LONG LATENCY REFLEXES UNDER

CHLORALOSE ANAESTHESIA

by

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1968.
Because of the complex procedures involved in this investigation, many results were obtained in collaboration with Drs. M.S. Devanandanan, Rosamond M. Eccles and D.M. Lewis. However, I was the principal investigator throughout and chapter V is almost entirely my own work.

DONALD STENHOUSE

...Donald Stenhouse...
The following papers have been published, or have been submitted for publication, as a result of the work done during the tenure of my research scholarship at the Australian National University:-


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CHAPTER I.

INTRODUCTION

Historical

In the early 1890's Hanriot and Richet, working in the Faculty of Medicine in Paris, were looking for methods of combining the hypnotic, chloral, with some other molecule, so that when ingested the chloral would be slowly released from its combined state and thus have a prolonged, gentle sedative action. Among the compounds they synthesised were the chloraloses—combinations of chloral with pentose or hexose sugars. One of these substances—derived from chloral and glucose—had been described as very toxic by Hefftter (1889). However, probably because they obtained it in a purer state, Hanriot and Richet (1893a) found that its α form had very interesting properties that could not be accounted for by assuming the steady release of chloral from its bound state. They named this α form, chloralose and the β form, parachloralose.

Parachloralose was physiologically inactive, but chloralose when administered to a dog had effects which seemed to them contradictory. It did induce sleep; but a sleep quite unlike that due to chloral in that it was
associated with an "exaggeration" rather than a depression of reflex activity. This led them to suppose that chloralose had a mixed anaesthetic and convulsant action, depressing cortical function and so producing the sleep while at the same time increasing the excitability of the spinal cord with resulting "exaggeration" of reflexes.

They extended and modified these initial observations and published a series of papers on the chemistry and physiology of chloralose and parachloralose (Hanriot and Richet, 1893b-f; 1897). Chloralose was given to dogs, cats, rats, rabbits, guinea-pigs, frogs, pigeons, ducks, chickens and man. The authors were most impressed with its ability to produce anaesthesia while leaving reflex functions intact and so recommended it as the anaesthetic of choice for animal experiments since both chloral and chloroform depressed reflexes and curare did not anaesthetise (Hanriot and Richet, 1893b; 1897). The "exaggerated" reflexes that they had observed are best described in their own words: In a dog, "le moindre attouchement détermine un soubresaut general, une sorte de convulsion presque strychnique" and a cat: "réagit par un soubresaut brusque et total à tout attouchement et surtout à tout ébranlement de la table. Il suffit de donner à la table le plus léger choc pour que l'animal
saute en l'air vivement. Même quand on frappe du pied par terre, ou qu'on ferme bruyamment une porte voisine, c'est assez pour que l'animal tressaute, comme strychnisé..." (Hanriot and Richet, 1893b). These are the first descriptions of what is often called the chloralose jerk. A more detailed account of its characteristics was given by Alvord and Fuortes (1954) who noted a rapid, stereotyped movement with closing of the eyelids and flexion of the neck, back and legs. This could be elicited by auditory, visual or mechanical stimulation provided the application of the stimulus was sufficiently abrupt and not repeated at less than 1 second intervals.

Although Hanriot and Richet initially thought that the chloralose jerk was consequent on increased spinal cord excitability, they later obtained experimental evidence against this interpretation (Hanriot and Richet, 1893c). Observing that after lumbar or thoracic cord transection, the characteristic jerk could be evoked in the forelimbs, but was completely abolished in the hindlimbs, they concluded that it was fairly probable ("assez vraisemblable") that the response was dependent on the medulla ("sous la dépendance du bulbe rachidien"). Moukhtar (1908) confirmed their experimental findings and, on the basis of his own studies in pigeons, suggested that
the jerk was of mid- and hind-brain origin ("d'origine bulbo-protuberantiale").

Thus, from the outset, it has been recognised that the brainstem might be of crucial importance in generating the chloralose jerk; but further analysis of its central and peripheral mechanisms had to await the development of more sophisticated experimental methods.

In recent years, the neurophysiological equivalent of the chloralose jerk has been taken as the reflex discharge into motor axons lasting some 10 to 25 msec and with a latency varying from 10 to 80 msec depending on the site of the stimulus and of recording (Alvord and Fuortes, 1954; Ascher, Jassik-Gerschenfeld and Buser, 1963; Ascher, 1965). With appropriate stimuli, such discharges have been recorded from limb muscles or their motor nerves (Alvord and Fuortes, 1954; Shimamura, Mori, Matsushima and Fujimori, 1964; Ascher, 1965), from ventral roots (Alvord and Fuortes, 1954; Shimamura, 1963; Shimamura et al., 1964; Ascher, 1965; Shimamura, Mori and Yamauchi, 1967a,b), from nerves to the intercostal muscles (Shimamura et al., 1964) and from the cranial motor nerves (Shimamura, 1963). The discharges can be elicited by a large range of afferent inputs: sudden noise, change in illumination or mechanical stimulation.
(Alvord and Fuortes, 1954; Ascher, 1965; Buser, St. Laurent and Menini, 1966), electrical stimulation of cutaneous and muscle nerves (Alvord and Fuortes, 1954; Shimamura et al., 1964; Megirian and Manning, 1967), the splanchnic nerve (Evans and McPherson, 1958) or the pelvic nerve (W.C. de Groat and R.W. Ryall - personal communication).

As Ascher (1965) pointed out, the chloralose jerk, or startle reaction ("la réaction de sursaut") as he called it, is doubly non-specific - evoked by a great variety of stimuli and discharging into widely dispersed efferent channels. It is natural to ask what central organisation subserves such a reflex pattern.

Adrian and Moruzzi (1939) concluded that it was mediated by the cerebral cortex as they were able to record an associated discharge in the region of the pyramidal decussation, and also found that the jerk could be completely abolished by bilateral destruction of the motor cortices or temporarily eliminated by briefly occluding the carotid arteries. Moruzzi (1944) disproved this hypothesis when he showed that the jerk, which he named the startle reflex ("riflesso del sobbalzo"), was only temporarily suppressed after decortication. However, he found that it did disappear permanently
following precollricular decerebration and therefore suggested that it was mediated by the corpus striatum.

The finding that the chloralose jerk survives decortication has been confirmed (Alvord and Whitlock, 1954; Ascher et al., 1963). Even so, although the cerebral cortex is not an essential element in elaborating the chloralose jerk, its role is not insignificant. The jerk discharge is smaller and more difficult to elicit after decortication (Ascher et al., 1963; Ascher, 1965). These authors have also shown that the sensory areas facilitate the jerk response and that this is a modality specific effect - e.g. the acoustic cortex facilitates only the response evoked by acoustic stimuli.

Moruzzi's hypothesis that the corpus striatum is the reflex centre for the chloralose jerk has not been confirmed. Evidence has accumulated that in fact the medial reticular formation of the medulla is the single essential component in the reflex elaboration of the jerk. Alvord and Whitlock (1954) stated "that the brain stem 'reticular formation' in particular functions in long reflex circuits subserving the chloralose reflex myoclonic reaction". In decerebrate unanaesthetised cats, Shimamura and Livingston (1963) were sometimes able to evoke long latency ventral root discharges which were
similar to those seen under chloralose anaesthesia. They found evidence that the medial bulbar reticular formation was the reflex centre for these discharges and so they called the system the spino-bulbo-spinal reflex (s-b-s reflex). The relationship of the spino-bulbo-spinal reflex to the chloralose jerk reflex is not clear. Shimamura (1963), Shimamura et al. (1964 and 1967a) implied that they are identical. Yet it seems improbable that the responses of a decerebrate animal (often with subconvulsive doses of strychnine) are the same as those observed under chloralose anaesthesia. It is tempting to think of the spino-bulbo-spinal reflex as the basic unit of the chloralose jerk—particularly as Ascher (1965) has shown that Moruzzi's (1944) observation was incorrect and that in fact decerebration of the chloralose anaesthetised preparation considerably modifies but does not abolish the jerk discharge. Further, he was able to demonstrate that in the intact preparation, destruction of the medial bulbar reticular formation in the region of the nucleus reticularis gigantocellularis completely abolishes the chloralose jerk. This most recent evidence takes us back full circle therefore and supports the pioneer work of Hanriot and Richet (1893c) and of Moukhtar (1908) who were the first to indicate the importance of the brain-stem in relation to the chloralose jerk.
A characteristic of the chloralose jerk that has received little attention from physiologists is its well known "fatigability" (Adrian and Moruzzi, 1939; Moruzzi, 1944; Alvord and Fuortes, 1954). Does this susceptibility to stimulus repetition rates in excess of 1/sec result from inhibitory processes and if so, what is the site and nature of the inhibition?

Alvord and Fuortes (1954) observed a late and long-lasting inhibition of test monosynaptic reflexes after conditioning stimuli that had evoked a chloralose jerk. Ascher (1965) reported cessation of spontaneous activity in both flexor and extensor efferent nerves following the chloralose-jerk discharge. Suppression of chloralose-jerk responses has been observed following stimulation of certain areas of cerebral cortex (Kaada, 1951; Hossmann and Lamarche, 1966a, b; Shimamura et al., 1967b) and the reticular formation (Moruzzi and Magoun, 1949). These results together with the suggestion by Monroe, Balis and Ebersberger (1963) that there is inhibition at the reticular level are the only indications of inhibitory processes in relation to the chloralose jerk, but detailed knowledge is completely lacking.

The two problems of the reflex organisation of the chloralose jerk and its "fatigability" are special aspects
of a third and more basic problem in the pharmacology of chloralose - its so called dual action, anaesthetic and convulsant. There has been no satisfactory explanation of this characteristic (Balis and Monroe, 1964; Winters and Spooner, 1966). An explanation will have to be in terms of detailed information about the effect of chloralose on CNS synaptic activity. Thus far the only hints have been the findings that chloralose suppresses the release of acetylcholine from the cerebral cortex (MacIntosh and Oborin, 1953; Mitchell, 1963) and possibly from the caudate nucleus (Beleslin, Carmichael and Feldberg, 1964; Beleslin and Polak, 1965). Krnjević and Phillis (1963) found that it depressed spontaneous and evoked firing of cholinceptive neurons in the cerebral cortex. There is also some evidence that spinal cord inhibitory activity is reduced by chloralose (Biscoe and Krnjević, 1963).

The fact that chloralose has a dual action, that it has a β isomer and that its purity was difficult to ensure have all proved fruitful sources of confusion and argument. For example, one finds Hanriot and Richet (1893e) refuting a claim by Mosso (1893) that β-chloralose (para-chloralose) was physiologically active and suggesting that Mosso's β-chloralose must have been impure. The dual
action has been explained by Chevalier and Cherbuliez (1924) and Kruger and Albe-Fessard (1960) by the postulate that chloralose is in fact a mixture of \( \alpha \) and \( \beta \) isomers and that the \( \alpha \) form has anaesthetic properties whereas \( \beta \)-chloralose is a convulsant. Kruger and Albe-Fessard (1960) recommended filtering chloralose at 60°C to remove the insoluble \( \beta \) form. Contradicting this, Monroe, Balis and Ebersberger (1963) showed that \( \alpha \)-chloralose had both anaesthetic and convulsant properties and that \( \beta \)-chloralose was inactive - so reaffirming the original claims of Hanriot and Richet (1893a,e). More recently, Winters and Spooner (1966) were unable to find any \( \beta \)-chloralose in three different commercial brands of \( \alpha \)-chloralose - nor could they detect any even after boiling chloralose solutions to dryness.

Such contradictions recur throughout the literature on chloralose - particularly in relation to autonomic functions. They are reviewed by Balis and Monroe (1964) who summarised the situation as follows - "...in spite of the considerable number of studies on alpha chloralose, the accumulated experimental evidence is not sufficient to allow a definitive statement regarding its action on the central nervous system. Some of the contradictory evidence on the pharmacological actions of alpha
氯醛可能由于这种药物已经在70年的时间里，许多次被使用了低效或过时的调查技术。其他研究如此明显地矛盾以至于让人怀疑研究者是否真的在研究相同的药物。鉴于在确定α氯醛的分子结构的困难以及研究药物的化学性质的缺乏，这是可能，但无法证明的，混淆来源。

研究

在这个学位论文中，我报告了一个试图更详细定义氯醛抽搐特征的研究。研究的目的是确定在突触水平上氯醛的作用模式。除非有特殊理由，否则刺激和记录都限于一只后肢，因为经验证实了Alvord和Fuortes (1954)的发现，即解剖减少了兴奋性。这项研究仅限于氯醛抽搐反射的传出成分，并且研究是倒退的，即首先观察到传入神经的放电。
(Chapter III), then intracellular studies of motoneurones (Chapter IV) and finally investigations on some descending tracts (Chapter VI). At all levels, a search was made for inhibitory processes that could provide a basis for understanding the "fatigability" of the chloralose jerk. As part of this search and also in the hope of understanding more of the central mechanisms of the jerk reflex system, the influence of the cerebellum on the chloralose jerk was investigated (Chapter V).
CHAPTER II

MATERIAL AND METHODS.

Preparation and Anaesthesia.

Experiments were performed on adult cats of both sexes weighing between 2.0 and 4.1 kg. Anaesthesia was induced with ether. After cannulation of the trachea, a polythene cannula was inserted into a forelimb vein, and 50 mg/kg α-chloralose (Fluka) was given intravenously. The chloralose for injection was freshly prepared as a 1% v/v solution in 0.9% saline at 80°C and cooled to about 40-50°C before administration. Occasionally, an extra 10 to 20 mg/kg of chloralose was required to reduce spontaneous activity - especially towards the end of experiments lasting 12 to 15 hours.

All preparations were paralysed with intravenous gallamine triethiodide (Flaxedil, May and Baker) and artificially respired.

A thermometer was placed between the scapula and the rib cage and the cat's temperature kept at 35 to 38°C by a hot water bottle and/or an electric heating pad underneath the abdomen.
Dissection and Experimental set up.

Limb nerves. In the left hindlimb, combinations of certain branches of the sciatic nerve were dissected. These branches are listed here, each followed in parenthesis by its abbreviation used in this thesis and its function; posterior biceps and semitendinosus (PBST - nerve to knee flexors); sural (Sur - cutaneous); gastrocnemius and soleus (GS - nerve to extensors of the ankle); posterior nerve to the knee joint (Joint); posterior tibial (Tib - a mixed cutaneous and muscle nerve to the hind paw); plantaris combined with flexor digitorum longus and flexor hallucis longus (P1FDHL - nerve to ankle and toe extensors); the nerve to tibialis anterior, extensor digitorum longus and the peronei muscles (PDP - supplying flexors of ankle and toes) and the superficial peroneal nerve (SP - cutaneous). Where possible the interosseus nerve was excluded from P1FDHL. The mixed nerve which supplies skin and extensor digitorum brevis was always excluded from PDP. In a few experiments a number of additional nerves were prepared: the nerve to the knee extensors - quadriceps (Quad) - was approached through a groin incision, dissected free and mounted in a buried electrode assembly. Sometimes branches of the sciatic nerve in the right hindlimb were required or the
cutaneous superficial radial nerve in the left forelimb (Sup Rad).

After completing any further dissection, the cat was mounted in the experimental frame and the edges of the skin incision were sewn up to form a pool which was filled with paraffin oil. Nerves were mounted on bipolar platinum wire electrodes and the temperature of the pool maintained by a heater coil.

**Spinal Cord.** The spines and laminae of vertebrae L1 to L7 were removed and the spinal cord exposed through a longitudinal incision in the overlying dura. In a few experiments the 7th lumbar and 1st sacral ventral roots were cut and the central end prepared for recording. The cat was rigidly mounted in the experimental frame by means of clamps on the iliac crests and the 13th thoracic vertebra and by side clamps on the body of the 5th or 6th lumbar vertebra. A paraffin filled pool was formed by sewing up the skin edges and the pool kept warm by glass enclosed heater coils. Ventral roots were mounted on bipolar platinum wire electrodes and cord dorsum potentials were recorded with a platinum ball electrode. This electrode also served to gently hold back dorsal roots so as to facilitate microelectrode tracking for motoneurones. Microelectrodes were attached to a
3-dimensional micromanipulator essentially similar to that described by Eccles, Fatt, Landgren and Winsbury (1954). Tracks for both motoneurones and tract fibres were made through a small hole torn in the pia overlying the dorso-lateral quadrant just lateral to the dorsal root entry zone.

**Pyramids.** The pyramids were exposed by a ventral approach. The trachea and oesophagus were cut and the larynx and pharynx reflected rostrally to expose the muscles over the base of the skull. These were reflected and a dental drill used to remove part of the basioc-ciput. The exposure was completed by cutting and reflecting the dura. After the animal was mounted and the head firmly fixed in the head frame (David Kopf Instruments, type 1204), platinum electrodes were manoeuvered into position with the aid of a mirror.

**Cerebellum.** The bony tentorium was exposed and removed after craniotomy and removal by suction of the occipital pole of the cerebral hemisphere. This procedure was normally confined to the left side (i.e. ipsilateral to the leg dissection) since exposure of the whole anterior lobe was usually attended by greater blood loss and consequently a poorer preparation. After fixing the head in the frame the dura was reflected. The edges of
the scalp incisions were sewn up to form a pool which was filled with warmed paraffin oil.

Methods of Stimulation.

