AN ELECTROPHYSIOLOGICAL INVESTIGATION OF SPONTANEOUS AND EVOKED ACTIVITY IN THE LATERAL GENICULATE NUCLEUS OF UNANAESTHETIZED, ANAESTHETIZED AND BLINDED CATS.

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MASTER OF SCIENCE

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CONTENTS

GENERAL INTRODUCTION

Many studies have been done in an attempt to establish the structural and functional properties of the various sensory systems in the body. In particular, there have been many reports of experiments carried out on the visual systems of various vertebrates. (For a recent review of much of the literature see Creutzfeldt and Sakmann, 1969). This thesis describes the results of some experiments on the visual system of unanaesthetized and anaesthetized cats.

The peripheral organ of vision is the eye, a structure that can be considered as an instrument capable of focusing rays of light from external objects on the retina and of regulating the amount of light falling on the retina. The retina arises embryologically as an outgrowth of the prosencephalon of the brain and it contains the nervous elements which are responsible for vision. Light falling on the retina activates the receptor cells (rods and cones) which are the only structures actually sensitive to photic stimulation. The receptor cells activate the bipolar cells, and these in turn activate retinal ganglion cells whose axons convey the nerve impulses established back to the brain.

When the axons of the ganglion cells leave the eye they are grouped together into a structure known as the optic nerve (ON). The two optic nerves come together after a short distance and form the optic chiasm, and here, in the cat, slightly more than half the fibers decussate (Fig. 1-1). The fibers which cross over are those from the nasal half of each retina and these join the uncrossed fibers from the temporal half of the other eye and proceed as the optic tract (OT) to the dorsal nucleus of the lateral geniculate nucleus (LGN) where they terminate. Hence, fibers from the nasal half of the retina terminate in the contralateral LGN, while those arising in the temporal half terminate ipsilaterally. The axons of cells in the geniculate then project as

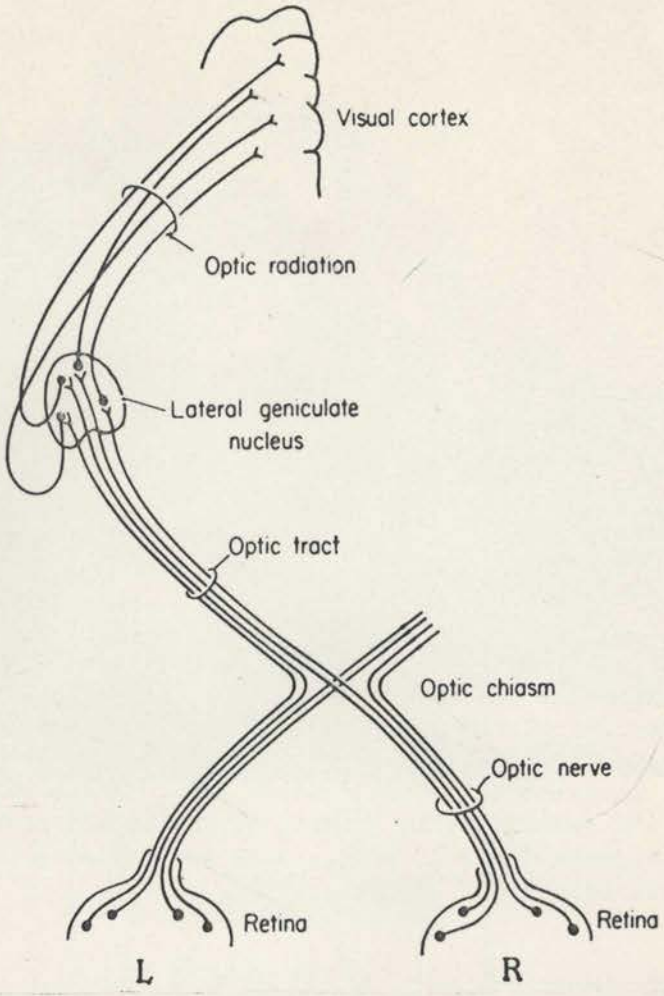
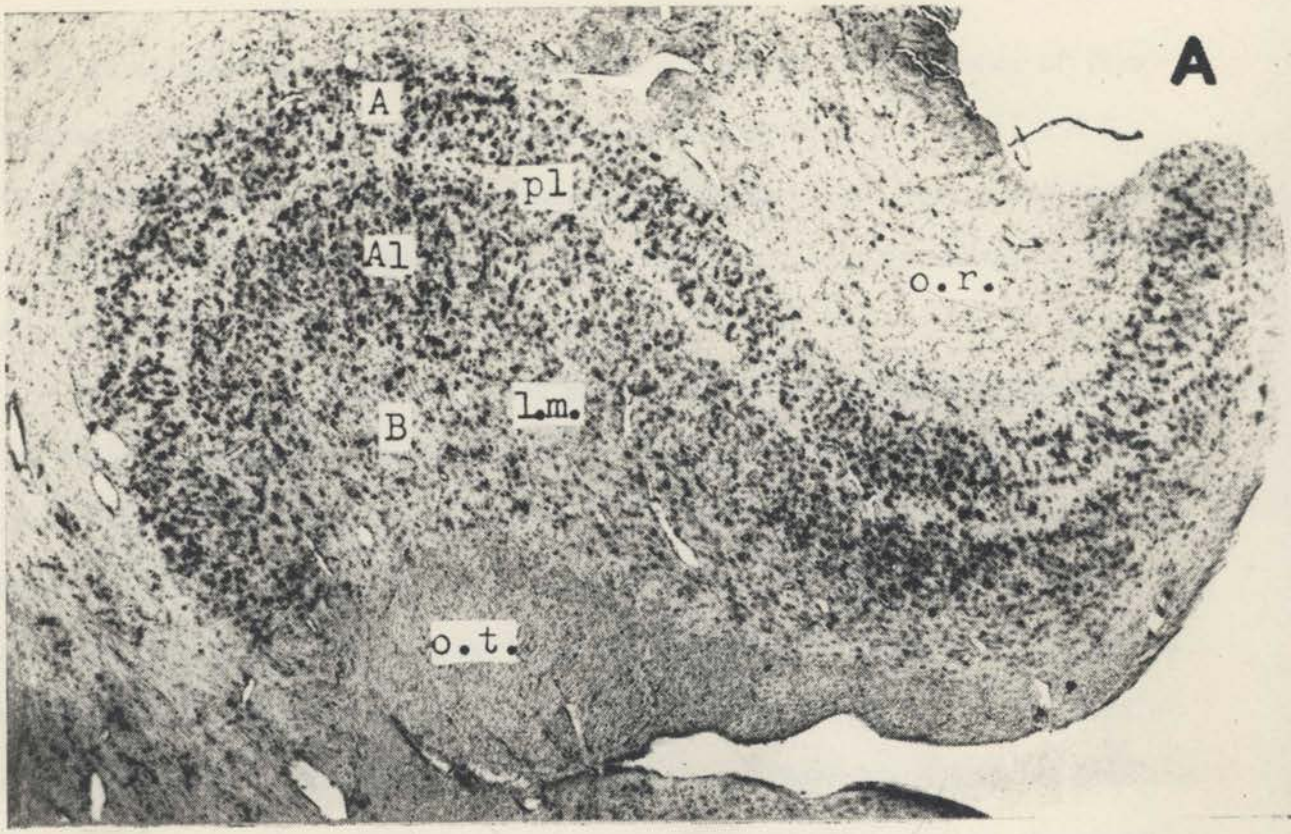


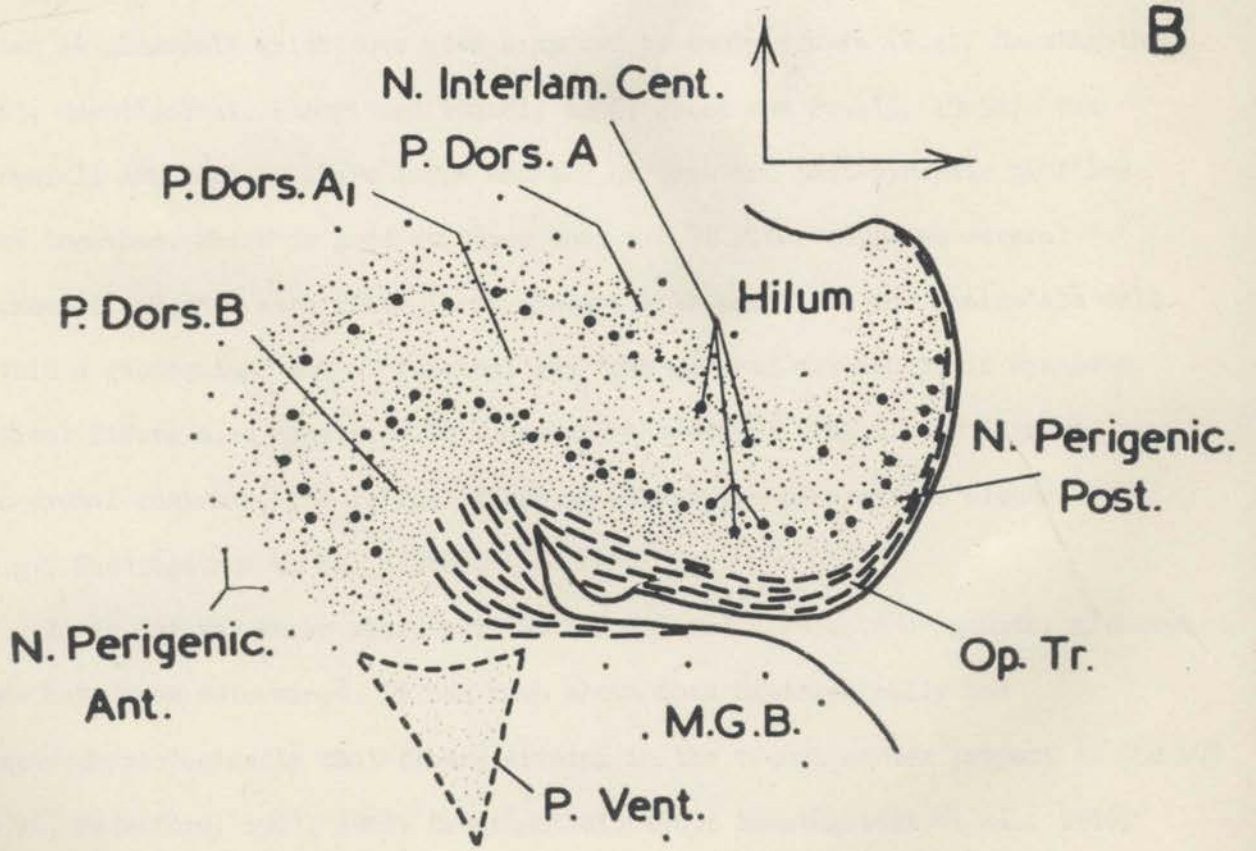
Fig. 1-1: Plan of the primary visual pathway from the level of the retinal ganglion cells through the lateral geniculate nucleus to the termination of the radiation axons in the visual cortex.

the optic radiations to the visual cortex. The part of the visual system which has been examined in the present study is the lateral geniculate nucleus, the main relay station in the primary visual pathway.

In the cat, the LGN is generally considered to consist of three distinct cellular laminae; these were called layers A, A_1, and B by Thuma in 1928. When the geniculate is viewed in parasagittal section it can be seen that these laminae are placed one above the other to give a flattened S-shaped structure (see Hayhow, 1958; Peters and Palay, 1966). Fig. 1-2 shows clearly the S-shaped appearance of the LGN and the three cellular laminae.

Fig. 1-2: (A.) Low-power view of a Nissl-stained parasagittal section through the central region of the lateral geniculate body (pars dorsalis) of the cat showing the three cellular layers, A, A_1, and B. o.r., optic radiation; o.t., optic tract; pl.,interlaminar fibre plexus; l.m., lamina magnocellularis. (B.) A semischematic drawing approximately corresponding to the photograph of A. P. Vent., pars ventralis; M.G.B., medial geniculate body. (Hayhow, 1958).


A
A
pl
Al
o.r.
B
l.m.
o.t.


B
N. Interlam. Cent.
P. Dors. A
P. Dors. A1
P. Dors. B
Hilum
N. Perigenic. Post.
N. Perigenic. Ant.
Op. Tr.
M.G.B.
P. Vent.

It is interesting that recently Guillery has reported the existence of five laminae in the cat's LGN which he refers to as layers A, A_1, C, C_1, and C_2. (Guillery, 1970). He says that neither C_1 nor C_2 have been recognized in earlier studies. In this thesis, however, the traditional nomenclature of A, A_1, and B is adopted.

The optic tract fibers approach mainly from below and pass up into the LGN approximately at right angles to the cell layers; the radiation fibers issue from the upper surface of the nucleus (see Bishop, 1953). The anterior half of the LGN is related to the superior retina and the posterior part to the inferior retina; the vertical meridian of the retina is represented along the medial edge of the nucleus and the area centralis a little behind the middle of its antero-posterior extent (see Bishop, Kozak, Levick and Vakkur, 1962). Within the geniculate OT fibers terminate exclusively in synaptic structures known as glomeruli which have been examined by many workers (e.g., Szentágothai, 1963; Szentágothai, Hámori and Tömböl, 1966; Jones and Powell, 1969a). The glomeruli are regions where large numbers of pre- and post-synaptic profiles come together. There is good evidence that one OT fiber supplies several glomeruli and that each fiber is in contact with more than one geniculate cell. Within a glomerulus, one OT terminal may form several axo-dendritic synapses. Retinal fibers also synapse with axons of non-retinal origin, but in such axo-axonal contacts, the retinal fiber is always the presynaptic element (e.g., Szentágothai et al., 1966).

It is not yet known where all the non-retinal afferents originate, although some have been determined. It has been shown both histologically and electrophysiologically that fibers arising in the visual cortex project to the LGN (e.g., Beresford, 1961, 1962; Szentágothai, 1963; Szentágothai et al., 1966;

Garey, Jones and Powell, 1968; Jones and Powell, 1969b; Holländer, 1970; Kalil and Chase, 1970) and presumably there are projections from subcortical areas as well (see Szentágothai, 1963; Peters and Palay, 1966; Jones and Powell, 1969b; also, see Introduction to Section I). In addition, there are cells in the LGN known as Golgi type II neurones whose axons are short and terminate within the nucleus (e.g., Tömböl, 1966/1967, 1969). Tömböl (1969) has subdivided these interneurones into two types (called type a and type b) mainly on the basis of the length of the axon. The type a cell has an axon which arborizes in a region restricted to the span of the dendritic tree, while the axon of the type b cell ramifies in a considerably larger area and connects different intranuclear regions and layers.

Although each LGN receives an input from each eye, the two inputs, for the most part, terminate in different layers. Fibers originating in the contralateral eye project to layers A and B, while the ipsilateral projection is to layer A_1 (see Hayhow, 1958; Laties and Sprague, 1966; Moore, Karapas and Frenkel, 1966; Stone and Hansen, 1966; Garey and Powell, 1968). However, it should be noted that in Guillery's new terminology for the layers of the LGN (Guillery, 1970), layer B has been subdivided and the divisions labelled C, C_1, and C_2; lamina C receives a contralateral input from coarse retinal fibers, C_1 receives fine ipsilateral fibers, and C_2 contains fibers passing through to other laminae. Although Guillery stated that layer C_1 had not been recognized in earlier studies, it should be stressed that in 1958, Hayhow indicated that there was a small ipsilateral projection to the medial part of layer B (see Fig. 7 of Hayhow's paper).

The regions where binocular overlap has been shown histologically are the central interlaminar nucleus (CIN), including the lamina magnocellularis, and

the medial interlaminar nucleus (MIN). The MIN is located on the medial side of the LGN and it separates the main body of the nucleus from the pulvinar. The large cells of the CIN separate the main layers of the LGN from each other (Fig. 1-2). As the MIN and CIN receive an input from each eye, it is conceivable that cells in these zones receive a dual innervation and therefore that at least in these regions, there is an anatomical basis for some degree of binocular interaction at the thalamic level.

As the projections to the main laminae of the LGN from each eye remain separated, it would appear that no binocular interaction would take place in these laminae. However, evidence is accumulating to the contrary. Bishop, Burke, Davis and Hayhow (1958), and Bishop, Burke and Davis (1959) obtained evidence from single unit recording that a small number of cells in the LGN could be influenced by electrical stimulation of either ON, and Bishop and Davis (1953) and Vastola (1961) reported that stimulation of one ON affected the orthodromic geniculate response evoked by stimulation of the other ON. (Details of responses are considered later.) In confirmation with these results are various reports that single units in the LGN can be activated by light flashes presented to either eye (e.g., Erulkar and Fillenz, 1960; Kozak, Rodieck and Bishop, 1965). It has also been found that if two separate light stimuli are presented, the response patterns of some neurones differ from those to monocular stimulation (Erulkar and Fillenz, 1960; Lindsley, Chow and Gollender, 1967; Chow, Lindsley and Gollender, 1968). Electrophysiological studies have often shown inhibitory binocular influences on cells in the LGN (e.g., Suzuki and Kato, 1966; Marchiafava, 1966; Bremer, 1967; Singer, 1970).

The demonstration of binocular interaction might appear surprising when the evidence from gross histological studies is considered - i.e., that the input

to each LGN from both eyes remains separate. However, clarification of the synaptic organization of the geniculate has, perhaps, revealed an anatomical basis for such interaction. Although the terminals of OT fibers do not cross the borders of the geniculate laminae (Guillery, 1967), the dendrites of some geniculate cells may (Guillery, 1966). Moreover, it is known that the visual cortex sends a projection to the LGN (e.g., Bishop et al., 1958; Szentágothai et al., 1966; Guillery, 1967;Jones and Powell, 1969b; Toyama, Matsunami and Ohno, 1969), and individual corticogeniculate axons may have terminals in more than one lamina (Guillery, 1966, 1967). It is therefore possible that the visual cortex may play a role in binocular interaction at the level of the LGN, although presumably such interactions could not be concerned with the initial stages of central visual integration. Tömböl's observation that the short axon Golgi type 2b interneurones in the cat's LGN project across the $A-A_1$ border (Tömböl, 1969) may provide a basis for a direct interaction mechanism within the geniculate. However, this needs further investigation as the connections of the type 2b interneurones were not clarified.

In an attempt to identify functional neuronal relationships in the LGN of the rat, Burke and Sefton carried out an extensive series of experiments in which they examined the behaviour of individual geniculate neurones following electrical stimulation of the optic nerve and visual cortex (orthodromic and antidromic activation respectively) (Burke and Sefton, 1966a, b and c). They found that all cells in the LGN could be classified into one of two clearly distinguished groups on the basis of their behaviour. The relationships between these two cell types are shown in Fig. 1-3; a similar relationship apparently exists in the LGN of the cat (e.g., Vastola, 1960; Suzuki and Kato, 1966, 1967; Sakakura, 1968) and the rabbit (Fuster, Creutzfeldt

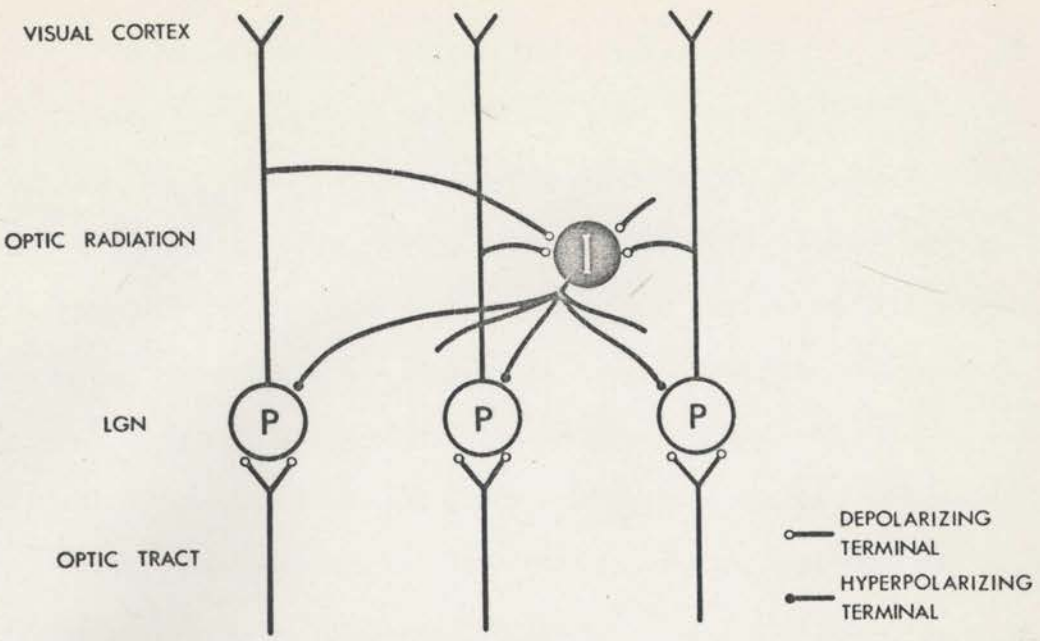


Fig. 1-3: Proposed neuronal circuit in the LGN of the rat; for details see text. (Burke and Sefton, 1966b).

and Straschill, 1965). Burke and Sefton obtained evidence which suggested that one group of cells (P cells) consisted of principal neurones which projected to the visual cortex, while the other group were interneurones (I cells) whose axons did not pass to the cortex. The model proposed by these workers indicates that the axons of the P cells give off collateral branches which activate the I cells, each I cell receiving inputs from several P cells. Each I cell then projects back to many P cells, producing hyperpolarization of the P cell membrane. Thus, as long as there are I cells discharging, the P cells are inhibited; with the cessation of I cell activity there is recovery of P cell discharges.

Histologically there is good evidence to support this neuronal model. Neurones with short axons ramifying extensively in the vicinity of their cell bodies have been reported in the LGN of the cat by many workers (e.g., Cajal, 1911; O'Leary, 1940; Szentágothai, 1963; Guillery, 1966; Tömböl, 1966/1967, 1969).

The ratio of geniculo-cortical relay cells to Golgi type 2 cells is roughly between 2:1 and 3:1 (Tömböl et al., 1969). Also, there are reports that the axons of the principal cells in the cat's LGN give off collateral branches (O'Leary, 1940; Szentágothai, 1963; Tömböl, 1966/1967). Similar models of recurrent inhibition have been proposed for other regions of the brain - e.g., for the spinal motor neurone - Renshaw cell circuit in the ventral horn of the spinal cord (Eccles and co-workers; see Eccles, 1961a, b and c), for the hippocampal pyramidal cell-basket cell circuit (Andersen, Eccles and Løyning, 1964a and b), for the thalamo-cortical relay cell-interneurone circuit in the somatic sensory thalamus (Andersen, Brooks, Eccles and Sears, 1964; Andersen, Eccles and Sears, 1964; Eccles, 1966) and for the cortical pyramidal cell - stellate cell circuit (see Eccles, 1965).

Brief reference has been made to the orthodromic field response which can be recorded from the LGN after stimulation of the afferent pathway. P.O. Bishop and his co-workers were largely responsible for the interpretation of this response and of the response which can be recorded from the ON after OT stimulation. In the present study these responses have been used to examine changes in responsiveness in the LGN of the cat under different experimental conditions. Whereas Bishop used acute, anaesthetized preparations, the technique of chronically implanting both stimulating and recording electrodes in various parts of the brain has been employed in the present study and therefore it has been possible to examine responsiveness in either the unanaesthetized cat or the anaesthetized cat. In the first section of this thesis, certain aspects of geniculate excitability are compared in the unanaesthetized, unrestrained cat and in the barbiturate anaesthetized cat. Comparison is also made between certain forms of spontaneous activity which occur in these two states.

Section II is concerned with the effects of disuse on geniculate excitability, and with the attempts made to reverse the effects of the disuse.

SECTION I

PGO WAVES IN THE SLEEPING AND THE BARBITURATE ANAESTHETIZED CAT.

INTRODUCTION

In this section, certain aspects of both spontaneous and evoked activity in the LGN of the cat are considered. During the phase of sleep known as low-voltage-fast activity (LVF) sleep, spontaneous potential changes appear in the LGN as well as in several other regions in the brain. These spontaneous waves have been called ponto-geniculo-occipital (PGO) waves. Details of the different states in the sleep-wakefulness cycle, and of the PGO waves found in LVF sleep will be considered shortly.

The results in the first part of this section consist of a detailed analysis of the PGO waves of LVF sleep and it will be shown that the evidence presented suggests that the waves are unitary events. Furthermore, essentially similar waves have been observed in the cat under barbiturate anaesthesia and the characteristics of these barbiturate PGO (or B-PGO) waves also support the concept of the PGO wave as a unitary event. The effects of PGO and B-PGO waves on geniculate excitability are also considered.

Before presenting the results of the experiments outlined above, however, it is necessary to briefly review the data at present available on certain aspects of these studies. The following pages therefore contain information relevant to the results, this information being presented under the following headings:

1. Phasic ponto-geniculo-occipital activity.
2. The effects of sleep and wakefulness on responsiveness in the LGN.
3. Functional relationships between the reticular formation and the lateral geniculate nucleus.
4. Additional evidence for extra-retinal inputs to the geniculate.

1. Phasic Ponto-Geniculo-Occipital Activity.

It need scarcely be pointed out that man naturally exhibits changes in his level of consciousness ranging from alertness to very heavy sleep from which it is often difficult to produce arousal. Cats likewise show distinct changes in their level of consciousness and like man, the sleep-wakefulness patterns have been found to have a cyclic occurrence. In this study, certain aspects of the sleep-wakefulness cycle have been examined in cats with chronically implanted electrodes.

Although details of the characteristics of the different levels of consciousness which can be clearly distinguished in the cat are deferred until the Preliminary Discussion, it is necessary at this stage to indicate the various states which have been considered. The state in which the animal is maximally aroused has been called alertness. As the level of arousal declines towards the state of sleep, it is possible to identify a distinct level of consciousness which will be called non-alertness. The state of sleep itself is not homogeneous and can be subdivided into two distinctly different levels; the lighter, and initial, state is known as slow-wave (SW) sleep, and the deeper state will be referred to as low-voltage-fast activity (LVF) sleep.

One obvious feature of the phase of LVF sleep is the spontaneous occurrence of phasic potential changes almost simultaneously in several places in the brain, including the pontine reticular formation (Jouvet, Michel et Courjon, 1959), lateral geniculate nucleus (Mikiten, Niebyl and Hendley, 1961; Bizzi and Brooks, 1963a and b; Brooks and Bizzi, 1963; Hendley, 1963), visual cortex (Mouret, Jeannerod et Jouvet, 1963), superior colliculus (Michel, Jeannerod,

Mouret, Rechtschaffen et Jouvet, 1964), oculomotor nucleus (Bizzi and Brooks, 1963a; Brooks and Bizzi, 1963), abducens nucleus (Brooks, 1966) and parietal cortex and pulvinar (Hobson, 1964). These potential changes have been termed "ponto-geniculo-occipital (PGO) waves" (Jouvet, 1967) and this terminology will be used here, although other workers have applied different names (e.g., "phasic activity", "deep sleep waves", and "lateral geniculate spikes").

PGO waves were originally described in association with the rapid eye movements of LVF sleep in cats. LVF sleep in man is also characterized by the occurrence of rapid eye movements, and when subjects are awakened from this phase of sleep they usually categorize their immediately preceding mental activity as "dreaming" (see Oswald, 1968). This prompted Dement and his coworkers (cited by Moskowitz and Berger, 1969) to suggest that the rapid eye movements represented the scanning responses of concurrent visual dream images. (Moskowitz and Berger obtained evidence against this suggestion when they were unable to find any correspondence between rapid eye movements and dream imagery). In view of the close correlation seen between the PGO waves and rapid eye movements of LVF sleep in the cat, Jouvet (1967) suggested that the waves were related to visual imagery during dreaming. However, it is now known that similar waves also occur during wakefulness (Calvet, Calvet and Langlois, 1965; Brooks, 1966, 1968a and b; Kasamatsu, Kiyono and Iwama, 1967; Jeannerod and Sakai, 1970) and slow-wave sleep (Kasamatsu and Iwama, 1966; Benoit,1967; Thomas et Benoit, 1967), and therefore it would seem that Jouvet's suggestion, if correct, does not indicate a unique relationship between the waves and LVF sleep. In confirmation with these findings in cats is the reported appearance of PGO waves in the PRF, LGN and visual cortex of both awake and sleeping monkeys (Cohen, 1966; Feldman and Bender, 1966; Feldman and Cohen 1968).

Many attempts have been made to determine the origin of these potential changes, and it will be shown that the waves in the visual system appear to be responses to a non-retinal input and that they may be concerned with some aspect of oculomotor-visual integration. Simultaneous recordings of spontaneous PGO waves in the pons and LGN during LVF sleep have shown that the pontine waves generally precede the geniculate waves (although the reverse can occur) and that the interval between the waves in these two structures never exceeds 5ms (Bizzi and Brooks, 1963b). The waves of LVF sleep are not suppressed in amplitude during complete darkness (Brooks, 1969), nor after coagulation of the retina (Berlucchi and Strata, 1965). Moreover, it has been reported that complete removal of the eyes and of the extraocular muscles is not followed by suppression of the waves, at least during 2-3 days post-operatively (Jeannerod, Mouret et Jouvet, 1965), and long-term persistence of PGO waves in the LGN, visual cortex and abducens nucleus has been found following bilateral optic nerve section (Brooks, 1967b). Thus, the waves found in LVF sleep are not a result of sensory feedback from the eyes. The fact that no trace of PGO wave activity has been found in the optic tract or optic chiasm (e.g., Brooks and Bizzi, 1963) is also evidence that the waves do not result from retinal feedback. Similarly, the lateral geniculate and pontine PGO waves in the awake cat are of non-retinal origin; however, the cortical PGO waves during wakefulness appear to be dependent on both retinal and non-retinal inputs to this region (Brooks, 1969).

The concept of a non-retinal origin for the PGO waves has been strengthened by the observation that apparently identical waves are evoked in the LGN during LVF sleep by stimulation of the pontine reticular formation (Bizzi and Brooks, 1963a and b). The synchrony observed between spontaneous waves in the

pons, LGN and visual cortex (Bizzi and Brooks, 1963b; Brooks, 1968a) suggests that these three areas are influenced by a common region, and the occurrence of bilateral wave synchrony at the level of both the LGN and cortex (Brooks, 1968a) suggests that the triggering region is a midline structure with bilateral efferent projections.

It has been found that neither the retina nor the visual cortex are the triggering regions for the LGN waves (see above; also, Bizzi and Brooks, 1963b), and from the evidence just presented this would suggest three possible alternative sites which might initiate them. These are (i) the LGN, (ii) the PRF, and (iii) some other region (excluding the retina and visual cortex) which influences both the PRF and the LGN. The first alternative would seem to be ruled out by the observation that waves are not evoked in the PRF following stimulation of the LGN (Bizzi and Brooks, 1963a and b). Moreover, bilateral coagulation in the LGN and adjacent areas of the optic radiations does not abolish cortical PGO waves (Hobson, Alexander and Frederickson, 1969), so the pathway responsible for the cortical waves is not directly localized in these thalamic regions. As PGO waves can be evoked in the LGN by stimulation of the PRF, the evidence would seem to favour this as a possible triggering site. However, against this idea is the precise synchrony between spontaneously occurring waves in the pons and geniculate (the interval between corresponding waves in these two regions never exceeding 5ms), whereas the waves evoked in the LGN by stimulation of the PRF have a latency of 25-35ms (see Bizzi and Brooks, 1963a and b).

Thus, the evidence available at present suggests that the PGO waves seen in the LGN during LVF sleep do not result from sensory feedback from the eyes, but that they represent some non-retinal input to the geniculate. Possibly this originates in the PRF, but further experiments are needed to establish this fact.

2. The Effects of Sleep and Wakefulness on Responsiveness in the LGN.

There have been many reports that natural changes occur in excitability in various parts of the brain during the sleep-wakefulness cycle (e.g., hippocampus: Noda, Manohar and Adey, 1969a and b; association cortex: Noda and Adey, 1970; hypothalamus: Findlay and Hayward, 1969; sensorimotor cortex: Evarts, 1963a; cochlear nucleus: Dunlop and Waks, 1968; medial geniculate body and auditory cortex: Wickelgren,1968a; mesencephalic reticular formation: Huttenlocher, 1961; Kasamatsu, 1969; somato-sensory system: Casati, Dagnino, Favale, Manfredi, Seitun and Tartaglione, 1969; vestibular nuclei: Lenzi, Pompeiano, and Satoh, 1969; nucleus ventralis lateralis and motor cortex: Steriade, Iosif and Apostol, 1969; Bremer, 1970; nucleus ventralis posterolateralis: Bremer, 1970; Dagnino, Favale, Loeb, Manfredi and Seitun, 1969; primary visual pathway: see below).

Most of the studies on the visual system have been concerned with changes in responsiveness in the sleeping animal, although there are also reported changes during different levels of arousal. Eisman, Hansen and Burke (1967) briefly reported that synaptic transmission in the LGN was affected by the degree of alertness of the cat, the postsynaptic response for any given presynaptic response being smaller in the non-alert than the alert state. Malcolm, Bruce and Burke (1970) re-examined this in more detail and found that accompanying the change from alertness to non-alertness there was a decrease in the excitability of both the OT nerve endings and the LGN cells. Examination of the excitability of the OT endings during sleep (Wall's method; Wall, 1958) has indicated that there are further naturally occurring changes in the level of presynaptic depolarization, this being reduced below the level in non-alertness (Malcolm et al., 1970).

Iwama, Kawamoto, Sakakura and Kasamatsu (1966) and Kasamatsu and Iwama (1966) reported that responsiveness was enhanced in LVF sleep (at times of no PGO waves) compared with SW sleep, but Malcolm et al. (1970) observed similar levels of tonic depolarization in these two states. The level of presynaptic depolarization is phasically enhanced during the PGO waves of LVF sleep to a level above that in the intervals between the waves (Sakakura and Iwama, 1965; Bizzi, 1965, 1966a; Iwama et al., 1966; Kasamatsu and Iwama, 1966; Malcolm et al., 1970). A similar enhancement is seen even in complete darkness during the rapid eye movements of LVF sleep (Dagnino, Favale, Loeb, Manfredi and Seitun, 1969); this is to be expected as PGO waves and rapid eye movements in LVF sleep frequently coincide (e.g., Brooks and Bizzi, 1963). Malcolm et al.(1970) report that presynaptic excitability during the PGO waves is enhanced to the normal tonic alert level. An increased level of presynaptic depolarization has also been found to accompany the tracking eye movements in the midpontine pretrigeminal preparation (Kawamura and Marchiafava, 1966, 1968).

In addition to these presynaptic changes, there are changes in postsynaptic responsiveness. Orthodromic activation of geniculate cells has revealed that excitability is minimal during SW sleep, and increases with changes to arousal and then to LVF sleep at times of no PGO waves (e.g., Iwama et al., 1966; Kasamatsu and Iwama, 1966; Malcolm et al., 1970). During the PGO waves of LVF sleep, postsynaptic excitability is enhanced above the level that exists in the intervals between the waves (Iwama and Kasamatsu, 1966; Iwama et al., 1966; Sakakura and Iwama, 1965; Bizzi, 1966a; Dagnino et al., 1969; Malcolm et al., 1970). As mentioned above, Malcolm et al. found that excitability was greater in the alert than in the non-alert state. In addition, they reported that during LVF sleep, (at times of no PGO waves), the postsynaptic excitability

was intermediate between the alert and non-alert levels, and that it was phasically enhanced above the alert level during the PGO waves. Antidromic activation of the LGN cells has also shown that postsynaptic excitability increases with changes from SW sleep to arousal to LVF sleep (Iwama and Kasamatsu, 1966). Comparable changes in geniculate excitability during the sleep—wakefulness cycle have also been observed by examining the appropriate components of responses in the optic radiation and visual cortex after optic nerve stimulation (e.g., Benoit, 1967; Okuma and Fujimori, 1963; Dagnino, Favale, Loeb and Manfredi, 1965; Walsh and Cordeau, 1965; Dagnino, Favale, Loeb, Manfredi and Seitun, 1966).

Concerning the behaviour of individual cells in the LGN during the sleep-wakefulness cycle, less is known. Studies on acute preparations have revealed changes in both spontaneous and evoked discharge patterns during sleep and waking (e.g., Arden and Söderberg, 1961; Taira and Okuda, 1962; Maffei and Rizzolatti, 1965); however, these are necessarily complicated by the effects of either neuromuscular blocking agents or transection of the brain stem. Nevertheless, results obtained using chronic animals (which are not complicated by the above factors) support the above-mentioned findings from field recordings. Thus, the rate of spontaneous discharge of individual principal neurones in the LGN decreases in the order, LVF sleep to arousal to SW sleep (see Hubel, 1960; Benoit, 1967; Sakakura and Iwama, 1967; Sakakura, 1968; Thomas, Groves and Verzeano, 1968; Mukhametov and Rizzolatti, 1968, 1970; Meulders, 1969), and during the PGO waves of LVF sleep there is phasic enhancement of the spontaneous activity of the LGN cells over the tonic level in that state (e.g., Thomas et Benoit, 1967; Gardner-Medwin, 1967; Sakakura, 1968; Bizzi, 1966b ; Mukhametov, Rizzolatti and Seitun, 1970). Excitability of

LGN cells during wakefulness and sleep assessed using light flashes has also revealed similar changes in responsiveness (e.g., Mukhametov and Rizzolatti, 1969, 1970).

With regard to naturally occurring changes in other regions of the visual system there are some relevant reports. For example, it is known that the excitability of the visual cortex varies according to the level of consciousness (e.g., Hubel, 1959; Fleming, Huttenlocher and Evarts, 1959; Evarts, 1960, 1962, 1963b; Palestini, Pisano, Rosadini and Rossi, 1964, 1965; Calvet, Calvet and Langlois, 1965; Walsh and Cordeau, 1965; Courtois and Cordeau, 1969). The effect of variations in the level of consciousness on the activity of the optic nerve is less certain. While some studies have reported that changes in the sleep-wakefulness cycle are not accompanied by changes in either spontaneous or evoked activity (e.g., Mouret et al., 1963; Mukhametov and Rizzolatti, 1969, 1970; Mukhametov et al., 1970; Taira and Okuda, 1962), Gardner-Medwin has recently observed changes in ON activity on awakening (Gardner-Medwin, 1970). There is no change in spontaneous OT fiber activity during the PGO waves or rapid eye movements of LVF sleep (Gardner-Medwin, 1967; Mukhametov et al., 1970).

The results discussed above therefore show that visual information is not only modified at the level of the cortex during different states of consciousness, but that modification also occurs more peripherally (namely at the LGN). The evidence presented also supports the concept of an extra-retinal input (or inputs) to the LGN, and therefore it adds weight to the evidence in favour of such an input which was given earlier (see the previous section on PGO waves). Whether or not similar modification of visual information also occurs in the retina is uncertain, although at the moment, most evidence does not support this idea. For such modification to occur in the retina it is necessary to

prove the existence of centrifugal influences on the retina. Although isolated descriptions have appeared of efferent fibers within the retinas of several mammals (e.g., for the monkey and cat, Brooke, Downer and Powell, 1965), these findings have not been substantiated and, in fact, neither histological nor physiological evidence has confirmed the existence of such fibers in the cat (e.g., Brindley and Hamasaki, 1961, 1966; Ogden, 1968). Thus it would appear that the LGN synapse is the first site at which visual information is modified by extra-retinal inputs according to the level of consciousness of the animal.

3. Functional Relationships Between the Reticular Formation and the Lateral Geniculate Nucleus.

As it is now known that a close relationship exists between an animal's level of arousal and the activity of the reticular formation, the possibility arises of the reticular formation being involved in the mechanism responsible for the sleep-wakefulness changes at the LGN. Therefore many workers have examined the effects of stimulating the reticular formation on synaptic transmission through the LGN. The first wave of the visual cortical response evoked by stimulation of either the ON or OT has been used to obtain an indirect indication about thalamic excitability. It has been found that this component of the response is enhanced following stimulation of the reticular formation in both the cat and the monkey (e.g., cat: Bremer et Stoupel, 1959; Long, 1959; Dumont and Dell, 1960; Redding, 1967; Chi and Flynn, 1968; monkey: Cohen, Feldman and Diamond, 1969). Direct evidence for reticular facilitation at the level of the LGN has been obtained by examining the orthodromic LGN response to either photic stimulation or to electrical stimulation of the ON or OT following a conditioning stimulus to the mesencephalic reticular formation (MRF)

(e.g., Bremer et Stoupel, 1959; Steriade and Demetrescu, 1960; Suzuki and Taira, 1961; Okuda, 1962: Hotta and Kameda, 1964; Pecci-Saavedra, Wilson and Doty, 1966; Chi and Flynn, 1968; Nakai and Domino, 1968; Cohen et al., 1969).

The effects of reticular stimulation on both pre- and postsynaptic changes have been identified. Suzuki and Taira (1961) reported that the postsynaptic (r) response was greatly enhanced by the reticular stimulation, whilst the presynaptic (t) response was either unchanged or was slightly reduced. Depression of the t response was also observed by Nakai and Domino (1968). Examination of the antidromic tract response to stimulation of the LGN has shown that the above presynaptic changes are due to a change in the level of presynaptic depolarization. Both in the cat (Angel, Magni and Strata, 1965) and the monkey (Cohen et al., 1969), this response is enhanced by reticular stimulation, and Angel et al. (1965) reported that the stimulation evoked a slow negative wave which could be recorded from the ON. Further evidence for such presynaptic depolarization was obtained by Kahn, Magni and Pillai (1967) when they observed an intracellular wave of depolarization in OT fibers following MRF stimulation. Thus, stimulation of the mesencephalic reticular formation leads to an increased level of presynaptic depolarization of the optic tract endings, and, in addition, there is facilitation of the postsynaptic geniculate neurones.

This facilitatory effect on the geniculate cells has been confirmed in unit studies, stimulation of the MRF leading to an increase in the spontaneous discharge of single LGN cells, and also to enhanced responsiveness of a large proportion of cells to both photic and electrical stimulation (e.g., Arden and Söderberg, 1961; Suzuki and Taira, 1961; Taira and Okuda, 1962; Ogawa, 1963; Motokawa and Suzuki, 1966; Satinsky, 1968; Melzack, Konrad and Dubrovsky, 1969; Meulders and Godfraind, 1969).

Whereas the above unit studies were concerned with changes in the activity of the principal (P) cells in the LGN, Fukuda and Iwama (1970) examined the effects of reticular stimulation on the interneurones (or I cells) in the LGN. They found that stimulation of the MRF usually led to a decrease in the I cell response to stimulation of the optic chiasm, and therefore they suggested that the facilitatory effect which MRF stimulation had on the LGN P cells (see above) was possibly due to inhibition of the I cells (i.e., P cell facilitation was in fact a result of disinhibition).

As mentioned earlier, the spontaneous activity of P cells in the LGN of the cat changes with variations in the level of consciousness of the cat, being greatest during LVF sleep, and decreasing during arousal and SW sleep. The activity of single cells in the MRF has also been found to change during sleep and wakefulness (e.g., Huttenlocher, 1961; Kasamatsu, 1969), and it has in fact been reported that the changes in unit activity in the MRF are in the same direction as in the LGN (that is, activity decreases from LVF sleep to arousal to SW sleep - Kasamatsu, 1969). By analogy, therefore, to the MRF stimulation experiments of Fukuda and Iwama (1970), where it was shown that such stimulation led to inhibition of the I cells (and thus, presumably, to the enhanced responsiveness of the P cells reported by other workers), it might be concluded that the greater the spontaneous MRF activity, then the greater the responsiveness of the P cells in the LGN due to the increased inhibition of the I cells. A similar explanation could also apply to the increase in P cell activity which accompanies the PGO waves of LVF sleep (see earlier), as Kasamatsu (1969) reported that the increase in MRF unit activity was most pronounced during these waves.

4. Additional Evidence for Extra-Retinal Inputs to the Geniculate.

The evidence presented so far suggests that the reticular formation and lateral geniculate nucleus are functionally related to each other. Apparently both the pontine and the mesencephalic reticular regions are involved in this functional relationship, evidence being obtained respectively from studies of the PGO waves of LVF sleep and of the natural changes in synaptic transmission which accompany changes in the sleep-wakefulness cycle. Such functional relationships could result directly from a reticulo-geniculate projection, or the pathway involved could be indirect and involve several other regions of the brain. Evidence that the relationship is direct has been obtained from the finding that fibers pass from the reticular formation to the LGN (Scheibel and Scheibel, 1958). The possibility that such fibers existed was predicted even earlier by Brodal and Rossi (1955). Further evidence that the PRF is involved directly in this relationship comes from studies of the effects of pontine lesions on both the sleep-wakefulness cycle (e.g., Batini, Moruzzi, Palestini, Rossi and Zanchetti, 1959; Favale, Loeb, Rossi and Sacco, 1961; Carli and Zanchetti, 1965) and the PGO waves of LVF sleep (Hobson, 1965).

The possibility exists, however, that stimulation of the PRF also activates adjacent fibers, including the ascending part of the medial longitudinal fasciculus which is composed of second order vestibular neurones (see Dagnino et al., 1969). Therefore it is possible that vestibular volleys may produce the response changes in the LGN following PRF stimulation. Pompeiano and his co-workers, and Papaioannou have, in fact, obtained evidence that the medial and descending vestibular nuclei provide an extra-retinal input to the LGN (see Bizzi, Pompeiano and Somogyi, 1964; Pompeiano and Morrison, 1965; Marchiafava and Pompeiano, 1966; Pompeiano, 1970; Papaioannou, 1969).

Moreover, changes occur in the transmission of labyrinthine volleys through the vestibular nuclei during sleep (Lenzi, Pompeiano and Satoh, 1969), and studies involving bilateral destruction of the medial and descending vestibular nuclei have revealed that vestibular discharges produce oculomotor and autonomic responses during LVF sleep (Morrison and Pompeiano, 1970).

Thus, the possibility exists for both direct and indirect influences of the reticular formation on the lateral geniculate nucleus. In addition to the effects of the reticular formation and vestibular nuclei on the LGN (and on the sleep-wakefulness cycle), there is evidence that possibilities exist for further modification of geniculate excitability. For example, Chi and Flynn (1968) observed that stimulation of the hypothalamus enhanced the orthodromic LGN response to OT stimulation. The effect did not appear to be due to indirect stimulation via the MRF as it persisted after bilateral lesions were made in the MRF, and it was suggested that the effect was mediated via the supra optic commissural fibers which connect the lateral geniculate bodies and superior colliculi to each other. These commissural fibers run in the ventral hypothalamus and at one point are adjacent to the fornix.

Lateral geniculate transmission can also be influenced by many other subcortical structures (e.g., Okuda, 1962), as well as by visual cortical activity (e.g., Iwama, Sakakura and Kasamatsu, 1965; Kwak, 1965; Suzuki and Kato, 1965; Angel, Magni and Strata, 1967; Vastola, 1967). Somatic and auditory stimulation have also been found to influence the LGN (e.g., Hotta and Kameda, 1963; Meulders, Colle and Godfraind, 1964; Meulders, Boisacq-Schepens, Godfraind et Colle, 1966; Melzack, Konrad and Dubrovsky, 1968, 1969; Godfraind et Meulders, 1969; Skrebitsky, 1969; Meulders, 1969), but it seems likely that such influences are mediated via

the reticular formation where there is known to be convergence of information from different sensory modalities on to single neurones (Scheibel, Scheibel, Mollica and Moruzzi, 1955; Palestini, Rossi and Zanchetti, 1957; Fessard, 1961; Bach-Y-Rita, 1964; Bell, Sierra, Buendia and Segundo, 1964; Bowsher and Petit, 1969).

