reportes of intracellular recording from elements other than the Purkinje cell in the cerebellar cortex of any animal. Purkinje cells in the alligator are difficult to penetrate and hold for any appreciable period of time, resembling the Purkinje cells of the cat and frog in this characteristic.

Figures 19 A to D show a series of intracellular records. The horizontal line above each record shows the extracellular potential so that the displacement of the record from this line indicates the resting potential of the cell. In all cases it is quite low, about 30mV, doubtless owing to penetration injury. Figure 19A shows the cell firing rhythmically with a characteristic injury response and a weak Loc stimulus may cause a slight increase in frequency of firing, but no more. Figures 19 B to D show the effect of an

increase in Loc stimulus strength. In Figure 19B, there is initially some increase in spike frequency then a marked diminution; in 19 C and D the effect is a single spike followed by inhibition and in 19D a definite hyperpolarization is seen. Figures 19 E to G show the results of another penetration. In this case, the Loc stimulation was stronger than in the first examples and the initial depolarization following the stimulus is clearly visible (as it is in 19D). Spikes are seen to arise from this depolarization in all cases. With the strongest stimulus (Figure 19G) the depolarization lasts about 50 msec. Following the depolarization there is, as in the first set of records, a hyperpolarization and then finally a resumption of the injury spikes. This same pattern is seen in records 19 H to K with a slightly expanded sweep in 19 I to K, in all of them there is an initial depolarization with an early spike and then the depolarization continues but no spikes are produced, finally a hyperpolarization is seen. The depolarization is produced by dendritic spikes initiated by the synaptic

action of parallel fibres, whilst the long-lasting hyperpolarization is caused by stellate inhibition.

Figure 19L illustrates another phenomenon. Three records are seen in two of which hyperpolarization begins about 40 msec after the Loc stimulus. In the third record, hyperpolarization begins earlier, about 20 msec after the stimulus, but the slope of commencement of the hyperpolarization is more gradual than that seen in the two records showing the delayed hyperpolarization. The cause of the more abrupt start to the hyperpolarization, after the prolonged depolarization, may be that the depolarization is caused by a barrage of dendritic spikes and gives rise to the type of post-tetanic hyperpolarization reported by Nakajima and Takahashi (1966a; 1966b) in the receptor neurone of the crayfish. Those workers showed that the hyperpolarization was caused by the

activation of an electrogenic sodium pump. There may also be a contribution from the usual after-hyperpolarization caused by increased potassium permeability (Eccles, 1957 - Chapter II).

4.5 The Effects of Polarizing Currents on the Intracellular Records from Purkinje Cells. Figures 20 A to E show the effect of Loc stimulation on the intracellularly recorded potential from a Purkinje cell. In Figure 20A, before the stimulus is applied, the cell fires rhythmically with an injury induced potential. Figures 20 B to E show the effect of increasing Loc stimuli which produce a depolarization having small amplitude spikes riding on it in 20D and E. After the depolarization the membrane potential returns to a hyperpolarized level due to the IPSP induced by stellate cells. This hyperpolarization also gives rise to an increase in the size of the injury spikes. The spikes riding on the depolarization make the waveform look like that induced intracellularly by a climbing fibre volley in the cat (Eccles, Llinás and Sasaki, 1966f) but that this is not a climbing fibre is shown in the next

series of records in Figures 20 F to J. Here the membrane has been hyperpolarized by passing a current through the recording electrode, using the Rockefeller bridge circuit amplifier (See Methods, Chapter II). This hyperpolarization prevents the occurrence of spikes so that only the EPSP is seen. A Loc stimulus is applied at increasing strengths as shown in Figures 20 F to J and the resulting depolarization is seen to be graded; this contrasts with a climbing fibre evoked EPSP which is all-or-none (Eccles, Llinás and Sasaki, 1966f). At the cessation of the hyperpolarization the injury spikes are seen to be very large, but to decay rapidly in size as the membrane returns to its resting potential. It is seen that as the Loc stimulus increases, the time taken for the injury potential to return to its normal size increases. This is caused by the additional hyperpolarization induced by the Loc evoked inhibition on the Purkinje cells. Finally, Figures 20 L to O show a set of records from another cell where there are no injury potentials and the level of the hyperpolarizing current is not sufficient to

prevent spikes. The spikes arising from the depolarization are seen to have several inflexions on the falling edge (Figures 20L, N, O); the phenomenon is also seen in Figures 20D and E. This is further evidence that the depolarization is caused by a barrage of dendritic spikes. Following the termination of the hyperpolarization, a rebound spike is seen in records 20 L to O.

4.6 Discussion. The results reported here show that WM stimulation in the alligator produces fields in the cerebellar cortex which are quite similar to those found in the cat (Eccles, Llinás and Sasaki, 1964b, 1966c; Eccles, Sasaki and Strata, 1967b). One feature, however, is different. The antidromic component does not invade the dendrites as readily in the alligator as in the cat. This fact is the more remarkable when it is considered that the dendrite can support active processes. In Figure 21A, a graph is shown of the size of the antidromic (centrifugal) and Loc evoked (centripetal) fields as a function of depth for one experiment. The stimulus strengths were

chosen so that the field at the level of the soma was about the same in each case. The centrifugal component clearly attenuates rapidly as it penetrates towards the surface, whilst the centripetal component, although diminished below 200μ, propagates to the soma. These results show that the dendrites tend to conduct spikes in one direction only, that is, towards the soma. Sometimes upward invasion, following orthodromic stimulation, does occur (Chapter III, Section 3, Figure 12), but the unidirectional tendency or "funnelling" is unmistakable. The two cases of centrifugal and centripetal invasion are illustrated diagrammatically in Figures 21B and C. In Figure 21B the dotted part of the Purkinje dendrite illustrates the extent of the active invasion following antidromic stimulation and the arrows indicate passive intracellular current flow. In Figure 21C the effect of a very narrow beam of parallel fibres (PFB) is shown, so narrow that only one tertiary dendritic branch is active. Spikes move down toward the soma (dotted region) but the other dendrites are

not invaded to an appreciable extent; instead they act as a passive current source. This means that each major dendritic branch has the possibility of acting independently, that is the initiation of spikes in one does not mean that the depolarization in other branches will be "erased" by spike invasion.

Turning now to the question of the output of the Purkinje cell, evidence has been presented that a Loc stimulus and a stimulus which evokes a climbing fibre input to the Purkinje cells may produce very similar outputs from the cell. In both cases, a burst of spikes may be seen and it is likely that this is produced by a barrage of dendritically arising spikes. A burst response in the soma to dendritic spikes evoked in the dendrite by a climbing fibre has also been noted by Fujita (1968) in the rabbit. In the cat it is only the activation of the climbing fibre which gives rise to a burst of spikes at the soma (Eccles, Llinás and Sasaki, 1964a, 1966f; Eccles Llinás, Sasaki and Voorhoeve, 1966). In the case

of Loc stimulation in the alligator, the stellate cells will be activated (Chapter III) and this might be expected to substantially reduce the number of dendritic spikes reaching the soma after a period of about 2 msec (synaptic delay and conduction time in the stellate cell). The records presented here show, however, that the inhibitory mechanism does not become fully effective in cutting off the Purkinje cell output until at least 10 msec after the stellate cell is activated. It could also be that the repetitive firing of the Purkinje cell arises at the somatic level rather than through a sustained barrage of dendritic spikes; it is to be recalled that the inhibitory mechanism in the alligator is restricted to the dendrites of the Purkinje cell and that the soma is devoid of inhibitory synapses.

The long lasting all-or-nothing negativities shown in Figure 18 cannot be explained with the data available from the present experiments. Such responses have also been seen in the cat and there they may have a climbing fibre origin (Llinás, personal communication); in any case, one may spec-

ulate that they represent some form of regenerative or reverberatory active process confined to a single cell. The climbing fibre response, as such, has not been investigated in this work. In order to do so an identifiable source of climbing fibres would have to be activated, since the possible similarity of Purkinje cell output, in this animal, to both climbing and parallel fibre excitation, precludes using the cell output alone as a criteria for climbing fibre identification. The olive should be a source of climbing fibres but, as mentioned in Appendix A, the olive has not been identified with certainty in the alligator; so that some further anatomical investigation would seem desirable before commencing the physiology of the climbing fibres.

The physiology of the Golgi cells has not been investigated in the present work. The histological evidence is that they are similar to those found in the cat (Appendix A) and so it would be expected that they perform a similar

functional role to that demonstrated by Eccles and his co-workers (Eccles, Llinás and Sasaki, 1964b, 1966c; Eccles, Sasaki and Strata, 1967a). The N_2 component of the WM evoked fields described in Section 4.1 has very likely a Golgi cell contribution.

The intracellular records from Purkinje cells demonstrate the familiar EPSP followed by an IPSP and thus confirm the presence of the active inhibitory process, caused by stellate cells, that was deduced from the field studies. The intracellularly recorded depolarization is seen to have many small peaks and in this respect resembles the climbing fibre EPSP seen in the cat. In the present case, the EPSP is not of climbing fibre origin but is evoked by parallel fibres, and the small peaks are almost certainly the result of somatic bombardment by dendritic spikes. That the depolarization is not caused by climbing fibres follows from the fact that it is graded in response to varying the strength of the Loc stimulus.

CHAPTER V

COMMUNICATIONAL ASPECTS OF NEURONAL CIRCUITRY IN THE CEREBELLAR CORTEX OF THE ALLIGATOR.

This chapter speculates on the functional implications of the findings presented in the last three chapters. In particular, the modes of action of the Purkinje cell are discussed in the light of a mathematical analysis of the cable properties of branching dendrites which is presented in detail in Appendix B. The functional and evolutionary significance of the inhibitory interneurones is discussed and the possible roles of climbing and mossy fibres compared.

5.1 The Purkinje Cell. The remarkable ramification and flattening of the Purkinje cell dendrite must have a considerable bearing on the functional properties of the cell and in particular on the relationship between the pattern of incoming synaptic bombardment and the outgoing sequence of spikes in the cell axon. Firstly, therefore, the anatomy of the Purkinje cell will be considered.

5.1-1 Morphology. Histological material, stained by a rapid Golgi technique and prepared by Dr. D.E. Hillman was used in this study. Figure 22 presents a photomontage of a Purkinje cell showing its general appearance. Figure 23 presents a drawing, made with the aid of a microprojection apparatus, of the cell shown in Figure 22. In the drawing the position and length of the branches are accurately represented but the thicknesses of the branches are not to scale and the dendritic spines have not been shown. To remedy these omissions Figure 24 shows six photomicrographs of various parts of the tree, taken under oil immersion and showing typical dendritic diameters and the distribution of spines on the smaller branches. One feature which is seen at once from Figure 23 is that the tree has a larger number of branches than is apparent from the direct photomicrograph (Figure 22). A count was made of the number of branches at different levels of the dendritic tree shown in Figure 23. The levels were chosen at 10μ increments along the main axis of the tree and the results

of the
/count are shown in Figure 25A. It can be seen that the branch density has a maximum between 300μ and 450μ and on either side of this region it falls quickly. Functionally it will be more important to ask how the branch density varies with path-length from the soma; that is with length measured along the dendritic tree. To measure this the tree was redrawn with path length as a vertical distance and the connectivity of the tree preserved; the number of branches at a given height were counted again and the results shown in Figure 25B. It is seen that the graph has a similar shape to that in Figure 25A with most branches lying between 350μ and 500μ from the soma. The similarity in shape suggests that height and path length may be linearly related in a rather precise manner. This is confirmed in Figure 26 which shows a scatter diagram for height versus path length for all branch points and terminal points of this tree (these points were chosen because they were easy to identify). This graph gives a good indication

of the "straightness" of the dendritic tree and shows that if the path length of a particular point in the tree is long then it will almost certainly be found high in the molecular layer. These results all refer to a dendritic tree which was chosen as a particularly good example, but similar results have been found for other, less well impregnated examples. Of course some Purkinje cells show considerable morphological distortion in the region where they conform to sharp curvature in the cerebellar cortex, but these are exceptional.

5.1-2 Passive Processes in a Branching Dendritic Tree. Although it has been shown that intense synchronised synaptic bombardment of the superficial dendrites (Loc stimulation) induces dendritic spikes it is likely that more diffuse, unsynchronised synaptic bombardment leads only to passive electrotonus in the dendrites. Furthermore an understanding of the passive properties provides a basis for discussion of active processes.

In the case of passive properties the important parameter will be the EPSP seen in the region of the soma, for this is where spikes will be generated. If a synapse was joined to the soma by a uniform cable the potential at the soma would show the well known waveform caused by conduction along a cable (Appendix B, Section B.1). The presence of extensive side branches on this cable could, however, modify the waveform substantially. The branched situation has therefore been analysed (Appendix B, Section B.2). The path-length of the dendritic tree from most distal synapse to soma was assumed to be 2λ for these calculations; this value is based on the 1.8λ used by Rall et al (Rall, Burke, Smith, Nelson and Frank, 1967) for the motoneurone. The result is that the presence of side branches will modify the waveform, but that the effect will be almost independent of the actual position at which the branch occurs on the synapse-soma path, unless the branch is within about 0.2λ of the synapse. In fact, λ will not be constant for the whole tree, but

will decrease towards the periphery of the dendritic tree because the diameter of the dendrites will be small there. This means that most synapses will be more than 0.2λ from most of the branches. Thus a good approximation to the dendritic system with one active synapse is to place all the branches at a single point on the electrotonic path. This considerably simplifies the dendritic geometry. The most noticeable effect of a branch is that it reduces the magnitude of an EPSP; the potential at the soma in a Y-shaped tree in response to synaptic activity at the tip of one branch is shown to be about 30% less than if the synapse and soma were joined by a single cable (Appendix B, Section B.2-1 and Figure 37). On the other hand, the time to peak is not significantly altered. To a first approximation then, the branched system may be represented by a single cable with a greater degree of attenuation than normal. In fact in Appendix B, Section B.3, it is shown that a branched cable can be completely replaced by a single infinite cable with a suitable system of

"image synapses; this helps significantly in solving some dendritic tree problems.

Reducing the tree to a single cable permits investigation of the question of the effects of synaptic position on the potential recorded at the soma (Appendix B, Section B.4). For a uniform cable of length 2λ there is little difference in somatic potential for synapses lying at least 1.5λ from the soma (Figure 39). At less than that distance effects increase rapidly and at a distance of λ from the soma the synapse will produce twice the EPSP that a synapse at 2λ will produce. This effect will be accentuated somewhat by the actual decrease in λ in remote regions. Despite this variation in effect, the EPSP produced at the soma is negligible for a synapse λ away compared to the effect produced by a synapse 0.2λ from the soma. In the latter case, the potential caused by the proximal synapse is 15 times that produced by the synapse at λ away. But from Figure 25B it is seen that most branches, and hence synapses, lie in the distal half of the

dendritic tree so that their EPSPs will be subject to great attenuation which will be accentuated still further by the effect of branching. Thus it would appear that there are no "dominant" synapses. Hillman (personal communication) has shown, however, that the initial dendritic stem does have some synapses with large deep lying parallel fibres; the significance of these fibres is not known at present but they could have a substantial effect on the soma. They are not numerous, however, and will be ignored in the following argument. If the majority of synapses have little effect on the soma then large numbers will have to be active to affect the output of the cell and the possibility of non-linear interactions between synapses becomes important (Appendix B, Section B.5). The effects are brought about by a decrease in λ caused by the presence of a large number of shunt conductances and a reduction in the effective ionic driving potential caused by a displacement of the resting potential in the

direction of the equilibrium potential for ion species responsible for the PSPs. In addition, Martin's correction (Appendix B, Section B.5) should be applied to obtain the correct conductance change; it is shown, however, in Appendix B that the results presented are valid for a wide range of changes in conductance. In the Purkinje cell dendrites of the alligator the extensive branching will ensure that most synapses are well separated. This means that non-linear effects in these dendrites will be small. It is only when a large number of adjacent synapses are active that non-linear effects could be really significant, but in this case active processes should occur and the whole situation will change (See Section 5.1-3). Some implications of the foregoing analysis can now be discussed.

If one assumes that there are times when the parallel fibre activity in a region of the cerebellum is random, perhaps

for instance when muscle groups are maintaining a constant tone, then one may assume that the sequences of spikes in different/parallel fibres are uncorrelated, or at least that there are a great number of bundles of fibres between which the spike sequences are uncorrelated, although some correlation may exist between the members within each bundle. Then, given that the vast majority of synapses have a similar effect on the soma, the following situation occurs. The sequence of intervals between PSP's in a Purkinje cell will be the resultant of a superposition of the actions of all the incoming spike trains, and it was first shown by Erlang (Khintchine, 1960), in connection with the problem of the random arrival of telephone calls at a central exchange, that the net process, that is the superposed sequence of PSPs, will have a Poisson distribution, independently of what the sequence of incoming inputs is in each parallel fibre, provided that the above conditions hold. This being so, the problem may be greatly simplified since a Poisson process is completely specified with only one

parameter, the mean rate; hence the entire mossy fibre induced excitatory input to any Purkinje cell will be described by a single quantity. In addition to the excitatory input each Purkinje cell is probably subject to the influence of many stellate cells, each of which might have a Poisson input similar to that of the Purkinje cell (though with a lower mean rate owing to the far fewer parallel fibres that contact the dendrites of these cells). The output of the cell would not be Poisson (see the argument below for the Purkinje cell output) but the stellate pooled/input to the Purkinje cell would, by the Erlang property, again resemble a Poisson process. The excitation and inhibition would combine linearly to determine the net output of the Purkinje cell and the only parameters which would govern the input would be the relative strengths of the excitatory synapses compared with the inhibitory synapses and the mean frequencies of the two processes. If one assumes: a) that the Purkinje cell has a certain threshold

relative to the depolarization produced by a synaptic input, b) that between inputs the membrane potential decays exponentially towards its resting potential, and/c) that the refractory period is small compared with the interval between output spikes,/then the output behavior of the neurone may be predicted. The exact calculation of the statistical parameters governing the distribution of output intervals is difficult but has been studied by Johannesma (1968); on a more intuitive level however it is fairly clear what will happen. The existence of the threshold of the neurone will mean that several EPSPs will have to add before an output can occur; thus the dominant short intervals of the Poisson distribution, that is the high frequencies, will be /filtered out by the neurone and a peak will occur for some non-zero value of the output interval. The higher the threshold of the neurone relative to the PSPs, the more the peak of the distribution will be shifted in the direction of longer intervals. A bell-shaped curve will result, since many EPSPs will have to add before the neurone fires; this effect will be accentuated by the presence of

inhibition. Using a similar argument, the inhibitory output of the stellate cells might also be computed as a function of mean parallel fibre activity, and so the whole parallel fibre input could be described by the single parameter of mean frequency, given the values of threshold and the appropriate data describing stellate-Purkinje cell relations. In turn the most probable output frequency of the Purkinje cell could be calculated. It is certainly untrue that a random input exists at all times in the cerebellum but it might furnish an adequate description of the background "tonic" activity of the organ. As more purposeful, coordinated motor movement takes place the mossy fibre input might take the form of synchronised bursts which would be transmitted to the parallel fibres and so change the whole mode of Purkinje cell output.

A possible criticism of the above ideas should be dealt with at this point. Braitenberg claims (Braitenberg, 1965; Braitenberg, et al, 1965), using an argument based on the central

limit theorem, that the randomness of the incoming parallel fibre spike sequences will lead to a Gaussian distribution of EPSPs. This result is at variance with the above argument and may stem from a failure to realise that when the spike trains are superimposed at the neurone the identity of the individual trains is quite lost, spikes from one train "fall" in the middle of another to give a pooled process.

5.1-3 Active Dendritic Processes

As parallel fibre volleys become more correlated and the density of synaptic activation rises, spikes will occur in the dendrites. The picture of dendritic spikes which has been drawn in the course of the experiments reported here is as follows. The probability of a spike being produced in any dendritic branch is a function of the intensity of synaptic impingement on that branch and of the polarization induced in this region by activities in its daughter branches. On reaching threshold, a spike is initiated in the dendrite and this spike propagates towards the soma without substantially

invading adjacent dendritic regions. The lack of centrifugal invasion by dendritic or WM stimulus evoked antidromic spikes implies a low safety factor for the process. This may be due to the large area of dendrites which must be invaded or the impedance mismatch at successive branch points. Some evidence for the low safety factor of the dendrites is provided by the observation of apparently discontinuous conduction (Chapter II). This may mean that not all of the membrane traversed by the spike is active as the spike propagates but rather that there are alternate active and passive regions, the coupling between active regions being made by passive electrotonus. The active sites might be very small and numerous, interlaced with passive sites of similar dimensions, or active sites might be found in specialised regions such as dendritic branch points. Such details cannot, however, be resolved by present recording techniques. When the dendritic spikes reach the soma-axon level the probably initiate a burst of spikes, resembling those produced by a

climbing fibre (Chapter IV). Some of the functional implications of these findings are discussed below.

Dendritic spikes converging on a bifurcation point from two different branches could have a variety of effects. If the safety factor were high the first spike to reach such a point would generate an action potential and a closely following spike in another branch would encounter a refractory membrane and so would be annihilated; in terms of a logical operation this would be an 'OR' operator. On the other hand if the safety factor were low it might require the summation of spikes from both branches to enable the propagation to continue, that is it would be an 'AND' operator. Such a possibility has already been pointed out by Wall (1965) in his discussion of the dorsal horn.
neurones of the/ This second situation with low safety factor would permit coincidence detection between inputs, because only when two inputs occurred with spatial and temporal relationships of such a nature that the spikes arrived at almost the same

time at the bifurcation point, would an impulse propagate to the soma. It remains to be determined whether a single impulse arriving at the soma is sufficient to fire the cell.

A further possibility may exist, namely that the spike generating process may become regenerative so that spikes oscillate backwards and forwards in certain regions of the dendrites as has been shown in the lobster stretch receptor (Grampp, 1966a, b). In this way the cell might continue to produce an output long after the input had ceased and this could account for bursts of spikes. Such a mechanism might, however, only operate with some form of tonic background to the dendrites which would cause a local reduction of threshold.

It is also to be noted that the presence of spikes in dendrites does not abolish the possible role of Purkinje cells in the timing mechanism in the cerebellar cortex proposed by Braitenberg (1961, 1962, 1967), since a well synchronised parallel fibre volley might be expected to produce

somatic activation after a rather precise delay so preserving the temporal integrity of the volley.

5.1-4 Conclusion. At low levels of uncorrelated parallel fibre activity the Purkinje cell dendrites probably behave passively and give rise to an output which has a relatively constant frequency; this may be called the tonic mode of activity. Under these conditions the behaviour of the cell could be modelled mathematically with a fair degree of accuracy. As the parallel fibre spike trains become correlated and more neighbouring fibres become active, spikes are likely to be generated in the dendrites and the whole mode of activity of the cell will change. In particular highly non-linear interactions within the dendrite now become feasible. Under these conditions the output of the cell is a burst of spikes, similar to that associated with climbing fibre activation in higher animals, followed by a silence, produced by stellate inhibition or after-hyperpolarization (Chapter IV); this is a phasic mode of activity.

5.2 The Role of the Inhibitory Interneurones.

The inhibitory interneurones of the alligator are the stellate and Golgi cells. The present study has presented evidence that the stellate cells can exert a powerful inhibitory influence on Purkinje cells; this had been inferred in the cat but not directly demonstrated because the basket cell inhibition was always evoked at the same time as the stellate effects. The effects of the Golgi cells have not been investigated in the course of the present work, but it can be assumed that they behave in a similar manner to the Golgi cells of the cat.

The inhibitory interneurones are the only elements which, so far, have been found to be missing in some cerebella. Golgi, stellate and basket cells exist in birds and mammals but the alligator lacks basket cells and the frog lacks all the inhibitory elements so far as can be seen. On these grounds Llinás (1968) has suggested that the inhibitory interneurones are an indication of cerebellar evolution and that as

cerebellar function became more sophisticated, the interneurones became progressively more specialized. It is true however, that the elasmobranchs, generally considered to be primitive fish and to have commenced evolution before the frog, have well developed stellate cells (Schaper, 1898; Houser, 1901) and this is true from the most primitive shark to the advanced rays (Llinás and Nicholson, unpublished observations). Two possibilities exist: either the frog is a specialised and, with respect to the cerebellum, a degenerate animal or there has been considerable parallel evolution during which the sharks have developed stellate cells independently of the main line of evolution. In this case it would be concluded that the main line did not develop stellate cells until the frog had split off. In this connection it is worth noting that the presence of basket cells in birds and mammals is probably also a case of parallel evolution, otherwise both would have to have a common ancestor, prior to the alligator which had basket cells.

In this case it would be necessary to regard the alligator cerebellum as unrepresentative.

5.2-1 Possible Functional Interpretation of Cerebellar Inhibition. The simplest type of inhibition is recurrent collateral inhibition where an axon collateral of an inhibitory cell returns to the soma or dendrite of that cell or another cell of the same type. No interneurone is interposed in the circuit and inhibition is a function of the output of the cell. Functionally, this type of inhibition will resemble negative feedback and where the recurrent collaterals contact the parent cell, or where the population of cells can be treated as a unit, will tend to stabilise the output frequency of the cell (MacKay, 1968). Under some conditions recurrent inhibition can bring about an effect similar to contrast enhancement in a visual system. For a long time histologists believed that Purkinje axon collaterals returned predominantly to Purkinje cells and since these cells are inhibitory the above consideration

would apply. Physiological investigation (Eccles, Llinás and Sasaki, 1966c) showed, however, that such effects are weak. Later histological work demonstrated that all the inhibitory interneurones receive Purkinje axon collaterals (Fox, Hillman, Siegesmund and Dutta, 1967; Hámori and Szentágothai, 1966b) and Llinás and Ayala (1967) demonstrated that Purkinje collaterals had a strong influence on the interneurones. This means that the effect of the Purkinje axon collateral is mediated predominantly through a complex disinhibitory pathway. At the present time it remains to be demonstrated in the alligator that Purkinje collaterals contact either stellate or Golgi cells.

Turning now to the Golgi cell system, the Golgi cells receive inputs from both mossy fibres and parallel fibres and inhibit the granule cells. This means that these cells can act both in a feed-forward and a feedback mode, but the dendrites and axons are both very diffuse and it is probable that their main effect is to reduce the output of granule cells as the mossy

fibre input activity increases in a particular region and therefore the question of it being a feed-forward or feed-back mechanism is largely irrelevant for so diffuse a system. The effect of this might be to "focus" granule cell activity, that is to prevent all but the most intense regions of incoming activity from being transmitted to the parallel fibres (Eccles, Ito and Szentágothai, 1967, Chapter XII). Alternatively / it might act by keeping the granule cells operating at the optimum region of their response characteristics, thus preventing them from being saturated by incoming activity, in effect controlling the operating point (MacKay, 1968). A further point about Golgi cells should be considered. A Golgi cell could be activated by granule cells upon which the Golgi cell itself could not act. This is made possible by the length of the parallel fibres. A Golgi cell, activated by a distant population of granule cells, would reduce the background activity of the population of granule cells under its own control which in turn could reduce the input to a further Golgi cell which would release its population of

granule cells from inhibition. Thus, provided a background of granule cell activity existed, a wave of neural activity would be spread across the cortex/alternate inhibition with and disinhibition of granule cells. Such a wave of activity would be "measured" by Purkinje cell output in response to the changing parallel fibre activity. It would need to be shown, however, that the Golgi-granule cell-parallel fibre system was sufficiently well coupled to permit the effect to exist.

The relationship of the stellate cell with the Purkinje cell can be described as one of delayed inhibition, the delay being produced by the addition of the interneurone. The effect is clearly seen in intracellular records (Chapter IV, Section 4.4) where initially an EPSP is seen and 2-20 msec later this gives way to an IPSP. In higher animals the basket cell-stellate cell inhibition usually becomes apparent within 2 msec of the EPSP

but is probably caused by the proximity of the basket cell synapses to the intracellular recording electrode, whilst in the case of the alligator the synapses are in the dendrites and the inhibition first makes itself felt as a reduction in dendritic spike size (Chapter IV, Figure 19). Such a delayed inhibitory system is well suited to producing an "ON" response in Purkinje cells following a well synchronised parallel fibre barrage. This would cause the Purkinje cell to fire when the excitation arrived and then to be cut off for the remainder of the barrage by the inhibition. The inhibition should outlast the excitation because of the long lasting time course of the IPSP (Chapter III) and because it is known that the inhibitory interneurones fire repetitively in response to a Loc stimulus (Eccles, Llinás and Sasaki, 1966d; Llinás and Nicholson, unpublished observations on elasmobranchs). Normally, however, it is unlikely that the input will be as well synchronised as that of a Loc stimulus and stellate cell inhibition might have simply a modulating

effect on the Purkinje cell; indeed it is probably true that this is the main function of dendritic inhibition in passive systems (Terzuolo and Llinás, 1967), rather than a dramatic switching function. Again, such modulation might be primarily to select the operating point of the cell. A further function in the alligator may be to control the point at which the cell switches from a passive to a spike mechanism, as diffuse inhibition may drastically reduce the probability of spikes being produced until an intense bombardment occurs.

Another point arises with basket cells which may apply to stellate inhibition. In the basket cells the axons spread predominantly laterally for a distance of some 10 Purkinje cell bodies and so the inhibition is mainly a lateral inhibition. Such a mechanism could have marked contrast enhancement properties such as those which have been explored in detail in sensory systems (Harmon, 1968). Eccles, Ito and Szentágothai (1967, Chap. XII) have put forward the idea that the normal mode of operation of the cerebellum involves the activation

of a localised focus of granule cells which causes excitation to spread in both directions down the length of the parallel fibres and induces on each side of it an inhibitory zone. This excitatory-inhibitory patch is regarded as the basic functional unit of cerebellar activity and the interaction of such patches leads to complex output patterns. Such a mechanism depends on a relatively localised parallel fibre focus, which may be achieved by Golgi cell inhibition as mentioned above, but at the present time there is no direct evidence for such a scheme. Again it is to be noted that although stellate cells are reported to have a laterally spreading axonal system it may not be as extensive or precise as that of the basket cells and more than one type of stellate cell may exist.

5.2-2 Conclusions. The inhibitory mechanisms of the cerebellum are activated concomitantly with the excitatory systems. This is true even of the climbing fibre input which sends collaterals to the inhibitory interneurones so that the inhibitory

system is not under the control of an independent afferent system. Furthermore, the inhibitory systems are influenced by the input to the cerebellar cortex, not the output; only the Purkinje axon collaterals provide a feedback system and they serve to augment the Purkinje cell output by disinhibition rather than to reduce it. Following some of the categories described by MacKay (1968) the most likely functions of inhibition in the cerebellar cortex may be summarised as follows: a) Control of operating point, preventing the neural elements from being swamped by large inputs and being driven to the point where they can no longer function. b) Control of resolving power by reducing the amount of extraneous synaptic noise or by a lateral inhibitory mechanism. c) Increasing the effective threshold of Purkinje cells so as to sharpen a coincidence detection role. d) Selecting the mode by which neural elements function, in particular controlling the point at which a Purkinje cell dendrite changes from a passive to an active device.

Finally, it should be recalled that the frog has no inhibitory mechanisms within its cerebellar cortex so that inhibition is not essential for all cerebellar function and probably is mainly there to improve performance rather than produce novel features.