Peripheral nerves were stimulated by condenser discharges. The strength of this stimulus was measured in multiples of threshold strength. Threshold being that strength which gave a just detectable action potential in the proximal part of the sciatic nerve or at the dorsal root entry zone. Thresholds were checked every 2 to 3 hours.

The cerebellum was stimulated through a pair of freshly chlorided silver balls about 2mm apart and placed on the surface. To stimulate the pyramid the electrode on the surface was made the cathode and the anode placed on a nearby bone edge. Stimuli to the cerebellum and the pyramid were either 0.2 msec rectangular pulses from a Grass (S4-E) stimulator or condenser discharges of total duration close to 0.2 msec. Stimulus current was monitored by observing the voltage drop across a 100Ω resistor in series with the anode of the stimulating circuit.

In order to stimulate the red nucleus, a pair of stainless steel needles coated except for their tips
were inserted into the mid-brain using Horsley-Clarke co-ordinates as given in Reinoso-Suárez (1961). Co-ordinates were chosen so that ideally the medial of the two electrodes would be in the mid-line close to the decussation of rubro-spinal fibres and the lateral electrode in the posterior pole of the red nucleus. Stimuli were 0.2 msec rectangular pulses from the Grass stimulator. After experiments, the electrode position was checked histologically by ferrocyanide stain for ferric ions.

**Methods of Recording.**

1.) Potentials in ventral roots, dorsal roots or peripheral nerves were recorded with bipolar platinum electrodes and the signals amplified by a capacity coupled amplifying system. To ensure monophasic records from peripheral nerves, cotton wool pellets soaked in a 1% solution of procaine hydrochloride (BDH) were placed for 10 to 15 minutes on their cut end.

2.) Microelectrodes were of pyrex glass tubing pulled to a fine tip by a vertical electrode puller (Winsbury, 1956). The filling solution was either 2M-potassium citrate (pH 7.0) or 3M-potassium chloride. The micro-electrode was connected through a cathode follower to both a capacity coupled amplifier and a low gain direct
coupled amplifier. The direct coupled amplifier drove a meter and a pen recorder (recti/riter - Texas Instruments). Both were calibrated in millivolts so that changes in DC level at the electrode tip could be directly observed and recorded. Potential changes were recorded relative to an earth electrode sewn onto the back muscles close to the recording site. In the micro-electrode records illustrated in this thesis the convention has been for positive potential changes to be upwards. Exceptions to this were the recordings from tract fibres in which the opposite convention was used.

3.) The coupling time constants of the amplifying system were always 200 msec or more and in all records on a slow time base (i.e. 300 msec or longer) the time constant was 1 second. Signals from the two independent recording channels were displayed on a double-beam cathode-ray tube and photographed by a Grass C4-k kymograph camera. Some figures have been touched up - particularly those showing records on a slow time base.

Finally, in this thesis, ipsilateral refers to the left side since records were always taken from nerves in the left hindlimb although other recording sites were occasionally used.
CHAPTER III.

CHLORALOSE-JERK RESPONSES
RECORDED FROM HINDLIMB NERVES.

INTRODUCTION

There has been no systematic investigation of chloralose-jerk responses evoked by electrical stimulation of afferent nerve fibres. Alvord and Fuortes (1954) stimulated muscle and cutaneous afferents and describe some characteristics of the jerk responses recorded from efferent nerves. Their description is illustrated by only one figure and is not very detailed. The paper of Shimamura and Akert (1965) deals with the hindlimb afferent and efferent nerves involved in the spino-bulbo-spinal reflex. However, since this reflex is unlikely to be identical to the chloralose-jerk reflex, the results obtained by Shimamura and Akert need not necessarily apply in the chloralose anaesthetised preparation.

There was therefore a need for a thorough examination of chloralose-jerk responses to establish in detail which somatic afferents elicit it and into which motor nerves it discharges. Such a study was necessary too as a preliminary to the central nervous system investigations to be described in succeeding chapters.
For the experiments described in this chapter, only the left hindlimb was dissected.

RESULTS

Cats anaesthetised with chloralose (50 mg/kg) showed a range of behaviour from complete quiescence to so much convulsive activity that dissection was impossible. Generally the animals were in an intermediate condition in which there was little spontaneous movement even though it was possible to evoke the characteristic jerk response by a light touch on the limb, body or head (particularly near the snout) or by a sudden noise or a tap on the operating table. Electrical stimulation of a peripheral nerve also produced a jerk which was indistinguishable from that observed on abrupt natural stimulation. Under these circumstances, a late reflex response of approximately 20 msec latency could be recorded in certain motor nerves. A series of responses is illustrated in Fig. 1, recorded from the nerves to knee flexors (PBST - upper traces) and extensors of the ankle and toes (PlFDHL - lower traces).

The late reflex response was elicited by stimuli applied once every 2 to 5 sec and had several characteristic features. It was most consistently recorded from
nerves to flexor muscles - appearing 10 to 15 msec after the flexor reflex. Late discharges into the nerves to extensors were occasionally observed (Figs. 1A, B, C, D, E and N; 2K, L, M, N and O). This extensor response had a slightly longer latency (25-45 msec) than that of the late response of flexors (20-40 msec), the duration of both responses varied from 5 to 20 msec. The peak amplitude of the extensor discharge of 0.2 to 0.4 mV was also smaller than that in the nerve to flexors (0.5 to 1 mV). Two phases were sometimes observed in the flexor late response on stimulation of cutaneous nerves at twice threshold intensity (Fig. 2A and E). Ascher et al. (1963) noted that in some preparations there was a similar double jerk response to auditory stimulation.

These late responses will be called chloralose-jerk responses, chloralose-jerk discharges or chloralose-jerk reflexes. Sometimes, so as to avoid monotony and where the context makes the meaning clear, the words "chloralose" or even "chloralose-jerk" will be omitted.

**Effective afferent stimuli for chloralose-jerk responses.**

(a) **Cutaneous:** On stimulating cutaneous nerves, the flexor chloralose-jerk discharge was sometimes evoked by stimuli of strength lower than those required to elicit
the flexor reflex (Figs. 1A; 2E). This phenomenon was noted by Alvord and Fuortes (1954). Shimamura and Akert (1965) also observed that the spino-bulbo-spinal reflex in decerebrate cats could similarly be evoked by stimuli to cutaneous afferents at strengths below the threshold for the flexor reflex. Increasing the stimulus strength resulted in very little alteration in the flexor chloralose-jerk discharge except that at 20 times threshold it usually decreased (Fig. 2D and H; Fig. 4C and F). This reduction was almost always observed on strong stimulation of sural and posterior tibial nerves but not of superficial peroneal (compare Fig. 4C and L).

Extensor chloralose-jerk discharges, when present, were evoked most effectively by low strength stimuli to sural and posterior tibial nerves (Fig. 1A, B, C and E; Fig. 4A and B), whereas stimulation of superficial peroneal was minimally effective (Fig. 1 I-L; Fig. 4 J-R).

(b) Muscle: Repetitive (250/sec) stimulation of low threshold muscle afferent fibres from either extensor (Fig. 1M) or flexor muscles (Fig. 2I) never evoked a chloralose-jerk response. Therefore activity in Group I muscle afferents (Ia fibres from primary spindle endings and Ib from Golgi tendon organs) is evidently not effective in generating a chloralose jerk. In contrast, a jerk
discharge (Fig. 1N; Fig. 2J) could be evoked by stimuli of 4 times threshold strength which will activate Group II afferent fibres as well as Group I (Eccles and Lundberg, 1959). A single stimulus at this strength most often proved inadequate, and in general 4 stimuli gave a maximum effect. Increasing the stimulus strength to 10 times threshold resulted in little change in the chloralose-jerk discharge (Fig. 10) but at 20 times threshold, when all Group III afferents will be activated, (Eccles and Lundberg, 1959) the discharge was reduced (Fig. 1P).

(c) Joint: Repetitive stimulation of the posterior nerve to the knee joint at twice threshold intensity sometimes evoked a chloralose-jerk response (Fig. 2M). However, at higher stimulus strengths, there was a more consistent response with a latency 5 to 10 msec longer than that evoked by cutaneous stimulation (Fig. 1R-T; Fig. 2M-0). Unlike high threshold muscle and cutaneous stimuli, strong stimuli to this joint nerve did not reduce the amplitude of the jerk discharge.

Variability in chloralose-jerk responses.

Types of variation in chloralose-jerk discharges are illustrated in Figs. 3, 4 and 5. In Fig. 3, single stimuli to sural and superficial peroneal nerves evoked a jerk
discharge in PBST nerve (lower traces) and this was simultaneously integrated (upper traces). Care was taken to integrate only the jerk discharge and exclude the flexor reflex. Particularly with sural stimulation, the integrated responses show wide variation from no response to a very large one (Fig. 3C). In these integrated records, the inability to maintain a plateau consistently is due to the 200 msec coupling time constant of the amplifier and may also be due in small measure to slightly biphasic peripheral nerve recording and/or drift in the integrator.

Fig. 4 shows a change that occurred in the pattern of chloralose-jerk responses in flexors and extensors over five hours. After the first 2 hours the jerk responses to superficial peroneal stimulation showed little variation (compare Fig. 4M-0 with J-L). Whereas jerk responses to stimulation of sural nerve were greatly altered, the more labile extensor response had disappeared while the flexor jerk response was increased in both amplitude and duration (Fig 4D-F compared with the control A-C). Between 2 and 5 hours there was a reduction in both flexor and jerk discharge evoked from sural or from superficial peroneal.

Two further examples of variability of extensor jerk responses are illustrated in Fig. 5. In A-D a single
sural stimulus evoked a discharge into one extensor, P1FDHL (lower trace) in the complete absence of any response in another extensor, GS (upper trace). Secondly, in the records of PBST and P1FDHL responses to a sural stimulus at twice threshold strength (Fig. 5E-L), the extensor responses (lower traces) were characteristically more variable than the jerk response simultaneously recorded from the flexor nerve (upper traces). Furthermore, in other experiments, if both flexor and extensor chloralose-jerk discharges were variable, their variation was independent, i.e. a large extensor discharge was not necessarily associated with a large flexor one or a small extensor one with a small flexor discharge.

Interactions of chloralose-jerk responses.

Fig. 6 illustrates the effect of increasing stimulus repetition rates on the chloralose-jerk response recorded from a flexor (A-F) and an extensor nerve (G-K). The more labile extensor response disappeared at lower stimulus frequencies than the flexor response. The frequency at which the flexor jerk discharge was significantly reduced varied considerably - in Fig. 6 between 3.0 and 4.5/sec but in other experiments at stimulus frequencies as low as 0.5/sec. In general, a small flexor jerk
response was more susceptible to increasing stimulus repetition rate than a large one.

The effect of activation of the chloralose-jerk reflex system on a subsequent flexor chloralose-jerk response is shown in Fig. 7. Series A-F and G-L are from different experiments. A and G are the respective control records of test responses recorded from the nerve to PBST and elicited by superficial peroneal stimulation at ten times threshold. When conditioned by a similar stimulus at progressively reduced condition-test intervals, the test jerk response (marked by arrows) was modified. In series B-F suppression began between 220 and 250 msec and was total at 150 msec. In contrast, the test jerk response in series H-L was much reduced even at 295 msec, but still detectable at 150 msec. These values varied from experiment to experiment - the period of total suppression ranged from 100 to 500 msec. The flexor reflex was also suppressed, particularly at the shorter condition-test intervals (e.g. Fig. 7F and L). There is too the emergence of a discharge some 50 msec after the test chloralose-jerk discharge in B, C and H. Such discharges were quite frequently observed when using condition-test intervals of 200 to 300 msec.
DISCUSSION

A most striking characteristic of chloralose-jerk responses is their variability. The only consistent finding has been that Group I muscle afferents do not evoke any chloralose-jerk discharge, confirming the observation of Alvord and Fuortes (1954). Apart from this, there have been many experiments in which there has been no extensor discharge. Sometimes there was no flexor jerk response to stimulation of low threshold cutaneous or Group II muscle afferent fibres. Also, flexor jerk discharges have occasionally been facilitated at stimulus frequencies between 1.5 and 3.0/sec or they may have shown random fluctuations, e.g. they may have been consistently large for five to ten minute periods, then become smaller for a similar period and finally returned to their original size. The causes of these pattern changes are not clear. However, as the chloralose anaesthetised preparation often responds to even a slight noise or very gentle mechanical stimulation, it is possible that the moment to moment variations as illustrated in Fig. 3 are due to the interplay of sensory inputs from the laboratory environment, e.g. camera and other noise, draughts, movements. Such stimuli might activate the jerk reflex pathways without causing any
overt response, though nevertheless affecting the response to deliberately applied stimuli.

There was a slight possibility that the chloralose-jerk discharge recorded from a peripheral nerve was a dorsal root reflex conducted to the periphery in afferent fibres rather than a reflex in efferent fibres. This was disproved by the following:—20 msec latency discharges were recorded only in muscle nerves and never in cutaneous fibres; jerk discharges recorded from ventral roots were of the same pattern as those recorded from muscle nerves, but with the expected reduction in latency; finally, chloralose-jerk discharges recorded from muscle nerves were abolished by sectioning the appropriate ventral roots.

The flexor chloralose-jerk response.

The chloralose-jerk response recorded from flexor nerves has been observed by other investigators (Alvord and Fuortes, 1954; Shimamura, 1963; Ascher, 1965) and its association with the predominantly flexor convulsive jerk seems clear cut. It can be evoked by electrical stimulation of all somatic afferents except Group I muscle afferent fibres, i.e. by activation of flexor reflex afferents (FRA) as defined by Oscarsson (1965). Low
threshold joint afferents which Oscarsson omits from the FRA are the single exception to this. The effectiveness of low threshold stimulation of cutaneous nerves indicates that the chloralose jerk is easily elicited by activation of large diameter cutaneous afferents which are from light-touch and hair receptors (Hunt and McIntyre, 1960). This accords with common experience that the chloralose anaesthetised cat is often very sensitive to even a puff of air.

The extensor chloralose-jerk response.

In decerebrate cats, Shimamura and Akert (1965) recorded late responses from extensor nerves only on stimulating the afferents from other extensor muscles. In these experiments on chloralose anaesthetised cats, extensor late responses were elicited from all afferent sources (cutaneous, joint, extensor and flexor muscle afferents).

It is interesting that a jerk discharge into extensors (if present) was obtained by stimulation of sural and posterior tibial nerves and very rarely from superficial peroneal. Posterior tibial is not simply a cutaneous nerve as it includes the fibres to the small muscles of the paw. However, superficial peroneal and
sural are both purely cutaneous. The difference in responses to activation of sural and superficial peroneal might be related to their supplying different skin areas, since Hagbarth (1952) showed that excitation or inhibition of extensor monosynaptic reflexes by cutaneous stimuli was dependent on the skin area stimulated.

Observations on extensor chloralose-jerk discharges by other investigators show an interesting gradation. Moruzzi (1944) used 35 mg/kg chloralose and Alvord and Fuortes (1954) worked on cats anaesthetised with as little as 30 mg/kg. These workers regularly recorded jerk responses from extensors. In contrast, Ascher (1965) used 80 mg/kg chloralose and never observed extensor jerk discharges on stimulating the ipsilateral hindlimb. The present observations using 50 mg/kg chloralose fit between these extremes and strongly suggest that the extensor jerk response is dependent on depth of anaesthesia.

When there is an extensor chloralose-jerk discharge, the simultaneously recorded flexor jerk discharge is often of unusually short duration (Fig. 1A-C, Fig. 4A-B). The transition from a mixed flexor and extensor discharge pattern to a pure flexor one (Fig. 4A-C, D-F) was accompanied by a broadening of the flexor jerk response. It is possible that certain descending impulses are
excitatory to extensor motoneurones and at the same time inhibitory to flexor neurones. If such a pathway ceased to operate, perhaps due to deepening anaesthetic level, there would be no late discharge into extensor nerves and a reduction in the inhibition onto the flexor neurones would permit a more prolonged excitation of flexor cells (by other pathways) and hence the longer flexor discharge. The alternative explanation is that while this descending pathway is still active, certain synapses in the spinal cord which are involved in this pathway have failed to operate due to local changes.

**Terminology.**

Because, in cats under 50 mg/kg chloralose, the jerk response is predominantly a discharge of flexor motoneurones and because through the bulk of this thesis the chloralose-jerk responses illustrated are records from the nerve to PBST, the words "chloralose-jerk response" etc., if used without qualification, will refer to this flexor discharge. Chloralose-jerk discharges recorded from extensor nerves will always be referred to as such.
CHAPTER IV.

RESPONSES RECORDED FROM ALPHA MOTONEURONES.

INTRODUCTION.

In the preceding chapter, characteristics of chloralose-jerk responses recorded from hindlimb flexor and extensor motor nerves were described. Although the technique of recording from peripheral nerves gives an indication of overall activity in a population of motoneurones, it does not reveal any details of excitatory and inhibitory influences at the cellular level which might be associated with the chloralose-jerk discharge. However, it is possible with intracellular recording from individual α-motoneurones to examine closely the responses of these motoneurones to stimulation of muscle, joint and cutaneous afferents.

