SLEEP AND STABLE MEMORY FORMATION.

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REM SLEEP AND STABLE MEMORY FORMATION.

Summary.

Preface.

This thesis was designed to investigate the hypothesis that there is a relationship between REM sleep and stable memory formation.

Chapter I.

Some of the major findings of sleep research are introduced. The EEG of sleep and sleep stages is discribed and some of the major differences between REM (rapid eye movement) and NREM (non rapid eye movement) sleep are shown. The major theories of sleep and of sleep function are also introduced.

Chapter II.

Some of the major theories which have been proposed in order to account for mechanisms of memory and learning are outlined. The most commonly held theory of memory storage i.e. the multistore theory of memory is discribed, with details of the levels of storage. Experimental evidence in support of this theory is given. Since this thesis is concerned with stable memory formation, theories for the mechanisms of storage at this level are discussed in greater detail.

Chapter III.

Postulated biochemical mechanisms which involve the nucleic acids in stable memory formation are discussed. Supporting evidence derived from experimental alteration of nucleic acid metabolism is reviewed.

Chapter IV.

Since protein biosynthesis is one of the central concepts of this thesis, the current known mwchanisms of protein biosynthesis are outlined. It is then proposed that the synthesis of protein and the occurrance of REM sleep are influenced and controlled, at least in part, by circadian rhythms.

Chapter V.

The role of neurohumoural transmitters in sleep and in memory is briefly outlined.

Chapter VI.

Postulated biochemical mechanisms involving cerebral protein biosynthesis in stable memory formation are discussed. Experiments where interference of cerebral protein synthesis using drugs and antobiotics resulted in memory impairments in animals are discribed in detail. Some possible biochemical models for stable memory formation are introduced,

Chapter VII.

Hypotheses for the function of REM sleep are considered. These are divided into two main categories :

(1) theories proposing a function for REM sleep other than memory formation.
(2) theories linking REM sleep to memory and learning.

Only the latter category of function is discussed in detail. Srudies investigating the effects of selective sleep deprivation on memory and learning in animal and human subjects are reviewed.

Chapter VIII.

Previous studies of the effects of altered visual input on sleep are critically discussed. There then follows a discription of my experiment to investigate the effect of distorted visual input on sleep. No significant effects were found and the reasons for this lack of effect are discussed.

Chapter IX.

The pilot experiment to investigate the effects of REM sleep deprivation on the retention of previously learned verbal material is discribed. One of the results of this experiment showed that there were inadequate controls for the level of arousal and for practice effect in the learning task. The design of the main study was therefore altered as a result of these pilot study findings.

Chapter X.

The main experiment to investigate the effects of REM sleep deprivation on memory is discribed. In addition, the effects of the antibiotic, doxycycline, on memory was investigated. Doxycycline is one of the tetracycline group of antibioticd and is hypothesised to act as a protein synthesis inhibitor. The use of this antibiotic was an attempt to carry out an experiment analogous to those where protein synthesis inhibitors were used with animal subjects. Results showed that when the antibiotic was administered in conjunction with techniques of REM sleep deprivation, there were significant memory impairments.

Chapter XI.

The effect of doxycycline on memory during the daytime was investigated. No significant memory impairments were found.

Chapter XII.

A hypothesis and a model for the memory formation function of REM sleep is proposed and results of experiments discribed in Chapters VII to XI are discussed. Some firther research proposals are suggested.

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Preface

Since the occurrence of rapid eye movement (REM) sleep was first reported by ASERINSKY & KLEITMAN (1953), there have been many theories put forward which try to ascribe a specific function to the REM phase of sleep. I shall be describing some of these theories in greater detail in chapter VII. One group of theories proposes that there is a relationship between long-term memory formation and REM sleep, and in my Thesis I set out to try to investigate this relationship.

This specific relationship which I have chosen to investigate is problematic as an experimental approach. All the measurements I have taken are indirect. Indirect measurement is often carried out in experiments within the psychological discipline, an approach which has been frequently referred to as the "black box approach". My choice of experiments is based on a number of assumptions. Does protein synthesis occur to a greater extent in REM sleep compared to NREM? Does the EEG distinction between REM and NREM sleep reflect a real difference between these two phases. Some sleep researchers would say that REM sleep is merely an integral part of sleep and it is therefore not necessary to propose a separate function for it.

Although I have not been able to demonstrate satisfactorily that REM sleep is involved in the formation of memories, I nevertheless still strongly suspect that a relationship of some sort does exist. Admittedly, I might not have chosen the most suitable method of investigating the phenomenon, and at best, my experiments have suggested/

other approaches to the problem, and I hope might inspire interest from other researchers in this and related fields.

Since the work for a Ph.D. Thesis involves a training in research methods, I have gained much valuable experience from carrying out the empirical work, and from the exercises of data analysis and writing.

The experiment on "Distorted Visual Input and Sleep", has already been published (ALLEN et al, 1972), and a short account of the experiments on REM deprivation and protein synthesis inhibition, will appear shortly in the "Proceedings of the European Sleep Research Society Conference", which was held in Rome (1974).

This Thesis is set out in the "funnel" manner. First of all, the broad disciplines on which the thesis draws ie, sleep, memory and protein biosynthesis are discussed. Certain aspects of these disciplines are then singled out for further consideration and are finally combined to form the hypothesis.

cknowledgements

I should especially like to express my deepest gratitude to Dr. I. Oswald, my supervisor. At times when the task of writing this thesis seemed too formidable for me to carry on, his continuing support, understanding, encouragement and guidance enabled me to complete it. I have also appreciated the support of many other members of the sleep research team both past and present, in particular Dr. S. Lewis who helped to supervise my first years, the secretarial staff Ms. C. Robb and Ms. D. Duncan and also the librarian Ms. M. Bonar. Technical assistance was unfailingly given by Mr. G. Burt and Mr. J. Fraser, and the typing of the thesis was mainly carried out by Ms. W. Timms.

In the distorted vision experiment, Dr. N. Wade from the University of Dundee assisted with the design of the reading task and thanks are also due to Butterworths (Edinburgh) Ltd for the manufacture of the spectacles. In the doxycycline, daytime experiment, the majority of the experimental data collection was undertaken by two psychology honours undergraduates, Ms. G. Frood and Ms. N. Falchikov. I can only hope that the experience was as valuable to them as their assistance was to me.

I should also like to thank my parents, family, and friends, especially Premananda and Lou, who often unknowingly, gave me every support I could wish for. Finally, the subjects who took part in the experiments, far too many to mention by name, who sacrificed a good nights sleep in their own beds for the advancement of knowledge.

ntroduction.

Sleep is studied primarily by polygraphic recording and EEG tracing but it must be remembered that sleep is first and foremost a behavioural state. KLEITMAN, from his publications and discussions defines sleep as a natural background state of an organism and its brain. A definition of sleep may thus be based on the information that the sleeper has decreased ability to react with his external environment. Sleep is a recurrant and easily reversible condition, characterised by an increased threshold for overt responses to external and internal stimulation. Although responses do occur in sleep, they are different from the ones occurring during wakefulness. The study of the behavioural state of sleep has yielded associations with certain patterns of EEG tracings which have come to be known as EEG sleep patterns.

Most adults sleep for seven and a half hours on average. Sleep has been shown to exist in reptiles, birds and mammals and KLEITMAN (1963), has theorised that sleep can first be seen as a feature of the cerbral cortex on which depends the integrative functions characteristic of waking life. However, the percentage of time spent sleeping does not correlate with the degree of cortical development in the various species.

As will be discussed more fully in the chapters to folloe, on sleep and on learning and memory, the behavioural observations and polygraphic recording revealed that sleep cannot be characterised as a state of quiescence alone since there are cyclical periods where there is activity

/activity and change throughout the night.

One of the major discoveries of recent years is that sleep is a cyclical phenomenon, comprising two kinds of sleep (see Chapter I):- REM sleep, which has also been called rapid eye movement sleep, desynchronised sleep, paradoxical sleep, fast sleep or active sleep, and NREM sleep, which may also be called non rapid eye movement sleep, synchronised sleep, orthodox sleep, slow wave sleep, SWS or quiet sleep. From these terms I have chosen REM sleep and NREM sleep for reasons of brevity and I shall use them throughout the Thesis.

Neurophysiological studies have shown that during REM sleep much of the forebrain is in a state similar to that of alert wakefulness. This suggests an active, central (possibly cortical) processing function for REM sleep which could involve memory and learning.

Mental activity during sleep has also been extensively studied. Although much of the original work on dreaming, confined dreaming to REM periods, it appears that the early work underestimated the absolute amount of NREM mental activity and rejected too hastily the evidence of NREM dreaming. Mental activity does occur in NREM as well as REM sleep and individual subjects may report mental activity on as many as 70% of their NREM awakenings. However, fewer reports of mental activity are elicited from subjects on NREM compared to REM awakenings. Also the proportion of reports labelled "thinking" rather than "dreaming" is higher for NREM reports. Even the/

/the NREM reports which are reported as "dreaming" tend to be less dream-like than the REM reports (FOULKES, 1962).

There has been a great deal of interest in the function of REM sleep particularly because of the association it has with dreaming. I have chosen to investigate the hypothesis that REM sleep is in some way related to the formation of permanent memories. One of the reasons for the choice of this particular hypothesis for REM sleep function, is from personal experience. Places, characters and objects appearing in dreams, do seem frequently to bear some relation to past experiences. It would seem realistic that past experiences may be re-examined to prevent them from being forgotten, particularly if these experiences contain some unresolved problems, which are so frequently encountered in every day waking life.

Learning can be broadly conceived of as a modification of response based on experience, and it is probably the single most important characteristic of higher animals. If the salient characteristic of learning is behaviour modification through experience, then this can be achieved only by means of some type of memory storage mechanism.

Most physiological theories of learning and retention assume that the initial storage is a temporary facilitation of synaptic transmission between a conditioned and unconditioned stimulus at the time of the initial learning experience. These functional modifications may decay rapidly and leave little or no trace of an electrophysiological/

/electrophysiological nature in the nervous system. Only when a particular combination of stimuli are repeatedly presented in a fashion such that the same central pathways are subjected to a recurrent or combined facilitation may a second process occur. This process in some way translates the pattern of temporary synaptic facilitation into permanent memory traces. Those permanent, stable memories would seem to depend on biochemical and anatomical changes which develop slowly and represent structural modifications in the CNS. In support of theory, ECCLES (1966), demonstrated experimentally that the use of a particular pathway can result in permanent alterations in synaptic relationships. He proposed that use strengthened neural transmission and disuse weakened it.

It has been suggested that this recurrent activity could be a result of "reverberatory" activity in neurones, so that a simple event could activate each synaptic link thousands of times within a few seconds, even though the primary input to the circuit no longer exists. In this manner a circuit could be set up which remains active until anatomical changes lay down the permanent memory trace (ECCLES, 1966; GERARD, 1963; HEBB, 1949; THOMSON, 1967)

Such a theory suggests a two stage memory storage process:-

1. short term storage, during which reverberatory activity takes place.
2. long term storage, by means of permanent structural changes.

The theory of short term storage gains support from studies with retrograde amnesia (RA), electro-convulsive shock (ECS) and drug effects. It has been reported that there are initial periods, varying in time between seconds and days depending on the type of experiment, following learning and during which consolidation takes place (DUNCAN, 1949; McGAUGH, 1966; STELLAR, 1957; THOMSON, 1967). This is thought to be the period in which the "reverberatory" or other temporary activity is involved in bringing about a more permanent structural change.

It is these structural changes in synaptic organisation, which I suspect could be occurring in REM sleep, and the stimulation of the neurones involved in the changes would thereby give rise to the mental content associated with REM periods. Many experiments have been carried out using techniques of REM deprivation. HARTMANN (1970), believes that it is possible to conclude from these deprivation studies that a function of REM sleep is in restoring mechanisms of learning or memory which are associated with attention. He believes, in addition, that REM sleep may also function to preserve emotional integrity and social adaptation. However, not all the REM deprivation studies have yielded results consistent with this hypothesis, some researchers have found that retention is better after REM deprivation than after REM sleep and have postulated that the mental events occurring during REM sleep might equally interfere/

/interfere with the laying down of particular memories (FOWLER et al, 1972), and stage 4 sleep might then be beneficial to memory. However, there are no experiments where subjects are deprived of stage 4 NREM sleep where this has a disruptive effect on memory consolidation. HARTMANN (1973) has also postulated that the restorative functions attributed to REM sleep might depend on proteins and other materials being made available in the previous SWS period. Thus REM deprivation effects in so far as they can be identified, might result from SWS deprivation as well as REM deprivation. Results of selective deprivation of either stage 4 or stage REM might then be difficult to separate in terms of their effects on memory.

A further reason for the need to ascribe a separate function to REM sleep is the presence of the REM rebound which was first reported by DEMENT (1960). It has been conclusively shown that if human or animal subjects are deprived of REM sleep, either by awakening at the time of entry into the REM state (DEMENT, 1960), or by the administration of certain drugs (OSWALD & THACORE, 1963) on termination of the deprivation treatment there occurs a rebound increase in the percentage of REM sleep. This would seem to indicate that organisms have an absolute requirement for the REM phase of sleep.

Chapter 1.

SLEEP.

(1) THEORETICAL CONSIDERATIONS.

The recording of polygraphic variables throughout the night has provided a full discription of sleep. Three separate variables are measured :- electroencephalogram (EEG), electromyogram (EMG) from the chin muscles, and electro-oculogram (EOG), a measure of eye movements. A major discovery revealed by all-night polygraphic recording was the existence of two separate types of sleep (OSWALD, 1962). ASERINSKY & KLEITMAN (1953; 1955) discribed many of the phenomena of REM sleep. DEMENT & KLEITMAN (1957) defined five electroencephalographic stages of sleep; stages 1 through 4, in which rapid eye movements are absent, and a fifth stage which is accompanied by rapid eye movements. It has now become recognised that a night's sleep does not simply consist of a transition from waking to deep sleep in the evening and then reversed back to waking in the morning, but that sleep is a cyclical phenomenon with four or five cycles occurring each night. Each sleep cycle may also be analysed into five different sleep stages.

When physiological measurements are also taken, further differences between REM and NREM sleep can be seen. These are summarised in Table 1.

There are many surprisingly constant factors which do occur in normal human sleep. REM sleep constitutes 20-25 % of a night of sleep. The appearance of NREM sleep

Table 1.

THE MAJOR CHARACTERISTICS OF REM AND NREM SLEEP IN MAN.

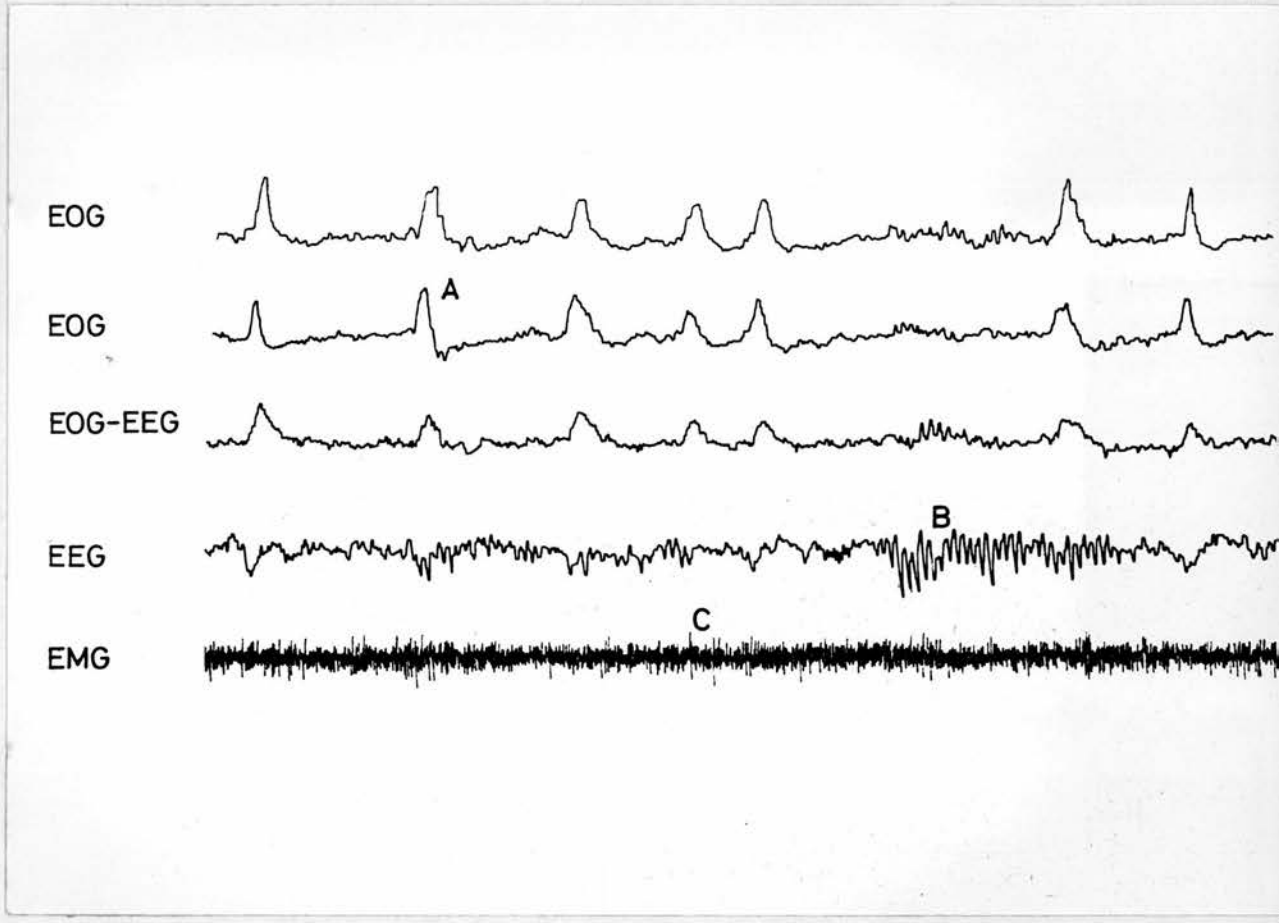
MEASUREMENT	NREM SLEEP	REM SLEEP
Scalp EEG (DEMENT & KLEITMAN, 1957).	slow waves and spindles	low voltage mixed frequency
Eye movements (ASERINSKY & KLEITMAN, 1953; 1954).	none, or a few slow ones	conjugate rapid movements
Chin EMG (BERGER, 1961; JOUVET, 1962; JACOBSON et al, 1964).	decreased from wakefulness	almost absent
Pulse (SNYDER et al, 1964).	low and steady	faster and more irregular
Respiration (SNYDER et al, 1964).	low and steady	faster and more irregular
Blood pressure (SNYDER et al, 1964; WILLIAMS & CARTWRIGHT, 1969).	low and regular	higher and irregular
Penile erections (FISHER et al, 1965; KARACAN et al, 1966).	absent	present
Body movements (JACOBSON et al, 1964).	a few gross movements	twitches
Galvanic skin response (BROUGHTON et al, 1965)	frequent	rare
Mentation (FOULKES, 1962).	thought-like, repetitive	dream-like, dramatic

always precedes the appearance of REM. Stages 3 + 4, the deep slow wave portions of NREM sleep always occur predominantly in the early part of the night or sleep period. The length of the REM - NREM cycle is about 90 minutes although BREZINOVA (1974) has shown that this cycle is always shorter early in the night.

(2) DESCRIPTION OF SLEEP STAGES AND THE EEG OF SLEEP.

I shall describe the different stages of sleep in detail, since in the REM deprivation experiments it was necessary to be able to immediately detect stage REM as it appeared.

As the subject passes from wakefulness (see Fig. 1.) to sleep there is a gradual slowing of the EEG activity. The waking EEG of low voltage fast activity as shown in Fig. 1 gradually subsides and is replaced by relatively low voltage, mixed frequency pattern with increased activity in the 2 - 7 Hz band. This pattern is called stage 1, (see Fig. 2). There are less than 50 % alpha waves, no spindles or K complexes and occasional slow rolling eye movements. After a varying length of time, usually of the order of a few minutes, bursts of 12 - 14 Hz sinusoidal waves, called sleep spindles, and isolated higher voltage biphasic waves, called K complexes appear. These define the presence of stage 2 NREM sleep (see Fig. 3). Following varying amounts of stage 2, high voltage delta waves point to the appearance of stage 3 (see Fig. 5), and for this sleep stage to be defined as stage 3, the delta waves must



not referenced.

Fig. 1.

AWAKE.

(A) Eye blink in EOG.

(B) Alpha rhythm in EEG.

(C) High muscle tone.

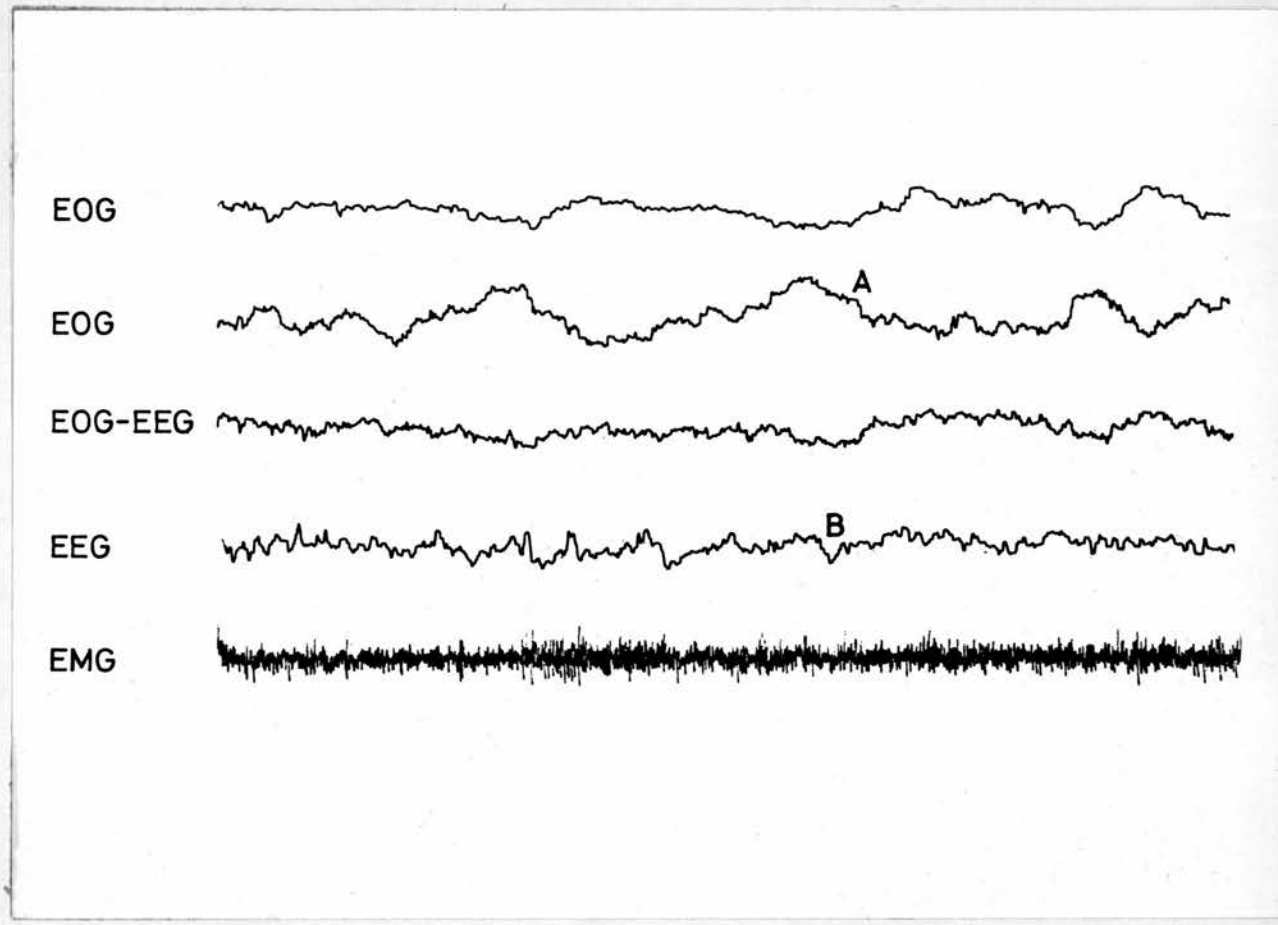


Fig. 2.

STAGE 1, DROWSY.

(A) Rolling eye movements.

(B) Low voltage EEG without spindles.

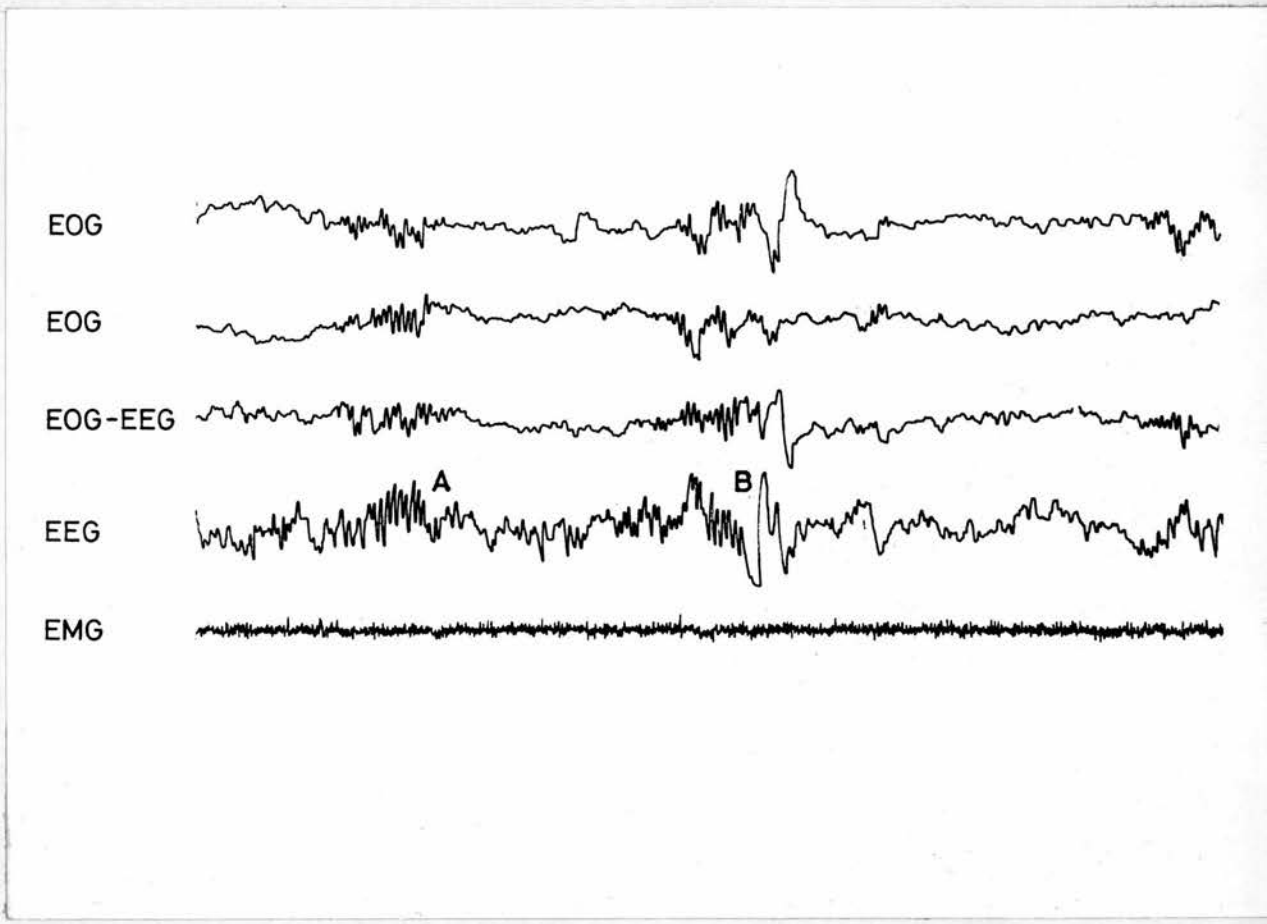


Fig. 3.

STAGE 2.

(A) Sleep spindle.

(B) Sleep spindle followed immediately by a K complex.