The evidence presented so far shows conclusively that it is no longer justifiable to consider the lateral geniculate nucleus as a straight-through relay system in the primary visual pathway. Instead, the concept of transmission through this nucleus must now be modified by the knowledge that extra-retinal inputs from more than one source influence both the optic tract nerve endings and the geniculate neurones. Thus, it would seem that the possibility exists for complex integration of visual and non-visual information at this thalamic level.

In the above discussion I have not attempted to cover all the structural and functional relationships which have been suggested to exist between the LGN and other regions of the brain. However, the connections I have considered, plus one or two additional ones, are summarized in Fig. 1-4.

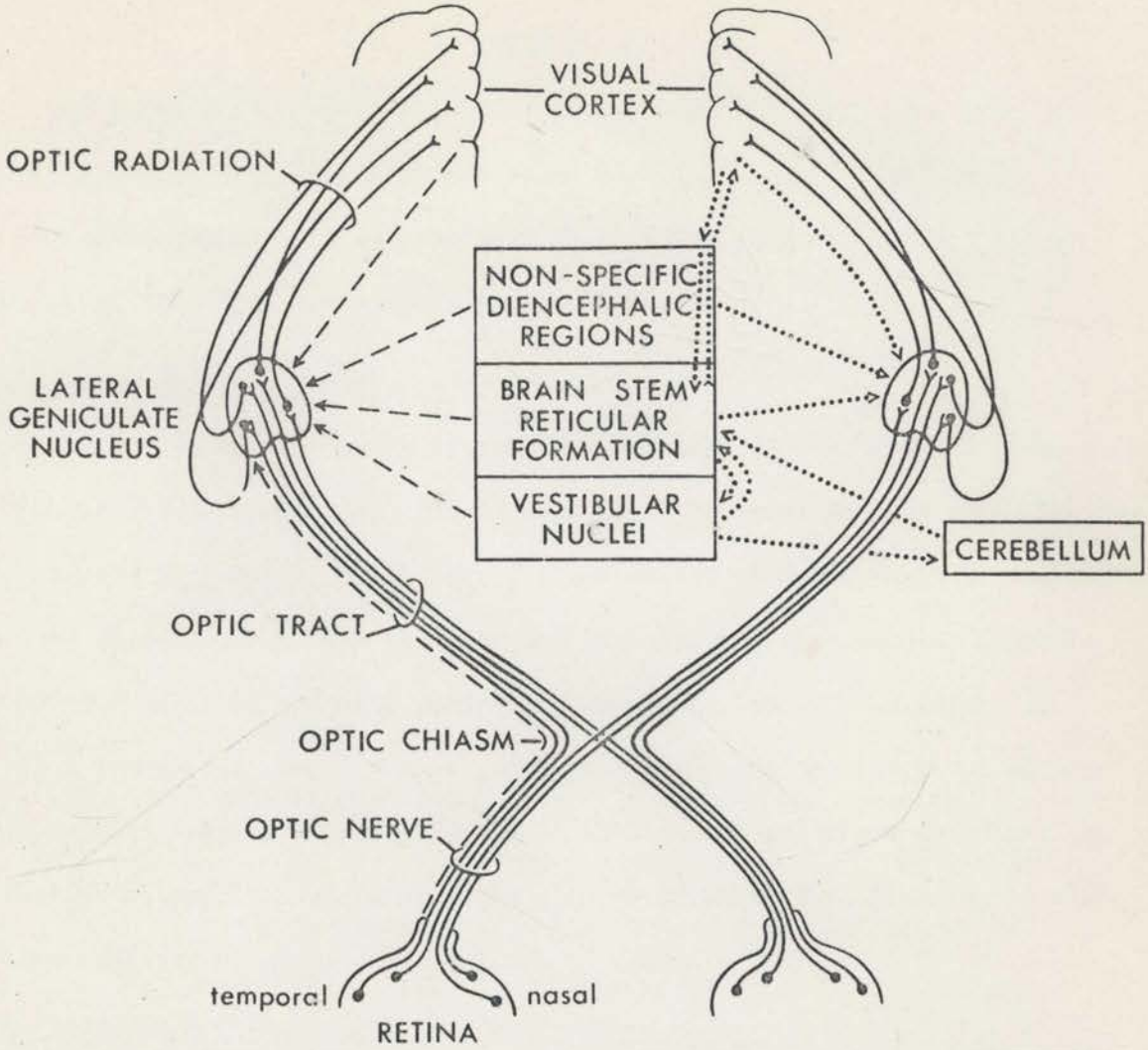


Fig. 1-4: Diagram summarizing the evidence now available for extra-retinal inputs to the LGN of the cat. --- Known functional relationships between the LGN and other parts of the brain. ····· Pathways identified histologically which may be responsible for the functional relationships shown on the left.

The dashed lines on the left hand side of the diagram show those regions of the brain which have been found to be functionally related to the LGN, while the right side of the diagram (dotted lines) shows the known structural relationships.

METHODS

The experiments reported in this thesis were carried out on adult cats with electrodes chronically implanted in various regions of the brain. The method of implanting the electrodes will be considered first, followed by an outline of the procedure adopted during experiments.

Preparation for the Implant.

Six adult cats, weighing between 2.7 and 4.5Kg, have been used during the course of these experiments. Female cats were preferred as they were usually more docile than males, particularly after months of confinement in cages. For the three or four days prior to the implant, an intramuscular injection of 300,000 units of procaine penicillin was given (300,000 units/ml. in aqueous suspension; Evans Medical, Australia), or the cat was given an oral chloromycetin capsule daily (125mg). In this way it was hoped to combat any infection already present in the cat, and to minimize infection at the time of the implant.

The Procedure for Implanting the Electrodes.

As it was hoped to obtain results from any one cat over long periods of time, the electrodes were implanted in a sterile operation.

The cat was anaesthetized with pentobarbitone sodium (Sagatal, May and Baker), an initial dose of 35mg/Kg being given by intraperitoneal injection. This was followed by additional doses, as needed, to produce and maintain the cat at a depth of anaesthesia where no withdrawal, whiskers or pinna reflexes could be elicited. To decrease the secretion of mucous a 1ml intraperitoneal injection of atropine sulphate was given (0.6mg/ml). Normal body temperature was maintained throughout the implant by an electric heating blanket which was controlled by a thermistor placed in the cat's rectum.

After the fur was shaved off the dorsal surface of the scalp, the cat was placed in a stereotaxic headholder and the skin and muscles were reflected to expose the skull. Two small sterilized stainless steel screws, to which insulated stainless steel wires had been attached, were firmly embedded in the supra- and infra-orbital ridges of one eye for recording the electro-oculogram (EOG). Larger sterilized stainless steel screws were embedded in the dorsal aspect of the skull. Four of these carried insulated stainless steel wires and they were placed over the sensory-motor and visual cortices to be used either for recording the electro-corticogram (ECoG), or to serve as indifferent recording electrodes or earthing points. One other large screw had no attached wire and was used only to help in securing the dental acrylic to the skull (details later).

The skull was then trephined over the proposed electrode positions. Usually two electrodes were placed in the right OT, one in each LGN and one in the left hippocampus. In four cats an electrode was also positioned in the right side of the pontine reticular formation (PRF). The dura mater was slit and reflected from all regions prior to insertion of the electrodes. The subcortical electrodes were insulated stainless steel wires of 0.16mm diameter which were sharpened and scraped free of insulation at the tip (approximately 0.5mm). Either two or three such wires were cemented to a supporting nichrome staff (diameter 0.24mm) with epoxy resin; the staff ended about 5mm above the electrode tips and the usual tip separation was about 1mm. Bipolar electrodes were positioned in the OT and hippocampus, a tripolar electrode in each LGN & either a bipolar or a tripolar electrode in the PRF.

The electrodes were positioned according to proposed Horsley-Clarke coordinates with the aid of the stereotaxic atlases of Jasper and Ajmone-Marsan

(1954) and Snider and Niemer (1964). They were then sterilized in a solution of Zephiran in 70% alcohol for at least 10 minutes before being inserted into the brain. The range for the final positions of all electrodes is shown in Table 1-1.

TABLE 1-1.

Range of the Final Horsley-Clarke Co-ordinates of Deep Electrode Positions.

	A.P.(mm)	Lat.(mm)	Vert.(mm)
Anterior OT (right)	+13.0 to +15.0	1.5 to 4.0	-7.8 to -4.8
Posterior OT (right)	+11.0 to +12.0	2.0 to 7.0	-8.4 to -4.2
LGN (right)	+ 6.0 to + 8.5	7.0 to 9.5	+3.0 to +5.3
LGN (left)	+ 6.0 to + 8.0	7.0 to 9.5	+2.5 to +4.6
Hippocampus (left)	+5.0	4.0 to 5.0	+6.7 to +9.6
PRF (right)	- 7.0 to - 5.5	1.7 to 2.0	-6.0 to -5.7

A.P., distances anterior (+) or posterior (-) to the interaural line;
Lat., distances lateral to the midline (side of brain shown in left column);
Vert., distances above (+) or below (-) the Horsley-Clarke zero position.

Although the electrodes were initially positioned according to the stereotaxic atlases, the final placements were, with one exception, determined by monitoring evoked or spontaneous activity as the electrode was lowered towards the desired location. The only electrode whose position was determined entirely from the atlases was the one placed in the PRF. This was lowered at an angle until the tip was at the stereotaxically determined location.

The two optic tract electrodes were inserted separately on two electrode holders, their final positions being determined by the field responses evoked by a bright flash of light. This response was accompanied by a characteristic "swishing" sound due to the asynchronous activity set up by the flash. Each bipolar electrode was left in a position where the "swish" and field potential

were evident from both leads. Thus it was hoped that the deeper electrode of each pair, which was usually used as the stimulating cathode, would lie among the large fibers located in the lower part of the tract, all small diameter fibers being confined to the upper regions (see Bishop, Jeremy and Lance, 1953).

The final position of the right geniculate electrode was determined by the characteristics of the response evoked by electrical stimulation of the OT. The electrode was usually placed in a fairly anterior position in the LGN as it is here that the small-fiber component of the response is least (see Bishop and Evans, 1956), and thus, the evoked response is not complicated by the activity of these more slowly conducting fibers. The electrically evoked response consists of two components, a presynaptic wave and a postsynaptic response. These will be called the 't' and 'r' waves respectively (further details are given later). Normally the electrode was left at a depth where the deep lead showed a t wave with moderate negativity as this indicates it is deep in the geniculate (Bishop and O'Leary, 1942). The r wave was identified by its changed amplitude to the second of two shocks applied to the OT at different intervals - at short intervals (about 3ms) the r response is facilitated, while at longer intervals (about 30ms) it is depressed (see Bishop and Davis, 1960; Eisman, Hansen and Burke, 1967). The facilitated response approximates the response which will be recorded from the unanaesthetized alert cat after single shock stimulation of the OT (Eisman et al., 1967).

To ensure that the three electrodes were in the right LGN a torch was waved in front of the cat's eyes, and, depending on the eye stimulated and the position of the electrode, a "swish" response could be heard. Details have already been given of the retinal projection to the three layers of the

LGN - crossed fibers project to layers A and B, and uncrossed fibers to layer A_1. By flashing a torch in each eye separately and listening for the "swish" response it is possible to obtain an idea of the approximate positions of the electrode tips.

A tripolar electrode was lowered into the LGN on the left side at the same antero-posterior and lateral co-ordinates as the right geniculate electrode. The final vertical co-ordinate was determined by the flash-evoked response. The electrode was usually left in such a position that the deep electrode was near the ventral surface of the nucleus.

The vertical co-ordinate of the hippocampal electrode was also determined by monitoring activity. As the electrode tip penetrates the hippocampus a "seizure discharge" can be recorded which probably represents mechanical stimulation of the cells by the advancing electrode. The electrode was usually lowered about 1mm beyond the point where the discharge was first recorded from the deep electrode, as this would place the upper electrode on the dorsal surface of the hippocampus.

When the electrodes had been satisfactorily positioned, they were cemented in place with dental acrylic (Texton). Unnecessarily long wires were then shortened and they were scraped free of insulation at their end and soldered to the terminals of either a 9-pin or 15-pin socket. Considerable care was taken to ensure that none of the wires were touching each other, and that each wire was identified as it was soldered to a terminal. The wires and sockets were then cemented in place with Texton after the skull had been carefully dried. The Texton was always built up into a mound so that none of the wires were exposed and so that the base of each socket was rigidly embedded in the cement. The skin wound was then dusted with Neosporin, a topical antibiotic powder, and

the muscles and skin were drawn up around the mound and clipped together. The cat was given 1ml of procaine penicillin (300,000 units) by intramuscular injection and was left on the electric heating blanket until fully recovered.

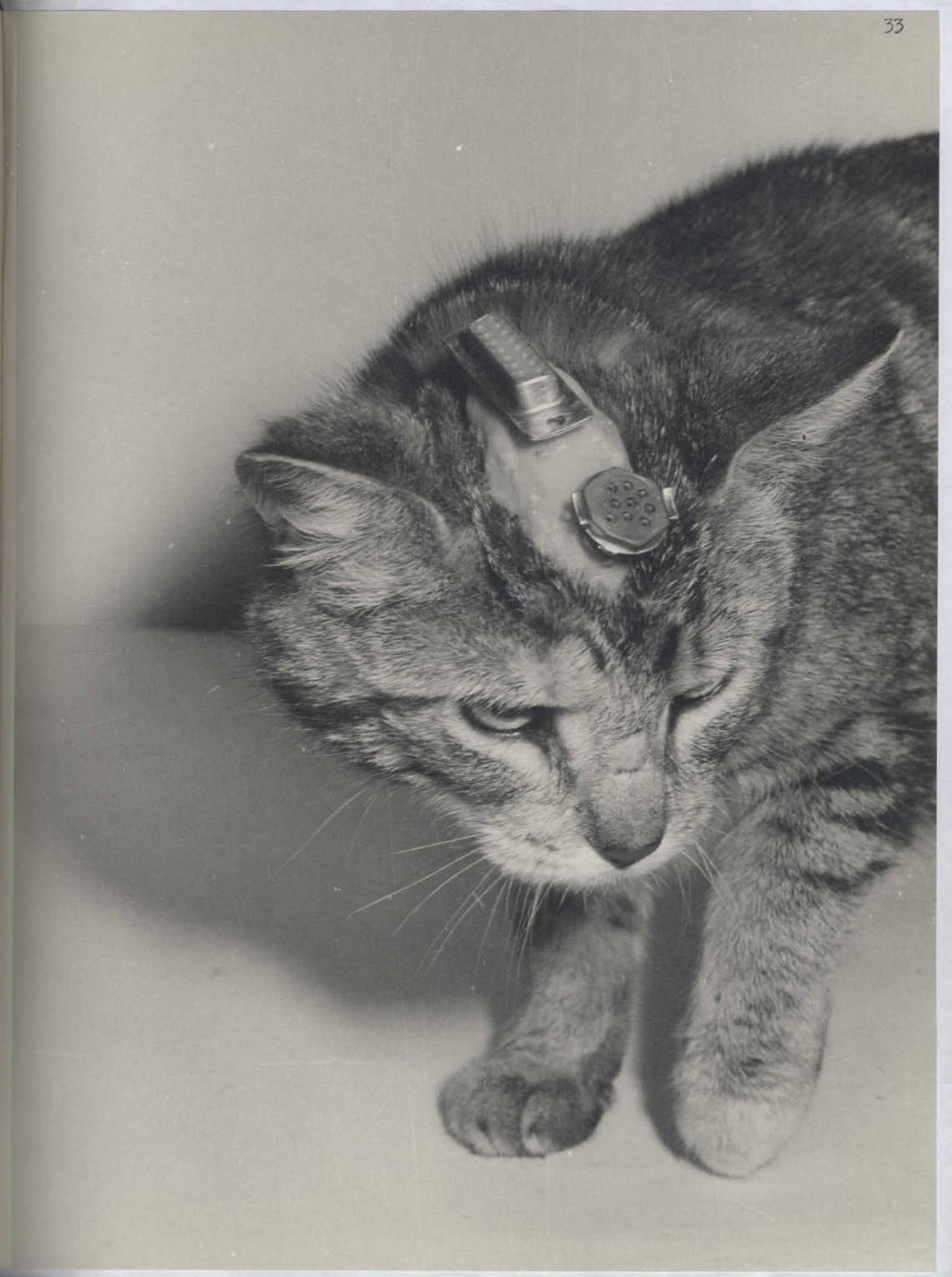
Treatment After the Implant.

Penicillin injections were continued daily usually until the fourth post-operative day. If the wound became infected Neosporin powder was applied, but it was found that the less the wound was touched, the more likely it was to heal completely, so causing the cat minimal discomfort.

In only one cat did the brain become infected and this was accompanied by marked psychological disorders - the cat was destroyed as a result.

Fig. 1-5 is a photograph of one of the cats after electrodes had been implanted as described above. The two sockets can be seen clearly, the 9-pin being the more anterior one. The individual terminals to which the electrodes were soldered can also be seen, particularly in the 9-pin socket. The mound of Texton which cements the electrodes and sockets firmly in place is visible between and around the sockets, and it is obvious that the skin has been drawn up firmly around the cement. The skin wound is completely clean and free from infection. This set-up does not bother the cats in any way, and we have been able to keep such implanted animals for well over twelve months. In this way, long-term experiments can be carried out on the one animal and any number of experiments can be done, thus reducing variability due to different electrode placements in different cats.

Fig.1-5: A photograph of one of the cats used in this study. The electrodes implanted in the brain were soldered to the two sockets (one 9-pin and one 15-pin socket) which can be seen embedded in a mound of dental acrylic (Texton).

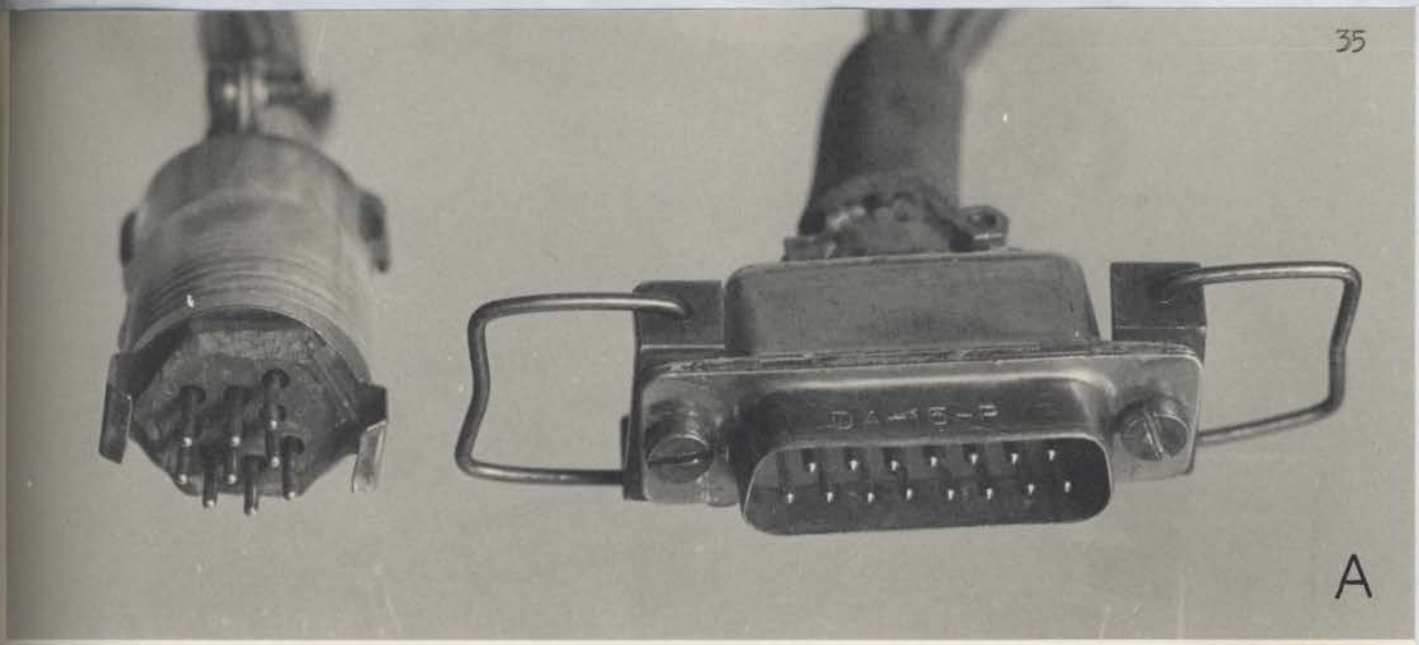


Experimental Procedure.

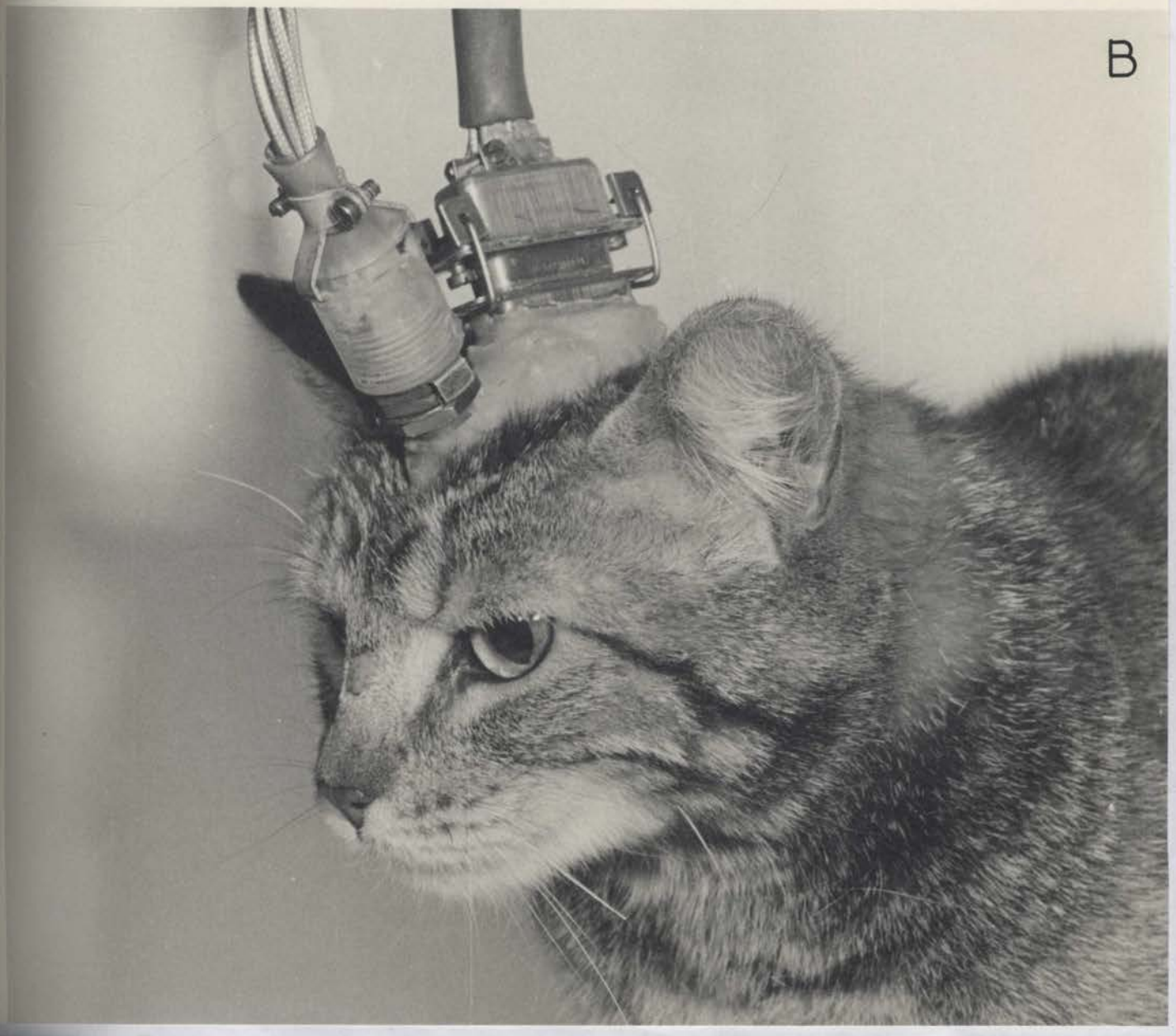
Experiments were performed on both unanaesthetized and anaesthetized cats. Those on the unanaesthetized animal were at any level of consciousness from alertness to sleep, but the two levels considered most frequently were alertness and low-voltage-fast activity (LVF) sleep. All experiments on the anaesthetized cat were done after giving pentobarbitone sodium (Sagatal, May and Baker). The initial dose was 35mg/Kg given by intraperitoneal injection. Supplementary doses were usually required to produce a deeper level of anaesthesia.

During all experiments the cat was placed in an observation box (3 feet x 2 feet x 1.5 feet) which could be internally illuminated or, alternatively, made completely dark. The normal level of illumination was 0.5 - 14.0 cds/m^2. The experimenter could observe the cat at any time during experiments carried out in the light, but usually it was not possible for the cat to see out of the box. Shielded leads about 18 inches long were soldered at one end to 9- and 15-pin plugs which could be firmly attached to the sockets on the cat's head. The two plugs can be seen in Fig.1-6A and they are shown connected to the cat in Fig.1-6B. The leads did not inconvenience the cat which could roam around the box or lie down as it chose. The other end of the leads was connected to the terminals of a distribution box which was placed directly above a small hole in the top of the observation box. The stimulating and recording equipment was then connected to the appropriate terminals of this box. The distribution box was completely enclosed and could be securely screwed to the top of the observation box so that no light could enter the observation box via the hole through which the leads left the box. It was therefore possible to record activity in both the light and the dark from a virtually unrestrained normal animal or from an anaesthetized animal.

Fig. 1-6: The upper photograph shows the two plugs (9-pin on the left, 15-pin on the right) which fit securely into the sockets on the cat's head. Shielded wires connect these plugs to the stimulating and recording equipment. The lower photograph shows the plugs connected to the sockets. Clips, which can be seen clearly in the upper photograph, hold the plugs firmly in place.



A



B

The electrical stimuli were always rectangular pulses and they were delivered via a stimulus isolation unit (Fein, 1960) to minimize the stimulus artefact. Pulse amplitude and duration and also the rate of stimulation were varied according to the site being stimulated and the response being recorded.

(i) When the orthodromic LGN response was being examined the pulse duration was 50μs and the rate of stimulation was 1/5s.

(ii) When the antidromic tract response (or t(anti) response) was evoked by stimulation of the ipsilateral geniculate, the stimuli were of 20μs duration and at a rate of 1/3s.

(iii) Sometimes the PRF was stimulated at various rates with square pulses of 2ms duration; paired shocks were also applied to the PRF over a wide range of intervals.

Evoked responses or spontaneous activity could be recorded either between two electrodes in the same structure (differential recording) or between an active electrode and an indifferent electrode located some distance from the active site (single-sided recording). The recorded activity passed via the leads and distribution box to a cathode follower. Then it was amplified and displayed on a cathode ray oscilloscope. Both high and low frequency filters could be varied independently so recordings were not complicated by irrelevant activity. Individual responses displayed on the oscilloscope were photographed with a Grass camera, and subsequently enlarged and projected onto graph paper for measurement.

Calibrating pulses were also photographed and enlarged so that

measurements could be converted to the appropriate units for time and amplitude.

Biological activity photographed on the oscilloscope was also monitored on another oscilloscope and over a loud speaker. If necessary, spontaneous activity recorded on the oscilloscope could be used to trigger stimuli to the optic tract, for example. An output from the back of the oscilloscope was taken to a Schmitt trigger which was then used to activate a digitimer at various delays. The digitimer output then triggered the normal stimulating circuit.

An electroencephalograph was also used to monitor electrical activity - this included the ECoG and EOG, and activity from the hippocampus, LGN, PRF, and OT. The activity was usually passed directly to the machine where it was amplified, and a time constant of 1 was applied so the unwanted high frequency components were removed. The activity was then used to drive ink writing pens and a permanent record obtained by having paper moving through the machine and under the pens at a known speed. Sometimes the activity was amplified before being led across to the electroencephalograph, and, if required, it could be observed and photographed on the oscilloscope at the same time as being recorded on the machine. Records so obtained could be used to determine the cat's level of consciousness or its depth of anaesthesia, or they could be used simply to observe the on-going activity in various regions of the brain.

Checking the Electrode Positions.

All subcortical electrode positions were checked histologically at the end of a series of experiments on a cat. The cat was deeply anaesthetized with sodium pentobarbitone and a current of 25μA was passed for 10s through the deep lead of each electrode. A similar current was also passed through the upper lead of tripolar electrodes. This results in the deposition of Fe^{3+} ions from the uninsulated tips of these wires. The brain was then perfused in situ

with warm, 0.9% saline, followed by 10% formol saline containing 1% w/v potassium ferrocyanide. The ferrocyanide reacts with the deposited ions leaving a blue spot at the site of the tip of each wire through which current was passed. After the elctrodes had been carefully removed, the brain was freed from the cranial cavity and placed in 10% formol saline until it was ready for embedding.

The brains were embedded in egg yolk by a technique which was modified from the egg albumin embedding procedure described by Snodgress and Dorsey (1963). After the brain had been fixed in formalin, it was washed overnight in running water and it was then embedded in beaten egg yolk. For embedding, an uncovered double-layered box was made which was about 2cm longer and wider than the brain. The inner layer of the box was made of vegetable parchment paper and the outer layer of aluminium foil. Six to eight small holes were made in the bottom of the box with a syringe needle - however, it is essential these holes are made only in the foil, the parchment paper remaining unperforated. The brain was placed in this box and completely covered with the egg yolk, care being taken to indicate the orientation of the brain and to ensure that no air bubbles were trapped under it. The box was then stood in a thin layer of 25% formalin that only wet its undersurface; hardening of the block occurs by formalin dialysis through the parchment paper. The total time needed to embed brains by this technique can be as little as 2 days.

To facilitate cutting, the block was left in a solution of 30% sucrose in 10% formol saline for at least 2 days before sectioning. The brain was sectioned coronally at 40μ and selected sections stained with cresyl fast violet. The sections were then examined histologically to check the placement of each subcortical electrode.

PRELIMINARY DISCUSSION

In this study, several forms of spontaneous and evoked electrical activity have been recorded from various regions of the brain, and before proceeding to the results, certain features of this activity will be considered briefly.

A. Characteristics of the Different Levels of Consciousness.

With the aid of the electroencephalograph it has been possible to monitor activity continuously in the nervous system and, as a result, different levels of consciousness have been identified and classified according to certain distinctive characteristics. It is now well established that cats pass through changes in their level of consciousness between the extremes of maximum alertness and deep sleep (e.g., Hess, Koella and Akert, 1953; Dement, 1958; Rossi, Favale, Hara, Giussani and Sacco, 1961; Okuma and Fujimori, 1963; Morrison and Pompeiano, 1966).

In the present study, four distinct states have been considered, these being alertness, non-alertness, slow-wave sleep, and low-voltage-fast activity sleep. Strict criteria have been adopted for distinguishing between the different states of the sleep-wakefulness cycle, including both behavioural and electroencephalographic features. The latter were based mainly on activity recorded from the cerebral cortex (ECoG), the hippocampus (HIPP), and the orbit of the eye (EOG). The characteristics of the four states are considered below and records obtained in each state are shown in Fig. 1-7.

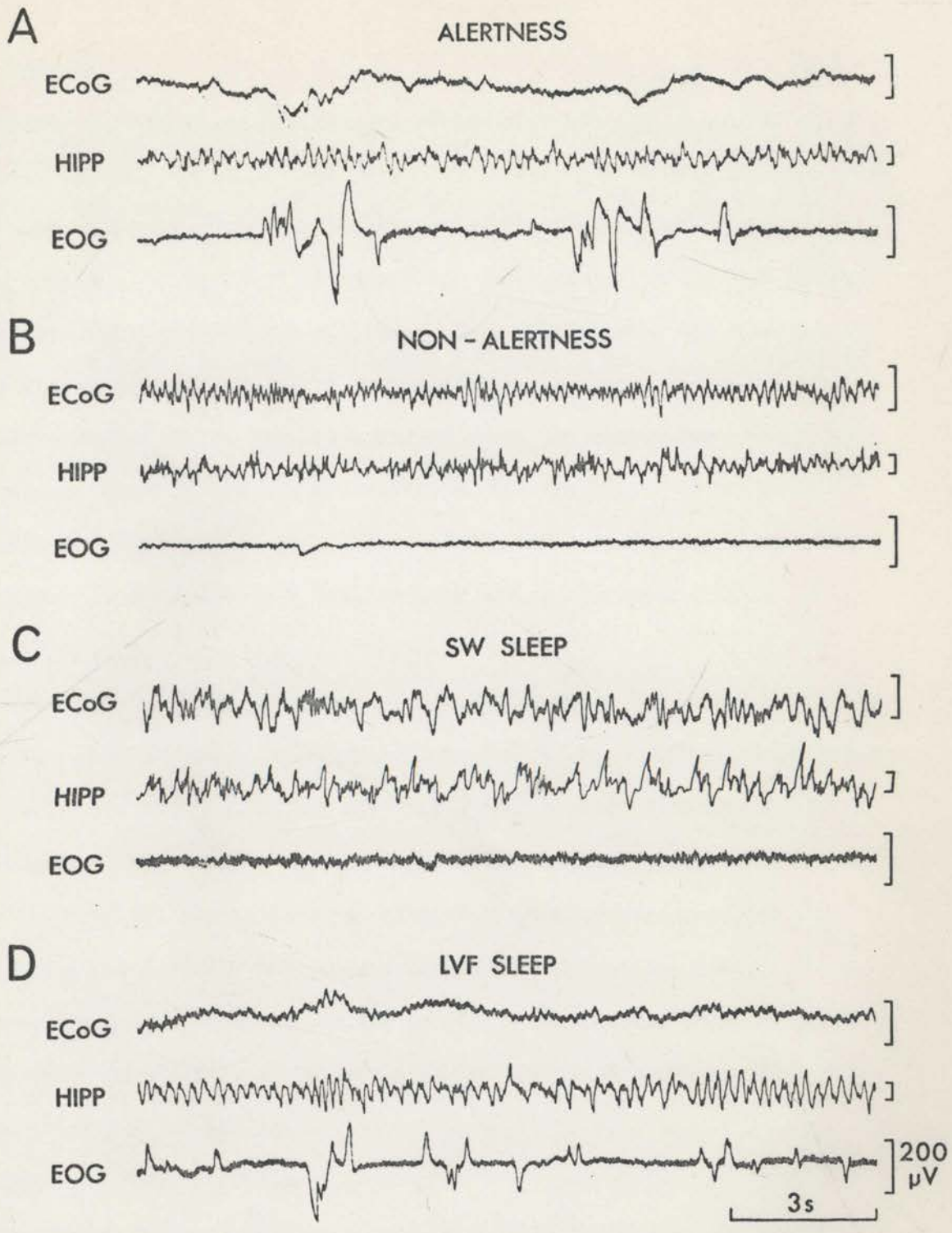


Fig. 1-7: Records obtained from the same cat on the same day, showing the characteristic activity recorded by the electroencephalograph during the four levels of consciousness - (A) alertness, (B) non-alertness, (C) slow-wave sleep, (D) low-voltage-fast activity sleep. The records shown were obtained simultaneously from the cerebral cortex (ECoG), hippocampus (HIPP) and the orbit of the eye (EOG).

(i) Alertness.

Maximum alertness was achieved by continually attracting the cat's attention. Usually visual and auditory stimuli were sufficient, but sometimes it was also necessary to employ tactile stimulation. For the cat to be classed as alert, it had to be standing up, or sitting with its head raised, and the eyes had to be open at all times. During alertness, the ECoG shows desynchronized activity of low voltage and the hippocampal record also shows irregular activity of low amplitude and of about 3-5 cycles per second. Many eye movements characterize the EOG record of the alert cat. Although the electromyogram was not recorded in this study, other studies have shown that in the alert animal there is a considerable amount of muscle tonus.

(ii) Non-Alertness.

As soon as the alerting procedures described above were stopped, the cat usually became non-alert in a short period of time. It could be either sitting or crouching with its head up and eyes open. The cortical activity is of larger amplitude than in the alert state, and 6-14c.p.s. spindle bursts can be seen in this record. The hippocampus exhibits irregular fast activity of moderate amplitude and the EOG shows a marked decrease in the number of eye movements. There is a reduction in the amount of neck muscle tonus compared with the alert level. This state is similar to the first stage of sleep (S_1) of Okuma and Fujimori (1963).

(iii) Slow-Wave Sleep or SW Sleep.

It is possible to divide sleep into at least two distinct phases, the first (SW sleep) being characterized by spindles and slow waves in the ECoG and the second (LVF sleep) showing desynchronized, low voltage activity in the cortex similar to that seen in the alert animal. The first of these two

phases has been given various names by different workers - "synchronised sleep", "the telencephalic phase of sleep", "light sleep", "LS_2 sleep", "slow sleep", "high-voltage-slow activity sleep" and "S_2 sleep". The term "slow-wave sleep" is used here because of the appearance of the ECoG. High voltage slow waves of 1-5c.p.s., and 6-14c.p.s. spindles characterize the cortical record. The hippocampus shows high voltage slow activity and spikes and irregular fast activity. There are no eye movements in this phase of sleep. Neck muscle tonus is further reduced, but it is not abolished. SW sleep normally occupies about 75% of behavioural sleep in the cat (see Delorme, Vimont et Jouvet, 1964; Jouvet, 1965; Sterman, Knauss, Lehmann and Clemente, 1965), and during this state the cat is lying down with its head down and eyes closed. The cat can be easily awakened from this state of sleep.

(iv) Low-Voltage-Fast Activity Sleep or LVF Sleep.

Dement and Jouvet were amongst the first workers to observe that the electroencephalogram of the sleeping cat could show periods of low voltage, fast activity (see Dement, 1958; Jouvet, 1961). Therefore it became apparent that although behavioural arousal is accompanied by a desynchronized ECoG, the reverse need not necessarily occur. This second phase of sleep, like SW sleep, has also become known by several names, including "desynchronized sleep", "the rhombencephalic phase of sleep", "deep sleep", "paradoxical sleep", "activated sleep", "low-voltage-fast activity sleep" and "rapid eye movement sleep". The term, "low-voltage-fast activity sleep" is used here because of the characteristic appearance of the ECoG. This resembles that seen in the alert animal. The hippocampal activity consists almost continuously of a very regular theta rhythm of 4-5 c.p.s. The EOG is characterized by frequent rapid eye movements, yet there is a complete loss of neck muscle tonus. LVF sleep normally occupies about 25% of

the total sleep time (Jouvet, 1965; Sterman et al., 1965), and it is difficult to arouse the cat from this state. Behaviourally, LVF sleep shows certain characteristic features. As in SW sleep, the cat is lying down in the typical posture of sleep, with head down and eyes closed, but, unlike SW sleep, there is now complete muscle atonia. At the same time there are bursts of rapid eye movements, twitches of the whiskers, ears, tail and extremities, and episodes of fast and irregular breathing. LVF sleep always follows SW sleep.

Although the above terminology will be adhered to in this thesis when differentiating between the different levels of consciousness in the sleep-wakefulness cycle, it is worth noting that, in confirmation with other workers, it has been possible to identify additional states of consciousness. However, these can usually be related to transitional stages in the cycle and therefore will not be considered here.

B. Phasic Slow-Wave Activity During the Sleep-Wakefulness Cycle.

In addition to the characteristics of LVF sleep which were outlined above, there is another very distinctive feature of this phase of sleep in the cat, namely, the spontaneous occurrence of phasic potential changes almost simultaneously in several places in the brain (pontine reticular formation, lateral geniculate nucleus,visual cortex, superior colliculus, oculomotor nucleus, abducens nucleus, parietal cortex and pulvinar). As mentioned earlier, these waves will be referred to as ponto-geniculo-occipital (PGO) waves. As the majority of this section concerns a detailed discussion of the characteristics of these waves and of similar waves found during barbiturate anaesthesia (barbiturate PGO, or B-PGO, waves), the known features of the waves of sleep

will be briefly presented here.It has been shown that each rapid eye movement (REM) occurring in LVF sleep is almost invariably accompanied by a PGO wave, but the reverse does not always hold true, as PGO waves can quite often be seen without a coincident REM.The PGO waves of LVF sleep found in the LGN have been described as predominantly monophasic spikes occurring at frequencies up to 8 c.p.s. Each wave has a duration of 35-100ms and an amplitude of 100-500μV. They can occur singly or in groups of up to eight (8) waves, but usually, they are in groups of three to four (3 to 4). The waves recorded from any one electrode position are always of the same polarity, but as the electrode is lowered through the geniculate, the polarity changes. Thus, the potential field associated with the waves is a dipole, the negative pole being within the LGN, and the positive pole being in the adjacent terminal part of the optic tract.

There have been reports that a second deflection of low amplitude and long duration, and of opposite polarity, may follow the first deflection. However, this second deflection is usually poorly defined, and it appears that it may be a manifestation of the recording procedure adopted.

The variable number of waves in a group, and the variable interval between groups, makes the PGO wave activity appear highly erratic. The sudden appearance of PGO waves is always the first indication of an approaching episode of LVF sleep, and after a delay of about 10-90 seconds, the other signs of LVF sleep become apparent (i.e., the characteristic cerebral cortical, hippocampal and neck muscle activity and eye movements appear). The waves are of greatest amplitude during the first 40 seconds of LVF sleep, after which they decline to approximately 70% of this initial value.

Various aspects of the above description were considered in the following papers: Mikiten, Niebyl and Hendley (1961), Brooks and Bizzi (1963), Brooks (1964, 1967a), Michel, Rechtschaffen and Vimont-Vicary (1964), Jouvet (1965,1967).

It will be shown in the Results that the characteristics listed above have been confirmed.In addition, similar waves have been observed during barbiturate anaesthesia (Malcolm, Burke and Watson, 1969; Malcolm, Watson and Burke, 1970), and evidence will be presented which suggests that the PGO waves of sleep and of barbiturate anaesthesia are unitary events. Before presenting the results, however, there is one form of evoked electrical activity which will be briefly considered.

C. Electrically Evoked Responses.

Although electrical stimulation of a nervous pathway produces synchronous volleys of impulses which would not be found under normal physiological conditions, the technique has proved very useful for testing the excitability of neurones or the efficacy of synaptic transmission. In this study, electrically evoked field responses have been used in an attempt to determine the effects of the PGO waves found in sleep and barbiturate anaesthesia on synaptic transmission in the LGN. Two evoked responses have been examined (see Fig. 1-8) and each will be considered briefly.

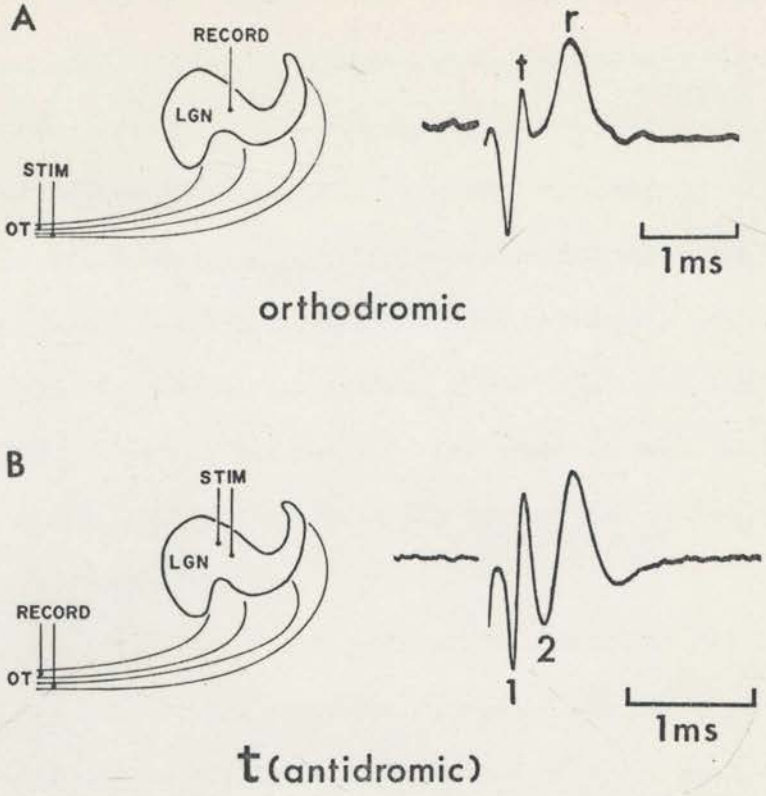


Fig. 1-8: Diagrams showing the stimulating and recording sites used in this study, and characteristic responses. OT = optic tract, LGN = lateral geniculate nucleus. (A.) The orthodromic LGN response has one presynaptic (t) and one postsynaptic (r) component. Negativity in LGN gives upward deflection. (B.) The antidromic response recorded differentially in the OT, t(antidromic), consists of two clear waves (1 and 2).

(i) The Orthodromic LGN Response.

One of the responses used for testing excitability was the field response evoked by electrical stimulation of the optic tract and recorded between an electrode in the ipsilateral LGN and an"indifferent" electrode over the visual cortex (Fig. 1-8A). The positions of the electrodes are shown diagrammatically on the left, and on the right is a typical response. The response consists of a positive/negative diphasic deflection, followed by a negative wave. The initial diphasic wave labelled 't' is the potential change associated with the arrival at the geniculate of impulses travelling along the optic tract fibers

(i.e., it is the presynaptic component of the response); the subsequent negative wave labelled 'r' represents the electrical activity of the postsynaptic geniculate neurones (see Bishop and McLeod, 1954, and Eisman et al., 1967). As the electrodes implanted in the OT were always carefully positioned in the lower part of the tract, and the geniculate electrodes were positioned in the anterior two-thirds of the nucleus, the response represents activity almost exclusively arising from the large, rapidly conducting tract fibers.

The presynaptic response was measured from peak to peak, and the postsynaptic response from the preceding dip to the peak of the negativity.

(ii) The Antidromic Tract Response.

To test the excitability of the optic tract endings in the LGN, stimuli were applied to the LGN and the antidromic response was recorded differentially between two electrodes in the tract (the method of Wall; Wall, 1958). On the left of Fig. 1-8B is a diagram of the stimulating and recording positions and on the right is a typical response. The response consists of two deflections, labelled 1 and 2, which represent activity propagating antidromically along two groups of OT fibers which have different conduction velocities (see Bishop, Jeremy and Lance, 1953).

Two measurements were made from this response. Wave 1 was measured from the baseline level after the artefact to the first peak of the wave, and wave 2 was measured from the preceding dip to the peak of the wave. However, as wave 2 was frequently superimposed on the"tail" of wave 1 it could therefore be affected by changes in the fast conducting group of fibers. Therefore, unless otherwise indicated, all results for this response are for wave 1.

Now, having dealt with these aspects of spontaneous and evoked electrical activity, I will proceed with the results of the experiments carried out.