The only published intracellular study of motoneurones in chloralose anaesthetised preparations is by Shimamura et al. (1967a). These authors recorded from extensor motoneurones but not from flexors. They illustrate one record from a gastrocnemius motoneurone showing excitation at the same time as the chloralose-jerk response. This occurred in 54% of a sample of 35 gastrocnemius motoneurones. In a further 20% there was no
excitation, but instead a slight membrane hyperpolarisation and the remaining 26% showed no membrane potential changes at all. In the present study, the response patterns recorded in extensor motoneurones were very different from those described by Shimamura et al. and probable reasons for this will be discussed.

Results are presented in two main sections. The first describes responses of flexor motoneurones. The second section is devoted to results obtained from extensor motoneurones.

METHODS.

Leg dissection and laminectomy were required for these experiments. Microelectrodes filled with 2M potassium citrate (DC resistance 5-10MΩ) were used throughout unless there was a special advantage in using electrodes filled with 3M-KCl (e.g. when it was desired to reverse the polarity of IPSPs by ejection of chloride ions from the microelectrode). Motoneurones were identified by antidromic and/or monosynaptic activation (Eccles, Eccles and Lundberg, 1957).

In all cases after completion of intracellular recording, the microelectrode was withdrawn from the cell and corresponding extracellular records taken using
identical stimulus parameters. For the sake of simplicity these extracellular records are not usually included in illustrations.

RESULTS.

A. Flexor Motoneurones.

Intracellular records were obtained from 90 ipsilateral hindlimb flexor motoneurones in the 7th lumbar and 1st sacral segments. A sequence of potential changes was initiated in these motoneurones on stimulating ipsilateral hindlimb afferents. For convenience, these potential changes have been studied in three groups:-(1) an early group of changes related to the segmental (flexor) reflex, (2) an intermediate group associated with the chloralose-jerk discharge and (3) a late group that followed the chloralose-jerk response and lasted several hundred milliseconds. The first two groups will be presented in detail in section 1 below. The last-mentioned will be illustrated in section 2. Section 3 deals with results obtained after spinal cord transection.

1. Potentials associated with the flexor reflex and the chloralose-jerk discharge.

In Fig. 8 are shown responses recorded intracellularly from a PBST motoneurone (top traces in each row)
together with the simultaneous record from the nerve to PBST (bottom traces). Stimulation of cutaneous, muscle and joint afferent fibres elicited a segmental flexor reflex and a chloralose-jerk discharge in the peripheral nerve as described in the preceding chapter. The intracellular records show excitation (excitatory postsynaptic potential, EPSP) with or without cell discharge in relation to the flexor reflex. This was followed by a larger EPSP with one or more spike discharges which correspond to the chloralose-jerk discharge recorded from the peripheral nerve. This second EPSP is named the chloralose-jerk EPSP to distinguish it from the early EPSP of the flexor reflex. Stimulation of muscle afferent fibres at Group I maximum strength (i.e. twice threshold) did not evoke any detectable chloralose-jerk EPSP (Fig. 8E), although there is a very small discharge in the peripheral nerve record - probably from activation of the lowest threshold Group II afferents. The interposed extracellular records (mid traces of each row) show that the intracellular potentials were true postsynaptic potentials.

The basic response pattern of flexor cells was fairly uniform and is illustrated by three typical PBST motoneurones in Fig. 9. In Fig. 9 (A-D) there is a
double depolarization: the first associated with the flexor reflex was followed by a larger chloralose-jerk EPSP. This chloralose-jerk EPSP may reach the threshold for cell firing (E to H) and an even more excitable cell (I to L) yielded responses in which only I showed any pause between the early segmental reflex firing and the later chloralose-jerk firing. It is possible that the cell shown in Fig. 9 (I-L) was damaged by the microelectrode and so fired very easily. However, the discharge recorded from the nerve to PBST was also unusually large when this cell was being examined.

Chloralose-jerk discharges recorded from peripheral nerves were shown to be easily depressed by increase in stimulus repetition rate (Ascher et al., 1963; Chapter III) and chloralose-jerk EPSPs recorded in flexor motoneurones were similarly affected. Fig. 10 (A-E) shows the response pattern of the same PBST cell illustrated in Fig. 9 (A-D) and below each record is the simultaneous response from the PBST nerve. As the chloralose-jerk discharge in the peripheral nerve was reduced; the second depolarization decreased until at a stimulus rate of 3.8/sec (E), there was neither a chloralose-jerk EPSP nor a chloralose-jerk discharge. Unlike the pattern sometimes seen in extensor motoneurones, there was no indication in
flexor motoneurons of a mixture of excitation and inhibition being "unmasked" at different stimulus rates. In another PBST motoneurone, Fig. 10 (F-J), there was reduction and eventually elimination of chloralose-jerk firing at increasing rates of stimulation until in J even the residual chloralose-jerk EPSP (I) had disappeared.

This same susceptibility to stimulus frequency may be observed by testing the chloralose-jerk reflex pathway after an earlier activation (Fig. 11). Single records were taken intracellularly from a PBST motoneurone (upper traces) and simultaneously from the nerve to PBST (lower traces). Conditioning and testing responses were to an SP stimulus at twice threshold strength. The condition-test interval was gradually reduced (B-G). A and H are controls for B and G respectively. Even at the longest interval illustrated, 330 msec (Fig. 11B), the intracellular jerk response showed a reduction from 4 to 3 spikes and the number of spikes was progressively reduced as the test interval diminished. Finally in G, there remain only EPSPs. A similar reduction occurred in the chloralose-jerk discharge recorded from PBST nerve. The intracellular record of Fig. 11E shows a very late EPSP. Such a late response has been observed in peripheral
nerve records when test stimuli were applied after a conditioning volley (e.g. Fig. 7B, C and H).

Activation of appropriate afferents evoked a double depolarization pattern in motoneurones of the ankle flexors, but with fewer spike discharges. This accords with the fact that in the chloralose jerk, proximal limb flexors contract more vigorously than other flexor muscles (Alvord and Fuortes, 1954).

Pentobarbitone sodium (Nembutal, Abbott Laboratories) is known to be an extremely effective depressant of the chloralose jerk and the spino-bulbo-spinal reflex (Shimamura et al., 1964; Shimamura and Akert, 1965). Five minutes after 2 mg/kg of pentobarbitone sodium was administered intravenously, the chloralose-jerk discharge in the PBST nerve vanished while the chloralose-jerk EPSP in the PBST motoneurone was diminished when compared with the control series (Fig. 12E-H compared with A-D). The flexor discharge into the peripheral nerve and the corresponding excitation in the motoneurone were also affected. Further doses of pentobarbitone sodium reduced the jerk responses and flexor reflexes until after a total dose of 10 mg/kg (Fig. 12M-P), only a flexor reflex EPSP in the motoneurone was evoked by the cutaneous stimuli.
2. Late group of potentials.

Late inhibitory potential changes were recorded from flexor motoneurones following stimuli that evoked a chloralose-jerk discharge. Fig. 13 (A-D) shows a late hyperpolarisation in a PBST motoneurone whose earlier responses are shown on a faster sweep and at lower amplification in Fig. 13 (E-H). In many cells, this late and long-lasting hyperpolarisation had three phases. A comparatively early and brief component followed the train of impulses evoked in time with the chloralose-jerk discharge. This phase of the hyperpolarisation is likely to be an after-hyperpolarisation following action potentials (Brock, Coombs and Eccles, 1952), a suggestion strengthened by the observation that it was absent in cells with little or no discharge. The initial phase was followed by a second negative potential which lasted 100 to 200 msec and then merged into the third phase which was a slow return to the base line. However, the recording time constant for the records in Figs. 13, 14 and 15 was one second, and hence the precise shape and time course of these late potential changes remain unknown. Extracellular records (illustrated below the intracellular traces of Fig. 13A-D) indicate that this late hyperpolarisation was not a field potential passively recorded across the cell membrane.
A late hyperpolarisation of smaller amplitude but similar time course could also be evoked by electrical stimulation of the superficial radial nerve of the ipsilateral forelimb and by acoustic stimuli (Fig. 14 B and C respectively).

It was hard to determine whether this late long-lasting hyperpolarisation was a true IPSP. In PBST motoneurones penetrated by 3M-KCl filled microelectrodes, "direct" inhibitory postsynaptic potentials were evoked on stimulation of the ipsilateral Group Ia quadriceps nerve fibres (e.g. Fig. 15A). These IPSPs have reversed polarity, no doubt due to leakage of chloride ions from the microelectrode (Coombs, Eccles and Fatt, 1955). However in these circumstances, the long late hyperpolarisation (Fig. 15B) was not reversed, so that this hyperpolarisation is unlikely to be an IPSP generated by inhibitory synapses having a similar distribution on the cell membrane to those responsible for the "direct" IPSP of Fig. 15A. On passage of a depolarising current across this cell membrane, the IPSPs generated by stimulating quadriceps Group Ia fibres reversed to a hyperpolarising potential (compare Fig. 15C and A) but the late hyperpolarisation remained unaffected (compare Fig. 15D and B). Nor was this late hyperpolarisation only an after-
hyperpolarisation (AHP) consequent upon cell firing. The AHP which followed six antidromic spikes had a much shorter duration than the long late hyperpolarisation (compare Fig. 15E and D). However, the AHP certainly contributed to its early phase as already suggested.

In a PBST motoneurone which was spontaneously discharging (Fig. 16E-H), stimulation of superficial peroneal nerve at ten times threshold strength evoked a burst of impulses in association with the chloralose-jerk discharge recorded from PBST nerve (Fig. 16A-D). This discharge was followed by a reduction in activity of the same duration as the hyperpolarisations illustrated in Fig. 13, 14 and 15. A rebound increase in activity followed. This rebound is more apparent in the single sweep traces (Fig. 16 B-D) than in the record of 10 superimposed traces (Fig. 16A).

Fig. 17 illustrates this process for a population of flexor motor units. In this preparation there was a considerable spontaneous asynchronous discharge into PBST nerve (Fig. 17 E-H). Stimulation of the superficial peroneal nerve resulted in the usual segmental and chloralose-jerk discharges which appear as one discharge because of the slow recording speed. These discharges were followed by reduction of the asynchronous activity for up
to one second. A spontaneous discharge (Fig. 17G) similar to evoked discharges was also followed by a period of reduced activity.

3. **Responses after spinal transection.**

In order to determine whether the late inhibitory responses were totally dependent on activity in long reflex pathways through higher centres, records were obtained from PBST motoneurones after spinal cord section at the first lumbar level. Fig. 18 (A-D) shows that the chloralose-jerk response in the intracellular record (upper trace) and in the nerve to PBST was completely abolished by the cord section. However, on a slow time base, a late long-lasting hyperpolarisation was recorded (Fig. 18E-H) and this was associated with a reduction of synaptic noise compared with the baseline activity (I-K). As in the intact animal, this late hyperpolarisation could not be entirely explained as an AHP following the flexor reflex firing, since four antidromic spikes did not result in a similar hyperpolarisation (compare Fig. 18L with E-H).

**B. Extensor Motoneurones.**

Intracellular records were obtained from 102 extensor α-motoneurones in the 7th lumbar and 1st sacral spinal
cord segments. 79 were gastrocnemius-soleus (GS) motoneurones. 14 belonged to the ankle and toe extensors - plantaris with flexor digitorum and hallucis longus (PlFDHL), and the remainder were motoneurones innervating semimembranosus or anterior biceps muscles.

Stimulation of hindlimb afferents evoked a series of complex membrane potential changes in ipsilateral extensor motoneurones. As with flexor motoneurones these changes have been most easily studied in three groups:- (1) that associated with the flexor reflex, (2) that associated with the chloralose-jerk discharge and finally (3) a late group. Extensor motoneurone responses in association with the flexor reflex have already been described in detail (Eccles and Lundberg, 1959). Therefore they will be considered in conjunction with the chloralose-jerk responses in section 1 below. The late group of potential changes is described in section 2. Section 3 is devoted to results obtained after spinal cord transection.

1. **Potentials associated with the flexor reflex and the chloralose-jerk discharge.**

In Fig. 19 are shown the responses of a GS motoneurone to stimulation of the three cutaneous nerves over a wide range of stimulus strengths:- superficial peroneal
(SP), sural (Sur) and posterior tibial (Tib). The lowermost records in each row are the flexor reflex and the chloralose-jerk discharge recorded from the nerve to PBST. The extracellular potentials (middle records) were photographed after withdrawal of the microelectrode from the motoneurone. A basic pattern of two hyperpolarisations followed by a depolarisation was generally observed. The first hyperpolarisation began with the flexor reflex discharge into the periphery and reached a maximum as the flexor reflex ended. The onset of the second hyperpolarisation usually coincided with the peak of the chloralose-jerk discharge and a maximum was reached as the jerk discharge died away. Exceptions to this were the responses to high stimulus strengths (Fig. 19D, H and L) in which the double hyperpolarisations tended to merge into one another. In this cell there was excitation following the first (flexor reflex) hyperpolarisation at the lowest stimulus strengths (Fig. 19A, E and I). This excitation disappeared on increasing stimulus strength above twice threshold. In some cells the flexor reflex hyperpolarisation was preceded by a small depolarisation (e.g. Fig. 19A-D).

It is well known that a hyperpolarising current passed across the cell membrane reduces or even reverses
hyperpolarising inhibitory postsynaptic potentials (IPSPs), whereas depolarising currents increase the size of the same IPSPs (Coombs et al., 1955). The double hyperpolarisations recorded in a PlFDHL motoneurone (Fig. 20A, B and I, J) were reduced by hyperpolarising currents (C and D) and increased by depolarising currents (K and L) and are thus true IPSPs. Control records taken after cessation of current flow are shown in the third row (Fig. 20E and F, M and N). The corresponding discharges recorded from the ventral root (row 4) indicate the time relationship between intracellular events and the flexor reflex and chloralose-jerk discharge.

Fig. 21 (upper records) illustrates the effect of increased rates of stimulation on the intracellular potentials of the same cell as in Fig. 20. The flexor reflex IPSP remained relatively uninfluenced whereas the second IPSP and the subsequent depolarisation diminished in parallel with the reduction of the chloralose-jerk discharge into the ventral root. At 4.3 stimuli per second there were only occasional small second IPSPs.

This second IPSP varied considerably in amplitude. It may be larger than the flexor reflex IPSP in some circumstances (Fig. 20B and F; Fig. 21 at 0.5/sec stimulus rate) but under other conditions it may be smaller.
or almost absent (Fig. 20J and N; Fig. 22E). Generally speaking, it was large when associated with a large chloralose-jerk discharge into flexor nerves and if the jerk discharge was reduced for any reason (e.g. spontaneous variation, increased repetition rate, etc.) then the second IPSP was correspondingly smaller. There were exceptions to this general rule, e.g. the small second IPSP in Fig. 22E was associated with a fairly large chloralose-jerk response.

In some extensor motoneurones, changes in repetition rate altered the response patterns in a very different way from that illustrated in Fig. 21. In Fig. 22 are responses from three gastrocnemius motoneurones (columns I, II and III). The stimulus parameters were identical for each cell. At a low stimulus rate the responses (A, E and I) showed a considerable diversity which was exaggerated at higher rates of stimulation (e.g. at 3.8/sec C, G and K). A fairly consistent feature of extensor motoneurone responses at these intermediate stimulus rates (2.8-3.8/sec) has been the accentuation of or emergence of a third hyperpolarisation with both latency and duration of 50-60 msec (seen best in Fig. 22B but also in Fig. 22C, G, J and K).

Although the double IPSP pattern of Fig. 22I was
fairly close to that of the P1FDHL motoneurone illustrated in Figs. 20 and 21, the response to increasing stimulus rate was totally different: the IPSPs seen in Fig. 22I were replaced by a complex excitatory pattern with cell discharge at higher stimulus rates (J-L). Discharges into GS nerve (upper records) and into PBST nerve (lower records, Fig. 22 column IV) were taken with identical stimulus parameters immediately after records I-L. The late responses into GS nerve (M-P) indicate that the cell under examination in I-L was not abnormal, for there were a sufficient number of GS motoneurones synchronously firing to give a peripheral nerve volley.

As a summary of the range of response patterns observed in extensor motoneurones, Fig. 23 illustrates records obtained from 8 different cells (G and H are from the same cell). The most frequent response pattern is shown in Fig. 23A (similar to that in Figs. 19B, 20B and 21). The rarest patterns were Fig. 23C with a triple hyperpolarisation and I with an almost pure depolarisation. Intermediate types of response showed two basic variations from the pattern of Fig. 23A:—(1) a depolarisation between the first and second IPSPs (D, E and F) which sometimes reached firing threshold (G); (2) a tendency for the depolarisation that followed the second IPSP to reach threshold (H).
2. **Late responses in extensor motoneurones.**

As in flexor motoneurones, intracellular recording revealed late inhibitory processes in extensor cells. In Fig. 24, slow time base records show potential changes in a GS motoneurone elicited by stimuli to cutaneous, joint and muscle nerves (extracellular records are once more positioned between the intracellular responses - upper traces - and responses from the nerve to PBST - lower traces). The very late hyperpolarisation (120 msec in latency) was evoked by all stimuli except that at Group I muscle afferent strength (Fig. 24I) and, in this respect, the late hyperpolarisation resembled other late responses seen in chloralose anaesthetised cats. This potential reached a maximum in about 100 msec and then gradually disappeared, although the recording time constant of 1 sec was too short to permit an accurate measure of its full time course.