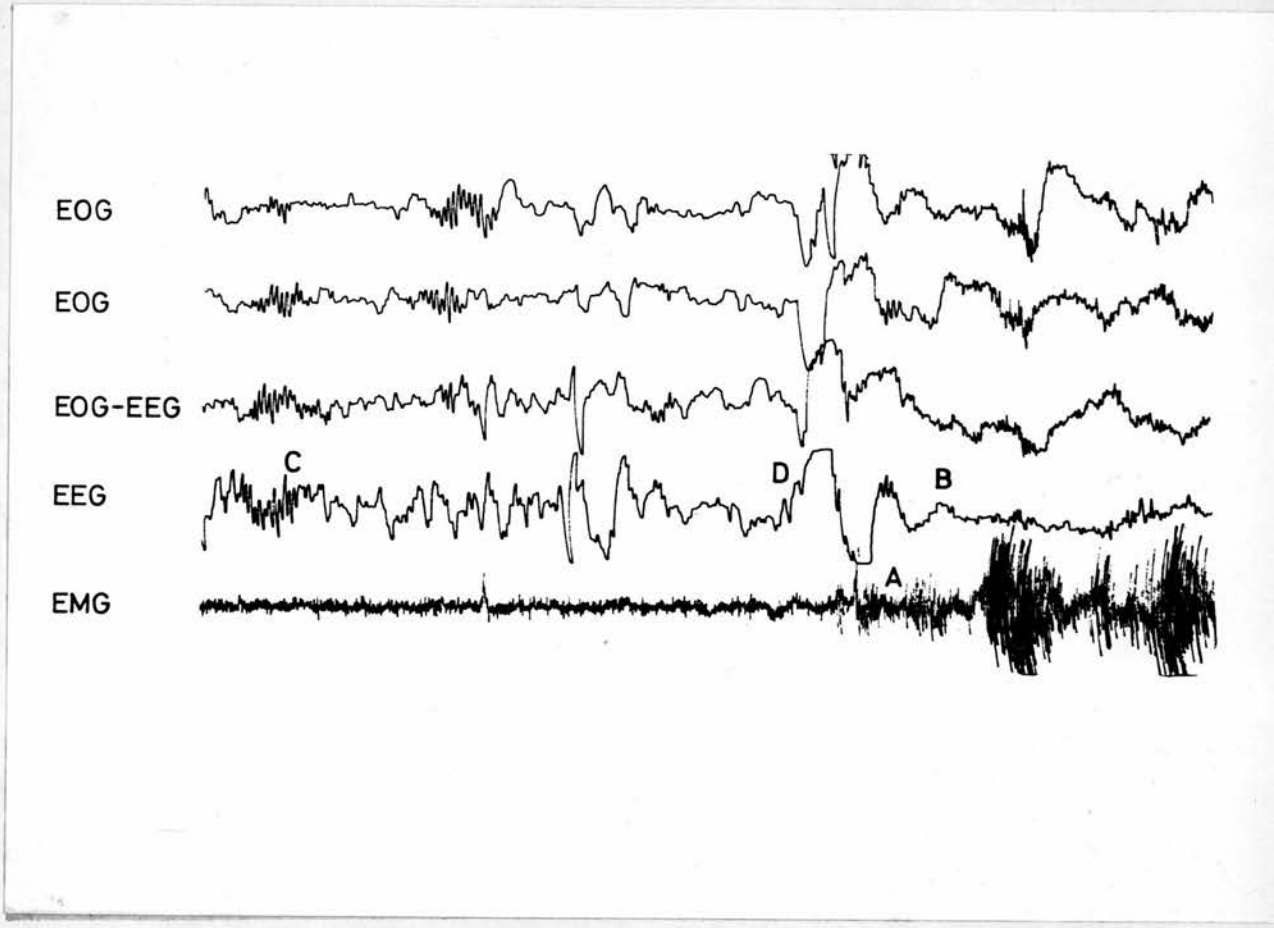


Fig. 4.

TRANSITION FROM STAGE 2 TO STAGE 1.

(A) Increase in muscle tone at the transition point.

(B) At the transition point, the EEG pattern changes to lower voltage, without spindles.

(C) Sleep spindle of Stage 2 sleep.

(D) K complex of Stage 2 sleep.

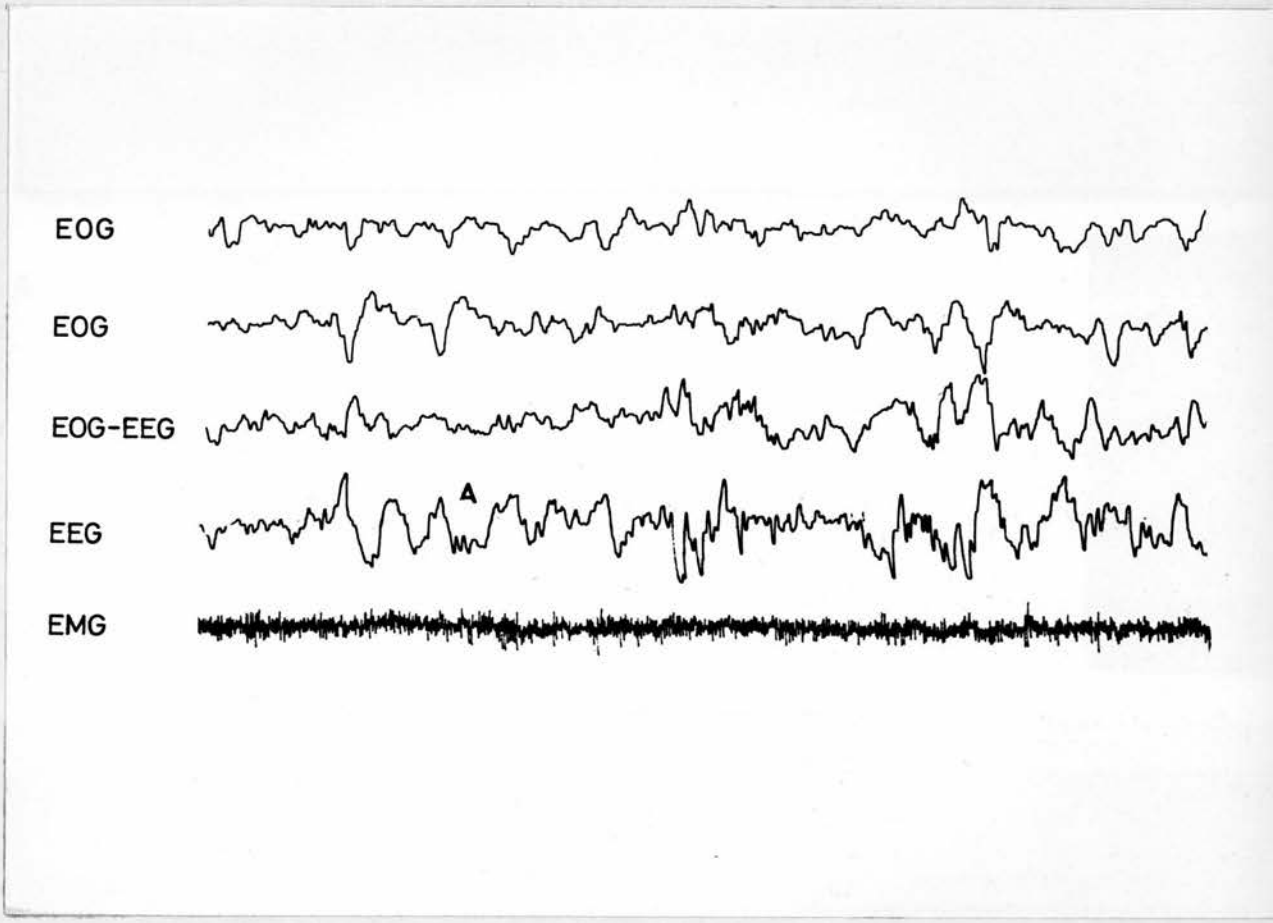


Fig. 5.

STAGE 3.

(A) Delta wave in EEG: there are between 20 and 50 percent delta waves in the EEG of Stage 3 sleep.

/must occupy 20% of the EEG recording. This delta activity increases gradually so that it occupies 50% or more of the EEG tracing and it is then called stage 4 (see Fig. 6). Stages 1-4 collectively are termed NREM sleep.

At some time, usually within the second hour following sleep onset, the sleeper enters REM sleep. The EEG changes to a relatively low voltage mixed frequency pattern sometimes containing the alpha rhythm. The chin EMG markedly decreases in activity up to a minute before the first rapid eye movements appear (see Fig. 7). Frequently a burst of theta activity precedes the onset of the REM period (DEMENT, 1967). Other waves of 2-3 cycles per second occur during REM. These sometimes have a notch on the rising or falling phase to give the appearance resembling a sawtooth (see Fig. 7) and hence, these have come to be known as sawtooth waves (SCHWARTZ & FISCHGOLD, 1960). Sawtooth waves are characteristic of REM sleep and frequently occur during or just before an eye movement (BERGER et al, 1963). When the eyes move, the dipole corneoretinal potential difference can be recorded by electrodes placed near the eyes. The conjugate eye movements occur through all sizes of arcs in any direction and start from whatever position the eyes are in as a result of the preceding movement (DEMENT, 1964). JACOBS et al (1971a), found that 25-35% of eye movements were in the vertical plane, 55-65% in the oblique/

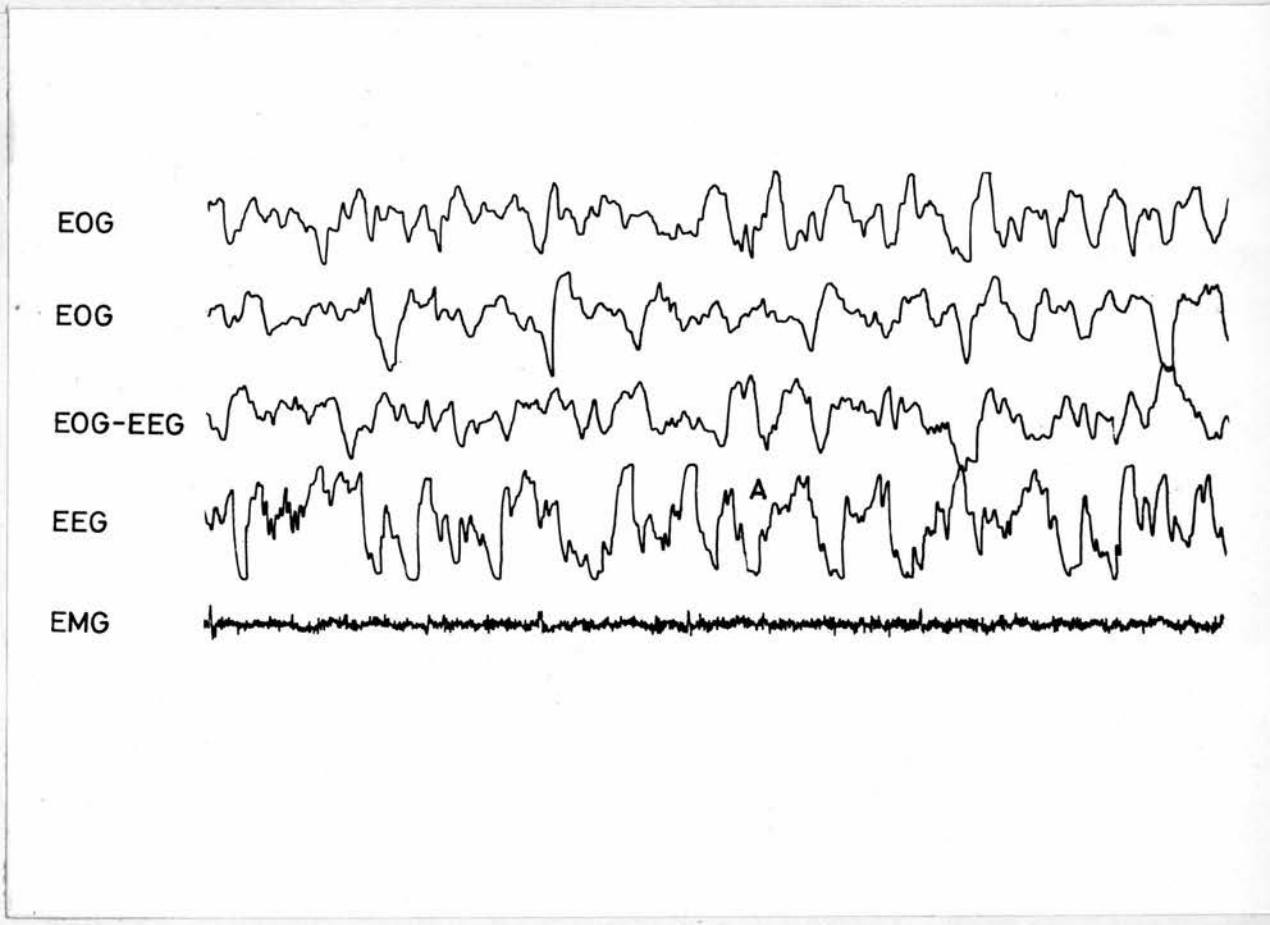


Fig. 6.

STAGE 4.

(A) Delta wave in EEG: Stage 4 has 50 percent or more delta waves in the EEG.

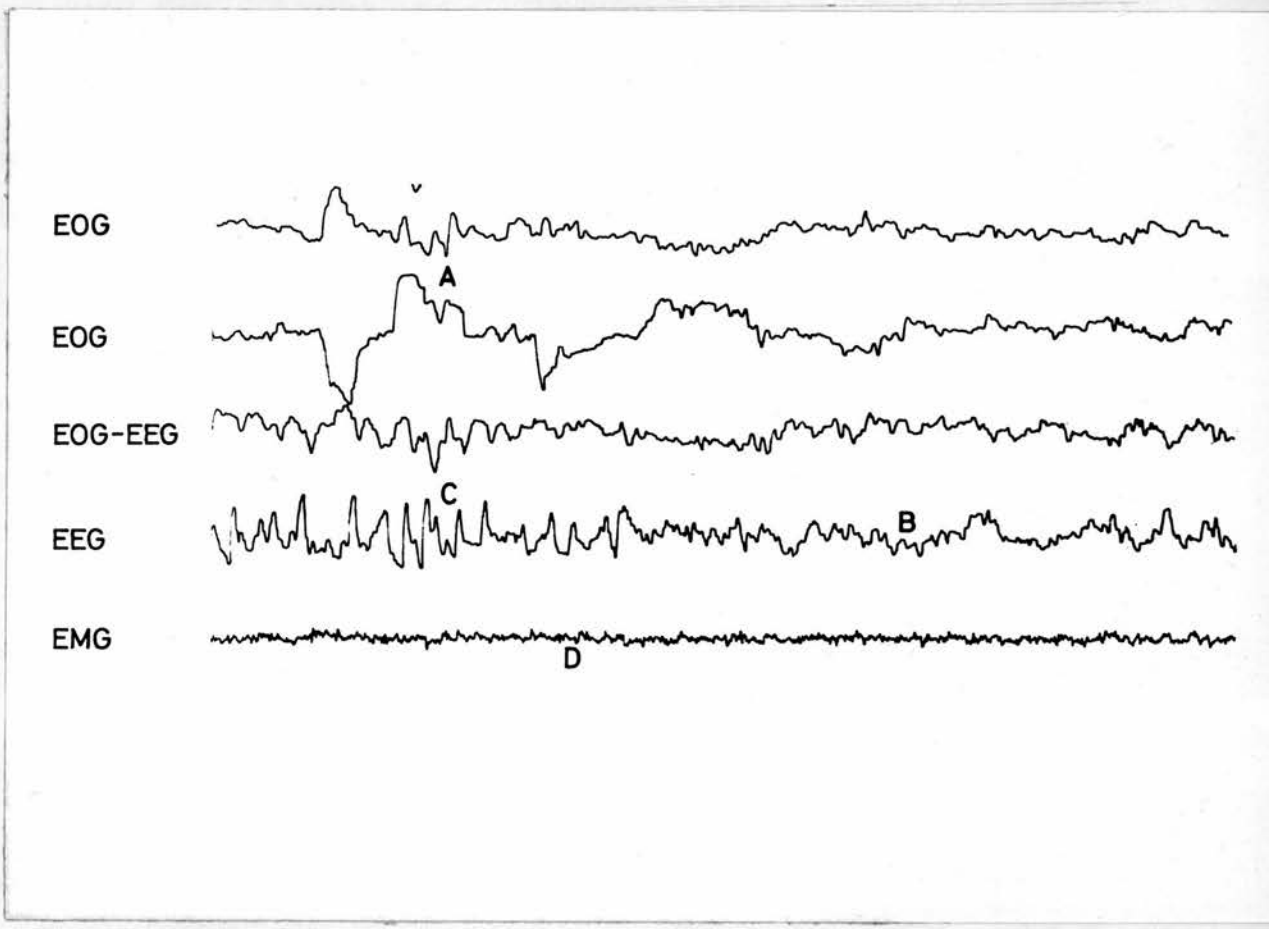


Fig. 7.

STAGE REM.

(A) Rapid eye movement in EOG.

(B) Low voltage non spindling EEG.

(C) Saw tooth waves in EEG.

(D) Low muscle tone.

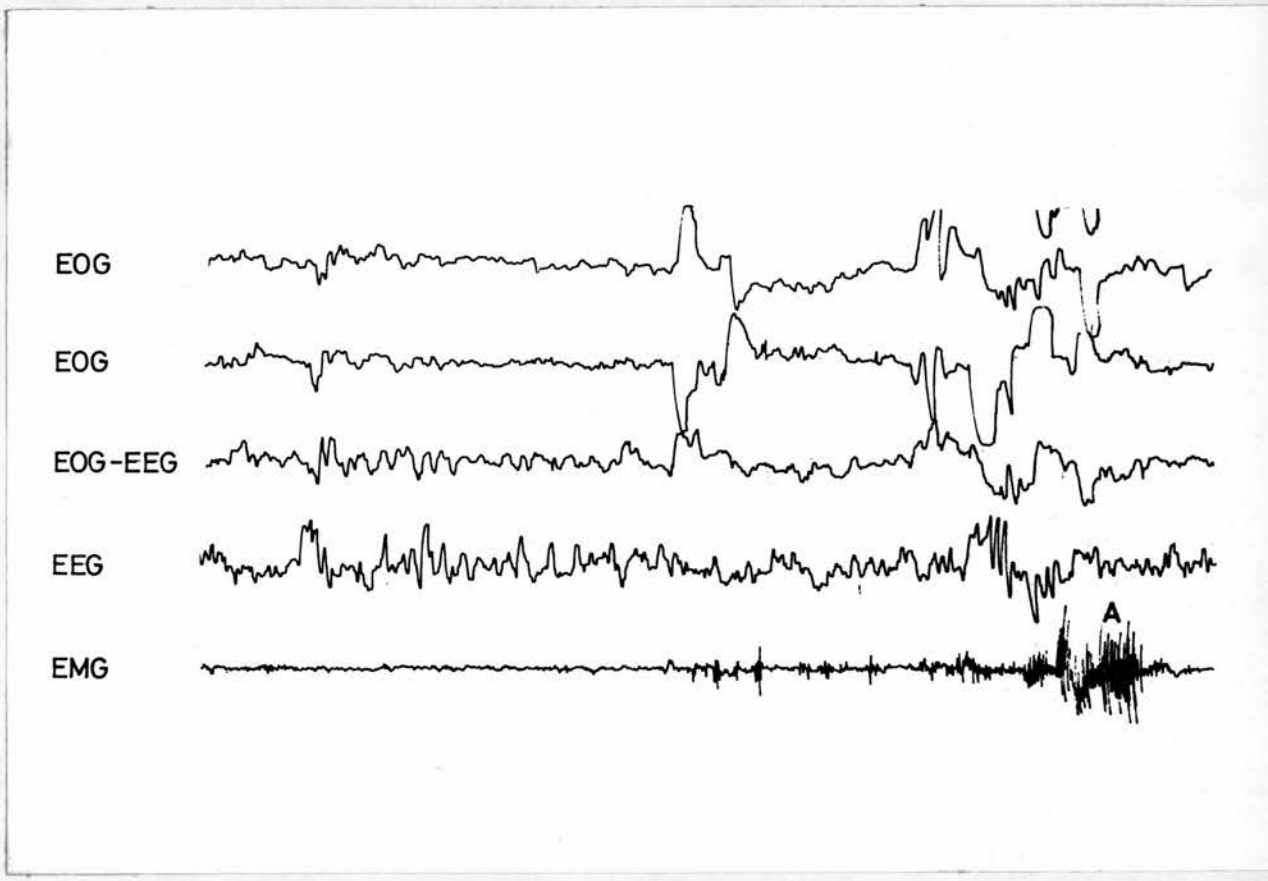


Fig. 8.

STAGE REM.

(A) Characteristic muscle twitch in EMG.

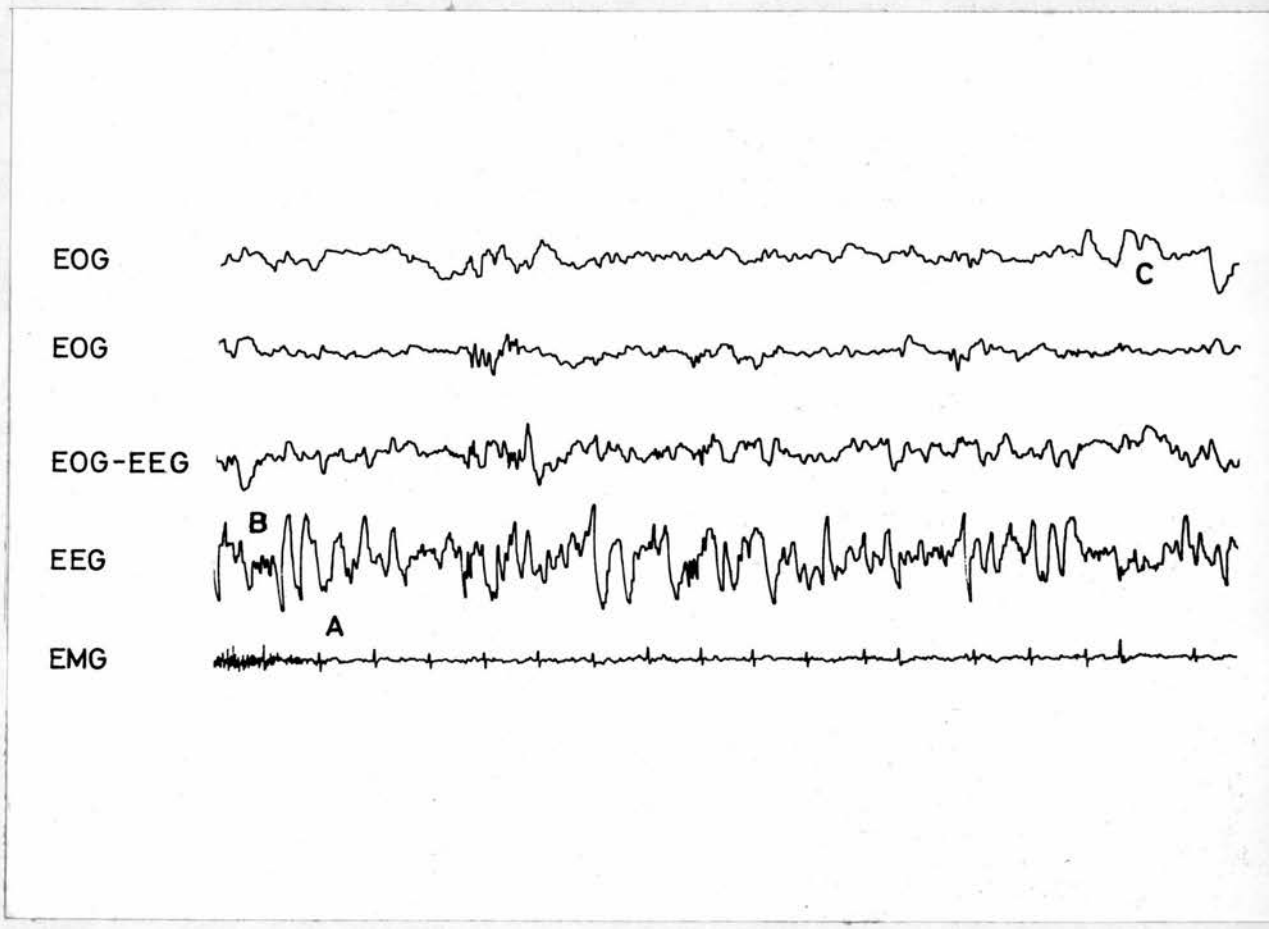


Fig. 9.

TRANSITION FROM STAGE 2 TO STAGE REM.

(A) Drop in muscle tone preceding the change in EEG pattern and the appearance of eye movements.

(B) Spindle of Stage 2 sleep.

(C) Eye movements of Stage REM.

/oblique plane, and 5-15% in the horizontal plane. Although JACOBS et al report that the eye movements during REM sleep are unlike any recorded during wakefulness, REYHER & MORISHIGE (1969) has described somewhat similar eye movements during day dreaming. Typical examples of EEG tracings of the different sleep stages can be seen in Figs. 1 to i.

Sleep stages are scored according to the criteria mentioned above and these have been set down by RECHTSCHAFFEN & KALES (1968). The stages of NREM sleep are arbitrary subdivisions based on the percentage of delta waves in the EEG channel of the record whereas the REM and NREM divisions of sleep is based on many physiological differences. The identification of an epoch as REM or NREM is based on all three polygraphic measurements.

NREM sleep in the early morning hours mainly consists of stage 2 sleep. REM sleep distribution also changes during the course of a night's sleep. The first REM period is shorter and has fewer eye movements than the subsequent ones (GOODENOUGH et al, 1965; VERDONE, 1965). Four to six REM periods usually occur per night.

An important technical detail in studying normal sleep patterns is the first night effect. The first night a subject sleeps in the laboratory, he spends more time awake, has a decreased REM percentage and a longer latency to the first period of SWS sleep and to the first REM period, than on subsequent nights/

/nights (AGNEW et al, 1966; MENDELS & HAWKINS, 1967; SCHMIT & KAEBLING, 1971). This slightly disturbed sleep pattern is thought to arise from the anxiety associated with the unfamiliar experimental surroundings and situation. Other environmental factors such as daytime exercise (BAEKELAND, 1970); thirst (KOULACK, 1970); or naps (KARACAN et al, 1970) may all cause changes in the polygraphic sleep pattern. Night 2 in the laboratory is also often regarded as abnormal.

Both SWS and REM sleep are on average slightly reduced with increasing age, but the most striking changes occur early on in life. A newborn child spends 16-18 hours asleep which appears to consist of two types. One type is characterised by no eye or body movements and regular respirations, while the other type is associated with eye and body movements, irregular respirations and spontaneous sucking (PARMALEE et al, 1968; PRECHTL, 1970; 1972). The first type develops into NREM sleep and the latter into REM. The REM percentage of the newborn is 40-50% of the sleeping time (ROFFWARG et al, 1966; PETRE-QUADENS, 1967). Preliminary studies in animals suggest that the foetus in utero has an even higher REM time than the new born. It has been suggested by OSWALD (1970) that this increased REM time in newborns could be related to the idea that this is the time when there is the largest amount of synthetic activity in the brain.

(3) THEORIES OF SLEEP AND SLEEP FUNCTION.

Sleep theories can be divided into two main types depending on whether they try to answer the question "why do we sleep?" or "how does sleep occur?" The "how?" theories attempt to delineate how we fall asleep, how we move from REM to NREM sleep, and how we awaken. "Why?" theories try to examine the function of one or both of the sleep states. I shall concern myself in this Thesis mainly with the theories relating to the functions of sleep.

One of the vaguer theories of the functions of sleep is that there is some compound or compounds which are used up during wakefulness at a rate faster than they can be replenished, thus building up a debt. During sleep the reverse would occur. The idea of a debt is quite usual in physiological explanation e.g. during intense exercise an oxygen debt is incurred by muscle tissue and rest is necessary for that debt to be repaid. This idea when extended to sleep has been called the "bottle" theory. As the day progresses, the level of this unknown substance falls, to rise again during sleep. Many chemical studies have been carried out in order to ascertain the "contents" of the "bottle". REICH et al, (1967) have found that in sleep there is a two to three fold increase in the incorporation of inorganic phosphate into the brain, some of which may be due to incorporation into phosphoproteins. Some is also due to incorporation into brain nucleotides and glycolytic intermediates (VAN DEN NOORT & BRINE, 1970).

These authors have also found that there is an increased concentration of adenosine triposphate (ATP), creatine phosphate, and fructose diphosphate in the brains of sleeping rats. It has also been suggested that neurotransmitters are used more rapidly during wakefulness, and their stores replenished during sleep. This neurochemical approach has been criticised most thoroughly by KLEITMAN (1963), who believed that the "grogginess" many people feel after awakening was not compatible with the "full bottle" idea.

Any theory for the function of sleep should take into account the already mentioned facts that there are two states of sleep and that under normal circumstances SWS always precedes REM. HARTMANN (1973) postulated the following pairs of functions.

(1) SWS produces, depletes or alters something which is then replaced by REM sleep.

(2) SWS facilitates one chemical reaction and REM sleep facilitates a second, further along a chain, which may then synthesise some important molecule.

(3) SWS is involved in the synthesis or activation of a substance which is then transported or located during REM sleep.

Whatever role sleep fills it seems to be required most by the young and this seems to be more the case with REM sleep than with SWS. The suggestion therefore seems logical that sleep might somehow function in the growth or maturation of the nervous system. HARTMANN however believes that a large number of tissues and/

/and organs show growth curves that could be comparable to the curve of sleep duration change with age, for sleep as a whole, or for SWS or REM sleep separately.

During REM sleep much of the forebrain is in a state similar to that of alert waking. The cortex shows desynchronised activity and blood flow is increased (SEYLAZ et al, 1970); there is a negative D-C potential shift (KARAMURA & SAWYER, 1964). Many species show prominent theta activity in the hippocampus (CADILLAC et al, 1961; WEISS & ROLDAN, 1964) and this finding may be linked to alert waking during learning (ADEY, 1966; SEGAL et al, 1972) and during motor activity (VAN DER WOLF, 1969). High levels of activity in the sensory cortex are also found during REM sleep (EVARTS, 1962), at a time when motor activity is strongly inhibited and sensory input is greatly reduced or occluded (POMPEIANO, 1970). Thus the central sensory-processing motor systems are strongly active while their usual inputs and outputs are blocked. This suggests active central processing, possibly cortical and therefore it is conceivable that memory and learning could be involved.