5.3 The Role of the Mossy Fibre Versus the Climbing Fibre Input. The very dissimilar nature of the climbing fibre and mossy fibre inputs to the cerebellum has caused much speculation on what their relative significance is; the present studies do not provide an answer to this question, but it seems worthwhile to list current conjectures and to comment on one of the findings in the present studies. Current hypotheses are: a) The timing mechanism hypothesis of Braitenberg. When a synchronised volley of parallel fibre activity reaches a Purkinje cell which is also being simultaneously activated by a climbing fibre the cell fires (Braitenberg, 1961, 1962, 1965). Physiological experiments have shown that whenever the climbing fibre is active it

fires the Purkinje cell so this mechanism is probably not feasible, although the parallel fibre system could still perform a timing function.
b) A read-out mechanism (Eccles, Llinás, Sasaki and Voorhoeve, 1966; Eccles, Llinás and Sasaki, 1966f). The climbing fibre is the only input which can break through the inhibitory silence imposed on the Purkinje cell by basket and stellate cells following Loc activation. The activation of the climbing fibre gives rise to between one and four spikes depending on the depth of inhibition and so the state of the cell as set up by the parallel fibre system is read out by the climbing fibres. As suggested above the inhibition probably modulates the background activity of the cell rather than imposing a total silence and under these conditions the "read out" following climbing fibre activation might be difficult to see.
c) The mossy and climbing fibres form independent systems which do not operate in conjunction but merely share a common target cell. The mossy fibre is responsible for a tonic cerebellar out-

put and the climbing fibre for a phasic output (Llinás, et al, 1968). d) A Purkinje cell reset mechanism. The state of polarization in a Purkinje cell set up by a previous barrage of mossy fibre activity is erased by a climbing fibre input. (Harmon, Kado and Lewis, 1968). e) A learning mechanism. The climbing fibre has a reinforcing or conditioning role in conjunction with the state of activity established by the mossy fibre system (Szentágothai, 1968).

In the earlier part of this Chapter it was suggested that in the alligator the mossy fibre input gave rise to a tonic Purkinje cell output, providing that the pattern of input activity was largely uncorrelated and of a relatively low density. This would be accompanied by purely passive dendritic processes. As the mossy input became more synchronised and dense the cell would be expected to switch to a phasic mode of output activity corresponding to the production of spikes in the dendrites. Under these circumstances, the output of the Purkinje cell would be very similar to that which might be produced by climbing fibres. So finally one could conclude that, in the alligator,

a tonic output would be caused only by mossy fibres, whilst a phasic output could be produced by either a mossy or climbing fibre input.

5.4 Concluding Remarks. This Chapter has presented arguments for believing that complex interactions can occur in Purkinje cell dendrites. Do such interactions occur under normal circumstances? Should the Purkinje cell dendrites be regarded as the real centre of the information processing capability of the cerebellum? Or, despite its potentiality for complex activity, does the cell in reality behave in a rather simple manner? If this were the case, then the basic information processing unit would have to be looked for in a network of neurones and the complexity would be found in the properties of such a network rather than in any constituent element. Finally, one cannot rule out the possibility that as regards the input-output relationship of the Purkinje cell it may be immaterial whether a passive or active process occurs, the effects could be functionally indistinguishable.

The experiments presented here do not throw light on these questions; for this it will be essential to study the cerebellum under far more natural conditions. The present work does, however, form the ground-work for these much more sophisticated experiments and provides guidelines for asking some important questions.

APPENDIX A

ANATOMY OF THE ALLIGATOR CEREBELLUM

A.1 Morphology

The brain of the alligator is rather sizable, the main bulk being contributed by the forebrain. The cerebellum is situated on the dorsal aspect of the brain, caudal to the mesencephalon or optic tectum, and curves backwards from its point of attachment to enclose the cerebellar ventricle and a part of the fourth ventricle, the closure being completed by the tela choroidea (Figure 27).

A.1-1 The Cerebellar Cortex. Two fissures can be observed on the external surface of the cerebellum, the fissura prima and the fissura secunda (Figure 28). In Caiman sclerops the fissura secunda is always deep (as has been reported also in the crocodile) (Larsell, 1967). These fissures divide the cerebellum into three lobes; anterior, medial and posterior. Larsell considered that these lobules may be most closely homologized with those of the birds as would be expected from the phylogenetic proximity of these two groups of animals.

The work of Larsell on the systematization of cerebellar morphology in a wide variety of animals enabled him to put the anterior lobule of the alligator into correspondence with lobules I to V of mammals, the medial lobule into correspondence with lobules VI to VIII and the posterior lobule into correspondence with lobules IX to X together with a part of the paraflocculus (Figure 29).

Several authors have called this region the auricle of the crocodilia, by analogy with the structure found in fish and amphibians, but Larsell (1932) suggests that the term flocculus be used in those animals where only the static vestibular projection is present, as is the case with the alligator which, in common with other reptiles, has no lateral line system.

Larsell (1932, 1967) suggested that the cerebellar cortex of the alligator, and several other reptiles, could be divided into a medial and lateral portion and that the relative sizes of these regions reflected the importance of trunk versus limb musculature in different species. Evidence for such a division of the alligator cerebellum was obtained by Goodman and

Simpson (1960) using electrical stimulation of the cortex in unanesthetized and unrestrained animals. The stimulation resulted in a stereotyped, rather complex pattern of limb and trunk movements, one pattern being evoked from the medial two-thirds of the medial lobe and medial half of the posterior lobule, and a mirror image of the pattern being produced by stimulation of the lateral third of the anterior lobule and the lateral third of the medial and posterior lobules. Larsell's hypothesis that limb musculature was represented laterally and trunk medially was not supported, however, since both patterns of activity involved the same types of limb and trunk movements. In these experiments no indication of a somatotopic localization was found; however, it must be kept in mind that it is difficult to assess how large an area of the brain is stimulated under such experimental conditions.

In mammals the cerebellum is usually divided into a medial zone, the vermis, an intermediate zone and a lateral zone; the latter two regions constituting the cerebellar hemispheres. In higher animals the lateral region is extensively developed but this is

not the case in the alligator where the homology should be between the medial region of the alligator and the mammalian vermis and between the lateral region of the alligator and the intermediate zone of the mammal.

A further gross morphological distinction between the alligator and the mammal is that the cerebellum of the latter is usually highly convoluted and folded into numerous folia in contrast to the simple morphology of the alligator.

The three characteristic layers of the cerebellar cortex are found in the alligator with the molecular layer forming the outermost stratum (Figure 28). In some reptiles the cerebellum tilts forward and the granular layer becomes uppermost, a fact which indicates the wide morphological variation amongst reptilian cerebella (Nieuwenhuys, 1967). A detailed description of the histology of the cerebellar cortex will be given in Section A.3.

A.1-2 The Deep Cerebellar Nuclei. Two nuclei are found in the central cerebellar white matter of the alligator and are named the medial and lateral cerebellar

nuclei. The lateral nucleus is found close to the superior vestibular nucleus (Figure 30). Larsell (1967) regarded the medial nucleus of the alligator as homologous with the fastigial nucleus of mammals and the lateral nucleus as homologous with a part of the intermediate or interpositus nucleus. In this connection it is to be noted that the lateral nucleus of the alligator is not homologous with the lateral nucleus found in higher animals. In mammals the nuclei become increasingly complex with phylogenesis. In all of them, however, there are three major structures, the fastigial, the intermediate or interpositus and the lateral nuclei, in that order, bilaterally from the midline. In the primates the lateral nucleus becomes the most prominent of the three and is more usually called the dentate nucleus.

In man the dentate nucleus is extremely large whilst the intermediate nucleus splits into two parts - the nucleus globosus and the nucleus emboliformis.

Kornieliussen (1968) has suggested (after studying cetacea and rat) that the intermediate nucleus in all mammals can be split into anterior and posterior

nuclei. This same author also advocates a more extensive system of lateral divisions in the mammalian cerebellar cortex.

A.2 Hodology

The few studies which have been made on the fibre tracts related to the cerebellum of the alligator have relied almost exclusively on normal material. Recently the Nauta technique has been employed in the lizard and on the spinal column of the alligator (Golby and Robinson, 1962; Ebbesson, 1967; Joseph and Whitlock, 1968a); these results will be discussed where appropriate. As a whole our knowledge of the afferent and efferent systems of the cerebellum in the alligator lacks precision, although most of the main pathways are probably known.

The Afferent Systems

A.2-1 The Vestibulo-cerebellar Tract. This contains both primary fibres from the vestibular root and secondary fibres from the vestibular nuclear complex. There is little agreement on the nuclei of origin of the secondary fibres. Larsell (1967) claimed that they originated in the superior vestibular nucleus,

Weston (1936) favoured the dorsolateral vestibular nucleus and the ventrolateral (Deiter's) vestibular nucleus, whilst Ariëns Kappers, Huber and Crosby (1936) regarded only the latter nucleus as the origin of the secondary vestibular fibres.

The fibres of the vestibulo-cerebellar tract terminate in the corpus (but not the anterior lobe) (Larsell, 1967), the flocculus and the medial nucleus of the cerebellum (Nieuwenhuys, 1967). Weston (1936) claimed that the direct fibres terminate mainly but not wholly in the auricular lobe and Larsell (1932) claimed a small bundle of vestibular fibres terminate in the contralateral flocculus.

In mammals both primary and secondary vestibular fibres are also found but the secondary fibres greatly predominate over the primary. The primary fibres again originate in the vestibular root fibres but the origin of the secondary fibres is still not known with complete certainty, although the best evidence currently available is that the nucleus inferior (descendens) and nucleus medialis are the origin of the secondary fibres (Larsell, 1967; Brodal, 1954). If this is so then the origin of the secondary fibres in mammals differs

from that in the alligator since the above mentioned nuclei are not homologous with any of the proposed origins in the alligator (Larsell, 1967). Some older work on the vestibulo-cerebellar tract suggested Deiter's nucleus and the nucleus of Bechterew as the origin of the secondary fibres in mammals, but this has not been substantiated (Brodal, 1954). The termination of the vestibular fibres in mammals is known with more certainty and is mainly in the flocculo-nodular lobe, the posterior region of lobules IX and X and lobule I. In addition, the fibres go to the fastigial nucleus, paralleling the projection to the medial nucleus of the alligator. Secondary vestibular fibres distribute to both sides of the cerebellum, primary fibres remain homolateral (Brodal, 1954; Bell and Dow, 1967).

Thus, apart from a possible and curious difference in the nuclei of origin of the secondary vestibular fibres, there is considerable similarity in the vestibulo-cerebellar projection in both alligator and mammal, perhaps because this is one of the oldest afferent systems of the cerebellum.

A.2-2 The Cochleo-cerebellar Fibres. Like the vestibulo-cerebellar fibres, there is both a primary and secondary component. The primary fibres come from the cochlear nerve whilst the secondary fibres originate in the nucleus laminaris (Weston, 1936). The nucleus laminaris varies greatly in different groups of reptiles and Larsell (1967) believed that its size might be related to the acuteness of hearing in the animal. It is to be noted, however, that the only path so far identified (in mammals) with the sense of hearing is a tecto-cerebellar path (Larsell, 1967) and that Weston (1936) commented that it was not certain if the so-called cochlear fibres were of cochlear origin. That author also noted that direct fibres were very sparse in the alligator compared with other reptiles.

In mammals, cochleo-cerebellar fibres have never been convincingly demonstrated (Brodal, 1954) and, although a nucleus laminaris is found in birds, it is not present in mammals (Larsell, 1967).

A.2-3 The Spino-cerebellar Tracts. A ventral spino-cerebellar tract (VSCT) has been identified by several authors in the alligator. This tract, after crossing over in the inferior cerebellar commissure,

ends in the cerebellar cortex of the contralateral side and according to Weston (1936) also in the homolateral medial nucleus although Larsell (1967) disputes the latter finding. A recent Nauta study in the tegu lizard (Tupinambis nigropunctatus) has thrown some more light on the termination of this tract in reptiles (Ebbesson, 1967). There the degeneration fragments were found everywhere except in the flocculus, but they predominated on the ipsilateral and not the contralateral side. Some degeneration was also seen in the deep nuclei. Ebbesson also pointed out that in the lizard the VSCT was the largest spino-cerebellar projection. Weston claimed, however, that the spinal projection in the alligator was less massive than in other reptiles such as the turtle (Weston, 1936).

The dorsal spino-cerebellar tract (DSCT) has been identified with much less certainty than the VSCT in reptiles. Weston claimed that such a tract existed and ended laterally and caudally with a possible projection to the homolateral auricular lobe. In the lizard, Ebbesson (1967) identified a

DSCT and mentioned that a few fibres from this tract ended in the lateral vestibular nucleus. An earlier Nauta study on the lizard (Lacerta viridis) by Golby and Robinson (1962) failed to reveal a DSCT.

In mammals, both VSCT and DSCT have been extensively studied. The DSCT arises in Clarke's column and the VSCT arises in the lateral part of the base and neck of the dorsal horn. It has recently been shown that a nuclear column, apparently homologous with Clarke's column, is present in the alligator (Joseph and Whitlock, 1968a).

It is now known that these tracts project from the hind limb region and end predominantly in the anterior lobes of the cerebellum (I to V) with a small amount of termination in the posterior lobes (Oscarsson, 1965; Bell and Dow, 1967). There is evidence that the DSCT carries information about single muscles or small areas of skin, whilst the VSCT conveys more general information concerning the whole limb (Bell and Dow, 1967).

In addition to the VSCT and DSCT, two other spino-cerebeller tracts are known in mammals--the rostral spino-cerebellar tract (RSCT) and the cuneo cerebellar tract (CCT). The RSCT arises from cells

rostral to Clarke's column and is the forelimb equivalent of the VSCT, and the CCT arises from the external cuneate nucleus and is the forelimb equivalent of the DSCT. The areas of termination are somewhat similar to the hindlimb tracts (Oscarsson, 1965).

A gracilo-cerebellar tract has been identified on physiological grounds in mammals, but little is known about it (Oscarsson, 1965).

All the above mentioned tracts have an intermediate neurone in the spinal colum but recently a direct projection from the dorsal root to the cerebellum has been demonstrated in the frog (Joseph and Whitlock, 1968b): such a pathway has not been found in reptiles (Ebbesson, 1967; Joseph and Whitlock, 1968a).

A.2-4 The Trigemino-cerebellar System. Direct and secondary fibres are found in this system. Direct fibres originate with the trigeminal nerve but are much less numerous in the alligator than in other reptiles (Weston, 1936). Secondary fibres arise in the chief sensory nucleus of the V nerve and pass

to the homolateral part of the cerebellum. Other fibres arise in the spinal nucleus of the V nerve passing to the contralateral side of the cerebellum in this case (Larsell, 1967; Weston, 1936). Weston also claimed that the chief sensory nucleus of the V nerve contributes to the crossed component and Larsell (1967) considered that a contribution arose from the somatic sensory nucleus of the IX and X nerves and that the trigemino-cerebellar system ended in the medial cerebellar lobe of the alligator.

Brodal (1954) stated that direct trigeminal fibres are very scarce in mammals. Secondary fibres have been traced from the nucleus of the spinal tract and chief sensory nucleus of the V nerve, as in the alligator. Fibres have also been observed from the radix mesencephalica of the V nerve; they end in the interpositus and emboliform nuclei and possibly also in the dentate nucleus in the human (Brodal, 1954).

A.2-5 The Tecto-cerebellar Fibres. The tecto-cerebellar fibres arise in the deep fibre layers of the optic tectum (Kawakami, 1954) and pass via

crossed and uncrossed fibres to the cerebellum where the crossed fibres terminate in the medial nucleus and cerebellar cortex, possibly as climbing fibres (Weston, 1936). Weston also believed that there was a contribution from the colliculus to this tract and suggested that there might also be isthmo-cerebellar fibres.

In mammals, tecto-cerebellar fibres have been described as arising in the inferior colliculus and are joined by other fibres from the mesencephalic root of the V nerve. Most of the fibres then cross over in the anterior medullary velum. The termination of this system is not known but it has been suggested that the tecto-cerebellar fibres mediate the auditory and visual projection to the cerebellum (Brodal, 1954) and if so the termination should be in lobules VI, HVI and VII (Dow and Moruzzi, 1958, Ch. 4; Fadiga and Pupilli, 1964).

A.2-6 The Reticulo-cerebellar Fibres. Weston (1936) includes a reticulo-cerebellar component amongst his nucleo or bulbar-cerebello fibres; he is not explicit

as to the origin of these fibres but he claims that they join the ventral superficial arcuate fibres. Ebbesson (1967) has recently shown in the lizard that there is a large spinal projection to the lateral reticular nucleus and thence to the cerebellum.

In mammals three cell groups of the reticular formation project to the cerebellum. These are the nucleus reticularis lateralis (nucleus funiculi lateralis), the nucleus tegmenti pontis of Bechterew and the paramedian reticular nucleus (Brodal, 1954).

A.2-7 The Olivo-cerebellar Fibres. There is some doubt as to whether or not an olive exists in the alligator. Larsell (1967) believed that it did but admitted that the cells were diffusely scattered and Weston (1936) acknowledged a cell mass in the region where the olive would be expected, but claimed that it could be a reticular nucleus. If such a cell mass is regarded as an olive, then the fibres arising from it accompany the spinocerebellar system to the cerebellum.

The olivo-cerebellar connections have been

studied in some detail in the mammal and a fairly precise somatotopic correspondence found between afferents which project to the cerebellum directly and those which go via the olive. One exception to the orderly convergence is found in the caudate nucleus--olivary cerebellar projection which ends in the floccular lobe of the cerebellum, that is the region in which vestibular fibres terminate (Walberg, 1954). It is now known that the olive is a primary (and possibly the only) source of climbing fibres in the mammal, all other afferents ending as mossy fibres (Szentágothai and Rajkovits, 1959; Eccles, Ito and Szentágothai, 1967, Ch. 2). Consequently, the olivo-cerebellar projection has aroused considerable interest in attempts to discover the relative significance of the climbing and mossy fibre input to the cerebellum. It may be noted, however, that Llinás and his co-workers (Llinás, Precht and Kitai, 1967) have shown that some of the direct vestibular fibres of the frog end as climbing fibres. No olive has been found in this animal.

A.2-8 The Ponto-cerebellar System. In man and higher animals, this is quantitatively the most significant afferent system, but it is wholly absent in the alligator. The absence is explicable because the majority of afferents to the pontine nuclei are from the cerebral cortex and this structure undergoes great development in higher animals. The pontine projection terminates in most parts of the mammalian cerebellum but predominantly in the lateral regions. In mammals, cerebellar afferents also arise in the para-hypoglossal nuclei and in the red nucleus (Brodal, 1954).

The Efferent Systems

In mammals the majority of cerebellar efferents originate in the deep cerebellar nuclei and only a few pass directly from the cerebellar cortex to other regions of the brain, and this probably holds true for the alligator. Thus, it becomes necessary to discuss the projection of the cerebellar cortex to the deep nuclei before describing the efferent system proper.

A.2-9 The Cortico-nuclear Projection of the Cerebellum.

No studies have been made on this projection system in the alligator so that it is necessary to rely on data from mammals.

As mentioned in Section A.1-2, the nuclei in mammals are three in number (four in man) arranged laterally beneath the cerebellar cortex. Jansen (1954) stated that the cortical projection to the nuclei is a rather orderly one. The most medial part of the cerebellum (the vermis) projects to the fastigial nucleus, the intermediate part of the cortex to the intermediate nucleus, and the most lateral cortex or hemisphere to the lateral nucleus. Such an organisation would explain the ever-increasing development of the lateral nucleus with the great extension of the hemispherical parts of the cerebellum in primate and man. Some doubt has been thrown on whether such a strict mapping exists (Eager, 1963a) but Walberg and Jansen (1964) in further studies reiterated that such an orderly mapping is essentially correct. Since the alligator cerebellum contains two nuclei, one might conjecture

that medial and lateral parts of the cortex project to them respectively.

Stefanelli (1943) noted that reptiles which relied on trunk musculature for movement (snakes, for example) had only a single cerebellar nucleus whilst in those animals which used lateral limbs two nuclei could be seen. This line of argument would suggest lateral parts of the cerebellum were concerned with limbs, and medial parts with the trunk, as postulated by Larsell; but the work of Goodman and Simpson (Section A.1-1) throws doubt on this.

A.2-10 The Brachium Conjunctivum. This tract is also referred to as the tractatus cerebello-tegmentalis mesencephali by Huber and Crosby (1926) and Kawakami (1954). The tract originates in the deep central gray matter of the cerebellum of the alligator, decussates and passes to the red nucleus and adjacent tegmental gray matter (Huber and Crosby, 1926). Another branch of the tract goes to the occulomotor and abducens nucleus according to Weston (1936).

In mammals, the brachium conjunctivum can be divided into a pars cerebellaris and a pars bulbaris. The pars cerebellaris originates in the homolateral intermediate and lateral nuclei and the contralateral fastigial nucleus. The tract then splits and probable regions of termination are the reticular formation, the red nucleus, occulomotor nuclei, Edinger-Westphal nucleus and thalamus. The most certain of these connections is the one with the red nucleus (Jansen, 1954).

A.2-11 The Tractus Cerebello-vestibularis and Cerebello-spinalis. This tract originates in the medial nucleus of both sides of the cerebellum and also contains a direct Purkinje cell component (Weston, 1936). The tract terminates partly in the nuclei of the vestibular complex and the region of the adjacent reticular gray matter and partly in the spinal column. The spinal component is probably joined by fibres from the pars dorsalis of the nucleus vestibularis ventrolateralis (Weston, 1936).

Weston claimed that the above tract was analogous to the uncinate fasciculus of Russel, found

in mammals. This tract originates in the fastigial nucleus and proceeds to the region of the vestibular nuclei and the reticular formation of the pons and medulla. It is believed that the specific termination is in the superior, lateral (Deiter's) and descending vestibular nuclei, dorsal part of the pontine reticular formation and the lateral reticular formation of the medulla. It has also been claimed that some fibres continue to the cervical region of the spinal cord (Jansen, 1954).

A.2-12 The Tractus Cerebello-motorius and Cerebello-tegmentalis. According to Weston (1936), this tract originates in the homolateral medial and lateral cerebellar nuclei and in addition, contains a direct Purkinje cell component. Some contralateral fibres also enter the tract. The cerebello-motorius component is strictly related to the motor nuclei of the cranial nerves, whilst the cerebello-tegmental component distributes to the more highly specialised gray matter of the tegmental region; Weston (1936) divides both these components into bulbar and mesencephalic parts. The tracts end in the reticular

gray, the III and IV nerve nuclei, the tegmental gray, the medial longitudinal fasciculus, the region of the primitive red nucleus and the V nerve motor nucleus (Weston, 1936; Huber and Crosby, 1926). Weston considered that some claim might be made for a mesencephalic component of the tractus cerebello-motorius and tegmentalis being analogous to the brachium conjunctivum of higher forms. He suggested that the tegmental component of the projection is the forerunner of the cerebello-rubro-thalamic path of higher forms. On these grounds, the mammalian equivalent may be considered to be a part of the brachium conjunctivum discussed in Section A.2-10.

A.2-13 The Cerebello-tectal System. Weston (1936) claimed that there is a projection from the Purkinje cells of the cerebellum to the periventricular fibre layer of the tectum and possibly the inferior colliculus in the alligator. Such a projection is not recorded in mammals.

A.2-14 The Cerebello-thalamic Projection. No such tract is found in the alligator (Larsell, 1967). In mammals, there is a cerebello-thalamic projection

within the brachium conjunctivum, originating in the dentate nucleus. The exact nuclei of termination are not well known but the principal terminal nucleus is the ventral thalamic nucleus (Jansen, 1954).

A.2-15 The Intra-cerebellar Connections. Larsell (1967) claimed that fibres connected the medial and posterior lobes of the alligator cerebellum, linking the granular layers of the two regions, but nothing more is known about such connections.

In mammals, the question of the so-called association fibres has arisen from time to time and was studied in detail by Eager (1963b) using cats. He found such pathways did not usually extend more than 2 or 3 folia and that the most extensive fibre systems were between the medial simplex lobule and the adjacent paravermal regions and hemispheres, and between the paramedian lobule and adjacent regions of crus II and hemispheres. The most likely origin of such connections are the axon collaterals of Purkinje cells.

A.3 Histology

It has long been recognised that the elements of the cerebellum form a unique ensemble. A strict and three dimensional pattern of geometrical relationships exists between axons and dendrites of the various cells found therein, and concomitant with this is an unusual specialisation of some of the elements. These facts have led to many attempts to quantify the histological relationships found in the cerebellum. Such quantification has been performed exclusively in mammals so that it will be necessary to discuss this data at some length, although this thesis is concerned with the alligator. Indeed, it is the mammalian cerebellum which has always received the most attention from histologists, although Cajal studied many of the lower animals as well. Recently Hillman has begun a continuing series of light and electron microscopic investigations of Caiman sclerops and the information to be presented on this animal is based on Hillman's studies.

A.3-1 The Cell Layers of the Cerebellum. In sagittal sections through the cerebellum of the alli-

gator, with suitable staining techniques, four layers of elements are visible. The outermost layer, called the molecular or plexiform layer, contains the dendrites of Purkinje cells (characteristically flattened), the dendrites of stellate cells, likewise flattened, together with the perikarya of these cells, the diffusely spreading dendrites of the Golgi cells and the terminal ramifications of the afferent climbing fibres. Passing into the plane of section in enormous numbers are the minute parallel fibres--the axons of granule cells. Below the molecular layer is found the ganglionic layer or layer of the Purkinje cell bodies. There lie the somas of Purkinje cells and immediately above and below the cell bodies, numerous fibres, which may be collaterals of the descending Purkinje axons. Beneath the ganglionic layer is the granule cell layer. This is a deep, densely packed mass of the minute granule cells interspersed here and there with Golgi cell bodies and structures known as "cerebellar islands", containing the glomeruli which are specialised synaptic structures peculiar to the cerebellum. Within this layer also are

found the diffuse axons of the Golgi cells and the terminal ramifications of the afferent mossy fibres. Finally, in the fourth layer of the cerebellum, the innermost medullary layer, a mass of afferent and efferent fibres is found. The depth of the molecular layer is about 400 to 450μ; the ganglionic layer has a thickness of about 50μ and the granular layer is rather variable with an average depth of about 300μ. Having located the principal elements of the cerebellum, a detailed description of these elements and their relationships will now be given. Figure 31 summarises the neuronal circuitry.

A.3-2 The Purkinje Cells. The Purkinje cell is the focal point of the cerebellum. Structurally it is one of the most amazing nerve cells known, whilst functionally it is the "final common path" of neural action in the cerebellar cortex since this cell furnishes the only output of the cortex. In the alligator the cell has a flask-shaped soma from the top of which a single, thick, dendrite emerges and heads towards the surface. After a short distance, the primary dendrite divides into

three or four second order branches which soon divide into third, fourth and even fifth order branches (Figure 22). This arborescence is very much confined to a single plane (sagittal in the alligator). All the dendrites are rather straight and the majority oriented towards the surface. A few branches may, however, turn round and descend to fill the space adjacent to the primary branch and the descending branch may occasionally dip below the level of the soma. The tertiary and higher order branches are densely covered with spines whilst the primary and secondary branches have only occasional spines. At the base of the Purkinje cell soma the axon emerges, often rather obliquely and descends through the granule cell layer to terminate in the deep cerebellar nuclei or pass directly out of the cerebellum to other centres of the brain. It is probable that this axon gives off collaterals shortly after leaving the cell body, but this has not yet been shown with certainty in the alligator.

The Purkinje cell in mammals has the same basic features as those of the alligator, notably the extensively arborizing and spine laden dendritic tree, flattened in a particular plane. The cerebellum of mammals, however, is convoluted and split up into many folia and the Purkinje cell tree is always oriented in the transverse plane of the folium. The Purkinje cell dendrite in mammals aquires a new feature, the spiny branchlet. The tree branches in a manner similar to that in the alligator, but the branches are less straight and from the higher order branches a multitude of thick, spine laden branchlets emerge, thus enormously increasing the total dendritic length (Cajal, 1911; Fox and Barnard, 1957; Fox, Hillman, Siegesmund and Dutta, 1967). Fox and Barnard have estimated that the total dendritic length is about 40 mm in the monkey and that this dendrite supports between 60,000 and 120,000 spines. The spines on the primary and secondary branches are rather sparse and are contacted by the climbing fibre whilst the rest of the dendritic spines are reserved for parallel fibre contacts. The dendritic

tree in the cat extends laterally for about 300μ, vertically 225μ and has a thickness of between 6 and 8μ. It is separated from the next tree by a distance of about 2μ (Eccles, Ito and Szentágothai, 1967, Ch. XI). Braitenberg and Atwood (1958) estimated that there were 300 Purkinje cells per mm^2 in the ganglionic layer in man; these same authors estimated a total cerebellar area of 50×10^3 mm^2 and thus the whole populate of Purkinje cells would be fifteen million. The soma in the cat has a minimum diameter of about 30 to 35μ and from the lower part of the flask-shaped body a thick axon emerges which becomes myelinated after a distance of some 30μ (Eccles, Ito and Szentágothai, 1967, Ch. I and XI). As the axon descends, it gives off collaterals which ascend obliquely through the granular layer to form the infraganglionic plexus just below the level of the Purkinje soma and the supraganglionic plexus just above (Cajal, 1911).

The lower plexus is predominantly orientated in a transverse direction (perpendicular to the direction of the parallel fibres) whilst the upper

plexus extends in a longitudinal direction (Cajal, 1911; Eccles, Ito and Szentágothai, 1967, Ch. IX). The collaterals terminate on the dendrite and somas of Golgi cells and on the somas of basket cells (Hámori and Szentágothai, 1968). Purkinje collaterals may also terminate on stellate and Purkinje cells, but the evidence for the latter statement is mainly physiological (Eccles, Ito and Szentágothai, 1967, Ch. IX; Hámori and Szentágothai, 1968).

The dendrites of the Purkinje cells in both alligator and cat receive inputs from three sources: the climbing fibres, the parallel fibres and the stellate cell axons.

A.3-3 The Climbing Fibre. This forms one of the two inputs to the cerebellum and its synaptic relationship with the Purkinje cell is of an unusual form. The climbing fibre enters the cerebellum and loses its myelin sheath at the level of the Purkinje cell soma in the cat (Eccles, Ito and Szentágothai, 1967, Ch. IX), then it proceeds to ramify greatly to achieve a spatial distribution matching that of the dendrite of a single Purkinje

cell. The climbing fibre and Purkinje cell synapse at multiple contacts with the primary and secondary branches of the cell. The synapses occur on spine projections from the branches in the alligator (Hillman, 1968). A similar relationship holds in the mammal (Cajal, 1911; Uchizono, 1967) but there are no synapses with spiny branchlets.

It is generally held that each climbing fibre only contacts a single Purkinje cell (Cajal, 1911), but recently it has been shown that in man the number of cells in the inferior olive is only one-fifteenth the number of Purkinje cells (Moatamed, 1966; Escobar, Sampedro and Dow, 1968). At the present time it is believed that all climbing fibres arise in the inferior olive in higher animals (See Section A.2-7); so that (a) a discrepancy may exist in the quantitative histological studies, (b) climbing fibres may contact several Purkinje cells, (c) some Purkinje cells may have no climbing fibre input, and (d) there may be another source of climbing fibres.

In mammals the climbing fibres give off side branches (Scheibel and Scheibel, 1954) which contact the dendrites of Golgi cells, the dendrites

and somas of basket cells and probably the stellate cells (Hámori and Szentágothai, 1966a).

A.3-4 The Granule Cells and the Parallel Fibres.

The second input to the cerebellum reaches the Purkinje cell dendrite by a more circuitous route than the climbing fibre. Its manifestation at the Purkinje cell is the parallel fibre synapse. The parallel fibres are the axons of the granule cells, the somas being situated in the granule cell layer (Figure 32,33). From the somas of the granule cells a thin vertical fibre projects through the ganglionic layer to the molecular layer where it bifurcates in the form of a T-shaped junction. Each branch of the junction proceeds longitudinally down the molecular layer dilating frequently to form vesicle filled bulges into which the spine of the higher order Purkinje cell branches and of the dendrites of the basket (in mammals), stellate and Golgi cells invaginate (Hillman, 1968).