RESULTS

1. General Characteristics of Spontaneous PGO Waves During Sleep and Barbiturate Anaesthesia.

(i) Waves occurring in the Unanaesthetized cat (PGO waves).

As mentioned earlier, one very distinctive feature of LVF sleep is the occurrence of spontaneous phasic potential changes (called PGO waves) in several places in the brain. The first indication of an approaching episode of LVF sleep is the appearance of the waves towards the end of SW sleep. The other signs of LVF sleep usually appear after a delay of between 10 and 90 seconds.

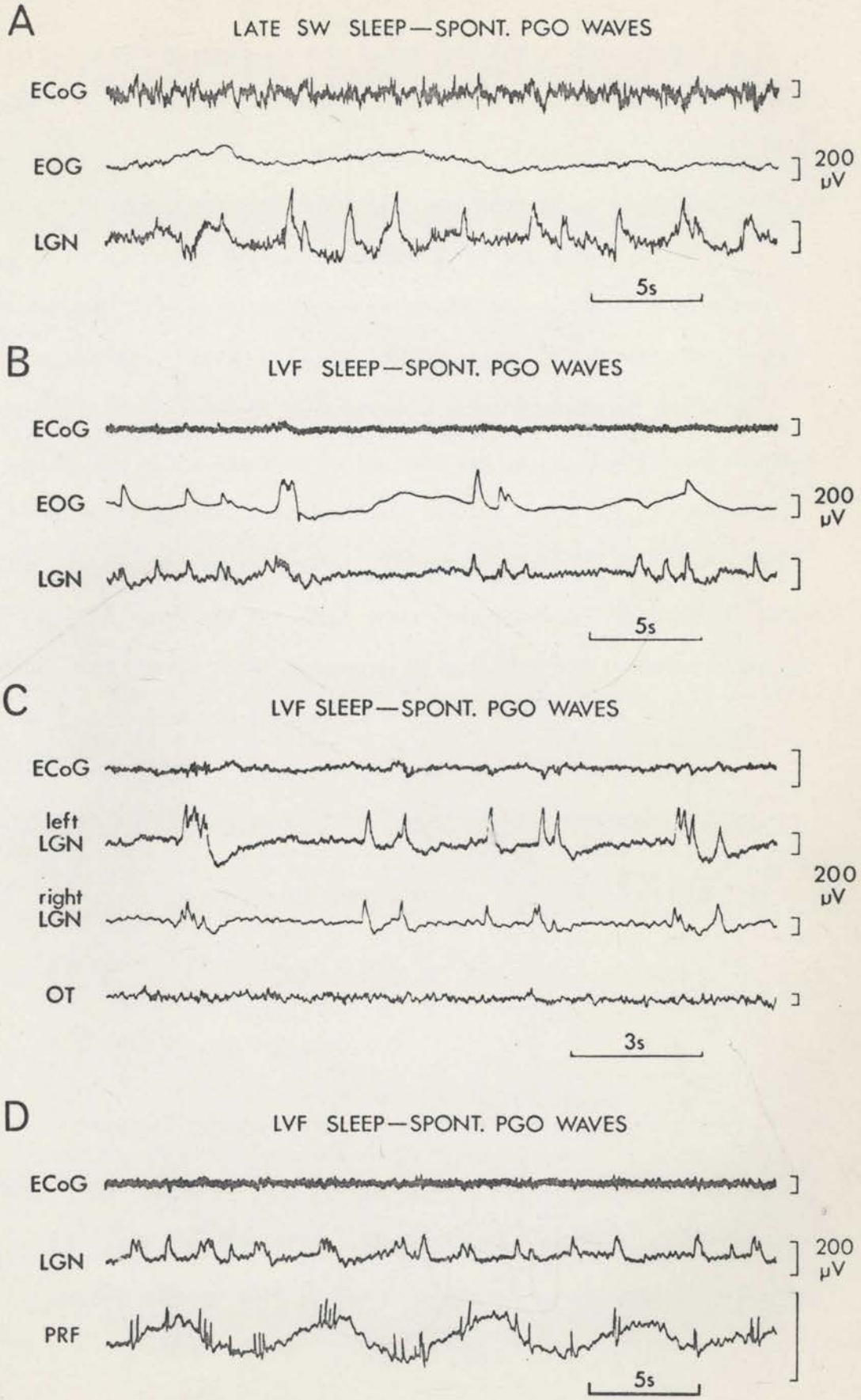
Certain basic characteristics of the waves are illustrated in Fig.1-9. The records in Fig. 1-9A were obtained with the cat in the dark towards the end of an episode of SW sleep. The upper record (the electrocorticogram) shows the high voltage slow wave activity which typifies cortical recordings during this phase of sleep, while the middle record (the electro-oculogram) shows that during SW sleep there is a complete absence of eye movements. The slight oscillation which can be seen in the EOG record was due to pick-up of activity from other electrodes. The PGO waves can be seen in the lower record (obtained from one LGN) as negative-going spikey-looking waves of approximately 200 to 300μV in amplitude. There are no PGO waves in the early part of the record, the first wave occurring approximately 8 seconds after the start of the record. A phase of LVF sleep began 30 seconds after the end of the records shown in A.

In Figs. 1-9B, C and D are various records obtained during LVF sleep. The upper record of each group shows clearly the typical low voltage, fast, desynchronized activity which is recorded from the cortex during this state. The middle record in Fig. 1-9B shows that the EOG during LVF sleep is characterized by frequent rapid eye movements,and when this record is examined in association with the lower record, it is apparent that each rapid eye movement (REM) is invariably associated with a PGO wave in the LGN.

Fig. 1-9: Activity recorded simultaneously from different regions during SW and LVF sleep. ECoG = electrocorticogram; EOG = electro-oculogram; LGN = lateral geniculate nucleus activity; OT = optic tract activity; PRF = activity recorded from the pontine reticular formation.

(A.) Activity recorded towards the end of an episode of SW sleep - an episode of LVF sleep began 30 seconds after the end of this record. Note the synchronized high voltage cortical activity and the lack of eye movements. In the LGN record clear PGO waves can be seen, heralding an approaching episode of LVF sleep.
(B.) Spontaneous PGO waves recorded in the LGN during LVF sleep. These records were obtained from the same cat on the same day as the records shown in A. The waves recorded after an episode of LVF sleep is well developed are considerably smaller in amplitude than the waves occurring late in SW sleep (see A). The EOG is characterized by frequent rapid eye movements, each of which is coincident with a PGO wave in the LGN. However, PGO waves can be seen without coincident eye movements.
(C.) Records obtained during LVF sleep from another cat. Spontaneous PGO waves can be seen occurring synchronously in the two geniculates. However, there is no evidence of any change in the activity recorded from the OT at the time of the LGN waves.
(D.) Spontaneous PGO waves recorded from another cat during LVF sleep. Note the high degree of synchrony between the waves in the LGN and the PRF.

Negativity in LGN gives upward deflection of pens. All records were obtained with the cat in the dark.


A
LATE SW SLEEP—SPONT. PGO WAVES
ECoG
EOG
LGN
200 μV
5s
B
LVF SLEEP—SPONT. PGO WAVES
ECoG
EOG
LGN
200 μV
5s
C
LVF SLEEP—SPONT. PGO WAVES
ECoG
left LGN
right LGN
OT
200 μV
3s
D
LVF SLEEP—SPONT. PGO WAVES
ECoG
LGN
PRF
200 μV
5s

However, PGO waves can be seen without coincident REM's. The records shown in B were recorded from the same cat and on the same day as the SW sleep records shown in A. It is evident that the PGO waves in B, which were recorded after the episode of LVF sleep was well developed, are reduced in amplitude compared with the waves heralding an approaching episode of LVF sleep (A).

As can be seen in Fig. 1-9C, spontaneous PGO waves occur synchronously in the two LGN's, but there is no evidence of any change in activity in the OT during the waves. In 4 different experiments, large numbers of the waves occurring simultaneously in each LGN were recorded on an electroencephalograph, so that the degree of synchrony between the two LGN's for the spontaneously occurring PGO waves could be determined. The results showed that there was between 92% and 95% synchrony for such waves (see Table 1-2) - that is, between 92% and 95% of spontaneous waves occurring in either LGN were accompanied by similar waves in the other geniculate.

TABLE 1-2.

Synchrony Between the Two Geniculates for Spontaneously Occurring PGO Waves.

Experiment	No. of Times a PGO Wave Appeared in one or in both LGN's.	Synchrony Between the two LGN's (as %).
1	305	95
2	782	93
3	1153	92
4	665	93
		Mean = 93%

PGO waves also occur synchronously in the pontine reticular formation and the LGN's during LVF sleep (see Fig.1-9D). Simultaneous recording of spontaneous PGO waves in the PRF and one LGN on the two beams of a dual-beam oscilloscope has shown that the waves may occur first in either region, the latency difference

between the two regions being up to 15ms. Sometimes the waves seem to occur simultaneously in the two structures.

Figs. 1-9B and C show clearly that the waves are predominantly monophasic and that they may occur singly or in groups (the average number of waves in a group was found to be 3). The appearance of the waves can be seen more clearly in Fig. 1-10, where four examples are shown of waves which were recorded on the cathode ray oscilloscope.

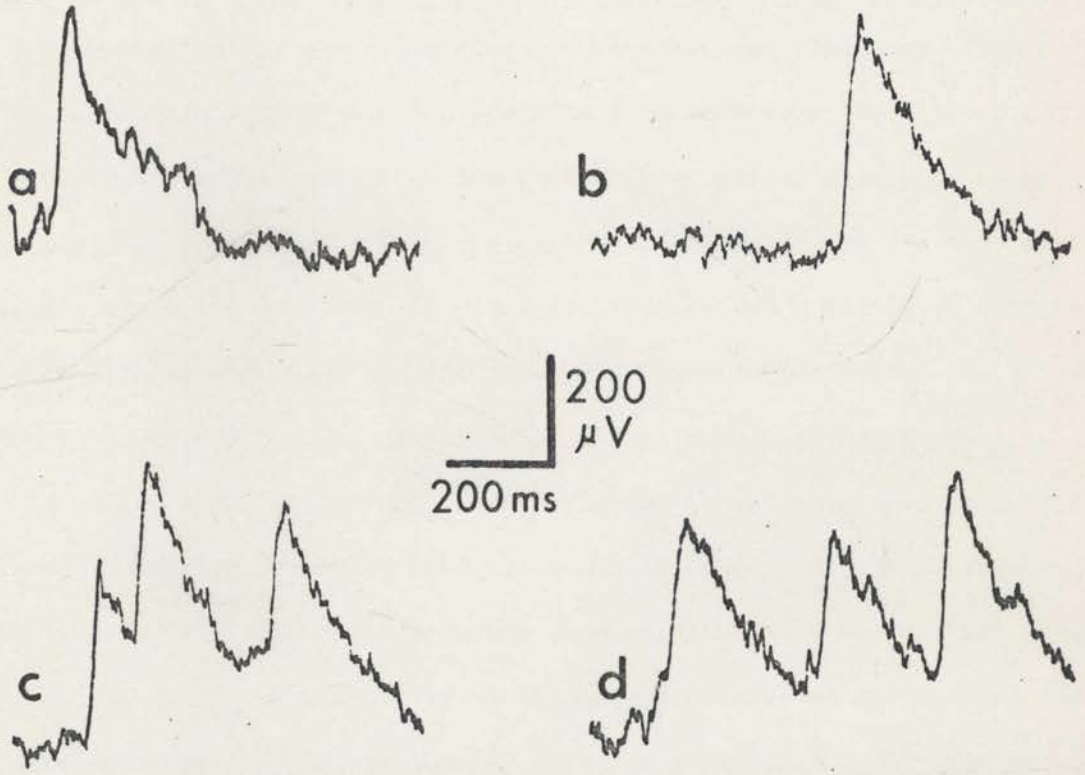


Fig. 1-10: Spontaneous PGO waves recorded during an episode of LVF sleep; the cat was in the dark. Negativity in LGN gives upward deflection. The voltage and time calibrations apply to all records.

Records a and b show single PGO waves, while in c and d can be seen groups of 3 PGO waves. The waves have a very rapid rising phase until the peak amplitude has been achieved, after which there is a slow, more-or-less exponential decay back to the baseline. The single waves are approximately 300-400ms in duration and have an amplitude of about 400μV. However, it must be stressed that the

amplitude of the waves depends on the position of the recording electrodes and therefore records obtained from different animals do not necessarily show waves of the same amplitude.

It is evident from traces c and d in Fig.1-10 that when more than one wave occurs at a time, the interval between the start of consecutive waves may be quite variable. In four experiments an attempt was made to determine the minimum interval between spontaneously occurring PGO waves.However, it was found that the recording conditions could influence the values obtained - e.g., much shorter minimum intervals were obtained when the waves were recorded on the oscilloscope than when measurements were made from the records on the electroencephalograph because of the greater resolution. Moreover, it was sometimes difficult to adhere to the strict criteria adopted for the identification of single and multiple PGO waves. It was therefore not possible to obtain reliable information about minimal intervals between spontaneous PGO waves, although the interval would appear to be approximately 70 to 100ms.

In addition to the changes which may occur in PGO wave amplitude when different recording sites are used, it was also found that the average amplitude of the waves could vary quite markedly from day to day in the one cat. For example, the average amplitude of 54 single waves recorded on the same day as the records shown in Fig.1-10 was 345.2μV. Two weeks later, the average amplitude of 166 PGO waves recorded from the same geniculate and with the same combination of electrodes was somewhat less (223.0μV). (The amplitude measurements in both experiments were made from the baseline to the peak of the single waves, and from the baseline to the peak of the first wave for groups of PGO waves.)

When large numbers of the waves were recorded it became apparent that those which were the largest in amplitude were also the longest in duration.

This can be seen in Fig. 1-11, where 166 PGO waves recorded from the one experiment were arbitrarily split into two groups based on their peak amplitude values.

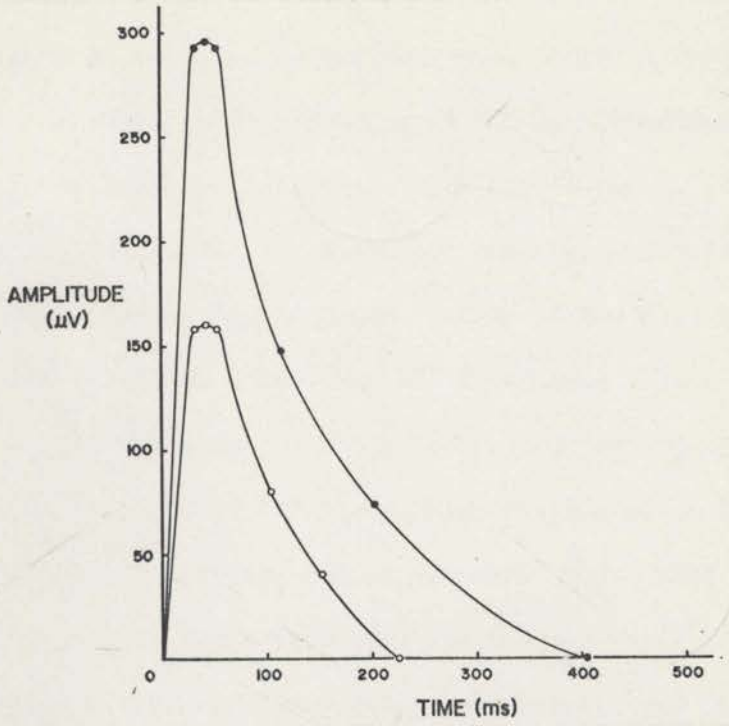


Fig. 1-11: Plot showing the average waveform and time-course of spontaneous single PGO waves recorded in the one experiment. The data were arbitrarily divided into two groups according to the peak amplitude of the wave. Closed circles (●), waves with a peak amplitude of > 223μV (N = 78). Open circles (○), waves with a peak amplitude of < 223μV (N = 88). The cat was in the dark. For further details see text.

Large waves (of which there were 78) were those with an amplitude of more than 223μV, while the 88 waves with amplitudes less than this fell into the small wave category. As many as possible of the following measurements were made on each single wave - (i) rise time (i.e., the time from the start of the wave to its peak), (ii) peak amplitude (measured from baseline to peak), (iii) the time for which this peak amplitude was maintained, (iv) half-decay time (i.e., the time at which the amplitude had fallen to half its peak value), (v) three-quarter-decay time (estimated in the same way as the half-decay time), and

(vi) total duration. The points plotted are the averages which were obtained for each of these six measurements. It can be seen from the graphs that the average peak amplitude values were 295 and 159μV for the large and small waves respectively, and that the larger waves were much longer in duration than were the small waves (the total durations averaged 405 and 226ms respectively).

Although PGO waves are a distinct feature of LVF sleep, there have been reports by other workers of similar potential changes during other phases of the sleep-wakefulness cycle. In the present study it has also been found that PGO waves may occur in states other than LVF sleep (see Fig. 1-12). However, the waves in these other states are not a consistent feature of geniculate or pontine activity as they are in LVF sleep. Fig. 1-12A shows spontaneously occurring potential changes in the LGN of the alert cat. These waves are greatly attenuated in amplitude in comparison with the waves seen in LVF sleep. It can be seen that the waves are associated with eye movements and hence they have been given the name of eye movement potentials (EMP's) by some workers (e.g., Feldman and Cohen, 1968; Jeannerod and Sakai, 1970). Brooks (1968a) simply refers to these waves by the same name as has been applied to the waves of LVF sleep (i.e., PGO waves). PGO waves were also seen in the non-alert cat (see Fig. 1-12B) and during drowsiness and SW sleep. In addition to the waves which occur at the end of SW sleep and which herald an approaching episode of LVF sleep (see Fig. 1-9A), PGO waves may occur sporadically throughout the course of SW sleep (see Fig. 1-12C).

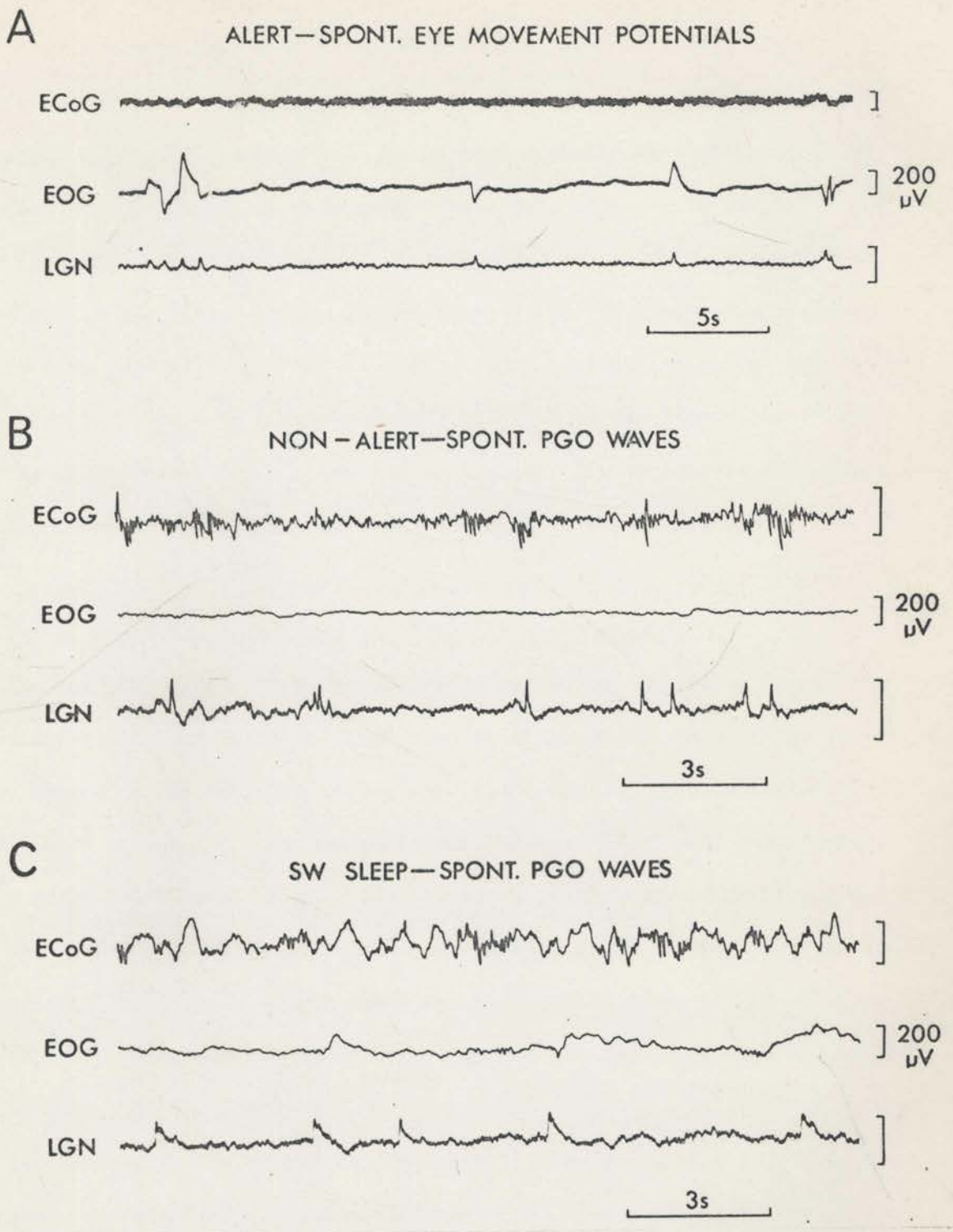


Fig. 1-12: Spontaneous potential changes recorded in the LGN on the electroencephalograph during (A) alertness, (B) non-alertness, and (C) slow-wave sleep. All records obtained with the cat in the dark.

It is apparent that even although PGO waves occur during all levels of consciousness, their high frequency (up to 60 or 70 per minute) and continual appearance during LVF sleep alone, makes them a distinctive characteristic of this state. Particularly with regard to sleep, it might be suggested that there are two distinct states, one including PGO waves (which would be equivalent to LVF sleep), while in the other phase (SW sleep) PGO waves are for the most part absent. Thus, it might appear that during sleep, PGO waves show a cyclic pattern of occurrence; on the basis of the proportion of time spent in each phase of sleep (see the Preliminary Discussion) it is apparent that the periods of waves only occupy about 25% of the total sleep time.

(ii) Waves occurring in the Anaesthetized Cat (B-PGO waves).

In addition to the PGO waves of LVF sleep (and of the other levels of consciousness in the unanaesthetized cat) which have been seen by the author and by several other workers, it was also found that spontaneous potential changes were a frequent occurrence in the LGN and PRF of cats anaesthetized with pentobarbitone sodium. When the existence of these waves was first reported they were termed "barbiturate waves" (see Malcolm et al., 1969), but in view of the essential similarity of PGO waves and barbiturate waves it now seems preferable to call them "barbiturate PGO waves" ("B-PGO waves"; see Malcolm et al, 1970).

PGO waves and B-PGO waves can be recorded from the same electrode positions, the waves recorded from any one position always being of the same polarity and similar waveform (see Fig. 1-13). The records shown in Fig. 1-13A were obtained during LVF sleep, while those in B are from a different experiment on the same cat after giving pentobarbitone sodium.

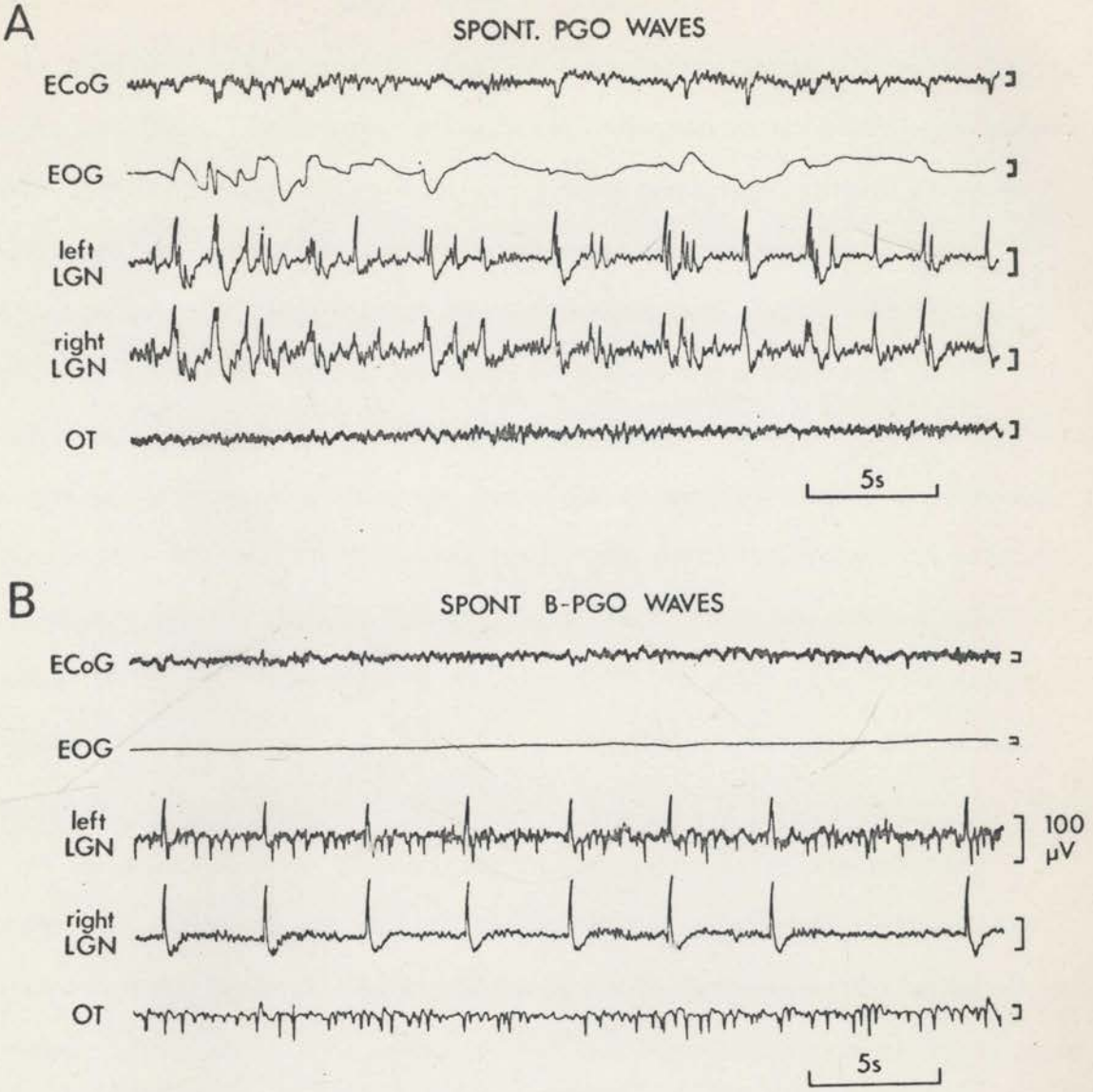


Fig. 1-13: (A) Spontaneous PGO waves recorded from each LGN during an episode of LVF sleep; the cat was in the dark. Note the high level of synchrony between the two LGN's for the waves, and the absence of potential changes in the OT. Rapid eye movements are evident in the EOG record. (B) Spontaneous B-PGO waves recorded from the same cat on a different day. In comparison to A, the waves in B are only occurring singly and there are no eye movements in the anaesthetized cat. Again, note the synchrony between the two LGN's.
Negativity in the LGN gives upward deflection of pens.

The recording arrangements were the same on the two days, although, as shown by the calibrations, the gains used were different. Like PGO waves, B-PGO waves have a fast rising phase followed by a slower decline back to the baseline.

As the waves recorded from any one position are always of the same polarity, it follows that B-PGO waves must be negative in polarity throughout most of the LGN (see Brooks, 1967a). Both types of wave occur synchronously in the two LGN's, as can be seen in Fig 1-13, and there is also synchrony between the waves occurring in the geniculate and the pontine reticular formation. It can be seen from the records that neither PGO nor B-PGO waves are associated with any change in activity in the OT. Unlike the waves of sleep, those occurring during barbiturate anaesthesia are obviously not associated with eye movements. Another very obvious difference between the two types of wave is clearly illustrated in Fig. 1-13 - whereas the PGO waves frequently occur in groups, the waves in the anaesthetized cat do not. Almost always the B-PGO waves occur singly, except when the cat is only very lightly anaesthetized, in which case the waves begin to appear in pairs and then groups (i.e., they become more like PGO waves in appearance).

In three experiments the activity in both geniculates was recorded simultaneously for long periods of time so that the degree of synchrony for spontaneous B-PGO waves in the two LGN's could be determined. The results, which are summarized in Table 1-3, indicate that the degree of synchrony was greater for B-PGO waves than for PGO waves (95-98%, average 97%, synchrony for B-PGO waves, against 92-95%, average 93%, synchrony for PGO waves; compare Tables 1-3 and 1-2).

TABLE 1-3

Synchrony Between the two LGN's for Spontaneously Occurring B-PGO Waves

Experiment	No. of times a B-PGO Wave Appeared in one or in both LGN's.	Synchrony Between the two LGN's (as %).
1	479	98
2	1258	98
3	461	95.5
		Mean =97%

As B-PGO waves tend to occur singly, it is frequently easier to count the number of such waves than it is to estimate the frequency of PGO waves (which, as mentioned above, may occur at very short intervals). This may account at least partially for the observed difference in synchrony for the two types of wave.

As for the PGO waves, an attempt was made to determine the minimum interval between spontaneous B-PGO waves, but again, difficulties were encountered when trying to establish strict criteria for classifying the waves. Although the LGN records shown in Fig. 1-13B suggest that all spontaneous B-PGO waves recorded from any one electrode position are very similar in appearance, this was not always so. As mentioned earlier, the waves recorded from any one position were always of the same polarity and were similar in waveform, but it was possible for slight variations to occur in the appearance of the waves - see Fig. 1-14.

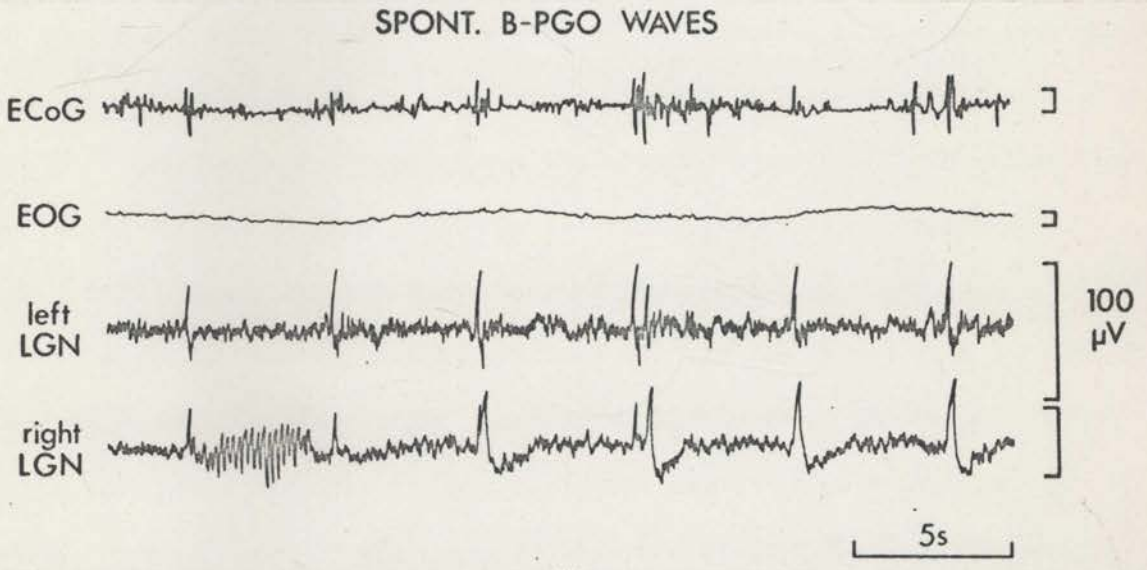


Fig. 1-14: Spontaneous B-PGO waves occurring in each LGN. The right LGN record shows the three waveforms which have been identified - for details, see text. Negativity in LGN gives upward deflection of pens.

The records shown in this figure were obtained from a different cat to that used in the experiments shown in Fig. 1-13. Comparison of the two LGN records in Fig. 1-14 again shows clearly that the waveform can vary when different recording

sites are used. However, the right LGN record also shows that not all waves recorded from any one position are identical in appearance. In fact, three different waveforms have been identified, all of which can be seen in Fig. 1-14. The first type is of rather smaller amplitude than the majority of the waves and it has a short duration. This type of wave was usually only seen very rarely, although in this particular record three such waves can be seen - these are the first two waves in the record and the first wave of the pair occurring just over half-way across the record. The second type of wave looked like a bigger and more long-lasting variety of the previously described wave type - see the last three waves of the record. The differences between these two types are seen most easily by comparing each wave in the pair just after the centre of the record. Finally, it is possible to record a wave which looks like a combination of the two previous types - see the third wave of the record - thus, this wave has an initial rapid phase followed by a second phase of larger amplitude and longer duration than the first phase. These three types of wave will be called types 1R, 1S and 2, referring respectively to their appearance as single rapid or single slow waves, or waves consisting of two parts.

As might perhaps be anticipated, not all records showed such a clear-cut distinction between these three types of wave. Moreover, the question arose as to how the type 2 wave should be classified. Should it be considered as a single entity, or as a type 1R and a type 1S wave (or even as two type 1S waves) at very brief intervals? As this problem was not resolved it has not been possible to determine the minimum interval between spontaneously occurring B-PGO waves, although on a few occasions what were thought to be two separate waves were seen occurring at an interval of 150ms.

Direct comparison of PGO waves and B-PGO waves recorded on the same day

(with the same electrodes) has revealed that the waves in the anaesthetized animal are larger in amplitude and longer in duration than are the waves of sleep. Records a and b of Fig. 1-15 were obtained during an episode of LVF sleep when the cat was in the dark; the waves shown in c and d were recorded on the same day with the cat in the light after giving barbiturate and the record shown in e was recorded with the cat in the dark. Although the B-PGO wave records obtained this day were quite clear and typical, the PGO waves were not as distinct as they were usually (e.g., see Fig. 1-10).

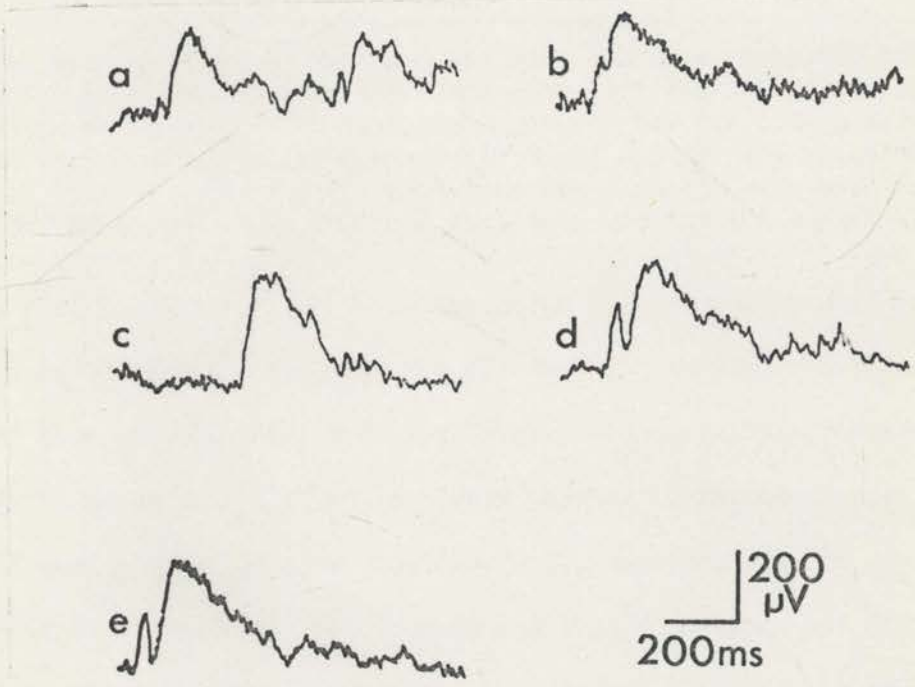


Fig. 1-15: Spontaneous PGO waves recorded on the oscilloscope from the same cat on the same day during LVF sleep (a and b) and barbiturate anaesthesia (c,d and e). Records a, b and e obtained with the cat in the dark, and c and d with the lights on. For details see text.

However, measurements made on large numbers of the waves during sleep and anaesthesia show clearly the increases in amplitude and duration for B-PGO waves which were stated above. The results of this experiment are summarized in Table 1-4.

TABLE 1-4.

Measurements for Spontaneous PGO and B-PGO Waves Recorded on the Same Day.

		Average Peak Amplitude(μV)	No.of Waves	Average Total Duration (ms)	No.of Waves
Single PGO Waves	Dark	129	106	307	19
Type 1S B-PGO Waves	Lights on	298	7	660	3
	Dark	318	15	500	2
Type 2 B-PGO Waves	Lights on	312	65	911	9
	Dark	318	54	1220	5

All waves were recorded on the same day with the same recording electrodes. As indicated, all records during LVF sleep were with the cat in the dark, while B-PGO waves were recorded in both the light and the dark. Data have been included for both the single slow B-PGO waves (type 1S) and for the waves consisting of two parts (type 2 B-PGO waves). Amplitude measurements were made from baseline to peak (for the type 2 waves the peak amplitude was for the second slow phase).

Several features of the B-PGO waves can also be pointed out from the data included in Table 1-4. Reference has already been made to the three different types of wave which may occur during barbiturate anaesthesia. The type 1R wave is a single rapid wave, and it is always smaller in amplitude than the other two kinds of wave. It can be seen from the Table, however, that in any one condition (i.e., lights on, or dark) the type 1S and type 2 waves do not differ in their average peak amplitudes, and that the type 2 wave is longer in duration than is the type 1S wave. This difference in duration is not surprising when it is considered that one wave consists of only a single component, while the other has, apparently, two components. Too much emphasis should not be placed on these total duration values, though, because of the relatively small number of observations. It is also apparent from Table 1-4 that B-PGO waves recorded in the light and in the dark do not show any consistent changes in amplitude. This last-mentioned

feature of the waves is evident if records c and d are compared with record e in Fig. 1-15.

As reasonably large numbers of type 2 B-PGO waves were recorded in both the light and the dark in the experiment summarized in Table 1-4, another aspect of the waves was also examined. It can be seen in Fig. 1-16 that the waves recorded with the lights on were divided into two groups according to their peak slow-wave amplitudes.

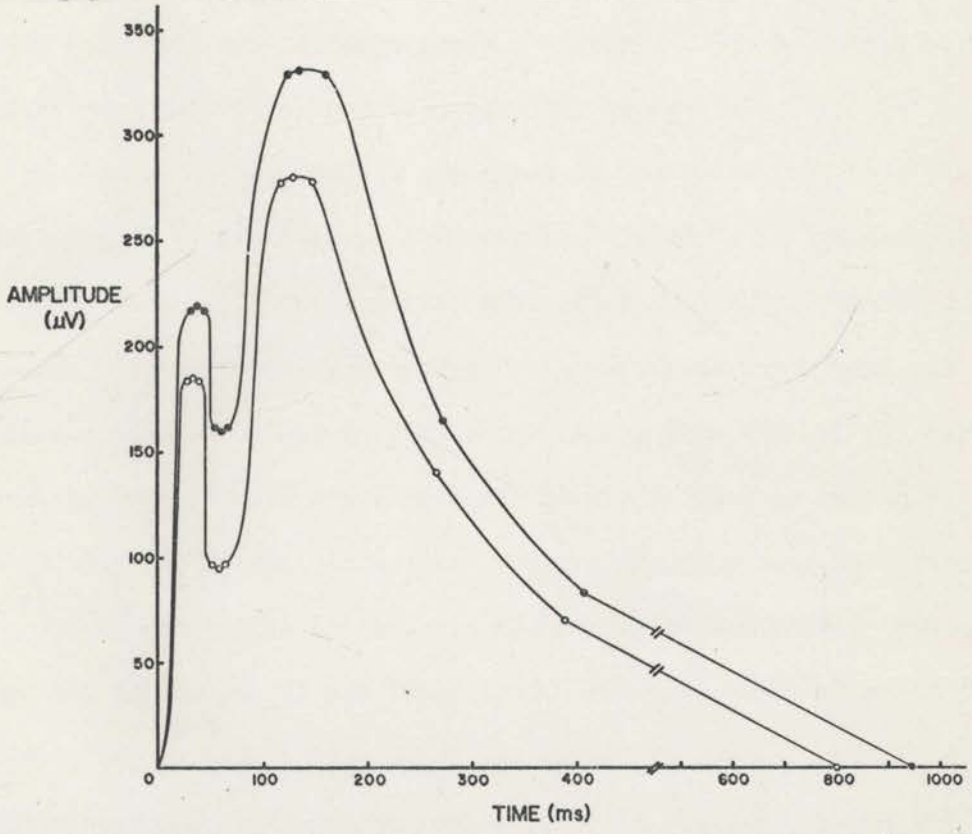


Fig. 1-16: Plot showing the average waveform and time-course of the spontaneous type 2 B-PGO waves recorded in the light during one experiment. Closed circles (●), waves with a peak amplitude of >300μV (N = 43). Open circles (o), waves with a peak amplitude of <300μV (N = 22). Note the break and changed scale after time 400ms. For further details, see text.

The amplitude division was made arbitrarily, in this case large waves (totalling 43) being considered as those with a peak slow wave amplitude of at least 300μV, while the small waves (totalling 22) were less than 300μV in amplitude. The waves

drawn are average plots obtained by making as many as possible of 12 different measurements on each wave. It is obvious from the graphs that both components of the large waves are greater in amplitude than the corresponding phases of the smaller waves. Associated with these amplitude differences are obvious differences in the total durations of the waves - the larger amplitude waves are also the ones of longer duration. It is also apparent that the peaks of the fast and slow components and the trough between these two peaks occur at slightly longer times after the start of the large waves than they do for the small waves; similarly for the half-decay and three-quarter decay times.

Before discussing the results presented in the preceding pages there is one further aspect of spontaneous B-PGO wave activity to be considered. It was found that not always could the waves be recorded in the anaesthetized animal, even when the usual combinations of recording electrodes were used. At the two extreme levels of anaesthesia in particular (i.e., when the cat was either very deeply or only very lightly anaesthetized) it was frequently impossible to record B-PGO waves. However, it was not until continuous long-term recordings were made of on-going geniculate activity in the anaesthetized animal that this phenomenon was explained. It was found that B-PGO waves show a cyclic pattern of occurrence - that is, periods in which the waves are present alternate with periods lacking waves. This can be seen clearly in Fig.1-17, where the frequency of B-PGO waves has been plotted for a continuous period of 80 minutes. During this 80 minute period there were 5 definite periods in which large numbers of waves were present (up to a maximum frequency of 50 per minute), while at other times there were none at all. The frequencies of B-PGO waves seen in this particular cat were unusually high. In 12 experiments on 3 other animals maximum frequencies have only been about 25 per minute (often less). Over most levels of anaesthesia,

cycle times (i.e., the time from the start of one B-PGO wave period to the start of the next B-PGO wave period) have proved extremely constant - e.g., in the cat used for the experiment shown in Fig. 1-17, the cycles were most frequently between fifteen and seventeen (15 and 17) minutes in duration.

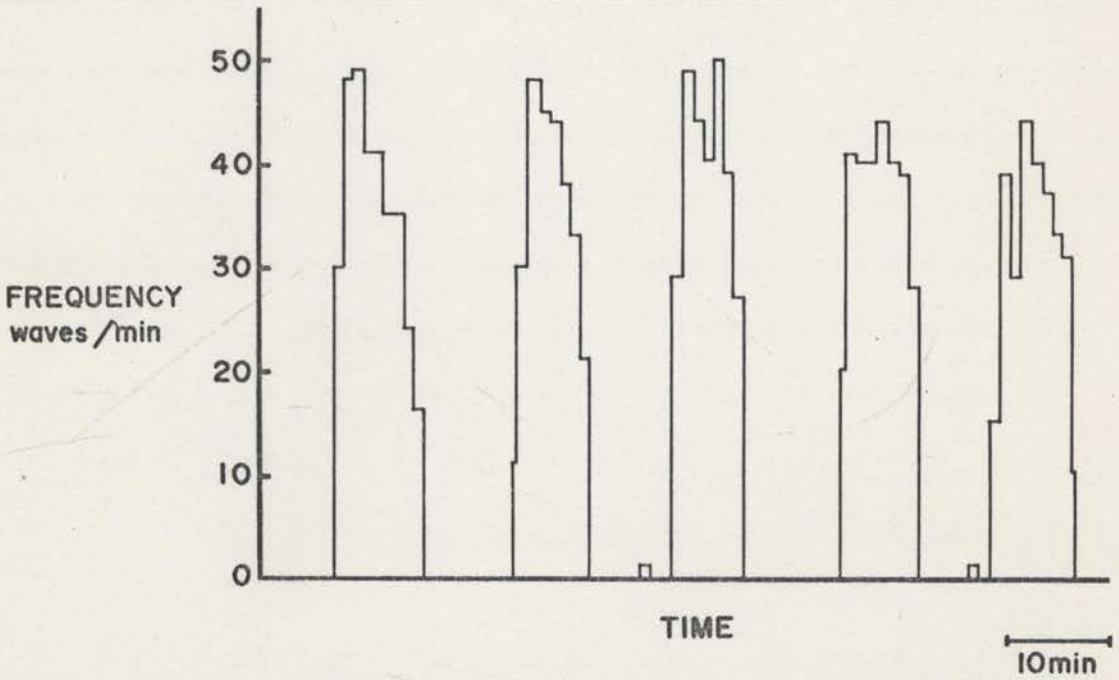


Fig.1-17: Graph showing the frequency of B-PGO waves recorded continuously for 80 minutes. Note the definite cyclic pattern of occurrence of the waves.

Similarly,there was little variation between cats, the range for cycle times for six experiments on four different cats usually being between fourteen and twenty (14 and 20) minutes. Frequently it was impossible to record B-PGO waves in very deeply anaesthetized cats, but as the depth of anaesthesia decreased slightly, the pattern of activity just described became apparent. Although the cycle time remains fairly constant, the proportion of time

for periods with waves tends to increase as the level of anaesthesia decreases. The time without waves decreases accordingly. Finally, there comes a point (usually when the animal is only lightly to very lightly anaesthetized) when the waves occur continuously for about one to two hours; this changed pattern of activity is associated with an increase in wave frequency due to the appearance of pairs and groups of waves (reminiscent of the activity in sleep). Usually the waves seem to vanish as the level of anaesthesia decreases even further. This final loss of the waves is usually at approximately the time when the cat begins to make frequent spontaneous movements, and at a time when the activity recorded from the cerebral cortex, hippocampus and lateral geniculate nucleus shows an increase in voltage and in the number of slow-wave components. When the effects of the anaesthesia have completely worn off, normal PGO waves can again be seen during the episodes of LVF sleep.