Few chemical studies give any clear indication as to the chemical changes occurring during sleep. Serum lactate and pyruvate levels have been shown to differ in sleep and wakefulness (REICH et al, 1972). One of the chemical changes that might point towards a functional role for sleep is the suggestion of increased synthesis of at least some macromolecules or proteins during sleep (REICH et al, 1967; OSWALD, 1969; 1970). Research/

Research has shown that there is an increase in the incorporation of P^{32} into brain tissue, most likely into phosphoproteins, during sleep in rats (REICH et al, 1967). The temporal patterns shown in these studies suggest that SWS is involved.

Growth hormone (GH) secretion has been shown to have a clear peak during stages 3 + 4 sleep (TAKAHASHI et al, 1968; SASSIN et al, 1969; HONDA et al, 1969) to shift with stages 3 + 4 when sleep is shifted to the daytime (SASSIN et al, 1969). GH release concomitant with SWS is increased by previous exercise (ADAMSON et al, 1974) as with waking release, though this effect was not observed by ZIR et al (1971). Deprivation studies suggest that GH secretion is related both to sleep onset and to the delta activity of stages 3 + 4 sleep (SASSIN et al, 1969; KARACAN et al, 1971; TUEN & PULLAN, 1974). Other hormones have not been studied as thoroughly as GH. Some preliminary studies link sleep with increased cortisol secretion (HELIMAN et al, 1970); testosterone (EVANS et al, 1971, BOYAR et al, 1974) antidiuretic hormone (MANDELL et al, 1966); lutenising hormone (RUBIN et al, 1972; BOYAR et al, 1974); prolactin (SASSIN et al, 1972; 1973; NOKIN et al, 1972; PARKER & ROSSMAN, 1973); FSH (BOYAR et al, 1973a, 1973b).

These chemical findings suggest an anabolic macromolecular synthesising role (OSWALD, 1972) at least for SWS since GH promotes the synthesis of RNA and protein (KORNER, 1965; CHEEK, 1973).

Chapter II.

LEARNING AND MEMORY.

(1) PSYCHOLOGICAL THEORIES.

Learning and memory cannot really be separated since by definition what is remembered has also at one time been learned. Learning generally refers to an indirectly observable phenomenon. It is an intervening variable linking antecedent external conditions with observed differences in behaviour. Learning requires attention, perception and selection of the material to be retained. Since I shall be using specific terms when describing some of the previous experiments involving memory and in the description of my own experiments, I should like to outline first of all some of the psychological terms and some of the ways in which learning and memory have been classified and investigated by psychologists.

Learning is a pre-requisite of memory and may be defined as the capacity of an organism to behave in a way which is modified by previous experience.

The first objective attempts to study the function of the brain in learning and memory were carried out using ablation techniques. Most of the work was performed using the cerebral cortex, partly because, commencing with the work of PAVLOV, the cortex was believed to be the area of the brain which endowed the animal with most, if not all, of its learning capacity.

LASHLEY (1950) studied maze learning and visual discrimination learning but his results showed no localisation of learning abilities apart from sensory functions. Maze learning seemed to be a function of the whole cortex. However, when the ablation techniques were used on the

rimate brain, as opposed to the rats used by LASHLEY, some spects of learning ability, particularly those measured y delayed responses, could be localised in the frontal obes of the cortex. What was specifically localised is still ot known, but MISHKIN (1964) presented strong evidence hat the primary effect of frontal lobe ablation was to increase the tendency to perseverate responses, an interpretation hich fits well with the observations on human frontal patients (MILNER, 1964). Certain other areas in the primate brain, are now known to have special functions in learning, for example the inferotemporal cortex (PRIBRAM & MISHKIN, 1955) and the ippocampus (DOUGLAS, 1967). Patterns of electrical activity in the hippocampus change during the course of learning (ELAZAR & ADEY, 1972) and it has also been possible to correlate changes in the rate of firing of single neurons of the parietal cortex with the acquisition of a task (JASPER et al, 1960).

Large bi-lateral ablations of the hippocampus in experimental animals have been shown to interfere with previously acquired conditioned responses and testing after bilateral ablation of the hippocampus in human patients led to the conclusion that while some forms of memory were preserved, the consolidation of recently learned habits was impaired (MILNER, 1962; DRACHMAN & OMMAYA, 1964). These experiments also indicate that events of memory and learning have an electrical counterpart.

It is possible to distinguish between certain kinds of memory, these will be discussed more fully in the section on multistore theories of memory, but I shall briefly mention them here.

a). Immediate memory. This is temporary, lasting no more

than a few seconds, sufficiently long to hold a conversation or perform a mental calculation.

b). Recent memory. This refers to events lasting from about ten minutes and extending back to a few months.

c). Long term memory. This includes memory of a more permanent nature, extending back as far as childhood.

d). Short term memory. This seems to be an ambiguous term in psychological literature since it may refer to immediate or recent memory or both.

The two main terms I shall be using when discussing my own experiments are :-

e). Labile memory. By this I mean short term memory which is thought to be an electrical event or trace.

f). Stable memory. This is postulated to be formed as a result of a stuctural change in the C.N.S. and approximately corresponds to the classical long term memory.

Memory has classically been regarded as involving several distinct mechanisms.

(i) acquisition (registration, impression); (ii) storage (retention); (iii) recall (retrieval, re-use); (iv) recognition. It is assumed that storage requires a physical alteration in the nervous tissue of the brain. This has been called the memory trace or engram. Recall may be effected by the activation of the memory trace but since recall is not the sole factor which may be measured when material is remembered, I shall employ the term re-use.

Memory always requires some connection between a present impression and a past event. Past experiences leave some kind of a trace which is revealed as a modification in the

behaviour of the organism. Thus memory can be seen to cover a complex group of mental activities some of which may be observed and others only inferred. Very little is known about acquisition and almost nothing about the active mental processes which must occur between acquisition and re-use. Of all the postulated stages of memory, re-use is the only one which can be measured.

As the storage of individual replicas of all impressions would be a physical impossibility, storage must involve transformation and coding. Impressions which voluntarily or involuntarily have been committed to memory may become temporarily or permanently unavailable for re-use and are then said to have been forgotten.

Factors which may influence forgetting are :-

(a). Delay between impression and re-use. The rapid forgetting following a single impression has been taken as evidence for the decay of the electrical trace i.e. that the trace tends to undergo deterioration solely with the passage of time. This is difficult to demonstrate since an interval empty of neural activity is needed. However, demonstration of this factor becomes partially possible when the interval between impression and re-use is one of sleep, brain injury or drug induced unconsciousness. JENKINS & DALLENBACH (1924) carried out experiments to show that some forgetting occurred when the intervening period was spent in sleep (see Chapter VII).

(b). Mental activities influencing forgetting: interference, facilitation and rehearsal. Numerous activities may take place during the interval separating impression and re-use.

An experimentally introduced task between learning and re-use causes a decrease in the amount of material remembered (retroactive interference; R.I.). The more similar the intervening activity to the original, the greater the forgetting of the original. Material learned prior to the original learning task also has a detrimental effect on the amount remembered (proactive interference; P.I.).

Items at the beginning of lists tend to be remembered better than those at the ends and those in the middle are remembered worst of all (UNDERWOOD, 1964). This suggests that P.I. is a more important cause of forgetting than the delay in time. Interference is considered to be the main factor responsible for forgetting but the reason for this effect is not clear. It is assumed that some period of consolidation is neccesary following learning to allow the material to become resistant to interference. The longer that period the greater the resistance. Consolidation may partly comprise an assimilation or recoding of the material into an individual's past learning experiences.

Rehearsal on the other hand keeps items in short term (labile) memory and affords an opportunity for storage in long term memory (stable storage) (KINTSCH, 1970). Thus the more rehearsal permitted the more likely the material will be stored in long term (stable) storage (HOWE, 1967).
(c). Receptiveness. Conditions neccesary for the reception or assimilation of an impression include the degree of alertness, attention and arousal. Verbal memory tests have been used in conjunction with physiological measures of arousal such as galvanic skin response (G.S.R.) and critical flicker

fusion (C.F.F.) (LEVONIAN, 1972). Receptiveness and the ability to receive, filter and integrate impressions have an important effect on the proportion of material that is subsequently forgotten (WALKER, 1962). Any differences in the level of arousal could therefore contribute to differences in the ability of subjects in my REM deprivation experiments to remember the previously presented material.

There does seem to be a limited relationship between memory capacity and intelligence. In view of this assumed relationship it is interesting to observe loss of memory in organically impaired or elderly people whose general level of intelligence shows little deterioration. With regard to age, it should also be remembered that the percentage of REM sleep also shows a decline with increasing age.

Emotion associated with a particular impression may also have an effect on what is later remembered. Word lists were remembered less well when associated with experimentally induced shock (HARDEN, 1930).

(d). Environment. It has been experimentally demonstrated that the relearning of lists of syllables is much faster when it takes place under exactly the same conditions as the original learning (GREENSPAN & RAYNAULD, 1957).

(e). Reconstruction and reorganisation. Memory is not a mirror reproduction of a past impression but seems to be a reconstruction based on partial remembering together with a knowledge of what is probable (BARTLETT, 1932).

The multistore theory of memory is the theory most popularly held for memory mechanisms. Before describing this theory, I should first like to briefly mention an

interesting theory put foreward by CRAIK & LOCKHART (1972), which is concerned with the encoding operations of memory formation. In this theory the rates of forgetting are a function of the type and depth of encoding. Perception, in this theory, involves the rapid analysis of stimuli at a number of levels or stages (SELFRIDGE & NEISSER, 1960; TREISMAN, 1964; SUTHERLAND, 1968). This concept of a hierarchy of processing stages is referred to as "depth of processing" where greater depth implies a greater degree of semantic or cognitive analysis. It is possible that dreaming could also be a mechanism wherby this analysis is permitted to occur. After the stimulus has been recognised it may undergo further processing by enrichment or elaboration coding (TULVING AND MADIGAN, 1970). This could also be carried out via dreaming and during REM sleep. Analysis proceeds through a series of sensory stages to levels associated with matching or pattern recognition and finally to the semantic-associative stages of stimulus enrichment resulting in the memory trace per se.

Such features of the trace as its coding characteristics and its persistence, arise essentially as by-products of the perceptual processing (MORTON, 1970). CRAIK & LOCKHART (1972) suggest that trace persistence is a function of the depth of analysis. Since the organism is normally concerned only with the extraction of meaning from stimuli, it is advantageous to store only the products of analysis but not the primary analysis. Highly familiar, meaningful stimuli are compatible, by definition, with the existing cognitive structures and these will be processed to a deep level

more rapidly than less meaningful stimuli and will also tend to be well retained. Since retention is a function of the depth of analysis,various factors such as the degree of attention, compatibility of the stimulus with the analysing structures, and the processing time available, determine the depth to which it is processed.

Memory, in this theory, is thus viewed as a continuum from the transient products of sensory analysis to the highly durable stable products of semantic associative operations. Superimposed on this basic memory system is a second way in which stimuli can be retained, by recirculating information at one level of processing. The theory of CRAIK & LOCKHART endorses the idea of MORAY (1967) that there is a limited capacity central processor which may be used in a number of different ways.

If this processing capacity is used to maintain information at one level, the phenomena of STM will appear. While limited capacity is a function of the processor itself, the number of items held will depend on the level at which the processor is operating. Repetition of analyses which have already been carried out are called type I, and this is in contrast with type II analyses, which are concerned with the deeper analysis of the stimulus. Only type II rehearsal should lead to improved memory performance.

(2) MULTISTORE THEORIES OF MEMORY.

The contrasting characteristics of STM and LTM have led to the hypothesis that there are two kinds of memory. The decay of traces in STM, in contrast to the permanence

of memory traces established through repetitive learning is the most universally held differentiation between the two stores.

One of the major criteria for distinguishing between stores is on the basis of their retention properties. For HEBB (1949) rapid decay of a trace was a correlate of the non-structural i.e. "activity" nature of a single perception that was given neither the "fixation" effect of repetition nor the opportunity for fixation through reverberation. For BROADBENT (1957; 1958) and BROWN (1958) the autonomous decay of the trace with time was a property of the postulated STM mechanism. CONRAD & HILLE (1958) postulated that time per se was a critical factor in the decay. BROADBENT (1958) proposed that information must be held transiently before entering the limited capacity processing channel. It could be held over the short term by recycling, following perception, through the same transient storage system. From there, information could be transferred to and subsequently retained in a more permanent long term store. BROADBENT'S ideas have been supported and developed by WAUGH & NORMAN (1965); PETERSON (1966) and ATKINSON & SHIFFRIN (1968). It is now quite widely accepted that memory can be classified into three levels of storage :-

(a) iconic memory
(b) short term memory
(c) long term memory.

(a) ICONIC MEMORY

Stimuli can enter the iconic (sensory) store regardless whether or not attention has been paid to the stimulus

(NEISSER, 1967; CROWDER & MORTON, 1969). BROADBENT (1970) defined iconic memory as a short lasting form of memory with a fairly peripheral locus. About a quarter of a second after the presentation of a visual stimulus, there appears to be some representation remaining in the CNS. The purpose of the iconic store is to act as a buffer and allow time for later selective operations which must be carried out within three and a half seconds (NEISSER, 1967). Further distinguishing features of the iconic store are its modality specific nature, moderately large capacity and the transience of its contents. Attention to the material in the iconic store is equivalent to "readout" of the material and transferrence to the STM store. STM stores are distinguishable from the iconic by their limited capacity (MILLER, 1956; BROADBENT, 1958), and coding features. For example, verbal items are coded in a phonemic fashion (SHULMAN, 1971) or in an auditory-linguistic-verbal way.

(b) SHORT TERM MEMORY.

There are at least two major sources of evidence about the STM store and its processing (WEISKRANTZ, 1970).

(i) from studies with human verbal memory

(ii) from studies of amnesia, whether experimentally induced in animals or as seen clinically.

Ther is a limited capacity of approximately seven items (MILLER, 1956) and there is rapid decay of such items. Experiments where human subjects have been required to remember just a single item, and where rehearsal was limited have shown that the amount remembered declines to a relatively low level within a matter of seconds (PETERSON & PETERSON,

1959). PETERSON & PETERSON determined the recallability of single consonant trigrams after intervals of three, six, nine, twelve, fifteen and eighteen seconds. The trigrams were presented auditorily in one second. A three digit number occurred during the next second and subjects counted backwards in threes and fours from that number until, after the appropriate interval, the suject received the cue to reproduce the trigram. Subjects were given up to fourteen seconds to reproduce the trigram thus avoiding any time pressure in the retrieval process. An appreciable amount of forgetting was found after the three and six second intervals.

BROADBENT (1957) in his dichotic listening experiments found that items presented to one ear but not recited until items presented to the other ear had been recited, similarly were available also only for a few seconds.

Assuming that there are two processes which have different temporal parameters and different capacities, then it should be possible to demonstrate that the two stores differ in content. GERARD (1963) showed that errors in the immediate remembering of digits and letters were largely confusable in terms of their acoustic similarity, even when these were presented visually. BADDELEY (1966) conformed this by showing that short words were acoustically confusible when tested by repeated learning trials followed by a time interval. Thus there do seem to be different types of coding characteristics in the two systems.

No animal studies provide data comparable with the studies of human digit span. There is evidence however, that treatments such as concussion, ECS (see later this

chapter), spreading cortical depression (see Chapter VI), drugs (PARFAIT & CORNELL, 1968; PEARLMAN et al, 1961; JOHNSON, 1970; PORTER, 1972) and anaesthetics (GRUBER, 1971; PAOLINO et al, 1966; ANGEL et al, 1972; CHERKIN, 1970; PEARLMAN et al, 1961) in animals and humans may impair memory of recent events leaving others unimpaired. If loss in retention of recent events following ECS is a genuine interference in memory and not one of the artifacts which have been suggested, then latest events may be represented in a physiologically different state from those prior to the ECS. The former may be termed STM and a gradual autonomous transfer to LTM may be postulated. This distinction may be linked to another, made on the physiological level, between electical dynamic activity i.e. labile memory and enduring structural change i.e. stable memory, either at the synaptic site or in some molecular substrate.

Although human short term verbal items become inaccessible within seconds, time constants in experiments using retrograde amnesia (R.A.) are very variable. They differ between experiments and experimental situations, from a few seconds to hours, and with clinical material even to months and years. There does however seem to be a correlation between the age of an event and its resistance to disruption. In some R.A. experiments, material thought to be lost, gradually returns (BICKFORD et al, 1968).

Amnesia in experimental situations is never total, it is merely that less is remembered compared with control groups. The minimum duration of the amnesia gives a measure

of the length of time activity must remain in the STM store before activity in the long term system is initiated. Long term traces in animals seem to be initiated almost immediately and WEISKRANTZ (1966) believes that RA treatments in humans mainly act on long term traces, by causing difficulty in retrieval. ECS probably also disrupts ongoing electrical activity and in this sense might affect labile traces only. Experiments using ECS.

In most experiments ECS given after a learning task has an RA effect. However there is recent evidence which suggest that the prescence of amnesia following ECS may depend on whether the response index of retention is skeletal or autonomic. Studies where autonomic responses have been investigated have shown that if heart rate were used as an index of learning, no amnesia resulted (MENDOZA & ADAMS, 1969; ROUTTENBERG & KAY, 1965; NAITOH, 1971), even when the same animals show responses indicating amnesia for inhibitory avoidance (HINE & PAOLINO, 1970) or classically conditioned motor response (NAITOH, 1971). It was suggested that ECS produced amnesia for punished responses, whereas changes in heart rate indicated that animals had learned to fear the experimental apparatus. McGAUGH & HERTZ (1972) suggested that different aspects of learning may thus be mediated by different neural structures. There are however some experiments (DAVIS & HOLMES, 1971; CAAL & BARRETT, 1972; DEVIETTI & KALLIONEN, 1972) where RA for heart rate responses has been found.

RA curves at best provide only indirect measures of memory storage since they reflect interference or susceptibility

and not consolidation. Behavioural or procedural variables can be shown to have an influence as strong or stronger, on the duration of the susceptibility of memories to ECS, than does the postulated time dependent change. ECS and its administration also have a stimulus value of their own. Learning in relation to this is as important as learning in relation to a milder aversive stimulus such as footshock. The nature of the acquired response to ECS, whether a conditioned avoidance response (CAR) or any other change in reactivity of the animals, then interacts with the behavioural test used and may show up as an apparent increase or decrease in memory retention. Much of this confounding may be removed by the use of discriminative rather than active or passive avoidance tests, although this type of task may require a greater number of trials so that the time between learning and ECS becomes unclear. The criterion of learning also has a strong effect on the duration of amnesia, an effect which also depends on the difficulty of the discrimination chosen. Absolute values of consolidation time therefore have very little significance concerning the kinetics of the consolidation process since behavioural factors are so powerful in influencing the results.

Passing large electrical currents through the brain may act directly on the biochemical processes required for memory formation. The action may be also on the organisation, perception, storage, classification or search processes occurring at a higher organisational level, but nevertheless essential for memory. It is important to discover which of these biochemical consequences of ECS are directly related

to the physiological consequences of memory loss.

Direct biochemical analysis has shown reduced RNA levels in the mouse brain following ECS (ESSMAN, 1966; 1967a). Various drugs protect against the ECS induced RA and these include :-

(i) TCAP which increases RNA levels presumably by accelerating RNA synthesis (ESSMAN & GOLOD, 1968).

(ii) Orotic acid which changes central nucleotide or RNA metabolism (OTT & MALLHIES, 1971).

(iii) Magnesium pemoline which increases RNA synthesis and may act as a mild stimulant (STEIN & BRINK, 1969).

All these drugs overcome the reduction in RNA levels caused by the ECS.

ECS causes an increase in the serotonin (5HT) turnover time (ESSMAN, 1967a; 1967b; 1968a; 1968b; ESSMAN et al, 1968). Treatment with the 5HT precursor, 5HTP, before learning plus ECS, or the administration of the 5HT synthesis stimulant, pipradel, reduced the ECS induced RA. The 5HT antagonists, reserpine, nialamide and amytriptyline protect against the ECS induced RA presumably by preventing the increase in 5HT production. p-Chlorophenylalanine (PCPA), a drug shown to decrease regional brain concentrations of 5HT by 60 % but having no effect on noradrenalin (NA) levels (REIGE, 1971) protects against ECS induced RA as does treatment with nicotine. 5HT treated animals show highly impaired retention from post training ECS, even when the ECS treatment is given as long as one hour following training. 5HT is thought to play an important role in the mediation of at least one phase of the memory consolidation process (ESSMAN, 1970).

ECS caused an increase in the acetylcholinesterase (AChE) activity and the release of bound acetylcholine (ACh) (ESSMAN, 1972). The anticholinergic drug, scopolamine, protected against ECS induced RA if given before retention trials (ADAMS et al, 1969; DAVIS et al, 1971) and had either no effect (DAVIS et al, 1971) or attenuated the ECS effect (KOPP et al, 1967) when given before learning. Scopolamine given without ECS had a disruptive effect on memory (DAVIS et al, 1972). Since these drugs may also have an effect on the retrieval and expression of memory, it is debatable whrther they are having an effect on memory formation. State dependent learning could also be used as an explaination of these results.

ECS increases the synthesis and utilisation of NA in the brain (ESSMAN, 1972), Increased tyrosine hydroxylase (the enzyme responsible for the rate limiting step in NA synthesis) concentrations cause an increase in NA turnover. Levels of the other biogenic amines dopamine and histamine are also increased following ECS (ESSMAN, 1972).

Increased neuronal activity necessitates an increased metabolic rate. Energy stores would be depleted during the convulsive activity associated with ECS. This would lead to a decrease in the levels of ATP, glycogen and glucose in the brain. Changes occurring as a result of ECS, also occur in the presence of the anaesthesia used to prevent the overt convulsions (ESSMAN, 1972). Thus it is not unexpected that macromolecular changes and alterations in the content and metabolism of the brain biogenic amines may also be observed.

The changes in brain proteins induced by the application of ECS are probably also significant for cognitive and informational processes. VESCO & GUIDETTA (1968) have shown in the rabbit that following ECS the number of free ribosomes was increased, accompanied by a comparable decrease in the polysome population. This strongly implicates protein synthesis in the cortex, either as a direct response to ECS, or following from the consequences of such treatments on other brain substrates.

Decrease in the specific activity of the protein bound glutamate in relation to aspartate in the total free protein fraction of ECS treated rats, suggest that there is a rapid turnover in protein following ECS (MINARD & RICHTER, 1968). ECS induces a rapid decrease in protein synthesis as measured by the difference in the rate of incorporation of labelled amino acids (AAs) into brain protein of ECS-treated compared to sham-treated mice (DUNN, 1971; DUNN et al, 1971; ESSMAN, 1972). The extent of the inhibition on whole brain incorporation produced by ECS occurs as a function of time after ECS. A single ECS exerts most of its profound effect on protein synthesis within the first 30 minutes of treatment. By one hour following ECS, there was no persisting inhibitory effect. Investigation of fractions of mouse brain at 15 minutes post ECS, showed the most marked effect in the cortex, in the presynaptic endings and mitochondria. COTMAN et al (1970)reported a short lasting inhibition of brain protein synthesis and an increase in the amount of free leucine as a result of ECS. They concluded that the ECS induced RA was unlikely to be due to

the inhibition of protein synthesis per se, but that brain seizures and inhibition of protein synthesis are signs that the co-ordinated functions of brain cells are altered to such an extent by the ECS, that the processes involved in memory storage are disrupted.

In view of the multiple effects of ECS, it cannot be concluded that any of them are a direct cause of the amnesia. It is also questionable whether these proposed changes have anything to do with the basis of memory formation and consolidation. These experiments have led WEISKRANTZ (1970) to conclude :-

(i) serial processing from STM to LTM is unlikely.

(ii) RA experiments give no information about the nature of STM.

(iii) human STM is qualitatively different from LTM in the verbal learning situation because it is probably invoked when the long term system is overworked. Under these circumstances it will depend heavily on the availability of a linguistic device.

(iv) an analogue of this type of STM may never be found in animals.

(v) although rapid decay is found in animal experiments, this may be due to interference in the hypothetical long term system, suggesting that STM in man is similarly found only in those situations.

(vi) the nervous system may be less complex than originally thought and if material can enter the permenent store then it will do so fairly rapidly without having to pass through buffers, filters and feedback loops

although some of these complexities may appear when entry into the long term store is made difficult. Thus the presence of STM results from stressing of the long term process.

(vii) the autonomous change in state of the long term trace and its consolidation, as inferred from a large number of experiments, is real, but does not necessarily require the label of being limited to the short term.

(viii) there may be short term behavioural processes which may perhaps be similar to those found in habituation.

(c) DIFFERENCES BETWEEN STM AND LTM STORES.

Despite the criticisms of WEISKRANTZ, mentioned above, there is well documented experimental evidence where differences in STM and LTM stores can be seen (WAUGH & NORMAN, 1965; BROADBENT, 1970).

Memory is worse for items which form part of a list of similar items, or which are preceded or followed by similar items. Acoustic similarity affects STM (WICKELGREEN, 1965), whereas other kinds of similarity such as semantic, have very little effect. Experiments using the same kind of material in LTM shows the reverse case to be true for retention (BADDELEY, 1966a; 1966b). In LTM it is the similarity of the stimulus which is important rather than the similarity of the response. This is in contrast to STM, where similarity of items to be remembered seems important rather than similarity between events which provoke the memory (BADDELEY (1968).

STM holds a fixed number of items. If items arrive,

they can only be held in STM by removing some item already present. The longer term memory is not restricted by the number of items and may still hold the item once it has been removed from STM (WAUGH & NORMAN, 1965; ATKINSON & SHIFFRIN, 1967).

(d) LONG TERM MEMORY, STABLE MEMORY.

As mentioned earlier, the classical theories of memory within the psychological discipline have used the terms STM and LTM. When discussing the electrophysiological and biochemical theories of memory and in my own experiments, I shall use the term stable memory to mean memory which is formed following a proposed structural change in the CNS.

MULLER & PILZECKER (1900) proposed the first perseveration theory in an attempt to account for retroactive interference (RI). The modern concept of the two stage theory of memory arose with HEBB (1949), who reformulated the perseveration theory in terms of neurophysiological substrates of memory.

In the previous section on STM, I have described experiments in which RA could be produced. The RA shrinks with time, but the special vulnerability of memories for events within a few minutes prior to the amnesic treatment suggests that memory progresses through a labile stage. At some time later, however, memories become resistant to treatments such as ECS and therefore must have become incorporated into the brain structure rather than into patterns of electrical activity. The importance of the structural change was affirmed with the discovery that antibiotics

which block DNA dependent RNA synthesis, or ribosomal protein synthesis, also blocked the formation of permanent memories.

The view that memory could be coded at the molecular level was put foreward by MONNÉ (1948) on the basis of the antigen-antibody reaction. He concluded that the engram was likely to depend on specific changes in the CNS proteins. VON FOERSTER (1948) favoured the view that proteins are concerned in the laying down of stable memory traces, because of the large number of possible ways by which a protein molecule may be altered. KATZ & HALSTEAD (1950) suggested that learning involved a change in the synthesis of a specific "memory protein" which permanently changed the electrical properties of the cell. They concluded that each individual memory trace was distinguished by the chemical composition and geometrical configuration of the proteins.

That either RNA or protein molecules could, by virtue of their unique coding potential, themselves represent individual engrams is considered unlikely because such a role would make redundant the known coding properties of the neuronal interconnections within the CNS (DINGMAN & SPORN, 1964). The patterns of synaptic interconnections make possible an intercellular, rather than an intracellular information storage system. Although the model of HYDÉN (1960) envisaged an RNA molecule, no biochemical role for RNA has been demonstrated, apart from acting as a way station for protein synthesis from an initial DNA template. Any effector memory would be protein albeit synthesised upon a specific RNA messenger. In addition, no very convincing model based on

"one protein is equivalent to one mnemon" system has ever been formulated.

The genetic analogy to memory has focussed attention primarily on changes in protein and RNA synthesis and the effect on memory consolidation. There is good evidence that in a large number of biological control systems, the initial step is the activation of RNA synthesis followed by new protein synthesis and subsequently by an alteration in the metabolic processes of the cell.

The main evidence in support of the molecular hypothesis of memory storage is that the nerve cell has an active RNA and protein metabolism which cannot otherwise be acounted for. The nerve cell is also exceptional in several aspects of its amino acid (AA) metabolism (RICHTER, 1962; GAITONDE et al, 1964). The brain differs from all other organs in its high content of glutamate and the manner in which it can utilise AAs as a source of energy. It is also unique in possessing active enzymic machinery for utilising glutamate through the gamma amino butyric acid (GABA) pathway, and is exceptional in its high rate of conversion of glucose carbon to glutamate. The nerve cell can synthesis protein in the cell body, but it also contains a proteinase, particularly active in the axon which is able to break down protein and liberate AAs (ANSELL & RICHTER, 1954). The nerve cell has little reserve of glycogen in relation to its high metabolic rate and activity, and the evidence suggests that it is specially adapted to utilise AAs and endogenous protein to provide the metabolites required for functional activity.

Another argument advanced for the molecular hypothesis of stable memory storage is that the number of traces that need to be stored in the brain is very large. It is therfore likely that the vast capacity of nucleoprotein macromolecules for storing genetic information would be utilised. HYDÉN (1962a) proposed that between 10^{15} and 10^{20} items could be in the memory store, although 10^{11} items was postulated as likely over a human lifetime of 70 years. 10^{11} items could be specified in terms of base sequences in nucleic acid macromolecules, but the mechanism allowing for the coding of the base sequences to correspond to the pattern of elecrtical impulses is difficult to explain. HYDÉN (1964)attributed the specificatioh of memory RNAs to the pre-existing DNA molecules. The mechanism of coding was not explained.