In mammals the diameter of the parallel fibres varies between 0.2 and 1μ, the diameter decreasing as the molecular layer is ascended (Fox and Barnard,

1957; Fox, Siegesmund and Dutta, 1964; Cajal, 1911). The length of the parallel fibres is about 1-1.5 mm on either side of the T-junction in the monkey (Fox and Barnard, 1957) and 0.5-5.0 mm in man (Braitenberg and Atwood, 1958). Parallel fibres occupy 60% of the space in the molecular layer (the other constituents are glia 20%, Purkinje cell dendrites 15%, other dendrites and axons 5% (Eccles, Ito and Szentágothai, 1967, Ch. XI)). Fox and Barnard (1957) calculated that there must be between 208,000 and 278,000 parallel fibres crossing each Purkinje cell in the monkey and probably at least half of this number must synapse with a given Purkinje cell. These authors suggested that a 3 mm long parallel fibre would traverse 450 Purkinje cells and make contact with 300 of them. The granule cell soma is about 5-8μ in diameter in the cat and has 3 to 5 dendrites extending 10-30μ from the body (Eccles, Ito and Szentágothai, 1967, Ch. I). The same also appears true for the alligator (Hillman, 1968). Because of the small size of the granule cells, it is possible to pack vast numbers into the granule

cell layer and it has been estimated that there are between 3 and 7 million per mm^2 in man, giving a total population of between 10^{10} and 10^{11} (Braitenberg and Atwood, 1958). The dendrites of granule cells end in a claw-like structure which forms part of a synaptic complex, the glomerulus, to be described later.

A.3-5 The Stellate Cells. Electrophysiological studies have shown that both the climbing and parallel fibres form excitatory synapses with Purkinje cells. There is, however, a third set of synaptic contacts on the dendrites of Purkinje cells in the alligator and these are inhibitory. The synapses are formed from the axons of stellate cells whose somas are found in the molecular layer of the alligator (Figure 32). The axons extend transversally with respect to parallel fibres, that is, in the plane of the Purkinje cell dendrites. Contacts are made with the primary, secondary and tertiary branches of the Purkinje cell, but rarely on the soma in the alligator (Hillman, 1968). The dendrites spread in the molecular layer in the spaces

between Purkinje cell dendrites; their dendrites are also flattened but lack the complexity of the Purkinje cell. The stellate cell receives its synaptic input from the parallel fibres.

Stellate cells are also found in mammals where they predominate in the centre two-thirds of the molecular layer (Cajal, 1911; Eccles, Ito and Szentágothai, 1967, Ch. I). Smirnow (1897) has divided them into several types in the cat as follows: Type a_1—a characteristic Golgi type II cell with very delicate and densely ramifying axons in the neighbourhood of the cell. The axonal terminal branches mainly descend. Type a_2—a somewhat larger cell than the a_1 type and also found deeper in the plexiform layer; it has both descending and ascending axons. The descending component may sometimes reach the axon hillock of a Purkinje cell, but goes no lower. Type b—this cell has a horizontal axon, running up to 900μ from the cell body, in a strictly transverse plane, terminal branches ascend or descend. The cell is quite small and found close to the surface. The axons of mammalian stellate cells contact

primary and secondary Purkinje cell branches. Some synapses are seen on the cell bodies of stellate cells and these may originate from climbing fibre collaterals or Purkinje axon collaterals (Hámori and Szentágothai, 1966a).

The stellate cell synapses complete the input to the Purkinje cell of the alligator but in mammals there is a fourth synaptic input functionally similar, but morphologically distinct from the stellate cells--this is the basket cell. The absence of this cell is an important difference between the histology of the alligator, on the one hand, and the birds and mammals on the other. Therefore the basket cell will be described.

A.3-6 The Basket Cells of Birds and Mammals. This cell occurs in the lower half of the molecular layer. It is about 15-20% more numerous than the Purkinje cell and its dendrites ramify within the molecular layer with a similar extent and flattening to those of the Purkinje cell. They lack, however, the characteristic Purkinje cell dendritic pattern (Cajal, 1911). The axons of these cells have a

highly characteristic form. They spread laterally for a distance of about 1 mm (equivalent to the distance occupied by 10 Purkinje cell somas) at the level of the top of the Purkinje cell soma. During its course, the main part of the axon gives off side branches in a longitudinal direction having an extent equivalent to the spacing of three or four Purkinje cells. From these side branches ascending and descending branches emerge. The destination of the ascending branch is not known, but it may terminate on Purkinje cell dendrites (Cajal, 1911). The descending branches make their way to the base of the Purkinje cell body where a sequence of brush-like endings begin (Cajal, 1911). These endings extend down to inclose the initial part of the axon of the Purkinje cell and form extensive synaptic contact in this region. One Purkinje cell may be contacted by 20-30 basket cells (Szentágothai, 1965a) and the total effect is a basket-like formation around the base of the cell. Electron microscopy has revealed that this synapse is very extensive and

that the neighbouring Bergmann glia also intimately participate in this ending (Palay, 1964; Eccles, Ito and Szentágothai, 1967, Ch. VI and Ch. XI). Estimates have been made of the number of Purkinje cells contacted by a single basket cell and give a figure of between 70 and 216 cells contacted (Eccles, Ito and Szentagothai, 1967, Ch. VI). Physiological studies have shown that basket cells, like stellate cells, inhibit the Purkinje cell.

A.3-7 The Golgi Cells. (Figure 33). In addition to the stellate cells, a second type of inhibitory mechanism exists in the alligator cerebellar cortex. This is the Golgi cell. These cells once again receive most of their input from the parallel fibre system, but distribute their axons, not to the Purkinje cells, but back to the granule cells. Golgi cells are almost always found in the granule cell layer and their population is only about 10% of that of the Purkinje cells in the cat. The dendritic tree extends into the molecular layer where it ramifies in all directions, in contrast to the flattened trees of the other dendrites in this region. The tree is rather sparse and is contacted

by parallel fibres (Cajal, 1911). In the cat, some dendritic branches of the Golgi cells apparently descend in the granule cell layer and receive a direct input from the mossy fibres (Cajal, 1911; Hámori and Szentágothai, 1966b). Some doubt has, however, been thrown on this finding by Fox and his colleagues (Fox, Hillman, Siegesmund and Dutta, 1967) in the monkey. The axons of the Golgi cells emerge into the granule cell layer and ramify enormously in all directions, extending over a volume similar to that occupied by the dendrites.

One cell may give rise to more than one axon (Cajal, 1911). Szentágothai claims that Golgi cells in the cat do not overlap, either axonally or dendritically with neighbouring Golgi cells to any appreciable extent (Eccles, Ito and Szentágothai, 1967, Ch. I). Fox, et al, believed, however, that there is considerable overlap between axonic plexi of neighbouring Golgi cells in the monkey (Fox, Hillman, Siegesmund and Dutta, 1967).

As has been described, the Purkinje, stellate, basket and Golgi cell receive their inputs from the granule cell. It now remains to describe the

input to the granule cell and its mode of synaptic contact.

A.3-8 The Mossy Fibres. The input to the granule cells and the second afferent system to the cerebellum is from the mossy fibres. These fibres enter the region below the granule cell layer and from then on branch many times as they ascend and terminate in the granule cell layer. The fibres remain myelinated for some of their passage in the granule cell layer. In mammals, where the cerebellum is foliated, a single mossy fibre may split up and distribute to more than one folium (Cajal, 1911). Within the folium the fibre makes contact with many glomeruli over a fairly large area. The region of termination of a given mossy fibre has aroused much interest, since it will have a considerable influence on the specificity of the input to a Purkinje cell, but no really conclusive results are available. There is evidence that, in the cat, the mossy fibre of the dorsal spinocerebellar tract (DSCT) terminates in a smaller region than the mossy fibre of the ventral spino-

cerebellar tract (VSCT) (Lundberg and Oscarsson, 1960; Lundberg and Oscarsson, 1962) and that, furthermore, the DSCT terminates more deeply in the granule cell layer than the input from the lateral reticular nucleus (Szentágothai, 1962; Eccles, Ito and Szentágothai, 1967, Ch. II). This latter finding could be significant because it seems that there is a direct relationship between the depth of a granule cell in its layer and the depth of its parallel fibre in the molecular layer (Cajal, 1911). Recently, it has been suggested that mossy fibres send collaterals to the deep cerebellar nuclei (Eccles, Ito and Szentágothai, 1967, Ch. XIII).

A.3-9 The Glomeruli of the Granule Cell Layer. The synaptic contact between mossy fibre and granule cell dendrites takes place in a complex structure known as the glomerulus. The structure also contains the terminals of the Golgi cell axons and those Golgi dendrites which make direct contact with mossy fibres. The mossy fibre ending, or rosette, forms the core of the glomerulus, around this the claw-shaped granule cell dendrites attach. The Golgi

axons contact the granule cell dendrite and the Golgi dendrite contacts the mossy fibre. The Golgi cell inhibition is thus post-synaptic rather than pre-synaptic. This whole complex is enveloped in a glial capsule (Hámori and Szentágothai, 1966b).

In the cat there are, on the average, 4-5 granule cells to one glomerulus and the glomerular diameter is about 20μ. Thus, the glomeruli are less numerous but larger than granule cells. In a single glomerulus, about 20 granule cell dendrites will be found (several from the same cell), but it is probable that only one Golgi cell contributes to a given glomerulus, owing to the dispersion of Golgi cells. A single mossy fibre may contact, via the glomeruli, as many as 800 granule cells (Fox, Hillman, Siegesmund and Dutta, 1967).

This completes the description of the cerebellar elements whose function will be described in the chapters on the physiology of the cerebellum. For completeness, however, there will be a short description of other cell types occasionally described by histologists in the cerebellum, the

cerebellar glia and the histology of the deep nuclei. Before this, an important finding of Uchizono should be mentioned. That author (Uchizono, 1965; Uchizono, 1967) using an appropriate fixation method and electron microscopy, discovered that the vesicles of some cerebellar synapses could be divided into two types--spherical and flattened. Spherical vesicles were found in the parallel fibres and climbing fibre synapses, with Purkinje cells, whilst the flattened vesicles were present in basket cell and Golgi cell axonal synapses. Thus, a correlation was discovered between vesicle shape and synaptic function, spherical vesicles being found in excitatory synapses and flattened vesicles in inhibitory ones. Moreover, excitatory synapses were found only on spines whilst inhibitory synapses occurred on the main dendritic branches or somas of cells. These results were confirmed by Larramendi and Victor (1967) in the mouse.

A.3-10 Other Types of Cells. Cajal (1911), Fox and Bertram (1954) and Fox (1959) describe several

varieties of fusiform cells in the cat, the most distinct being the intermediate cells of Lugaro whose axons distribute in the molecular layer in the same direction as the parallel fibres. Szentágothai (Eccles, Ito and Szentágothai, 1967, Ch. II) also mentions the intercalated cells described by Pensa, deep Golgi type II cells and stellate cells with long axons which descend deeply in the granule cell layer. No evidence has been found to date that these cells, if they exist, play a significant part in the operation of the cerebellum.

A.3-11 The Glia. In mammals, the astroglia of the cerebellum are rather specialised and the predominant type are the Bergmann cells. These are located between Purkinje cells and send two, three, or more long processes to the outer surface of the cerebellum, where they terminate in conical end feet beneath the pia mater (Sotelo, 1967; Fox, Siegesmund and Dutta, 1964; Fox, Hillman, Siegesmund and Dutta, 1967). These cells encapsulate the Purkinje cells and their dendrites. In the lower regions of the molecular layer, another form of

astroglia, the Fañanás cells, are found. These are similar to Bergmann cells but their processes do not reach as far as the pia mater. The astroglia are believed to aid the metabolism of the Purkinje cells and may contribute to the synaptic organisation by insulating parts of the dendritic tree from accidental synaptic contact (Eccles, Ito and Szentágothai, 1967, Ch. XI).

Oligodendroglia seem to be found throughout the cerebellum (Sotelo, 1967) and Fox, et al, suggested that one role for these cells is in association with supra- and infraganglionic plexi (Fox, Hillman, Siegesmund and Dutta, 1967).

A.3-12 The Deep Cerebellar Nuclei. The deep nuclei of the cerebellum have received comparatively little attention until recently, and even now their histology is far from clear. In the alligator, as mentioned in Section A.1-2, there are two nuclei. The medial nucleus contains both large and small cells whilst the lateral nucleus has medium and small cells within it (Weston, 1936). In mammals, as mentioned in Section A.1-2, three nuclei are present (four in man).

All nuclei contain large multipolar cells, but the dendritic ramification is greatest in the lateral nuclei. Numerous collaterals terminate in all nuclei, except in the fastigial nucleus. In addition to the large multipolar cells, some small cells are also present (Eager, 1968). One of the most impressive features of the nuclei is the columinar axonal ramification of the terminating Purkinje cell axons. This is most marked in the lateral nucleus (Cajal, 1911; Eccles, Ito and Szentágothai, 1967, Ch. XIII). Recent evidence suggests that the two types of cerebellar afferent systems, the climbing and mossy fibre, send collaterals to the deep nuclei (Eccles, Ito and Szentágothai, 1967, Ch. XIII). No information is available about the relative termination within the nuclei of such collaterals and the axons of Purkinje cells which are influenced by the fibres contributing to these collaterals; such a system must be, however, of considerable physiological significance.

APPENDIX B

THE MATHEMATICAL ANALYSIS OF DENDRITIC SYSTEMS

Dendrites may be considered as a system of branching cables. The term cable implies that current not only flows along its core, but also through its wall. The core of a nerve cell is treated as purely resistive (Cole and Hodgkin, 1939) but the wall, that is the membrane, is considered to have a capacitative and resistive component in parallel (Cole, 1949).

Several papers have dealt with the mathematical theory of electrotonus in neural elements, for example, Davis and Lorente de Nó (1947); Clark and Plonsey, 1967, 1968; Hellerstein, 1968. The theory has also been applied to dendrites, notably by Rall (1959, 1962a, 1962b, 1964, 1967) and recently by MacGregor (1968). The work of Rall has relied mainly on extensive computer models of symmetrically activated dendritic trees; the significance of dendrites is more fully seen when the synaptic activation is asymmetric. In this appendix analytical techniques, based on the

Laplace transform, are used to explore a variety of relevant problems and the mathematical formalism is interpreted in terms of neural structure and function. Explicit expressions for electrotonic potential as a function of space and time are derived and in many cases they have been evaluated with the aid of a LINC-8 computer using the FOCAL algebraic language.

B.1 The Cable Equation for a Synaptically Activated Nerve Process. A small region of the membrane can be represented by the circuit shown in Figure 34A (Hodgkin, 1958). A current $\underline{i}$ flows between the inside of the membrane, at potential V_I and the outside at potential V_E. The current flows through the network of the membrane capacity C, the membrane conductance G_R and the excitatory and inhibitory conductances G_1 and G_2 respectively. In series with each of the conductances is the appropriate driving potential E_R, E_1 and E_2. Then the total current $\underline{i}$ is given by

$$i = C\frac{\partial}{\partial t}(V_I - V_E) + G_R(V_I - V_E - E_R) + G_1(V_I - V_E - E_1) + G_2(V_I - V_E - E_2)$$

Let the deviation of the transmembrane potential from the resting potential be V, that is

$$V = (V_I - V_E) - E_R$$

then,

$$i = C\frac{\partial V}{\partial t} + G_R V + G_1(V + E_R - E_1) + G_2(V + E_R - E_2) \quad \text{I}$$

Now G_1 and G_2 are zero except when excitation or inhibition is occurring in the patch of membrane. These changes in the conductances provide the driving potential for the change in V. It is convenient to set $G_1 = k_1 G_R$ and $G_2 = k_2 G_R$, so that the excitatory and inhibitory conductances are expressed in terms of the resting membrane conductance, then the part of equation I which provides the driving potential may be written as W where,

$$W = \{k_1(V + U_1) + k_2(V + U_2)\} G_R$$

and

$$U_1 = E_R - E_1 \qquad U_2 = E_R - E_2 \quad \text{II}$$

so that

$$i = C\frac{\partial V}{\partial t} + G_R V + W$$

A length of dendrite consists of membrane surrounding a cylindrical conductive core. Within the membrane the current flows radially, whilst within the core the current flows almost exclusively in a longitudinal direction. Outside

the membrane current will flow both radially and longitudinally in the extracellular medium. It has been shown to be a valid approximation to regard the extracellular potential as zero (Clark and Plonsey, 1967) so long as the variable of interest is the transmembrane potential. One may therefore take a small element of a cylindrical nerve process, such as a dendrite, and represent it by the scheme of Figure 34B. A current $j(x)$ flows through the core resistance, whose value for an element of length Δx is $Q\Delta x$. The internal potential is $V(x)$ and the external potential zero. So:

$$V(x+\Delta x) - V(x) = -Q\Delta x \cdot j\left(x + \frac{\Delta x}{2}\right)$$

which in the limit gives $\frac{1}{Q}\frac{\partial V}{\partial x} = -j$ III

and $j(x+\Delta x) - j(x) = -i\,\Delta x$

for a current $i\Delta x$ leaving per unit element.

so $i = -\frac{\partial j}{\partial x}$ IV

finally from II, setting $G_R = \frac{1}{R}$ where R is the

transmembrane resistance and combining with III and IV one obtains

$$\frac{1}{\varrho}\frac{\partial^2 V}{\partial x^2} - C\frac{\partial V}{\partial t} - \frac{V}{R} - W = 0 \qquad \text{V}$$

This is the well known cable equation. It is convenient to multiply through by R and to set $\lambda = \sqrt{\frac{R}{\varrho}}$ $\tau = RC$, then one defines new variables

$$X = \frac{x}{\lambda} \quad \text{and} \quad T = \frac{t}{\tau}$$

so that equation V becomes

$$\frac{\partial^2 V}{\partial X^2} - \frac{\partial V}{\partial T} - V - RW = 0 \qquad \text{VI}$$

B.1-1 The Choice of a Function to Represent the Conductance Change. Recalling the definition of W from II one sees that it is a sum of terms of the form

$$k_n G_R (V + U_n)$$

where k_n will represent the conductance change associated with a synapse for the nth ion species $U_n = E_R - E_n$ represents the difference between the equilibrium potential for the ion involved and the resting potential of the membrane. Un will thus be a constant whilst k_n will be a function

of both time and space. Some idea of the data involved is given by the following figures taken from Eccles (1964, Chap. IV and Chap. IX).

E_R (Resting potential) -70 MV

E_1 (Equilibrium potential for excitatory ions) 0 MV

E_2 (Equilibrium potential for inhibitory ions) -80 MV

R (Membrane Resistance) $1{\cdot}2 \times 10^6$ Ohms

C (Membrane Capacity) $2{\cdot}5 \times 10^{-9}$ Farads

G_1 (Excitatory Conductance) Peak $0{\cdot}2 \times 10^{-5}$ Mhos

G_2 (Inhibitory Conductance) Peak $0{\cdot}14 \times 10^{-5}$ Mhos

The waveform of the postsynaptic current caused by the synaptic activation is triangular with a base of approximately 2 msec duration for both excitation and inhibition (Eccles, 1964 - Chap. IV, Figure 14A and Chap. IX, Figure 58A).

Now it is reasonable to regard a synapse as being localised at a single point, since its dimensions are very small compared to λ (which will be of the order of 200-300μ if one takes the value of 1.8λ used for the maximum path length

in the motoneurone (Rall, Burke, Smith, Nelson and Frank, 1967) as a reasonable value for the Purkinje cell). Thus the space dependence of the conductance change may be adequately represented by a Dirac delta function $\delta(X-a)$ for a synapse at $\underline{a}$. On the other hand, the duration of the conductance change is an appreciable fraction of τ so that a delta function approximation is not, at first sight, accurate. Furthermore, if the conductance change occupies a finite period the transmembrane potential V in the vicinity of the synapse will diminish with time. This makes differential equation VI non-linear and consequently difficult to solve. This non-linearity may be avoided by the use of a delta function for the time dependence, so it becomes important to explore the delta function solution in comparison with the solution for a conductance change of finite duration to see under what conditions, if any, the simpler approximation is valid.

If the finite conductance change is chosen to give a constant rectangular pulse of current, then the non-linearity will be excluded since the current will not depend on V. Although this is not a true representation of the situation it will provide the data sought, namely a comparison with the delta function since it will in fact over emphasise the effect of a finite conductance change. Both these cases will now be considered and it will then be seen that the non-linear case can also be approximated arbitrarily well from the results.

B.1-2 Comparison of Delta Function and Rectangular Pulse Conductance Changes. For this comparison the dendrite is regarded as a uniform cylinder of constant λ and τ. This model forms the basis of the branched system to be considered later. The problem analysed here is to find the potential distribution in the dendrite following the activation of a synapse situated at $X = a$, where X is measured from the distal most tip of the dendrite. At $X = 0$ it is assumed that no current

leaves the dendrite. In both cases to be considered the function W is independent of the membrane potential, for in the case of the delta function the conductance change takes place at T = 0 when V = 0 and in the case of the pulse it is defined to be independent of V. Furthermore, the space dependence in both cases is a delta function so the term RW in equation VI may be written as

$$RW = P(T)\,\delta(X-a)$$

where P(T) is the conductance change, then equation VI becomes

$$\frac{\partial^2 V}{\partial X^2} - \frac{\partial V}{\partial T} - V - P(T)\,\delta(X-a) = 0 \qquad \text{VII}$$

This may be readily solved by using the Laplace transform method. Starting with the time dependence one may define the Laplace transforms of V and P with respect to time as

$$\overset{*}{V}(X,s) = \int_0^\infty e^{-sT} V(X,T)\,dT \; ; \; \overset{*}{P}(s) = \int_0^\infty e^{-sT} P(T)\,dT$$

hence VII transforms to

$$\frac{\partial^2 \overset{*}{V}}{\partial X^2} - (s+1)\overset{*}{V} + V(X,0+) - \overset{*}{P}(s)\,\delta(X-a) = 0$$

where $V(X,0+) = \lim_{\varepsilon \to 0} V(X,\varepsilon)$ for positive ε,

hence $V(X,0+) = 0$ because the whole system is initially at the resting potential. Now transforming the space dependence and defining

$$\bar{V}(r,s) = \int_0^\infty e^{-rX} \overset{*}{V}(X,s)\, dX$$

one obtains:

$$(r^2 - s - 1)\bar{V} - r\overset{*}{V}(0+,s) - \frac{\partial \overset{*}{V}(X,s)}{\partial X}\bigg|_{X=0+} - \overset{*}{P} e^{-ra} = 0$$

now, $\overset{*}{V}(0+,s)$ remains to be determined so let it be $A(s)$;

$$\frac{\partial \overset{*}{V}(X,s)}{\partial X}\bigg|_{X=0+} = \left\{ \int_0^\infty e^{-sT} \frac{\partial V}{\partial X}(X,T)\, dT \right\}\bigg|_{X=0+}$$

but

$$\frac{\partial V}{\partial X}(X,T)\bigg|_{X=0+} = \lambda \frac{\partial V}{\partial x}\bigg|_{x=0+} = \lambda Q\, j(0+,t)$$

$= 0$ by the required boundary condition so:

$$\bar{V} = \frac{r A(s) + \overset{*}{P}(s) e^{-ra}}{r^2 - (s+1)}$$

The inverse Laplace transformation of this expression is:

$$\overset{*}{V} = A(s) \cosh \sqrt{s+1}\, X + \frac{\overset{*}{P}(s) \sinh \sqrt{s+1}\,(X-a)}{\sqrt{s+1}}$$

for $X > a$

(Korn and Korn, 1968, Section 8.3-2 Theorem 9 and Table D7, transforms 17 and 18). This may be rewritten as

$$\overset{*}{V} = \frac{e^{\sqrt{s+1}\,X}}{2}\left\{\frac{\overset{*}{P}(s)\,e^{-\sqrt{s+1}\,a}}{\sqrt{s+1}} + A(s)\right\} + \frac{e^{-\sqrt{s+1}\,X}}{2}\left\{\frac{-\overset{*}{P}(s)\,e^{\sqrt{s+1}\,a}}{\sqrt{s+1}} + A(s)\right\}$$

As the recording position, X, becomes further and further from the synapse, the potential must become less, for any given instant, so the coefficient of the positive exponential must be zero. So:

$$A(s) = \frac{-\overset{*}{P}(s)\,e^{-\sqrt{s+1}\,a}}{\sqrt{s+1}}$$

then

$$\overset{*}{V} = -\overset{*}{P}(s)\left\{\frac{e^{-\sqrt{s+1}\,(X-a)} + e^{-\sqrt{s+1}\,(X+a)}}{2\sqrt{s+1}}\right\} \quad \text{VIII}$$

to proceed further it is necessary to define $\overset{*}{P}(s)$.

B.1-3 Delta Function Representation of Conductance Time Dependence. In this case

$$P(T) = R \cdot kU\,G_R\,\delta(T-t) = kV\,\delta(T-t)$$

for synaptic activation at T = b. The Laplace transform of this is simply

$$kUe^{-bs}$$

so $\overset{*}{P}(s) = kUe^{-bs}$ and then the inverse time transform of X is

$$V = \frac{-kU}{2\sqrt{\pi(T-b)}}\, e^{-(T-b)} \left\{ e^{\frac{-(X-a)^2}{4(T-b)}} + e^{\frac{-(X+a)^2}{4(T-b)}} \right\} \qquad IX_A$$

for X > a

(Korn and Korn, 1968, Table D7, transform 84)

This may also be written as

$$V = \frac{-kU}{\sqrt{\pi(T-b)}}\, e^{-\left(\frac{X^2+a^2}{4T} + (T-b)\right)} \cosh\left(\frac{aX}{2(T-b)}\right) \qquad IX_B$$

In the case of a = b = o, i.e., a distally situated synapse activated at time zero

$$V = \frac{-kU}{\sqrt{\pi T}}\, e^{-T}\, e^{-\frac{X^2}{4T}} \qquad X$$

B.1-4 Rectangular Function Representation of Conductance Time Dependence. Let the pulse begin at time b and end at time d, then the waveform

is just the difference of the two step functions

$$H(T-b) = 1 \quad T \geq b$$
$$= 0 \quad \text{otherwise}$$
$$H(T-d) = 1 \quad T \geq d$$
$$= 0 \quad \text{otherwise}$$

Let the magnitude of the pulse be lU

then the Laplace transform will be

$$\overset{*}{P}(s) = lU\left\{\frac{e^{-bs} - e^{-ds}}{s}\right\}$$

then from VII

$$\overset{*}{V} = -lU\left\{\frac{e^{-bs} - e^{-ds}}{s}\right\}\left\{\frac{e^{-\sqrt{s+1}(X-a)} + e^{-\sqrt{s+1}(X+a)}}{2\sqrt{s+1}}\right\}$$

To find the inverse of this consider a function

$$\overset{*}{F}(s) = \frac{e^{-ds}}{s}\,\frac{e^{-\sqrt{s+1}(X-\beta)}}{\sqrt{s+1}}$$

$$= e^{-ds}\, g(s+1)$$

Denote the Laplace transform operation by the symbol $\rightarrow$, then one seeks the function F(T) such that

$$F(T) \rightarrow \overset{*}{F}(s) = e^{-ds}\, g(s+1)$$

but

$$e^{T} F(T) \rightarrow \overset{*}{F}(s-1) = e^{-\alpha(s-1)}\, g(s)$$

(Korn and Korn, 1968, Table 8.3-1, Theorem 11)

and then

$$e^{T+\alpha} F(T+\alpha) \longrightarrow e^{\alpha s} \bar{F}(s-1) = e^{\alpha} g(s)$$

(Korn and Korn, 1968, Table 8.3-1, Theorem 5)

e^{α} can be cancelled since it remains invariant under the transformation so finally:

$$e^{T} f(T+\alpha) \longrightarrow g(s)$$

where g(s) is obtained by substituting s-1 for s in

$$\frac{e^{-\sqrt{s+1}\,(X-\beta)}}{s\sqrt{s+1}}$$

i.e.

$$g(s) = \frac{e^{-\sqrt{s}\,(X-\beta)}}{(s+1)\sqrt{s}}$$

$$= \frac{e^{-\sqrt{s}\,(X-\beta)}}{2\sqrt{s}} \left\{ \frac{1}{\sqrt{s}-1} - \frac{1}{\sqrt{s}+1} \right\}$$

so using Transform 87 of Table D7 in Korn and Korn, 1968.

$$e^{T} F(T+\alpha) = \frac{e^{T}}{2} \left\{ e^{-(X-\beta)} \operatorname{erfc}\left(-\sqrt{T} + \frac{(X-\beta)}{2\sqrt{T}}\right) - e^{(X-\beta)} \operatorname{erfc}\left(\sqrt{T} + \frac{(X-\beta)}{2\sqrt{T}}\right) \right\}$$

hence

$$F(T) = \frac{1}{2}\left\{ e^{-(X-\beta)} \operatorname{erfc}\left(-\sqrt{T-\alpha} + \frac{(X-\beta)}{2\sqrt{T-\alpha}}\right) - e^{(X-\beta)} \operatorname{erfc}\left(\sqrt{T-\alpha} + \frac{(X-\beta)}{2\sqrt{T-\alpha}}\right)\right\}$$

where erfc Z is the complementary error function defined by

$$\operatorname{erfc} z = \frac{2}{\sqrt{\pi}} \int_{z}^{\infty} e^{-\zeta^2} d\zeta$$

so

$$V = -\frac{1U}{4}\left\{ e^{-(X-a)} \operatorname{erfc}\left(-\sqrt{T-b} + \frac{(X-a)}{2\sqrt{T-b}}\right) - e^{(X-a)} \operatorname{erfc}\left(\sqrt{T-b} + \frac{(X-a)}{2\sqrt{T-b}}\right) + e^{-(X+a)} \operatorname{erfc}\left(-\sqrt{T-b} + \frac{(X+a)}{2\sqrt{T-b}}\right) - e^{(X+a)} \operatorname{erfc}\left(\sqrt{T-b} + \frac{(X+a)}{2\sqrt{T-b}}\right)\right\} + \frac{1U}{4}\left\{ e^{-(X-a)} \operatorname{erfc}\left(-\sqrt{T-d} + \frac{(X-a)}{2\sqrt{T-d}}\right) - e^{(X-a)} \operatorname{erfc}\left(\sqrt{T-d} + \frac{(X-a)}{2\sqrt{T-d}}\right)\right.$$

$$+ e^{-(X+a)} \operatorname{erfc}\left(-\sqrt{T-d} + \frac{(X+a)}{2\sqrt{T-d}}\right)$$

$$- e^{(X+a)} \operatorname{erfc}\left(\sqrt{T-d} + \frac{(X+a)}{2\sqrt{T-d}}\right)\Bigg\}$$

for a synapse at X = 0 and the pulse beginning at T = 0 this simplifies to

$$V = -\frac{1U}{2}\left\{ e^{-X}\left[\operatorname{erfc}\left(-\sqrt{T} + \frac{X}{2\sqrt{T}}\right) - \operatorname{erfc}\left(-\sqrt{T-d} + \frac{X}{2\sqrt{T-d}}\right)\right] - e^{X}\left[\operatorname{erfc}\left(\sqrt{T} + \frac{X}{2\sqrt{T}}\right) - \operatorname{erfc}\left(\sqrt{T-d} + \frac{X}{2\sqrt{T-d}}\right)\right]\right\} \quad \text{XII}$$

It is to be noted in passing that if a unit delta function input occurs at T = 0, then $\overset{*}{P}(s) = 1$, V is then just the inverse Laplace transformation of

$$\frac{e^{-\sqrt{s+1}\,(X-a)} + e^{-\sqrt{s+1}\,(X+a)}}{2\sqrt{s+1}} = \overset{*}{Z}(X,s)$$

for functions other than the unit delta function V is the inverse transformation of $\overset{*}{P}(s)\ \overset{*}{Z}(X,s)$; this represents the convolution of the new input P(T) with the unit delta function response.

i.e.

$$V(X,T) = \int_0^\infty P(\eta)\, Z(X, T-\eta)\, d\eta$$

This is the so-called superposition integral (Mason and Zimmerman, 1960, Chap. 7)

B.1-5 Comparison of the Delta Function and Pulse Function Results. In order to evaluate the above formulae it is necessary to put the same quantity of charge into both systems.

$$\text{Charge} = q = \int_0^\infty \text{current}.\, dT$$

so for the delta function

$$q = \int_0^\infty kU\, \delta(T)\, dT = kU$$

for the rectangular pulse

$$q = \int_0^\infty lU \{H(T) - H(T-d)\} dT = lUd$$

so by setting $l = \frac{k}{d}$ results may be compared. To compare these functions the expressions given in X and XII were evaluated using a computer and the results are shown in Figures 35,36. In

kU
all calculations/is set to -100 for convenience. Figure 35A compares the potential produced by a delta function input and a rectangular pulse of 0.04τ duration measured 2λ away from the input. The curves were found to be virtually identical for so short a pulse and it was necessary to look at the potential 0.2λ from the source with the time scale expanded (Figure 35B) for an appreciable effect. A realistic value for the duration of the conductance change might be 0.5τ, given $\tau=5$ msec (Rall, Burke, Smith Nelson and Frank, 1967) and Figure 36 shows a comparison of the results for a delta function input and a rectangular pulse of this duration. Figure 36A shows the results at 2λ away from the synapse. A difference can be seen in the time course, the peak of the 0.5τ duration pulse occurring about 0.3τ after the impulse response peak, but the amplitudes are very similar. At λ from the source (Figure 36B) the peaks are separated by about 0.35τ but the amplitudes are again similar. Finally, Figure 36C shows the effect of recording the potentials 0.5λ

from the source, the peaks are about 0.43τ apart and the amplitude and waveform are significantly different. The conclusions drawn from this are that the general characteristics of the cable response to an impulse function show the same features as those of a more realistic conductance change provided that distance between source and recording position is at least λ. The amplitudes of the potentials are quite similar, although the time to peak is significantly slowed with the long duration input. The delta function is thus a useful "testing function" for more complex dendritic systems.