Some indication of the nature of the late hyperpolarisation was obtained on penetration of cells with 3M-KCl filled microelectrodes. The depolarising potentials of Fig. 25A are presumably IPSPs reversed by leakage of chloride ions, the potentials being readily reversed into hyperpolarising potentials (Fig. 25C) when a depolarising current was passed across the cell membrane (Coombs et al.,
1955). However, the late hyperpolarisation (Fig. 25B) remained substantially the same during the passage of depolarising current (compare B and D). Thus, as with flexor motoneurones, this hyperpolarisation was unlikely to be an IPSP generated on the cell soma. In the same motoneurone there was considerable background synaptic noise (Fig. 25F), which was practically abolished during the late hyperpolarisation (Fig. 25E). Similar evidence was obtained from most extensor motoneurones for a diminution of background synaptic activity during the hyperpolarisation.

This late inhibitory process could also be studied in a population of extensor motoneurones by the technique of monosynaptic reflex testing. Fig. 26A illustrates the depression of the gastrocnemius-soleus monosynaptic reflex after a conditioning volley in the superficial peroneal nerve. The time course of this depression was very similar to that of the late hyperpolarisation. Control records of the test response (CON) taken before and after the condition-test series show that the monosynaptic reflex was sometimes depressed by as much as 60%. An identical series taken after recovery from spinal cord transection (Fig. 26B) shows that although the size of the test reflex was unaltered, the time course and extent of depression
of the test reflex after a conditioning volley were considerably changed. It is interesting however, that a fairly long-lasting depression was still present in the spinal preparation.

3. Responses after spinal transection.

It seemed possible that the range of extensor cell response patterns could be partly accounted for by the temporal overlap of long lasting spinal influences and the effects of descending tract activity. Also, in view of the depression of the monosynaptic reflex shown in Fig. 26B, it was of interest to determine whether the intracellularly recorded late long-lasting hyperpolarisation had a contribution of spinal origin. Accordingly, extensor motoneurones were examined after recovery from spinal cord section at the 1st lumbar level. Fig. 27 illustrates responses in GS motoneurones to cutaneous stimulation over the usual range of stimulus strengths. In A-D the intracellular records (upper traces) show a flexor reflex IPSP associated with the discharge into the nerve to PBST and subsequently a considerable depolarisation lasting about 50 msec. Not all extensor motoneurones showed this pattern - sometimes only the flexor reflex IPSP was recorded. Thus even in the spinal chloralose anaesthetised preparation there was no uniform response
pattern. It is interesting that the potential changes seen in Fig. 27A-D are very similar to some of the graphs of excitability changes in extensor motoneurone pools that Hagbarth and Naess (1950) and Hagbarth (1952) observed after stimulation of cutaneous nerves. Fig. 27E-H shows records on a slower time base taken from another GS motoneurone. These demonstrate that there was a late long-lasting hyperpolarisation and an associated reduction of the background synaptic activity which was quite considerable in the absence of any stimulus (I-L). This has been a consistent finding, although the hyperpolarisation has usually been smaller and earlier in onset than in preparations with an intact spinal cord.

DISCUSSION

1. **Flexor reflex and chloralose-jerk responses of flexor motoneurones.**

   In flexor α-motoneurones, a stimulus to an afferent nerve (from muscle, joint and skin) evokes first an EPSP in association with the flexor reflex and then an EPSP which corresponds with the chloralose-jerk discharge recorded from nerves to flexor muscles and has been called the chloralose-jerk EPSP.

   The early EPSP evoked by flexor reflex afferents has
been fully investigated (Eccles and Lundberg, 1959). It remained after spinal cord transection (Fig. 18) and was relatively unaffected by pentobarbital (Fig. 12) and by varying the frequency of stimulation (Fig. 10).

Chloralose-jerk responses are particularly sensitive to pentobarbital and are thus not present in animals anaesthetised with this agent. This sensitivity is shown in Fig. 12 where the administration of a dose approximately 5% of that normally used for anaesthesia greatly reduced both the chloralose-jerk EPSP and the chloralose-jerk discharge.

As would be expected, a small jerk response in the peripheral nerve was more likely to be associated with only chloralose-jerk EPSPs in the motoneurones (e.g. Fig. 9A-D). If the jerk discharge was larger, then it was more common to find motoneurones in which the chloralose-jerk EPSP reached firing threshold (e.g. Fig. 9 E-H and I-L).

2. Flexor reflex and chloralose-jerk responses of extensor motoneurones.

In extensor motoneurones the IPSP associated with the flexor reflex has been called the flexor reflex IPSP. Then in most extensor motoneurones, a second IPSP was recorded which can be named the chloralose-jerk IPSP.
This nomenclature is justified by the following facts. There was a close relationship between the chloralose-jerk response recorded from flexor nerves and the second IPSP in extensor motoneurones. When the chloralose-jerk discharge into the periphery was reduced by increased stimulus repetition rates or spontaneous variations, a corresponding reduction was noted in the second IPSP. Transection of the spinal cord abolished both this second IPSP of extensor cells and the discharge into flexor nerves (Fig. 27 A-D).

On the basis of their records from extensor motoneurones, Shimamura et al. (1967a) concluded that their results "do not support the hypothesis that the SBS reflex volley exerts postsynaptic inhibition of extensor motoneurones". This is completely at variance with the evidence presented here for a chloralose-jerk IPSP in extensor motoneurones. The reason for this is not that the spino-bulbo-spinal (SBS) reflex is a different entity from the chloralose-jerk, since Shimamura et al. used the term SBS reflex for typical jerk discharges recorded in their chloralose anaesthetised cats. There are four probable reasons for the divergence. (1) They used 20 mg/kg chloralose (as against 50 mg/kg). As discussed in Chapter III, extensor chloralose-jerk responses are more prominent
when such low doses of chloralose are used; (2) Seven out of 32 of their experimental animals were unanaesthetised-decerebrate and cerebellectomised; (3) They do not mention the strength of sural nerve stimulation that they used to elicit responses in extensor motoneurones. As Fig. 19 (E-H) shows, the response pattern of an extensor motoneurone is dependent on the strength of afferent stimulation. (4) Shimamura et al. used microelectrodes filled with either 3M-KCl or 2M-NaCl. Their results may have been complicated by leakage of chloride ions into the motoneurones and in their paper they do not indicate any awareness of this possibility.

The complexity of extensor motoneurone responses has been emphasised (Figs. 22 and 23) and a variety of factors could account for this:-

(a) The inherent variability of preparations under chloralose anaesthesia and the influence of local conditions such as spinal cord circulation.

(b) The temporal overlap of spinal influences with the effects of activity in descending tract systems.

(c) The probability that these descending tract influences are a mixture of excitation and inhibition.
Detailed information about these factors is lacking, but consideration of each in turn may explain the range of extensor cell response patterns.

(a) The variability of extensor discharges in preparations under chloralose anaesthesia has been demonstrated in some detail (Chapter III). In view of this, it is perhaps not surprising that extensor cell responses are very varied. Eccles and Lundberg (1959) noted that the synaptic actions of high threshold muscle afferents on extensor motoneurones varied considerably, particularly if there were local disturbances in spinal cord circulation.

(b) Fig. 28 (solid line) is a drawing of a type of response very often seen in the extensor motoneurones of a cat with an anatomically intact central nervous system (e.g. the cell in Figs. 20 and 21). In contrast, the broken line of Fig. 28 illustrates the response of an extensor cell after spinal cord section (e.g. Fig. 27A-D). One may not assume that the pathways involved in the generation of the late spinal effects would necessarily be operating when the central nervous system is intact, since they could be under tonic inhibitory influence. However, it seems probable that some of the variations seen in extensor cells (e.g. Fig. 22A, E and I, Fig. 23) could be due to differences in the balance of spinal and
descending effects, so that, for example, where the spinal influence is strong, a pattern such as in Fig. 22E and Fig. 23 F, G and I emerges. This same argument may partially account for the effects of increased stimulus repetition rate recorded in certain extensor cells (e.g. Fig. 22I-L). The inhibitory pattern in Fig. 22 I possibly generated mainly by descending tract activity gives way to excitation which may be predominantly spinal. It is as if the solid line pattern of Fig. 28 approximates the broken line when an increase in stimulus frequency results in reduced effectiveness of descending influences.

(c) Variations in response patterns would also arise if activity in the descending tracts was both excitatory and inhibitory to extensor motoneurones. The effects of descending activity have been extensively studied, most recently by Willis, Willis and Thompson (1967), and are known to be complex. Unfortunately there is yet no detailed investigation of the descending tract systems activated during the chloralose jerk. Certainly descending fibres in the lateral funiculus of the lumbar cord (pyramidal, rubro-spinal and medullary reticulo-spinal) discharge before and during the chloralose-jerk (Chapter VI). As summarised by Nyberg-Hansen (1966), activity in these three tracts is mainly excitatory to flexors and
inhibitory to extensors. They therefore seem likely candidates for the descending tracts involved in generating the chloralose-jerk excitation of flexor motoneurones together with the chloralose-jerk IPSP in extensors. The principal descending tracts localised in the ventral funiculus are the vestibulo-spinal (Nyberg-Hansen and Mascitti, 1964) and the pontine reticulo-spinal (Nyberg-Hansen, 1965). These give excitation in extensors and inhibition in flexors (Nyberg-Hansen, 1966). Should these tracts be activated during the chloralose-jerk, they would contribute to the excitation sometimes seen in extensor motoneurones. Presumably any corresponding inhibition in flexor cells is completely masked by strong excitation from other sources.

3. The late responses of flexor motoneurones.

Following a flexor chloralose jerk, there is a long period of reduced excitability of flexor motoneurones which may be observed in a number of different ways: the subnormality of a test response after a conditioning stimulus (Figs. 7 and 11), the late long-lasting hyperpolarisation (Figs. 13 A-D; 14; and 15B and D) and reduction of spontaneous firing following a jerk discharge (Fig. 16A-D; Fig. 17A-D). Although illustrated here in a cell from a spinal preparation (Fig. 18E-H), the reduction in synaptic
noise associated with the late long-lasting hyperpolarisation may also be observed in some flexor motoneurones of cats with an intact spinal cord. Like other phenomena observed in chloralose anaesthesia, this hyperpolarisation is variable - almost absent in some motoneurones and up to 5 mV in others. It is probable that only the initial phase of this potential is an after-hyperpolarisation following cell firing (Fig. 15E compared with D). The late hyperpolarisation did not reverse polarity in cells in which increased intracellular chloride concentration has reversed IPSP's (compare Fig. 15B and A). This suggests that the hyperpolarisation is not an inhibitory polarisation but is produced by the reduction of a tonic excitatory synaptic bombardment - a disfacilitation. The associated reduction in synaptic noise indicates that in some cells there is a withdrawal of background synaptic activity. Such a withdrawal might result from reduced activity of tonically excitatory interneurones and/or a presynaptic inhibition operating on the axons from those excitatory interneurones. Alternatively, there may be IPSPs generated on the motoneurone dendrites sufficiently remote from the site of electrode penetration that they are unaffected by changes of chloride ion concentration within the main cell body or by polarising currents applied through the electrode.
Because this late long-lasting process is seen in the acute spinal preparation (Fig. 18E-H), the question arises as to what reflex pathways subserve it. Lund, Lundberg and Vyckicky (1965) observed a long-lasting (500 msec) inhibition of transmission to Ia primary afferent fibres on stimulation of flexor reflex afferents in chloralose-anaesthetised, spinal cats. They postulated that this inhibition occurred on the Ia to Ia presynaptic inhibitory pathway and as it was unaffected by large doses of strychnine, they speculated whether the process could be a presynaptic one with depolarisation of interneurone axon terminals. A similar mechanism may well be involved in the generation of the late long-lasting hyperpolarisation and reduced synaptic noise seen in motoneurones of spinal, chloralose-anaesthetised cats. However, it is also clear that in preparations with an intact spinal cord, descending tract systems make some contribution to this hyperpolarisation as it was evoked by auditory stimuli (Fig. 14C). Any explanation for this late hyperpolarisation must also take into account that an exactly similar phenomenon is observed in extensor motoneurones of chloralose anaesthetised cats.

The finding of a long-lasting inhibitory process in flexor motoneurones allows us to understand at least in
part why the chloralose jerk is "fatigable". If the hyper-
polarisation was the only basis for the "fatigability" of
the chloralose jerk, then one would expect that the
flexor reflex would be equally affected by increasing
stimulus repetition rates. As this is not the case, it
seems likely that there are inhibitory processes at other
points on the chloralose-jerk reflex pathway which also
contribute to "fatigability" of the chloralose jerk.

4. The late responses of extensor motoneurones.

The late long-lasting inhibitory process in extensor
motoneurones can be observed as a membrane hyperpolarisa-
tion (Figs. 24 and 25), a reduction of test monosynaptic
reflexes (Fig. 26; also Alvord and Fuortes, 1954) or a
reduction in spontaneous activity (Ascher, 1965).

The characteristics of the late hyperpolarising
potential in extensor motoneurones are similar to the
hyperpolarisation recorded from flexor cells - a delayed
onset, a duration of some 300-400 msec, an amplitude of
up to 5 mV, a frequently associated reduction in synaptic
noise and presence in spinal, chloralose-anaesthetised
preparations. As in flexor motoneurones, increased intra-
cellular chloride ion concentration did not reverse the
polarity of this late potential. All these similarities
indicate that the mechanism for generating the hyper-
polarisation in both extensor and flexor motoneurones is the same and that like the flexor late potential, the late potential recorded from extensor cells is probably a disfacilitation.

A general point emerges from the observation of these late potentials. If, as seems most likely, the late hyperpolarisation is a disfacilitation, then this implies that in chloralose-anaesthetised cats there is a maintained depolarisation of flexor and extensor motoneurones. This tonic depolarisation presumably results in the motoneurones being in a more excitable state under chloralose anaesthesia and so being more readily fired by activity in descending tracts. Hence we have at least a partial knowledge of the underlying mechanism of chloralose hyperexcitability. How chloralose acts to produce this condition remains unknown.
CHAPTER V.

THE CEREBELLUM AND THE CHLORALOSE JERK.

INTRODUCTION.

In their paper of 1897, Löwenthal and Horsley credited Löwenthal with the discovery that repetitive stimuli to the anterior lobe of the cerebellum abolished decerebrate rigidity ("acerebral" tonus). Sherrington (1897, 1898) independently discovered the same phenomenon. Since these original descriptions, there has grown a voluminous literature on the effects of stimulating the anterior cerebellar lobe (Ch. 3 in Dow and Moruzzi, 1958; Brookhart, 1960).

Following the work of Bremer (1922), it has been generally agreed that the inhibition of decerebrate rigidity results from activation of the Purkinje cells of the cerebellar cortex. Bremer (1922) was also the first to demonstrate the phenomenon of post-stimulation "rebound" of extensor rigidity. He observed too that the inhibition was mainly ipsilateral, and that contralateral effects varied - sometimes inhibition, sometimes increase of extensor tonus. Subsequent workers (e.g. Denny-Brown, Eccles and Liddell, 1929) found variations on these results and these have been attributed to variation in
site, strength and frequency of cerebellar stimulation (Brookhart, 1960).

In recent years, it has been accepted that cerebellar inhibition of decerebrate rigidity is mediated by reticular relays (Snider, McCulloch and Magoun, 1947; Mollica, Moruzzi and Naquet, 1953; Rossi and Zanchetti, 1957; Terzuolo, 1959; Llinás, 1964). More recently still, the detailed physiological investigations of Ito and his co-workers have given us a much more accurate picture of the functional relationships between the cerebellum and brain-stem structures (chs 13 and 14 in Eccles, Ito and Szentágothai, 1967).

In chloralose anaesthetised cats, Moruzzi (1950) found postural effects following cerebellar stimuli and also observed that cerebellar stimulation could suppress movements evoked from the cerebral cortex. However, he did not study the influence of the cerebellum on the chloralose jerk itself.

In the introduction to this thesis, evidence has been cited that the chloralose jerk is a reflex mediated largely by the brain-stem reticular formation. It seemed possible that on an analogy with cerebellar inhibition of decerebrate rigidity, stimulation of the cerebellum might influence the chloralose jerk.
RESULTS.

A. Stimulation Experiments.

Fig. 29. illustrates the effect on the chloralose-jerk discharge of stimulating the anterior lobe of the cerebellum. Stimulation of the left sural nerve at 4 times threshold strength elicited flexor reflex and chloralose-jerk discharges in the ipsilateral nerve to PBST (Fig. 29B). On conditioning with 4 (250/sec and 2.5 mA) stimuli to the ipsilateral anterior lobe of the cerebellum, the chloralose-jerk response disappeared without any alteration in the flexor reflex (Fig. 29C). Fig. 29D shows the response to the 4 cerebellar stimuli alone. The most effective stimulus site was straddling the paravermian vein on lobule IV or on lobule V close to lobule IV (e.g. the black dots in the diagram of the anterior cerebellum - Fig. 29A). This position corresponds with the region where potentials evoked from the ipsilateral hindlimb are largest (Morin and Haddad, 1953; Harvey, 1967). Here, the current strength needed for complete suppression of the chloralose-jerk response was usually about 5.0 mA, although values ranged from 2.5mA (e.g. in Fig. 29) up to 10mA. No reason could be found for this variation. The condition of the cerebellum was
monitored by recording evoked potentials from the surface of the anterior lobe in response to stimulation of ipsilateral hindlimb nerves. These potentials did not differ significantly when small or large currents were needed. Results were rejected if stimulus currents greater than 5.0mA were necessary to suppress the chloralose jerk. Such stimuli influenced the jerk no matter where they were applied. In addition, Armstrong and Harvey (1966) found that on stimulating the surface of the paramedian lobule with 0.2 msec pulses, significant spread of current occurred with intensities beyond 4 to 5 mA.