A further problem is how to accumulate the quantity of DNA required for memory coding. The number of genes in the chromosomes is between 10^6 and 10^7, and if the efficiency of storage and re-use of information from the memory DNA were no better than the genes, the amount of DNA required for the storage of 10^{11} specific memory items would be 1,000 to 10,000 times that required for the recognised structural genes. A further problem is the "read out" of information stored in 10^{11} different proteins. The similarity of memory based on learning to phylogenetic memory is questionable. Although complex behavioural patterns can be inherited and therefore encoded in the DNA molecules, these mechanisms appear to operate by influencing growth and structural organisation of the neural network, thus

phylogenetic mechanisms seem too slow. The concept of "neurobiotaxis" was introduced by KAPPERS (1917). He proposed that new neural processes grew between cells as they responded to stimuli, and thus storage of information and experience was mediated by this structural change.

Whether information is stored by the formation of new connections between cells, or by the synthesis of substances within neurons or glial cells, these processes must require changes in brain constituents to occur. Since stored information may persist throughout a lifetime, it should be possible to demonstrate a stable brain component which would be a counterpart to the stability of the stored information. Radio-isotope turnover measurements provide information as to whether any cerebral molecular species are static. However there seems to be no significant compound in the brain which does not display a remakably high turnover rate (LAJTHA, 1961; GAITONDE & RICHTER, 1953; 1956; SMITH, 1968; WARREN & GLICK, 1969; SCHMITT, 1964; 1973). The permanence of memory cannot therefore be attributed to new intra or extracellular structures laid down by permanent molecules. A stable representation of experience might be due to some change in configuration, or some substance mediated by a chemical system, which although in itself not stable, is characterised by the fact that the molecules which break down are then resynthesised in a specific way so as to maintain the essential features of the change. Such template functions are known to be served by the nucleic acids.

DNA is completely localised in the nucleus of the cell,

but RNA is found not only in the nucleus but also distributed on the microsomes and throughout the cytoplasm. Since stimuli impinge on the outer surfaces of the cells, the chemical systems which permanently alter cellular responses probably modify substances found near the cellular surfaces or in the subadjacent cytoplasm. This has led to theoretical formulations about the possible role of DNA and proteins in the mediation of stable memory storage. Modifications of DNA acivity could also play a part, despite its nuclear location and greater resistance to alteration. This mechanism could be "instructional" i.e. a change in the structure of existing molecules specified by the information to be stored. Alternatively it may be "selectional" i.e. a release of existing molecules or potential molecules is allocated for a given representational function.

(i) The proteins.

Protein formed from altered RNA function, could alter synaptic transmission functions in a number of ways :-
(a) presynaptically, by increasing the synthesis or release of transmitter (BARONDES, 1965; BRIGGS & KITTO, 1962; HYDÉN, 1959; 1960; HYDÉN & LANGE, 1965; 1966), or by altering the release of transmitter making it available only with certain characteristics of stimulation (SCHMITT, 1962).
(b) by changing the receptor properties of the post synaptic membrane by increased sensitivity or synthesis of receptor molecules, increased permeability to transmitter, or decreased degradation of the transmitter (BARONDES, 1965).
(c) by altering both pre and post synaptic properties by glycoproteins or glycolipids (BOGOSCH, 1968), or by

altering the proximity of the two membranes with protein (ELUL, 1966).

(d) by production of an antibody which would cause cells to tire (GRIFFITHS & MAHLER, 1969), or by the introduction of specific memory antibody in certain neurons causing facilitated synaptic transmission (SZILARD, 1964). HYDÉN & LANGE. (1970) have implicated the specific protein S100, in learning.

(ii) DNA and RNA.

Many theories invoke an action of an RNA synthesised in response to gene activation on the DNA molecule, brought about by a learning experience. Gene activation has been postulated to occur by :-

(a) modulated frequencies of nerve impulses cause the specific protein eventually synthesised to respond to this frequency thus leading directly to increase in transmitter (HYDÉN, 1960; SCHMITT, 1962). Electrical impulses could alter DNA, RNA or AA sequences (GAITO, 1961), or produce methylation or demethylation of a "ticket" on the DNA molecule leading to a synaptic change (GRIFFITHS, 1966). HYDÉN & LANGE (1965; 1966), have suggested that the DNA sites are stimulated by environmental factors so that unique RNA is synthesised with a different base ratio composition.

GAITO (1966) proposed that during learning, stimulation of specific nerve cells causes a modification on the DNA complex such that DNA is activated to produce more RNA leading to more protein synthesis in synaptic regions.

(b) the neurotransmitter induces gene activation (BARONDES, 1965; BRIGGS & KITTO, 1962; SMITH, 1962).

(c) gene activation is by a substance released by the transmitter by repression or derepression of the genes (BARONDES, 1965; UNGAR, 1968).
(d) the protein or its product induces gene activation (FLEXNER et al, 1967; ROBERTS et al, 1970).
(e) a foreign RNA is transferred from glia to neuron and this depresses DNA (PRIBRAM, 1966).

Other hypotheses do not require gene activation for the learning process. In these the change is postulated to be brought about by :-
(a) production of a specific RNA template by glial-released RNA or methylation of RNA influenced by the biogenic amines (LANDAUER, 1964) or another RNA (CORNING & JOHN, 1961; McCONNELL, 1964a; 1964b).
(b) changes in electrical frequency directly changing the base ratio of RNA (HYDÉN, 1959; 1960).

Disagreement exists about the reproducibility of experiments which give rise to the theories involving the transfer of behaviour between animals by the injection of brain and other tissues. Although the transfer of chemical components from animals having learned a response to naive animals seems a logical way to investigate the chemical components of memory and learning, problems arise because the material transferred is often a complex molecule. Complex molecules may be broken down in the recipient animal which may be resynthesised to form a similar but not identical molecule.

The acceleration of brain RNA or protein synthesis by environmental stimulation is easier to replicate but

the relevance of this to learning and memory is unresolved.

Detailed biochemical responses to long term alterations in the environment have been investigated by BENNETT et al (1964); KRETCH et al (1960; 1966) and DIAMOND et al (1964). Rats were reared in conditions of environmental complexity or in conditions of greater or lesser isolation. Brains were frozen, stained with threonine and then sections were sampled for variables such as cholinesterase (AChE) levels, brain weight and cortical thickness. Results of these experiments showed an apparent increase in cortical weight and thickness in the enriched group compared with the impovrished controls. AChE activity decreased especially in the visual cortex. In contrast, the enzyme increased, at least in total quantity, in the sub cortical regions. ROZENZWEIG et al (1968) have shown, that as little as one hour per day exposure to the enriched condition, is sufficient to produce a statistically significant change. GYLLENSTEN (1959) and GYLLENSTEN et al (1965) showed that the visual cortex of mice reared in the dark was less developed than that of normally reared litter mates. DIAMOND et al (1966) found the increase in cortical depth to be greater in the medial aspects of the visual cortex than in the lateral.

Glial cells are thought to supply additional nutritional needs; to support newly formed fibres; to give additional specificity to synaptic membranes; to respond to axoplasmic movement; to encode experience or to supply either substrates for energy or the energy itself, to axons. The glial to neuronal ratio was 12 % higher in the

enriched condition (DIAMOND et al, 1966).

TAGNEY (1972) performed a series of experiments with rats to attempt a correlation between brain activity, cerebral protein synthesis and sleep. Rats were reared in either enriched or isolated environmental conditions. Rats in the enriched condition were found to have significantly more sleep than their litter mates reared in the isolated condition. This difference was seen in SWS and REM time, although the percentage of REM sleep in the total sleep time was not greater in the enriched condition. It was postulated that the need for extra sleep as a result of increased mental rather than increased physical activity was that whereas restorative processes in skeletal muscles could take place while the organism was awake, conditions of sleep are required for brain restoration. It is possible that metabolic activity in cerebral neurons could not lower itself to the level at which macromolecular synthetic pathways begin to operate (BOGOSCH, 1970) until consciousness is lost and the low average firing rate of both cortical and subcortical neurons seen in SWS is reached (BALZANO & JEANNEROD, 1970; DESIRAJU, 1972; MANDHAR et al, 1972; NODA & ADEY, 1970; NODA et al, 1969). In conjunction with other factors supporting the possibility of increased synthetic activity of cerebral neurons during sleep (see Chapter VII), increased sleep may occur in rats reared in enriched conditions, because they have increased levels of cerebral activity and require longer periods of optimal conditions for restorative brain ptotein synthesis.

(iii) Electrophysiology and neuroanatomy.

ROSE et al (1961) on the basis of laminar lesions produced by radiation, found that after a period of time following the lesion formation, large numbers of nerve fibres could be seen. These fibres appeared sooner if the radiation dose was relatively mild and later if the dose was strong. They concluded that these fibres were new sprouts and interpreted this growth as being due not only to regeneration i.e. after injury, but to be the result of normal continuous growth of these fibres, which was still possible after the radiation doses. Growth must take place at the terminal or paraterminal regions of central axons since this is the only assumption concordant with the massive growth in the laminar lesion.

All central neurons might normally have the capacity for continuous growth in the paraterminal region of every axon, and thus the activity of the neuron itself could be a factor in modulating the rate and extent of growth. Following these assumptions, all synaptic endings have to be metabolised, and therfore any given ending could be expected to be, not a permanent structure existing throughout life, but only an evanescent morphological structure. Continuous growth of nerve fibres has been formulated by WEISS & HISCOE (1948), and on the basis of experiments with peripheral nerves. This was later supported by GERARD (1948); HYDÉN (1950); SANDERS (1948) and YOUNG (1945). If continuous growth occurs in peripheral fibres, it seems reasonable to suggest that the same holds true for central axons.

HYDÉN (1959; 1960; 1961) proposed a model for LTM formation based on the genetic analogy which has become known as his "instructional" model. He postulated the formation in the nerve cell of :-

(1) a new form of RNA with a specific base sequence coded to correspond to the pattern of impulses in the existing sensory neuron.

(2) this specified RNA serves as a template for the synthesis of a protein replica which is postulated to have the property of dissociating rapidly in response to the same electrical pattern of stimulation originally specifying the RNA template.

(3) the dissociating molecule liberates a smaller molecule.

(4) this molecule reacts with a complementary one already present in the cell.

(5) a transmitter substance is released at the synapse.

(6) the transmitter is released in a sequence of bursts at frequencies close to the original pattern of electrical stimulation.

The modulated release of transmitter can pass on the original stimulus in this manner. Each specified type of RNA continues to replicate itself and to produce protein replicas in the nerve cell as long as the memory persists. The presence of the specified protein in the cell makes the cell respond differently to stimuli depending on whether the incoming pattern causing the protein to dissociate is novel or familiar. Neuronal paths may grow by successive specification of the RNA molecules and the proteins formed from them.

HYDÉN later (1965) proposed his "selectional" mechanism utilising RNA as a possible stable configuration for experiential coding. Under physiological conditions an increase in neural stimulation produces an increase in the neuronal content of RNA, protein and enzymic activity. The following occasion at which the same modulated frequencies enter the neuron, the specific protein leads to an avtivation of the transmitter substance. Thus there could occur a chemical specification of neurons situated within the phylogenetically given pathway where the stimulus entered, and also the specification of millions of neurons situated outside the first area. He suggested that when learning took place, the neuronal glia regulate the induction of RNA synthesis in the neuron. Kinetic studies of enzymic activity and RNA in the glia around Deiter's nucleus have shown that glial RNA can react more rapidly than the neuron and also precedes the reaction of the neuron. In an acute learning situation, the modulated frequencies set up by the neuron are transferred to the glia. When the neural frequency is changed, there is a coupling of frequencies between neuron and glia i.e. a "phase lock in" effect. The glial ionic equilibrium was disturbed. Substrates in the form of nucleotides are transferred from glia to neuron. The repressed chromosome region is released and the necessary enzyme synthesis for RNA production is induced.

Restriction of sensory experience early in post natal life has been found to change cell morphology (CRAGG, 1969; GLOBUS & SCHEIBEL, 1967; MOLGAARD et al, 1971;

ROSENZWEIG, 1971) and alter the pattern of functional connectivity within the brain (BLAKEMORE & COOPER, 1970; HUBEL & WEISEL, 1970). Rearing mice in darkness produced a loss of spines in pyramidal cells of the visual cortex (VALVERDE, 1967). Rearing complexities also affect the branching of dendrites (VOLKMAR & GREENOUGH, 1972). Factors within the cell regulating growth and maintanance of cytoplasmic processes and synaptic connections must therefore play a central role in bringing about some of the long term changes that arise frim learning or early experience. If stable memory is formed in the brain as an altered growth pattern, then storage must involve some alteration of membrane structure wherby molecular constituents are either resorbed or incorporated locally into the membrane to produce a functionally altered pattern of synaptic connectivity (DEUTSCH, 1972).

Brain microtubules are important in mediating fast transport of materials along axons (OCHS, 1972). Microtubules and microfilaments may be implicated in regulating the supply of materials within cells to locations where new materials are required to promote the growth or maintainance of cell cytoplasmic processes, and perhaps also for the formation or maintainance of synapses. ROISEN et al (1972) have shown that axonal elongation depends on microtubule formation. Drug inhibition of fast axonal transport mimicks the effects of axotomy (PILLARD & LANDMESSER, 1972) and leads to the functional depression of synapses (PERISIC & CUENOD, 1972) and morphological signs of synaptic degeneration (CUENOD et al, 1972). Microtubules

might therefore be involved in conveying the materials necessary to bring about local changes in membrane structure or cell morphology resulting from learning or early experience.

Colchicine, a plant alkaloid, combines with protein subunits of microtubules (OCHS, 1972; SJORSTRAND & HANDSON, 1972) and has been used to inhibit fast axonal transport (CRONLY-DILLON et al, 1974). When administered intracranially it prevented the memory of a recently acquired task from entering long term storage.

The effect of colchicine was similar to that seen with protein synthesis inhibitors. However gross protein synthesis in the brain was not detectibly affected over a one hour period following training. STM was unaffected by colchicine. Thus cellular mechanisms responsible for transferring learned information from STM to LTM probably comprises several components (MARK & WATTS, 1970; WATTS & MARK, 1970), one of which is susceptible to interference by the same agents as interfere with the fast transport of materials within cells. A converse effect on growth of cellular processes and memory fixation has been reported for agents which promote the stabilisation or formation of microtubules. D_2O has been found to accelerate the growth of cell processes in isolated nerve tissue (MURRAY & BENITEZ, 1968) and facilitates learning in goldfish (LEHR et al, 1970).

Cyclic AMR initiates the "in vitro" polymerisation of microtubule subunits (GOODMAN et al, 1972) and a dopamine sensitive adenyl cyclase was activated by synaptic

activity (KEBABIAN & GREENGARD, 1971; KEBABIAN et al, 1972). Thus a possible component of the memory fixation process is a local increase in the intracellular cyclic AMP produced by synaptic activation of adenyl cyclase, which may then, in some synapses, initiate tubule assembly and hence increase the number of "supply lines" from the cell soma. In modifiable neurons CRONLY-DILLON et al (1974) suggest that these could serve to direct the movements of various materials to the vicinity of synapses recently activated. Thus colchicine could act by interfering with an immediate link between STM and the stage requiring the synthesis of protein. Microtubules could thus have a role of mediating the fast transport of some intracellular material needed to bring about a structural change at certain synapses in the brain that are involved in the original learning.

(iv) Effects of learning on the synthesis of RNA and protein.

SMIRNOV (1955) found that after the establishment of a conditioned response, presentation of an auditory conditioned stimulus caused an increase in the turnover of RNA in the auditory cortex. This was not observable in the adjacent cortical regions. Such increased turnover was not elicited by neutral stimuli.

EIDUSON (1961) found a negative correlation between the effectiveness of imprinting in the chick brain and the amount of available RNA. This suggests that as the amount of available RNA increases, the more possible it becomes for the chick to establish alternative behaviours to the

rigid responses represented by the results of imprinting.

CORNING (1966) studied the incorporation of P^{32} into RNA during the establishment of a conditioned response. Conditioned worms incorporated more P^{32} during the conditioning process than did controls.

Using labelled uridine, increased RNA synthesis has been demonstrated in the brains of goldfish which learned a CAR, but not in the brains of controls (GLASSMAN et al, 1966). Analysis of RNA from the nuclear and ribosomal fractions of centrifuged brains of mice trained to perform the CAR, showed greater incorporation of the label than controls (WILSON et al, 1966). Further analysis showed that it was the rate of synthesis of a messanger like RNA which was increased during the learning.

ROSE (1967; 1968a) and ADAIR (1968a; 1968b) using the first exposure of animals to light, found an increase in the rate of incorporation of AAs into proteins of the visual cortex at three hours after the emergence of the animals to light. This effect was specific to the visual cortex. When the light exposure was increased to six hours the effect on incorporation was abolished. Periods of nine to 99 hours depressed the incorporation compared with controls. By 99 hours, incorporation was normal. The transient increase in incorporation and presumably protein synthesis in the visual cortex, was thus followed by a more prolonged decline. The activity of animals in the condition of darkness was shown initially to be greater, and ROSE (1967) found that intermittent periods of exercise, produced significant decreases in incorporation

in whole cortex samples. This is an inversion of the effect due to light alone. JACOUBEK & GUTMAN (1968) found a decrease in protein synthesis in spinal motoneurons in rats following 90 minutes of enforced exercise. ALTMAN & DAS (1966); ALTMAN et al (1966) and METZGER et al (1967) detected no changes in protein synthesis, and TALWAR et al (1966) and SINGH & TALWAR (1966) have found substantial increases in lysine incorporation following exposure to flashing light, compared with controls. The effects measured therefore, seem both labile and reversible, depending on the nature and duration of the stimulus chosen.

In few of these experiments has the full time course of the effect been analysed. Such environmentally determined changes in protein synthesis rate do not by themselves indicate a change as a consequence of learning. They could equally well be due to a generalised alteration in the pattern of information arriving at the brain.

Other memory hypotheses.

One of the attractions of the synaptic change hypothesis is that large amounts of information can be stored. If there are 10^{10} neurons in the human brain, each with 10^{3} synapses, then there are 10^{13} possible synapses. The number of alternative patterns made by an all or none change involving any 10 synapses would be in the order of 10^{130}. Changes at the synapse could take place by :-

(1) neuronal activity promoting the growth of nerve endings with the formation of new synaptic connections.

(2) a change in lipoproteins of the synaptic membranes occurring so that transmission is either facilitated or

impaired. These changes might involve the synthesis of RNA and protein, but they might equally result from a change in the quality or distribution of the lipid content.
(3) prolonged activity of neuronal circuits causing the amount of a transmitter substance to change. In the adrenal medulla, prolonged stimulation causes a change in the proportions of adrenalin and NA that are released.
A change in the position of a synapse on the receiving neuron could also change the behaviour of a neuronal circuit. There is a greater possibility of a receiving neuron firing when the synapses are near the cell body. PURPURA & SHIFFER (1965) have shown that synapses move towards cell bodies as the brain undergoes maturation.

Frequency modulation in the CNS can modify a response and this is supported by experiments with inserted microelectrodes. Transmitter released from one neuron could result in the formation of transmitter in a second, and a facilitated pathway could develop (BRIGGS & KITTO, 1962). This would depend on the formation of a specific RNA in the first place, and would continue as long as RNA dependent protein synthesis was maintained. Changes in RNA expected to result from stimulation might be similar to those reported in learning experiments by HYDÉN. Unlike the nucleotide hypothesis of memory coding, this hypothesis involves no special assumptions or biochemical mechanisms which have not been previously been demonstrated in other systems.

The brain has a very active metabolism. It represents only 3% of the body weight but acounts for 20% of the

total heat production when the body is in the normal resting state. Much of the energy requirement of the nerve cell is for the maintainance of an irritable membrane, the Na^+ pump. Unlike muscle, which is versatile in using fats as well as carbohydrates, as a source of energy, the brain itself relies almost solely on glucose. This is oxidised directly to CO_2, or converted to AAs or protein.

The active protein metabolites of the brain are not excreted much although some nerve cells do have a neurosecretory function. Protein must therefore be conserved in the brain. Part of the protein is used in the production of transmitters, trophic substances and enzymes. Protein is also broken down at the periphery of the cell by proteinase and utilised for energy requirements of axons and dendrites. Protein synthesis may be involved in the mechanism of memory storage and re-use, but it seems more reasonable to relate the active RNA and protein metabolism of the neuron to normal processes of neuronal and synaptic transmission. Molecular hypotheses do not acount for the specification of RNA by electrical impulses nor for the subsequent "read out" of information from the memory store.

The enhancement of protein and RNA synthesis by neural activity and the decrease in the amount of these substances resulting from sustained inactivity, suggest the existence of a rate limiting factor. The increase in the RNA content and the synthesis rate accompanying learning may additionally reflect the activation of new feedback loops involving repression. Changes in the base ratio of neuronal RNA with learning, could reflect the production

of a new RNA, with its own characteristic composition, rather than alteration in base ratios of existing RNA species.

The mechanism which mediates the early, labile phase of information storage could be a frequently repeated reverberation of a loop involving relatively small numbers of neurons. Alternatively the circulation maintaining this loop might produce repeated discharge throughout the consolidation period. These cells must be protected against capture by circuits engaged in other ongoing activity.

This protection function could be one of the factors provided by the occurrance of REM sleep, since the state of the brain is one of activity similar to the relaxed waking state. In addition, some of the other processes of a cognitive nature normally present during wakefulness are diminished during REM sleep.

Sustained neural activity will cause the movement of K^+ out of the cell and HERTZ (1965) has presented evidence for the presence of K^+ activated ATPase on the outer surface of glial cells. Addition of K^+ to an incubation medium causes a sharp increase in the respiration rate of brain slices in vitro. The effect was specific to the brain grey matter. Glial cells also comprise a system of active K^+ transport, and changes do occur in both glial and neuronal cells during stimulation and learning (HYDÉN, 1962b; HYDÉN & EGYHAZI, 1963).

The electrolyte shift could affect the stable storage of information in a group of cells since changes in electrolyte concentration may be a rate determining factor

in the synthesis of protein (RUDENBERG & TOBIAS, 1960; GURD & WILCOX, 1956). Extracellular mechanisms might govern protein synthesis or cell growth through the mechanism of K^+ transport (LUBIN, 1963; 1964). LUBIN & ENNIS (1963) reported that NH_4^+, a product of neural excitation (VRBA et al, 1974), also had a marked effect. NH_4^+ fixes amino acyl t RNA to the ribosomes (CONWAY, 1964).

The ionic shift resulting from a sustained pattern of neural activity, or the effect of enzyme activity on protein synthesis may act as effector. Change in protein synthesis rate could hypothetically maintain the feedback loop. The altered protein synthesis could alter the response characteristics of the neuron in such a way as to provide for the retrieval of the stored information which it represents.

It is also important to consider what that information might be. Members of a subset of cells receiving afferent input could share a sustained pattern of increased or d creased activity, the configuration of which could constitute a particular response. Sustained activity i.e. a non-random occurance in that specific response mode, could represent the effect of the afferent input on the population of neurons. Membrane characteristics of the neuron in the representational subset could be altered by a change in protein synthesis caused by the same activity. Cells receiving those particular temporal patterns would be more capable and likely to display a sustained response. If tuning to a particular pattern could be established in this subset, activity in the relevant mode would be increased

when the total population received that input. This would occur by the binding of K^+ ions by the newly synthesised protein (analogous to the K^+ binding by muscle protein). Increase in coherent responses in a particular mode would constitute the essential features of information retreival in neural networks.

It is assumed that these associated groups of cells are constucted not only within the boundaries of various anatomical brain regions, but also that the afferent and efferent circuits between these regions join them into anatomically larger and extensive systems. Anatomical differentiation and organisation could distinguish the response to familiar events, providing an adequate basis for a functionally significant read out to occur.

Thus there could be two kinds of cellular organisation in the brain :-

(1) stable cells or networks which display a relatively constant and invariant response to specific inputs.

(2) plastic cells or networks which display a variable response to specific inputs.

If these two ouputs converge and if they are of compatible mode and non random magnitude, this would indicate that the stimulus producing the activity in the stable network was a familiar event (YOSHII & OGARA, 1960; MORRELL et al, 1966).

An advantage of this model is that the proposed memory mechanism involves the alteration in rate of one of the potential processes of chemical synthesis in cells by "selectional" processes rather than the synthesis of a unique representational configuration (MORRELL, 1962).

Chapter III.

BIOCHEMICAL CONSIDERATIONS OF STABLE MEMORY FORMATION I: THE NUCLEIC ACIDS.

The action of a stimulus on living tissue causes a number of biochemical changes, thus meeting one criterion of an engram. A second criterion, that the change must be permanent or at least durable, is more likely to be satisfied by a change in a structural lipoprotein or lipid, than by a constituent as labile as RNA. The engram could also be represented by a process which influences neural transmission.

The experimental evidence showing that short term changes in RNA and protein synthesis may result from stimulation and learning are described in the previous chapter, HYDÉN & EGYHAZI (1963; 1964) found significant changes in the base ratios of nuclear but not cytoplasmic RNA in cats that had undergone learning. The experimental group had more adenine and less uracil, although there were also qualitative changes in control cats.

The protein S100 unique to the brain, has been found in the glial cell membrane and cytoplasm and in the neuronal nucleus. HYDÉN (1966) suggested that this protein moves from glia to neurons since the loss in glial RNA as a result of activity, exactly balances the increase in neuronal RNA, with the same base ratio. The protein could act as an inhibitor of neuronal repressor RNA. Functional demands could be met by blocking this RNA, which would result in enzyme induction and specific protein synthesis. Also the neuronal RNA might partly be used to synthesise enzymes involved in the

/the Na^+ and K^+ transport at the cell membrane. Glial cells might therefore specify part of the neuronal protein synthesis (HYDÉN, 1966).

Although there has been much criticism of HYDÉN'S work (BOWMAN & HARDING, 1969; JOHN, 1967; MANDEL, 1969; KUFFLER & NICHOLS, 1969; ROSE, 1968), his broader conclusions, i.e. that behavioural manipulations lead to changes in the rates of synthesis, and the total amounts of RNA and protein in specific regions of the CNS, seem to be substantiated (WATSON, 1965; LAMBERT & DUNHOLT, 1968; SJORSTRAND, 1966).

BRIGGS & KITTO (1962) have prescnted objections to the formulations involving the synthesis of specific proteins by specifically coded RNA. Their model suggests that during learning, the increased demands made on the cell for the release of transmitter deplete the endogenous store of transmitter substance. The depletion alters the precursor concentrations to create a situation analogous to enzyme induction. Altered concentrations feed back onto the DNA in the nucleus, inhibit the repressor sites on the DNA and permit the synthesis of new RNA. This in turn will make greater amounts of transmitter available. Enzyme induction hypotheses were also put forward by SMITH (1962) and SZILARD (1964). Retrieval of stored information is postulated to be mediated by enhanced release of transmitter substances.

Derepressor hypotheses rely heavily on the postulated, but not demonstrated, ability of the CNS to discriminate/

discriminate between modes of activity in neuronal ensembles. They invoke the synthesis of new RNA as a result of the depression of DNA, despite the evidence that learning can take place while new RNA synthesis is inhibited by Actinomycin D, (see section (2)).

(1) DESTRUCTION OF RNA

Intracerebral injection of RNA-ase, trypsin or serum albumen, blocked retention of a conditioned defensive reflex in mice (KRYLOV et al, 1965). CORNING & JOHN (1961) used head and tail segments of transected planaria which were regenerated in a low concentration ribonuclease solution. Worms regenerated from head segments retained a CAR equally well, whether regenerated from the RNA-ase solution or not. In contrast, tail segments regenerated in the RNA-ase performed the CAR at random levels, while controls performed indistinguishably from head segments. Since these animals are dominated in their behaviour by the cephalic ganglion, the regenerating tail would seem to produce head tissue, containing the cephalic ganglion. The information stored in the head segment during training seems to be transferred to the new cephalic ganglion tissue. This could be achieved by:-

(a) "trained" cells migrating from the tail to the cephalic ganglion.

(b) the chemical configuration of molecules being synthesized to constitute the regenerating head is specified by/

/by "training" RNA in the tail tissue.

(c) patterns of nervous discharge, established by training in the tail segment, are transmitted to nerve cells formed in the regenerating head segments, thus "teaching" the new head tissue.

CORNING & JOHN'S experiments do not distinguish between these three possibilities. Thus the difference may be due not to the destruction of RNA-ase serving a storage function, but to some less specific interference.

(2) INHIBITION OF MEMORY FORMATION BY DRUGS AFFECTING SYNTHESIS.

(a) cytosine arabinose.

No effect on learning was produced by the injection of cytosine arabinose into goldfish brain although this resulted in inhibition of 95% of DNA synthesis at the time of learning (CASOLA et al, 1968). This drug is postulated to act by inhibiting DNA polymerase and has no effect on protein or RNA synthesis. The lack of interference with avoidance responding suggests that the formation of memory does not depend on DNA synthesis.

(b) hydroxylamine.

Hydroxylamine is without effect when injected before training. However, if injected intracranially from four hours to three weeks after training, it interferes with the retention of the learned task, (REINIS 1970; 1971a; 1971b). Hydroxylamine is postulated to act specifically on activated or derepressed DNA, leaving behind the inactive, repressed one.

REINIS suggested hydroxylamine, by altering the derepressed DNA, causes misreading of the code, leading to the production of a defective protein. Biochemical proof of this hypothesis is difficult and other explanations are possible.

(3) INHIBITION OF MEMORY FORMATION BY DRUGS AFFECTING RNA SYNTHESIS.

(a) 8-azaguanine.

This is incorporated into the RNA molecule forming an abnormally-functioning structural analogue of RNA.