One feature of the delta function response should be pointed out. The response is

$$V = \frac{kU}{\sqrt{\pi T}} e^{-\frac{X^2}{4T} - T}$$

and as the origin is approached in the X-T plane this function can become unbounded -- for example, if the origin is approached along the curve $X = \sqrt{T}$, thus for recording positions very close to the source this function gives unrealistic

results, it is however only in immediate neighbourhood of the origin that this is so.

B.1-6 Non-linear Conductance Changes and Their Approximation. The foregoing results enable the potential at any point in a cylinder to be calculated for a rectangular conductance change with a constant driving potential. If the driving potential is a function of the membrane potential at the position of the conductance change, then it may be divided up into a large number of small rectangular pulses during which the driving potential is held constant and at the termination of each pulse a new driving potential is calculated and used. The superposition of the solutions to this sequence of pulses will be the non-linear response. When the recording position is a significant distance away from the source a sequence of delta function approximations to the rectangular pulses will be quite adequate, with consequent simplification of the mathematics.

B.2 Potentials in a Branched Dendritic System in Response to the Activation of a Single Synapse. Having established the validity of the impulse function approximation this can now be used to derive results for dendrites. The most important question is the effect of branching.

B.2-1 The Y-Shaped Dendritic Tree. The problem is: under what circumstances, if any, is it justifiable to regard the branched tree as a single cylindrical dendrite? The tree is defined to be Y-shaped with each of the upper branches of the Y equal to a in length and terminated by the usual boundary condition $j = 0$. A synapse is activated on the tip of one of the dendrites at time $T = 0$.

Let the upper left-hand branch be assigned the variables X_1, V_1, the upper right-hand branch the variables X_2, V_2 and the stem of the Y be assigned variables $X_3 V_3$. Then the equations governing the system are

$$\frac{\partial^2 V_1}{\partial X_1^2} - \frac{\partial V_1}{\partial T} - V_1 - k\,\delta(X_1)\,\delta(T)(V+U) = 0$$

$$\frac{\partial^2 V_2}{\partial X_2^2} - \frac{\partial V_2}{\partial T} - V_2 = 0$$

$$\frac{\partial^2 V_3}{\partial X_3^2} - \frac{\partial V_3}{\partial T} - V_3 = 0$$

$$X_3 = X_1 - a$$

$$\left.\frac{\partial V_1}{\partial X_1}\right|_{X_1=a} = \left.\frac{\partial V_2}{\partial X_2}\right|_{X_2=a} = 0 \qquad \text{current boundary condition}$$

$$V_1(a,T) = V_2(a,T) = V_3(0,T) \qquad \text{potential continuity}$$

$$\frac{\partial V_1}{\partial X_1} + \frac{\partial V_2}{\partial X_2} = \frac{\partial V_3}{\partial X_3} \qquad \text{current conservation}$$

λ and τ are assumed constant for the whole system.

Then taking the successive temporal and spatial Laplace transforms of the three partial differential equations one obtains

$$\bar{V}_1 = \frac{r_1 A_1(s) + k U}{r_1^2 - (s+1)}$$

$$\bar{V}_2 = \frac{r_2 A_2(s)}{r_2^2 - (s+1)}$$

$$\bar{V}_3 = \frac{r_3 A_3(s) + B(s)}{r_3^2 - (s+1)}$$

where V_1, V_2, V_3 are the respective Laplace transform variables; A_1, A_2 and A_3 have the same significance as in Section B.1-3 and $B(s) = \frac{\partial \overset{*}{V}_3}{\partial X_3}\Big|_{x_3 = 0}$ also remains to be determined in this case.

Next taking the inverse Laplace transform of the spatial dependence one obtains

$$\overset{*}{V}_1 = A_1(s) \cosh \sqrt{s+1}\, X_1 + \frac{Uk \sinh \sqrt{s+1}\, X_1}{\sqrt{s+1}}$$

$$\overset{*}{V}_2 = A_2(s) \cosh \sqrt{s+1}\, X_2$$

$$\overset{*}{V}_3 = A_3(s) \cosh \sqrt{s+1}\, X_3 + \frac{B(s) \sinh \sqrt{s+1}\, X_3}{\sqrt{s+1}}$$

now considering $\overset{*}{V}_3$ one asks that

$\overset{*}{V}_3 (X_3, s) \to 0$ as $X \to \infty$ which implies, when the expression is written in terms of exponentials that

$$A_3(s) = \frac{-B(s)}{\sqrt{s+1}}$$

so that

$$\overset{*}{V}_3 = \frac{-B(s)}{\sqrt{s+1}}\, e^{-\sqrt{s+1}\, X_3}$$

now potential continuity demands that

$$A_1(s)\cosh\sqrt{s+1}\,a + \frac{Uk\sinh\sqrt{s+1}\,a}{\sqrt{s+1}} = -\frac{B(s)}{\sqrt{s+1}}$$

and

$$A_2(s)\cosh\sqrt{s+1}\,a = -\frac{B(s)}{\sqrt{s+1}}$$

so that expressions for $A_1(s)$ and $A_2(s)$ are obtained. Now using the current conservation condition, which transforms to

$$\frac{\partial \overset{*}{V}_1}{\partial X_1} + \frac{\partial \overset{*}{V}_2}{\partial X_2} = \frac{\partial \overset{*}{V}_3}{\partial X_3}$$

one obtains

$$A_1(s)\sqrt{s+1}\sinh\sqrt{s+1}\,a + Uk\cosh\sqrt{s+1}\,a + A_2(s)\sqrt{s+1}\sinh\sqrt{s+1}\,a = B(s)$$

substituting for $A_1(s)$ and $A_2(s)$ and solving for B(s) one obtains

$$B(s) = \frac{2Uk\,e^{-\sqrt{s+1}\,a}}{3 - e^{-2\sqrt{s+1}\,a}}$$

hence

$$\overset{*}{V}_3(X_3, s) = -\frac{Uk\,e^{-\sqrt{s+1}\,X_3}}{\sqrt{s+1}} \cdot \frac{2\,e^{-\sqrt{s+1}\,a}}{3 - e^{-2\sqrt{s+1}\,a}}$$

but $X_3 = X_1 - a$

so $$\overset{*}{V}_3(X_1, s) = \frac{-Uk\, e^{-\sqrt{s+1}\,X_1}}{\sqrt{s+1}} \cdot \frac{2}{3 - e^{2\sqrt{s+1}\,a}}$$

$$= \frac{-Uk\, e^{-\sqrt{s+1}\,X_1}}{\sqrt{s+1}} \cdot D(s)$$

Now D(s) may be expanded using the Binomial Theorem, so that

$$D(s) = \frac{1}{3}\left\{1 + \frac{e^{-2\sqrt{s+1}\,a}}{3} + \frac{e^{-4\sqrt{s+1}\,a}}{9} + \cdots\cdots\right\}$$

so

$$\overset{*}{V}_3(X_1, s) = -\frac{2}{3} Uk \frac{e^{-\sqrt{s+1}\,X_1}}{\sqrt{s+1}} \sum_{\nu=0}^{\infty} \frac{1}{3^\nu} e^{-2\sqrt{s+1}\,a\nu}$$

This may be transformed term by term to yield

$$V_3 = -\frac{2}{3} \frac{Uk\, e^{-T}}{\sqrt{\pi T}} \sum_{\nu=0}^{\infty} \frac{1}{3^\nu} e^{\frac{-(X+2a\nu)^2}{4T}} \qquad \text{XIII}$$

B.2-2 The Evaluation of the Formula for a Branched System. Expression XIII was evaluated on the computer for various values of a, the position of the branch. The direct path length between synapse and recording position was 2λ

and Uk was -100 as before. The results are shown in Figure 37 . It is seen that for a greater than 0.25λ the potentials are very similar with a reduction in amplitude compared with the result for a straight cable (a = 0). The time to peak is virtually constant in all cases. So it can be concluded that unless the recording position is very close to the synapse the effect of branching is the same no matter where the branch is and results in an attenuation of the potential.

B.2-3 The Total Charge Passing Any Point in a Branched Tree. Consider the total charge at a given point X in the tree, X > a.

Total charge = $q(X) = \int_0^\infty j(X,T)\,dT$

but

$$j(X,T) = \frac{\lambda}{Q}\frac{\partial V}{\partial X}$$

so

$$q(X) = \frac{\lambda}{Q}\int_0^\infty \frac{\partial V}{\partial X}\,dT = \frac{\lambda}{Q}\int_0^\infty \lim_{s\to 0} e^{-sT}\frac{\partial V}{\partial X}\,dT$$

$$= \frac{\lambda}{Q}\lim_{s\to 0}\frac{\partial}{\partial X}\int_0^\infty e^{-sT}V\,dT = \frac{\lambda}{Q}\lim_{s\to 0}\frac{\partial V^*}{\partial X}$$

so that the total charge passing a point X may be found by differentiating $\overset{*}{V}(X,s)$ with respect to X and then letting $S \to 0$; in the present case one obtains

$$q(X) = \frac{2\,Uk\,e^{-X}}{3 - e^{-2a}}\,\frac{\lambda}{Q}$$

one notes at once that this is least when $a \to \infty$ and greatest when $a = 0$

$$q_{a=0}(X) = Uk\,e^{-X}\,\frac{\lambda}{Q}$$

$$q_{a\to\infty}(X) = \frac{2}{3}\,Uk\,e^{-X}\,\frac{\lambda}{Q}$$

so that the presence of the branch diminishes the total charge passing the point by, at most, one third.

B.3 A Geometrical Interpretation of the Foregoing Results - Formal Equivalence to an Infinite Cable with Image Synapses. In this section it will be shown that the expressions derived above for the potential caused by a synapse at an arbitrary point in a cylindrical cable and for the synapse at one end of a Y-shaped tree can be interpreted geometrically in a manner similar

to the well-known image method of solving the potential problems of physics (Jackson, 1962, Chapter 2).

B.3-1 The Single Synapse at an Arbitrary Position in a Cylindrical Dendrite. Comparison of equations IX_A and X gives a new physical interpretation of the situation.

Figure 38A shows a current source of total strength I situated at X = a and emitting current to the right (I_R) and to the left (I_L). The two currents are unequal and thus represent the problem whose solution is given by equation IX_A. Figure 38B shows the case when the current source is situated at one end of the cable at X = 0. In this case current is emitted in one direction only so that the current to the right is I; this corresponds to the situation whose solution is given by equation X. Now if current sources are placed at X-a and X+a units from the recording position, both on the same side, and allowed to emit current in both

directions (Figure 38C), the potential at the recording position will be half that due to the superposition of the effects of one sided sources in the same positions. Moreover at a position midway between the two sources the longitudinal currents will be equal and opposite and so the net current will be zero. From this it is seen that the systems in Figure 38A and 38C are mathematically identical and hence the configuration of sources in 38C will give the same result as that in A.

It follows that, using the diagram of Figure 38C, the solution of the single synapse problem in the region $o < X < a$ can be written down by inspection as

$$V = -\frac{kU}{2\sqrt{\pi T}}\, e^{-(T-b)} \left\{ e^{\frac{-(X-a)^2}{4(T-b)}} - e^{\frac{-(X+a)^2}{4(T-b)}} \right\}$$

for $o < X < a$. IX$_c$

B.3-2 The Geometrical Interpretation of the Branched System. Rewriting the solution of the branched system (Expression XIII) as

$$V = -\sum_{\nu=0}^{\infty} \frac{2}{3}\frac{1}{3^{\nu}} \frac{Uk\, e^{-T}}{\sqrt{\pi T}}\, e^{-\frac{(X+2a\nu)^2}{4T}}$$

it is seen by the argument of B.3-1 that this may be interpreted as a sequence of sources placed in an infinite cable in the following configuration:

Source of strength $\frac{2}{3} \cdot \frac{1}{3}$ at $X = 0$

Source of strength $\frac{2}{3} \cdot \frac{1}{9}$ at $X = -2a$

Source of strength $\frac{2}{3} \cdot \frac{1}{3^{\nu}}$ at $X = -2\nu a$

and so on,
that is, an infinite sequence of sources of diminishing strength. So, in this way the branched cable is reduced to a single infinite cable.

B.4 The Effect of Synaptic Position on the Potential Recorded at the Soma. It has been shown in Section B.2-2 that the effect of a branch is mainly to attenuate the effect which would be produced in single cable by an impulse (although the waveforms do differ in shape if one examines them in detail). It is therefore legitimate to consider only a single cable in

the investigation of the effect of synaptic position. In this case the required expression is already available (IX_A). This expression has been evaluated on the computer and the results are shown in Figure 39 for a range of synaptic positions for $X = 2\lambda$ at $Uk = -100$. It is seen that for a synapse at λ from the recording position the potential is twice that for a synapse at the distal end of the tree. The time to peak is also shortened. Between the distal end and $\underline{a} = 0.5\lambda$ the effects of synaptic position are much less. These results should be seen in perspective. If the distance of the synapse from the recording position is 0.2λ the potential will be fifteen times that for a synapse λ away and so the variations shown in Figure 39 are relatively small.

This concludes the main results. It is of interest, however, to comment on the question of synaptic interaction since all the tools for this have been developed.

B.5 The Effect of a Preceding Synaptic Activation on Another Active Synapse. If two synapses are active, one at X = 0, T = 0 and the other at X = a, T = b and the first is specified by k_1 and V_1 and the second by k_2 and V_2, then the superposition of sources enables the solution to be written down. For in the interval $o < T < b$ only the distal synapse will be active and the potential recorded at X will just be, from X

$$V = -\frac{k_1 V_1}{\sqrt{\pi T}} e^{-T} e^{-\left(\frac{X^2}{4T}\right)}$$

$$o < T < b$$

When the synapse at a becomes active, the time will be b and the potential at this point will be V(a,b). This potential must be algebraically added to the driving voltage of this synapse so that U is replaced by $U_2 + V(a,b)$. Now using IX_A with the new driving potential and adding to this the potential set up by the first the

solution of the problem is

$$V = \frac{-k_1 U_1}{\sqrt{\pi T}} e^{-T} e^{-\left(\frac{X^2}{4T}\right)} - \frac{k_2}{2\sqrt{\pi(T-b)}} \cdot$$

$$\left[U_2 + V(a,b)\right] e^{-(T-b)} \left\{ e^{\frac{-(X-a)^2}{4(T-b)}} + e^{\frac{-(X+a)^2}{4(T-b)}} \right\}$$

but

$$V(a,b) = \frac{-U_1 k_1}{\sqrt{\pi b}} e^{-b} e^{\frac{-a^2}{4b}}$$

so that

$$V = \frac{-k_1 U_1}{\sqrt{\pi T}} e^{-T} e^{-\frac{X^2}{4T}} - \frac{k_2 e^{-(T-b)}}{2\sqrt{\pi(T-b)}}$$

$$\left[U_2 - \frac{U_1 k_1}{\sqrt{\pi b}} e^{-b} e^{-\frac{a^2}{4b}}\right] \left\{ e^{\frac{-(X-a)^2}{4(T-b)}} + e^{\frac{-(X+a)^2}{4(T-b)}} \right\}$$

Finite conductance change durations can also be accommodated and Martin's correction (Martin, 1955) can be applied if desired to the expression for the second synapse; this correction is discussed at length by Hubbard, Llinás and Quastel (1969, Chap. II). This leads,

however, to the discussion of better models for synaptic activation which is outside the scope of this Appendix.

REFERENCES

Andersen, P. (1960): Interhippocampal impulses. II. Apical dendritic activation of CA1 neurons. Acta Physiol. Scand. 48: 178-208.

Andersen, P., J.C. Eccles and P.E. Voorhoeve (1963): Inhibitory synapses on somas of Purkinje cells in the cerebellum. Nature 199: 655-656.

Andersen, P., J.C. Eccles and P.E. Voorhoeve (1964): Postsynaptic inhibition of cerebellar Purkinje cells. J. Neurophysiol. 27: 1138-1153.

Andersen, P., Holmqvist and P.E. Voorhoeve (1966a): Excitatory synapses on hippocampal apical dendrites activated by entorhinal stimulation. Acta Physiol. Scand. 66: 461-472.

Andersen, P., B. Holmqvist and P.E. Voorhoeve (1966b): Entorhinal activation of dendate granule cells. Acta Physiol. Scand. 66: 448-460

Andersen, P. and T. Lømo (1966): Mode of activation of hippocampal pyramidal cells by excitatory synapses on dendrites. Exp. Brain Res. 2: 247-260.

Ariëns Kappers, C.U., G.C. Huber and E.C. Crosby (1936): The Comparative Anatomy of the Nervous System of Vertebrates, Including Man. New York: MacMillan.

Bell, C.C. and R.S. Dow (1967): Cerebellar circuitry. Neurosciences Res. Prog. Bull. 5: 121-222.

Braitenberg, V. (1961): Functional interpretation of cerebellar histology. Nature 190: 539.

Braitenberg, V. (1965): What can be learned from spike interval histograms about synaptic mechanisms? J. Theoret. Biol. 8: 419-425.

Braitenberg, V. (1967): Is the cerebellar cortex a biological clock in the millisecond range? IN: Progress in Brain Research 25: The Cerebellum. Ed. C.A. Fox and R. Snider Amsterdam: Elsevier Publishing Co., 334-346.

Braitenberg, V. and R.P. Atwood (1958): Morphological observations on the cerebellar cortex. J. Comp. Neurol. 109: 1-27.

Braitenberg, V., G. Gambardella, G. Ghigo and U. Vota (1965): Observations on spike sequences from spontaneously active Purkinje cells in the frog. Kybernetic 2: 197-205.

Braitenberg, V and N. Onesto (1962): The cerebellar cortex as a timing organ. Discussion of a hypothesis. Atti. 1° Congr. Int. Medicina Cibernetica, Napoli, Edz.: Giannini.

Brodal, A, (1954): Afferent cerebellar connections. IN: Aspects of Cerebellar Anatomy, Ch. II, Ed. J. Jansen and A. Brodal. Oslo: Johan Grundt.

Brookhart, J.M. (1960): The Cerebellum. IN: Handbook of Physiology, Sect. I, Neurophysiology, II. Washington, D.C.: American Physiological Society, 1245-1280.

Calvin, W.H. and D. Hellerstein (1968): Dendritic spikes vs. cable properties-- Technical Comment. Science (In press).

Clark, J. and R. Plonsey (1968): The extracellular potential field of the single active nerve fibre in a volume conductor. Biophys. J. 8: 842-864.

Clark, J. and R. Plonsey (1967): A mathematical evaluation of the core conductor model. Biophys. J. 6: 95-112.

Cole, K.S. (1949): Dynamic electrical characterisitcs of the squid axon membrane. Arch. Sci. Physiol. 3: 253-258.

Cole, K.S. and A.L. Hodgkin (1939): Membrane and protoplasm resistance in the squid giant axon. J. Gen. Physiol. 22: 671-687.

Cragg, B.G. and L.H. Hamlyn (1955): Action potentials of the pyramidal neurones of the hippocampus of the rabbit. J. Physiol. (Lond.) 129: 608-627.

Dow, R.S. (1949): Action potentials of cerebellar cortex in response to local electrical stimulation. J. Neurophysiol. 12: 245-256.

Dow, R.S. and G. Moruzzi (1958): The Physiology and Pathology of the Cerebellum. Minneapolis: University of Minnesota Press.

Eager, R.P. (1963a): Efferent cortico-nuclear pathways in the cerebellum of the cat. J. Comp. Neurol. 120: 81-104.

Eager, R.P. (1963b): Cortial association pathways in the cerebellum of the cat. J. Comp. Neurol. 121: 381-394.

Eager, R.P. (1968): Some fine structural features of the neuronal elements composing the cerebellar nuclei in the cat. J. Comp. Neurol. 132: 235-262.

Ebbesson, S.O.E. (1967): Ascending axon degeneration following hemisection of the spinal cord in the tegu lizard (Tupinambis Nigro punctatalus). Brain Res. 5: 178-206.

Eccles, J.C. (1957): The Physiology of Nerve Cells. Baltimore: Johns Hopkins Press.

Eccles, J.C. (1964): The Physiology of Synapses. Berlin: Springer-Verlag.

Eccles, J.C. (1965): Functional meaning of the patterns of synaptic connections in the cerebellum. Perspect. Biol. Med. 8: 289-310.

Eccles, J.C., M. Ito and J. Szentágothai (1967): The Cerebellum as a Neuronal Machine. New York: Springer Verlag.

Eccles, J.C., B. Libet and R.R. Young (1958): The behaviour of chromatolysed motoneurones studied by intracellular recording. J. Physiol. (Lond.) 143: 11-40.

Eccles, J.C., R. Llinás and K. Sasaki (1964a): Excitation of cerebellar Purkinje cells by the climbing fibres. Nature 203: 245-246.

Eccles, J.C., R. Llinás and K. Sasaki (1964b): Golgi cell inhibition in the cerebellar cortex. Nature 204: 1265-1266.

Eccles, J.C., R. Llinás and K. Sasaki (1965): Inhibitory systems in the cerebellar cortex. Proc. Aust. Assoc. Neurol. 3: 1-14.

Eccles, J.C., R. Llinás and K. Sasaki (1966a): Parallel fibre stimulation and the responses induced thereby in the Purkinje cells of the cerebellum. Exp. Brain Res. 1: 17-39.

Eccles, J.C., R. Llinás and K. Sasaki (1966b): Intracellularly recorded responses of the cerebellar Purkinje cells. Exp. Brain Res. 1: 161-183.

Eccles, J.C., R. Llinás and K. Sasaki (1966c): The action of antidromic impulses on the cerebellar Purkinje cells. J. Physiol. (Lond.) 182: 316-345.

Eccles, J.C., R. Llinás and K. Sasaki (1966d): The inhibitory interneurones within the cerebellar cortex. Exp. Brain Res. 1: 1-16.

Eccles, J.C., R. Llinás and K. Sasaki (1966e): The mossy fibre-granule cell relay of the cerebellum and its inhibitory control by Golgi cells. Exp. Brain Res. 1: 82-101.

Eccles, J.C., R. Llinás and K. Sasaki (1966f): The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. J. Physiol. (Lond.) 182: 268-296.

Eccles, J.C., R. Llinás, K. Sasaki and P.E. Voorhoeve (1966): Interaction experiments on the responses evoked in Purkinje cells by climbing fibres. J. Physiol. (Lond.) 182: 297-315.

Eccles, J.C., K. Sasaki and P. Strata (1966): The profiles of physiological events produced by a parallel fibre volley in the cerebellar cortex. Exp. Brain Res. 2: 18-34.

Eccles, J.C., K. Sasaki and P. Strata (1967a): A comparison of the inhibitory actions of Golgi cells and of basket cells. Exp. Brain Res. 3: 81-94.

Eccles, J.C., K. Sasaki and P. Strata (1967b): Interpretation of the potential fields generated in the cerebellar cortex by a mossy fibre volley. Exp. Brain Res. 3: 58-80.

Escobar, A., E.D. Sampedro and R.S. Dow (1968): Quantitative data on the inferior olivary nucleus in man, cat and vampire bat. J. Comp. Neurol. 132: 397-404.

Fadiga, E. and G.C. Pupilli (1964): Teleceptive components of the cerebellar function. Physiol. Rev. 44: 432-486.

Fatt, P. (1957a): Electric potentials occurring around a neurone during its antidromic activation. J. Neurophysiol. 20: 27-60.

Fatt, P. (1957b): Sequences of events in synaptic activation of a motoneurone. J. Neurophysiol. 20: 61-80.

Fox, C.A. and J.W. Barnard (1957): A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibres. J. Anat. (Lond.) 9: 299-313.

Fox, C.A. and E.G. Bertram (1954): Connections of the Golgi cells and the intermediate cells of Lugaro in the cerebellar cortex of the monkey. Anat. Rec. 118: 423.

Fox, C.A., D.E. Hillman, K.A. Siegesmund and C.R. Dutta (1967): The primate cerebellar cortex: A Golgi and electron microscopic study. IN: Progress in Brain Research 25: The Cerebellum Ed. C.A. Fox and R.S. Snider, Amsterdam: Elsevier Publishing Co., 174-225.

Fox, C.A., K.A. Siegesmund and C.R. Dutta (1964): The Purkinje cell dendritic branchlets and their relation with the parallel fibres: Light and electron microscopic observations. IN: Morphological and Biochemical Correlates of Neural Activity. Ed. M.M. Cohen and R.S. Snider, New York: Hoeber, 112-141.

Fujita, Y. and H. Sakata (1962): Electrophysiological properties of CA1 and CA2 apical dendrites of rabbit hippocampus. J. Neurophysiol. 25: 209-222.

Fujita, Y. (1968): Activity of dendrites of single Purkinje cells and its relationship to so-called inactivation response in rabbit cerebellum. J. Neurophysiol. 31: 131-141.

Golby, F. and L.R. Robinson (1962): The central connections of dorsal spinal nerve roots and the ascending tracts in the spinal cord of Lacerta viridis. J. Anat. (Lond.) 96: 153-170.

Goodman, D.C. and J.T. Simpson, Jr. (1960): Cerebellar stimulation in the unrestrained and unanesthetized alligator. J. Comp. Neurol. 114: 127-135.

Grampp, W. (1966a): Multiple-spike discharge evoking after-depolarizations in the slowly adapting stretch receptor neurone of the lobster. I. The labile and the fast after-depolarization. Acta Physiol. Scand. 67: 100-115.

Grampp, W. (1966b): Multiple-spike discharge evoking after-depolarization in the slowly adapting stretch receptor neurone of the lobster. II. The slow after-depolarization. Acta Physiol. Scand. 67: 116-126.

Granit, R. and C.G. Phillips (1956): Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. J. Physiol. (Lond.) 133: 520-547.

Hámori, J. and J. Szentágothai (1964): The "Crossing Over" synapse. An electron microscope study of the molecular layer in the cerebellar cortex. Acta biol. Sci. hung. 15: 95-117.

Hámori, J. and J. Szentágothai (1965): The Purkinje cell baskets: ultrastructure of an inhibitory synapse. Acta biol. Acad. Sci. hung. 15: 465-479.

Hámori, J. and J. Szentágothai (1966a): Identification under the electron microscope of climbing fibres and their synaptic contacts. Exp. Brain Res. 1: 65-81.

Hámori, J. and J. Szentágothai (1966b): Participation of Golgi neurone processes in the cerebellar glomeruli: An electron microscope study. Exp. Brain Res. 2: 25-49.

Hámori, J. and J. Szentágothai (1968): Identification of synapses formed in cerebellar cortex by Purkinje axon collaterals: An electron microscope study. Exp. Brain Res. 5: 118-128.

Harmon, L.D. (1968): Modeling studies of neural inhibition. IN: Structure and Function of Inhibitory Neuronal Mechanisms. Ed. C. von Euler and S. Skoglund and U. Söderberg, Pergamon Press, 537-563.

Harmon, L.D., R.T. Kado and E.R. Lewis (1968): Cerebellar modeling problems. Neurosciences Res. Prog. Bull. (In press).

Hellerstein, D. (1968): Passive membrane potentials. A generalization of the theory of electrotonus. Biophys. J. 8: 358-379.

Hild, W. and I. Tasaki (1962): Morphological and physiological properties of neurones and glial cells in tissue culture. J. Neurophysiol. 25: 277-305.

Hillman, D.E. (1968): Morphological basis for a model of the cerebellar cortex. Fifth Internat. Congr. of Cybernet. Naples, (In press).

Hodgkin, A.L. (1958): The Croonian Lecture: Ionic movements and electrical activity in giant nerve fibres. Proc. Roy. Soc. (Lond.) Ser. B 148: 1-37.

Houser, G.L. (1901): The neurones and supporting elements of the brain of a selachian. J. Comp. Neurol. 11: 65-175.

Hubbard, J.I., R. Llinás and D.M.J. Quastel (1969): Electrophysiological Analysis of Synaptic Transmission. London: Edward Arnold Press.

Huber, G.C. and E.C. Crosby (1926): On thalamic and tectal nuclei and fibre paths in the brain of the American alligator. J. Comp. Neurol. 40: 97-227.

Jackson, J.D. (1962): Classical Electrodynamics. New York: Wiley.

Jansen, J. (1954): Efferent cerebellar connections. IN: Aspects of Cerebellar Anatomy, Ch. III. Ed: J. Jansen and A. Brodal; Oslo: Johan Grundt.

Jansen, J. and A. Brodal (1954): Aspects of Cerebellar Anatomy. Oslo: Johan Grundt Tanum.

Johannesma, P. (1968): Diffusion model for the stochastic activity of neuron. Proc. of the Summer School on Mathematical Models of Neuronic Network. Ravello, 1967, Springer (In press).

Joseph, B.S. and D.G. Whitlock (1968a): Central projections of selected spinal dorsal roots in anuran amphibians. Anat. Rec. 160: 279-288.

Joseph, B.S. and D.G. Whitlock (1968b): Central projections of brachial and lumbar dorsal roots in reptiles. J. Comp. Neurol. 132: 469-484.

Katz, B. and S. Thesleff (1957): On the factors which determine the amplitude of the "miniature end-plate potential". J. Physiol. (Lond.) 137: 267-278.

Kawakami, M. (1954): Contributions to the comparative anatomy of the cerebellar fibre connections in the reptiles. Hiroshima J. Med. Sci. 2: 295-317.

Khintchine, A.Y. (1960): Mathematical methods in the theory of queuing. Griffin's Statistical Monographs and Courses. No. 7, Griffin, London.

Korn, G.A. and T.M. Korn (1968): Mathematical Handbook for Scientists and Engineers. 2nd Edition. New York: McGraw-Hill.

Korneiliussen, J.K. (1968): Comments on the cerebellum and its division. Brain Res. 8: 229-236.

Larramendi, L.M.H. and T. Victor (1967): Synapses on the Purkinje cell spines in the mouse. An electron microscopic study. Brain Res. 5: 15-30.

Larsell, O. (1932): The cerebellum of reptiles chelonians and alligator. J. Comp. Neurol. 56: 299-345.

Larsell, O. (1967): The Comparative Anatomy and Histology of the Cerebellum from Myxinoids through Birds. Minneapolis: University of Minnesota Press.

Llinás, R. (1968): Functional aspects of interneuronal evolution in the cerebellar cortex. IN: The Interneuron, Ed. M.A. Brazier, U.C.L.A. (In press).

Llinás, R. and J.F. Ayala (1967): Purkinje axon collateral action upon interneurones in the cerebellar cortex. IN: Neurophysiological Basis of Normal and Abnormal Motor Activities. Ed. M.D. Yahr and D.P. Purpura., Hewlett, N.Y.: Raven Press.