The effect of varying the number of cerebellar stimuli is shown in Fig. 30 where a single stimulus was ineffective and four gave total suppression of the jerk discharge.

Fig. 31 illustrates that unilateral stimulation of the cerebellum could suppress both ipsilateral and contralateral jerk discharges - regardless of whether these were evoked by ipsilateral or contralateral stimuli. Fig. 31R is a diagram of the experimental design. Discharges were recorded from the ipsilateral and contralateral nerves to PBST (upper and lower traces in each row) - in the diagram, R₁ and R₂ respectively. These discharges were evoked by stimuli to ipsilateral (A-D) and contralateral (I-L) sural
nerves — in the diagram $S_1$ and $S_2$ respectively. When conditioned by 4 (250/sec and 5.0mA) stimuli to the ipsilateral anterior cerebellum, all chloralose-jerk responses were suppressed (Fig. 31 E-H and M-P). Such total elimination of all jerk discharges no matter the side of stimulating or recording, did not always occur. For example, in another experiment, both the flexor reflex and the chloralose-jerk response evoked and recorded in the ipsilateral hindlimb (Fig. 32 B) were enhanced by stimuli to the contralateral cerebellar cortex (Fig. 32C). The experiment of Fig. 32 is identical in design to that of Fig. 31 O and K (lower traces) so that in diagram R of Fig. 31, it is C.S. conditioning responses evoked by $S_2$ and recorded from $R_2$.

Jerk discharges recorded from an extensor nerve were also affected by cerebellar stimulation. Fig. 33 shows results recorded simultaneously from the ipsilateral and contralateral nerves to P1FDHL (upper and lower traces in each row respectively). Responses in these nerves were evoked from both ipsilateral (A-D) and contralateral (I-L) sural stimulation. Regardless of the side of stimulating or recording, unilateral cerebellar stimulation suppressed these extensor discharges (Fig. 33 E-H and M-P).

Results from 2 different experiments of identical
design illustrate time courses for cerebellar suppression of the chloralose-jerk response (Fig. 34 A-G and H-N). In series A-G, there was a marked enhancement or rebound of the jerk discharge at the longer intervals after cerebellar stimulation (Fig. 34B and C). Suppression of the jerk response began at about 75 msec (Fig. 34 E) and was present even at 18 msec (Fig. 34 G). As a contrast, in series H-N, there was no enhancement of the jerk discharge at the longer condition-test intervals. Instead, suppression began at 160 msec (Fig. 34 I) and reached a maximum at 80 msec (Fig. 34 L). The suppression then diminished at the shorter intervals (Fig. 34 M and N). This sequence is more clearly displayed in Fig. 34 (O-U) which is identical to series H-N but with the test responses expanded in time. The jerk discharge in Fig. 34 U, which otherwise closely resembled the control response (Fig. 34 O) had a latency 10 msec less than that of the control. No reason could be discovered for so significant a reduction in latency, nor was it clear what determined the different time courses of series A-G and H-N.

It is important to note that although the flexor reflex discharge was occasionally increased slightly by cerebellar stimulation (Figs. 30 and 31), it was usually unaffected and never reduced (Figs. 29 and 34). This relative
lack of effect on a purely spinal reflex suggested that the mechanism for cerebellar suppression of the chloralose jerk did not operate at the spinal cord level. The suggestion could be further tested by intracellular recording from motoneurones.

Cerebellar stimulation totally suppressed the chloralose-jerk EPSP recorded from a PBST motoneurone (upper traces Fig. 35 D-F compared with controls A-C) - yet there were no postsynaptic potentials generated by the cerebellar stimulation (upper trace Fig. 35 G). The abolition of the chloralose-jerk EPSP was reflected in suppression of the chloralose-jerk discharge recorded from the nerve to PBST (lower traces Fig. 35 D-F). In Fig. 36 (E-H), both the chloralose-jerk IPSP in a gastrocnemius motoneurone and the jerk response recorded from the nerve to PBST were partially suppressed by cerebellar stimuli (compare Fig. 36 E-H with the control records A-D). Cerebellar stimulation itself did not consistently evoke postsynaptic potentials (Fig. 36 I-L upper traces).

B. Ablation Experiments.

As described in the preceding chapter, the late long-lasting hyperpolarisation recorded from flexor and extensor motoneurones has characteristics of a disfacilitation.
Although a similar hyperpolarisation was observed in a spinal cat, the fact that cerebellar Purkinje cells are inhibitory neurones (Ito and Yoshida, 1964; Ito, Yoshida and Obata, 1964) suggests that the cerebellar cortex might be implicated in this disfacilitation – for example by suppressing the spontaneous activity of reticulo-spinal neurones. This hypothesis was tested by recording from motoneurones after total cerebellectomy. Records from a gastrocnemius motoneurone in such an experiment are shown in Fig. 37. A late long-lasting hyperpolarisation was present in the complete absence of the cerebellum (Fig. 37 E-H).

Because any operative interference with the CNS modifies the chloralose-jerk response, it was hard to determine whether cerebellectomy had any specific effect on the jerk discharge or the response pattern of motoneurones. Fig. 37 (A-D) shows a minimal jerk discharge recorded from the nerve to PBST (lower traces) and rather atypical responses from the extensor cell. On the other hand, Fig. 38 (G-L) illustrates the responses of another extensor motoneurone following cerebellectomy and the response pattern to increasing stimulus repetition rates was similar to that in Fig. 22 (A-D). Similarly, the responses of a flexor cell at various stimulus repetition
rates did not present any unusual features after cerebellectomy (Fig. 38 A-F).

DISCUSSION.

The main questions that arise from the results presented in this section are: (1) what is the mechanism for cerebellar suppression of the chloralose jerk and (2) if we know this, what light does it throw on the reflex organisation of the jerk response?

It is unlikely that cerebellar suppression of the chloralose jerk could be at the spinal level. The evidence for this is twofold. There was no suppression and sometimes slight enhancement of flexor reflex responses recorded from the nerve to PBST (Figs. 29, 30, 31, and 34) and from motoneurones (Figs. 35 and 36). Secondly, cerebellar stimuli which suppressed the chloralose jerk produced no significant postsynaptic changes in the motoneurones themselves (Figs. 35 and 36). The most likely site for cerebellar suppression is therefore in the supraspinal path of the chloralose-jerk reflex arc. It is tempting to suppose that this lies in the brain-stem reticular formation - and evidence can be brought forward in support of this.

Stimulation of the cerebellum at the optimum position
and current strength for suppression of the chloralose jerk will directly and synaptically activate a variety of cerebellar neural components, in particular Purkinje cells, climbing fibres and mossy fibres. Purkinje cells are inhibitory to their target neurones (Ito and Yoshida, 1964; Ito, Yoshida and Obata, 1964) and from the stimulated area project ipsilaterally to Deiters' nucleus, to the fastigial nucleus and the nucleus interpositus (Jansen, 1954). Climbing fibres from the contralateral inferior olive will be antidromically fired (Armstrong and Harvey, 1966) as will the mossy fibres from the cuneocerebellar and spino-cerebellar tracts (Oscarsson, 1965), from the ponto-cerebellar projection (Brodal and Jansen, 1946; Brodal, 1954) and from reticulo-cerebellar fibres (Brodal, 1954). Antidromic activity set up in these afferent fibres to the cerebellum will also spread via collaterals to the cerebellar nuclei. In addition, there will be excitation of granule cells, basket cells, Golgi cells and stellate cells of the cerebellar cortex. The net effect of stimulating the cerebellum is therefore a complex pattern of excitatory and inhibitory activity affecting the cortex, cerebellar nuclei and brain-stem structures. This compels caution in interpreting these results. Nevertheless it seems to me plausible to argue that
Purkinje cell activation can account for the suppression of the chloralose jerk.

In chloralose anaesthetised cats, Massion and Albe-Fessard (1963) showed that stimulation of the cerebellar cortex resulted in an inhibition of red nucleus cells followed by a rebound increase in their activity. Tsukahara, Toyama, Kosaka and Udo (1965) have shown that the inhibition observed by Massion and Albe-Fessard is in fact a disfacilitation. Red nucleus neurones are under tonic monosynaptic excitatory bombardment from the nucleus interpositus (Tsukahara, Toyama and Kosaka, 1964; Tsukahara et al., 1965). Stimuli to the cerebellar cortex remove this tonic excitation by activating Purkinje cells which monosynaptically inhibit nucleus interpositus neurones (Ito et al., 1964). The rebound excitation following the disfacilitation produced by cerebellar stimulation has been shown to be a true excitatory postsynaptic potential and is named a late facilitation (Eccles, Ito and Szentágothai, 1967). It has been attributed to a late inhibition of Purkinje cells following cerebellar surface stimulation. There is a resulting removal of tonic inhibitory bombardment from Purkinje cells to the cells of the nucleus interpositus which are therefore disinhibited and so increase their rate of spon-
taneous activity. This then results in the late facilitation of red nucleus neurones (Eccles, Ito and Szentágothai, 1967).

Although they have not been studied in such detail as red nucleus neurones, cells of the medullary and pontine reticular formation also show a disfacilitation and late facilitation following cerebellar cortical stimulation (Ito, Udo and Mano, 1967). These authors have shown this to be mediated by a pathway from the Purkinje cells to neurones of the fastigial nucleus which in turn project to cells of the reticular formation.

If the chloralose jerk is mediated by cells of the red nucleus and/or the ponto-medullary reticular formation, then the disfacilitation and the late facilitation induced in those cells by cerebellar stimulation provide a basis for explaining the suppression of the chloralose jerk and the rebound phase seen in Fig. 34 (A-G). The proposed mechanism is shown schematically in Fig. 39. The situation at rest is shown on the left, with spontaneous discharge of Purkinje cell (P.cell) and fastigial nucleus cells (FN) in A and B respectively. The tonic depolarisation of reticular formation (RF) neurones is represented in C. Afferent stimulation sufficient to evoke a chloralose jerk excites reticulo-spinal neurones
(Ret Sp) which discharge down the reticulo-spinal tract (D) and so through spinal cord interneurones (i.n.) excite motoneurones (MN). The consequent chloralose-jerk discharge (CJ) is shown in E. The situation following 4 stimuli (C.S.) to the cerebellar surface is shown on the right of the diagram. The 4 vertical bars beside C.S. and in rows A and E represent the cerebellar stimuli. Stimulation sets in train the series of events described by Eccles, Ito and Szentágothai (1967):- **excitation** of Purkinje cells (A) with resulting postsynaptic **inhibition** of FN cells (B); these cease their spontaneous discharge and hence the membrane potential of RF cells tends towards resting levels - the **disfacilitation** in C. Under these circumstances, an afferent input which normally evokes a chloralose jerk is unable sufficiently to excite reticulo-spinal neurones (D) so that there is **suppression** of the chloralose-jerk response (E). Following this phase, there is **inhibition** of Purkinje cells (A) and so a **disinhibition** of FN cells (B) which attain a higher rate of spontaneous firing with resulting **late facilitation** of RF cells. In such conditions, reticulo-spinal neurones are more excitable and so an afferent stimulus elicits more intense reticulo-spinal activity (D) and thus a **rebound** in the chloralose-jerk response (right hand side CJ in E).
The diagram has been based on the fastigio-reticular projection rather than on the projection from the nucleus interpositus to the red nucleus because evidence will be brought forward in Chapter VI which strongly indicates that the red nucleus is an insignificant element in the chloralose-jerk reflex pathway.

In connection with the proposed mechanism for suppression of the chloralose jerk, it is interesting that the fastigio-reticular projection is particularly dense to the nucleus reticularis gigantocellularis (Walberg, Pompeiano, Westrum and Hauglie-Hanssen, 1962). As we have seen, there is evidence that this nucleus is of particular importance in the chloralose-jerk reflex. This evidence is now strengthened by the finding of cerebellar suppression of the chloralose jerk and its possible explanation based on disfacilitation of reticulo-spinal neurones by activation of cerebellar Purkinje cells.

The mechanism for suppression of jerk discharges recorded from extensor nerves may be tentatively assumed to be similar to that for the flexor jerk discharge. The known descending tracts that are excitatory to extensor cells are the vestibulo-spinal and pontine reticulo-spinal (Nyberg-Hansen, 1966). Cerebellar stimulation results in IPSPs and disfacilitation in lateral vestibular
neurones (Ito, Kawai, Udo and Sato, 1967), and disfacilitation in cells of the pontine reticular formation (Ito, Udo and Mano, 1967). However, there have been no opportunities for observing the effect of cerebellar stimulation on the membrane potential of an extensor cell that showed chloralose-jerk firing. Therefore, there remains the possibility that the inhibition observed in Fig. 33 might be at the level of the extensor motoneurones themselves.

There is no obvious explanation for the fact that the effect of cerebellar stimulation on the chloralose-jerk response recorded from contralateral nerves was sometimes suppression (Fig. 31 lower traces M-P) and sometimes enhancement (Fig. 32 C). Just as with the rather variable contralateral cerebellar effects in decerebrate preparations, this may be a function of stimulus site, strength and frequency.

Finally, does the cerebellum ordinarily affect the characteristics of the chloralose jerk? Apparently not to any great extent, since there remained relatively unchanged jerk responses after recovery from cerebelllectomy (Fig. 38). However, these results do not rule out more subtle influences not detected by the means used here.
CHAPTER VI.

DESCENDING TRACTS AND THE CHLORALOSE JERK.

GENERAL INTRODUCTION.

Microelectrode recording from single spinal cord tract fibres has yielded valuable information about nervous system organisation (e.g. Laporte, Lundberg and Oscarsson, 1956; Oscarsson, 1957; Jansen and Rudjord, 1965). This technique was therefore applied here to relate activity in descending tract fibres to the chloralose-jerk discharge.

A cross-section diagram of the spinal cord shows the principal descending tracts (Fig. 40). This figure is based on the work of Nyberg-Hansen and his colleagues: cortico-spinal fibres (Nyberg-Hansen and Brodal, 1963); rubro-spinal fibres (Nyberg-Hansen and Brodal, 1964); vestibulo-spinal fibres (Nyberg-Hansen and Mascitti, 1964); pontine and medullary reticulo-spinal fibres (Nyberg-Hansen, 1965). Smaller descending tracts and the propriospinal tracts have been omitted. Records were taken exclusively from fibres in the lateral funiculus, i.e. in the region of the cortico-spinal, rubro-spinal and medullary reticulo-spinal tracts. The reason for this was that as Nyberg-Hansen (1966) emphasised, the
three descending tracts in this region are mainly excitatory to flexor and inhibitory to extensor motoneurones, whereas both the pontine reticulo-spinal and vestibulospinal tracts have the opposite action. Therefore, the tracts of the lateral funiculus seemed the most likely to be involved in generating the largely flexor chloralose jerk.

Although the results described in the preceding chapter could not be over-confidently interpreted in terms of where and how cerebellar suppression of the chloralose jerk operated, the phenomenon in itself is a tool for analysing the chloralose-jerk reflex. If a descending tract is part of the chloralose-jerk reflex pathway, one would expect that cerebellar stimulation which suppressed the chloralose jerk would also reduce or suppress activity in the fibres of that tract. Provided the type of each descending fibre was known, such experiments might yield accurate information about the descending limb of the chloralose-jerk reflex pathway. However, it was difficult to obtain results because the extensive dissection required usually resulted in a depressed jerk response and/or a cerebellum in poor condition.

Microelectrodes filled with 3M-potassium chloride
(DC resistance 2-5 MΩ) were used to record from single fibres of the left (ipsilateral) lateral funiculus in the second and third lumbar segments. Although the three tracts were studied concurrently, they are considered below in separate sections.

1. THE PYRAMIDAL TRACT AND THE CHLORALOSE JERK.

Introduction

Adrian and Moruzzi (1939) were the first to record a discharge in the region of the pyramidal decussation in association with chloralose jerks. The characteristics of reflexly elicited pyramidal tract activity have since been extensively investigated (Buser and Ascher, 1960; Patton and Amassian, 1960; Casey and Towe, 1961; Patton, Towe and Kennedy, 1963; Towe, Patton and Kennedy, 1963, 1964; Megirian and Troth, 1964; Ascher, 1965).

Studies at the unitary level (e.g. those of Patton et al., 1962; Towe et al., 1963, 1964) have been mainly concerned with the afferent input to pyramidal tract cells, whereas the relationship of pyramidal tract discharges to motor activity has been studied by recording the mass activity of the pyramidal tract (Buser and Ascher, 1950; Ascher, 1965). The information obtained by these
separate methods is difficult to interrelate, particularly in respect of latency relationships between activity of pyramidal tract fibres and the chloralose-jerk response. Therefore records were obtained from a sample of pyramidal fibres so as to build up a more detailed picture of what happens in the pyramidal tract in relation to the chloralose jerk.

Results.