When administered before learning, it impaired acquisition in goldfish, but had no affect on retention when administered after training (DINGMAN & SPORN, 1961). This may, however, be impairment of aspects of performance other than those involved in memory processes. The same difficulty in interpretation also applies to the effects on spinal fixation time (CHAMBERLAIN et al, 1963), on the learning of a fixed interval schedule (JEWETT et al, 1965) and on the acquisition of a new operant schedule (WARBURTON & RUSSELL, 1968). 8-azaguanine also depresses motor activity as assessed by lower bar pressing rates (JEWETT et al, 1965), and reduced running in an activity wheel (CHAMBERLAIN et al, 1963). It seems that 8-azaguanine has not been sufficiently investigated to warrant conclusions as to its effect on memory.

(b) actinomycin D.

Almost all micro-organisms inhabiting a natural environment are subject to antagonistic or favourable reactions/

reactions owing to the presence of other types of living organism. If the association is unfavourable, an antibioisis occurs. The term antibiotic can be applied to a chamical substance which has a lethal or inhibitory action on cell growth or division. Antibiotics may be derived from living bacteria, yeasts, moulds or other plants, or may be chemically synthesised. They act by interfering with various mechanisms of the DNA, RNA or protein synthesising apparatus of cells or may act on the cell membrance.

The antibiotic, actinomycin D, blocks transcription by binding the guanine nucleotides on the DNA molecule so that RNA synthesis ceases (GOLDBERG et al, 1962; REICH et al, 1962). The effect on protein synthesis does not occur in fish brain until several hours after injection (AGRANOFF et al, 1967).

Actinomycin D injected before learning in doses producing 80-95. inhibition of RNA synthesis, had no effect on acquisition or retention up to four hours after learning (BARONDES & JARVIK, 1964). Higher doses caused severe illness ten hours following injection, and death within 24 hours. Memory seen at four hours could be STM, and therefore not affected by RNA synthesis inhibition. Smaller doses had no effect on retention 24 hours after training (BARONDES & COHEN, 1967b; GOLDSMITH, 1867), but the level of inhibition of RNA achieved may have been too low to permit the conclusion that RNA is not required for LTM formation.

Using low doses, SQUIRE & BARONDES (1970) found retention deficits with injections prior to learning and also 24 hours after learning when testing was carried out one day following drug administration. When given one week later, actinomycin D did not produce retention deficits. Because the drug had an effect as long as 24 hours after training, it was concluded that the loss in memory was not due to a lack of RNA synthesis. CHEN (1970) using a more sensitive task, found little retention 24 hours following a low dose of actinomycin D given prior to training. Retention was howeve affected when the drug was given 30 minutes after training.

Studies by COHEN (1971) and AGRANOFF (1972) support the involvement of RNA synthesis in memory. However, NAKAJIMA (1969) reported that five days after a very small intracranial dose of actinomycin D, both acquisition and retention of a spatial task were impaired. There was evidence of abnormal cerebral electrical activity and extensive chromatolysis in the hippocampus in addition to other cerebral abnormalities (APPEL, 1965). Thus the behavioural effects of actinomycin D may be due to actions other than the suppression of RNA synthesis alone.

Actinomycin D differs fron 8-azaguanime in that it has no effect on acquisition of a task (BARONDES & JARVIK, 1964; COHEN & BARONDES, 1968b; SQUIRE & BARONDES, 1970). The toxic effect of doses of actinomycin D sufficiently large to cause marked inhibition of RNA synthesis, make interpretation of the actions of this drug difficult.

(c) Other nucleic acid antimetabolites.

2,6-diaminopurine and 5-iodouracil are thought to interfere with RNA synthesis and produce defective proteins whose functional specificity is lost. These drugs produced impaired retention of passive avoidance learning when given before learning and up to one but not two hours following learning. Evidence has failed to demonstrate conclusively that RNA synthesis is necessary for memory storage. Agents used in these experiments produce multiple effects making it difficult to obtain an adequate test of the hypothesis that RNA is involved in the formation of LTM.

(4) THE EFFECTS OF INCREASED RNA ON LEARNING.

(a) Facilitation of RNA synthesis.

CHAMBERLAIN et al (1963), found that the administration of trichloroaminopropene (TCAP), known to facilitate RNA synthesis, decreased consolidation time of hind limb asymmetry. Lower avoidance latency and higher numbers of avoidance responses were also found. Accelerated rates of consolidation as a consequence of chemical stimulation of RNA synthesis was also found by ESSMAN (1965; 1966).

Strychnine increases the concentration of brain RNA but not DNA. It also prolongs the action potential (AP), and produces repetitive spike discharge in isolated nerve cells (WASHIZU et al, 1961). Strychnine sulphate produces marked diminution in H-bonding of RNA in ribosomes of cortical slices (DATTA & GHOSH, 1964). Strychnine facilitated/

/facilitated consolidation whether administered before or after a learning experience (CARLINI & CARLINI, 1965). This may be mediated by an increase in RNA content of neurones owing to their increased rate of activity (AHMED & McKENZIE, 1963).

Magnesium pemoline which stimulated RNA polymerase activity in vivo (GLASKY & SIMON, 1966), enhances acquisition and retention of avoidance responses in rats (PLOTNIKOFF, 1966a). Animals treated with magnesium pemoline also relearned a CAR faster than controls, following disruption of conditioned performance by ECS administered immediately after training (PLOTNIKOFF, 1966b). These effects may reflect enhancement of consolidation.

Methylphenidate and metamphetamine administration did not affect gross operant behaviour, although they facilitated the acquisition of some instrumental responses (GOLUB & BRADY, 1965).

As the facilitation of learning by some drugs may be restricted to certain tasks only, this suggests a non-specific action on anxiety levels, rather than on mechanisms specifically related to learning and memory.

(b) Injection of RNA.

Aged people receiving large doses of RNA but not DNA were claimed to show a marked improvement in memory (CAMERON & SOLYON, 1961), involving almost total retention in some cases. The defects returned on termination of the RNA injections. However, the changes may also reflect alterations in metabolism due for example to improving/

/improving the nutritional state, and not due specifically to the effects on the brain RNA. It may also be that the constituents of the RNA, i.e. the nucleotides themselves, are the responsible agents.

Memory in rats was improved by injections of yeast RNA (KRAL & SVED, 1963). This was postulated to occur because more of the endogenous RNA was allowed to survive and accumulate, but more general effects on the brain may again be possible. COOK et al (1963) injected rats intraperitoneally with yeast RNA before learning sessions; these acquired a CAR more rapidly than controls. RNA injected animals also showed a significantly greater resistance to extinction of the response. Maze learning in rats showed similar facilitation after subcutaneous injection of yeast RNA (GORAN, 1965). CORSON & ENESCO (1966), found no enhancement using a different task. Thus it appears that the response required is a critical variable in the determination of the RNA effect.

SOLYOM (1965) injected rats systemically with 10% RNA solution. Naïve animals placed in a Skinner box with trained animals acquired a CAR significantly faster than controls. This data indicates that the ease of acquisition of new responses and the retrieval of stored information may be increased as the availability of RNA is increased. More general factors such as increased excitability or improved nutrition may however also be responsible for the observed effect of the RNA injection.

As with the regeneration experiments with planaria/

/planaria (see earlier this chapter), "cannibalism experiments", using the same experimental animal, do not provide any firm conclusions as to whether information about a specific learned stimulus response relationship has been transferred. Sensitisation to shock, light, or generally increased reactivity may also be responsible.

The possibility that substances carrying information were found in nervous tissue led to attempts to extract RNA from trained animals and injecting this into naive animals. Experimentally injected animals were found to respond more frequently to a conditioned stimulus than a control group (ZELMAN et al, 1963). BABICH et al (1965) injected RNA from trained rats intraperitoneally into naive rats. These then showed a greater approach tendency than controls receiving RNA from naive animals. GROSS & CAREY (1965) were unable to replicate these results but positive findings have been reported (FJERDINGSTAT et al, 1965; ROSENBLATT et al, 1966a, 1966c; UNGAR & COHEN, 1965; UNGAR & OCEGUERA-NAVARRO, 1965; UNGAR, 1966; JACOBSON et al, 1965; 1966; NISSEN et al, 1975; BABICH et al, 1965b; ESSMAN & LEHRER, 1966). These results could be attributed to general consequences of the injection such as metabolic influences, changes in overall reactivity to the stimulus, changes in excitability or other similar factors.

ALBERT (1966a; 1966b) using CSD and the removal of one hemisphere, found the retention of an avoidance response. This response was blocked when the remaining hemisphere was disturbed by the CSD. After ablation, these animals/

/animals failed to perform the CAR when tested with the remaining hemisphere functioning normally. If the removed tissue were homogenised and injected intraperitoneally, significant savings in learning were obtained on retraining. Injection of the other cortical regions did not have this effect. Thus the results were not due to general consequences of tissue injection, and the savings were specific to the previously learned task. It was also indicated that the results did not reflect general increases in excitability or other non-specific factors.

Furthermore, savings were not obtained after injection of homogenised medial cortex unless consolidation processes were permitted to continue for several hours immediately following the acquisition session. Chemical analysis of the excised tissue showed that the active constituent was probably RNA. Negative results were obtained when the substances were injected into animals other than the donor. This could have been due to the fact that the RNA unaccompanied by other essential compounds in the tissue.

REINIS (1965) made brains of trained animals radioactive with P^{32}. Radioactive brain homogenate was then injected into naive animals but no radioactivity could be detected in the injected brain tissue. The injected extract did not therefore seem to cross the blood-brain barrier (LUTTGES et al, 1966). ALBERT (1966c) cites evidence that large molecules can enter the brain/

/brain (SHERWIN et al, 1963) and also claims that the lesions made by injections would disturb the blood brain barrier so as to increase the ease of entry of injected substances into the brain (LAJTHA, 1962).

ROSENBLATT & MILLER (1966); ROSENBLATT et al (1966); UNGAR & IRWIN (1967) and UNGAR et al (1966) injected recipient rats with extracts from trained donors and found the best results with a soluble fraction not destroyed by tripsin. Purification of the transfer fraction suggested that it was neither RNA or protein, but a low molecular weight peptide, perhaps eight AAs in chain length (UNGAR et al, 1970). If the factor is of this chemical class the many of the biochemical objections to the transfer experiments may be modified.

CHAPTER IV.

PROTEIN BIOSYNTHESIS

(1) MECHANISMS.

Since the concept of protein synthesis inhibition is central to this Thesis, I shall include here a description of the current known mechanism of protein biosynthesis. I shall not describe any of the experiments which gave rise to the discovery of this scheme, but merely present the scheme as it finally came to be formulated (the scheme is represented diagrammatically in Fig. 10).

The specific structure of protein is determined by DNA, a long and linear molecule consisting of two mutually coiled polymer chains. The monomers of these chains are four types of deoxyribose nucleotides, the sequence of which along the chain is unique and specific for each DNA molecule, and each region of it. Different regions of the DNA molecule are responsible for the synthesis of different proteins. Thus one DNA molecule can determine the synthesis of a large number of functionally and chemically different proteins. A definite region of the DNA (cistron) is responsible for the synthesis of each type of protein.

DNA does not participate directly in the construction of proteins. A chemically-related polymer messenger RNA (mRNA) is first synthesised on the template DNA (transcription). mRNA is a single chain, the monomers of which are four types of nucleotide, slight modifications of the four deoxyribose nucleotides of DNA. The sequence of the four types of ribonucleotides of the mRNA exactly repeats/

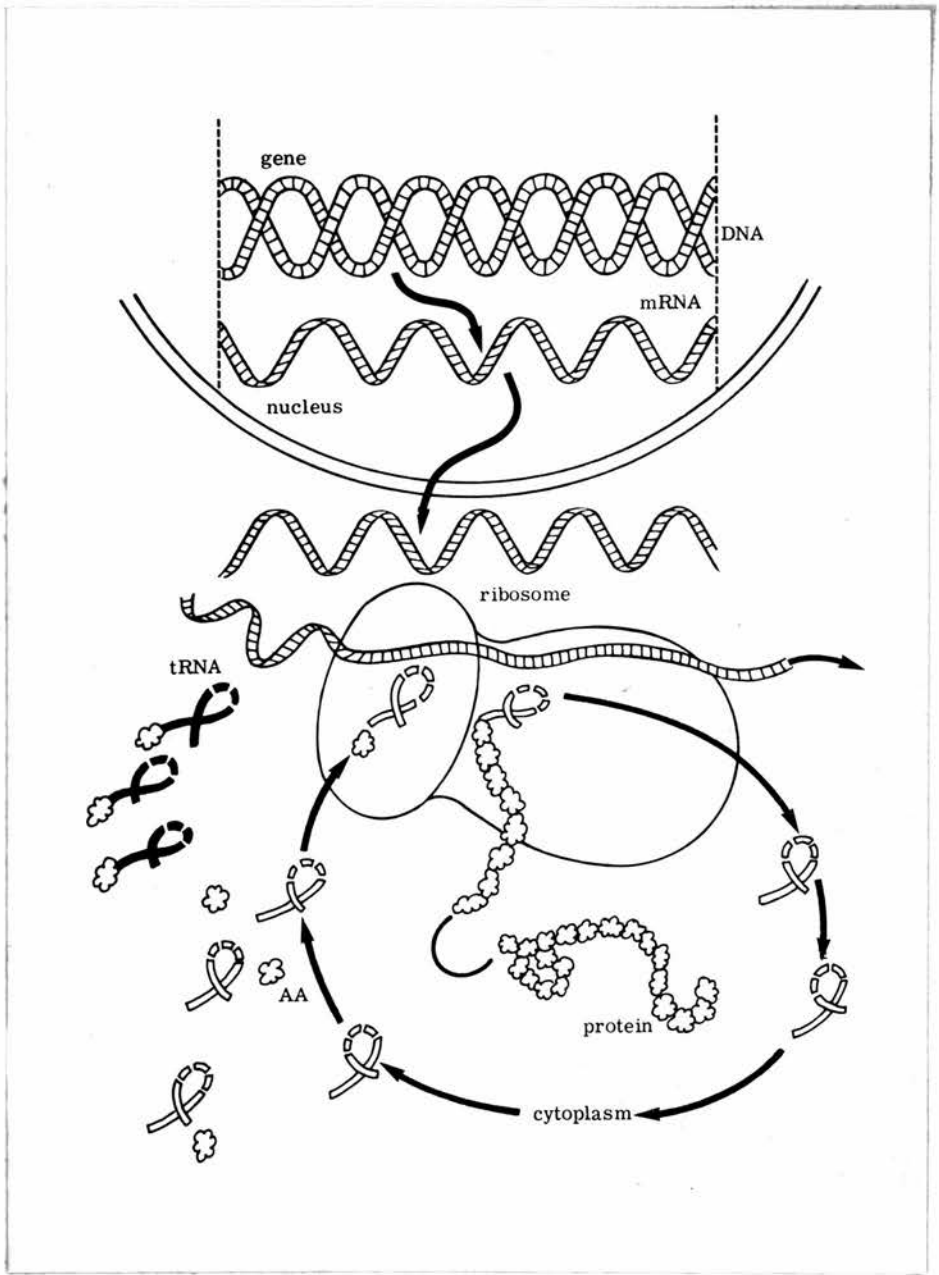


Fig. 10.

GENERAL SCHEME OF PROTEIN BIOSYNTHESIS.

Reproduced from "Molecular Biology, Biochemistry and Biophysics. No. 4. The Ribosome." Spirin, A.S. & Gavolova, C.P. (Eds.). New York, Heidelberg and Berlin: Springer Verlag. (1969).

repeats the sequence of the corresponding deoxyribonucleotides of the DNA. mRNA molecules which are thus multiple copies of the genes, are sent out through the cells, carrying the same information as the genes. These act directly with the protein-synthesising particles of the cell (ribosomes) in the construction of proteins.

For the synthesis of protein, free AAs present in the cell are brought into a suitable flow directed to the ribosome and there assembled into a chain in a definite, unique way, directed by mRNA. AAs are added to the ends of small chains of adaptor or transfer RNA (tRNA), one AA per tRNA. Each AA, of which there are 20 different kinds, has its own tRNA, and in this way AAs are delivered directly to the ribosomes.

The central factor in protein biosynthesis is the merging of the flow of information and the flow of raw materials in the ribosome. The ribosome contains ribosomal RNA (rRNA), and structural ribosomal protein. They can "read" the information coded on the mRNA chain, and release it as a protein molecule with a specific structure. Thus the linear assembly of the 20 types of AA in the protein chain is determined by the arrangement of the four types of nucleotides in the mRNA, (Translation). The ribosome is the minimum biological particle within which the requisite organisation of all stages of synthesis is carried out.

Coding of information (the genetic code).

There are 20 different AAs but only four different nucleotides. Each AA has its own triplet of nucleotides (CRICK et al, 1961; CRICK, 1963). The establishment of the nucleotide composition and sequence of triplets for all 20 AAs has been determined (SPEYER et al, 1962; MORGAN et al, 1966; WHITMAN & WHITMAN-LIEBOLD, 1963; YANOFSKY, 1963; BRENNER et al, 1965). Most AAs are coded by more than one triplet (codon).

Storage and replication of the coded material.

DNA has a limited localisation in the nucleus of cells of higher organisms, and there is a genetically autonomous system within a single cell. WATSON & CRICK (1953a; 1953b) proposed a model for the molecular structure of DNA whereby two polynucleotide chains are joined together through the interaction of their opposite nucleotides which are sterically "complementary". This is possible only between nucleotides A and T, and G and C, so a sequence of four types of nucleotide in one chain of the DNA molecule will determine the nucleotide sequence in the second. Synthesis of new DNA molecules occurs only on the basis of the DNA already present. The two chains of the initial molecule separate and a second chain is assembled on each of the separated single stranded sections, in exact correspondence to the principle of "complementariness". Two DNA molecules identical with the original appear in place of the original.

Replication requires the enzyme DNA polymerase (ESSMAN et al, 1968; LEHAMNN, 1963). This enzyme brings about the separation of the two chains which occur successively from one end of the DNA molecule to the other, with simultaneous polymerisation of free nucleotides, according to the principle of complementariness.

Transfer of information (transcription).

mRNA is synthesised directly using the corresponding section of the DNA template (WEISS & NAKAMOTO, 1961; GEIDUSCHEK et al, 1961; HURBITZ et al, 1962; HURVITZ & AUGUST, 1963). The mRNA chain produces copies of the nucleotide sequence of the parent DNA adhering to the complementariness principle. An enzyme, DNA polymerase, is involved in the resulting transcription of information from the DNA to mRNA. The template DNA remains unaltered, and is always ready for the transcription of mRNAs from it. The flow of mRNA from DNA represents the flow of information which provides the entire programming of protein synthesis.

Involvement of AAs in protein synthesis.

The principal problems in biosynthesis are:-

(a) the energy provision of the process

(b) the primary "recognition" by AAs of the nucleotide combinations corresponding to them.

These problems are solved by the joining of AAs with tRNA (HOAGLAND et al, 1957; 1958; OGATA et al, 1957; HOAGLAND, 1960). Firstly there is energetic activation of AA by enzymic reaction with ATP. The activated aminoacyl combines with the end of tRNA. The increase in the/

/the chemical energy content of the activated AA is conserved in the form of a chemical bond between the AA and the tRNA. This free energy is sufficient for the subsequent formation of peptide bonds between the AAs in the construction of proteins.

The enzyme, amono acyl tRNA synthetase, brings about the reaction between the AA and tRNA. There is one type of specific enzyme for each of the 20 AAs. Each of the enzymes can bring about a reaction only with those tRNAs that carry a strictly defined combination of nucleotides in their chain. These are different for each enzyme. Synthesis of protein on the ribosome, translation.

The energising of AAs on the tRNA, before they are delivered to the ribosomes, make it possible for the tRNAs to interact with mRNA triplets during translation.

The ribosome is first programmed with mRNA. At a given moment, only a short section of the mRNA chain is on the ribosome, and this can interact with the tRNA. Again the principle of complementariness is involved. If a section of mRNA situated in the corresponding ribosomal site has a specific nucleotide sequence, then a chain of tRNA carrying a complementary triplet, is anatomically attached. Since a tRNA with this triplet can carry only a specific AA, the mRNA triplet determines delivery into the ribosomes and bonding according to complementariness.

In addition to the tRNA molecule charged with AA, there is another tRNA with the end of its molecule connected to the end of the growing peptide chain. Considering/

/Considering a protein chain which is already being constructed with tRNA bonded to it, the new tRNA charged with its AA enters the ribosome and is attached to the triplet. Attachment of tRNA to the triplet at a given site on the ribosome causes a covalent peptide bond to arise between them and joins the protein chain under construction with the AA residue of the arriving chain and the chain is lengthened by one AA, which is connected to the other terminal of the tRNA. After this the two ends of the tRNA are interchanged. The previous molecule of tRNA is free, is then displaced and leaves the ribosome. The new tRNA, charged with the protein chain under construction takes its place, and the mRNA chain will be shifted relative to the ribosome by one triplet, thus exposing the previously occupied triplet for attachment to commence once more.

A sequential triplet after triplet passing of the mRNA chain through the ribosome is accomplished resulting in the mRNA chain being read completely by the ribosome. Coupled with this is the sequential AA after AA build up of the protein chain. Correspondingly, tRNA molecules charged with AAs enter the ribosome one after another and tRNA molecules without AAs leave the ribosome. In solution outside the ribosome, the free tRNA molecules again combine with AAs and carry them into the ribosome, themselves returning in a cyclic manner without breakdown or change.

(2) CIRCADIAN SYSTEMS OF SYNTHESIS.

I should like now to show that biological rhythms, and therefore probably the cyclic appearance of REM sleep, may also be regulated by the synthesis of protein.

Mechanisms responsible for programming biological rhythms, i.e. the timimg mechanism, may be distinguished from the system responsible for accepting external signals from the phase shifting e.g. light, stimulus. The timing mechanism is probably the biological clock per se. GOODWIN (1963) has suggected a biochemical model using bacteria, and has indicated that the mechanism may reside in a long feedback loop involving macromolecular biosynthesis. The rhythms can be abolished in bacteria using puromycin, and may be regained by washing out this inhibitor. The resulting phase shifting is then synchronous with a control uninhibited culture, indicating that a feedback loop does exist.

Temporal regulation at the biochemical level is not restricted to circadian systems. In embryological development, for example, a compli-vated series of biochemical events follow each other in a well ordered way. A hypothesis for the dependent activation in developmental processes

is that which assumes sequential activation of genes. Specific mRNA is formed in each gene and this possesses a lifetime appropriate to its function and to the time scale involved. Stability of mRNA can differ greatly in different systems, and stability would dictate the time interval during which a protein would be synthesised by the cell. This stability corresponds with gene activation and constitutes a timing mechanism. The only verified role for mRNA is the production of protein. It is hypothesised that the sequence derives from a linear ordering on the DNA molecule and that the readout mechanism proceeds along the DNA molecule forming a feedback loop.

Circadian systems exhibit similarity to differentiating cells in the sense that there is daily differentiation. In the embryological situation the activity is restricted to a specified sequence of expression. In the circadian cycle there is temporal control of activity. There is a ssumed to be turnover or instability of some sort which is inherent in the system.

A number of inhibitors of macromolecular synthesis have been found to have an effect on rhythmicity in bacterial cells. KURAKASIAN & HASTINGS (1962; 1973) found that actinomycin D had a pronounded effect on the slow rhythm in the insect Gonyaulax, suggesting the involvement of mRNA in the clock mechanisms. Low concentrations are effective suggesting that the effect is highly specific.

If the newly synthesised RNA, whose inhibition blocks the rhythm, normally functions as a messenger for the synthesis of protein controlling the rhythm, then similar effects should be observed with inhibitors of protein synthesis such as puromycin and chloramphenicol. Puromycin has been found to block the rhythm without any lag. Chloramphenicol administration resulted in a marked increase in amplitude of rhythm. Chloramphenicol should result in an RNA accummulation, and since the newly synthesised RNA may function not only as a messenger but also as a control RNA in another biochemical system, then the puromycin effect could be interpreted in terms of its ability to mimic this functional RNA or to stimulate its breakdown.

If a clock function relates to newly synthesised RNA, then inhibition or new DNA synthesis should not disturb the rhythm. Rhythms continue in starved cultures where no new DNA synthesis occurs, and this supports the conclusion concerning the role of RNA in rhythmicity. In addition, both ametophorin and norobiocin inhibit DNA/

/DNA synthesis, but do not inhibit rhythmicity. The delayed inhibitory effect found when mitocin C is used to inhibit DNA synthesis, is attributed to the slow breakdown of DNA in the presence of this inhibitor making it no longer available as a primer in RNA synthesis.

In many higher organisms, locomotor output is used as a measure of the properties of biological clocks. Such output is controlled by the CNS since spinal cord neurones innervate limb muscles. Such motoneurones are in turn under the control of the descending axons of neurones situated in the midbrain or cortex. STRUMWASSER (1969) has demonstrated a circadian rhythm within a single neurone. He used the parietovisceral ganglion (PVG) of Aplysia calafornica, the sea hare, and has outlined a possible model to account for the circadian oscillations of spike output in this neurone. From the evidence that actinomycin D selectively binds to DNA (REICH & GOLDBERG, 1964), STRUMWASSER proposed two phases in the normal circadian cycle of the PVG.

(1) mRNA units are produced from DNA templates in the nucleus

(2) mRNAs are released from the DNA and act in the cytoplasm as instructional units for the synthesis of polypeptides and proteins.

When actinomycin D binds to the DNA, it displaces these mRNA units thus prematurely releasing the intact message. The messenger units initiate the production of a substance in the cytoplasm, which depolarises the neuronal membrane/

/membrane causing spike discharge.

In the dark sensitive period of the Aplysia cells, most of the message is already produced (STRUMWASSER, 1965). Its premature release with the administration of actinomycin D causes a phase advance of a complete circadian activity peak. A few hours after the normal occurrence of the activity peak, new messenger units are in production. Premature release of message at this time causes a weak effect since only a small amount of the nuclear message units are available to the synthesising system in the cytoplasm.

Thus it can be seen that the mechanism of DNA, RNA and protein synthesis of neuronal cell is intimately concerned with the production and regulation of circadian rhythms in neurones and single cell bacteria.

The time of occurrence of REM periods also determined by a circadian rhythm (ROFFWARG, 1966). It is therefore not untenable that protein synthesis is also instrumental in causing and maintaining REM period occurrence. It should be possible therefore to disrupt synthesis without disturbing other ongoing activities within the REM period. The observation that the second REM period of the night is lengthened when a protein synthesis inhibitor was given (OSWALD, unpubl.) could be consistent with the idea that the protein synthesising apparatus would attempt to compensate for the inhibition, by having more REM sleep and therefore increasing the possibility of the resumption of protein synthesis.

Conversely when REM deprivation takes place, the effects of protein synthesis disruption on memory may not be immediately apparent, as there would be a time lag between the learning experinece and the time taken for the modification of synapses or for the completion of specific connections synthesised for that experience. In the meantime, the experiences could still be in the short term store. Alternatively the circadian mecanisms involved in the occurrence of REM sleep, and the circadian effect of protein synthesis for LTM formation operate by separate mechanisms occurring synchronously, directed by the same synthesising apparatus. Even though these might occur at the same time, there may or may not be any relationship between the outcomes of these separate processes. The relationship is represented diagrammatically in Fig. 10a.

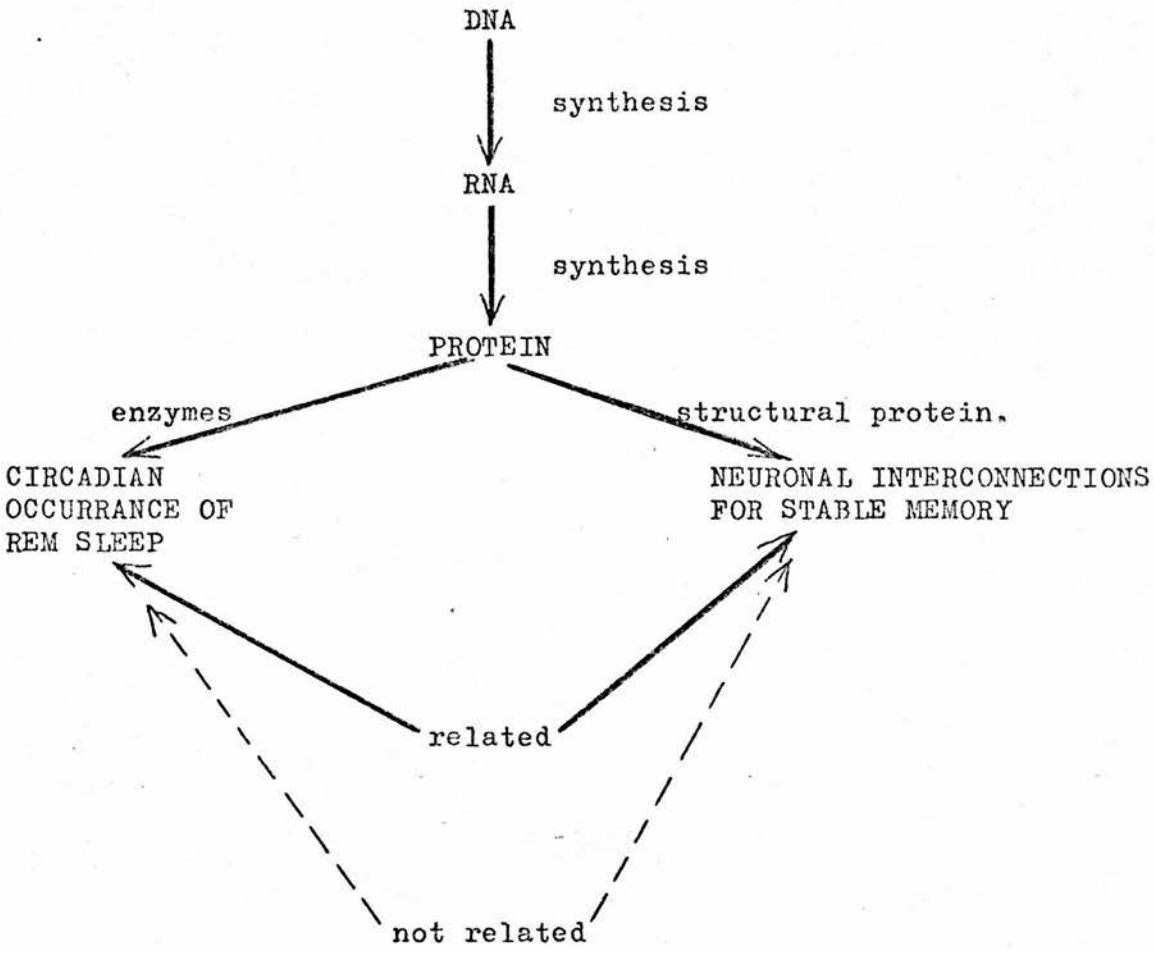


Fig. 10a.