Llinás, R. and J.R. Bloedel (1967): Frog Cerebellum: Absence of long-term inhibition upon Purkinje cells. Science 155: 601-603.

Llinás, R., D.E. Hillman and W. Precht (1968): Functional aspects of cerebellar evolution. IN: The Cerebellum in Health and Disease (In press).

Llinás, R., W. Precht and S.T. Kitai (1967): Climbing fibre activation of Purkinje cell following primary vestibular afferent stimulation in the frog. Brain Res. 6: 371-375.

Lorente de Nó, R. (1947a): A study of nerve physiology. Studies Rockefeller Inst. Med. Res. Ch. 16.

Lorente de Nó, R. (1947b): Action potential of the motoneurones of the hypoglossal nucleus. J. Cell. Comp. Physiol. 29: 207-288.

Lundberg, A. and O. Oscarsson (1960): Functional organization of the dorsal spino-cerebellar tract in the cat. VII. Identification of units by antidromic activation from the cerebellar cortex with recognition of five functional subdivisions. Acta Physiol. Scand. 50: 356-374.

Lundberg, A. and O. Oscarsson (1962): Functional organization of the ventral spino-cerebellar tract in the cat. IV. Identification of units by antidromic activation from the cerebellar cortex. Acta Physiol. Scand. 54: 270-286.

MacGregor, R.J. (1968): A model for responses to activation by axodendritic synapses. Biophys. J. 8: 305-318.

MacKay, D.M. (1968): Possible information-processing function of inhibitory mechanisms. IN: Structure and Function of Inhibitory Neuronal Mechanisms. Ed. C. von Euler, S. Skoglund and U. Söderberg, Pergamon Press.

Martin, A.R. (1955): A further study of the statistical composition of the end-plate potential. J. Physiol. 130: 114-122.

Mason, S.J. and H.J. Zimmerman (1960): Electronic Circuits, Signals and Systems. New York: Wiley.

Mellon, D. Jr. and D. Kennedy (1964): Impulse origin and propagation in a bipolar sensory neuron. J. Gen. Physiol. 47: 487-499.

Moatamed, F. (1966): Cell frequencies in the human inferior olivary nuclear complex. J. Comp. Neurol. 128: 109-116.

Nakajima, S. and K. Takahashi (1966a): Post-tetanic hyperpolarization in stretch receptor neurone of crayfish. Nature 209: 1220-1221.

Nakajima, S. and K. Takahashi (1966b): Post-tetanic hyperpolarization and electrogenic Na pump in stretch receptor neurone of crayfish. J. Physiol. (Lond.) 187: 105-128.

Nelson, P.G. and K. Frank (1964a): Extracellular potential fields of single spinal motoneurones. J. Neurophysiol. 27: 913-927.

Nelson, P.G. and K. Frank (1964b): Orthodromically produced changes in motoneuronal extracellular fields. J. Neurophysiol. 27: 928-941.

Nieuwenhuys, R. (1967): Comparative anatomy of the cerebellum. IN: Progress in Brain Research 25, The Cerebellum. Ed. C.A. Fox and R.S. Snider Amsterdam: Elsevier, pp. 1-93.

Nieuwenhuys, R. and C. Nicholson (1967): Cerebellum of mormyrids. Nature 215: 764-765.

Oscarsson, O. (1965): Functional organization of the spino- and cuneo-cerebellar tracts. Physiol. Rev. 45: 495-522.

Palay, S.L. (1964): The structural basis for neural action. IN: Brain Function, Vol. II. RNA and Brain Function; Memory and Learning. (U.C.L.A. Forum Med. Sci. No. 2). Ed. M.A. Brazier, Los Angeles: University of California Press, pp. 69-107.

Purpura, D.P. (1967): Comparative physiology of dendrites. IN: The Neurosciences. Ed. G.C. Quarton, T. Melnechuk and F.O. Schmitt. New York: Rockefeller University Press, pp. 372-393.

Purpura, D.P. and A. Malliani (1966): Spike generation and propagation initiated in dendrites by transhippocampal polarization. Brain Res. 1: 403-406.

Purpura, D.P., J.G. McMurtry, C.F. Leonard and A. Malliani (1966): Evidence for dendritic origin of spikes without depolarizing prepotentials in hippocampal neurones during and after seizure. J. Neurophysiol. 29: 954-979.

Rall, W. (1959): Dendritic current distribution and whole neurone properties. Research Report NM 01 05 00.01.02, Naval Medical Research Institute, Bethesda, Maryland.

Rall, W. (1962a): Theory of physiological properties of dendrites. Ann. N.Y. Acad. Sci. 96: 1071-1092.

Rall. W. (1962b): Electrophysiology of a dendritic neurone model. Biophysical. J. 2: 145-167.

Rall. W. (1964): Theoretical significance of dendritic trees for neuronal input-output relations. IN: Neural Theory and Modelling. Ed. R.F. Reiss. Stanford: Stanford University Press, pp. 73-97.

Rall, W. (1967): Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. J. Neurophysiol. 30: 1138-1168.

Rall, W., R.E. Burke, T.G. Smith, P.G. Nelson and K. Frank (1967): Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurones. J. Neurophysiol. 30: 1169-1193.

Ramón Y Cajal, S. (1909-1911): Histologie du système nerveux de l'homme et des vertébrés. 2 Vols. Paris: A. Maloine.

Romer, A.S. (1959): The Vertebrate Story. Chicago: University of Chicago.

Schaper. A. (1898): The finer structure of the selachian cerebellum (Mustelus vulgaris) as shown by chrome-silver preparations. J. Comp. Neurol. 8: 1-20.

Scheibel, M.E. and A.B. Scheibel (1954): Observations on the intracortical relations of the climbing fibres of the cerebellum. A Golgi study. J. Comp. Neurol. 101: 733-764.

Segundo, J.P., T. Takenaka and M. Encabo (1967): Electrophysiology of bulbar reticular neurones. J. Neurophysiol. 30: 1194-1220.

Sotelo, C. (1967): Cerebellar neuroglia: morphological and histochemical aspects. IN: Progress in Brain Research 25, The Cerebellum. Ed. C.A. Fox and R.S. Snider; Amsterdam: Elsevier, pp. 174-225.

Smirnow, A.E. (1897): Über eine besondere Art von Nervenzellen der Molecularschicht des Kleinhirns bei erwachsenen Säugetieren und beim Menschen. Anat. Anz. 13: 636-642.

Spencer, W.A. and E.R. Kandel (1961): Electrophysiology of hippocampal neurones. IV. Fast pre-potentials. J. Neurophysiol. 24: 272-285.

Stefanelli, A. (1943): Osservazioni comparative sui nuclei cerebellari dei rettili in relazione al differente modo di lomomozione. Monit. Zool. ital. 54: 65-72.

Szentágothai, J. (1962): Anatomical aspects of junctional transformation. IN: Information Processing in the Nervous System. Ed. R.W. Gerard and J.W. Duyff. Exerpta Medica Congress Series No. 49, pp. 119-136.

Szentágothai, J. (1965): The use of degeneration methods in the investigation of short neuronal connections. IN: Progress in Brain Research 14, Degeneration Patterns in the Nervous System. Ed. M. Singer and J.P. Schadé; Amsterdam: Elsevier, pp. 1-34.

Szentágothai, J. (1968): **Structuro-functional considerations of cerebellar neuronal networks.** Proc. I.E.E.E. 56:960.

Szentágothai, J. and K. Rajkovits (1959): Über den Ursprung der Kletterfasern des Kleinhirns. Z. Anat. Entwichl.-Gesch. 121: 130-141.

Terzuolo, C.A. and R. Llinás (1967): Distribution of synaptic inputs in the spinal motoneurone and its functional significance. IN: Muscular Afferents and Motor Control. Ed. R. Granit. Stockholm: Almqvist and Wiksell, pp. 373-384.

Uchizono, K. (1965): Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. Nature 207: 642-643.

Uchizono, K. (1967): Synaptic organization of the Purkinje cells in the cerebellum of the cat. Exp. Brain Res. 4: 97-113.

Walberg, F. (1954): Descending connections to the inferior olive. IN: Aspects of Cerebellar Anatomy, Ch. IV. Ed. J. Jansen and A. Brodal. Oslo: Johan Grundt.

Walberg, F. and J. Jansen (1964): The cerebellar cortico-nuclear projection. An experimental study with silver methods. J. Hirnforsch. 6: 338-354.

Wall. P.D. (1965): Impulses originating in the region of dendrites. J. Physiol. (Lond.) 180: 116-133.

Weston, J.K. (1936): The reptilian vestibular and cerebellar gray with fibre connections. J. Comp. Neurol. 65: 93-199.

Figure 1. Evolution of vertebrates with a cerebellum. Cyclostomes would appear at the base of the tree (450 million years ago) but are omitted because their cerebellum is minute. Figure 1 is based on the classification of Romer (1959).

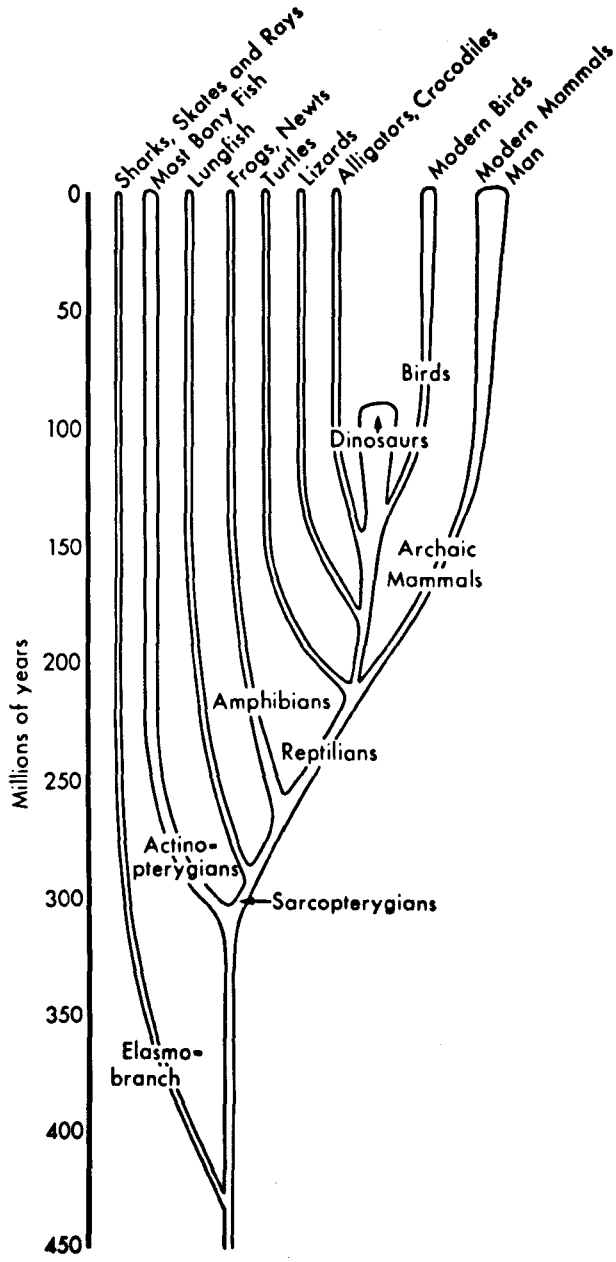

Sharks, Skates and Rays
Most Bony Fish
Lungfish
Frogs, Newts
Turtles
Lizards
Alligators, Crocodiles
Modern Birds
Modern Mammals
Man
Birds
Dinosaurs
Archaic Mammals
Amphibians
Reptilians
Actino-pterygians
Sarcopterygians
Elasmo-branch
Millions of years
0
50
100
150
200
250
300
350
400
450

Figure 2. Diagram of positions of stimulating and recording electrodes in cerebellar cortex. Local (Loc) or white matter (WM) bipolar stimulating electrodes excite neural elements in the molecular layer (ML), Purkinje cell layer (PC) or granular cell layer (GL). Resulting electrical activity recorded with as many as four micropipettes (1-4) feeding FET negative capacity headstages. FP: Fissura Prima, FS: Fissura Secunda. Anatomy described in detail in Appendix A.

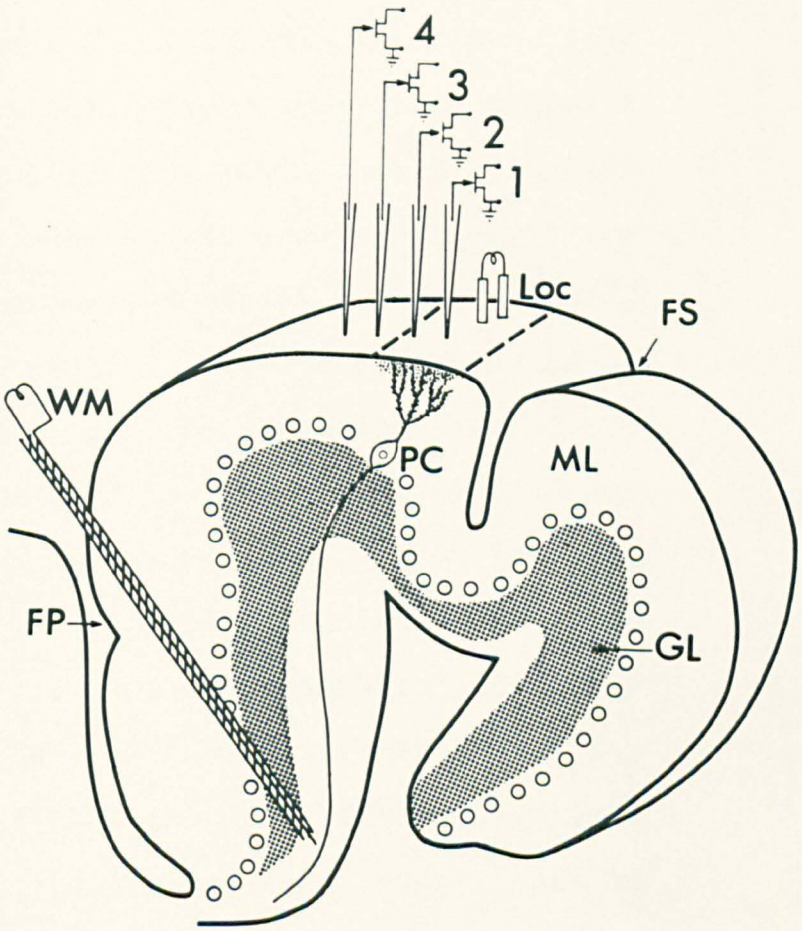

4
3
2
1
Loc
FS
WM
PC
ML
FP
GL

Figure 3 A-C. Loc evoked field potentials. A and B show fields following Loc stimulus (stimulus artefact indicated by dotted line with arrow beneath it). In A several oscilloscope sweeps were superimposed, in B 16 sweeps were averaged on an instrument computer. C shows graph of time of occurrence of foot of B-component of wave in A and B and time of occurrence of peak of B-component as function of depth of recording electrode tip. Open and closed circles correspond to symbols and dotted lines in A and B. Depth of recording electrode tip indicated to left of each figure. In all extracellular records a negative potential is displayed as an upward deflection.

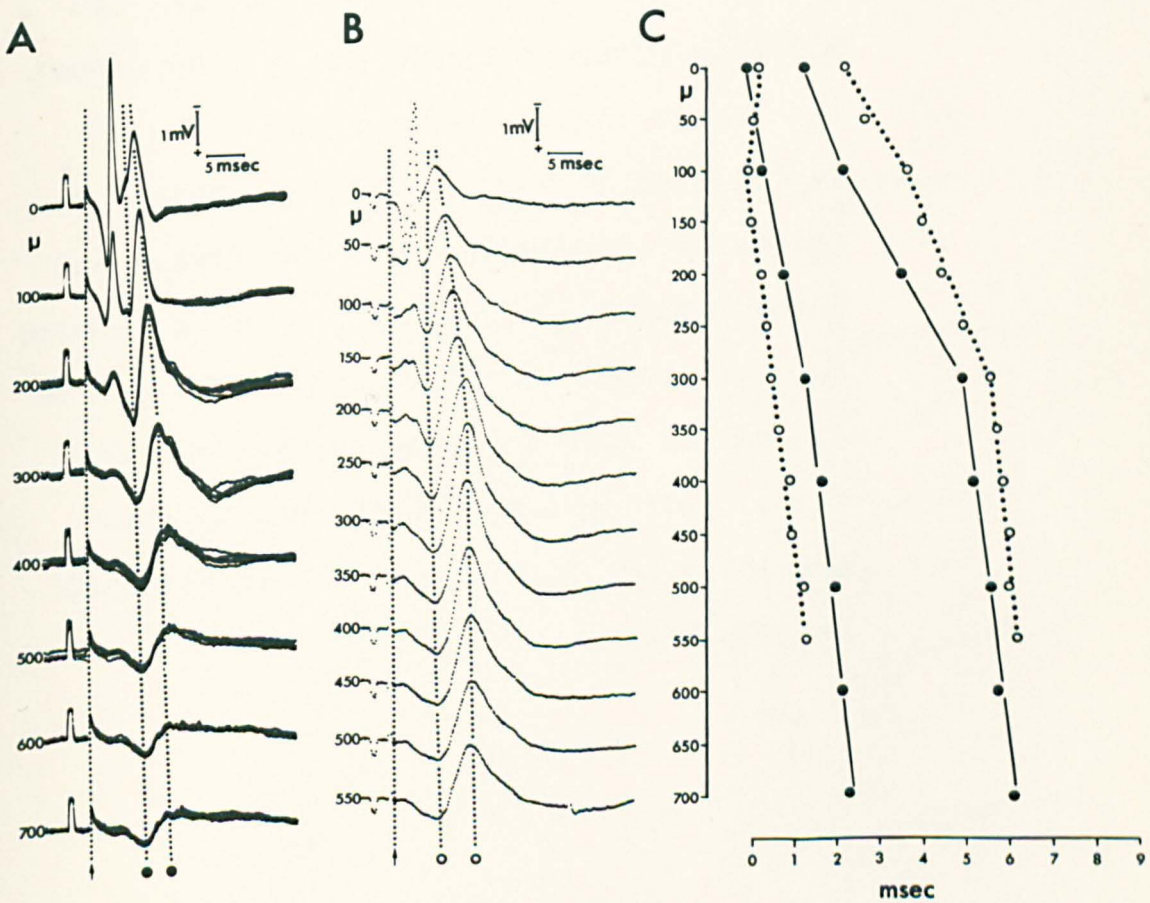

A
B
C
1mV
5 msec
μ
0
100
200
300
400
500
600
700
50
150
250
350
450
550
msec
1
2
3
4
5
6
7
8
9

Figure 4 A and B. Effect of preceding a Loc stimulus by another Loc with a short interval between them. In A the control response (CON) is shown for a single Loc stimulus given at time indicated by arrow and dotted line. In B the Loc stimulus is preceded 20 msec before by another Loc (times of stimuli indicated by arrows and dotted lines). A-component of wave remains but B-component is abolished. Time course of control wave indicated by dotted line in B. Note EPSP wave uncovered by the preceding Loc in B at 0μ and its reversal at 300μ.

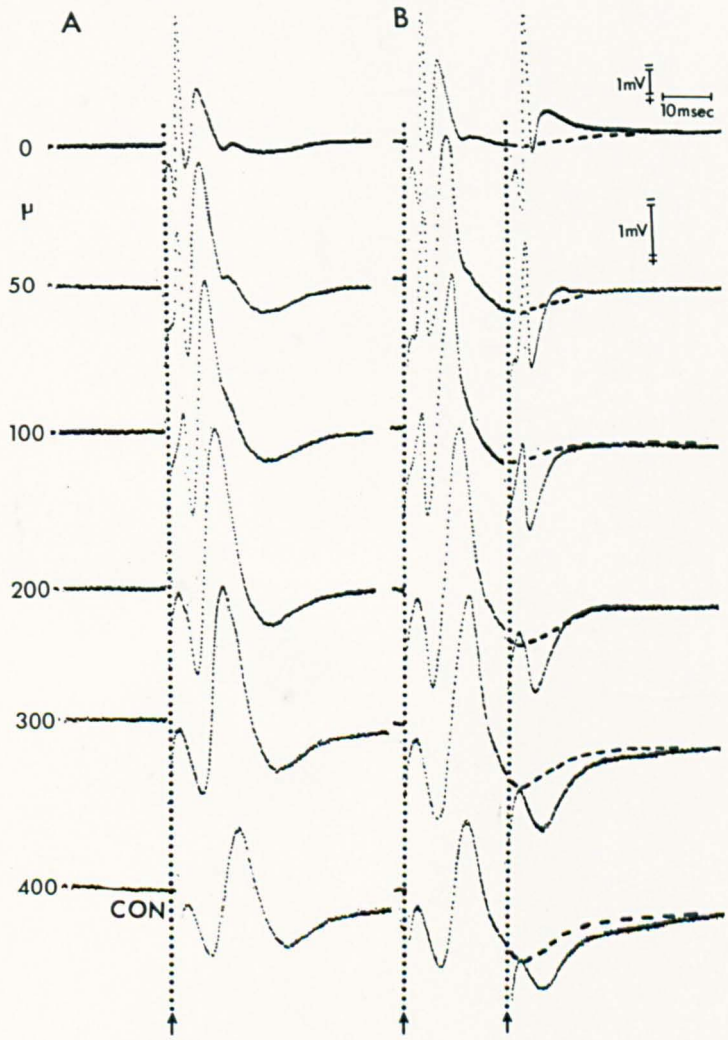

A
B
1mV
10msec
0
μ
50
100
200
300
400
CON
1mV

Figure 5 A and B. Conduction velocity of the A-component (parallel fibre volley) of Loc evoked field. A shows A-component recorded near surface and at different distances from the stimulating electrode. B shows graph of the recording position as a function of time of peak (open circles in Figures A and B) relative to stimulus, indicated by arrow in A. Closed circles and squares in Figure B indicate results from other experiments. Slope of graph in Figure B gives a conduction velocity of 0.18m/sec.

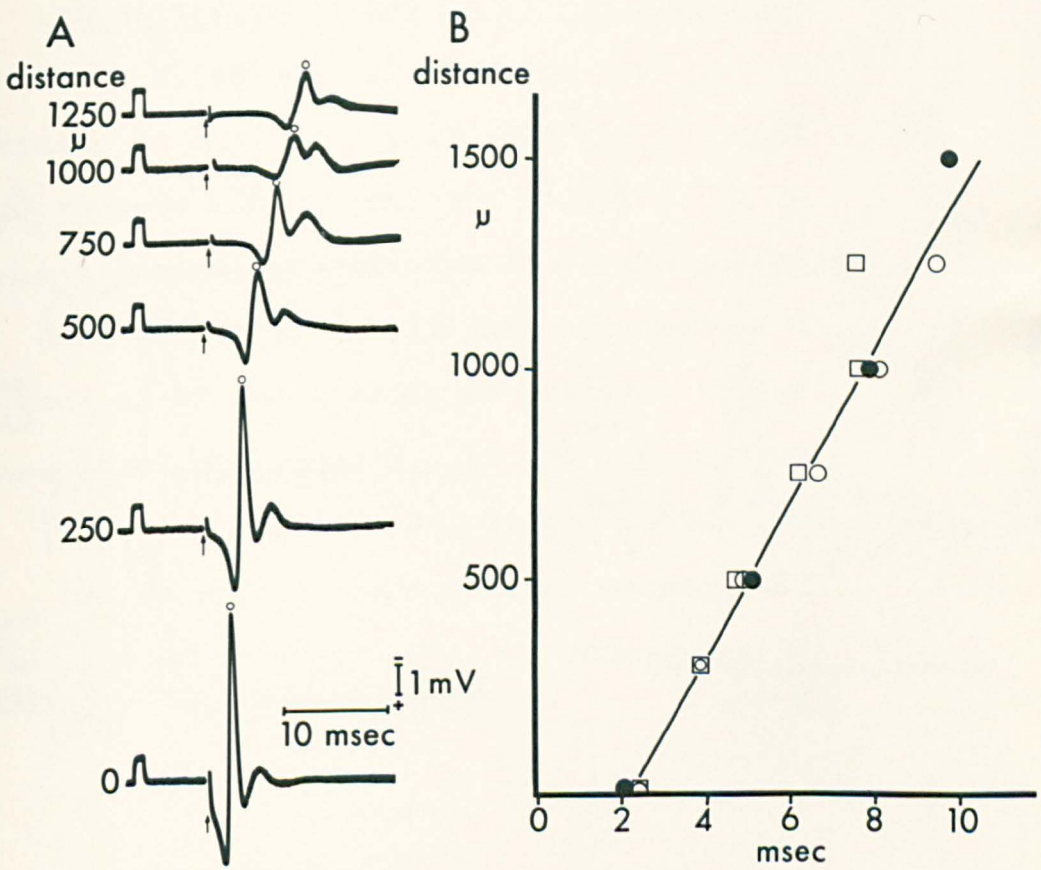

A
distance
1250
μ
1000
750
500
250
0
1 mV
10 msec
B
distance
1500
μ
1000
500
0
2
4
6
8
10
msec

Figure 6 A to E. Extracellularly recorded dendritic spikes in the molecular layer. In A a weak Loc stimulus (arrow) evokes only a field potential, but in B an increase in Loc strength produces an all-or-none spike. C shows superimposed spikes and E demonstrates inhibition of spikes when Loc is preceded by another Loc (D is the control Loc for E). In one case in E, the second Loc is not inhibited. Note the notched falling phase of the spikes in all cases.

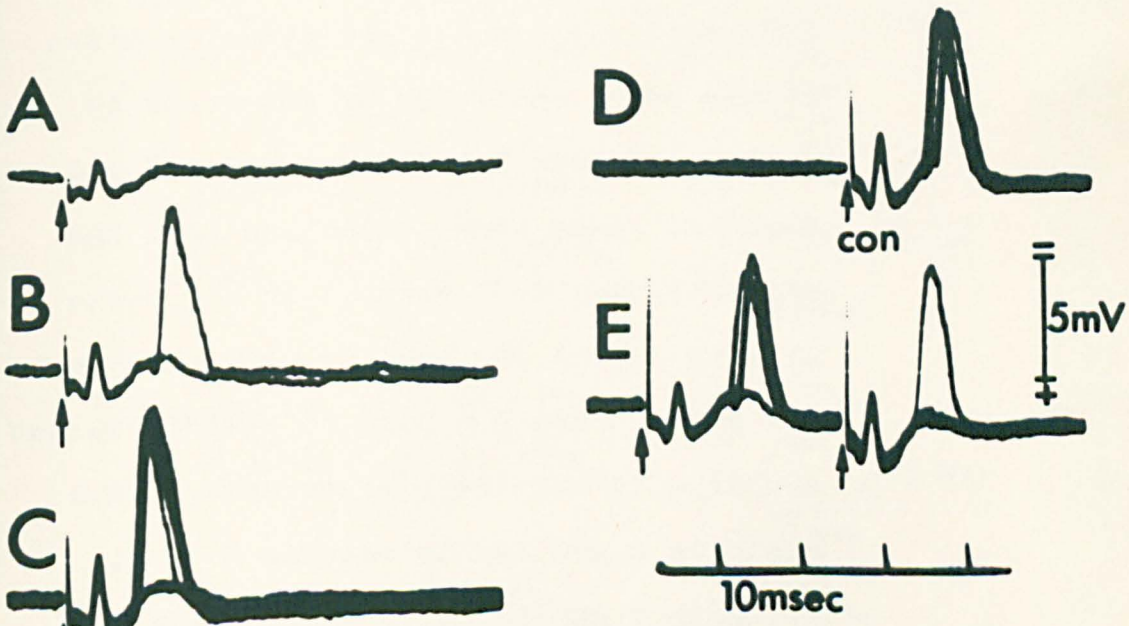

A
B
C
D
con
E
5mV
10msec

Figure 7 A -L. Dendritic spikes recorded simultaneously with two micropipettes. The micropipette tips were separated by 200μ vertically and a Loc stimulus given at the point indicated by the arrow in A to C. Sweeps D to L were delayed and expanded (note time scales) so that the stimulus does not appear. In all cases similar spikes are seen on both electrodes and in I, K and L a pair of similar spikes are seen in both sets of records. Time scale in C applies to records A to C, scale in F applies to D to F, scale in I applies to G to I and scale in L applies to J to L.

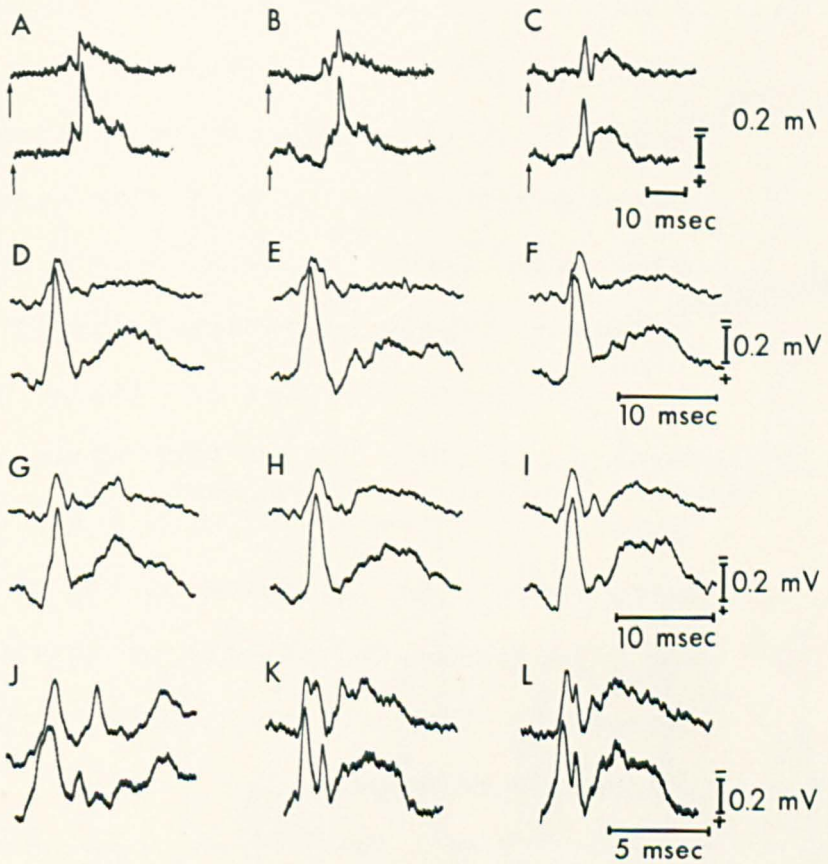

A
B
C
0.2 mV
10 msec
D
E
F
0.2 mV
10 msec
G
H
I
0.2 mV
10 msec
J
K
L
0.2 mV
5 msec

Figure 8 A to P. Pairs of dendritic spikes recorded with two micropipettes. The technique used to obtain these records was the same as that used in Figure 7. One electrode recorded a particularly clear potential in these experiments. A to F show spike at slow sweep speed (scale in F). G to L show spikes at faster sweep speed (scale in L). Note lability of spikes is similar on both electrodes (L). M to P show histograms of latency of spike recorded on one electrode with respect to the spike on the other. M to O represent the effects of increasing strengths of Loc stimuli, whilst P is the composite histogram.