Eighty pyramidal tract fibres were identified by their constant latency response to high frequency stimulation of the contralateral pyramid (e.g. Fig. 41 - bottom right). The commonly observed response pattern is illustrated in Fig. 41, with firing to stimulation of the full range of afferent fibres - even of a muscle nerve at twice threshold (Fig. 41M). This latter was presumably due to activation of the lowest threshold Group II afferents since hindlimb Group I afferent inputs do not reach the cerebral cortex (Mountcastle, Covian and Harrison, 1952; McIntyre, 1953).

It was rarely possible to investigate fibres as thoroughly as in Fig. 41. Therefore, a 10 times threshold stimulus to the superficial peroneal (SP) nerve was chosen as a standard afferent input. A second reason for this was that in animals with jerk discharges reduced by exten-
sive dissection, this particular stimulus was the most effective in evoking a jerk response. Half the sample of pyramidal fibres studied in this way responded with a burst of between 4 and 6 impulses (e.g. Fig. 41C). Thirteen fibres fired 7 to 10 impulses and the rest fired between 1 and 4. Firing rates were of the order of 400 to 700/sec. These values are close to those given by Patton and Amassian (1960) - between 1 and 11 impulses at 500 to 700/sec.

Conduction velocities of 68 fibres were measured. These ranged from 20 to 100 m/sec, with the majority between 30 and 70 m/sec and a peak in the 40-50 m/sec group (Fig. 42A). The maximum value of 100 m/sec is higher than has been found for bulb to spinal cord conduction velocities of pyramidal fibres by the method of recording mass discharges (Lloyd, 1941; Lance, 1954). This may be attributed to selection of the largest fibres by microelectrode recording. Even so, the conduction velocity range is very similar to that obtained by Lloyd (1941) and by Lance (1954) - 18-65 m/sec and 22-70 m/sec respectively. Lance (1954) also recorded the mass activity of pyramidal fibres with conduction velocities between 8 and 22 m/sec. Towe et al. (1963) and Takahashi (1965) similarly found two separate groups of pyramidal
tract neurones - those with axon conduction velocities greater than 20 m/sec and those with conduction velocities in the 10 to 20 m/sec range. Clearly the use of micro-electrodes has excluded the slower conducting pyramidal tract units and the fibre sample obtained here must be confined to the fast conducting units - i.e. those 7% of pyramidal tract fibres of greater than 4μ diameter (van Crevel and Verhaart, 1963).

The latency relationship between the first impulse in pyramidal tract fibres and the onset of the chloralose-jerk discharge as recorded from the nerve to PBST is shown in Fig. 42B. The black bar at the top right represents a jerk response with onset at zero time and lasting 20 msec. Plotted are the number of fibres that fired their first impulse at the indicated intervals before, during and after the onset of the chloralose-jerk discharge. This histogram requires a correction factor before accurately revealing what latency relationship exists between the onset of pyramidal tract activity and the chloralose jerk. Fibre activity was recorded in the upper lumbar regions. There will therefore be a time lag of some 4-5 msec between that activity and any consequent discharge recorded from the nerve to PBST. This allows approximately 1.5 msec each for conduction to the L7-S1 region, for
interneurone relays to PBST motoneurones and for conduction in the PBST nerve. Therefore, only fibre activity earlier than -4 msec can contribute to the onset of jerk discharges. However, firing later than -4 msec will be part of the sustained barrage of descending excitatory activity that results in chloralose-jerk discharges of 20 msec or more.

The effect of cerebellar stimulation was tried on 8 pyramidal tract fibres. Results are difficult to assess, because in these experiments, the effect of cerebellar stimuli on the chloralose jerk was variable. In all 8 fibres there was a reduction in firing of the order of 30%. Fig. 43 (E-H) shows a reduction to 3 or 4 impulses compared with the 4 to 6 in the control series (A-D). At the same time the chloralose-jerk discharge was abolished (E-H upper traces) except for a small discharge in H. Assessment of these results is further complicated since the cerebellar stimuli often produced some sporadic firing (Fig. 43 I-L).

Discussion.

The pyramidal tract is not an essential component of the chloralose-jerk reflex system since the jerk response is present after section of both pyramids (Ascher et al., 1963; Megirian and Troth, 1964; Shimamura et al.,
1967b). Nevertheless, the evidence presented here indicates that activity evoked in the largest pyramidal tract fibres is sufficiently early to influence the jerk responses of motoneurones. Since the pyramidal tract is predominantly excitatory to flexor and inhibitory to extensor motoneurones (Lundberg and Voorhoeve, 1962; Agnew, Preston and Whitlock, 1963; Uemura and Preston, 1965), the firing of cortico-spinal fibres must contribute to chloralose-jerk EPSPs and IPSPs. Activity evoked in the slower conducting pyramidal tract fibres (Towe et al., 1963) is presumably too late to have any influence on chloralose-jerk responses.

The effects of cerebellar stimulation on cortico-spinal fibres were consistent although observed in too small a sample to permit confident generalisation. In chloralose anaesthetised cats, Casey and Towe (1961) observed the effect of cerebellar stimulation on reflexly elicited pyramidal activity. They found initial facilitation lasting about 10 msec followed by some 400 msec depression which in turn was succeeded by facilitation lasting about 600 msec. Casey and Towe's results are difficult to link in with the present investigation as there are important differences in experimental procedure between the two studies - they recorded the mass discharge
of the pyramid, stimulated mid-line cerebellar cortex and used 40 msec trains of 0.05 msec pulses at 312/sec.

The most direct pathway from the cerebellum to the fast pyramidal neurones is the disynaptic excitatory path-way from the nucleus interpositus to the ventro-lateral thalamic nucleus of the opposite side and from there to the pyramidal neurones (Yoshida, Yajima and Uno, 1966). It is possible that the sporadic firing of pyramidal fibres induced by cerebellar stimulation results from activation of this pathway. However, the observed reduc-tion of the firing associated with the chloralose-jerk discharge remains inexplicable unless one postulates that there is tonic excitation of fast pyramidal-tract neurones from the nucleus interpositus via the thalamic relay, and that cerebellar stimulation cuts down this background excitation and so renders pyramidal cells less excitable. Intracellular recording from fast pyramidal tract neurones is required to establish whether cerebellar stimulation that reduces their firing does so by such a disfacilita-tion.
2. THE RUBRO-SPINAL TRACT AND THE CHLORALOSE JERK.

Introduction

In chloralose anaesthetised cats, Massion (1961) and Massion and Albe-Fessard (1963) observed that red nucleus cells had a typical three phase response pattern to somatic, visual or auditory stimuli. A short latency firing was followed by some 200-300 msec of inhibition which in turn was succeeded by renewed and prolonged firing. The phases of inhibition and late excitation were dependent on the integrity of the cerebellum and could be mimicked by stimuli to the cortex of the contralateral anterior lobe.

The predominant effect of rubro-spinal activity on motoneurones is excitation of flexors and inhibition of extensors (Sasaki, Namikawa and Hashiramoto, 1960; Hongo, Jankowska and Lundberg, 1965). Therefore, if suitably timed, the short latency response described by Massion (1961) and by Massion and Albe-Fessard (1963) could contribute to the chloralose-jerk responses of flexor and extensor motoneurones.

Results

Identification criteria were not rigorously strict
for all the 42 rubro-spinal fibres studied. Ten were identified by their response to high frequency stimulation of the red nucleus. Another 11 were taken as rubro-spinal because of their typical response to peripheral and cerebellar stimulation. The remaining 21 were judged rubro-spinal on the basis of their typical response pattern to a peripheral stimulus.

It is possible that a small number of reticulo-spinal fibres are included in this sample as some units in the reticular formation in chloralose anaesthetised cats show a response pattern similar to that of rubro-spinal neurones (Amassian and De Vito, 1954; Albe-Fessard, Bowsher and Mallart, 1962; Bach-y-Rita, 1964). The probability of having a mixed sample is difficult to assess but may not be very high as it is neurones of the nucleus reticularis pontis oralis which most frequently show the "rubro-spinal" response pattern (Bach-y-Rita, 1964). Pontine reticulo-spinal fibres descend in the ventral funiculus (Nyberg-Hansen, 1965) and will not therefore complicate the present study.

Fig. 44 shows responses of 4 fibres identified by high frequency stimulation of the red nucleus (Fig. 44A, D, G and J). Their responses to a peripheral stimulus are shown on both a fast (Fig. 44B, E, H and K) and slow
time base (Fig. 44C, F, I and L). In the sample of 42 fibres, the short latency response of Massion (1961) and Massion and Albe-Fessard (1963) was usually 1 or 2 impulses before or during the chloralose-jerk discharge (e.g. lower traces of Fig. 44 E and H). Many did not fire at short latencies (e.g. lower trace of Fig. 44B) and only 2 fired 4 or more times (e.g. lower trace of Fig. 44K). The characteristic period of inhibition and late firing is illustrated on the slow time-base records (lower traces of Fig. 44C, F, I and L). Spontaneous activity was also apparent (Fig. 44 C and L). Such activity was more evident in some preparations than in others, but it was characteristic of rubro-spinal units in contrast to other descending tract fibres which were seldom spontaneously active.

The conduction times of the 10 units identified by red nucleus stimulation varied from 2.5 to 5.1 msec (average 3.4 msec). The conduction distance of approximately 30 cm gives conduction velocities in the range 60-120 m/sec. Although the sample was small and the figures only approximate, they correspond fairly well with the range 41-123 m/sec obtained by Tsukahara, Toyama and Kosaka (1967).

Lower traces in each row of Fig. 45 show records of a
rubro-spinal fibre identified by its response to peripheral stimulation (Fig. 45 E-H) and stimulation of the cerebellum (Fig. 45 I-L). Like other fibres (Fig. 44 A-C and J-L), this unit was spontaneously active (Fig. 45 A-D). The cerebellar stimulus was at the same site and of the same strength as used for suppression of chloralose-jerk responses and in confirmation of Massion (1961) and Massion and Albe-Fessard (1963) it effectively induced 400 to 500 msec of quiescence in the background discharge, followed by a considerable rebound activity.

**Discussion**

The short latency rubro-spinal activity will make some contribution to chloralose-jerk EPSPs and IPSPs of flexor and extensor motoneurones respectively. However, the extent of this contribution is difficult to assess. From the evidence presented here, it seems probable that it is of relatively minor importance. The intensity of activity in this pathway before and during chloralose-jerk responses is negligible compared with the late firing - yet the late firing has no detectable influence on motoneurones. This late rubro-spinal discharge, beginning some 150-300 msec after a peripheral stimulus, corresponds roughly in time with the late long-lasting hyperpolarisation recorded from both flexor and extensor cells. One
would expect the late rubro-spinal influence to be prolonged postsynaptic excitation of flexor motoneurones and a postsynaptic inhibition of extensors, whereas one observes what is most probably a disfacilitation in both types of motoneurone.

3. "RETICULO-SPINAL" FIBRES AND THE CHLORALOSE JERK.

Introduction

As summarised in the introductory chapter, there is evidence that the medial reticular formation of the medulla is the most important reflex centre for the chloralose jerk. This evidence is given some support by the finding of cerebellar suppression of chloralose-jerk responses which I have argued might operate by a disfacilitation of reticular neurones. A convincing demonstration of this point would be cerebellar suppression of the firing of reticulo-spinal fibres in association with suppression of jerk responses.

There were three possible methods for identifying reticulo-spinal fibres:— (1) to identify directly by stimulating the reticular formation; (2) to record from units in the reticular formation and identify by antidromic firing — i.e. the technique of Magni and Willis
(1963) and of Wolstencroft (1964); (3) to identify indirectly by assuming that descending fibres in the lateral funiculus which were neither cortico-spinal nor rubro-spinal had a high probability of being medullary reticulo-spinal. In spite of the risk of studying a mixture of fibre types, the third method was chosen as the most suitable in this investigation. As discussed in connection with identification of rubro-spinal fibres, the risk of obtaining a mixed sample is difficult to assess but it is possible that there is a small overlap of rubro-spinal and reticulo-spinal fibres.

**Results**

Records were obtained from 51 fibres that were neither rubro-spinal nor pyramidal tract units which I call "reticulo-spinal". In any microelectrode track through the lateral funiculus, "reticulo-spinal" units were the most frequently encountered descending fibres. The sample size is small, since all but pyramidal and rubro-spinal fibres were rejected in the initial experiments. As with the other tract fibres, a 10 times threshold stimulus to superficial peroneal nerve was used as a standard afferent input.

The range of responses in "reticulo-spinal" fibres is illustrated in Fig. 46. Half the sample fired 5 or
more impulses (e.g. Fig. 46A and B). The remainder usually fired 2 to 4 times (e.g. Fig. 46C). Unlike rubro-spinal fibres, "reticulo-spinal" fibres rarely showed spontaneous activity.

Fig. 47 shows the latency relationship between the first impulse in "reticulo-spinal" fibres and the chloralose-jerk discharge recorded from the nerve to PBST. As with the similar analysis for cortico-spinal fibres in Fig. 42B, allowance must be made for the delay of about 4 msec between the third lumbar segment and the nerve to PBST. Even so, it is apparent that the jerk discharge is preceded by activity in a considerable number of "reticulo-spinal" fibres.

The effects of cerebellar stimulation were observed in 6 "reticulo-spinal" fibres (e.g. Fig. 48). In any trial in which there was complete suppression of the jerk discharge, all the fibres showed a 50-60% reduction in the number of impulses. Generally, the firing was reduced in parallel with the extent of suppression of the chloralose-jerk discharge, so that when the jerk discharge was only partially suppressed, there was a lesser reduction in fibre firing than when the jerk was completely suppressed.
Discussion

On the basis of anatomical studies (Nyberg-Hansen, 1965, 1966), fibres in the lateral funiculus which are neither cortico-spinal nor rubro-spinal are most likely to belong to the reticulo-spinal projection from the medulla - so it seems reasonable to assume that most of the 51 "reticulo-spinal" fibres belong to this group.

There have been a number of microelectrode studies of units in the ponto-medullary reticular formation of chloralose anaesthetised cats (Amassian and De Vito, 1954; Albe-Fessard, Bowsher and Mallart, 1962; Bach-y-Rita, 1964; Hossmann and Lamarche, 1966b). These have shown that there is wide sensory convergence onto reticular cells and that these cells which are often spontaneously active have a variety of response patterns to afferent stimuli. In the present investigation, responses of "reticulo-spinal" units to electrical stimulation of a cutaneous nerve were rather stereotyped and spontaneous activity was rarely observed. At least two factors may account for the discrepancies. The recordings made by other investigators were from all types of reticular cell and it is possible that reticulo-spinal neurones have the special characteristics described here. It is also probable that the indirect method of identifying
"reticulo-spinal" fibres has excluded those units with firing patterns resembling rubro-spinal fibres.

The evidence presented here is quite strongly suggestive that these "reticulo-spinal" fibres are of importance in the chloralose-jerk reflex. As studied by microelectrodes, they are the most numerous descending axons in the lateral funiculus. Their firing is correctly timed in relation to the chloralose-jerk response and this firing is depressed by cerebellar stimulation. Although studied in only 6 fibres, the cerebellar depression of "reticulo-spinal" activity favours the hypothesis put forward in chapter V that cerebellar suppression of chloralose-jerk responses operates at the level of the reticular formation. A further test of this hypothesis would be to observe a late rebound of "reticulo-spinal" firing in association with a rebound increase in the chloralose-jerk discharge. However, attempts to do this were unsuccessful.
CHAPTER VII.

GENERAL DISCUSSION AND CONCLUSIONS

The events initiated by a stimulus that evokes a chloralose jerk are of a more complex order than those involved in spinal reflex systems. In cats anaesthetised with 50 mg/kg chloralose, the jerk response elicited by electrical stimulation of hindlimb afferent nerves is consistently a flexor response and only occasionally is it recorded from nerves to extensor muscles. The fact that chloralose-jerk discharges of extensor motoneurones are commonly present in cats lightly anaesthetised with chloralose (Moruzzi, 1944; Alvord and Fuortes, 1954) and are not seen in preparations under 80 mg/kg (Ascher, 1965) indicates that animals under light chloralose anaesthesia are in a very different reflex state from the deeply anaesthetised. This implies that when comparing results obtained under chloralose, care must be taken to rule out anaesthetic dose as a source of contradictory findings. Furthermore, variation in depth of anaesthesia at various times after administering the drug (Winters and Spooner, 1966) will be another source of apparently contradictory results. It is therefore not surprising that the responses of extensor motoneurones (Chapter IV) should be
rather variable and also that they differ from those obtained by Shimamura et al. (1967a) who used only 20 mg/kg chloralose.

Intracellular recording from motoneurones revealed that the chloralose-jerk response of flexor motoneurones was always an EPSP with or without superimposed spike discharges. The responses of extensor motoneurones were more complex than those of flexors, but a chloralose-jerk IPSP was observed in all but one of the 102 extensor cells studied. Basically therefore, the jerk response of cats anaesthetised with 50 mg/kg chloralose is a discharge of flexor motoneurones with a reciprocal inhibition of extensor motoneurones.

The present study, together with other anatomical and physiological data, provide fairly firm evidence that the chloralose jerk is mediated by a number of reflex centres, but primarily by the medial medullary reticular formation. The observation that a chloralose jerk is present in the acutely or chronically decorticate animal (Alvord and Whitlock, 1954; Ascher, 1965) rules out the pyramidal tract as being of paramount importance in the jerk reflex pathway. Since intercollicular decerebration which will destroy the red nucleus does not completely abolish jerk responses (Ascher, 1965), the rubro-spinal
tract is likewise a non-essential component of the chloralose jerk reflex pathway.