A POSSIBLE RELATIONSHIP BETWEEN THE CIRCADIAN OCCURRANCE OF REM SLEEP AND THE SYNTHESIS OF PROTEIN FOR STABLE MEMORY FORMATION.

Chapter V.

TRANSMITTERS.

Since many of the drugs administered as protein synthesis inhibitors also have an effect on transmitters and since, in addition, there may be a relationship between protein synthesis and transmitters, I shall include a brief chapter on the involvement of transmitters in memory and in sleep.

(1) TRANSMITTERS AND MEMORY.

Following a learning experience there is a graded change in the level of transmitter released at a synapse during transmission. The level of ACh is higher immediately after learning, during STM storage, it then rapidly declines to a low point, from which it slowly increases to a new, higher level underlying stable memory storage (WEINER, 1970).

(a) anticholinesterase drugs.

Di-iso-propyl-flurophosphate (DFT) and physostigmime (PS) inacivate cholinesterase and so prevent the destruction of ACh. In sub-maximal dosage these druge inactivate not all, but only a part of the cholinesterase present, and hence only slow down but do not stop the destruction of ACh. The effect of sub-maximal levels of AChE is to increase the lifetime of any ACh emitted into the synapse and to increase ACh concentrations resulting from a greater rate of emission. Up to a certain level, the greater this concentration the greater is the efficiency of transmission i.e. conduction across the synapse. Above that level, which is set by the sensitivity of the post synaptic

membrane, any further increase in the ACh concentration produces a synaptic block (GOODMAN & GILMAN, 1965). Thus the application of a given dosage of anticholinesterase will (by protecting ACh from destruction) have different effects on the efficiency of synaptic conduction depending on the rate of ACh emission during transmission and on the sensitivity of the post synaptic membrane. At low levels of emission of ACh or low sensitivity of the post synaptic membrane, application of anticholinesterase will make transmission more efficient.

If there are changes with time following learning in the level of ACh emitted at the modified synapse, then such a synapse should show either facilitation or block, depending on the time following learning when the same dose of anticholinesterase is injected. A similar arguement may be applied if it is assumed that instead of a pre synaptic increment in transmitter, it is the post synaptic membrane which becomes more sensitive to transmitter as a function of time following learning. The use of anticholinesterase drugs does not allow a decision as to which of these alternatives operates in the learning situation.

DEUTSCH et al (1966) and DEUTSCH & LEIBOVITZ (1966) showed that facilitation or block of a memory could be obtained with the same dose of anticholinesterase simply as a function of the time of injection following the original learning. Anticholinesteases, if administered just before test trials have little or no effect soon after learning but cause a deficit in relearning as the synaptic conduction improves with time (DEUTSCH et al, 1966; HAMBERG, 1967;

SQUIRE et al, 1971).

Altering the task difficulty, the amount of training, or spacing of the learning trails results in a different amount of learning and a different synaptic conduction. The effects of DFP with untrained habits, difficult tasks or spaced trials, facilitates the relearning at a time when over-training, simple tasks or massed trials are impaired (DEUTSCH, 1966; 1969; DEUTSCH & LEIBOVITZ, 1966; DEUTSCH & LUTSKY, 1967). The amnesic DFP effect is temporary, diminishing with the time of injection to test interval, and this effect is in agreement with the hypothesis that amnesia is due to synaptic block.

The puromycin effect on memory could also be due to an altered ACh/ChE balance, since it has been shown that puromycin affects this balance (BURKHALTER, 1963).

(b) anticholinergic drugs.

By reducing the quantity of transmitter which can combine with receptors, these drugs exert most of their effects when synaptic conduction is low, and they have no effect when it is high. Anticholinergic drugs produce a deficit in retention. These results support the hypothesis of DEUTSCH (1971) that the basis of memory is a synaptic change. These drugs however, may not have a specific effect on the cortical neurons recently involved in memory since they must have an effect on the total population of cortical neurons. Anticholinergic drugs tend

/to have differentiated effects related to dose, and WEISS & HELLER (1969) criticised the DEUTSCH experiments for the use of a single dose only. Varied doses of PS amd scopolamine produce normal dose responses in relation to retention (SQUIRE et al, 1971). The drugs may also produce state dependent learning (BERGER & STEIN, 1969) or there may be an effect on acquisition (MEYERS, 1965; STARK, 1967), suggesting that the drugs are affecting performance.

Correlations between specific memory functions and substances having as general a distribution as ACh are speculative, since these substances are also involved in the nature of the nerve impulses. A distinction between central and peripheral effects is also important, as are the side effects of these drugs (SILVER, 1967; 1971; KASA, 1971).

(c) Catecholamines and 5HT.

Catecholamine systems might be used in learning either as reinforcement mechanisms or to modify neuronal metabolism (KETY, 1970). Compounds having an effect on mood in man, also have an effect in animals of altering the levels of biogenic amines. Agents such as ECS, which disrupt memory, increase levels of 5HT and catecholamines in the brain. Chlorpromazine had no effect on retention when given 30 minutes before training (MADDEN & GREENOUGH, 1972). Amphetamine produces behavioural excitement (DISMUKES & RAKE, 1972), and counteracts the amnesic effect of scopolamine (PAZZAGLI & PEPPEN, 1964).

Reserpine, which depletes catecholamine containing neurones, produces a loss in savings on retention, when given one minute but not 24 hours after learning, an effect which was counteracted by giving excess DOPA (DISMURKES & RAKE, 1972). DOPA alone enhanced acquisition in fish, but when pargyline (prevents NA breakdown) is used, response acquired under the drugs were not apparent on retest (STAHL et al, 1971). Inhibition of NA synthesis by diethyldithiocarbamate (DDC), given 30 minutes before or immediately after learning, impaired retention at 24 hours (RANT et al, 1971). DDC has many side effects on activity. DDC also antagonises the effect of AXM, suggesting that AXM could act by reducing amine levels rather than blocking protein synthesis (FLEXNER & FLEXNER, 1970b; ROBERTS et al, 1970; SEROTA et al, 1972).

As with ACh, the true physiological role of the catecholamines in the CNS is not conclusively defined. Adrenergic neurones in the cortex and hypothalamus may innervate small arterioles, and this may be their sole function (HARTMANN et al, 1972). Agents that interfere with amine metabolism may exert their action entirely through brain microcirculation. Even if a neuronal system using identified transmitter is uniquely involved in memory, it may be extremely difficult to alter such a system by drug action. In peripheral synapses, procedures which liberate excess transmitter are as liable to block by desensitisation, as to potentiate transmission, in all but a very narrow dose range. Agents chronically/

/chronically blocking transmitter release affect the sensitivity of the post synaptic membrane. Also, at peripheral adrenergic and cholinergic synapses, the release of transmitter may affect the release of the same or different transmitters, from the same or adjacent presynaptic terminals. Thus the release of NA may inhibit further release from the same terminals (ENERO et al, 1972). ACh may facilitate or inhibit the release of NA (MUSCHOLL, 1970), and NA may facilitate or inhibit the release of ACh (CHRIST & NISHI, 1971).

Higher catecholamine levels are generally associated with increased performance and low levels with decreased performance (SEIDEN & PETERSON, 1968; FUXE et al, 1971; HARTMANN, 1970). This is often seen in, but not restricted to, conditions of sleep deprivation or states of reduced cortical functioning (WEISS & LATIES, 1962). Increased catecholamine levels facilitate new learning or STM (DOTY & DOTY, 1966; KULKARNI, 1968; LATZ et al, 1967; STEIN, 1965). NA is probably involved in the registration of information and STM, as well as the consolidation of LTM (OLIVERIO, 1965).

Human studies suggest that amphetamines and related compounds produce increased motor co-ordination and psychomotor performance. The well known effects of amphetamines suggest that catecholamines are involved in functions such as vigilance or directed attention as well as arousal or counteraction of fatigue. In self stimulation studies with rats, catecholamine/

/catecholamine containing neurones in the medial forebrain bundle may be involved not only in positive reinforcement learning, but also in avoidance learning (STEIN, 1965; 1967).

There may be several separate, but closely related effects and behaviours subserved by brain catecholamines: a tendency to optimistic or euphoric mood and to increased energy and motivation, motor co-ordination, new learning and STM especially in reward systems and motivated learning, vigilance, attention, and task orientation. Catecholamines therefore play a part in integrated adaptive functions during waking.

(2) TRANSMITTERS AND SLEEP.

Groups of neurones containing monoamines have been shown to correspond to systems involved in sleep. There has been much investigation of these to attempt a biological explanation of the REM rebound. The monoamines, NA, DA and 5HT do not cross the blood brain barrier and most of the drugs used to alter one of the amines interfere in some way with the metabolism of the others.

Brain 5HT may be increased by injecting 5HTP, or the catabolism of 5HT may be inhibited by use of an MAOI. Increasing 5HT in the cat produces an increase in NREM sleep with an accompanying decrease in REM sleep (JOUVET, 1969). The natural dietary precursor of 5HT, L-tryptophan, has been found to produce no changes in %SWS of normal/

/normal subjects (HARTMANN, 1970) and also to increase the %SWS (WILLIAMS et al, 1969; ZARCONE et al, 1968a, 1968b). REM sleep may be decreased (CAZULLO et al, 1969), increased (HARTMANN, 1967b; ZARCONE et al, 1968a; 1968b; WILLIAMS et al, 1969) and remain unchanged (HARTMANN, 1970b).

PCPA inhibits the enzyme tryptophan hydroxylase (KOE & WEISSMAN, 1966), and may be used to selectively decrease 5HT without interfering with NA or DA metabolism. PCPA has been administered to the cat (DELORMÉ et al, 1966; KOELLA et al, 1968; DEMENT et al, 1968), the rat (RECHTSCHAFFEN et al, 1969), the rabbit (FLORIA et al, 1968) and monkeys (WEITZMAN et al, 1968). In all animal studies NREM sleep was markedly decreased or totally absent and REM sleep was increased with the exception of monkeys. In man, however, PCPA administration produced a decrease in REM sleep without appreciably affecting NREM sleep (WYATT, 1972). The apparent differences in response to PCPA administration between humans and animals might be due to different drug dosage, since in humans the dose was never greater than 50 mg/kg whereas in animals, the dose administered was between 50 and 100mg/kg. PCPA has also been found to lower spinal fluid 5HIAA (the principle metabolite of serotonin) concentrations. Concentration of this metabolite presumably reflects the general nervous system concentration of 5HT (GUILDBERG & YATES, 1969). The blocking action of PCPA can be antagonised by injection of 5HTP (JOUVET, 1969).

It therefore appears that 5HT may have an important role in both REM and NREM sleep.

Neurones containing 5HT are almost completely located in the raphe system. Those containing NA are more widespread, and DA containing neurones are located almost exclusively in the ventral part of the mesencephalon. Eight to ten days after destruction of monoamine cell bodies, 5HT and NA disappear completely from terminals. The content of 5HT or NA in the brain can be altered selectively by destroying neurones containing these substances (DAHLSTROM & FUXE, 1964). Following a subcortical 80-90% destruction of the raphe system, a state of permanent insomnia is apparent for three to four days. There then followed a period where no REM sleep occurred and NREM sleep was less than 10% of the recorded time. Partial lesions in the Raphe system resulted in a less pronounced insomnia and there was a significant correlation between the extent of the raphe system destruction and the percentage of sleep (JOUVET, 1969).

JOUVET (1969) proposed that the adrenergic system is in part responsible for the production of the tonic phenomena (low voltage EEG and muscle inhibition, but not PGO spikes or eye movements) associated with REM sleep. Alphamethyl para tyrosine (AMPT) impairs NA synthesis (TORCHIANA et al, 1970). MARANTZ & RECHTSCHAFFEN (1967) reported that AMPT administration produced no sleep changes in rats and did not prevent a REM rebound in REM deprived/

/deprived rats (MARANTZ et al, 1968). NREM sleep in rats was increased (BRANCHEY & KISSIN, 1970). When given to cats, AMPT was found to produce initial sedation associated with an increase in REM (KING 1971). Another study, however, showed an initial decrease in REM which was followed by a large REM increase (ISKANDER & KAEBLING, 1970). AMPT decreased REM sleep in the monkey CROWLEY ET AL, 1969; WEITZMAN et al, 1969).

In experiments using human patients, AMPT administration increased REM sleep, with a concomitant daytime sedation during treatment (WYATT, 1972). When treatment was discontinued, there was a period of insomnia with decreases in REM sleep lasting considerably longer than decreases in NREM sleep.

At low dose levels, administration of L-DOPA, a precursor of NA and DA, to humans caused a decrease in REM sleep in the early part of the night with a subsequent rebound in the second half of the night. At moderate dosages, REM sleep was decreased for the whole night, and at high doses both REM and NREM sleep were reduced. Thus it appears that in man, high brain catecholamine concentrations decrease REM sleep, while lowering catecholamine concentrations promotes REM sleep (WYATT, 1972).

Bilateral destruction of NA containing neurones located in the locus coeroeleus impaired REM but not NREM sleep and therefore JOUVET (1969) postulated that the triggering structures for NREM sleep was independent from those involved in REM. Results of the human/

/human studies however indicate that the adrenergic system interacts with the serotonergic system to produce REM sleep (WYATT, 1972). There is evidence that adrenergic substances can modify brain 5HT metabolism (NIG et al, 1970; EVERETT & BORCHERDING, 1970) and serotonin may then augment REM sleep and an adrenergic substance may reduce it.

NREM sleep must constitute at least 15% of the night before REM appears. This is usually between 60 and 90 minutes in humans (OSWALD, 1969). Since there is some correlation between NREM sleep and 5HT, REM sleep could be triggered by first priming the 5HT mechanism, which acts through the deaminated metabolites on the cholinergic mechanisms. These in turn, may trigger the final NA mechanisms of REM sleep (JOUVET, 1967).

Turnover of tritiated NA is high during both REM deprivation and recovery following deprivation (PUJOL et al, 1968; SCHILDKRAUT & HARTMANN, 1972). The retained higher levels during recovery periods, suggest that synthesis rates exceed utilisation, but this may be a non specific effect due to stress (HARTMANN, 1973). Tyrosine hydroxylase is the rate limiting enzyme in catecholamine synthesis and there were decreases in activity of this enzyme as a result of REM deprivation and a small increase in activity of the enzyme in recovery from the deprivation (HARTMANN & POPPER, 1972).

REM deprived animals show a deficit in some waking functions requiring catecholaminergic neural systems/

/systems and these can be reversed at least temporarily, by increasing available catecholamines (STEIN & WISE, 1969; HARTMANN & STERN, 1972).

Administration of AMPT and 6 hydroxydopamine caused REM sleep time to be increased at the expense of waking time, whereas NREM time remained unchanged. Catecholamines may therefore have a role in regulating the balance between REM sleep and waking (HARTMANN, 1973). This could be important as there are clear neurophysiological similarities between REM sleep and waking. In HARTMANN'S theory, the waking state is characterised by excitation, learning and memory formation, and in REM sleep there is a reappraisal or re-arousal of the same areas, pathways and mechanisms, though why this occurs is not clear.

The functioning of the catecholamine system could be improved or restored especially during REM sleep, and HARTMANN suggests some ways in which this could be accomplished:-

(a) the synthesis of catecholamines in relevant neurones could be increased. Tyrosine hydroxylase could be involved, especially since it has been shown to be responsible to stress and to drugs such as reserpine.

(b) catecholamines at brain synapses could be rendered more active by preventing re-uptake into cells; stimulation of release; or preventing enzymatic catabolism of the amines.

(c) receptors for catecholamines are less sensitive during the day and are restored during REM sleep.

Presynaptic axonal endings with their storage vesicles or tubules used in channelling catecholamines to endings might also be restored.

All these mechanisms suggest structural restoration and would involve the laying down of new structural as well as new enzymic protein. HARTMANN hypothesised a relationship between the known data on catecholamine effects on sleep, and catecholine effects on memory and learning. Thus REM sleep can be conceptualised from the psychological point of view, as having a role in the restoration of mind or brain after new learning or difficult, stressful and emotional experiences.

On a physiological level, this may occur after intense stimulation of the reticular activating system, and on decrease in functional brain catecholamines. If there have been emotionally and intellectually demanding experiences during the daytime waking activity, this would produce much activity in the cortex and higher brain centres, which would also be a result of activity in the reticular activating system. Catecholamine synapses especially in the cortex might then become depleted. Sleep and in particular REM sleep might therefore provide recuperation for those catecholamine dependent systems which are involved in learning, memory, attention and emotional equilibrium during waking.

CHAPTER VI.

BIOCHEMICAL CONSIDERATIONS IN STABLE MEMORY FORMATION II: THE PROTEINS.

The mechanism of protein biosynthesis has been described in Chapter IV. Experiments involving measurements of the rates of incorporation of labelled AAs into protein have also been described in Chapter II. The overall rate of protein synthesis was found to be greater in young animals than in adults, and the incorporation of AAs into protein is more active in the cerebral grey matter than the white, being most active in the cell bodies of nerve cells (LAJTHA et al, 1957), although different proteins differ widely in their turnover rates. Rearing animals in different environments has shown that exposure to sensory stimulation increased the overall weight and protein content of the brains of these animals when compared to controls (see Chapter II).

Electrical stimulation causes changes in chemical properties of individual proteins in the brain and nerve cells. There can be partial denaturation of brain proteins and an increase in the proportion of protein bound - SH groups. The staining properties of the proteins were found unchanged (UNGAR & ROMANO, 1963; FISCHER et al, 1961; FREUNDL et al, 1964). Prolonged physical exercise causes small decreases in protein bound amide groups in rat brain (VRBA & FOLBERGROVA, 1959) and peripheral nerve JACOUBEK et al, 1963). Stimulation by administration of camphor increases the incorporation of P^{32} into brain phosphoporteins (VLADMIROV, 1953;/

/(VLADMIROV, 1953; HEALD, 1959). The phosphoproteins of nerve cells are located mainly in cell membranes and they may be concerned with the transport of electrolytes across the cell membrane. Studies of protein metabolism in peripheral nerve have shown that axoplasm generated in the cell body of the neurone passes into the axon where it moves outwards at a rate of about 1 mm. per day.

Stimulation causes the release of transmitter substances and an increased energy requirement associated with the rapid reversible changes in a number of labile metabolites. The overall rate of protein turnover is not greatly affected by stimulation in acute experiments, but prolonged stimulation appears to increase the rate of generation of axoplasm as it affects the rate of incorporation of metabolites into some of the structural elements of the nerve cell. There is some evidence from chronic experiments that sensory stimulation can increase the total quantity of structural and other proteins in rat brain.

HYDÉN'S experiments have shown that changes do occur in RNA and proteins of the nerve cell bodies although these changes are not completely specific to learning.

(1) INHIBITION OF MEMORY FORMATION BY DRUGS AFFECTING PROTEIN SYNTHESIS.

(a) Puromycin.

(a) Puromycin.

Puromycin is an antibiotic capable of inhibiting protein synthesis (DARKEN, 1964; YARMOLINSKY & DE LA HABA, 1969). Puromycin structurally resembles the amino acyl terminal of the tRNA and acts by mimicking the naturally occurring charged tRNA. It becomes incorporated into the carboxyl ends of the growing polypeptide chains and causes them to become prematurely released from the ribosomes as peptidylpuromycin (ALLEN & ZAMECNIC, 1962; NATHANS, 1964), all bearing a puromycin residue instead of a carboxyl terminal (WILLIAMSON & SCWEET, 1965). This leads to a breakdown of polysomes and an average decrease in polysome size (VILLA-TREVINO et al, 1964).

Other known effects of puromycin on the cell are:-

(a) a lowering of the resting potential, indicating changes in the membrane (STRUMWASSER, 1968).

(b) an effect on the ultrastructure of brain cells (GAMBETTI et al, 1968a).

(c) mitochondrial abnormalities (GAMBETTI et al, 1968b)

(d) depression of cholinesterase activity and synthesis (BURKHALTER et al, 1963).

(e) direct inhibition of 3'5' cyclic AMP phosphodiase, the enzyme which degrades cyclic AMP. Activation of phosphorylase is thereby affected (APPLEMAN & KEMP, 1966).

(f) intracerebral injection inhibits the incorporation of H^3 sphingosine into gangliosides by 73% within three hours, whilst incorporation into sphingomyelin is/

/is increased (KAFNER et al, 1967).

(g) direct action on glycogen synthetase (SOVIK, 1967).

Puromycin has no effect on STM in mice and goldfish. This is taken to indicate that protein synthesis plays no role in the STM formation.

The first studies showing quantifiable inhibition of protein synthesis were carried out using mice (FLEXNER et al, 1962). Subcutaneous injections of puromycin were given, producing 83% of protein synthesis inhibition, for several hours. This had no demonstrable effect when injected either before or after learning.

Intracerebral injections were found to produce complete amnesia when injected bilaterally into the hippocampus, ventricular and frontal regions, or only into the temporal regions of mouse brain, 24 hours after training. Frontal and/or ventricular injections had no disruptive effect. When the injection time was delayed beyond three days, all areas had to be injected simultaneously to disrupt a learned response. It was concluded that the hippocampal zone was the site of recent memory storage and this locus then spread extensively in the neocortex which was concerned in the longer term storage.

The loss in memory required from 12 to 20 hours to develop, but once established it persisted for at least three months. Overtraining protected the response from puromycin (FLEXNER et al, 1967). Puromycin affected only recent learning and when injected after/

/after reversal training, the original learning remained intact. Therefore puromycin did not solely produce an inability to perform (FLEXNER et al. 1963). The puromycin effect was dependent on the dose and hence the degree of protein synthesis inhibition (FLEXNER et al, 1965). Substances structurally related to puromycin but without protein synthesis inhibition properties e.g. hydrolysed puromycin and the aminonucleoside of puromycin, were without effect (FLEXNER et al, 1965).

Acetoxycycloheximide (AXM), which alone in this situation had no effect on memory, protected the memory against the puromycin-induced retention deficit (RD) when both drugs were administered together (FLEXNER & FLEXNER, 1966) by protecting against the formation of the abnormal peptidyl-puromycin. mRNA was preserved and could resume synthesis of new protein after the drug level had fallen. However, puromycin injected with AXM in goldfish induced a greater amnesia than other drugs alone (LIM et al, 1970).

Memory deficits seemed to be due to the formation of these abnormal peptides which survived in the brain for at least eight weeks post intracerebral injection as demonstrated with tritiated puromycin (FLEXNER & FLEXNER, 1968a). The period in which the peptidyl-puromycin concentration reached its peak, coincided with the decay in responding after injection.

There was no amnesia effect when puromycin was/

/was neutralised with bases of cations other than N^{+}_{a} e.g. K^{+}, Li^{+}, Ca^{++} and Mg^{++} (FLEXNER & FLEXNER, 1969b). These cations are thought to bind to the anionic sites of the neuronal membrane and thereby protect from the disruptive effects of peptidyl puromycin.

Memory deficits after injection of puromycin 24 hours after training were found to be reversible as intracerebral injections of isotonic saline or water reversed the retention deficit (FLEXNER & FLEXNER, 1967; 1969a; 1970a; ROSENBAUM et al, 1968). It was concluded that puromycin blocked the expression of memory without altering the process maintaining the memory trace. However, if puromycin was given within minutes of training with a simple task, saline injections were not effective in restoring memory (FLEXNER & FLEXNER, 1968b). Further studies using saline to reverse the puromycin deficits revealed that the memory trace appeared to be widely spread in the cortex, despite the suppression of recent memory in the hippocampus (FLEXNER & FLEXNER, 1969a). When puromycin blockage of expression was reversed, the puromycin did not interfere with consolidation. It was suggested that the peptidyl puromycin interfered with the action of peptides normally responsible for maintenance and retrieval. The possibility that saline served as a reminder was considered unlikely, since injection of other salts did not induce the recovery of memory (FLEXNER & FLEXNER, 1970a).

It was also suggested that puromycin might block the expression of memory by interference of peptidyl-puromycin with adrenergic receptors (FLEXNER & FLEXNER, 1970b; ROBERTS et al, 1970; SEROTA et al, 1972; LANDE et al, 1972; FLEXNER & FLEXNER, 1971).

BARONDES & COHEN (1966) trained mice five hours after intracerebral bilateral injection of puromycin, during the peak of protein synthesis inhibition, to ascertain whether protein synthesis was required for both learning and consolidation. Acquisition by puromycin treated mice was indistinguishable from saline treated controls. Fifteen minutes after training, mice exhibited normal retention, but had total amnesia three hours later. Thus their capacity for learning and STM was retained while cerebral protein synthesis was inhibited. This supported the idea of an initial stage of memory storage which is independent of cerebral protein synthesis.

Puromycin has been shown to produce occult seizures in the hippocampus and therefore some of the amnesia-producing properties of puromycin might be due to a general cerebral abnormality, rather than to the inhibition of protein synthesis (COHEN et al, 1966; COHEN & BARONDES, 1967a).

Using goldfish, puromycin was found to produce amnesia three days later (AGRANOFF & KLINGER, 1964; DAVIS et al, 1965; DAVIS & AGRANOFF, 1966; AGRANOFF et al, 1965). If the inhibitor was administered/

/administered either immediately before or immediately after training, memory on testing was poor. If injections were delayed for more than one hour after training, or given 20 minutes before training, no effect on memory was observable, (AGRANOFF & KLINGER, 1964). Intracranial injections were given to fish at various times relative to the training period. Control fish were un-injected and retention was tested at 72 hours after the original training. Practically no forgetting was shown by the control group. Significantly greater forgetting was shown by those injected immediately prior to, or immediately after, the initial training. Fish injected at 20 minutes prior to, or 30 minutes following training showed less forgetting. Injections preceding training did not seem to influence the acquisition phase of learning (DAVIS & AGRANOFF, 1966).

Metrazol convulsions were potentiated in fish by intracranial puromycin. This was not found with AXM, in direct contrast to the COHEN & BARONDES (1967a) experiments. Puromycin aminonuclease did not show the potentiation effect, suggesting that the convulsant activity of puromycin was not responsible for amnesia in fish. Furthermore, peptidyl puromycin was thought not to be the convulsant agent since pretreatment with AXM, which should block peptidyl puromycin effects, did not protect against convulsions. Peptidyl puromycin was not formed when the aminonucleoside was used (AGRANOFF, 1969).

The time course of puromycin-induced forgetting was studied to attempt to produce maximal forgetting. Fish were given retention tests at intervals following the original learning. Only after 48 hours did fish exhibit statistically greater forgetting than controls (DAVIS & AGRANOFF, 1966).

ECS produced memory loss in goldfish but the disruptible period was longer than that due to puromycin (DAVIS et al, 1965). ECS can be given up to 90 minutes after learning and still produce amnesia. Maximum inhibition of protein synthesis would not, however, be produced immediately after injection. Puromycin had no effect on the rate of learning when injected before learning and did not produce any postural or locomotion impairments.

In an operant conditioning situation in goldfish, puromycin immediately following a training session did not disrupt performance of a well established response (GOLLUB et al, 1972). Thus memory shown by improvement in performance during training is not puromycin susceptible and is STM. Injection of puromycin immediately after learning and retesting at various following intervals, showed a gradual decay in retention after six hours and a total amnesia developed over the next two days (DAVIS & AGRANOFF, 1966).

The puromycin moieties, puromycin aminonucleoside and methyl tyrosine, did not block protein synthesis or produce memory deficits. Consolidation could be/

/be delayed by keeping the fish in the learning situation after training and injecting puromycin before returning them to the home tank (DAVIS & AGRANOFF, 1966; DAVIS, 1968; DAVIS & KLINGER, 1969). It was postulated that arousal invoked during training inhibited fixation and the visual stimuli in the testing situation maintained the arousal after training. If fish were replaced in the training situation for a brief period prior to puromycin injection, KCl or AXM amnesia could be induced as long as 24 hours after training (DAVIS & KLINGER, 1964).

Post session puromycin injections produced RDs in goldfish in a discriminative avoidance task, but there was a continuous improvement in performance over weekly training sessions. The complete loss of retention that would have been predicted from AGRANOFF'S experiments was not seen. POTTS & BITTERMAN (1967) concluded that the impaired retention was due to lack of consolidation of conditioned fear. These studies do not rule out the alternative possibility that puromycin interferes with consolidation of information more generally relevant to the instrumental behaviour. In a more direct measure of emotionality, measuring heart rate decelleration to a "light off" signal paired with shock, intracranial puromycin injected just before or immediately after a training session, did not appear to block the formation of memory (SCHOEL & AGRANOFF, 1972). This result is incompatible with puromycin interference/

/interference with conditioned fear consolidation.