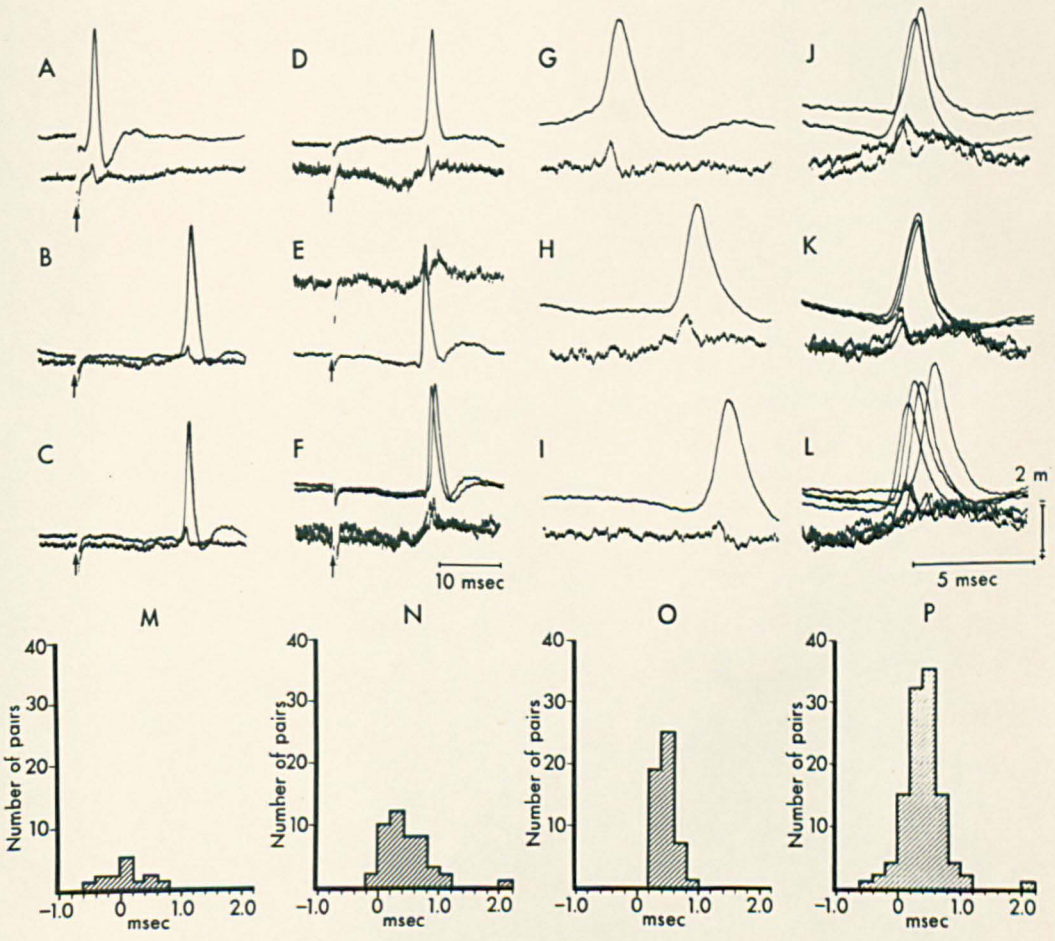

A
B
C
D
E
F
G
H
I
J
K
L
10 msec
2 m
5 msec
M
N
O
P
Number of pairs
40
30
20
10
-1.0
0
1.0
2.0
msec

Figure 9 A to C. Time course of stellate cell inhibition. Top record of A shows control Loc induced field (stimulus at arrow) recorded by a micropipette at 200μ depth. Below this are seen the effects of preceding a similar stimulus (TEST) by an identical Loc (COND) at the intervals shown to the left of the records. In each case the responses have been averaged and the field of the conditioning stimulus alone subtracted. B shows pairs of stimuli and responses without averaging or subtraction (same delays as in A). C shows a graph of the percentage height of the averaged test response after conditioning as a function of the averaged conditioned negativity (COND and TEST stimuli were identical).

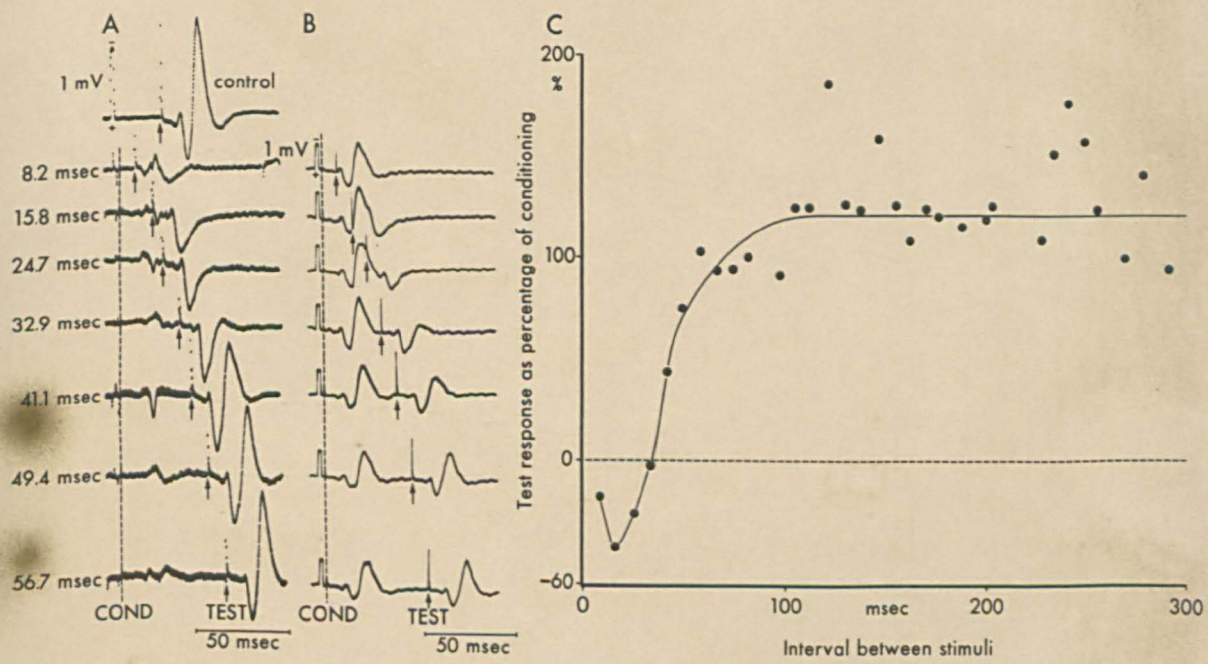

A
B
C
1 mV
control
1 mV
8.2 msec
15.8 msec
24.7 msec
32.9 msec
41.1 msec
49.4 msec
56.7 msec
COND
TEST
50 msec
COND
TEST
50 msec
200
%
100
0
-60
Test response as percentage of conditioning
0
100
msec
200
300
Interval between stimuli

Figure 10 A to C. Summary of stellate cell inhibitory effects. A shows a Loc (given at time indicated by circle) evoked field. B shows a sequence of such fields given at varying intervals after a conditioning Loc (first closed circle). The dendritic spike component is seen to be markedly reduced at short intervals after the conditioning stimulus. Note that the field caused by the second stimulus rides on the potential caused by the first. Calibration for A and B given in A. C summarises the relationship between stellate cell (SC) and Purkinje cell (PC). PF is parallel fibre, DS dendritic spine and IS is the inhibitory synapse.

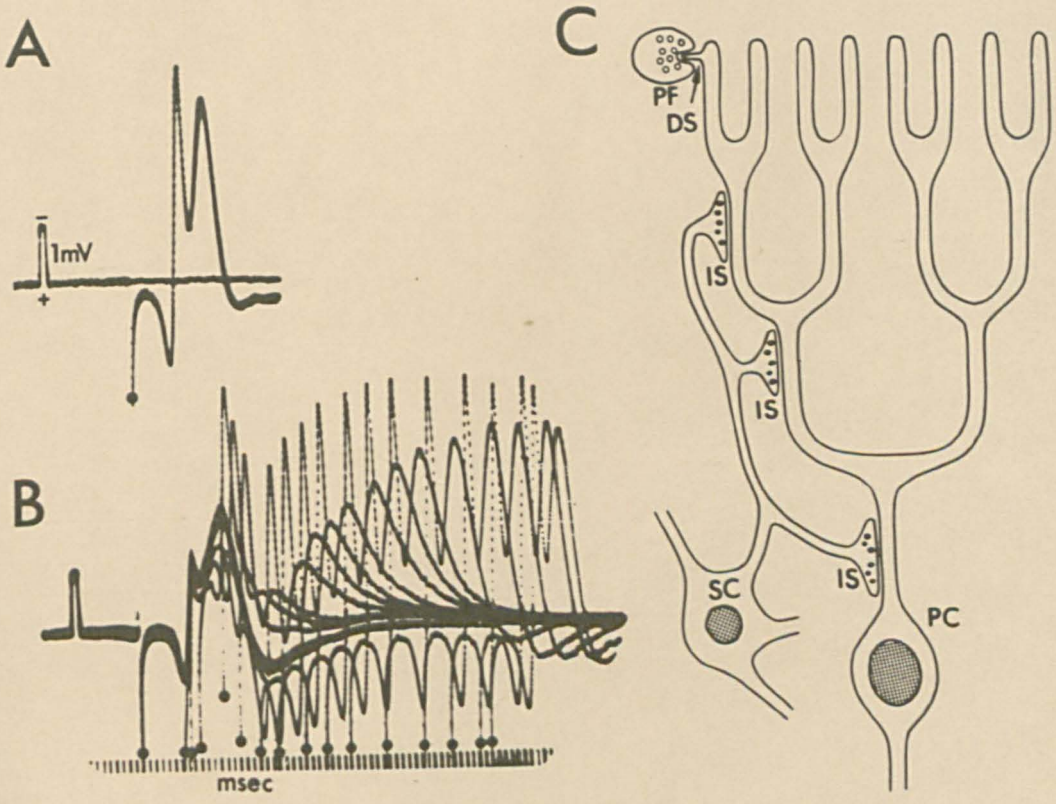

A
1mV
B
msec
C
PF
DS
IS
IS
IS
SC
PC

Figure 11 A and B. Fields evoked by Loc stimulus and recorded perpendicular to parallel fibres. Three micropipettes with their tips at the same level and orientated in a plane perpendicular to the direction of the parallel fibres; the distance between the tips of the electrodes was 200 to 250μ. A and B show two sequences of records at increasing Loc stimulus strengths. Strength relative to threshold indicated beneath each set of records. The lower records in each case are from the micropipette closest to the centre of the parallel fibre beam.

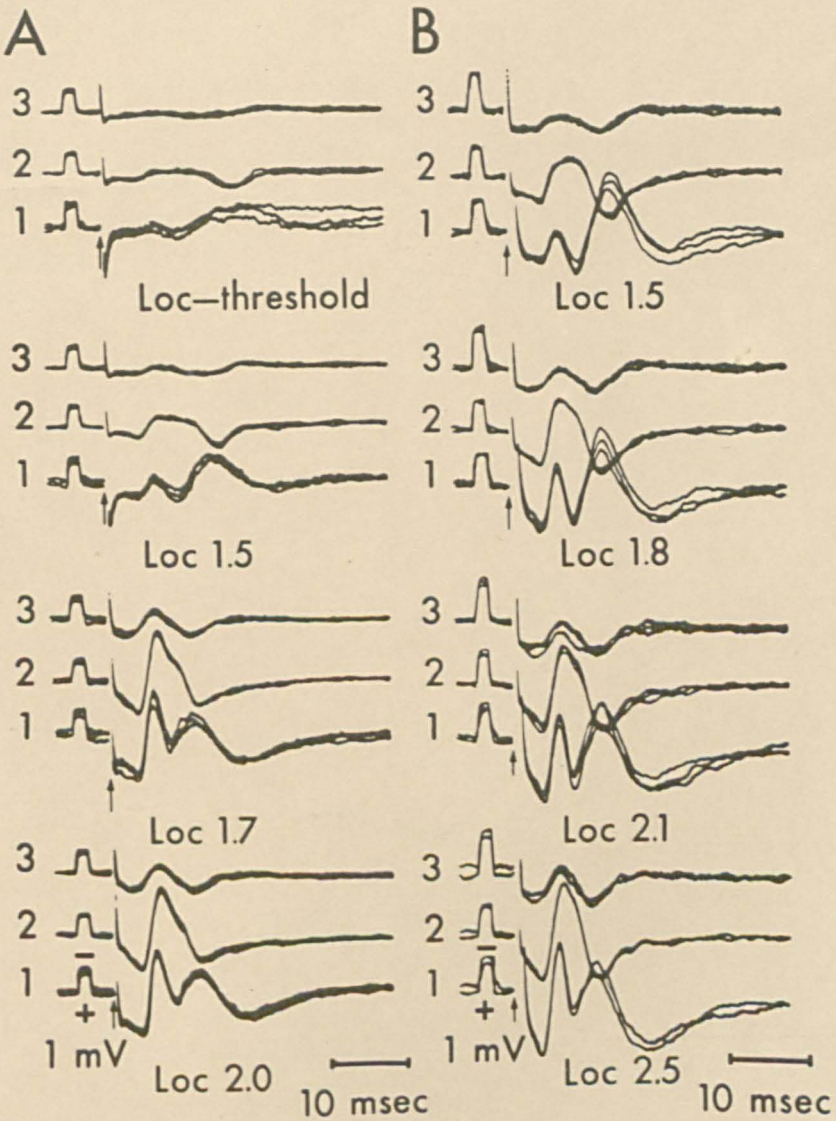

A
B
3
2
1
Loc—threshold
Loc 1.5
Loc 1.5
Loc 1.8
Loc 1.7
Loc 2.1
1 mV
Loc 2.0
10 msec
1 mV
Loc 2.5
10 msec

Figure 12 A and B. Loc evoked fields recorded simultaneously with four micropipettes. The pipettes were assembled in a plane perpendicular to the direction of the parallel fibres (see Figure 2) with tips on the same level 200μ apart. Pipette 1 was in the centre of the parallel fibre beam. A shows sets of records at different depths, records assembled one above the other to show temporal relationships. Downward arrow indicates parallel fibre volley. B shows records set out to indicate spatial pattern of fields. Note dendritic spike component propagates down in records from pipette 1 but up in records from pipette 2 and note also individual spikes at 300-450μ. Upward arrow indicates Loc stimulus.

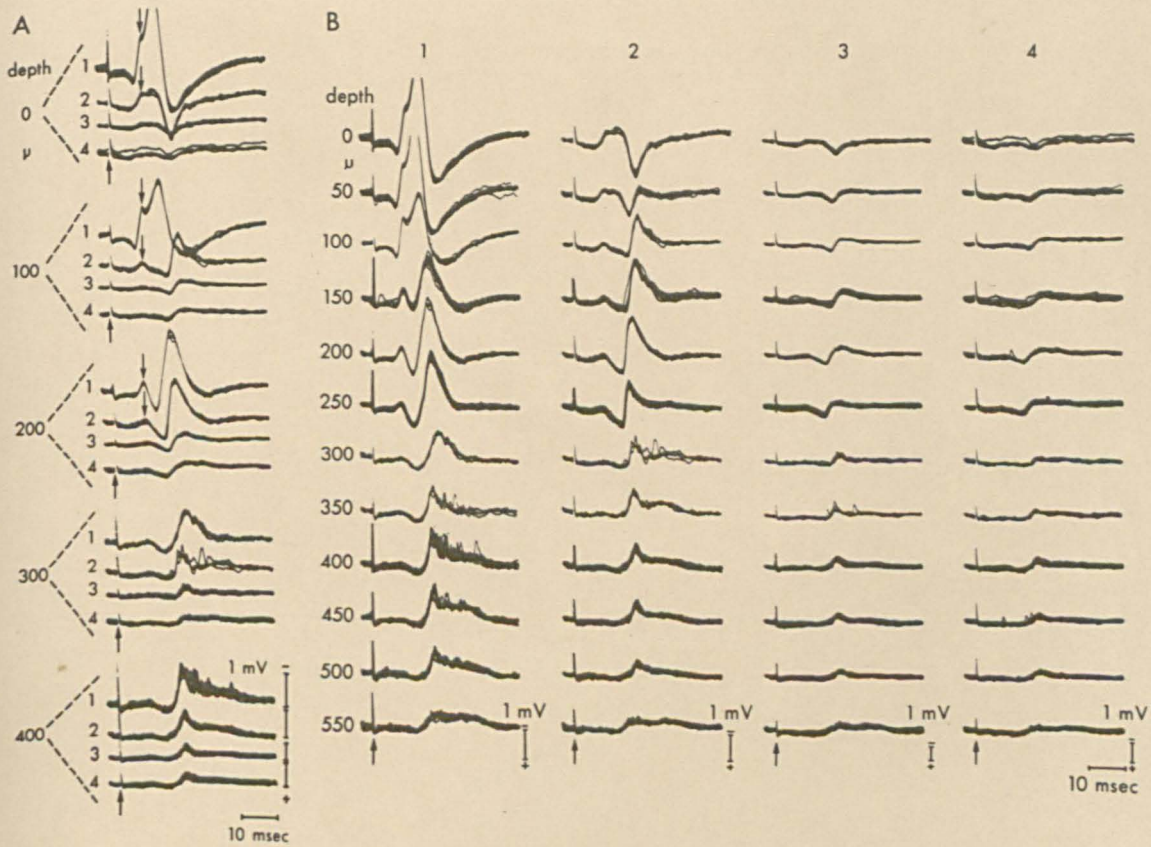

A
depth
0
μ
100
200
300
400
1
2
3
4
1 mV
10 msec
B
1
2
3
4
depth
0
μ
50
100
150
200
250
300
350
400
450
500
550
1 mV
10 msec

Figure 13 A and B. Fields evoked with weak Loc stimulus. Stimulus in A very weak (at arrow). Dotted line with filled triangle beneath it indicates time of negativity peak at 0μ in A and B. B shows effect of slight increase in stimulus strength. Between 0 and 100μ the negativity propagates down with a velocity of 0.025 m/sec. Eight successive records a averaged to give fields in each case.

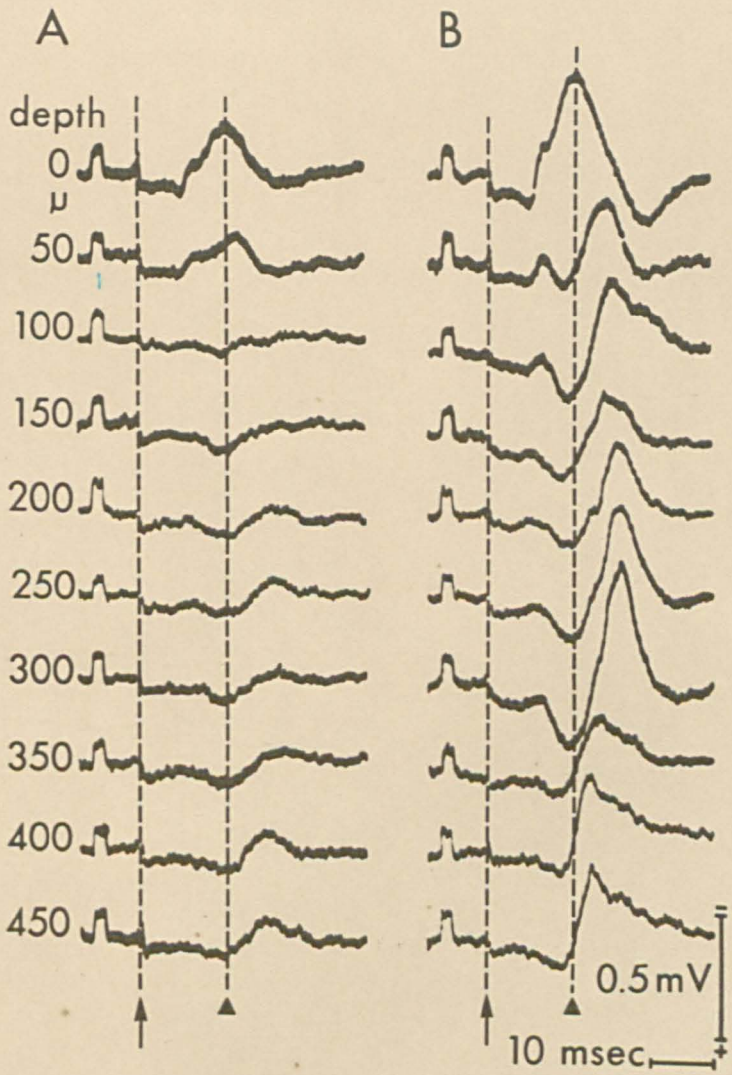

A
B
depth
0
μ
50
100
150
200
250
300
350
400
450
0.5 mV
10 msec

Figure 14 A and B. Fields evoked by white matter (WM) stimulation. A shows fields following WM stimulus, recorded at depths shown, with a micropipette (see Figure 2 for position of WM electrode). B shows the effect of a preceding Loc st stimulus on the WM evoked fields; the late negativity is reduced superficially. Note negativity caused by antidromic Purkinje cell invasion immediately following WM stimulus at 450-250μ.

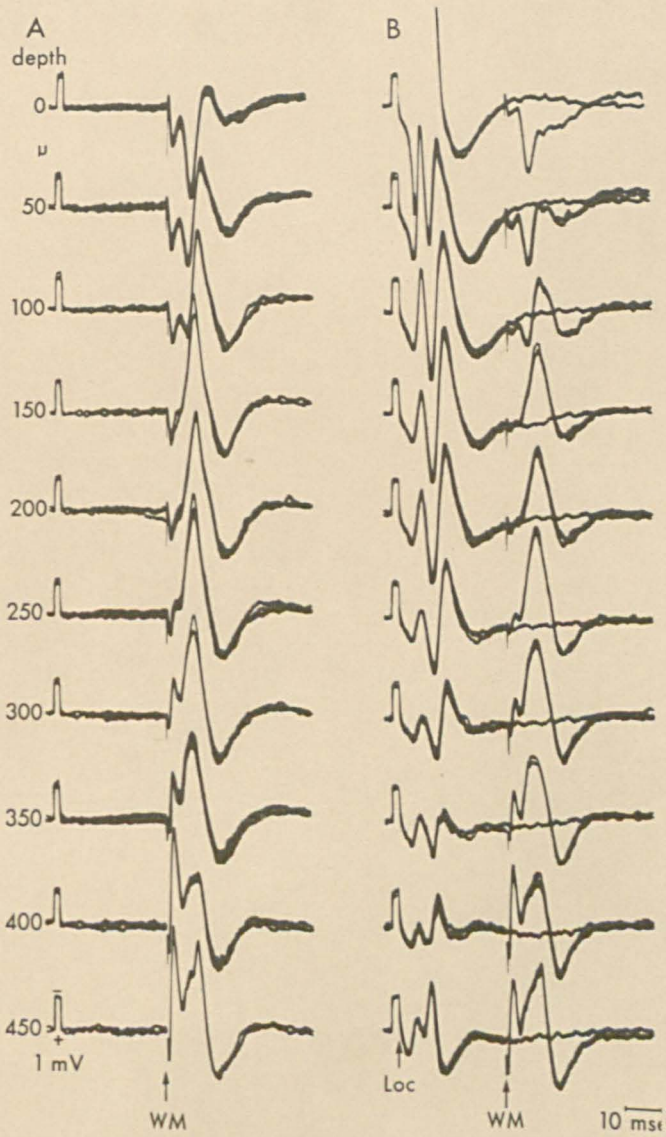

A
depth
0
μ
50
100
150
200
250
300
350
400
450
1 mV
WM
B
Loc
WM
10 mse

Figure 15 A and B. Fields caused by antidromic invasion of Purkinje cells. WM stimulus given at arrow in A. Fast oscilloscope sweep speed shows negativity caused by antidromic invasion of Purkinje cells. B shows level of upward penetration as a function of time; invasion fails at 200μ.

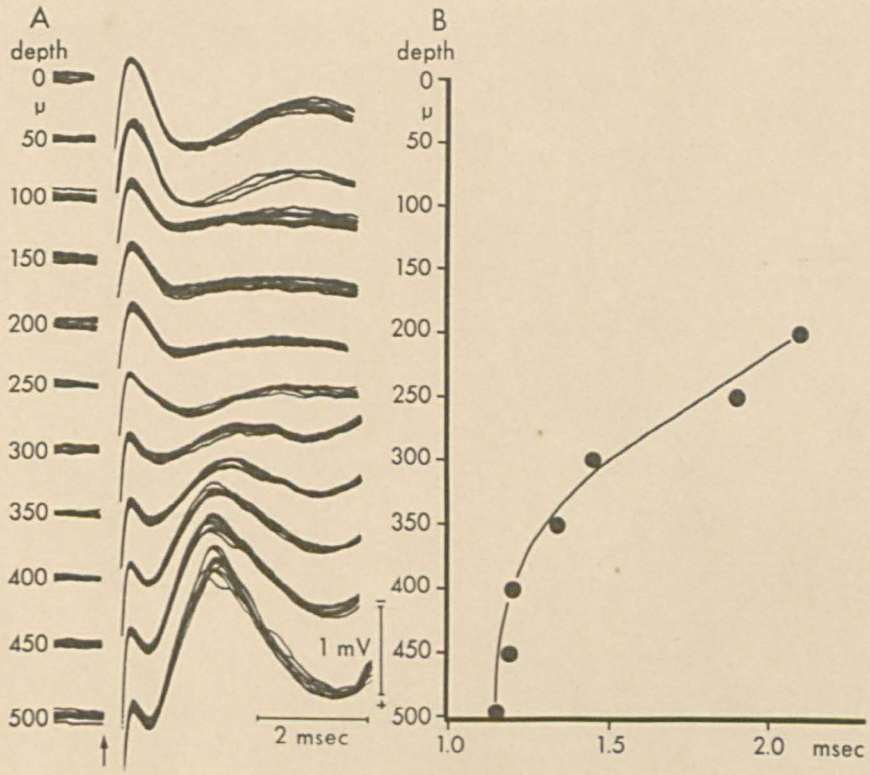

A
depth
0
μ
50
100
150
200
250
300
350
400
450
500
1 mV
2 msec
B
depth
0
μ
50
100
150
200
250
300
350
400
450
500
1.0
1.5
2.0
msec

Figure 16 A and B. WM evoked fields recorded simultaneously with three micropipettes. Pipettes were spaced 200μ apart perpendicular to the parallel fibres with tips at the same level. A shows WM evoked fields alone, B shows effect of preceding Lcc. Lower record of each set corresponds to pipette near centre of parallel fibre beam.

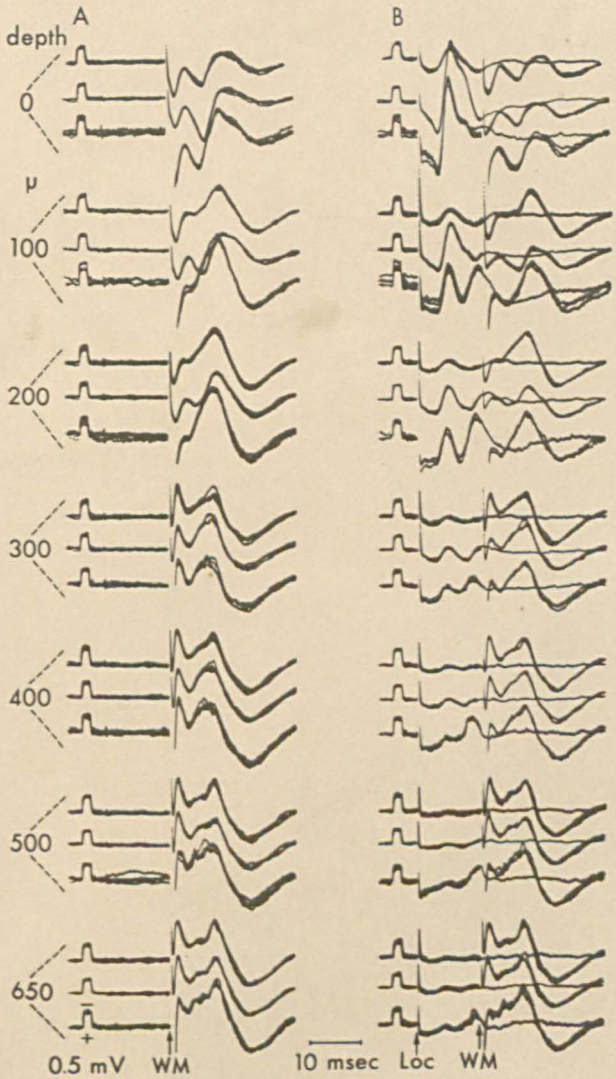

depth
A
B
0
μ
100
200
300
400
500
650
0.5 mV
WM
10 msec
Loc
WM

Figure 17 A to L. Purkinje cell spikes recorded at soma-axonal level. A to C show Loc evoked spikes (artefact marks position of stimulus). D shows effect of preceding Loc with WM stimulus (arrow). Calibration for A to D shown beneath D. E to G show effect of WM stimulus alone, in G a spontaneous spike occurs before the stimulus in one case and the negative spike component following the stimulus is replaced by a long-lasting negativity in all cases except one. Calibration for E to G given in G. H shows spike followed by small oscillation, after WM stimulus. I to K show effect of preceding WM stimulus (arrow) by a Loc, I is control for this sequence, calibration in K applies to I-K. L shows some spontaneous spike activity of a Purkinje cell.

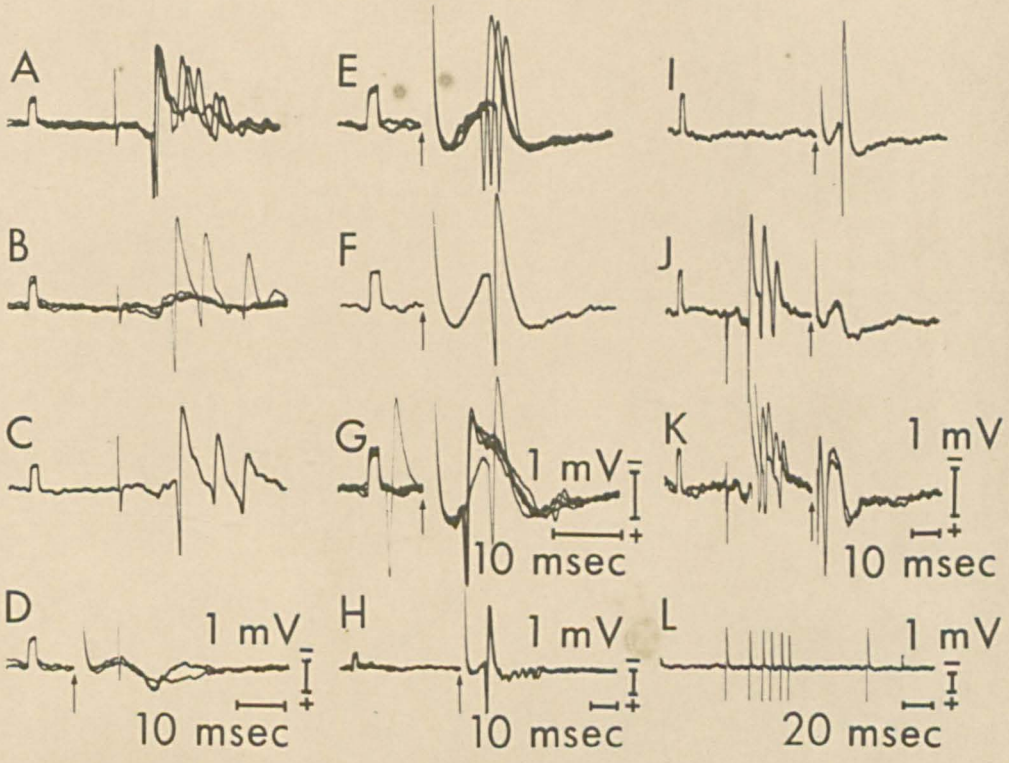

A
B
C
D
1 mV
10 msec
E
F
G
1 mV
10 msec
H
1 mV
10 msec
I
J
K
1 mV
10 msec
L
1 mV
20 msec

Figure 18 A to I. Long lasting all-or-none negativity recorded from region of Purkinje cell bodies. A and B show Loc evoked responses. Calibration shown in B applies to A also. C to E show WM (arrow) stimulus evoked long-lasting negativity. C and E show negativity is all-or-none. G to I show effect of preceding Loc stimulus (first arrow) on negativity evoked by WM stimulus (second arrow), at different intervals. Calibration for G to I given in I.