There are several lines of positive evidence indicating the importance of the medial medullary reticular formation and in particular, the nucleus reticularis gigantocellularis. 1) Shimamura and Livingston's (1963) finding of a spino-bulbo-spinal reflex in decerebrate cats. 2) Ascher's (1965) observation that chloralose jerks elicited by all types of afferent stimuli were abolished if the region of the nucleus reticularis gigantocellularis was destroyed by an electrolytic lesion, by local injection of KCl or by transverse cutting. 3) The fact that the spinal projection from the medullary reticular formation is principally from the nucleus reticularis gigantocellularis (Torvik and Brodal, 1957; Nyberg-Hansen, 1965; Petras, 1967). 4) The fact that the medullary reticulo-spinal projection is the only major bilateral descending tract system (Torvik and Brodal, 1957; Nyberg-Hansen, 1965, 1966). This provides an anatomical substrate for a reflex such as the chloralose jerk in which there is bilateral excitation of flexor motoneurones. 5) The nucleus reticularis gigantocellularis receives a greater proportion of ascending spinal afferents than other reticular nuclei (Petras, 1967). Finally, the results presented in this thesis give
added weight to the evidence outlined above. The finding of cerebellar inhibition of chloralose-jerk responses is most satisfactorily explained on the basis of a reflex mediated by the reticular formation. Observations on "reticulo-spinal" fibres in the lateral funiculus indicate that these fibres, most of which are likely to derive from the nucleus reticularis gigantocellularis, are of importance in generating the chloralose-jerk discharge.

Although it is tempting to think exclusively in terms of the nucleus reticularis gigantocellularis, this may be too simple a view. The investigation of descending tract fibres (Chapter VI) has not been carried through to a study of the pontine reticulo-spinal tract descending in the ventral funiculus. Although Nyberg-Hansen (1965, 1966) concluded on the basis of accumulated experimental evidence that this tract is excitatory to extensor motoneurones and inhibitory to flexors, there are studies which contradict this. For example, Sasaki, Tanaka and Mori (1962) found that stimulation of the pontine reticular formation resulted in flexor excitation and inhibition of extensor motoneurones. Therefore the conclusion that the nucleus reticularis gigantocellularis is the reflex centre for the chloralose jerk is tentative and provides a useful starting point for further investigations on the descending
tracts activated in association with chloralose jerks.

As studied here, the flexor reflex and the chloralose-jerk reflex recorded from flexor nerves have at the least two components of their reflex paths in common - the flexor reflex afferents (FRA) and the flexor motoneurones. (Preliminary studies indicate that some spinal cord interneurones are also common to both pathways). If the mechanisms that underlie the ready "fatigability" of the chloralose jerk are confined to neural elements common to both pathways, one would expect the flexor reflex and the chloralose jerk to be equally susceptible to increase in stimulus repetition rates. Indeed in these circumstances, the flexor reflex might be the more susceptible as it is so often smaller than the chloralose-jerk discharge. Clearly this is not the case (Fig. 6A-F and Fig. 10). Therefore, as discussed in Chapter IV, although the late long-lasting hyperpolarisation of flexor motoneurones must partly explain the "fatigability" of the jerk, it is not a sufficient explanation. By the same reasoning, presynaptic inhibition of transmission from flexor reflex afferents cannot account for this phenomenon. Hence, there must be processes at other levels in the chloralose-jerk reflex pathway which will account for the "fatigability". What these are is not revealed by the present investigation.
The fact that chloralose jerks are "fatigable" which-ever afferent system elicits them (Alvord and Fuortes, 1954) suggests that the "fatigability" arises from processes in components of the jerk reflex pathway common to all chloralose jerks - no matter how elicited, i.e. in the motoneurones and in the cells of origin of the descending pathway(s) - most probably medullary reticulo-spinal neurones. As we have seen, the hyperpolarisation of the motoneurones cannot provide a complete account of the "fatigability" of the jerk response. Therefore, on the basis of the above argument, it is probable that the reticular formation is another site for processes underlying chloralose jerk "fatigability". Available evidence suggests two likely mechanisms for these processes. 1) the high degree of "attenuation" or "habituation" in reticular neurones on repetitive afferent stimulation. 2) the finding of a monosynaptic inhibition of reticular neurones, possibly from recurrent collaterals of a reticulo-spinal projection.

Scheibel and Scheibel (1963, 1965) first reported the response characteristics of reticular neurones to repeated stimulation - 1/sec stimuli to the sciatic nerve resulted in a marked "habituation" in 75% of their sample of reticular units in unanaesthetised cats. Recently,
Segundo, Takenaka and Encabo (1967a and b) have recorded intracellularly from reticular neurones in unanaesthetised cats and in some preparations under 70 mg/kg chloralose or Nembutal. They observed some response "attenuation" on afferent stimulation at 1/sec and more marked "attenuation" at 2.5 and 5/sec stimulus rates. As they were unable to account for this by demonstrable changes in the reticular neurones themselves (IPSPs, alteration in spike threshold, etc.), Segundo et al. (1967a) proposed "that attenuation is determined at intrareticular junctions where activity is followed by prolonged subnormality of the presynaptic terminal". The finding of 'attenuation' of reticular neurone responses at stimulus rates in the range 1 to 5/sec implies that this mechanism is very probably an important underlying factor in the "fatigability" of chloralose-jerk responses. However, these observations were made on a miscellaneous sample of reticular cells and although almost certainly applicable to reticulo-spinal neurones, records from identified reticulo-spinal cells are needed to establish this beyond dispute.

It is interesting that Segundo et al. (1967a) did not observe any inhibitory potential changes in reticular cells repetitively activated. Willis and Magni (1964) found
no evidence for recurrent inhibition in reticulo-spinal neurones. It would seem then that a feed-back inhibition onto reticulo-spinal neurones is not a factor in chloralose-jerk "fatigability". However, there is recent evidence which does point to this possibility. Ito, Udo and Mano (1967) have found that monosynaptic IPSPs were recorded in a large sample of ponto-medullary reticular neurones on stimulating the ventro-lateral cervical cord. They also showed that this monosynaptic inhibitory pathway most frequently affected reticular neurones in the ventro-caudal regions of the medulla which projected down the ventrolateral cord, i.e. cells which are most probably in the nucleus reticularis gigantocellularis. Ito et al. postulate that the inhibitory pathway is mediated by recurrent collaterals of reticulo-spinal fibres. Activation of such a pathway during the chloralose jerk would provide a feed-back of inhibition onto reticulo-spinal neurones and hence be another mechanism underlying the "fatigability" of chloralose jerks.
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Fig. 1. (opposite) Reflex discharges simultaneously recorded from the nerves to PBST (upper traces of each row) and PlFDHL (lower traces) following electrical stimulation of the ipsilateral hindlimb nerves shown on the left of each row. Stimulus strengths are given in multiples of threshold above each column. Four stimuli at 250/sec were applied to GS (M-P) and joint (Q-T). Each record is of about 10 superimposed traces. The onset of the chloralose-jerk discharge is marked by an arrow. The voltage and time calibrations apply to all records.
Fig. 2. Single traces of reflex discharges simultaneously recorded from the nerves to PBST (upper traces of each row) and GS (lower traces). Stimuli were applied to the ipsilateral hindlimb nerves shown on the left of each row at the strengths above each column. PDP (I-L) and joint (M-O) were stimulated by 4 volleys at 250/sec. The onset of the chloralose-jerk discharge is marked by an arrow. The voltage and time calibrations apply to all records.
Fig. 3. Reflex discharges recorded from PBST nerve (lower traces) evoked by single stimuli to sural and superficial peroneal nerves at 2, 4 and 10 times threshold. Above each response is the simultaneously integrated record of the chloralose-jerk discharge. The deflection in association with the flexor reflex is an artefact. The onset of the chloralose-jerk discharge is marked by an arrow. About 10 to 15 consecutive responses were superimposed for each record. The voltage calibration applies only to the response recorded from the nerve. The time scale applies throughout.
Fig. 4. A-I. Simultaneous records of responses in the nerves to PBST (upper traces of each row) and GS (lower traces) on application of a stimulus to sural nerve at 2, 4 and 20 times threshold. The responses evoked by a stimulus to superficial peroneal nerve are illustrated in J-R - these are from the same experiment and were recorded immediately after the corresponding responses to sural stimulation. Series D-F and M-O were taken 2 hours after the respective control series A-C and J-L. G-I and P-R were taken 5 hours after the control series. All records are composed of about 10 superimposed traces. Arrows mark the onset of the chloralose-jerk discharges. The voltage and time calibrations apply to all records.
Fig. 5. A-D. Single traces of responses simultaneously recorded from the nerves to P1FDHL (lower traces) and to GS (upper traces) and evoked by a stimulus to sural nerve at 2, 4, 10 and 20 times threshold. E-L. A series of consecutive responses to a twice threshold sural stimulus recorded simultaneously from the nerves to PBST (upper traces) and to P1FDHL (lower traces). Arrows mark the onset of chloralose-jerk discharges. The time calibration applies to all records. The voltage calibrations are at the end of each row.
Fig. 6. (opposite) A-F. 10 superimposed traces of responses recorded from the nerve to PBST elicited by a stimulus to superficial peroneal nerve at 4 times threshold strength. The stimulus repetition rate for each record is given in number of stimuli per sec. From the same preparation, G-K is a series of responses recorded from GS nerve to increasing repetition rates of single stimuli to posterior tibial nerve at twice threshold strength. Arrows mark the onset of chloralose-jerk discharges. The voltage and time calibrations apply to all records.
Fig. 7. (opposite) Approximately 10 superimposed traces of responses recorded from the nerve to PBST. A-F and G-L are series from different experiments. In both series the response to a superficial peroneal nerve stimulus at 10 times threshold strength was conditioned by a stimulus in the same nerve (also at 10 times threshold strength). The intervals between conditioning and testing stimuli are given in msec with each record. A and G are controls of the test responses for their respective series. The test chloralose-jerk discharges are marked by an arrow. The voltage and time calibrations apply to all records.
Fig. 8. Records obtained simultaneously from a PBST motoneurone (upper traces of each row) and from the nerve to PBST (lower traces) following stimulation of the ipsilateral hindlimb nerves shown on the left of each row. The stimulus strengths in multiples of threshold are given above each column. Four stimuli at 250/sec were applied to GS (E-H) and joint (I-L). Corresponding extracellular records (taken just outside this motoneurone) constitute the middle traces of each row. Resting potential, -70 mV. The 25 mV calibration applies to the intracellular and extracellular records. The 1 mV calibration applies to the peripheral nerve records and the time scale applies to all records.
Fig. 9. Intracellular records (top traces) from 3 PBST motoneurones in A-D, E-H and I-L. E-H and I-L were from the same animal. Each response is accompanied by the corresponding discharge recorded from the nerve to PBST (lower traces). Single stimuli were applied to superficial peroneal nerve at the strengths given above each column. Records I-L are from a cell penetrated by a 3M-KCl filled microelectrode; all other responses being recorded by a 2M-K citrate filled microelectrode. Resting potentials, (A-D) -75 mV; (E-H) -70 mV; (I-L) -60 mV. The voltage calibrations are at the end of their respective rows and the time scale applies throughout.
Fig. 10. (opposite) A-E. 10 superimposed traces of responses recorded simultaneously from a PBST motoneurone (upper traces) and from the nerve to PBST (lower traces). Stimuli at 4 times threshold strength were applied to superficial peroneal nerve at the rates shown on the left for each record - the rates are in stim/sec. Resting potential, -75 mV. The 10 mV and 200 µV calibrations refer to the intracellular and peripheral nerve records respectively. F-J. An identical series recorded from another PBST motoneurone but to stimulation of sural nerve at 4 times threshold. Resting potential, -50 mV. The 20 mV calibration applies to the intracellular records and the 0.5 mV to the records from the nerve to PBST. The time scale applies to both series.
Fig. 11. (opposite) A-H. Records simultaneously obtained from a PBST motoneurone (upper traces) and the nerve to PBST (lower traces). In B-G the response to a stimulus to superficial peroneal nerve at twice threshold strength was conditioned by a volley in the same nerve (also at twice threshold strength). The condition-test interval is in msec with each record. A and H are controls for the test responses in B and G respectively. Resting potential, -60 mV. The time scale applies throughout and the 25 mV and 1 mV calibrations apply to the intracellular and peripheral nerve records respectively.
Fig. 12. Records simultaneously obtained from a PBST motoneurone (top traces) and the nerve to PBST (bottom traces). A-D is a control series taken to a stimulus applied to the sural nerve at the strengths indicated above each column. E-H. The series repeated 5 minutes after intravenous injection of 2 mg/kg Nembutal. I-L following a further 2 mg/kg and M-P after a total of 10 mg/kg had been injected. Resting potential, -65 mV. The time scale applies throughout and the 10 mV and 0.5 mV calibrations apply to the intracellular and peripheral nerve records respectively.
Fig. 13. Simultaneous records from a PBST motoneurone (top traces) and the nerve to PBST (lowermost traces) on stimulating superficial peroneal nerve at 2, 4, 10 and 20 times threshold strength. The coupling time constant of the amplifier was 1 sec. In A-D the corresponding extracellular responses are interposed between the intracellular and peripheral nerve records. Resting potential, -65mV. The 100 msec time scale applies to A-D and the 10 msec to E-H. The 1 mV calibration is for the PBST nerve records; the 5 mV for the intracellular and extracellular records in A-D and the 25 mV calibration for the intracellular records in E-H.
Fig. 14. Records taken simultaneously from a PBST motoneurone (top traces) and the nerve to PBST (bottom traces). A and B are responses to 4 times threshold stimulation of superficial peroneal nerve and of the superficial radial nerve of the ipsilateral forelimb respectively. C is a record of a response to a loud handclap. The coupling time constant of the amplifier was 1 sec. Resting potential, -65 mV. The time scale applies throughout and the 5 mV and 1 mV calibrations refer to the microelectrode and peripheral nerve records respectively.
Fig. 15. (opposite) A-E. Intracellular records from a PBST motoneurone penetrated by a 3M-KCl filled microelectrode. In B and D, lower traces are simultaneously taken records from the PBST nerve on stimulating superficial peroneal nerve at twice threshold intensity. A and C are responses to twice threshold stimulation of the ipsilateral nerve to the quadriceps. F was obtained by 6 stimuli to the nerve to PBST thereby antidromically activating the motoneurone. C and D were taken while passing depolarising current across the cell membrane. A and B, the controls, were taken in the absence of such current passing. The coupling time constant of the amplifier was 1 sec. Resting potential, \(-60 \text{ mV}\). The 10 msec time scale applies to A and C. The 100 msec scale to B, D and E. The 5mV and 2 mV calibration scales refer to the intracellular and peripheral nerve records respectively.
Fig. 16. Records obtained intracellularly from a PBST motoneurone (upper traces) together with the simultaneous records from the nerve to PBST (lower traces). A-D on stimulating superficial peroneal nerve at 10 times threshold strength and E-H in the absence of any applied stimuli. A and E are about 10 superimposed traces. Remaining records are of single responses. Resting potential, -50 mV. The 100 msec scale applies throughout and the 50 mV and 0.5 mV calibrations respectively refer to the intracellular and peripheral nerve records.
Fig. 17. A-D. Single trace records from the nerve to PBST on stimulating superficial peroneal nerve at 2, 4, 10 and 20 times threshold strength. E-H. Records obtained in the absence of applied stimuli. The time and voltage calibrations apply throughout.
Fig. 18. Records obtained after recovery from spinal transection. A–D and E–H are responses recorded simultaneously from a PBST motoneurone (upper traces) and the nerve to PBST on stimulating superficial peroneal nerve at 2, 4, 10 and 20 times threshold strength. I–K are records in the absence of applied stimuli. L is a record of antidromic firing of the cell by 4 (250/sec) stimuli to the PBST nerve. The coupling time constant of the amplifier was 1 sec. Resting potential, -65 mV. The 10 msec time scale applies to A–D and the 100 msec to E–L. Voltage calibrations are beside their respective rows.
Fig. 19. Approximately 10 superimposed traces of intracellular records from a GS motoneurone (top traces of each row) and the simultaneously taken records from the PBST nerve (bottom traces). Stimuli were applied to the nerves shown beside each row at the strengths indicated above each column. Resting potential, -75 mV. Corresponding extracellular records are inserted between the intracellular and peripheral nerve records. The 10 mV calibration applies to the intra- and extracellular records, the 1 mV to the peripheral nerve records and the time scale applies throughout.
Fig. 20. Single responses recorded from a PlFDHL motorneurone on applying a stimulus to superficial peroneal nerve at 2 and 4 times threshold strength (A-F) and on giving 4 stimuli at 250/sec to the PDP nerve at 3 and 6 times threshold strength (I-N). Records C and D were obtained during the passage of hyperpolarising current across the cell membrane; A and B, E and F being controls taken respectively before and after current passage. Similarly I and J, M and N are controls taken respectively before and after records obtained while passing depolarising current across the cell membrane (K and L). G, H, O and P are corresponding records from the ventral root. Resting potential, -70 mV. The 5 mV and 0.5 mV calibrations apply to the intracellular and ventral root records respectively. The time scale applies throughout.
Fig. 21. Superimposed traces of records simultaneously taken in a PlFDHL motoneurone (upper traces) and from the ventral root on stimulating superficial peroneal nerve at 4 times threshold strength at increasing stimulus repetition rates - the rates in stimuli/sec are given beside each record. Resting potential, -70 mV. The 5 mV and 0.5 mV calibrations apply to the intracellular and ventral root records respectively. The time scale applies throughout.
Fig. 22. (opposite) Intracellular records of the responses of 3 different GS motoneurones to increasing stimulus repetition rates are shown in columns I, II and III. The simultaneously taken records from the nerve to PBST are shown below each intracellular trace. The cells of columns II and III are from the same experiment. In each series, stimuli at 4 times threshold strength were applied to superficial peroneal nerve at the repetition rates shown in stimuli/sec at the left of each row. Records are of approximately 10 superimposed traces. At the bottom of each column, the upper calibration is 5 mV and the lower 1 mV and these refer respectively to the intracellular and peripheral nerve records of that column. Resting potentials, I -70 mV; II -70 mV; III -65 mV. Series M-P in column IV was recorded immediately after the series of column III using the same stimulus parameters but recording from the nerve to GS (upper records) instead of in the GS motoneurone. At the foot of column IV the upper calibration is 200 μV and refers to GS nerve records, the lower is a 1 mV calibration for the PBST nerve records. The time scale applies throughout.
Fig. 23. (opposite) Responses recorded in 8 gastrocnemius-soleus motoneurones on stimulating superficial peroneal nerve at 4 times threshold strength. Records G and H are from the same cell. Resting potentials, A not reliable; B -55 mV; C -70 mV; D -75 mV; E not taken; F -75 mV; G and H -60 mV; I -70 mV. Below G, H and I are diagrams of the flexor reflex and chloralose-jerk response (CJ). Each intracellular record is accompanied by a 4 mV calibration. The time scale applies to all records.
Fig. 24. Single traces of records taken simultaneously in a GS motoneurone (top traces of each row) and from the nerve to PBST on stimulating the nerves designated beside each row at the multiples of threshold strength shown above each column. The 4 stimuli applied to the joint and PlFDHL nerves were at 250/sec. Corresponding extracellular records are inserted between the intracellular and peripheral nerve traces. The coupling time constant of the amplifier was 1 sec. Resting potential, -70 mV. The 5 mV and 1 mV calibrations apply respectively to the microelectrode and the peripheral nerve records. The time scale applies throughout.
Fig. 25. Single traces of responses evoked in a GS moto-
neurone penetrated by a 3M-KCl filled microelectrode
(upper records) and the corresponding discharge recorded
from the nerve to PBST (lower records). One stimulus at
4 times threshold was applied to superficial peroneal nerve
in A-E. A and B are respective control records for C and
D which were taken during passage of depolarising current
across the motoneurone membrane. F is a single trace of
synaptic noise in the absence of applied stimuli. The
coupling time constant of the amplifier was 1 sec. Resting
potential, -80 mV. The 5 mV calibration refers to the
intracellular records in E and F, and the 2 mV to the
intracellular records of A-D. The 0.5 mV calibration
refers to the peripheral nerve records in A-D. Appropriate
time scales are below each column.
Fig. 26.  A. Time course of suppression of the GS unfacilitated monosynaptic reflex following a 4 times threshold conditioning stimulus to superficial peroneal nerve. CON are control records of the monosynaptic reflex taken before and after the condition-test series. Each vertical line represents one monosynaptic reflex response. The flexor reflex and chloralose-jerk discharge are represented by the filled-in double peak.