Discussion

Only one other report exists of similar phasic activity in the LGN of the barbiturate anaesthetized cat. Jouvet and Delorme (1965) reported that between the second and fourth hours after giving 35mg/Kg of pentobarbitone sodium

isolated spikes could be seen at the level of the LGN. Although in the present study waves have usually been observed at much shorter times after giving the anaesthetic (usually after only about 15 minutes), the waves described here are probably identical with those reported by Jouvet and Delorme. During recovery after prevention of LVF sleep for several days there is an increase in the proportion of time spent in this phase of sleep, and an associated increase in the frequency of groups of PGO waves in the LGN (Jouvet, Vimont, Delorme et Jouvet, 1964; Kiyono, Kawamoto, Sakakura and Iwama, 1965). Jouvet and Delorme (1965) observed that the waves in the anaesthetized animal were similarly enhanced after prior prevention of LVF sleep. Therefore they concluded that the periods of PGO waves at the level of the LGN which were specific for LVF sleep could persist under anaesthesia in the absence of all other signs of that phase of sleep.

The spontaneously occurring waves which have been observed in barbiturate anaesthetized animals in the present study bear many similarities to the PGO waves found in the LGN during sleep. Both types of wave may be recorded from the same electrode positions and both are of the same polarity at any one recording site. Moreover, the waves are similar in appearance, being essentially monophasic with a rapid rising and a slower falling phase. Both PGO and B-PGO waves have a cyclic pattern of occurrence, periods in which the waves are present alternating with periods lacking such activity. However, this cyclic behaviour is far more definite for the B-PGO waves than for the PGO waves of LVF sleep, due to the occasional appearance of PGO waves during SW sleep. Moreover, the proportion of time in which the waves are present is greater in the anaesthetized than in the sleeping cat. Simultaneous recordings from the two LGN's during sleep and anaesthesia have revealed that in both states a high proportion of the waves

occurring in one geniculate are accompanied by similar activity in the other LGN, and synchrony also exists between the waves in the LGN and the PRF. Neither type of wave is associated with any change in the electrical activity of the optic tract, while during both kinds of wave it is possible to detect discharges of LGN cells over a loudspeaker.

In view of these similarities, and of others which will be considered later in the results, it has been suggested that these two forms of spontaneous activity are in fact a result of the one mechanism, (see Malcolm et al., 1970). However,there are also some differences in these two kinds of wave. For example, PGO waves are smaller in amplitude and shorter in duration than are B-PGO waves recorded on the same day, and there are also slight differences in the appearance of the waves. The most obvious difference, however, is that, whereas the waves in the anaesthetized cat almost invariably occur singly, those in the sleeping animal may occur singly or in bursts of up to eight waves. This change in the pattern of occurrence of the waves seen during barbiturate anaesthesia accounts for the different characteristic frequencies of the waves in the two states.

On the basis of the close correlation seen between the PGO waves and rapid eye movements of LVF sleep, the suggestion has been made that the waves are related to visual imagery during dreaming (Jouvet, 1967). However, this hypothesis was questioned when similar waves were observed in the awake animal (see the Introduction). If the waves described in the anaesthetized animal represent the same phenomenon as those seen during LVF sleep, then they also provide evidence against Jouvet's hypothesis. The lack of eye movements in the anaesthetized animal indicates that certainly in this state there is no relationship between the waves and the activity of the eyes, such as seems to exist in LVF sleep and alertness.

Therefore, the question arises as to what is the functional significance of this phasic activity. Brooks (1968a) suggested that PGO waves were concerned with some aspect of oculomotor-visual integration. This hypothesis was advanced in view of the association between the waves and eye movements, and the known localization of the waves to structures of both the visual and oculomotor systems (Brooks and Bizzi, 1963). However, such a hypothesis would not seem to be applicable for the B-PGO waves described here, certainly insofar as an association with eye movements is concerned.

In the following pages, further features of the waves of sleep and barbiturate anaesthesia are considered and it will become increasingly apparent that these two types of wave show many similarities to each other.

2. The Effects of Pontine and Auditory Stimulation on the Phasic Geniculate Activity.

In the preceding pages were presented the characteristics of the waves occurring spontaneously in the LGN of the sleeping and anaesthetized cat. As the pontine reticular formation may be the triggering site for these waves (see the Introduction), the effects of electrical stimulation of the PRF on the phasic LGN activity were examined. Electrical stimuli of 2ms duration were applied to the PRF at various rates. The unanaesthetized cat was always in the dark during such experiments.

During LVF sleep such pontine stimulation evokes a response in the LGN which resembles the waves occurring spontaneously in this phase of sleep. An example of PGO waves evoked by stimulation of the PRF can be seen in Fig. 1-18A. The stimuli were applied between two electrodes in the pons at a rate of 1/3 seconds and each was 2.8 volts in amplitude. The black dots below the right geniculate record indicate the moment of application of each stimulus, and it is obvious that in this record the level of success for the evoked waves was 100%.

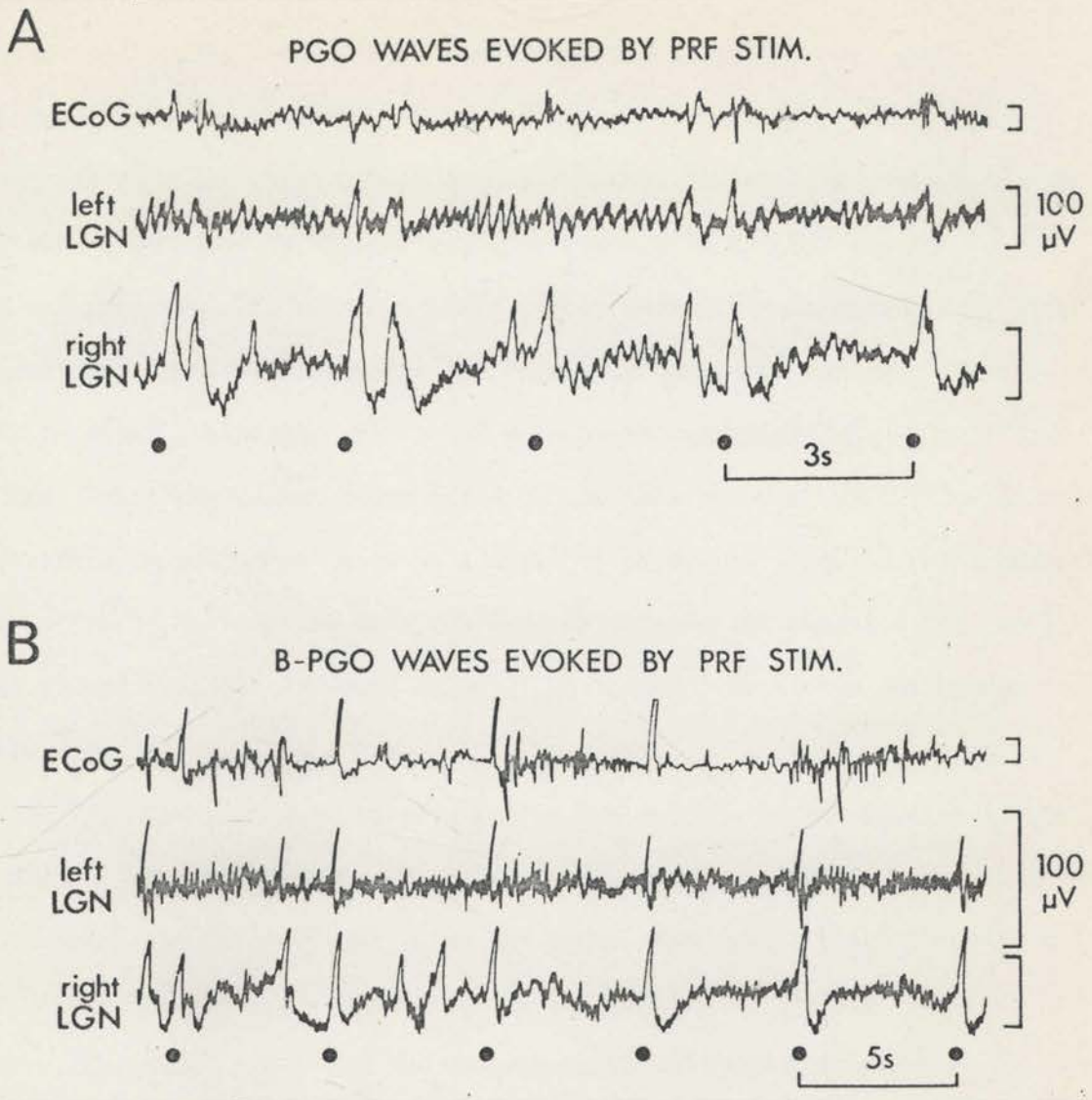


Fig. 1-18: PGO waves evoked by stimulating the PRF (A) during LVF sleep, and (B) during barbiturate anaesthesia. Records obtained from the same cat on different days. The closed circles (●) indicate when each stimulus was applied. In both records note the similarity in appearance of the evoked and spontaneous waves.

Spontaneous PGO waves can also be seen in the right LGN. The record obtained from the left geniculate does not show obvious waves like the right LGN record because the gain of that channel of the electroencephalograph was not great enough. However, small deflections can be seen at the same time as both the spontaneous and evoked waves. Other experiments have shown more clearly than

this that the evoked waves, like those occurring spontaneously, occur synchronously in the two LGN's. When the degree of synchrony was determined between the left and right LGN in one experiment, in only 3 out of 59 trials did a single stimulus to the PRF evoke a response in one LGN but not in the other. Thus, approximately 95% of the stimuli evoked waves in each geniculate. It will be remembered that this resembles the degree of synchrony for the spontaneously occurring waves (values of 92% to 95% were quoted earlier).

The evoked PGO waves resemble the spontaneous waves in amplitude, polarity and waveform. Measurements made on a total of 62 evoked waves in the experiment shown in Fig.1-18A revealed that the latency between the stimulus and the start of the evoked response averaged 12.5ms. The latency was always determined from oscilloscope records of the waves (time base 50ms/cm) as this was far more accurate than taking the measurements from the records on the electroencephalograph.

Stimulation of the PRF also evokes responses in the LGN of the barbiturate anaesthetized cat (Fig. 1-18B). Like the evoked PGO waves, these PRF-evoked B-PGO waves resemble those occurring spontaneously in amplitude, polarity and waveform. The waves evoked in the anaesthetized cat have a longer latency than those evoked during LVF sleep; the average latency for 20 evoked B-PGO waves was 50.8ms (in contrast to the average of 12.5ms given above for evoked PGO waves). The black dots in Fig. 1-18B indicate when the stimuli were applied to the PRF. Each stimulus was of 2.8 volts, and at this strength of stimulation there was always an evoked response. The synchrony between the two LGN's which is evident from this record was better examined by recording responses to large numbers of PRF stimuli. In this experiment, after all but 1 of 33 single shocks applied to the PRF at a rate of 1/5 seconds, there was a response evoked in each geniculate. Thus, there was 97% synchrony for the evoked B-PGO waves. In Fig. 1-18B it is also apparent that pontine stimulation frequently evokes a clear response in the

cerebral cortex.

As spontaneous waves occur in stages of the sleep-wakefulness cycle other than LVF sleep, an attempt was made to evoke similar waves during alertness, non-alertness and slow-wave sleep. Single shocks (pulse width 2ms) were applied to the PRF via the same electrodes used in the LVF sleep study. The data described below were obtained on the same day as that shown in Fig. 1-18A. When the cat was alert and in the dark, the PRF stimuli evoked small amplitude, short-lasting waves in the LGN (approximately 70μV and 50ms respectively). These waves can be seen in Fig. 1-19A, where the moment each stimulus was applied has been indicated. The waves are of the same polarity as the spontaneous and evoked PGO waves of LVF sleep, but comparison of Fig. 1-19A with Figs. 1-9B to D and Fig. 1-18A shows that the waves evoked in the alert cat are not the same as those during sleep. Moreover, the waves evoked during alertness do not resemble the spontaneous eye movement potentials of the same state (compare Fig. 1-19A with Fig. 1-12A).

Stimulation of the PRF also evokes a response in the LGN during non-alertness (Fig. 1-19B), but again, the evoked waves, although of the same polarity, do not resemble those occurring spontaneously in either non-alertness (see Fig. 1-12B), or LVF sleep (see Fig. 1-9B to D). The waves evoked in the non-alert animal tend to be larger in amplitude than those evoked in alertness for a given stimulus strength. Also, the response sometimes consists of a slow wave superimposed on fast wave activity. During SW sleep, the effects of PRF stimulation are difficult to determine because of the high level of background activity (Fig. 1-19C). It would appear that the stimuli were again evoking a response but that this response was very variable in amplitude. The stimuli never evoked a response resembling the spontaneous PGO waves of SW sleep (see Fig. 1-12C).

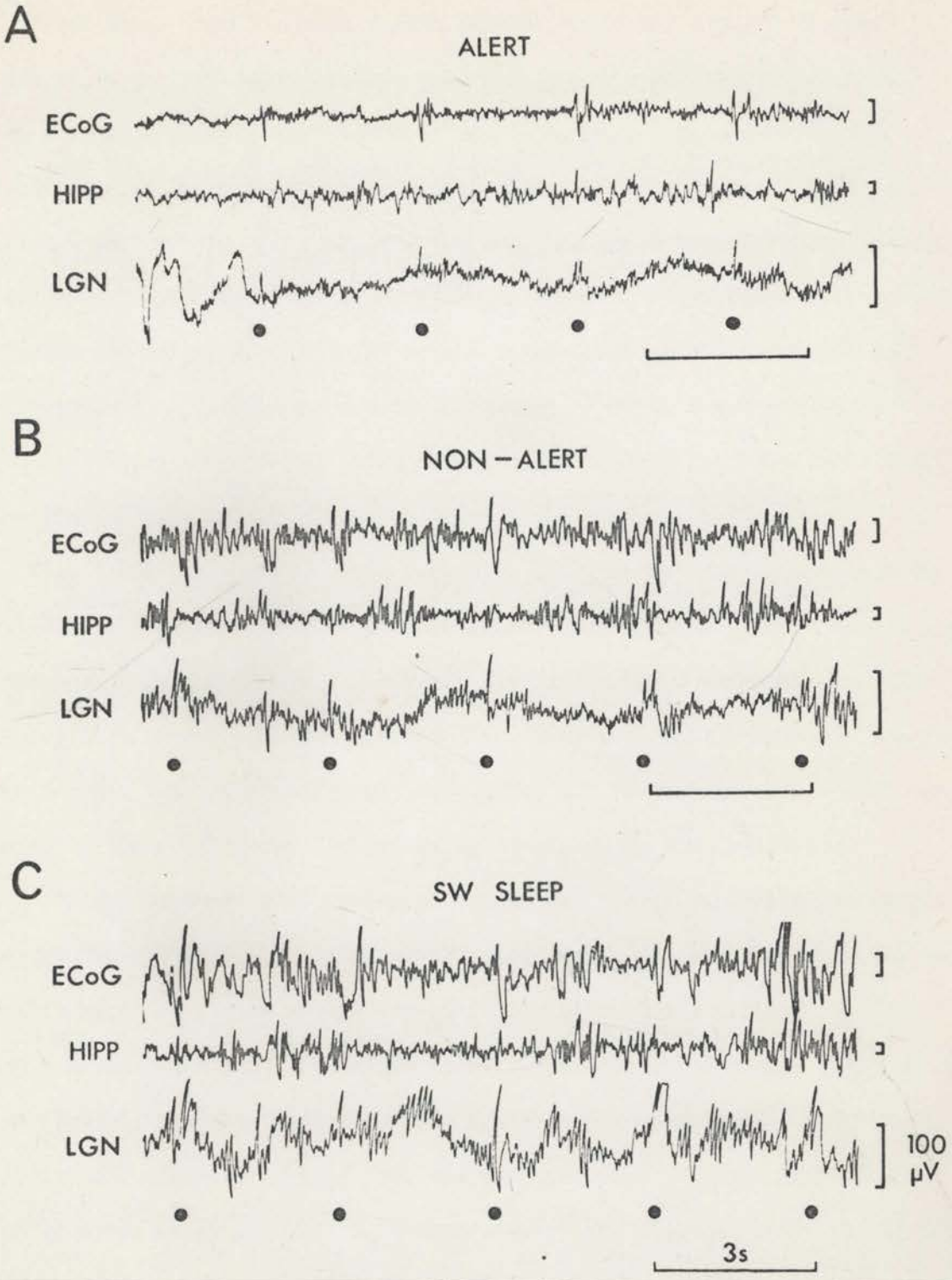


Fig. 1-19: Potential changes evoked in the LGN by stimulating the PRF (A) during alertness, (B) during non-alertness, and (C) during slow-wave sleep. All records were obtained in the dark from the same cat and on the same day. The closed circles (●) indicate when each stimulus was applied.

B-PGO waves were frequently also evoked in the PRF and LGN by making sudden sharp noises. However, this was only the case when the stimuli were given during periods of spontaneously occurring waves. Noises which were effective included clapping, banging two pieces of wood or metal together, and banging a pair of forceps against a tea cup. The noise of the camera and clicks given via a loudspeaker were also effective stimuli. These noise-evoked waves resembled the spontaneous and PRF-evoked waves in polarity, amplitude and waveform. In Fig. 1-20A can be seen an example of B-PGO waves evoked by banging together two pieces of metal - the moment of each noise has been indicated below the record.

Fig. 1-20B shows B-PGO waves evoked in the same cat and on the same day as Fig.1-20A by giving click stimuli through an audioamplifier placed beside the cat in the box. Although the waves shown in Figs.1-20A and B are somewhat different in appearance to all examples shown so far, they were fairly typical looking waves for this cat. This different appearance and reduced amplitude would be related to the position of the recording electrode in the geniculate.

Auditory stimulation by means of clicks was not as successful in evoking waves as the other forms of such stimulation (e.g., claps and bangs). The reason for this seems to lie in the different intensities of the various stimuli. Using a Brüel and Kjær sound level meter (type 2204), the normal background noise level in the observation box was found to be somewhat less than 72 decibels (db) - the reading of 72db was obtained with the doors of the box slightly open, whereas during experiments they were fastened shut, hence reducing the noise level to a value below that determined. The maximum intensity of the click stimulus which could be given via the audioamplifier was only 85db, representing an increase of only 13db over the noise level. Hard hand claps and

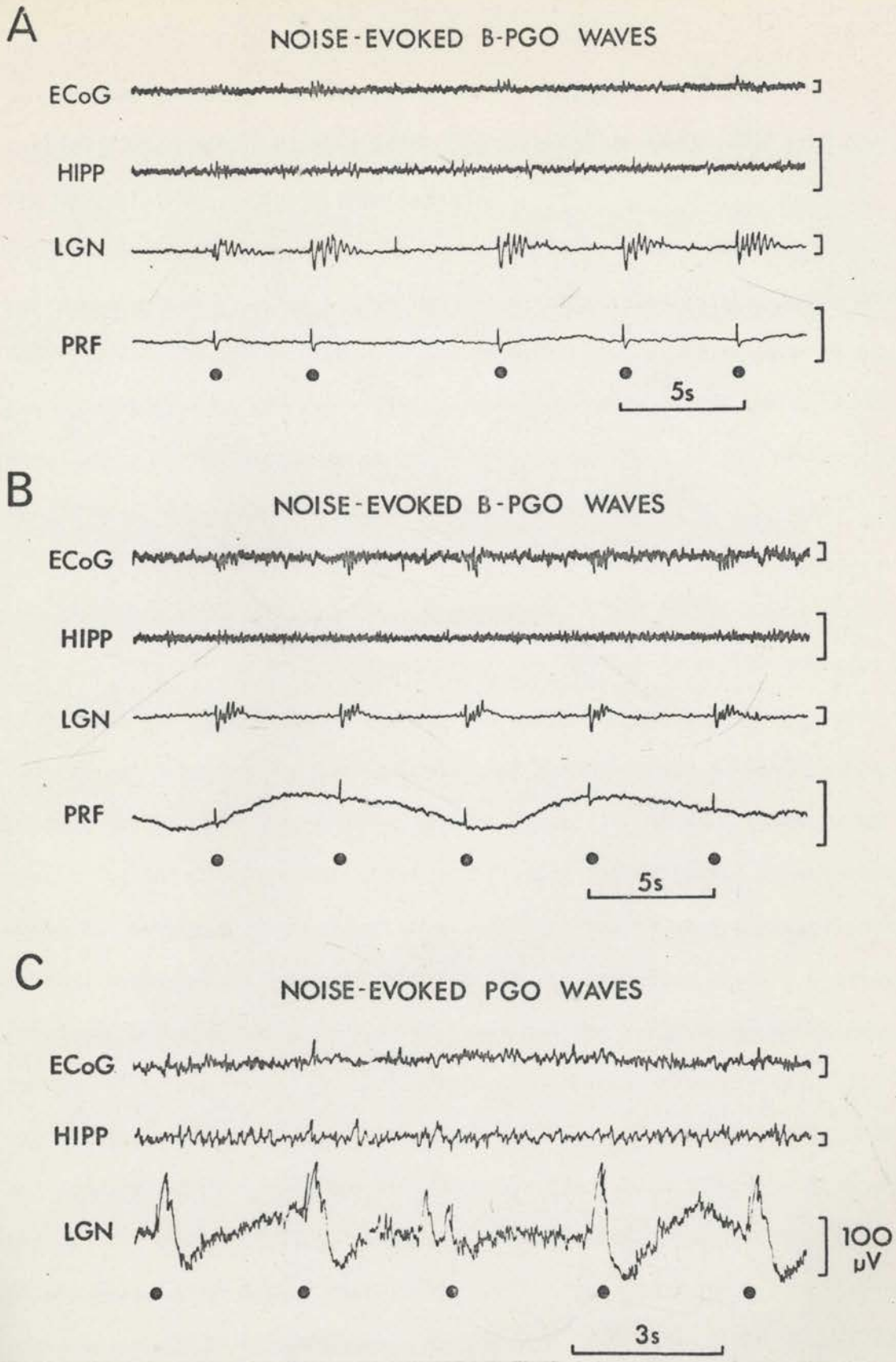


Fig. 1-20: PGO waves evoked by auditory stimulation in the anaesthetized cat (A and B) and the sleeping cat (C). Records in A and B obtained from the same cat, those in C from another cat. For details see text.

banging two pieces of metal together each produced a much larger increase in intensity over the background level (an increase of about 48db was registered for each of these forms of stimulation).

It has also been possible to evoke PGO waves during LVF sleep with the above-mentioned auditory stimuli. Although it was often difficult to evoke waves in the LGN, PRF waves could be evoked with little difficulty. An example of one experiment where click stimuli did successfully evoke PGO waves in the LGN is shown in Fig. 1-20C. As for the noise-evoked B-PGO waves, those in the sleeping cat can be seen to resemble the spontaneously occurring waves.

Discussion

The observation that stimulation of the PRF may evoke PGO waves in the LGN of the cat during LVF sleep supports the evidence presented in the Introduction to this section that the pontine reticular formation and lateral geniculate nucleus are functionally related to each other. It was also found in this study that stimulation of the PRF in the barbiturate anaesthetized animal evoked waves in the LGN which appeared identical with the waves occurring spontaneously in this state. Thus, evidence that the PRF may influence the LGN functionally has been obtained from both the PGO waves and the B-PGO waves which are evoked in the LGN during sleep and barbiturate anaesthesia respectively.

In the present study, it was found that the waves evoked in the sleeping cat occurred after an average latency of 12.5ms (the number of measurements made was 62). On the other hand, Bizzi and Brooks (1963b) reported that the latency of the response evoked by stimulating the PRF was 25-35ms. It is not known why there is this discrepancy between the results of Bizzi and Brooks and those reported here. Perhaps, however, it may be related to different recording

conditions. In the present study all latency measurements were made from oscilloscope records obtained with a time base of 50ms/cm. Moreover, a very narrow pulse was always fed into the record at the moment of stimulation so that the latency could be measured accurately from each sweep as the time between this pulse marker and the start of the evoked response. The figure included by Bizzi and Brooks showing an oscilloscope tracing of a PGO wave evoked by stimulation of the PRF gives no indication that such a procedure was followed by these workers (see Fig. 4 of Bizzi and Brooks, 1963b). The waves evoked by pontine stimulation in the anaesthetized cat in the present study had a longer latency than those evoked in the sleeping cat (average latency of 20 evoked B-PGO waves was 50.8ms).

In the Introduction to this section, evidence was presented that the pontine reticular formation might be the site where the triggering mechanism for the PGO waves of LVF sleep was located. However, Bizzi and Brooks, in addition to proposing this hypothesis, also put forward some evidence against this idea (Bizzi and Brooks, 1963a and b). They reported that there was precise synchrony between the waves occurring spontaneously in the PRF and LGN, with the interval between corresponding waves in these two regions never exceeding 5ms. On the other hand, the waves evoked in the LGN by stimulating the PRF had a latency of 25-35ms. If the PRF does represent the site where the waves are initiated, it is certainly surprising that the evoked waves in the LGN do not occur after a latency which is comparable to the interval between the spontaneously occurring waves in the LGN and PRF. The results obtained in the present study, however, contribute to the understanding of this problem. It was reported in the Results that the latency difference between the spontaneous PGO waves in the pons and the geniculate could be up to 15ms, while the evoked waves had an average latency

of 12.5ms. The close correspondence between these two values is certainly in accordance with the idea that the PRF is the site where the triggering mechanism for these waves resides.

In confirmation with the observation made by Bizzi and Brooks (1963b) it was not possible to evoke PGO waves with PRF stimuli in any state of the sleep-wakefulness cycle other than LVF sleep. However, as shown in Fig. 1-19, stimulation of the pontine reticular formation in the three states of alertness, non-alertness and slow-wave sleep did evoke a short-lasting response in the LGN. Nevertheless, the responses evoked in these three states do not resemble the waves occurring spontaneously in the same states. It is therefore evident that by some mechanism, as yet unknown, the stimuli applied to the PRF will only evoke a PGO wave in the LGN if the cat is in a specific state of the sleep-wakefulness cycle (i.e., LVF sleep). If in fact the waves are triggered by some mechanism located in the pons, it may be that during the states of the sleep-wakefulness cycle other than LVF sleep there is a marked increase in the threshold of the pontine triggering mechanism. Alternatively, during these other states there may be an increased threshold in the geniculate for the signals arriving there in response to the pontine stimulus. Finally, it is possible that both these alternatives may contribute in some way to the observed failure of the PRF stimuli to evoke PGO waves in alertness, non-alertness and slow-wave sleep. The fact that both geniculates behave in an identical way, however, would tend to favour the idea that the mechanism responsible for the failure of the stimuli to evoke waves in states other than LVF sleep resides in the PRF, not in each LGN.

Evidence was also presented that PGO (and B-PGO) waves may be evoked in the LGN and PRF by auditory stimulation. These noise-evoked waves resemble the

waves occurring spontaneously and the waves evoked by stimulation of the PRF. As mentioned earlier, there have been manyother reports that peripheral stimuli may influence the LGN, and it would appear that such influences may be mediated via the reticular formation where information from different sensory modalities has been shown to converge on to single neurones.

Frequently it was difficult to evoke responses in the LGN of the sleeping cat using the different forms of stimulation mentioned in the Results, while at the same time the stimuli did evoke pontine waves. Therefore it is possible that the mechanism responsible for triggering the geniculate waves (which,as mentioned above, may very probably reside in the PRF) may pass through periods of responsiveness alternating with periods in which the responsiveness of the triggering mechanism is very greatly reduced. In accordance with this idea is the observation made by Scheibel and Scheibel (1965b) that, in cats immobilized with Flaxedil, single cells in the reticular formation may show periods of maximum driving responses to sciatic nerve stimulation, whisker pulls and flash and click stimuli, alternating with periods of relative or absolute non-responsiveness to the same stimuli. There is another factor, however, which may have contributed to the difficulty experienced in evoking waves (both PGO and B-PGO) with auditory stimuli. Frequently, it was noticed that when the stimulation was commenced, each stimulus evoked a response in the LGN. However, after a short time, the responses appeared to decrease rapidly in amplitude to successive stimuli until no further evoked responses were seen - i.e., it appeared that the response was habituating rapidly to the stimulus. Such habituation could, conceivably, occur in the LGN or in the region responsible for triggering the evoked waves, or in the auditory pathway.

In the present study no attempt was made to examine this problem further,

so it is not possible to conclusively isolate any one structure as being the site responsible for the observed effect. However, there is evidence that, in some conditions at least, habituation may occur in the reticular formation and in the auditory system. Scheibel and Scheibel (1965a) observed that in awake, immobilized cats, 75% of reticular units showed habituation to a sciatic nerve stimulus repeated slowly over time; intervals of 30 to 100 minutes were needed for the return of full responsiveness to most of the units. No such habituation was observed when the cats entered SW sleep, and no observations were made on cats in LVF sleep. Although these results show that reticular units may show habituation to sciatic nerve stimuli, it is not feasible to conclude that similar habituation would occur following auditory stimulation. Nevertheless, the results do suggest that this possibility is worth investigating. There have been many reports that evoked responses recorded from various regions of the auditory pathway show habituation to repeated acoustic stimuli (e.g., Wickelgren, 1968b; Holstein, Buchwald and Schwafel, 1969; Jaffe, Bourlier and Hagaman, 1969; Webster, 1969; Fruhstorfer, Soveri and Järvilehto, 1970). Therefore it seems likely that such auditory habituation may have been responsible at least in part, for the difficulty experienced in the present study when trying to evoke PGO and B-PGO waves with auditory stimuli.

In the next part of this section the effects of the two kinds of wave on synaptic transmission in the LGN are examined. It will be shown that both PGO and B-PGO waves have similar effects on responsiveness, regardless of whether the waves are spontaneous or are evoked by stimulation of the PRF.

3. The Effects of Spontaneous and Evoked Waves on Synaptic Transmission in the LGN of the Sleeping and Anaesthetized Cat.

(i) Excitability changes associated with the PGO waves of LVF sleep.

There have been reports by several other workers that spontaneous PGO waves modify LGN excitability (see the Introduction). Therefore, the changes in excitability produced by these waves will only be presented briefly before the effects of evoked PGO waves are considered.

It was mentioned earlier that PGO waves appear before the other electroencephalographic and behavioural signs of LVF sleep become evident. For this reason, all the experiments in which the effects of the waves on geniculate excitability were examined were conducted exclusively on the PGO waves occurring after LVF sleep was well developed. As the waves frequently coincide with rapid eye movements, it is essential to avoid photic stimulation when examining the effects of the waves on responsiveness. Therefore, all studies during LVF sleep were done with the animal in the dark. The responses of LVF sleep were separated into those occurring during PGO waves and those occurring between the waves. To avoid interaction between the effects of adjacent waves, an evoked response was classified as occurring during a PGO wave only if the wave was separated from the preceding wave by at least 1 second. Thus, responses evoked during the second and later waves of a burst of PGO waves were excluded. For a response to be considered as being unaffected by the waves there had to be no PGO wave in the preceding 1 second. The above criteria were adopted when examining the effects of either spontaneous or evoked PGO waves on responsiveness.

The magnitude of the changes in both the orthodromic and t (antidromic) responses were assessed by recording a series of approximately half-maximal responses during and between the PGO waves. The responses obtained in each of these conditions were averaged, and those occurring during the PGO waves were

expressed as a percentage of the mean response amplitude between the waves. Details have previously been given of (i) the positions of the stimulating and recording electrodes, (ii) the parameters of the stimulating pulses, and (iii) the components of the evoked responses. Briefly, the orthodromic LGN response was evoked by applying square pulses of 50μs duration between two electrodes in the optic tract. As shown in Fig. 1-8A, the response consists of a presynaptic and a postsynaptic component (the t and r waves respectively). The t(antidromic) response was evoked by applying stimuli of 20μs duration between two electrodes in the LGN. The response recorded from the ipsilateral OT consists of two waves (see Fig. 1-8B) due to impulses travelling along two groups of fibers with different conduction velocities.

In Fig. 1-21 can be seen the changes found in a single experiment in the orthodromic response during the spontaneous PGO waves of LVF sleep.

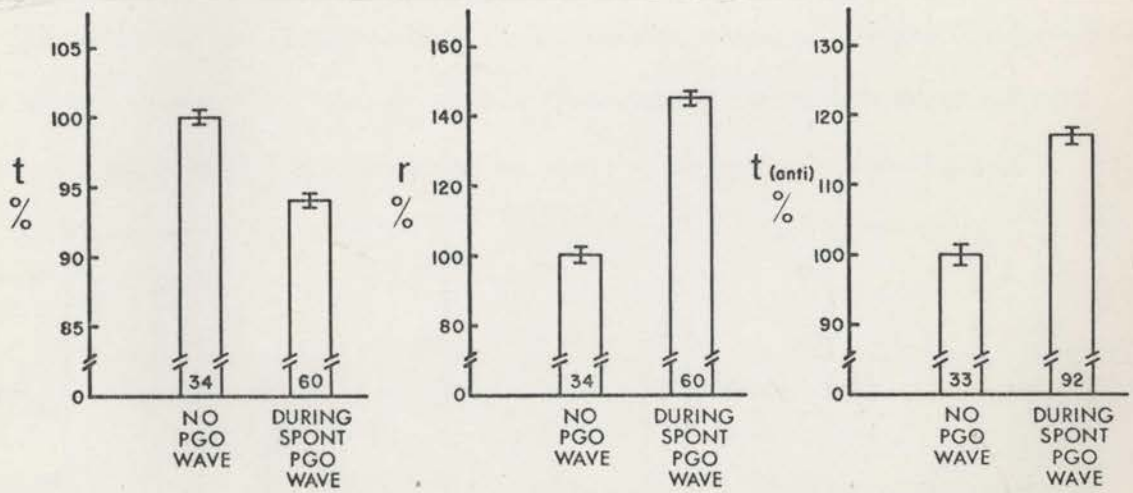


Fig. 1-21: The effects of spontaneous PGO waves on the t, r and t(anti) responses in the sleeping cat. Results for t and r from a single experiment and for t(anti) from another experiment on the same cat. The mean of the responses during the waves is expressed as a percentage of the responses evoked when there was no wave in the preceding 1s. The vertical bars indicate ±1 standard error of the mean. The figure at the base of each column is the number of responses averaged.

During the waves, the presynaptic (t) response was depressed to 94% of the control level. The r response was greatly enhanced, the average amplitude being 145% of

the level between the waves. It was shown using Wall's method (Wall, 1958) that the presynaptic change was due to a change in the level of presynaptic depolarization. When the excitability of the optic tract endings in the LGN was tested by stimulating the LGN and recording the antidromic response in the tract, it became evident that excitability was phasically enhanced during the waves. The results obtained in one such experiment are shown in the graph on the far right of Fig. 1-21; only the change in the first wave of the response has been considered. During the PGO waves the t(anti) response was increased to 117% of the control level, indicating that the waves were associated with phasic enhancement of the level of depolarization of the OT endings. Thus, during the waves of LVF sleep the excitability of both the LGN cells and the OT endings is phasically enhanced.

As stimulation of the PRF during LVF sleep evokes waves in the LGN which look identical to the spontaneously occurring PGO waves, it seemed likely that these evoked waves might produce similar changes in geniculate responsiveness. Therefore, excitability was examined as above - for results see Fig.1-22.

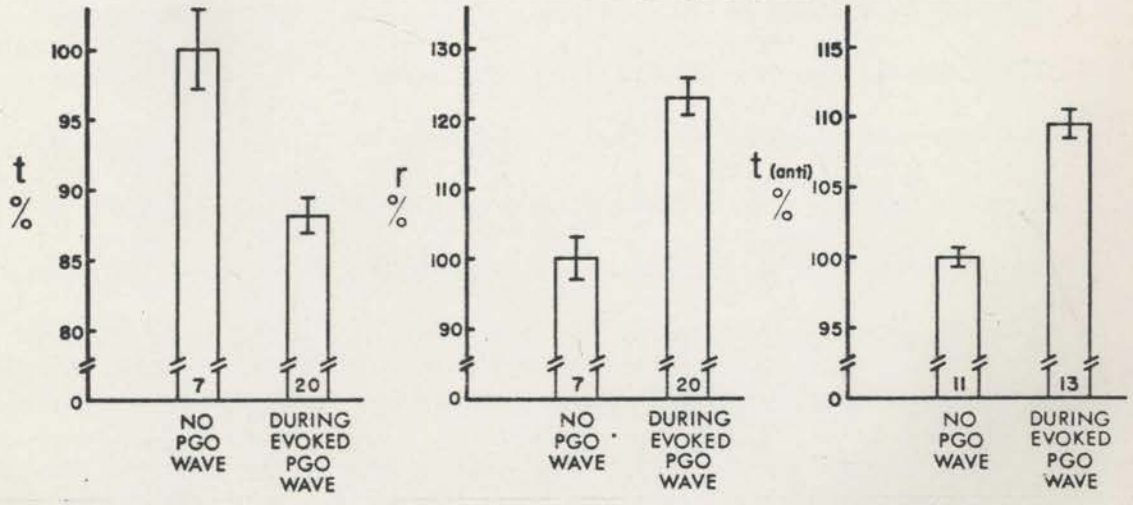


Fig.1-22: The effects of evoked PGO waves on the t,r and t(anti) responses in the sleeping cat. Results for t, r and t(anti) obtained from single experiments done on the same day. Further details as for Fig. 1-21.

Conditioning stimuli were applied to the PRF and after a delay of 100ms the test stimulus was applied to either the OT (for the orthodromic response) or the LGN (for the t(antidromic) response). This 100ms delay meant that the test stimulus was applied very close to the peak of the evoked wave. Control responses were obtained by giving single test stimuli without a preceding conditioning stimulus to the PRF. For all the control responses there had to be at least 1 second with no spontaneous PGO wave preceding the stimulus.

Comparison of the changes in t and r in Figs. 1-22 and 1-21 reveals that the evoked PGO waves affect the orthodromic LGN response in the same way as the spontaneously occurring waves. In the experiment shown in Fig. 1-22 the t response was reduced to 88.1% of the control level when it was recorded during an evoked PGO wave. At the same time there was enhancement of the r response to 122.9% of the control level, and of the first wave of the t(anti) response to 109.3% of the control level.

(ii) Excitability changes associated with B-PGO waves.

Because of the similarity of the waves found in the LGN of the sleeping and anaesthetized cat, the effects of both spontaneous and evoked B-PGO waves on geniculate responsiveness were determined. The waves in the anaesthetized cat are not associated with eye movements, and for this reason it was not necessary to carry out the experiments with the cat in the dark.

Examination of the time-course of the effects of the B-PGO waves on the postsynaptic orthodromic response has revealed that these are more long-lasting than are the changes associated with the waves of sleep. By triggering the OT stimulus at various intervals after the start of the spontaneous waves it can be seen that the postsynaptic changes usually last slightly less than 3.5 seconds. An example of one experiment in which the time-course of the change in the

orthodromic response was examined is shown in Figs. 1-23 and 1-24.

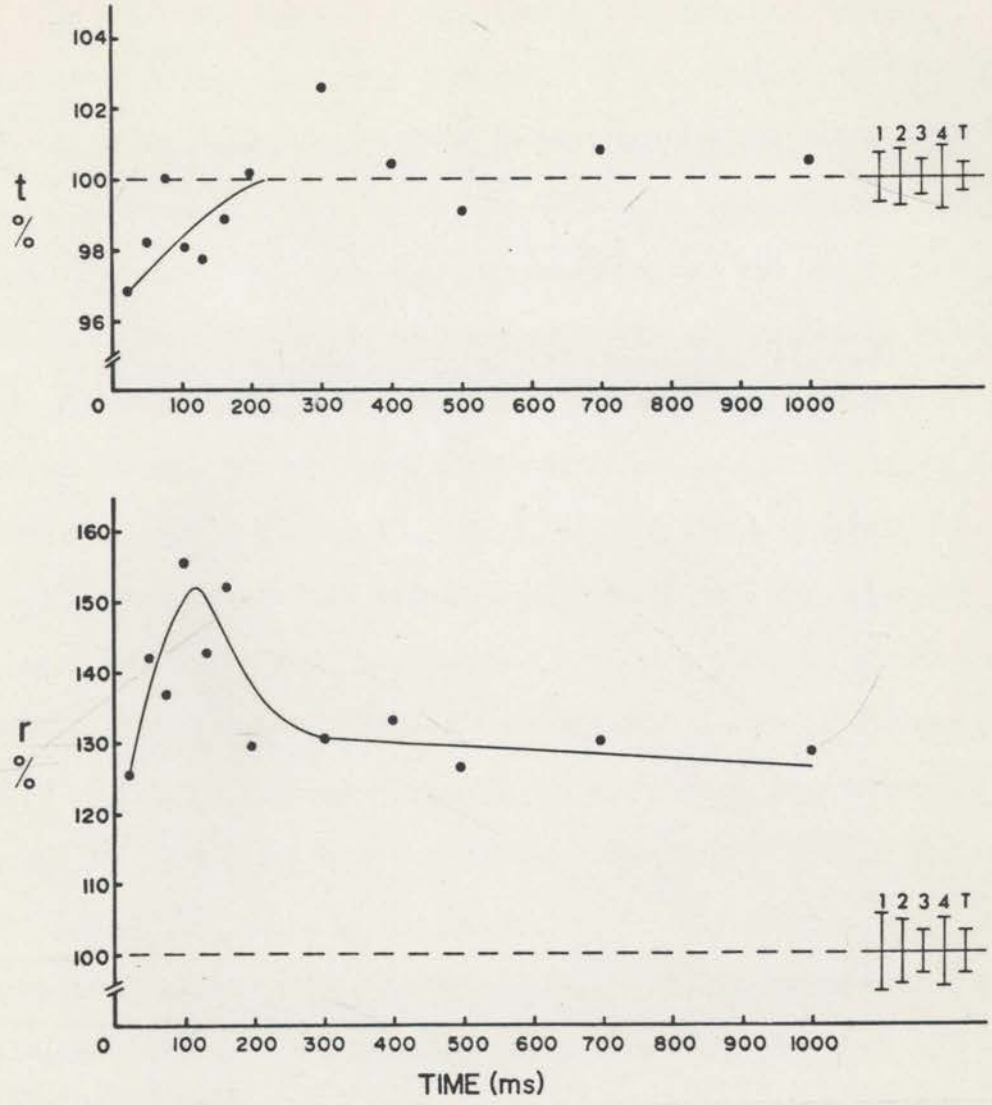


Fig. 1-23: Time-course of the effects of B-PGO waves on the orthodromic response. For details, see text.

Each point on the graphs represents the average value of between 5 and 22 responses. As it took two hours to complete this particular time-course study, the data were divided into 4 groups, each group consisting of the responses in a particular half-hour period. The control responses in each half-hour period were those responses which, during that time, were evoked at intervals of greater than 5 seconds after the start of the nearest preceding B-PGO wave.

All other responses in any particular half-hour period were subdivided according to the time after the start of a wave when the OT stimulus was given, and each response was then expressed as a percentage of the control level for that half-hour period.All the percentage values so obtained for responses evoked at the different intervals after the start of a wave were then averaged to obtain the mean percentage value for that time. By analysing the results in this way it was hoped that any effects due to changing levels of anaesthesia would be removed. The bars at the right hand end of the 100% levels in each graph show the standard errors for the means of the different groups of control responses; the first four bars (numbered 1, 2, 3, 4) are the standard errors for the responses in each of the four half-hour periods (1 to 4 respectively), and the final bar (labelled T) shows the standard error for the mean of all the control responses taken together. In Fig.1-24 the standard errors of the means of the responses at times 3.5, 4 and 5 seconds are also shown. The curves drawn in each graph were estimated by eye to be the lines of best fit for the plotted points.

Looking first at the lower graph in Fig. 1-23, it can be seen that the postsynaptic response was enhanced to slightly greater than 150% of the control level at approximately 100ms after the start of a B-PGO wave. The curve is still considerably elevated at time 1 second (about 125%). Fig. 1-24 shows the same results plotted on a different time-scale so that the full time-course of recovery can be seen. It is obvious that in this particular experiment the effects of the waves on the r response were not complete until 4.5 to 5 seconds after the start of a wave. However, similar experiments on 3 other occasions have shown that the effects of the waves on the postsynaptic response usually last slightly less than 3.5 seconds.

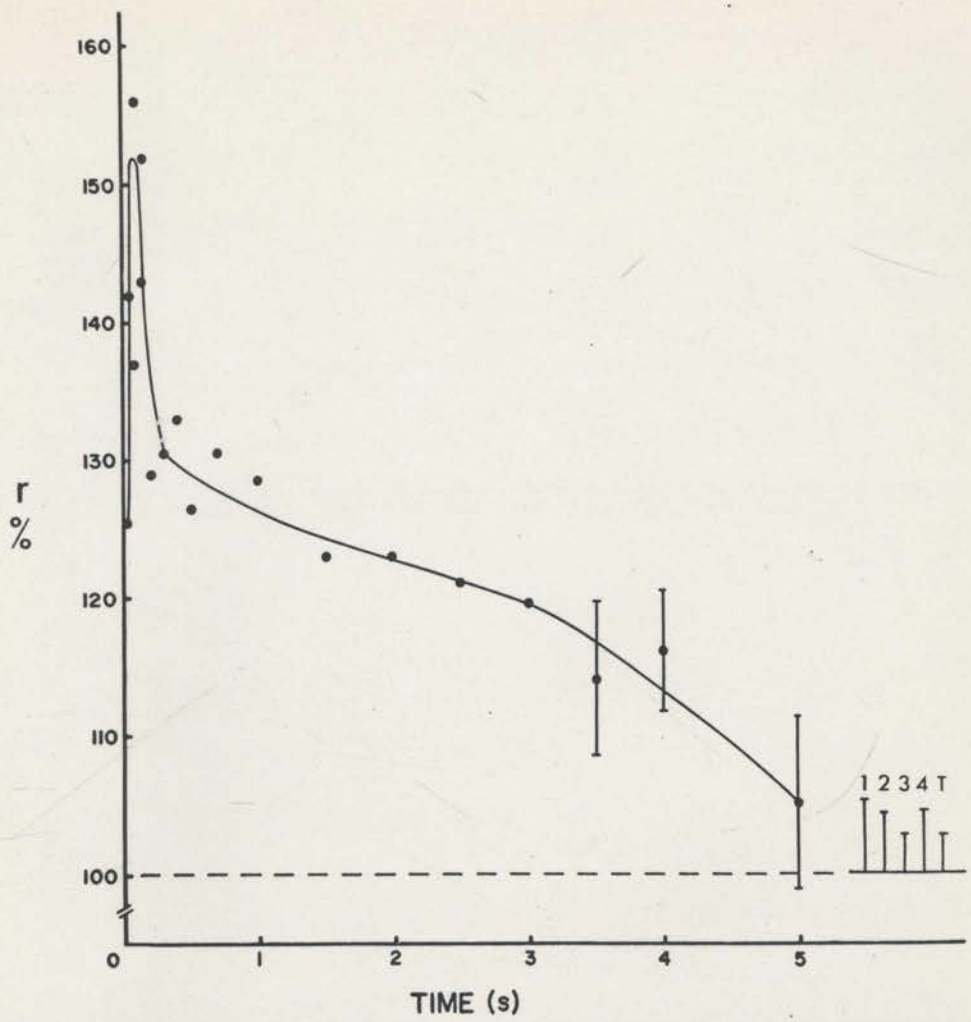


Fig. 1-24: Time-course of the effects of B-PGO waves on the r response. Same experiment shown in Fig. 1-23, but time scale altered to show full time-course of the effects of the waves on the postsynaptic response.

Turning now to the effects of the B-PGO waves on the presynaptic response it can be seen from the upper graph of Fig. 1-23 that during the first 200ms after the start of the wave there is depression of the t response. In this experiment the response was only depressed to slightly less than 97% of the control level, but depression to 92% was seen in another experiment. Preliminary observations on the effects of the B-PGO waves on the t(anti) response showed that this response was enhanced during the waves. Therefore, the time-course of the effects of the waves on this response was also determined (Fig. 1-25).