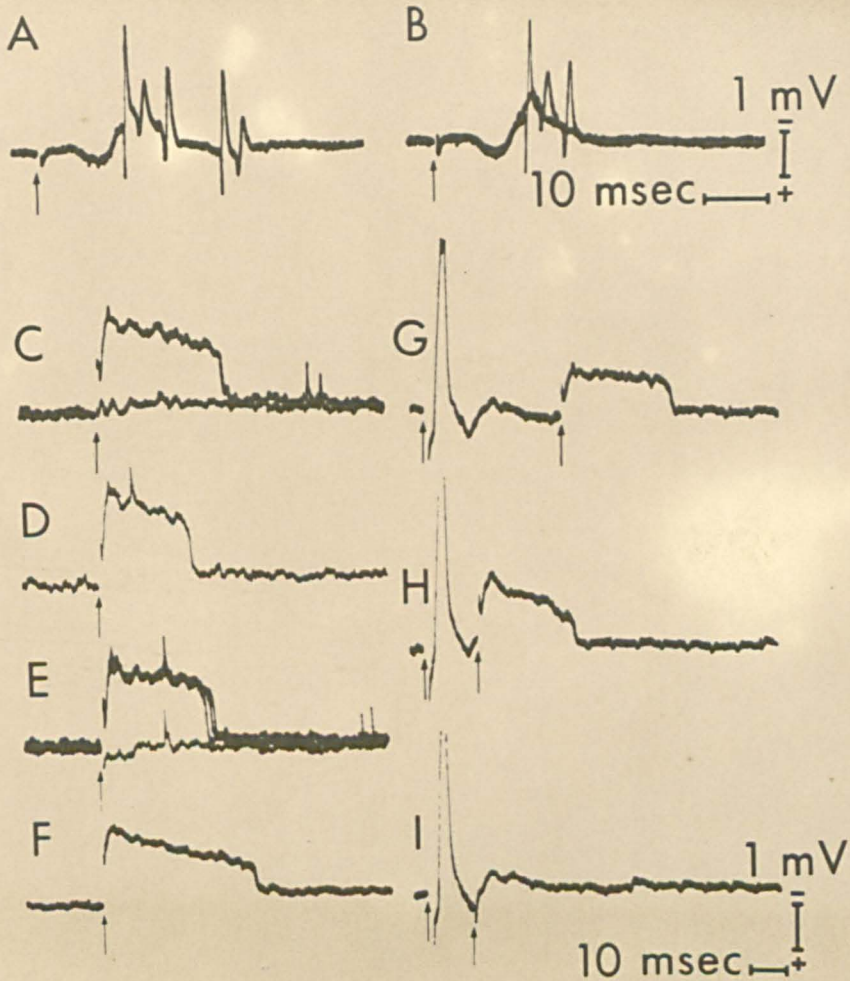

A
B
1 mV
10 msec
C
G
D
H
E
F
I
1 mV
10 msec

Figure 19 A to L. Intracellular recording from a Purkinje cell. A to D show the effects of increasing Loc stimulus strength on intracellularly recorded potentials (Loc given at arrow). Injury spikes seen in A are first accentuated (B) by depolarization then inhibited by hyperpolarization (B to D). E to G show another penetration, stronger Loc gives more pronounced depolarization on which are seen many small peaks. Hyperpolarization follows depolarization. Calibration in G applies for A to G. Records H to K show similar effects, calibration for I to K given in K; note expanded sweeps to show peaks on depolarization in J and K. L shows two different rates of onset of hyperpolarization, encountered on three successive sweeps; see text for discussion. In all intracellular records a positive potential is displayed as an upward deflection.

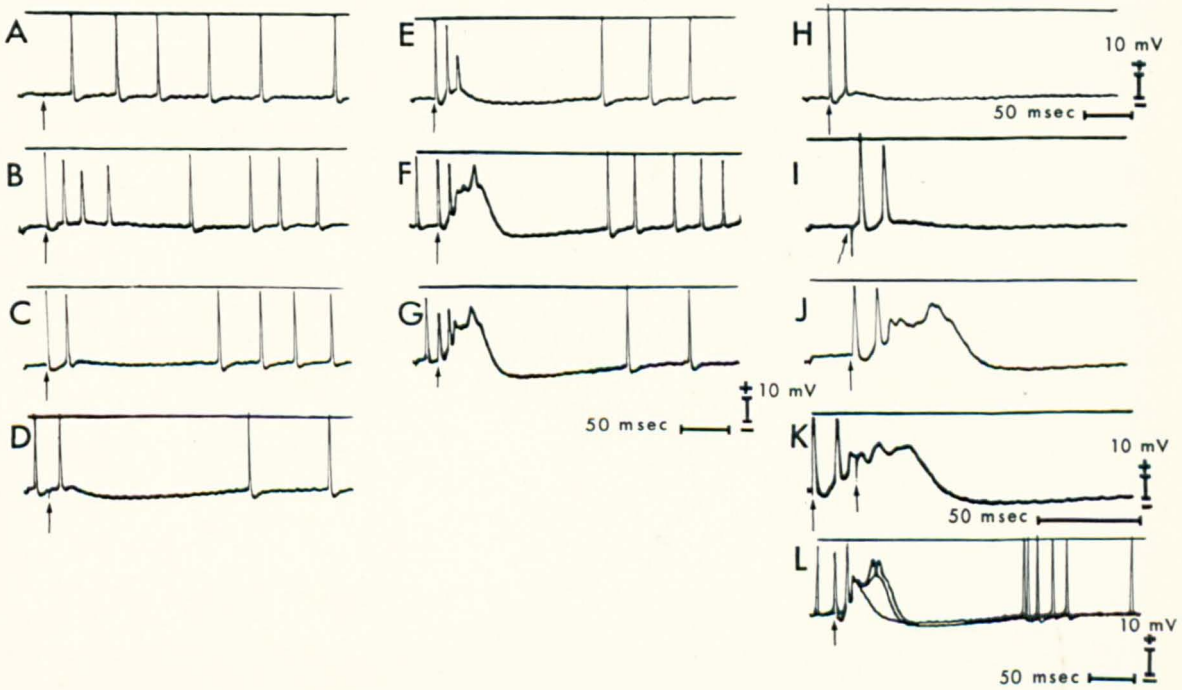

A
B
C
D
E
F
G
10 mV
50 msec
H
10 mV
50 msec
I
J
K
10 mV
50 msec
L
10 mV
50 msec

Figure 20 A to O. Effect of hyperpolarizing current on intracellular records from Purkinje cells. A shows background injury response. B to E show effect of a Loc stimulus of increasing strength; as in Figure 19, one sees a depolarization with small peaks followed by a hyperpolarization. F to G show effect of a pulse of hyperpolarizing current (between triangles) applied through the micropipette; Loc stimulus (arrow) is increased from F to J. These records show that Loc evoked depolarization is graded. L to O show similar effects of hyperpolarizing current; a rebound spike occurs at cessation of current pulse. Note increased size of injury spikes when they occur during hyperpolarization (B to J). Calibration for all records given in O.

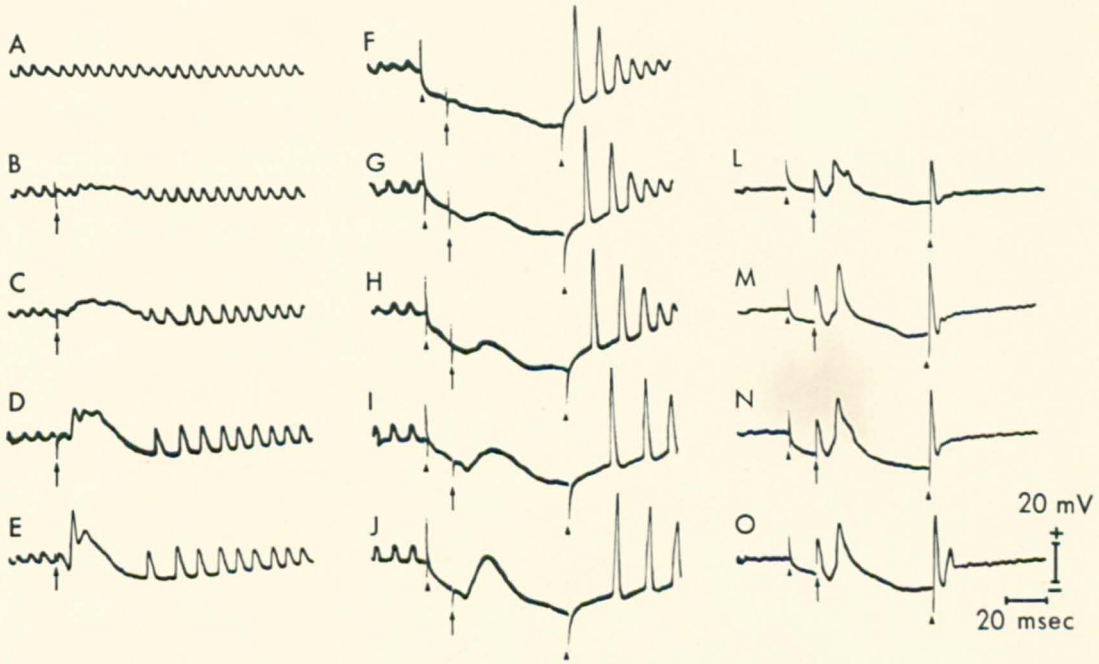

A
B
C
D
E
F
G
H
I
J
L
M
N
O
20 mV
20 msec

Figure 21 A to C. Unidirectional tendency for spike propagation in dendrites. A shows graph of the magnitude of the dendritic spike component of a Loc evoked field (filled circles) and the magnitude of the negativity caused by antidromic invasion of Purkinje cell (open circles). Both negativities have same magnitude in soma region (450μ) but centrifugal negativity attenuates rapidly whilst centripetal negativity reaches soma. Diagrammatic illustration of antidromic invasion of Purkinje cell (PC) shown in B; shaded region shows limit of active invasion, arrows indicate passive current flow. C shows situation when dendritic spike initiated in one distal branch of cell by parallel fibre beam (PFB), other secondary branches are not invaded. Shading and arrows have same significance in B and C.

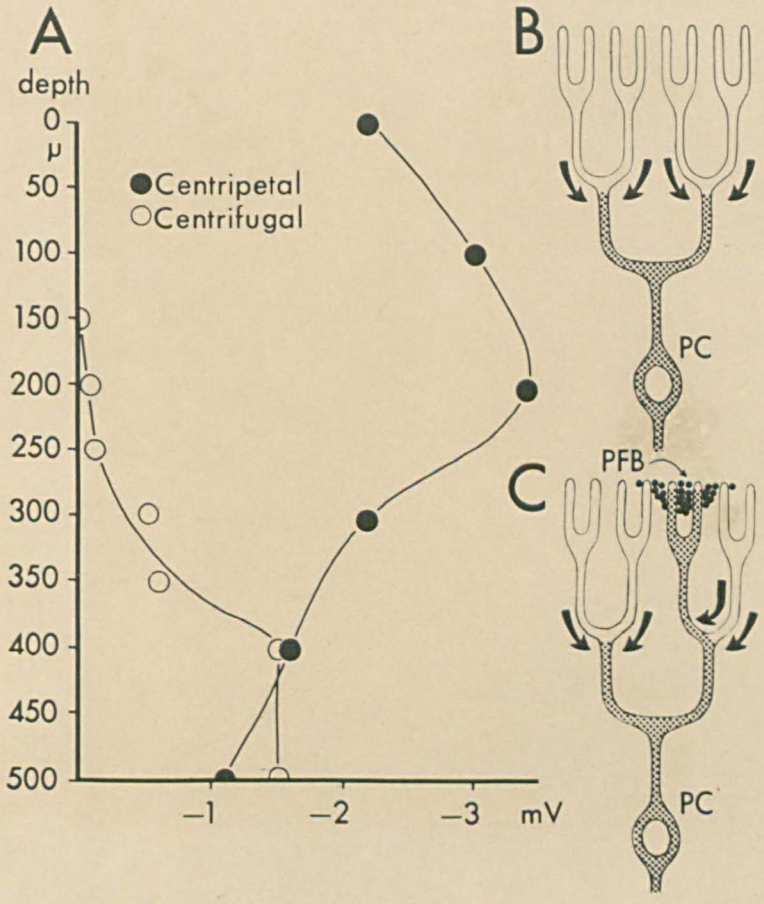

A
depth
0
μ
50
100
150
200
250
300
350
400
450
500
Centripetal
Centrifugal
−1
−2
−3
mV
B
PC
PFB
C
PC

Figure 22. Photomontage of Purkinje cell. Note the single initial branch arising from the soma and the rather straight second order and higher order branches. Golgi preparation by Dr. D.E. Hillman.


100 μ

Figure 23. Drawing of Purkinje cell shown in Figure 22. The lengths of the branches and the connectivity are reproduced accurately, but the branch diameters are not to scale and the dendritic spines are not shown. Drawing made with the aid of microprojection equipment.

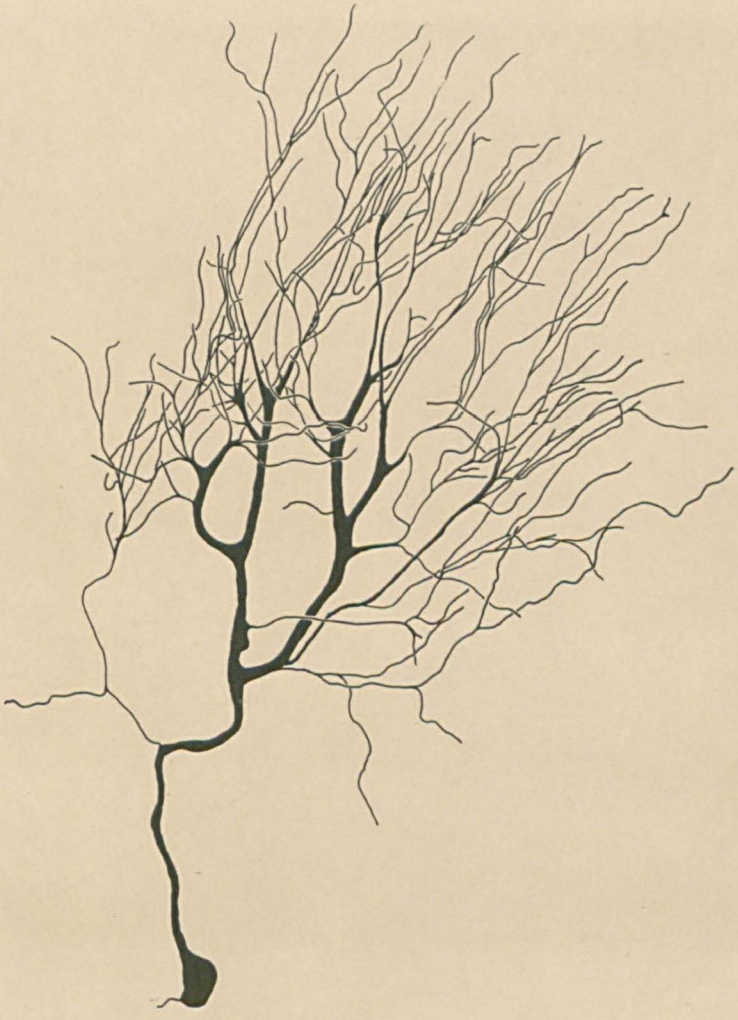
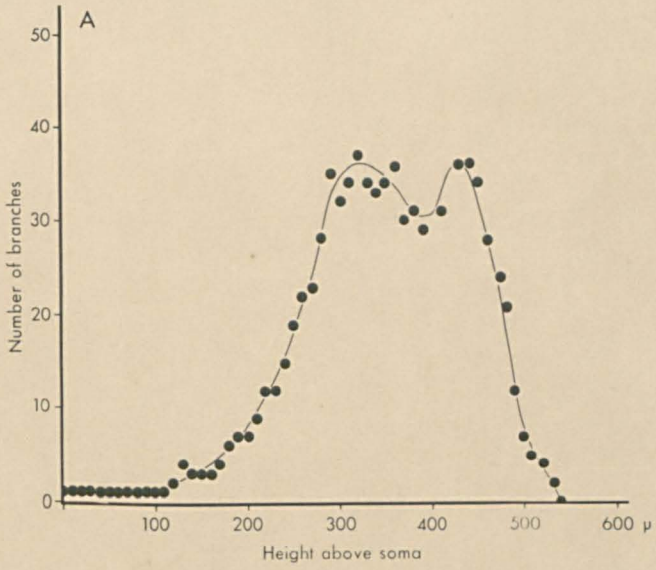


Figure 24. Details of Purkinje cell shown in Figures 22 and 23. The soma (lower left) and five branch points have been photographed under oil-immersion to show details of diameter of branches, branch points and dendritic spines.


25μ

Figure 25 A and B. Degree of branching of Purkinje cell dendritic tree.

Using the drawing shown in Figure 23 a series of lines were drawn 10μ apart and perpendicular to the vertical axis of the cell. The number of branches at each level were counted and the results are shown in A. For B the tree was redrawn so that the distances from point to point within the tree was represented by a vertical line and the branch counting procedure, outlined above, repeated. This gave the number of branches as a function of path length within the tree.


A
50
40
30
20
10
0
Number of branches
100
200
300
400
500
600 μ
Height above soma

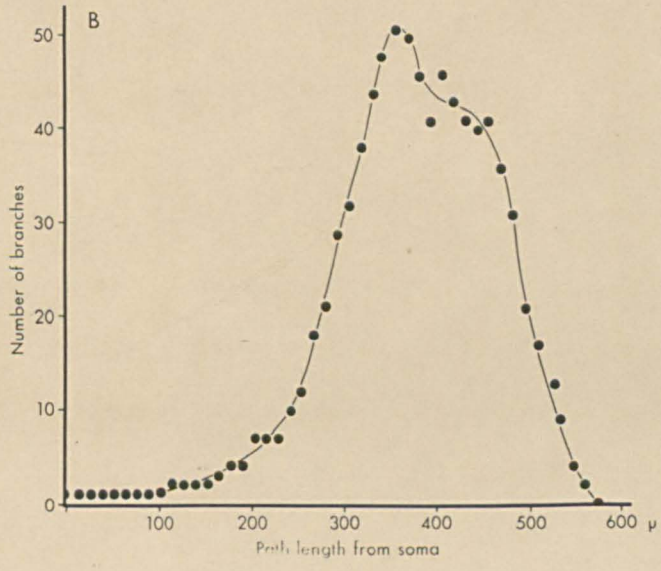

B
50
40
30
20
10
0
Number of branches
100
200
300
400
500
600 μ
Path length from soma

Figure 26. Scatter diagram for path length versus vertical height for points on Purkinje cell dendrites. Using the data compiled for Figure 25 the path length was plotted against height above soma for branch points (open squares) and terminal points (filled circles); the resulting diagram gives a measure of the "straightness" of the dendritic tree.

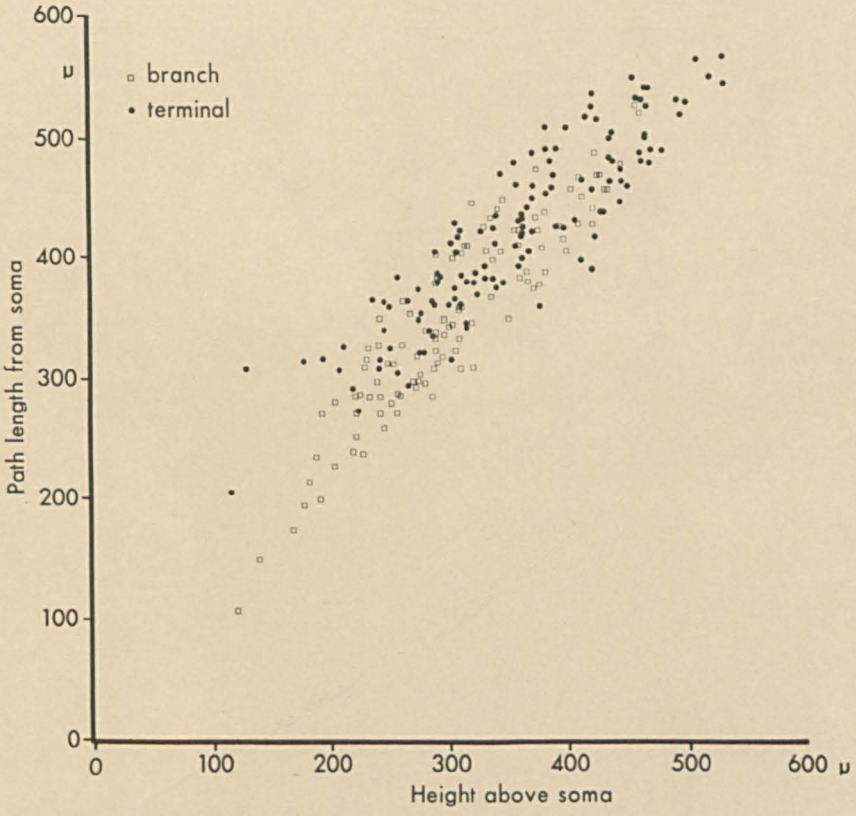

600
μ
500
400
300
200
100
0
branch
terminal
Path length from soma
0
100
200
300
400
500
600 μ
Height above soma

Figure 27 A and B. The brain of the alligator. A shows dorsal view. B shows median section. From Nieuwenhuys (1967); auric. = auricular lobe of the cerebellum; cereb. = cerebellum; corp. cer. = corpus cerebellum; tect. mes. = mesencephalic tectum; tel. = telencephalon; tr. ol. = olfactory tract; IV, V, VII and VIII = cranial nerves.

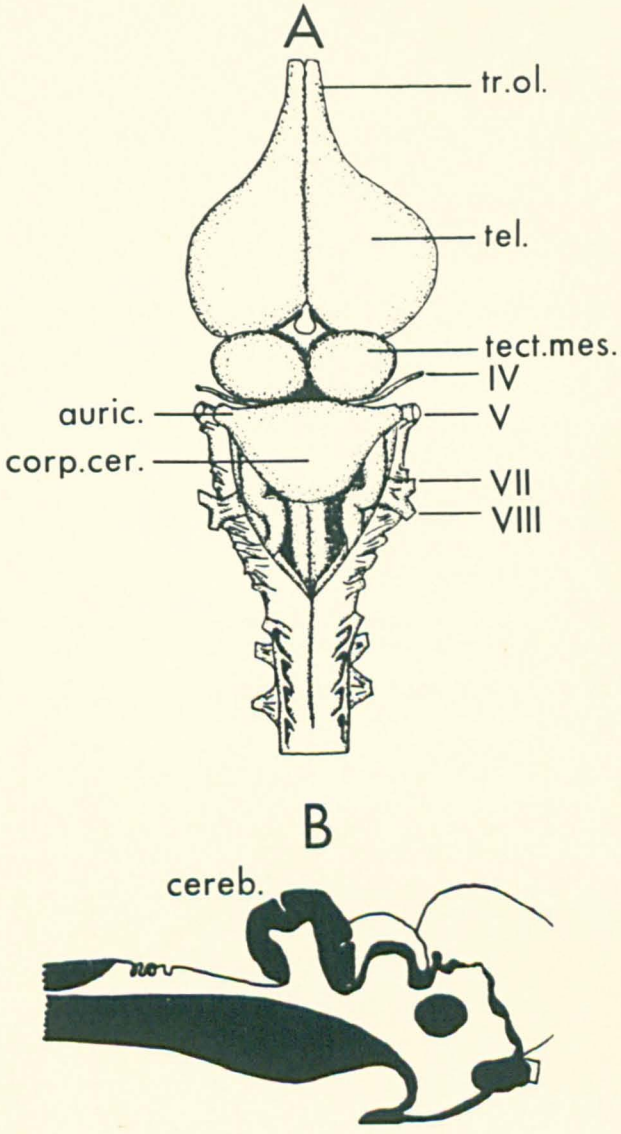

A
tr.ol.
tel.
tect.mes.
IV
V
auric.
corp.cer.
VII
VIII
B
cereb.

Figure 28. Diagram of alligator cerebellum cut sagittally. Note the three lobes and the typical arrangement of the cerebellar layers; the alligator cerebellum is not foliated however.

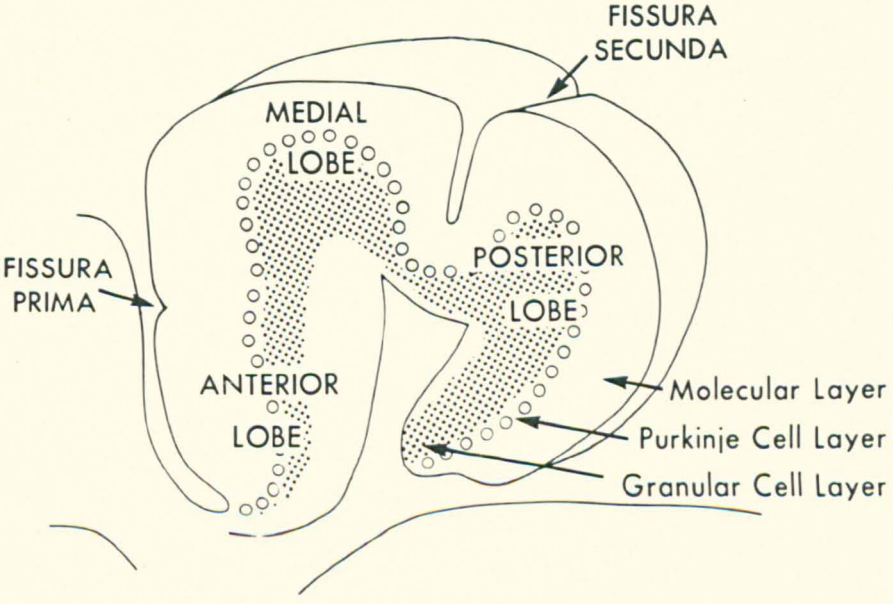

FISSURA
SECUNDA
MEDIAL
LOBE
FISSURA
PRIMA
POSTERIOR
LOBE
ANTERIOR
LOBE
Molecular Layer
Purkinje Cell Layer
Granular Cell Layer

Figure 29. Comparison of mammalian and crocodilian cerebellar divisions. Mammalian scheme taken from Brookhart (1960); crocodilian scheme based on Larsell (1967).

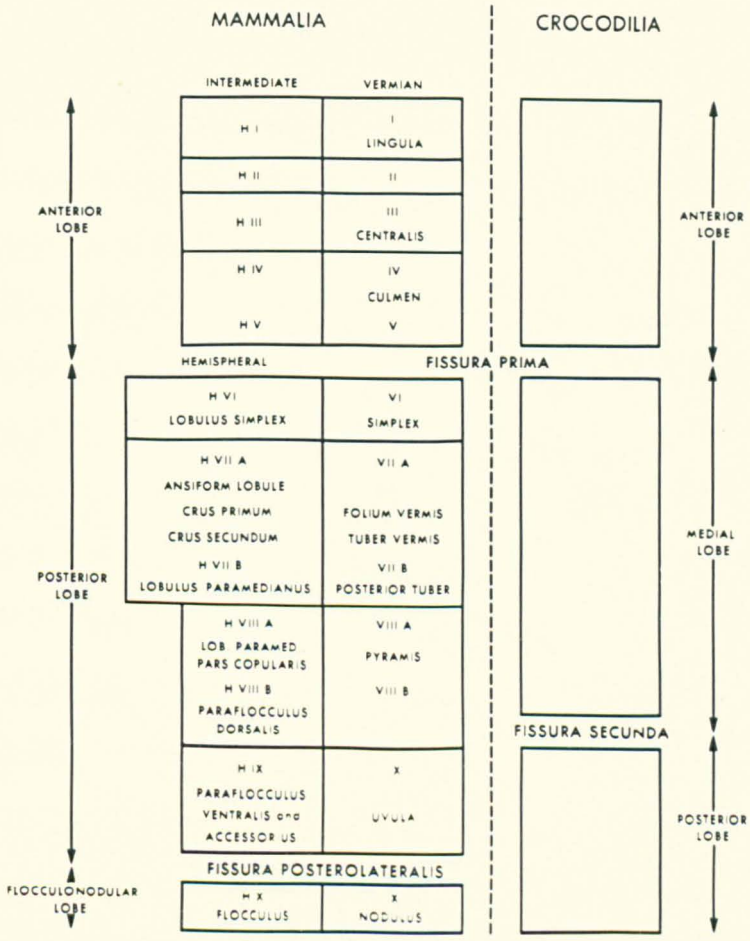

MAMMALIA
CROCODILIA
INTERMEDIATE
VERMIAN
H I
I
LINGULA
H II
II
H III
III
CENTRALIS
H IV
IV
CULMEN
H V
V
ANTERIOR LOBE
ANTERIOR LOBE
HEMISPHERAL
FISSURA PRIMA
H VI
LOBULUS SIMPLEX
VI
SIMPLEX
H VII A
ANSIFORM LOBULE
CRUS PRIMUM
CRUS SECUNDUM
H VII B
LOBULUS PARAMEDIANUS
VII A
FOLIUM VERMIS
TUBER VERMIS
VII B
POSTERIOR TUBER
POSTERIOR LOBE
MEDIAL LOBE
H VIII A
LOB. PARAMED. PARS COPULARIS
H VIII B
PARAFLOCCULUS DORSALIS
VIII A
PYRAMIS
VIII B
FISSURA SECUNDA
H IX
PARAFLOCCULUS VENTRALIS and ACCESSORIUS
X
UVULA
POSTERIOR LOBE
FISSURA POSTEROLATERALIS
H X
FLOCCULUS
X
NODULUS
FLOCCULONODULAR LOBE

Figure 30. Frontal section through cerebellar cortex and deep cerebellar nuclei of the alligator. Toluidine-blue stain. x 10. From Weston (1936); f.l.m. = median longitudinal fasciculus; fl. = flocculus (auricular lobe); nuc. lat. cb. = lateral cerebellar nucleus; nuc. med. cb. = medial cerebellar nucleus; nuc. V.s. = sensory V nucleus; str. gr. = granular layer; str. mol. = molecular layer; str. P.c. = Purkinje cell layer; r. V. = root of V nerve; v. cb. = cerebellar ventricle.

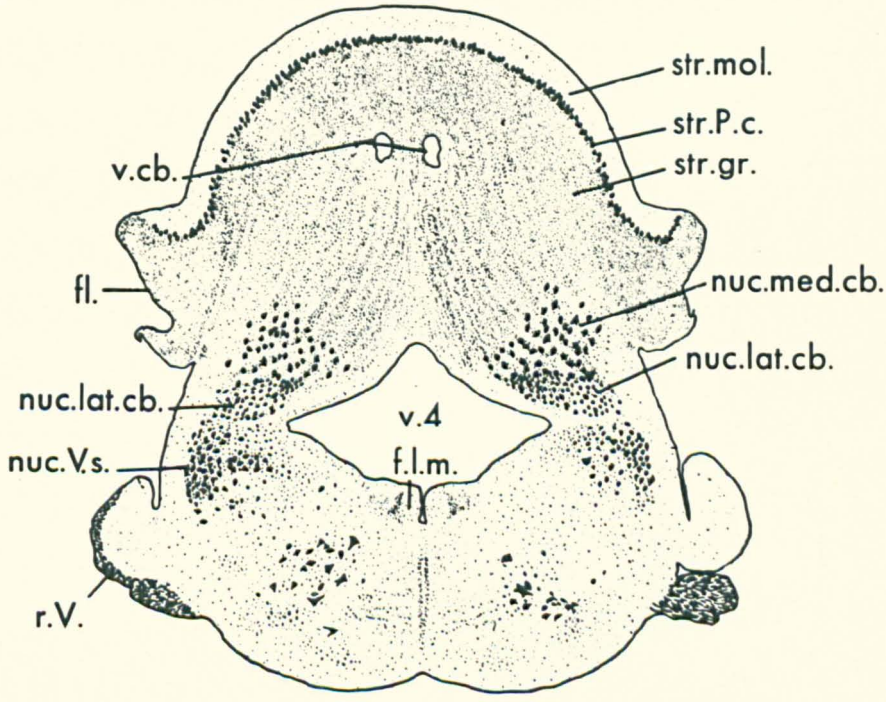

str.mol.
str.P.c.
str.gr.
v.cb.
fl.
nuc.med.cb.
nuc.lat.cb.
nuc.lat.cb.
v.4
nuc.V.s.
f.l.m.
r.V.