B. An identical series from the same preparation taken after recovery from spinal cord transection at the first lumbar level. The voltage scale applies to both A and B.
Fig. 27. (opposite) Records after recovery from spinal cord transection at the 1st lumbar segment. A-D. 10 superimposed traces of responses recorded in a GS motoneurone (upper records) and from the nerve to PBST on stimulating superficial peroneal nerve at 2, 4, 10 and 20 times threshold. Resting potential, -70 mV. The 10 msec time scale applies to these records. E-H. Single responses from another GS motoneurone (upper records) and the nerve to PBST using the same stimuli as in A-D. I-L are records from the same cell as in E-H in the absence of applied stimuli. The coupling time constant of the amplifier was 1 sec. The resting potential record was unreliable. The 100 msec time scale applies to records E-L. Voltage calibrations are at the end of their respective rows.
Fig. 29. Effect of stimulating the cerebellum on the flexor chloralose-jerk discharge. Records are of about 10 traces superimposed. B. Response recorded from the left nerve to PBST on stimulating the left sural nerve at 4 times threshold intensity. C. Conditioning the response in B by surface stimulation of the ipsilateral anterior lobe of the cerebellum - 4 stimuli at 250/sec and a stimulus current of 2.5 mA. D. Effect of cerebellar stimulation alone. A. Frontal view of the anterior lobe of the cerebellum with black dots indicating the position of the bipolar stimulating electrode. PV - paravermian vein; LS - lobus simplex; FP - fissura prima; C₁ - crus 1; IC - inferior colliculus; SC - superior colliculus. Roman numerals show lobuli, numbered after Larsell (1953). Time and voltage calibrations apply to A, B and C.
Fig. 30. Number of cerebellar stimuli and suppression of the flexor chloralose-jerk discharge. Records are of about 10 superimposed traces. The response recorded from the nerve to PBST on stimulating superficial peroneal nerve at 10 times threshold strength (CON) was conditioned by 4, 3, 2 and then 1 stimulus to the ipsilateral anterior cerebellum. Stimulus frequency was 250/sec and current strength 5.0 mA. Time and voltage calibrations apply throughout.
Fig. 31. (opposite) Records taken simultaneously from the left (ipsilateral - top traces of each row) and the right nerve to PBST (contralateral - bottom traces of each row). Each record is of about 10 superimposed traces and responses were evoked by a stimulus to the left (ipsilateral) sural nerve (A-D) and the right (contralateral) sural nerve (I-L). The strength of sural stimulation is given above each column. In (E-H) and (M-P), the responses elicited in (A-D) and (I-L) respectively were conditioned by 4 stimuli to the left (i.e. ipsilateral) anterior lobe of the cerebellum. The cerebellar stimuli were 5.0 mA at 250/sec. The effect of cerebellar stimulation alone is shown in Q. The 0.5 mV and 200 µV calibrations refer respectively to the top and bottom line of each row and the time scale applies to all records.

Diagram R shows the design of the experiment. A-D. $S_1$ evoking responses in $R_1$ (top traces) and $R_2$ (bottom traces). E-H. Responses of series A-D conditioned by cerebellar stimulation (C.S.). I-L. $S_2$ evoking responses in $R_1$ (top traces) and $R_2$ (bottom traces). M-P. Responses of series I-L conditioned by C.S. Q. Responses in $R_1$ and $R_2$ to C.S. alone.
Fig. 32. Each record is of about 10 superimposed traces of responses recorded from the left nerve to PBST. B. Response elicited by a stimulus to the left superficial peroneal nerve at 10 times threshold strength. C. The response in B conditioned by 4 stimuli to the right (i.e. contralateral) anterior cerebellar lobe. The stimuli were 5.0 mA at 250/sec and their effect alone is shown in D. A. Diagram with black dots marking the site of the cerebellar stimulating electrodes. The time and voltage calibrations apply to B, C and D.
**Fig. 33.** (opposite) Effect of cerebellar stimulation on extensor chloralose-jerk responses. Identical in design to Fig. 31 except that responses were recorded from the left (ipsilateral - upper traces) and right (contralateral - lower traces) nerves to P1FDHL instead of from the nerves to PBST. All records are of approximately 10 traces superimposed. A-D. Responses evoked by stimulation of the left sural nerve. I-L. Responses evoked from the right (contralateral) sural nerve. E-H and M-P show the effect of 4 (250/sec, 5.0 mA) conditioning stimuli to the left (ipsilateral) anterior cerebellum on the responses of A-D and I-L respectively. Q shows the effect of cerebellar stimuli alone. The upper and lower 200 μV calibrations refer to the ipsilateral and contralateral records respectively. The time scale applies throughout.

Diagram R summarises the design of the experiment. A-D. S₁ evoking responses in R₁ (top traces) and R₂ (bottom traces). E-H. Responses of A-D conditioned by cerebellar stimulation (C.S.). I-L. S₂ evoking responses on R₁ (top traces) and R₂ (bottom traces). M-P. Responses of I-L conditioned by C.S. Q. Responses in R₁ and R₂ to C.S. alone.
4 CEREBELLUM → CHLORALOSE JERK DISCHARGE

A  CON
B  150
C  120
D  100
E  75
F  45
G  18
H  CON
I  160
J  130
K  110
L  80
M  30
N  18
O
P
Q
R
S
T
U

10 msec 10 msec
Fig. 34. (opposite)  Time course of cerebellar suppression of the flexor chloralose-jerk response. Series (A-G) and (H-N) are from two different experiments. In each, 4 (5.0 mA, 250/sec) stimuli to the ipsilateral anterior lobe of the cerebellum conditioned a test response recorded from PBST nerve and elicited by a stimulus to superficial peroneal nerve at 4 times threshold (B-G) and at 10 times threshold (I-N). A and H are control responses for their respective series. Each record is of 10 superimposed traces. The condition-test interval measured from the first cerebellar stimulus is in msec with each record. (O-U) is a series taken immediately after (H-N) using identical stimuli and condition-test intervals but with the test responses expanded in time by a strobe step. Voltage calibrations are 0.5 mV and are at the foot of their respective columns. The time scale below N refers to A-N and below U to O-U.
Fig. 35. Each record is of 10 superimposed traces of responses recorded intracellularly from a PBST motoneurone (upper traces) and simultaneously from the nerve to PBST. A-C. Responses to a sural nerve stimulus at 2, 4 and 10 times threshold strength. D-F. These responses were conditioned by 4 (5.0 mA, 250/sec) stimuli to the ipsilateral anterior cerebellar lobe. G. Effect of cerebellar stimuli alone. Resting potential, -55 mV. The 5 mV and 0.5 mV calibrations apply to the intracellular and peripheral nerve records respectively. The time scale applies throughout.
Fig. 36. Intracellular records from a GS motoneurone (top traces in each row) and the simultaneous responses recorded from the nerve to PBST. A-D. Responses elicited by a stimulus to sural nerve at 4 times threshold strength. In (E-H), these responses were conditioned by 4 (5.0 mA, 250/sec) stimuli to the ipsilateral anterior cerebellar lobe. I-L. Responses to cerebellar stimulation alone. All are single traces except D and H which are of about 10 traces superimposed. No record of resting potential. The 5mV and 0.5 mV calibrations apply to the intracellular and peripheral nerve records respectively. The time scale applies throughout.
Fig. 37. Responses after cerebellectomy. Single traces of intracellular responses from a GS motoneurone (top records) and the corresponding record from the nerve to PBST (bottom records). A-D on a relatively fast time base. E-H on a slower time base and at higher amplification. Responses were evoked by a sural stimulus at the indicated multiples of threshold strength. The coupling time constant of the amplifier was 1 sec. Resting potential, -65 mV. Voltage and time calibrations are with their respective rows.
Fig. 38. (opposite) Responses after cerebellectomy. Each record is of about 10 superimposed traces. Intracellular recording from a PBST (A-F) and an SMAB motoneurone (G-L). The corresponding records from the nerve to PBST are below the intracellular ones. Responses were elicited by a stimulus to superficial peroneal nerve at 4 times threshold strength and at increasing repetition rates (given in stimuli/sec with each record). Resting potentials, (A-F) -50 mV; (G-K) -50 mV. The 0.5 mV calibration applies to all peripheral nerve records. The 10 mV and 5 mV calibrations apply to the intracellular records in (A-F) and (G-L) respectively. The time scale applies throughout.
RESTING STATE  AFTER CEREBELLAR STIMULATION

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P. CELL

Excitation  Inhibition

F N

Inhibition  Disinhibition

Disfacilitation  Late facilitation

RF

Ret Sp

Suppression  Rebound

L.n.

MN

C J

10 msec
Fig. 39. (opposite) Diagramatic representation of the proposed mechanism for cerebellar suppression of flexor chloralose-jerk responses. P. cell - Purkinje cell; FN - fastigial nucleus cell; RF - reticulo-spinal neurone of the ponto-medullary reticular formation; Ret sp - reticulo-spinal axon; i.n. - spinal cord interneurone; MN - motoneurone. A and B represent activity in Purkinje cell and fastigial nucleus cell axons respectively. C represents an intracellular record from reticulo-spinal neurones and shows the tonic membrane depolarisation. D shows activity evoked in the reticulo-spinal tract by a stimulus that generates a chloralose jerk. E represents records obtained from motor axons showing the flexor reflex and the chloralose-jerk discharge (CJ). On the left of the cells is shown the "resting state" i.e. in the absence of cerebellar stimulation. To the right is the sequence of events following cerebellar stimulation (C.S.). The cerebellar stimuli are represented as 4 vertical bars beside C.S. and in rows A and E.
Fig. 40. Diagram of spinal cord cross-section at the third lumbar level showing the principal descending tracts. Tracts that project from contralateral structures are shown on the left as crossed tracts. On the right are uncrossed tracts descending from ipsilateral sources. Note that the medullary reticulo-spinal tract has both crossed and uncrossed components.
Fig. 41. (opposite) Responses recorded simultaneously from a pyramidal tract fibre (lower traces of each row) and from the PBST nerve (upper traces) following stimulation of the nerves shown on the left of each row. Four stimuli at 250/sec were applied to P1FDHL, PDP and joint nerves. Stimulus strengths in multiples of threshold are given above each column. Bottom right is the response of the fibre to 4 (250/sec) stimuli to the contralateral pyramid. The 1 mV calibration applies to the PBST records in A-D and the 0.5 mV to all other PBST records. The 5 mV calibration refers to the fibre records. The time scale applies throughout.
Fig. 42. (opposite) A. Histogram of the conduction velocities of 68 pyramidal tract fibres. Conduction distance was measured from the stimulating electrode on the surface of the contralateral pyramid to the recording microelectrode.

B. Histogram of latency relationship between the first impulse in pyramidal tract fibres and the onset of the chloralose-jerk discharge recorded from the nerve to PBST. The time of onset of the chloralose-jerk discharge was taken as zero. The time in msec of the first impulse in each pyramidal tract fibre was measured in relation to this zero and was negative or positive according to whether the fibre first fired before or after the onset of the jerk discharge. The black bar (top right) represents the duration of a chloralose-jerk response.
**Fig. 43.** Responses simultaneously recorded from a pyramidal tract fibre (lower traces) and from the nerve to PBST (upper traces). A–D. Responses to stimulation of superficial peroneal nerve at 10 times threshold strength. E–H. The responses of (A–D) conditioned by 4 (250/sec, 5.0 mA) stimuli to the ipsilateral anterior lobe of the cerebellum. I–L. Responses to the cerebellar stimuli alone. The 1 mV and 5 mV calibrations refer respectively to the PBST and fibre records. The time scale applies throughout.
Fig. 44. (opposite) Responses recorded from 4 rubrospinal fibres; A-C, D-F, G-I and J-L respectively. A, D, G and J show their response to high rate stimulation of the contralateral red nucleus - each record has a 1 msec time scale. B, E, H and K are records on a relatively fast time base of responses to a stimulus to superficial peroneal nerve at 10 times threshold strength - the simultaneously taken record from the nerve to PBST is shown above the fibre records. Similarly, C, F, I, and L are responses to superficial peroneal stimulation but recorded on a slower time base. The 0.5 mV calibration refers to the peripheral nerve records. The voltage calibration for the fibre records are beside their respective traces. The 10 msec scale refers to B, E, H and K and the 100 msec to C, F, I and L. Note that in this experiment this latter time base was not linear.
Fig. 45. Responses simultaneously recorded from a rubrospinal fibre (lower traces) and from the nerve to PBST (upper traces). A-D shows spontaneous fibre firing. E-H. Responses to stimulation of superficial peroneal nerve at 10 times threshold strength. The chloralose-jerk discharges recorded from PBST nerve are marked by an arrow. I-L. Responses to 4 (250/sec, 5.0 mA) stimuli to the ipsilateral anterior cerebellar lobe. The 2mV and 5 mV calibrations refer respectively to the PBST and fibre records. The time scale applies throughout.
Fig. 46. Responses recorded from 3 different "reticulo-spinal" fibres - lower trace of A, B and C respectively. With each is the simultaneously taken record from the nerve to PBST (upper trace). Responses were to a stimulus to superficial peroneal nerve at 10 times threshold strength. Voltage calibrations are with each trace. The time scale applies throughout.
Fig. 47. Histogram of the latency relationship between the first impulse in "reticulo-spinal" fibres and the onset of the chloralose-jerk response recorded from the nerve to PBST. The analysis was identical to that of Fig. 42 B. The black bar (top right) represents the chloralose-jerk response.
Fig. 48. Responses recorded simultaneously from a "reticulo-spinal" fibre (lower traces) and from the nerve to PBST (upper traces). A-D. Responses to stimulation of superficial peroneal nerve at 10 times threshold strength. E-H. The responses of A-D conditioned by 4 (250/sec, 5.0 mA) stimuli to the ipsilateral anterior lobe of the cerebellum. I-L. Responses to the cerebellar stimuli alone. The 1 mV and 5 mV calibrations refer to the PBST and fibre records respectively. The time scale applies throughout.