Either overtraining, or injection of a very high dose of puromycin up to 48 hours after training, did not alter the RD (HUBER & LONGO, 1970). Puromycin injected 90 minutes before training blocked the memory of a new swimming pattern in goldfish when tested 22 hours later (SHASHOUA, 1969). RA was produced in the Japanese Quail when puromycin was injected 5 minutes after training. Amnesia was not found with AXM or with reversal training. These authors concluded that amnesia was due to peptidyl puromycin formation.

FELDMAN (1969) believes that experiments with puromycin do not demonstrate that a consolidation process follows learning, but that some process occurring after learning becomes increasingly difficult to disrupt with time. He believes the conclusions from experiments with puromycin concerning consolidation are tentative for the following reasons:-

(a) If the effect of a memory blocking agent is temporarily reversible by another agent, retrieval from storage has been affected rather than the process of storage. This is supported by the experiments where the effects of puromycin can be completely reversed with saline injection following puromycin but before recall.

(b) Processes taking place during the original learning seem to consolidate the task-related memory against puromycin disruption and this is shown when 60 trials/

/trials of overtraining were given (FLEXNER & FLEXNER, 1967). In this case puromycin did not disrupt memory, whether this be consolidation or retrieval.

(c) The savings method frequently used to assess retention is biased in favour of showing increased forgetting (DEUTSCH, 1969). The FLEXNERS' data indicates that puromycin caused some animals to have more difficulty in relearning than in the original learning trials. Puromycin-induced forgetting and the drop in savings percent, may result from a disruption in learning rather than retention failure.

Other data shows that puromycin might not be affecting memory through a direct effect on protein synthesis. Thus in the work of FLEXNER & FLEXNER (1966) and BARONDES & COHEN (1967b), mice trained to a nine out of ten criterion showed a memory deficit with puromycin and not AXM, even though AXM inhibits protein synthesis more effectively. A similar effect was found in vitro when AXM protected polysomes from disaggregation by puromycin (GODCHAUX et al, 1967). To account for their data, FLEXNER & FLEXNER (1966) postulated that proteins involved in the maintenance and expression of memory, or their products, act as inducers for the synthesis of mRNA. Protein level is thereby maintained by self induction. By this theory, the puromycin effect on memory could be caused by the destruction of mRNA. However, when 12 μ litres of saline were given up/

/up to 60 days after training, with puromycin injection the memory deficit was restored. The deficit caused by AXM was not restored with saline. Thus the mRNA induction theory was incorrect. KERKUT et al (1970) suggests that the trauma of injection could be having an effect, since it is difficult to envisage how such a small amount of saline could have such a large effect.

In summary, it can be seen that puromycin has multiple effects in the brain. Behavioural studies with this drug cannot therefore be conclusive in demonstrating the role of protein synthesis in stable memory formation. The release of peptidyl puromycin into cells seems to be the most plausible explanation of the behavioural effects of puromycin given long after training. Peptidyl puromycin remained in the brain for at least 58 days (FLEXNER & FLEXNER, 1968b), the memory trace may therefore be dependent on the synthesis of long lasting proteins. Some of the amnesic effects are reversible and the only major conclusion which can be drawn from these experiments is that puromycin is not a satisfactory agent with which to study the participation of cerebral protein biosynthesis in stable memory formation. Since these experiments constituted most of the pioneering work in this field I felt them worthy of a lengthy description in this Thesis.

(b) Acetoxycycloheximide (AXM)

AXM seems free of any of the side effects seen with puromycin. It is a protein synthesis inhibitor of greater potency than puromycin and exerts its effect by inhibiting the transfer of AAs from tRNA to the polypeptides, and prevents the subsequent release of polypeptides from polysomes without apparently damaging the ribosome (ENNIS & LUBIN, 1964). It does not directly affect peptide bond formation (PESKA, 1971).

FLEXNER & FLEXNER (1966) failed to show the amnesia with AXM injected 24 hours after training. When they administered AXM before training, they found the mice very difficult to train. RA was produced if reversal training was given after AXM or if AXM was given 5 minutes after training. Memory for the task persisted for periods extending up to three hours, was temporarily lost for three days and then returned. Thus the hypothesis that the establishment of memory is dependent on one or more species of mRNA which alters the rate of synthesis of one or more of the proteins essential for the expression of memory, was supported. These proteins or their products could act as inducers of inducer protein. In the presence of a protein synthesis inhibitor, the concentration of essential proteins could fall to levels too low for the expression of memory. The loss of memory could be temporary if the mRNA was conserved to direct the protein synthesis after the inhibition had ceased. According to this hypothesis,/

/hypothesis, the mRNA required for the expression of memory is degraded by puromycin but conserved in the presence of AXM.

Transient amnesia was found in rats when trained under the influence of AXM (SEROTA, 1971). Amnesia was present one day after training but the memory returned spontaneously within six days. This transient deficit in retrieval was interpreted as a deficiency in neurotransmitters.

RA was not produced in mice trained to a criterion of nine out of ten correct responses when AXM was given before training. If less training trials were given and the drug administered before training, amnesia was seen the next day. Given after training there was only very slight amnesia (BARONDES & COHEN, 1966b). Thus deficits could be produced with AXM as long as extensive overtraining was not given (BARONDES & COHEN, 1967b; COHEN & BARONDES, 1967b). Doses of AXM which inhibited cerebral protein synthesis by more than 90% were required to show this effect (COHEN & BARONDES, 1967b). The amnesic effect was thought not to be due to systemic illness since subcutaneous injections of an equivalent dose, which inhibited cerebral protein synthesis far less did not impair memory. Subcutaneous injections as opposed to intracerebral injections of the larger doses of AXM avoided the possibility of erroneous interpretation due to such factors as increased intracranial pressure, formation of brain lesions and scars,/

/scars, and heterogenious distribution of the drug, (BARONDES & COHEN, 1968a). One additional advantage of subcutaneous injection was a much more rapid onset of the inhibition. Mice could then be trained shortly after injection when they appeared to be completely healthy and tested when they had completely recovered. As with intracerebral injections before training, mice were amnesic by six hours. If the drug were given immediately after training, a smaller amnesic effect was seen and if injection was delayed to 30 minutes, AXM was ineffective.

The relative lack of effects of the doses of AXM which inhibited protein synthesis by nearly 90% was explained by postulating that the residual protein synthesis was sufficient to store the information. This was based on the idea of redundancy and the adaptive value of the particular learning task in the natural environment. A small amount of protein synthesising capacity might be sufficient to mediate memory storage, if prolonged repetition rather than brief training were given. With a more difficult task, e.g., a light-dark discrimination, training to a criterion of nine out of ten was not sufficient to overcome the amnesic effect of AXM (COHEN & BARONDES, 1969a), overtraining to 15 out of 16 was then necessary. AXM-induced amnesia was not reversed by saline injections (ROSENBAUM et al, 1968).

Although the protein synthesis which appears to be required for memory may commence during or within/

/within minutes after training, inhibition of protein synthesis during that period is not reflected in impaired memory until three or six hours later (BARONDES & COHEN, 1967a; 1967b; 1968a; COHEN & BARONDES, 1967b; 1968a). SQUIRE & BARONDES (1970) using bitemporal injections of AXM one day following training on a shock avoidance discrimination task in rats showed no effect on retention measured 27 hours later. For at least three hours after learning a process other than the synthesis of new protein seems to be responsible for the storage of memory. Because the injection of the drugs has to be given at or near the time of training to be effective in impairing memory, it seems that both the short and the long term phases of memory begin at the same time. LTM becomes more permanent as STM decays (DANIELS, 1971b; 1972). Until the STM decays, the effects of prior inhibition of protein synthesis for long term storage cannot be detected.

AXM was injected into the hippocampus of rats before training in a brightness discrimination task and an appetitive learning task. There was no amnesia for three hours following training but it became apparent at four to six hours later and was still present after seven days. This amnesia was therefore not transient. A decay presumably in STM occurred in a one trial inhibitory avoidance task with mice. The RA increased as testing was delayed from two hours to two days (SWANSON et al, 1969). Further positive evidence that protein required/

/required for stable storage must be synthesised near the time of training comes from experiments with goldfish (AGRANOFF et al, 1966a; 1966b; 1967; AGRANOFF, 1970b; CASOLA et al, 1968; LIM et al, 1970). Fish injected immediately after avoidance training were amnesic when tested, whereas injections delayed more than one hour were ineffective in blocking memory formation. AXM administered four hours before training, similarly was without effect. Amnesia developed over two days when AXM was injected immediately before training (AGRANOFF, 1970b). From this result, it was suggested that although STM appears to be converted to LTM, protein synthesis is also necessary for STM to decay. Repeated AXM injections resulted in only partial amnesia at a time when one injection produced complete amnesia.

In these experiments, delayed injections of AXM were not effective in producing amnesia but nevertheless, a possible requirement for the maintenance of long term memories cannot be excluded. Protein required for memory maintenance might have such a slow turnover rate as to have been missed in these inhibitor studies, since the effect of inhibitors wears off within hours or days and cannot be maintained for any length of time without seriously affecting several other behaviours of animals.

(c) Cycloheximide (CXM)

CXM exerts its action of protein synthesis inhibition in the same manner as AXM. CMX shows less sustained inhibition of cerebral protein synthesis than AXM,/

which allows less time for the possible development of a non specific interference with cerebral protein.

Amnesia from CXM, like AXM, depends on the time of injection relative to training. The drug can be given 45 minutes before or 10 minutes following training and produce amnesia (GELLER et al, 1969; MARK & WATTS, 1971; WATTS & MARK, 1971a). Memory, presumably STM, is seen following training for between 90 minutes and six hours, after which time the amnesia develops (COHEN & BARONDES, 1968b; BARONDES & COHEN, 1968b; GELLER et al, 1969; SQUIRE & BARONDES, 1972; QUARTERMAIN & McEWAN, 1970; WATTS & MARK, 1971b; QUINTON, 1971; 1972). CXM has been used in a number of training situations and has been found to produce amnesia at a time after STM was decayed. In mice, amnesia has been produced using an appetitively motivated task (COHEN & BARONDES, 1968b), a one trial passive avoidance step-through task, (BARONDES & COHEN, 1968b; QUINTON, 1971; QUARTERMAIN et al, 1972) and in chickens in a one trial passive avoidance task (MARK & WATTS, 1971; WATTS & MARK, 1971b). Exploratory habituation was not vulnerable to interruption by CXM (SQUIRE et al, 1970).

The degree of training is also an important variable in the amnesia seen with CXM injection. The number of trials in the original learning also affects the extent of amnesia, as doses delay footshock following the response (BARONDES & COHEN, 1968b; SQUIRE & BARONDES, 1972a),/

/1972a), or when footback is varied in intensity in a one trial step through task (GELLER et al, 1970). The number of learning trials also affects the rate of decay of STM (SQUIRE & BARONDES, 1972b). Other psychological variables, important in ECS-induced amnesia also seem to effect CXM-induced amnesia (DAVIS & AGRANOFF, 1966; DAVIS, 1968; DAVIS & KLINGER, 1969; GELLER et al, 1971; BARONDES & COHEN, 1968b).

Using mice with CXM-induced protein synthesis inhibition, BARONDES & COHEN (1968b) found that arousal generating manipulations could lead to the development of stable memory if these were introduced at a time when STM still persisted and protein synthesis had recovered. This arousal producing treatment could serve as a reminder of the original training and would be effective in protecting against the protein synthesis inhibitor only so long as STM had not decayed, and the synthesis of protein had been re-established. That STM remains available for manipulation affecting consolidation for the duration of the period of arousal is demonstrated when goldfish are detained in the training apparatus after training. Under normal post-training conditions in the home tank, susceptibility diminishes within one hour (DAVIS & AGRANOFF, 1966; DAVIS, 1968; DAVIS & KLINGER, 1969). Continued arousal might hold the information in the short term state. For stable storage, in addition to the information acquired during training and an intact protein synthesising capacity,/

/capacity, the appropriate state of arousal, which specifically directs the establishment of permanent memory may be necessary. Arousal could also have a role as an order for neurones recently activated to "print" information in a stable form (LIVINGSTONE, 1967). If amphetamine was given six hours after training, stable memory was again not formed. Optimal doses of amphetamine have facilitative effect on LTM (RAHMAN, 1970). This is thought to be due to increase in concentration, attention, and motivation. An acceleration and increase in the intensity of activity has also been shown. No potentiation of STM was found with amphetamine and thus either the process of consoldiation could be facilitated, or the effects may be on the long term processes of memory (CROW & BURSILL, 1970).

Activity for 30 minutes following CXM injection was increased, and was then depressed for one to three hours (SQUIRE et al, 1970). Isocycloheximide (IXM) which does not inhibit protein synthesis and did not produce amnesia, depresses activity to the same extent as CXM. Activity level changes do not therefore seem to be related to CXM amnesic effect (SEGAL et al, 1971). Amphetamine, which can antagonise the effects of CXM (BARONDES & COHEN, 1968;) did not antagonise the depression of activity (SEGAL et al, 1971).

(d) Anisomycin (AIM).

AIM represents a third class of protein synthesis inhibitor which has amnesic effects. AIM is structurally/

structurally different from CXM or puromycin and has different effect on the protein synthesis process. It acts by blocking peptide bond formation (CROLLMAN & HUANG, 1973). SQUIRE & BARONDES (1973) studied the effects of AIM and CXM using discrimination training in mice.

They found:-

(a) both drugs can be used to inhibit protein synthesis to the same extent and have identical amnesic effects.

(b) although AIM has no effects on acquisition with brief training, it impairs further acquisition with prolonged training as does CXM (SQUIRE & BARONDES, 1973, SQUIRE et al, 1973).

(c) AIM and CXM have different effect on activity.

(d) two structural analogues of AIM inhibit protein synthesis to a lesser degree but do not produce amnesia. One of these has effects on activity similar to those of AIM.

Results from work with AIM considerably strengthen the contention that cerebral protein synthesis is involved in stable memory formation. Since there are structural differences between AIM and CXM and they interact differently with the protein synthesising apparatus of the cell, it is unlikely that they would share the same side effects unrelated to the inhibition of protein synthesis. It is possible that these drugs impair memory by producing some non-specific effect due to protein synthesis inhibition itself rather than by/

/by inhibiting the synthesis of protein specifically required for memory storage. It is possible that inhibition of protein synthesis 30 minutes before training leads to an abnormal brain state at the time of learning (BARONDES, 1974; SQUIRE, 1974). Results of studies comparing the amnesic effectiveness of different levels of inhibition at different times before training render this possibility unlikely since it was found that initiating inhibition of 95% of cerebral protein synthesis five minutes before training impaired memory formation (BARONDES & COHEN, 1968a). In contrast, initiating inhibition of 85% of cerebral protein synthesis for hours before training and maintaining this level of inhibition throughout the training period,had no effect on LTM (BARONDES & COHEN, 1967a). An alternative explanation is that a state-dependent effect could be operating but as the shorter period with 95% inhibition had had a more potent effect than the longer period of 95% inhibition this suggests that the inhibitors prevent synthesis of protein required for memory, rather than producing non-specific effects.

CXM and AIM were studied concurrently and had identical effects on memory. However, in the two studies carried out with the two drugs there were a number of differences between the results where CXM was used (SQUIRE & BARONDES, 1972; 1973). Some of the differences could have been due to the use of different strains of mice in the two studies since different strains may not have equivalent/

/equivalent learning capabilities. Differences in initial learning scores, the sensitivity of the amnesic effects to the extent of training, and the recovery of memory are all related. However, the initial variable in producing these changes has not been identified. Differences in the relationship between the degree of training and the amnesic effects of an inhibitor have also been observed (COHEN & BARONDES, 1968a), and have been related to differences in the difficulty of the task (SQUIRE & BARONDES, 1973). Recovery of memory has been observed in some experiments (QUARTERMAIN & McEWEN, 1970; SEROTA, 1971), but not in others (AGRANOFF, 1972; BARONDES, 1970; FLOOD et al, 1972; GELLER et al, 1969; SQUIRE & BARONDES, 1972b). The recovery of memory under certain circumstances does not contradict an absolute requirement for cerebral protein synthesis in the development of stable memory (BARONDES, 1974; BARONDES & SQUIRE, 1972). Mice treated with CXM showed retention impairments at six but not at three hours after training in a t maze discrimination task. SQUIRE et al (1973) used an alternative training procedure and showed a CXM-sensitive component of memory storage which could be identified within minutes after the beginning of training. Thus under some circumstances, cerebral protein synthesis may be required for the normal expression of memory within minutes after learning has begun.

(2) CONCLUSIONS FROM EXPERIMENTS WITH PROTEIN SYNTHESIS INHIBITORS.

(i) Behavioural.

Some of the problems encountered in ECS studies with regard to training variables are also apparent with the use of antibiotics. The amount of training and the difficulty determines the occurrance and the extent of amnesia. The greater the degree of learning, the less vulnerable the memory to interference. Most of the learning tasks used have required more than one trial and thus clear cut descisions concerning the susceptibility of behavioural components to chemical interference are difficult to make.

Important also is the dosage of inhibitor. Different experiments use different dosages, although dose responses have been reported in some experiments with puromycin (AGRANOFF et al, 1965; FLEXNER et al, 1965); with AXM (FLEXNER & FLEXNER, 1966; COHEN & BARONDES, 1967b; BARONDES & COHEN, 1967b) and with actinomycin D (BARONDES & JARVICK, 1964; COHEN & BARONDES, 1966b). To show that the inhibitors are interfering with one mechanism alone, it is important to show that the dose response relationships are simple and graded.

The report of the remainder shock raised the question of whether CXM was suppressing or inhibiting the expression of an intact memory trace or whether some part of the trace was remaining intact. It is possible that the level of arousal could be exerting an important effect. Time of injection relative to training is of great importance.

It has been assumed from the work of the FLEXNERS, that there was an interruption of memory formation when injections were given at one day or more after training but the return of the memory after saline injections cast serious doubts on the consolidation hypothesis. However, as has been frequently pointed out, this work is based on interference with the expression of memory once it has been formed and if the injection of inhibitor is close enough to the learning then memory loss is not reversible by the saline.

(ii) Pharmacological.

To draw the conclusion that the synthesis of protein is specifically required for memory storage, the drugs used should specifically inhibit the synthesis of these molecules and should not have any other actions on the brain. The drugs used so far in these studies do not fulfill this requirement. They inhibit the synthesis of all proteins, both those required for normal maintenance of cell functions which may be normally degraded or have a rapid turnover rate and therefore need replacement, as well as the synthesis of new proteins which may subserve adaptive processes such as memory storage. A diminution of constitutive proteins may result from prolonged inhibition of cerebral synthesis and any behavioural deficit may be due to this rather than to inhibition of protein synthesis specifically required for memory formation. This problem may not be so important when inhibition is fairly brief and when no detectable behavioural or electrophysiological abnormalities are present.

The second problem is that the drugs may have some other action besides the one for which they are being used. Thus puromycin seems to have many other effects aside from the inhibition of protein synthesis, as has been previously described in this chapter.

AXM, CXM and AIM have apparently a much more specific action in inhibiting protein synthesis and produce no abnormal peptides. CXM is preferable to AXM in most experiments because of the rapid onset of inhibition and the shorter duration of synthesis inhibition.

The brain seems to be able to function well enough for learning to take place despite the marked initial inhibition of either RNA or protein synthesising capacity. However, stable memory storage is apparently dependent on cerebral protein synthesis during, or within minutes after training. Evidence for the participation of cerebral protein synthesis in memory formation comes mainly from results of experiments with the glutarimide derivatives. The two major ojections to these results are:-

(a) Glutarimides not only inhibit cerebral protein synthesis, but may also have other actions on the brain which are responsible for the effects on memory.

(b) Inhibition of "constitutive" protein synthesis impairs replacement of brain proteins undergoing degradation, and this produces abnormal cerebral functioning which impairs memory storage.

BARONDES (1970) argues that the first of these objections might be invalid because of the relative effectiveness of AXM and CXM. These drugs are closely related structurally but differ markedly in the degree to which they inhibit protein synthesis. It is necessary to give a 10 to 20 times larger dose of CXM to AXM to achieve the same degree of inhibition. The amnesic effects of the drugs are therefore correlated with the degree of inhibition produced rather than with the administered dose. It would be expected that the side effects would also have the same dose response relationship. Furthermore, other derivatives of the glutarimide series, which have little or no effect on protein synthesis, have no effect on memory when administered in equivalent doses.

Objection (b) above, requires that there are critical brain proteins, present only in limited amounts, and having a very rapid turnover (BARONDES, 1970). The existence of such proteins is not suggested, however, by the apparently normal learning found even hours after prolonged inhibition of cerebral protein synthesis found in some experiments (BARNONDES & COHEN, 1967b; 1968a). In contrast, the possibility that the protein is specifically synthesised to store each memory, implies the existence of a highly efficient mechanism used by cells in a variety of situations for regulatory purposes only. It is for this reason that the results are interpreted as indicating that cerebral protein synthesis during training and/

/and within minutes after training is specifically required for the stable storage of memories.

(iii) Overall conclusions and summary.

Experiments involving physical treatments to the brain such as ECS, and the animal experiments using protein synthesis inhibition indicate that memory passes through a short term stage, which then decays unless replaced by the permanent storage process. The distinction between the physiological short and long term storage systems refers only to the physical nature of the memory trace. Short, intermediate and long term memory, are also terms used by psychologists to separate out the stages in the processing of information, particularly in human verbal learning experiments (BROADBENT, 1970; CRAIK & LOCKHART, 1972). The animal experiments mostly deal with time intervals longer than the decay of human STM. Physiological STM probably corresponds to the initial form of storage of information in psychological LTM.

A central question has been whether the behavioural experiments can be used to determine the transformation in the nature of the storage material of memory. In the case of ECS in man and animals, it seems that the essential information may be impossible to obtain because of the interfering behavioural effects due to the ECS administration. With the antibiotic block of stable memory formation in animals, it may be possible to determine the storage material, since drugs can be administered before learning and do not produce any obvious behavioural change beyond their/

/their effect on memory. Nevertheless differences in task, training, testing and the timing of these, all have effect on the time course and rate of decay of memories. Bheavioural analysis must be given weight equal to methods of a pharmacological type. As is clear from the ECS experiments, the choice of an apparently simple learning task does not mean that the behavioural variable can be ignored. A more complicated psychological situation may be necessary in order to attribute behavioural change to drug action on memory.

As far as agents used to interfere with memory are concerned, ECS by itself appears to have reached a plateau of usefulness until more is known about the physiology of its action. It would be helpful to know exactly which of the cellular components are directly responsible for the amnesia and which of the consequences of ECS and other convulsants are effective. Whether the decline of memory measured after learning in an animal in which cerebral protein synthesis is inhibited, corresponds to the decline in labile memory as deduced from ECS and other non specific experiments, is not yet known.

The reason that the cerebral processes underlying learning and memory are not as well understood as other physiological functions, e.g. digestion, blood circulation, is that a good in vitro preparation of the brain has not been devised in animals and cannot be considered as a possibility with human subjects. Therefore the brain of the learning animal must be observed with implanted/

implanted instruments while the animal is conscious, or else indirect methods of observation must be used. This is particularly important with human subjects such as those used in my experiments.

As can be seen from the experiments where agents are used to interfere with memory, the brain is "well insulated" from the outside world. The scalp, skull and meningeal layers that protect the brain from external physical assault also offer an obstacle to research. Electrical current can penetrate, and the effectiveness of ECS and the availability of the EEG depend on this property. The consequence of learning are not grossly observable in the brain (JARVICK, 1972). Either prolonged environmental changes are needed to induce measureable modifications in brain structure (ROSENZWEIG & LEIMAN, 1968) or else the use of sensitive methods involving radio active tracers must be employed to detect changes resulting from a brief learning experience.

Some chemicals can be administered parenterally and can pass the blood brain barrier, yet other drugs must be put directly onto the brain. Most of these drugs used in animal studies cannot be used on human subjects and the administration of drugs to humans is limited to those which are clinically prescribed. Since these drugs are prescribed to treat abnormalities in patients they presumably have effects in addition to and other than those required for experimental manipulation. Many drugs having a marked effect on behaviour act by mechanisms which are/

/are as yet unidentified (ESSMAN, 1971).

If an agent were available which was both specific and effective in influencing memory and the relevant biological processes underlying memory, then the task of identifying the biological basis of memory would be simplified. The likelihood of such an agent is remote since learning is protected not only by physical barriers but also the greater susceptibility of other physiological and psychological factors to various treatments. Thus arousal, motivation, activity and perception may easily be impaired by low levels of a drug which do not impair learning or retreival but may indirectly affect it. Higher levels of drugs may render the animal inactive or unconscious. Non specific effects of drug administration in human subjects such as a tendency to cause illness or nausea may also produce decrements in learning or memory.

The observed effects of administered drugs may be considered positive, i.e. exerting some degree of facilitation; negative, i.e. inducing impairment; or without effect. One critical determinant of such outcome is the behavioural technique employed, e.g. appetitive reinforcement, avoidance behaviour etc; the measure of behaviour utilised as a "process" index, e.g. response rate, response errors, response latency etc; and some implicit relationship between drug action and the process being acted upon. When human verbal memory is investigated, the situation becomes even more complex as will be seen in the final chapter. Phenomenological studies seldom offer much/

/much in the way of understanding the mechanism of drug action or mechanisms involved with the learning or memory process. To be taken into account also, are questions concerning acute versus chronic treatment conditions and their respective effects, and the nature of possible contributions provided by variables such as species, strain and sex differences, population density, and age in animal studies.

There is also a more fundamental question concerning the relative generality of the cogntivie processes studied in animals and the comparable events in man, particularly insofar as drug action alteration of these processes is concerned. Data from studies of neuropharmacological effects on the memory and learning process in animal experiments, can provide hypotheses which may be tested in man.

CHAPTER VII.

THEORIES OF REM SLEEP FUNCTION.

(1) THEORIES PROPOSING FUNCTIONS OTHER THAN MEMORY FORMATION.

I shall differentiate when talking about theories of REM sleep function between REM sleep itself and dreaming, although there is a well known and statistical connection between the two. The psychoanalytic theories will not be discussed and most of the theories outlined will be those that have followed the discovery of the two states of sleep.

Several theories suggest that SWS has the function of providing rest and inactivity for cortical neurones and that the function of REM sleep is to interrupt this state of quiescence. EPHRON & CARRINGTON (1966), have suggested that SWS is a state of sensory deprivation or cortical deafferentation, necessary for unspecified reasons. Long periods of such deprivation may be evolutionarily maladaptive since the organism is unprotected. Therefore REM sleep has a homeostatic function, whereby the cortex is "reafferented", bringing cortical excitation back to some predetermined necessary level. Similarly, WEISS (1966), suggested that REM sleep reorganises CNS firing patterns which have become disorganised during previous SWS sleep periods. HAWKINS (1966), suggested that REM sleep serves to re-establish patterned operations. These theories are for the most part rather vague and have not been empirically affirmed.

An arousal function for REM sleep was proposed by SNYDER (1966). His view is based on phylogenetic considerations and the relatively large amount of REM sleep found in some primitive mammals. Sleep functions to conserve energy since an animal uses less energy when sleeping. Sleep also may keep an animal protected for some period out of 24 hours. However, SNYDER suggests that an animal sleeping for a long period is subject to danger and a periodic arousal mechanism is useful. This role, he attributes to the periodically occurring REM sleep, which does have certain characteristics of cortical arousal. This theory is consistent with the brief periods of arousal found in some species, following every REM period. However, this could theoretically be replaced by a period of actual arousal so the theory does not explain the necessity for REM sleep as such. The mental content associated with REM sleep in humans, the presence of the eye movements, and penile erections are not accounted for in this theory. As well as having characteristics similar to arousal, REM sleep also has unique features, such as loss of muscle tone. The animal may also sometimes be more difficult to arouse from REM sleep than from SWS (BENOIT & BLOCH, 1960; DILLON & WEBB, 1965). There are also sometimes lower thresholds of arousal during REM sleep if an auditory stimulus is familiar such as a human's own name (OSWALD, 1966). It would seem that if the only function of REM sleep were arousal, it would be especially important that an animal be more easily/

/easily aroused by unfamiliar or unknown stimuli. The function of REM sleep in humans by this theory would be merely an extrapolation of its function in animals.

The theory of FREEMON (1970), suggests that there are two arousal systems and the continued need for vigilance accounts for the two sleep states. The evidence he produces is on a neuroanatomical basis since there are slow waves in one system and fast in the other. This theory does not deal directly with the function served by sleep which FREEMON refers to as "renewal", and inspite of this renewal the organism has still to be protected by these two vigilance systems.

Related to the afferentation or cortical excitation view of REM sleep is a theory of ROFFWARG et al (1966), derived from ontogenetic data. There is a high percentage and large amounts of REM time in young and newborn animals and humans and this is likely to be even higher in utro. A certain amount of stimulation is necessary for the proper development of the cortex (ROSENZWEIG et al, 1968), and since the newborn mammal sleeps for a large percentage of time and has need for more cortical stimulation than can be provided from the external environment, REM periods provide an internal, quasi-sensory bombardment of the cortex.

Several other biological theories do not fit into the deafferentation/reafferentation models or the other major category of REM sleep function theories, proposing a memory consolidation function which will be/

/be discussed in greater detail to follow.