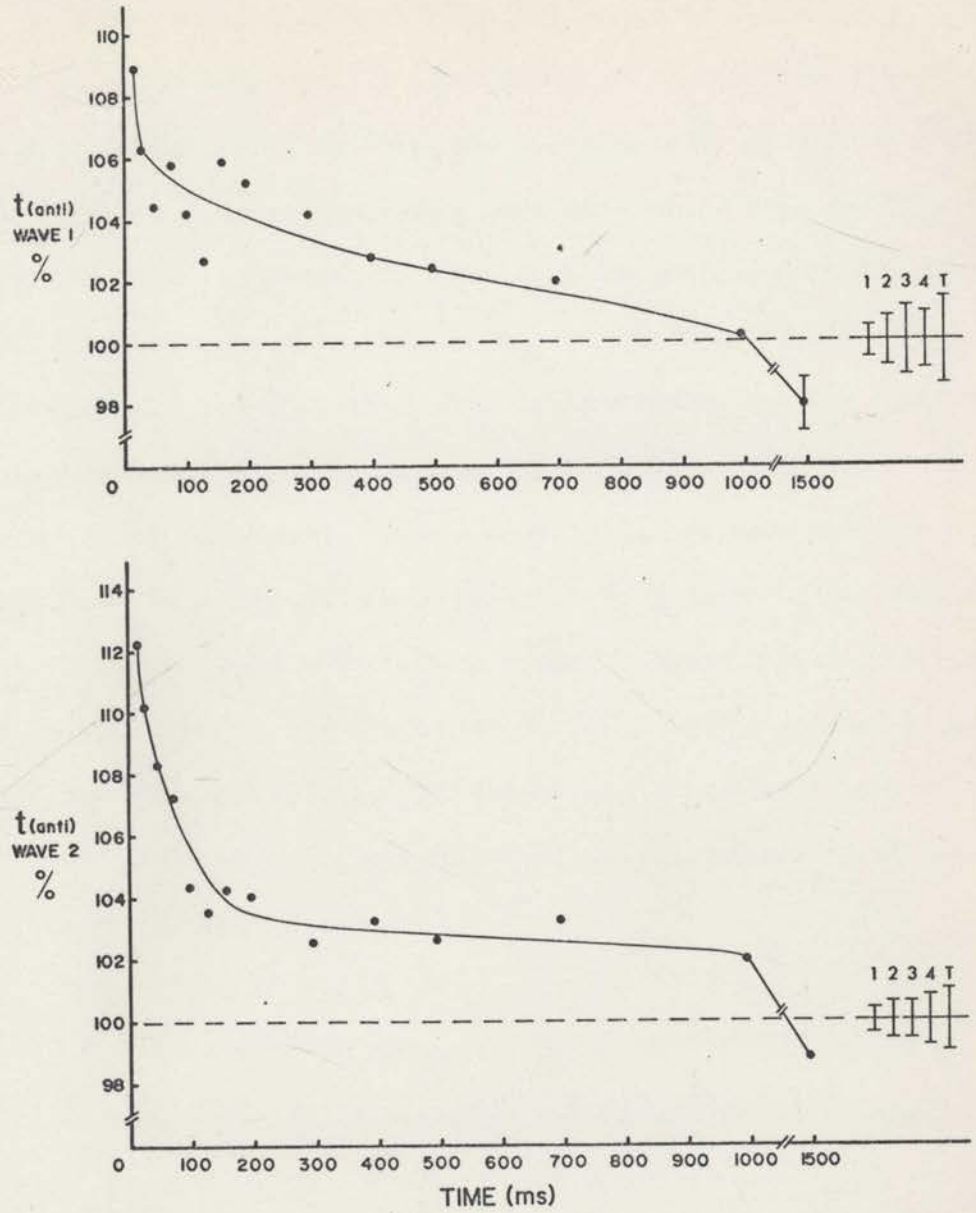


Fig. 1-25: Time-course of the effects of B-PGO waves on the t(antidromic) response. For details, see text.

In Fig. 1-25, the upper graph shows the changes in the first wave of the response, and the lower graph the changes in wave 2. The results of this experiment (which was done on the same cat, but on a different day to the experiment shown in the two previous figures) were analysed in exactly the same way as described for the orthodromic time-course study. The data were split into four half-hour

periods, each with its own control level, and responses at different times after the start of a wave were expressed as a percentage of the control level for the particular half-hour period in which the response fell. As before, the control responses during the time-course study were those which were preceded by at least 5 seconds without a B-PGO wave. Each point on the graphs represents an average of between 5 and 18 responses. The curves were estimated by eye to be the lines of best fit for the plotted points. The standard errors for the means of the control responses are shown in the same way as in Figs. 1-23 and 1-24, and the standard error for the mean of the responses at 1.5 seconds has also been indicated. From the graphs it can be seen that both waves of the response were significantly enhanced above the control level at short intervals after the start of the B-PGO waves. The first wave of the t(anti) response was enhanced to 109% of the control level, and the second wave to slightly more than 112%, at time 20ms. The effects of the B-PGO waves on both components of the t(anti) response were over within 1 second.

As for the studies during LVF sleep, the extent of the changes in excitability in the anaesthetized cat were assessed by recording a series of approximately half-maximal responses. These responses were separated into those occurring during & between the B-PGO waves. In view of the results just presented (Figs. 1-23 to 1-25), in the bar graphs which follow, showing the effect of the B-PGO waves on geniculate excitability, the r responses were classified as occurring during a B-PGO wave if there was a wave in the preceding 3.5 seconds; to avoid interaction between the effects of adjacent waves, the wave had to be separated from the preceding wave by at least 3.5 seconds. The control r responses (100%) were those which were preceded by at least 3.5 seconds with no spontaneously occurring waves;

these control responses included those evoked during the periods of B-PGO waves and at times of no waves. Regarding the effects of the waves on the t and t(anti) responses, the 100% level was determined by the average amplitude of all responses occurring at an interval of greater than 1 second after the start of a wave.

Changes in the orthodromic and antidromic responses during spontaneous B-PGO waves are shown in Fig. 1-26. The results are from single experiments done on the same cat on different days.

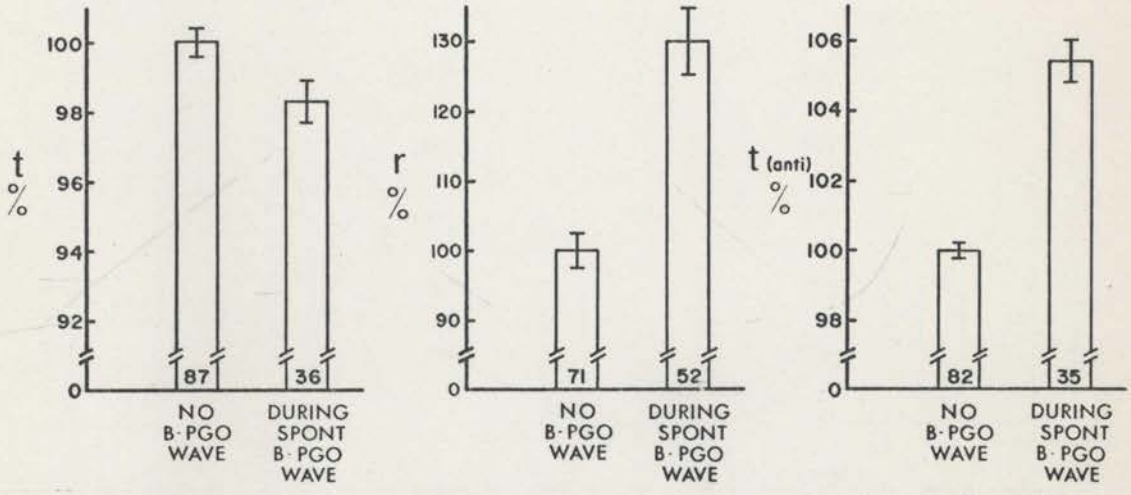


Fig. 1-26: The effects of spontaneous B-PGO waves on the t, r and t(anti) responses. Data for the orthodromic response changes obtained from a single experiment and for the antidromic response from a single experiment done onthe same cat but on a different day. The mean of the responses during the waves is expressed as a percentage of the responses evoked when there was no preceding wave for 1s (t and t(anti) responses) or for 3.5s (r response). The vertical bars indicate ±1 standard error of the mean. The figure at the base of each column is the number of responses averaged.

In the experiment shown, the t response evoked during the waves was reduced to 98.3% of the control level. The r response was enhanced to 130% of the control level during the B-PGO waves. When the t(anti) response was used to examine the excitability of the optic tract endings it was found that the spontaneous B-PGO waves were associated with phasic enhancement of the level of depolarization; during the waves the average amplitude of the t(anti) response was enhanced to 105.4% of the control level.

The effects on LGN excitability of B-PGO waves evoked by PRF stimulation have also been determined. As for the experiment on the sleeping cat, the interval between the conditioning and test stimuli was 100ms. The control responses were those preceded by neither a spontaneous wave nor a PRF stimulus. It can be seen from Fig. 1-27 that the effects of the evoked B-PGO waves on LGN excitability were the same as the changes seen following the spontaneous waves; the t response was reduced in amplitude, while both the r and t(anti) responses were enhanced (97.6%, 150.7%, 109.5% respectively).

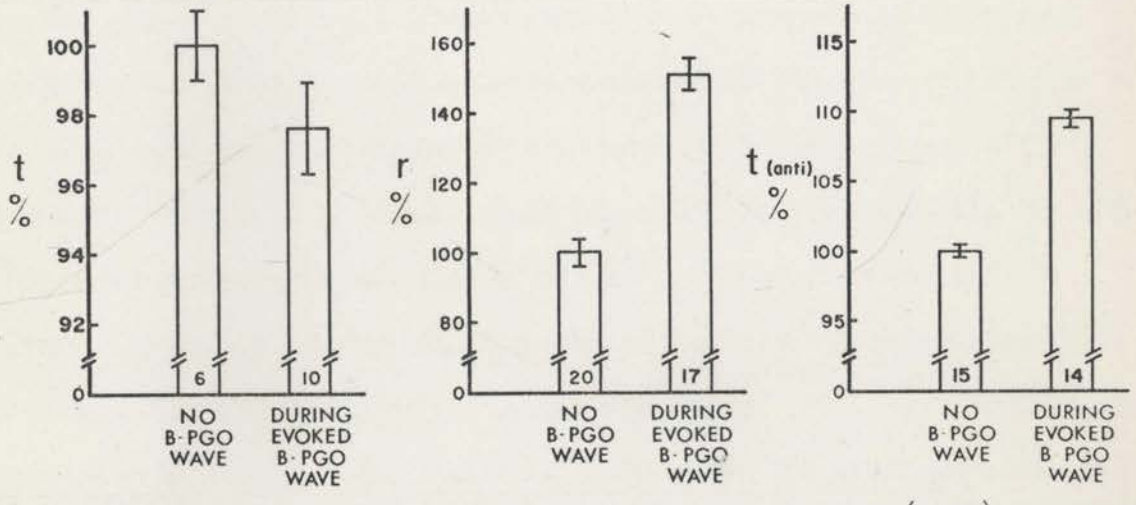


Fig. 1-27: The effects of evoked B-PGO waves on the t, r and t(anti) responses. Data obtained from single experiments done on the same day. Results plotted in the same way as Fig. 1-26.

Discussion

In the preceding pages the effects of four types of phasic slow-wave activity on synaptic transmission through the LGN were considered. These four types of activity were (i) spontaneously occurring PGO waves, (ii) PGO waves evoked by stimulation of the PRF, (iii) spontaneously occurring B-PGO waves, and (iv) B-PGO waves evoked by stimulation of the PRF. It was shown in the Results that these four types of wave all produced similar changes in the

orthodromic LGN and antidromic OT responses - that is, the waves were accompanied by a decrease in the amplitude of the t response, and increases in the amplitudes of the r and the t(antidromic) responses.

The observed changes in the orthodromic and t(antidromic) responses during the spontaneous PGO waves of LVF sleep are in confirmation with the findings of other workers (e.g., Sakakura and Iwama, 1965; Bizzi, 1965, 1966a; Iwama and Kasamatsu, 1966; Iwama et al., 1966; Kasamatsu and Iwama, 1966; Dagnino et al., 1969; Malcolm et al., 1970). These workers reported that during the PGO waves there was phasic enhancement of the level of presynaptic depolarization to a level above that in the intervals between the waves. This phasic change in the level of presynaptic depolarization is accompanied by an increase in the postsynaptic excitability. Sakakura and Iwama (1965), when examining the effects of PGO waves evoked by stimulation of the PRF on the orthodromic LGN and antidromic OT responses, also observed that the effects of such evoked waves were similar to the effects of the spontaneous waves.

In view of the essential similarity between the PGO waves recorded in the LGN of the sleeping and barbiturate anaesthetized cat, it was not surprising to find that the spontaneous B-PGO waves affected synaptic transmission in the same way as the spontaneous waves occurring in sleep. Similarly, the effects of the B-PGO waves on geniculate responsiveness were not unexpected.

Although PGO waves and B-PGO waves have similar effects on the LGN, the time-course of these effects differs. Bizzi (1965, 1966a) has reported that the waves of sleep alter the presynaptic response (recorded either from the LGN or the OT) for only about 60-65ms. Sakakura and Iwama (1965), on the other hand, observed depression of the t response for 200ms after an evoked wave. Sakakura and Iwama also reported that the evoked waves affected the postsynaptic response

for approximately 300ms. In the present study the time-courses of the effects of spontaneous B-PGO waves on the orthodromic and antidromic responses were examined and it was found that the presynaptic effects lasted about 1 second (see Fig. 1-25) while the postsynaptic effects were much more long lasting, usually having a duration of 3.5 seconds.

Thus, from the results presented so far in this section, it is evident that the PGO and B-PGO waves found respectively in the sleeping and the anaesthetized cat are essentially similar events. Both types of wave are recorded from the same electrode positions,and they have the same polarity and similar waveforms. Moreover, both types of wave are evoked by stimulation of the pontine reticular formation and by auditory stimuli. Finally, both the spontaneously occurring and the evoked waves (PGO and B-PGO) are associated with the same excitability changes in the lateral geniculate nucleus (that is, an increased excitability of both optic nerve endings and principal LGN cells). In the Results presented in the next part of this section it will be shown that a more detailed analysis of certain of the characteristics of the waves also points to their essential similarity.

4. PGO Waves as Unitary Events.

Brooks and Bizzi obtained some evidence suggesting that the PGO waves found in the LGN during LVF sleep might be regarded as unitary events. For example, it was reported that although the spontaneous waves did show some variation in amplitude, "at a given electrode position, however, the amplitude was rather constant from wave to wave" (Brooks and Bizzi, 1963). Secondly, these same workers found that PGO waves could be evoked in the LGN by stimulating the PRF (Bizzi and Brooks, 1963b). They reported that the evoked waves "seemed

to be 'all or none' in nature". With the stimulus intensities normally used by Bizzi and Brooks, about four out of every five PRF stimuli during any given episode of LVF sleep were effective in producing responses in the LGN, and the amplitude of these responses was rather uniform and comparable to that of the spontaneously occurring waves. When the intensity of the stimulus to the pons was reduced from the usual value it was found that the amplitude of the evoked waves was not altered, the only effect being a reduction in the proportion of stimuli evoking PGO waves in the LGN.

In the present series of experiments, additional evidence has been obtained for the unitary nature of the PGO waves found in the sleeping cat. The waves found in the barbiturate anaesthetized cat also appear to be unitary events. In the following pages the evidence obtained to support this concept of the unitary nature of the PGO waves found in the sleeping and barbiturate anaesthetized cat is presented.

(i) Experiments on the Sleeping Cat.

In four experiments large numbers of spontaneous PGO waves were recorded in the LGN during LVF sleep. Histograms were then plotted showing the number of waves having the different amplitudes observed. All experiments were carried out with the cat in the dark. In Fig, 1-28 are shown two such plots obtained from two different cats. It will be remembered that PGO waves may occur singly or in groups of up to about 8 waves (see Fig. 1-10). In the two histograms shown in Fig 1-28, the only amplitude measurements included were made from the baseline to the peak of the single waves, and from the baseline to the peak of the first wave for the groups of PGO waves. Thus, these two histograms show the amplitude variations of single PGO waves in two different cats. Obviously, if the peak amplitudes of the groups of waves were included, the true nature of the range of amplitudes of the single waves would not be revealed.

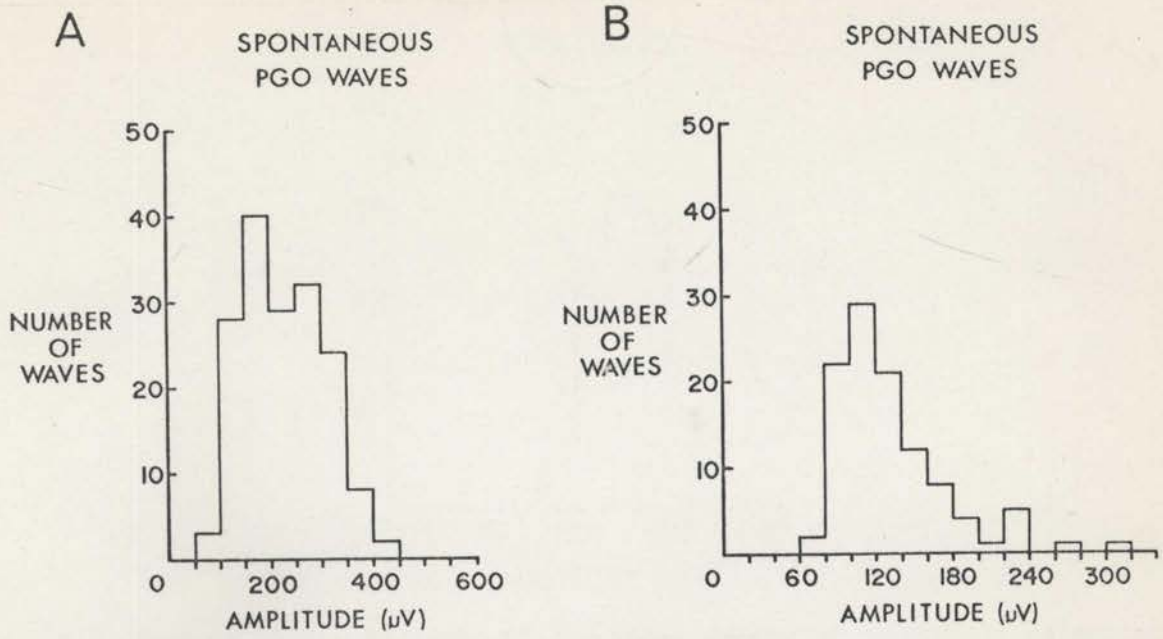


Fig. 1-28: Histograms of amplitudes of spontaneous PGO waves recorded in single experiments on two different cats. Both experiments done with the cats in the dark. (A) Number of waves = 166; (B) number of waves = 106.

Although in the experiment shown in Fig. 1-28A the PGO waves ranged in amplitude from 80 to 432μV (average 223μV; number of waves = 166), this variation is not great. The histogram of amplitudes plotted in Fig. 1-28B shows that the waves recorded could show even less variation; in this particular experiment the range of amplitudes of 106 waves was only 70 to 310μV (average 129μV). The coefficients of variation of amplitude were determined in these two experiments and in the two other experiments in which large numbers of spontaneous PGO waves were recorded. The values obtained were less in the two experiments not shown (29.3% and 29.9%) than in those illustrated in Fig. 1-28 (35.4% and 33.4% for A and B respectively).

The histogram of amplitudes of PGO waves evoked by stimulating the PRF with supramaximal shocks was also determined in one experiment (Fig. 1-29). The range of amplitudes of the 105 evoked waves was 90 to 235μV (average 150μV); the coefficient of variation in this experiment was only 15.7%.

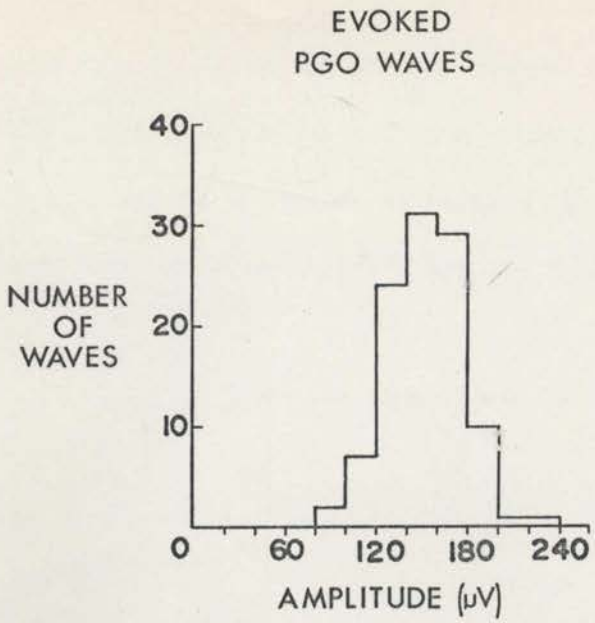


Fig. 1-29: Histograms of amplitude of PGO waves evoked by electrical stimulation of the PRF (pulse width = 2ms). N = 105.

From the above evidence, therefore, it is apparent that the amplitudes of PGO waves (both spontaneous and evoked) are not widely scattered.

Although Fig. 1-29 shows that PGO waves evoked by applying single supramaximal shocks to the PRF are rather constant in amplitude, it does not give any indication as to whether or not the amplitude of the evoked waves is independent of stimulus strength. In view of Bizzi and Brooks' observation that the amplitude of the evoked waves was not altered by changing the stimulus intensity (Bizzi and Brooks, 1963b), an experiment was done in which stimuli of different intensities were applied to the PRF and the amplitudes of the responses so evoked were measured. In confirmation with Bizzi and Brooks, it was found that as the stimulus intensity was reduced below the level normally used, the amplitude of the response remained the same as when the supramaximal shocks were applied. Thus, the amplitude of evoked PGO waves is not in any way dependent on the intensity of the stimulating pulses. Changes in the strength of the stimulating pulses do, however, affect the proportion of evoked responses.

The relationship between the strength of the stimuli applied to the PRF and the level of success with which the stimuli evcked waves in the LGN is shown in Fig. 1-30. The percentage success at each stimulus strength was determined by expressing the number of times the stimulus evoked a PGO wave in the LGN as a percentage of the number of stimuli applied to the PRF.

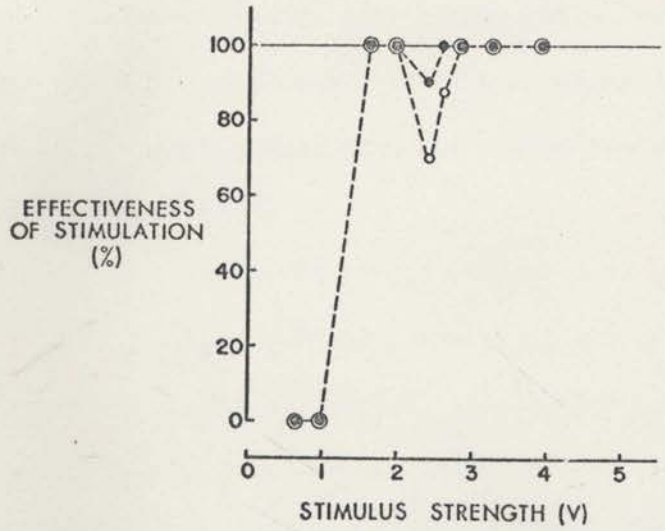


Fig. 1-30: Stimulus-response relationship for PGO waves evoked by electrical stimulation of the PRF. The success of the stimulation is shown as the percentage of stimuli at each strength that evoked waves. Evoked waves were recorded from each LGN; closed circles (●), right LGN; open circles (○), left LGN.

The points at stimulus strengths 1.6 and 1.95 volts were obtained from 3 trials while for all other points there were between 6 and 10 trials. The only trials included were those where the PRF stimulus was preceded by at least 0.5s with no spontaneous PGO wave. From the graph it is apparent that each stimulus of strength 1.6 volts evoked a wave in each geniculate, but when the stimulus strength was reduced to 1.0 volts none of the stimuli evoked a response in either LGN. Similarly, at 0.65 volts all stimuli were ineffective. It is unfortunate that no stimuli were applied between strengths 1.0 and 1.6 volts, as the exact strength at which the response failed remains unknown. Above a

strength of 1.6 volts the level of success remained at 100%.

It was noted by Bizzi and Brooks (1963b) that sometimes a supramaximal stimulus to the PRF failed to evoke a response in the LGN. In the experiment shown in Fig. 1-30 the points plotted at strengths 2.4 and 2.6 volts confirm this observation made by the above workers. At 2.4 volts, one of the ten stimuli failed to evoke a response in either LGN, while two stimuli evoked waves in the right LGN only. At strength 2.6 volts, eight stimuli were applied. All evoked a wave in the right geniculate, but following one stimulus no response could be detected in the left LGN.

The recovery of excitability for evoked PGO waves was determined by applying paired stimuli of supra-maximal strength to the PRF. The interval between the two shocks of a pair was varied, and the proportion of times was determined when a response appeared to the second of the two stimuli applied to the PRF. It was found that there was a long interval following the first shock in which the second shock was less effective than the first in eliciting a PGO wave. In Fig. 1-31, the success of the second stimulus, shown as the percentage of stimuli that evoked waves, has been plotted against the interval between the stimuli. The number of paired stimuli given at each interval varied from 7 to 22. The horizontal lines at 86% and 100% show the range of responsiveness to the first of the paired stimuli. It can be seen from the graph that at intervals of less than about 100ms the second shock was ineffective. As the interval was increased beyond 100ms the proportion of responses to the second shock progressively increased until full recovery at about 150ms. When the amplitudes of the responses to the second shocks were examined it was found that at intervals less than about 400ms there was a reduction in evoked PGO wave amplitude. However, the significant feature of these results is that the second response failed in an all-or-nothing manner in the intervals between 100 and 150ms.

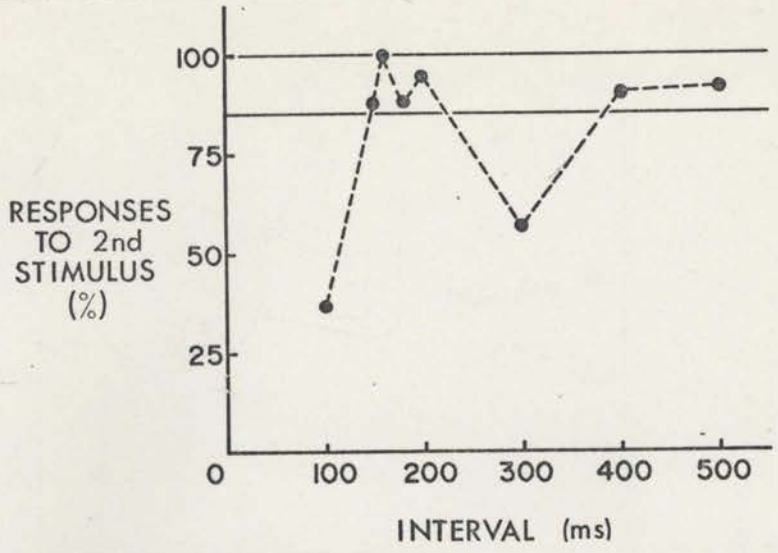


Fig. 1-31: Recovery of excitability for evoked PGO waves recorded from the right LGN. Paired stimuli of pulse width 2ms were applied to the PRF and the interval between the stimuli was varied. The success of the second stimulus, shown as the percentage of stimuli that evoked waves, has been plotted against the interval between the stimuli. Between 7 and 22 paired stimuli were given at each interval. The horizontal lines show the limits of success of the first stimulus (86%-100%).

(ii) Experiments on the Anaesthetized Cat.

It was shown earlier in this section that during barbiturate anaesthesia it is possible to record waves that resemble those occurring in the LGN of the cat during LVF sleep. As the barbiturate-PGO waves appeared to be essentially similar to the PGO waves in the sleeping cat, certain aspects of the B-PGO waves were examined to see whether they were also unitary events.

Large numbers of spontaneous B-PGO waves were recorded in seven experiments and, as described above for the PGO waves, histograms were plotted of the amplitudes of the waves. The results obtained from one experiment are shown in Fig. 1-32A. All waves (of which there were 86) were recorded from the one period of B-PGO waves (c.f., Fig. 1-17 for the cyclic occurrence of the waves). The cat was in the light throughout this time.

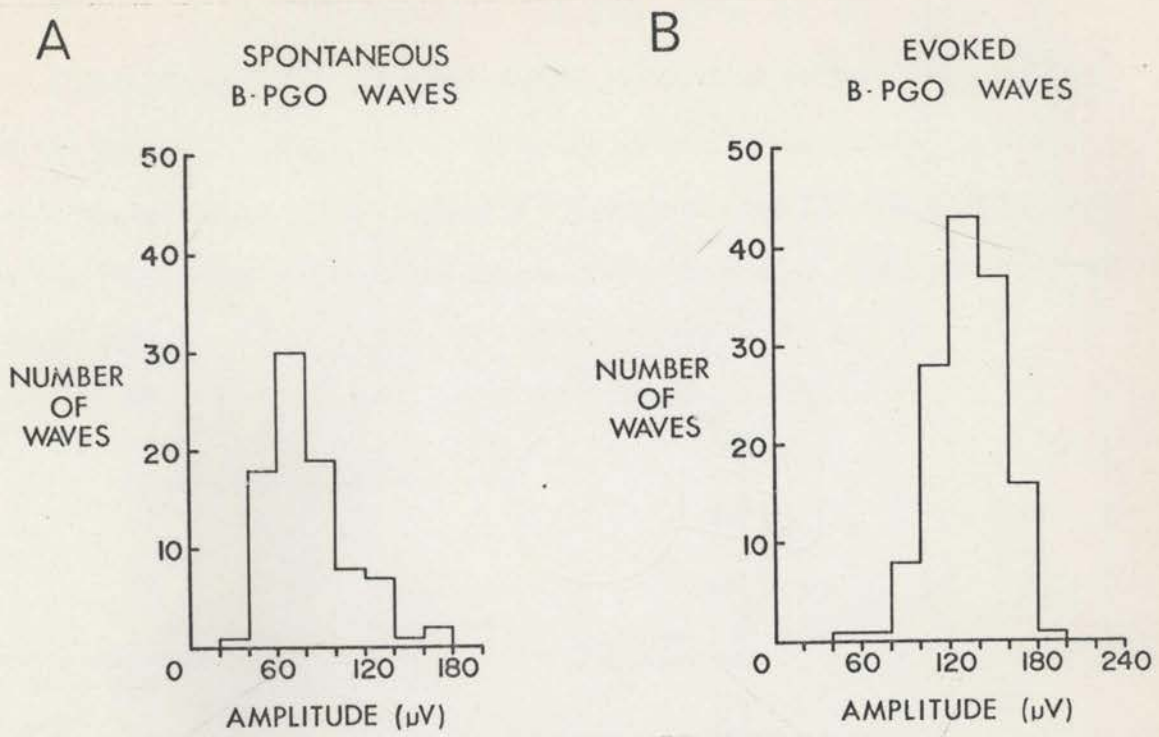


Fig. 1-32: Histograms of amplitudes of (A) spontaneous B-PGO waves (N = 86), and (B) B-PGO waves evoked by electrical stimulation of the PRF (N = 135). Data obtained from the same cat on the same day.

The waves ranged in amplitude from 30 to 165μV (average 79.9μV). The coefficient of variation of amplitude was 34.4%. The six other experiments in which similar data were obtained also showed that the spontaneous B-PGO waves, like the PGO waves, are only graded in amplitude over a limited range (the coefficient of variation in these other experiments ranged from 11.1% to 27.0%). B-PGO waves evoked by stimulation of the PRF are also graded in amplitude over a limited range (Fig. 1-32B). In the experiment shown, a total of 135 evoked waves were recorded and these had an average amplitude of 132μV (range 50 to 180μV; coefficient of variation 17.6%). As this experiment was done on the same cat and on the same day as the experiment shown in Fig. 1-32A, it is somewhat surprising that the average amplitude of the evoked waves was approximately 50μV greater than that of the spontaneous waves.

The effects that alteration of the strength of the stimulating pulses had on the evoked B-PGO waves in the LGN was similar to that reported earlier for the evoked PGO waves. As the stimulus intensity was reduced below the normal level, the amplitude of the evoked B-PGO waves in the LGN remained unchanged, but the proportion of responses progressively decreased. These findings are shown in Fig. 1-33.

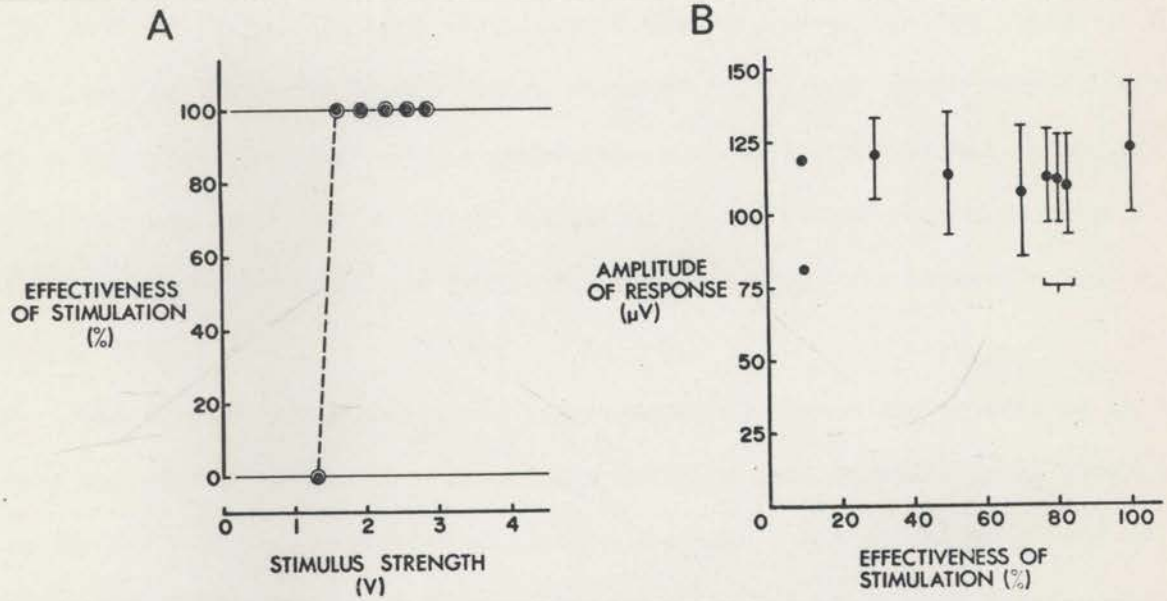


Fig. 1-33: (A) Stimulus-response relationship for B-PGO waves evoked by electrical stimulation of the PRF. The success of the stimulation is shown as the percentage of stimuli at each strength that evoked waves. Evoked waves were recorded from each LGN; closed circles (●), right LGN; open circles, (o), left LGN. (B) The effects of different levels of success for B-PGO waves evoked by PRF stimulation on the amplitude of the evoked waves. Each point represents the mean of between 3 and 15 evoked responses, ±1 standard deviation of the mean.

In A, the effectiveness of the stimulation (i.e., the number of times the pontine stimuli evoked a B-PGO wave in the LGN expressed as a percentage of the number of stimuli applied) is plotted against the stimulus strength. Between 5 and 20 trials contributed to each data point. It can be seen from the graph that every stimulus between 1.6 and 2.85 volts evoked a response in each LGN, whereas when the intensity was reduced by only 0.3 volts (to 1.3 volts) there were no evoked responses. In Fig. 1-33B, the amplitude of the response has been

plotted against the effectiveness of the stimulation. The results used in this plot were obtained some minutes after those shown in Fig. 1-33A, when a second stimulus-response run was done. In this later stimulus-response experiment several points were obtained between the 0% and the 100% levels of success. Each amplitude value on the graph, with the exception of the two points at 10% effectiveness of stimulation, represents the average of between 3 and 15 responses. The bars show ± the standard deviation of the responses. The two points at the 10% level of success are each single response amplitudes. It is very obvious from the graph that even as the percentage success for the evoked waves fell to 30%, there was no decrease in the amplitude of the evoked responses. Similarly, the two responses at the 10% level of success were not significantly reduced in amplitude.

The recovery of excitability for evoked B-PGO waves was determined in the same way as for the evoked waves in the sleeping cat. Paired stimuli were applied to the PRF and the proportion of times a response appeared to the second of the two stimuli was determined. The results shown in Fig. 1-34 were obtained from such an experiment. The graph shown in A has been plotted in the same way as Fig. 1-31, although results from both geniculates are shown in Fig. 1-34. It can be seen from the data that the recovery of excitability occurred in an almost identical way in both LGN's. Comparison of Figs. 1-34A and 1-31 shows that the "refractory period" was longer in the anaesthetized than in the sleeping cat. No B-PGO waves were evoked by the second stimulus when the interval was 200ms, but there was recovery almost to normal values by 300ms. There was then a slower recovery lasting up to 1 second. As in other experiments there was not always a response to a single stimulus, even when this was well above threshold; the horizontal lines at 100% and 91% show the range of responsiveness to the first of the paired stimuli.

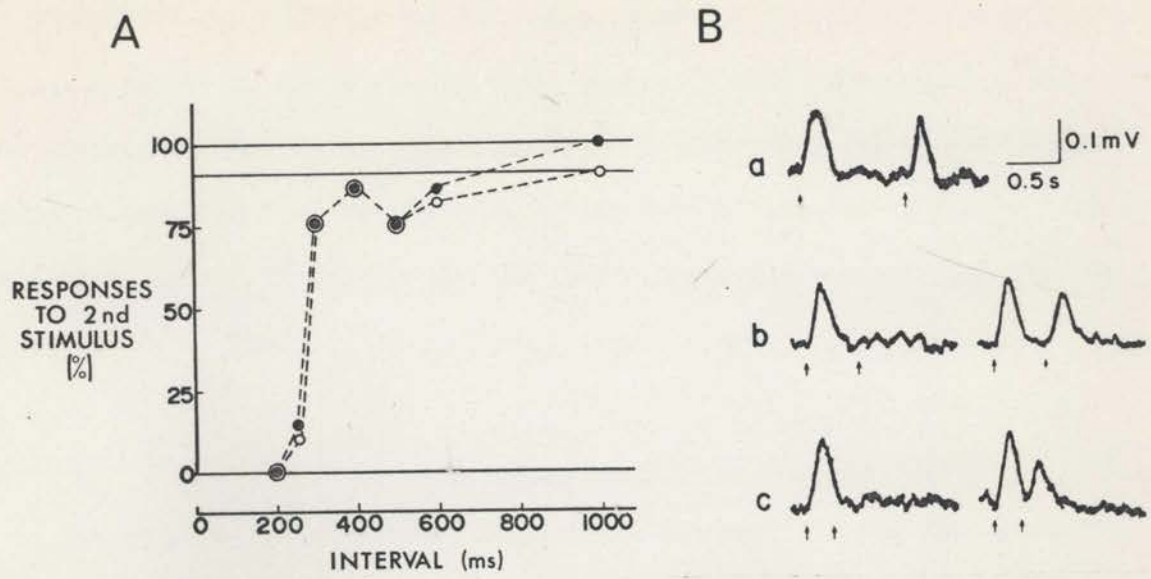


Fig. 1-34: Recovery of excitability for evoked B-PGO waves. Paired stimuli of pulse width 2ms were applied to the PRF and the interval between the stimuli varied. (A) The success of the second stimulus, shown as the percentage of stimuli that evoked waves, has been plotted against the interval between the stimuli. The number of paired stimuli given at each interval varied from 11 to 29. B-PGO waves were recorded from each LGN; closed circles (●), right LGN; open circles (o), left LGN. The horizontal lines show the limits of success of the first stimulus (96-100% for right LGN, 91-100% for left LGN). (B) Examples of evoked B-PGO waves recorded in the right LGN. Stimuli given at arrows. In (a) two stimuli at 1 second interval (100% success to 2nd stimulus), (b) 500ms interval (75% success), (c) 250ms interval (14% success). Note the all-or-nothing nature of the response to the 2nd stimulus at 250 and 500ms.

At all intervals the response to the second stimulus was either absent or clearly present, although at short intervals the second response was reduced in amplitude. However, there was never any difficulty in deciding whether or not a wave had been evoked. It was found, for example, that even when the percentage of second responses had fallen to 10-14% at 250ms, the mean amplitude of the responses was still 47% of normal. In Fig. 1-34B can be seen specimen records obtained during this experiment. The evoked waves shown were recorded in the right LGN. In (a) can be seen the responses evoked by two stimuli at an interval of 1 second; as shown in A, there was 100% success in the right LGN to the second stimulus at this interval. The two records in (b) were obtained at an interval

of 500ms (75% success); in the left hand record it is obvious that the second stimulus failed to evoke a B-PGO wave, whereas a clear response is seen after the second stimulus in the right hand record. Similarly, at an interval of 250ms (records in (c)) the response to the second stimulus is clearly all-or-nothing in nature, even although the level of success was only 14% at this interval.

Discussion

The results just presented support the concept that the PGO waves found in the sleeping and barbiturate anaesthetized animal are unitary events. Firstly, although the waves show a variation in amplitude, the range of values is not great. Considering the results of all experiments together (i.e., data from both spontaneous and evoked PGO and B-PGO waves), the coefficient of variation ranged from 11.1% to 35.4% in 13 experiments. These values are similar to the coefficients of variation of amplitude obtained for miniature endplate potentials in the frog and the cat (30% and 26-34% respectively; see Fatt and Katz, 1952, and Boyd and Martin, 1956 respectively).

Secondly, there is evidence from the PGO (and B-PGO) waves which may be evoked in the LGN by stimulating the PRF. When the stimulus was well above threshold it was sometimes not possible to detect a response in the LGN. Although Bizzi and Brooks (1963b) say that about one stimulus in five (i.e., about 20% of stimuli) failed to produce any detectable response in the LGN, in the present study, a higher proportion of stimuli were effective with the intensities normally used. For example, in two experiments when 23 and 34 stimuli were applied to the PRF, the effectiveness of the stimulation in evoking waves was found to be 96% and 97% respectively. Regardless of the actual values, though, it has been found by both Bizzi and Brooks (1963b) and the author that even at

supramaximal stimulus strengths the response is all-or-nothing in nature. This all-or-nothing nature of the response was also seen when the stimulus intensity was reduced below the usual value - as the intensity of the stimulus was reduced the amplitude of the response remained unaltered, the only effect being a decrease in the proportion of stimuli evoking PGO (or B-PGO) waves.

The third piece of evidence favouring the idea of PGO waves as unitary events comes from experiments in which the recovery of excitability for the waves was determined by applying paired stimuli to the PRF. In the sleeping cat, it was found that at intervals less than about 100ms the second shock was ineffective, while in the intervals from about 100 to 150ms the second response failed in an all-or-nothing manner (Fig. 1-31). The recovery of excitability for evoked B-PGO waves occurred in an almost identical way (Fig. 1-34A), but the'refractory period' was longer than in the sleeping animal. At intervals of 200ms the second shock failed to evoke any response, but by 300ms there was almost complete recovery. In the intervals from 200 to 300ms the second response failed in an all-or-nothing manner (Fig. 1-34B).

Although these results support the concept of the PGO wave in both the sleeping and anaesthetized animal as a unitary event, they do pose two major problems. The first relates to the significance of the unitary nature of the PGO wave. Secondly, what is the mechanism which determines the PGO wave as an all-or-nothing event? It is the second problem which will be considered here.

It was pointed out above that reduction in the amplitude of the pontine stimulus did not lead to a reduction in the amplitude of the response (as might have been expected with an evoked response), but rather that there was a reduction in the proportion of stimuli which were effective in producing a response in the LGN. Thus, even when the effectiveness of the stimulation in evoking a response was greatly reduced, there was no change in the response

amplitude. This relationship holds true for both PGO and B-PGO waves evoked by stimulating the PRF. This result would be obtained if the signal arriving at the LGN was all-or-nothing, and if, by decreasing the stimulus strength, there was a reduction in the probability of the PRF stimulus generating this signal. In the Introduction to this section, evidence was presented that the PRF and LGN are both structurally and functionally related. The known extra-retinal inputs to the LGN were summarized in Fig. 1-4, and it can be seen clearly from the right hand side of the diagram (where the structural relationships are shown) that the possibilities exist for both direct and indirect inputs to the LGN from the brainstem reticular formation. However, as it is not known what the exact anatomical connections are between the PRF and LGN which are responsible for the known functional relationships between these two regions, it is not possible at this stage to identify the places where the PGO wave signal arriving at the LGN finally assumes its all-or-nothing character. There may be any number of synapses included in the reticulo-geniculate pathway at which the signal assumes such a character. There is, however, a possible alternative explanation for the mechanism producing the observed relationships between the strength of the PRF stimulus and the nature of the evoked response. Perhaps the signal arriving at the geniculate is graded according to the strength of the stimulus applied to the PRF, and the mechanism responsible for the all-or-nothing nature of the response resides in the LGN.

In attempting to decide between these two possibilities it has been instructive to compare the occurrence of PGO waves in each LGN. In both the sleeping and anaesthetized cat there is a high proportion of synchrony for evoked PGO waves (about 95%) between the two LGN's. Thus, about 95% of stimuli applied to the PRF have the same result in each geniculate (i.e., the stimulus either does or does not succeed in evoking a PGO wave). The remaining 5%

(approximately) of stimuli produce a response in one LGN but not in the other. This high degree of synchrony between the two geniculates is maintained even when the probability of response is reduced. Thus, in Fig. 1-34A, from the 29 pairs of stimuli applied at an interval of 250ms, on 25 occasions there was no response to the second stimulus in either LGN; on 3 occasions a response was evoked in each LGN and once there was a response in only one geniculate. If the all-or-nothing mechanism resided in each LGN, then with a 10-14% probability of response (c.f. Fig. 1-34A), 6 asynchronous responses would be predicted.

A similar degree of synchrony between the two LGN's has been observed when the strength of a single shock is reduced to a level at which the probability of a response is low. For example, in one experiment on an anaesthetized cat 90 stimuli were applied at various suboptimal strengths. Of these, 48 evoked synchronous B-PGO waves in each LGN, one evoked a wave in one LGN but not in the other, and 41 evoked no response in either geniculate.

It is unlikely that this high degree of synchrony between the two LGN's could occur if the signal arriving at each geniculate were graded and each LGN possessed some mechanism which was responsible for the all-or-nothing nature of the responses. Certainly, for this to be the explanation for the all-or-nothing nature of the responses, it would seem necessary to postulate an additional mechanism, for example synchronizing signals between the two LGN's. Such synchronizing signals do not appear to exist for two reasons. Firstly, in other respects the activity of the two geniculates is not synchronized, and secondly, at no time in either the sleeping or the barbiturate anaesthetized cat has electrical stimulation of one LGN produced a PGO wave in the other LGN.