Figure 31. Neuronal circuitry of the alligator cerebellar cortex. Purkinje cells (PC) have flattened dendrites which are contacted by parallel fibres (PF). Parallel fibres are the axons of granule cells (GrC) on which synapse the mossy fibres (MF), one of the cerebellar afferents, and the axons of Golgi cells (GC). Purkinje cell dendrites also make synaptic contact with stellate cell (SC) axons and the second afferent system to the cerebellum, the climbing fibres (CF). The sole output of the cerebellar cortex is via the Purkinje cell axons (PA). Further details in Appendix A. Figure from Hillman (1968).

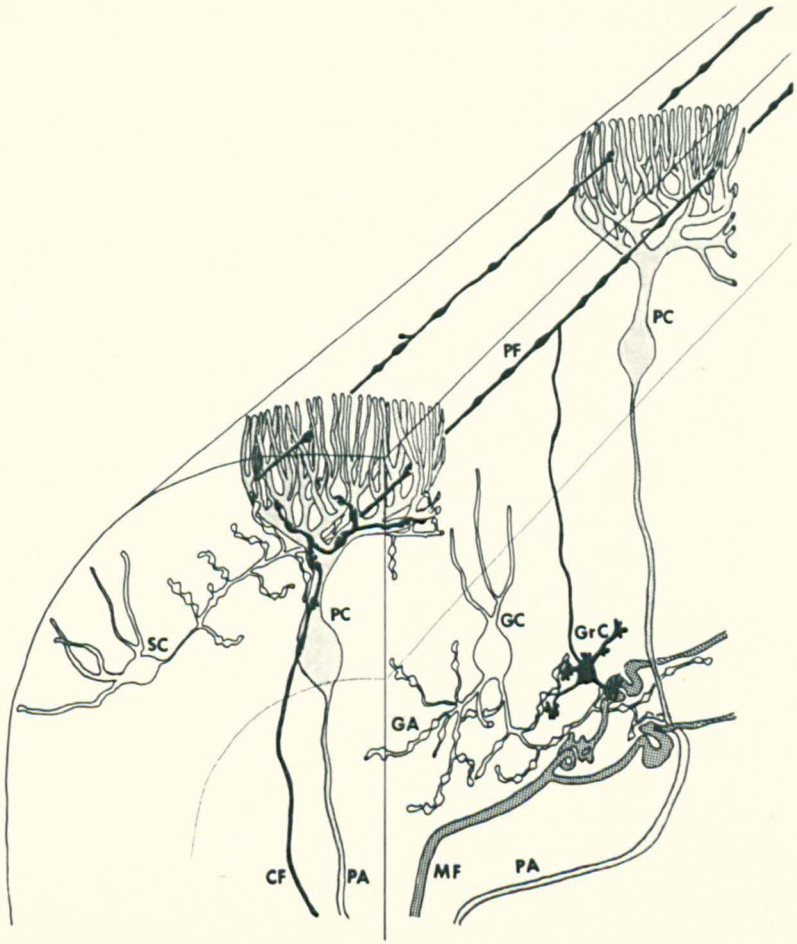
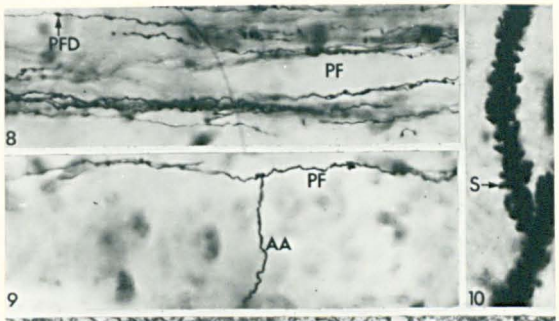
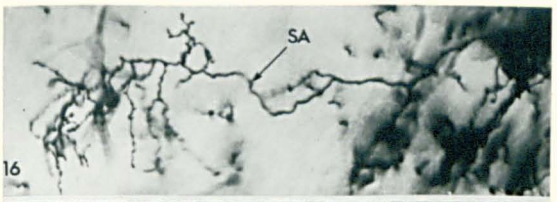

PC
PF
PC
SC
GC
GrC
GA
CF
PA
MF
PA

Figure 32. Parallel fibres and stellate cells. Micrographs 8 and 9 show Golgi impregnation of parallel fibres (PF) and ascending portion of granule cell axon (AA) in molecular layer. Synaptic enlargements (PFD) are seen on the parallel fibres, 300x. Micrograph 10 shows segment of branchlet from Purkinje cell with numerous spines (S), 1500x. Electron micrograph 11 shows spiny branchlet (SB) with spines (S). Note synaptic contact with parallel fibre dilation (PFD), non-synapsing parallel fibres are also seen (PF), 19,000x. Micrograph 16 shows axon of stellate cell (cell body not shown, would be to extreme right), 600x. Electron micrograph 17 shows bouton of stellate cell axon (SA) synapsing on spine (S) of large Purkinje cell dendrite (PD). A climbing fibre is also seen (CF), 22,000x. Micrographs from Hillman (1968).

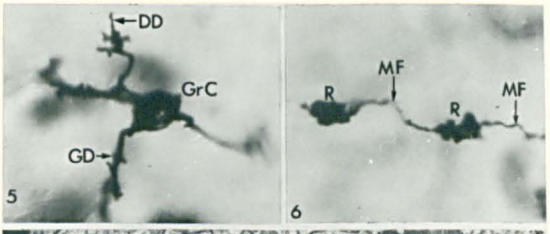

PFD
PF
8
PF
AA
9
S
10

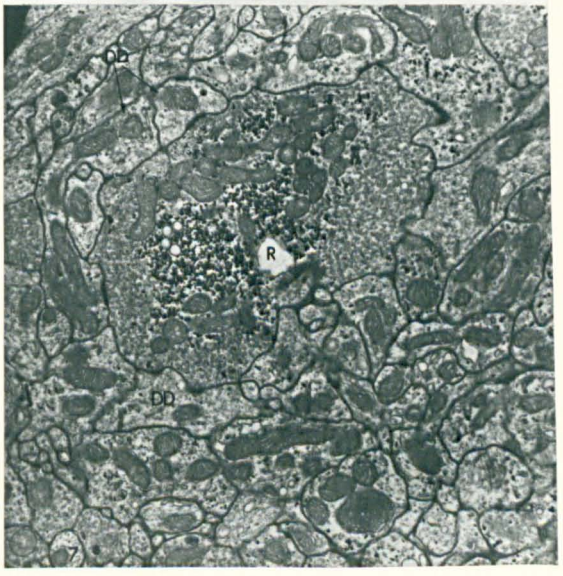

SA
16

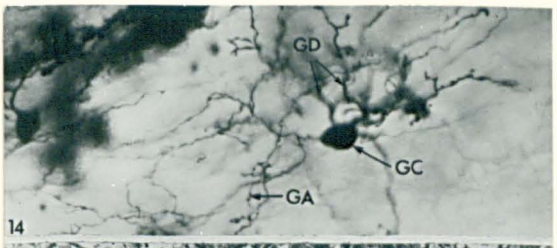

PFD
PF
SD
S
PFD
PF
SA
PF
11


PFD
PFD
S
PD
SA
CF
G
17

Figure 33. Granule cell, mossy fibre and Golgi cell. Micrograph 5 shows Golgi stained granule cell (GrC) with four dendrites (GD) from which dendritic digits (DD) emerge, 900x. Micrograph 6 shows Golgi preparation of mossy fibre (MF) with 2 rosettes (R), 900x. Electron micrograph 7 shows mossy fibre rosette (R) surrounded by dendritic digit (DD), 14,000x. Micrograph 14 shows Golgi preparation of Golgi cell (GC) with dendrites GD) and ramifying axons (GA), 400x. Electron micrograph 15 shows relationship between mossy fibre rosette (MFR), Golgi cell axonal beads (GA) and the dendritic digits of granule cells (DD), 22,000x. Micrographs from Hillman, 1968.


DD
GrC
GD
5
MF
R
R
MF
6


DD
R
DD
7


GD
GC
GA
14

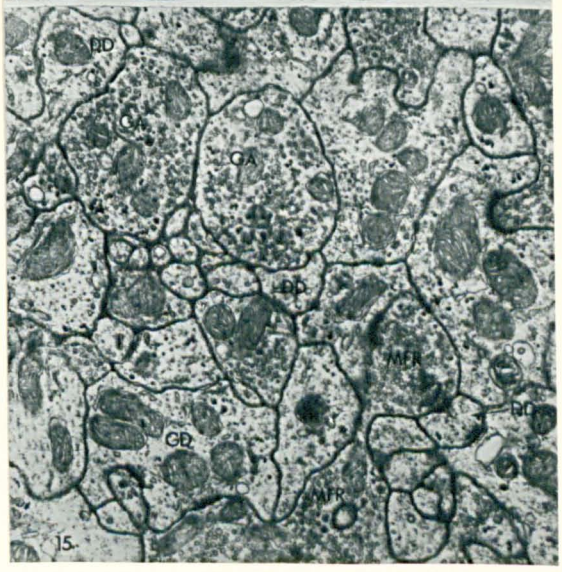
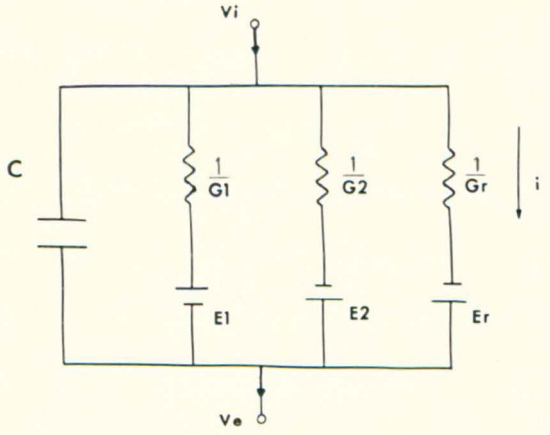

DD
GA
GA
GA
DD
MFR
RD
GD
MFR
15

Figure 34. Electrical analogue of membrane and section of dendrite. A shows equivalent circuit of membrane. C = membrane capacity, E_1 = equilibrium potential for ion species 1, E_2 = equilibrium potential for ion species 2, E_r = resting membrane potential, G_1 = conductance for ion species 1, G_2 conductance for ion species 2, Gr = membrane conductance, Vi = internal potential, Ve = external potential. B. shows equivalent circuit of element of a dendritic cable of length Δx. $\underline{i}\ \Delta x$ is transmembrane current, j(x) is internal longitudinal current, $Q\ \frac{\Delta x}{2}$ is half the internal longitudinal resistance, V(x) is transmembrane potential. Box represents membrane circuit shown in A.

A



B

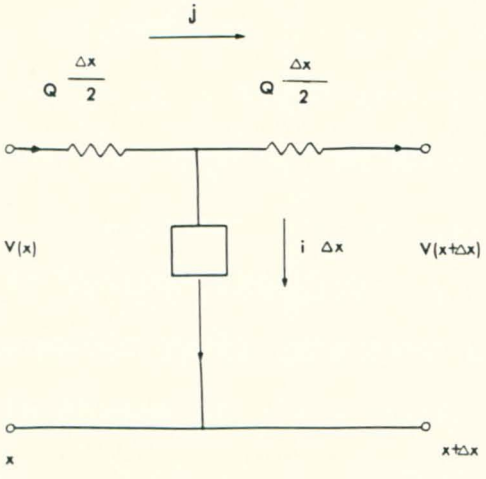
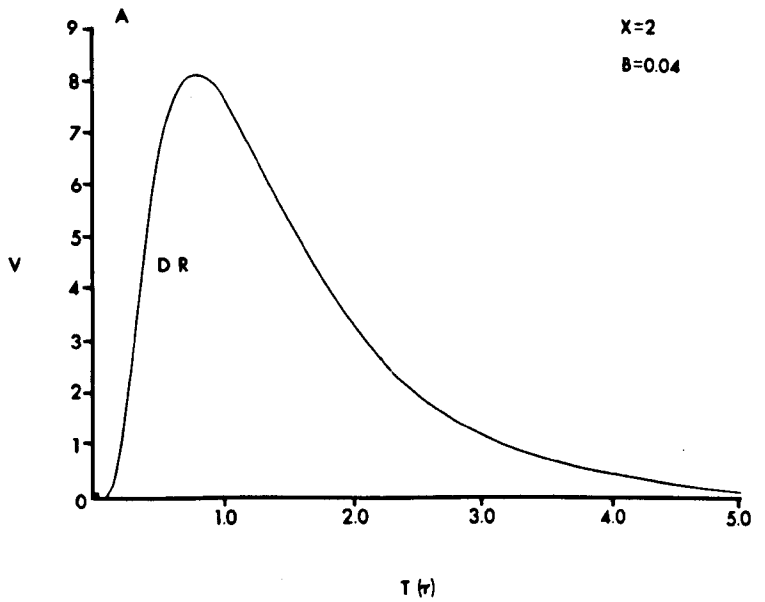


Figure 35 A and B. Theoretical comparison of potentials generated by delta function and brief rectangular pulse conductance changes in a cylindrical dendrite. A shows potential V (arbitrary units) recorded at 2λ from the site of the conductance change (X = 2) for a delta function input (D) and a rectangular pulse (R) of duration 0.04τ (B = 0.04). Duration indicated by black bar on abscissa. In each case the total charge put into the system is the same (100 arbitrary units). It is seen that the two potentials coincide. B shows the potentials recorded at 0.2λ (x = 0.2) following the same conductance changes as used for A. Now a difference in potential can be seen. Note the different potential and time scales in A and B.


A
X=2
B=0.04
9
8
7
6
5
4
3
2
1
0
V
D R
1.0
2.0
3.0
4.0
5.0
T (τ)

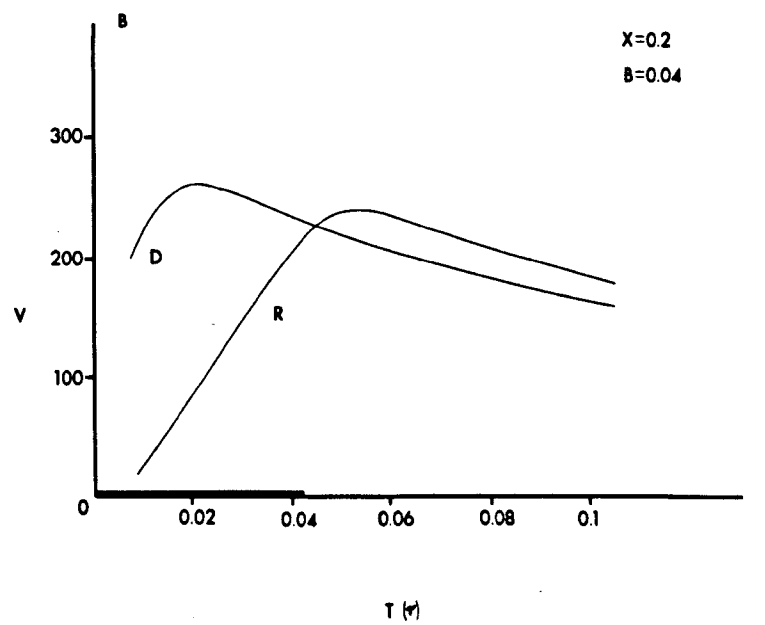
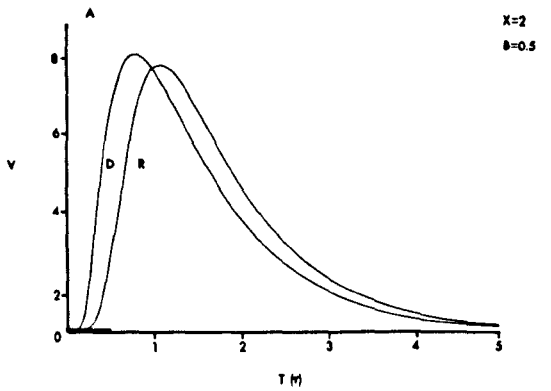
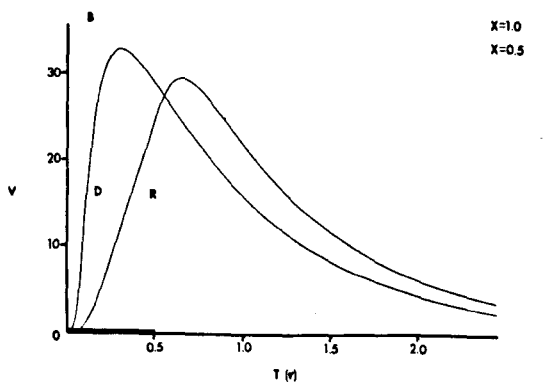

B
X=0.2
B=0.04
300
200
100
0
V
D
R
0.02
0.04
0.06
0.08
0.1
T (τ)

Figure 36 A-C. Theoretical comparison of potentials generated by a delta function and a 0.5τ duration rectangular conductance change in a cylindrical dendrite. The conventions of Figure 35 apply here. In the present case the rectangular pulse conductance change had a duration of 0.5τ (B = 0.5). Duration indicated by black bar on abscissa. Potentials were recorded at 2λ (Graph A), 1λ (Graph B) and 0.5λ (Graph C) from the site of the conductance change. Note different potential and time scales in each case. It is seen that the closer the recording point to the source, the greater the difference between the delta and rectangular pulse generated waveforms. At 2λ the waveforms are quite similar however. Total charge entering system is 100 arbitrary units.


A
X=2
B=0.5
V
D
R
0
2
4
6
8
1
2
3
4
5
T (Y)


B
X=1.0
X=0.5
V
D
R
0
10
20
30
0.5
1.0
1.5
2.0
T (Y)

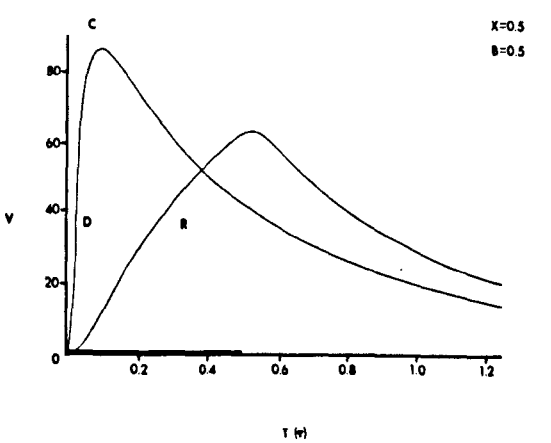

C
X=0.5
B=0.5
V
D
R
0
20
40
60
80
0.2
0.4
0.6
0.8
1.0
1.2
T (Y)

Figure 37. Effect of branch position on potentials in Y-shaped dendritic tree. A delta function conductance change occurs at the tip of one of the upper branches of the Y-shaped tree. The potential is recorded 2λ away on the stem of the tree. The branch position is varied from no branch ($A = 0$) to a branch 1.5λ from the source ($A = 1.5$). It is seen that for A greater than 0.25λ the potentials depend little on the position of the branch. Total charge entering system is 100 arbitrary units.

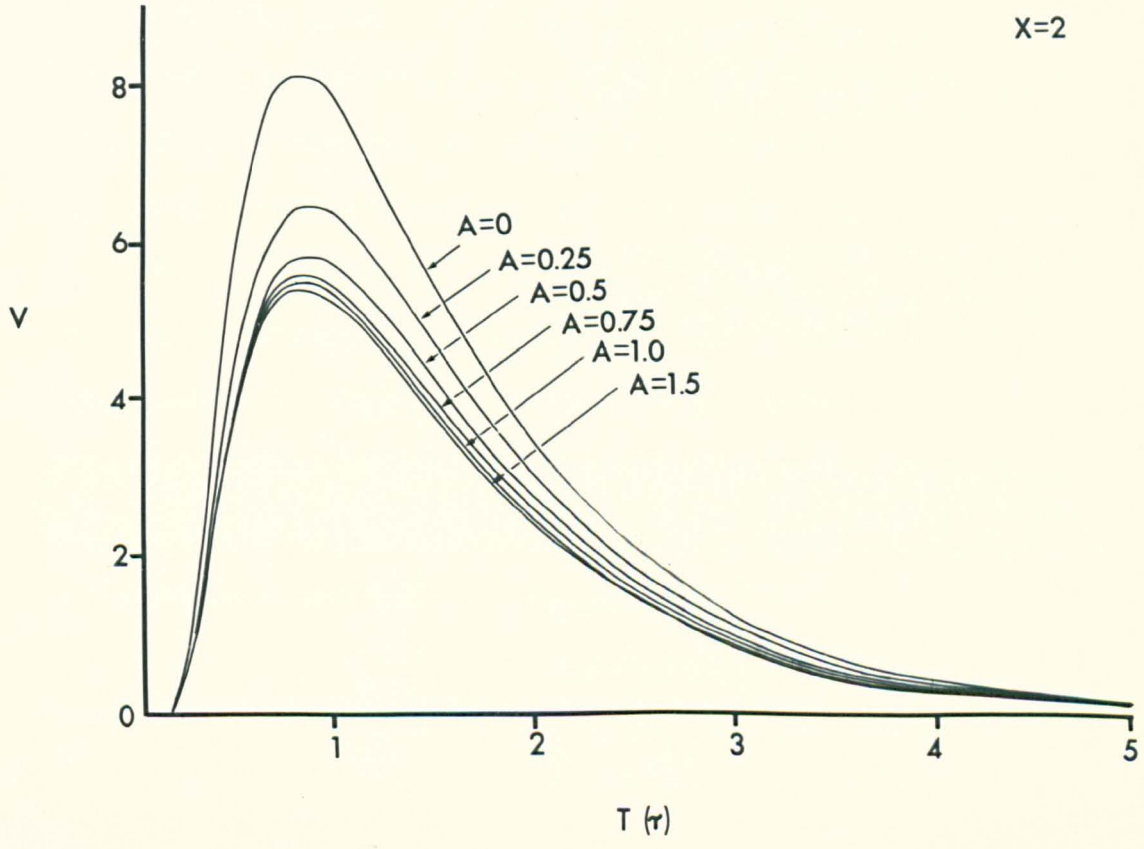

X=2
V
8
6
4
2
0
A=0
A=0.25
A=0.5
A=0.75
A=1.0
A=1.5
1
2
3
4
5
T (τ)

Figure 38. Geometrical interpretation of synapse at an arbitrary position on a dendritic tree in terms of a system of image synapses. A represents synapse at distance a from left-hand end of a cylindrical dendrite. Total current entering system is I, Il goes to the left, Ir to the right. B shows same amount of current entering at tip of dendrite. C shows system of image synapses in an infinite cable which give the same potential distribution as the synapse in A. Further description in Section B.3.

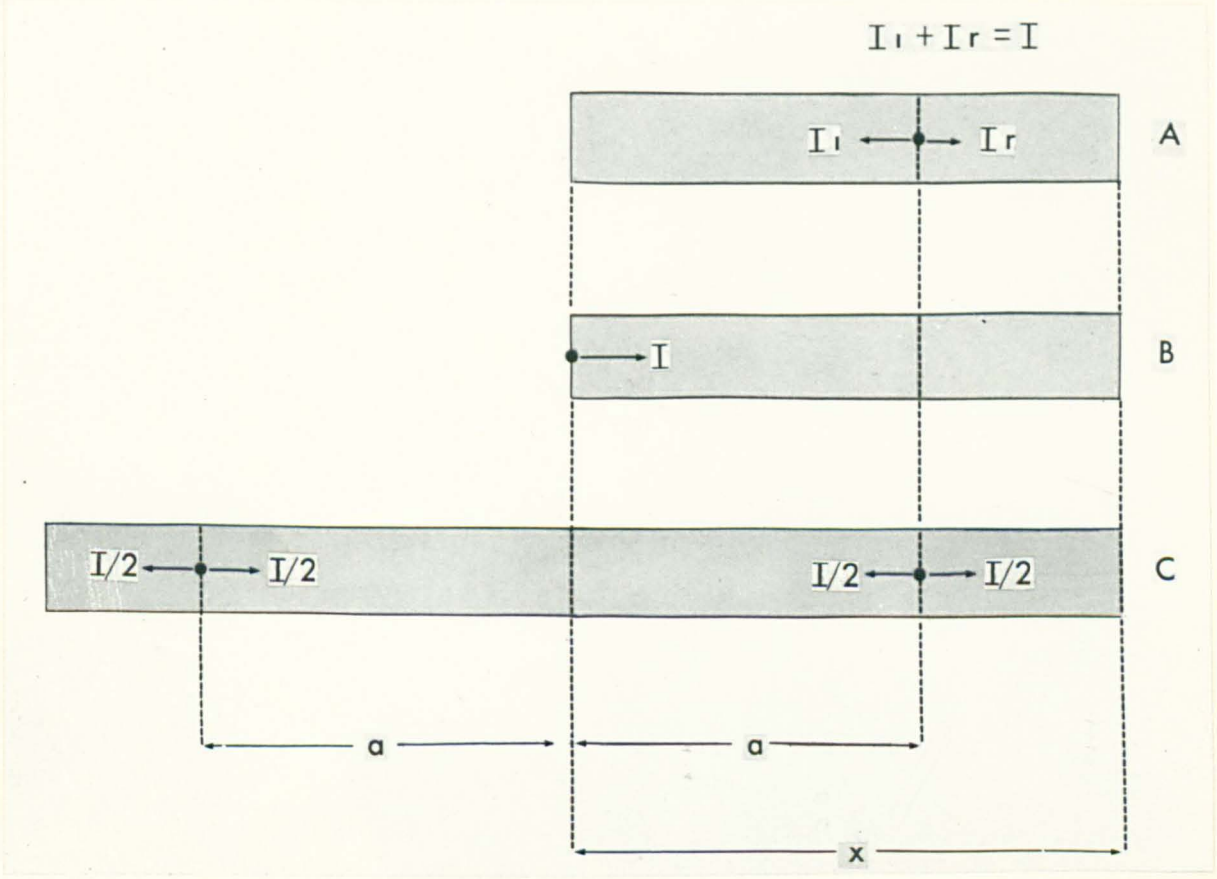
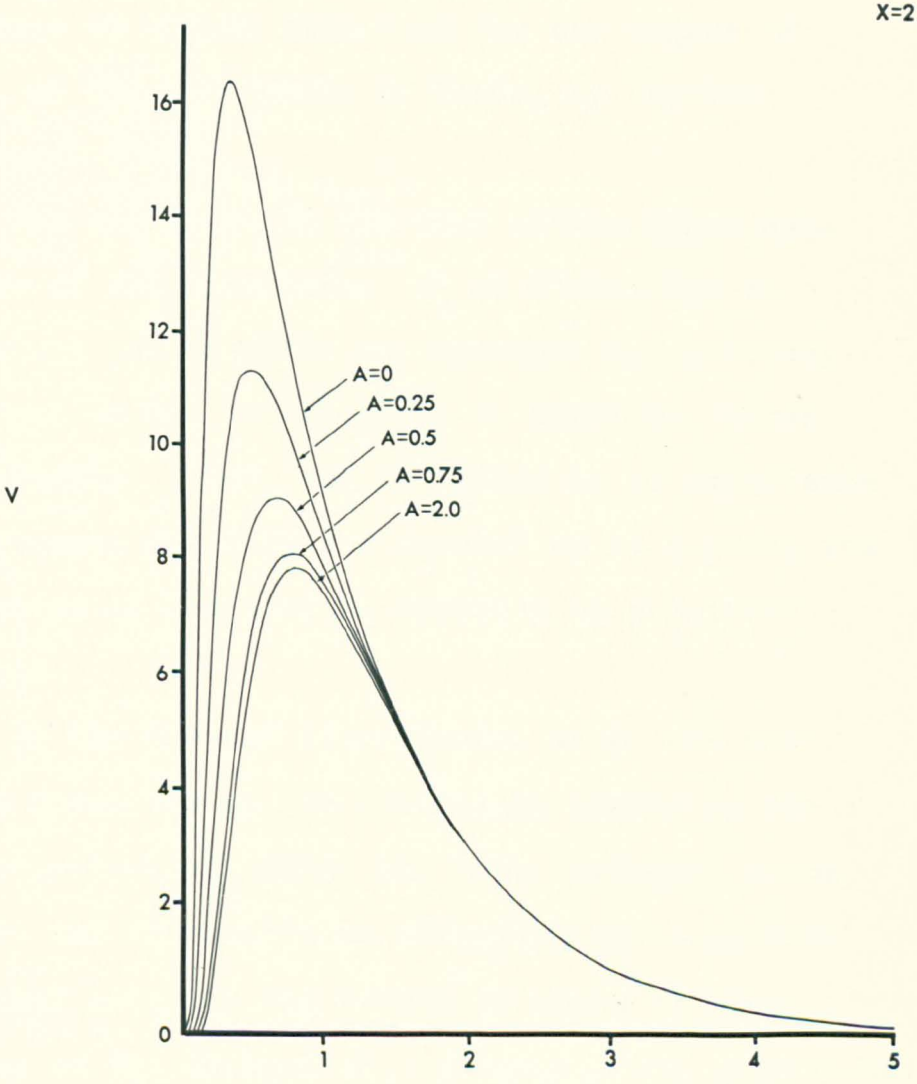

Il + Ir = I
Il
Ir
A
I
B
I/2
I/2
I/2
I/2
C
a
a
x

Figure 39. Effect of synaptic position on the potential recorded in a cylindrical dendrite. The potential is recorded at 2λ from the tip and the synapse is moved from the tip of the dendrite ($A = 0$) to a position 1λ ($A = 1$) from the recording position. It is seen that for A between 0 and 0.5 the variation of the potential is small but thereafter it becomes more marked. 100 arbitrary units of charge put into the system as a delta function conductance change.

X=2



Communicational Aspects of Neuronal Circuitry in the Cerebellar Cortex of the Alligator.

Abstract of thesis submitted for the degree of Ph.D. at the University of Keele, by Charles Nicholson (1968).

The alligator is the only living close relative of the dinosaurs. It is classified as a reptile and occupies an important evolutionary position in its relationship to amphibia, birds and mammals. Thus, the alligator is of importance in attempts to study the cerebellum by tracing its evolution as revealed by research on primitive animals.

In the present experimental work the neuronal circuitry of the cerebellar cortex in the anaesthetised alligator has been explored, using extra- and intracellular recording micropipettes. Neuronal activity was evoked by means of electrodes on the surface of, or within, the cerebellum. The techniques used were similar to those employed by Eccles and co-workers in the cat.

The histology of the cerebellum in both cat and alligator is basically similar except that the latter animal lacks basket cells. The present work has shown that, in general, the neuronal physiology of the two animals is similar. Nevertheless, at least one significant difference has been found. In the alligator, electrical stimulation of the parallel fibre system evokes action potentials in the dendrites of Purkinje cells. This contrasts with the cat where Purkinje cell dendrites behave passively under similar conditions. Moreover, it has been shown in this work that the dendritic action potentials of the alligator propagate preferentially in a centripetal, rather than a centrifugal direction. These results suggest that Purkinje cells in the cat and the alligator may function in different ways.

The present studies have also shown that the stellate cells of the molecular layer in the alligator inhibit Purkinje cell dendrites. These cells are found also in the cat, but in that animal their effect is masked by the parallel in-

hibitory action of the basket cells.

Finally, the communicational aspects of the above findings have been considered. Passive and active dendritic properties have been compared with the aid of a specially developed mathematical analysis of the properties of passive branched dendritic systems. The hypothesis has been advanced that the Purkinje cells of the alligator have two basic modes of operation. Firstly, a tonic or constant output frequency mode. This corresponds to weak, random, synaptic activation and passive dendritic processes. Secondly, a phasic mode with an output consisting of a burst of spikes followed by silence. This corresponds to strong, synchronised synaptic activation and active dendritic processes. The inhibitory interneurone system of the cerebellar cortex has also been discussed in the light of comparative studies and it is concluded that it may represent a refinement rather than a fundamental cerebellar mechanism.

The present work also includes an appendix reviewing the anatomy, hodology and histology of the alligator cerebellum and comparing them with the cat. A second appendix presents the mathematical analysis of branched dendritic systems, referred to above.