The evidence available, therefore, tends to favour the possibility that the signal to the LGN is all-or-nothing. As mentioned earlier, it is not known where the signal might finally assume its all-or-nothing character; in fact,

it is not known what pathway mediates these functional relationships between the reticular formation and the lateral geniculate nuclei. Therefore, before the mechanism responsible for the all-or-nothing nature of the PGO waves can be satisfactorily identified, it will be necessary to obtain much more information about the pathway (direct or indirect) from the PRF to the LGN. At the same time, the possibility that the all-or-nothing mechanism resides in the geniculate and that some additional mechanism synchronizes the activity in the two LGN's should be examined in more detail. Certainly for this to be excluded as a possible mechanism, stronger and more direct evidence is needed than that available at present.

FINAL DISCUSSION

The results presented in this section all point to the essential similarity of the PGO waves found in the sleeping and the barbiturate anaesthetized cat. Both types of wave are recorded from the same electrode position, and from any one position the waves are of the same polarity and similar waveform. For both types of activity there is a high degree of synchrony between the waves occurring in each LGN and between these waves and those in the PRF. Both the spontaneous and the evoked waves seen in the sleeping and the anaesthetized cat are associated with the same changes in excitability of the optic nerve endings and the postsynaptic cells and there is a discharge of the LGN cells during both kinds of wave. Finally, evidence was presented that the waves occurring in these two states are unitary events. It is therefore apparent that these waves might be due to a common mechanism which is functional during both LVF sleep and barbiturate anaesthesia.

There seems to be some disagreement about the structure in which the PGO waves might originate. Bizzi (1965) and Jeannerod, Mouret and Jouvet (1965) reported that the waves in the LGN disappeared several days after enucleation of both orbits or retinal photocoagulation. This time course of wave disappearance corresponds to that of optic nerve degeneration, suggesting that the optic tract terminals are involved in the genesis of the geniculate PGO waves. Brooks, on the other hand, reported that the waves were still present 147 days after bilateral optic nerve section, and it was concluded that the waves were not generated by the optic tract fiber terminals as suggested above, but that the wave-generating structures were lateral geniculate neurones which send axons into the optic tract (Brooks, 1967b). However, it should be noted that although Brooks observed that the waves were not completely abolished by bilateral optic nerve section, they were confined to the first 10-15 seconds of each LVF

sleep episode.

Brooks (1967a) observed that the potential field associated with the monophasic waves is a dipole, with its negative pole within the LGN, and its positive pole in the terminal optic tract. Such a potential field could be generated by depolarization of either the optic tract terminals or of the postulated centrifugal cells. Certainly, the centrifugal axon hypothesis of lateral geniculate PGO wave generation needs further investigation, for, although both anatomical and physiological evidence has been presented for such fibers, their presence has also been denied. Elsewhere in the nervous system, presynaptic depolarization has been found to accompany presynaptic inhibition, and although a similar mechanism may be operating in the LGN it is important to remember that at present there is no anatomical evidence for presynaptic inhibition in the LGN (see Szentágothai, 1968).

The presynaptic depolarization which accompanies presynaptic inhibition elsewhere in the nervous system is enhanced by barbiturates (see Chapter 15 of Eccles, 1964). If the PGO waves of LVF sleep are due to depolarization in the LGN occurring in the optic nerve terminals, and if this has a similar mechanism to the presynaptic depolarization referred to by Eccles, it might be expected that it too would be enhanced by barbiturate. Therefore, it is possible that the larger amplitude and longer duration of the B-PGO waves compared with the PGO waves is due to an action of the barbiturate which enhances and prolongs the presynaptic depolarization. However, before such a conclusion can be reached, it is essential that the question of the existence of presynaptic inhibition in the LGN be settled.

Although the above evidence might seem to suggest that the PGO waves occurring in the sleeping and the anaesthetized cat are due to depolarization of either the optic tract terminals or of the centrifugal cells, it does not

explain why the effects of the waves postsynaptically are more long-lasting than are the presynaptic effects. Also, it is necessary to explain how the LGN cells become depolarized and fire during either the PGO waves or the B-PGO waves. It is possible that the wave of presynaptic depolarization releases sufficient transmitter substance to produce a prolonged burst of firing. However, it would seem that a more likely explanation is that there is a separate excitatory action to the LGN cells. If this excitatory action were also to be enhanced by the barbiturate, then it would be easy to understand why the time-course of the effects of the B-PGO waves on the r-response was more prolonged than that of the PGO waves.

The possibility should also be considered that the action of the barbiturate on the PGO waves is not exerted in the LGN. Throughout this section, evidence has been presented suggesting that the PGO waves occurring in the LGN are triggered by some mechanism residing not in each geniculate, but in the pontine reticular formation. It is quite feasible, therefore, that the barbiturate exerts its effect at the PRF.

Regardless of the mechanism responsible for the prolonged action of the B-PGO waves on geniculate responsiveness, it is possible from the above discussion to propose a model which may prove helpful in understanding the mechanism of the effects of the PGO waves and B-PGO waves on synaptic transmission in the LGN. This model, shown in Fig. 1-35, includes the simple neuronal circuitry proposed by Burke and Sefton (1966a, b and c) to exist in the LGN of the rat (see Fig. 1-3 in the General Introduction). It is now known that a similar relationship exists in the LGN of the cat (e.g., Vastola, 1960; Suzuki and Kato, 1966, 1967; Sakakura, 1968). In view of the evidence presented throughout this section that the PGO waves in the LGN may be triggered by a mechanism residing in the PRF, the extra-retinal input to the geniculate which is

responsible for the waves is shown as a pathway originating in the reticular formation.

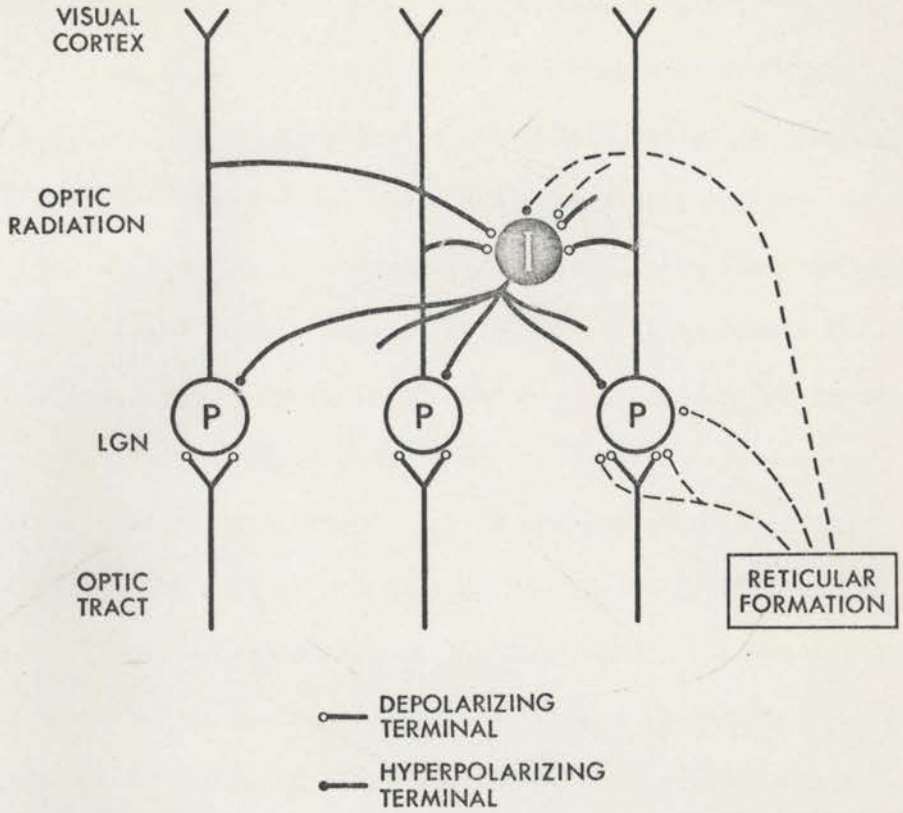


Fig. 1-35: Diagrammatic representation of the postulated relationships of neurones in the LGN. The model is based on the model of Burke and Sefton (1966b) shown in Fig. 1-3 in the General Introduction to this thesis. The dashed lines represent extra-retinal inputs to the geniculate which modify synaptic transmission at both the presynaptic and the postsynaptic level. For details see text.

However, it is important to realize that other pathways from other structures may be implicated in the effects of the waves on geniculate responsiveness. Also, as nothing is known about the anatomical connections between the PRF and LGN, the pathway drawn does not imply that the projection is a direct one between these two structures.

As mentioned in the preceding pages, it seems necessary to account separately for the changes seen presynaptically and postsynaptically during the waves. Although there is as yet no anatomical evidence for presynaptic

inhibition in the LGN, the results obtained electrophysiologically do support the idea that the presynaptic changes are mediated by fibers which terminate on and depolarize the optic tract terminals. To explain the prolonged effects of the waves on the postsynaptic response, it was suggested that there was a separate excitatory action directly to the geniculate cells. As shown in Fig.1-35 such an effect could be mediated by fibers which terminate on, and depolarize, the principal (P) cells in the LGN. However, in view of the findings of Fukuda and Iwama (1970), I would like to suggest an alternative mechanism for the postsynaptic effects. These workers found that stimulation of the mesencephalic reticular formation usually led to a decrease in the I cell response in the LGN to stimulation of the optic chiasm, and it was suggested that the facilitatory effect which similar stimulation had on the P cells in the geniculate (for references, see Part 3 of the Introduction to this section) may have been due to inhibition of the I cells. Such a mechanism could be operating during the PGO waves of sleep and barbiturate anaesthesia,and therefore it has been included in Fig. 1-35. As shown in the diagram, such inhibition of the I cells could be due to fibers terminating presynaptically on (and depolarizing) the terminals of the collateral branches of the radiation axons. Alternatively, the I cell inhibition could be due to fibers which hyperpolarize the I cell itself. It is possible, therefore, that the postsynaptic effects of the waves could be mediated via any combination of the three routes outlined above.

It was shown in the Introduction that during the different phases of the sleep-wakefulness cycle there are different degrees of presynaptic depolarization and postsynaptic facilitation. Quite possibly it is the mechanism shown in Fig.1-35 which operates to produce these characteristic changes in excitability. Obviously though, it is necessary to examine in more detail the origin of and route taken

by pathways from extra-retinal sites which terminate in the LGN. It is also necessary to establish whether or not presynaptic inhibition does take place in the geniculate.

SUMMARY OF RESULTS

1. Using cats with chronically implanted electrodes, a study has been made of the PGO waves occurring in the LGN of the unanaesthetized and the anaesthetized animal. During all phases of the sleep-wakefulness cycle in the unanaesthetized cat, it is possible to record PGO waves from electrodes positioned in the geniculate. However, these waves are a distinctive feature only of LVF sleep, and it was the waves occurring in this state of sleep which were examined in detail in this study. Similar waves (which have been called B-PGO waves) were recorded from the LGN of cats anaesthetized with pentobarbitone sodium, and the characteristics of these barbiturate PGO waves were compared with those of the waves of sleep.

2. Both types of wave are essentially monophasic spikes which are recorded from the same electrode positions and which are of the same polarity and similar waveform. During both LVF sleep and barbiturate anaesthesia there is a high degree of synchrony between the waves occurring in each LGN. Similar synchrony was observed for waves occurring in the LGN and PRF. During the two kinds of wave there is no indication of any change in the activity of the optic tract. Both kinds of wave show a cyclic pattern of occurrence, but this is more pronounced in the case of the B-PGO waves.

3. B-PGO waves, like PGO waves, are associated with characteristic excitability changes in the LGN. In order to detect the presynaptic and the postsynaptic changes at the geniculate, two electrically evoked field responses were examined; these were the orthodromic response recorded in the LGN and the antidromic response recorded in the optic tract. During the waves there is a phasic increase

in the level of presynaptic depolarization of the optic nerve endings. This increased excitability of the endings is associated with an increased excitability of the principal LGN cells. The change seen postsynaptically was more prolonged than that seen presynaptically.

4. In addition to these similarities, there are some differences between the waves. Whereas PGO waves frequently occur in groups, it is only rarely that the waves in the anaesthetized animal are seen occurring other than singly. PGO waves are smaller in amplitude and shorter in duration than are the B-PGO waves, and their effects on geniculate excitability are likewise of shorter duration.

5. It is possible to evoke both types of wave at certain times by stimulation of the PRF, and these evoked waves are similar in appearance to the spontaneously occurring waves. Moreover, the evoked waves are associated with the same excitability changes in the LGN as the spontaneously occurring waves (an increased excitability of optic nerve endings and LGN cells). Both types of wave can also be evoked at certain times by auditory stimulation.

6. Evidence was presented supporting the concept that the waves found in the sleeping and the anaesthetized animal are unitary events. Firstly, although the waves show a variation in amplitude, the range of values is not great. Secondly, the amplitude of the PGO (or B-PGO) wave evoked in the LGN by stimulation of the PRF is independent of stimulus strength, and the response seems to be all-or-nothing. Finally, when paired shocks of supramaximal strength are applied to the PRF there is a long interval following the first shock in which the second shock is less effective in eliciting a PGO (or B-PGO) wave than the first; in the intervals between complete failure and 100% success to the second shock, the

second response fails in an all-or-nothing manner.

7. From several lines of evidence it was suggested that the pontine reticular formation may be the structure where the triggering mechanism for the geniculate waves is located. As the waves appear to be due to depolarization of the optic tract terminals, it was suggested that there is an extra-retinal input to the LGN from the reticular formation and that the fibers involved in this pathway terminate on the OT terminals. In order to explain the prolonged facilitatory effects of the waves seen postsynaptically, a separate pathway from the reticular formation has been proposed; it was suggested that the postsynaptic facilitation could be caused either by a direct facilitatory influence onto the P cells, or by inhibition of I cell discharges or by a combination of these effects.

8. Finally, it was suggested tentatively that the larger amplitude and longer duration of the B-PGO waves compared with the PGO waves might be due to an action of the barbiturate which enhances and prolongs the presynaptic depolarization. Such an effect would also explain the longer duration of the effects of the B-PGO waves than the PGO waves on the presynaptic excitability.

SECTION II

THE EFFECTS OF DISUSE AND OF PROLONGED PERIODS OF OPTIC TRACT STIMULATION ON SYNAPTIC TRANSMISSION IN THE LATERAL GENICULATE NUCLEUS.

INTRODUCTION

There are three main reasons why the effects of use and disuse have been examined by so many physiologists. The first is that there is obviously clinical significance in knowing what happens when some part of the body is subjected to prolonged changes in activity. The second relates to the effects of prolonged periods of sensory deprivation on the structure and function of sensory systems - a knowledge of how sensory systems are affected by deprivation is essential, for example, in space research programs. Finally, attempts have been made to explain memory and learning in terms of the amount of synaptic usage. The results of some studies which are particularly relevant to these problems are considered in the following pages.

It is well known that prolonged periods of muscular inactivity are accompanied by muscular weakness and atrophy. Many experimentors have examined changes in the mechanical and metabolic properties of muscles following periods of muscular inactivity in an attempt to elucidate the mechanisms involved (e.g. Eccles, Eccles and Kozak, 1962; Fischbach and Robbins, 1969; Goldberg and Goodman, 1969; Guth, 1969a and b; Miledi and Slater, 1969; Nelson, 1969; Wells, 1969). The techniques used in these studies include denervation, tenotomy, spinal cord isolation and limb fixation, all of which have been suggested to produce disuse of the neuromuscular junction. However, the mechanical responses of muscles subjected to such disuse have varied, depending on the technique employed, and in many studies (for example, those involving direct injury to nerve fibers) it has been impossible to distinguish effects due to disuse from those due to degenerative changes. Only rarely in such studies has impulse activity been monitored, so, in most studies,it is not even possible to conclude that disuse has in fact been produced.

Repeated muscular exercise also produces marked changes in skeletal muscles, and again, attempts have been made to examine this experimentally by producing excess use (e.g., Binkhorst, 1969; Gutman, Hájek and Horský, 1969). Changes in the speed of muscle contraction have also been examined following long-term stimulation (Eccles,Eccles and Kozak, 1962; Vrbová, 1966; Salmons and Vrbová, 1967). Thus it is apparent that changes in neuromuscular activity are accompanied by changes in muscle contractile properties, although, at present, it does not seem possible to attribute changes solely to the effects of disuse or excess use. One of the main reasons for this is that nerves have been shown to exert a trophic influence on the muscles they innervate.

The trophic influences of nerve on muscle refer to those inter-relationships between the tissues which are not concerned in the transmission of electrical impulses. Many techniques exist for examining such influences, including nerve degeneration and regeneration, cross-innervation of nerves to fast and slow muscles, and changes in motor nerve fiber activity in the absence of degenerative changes. Details of the trophic functions of nerves on muscles can be found in many studies (e.g., Luco, 1963; Eccles, 1964,1967; Miledi, Stefani and Zelená, 1968; Guth, 1969b; Mark, 1969; Marotte and Mark, 1970a and b). Apart from these neuromuscular interactions it is also known that neuroneural influences exist. For example, following transection of the optic nerve of amphibians, degeneration occurs such that the regenerating fibers re-establish their correct connections in the optic tectum (see Eccles,1964; Kandel and Spencer, 1968). Finally, it has been shown that muscle fibers exert a trophic influence on the neurons that innervate them (see Eccles, 1964). While a detailed examination of the trophic functions of synapses is not directly relevant to the present project, brief reference has been made to them

"because they provide models of long-term, neural regulatory processes" (Kandel and Spencer, 1968) and so may be of relevance to the study of learning. Postulated mechanisms involved in learning are considered later in the Introduction.

Although the effects of prolonged muscular inactivity are well known, it is far less widely known that severe visual defects can result from distortion of normal form vision early in life. In man, for example, an uncorrected squint in the first few years of life may lead to failure to develop stereoscopic vision and to a loss of visual acuity in the deviated eye. Experiments on animals have recently been done to elucidate the mechanism underlying binocular vision (see Nikara, Bishop and Pettigrew, 1968; Pettigrew, Nikara and Bishop, 1968a and b) and some of the results were related to the above-mentioned visual defects resulting from an uncorrected squint (see Bishop, 1965b; Pettigrew et al., 1968c). Whereas the work of Bishop and coworkers was done on normal animals, experiments have been done on kittens with artificially produced squint in an attempt to investigate directly the mechanisms involved (Hubel and Wiesel, 1965). Recordings from the visual cortex revealed a marked decrease in the proportion of binocularly driven cells - out of a total of 384 cells recorded in 4 kittens with squint, 21% were binocularly driven, compared with 85% in the normal cat (Fig. 2-1). (For details of the functional organisation of the visual cortex in normal adult cats, see Hubel and Wiesel, 1959, 1962,1963a). From the experiments, it was concluded that squint leads to a profound disruption in the connections subserving binocular interaction.

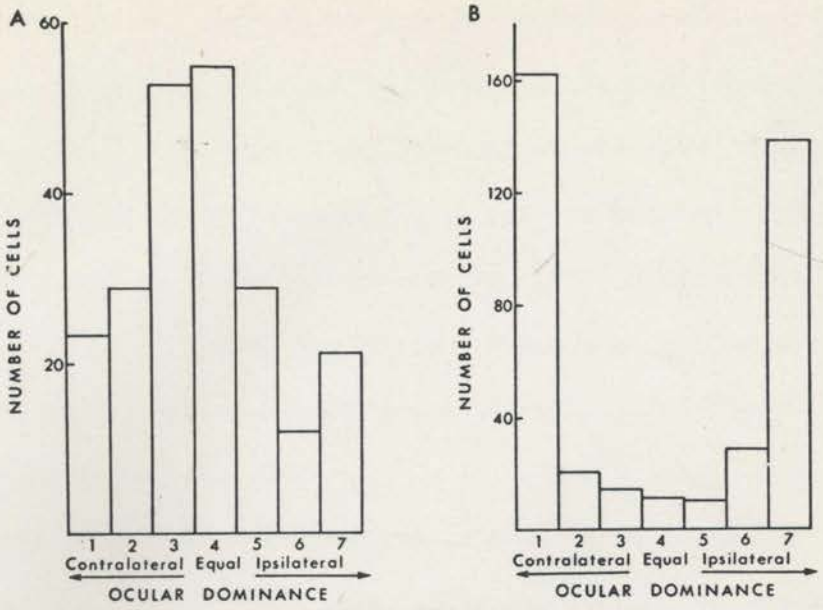


Fig. 2-1: Comparison of ocular-dominance distributions of cells recorded from the striate cortex of (A), normal adult cats, and (B), kittens reared with artificial squint. (Hubel and Wiesel, 1962, 1965, respectively).

The preceding paragraph indicated the clinical importance in examining experimentally the effects of distortion of sensory input. One aspect of sensory deprivation which has been quite extensively examined is the effect of light deprivation on behaviour and on the structure and function of the various parts of the primary visual pathway. Animals raised in darkness or under diffuse light conditions from birth for varying periods of time behave abnormally when placed in a normally illuminated environment (for example, Goodman, 1932; Chow, Riesen and Newell, 1957; Wiesel and Hubel, 1963b, 1965a and b; Riesen, 1966; Ganz and Fitch, 1968; Van Hof, 1969; Dews and Wiesel, 1970). These behavioural manifestations of defective vision are accompanied by histological and physiological changesin the visual system. Structural changes have been reported in the retinas of dark-reared animals (Chow et al., 1957; Rasch, Swift, Riesen and Chow, 1961; Cragg, 1969b) and of adult animals following short periods of visual deprivation (De Robertis and Franchi, 1956; De Robertis, 1958; Cragg, 1968). Significant

histological changes are seen in the lateral geniculate nucleus (LGN) of animals raised for several months after birth in the dark (Cragg, 1968,1969a), and, following visual deprivation by monocular or binocular lid suture or by placing a translucent contact occluder over one eye (Kupfer and Palmer, 1964; Wiesel and Hubel, 1963a, 1965a and b; Fifková, 1967; Fifková and Hassler,1969; Hubel and Wiesel, 1970). Light deprivation produces only minor structural changes in the visual cortex (Globus and Scheibel, 1967; Fifková, 1967; Fifková and Hassler, 1969).

The b-wave of the electroretinogram is reduced in amplitude in kittens raised in the dark (Baxter and Riesen, 1961; Ganz, Fitch and Satterberg, 1968) or with unilateral eyelid suture (Ganz et al., 1968), but it appears unchanged following monocular deprivation with a translucent occluder (Wiesel and Hubel, 1963b). Cornwell, Sharpless and Kanor (1962) and Cornwell and Sharpless (1968) also report that the b-wave is reduced in adult cats following brief periods of visual deprivation. It has been shown in kittens, that up to the level of the visual cortex connections are present and functional at birth and that visual experience is not necessary for their development (see Wiesel and Hubel, 1963a; Hubel and Wiesel, 1963b). However, visual deprivation in early life leads to subsequent physiological abnormalities which are only minor at the level of the LGN (Wiesel and Hubel, 1963a) but which are marked in the striate cortex (Wiesel and Hubel, 1963b, 1965a and b; Ganz et al., 1968; Wickelgren and Sterling, 1969; Hubel and Wiesel, 1970).

Reference has been made to three types of visual deprivation in the above two paragraphs. Firstly, animals may be reared in total darkness, in which case they receive neither form nor light stimulation. This is the most severe type of deprivation. Secondly, animals may be raised with the lids of one or both

eyes sutured together. This also abolishes form stimulation, while the amount of light entering the eye is reduced by 4 to 5 log units (see Hubel, 1967). Finally, the least severe form of visual deprivation involves the use of translucent contact occluders which only reduce the incident light by 1 to 2 log units.

It has been shown already that visual deprivation in the early months of life leads to changes at all levels of the primary visual pathway, and to this it must be added that the damage produced is greater, the more severe the nature of the deprivation. However, the effects do not appear to be due entirely to disuse, as there is evidence that the damage may also depend to a large extent on interaction of the two pathways (see Wiesel and Hubel, 1965a and b). The changes produced by form and light deprivation are greatest in the early months of life and decline rapidly with age, so that no changes are seen in adult cats similarly deprived (Wiesel and Hubel, 1963a and b; Hubel and Wiesel, 1970).

Several workers have failed to observe some of the changes just described following visual deprivation, but this can usually be attributed to either the techniques employed or to the use of adult animals. It is interesting to note that, recently, auditory deprivation has been shown to lead to physiological changes in the auditory system (Batkin, Groth, Watson and Ansberry, 1970), and perhaps it will eventually be seen that deprivation of any sensory input at an early age produces damage to that particular sensory system. Assuming that deprivation does affect other systems, it will be interesting to learn whether the effects decline with age, as has been found in the visual system. These studies may prove highly relevant to space research programs where men are placed in completely different environments devoid of normal external

stimuli for long periods of time.

Finally, the effects of use and disuse have been examined by many workers in an attempt to explain the mechanisms involved in memory and learning. An understanding of these phenomena is obviously of great interest. It has been proposed by Eccles that repeated use of a synapse facilitates transmission through that synapse and that this may contribute to the process of learning (see Eccles, 1953, 1961, 1964). This hypothesis of Eccles is summarised in Fig. 2-2. The quotation below, taken from Eccles (1961, 1964), indicates his

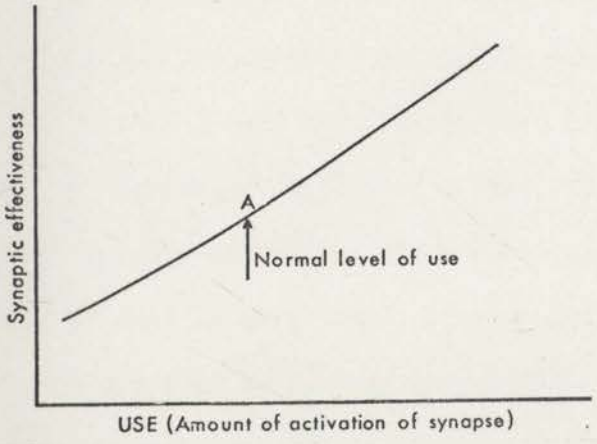


Fig. 2-2: Hypothetical relationship between synaptic use and function. (Taken from Kandel and Spencer, 1968).

views on this problem:

"Two explanations, not mutually exclusive, have been proposed for the neurological basis of learning and conditioning....According to one, learning is a dynamic process, due to continuously circulating patterns of impulses in closed neural chains....As a consequence, the reaction of the nervous system to any particular sensory input is changed in a unique way so long as this circulation of impulses continues. Conceivably, this explanation could apply at brief intervals - seconds or minutes - after some initial conditioning stimulus. It certainly cannot account for memories or conditioned behaviours

that survive either a virtual suppression of all activity in the cerebral cortex - e.g. deep anaesthesia, concussion, coma, extreme cold or even deep sleep - or the converse, convulsive seizures of the whole cortex.

The alternative explanation is that activation of synapses increases their efficacy by some enduring change in their fine structure.... We may assume that a given sensory input results in a uniquely patterned activation of central neurones, and, according to this explanation, a subsequent re-presentation of this input would tend to be channelled along the same pathways because of the increased efficacy of the synaptic actions exerted by all those neurones activated initially. There would thus be a further reinforcement of the synapses responsible for the unique pattern of activation and response, with consequently a more effective channelling; and so on, cumulatively, for each successive application of that sensory input. Necessarily, the postulated changes in synaptic efficacy must be of very long duration - days or weeks. There is no way in which relatively brief durations of synaptic change for each synapse of a serial arrangement can sum to give a more prolonged change."

Of the two alternatives just given, it is the second which has gained more support with regard to the mechanism of learning. Following periods of tetanic stimulation, many synapses show brief periods in which responsiveness is potentiated, lending some support to this hypothesis. However, such a short-lasting effect can scarcely explain the long-lasting, and sometimes permanent, changes which must be associated with learning. In examining experimentally Eccles' hypothesis, it has been easier to test for changes produced by lack of use than to examine the effects of repeated use of a synapse. The argument adopted was that if repeated use of a synapse led to an increase in synaptic efficacy, then disuse might be expected to produce depression of synaptic

function.

The various studies done to test this hypothesis will be considered later in this section (see the Final Discussion), and, at present, only those observations of Eccles and his colleagues which are directly pertinent to this as a possible mechanism involved in learning will be considered. The difficulties inherent in the methods used in these studies will not be considered at this point.

The first evidence in favour of the hypothesis came from studies on the effects of prolonged total disuse of monosynaptically activated motoneurones. Following transection of the afferent nerve fibers just peripheral to the dorsal root ganglion, there was a loss or decrease in the amplitude of the monosynaptic reflexes on the experimental side relative to the control side. (Fig. 2-3; Eccles and McIntyre, 1953).

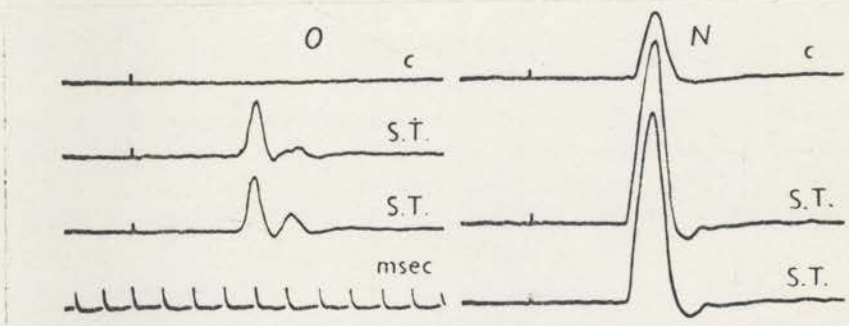


Fig. 2-3: Reflex discharges into gastrocnemius nerves in response to dorsal root volleys. O, reduced responses on operated side 40 days after dorsal root section; N, responses from normal (control) side of same animal. C, after single shock; S.T., after a short tetanus. (Eccles and McIntyre, 1953).

These findings were subsequently confirmed after severing the nerves to muscles peripherally and recording intracellularly from the motoneurones (Eccles, Krnjević and Miledi, 1959). The results were therefore taken as evidence that disuse leads to a depression of synaptic transmission. Attempts were then made

to examine the effects of increased synaptic activity (R.M.Eccles and Westerman, 1959; R.M.Eccles, Kozak and Westerman, 1962). All the muscles but one of a synergic group were denervated, the assumption made being that the remaining innervated muscle was placed under greater mechanical stress and that this should have led to increased synaptic activation. After some weeks, an increase in the monosynaptic reflex from each residual muscle nerve of the synergic group was seen consistently, (Fig. 2-4), suggesting that excess use had been produced and that this had been responsible for the observed enhancement of synaptic function.

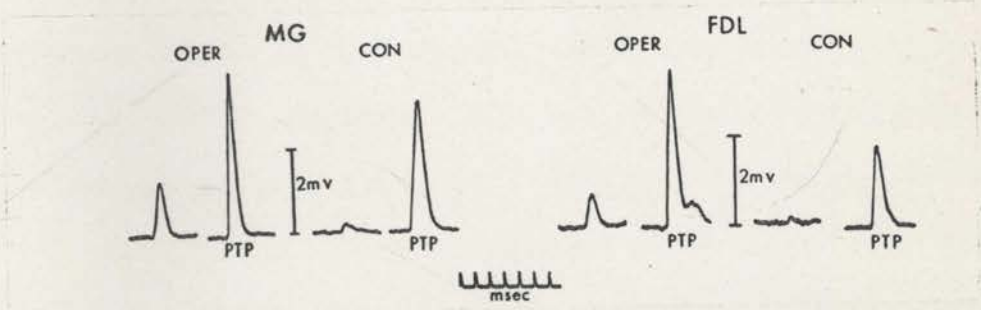


Fig. 2-4: Reflex responses evoked from medial gastrocnemius (MG) and flexor digitorum longus (FDL) muscle nerves. These were left intact, and nerves to synergic muscles were sectioned. Response on operated side (oper) is enhanced over control response (con) after single shocks and during post-tetanic potentiation (PTP). (Eccles et al., 1962).

Although Eccles has used these data to support his explanation of learning it will be shown later that, in fact, there is now a vast quantity of evidence favouring the view that disuse increases rather than decreases synaptic efficacy. It appears that the changes seen following disuse of the Ia /motor neurone synapse may have been due not to the disuse, per se, but to degenerative changes resulting from nerve injury. With this in mind, Burke and Hayhow (1960, 1968) attempted to produce disuse at the synapse between the optic tract fibers

and the principal neurones in the lateral geniculate nucleus without directly injuring the presynaptic fibers or the geniculate cells. This was achieved by giving a drug which destroyed the receptor cells in the retina and thus produced a decrease in the discharge of the retinal ganglion cells. Their results, however, did not support Eccles' hypothesis that disuse leads to a decrease in synaptic efficacy - if anything, there was increased responsiveness in the LGN following prolonged periods of disuse.

In the present study, the effects of disuse in the LGN have been re-examined using a chronic cat. It is well known that the amplitude, waveform and characteristics of the orthodromic geniculate response vary considerably in different cats because of the different combinations of positions for the stimulating and recording electrodes. This is a feature which may therefore have influenced some of Burke and Hayhow's results from acute experiments and which is not involved in the present study. Most of the observations made in the two studies are in fact comparable, although certain differences will be shown.

Using this same animal, attempts were then made to reverse the effects of the disuse by repetitively stimulating the optic tract for long periods. It will be shown that these attempts were not successful and possible explanations for this are given.

The results presented in detail in this thesis have already been reported briefly (Watson, Burke and Malcolm, 1969).

METHODS

All the experiments reported in this section were carried out on the one cat. The following electrodes had been implanted in the cat's brain in a sterile operation before commencing the series of experiments to be discussed: 2 bipolar electrodes in the right OT, 1 tripolar electrode in each LGN and one bipolar electrode in the hippocampus (left side) and the pontine reticular formation (right side). Various screws had also been inserted and carefully secured to the skull - 2 for recording the electro-oculogram, 2 over the left sensorimotor cortex and 1 over each visual cortex. These were used to monitor the cat's state of alertness and the depth of the anaesthesia during experiments. Details of the procedure for implanting the electrodes are given in the previous section.

Destruction of the Receptor Cells.

It was concluded by Burke and Hayhow (1960, 1968) that the drug 1,5-di(p-aminophenoxy)pentane dihydrochloride (M and B 968A) produced a state of disuse in the LGN. This drug selectively destroys the pigment epithelium of the retina which in turn leads to degeneration of the receptor cells, but it does not appear to have any action elsewhere in the visual system (Edge, Mason, Wien and Ashton, 1956; Ashton, 1957; Burke and Hayhow, 1960, 1968). Because the effects of this drug have been well documented both histologically and physiologically, it was therefore used in the present study to produce destruction of the retinal receptor cells and hence disuse in the geniculate.

The drug was given on 4 occasions spaced over a period of almost 3 months. Doses 1, 2 and 4 were each 50mg/Kg of M and B 968A dissolved in 5ml of boiled distilled water and given by slow intravenous injection over a

period of about 3 to 5 minutes. Dose 3 was 100mg/Kg dissolved in 10ml of boiled distilled water and likewise injected slowly.

Details of why it has been concluded that M and B 968A leads to a state of disuse in the LGN are given in the Preliminary Discussion.

A careful watch was kept on the cat's level of blindness at all times. Although some experiments will be described which were done before a steady level of blindness had been achieved, for all those carried out after the fourth dose of the drug, the cat was behaviourally blind and the level of blindness appeared to remain constant.

Technique for Attempting to Reverse the Effects of Disuse.

It is clear that M and B 968A greatly alters transmission through the LGN (see the Results; also Burke and Hayhow, 1968) and it was thought that this effect was due to the establishment of a state of disuse in the geniculate. It was therefore hoped that the effects of this pharmacological disuse could be reversed by repeated stimulation of the OT and thus, repeated activation of the geniculate synapses.

Two forms of prolonged OT stimulation have been tried and these will be referred to as regular and random stimulation. Each stimulating session lasted one week and was carried out on the unanaesthetized cat, but no attempt was made to maintain a steady level of consciousness during this time. Each day of a session, stimuli of alternating polarity were delivered to the OT for a constant period of time. The stimuli were always 50μs in duration and of sufficient intensity to evoke a maximal orthodromic response. At all times, the response to each stimulus was monitored on an oscilloscope.

1. Regular Stimulation. During five sessions, stimuli of alternating polarity were delivered to the optic tract at a regular rate of 30 per second. One session involved OT stimulation for $2\frac{1}{2}$ hours each day, another for 4 hours each day, and during three other sessions, stimulation was continued for 8 hours a day. The rate of stimulation was controlled as follows. The output of a Tektronix waveform generator (Pulse interval 66ms) was used to trigger two pulse generators, one of which produced a pulse at time zero, the other after a delay of 33ms. The pulses from each pulse generator passed to separate stimulus isolation units and then to a pulse mixer (see Eisman, 1963-64, for details). Thus, the pulse mixer received one incoming pulse every 33ms. One isolation unit was connected to the pulse mixer in the correct way (that is, cathode to cathode, anode to anode), while the connections of the other were reversed (cathode connected to anode and vice versa). In this way, the pulses leaving the pulse mixer every 33ms (or at a rate of approximately 30 per second) were of alternating polarity. It was these pulses which were led to the two optic tract stimulating electrodes.

2. Random Stimulation. The temporal pattern of the stimulating pulses in three sessions was determined by the firing of a retinal ganglion cell recorded from a normal cat anaesthetized with nitrous oxide. The discharge of this cell had been stored on magnetic tape and it was used to trigger the stimulating equipment. The three sessions all involved OT stimulation for 8 hours per day, so that the tape, which ran for only 66 minutes, was replayed several times each day. As far as possible, the stimulation was continued uninterrupted for the required period each day. However, the cat sometimes had a violent fit during the period of stimulation and when this happened, the tape was stopped for a short time until she had recovered. Similar fits were also seen during the regular

stimulating sessions. It is known that, for part of its course, the optic tract lies close to the amygdaloid body and there is evidence to suggest that this structure has a definite effect on emotional and behavioural responses in animals (see Ranson and Clark, 1959). It is possible that the fits which occurred during the stimulating sessions resulted from current flow from the OT electrodes activating the amygdala. Goddard, McIntyre and Leech (1969) found that daily electrical stimulation of the amygdala of rats, cats and monkeys led to clonic convulsions during stimulating sessions and the characteristics of these convulsions resemble the fits seen in the present study.

The set-up used to produce the stimulating pulses from the cell's discharge can be seen in Fig. 2-5A. The output of the tape recorder was led to a Schmitt trigger (Schmitt 1) which in turn activated a specially designed flip-flop circuit (for details of the circuit see Fig. 2-5B). This flip-flop circuit, by separating every alternate incoming pulse from its neighbours, produced two temporally alternating outputs which were led via separate Schmitt triggers (numbered 2 and 3 in the diagram) and pulse generators to a pulse mixer. As described for the regular stimulation, the two inputs to the pulse mixer were connected in reverse ways so that the pulses leaving it and passing to the cat were of alternating polarity. The stimulating pulses were constantly monitored on an oscilloscope, as were the responses evoked by each stimulus. Biological activity was also monitored over a loud speaker. Stimuli were not delivered via stimulus isolation units during the sessions of random OT stimulation.

The temporal characteristics of the ganglion cell discharge were determined by feeding the output of the tape recorder into a Computer of Average Transients. In Fig. 2-6A can be seen the firing pattern of the cell for the duration of the tape (66 minutes).

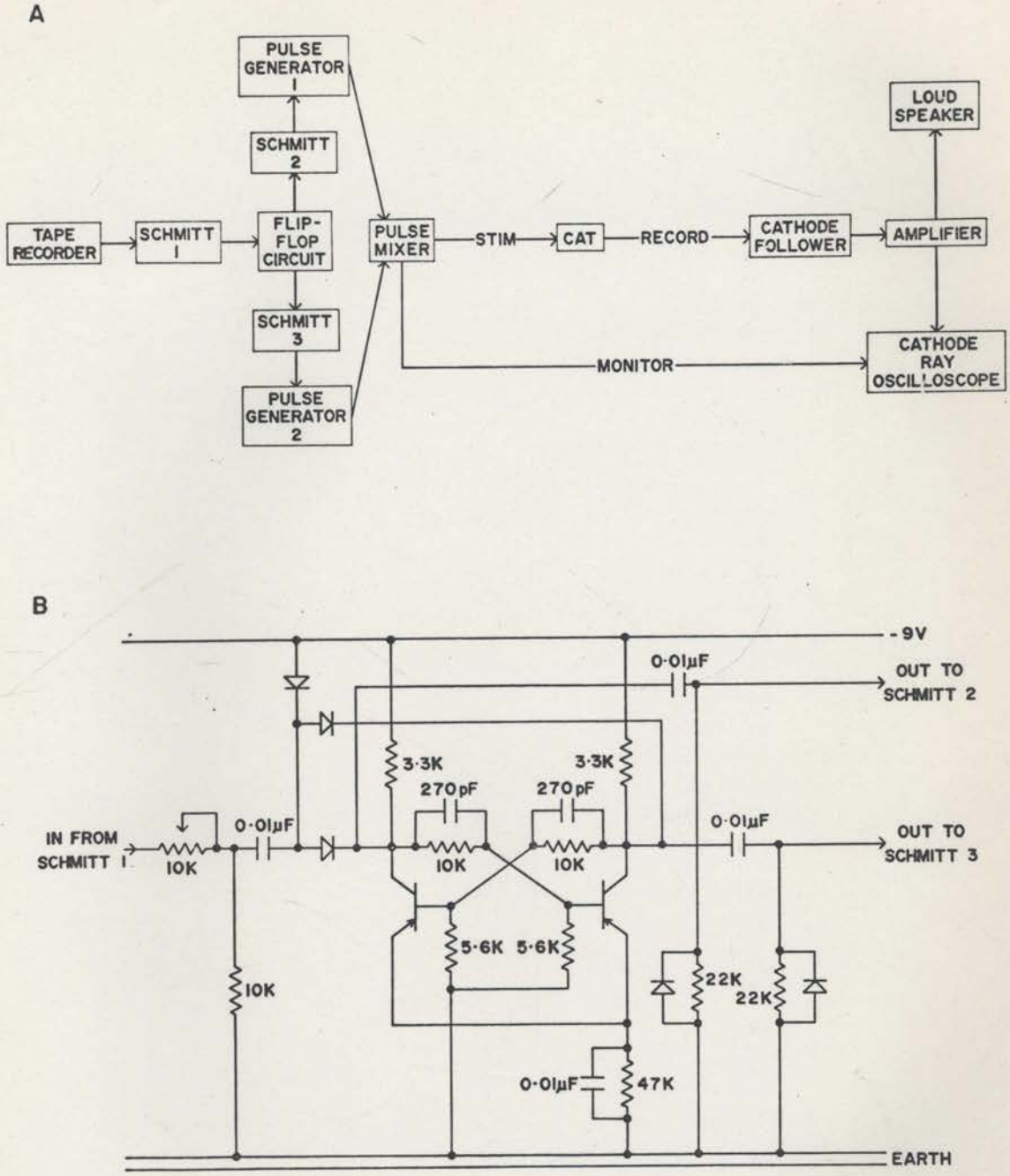
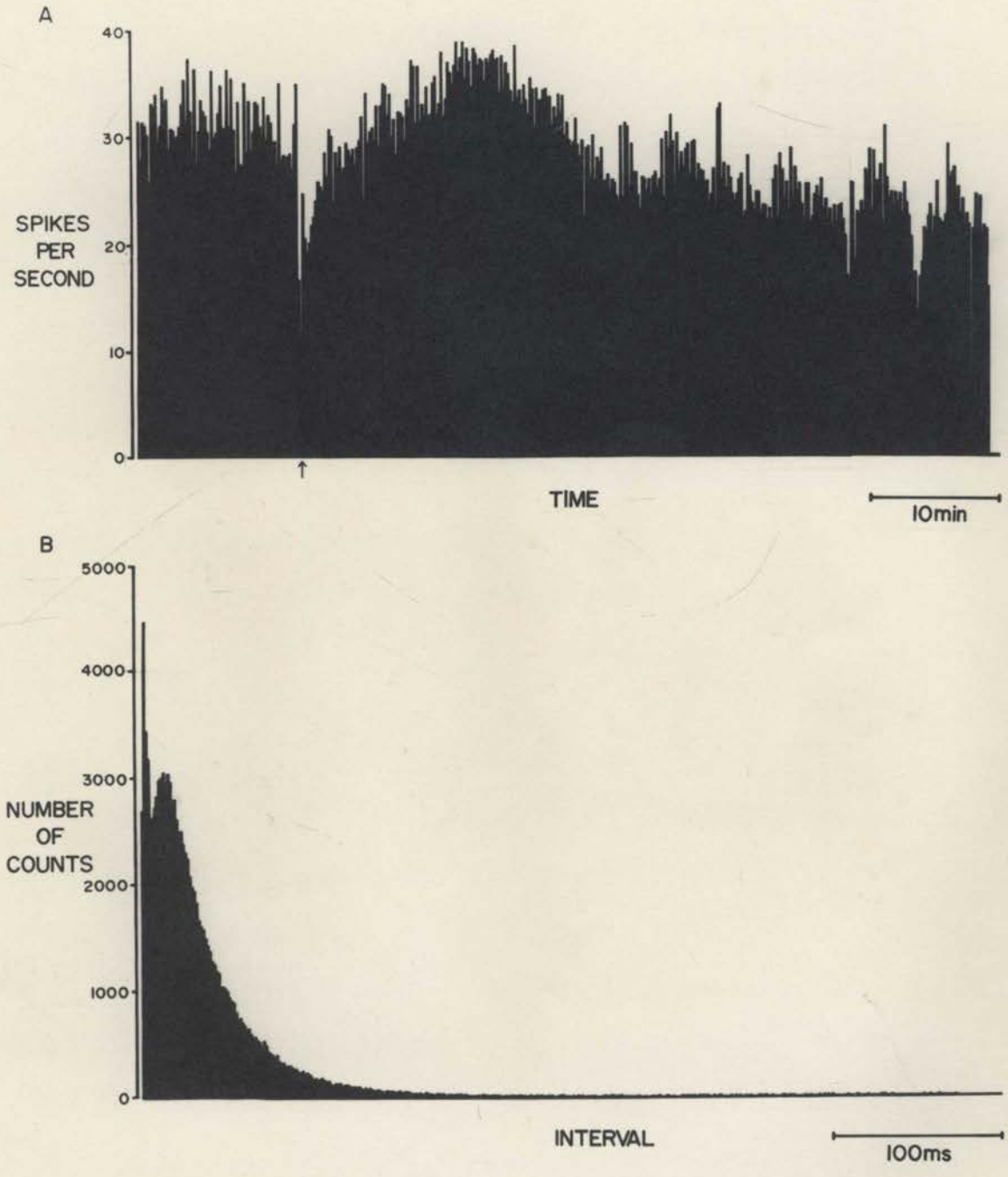


Fig. 2-5: (A.) Block diagram of the equipment used in the random stimulating sessions. (B.) Details of the flip-flop circuit which was used to split the output of the tape recorder into two trains of pulses.

Fig. 2-6: Characteristics of the ganglion cell discharge used in the random stimulating sessions. (A.) Histogram showing the frequency of the unit's discharge for the duration of the tape (66 minutes); bin width 10s. (B.) Interspike interval histogram; bin width 1.25ms.


A
40
30
20
10
0
SPIKES
PER
SECOND
TIME
10min
B
5000
4000
3000
2000
1000
0
NUMBER
OF
COUNTS
INTERVAL
100ms

The histogram was compiled by obtaining the total number of spikes every 10 seconds (that is,the bin width was 10s), and converting the values to the frequency shown on the ordinate. It can be seen that the cell was firing continuously for the duration of the tape. The average discharge rate was 28.2 per second (range 7 to 39 spikes per second). The discharge recorded initially was obtained with the cat in the light. The sudden change in activity 18 minutes after the start of the tape (marked by an arrow below the abscissa) was due to the cat being placed in the dark. Most of the discharge was therefore obtained with the cat in the dark.

The distribution of the interspike time intervals of the discharge of this cell is shown in Fig. 2-6B. Although the most frequent intervals were between 2.5 and 3.75 ms, the mean interval was approximately 36ms, corresponding to the average discharge frequency of approximately 28 per second.

Experimental Procedure.

Geniculate excitability was examined in the alert and the barbiturate anaesthetized cat after single shock stimulation of the ipsilateral optic tract and in the anaesthetized cat after tetanization of the tract. The orthodromic response which was examined and the stimulating and recording parameters have already been discussed (see Section I). Usually a series of responses was obtained in the alert cat so that a comparison could be made with the control barbiturate responses. The cat was anaesthetized with pentobarbitone sodium (Sagatal, May and Baker,), 35 mg/Kg being given by intraperitoneal injection. When necessary, supplementary doses were given to maintain the cat at a fairly deep level of anaesthesia. Usually a series of control barbiturate

responses was obtained before beginning the examination of recovery of geniculate excitability. In all experiments during barbiturate anaesthesia, the on-going geniculate activity was monitored on an electroencephalograph and responses were only considered if there had been at least 3.5 seconds before the test stimulus during which no barbiturate waves had occurred (see Section I for details).

The cat's body temperature was maintained within normal limits by means of an electric blanket controlled by a thermistor placed in the rectum.

The recovery of geniculate responsiveness was initially examined before the cat was given M and B 968A. It was then re-examined in the same way after giving the drug, but before commencing any of the attempted reversal studies. The results obtained from the normal cat served as references for the post-M and B studies so that the effects of disuse could be evaluated. Geniculate excitability was also examined before and after each stimulating session. The results obtained before starting the session served as the controls for those obtained at the end of that particular session, and therefore, any changes produced by the week of stimulation could be determined. Initially, the experiments were done one day after completion of a session. However,it was felt that any change which may have been produced could be transient and thus very much reduced or no longer evident after this 24 hour delay. Therefore it was decided to examine excitability both immediately after, and one day after, ending the stimulating session. An additional experiment was often done one week later.

The recovery of responsiveness following single shock stimulation of the OT was not examined following sessions done towards the end of this project because virtually no change was seen in the recovery cycle after giving

M and B 968A. Post-tetanic recovery cycles were always studied.

Histology.

At the end of the series of experiments, the brain and retina of the cat were examined histologically. The cat was deeply anaesthetized and Fe^{3+} ions were deposited from the electrode tips. The brain was then perfused in situ with 0.9% saline, followed by 10% formol saline containing 1% w/v potassium ferrocyanide. The brain was removed and placed in 10% formol saline until ready for embedding. It was embedded in egg yolk, as described in Section I, and the block was subsequently frozen with CO_2 and sectioned coronally at 40μ. Selected sections were stained with cresyl fast violet.

After the eyes were removed from the orbit, a small amount of formol saline (about 0.2ml) was injected into the vitreous humor before placing the whole eye in 10% formol saline. About two days later, the cornea and lens were carefully removed and the eye was then returned to the fixative for some weeks. One eye was embedded in egg yolk,as described for the brain. However, this proved unsatisfactory, as the egg yolk penetrated the retina and made identification of retinal components impossible. The other eye was washed overnight in running water, dehydrated in graded ethyl alcohols and embedded in low viscosity nitrocellulose (LVN). LVN sections were cut horizontally at 20μ and stained with Masson's trichrome stain (Jones, 1950). The sections were compared with those from a normal adult cat's retina shown by Donovan (1966) and with those from other cats treated with M and B 968A (Ashton, 1957; Burke and Hayhow, unpublished data).

PRELIMINARY DISCUSSION

As retinal ganglion cells normally show a maintained discharge even in the complete absence of light (e.g., Granit, 1947, 1955; Kuffler, 1953; Kuffler, Fitzhugh and Barlow, 1957; Erulkar and Fillenz, 1960; Arduini, 1963; J.E. Brown, 1963; Rodieck and Smith, 1966; Rodieck, 1967; Creutzfeldt and Sakmann , 1969), it follows that, even in total darkness, there is bombardment of geniculate synapses with impulses from the retina. Thus, regardless of the level of dark or of light adaption, and irrespective of whether or not any visual stimulus is present, the synapses between the optic tract fibers and the principal cells of the geniculate are in a continual state of use. Even after several hours in darkness, the ganglion cells still show a maintained dark discharge (Kuffler et al., 1957; Rodieck and Smith, 1966; Rodieck, 1967) and it has been suggested that it may continue indefinitely (Burke and Hayhow, 1960, 1968).

Although Hughes and Maffei (1965) concluded that the dark discharge was "the expression of the autochthonous activity of the deafferented ganglion cells or of neural nets not including the bipolar cells or the receptors", there is a considerable amount of evidence to suggest that this discharge is dependent on intact receptor cells. For example, Noell (1953) observed that there was a lack of ganglion cell discharge in cats given iodoacetate, a drug which is known to produce primary and selective destruction of the visual receptor cells (for references, see Ashton, 1957). A loss of the maintained ganglion cell activity has also been reported after administration of 1,5-di(p-aminophenoxy)pentane dihydrochloride (M and B 968A). This drug produces degeneration of the pigment epithelium of the retina, which in turn leads to degeneration of the receptor cells. The retinal bipolar cells and

ganglion cells, the optic nerve fibers and the cells of the lateral geniculate nucleus do not appear to be damaged by the drug (Edge et al., 1956; Ashton, 1957; Burke and Hayhow, 1968).

Rodieck observed that the retina of a cat given 50 mg/Kg of this drug three days earlier was characterized by an almost complete lack of maintained activity, whereas, penetration of the retina of a normal cat revealed massed fiber discharge and unit activity (Rodieck, 1967). Of the 8 units recorded from a total of 50 penetrations in the treated cat, all showed abnormal maintained and light evoked activity. Histologically, it was found that, except for a few "islands" of apparently normal retina, there was complete degeneration of the receptor cells. Ashton (1957) also reported the existence of such "islands" of undamaged retina. From these observations, Rodieck suggested that intact receptor cells were necessary for the occurrence of the normal maintained retinal discharge.

Burke and Hayhow (1968) have also reported that M and B 968A leads to a marked decrease in the retinal ganglion cell discharge. In experiments on two treated cats given the drug 2 to 3 weeks earlier, one showed no retinal discharge either in the dark or in response to steady or modulated light, while the other showed no discharge in some penetrations and only a weak discharge in others. The results from this second treated cat are shown in the upper three records of Fig. 2-7.

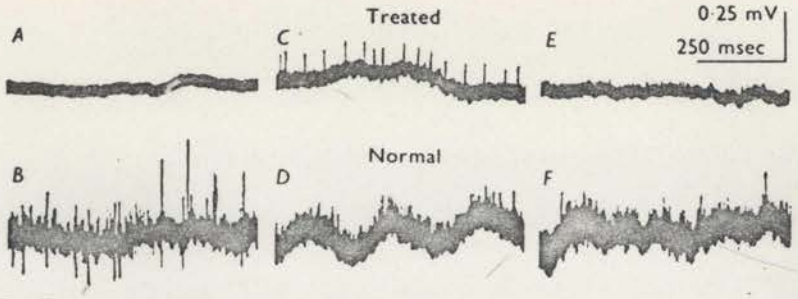


Fig. 2-7: Massed discharge of retinal ganglion cells from 3 different regions in a treated cat (14 days after giving M and B 968A) and in a normal cat. A,B in total darkness; C,D in diffuse moderate illumination; E,F in modulated light. (Burke and Hayhow, 1968).

In contrast to these results were those from four normal cats, all of which showed a strong massed unit discharge - the lower three records of Fig. 2-7 show examples of the discharge from one of the normal cats.

It would therefore seem that, although M and B 968A does not have a direct effect on the LGN, it should lead to a state of disuse in this nucleus due to its effect at the retina. That is, by destroying the receptor cells and thereby abolishing the maintained ganglion cell discharge, there is loss of the normal maintained input to the LGN, leading to inactivity at the optic tract/lateral geniculate synapses.

However, it has been frequently observed that many geniculate cells continue to show a maintained discharge, even after destruction of both retinas (e.g., Bishop, Burke and Davis, 1962a; Arduini and Pinneo, 1963; Levick and Williams, 1964; Sakakura and Iwama, 1967; Arduini, 1969). Thus, in addition to receiving a maintained input of retinal origin, geniculate cells must be either spontaneously active or they must receive inputs of non-retinal origin. Alternatively, this residual discharge could arise from the spontaneous release of transmitter substance from the optic tract terminals. Extra-retinal inputs to the LGN are known to exist (see Section I of this thesis for a brief

discussion of the evidence in this regard) which therefore makes identification of spontaneous geniculate discharge difficult. However, regardless of the mechanism responsible for this residual geniculate activity following retinal inactivation, it should be possible to observe similar activity in the geniculate of M and B treated cats. In fact, maintained activity has been reported in the geniculate of both acute (Burke and Hayhow, 1968) and chronic (Bruce, 1964-65; Hansen, 1965) treated preparations.

It would therefore seem pertinent to ask whether M and B 968A does lead to a state of disuse in the LGN. The fact that residual optic nerve activity may remain after giving the drug would suggest that the disuse was not complete, as this residual discharge would activate the geniculate synapses. However, it was mentioned earlier that one of Burke and Hayhow's treated cats did not show any retinal discharge. Although these authors made no comment about the level of blindness in this cat, they stated that the cat which showed the weak discharge had slightly recovered some vision. Perhaps a total lack of retinal activity is characteristic of cats which have been completely blinded by the drug. If so, then the cat used in the present study should not have received geniculate activation from residual retinal discharge as behaviourally it was completely blind.

If the maintained geniculate discharge, which is seen in the absence of retinal input, were to arise spontaneously in the LGN or by activation from an extra-retinal source, it would not influence the OT/LGN synapses. However, if a spontaneous release of transmitter from the nerve endings is responsible for the discharge, then M and B 968A would not produce complete disuse of the OT/LGN synapses. Nevertheless, a state of at least relative disuse must be achieved in the geniculate, following administration of this drug.

Whether or not M and B 968A has effects other than those considered above is not known, although at present there is no direct evidence to suggest that this is so. The possibility does remain, however, that other sites of action exist and therefore, that the effects of disuse alone are not being examined in the results that follow. This point is considered in more detail later.

RESULTS

1. The Effects of M and B 968A on the Eye.

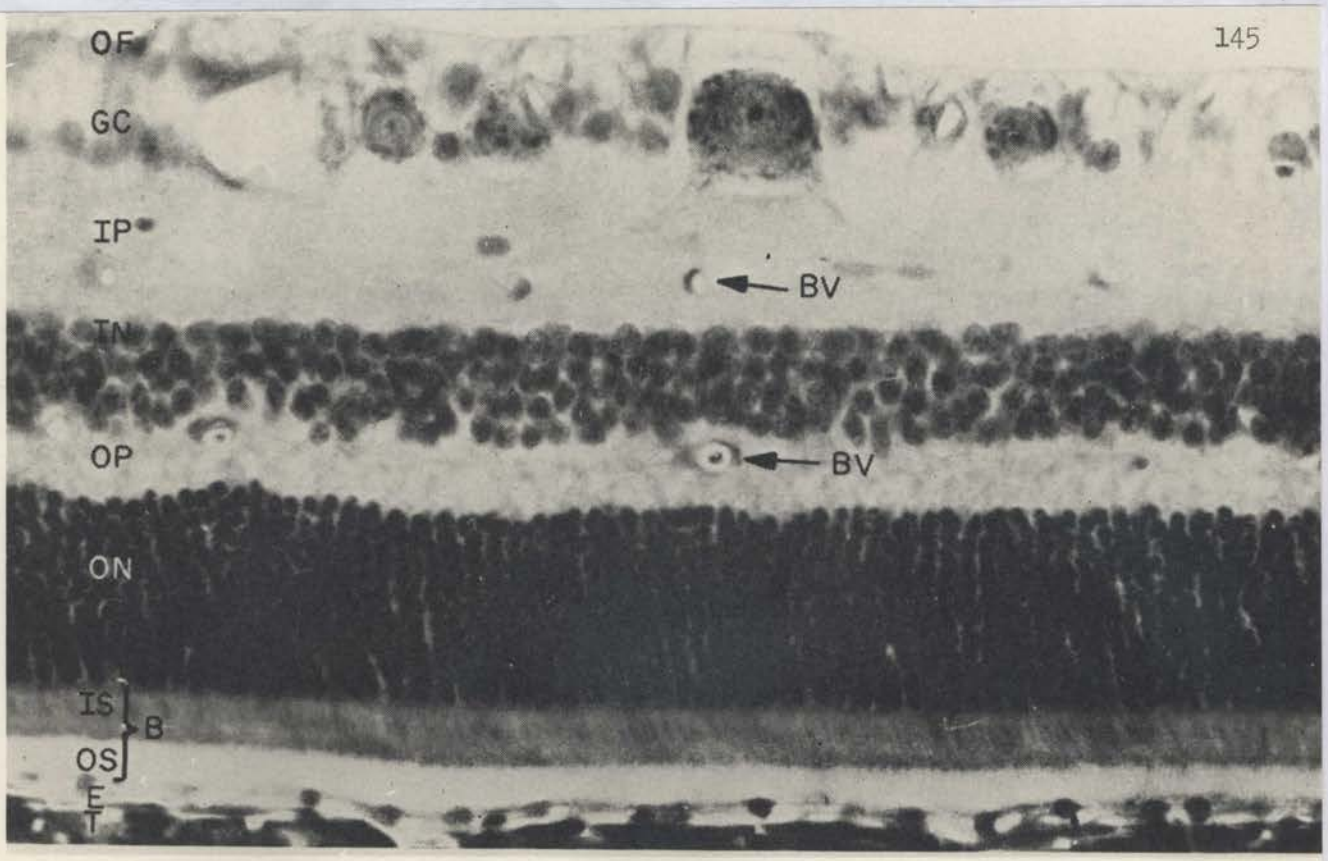
Within 48 hours of giving the first dose of the drug, there was marked dilation of the pupils such that, even in normal room illumination, they were fully dilated. The pupillary reflex to a bright light shone in the eye was also affected by the drug: whereas in the normal cat such stimulation produces constriction of the pupil to a mere slit, 48 hours after giving M and B 968A, there was no pupillary constriction at all. One week later, the cat appeared to be behaviourally blind, but, during the following week, there was a slight recovery of vision. A second dose of the drug was given two weeks after the first. Within two weeks, there was again partial recovery of the pupillary reflex and, by five weeks, it was almost normal and, behaviourally, the cat did not appear to be seriously affected.

A satisfactory level of blindness was reached after two further doses were given, and this appeared to remain steady during the subsequent eight months. The pupils remained fully dilated in normal room illumination and they only constricted to about two-thirds of this diameter when a bright light was shone in them. When viewed with an ophthalmoscope, each retina appeared very pale in the region of the optic disc and the retinal blood vessels appeared white in colour and very constricted. Behaviourally, the cat appeared to be completely blind; when moving around the laboratory, she walked into anything in her path (e.g., chairs, tables, equipment racks and walls), and, on one occasion, she walked off the edge of a table. She was unaware of both still and moving objects in her visual field and she jumped back whenever she bumped into things and when there was a sudden noise.

Histological examination of horizontal sections of the retinas of this M and B treated cat confirmed observations made by previous workers on similar material (e.g., Edge et al., 1956; Ashton, 1957; Bishop, Burke, Davis and Hayhow, 1960; Rodieck, 1967; Burke and Hayhow, 1968). Most of the sections examined showed complete degeneration of the pigment epithelium and the photoreceptors, while other regions of the retina(including the ganglion cells) appeared normal. In some sections there were isolated regions in which the receptor cells were still intact. The degenerated pigment cells had aggregated into large irregular clusters, many of which had migrated forwards into the retinal tissue. This migration is evident in Fig. 2-8B, a photograph of a horizontal section of the retina of the M and B treated cat. The extent of the abnormalities becomes very obvious when this photograph is compared with one of a similarly oriented retina of a normal adult cat (Fig. 2-8A).

Fig. 2-8: Horizontal sections of the retinas of (A) a normal adult cat (from Donovan, 1966), and (B) the M and B treated cat used in this study. Both sections stained with Masson's trichrome stain.
Abbreviations used in (A):
B - bacillary layer; BV - blood vessels of deep capillary net;
E - pigment epithelium; GC - ganglion cell layer; IN - inner nuclear layer; IP - inner plexiform layer; IS - inner segments;
OF - optic nerve fiber layer; ON - outer nuclear layer;
OP - outer plexiform layer; OS - outer segments;
T - tapetum cellulosum.

A



B

2. The Effects of Disuse on Geniculate Excitability.

The waveform of the orthodromic geniculate response to a single optic tract stimulus was not altered by giving M and B 968A. The relationship between stimulus strength and response amplitude was examined only in a few experiments before and after giving the drug; experiments were done when the cat was alert and under barbiturate anaesthesia. The shapes of such stimulus-response curves were comparable in the normal and in the treated cat, but, after giving the drug, there was a slight rise in the threshold of the response (by about 10%). Direct comparison of graphs, plotting the amplitude of the postsynaptic response against that of the presynaptic response, revealed that the synaptic responsiveness was not greatly altered by giving M and B 968A.

In almost all experiments, the amplitudes of approximately half-maximal responses were directly compared in the alert and anaesthetized state. Sometimes this comparison was made using data from the stimulus-response curves, but usually the response amplitude was less accurately determined - the stimulus strength was increased until the response on the oscilloscope appeared to have reached a maximum amplitude, and it was then reduced until the response was about half-maximal. Although this procedure prevents satisfactory quantitative comparisons from being made, the results did indicate that the amplitude of the presynaptic (t) response was reduced in the anaesthetized cat after giving M and B 968A. When the barbiturate t response was expressed as a percentage of the alert t response, it was reduced from 126% in the normal cat to 114.8% in the partially blind animal. The response was further reduced when the cat was completely blind (to 101.7% of the alert level). Thus, in the M and B treated cat, the barbiturate produced far less enhancement of the response over the alert level than it did in the normal cat. There were no definite changes

in the postsynaptic response after giving M and B 968A.

A. Responsiveness Following Single Shocks to the Optic Tract.

In addition to the results just discussed, recovery cycles were examined by the two-shock technique in both the alert and the barbiturate anaesthetized cat before and after giving M and B 968A. Paired stimuli of 50μs duration were applied to the optic tract at varying intervals and the response recorded in the geniculate to the second (i.e., the test) stimulus was used as a measure of geniculate excitability. The first (or conditioning) stimulus was of supramaximal strength, while the strength of the test stimulus was adjusted to produce an approximately half-maximal response. Immediately before and after examining the excitability at any particular interval, a small number of unconditioned test stimuli were given; the conditioned responses were expressed as a percentage of these unconditioned responses obtained at approximately the same time. The amplitude of the postsynaptic response was always corrected for changes in the presynaptic response by simple proportion (i.e., $\frac{r\%}{t\%} \times 100 = r\%$ (corrected)).

The results will be discussed with reference to several graphs (Figs. 2-9 to 2-13) showing the amplitude of the conditioned response (expressed as a percentage of the unconditioned response amplitude) plotted against the interval (in milliseconds) between the two stimuli; the graphs are plotted with a logarithmic time scale. Each point on the graphs is an average of between 2 and 9 responses (usually of 3 or 4). In each figure, the upper graph shows the recovery of the t response, and the lower graph, the r recovery curve corrected for changes in t.

Normal Cat: Fig. 2-9 shows the results of an experiment on the normal, alert cat.

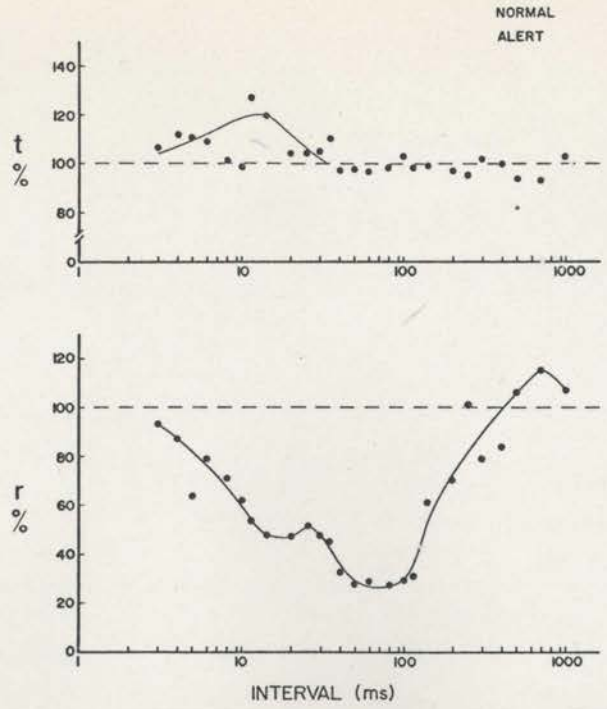


Fig. 2-9: Recovery of t and r responses following a single supramaximal shock to the optic tract of the normal, alert cat. For details, see text.

The presynaptic response shows a period of supernormality (amplitude 120%) lasting to an interval of 34ms. Usually there is an early phase of subnormality preceding this supernormality, but, as no responses were obtained at an interval less than 3ms, this cannot be seen in this diagram. The recovery of the postsynaptic response is more complex. There is an initial phase of relative supernormality (to 93%) followed by a profound depression to 26% at 70ms. The period of subnormality lasts about 400ms and is followed by another period of facilitation.

The results from another alert two-shock study done before giving M and B 968A were comparable with the above experiment. These results and the results from other two-shock studies are summarised in Tables 2-1 and 2-2.

TABLE 2-1

Recovery of the Presynaptic Response after a Single Stimulus to the Optic Tract.

		Supernormality			Number of Experiments
		Peak (%)	Peak Time (ms)	Duration (ms)	
ALERT	Normal	123 (120-126)	10 (8-12)	27 (20-34)	2
	Partially Blind	122	6	37	1
BARBITURATE	Normal	113 (112-114)	9 (6-12)	20	2
	Partially Blind	118	6	10	1
	Blinded	111.5 (111-112)	6 (4-8)	13 (11-15)	2

Amplitudes of the conditioned test responses have been expressed as percentages of the unconditioned response amplitude. Values are the average for the number of experiments shown; the range of values for individual experiments are given in brackets. The time values indicate the intervals between the conditioning and test stimuli.

TABLE 2-2

Recovery of the Postsynaptic Response after a Single Stimulus to the Optic Tract

		Initial Supernormality		Subnormality		Terminal Supernormality		Number of Experiments
		Peak (%)	Time (ms)	Peak (%)	Time (ms)	Peak (%)	Time (ms)	
ALERT	Normal	83 (73-93)	3.5 (3-4)	19 (12-26)	70	115-?	700->1000	2
	Partially Blind	89	4	13	100	?	?	1
BARBITURATE	Normal	223 (173-273)	3.5 (3-4)	26.5 (20-33)	27 (24-30)	110-?	300-?	2
	Partially Blind	152	4	13	25	?	?	1
	Blinded	162-?	3	40 (32-48)	16 (10-22)	?	?	2

Table prepared in the same way as Table 2-1. The postsynaptic response was corrected for changes in the presynaptic response. Question marks (?) indicate either that the peak amplitude was not obtained (initial supernormality) or that the interval between the two stimuli was not sufficient to reveal the response change (terminal supernormality).

In Fig. 2-10 can be seen the t and r recovery curves when the cat was under barbiturate anaesthesia. This experiment was done on the same day as the alert study shown in Fig. 2-9.

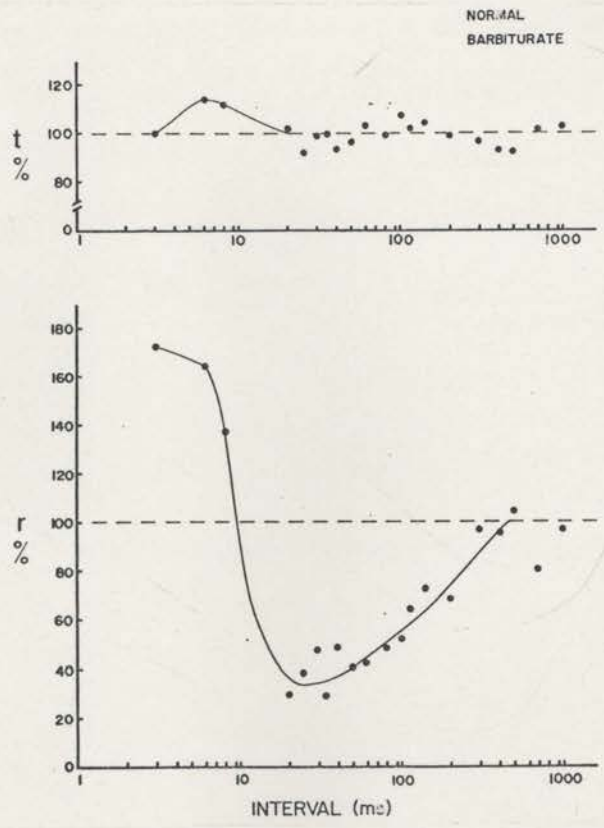


Fig. 2-10: Recovery curves in the normal cat under barbiturate anaesthesia; as for Fig. 2-9.

The recovery of the presynaptic response is similar to that seen in the alert cat - there is a small enhancement of the response, followed by a return to the control level by 20 ms. The r response is initially enhanced well above the control level (173% in this experiment, 273% in another experiment - see Table 2-2), whereas, in the alert cat, this early supernormality was only relative, being superimposed on a phase of depression (Fig. 2-9, Table 2-2). As in the alert cat, the response is then greatly depressed. This graph shows no late supernormality of the postsynaptic response, but, in another experiment,

the r response was subsequently enhanced to 110% of the control amplitude at 300ms (Table 2-2).

M and B Treated Cat: Fourteen days after giving the first dose of the drug, geniculate excitability was re-examined in both the alert and the anaesthetized cat (Figs 2-11 and 2-12, respectively; Tables 2-1 and 2-2).

Comparison of Figs 2-11 and 2-9, for the alert cat, reveals no great differences in the amplitudes or durations of any changes in t and r after producing a state of partial blindness.

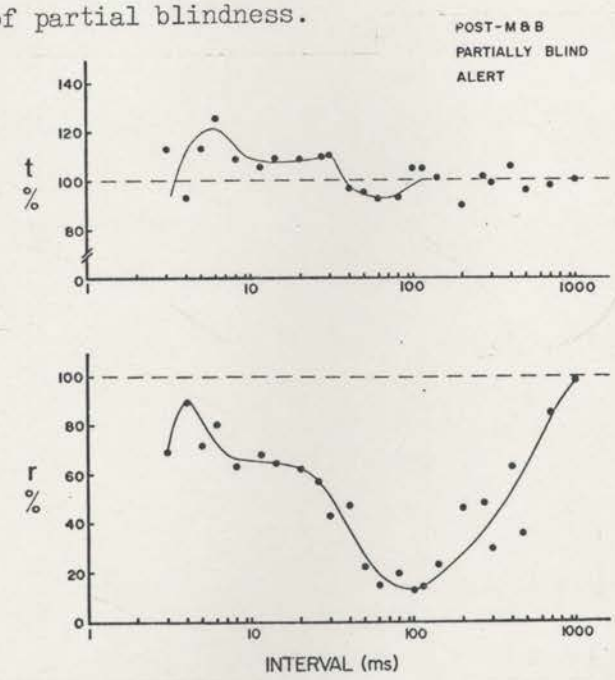


Fig. 2-11: t and r recovery curves in the alert cat 14 days after giving M and B 968A.

Although the shape of the t curve in Fig. 2-11 at the time of the supernormality does differ from that seen in both the experiments prior to giving the drug, this would not be a significant difference. The r recovery curve in the partially blind cat is very similar to that obtained normally. It is particularly evident that there has been no decrease in the amount of subnormality in the treated cat.

Similarly, under barbiturate anaesthesia, the two-shock recovery curves obtained in the normal and the partially blind cat (Figs 2-10 and 2-12, respectively) are very similar. Responsiveness following a single shock was also re-examined in the anaesthetized animal after a steady level of blindness had been reached (Fig. 2-13, Tables 2-1 and 2-2); again, it was obvious that the drug had not produced any marked changes in the recovery curve.

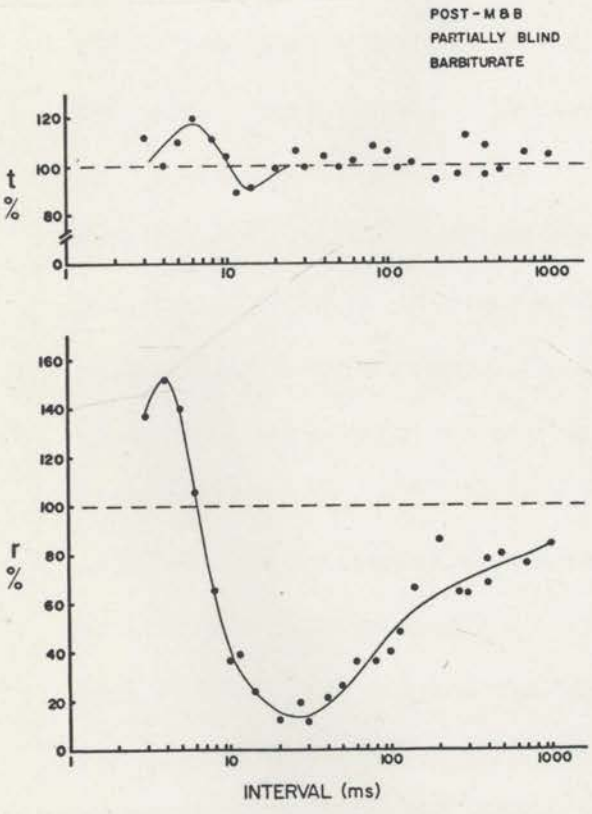


Fig. 2-12: t and r recovery curves 14 days after giving M and B 968A; cat under barbiturate anaesthesia.

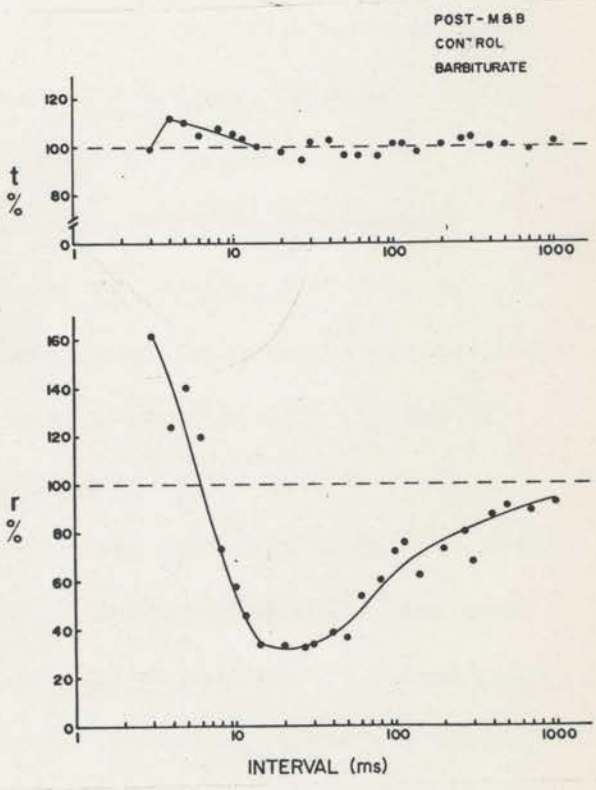


Fig. 2-13: Two-shock recovery curves in the anaesthetized cat after a steady level of blindness had been reached.

Table 2-2 suggests that there may have been a slight decrease in the amplitude of the subnormality in the blinded cat compared to the level seen normally.

Discussion

The results obtained in the present study from a chronic anaesthetized cat confirm observations made in acute anaesthetized preparations that geniculate excitability shows periods of supernormality and subnormality following single shock stimulation of the optic tract (e.g., Marshall, 1949; Bishop and Davis, 1960). Moreover, the results indicate that the recovery of geniculate responsiveness after single shocks to the optic tract is different in the normal alert cat and in the normal cat under barbiturate anaesthesia. This observation agrees with results obtained by Morlock and Marshall (1964) and by Eisman, Hansen and Burke (1967) using chronic preparations. In the alert cat, there is no real phase of early supernormality of the postsynaptic response - although there is some enhancement of the response, this is only relative and does not exceed the 100% control level. Under barbiturate, there was a real enhancement of the r response to an average of 223% for the two experiments (Table 2-2). This large supernormality in the anaesthetized cat is related to the amount of depression produced by the barbiturate (see Eisman et al., 1967, and Section I of this thesis). A late supernormality has been seen in both the alert and the anaesthetized cat, as reported by Eisman et al. The results therefore suggest that, in both these states, there is a subliminal fringe in the LGN, but that this is greater during barbiturate anaesthesia.

The recovery of the t response in the normal animal appears to be the same, whether the cat is alert or anaesthetized. There is an initial phase of subnormality, due to the absolute and relative refractory periods of the nerve fibers, and a subsequent phase of supernormality due to synchronous negative after-potentials of the fibers (Bishop, Jeremy and Lance, 1953). With regard to the initial supernormality of the postsynaptic response, it should be

pointed out that this normally has two phases (see Eisman et al.,1967), although, in this study, no distinction was made between these. The first phase appears to be due to summation of synaptic potentials at subliminally excited geniculate synapses (Eisman et al., 1967), while the second phase may be due to negative after-potentials in the principal (P) cells of the LGN (Bishop and Davis, 1960). As summation of synaptic potentials should still be effective in the alert animal, Eisman et al. (1967) suggested that barbiturate reduces or abolishes an inhibitory process which, in the alert animal, opposes this facilitation. The subnormality of the r response, which is seen in both the alert and the anaesthetized state, is probably due to the hyperpolarizing influence of the inhibitory interneurones (I cells) on the P cells (see Vastola, 1960; Burke and Sefton, 1966a, b and c).

Burke and Hayhow (1968) reported a significant decrease in the amount of r subnormality in acute anaesthetized cats which had previously been given M and B 968A. They quoted values of r subnormalities ranging from 20 to 44% of the control level in normal cats, and from 43 to 100% in the treated cats. In contrast to Burke and Hayhow's finding, the results obtained in the present study do not indicate that the amount of r subnormality was greatly reduced in the treated cat: under barbiturate, the levels of depression seen postsynaptically were 20 to 33% (normal), 13% (partially blind) and 32 to 48% (blinded). There is certainly no lessening of the depression in the partially blind animal, but, after a steady level of blindness had been reached, there is a suggestion of a slight decrease in the amount of depression. In the alert animal, there was also no evidence of a marked reduction in the amount of r subnormality in the M and B treated cat - before giving the drug, peak subnormality values of 12 and 26% were obtained, while, in the partially blind

animal, the single experiment done indicated depression to 13%.

The difference between these results and those of Burke and Hayhow is probably due to the preparations used. In the present study, all data were obtained from the same animal, which had electrodes chronically implanted in its brain, and, in each experiment, identical combinations of electrodes were used for stimulating and recording. Thus, variability due to different electrode placements did not contribute to the results. On the other hand, Burke and Hayhow used acute preparations, so their results may have been contaminated by such variability. It is obvious that the use of a chronic preparation is desirable in experiments such as this, where direct comparisons are to be made between two different conditions.

Finally, I wish to draw attention to preliminary observations made by Bruce (1964-65) and Hansen (1965) on the effects of M and B 968A on the recovery cycles after single OT shocks in chronic cats - the same cats were used by these two workers. In the alert preparation, Bruce gives values for the amount of depression of the r response ranging from 16 to 74% (average 47%) for normal cats and 70 to 89% (average 81%) for the treated cat; under barbiturate annesthesia, the values were 3.3 to 94% (average 48%) and 45 to 70% (average 58%) for normal and treated cats respectively. Data from more experiments contributed to these results than to those obtained by the author, so it might appear that these results support those of Burke and Hayhow - namely, that the amount of depression of the postsynaptic response is reduced in the M and B treated cat. However, it is possible that the discrepancy between Bruce's and Hansen's results and the results obtained in the present study is also related to the procedure adopted - even although chronic cats were used by Bruce and Hansen, different animals were used for the normal and the post-M and B studies.

Thus, the possibility remains that M and B 968A does produce a slight change in geniculate excitability following single shock stimulation of the optic tract, but to examine this effect properly, control and experimental data need to be obtained from the one animal.

B. Responsiveness Following Tetanic Stimulation of the Optic Tract.

Changes in geniculate excitability resulting from disuse of the OT/LGN synapses were further examined by applying a brief tetanus to the optic tract and following the time-course of the recovery of responsiveness. The tetanus was a 15 second train of pulses of alternating polarity at a rate of 500 stimuli per second. Before giving the tetanus, single test stimuli were applied to the OT via the same electrodes every 5s, until about 20 control responses had been obtained. Test stimuli were also given at 5 second intervals after the tetanus, and each post-tetanic response was expressed as a percentage of the average pre-tetanic control response amplitude. All stimuli were 50μs in duration and of supramaximal intensity. Post-tetanic excitability was only examined in the barbiturate anaesthetized cat.

The results of all experiments were plotted to show the amplitude of the post-tetanic responses in relation to the time after the end of the tetanus. In all illustrations, the recovery of the presynaptic response is shown in the upper graph, and of the postsynaptic response in the lower graph.

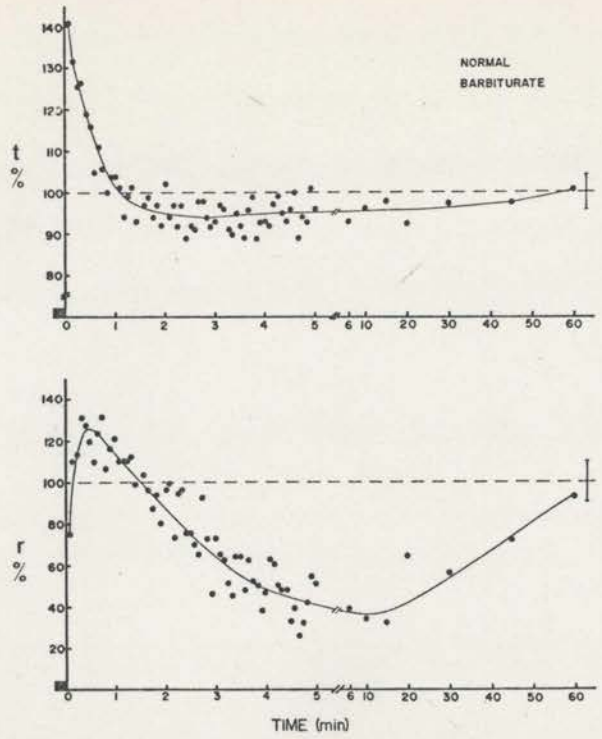


Fig. 2-14: Effect of 15-s tetanus (500/s) on presynaptic and postsynaptic responses in the LGN of the normal cat. Cat under barbiturate anaesthesia. For details, see text.

The black box to the left of the intersection of the two axes represents the 15 second tetanus, while the dashed line indicates the average pre-tetanic response amplitude (taken as the 100% control level). The vertical bar on the right hand end of the line through 100% indicates ± the standard deviation of the control responses. Each point on the graphs between 0 and 5 minutes after the tetanus represents the amplitude of a single response, while later points are the average of between 3 and 12 responses. The curves were estimated, by eye, to be the lines of best fit for the points.

Fig. 2-14 shows the recovery of excitability in the normal (anaesthetized) cat. Post-tetanically, the t response shows a period of enhancement lasting for only 1 minute, followed by a prolonged but very shallow phase of depression. The r response shows a brief period of post-tetanic depression followed by

potentiation of the response to 125% of the control level. There is then a deep and prolonged depression of the response to 36% of the control amplitude - this second phase of depression of the r response is known as post-tetanic delayed depression (PTDD). The results of this and other post-tetanic recovery experiments are summarized in Table 2-3.

TABLE 2-3

Recovery of the Presynaptic and Postsynaptic Responses after Tetanic Stimulation of the Optic Tract.

	Supernormality of t (%)	Subnormality of t (%)	Supernormality of r (%)	Delayed Subnormality of r (%)	Number of Experiments
Normal	137 (133-141)	94	109 (93-125)	45 (36-54)	2
Partially Blind	121 (116-125)	90 (86-95)	126 (114-150)	76 (60-87)	4
Blinded	121 (112-127)	95 (90-100)	106 (95-116)	94 (86-100)	6

All values are expressed as a percentage of the control amplitude (i.e., of the average pre-tetanic test response amplitude). Values given are averages for the number of experiments indicated, and, in brackets is given the range of values for individual experiments. All post-tetanic excitability studies were done on the barbiturate anaesthetized animal.

From the table, it can be seen that the results of the other experiment done before giving M and B 968A were essentially the same as those just described, except that the phase of r supernormality was only relative, the response not exceeding the control level.

Fourteen days after giving the first dose of the drug, there was a marked change in the post-tetanic recovery cycle (Fig. 2-15). This change was a decrease in the amount of PTDD, the depression now being to only 87% of the control level. The r supernormality and t recovery appear normal in the partially blind cat.

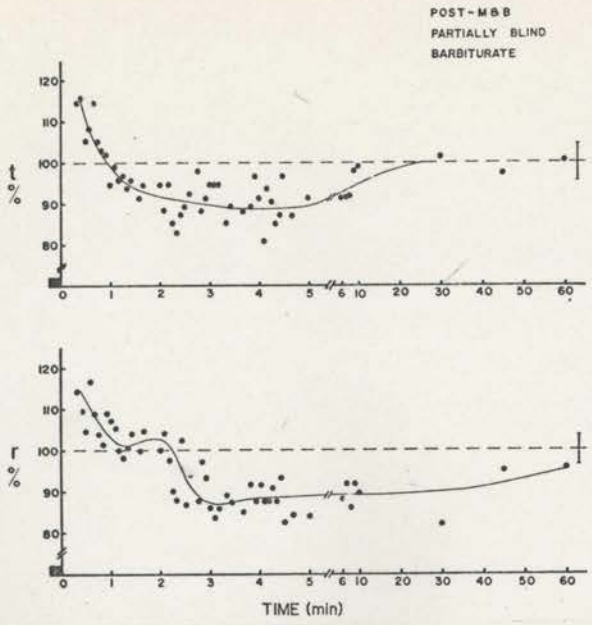


Fig. 2-15: Post-tetanic recovery curves for t and r, 14 days after giving M and B 968A.

Three other control experiments (i.e., experiments not following stimulating sessions) were done over the next two months, during which time the cat was only partially blind. As shown in Table 2-3 , the amount of PTDD was always less than in the normal cat. As the cat began to recover behaviourally from the drug, there was a tendency for the amount of PTDD to increase (i.e., to approach the normal range for such depression).

Further doses of M and B 968A were given and, when a steady level of blindness had been reached, it was found that the amount of PTDD had decreased even further. For example, in the experiment shown in Fig. 2-16, the postsynaptic response was only depressed to 98% of the control amplitude. Table 2-3 shows that the five other control experiments on the blinded cat gave similar results. Again, the potentiation of the r response and recovery of the t response did not appear to have been affected by the drug.

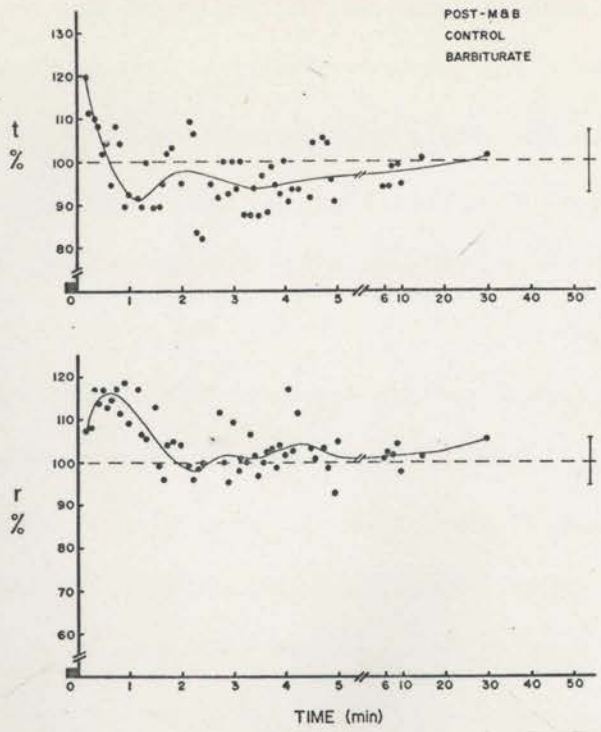


Fig. 2-16: Post-tetanic recovery curves in the blinded cat.

Discussion

Post-tetanic recovery cycles in the geniculate have been examined by other workers (e.g., in acute preparations, Hughes and Evarts, 1955; Evarts, Hughes and Marshall, 1956; Hughes, Evarts and Marshall, 1956; Evarts and Hughes, 1957a and b; Bishop, Burke and Hayhow, 1959; Burke and Hayhow, 1968; and in chronic preparations, Morlock and Marshall, 1964; Eisman, 1963-64; Hansen, 1965). The results obtained in the present study on the normal cat are in agreement with the findings of these earlier workers.

Following supramaximal testing stimuli, the t response showed an initial phase of potentiation. Three factors have been suggested to contribute to this supernormality: (i) an enhanced amplitude of the OT action potentials, (ii) a decrease in conduction velocity along the fibers, and (iii) an increase in the

duration of the action potentials in the tract endings (see Bishop et al., 1959). Bishop et al. reported that, if the test shock was submaximal, the t response showed a prolonged, shallow phase of depression, while, if it was barely maximal, the post-tetanic response could show both an early brief potentiation and a later prolonged depression. As a shallow phase of depression was seen to follow the potentiation in the present study (e.g., Fig. 2-14), this might indicate that the stimuli used were not supramaximal, as was thought, but that they were only just maximal. Bishop et al. suggested that the prolonged depression represented a decrease in the number of fibers being stimulated. This may have resulted from decreased excitability at the site of stimulation, perhaps due to hyperpolarization following the tetanus.

The initial phase of depression of the r response is due to refractoriness of the geniculate neurons. The subsequent phase of post-tetanic potentiation (PTP) seems to depend on the existence of a fringe of subliminally excited cells. The mechanism responsible for PTP is generally agreed to be presynaptic in origin (see Hughes, 1958). Hughes et al. (1956) suggested that PTP might involve an increase in the amount of transmitter substance liberated, as it could not be satisfactorily accounted for by changes in soma excitability or changes in the presynaptic response. PTP also occurs at neuromuscular junctions and this also appears to have its origin presynaptically and to be due to increased transmitter release. It has been suggested that this increased release at the neuromuscular junction is due to "transmitter mobilization" and therefore to an increased availability of transmitter substance (see Eccles, 1964; also, Maeno, 1969; Maeno and Edwards, 1969). On the other hand, Rosenthal (1969) believes that PTP is due to an increase in the fraction of transmitter actually released from each vesicle by an action potential, not to an increase

in the amount available for release. It is also possible that an increase in the presynaptic spike potential due to a prolonged after-hyperpolarization of the presynaptic fibers would influence the amount of transmitter released and therefore, the amount of PTP (see Bishop et al., 1959; Eccles, 1964; Sharpless, 1964).

The results of most workers seem to indicate that PTDD in the LGN is secondary to barbiturate anaesthesia (although Bishop et al., 1959, did see PTDD in cerveau isolé preparations). PTDD, like PTP, appears to have its origin presynaptically (Morlock, Pearlman and Marshall, 1965). Possibly, it is due to depletion of transmitter substance or to desensitization of the receptor sites on the postsynaptic membrane resulting from the prolonged action of the transmitter substance during PTP (see Bishop et al., 1959; Eccles, 1964). Løyning, Oshima and Yokota (1964) reported that barbiturates act mainly on the afferent nerve terminals of the monosynaptic spinal reflex pathway, resulting in a reduction of their spike amplitude and thus, decreased transmitter release and a reduced synaptic potential. This action of barbiturate must therefore be considered along with the direct effects of the tetanus as one factor contributing to the existence of PTDD.

Disuse at the OT/LGN synapses resulted virtually in abolition of the PTDD, while the other components of the recovery cycles were unaltered. From the preceding paragraph, it would appear that there are three possible explanations for this loss of depression. Firstly, it was suggested that tetanization might lead to depletion of the supplies of transmitter substance. It was shown earlier that, after giving M and B 968A, there was a marked loss in maintained optic tract discharge, and thus a state of disuse was said to have been produced in the OT/LGN synapses. It is feasible that, with this loss of synaptic activity,

there is an accumulation of transmitter substance in the tract terminals and therefore, that increased amounts of transmitter are released during subsequent stimulation. Brown, Davies and Ferry (1961) and Brown, Dearnaley and Geffen (1966) have obtained results which support this hypothesis in experiments on the splenic nerve.

If this explanation were correct, it would be expected that prolonged periods of OT stimulation prior to tetanization would deplete the accumulated supplies of transmitter towards the normal levels and so lead to an increase in the amount of post-tetanic depression. However, it will be shown shortly that, even after stimulating the OT for 8 hours a day for a week, there was no such increased depression, so this would not seem to be the entire explanation for the lack of PTDD in the LGN following disuse.

Secondly, it is possible that, during disuse, the postsynaptic membrane may become supersensitive to transmitter substance. Following denervation of skeletal muscle, the sensitivity to acetylcholine becomes greatly enhanced along the entire muscle membrane (e.g., Nicholls, 1956; Thesleff, 1960; Albuquerque and McIsaac, 1970). Denervation supersensitivity also occurs in other tissues deprived of their normal input - for example, smooth muscle and glands (Vera, Vial and Luco, 1957; Emmelin, 1960; Luco, 1963; Trendelenburg and Langer, 1965), ganglia (see Sharpless, 1964) and the central nervous system (see Stavraky, 1961). Supersensitivity also results from less direct procedures which abolish or alter the normal input to a tissue; for example, sectioning the preganglionic fibers leads to increased sensitivity in autonomic effectors (e.g., Emmelin, 1959) even although the post-ganglionic fibers are intact (decentralization supersensitivity). Effector organs also become supersensitive if drugs are used which prevent the liberation of transmitter substance from

the nerve terminals or which inhibit the combination of transmitter with the receptor sites on the postsynaptic membrane (e.g., Emmelin, 1959, 1960, 1961). This phenomenon of denervation supersensitivity is considered in detail in several reviews (Cannon and Rosenblueth, 1949; Emmelin, 1961; Stavraky, 1961; Trendelenburg, 1963; Sharpless, 1964).

Finally, it is possible that the action of barbiturate might be affected in some way by giving M and B 968A. From experiments conducted previously by other workers in our laboratory, it appeared that M and B had "the effect of reducing the action of barbiturate at the LGN" (see Bruce, 1964-65). A similar conclusion may be drawn from results given earlier in this section. It was shown that the t response to single shocks in the anaesthetized animal was reduced in amplitude after giving M and B 968A, and that the threshold of the response (both alert and anaesthetized) was slightly increased by the drug. Perhaps this effect is mediated at the retina as both barbiturate and M and B 968A are known to act here. In confirmation with other workers, the optic disk of the treated cat appeared very pale and the retinal blood vessels were greatly attenuated. The reduced blood supply may have affected the metabolism of the retinal ganglion cells and optic nerve fibers, and so may have been partly responsible for the changes seen in the presynaptic response. However, some other factor must also be involved, as the response was only reduced in amplitude when the cat was anaesthetized.

No obvious changes were seen in the postsynaptic response to single OT stimuli after giving M and B 968A. It is interesting to note that Burke and Hayhow (1968) observed a slight decrease in the amount of PTP of the r response in acute anaesthetized cats previously given M and B 968A. They suggested that the response to a single shock may have increased slightly in amplitude

and so obliterated the subliminal fringe which is necessary for the production of PTP. In fact, an increase in the amplitude of the postsynaptic response in the treated cat would be anticipated from the observation that the retinal discharge normally produces tonic inhibition in the LGN (Hansen et al., 1967; Kasamatsu, Kiyono and Iwama, 1967; Suzuki, 1967; Suzuki and Ichijo, 1967; Nakai and Domino, 1968).

From this discussion, it would seem that the state of disuse that we are examining may, in fact, be contaminated by other variables. Although the loss of PTDD seen after giving M and B 968A may be a direct result of the disuse which the drug produces at the OT/LGN synapses, the possibility of other effects of the drug cannot be excluded entirely. There is evidence that M and B 968A has a potent inhibitory effect on the aerobic respiratory enzyme, malic dehydrogenase, which is present in the retina (see Bishop, Burke, Davis and Hayhow, 1960). Although this enzyme is present in other cells throughout the body, the susceptibility to inhibition of the enzyme must be greater in certain parts of the retina than it is elsewhere and hence, the apparently exclusive action of the drug which was described earlier. If M and B 968A does have effects other than those described at the retina, they may result from a much more subtle action of the drug on malic dehydrogenase in other cells and this could be the reason that histological studies have failed to reveal any other sites of action of the drug.

3. Attempted Reversal of the Effects of Disuse in the LGN.

It is evident from the results just reported that disuse in the LGN does not lead to a decrease in synaptic efficacy as Eccles postulated and found (see the Introduction). To the contrary, there appeared to be enhanced excitability of the cells in the geniculate. This was particularly evident following brief tetanization of the OT, when the amount of PTDD was found to be greatly reduced in the treated cat compared to the level seen normally. The responsiveness following single shock stimulation of the OT was little affected by disuse, there being only a suggestion of a slight decrease in the amount of depression of the postsynaptic response after giving M and B 968A. If these effects were due to disuse and not to some other action of the drug, then it should have been possible to reverse the effects of disuse by re-activating the geniculate synapses. This possibility was examined by electrically stimulating the optic tract for long periods of time and so artificially providing an input to the synapses.

In the initial stimulating sessions, regular optic tract stimulation at a rate of 30 stimuli per second was used. This rate was chosen because it approximates the rate of maintained ganglion cell discharge under constant conditions. The two-shock recovery cycle was re-examined after only two of the stimulating sessions, while post-tetanic excitability was examined after each of the five sessions. Details of the stimulation were given in the Methods. The results of all experiments are summarised in Tables 2-4 and 2-5.

In view of the small effect of disuse on the two-shock recovery cycle, it is not surprising that regular OT stimulation likewise had little effect on the recovery. Table 2-4 shows that regular stimulation for 4 hours per day for a week did not lead to a change in the amount of subnormality of the r response following a single OT stimulus.

TABLE 2-4

The Effects of Prolonged Periods of Optic Tract Stimulation on the Two-Shock Recovery Cycle.

	Supernormality of t (%)	Initial Supernormality of r (%)	Subnormality of r (%)	Terminal Supernormality of r (%)	Number of Experiments
Normal	113 (112-114)	223 (173-273)	26.5 (20-33)	110-?	2
Blinded controls	111.5 (111-112)	162-?	40 (32-48)	?	2
) after 1 week of regular imulation 4hrs/day.	117	124	42	110	1
) after 1 week of regular imulation 8hrs/day.	116	126	34	105	1

Table prepared in the same way as Tables 2-1 and 2-2, except that no time values have been included. All experiments were done under barbiturate anaesthesia. The values from experiments on the normal cat and the blinded cat (controls) which were shown in Tables 2-1 and 2-2, are included here for easy reference. Experiments following prolonged OT stimulation were done 1 day after the end of the session.

Similarly, the 8 hour per day stimulating session was ineffective (Fig. 2-17, Table 2-4). The control experiment done prior to the 8 hour per day session is shown in Fig.2-13 - in this experiment, the postsynaptic response was depressed to 32%, whereas, after the stimulation, there was depression to 34% (Fig. 2-17).

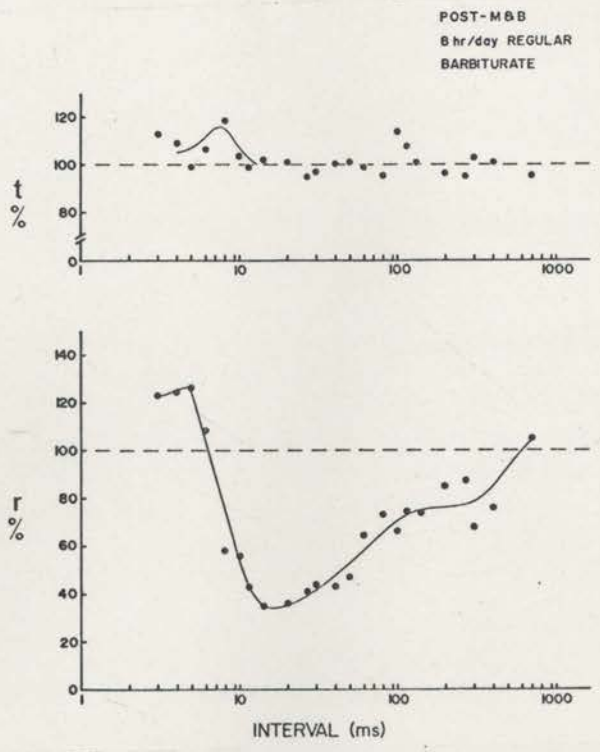


Fig. 2-17: Recovery curves after a single optic tract stimulus in the blinded cat; experiment done 1 day after finishing 1 week of OT stimulation at 30/s for 8 hours/day. For control experiment, see Fig. 2-13.

Although the two-shock results were not unexpected, the studies of post-tetanic excitability were a little more surprising. Following none of the regular stimulating sessions was there a reversal of the recovery cycle towards that seen in the normal animal. Daily stimulation was for either $2\frac{1}{2}$, or 4, or 8 hours during the week. Fig.2-18 shows the results of the experiment after stimulating the OT for $2\frac{1}{2}$ hours per day for a week when the cat was partially blind (compare with the control experiment shown in Fig. 2-15).

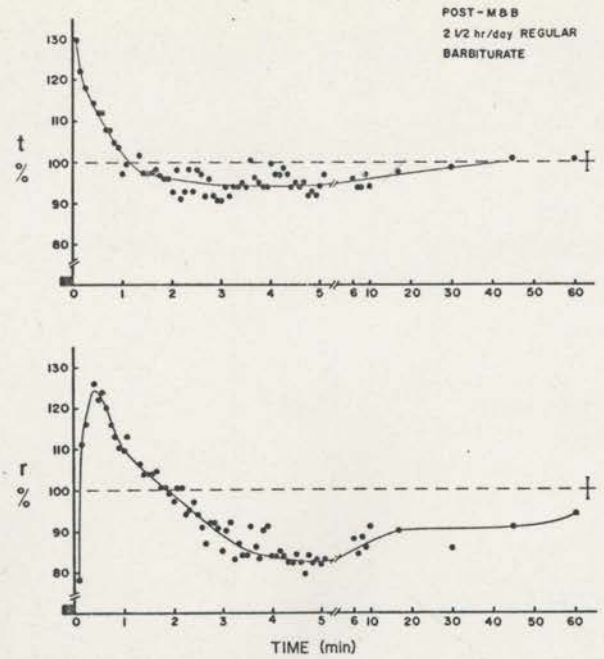


Fig. 2-18: Post-tetanic excitability in the partially blind cat; 1 day after finishing a week of 30/s OT stimulation for $2\frac{1}{2}$ hours/day. For control experiment, see Fig. 2-15.

The t and r recovery curves in these two figures are very similar, and it can be seen from Table 2-5 that the levels of supernormality and subnormality seen after the period of stimulation lie within the range for the partially blind controls. Two 8 hour per day sessions were also carried out while the cat was only partially blind and again, there was no increase in the amount of post-tetanic delayed depression.(Table 2-5).

TABLE 2-5

The Effects of Prolonged Periods of Optic Tract Stimulation on Post-Tetanic Excitability.

	Supernormality of t (%)	Subnormality of t (%)	Supernormality of r (%)	Delayed Subnormality of r (%)	No.of Expts.
Normal	137 (133-141)	94	109 (93-125)	45 (36-54)	2
Partially Blind -					
(i) controls	121 (116-125)	90 (86-95)	126 (114-150)	76 (60-87)	4
(ii) 1 day after a week of regular stimulation for 2½hrs/day.	130	94	124	82	1
(iii)1 day after a week of regular stimulation for 8 hrs/day.	123 (116-130)	91.5 (88-95)	132 (128-136)	89.5 (89-90)	2
Blinded -					
(i) controls	121 (112-127)	95 (90-100)	106 (95-116)	94 (86-100)	6
(ii) 1 day after a week of regular stimulation for 4 hrs/day.	103	91	129	99	1
(iii) immediately and (iv) 1 day after a week of regular stimulation for 8 hrs/day.	123 / 117	92 / 93	104 / 113	87 / 92	1 / 1
(v) immediately and (vi) 1 day after a week of random stimulation for 8 hrs/day.	118.5 (118-119) / 123.5 (123-124)	93.5 (92-95) / 96.5 (96-97)	111 (104-118) / 106.5 (96-117)	91.5 (84-99) / 91.5 (87-96)	2 / 2

Table prepared in the same way as Table 2-3. Data from Table 2-3 included here for easy reference.

Following the above series of experiments, more M and B 968A was given until a steady level of blindness was reached; two more regular stimulating sessions were then done, one for 4 and the other for 8 hours per day for a week. Fig 2-19 shows the results of the control experiment just before beginning the 4 hour session, and Fig. 2-20 the results 1 day after the end of the week of stimulation. It is not known why the t recovery curve in Fig. 2-19 did not show the normal phase of depression - this was the only experiment in the entire study in which such a result was obtained.There was no increase in the amount of PTDD after the stimulating session (Fig.2-20).

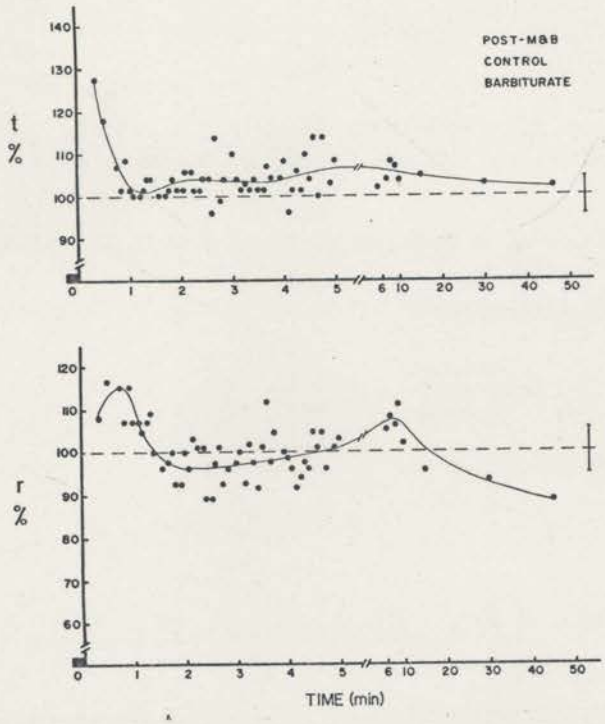


Fig. 2-19: Post-tetanic recovery curves in the blinded cat. Control experiment done before beginning the week of stimulation shown in Fig. 2-20.

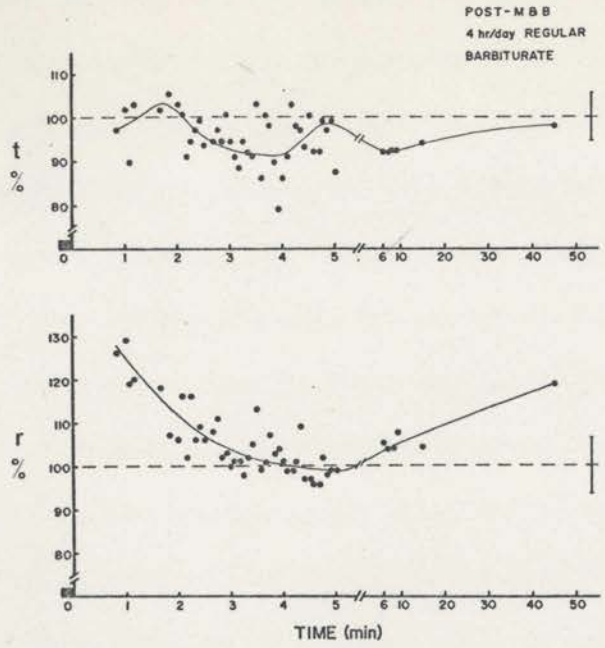


Fig. 2-20: Post-tetanic excitability in the blinded cat; experiment done 1 day after finishing a week of regular OT stimulation for 4 hours/day.

Similarly, after stimulating for 8 hours a day for a week, there was no increase in the amount of depression of the r response towards the normal level (Fig.2-21).

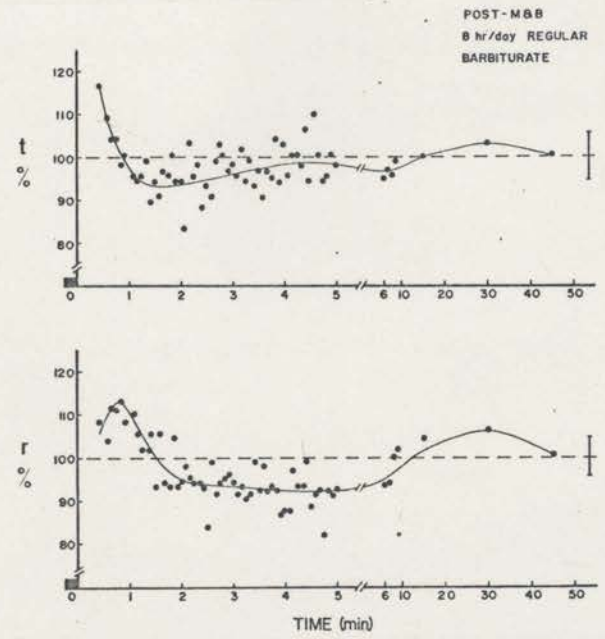


Fig. 2-21: Effect of regular OT stimulation for 8 hours/day for a week on post-tetanic excitability in the LGN of the blinded cat.

The experiment shown in Fig. 2-21 was done 1 day after ending the stimulating session, but, as shown in Table 2-5, an experiment was also done on the previous day, immediately after stopping the OT stimulation. This procedure was adopted following this and subsequent stimulating sessions because it was felt that any changes in excitability might be short-lasting and so not evident after a 24 hour delay. Although the amount of PTDD was slightly greater immediately after the session than it was 1 day later (87% compared with 92%), it was doubtful whether such a difference would prove significant.

As no definite changes had been seen after any of the above sessions of regular stimulation, an attempt was made to use a more physiological form of stimulation. The "random" firing of a normal cat's retinal ganglion cell was used to trigger the stimuli. In each session, stimulation was continued for 8 hours each day. Details of the stimulating set-up and the characteristics of the ganglion cell discharge used have already been given. Results of all experiments are included in Table 2-5. Fig. 2-22 shows the results obtained 1 day after ending one such stimulating session.

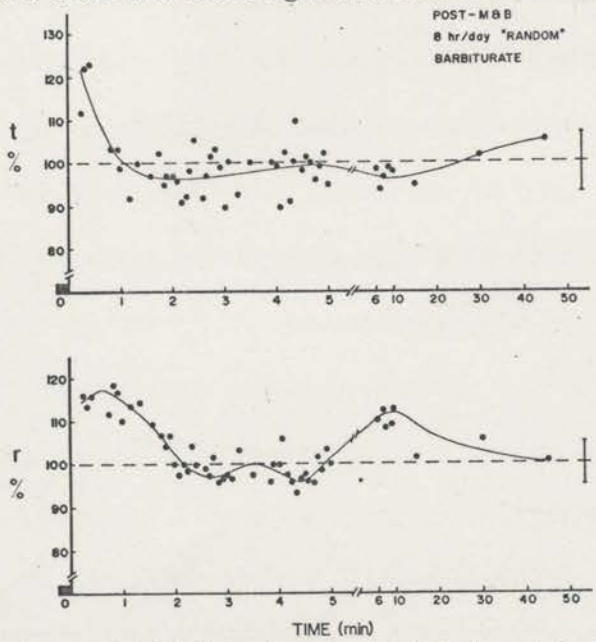


Fig. 2-22: Post-tetanic excitability in the blinded cat 1 day after finishing a week of random OT stimulation for 8 hours/day.

(The results from the other session were similar to these). These graphs should be compared with those shown in Fig. 2-16, as this was the control experiment done prior to this particular week of stimulation. Post-tetanically, both the t and the r recovery curves are similar to the control curves - in particular, there is no evidence of an increase in the amount of PTDD. There was also no indication of increased levels of PTDD when the experiments were done immediately after ending the week of random stimulation (Table 2-5).

Discussion

The results just described indicate that disuse at the LGN is not a readily reversible phenomenon. It had been hoped that, by artificially producing optic tract activity and so reactivating the optic tract/LGN synapses, the recovery cycles following single shock or tetanic stimulation of the OT would return to the curves seen in the normal cat. The change in the two-shock recovery cycle was very slight following disuse, making identification of any change produced by the stimulation difficult. On the other hand, the depression seen post-tetanically in the normal cat (average 45%) was virtually abolished in the blinded cat (average 94%), so a return towards the normal values following re-use of the synapses should have been obvious. It must therefore be concluded that the stimulating sessions were without effect on geniculate excitability, certainly as far as the parameters examined are concerned.

Burke and Hayhow (1968) also reported that the degree of post-tetanic delayed depression was not altered by prolonged optic nerve tetanization - in their experiments, stimulation lasted for either 10 minutes or 1 hour at 500/second. However, Eisman (1963-64) and Hansen (1965) reported that the effects of disuse on post-tetanic excitability were partially reversed by

optic tract stimulation (a total of $23\frac{3}{4}$ hours of stimulation was done at a rate of about 60/second over a period of 3 days), and that"these reversals were themselves reversed by a further 4-week period of optic tract quiescence". These observations were not confirmed in the present study, and, in fact, the values quoted from the graphs shown by these workers are a little dubious.

Physiological changes following long-term stimulation of some parts of the brain have been reported by other workers (e.g., Delgado, 1959;Willey and Freeman, 1968; O'Connor, Rosenthal and Jöbsis, 1969). It has been shown that continuous stimulation of a peripheral nerve does not produce any visible histological changes (Hershberg, Sohn, Agrawal and Kantrowitz, 1967), so the effects which were seen centrally may not have been manifestations of structural change. O'Connor et al. (1969) reported that the changes they observed resembled memory.

Why the prolonged optic tract stimulation has proved unsuccessful in reversing the effects of disuse in the LGN is not known, but several possible explanations will be briefly considered. Firstly, the stimulation may not have been continued for long enough each day and the duration of the stimulating sessions may also have been too short. This may have applied particularly towards the end of the series of experiments when the LGN had been in a state of disuse for so long (almost 10 months) that such relatively short periods of stimulation were negligible compared to the period of inactivity. Possibly, if a state of complete blindness were produced quickly and a stimulating session done immediately, there might be some reversal of the effect of disuse. An obvious way to examine these two possibilities (i.e., that the daily duration of stimulation was too small, and that the LGN had been in a state of disuse for too long) would be to produce a state of total blindness very rapidly and

then to stimulate the OT continuously for 24 hours a day for a week. If there were still no reversal of the effect of disuse, then another explanation would have to be sought.

A second possible explanation for the lack of reversal is that the stimulating parameters were unsuitable. It was for this reason that the regular 30 per second stimulation was replaced by the "random" stimulation. This form of stimulation may also be too unphysiological to mimic the effects of the normal maintained discharge of the retinal ganglion cells as a whole. For example, even the random stimulation which was used led to synchronous activation of all the OT fibers, whereas, normally, these are firing asynchronously. It might be interesting to see if direct stimulation of the LGN would prove successful in reversing the effects of the disuse, but it is difficult to imagine how this could be effective when OT stimulation has so far, at least, proved so unsatisfactory.

In the normal cat, there is a maintained discharge of the ganglion cells at all times, including the times when the excitability studies are being carried out. This is not so, however, in the M and B treated animal, either in experiments done before or after a stimulating session. It might therefore be suggested that it is the absence of this normal discharge at the actual time of an experiment which is partly or wholly responsible for the lack of PTDD in the treated animal. This suggestion is not relevant, though. Post-tetanic excitability has been examined in acute enucleated preparations (e.g., Burke and Hayhow, 1968) which, like the M and B treated cat used in the present study, have no maintained ganglion cell activity; yet, the recovery cycles in such enucleated preparations resemble those in normal chronic cats. Therefore, this would not seem to be the explanation for the loss of PTDD after giving

M and B 968A.

Finally, a control experiment still remains to be done - the effects of prolonged optic tract stimulation on geniculate excitability in the normal cat have yet to be determined. An attempt was made to examine this, but the stimulation evoked such violent fits (see the Methods) that it had to be discontinued. Perhaps such prolonged stimulation in the normal cat may itself lead to a reduction in the amount of PTDD following a 15 second tetanus. If so, it is pointless to try to increase the amount of depression in the treated cat by using such stimulation.

It would seem from this discussion, that two experiments still need to be carried out - the first is to constantly stimulate the OT for 24 hours a day for a week and the second is to examine the effects of such prolonged periods of OT stimulation on excitability in the LGN of a normal cat. Possibly the loss of PTDD will not be reversed by continuous stimulation for a week, and the experiment on the normal animal may not reveal that the method used to try to reverse the effects of the disuse is unsuitable. If so, the hypothesis that M and B 968A has some other action besides that at the retina must be considered. As mentioned earlier, the drug may have a direct effect at the geniculate, but this may be of such a nature that it cannot be identified by light microscopy. If some change does occur at the LGN other than the indirectly produced disuse, then re-activation of the synapses would not be expected to return the excitability to normal. Obviously, the way to examine this possibility is to try to produce disuse at the OT/LGN synapses by other means. After determining the effects of the disuse, the synapses could be re-activated as described here, in an attempt to reverse any changes resulting from the disuse. Other techniques which could be used are considered in the Final Discussion.

FINAL DISCUSSION

Non-Visual Studies on the Effects of Use and Disuse.

Evidence in favour of the view that use enhances and that disuse depresses synaptic transmission has been derived mainly from studies of monosynaptic spinal reflexes carried out by Eccles and co-workers. In the Introduction to this Section, only those aspects of the experiments which were directly relevant to the possible mechanism of learning were considered. These experiments will be considered in more detail in the following pages, and the results of other studies which have tested Eccles' hypothesis will be discussed.

Eccles and McIntyre (1953) obtained disuse of the Ia/motoneurone synapse in the cat by sectioning the dorsal root fibers just distal to the ganglion cells and thereby abolishing impulses in the presynaptic nerve fibers. Thirty to forty days after the operation, it was found that the monosynaptic reflexes of both flexor and extensor muscles were absent or very much reduced in size. In Fig. 2-3, an example of one such experiment, can be seen the monosynaptic reflex discharges in the nerves of the gastrocnemius muscle (an extensor) on the operated (O) and normal (N) side of the animal. Eccles,Krnjević and Miledi (1959) obtained disuse of this synapse in a different way. The nerves to muscles were severed peripherally 13-25 days before changes in synaptic function were tested by recording from motoneurones with intracellular electrodes. As only one nerve of a pair supplying synergic muscles was severed, it was possible to compare in the same motoneurone, responses evoked by the normal afferent and the surgically transected pathways. In agreement with the results of Eccles and McIntyre, disuse was found to cause depressed motoneurone responses.

It was concluded from the above studies that the observed changes were due at least partly to the disuse produced at the synapses between the Ia fibers

and the motoneurones. However, the operative procedures followed in these experiments were accompanied by degenerative changes due to direct injury of the nerves. Following sectioning of the afferent nerves, there was shrinkage of the dorsal root fibers and evidence of degeneration in the operated ganglion. Such degenerative changes may conceivably have extended to the synaptic terminals and contributed to the depressed synaptic transmission. Okamoto and Riker (1969) and Miledi and Slater (1970) have recently shown that there are functional changes in motor nerve terminals within hours of sectioning the nerve, and it may be that, even when afferent nerve section was not accompanied by obvious degenerative changes, there were functional changes in the presynaptic terminals. The complications of degeneration also arise when the nerve section involves damage to the axons of the motoneurones. It is possible, therefore, that none of the observed changes were due to disuse, but that they resulted from nerve injury.

In the experiments of Eccles and McIntyre (1953) and Eccles et al.(1959), it was also found that there was an increase in the amplitude and duration of post-tetanic potentiation (PTP) on the operated side relative to the normal side (see Fig.2-23).

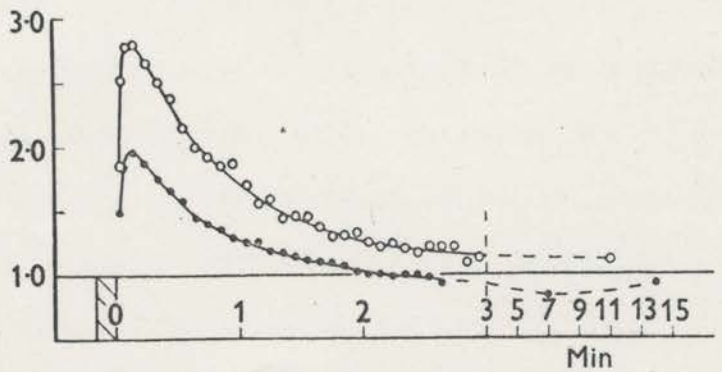


Fig. 2-23: Time course of PTP of EPSP's after a conditioning tetanus of 10s at 400/s (indicated by hatched column). Amplitude of EPSP's plotted relative to control amplitude; open circles - operated side, closed circles - control side. (Eccles et al., 1959).

Following disuse in the LGN, there is a decrease in the amount of depression following tetanic stimulation (Burke and Hayhow, 1968; this study), and, although no change was seen in the amount of post-tetanic potentiation, the results do not necessarily conflict with those of Eccles and co-workers in this regard (see Burke and Hayhow). Therefore, these may be real changes resulting from disuse, and the decreased amplitude of the motoneurone response may be a direct result of injury to the afferent fibers.

Electrical stimulation of the unmyelinated afferent fibers in the abdominal vagus nerve of the rabbit is known to evoke a reflex rise in blood pressure. Cragg (1965) therefore used this reflex to study the effect of distal section of the nerve on the efficiency of the synapses made by the afferent fibers in the medulla. Experiments done 40 to 160 days after the operation revealed a smaller and slower reflex rise in blood pressure, indicating a decrease in the central action of the nerve fibers. If disuse does lead to reduced synaptic efficacy, which is how the above-mentioned groups of workers interpreted their results, then excess use would be expected to produce enhancement of synaptic function above the normal level.

R.M. Eccles and Westerman (1959) and R.M. Eccles, Kozak and Westerman (1962) denervated all muscles but one in a synergic group and subsequently examined the monosynaptic reflex responses from the muscle with the intact innervation. It was found that, after some weeks, the reflex was always larger on this side than was the corresponding control reflex on the other side (see Fig. 2-4). The initial operation was presumed to have placed excess stress on the remaining intact muscle, with a consequent increase in the discharge from its stretch receptors. Thus, it was postulated that the operation led to an increase in the activation of the central synapse, and the results were

explained in these terms - that is, the enhanced synaptic efficacy was a result of the increased synaptic usage. However, as pointed out by Sharpless (1964), alternative explanations could exist, such as nonspecific collateral growth or denervation supersensitivity. In fact, it would appear that the observed increase in synaptic efficacy could not be due to excess use because the same result was obtained even when the residual muscles were carefully protected from all mechanical stress.

Attempts have been made to produce disuse without directly injuring the presynaptic nerve fibers. One technique employed by several workers has been to sever the muscle tendons, the suggestion made being that tenotomy prevents the muscle from exerting effective tension and so, leads to disuse of the Ia/motoneurone synapses. Following tenotomy, Beránek and Hnik (1959) observed a considerable increase in the amplitude of the monosynaptic response on the operated side compared with the control (unoperated) side. There was also a decrease in the latency of the response, which was attributed to a change in the central synaptic delay. These findings can be seen in Fig. 2-24, where the response of the operated side is 3.25 times larger than that of the non-operated side and occurs 0.31ms earlier. Although it was suggested that these effects were brought about by disuse, no attempt was made to monitor the proprioceptive impulse activity from the muscle.

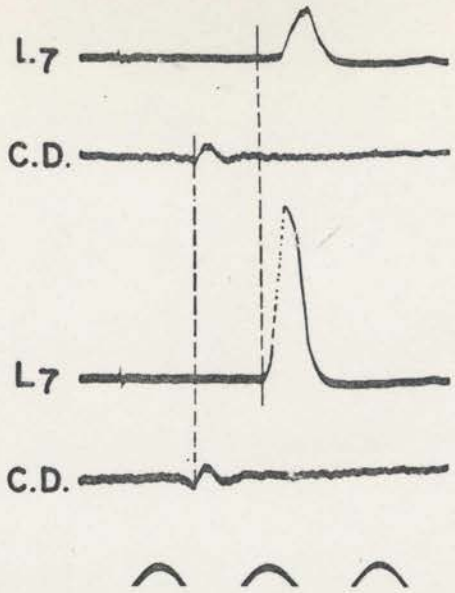


Fig. 2-24: Monosynaptic responses recorded from ventral root L_7 and cord dorsum potential (C.D) after single stimuli to the gastrocnemius nerves in a cat 32 days after unilateral section of the Achilles tendon. Upper two traces - control side; lower two traces - operated side. Time, 500c.p.s. (Beránek and Hnik, 1959).

Kozak and Westerman (1961) also observed an increased response after tenotomy. In addition, they reported that this enhancement was accompanied by increased afferent spike discharges. It was therefore postulated that the effect might, in fact, be due to excess use, not to disuse. The excess use was presumed to be due to an over-active gamma (γ)-loop mechanism as shown in Fig. 2-25. However, subsequent experiments revealed that the changes in the monosynaptic response and in the resting afferent discharge were due to muscle atrophy (see Kozak and Westerman, 1965). This problem was recently re-examined by April and Spencer (1969) who found that tenotomy, in combination with de-efferentation had similar effects to tenotomy alone - that is, increased synaptic efficacy ensued.

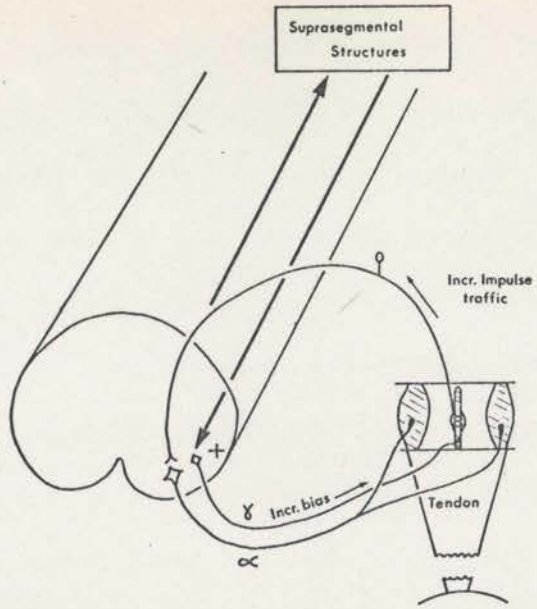


Fig. 2-25: Mechanism by which Kozak and Westerman (1961) thought that tenotomy could lead to an increased usage of the IA afferents. Suprasegmental structures were suggested to cause increased tonic activity of γ fibers supplying the tenotomized muscle; this increased the IA fiber activity. (Taken from Kandel and Spencer, 1968).

Because ventral root section causes degenerative changes in the cell bodies of the α motoneurones, April and Spencer did not examine responsiveness at the Ia/α motoneurone synapse. Instead, they recorded the ascending monosynaptically relayed discharges in Clarke's column. As de-efferentation conclusively abolishes the γ-loop mechanism, April and Spencer's results refute the suggestion made by Kozak and Westerman (1961) that the changes seen after tenotomy alone were due to excess use mediated via the γ-loop. These findings therefore favour the hypothesis that prolonged disuse is associated with increased synaptic effectiveness.

There is a considerable amount of evidence from other studies on the effects of use and disuse that this hypothesis also seems to apply to other junctions. For a comprehensive review of the data supporting this hypothesis, see Sharpless (1964). Perhaps further experimentation will reveal that, at

least at some junctions, Eccles' hypothesis does hold true - namely, that use enhances and that disuse depresses synaptic function. Therefore, in time, it may be possible to explain learning and memory in terms of the amount of use at a particular synapse, but the data available at present would suggest that another explanation be sought. Jasper and Doane (1968) have examined some of the more recent hypotheses developed from experimental evidence to explain the neurophysiological mechanisms involved in learning and memory. Their review indicates that the mechanisms are extremely complex, involving far more than just neuronal activity, which forms the basis of Eccles' hypothesis. It should not be forgotten that, although many of the experiments which have been carried out on the effects of use and disuse on synaptic function were directed towards testing Eccles' hypothesis of learning, it is probable that learning does not, in fact, take place at any of the synapses examined. Finally, when examining the effects of use and disuse in the central nervous system, there is an important question which should be kept in mind at all times: do "the observed changes represent primary processes at the cellular level, or secondary changes dependent on the organization of the system"? (Sharpless, 1964). Possibly, an answer to this question will not always be found.

Other Techniques for Producing Disuse in the Visual System.

Before concluding this section, I will return briefly to the studies carried out on the effects of disuse and re-use in the visual system. Disuse was produced at the OT/LGN synapse by giving the drug 1,5-di(p-aminophenoxy) pentane dihydrochloride, which indirectly leads to a loss of the normal maintained retinal ganglion cell discharge. As a result of this disuse, there was a change in geniculate excitability when examined after brief tetanization

of the optic tract. The change was a loss of the post-tetanic delayed depression which normally follows such tetanization. It was suggested that, if this loss of PTDD were due to the disuse, then re-use of the synapses should reverse the effects of the disuse and so return the synapses to normal. However, the forms of re-activation employed were without effect on the geniculate responsiveness. As the stimuli used were of sufficient intensity to produce maximal responses in the geniculate, and so the synapses were being re-used, it is surprising that the synapses should have been completely unaltered by this procedure. It was therefore suggested that the drug might have an additional action at the geniculate which was responsible for the lack of reversal after re-use.

Perhaps the loss of PTDD after giving M and B 968A is a direct result of the disuse which follows administration of the drug, but there may also be an additional change at the optic tract endings. If so, the PTDD should also be abolished when other techniques are used to produce disuse at the OT/LGN synapses. Obviously, OT transection is one technique which cannot be employed as this involves direct injury to the presynaptic fibers and so is followed by degenerative changes. Another technique which is unsuitable, at least for experiments on adult animals, is visual deprivation (see the Introduction). Not only do retinal ganglion cells appear to continue firing indefinitely in the dark, but form and light deprivation after a critical age (approximately at the end of the third month) produce no detectable morphological or physiological abnormalities at least in the LGN and visual cortex.

I will briefly consider three ways in which disuse could perhaps be produced, so that it can be determined whether the effects seen after giving M and B 968A were due to disuse or whether some other factor was involved.

The first involves locally anaesthetizing the optic nerve for prolonged periods of time so that impulses arising in the retina are prevented from activating the geniculate synapses. A state of disuse would thus be produced in the geniculate and the effects of this could be determined in the same way as in the present study. If the conduction block were then removed, the normal level of synaptic activation would presumably be returned and experiments could then be done to establish whether or not excitability had also returned to normal. Denervation-like changes have been reported in innervated skeletal muscle, following treatment with a local anaesthetic (Sokoll, Sonesson and Thesleff, 1968; Libelius, Sonesson, Stamenović and Thesleff, 1970). After only 10-20 hours of local anaesthesia produced by Marcaine (1-n-butyl-DL-piperidine-2-carboxylic acid-2,6-dimethylanilide hydrochloride) quite distinct changes were seen in the membrane properties of the extensor digitorum longus muscle of rats. Most of the changes observed were reminiscent of effects produced by surgical denervation. Recently, Robert and Oester (1970) re-examined the effects of prolonged pharmacologically induced nerve-impulse deprivation of skeletal muscle in a slightly different manner. The conduction block was maintained for up to 14 days by a lidocaine-silicone polymer implant around the sciatic nerve of rabbits. Following this treatment, however, it was reported that there was never development of supersensitivity to acetylcholine, of fibrillations or of muscle atrophy. Robert and Oester suggested that the discrepancy between their results and those of Sokoll et al. (1968) and Libelius et al. (1970) may have been due to a direct action of the drug used by the other workers on the metabolic processes in the muscle, or that in the experiments of Sokoll et al. and Libelius et al., the muscle paralysis may not have been completely maintained. Regardless of the explanation for the

discrepancy between these studies, it would be interesting to examine the effects of disuse produced by a similar technique in the LGN.

Another way of producing disuse would be to destroy the blood supply to the receptor cells of the retina, which would therefore lead to their degeneration. As the blood supply to the retinal ganglion cells originates independently from that to the receptor cells, they should not be directly affected by such a procedure, yet indirectly they would be affected, because receptor cell degeneration would be followed by a loss of the maintained discharge of the ganglion cells. Thus, a state of disuse would be produced at the geniculate and the effects of this could then be determined. This procedure might not prove practical, however, as it would involve extensive surgery, and a great deal of damage would presumably be done during the operation. Moreover, the animal would be subjected to an unreasonable amount of pain and trauma during the recovery period.

Finally, it would be interesting to approach the problem from an immunological point of view. If protein were extracted from the receptor cells of a normal cat and injected into a rabbit, the rabbit would develop antibodies against the protein. Introducing these antibodies into another normal cat would lead to an antibody-antigen reaction and, as a result, degeneration of the receptor cells would follow. Thus, the ganglion cell discharge would be abolished and a state of disuse would result at the OT/LGN synapses. Although technically, it would be very difficult to carry out the extraction and purification procedures necessary for such a study, this would possibly be the most physiological means for producing disuse.

Possibly the effects of disuse produced by some of the above procedures may prove the same as the effects seen after giving M and B 968A - that is, there may be a loss of post-tetanic delayed depression and, moreover, it may

be impossible to reverse this effect by re-activating the geniculate synapses. If so, an explanation will have to be sought for the lack of reversal in the M and B study other than that a direct change has been produced at the LGN.As a preliminary hypothesis, the results might indicate that the normal functioning of a synapse is not only dependent on normal impulse transmission, but is also dependent on normal biochemical processes. In the Introduction, brief reference was made to the trophic properties of synapses - that is, to those properties not concerned in the transmission of electrical impulses - and it was shown that trophic influences do exist. Perhaps, in our study, the administration of M and B 968A was accompanied not only by a loss of retinal ganglion cell impulse activity, but that there was also some biochemical change. For example, it may be that normal biochemical function in the retinal ganglion cells is dependent on some input from the bipolar cells. This input would be lost by giving the drug and would not be replaced by optic tract stimulation. Possibly, direct stimulation of the retina (which would activate the bipolar cells) would be sufficient to provide an input and so biochemical (and impulse) activities might return to normal. However, this could probably not be done in an unanaesthetized animal, as it would be associated with an immense amount of pain.

If this were the explanation for the lack of reversal in the M and B treated cat after OT stimulation, then disuse produced by destruction of the blood supply of the receptor cells or by immunological destruction of the receptor cells should yield the same results. On the other hand, locally anaesthetizing the OT might be expected to yield different results, as the receptor cells (and therefore the bipolar cells) would not be affected. It is known that normally, there is a proximo-distal transport of material along

nerve axons, and possibly this normal axoplasmic transport provides the basis for the normal trophic influences of the synapse. Although local anaesthesia of the OT abolishes nerve impulses, it probably does not affect axoplasmic flow. Therefore, it would be expected that disuse produced in this way would only be disuse with respect to loss of nervous impulses and thus, it should be possible to reverse such disuse by replacing the impulse activity. If this proves to be the case, it would be interesting to determine the effects of blocking axoplasmic flow in the OT on geniculate excitability. This could possibly be done by locally applying colchicine, a drug which seems to interrupt the fast axonal transport of amine storage granules (Dahlström, 1968, 1969). If colchicine were also to block the transport of synaptic vesicles, it would presumably also lead to disuse in the sense of the loss of synaptic transmission. However, as the effects of colchicine are not well known, extensive preliminary observations would need to be made before the drug could be used in studies similar to those described in this section.

From the data presented in this section it may be concluded that although a state of disuse was produced in the LGN, the effects of this disuse on geniculate excitability were not readily reversible. The lack of reversal may indicate either that the drug has another site of action which so far remains unidentified, or that associated with the loss of impulse activity is some change in the trophic properties of the neurones.

SUMMARY OF RESULTS

1. Cats with permanently implanted electrodes have been used in an attempt to reverse the effects of disuse produced experimentally in the lateral geniculate nucleus. The effects of the disuse and re-use of the geniculate synapses were assessed by examining the recovery of the orthodromic LGN response after applying a single shock or a brief tetanus to the optic tract.

2. Disuse was produced in the synapses between the optic nerve fibers and the principal cells of the lateral geniculate nucleus by giving the drug 1,5-di(p-aminophenoxy)pentane dihydrochloride (M and B 968A). This drug leads to destruction of the receptor cells in the retina and therefore indirectly silences the normal maintained retinal ganglion cell discharge.

3. After producing disuse in this way there was no appreciable change in the LGN response to a single stimulus to the optic tract. There was some evidence that there may have been a slight decrease in the amount of depression which normally follows a single OT stimulus. The disuse was accompanied by a marked reduction in the amount of depression that is normally seen following brief tetanic stimulation of the optic tract.

4. Attempts were then made to reverse this change by electrically stimulating the optic tract for long periods. Regular stimulation at a rate of 30/second for $2\frac{1}{2}$ hours/day for a week did not alter the enhancement. Similar stimulation for 4 hours/day and 8 hours/day for a week was also ineffective. "Random" stimulation, produced by triggering the optic tract stimuli with the discharge of a normal cat's retinal ganglion cell, was also used. Such stimulation for 8 hours/day for a week did not alter geniculate excitability.

5. The results suggest that disuse at the LGN is not a readily reversible phenomenon. Several possible explanations for this lack of reversal were considered. One likely explanation seems to be that the drug exerts some action at the geniculate, as yet unidentified, in addition to its known effect on the retina.The relevance of these results to other studies on the effects of use and disuse of various synapses was discussed.

6. Finally, several other techniques were suggested whereby disuse could be produced at the LGN synapses.

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