BERGER (1969) proposes that REM sleep has a function in restoring the neuronal and neuromuscular apparatus necessary for binocular vision. His hypothesis is primarily concerned with the eye movement aspects of REM sleep. Thus REM sleep provides a mechanism for the establishment of neuromuscular pathways serving voluntary conjugate eye movements and maintains their integrity during the extended periods of sleep. The degree of fine neuromuscular control involved in the execution of voluntary conjugate eye movements in higher mammals exceeds that of any other musculature system in the body. During NREM sleep the eyes adopt an upward divergent resting position with occasional disconjugate rolling eye movements. The EMG of eye muscles is almost entirely absent and innervation of the oculomotor system is at a minimum. Lack of afferent input can lead to a degeneration of neural processes (REISEN, 1967). BERGER proposes that without the periodic innervation of the oculomotor system provided by REM sleep periods, the integrity of the CNS processes involved in the co-ordination of eye movements would be temporarily lost after extended periods of sleep.

The evolution of REM sleep for this reason is supported by a high correlation between the amounts of partial decussation in the optic chiasma and the percentage of the total sleep time spent in REM sleep. It was originally thought that all mammals exhibited REM sleep, However, this sleep stage is absent in the spiny anteater/

/anteater (ALLISON & GOFF, 1968). This animal exhibits no waking eye movements either.

BERGER believes that the large amounts of REM sleep seen in infancy and the decline in the percentage of this sleep stage with old age, represents a change in the function of REM sleep e.g. in the developing organism REM sleep is involved in establishing neural pathways serving co-ordinated eye movements, and thereafter, it merely maintains the pathways during extended sleep periods. BERGER also found that it was possible to detect degenerative changes in occulomotor co-ordination during wakefulness or when normal innervation is disrupted during episodes of NREM sleep when innervation is at its lowest level. Marked deterioration in depth perception was found in subjects experiencing alternating monocular occlusion of each eye every two or three hours for a period of 24 hours (WALLACH & KURSCH, 1963). The period required for recovery was about 20 minutes, a time equal to the mean duration of REM periods throughout the night.

BERGER & SCOTT (1971) showed that the accuracy of binocular depth perception was significantly less at the onset compared to the end of a REM period, whilst monocular depth perception showed no systematic variation. The accuracy of monocular depth perception was significantly worse after final awakening in the morning than prior to falling asleep initially, while binocular depth perception showed a significant increase in accuracy from evening to the following morning. These results were predicted/

/predicted from the assumption that if binocular co-ordination is impaired during NREM sleep then binocular depth perception should be dependently affected. BERGER (1969) has shown that the eyes are poorly co-ordinated during oculomotor tracking tasks following awakenings at REM period onset while co-ordination is intact at the ends of REM periods.

Some of the most extreme changes in electrical events which can be recorded during REM sleep are associated with eye movements. During REM sleep, as during waking, the eye movements are accompanied by bilaterally synchronous monophasic waves in many areas of brain visual pathways (BROOKS & BIZZI, 1963; MIKITEN et al, 1961; MOURET et al, 1963). REM characteristics are remarkably constant from one period to another as are the frequency of monophasic waves from one episode to another, showing rigid organisation of the occulomotor system during REM.

REM sleep may appear periodically to clear the CNS of some endogenous substance or toxin that builds up during SWS or waking (DEMENT, 1964). There is no empirical data supporting this possibility, and the complex events comprising REM sleep are not explained.

FISHER (1965a; 1965b) proposed a theory based on FREUD'S views. Dreams and therefore REM sleep functions to discharge instinctual drives in adults. FISHER believes that in children, where true instinctual drives of the/

/the same kind may not be present, REM sleep discharges physiological drives. This is consistent with the build-up and discharge model of sleep which WOLFF (1966) believes may characterise the entire life of the newborn and infant. It is also consistent with the observation that REM deprivation produces hyperphagic and hypersexual cats (DEMENT, 1970). Otherwise the theory seems difficult to prove or refute experimentally.

Other theories are based on the implication that REM sleep allows the process of dreaming to occur. The specific function of dreaming is proposed as a means of mastering current problems and conflicts, thus achieving better psychological adaptation for future waking life (FRENCH & FROMM, 1964; JONES, 1962; GARMA, 1966).

The state of the brain in REM sleep shares many characteristics in common with relaxed waking behaviour and there is increased cortical excitation compared with NREM sleep. OSWALD (1969; 1970) hypothesised that REM sleep was a time for non specific synthesis within cerebral neurones. He suggested that the presence of the REM rebound after drug withdrawal indicates increased protein synthesis and he cites the evidence of the high proportion of REM sleep seen before and immediately after birth, a time when the brain is undergoing fine differentiation. Increased protein synthesis during REM sleep would also be consistent with increased bloodflow and temperature during REM, and the theory is also supported by evidence that REM sleep in senility and mental defect is reduced, coupled with/

with reduced learning capacity.

(2) THEORIES LINKING REM SLEEP TO MEMORY AND LEARNING.

Theories in this category are amenable to experimental testing. Consequently a large amount of data has been generated which is often contradictory. The simplest theory based on a superficial view of dream content and a computer analogy, suggests that REM sleep may have the function of clearing the memory "tapes" of irrelevant information gathered during the daytime. This allows for new information to be absorbed the next day (NEWMAN & EVANS, 1965). Some theories suggest that REM sleep may actively consolidate new and useful memories (BREGER, 1967). Thus REM sleep and dreaming are especially necessary for perceptual learning and memory consolidation.

MORUZZI (1966) suggested that sleep permitted the recovery of "plastic synapses" in the brain and that it was involved in the formation of engrams. This is a neuro-chemical and neuro-anatomical model on which many of the later theories are based. GAARDER (1966) similarly proposes that memories may be processed and coded during REM sleep. HENNEVIN & LECONTE (1971) also suggest an information-processing and memory consolidation function for REM sleep.

I shall consider the "Programming (P) hypothesis" of DEWAN (1970) in some detail since it has given rise to a great number of experiments including one of my own. The hypothesis is based on a number of general observations:-

(a) biological phenomena ranging from adaptation,/

/adaptation, evolution and homeostasis through learning behaviour, all exhibit "error correction" or "optimisation", common in any control system. Higher animals are able to learn and to adapt to changing situations throughout their lifetimes. Nature is also "economical" and brains are able to perform functions relevant to their current needs. Information no longer required could be stored "economically" at the price of "accessibility". The hypothesis states that brains of higher organisms are in a state of constant alteration, their functional structure being constantly revised for current situations. This is analogous to programming and reprogramming in a computer. DEWAN believes that during sleep, an animal "disconnects" from the environment, which is necessary as "reprogramming" is carried out "off line".

(b) REM sleep exhibits phylogenetic development (HARTMANN, 1966).

(c) REM sleep exhibits ontogenetic development (ROFFWARG et al, 1966). % REM is largest at birth and early development when the animal is most "plastic" and able to learn.% REM sleep decreases from adulthood to senility, when learning and memory recall are at their lowest values. It has been estimated (PARMALEE & STERN, 1972) that at about 24 to 30 weeks gestation age in humans, REM sleep is 100% in the human foetus. The initial programming of the brain occurs presumably at a maximum rate during early embryological development.

(d) REM sleep is associated with dreaming (DEMENT &/

DEMENT & KLEITMAN (1967).

(e) there is a REM rebound following REM suppression. This implies that REM sleep is in some way important to the organism (DEMENT, 1960).

In modern computers, there are two methods employed in setting up programmes of functional structures (DEWAN, 1970). Firstly, the components must be connected to a "patch panel" and secondly, instructions must be stored as coded numbers (memory stored control). During operation, these instructions may be called forth in sequence. Changing the programme consists of replacing these instructions in the memory device. In either type of programming there is an alterable functional structure. DEWAN suggested that in the brain, the functional structure, i.e. the pathway, can also be altered. Thus the brain has a method of reprogramming itself spontaneously and automatically.

Six catagories of programming (P) were considered, as well as experimental evidence in support of each catagory.

(1) physical aspects including the following functions :-

(a) establishing new pathways as neurons die with age and are not replaced.

(b) initial programming of the embryo brain.

(c) establishing new functional pathways after brain damage.

DEWAN proposed that long term deprivation of REM sleep would cause permanent damage and that REM deprivation in the embryo e.g. by the use of drugs, would inhibit the natural anatomical brain growth. An experimental

approach which has been used is to measure the percentage of REM sleep in aphasic patients. Those who were rapidly recovering showed a significantly higher percentage of REM sleep than those who did not improve (GREENBERG & DEMENT, 1969).

(2) memory P.

In a computer, the use of memory devices has a hierarchical organisation (VON NEUMANN, 1958). Material less frequently used is stored as progressively slower and more economical memories. Memories can also be shifted as priorities change and this seems to be analogous to memory in the brain. As information is utilised less often it becomes no longer recallable, but may still be recognisable and finally the memory may manifest itself only as a savings in relearning trails.

Imprinting, a special example of learning and memory, is one example of the memory type processing. Higher amounts of REM sleep could be predicted during this critical phase.

DEWAN also made the following predictions involving memory and learning:-

(a) Korsakoff's patients, who show practically no ability to recall, should have no REM sleep. However it has been experimentally found that these patients did show REM sleep but it was of an abnormal type, often without eye movements (GREENBERG et el, 1967). It was suggested that this type could occur in the absence of P.

(b) ECS, which causes amnesia, should reduce REM pressure. REM deprivation did not produce a rebound on successive

/successive nights if ECT had intervened (COHEN & DEMENT, 1966; COHEN et al, 1967).

(c) lower organisms e.g. birds, when subjected to a high regimen of learning e.g. operant conditioning experiments, should show more REM sleep.

Memory P has been investigated by many experimenters, some of which will be discussed later in this chapter. GREENBERG & LEIDERMAN (1969); PEARLMAN (1968); FISHBEIN (1968); and FELDMAN & DEMENT (1968) were among the first to obtain evidence that REM sleep may involve both a necessary preparation for learning i.e. a form of "meta programming", and also perform the function of LTM consolidation.

(3) input-putput aspects of P

These aspects comprise programmes involved with the organisation of perception, co-ordination of motor activity, e.g. acquisition of motor skills, co-ordination of sensory motor mechanisms, co-ordination of motor activity controlling perception, especially eye movement in visual perception, and attention programmes which automatically filter the sense modalities and present the brain with the most relevant incoming information.

Experiments designed to test this have involved visual field rotation. Subjects can adapt to visual distortion provided they exercise willed motor activity over a certain interval of time (KOHLER, 1964; HELD, 1963). Thus DEWAN predicted that visual field inversion would enhance REM time and intensity. These experiments/

/experiments will be discussed more fully in Chapter VIII. By and large, the prediction was only satisfactorily supported by one set of findings that have not been replicated.

(4) the organisation of programming by drives and goals.

A surplus amount of ability to "cope" gives rise to a pleasant emotional experience and vice versa (SIMONOV, 1969). It is associated with the drive strength, and therefore the amount of emotionality experienced by a subject should affect REM pressure. This could be an explanation of how tranquillisers which decrease emotionality, e.g. anxiety, also decrease the REM % (KRAMER et al, 1966). REM deprivation should also increase emotionality given that the subject would have to cope with a situation for which he was unprepared. This prediction was supported by experiments where subjects were shown stressful films at bed time (GREENBERG et al, 1968).

Emotion may play an additional role in programming since it may "trigger" or "label" memories and programmes for the purpose of consolidation. Each goal or drive would have an associated set of feelings which would label the perceptions and experiences occurring at any given time. DEWAN hypothesised that all memories, programmes etc., relevant to a current important need can be brought together and "filed" in one place, by making use of these labels. This is analagous to computer "associative memory" where the address number of each memory location includes a coded numerical label to identify the type/

/type of material stored. State dependent memory is consistent with this hypothesis. The "state" can be regarded as a constellation of feelings.

(5) REM periods, biological rhythms and homeostasis.

Programming is regarded as a form of homeostasis which involves not only the ability to cope but also the entire range of mental and physical bodily processes. Since REM sleep is "locked in step" with bodily rhythms, programming processes can also affect these rhythms. STROEBEL (1968) suggested that the body could be programmed for a certain emotion as a function of the time of day. Thus programming can control hormone release, and other somatic and homeostatic anticipatory activity to prepare the animal to cope. REM deprivation should tend to prevent this.

(6) breakdown of the P system.

Breakdown of P can result in bad health on a physical level and "oscillation" and "self destruction" on a psychiatric level. I shall not consider these aspects of P since they are not relevant to REM sleep, I shall merely state that one finding has been that there is a lack of REM rebound in schizophrenics (ZARCONE et al, 1967).

According to JONES (1970), there are five different biological functions hypothetically ascribed to REM sleep which can also be extended to include dreaming:-

(1) a neutralising function which should counteract some noxious impulses or memories.

(2) a stimulating function, in compensation for the/

The periodic sensory deprivation of SWS. As REM sleep may stimulate the cortex in a state of afferent deprivation, dreaming may stimulate emotion or memory in a state of emotional deprivation.

(3) a reorganising function, in response to the disorganising effects of sleep on the CNS. Thus REM sleep may reorganise firing patterns in the CNS and dreaming may serve to reorganise patters of ego defence in response to the disorganising effects of waking experiences.

(4) a vigilance function in preparation for "fight or flight" responses to potential threats to physical integrity. Dreaming may also serve this function with respect to potential threats to psychosocial integrity.

(5) an innervating function serving visual depth perception. Dreaming may help to maintain and establish perception of self.

JONES believes that the hypotheses of SNYDER, discussed above and ULLMAN (1961), which is concerned with the adaptive value of the vigilance function of dreaming for psychosocial behaviour, could be combined to form a powerful psychobiological theory.

KLEITMAN (1970) points out that REM sleep occurs as a phenomenon of the basic rest activity cycle (BRAC) and thus it is not necessary to postulate a purposive explanation of it. Dreaming is not only a function of the activity phase since KLEITMAN states that the short memory span of a dream experience resembles the inability to recall events during alcohol intoxication. Thus, although dream/

/dream reports may be of diagnostic and therapeutic value to a pschoanalyst, dreaming as a process is as inevitable in the activity phase of the BRAC, as thinking during wakefulness.

HARTMANN (1973) believes that there is no need to question the common sense notion that sleep is involved in restoration and restitution. However, there does seem to be a separate requirement for the two states of sleep. SWS, he believes, is the deeper and most intensive part of sleep which has a physically restorative function which becomes necessary after exercise (BAEKELAND & LASKY, 1966; HOBSON, 1968; BOLAND & DEWSBURY, 1971; HAURI, 1968) or when catabolism has been increased. He considers SWS as an anabolic phase of sleep and suggests that macromolecules e.g. protein and RNA, are synthesised especially in the CNS. These macromolecules can then be used in the functions of REM sleep. Although increased requirements for SWS may be produced by states of general body tiredness or increased catabolism, the increased synthetic processes probably occur especially in the brain. This would indeed seem logical since secretion of all hormones and releasing factors which initiate cellular processes are under hypothalamic-pituitary control. Thus HARTMANN believes that SWS plays a preparatory role for the functions of REM sleep.

The functions of REM sleep according to HARTMANN are more complex than those of SWS. From studies of sleep deprivation (NAITOH et al, 1971; TECCE, 1972) and/

/and the psychology of tiredness (HARTMANN, 1973) REM sleep acts by restoring systems of focussed attention and systems involving the maintainance of optimistic mood, energy and self confidence, and systems involving processes of emotional adaptation to the physical and social environment.

From studies of short, long and variable sleepers, HARTMANN concludes that REM sleep is needed in higher quantities after days of stress, worry and intense new learning especially if the new learning is itself somewhat stressful. REM sleep may thus have a role in the consolidation of new learning but stress is also important and more REM sleep is needed if there have been changes during the day involving emotional reactions.

Systems not functioning during REM sleep may be undergoing repair at that time. Focussed attention, energy amd a continuing sense of self are not present in REM and HARTMANN suggests that the same processes that wear down during tiredness are exactly the same ones which are absent from REM sleep, and so REM sleep is a time when they can be restored.

HARTMANN then attempts to specify the physiology and chemistry of the processes taking place in REM sleep. HARTMANN believes that pathways which might require REM sleep for restoration are likely to involve particularly the frontal lobe of the cortex; probably the ascending pathways from midbrain to cortex: and specifically the catecholamine containing pathways of the medial forebrain/

/forebrain bundle (HARTMANN et al, 1971a; 1971b). Low catecholamine levels increase REM time, while high ones tend to decrease REM time (HARTMANN et al, 1971a; 1971b). Thus sleep has a homeostatic feedback role in restoring brain catecholamine systems. The most likely way in which restoration of these cortical systems could be accomplished is by changing receptor mechanisms, or other aspects of the physical structure of the synapse, in order to render it again more sensitive to catecholamines or more efficient in its activity.

In addition to making use of proteins or other macromolecules previously synthesised, the restructuring of the synapse is definitely a mechanism by which cortical systems depending on catecholamines could not only be restored to previous sensitivity, but could be altered by changing the conductivity or the formation of new inter-connections. This could therefore easily be related to the reconnecting and memory functions of REM sleep. This theory, however, cannot be experimentally verified directly, and HARTMANN'S theory is based on a chemical mechanism whereby he believes that REM sleep could function in this restoration, perhaps by means of synaptic rebuilding using macromolecules previously synthesised, of the catecholamine dependent systems which play a role in physiological mechanisms including attention, and guidance during waking. I have considered this theory in detail, since one of the important points is that it does take into account the existence of the two states of sleep, and their temporal relationship.

separate functions are suggested for REM and NREM sleep and they are related in such a way that NREM sleep, responsible for anabolism and the production of macromolecules, must precede REM. During REM sleep, some of the products synthesised in NREM may then be used. The theory is compatible with phylogenetic aspects of the states of sleep since it is only in mammals in particular that cortical and corticopetal connections involving the flexible "self-guidance" systems can be assured to be present.

Within mammals, however, there are no clear differences in the amount of time spent in REM sleep per 24 hours, that can be related to the complexity or flexibility of behaviour of the particular species. In man, the changes occuring in mental deficiency and senility are consistent with this theory. The theory is also compatible with the ontogeny of sleep states since a young animal or human probably carries out more programming and rearrangement of the CNS than in old age. The higher levels of cortical and forebrain arousal during REM sleep are compatible with these views, since times of increased memory consolidation and learning have been associated with high levels of cortical arousal.

One of the predictions made by HARTMANN is that inhibitors of macromolecule or protein synthesis would grossly interfere with both the functions of REM and NREM sleep. However, PEGRAM et al (1972) and STERN et al (1972)

/al (1972) have reported conflicting results. Since it is postulated that the restorative functions attributed to REM sleep depend on materials being made available during the previous SWS period, REM deprivation effects might also result from SWS deprivation making the conditions difficult to separate. Thus a control for REM deprivation would not be stage 4 deprivation but perhaps awakening from stage 2, later on in the night, at approximately the same time as the REM deprivation awakenings. The restorative functions of SWS are postulated to precede REM, therefore if appropriate tests were carried out to investigate the physical restoration produced by SWS defects should be found after SWS deprivation but not after REM deprivation.

(3) EXPERIMENTS USING SELECTIVE SLEEP DEPRIVATION I: ANIMAL STUDIES.

DEMENT (1965) provided a preliminary description of a learning impairment following REM deprivation, using cats in a Y-maze. This study contained few subjects and control data were not reported. Studies with animals for the most part suffer from design faults since it is often difficult to provide adequate controls. A technique frequently used for REM deprivation is to allow the animals to sleep on a small platform. The muscle atonia accompanying REM sleep causes the animal to roll over onto its side and thus fall off the platform, usually into surrounding water. Since this occurs every time they/

enter the REM state, they are efficiently deprived of REM sleep. ALBERT et al (1970) suspected that inferior performance by REM deprived animals might be due to exposure to water and he subsequently showed that rats soaked in water for one hour prior to training performed at a level significantly lower than animals not soaked. Results from animal experiments where this technique has been used should be regarded with extreme caution.

JOY & PRINTZ (1967) carried out some of the pioneering animal experiments using rats. Rats were deprived during 24 hours following a seven day acquisition period (AQP) during which they received either one or two trials on a shock avoidance task (SAT). One control group was put through a stress-inducing large platform procedure and a second control group lived in sawdust filled cages. These two groups plus the experimental group were not found to differ in task performance during the seven day AQP. Following the AQP each rat in each group was given a retention test (RT) at two, four, six or eight weeks following the original learning (OL). All rats were kept in sawdust-filled cages for the periods preceding the first and second RTs. On both these occasions, rats exhibited retention significantly inferior to either control group. Rats were kept in the sawdust cages until four days prior to the third RT. Each group of rats was then put through the same treatment that they had received during the AQP. The third RT failed to show differences in performance in the experimental and control groups./

For the following two weeks rats again lived in sawdust filled cages, and on the fourth RT again there were no significant differences between the three groups. Thus REM sleep was not related to performance or long term retention within the experimental conditions and it was concluded that the experimental and control RDs found on the first two testing sessions were a result of state dependent learning rather than a consequence of impaired memory consolidation. Differences between experimental and control rats might have become apparent had an extensive number of learning trials been given every day, had the REM sleep deprivation not preceded the final acquisition trial, or had the learning not been both preceded and followed by REM deprivation.

In a second experiment, JOY & PRINTZ used two groups of rats subjected to a control procedure during the acquisition of an SAT. One group received normal sleep prior to testing and the second group were REM deprived for several days prior to testing. The two groups again did not show unequal retention. Thus if state dependency were operating in the first experiment, then it was only unidirectional, which is contrary to the two-way nature of state dependency traditionally described.

ALBERT et al (1970) demonstrated that learning in rats is not affected by prior REM deprivation. He also showed that retention in these animals is not influenced by REM deprivation following OL. Small platform experimental rats, stressed large platform controls,/

and unstressed controls were put through their respective procedures for five days prior to learning an SAT. The experimental and large platform groups were found not to differ in acquisition performance, and unstressed controls exhibited superior performance. The RD could therefore be due solely to the exposure to water. ALBERT then trained the rats in a multiple SAT to assess the relationship between REM sleep and retention. Rats were put through three days of small platform experimental procedure, large platform control or unstressed control procedure. Rats were tested without the intervention of recovery sleep. Retention did not vary between the groups and thus the JOY & PRINTZ findings were supported.

FISHBEIN et al (1971) claimed that REM deprivation caused memories to be more labile. The susceptibility of the memory trace to disruption by ECS decreases as the interval between learning and the application of the amnesic treatment is increased (see Chapter II). Mice in FISHBEIN'S experiment were subjected to continuous REM deprivation for 48 hours after training. On a 24 hour RT, mice displayed an RA gradient that varied with the interval between the termination of REM deprivation and the administration of the ECS. Mice receiving ECS up to one hour after being removed from the REM deprivation situation displayed a marked RT when compared with mice given ECS without REM deprivation. Mice given ECS between three and twelve hours after REM deprivation displayed no memory loss. Evidence from this study indicates that the/

memory trace of a previously learned experience can remain susceptible to disruption for several days after training if the animals are REM deprived in the interval, although memory processes are normally susceptible to disruption only for brief periods of a few hours after training. FISHBEIN (1970; 1971) has given evidence that neurochemicals important for maintaining LTM could be synthesised and used during REM sleep in mice. FISHBEIN et al (1971) have provided further evidence that processes occuring during REM sleep influence the mechanism underlying the storage of LTM.

The simplest form of "coping" such as the avoidance situation used by JOY & PRINTZ and ALBERT, might not be dependent upon REM sleep since this would not be consistent with survival (PEARLMAN, 1971). The utilisation of more complex bits of information based on previous experiences which have recently become important to the organism might involve REM. As formulated by DEWAN, reprogramming of this nature would interfere with performance of existing programmes if both were to occur simultaneously during waking life. Thus reprogramming could be accomplished more effeciently during REM sleep. Results from FISHBEIN'S experiments therefore support DEWAN'S hypothesis.

PEARLMAN (1971) used a latent learning situation where rats were given daily trials with no reward. These showed little reduction in running speed or entry into a blind alley when compared with rewarded animals. If the unrewarded group were then rewarded, their performance on/

the following day improved and it was then indistinguishable from the regularly rewarded group. Thus the animals, unrewarded initially, seemed to have acquired a latent knowledge about the maze. PEARLMAN then interpolated REM deprivation between the rewarded trial and the one the following day. REM deprivation prevented the integration of the unrewarded maze exploration with the reinforcement experience. Results of a one trial appetitive procedure plus REM deprivation did not produce a RD. However, REM deprivation would produce a drive state, sensitising the animal to environmental stimuli with a resultant increase in exploratory behaviour (ALBERT et al, 1970).

STEINER & ELLMAN (1972) found an increased rate of hypothalamic self stimulation after REM deprivation, which suggested an alteration in motivational state. However, another plausible explanation is that the learned activity closely resembled instinctive behaviour, and could be thus unaffected by REM deprivation. The REM state therefore appears to be involved at a more complex level such as in latent learning and two-way-avoidance (PEARLMAN, 1971).

STERN (1971a; 1971b) conducted five well-controlled experiments to investigate acquisition following REM sleep deprivation in rats. Rats were REM deprived for five days by small platform confinement. Acquisition of three tasks, passive avoidance, active avoidance and appetitive alternation was slower following REM deprivation after normal or stress control conditions. Sleep loss controls, having reduced NREM sleep, performed as well as normals and better than REM deprived rats on the two SATs. Thus five days of/

REM deprivation did appear to produce RDs which were not due to non-specific stress, NREM sleep, or changes in activity level. Disruption of the hippocampal or temporal lobe activity by lesions or electrical stimulation has been reported to impair acquisition of passive avoidance and alternation responding in rats (GODDARD, 1964; McLEARY, 1966), and STERN suggested that perhaps REM deprivation acts in a similar manner. During REM sleep, the hippocampus exhibits theta activity. Absence of this activity with REM deprivation could result in altered hippocampal excitability in the waking state and consequently impairments in acquisition. Since the learning impairments occurred in three different tasks, it is also unlikely that these effects were due to some unique property of the testing situation.

Secondary effects of REM deprivation make it difficult to determine whether the deficits following deprivation are the results of impaired memory and learning or simply impaired performance (WOLFOWITZ & HOLDSTOCK, 1971). These authors found that three days of REM deprivation followed by a 48 hour recovery period did not interfere with retention of avoidance. If ECS was administered immediately after the REM deprivation, then animals exhibited no memory of the task following the 48 hour recovery period. Their results supported the hypothesis that memory is held in a labile form during REM deprivation, and permanently consolidated following the deprivation. It could be argued that the ECS and not the REM deprivation was the critical factor in the disruption of consolidation. However, ECS was/

administered 72 hours following learning and it has been shown that ECS does not interfere with consolidation unless administered less than one hour after learning. REM sleep deprivation may also have rendered the ECS a more effective amnesic agent.

Another factor to be considered is that certain forms of learning such as passive avoidance may be inappropriate, since increases in non-specific levels of activity can produce results indistinguishable from impaired acquisition of passive avoidance behaviour (HARTMANN & STERN, 1972). Animals are also more active at the end of deprivation (STERN, 1971a; 1971b). HARTMANN & STERN used a one-way active avoidance task, and here the increased spontaneous activity would, if anything, have produced an outcome which could be interpreted as better performance or faster learning. Thus, the acquisition impairments found following REM deprivation must have overcome this non-specific activity effect.

HARTMANN & STERN (1972) were interested in discovering whether the REM deprivation effect would be altered by administration of L-dopa. L-dopa has been shown to have multiple effects on brain neurochemistry (CHALMERS et al, 1971; ORZECK & BARBEAU, 1970). The learning deficit produced by REM deprivation was reversed by administration of L-dopa. L-dopa, given to normals also produced an avoidance acquisition deficit, but when L-dopa administration was combined with REM deprivation normal acquisition resulted. L-dopa was also found to counteract the deficit in avoidance acquisition following α-methyl paratyrosine (AMPT) /

administration (MOORE & REICH, 1967); SCHOENFELD & SEIDEN, 1969), therefore the mechanism could operate via the increase in brain catecholamines produced by L-dopa. The reason L-dopa alone interferes with acquisition in normals is uncertain but it is known to have a variety of peripheral effects (BUTCHER & ENGEL, 1969). Drugs which enhance catecholamine activity such as amphetamine, imipramine and the monoamine oxidase inhibitors (MAOIs), were able to restore memory in mice treated with puromycin one day after training (ROBERTS et al, 1970). Amphetamine and other antidepressent drugs also facilitate learning (DOTY & DOTY, 1966; KULKARNI, 1968; LATZ et al, 1966; MARK et al, 1969). HARTMANN & STERN'S results support the view that catecholamines participate in new learning. Moreover, their results are consistent with the view that REM deprivation produces a deficit in the functioning of neuronal systems, a condition which is reversed by L-dopa administration.

SLOAN (1972) investigated possible permanent neurological and behavioural alterations resulting from REM sleep deprivation. Maze-learning post-deprivation performance combined with a histological examination of the brain did not reveal the presence of any deprivation induced neuropathy. SLOAN also attempted to discover whether a rise in shock-induced aggression during deprivation (MORDEN et al, 1968) was paralleled by a corresponding rise in spontaneous aggression. This was found to be the case although SLOAN investigated induced rather than spontaneous aggression. However REM deprivation produced increased plasma corticosteroid levels (MORDEN et al, 1968). These may be the cause of/

the induced aggression since corticosteroids affect neural excitability which is related to aggression (WELCH, 1965). There were consistent increases in REM time following the deprivation period, thus the REM phase must have some neurological significance. SLOAN suggested that both REM and NREM sleep provide optimal conditions for neurochemical and metabolic processes but the results of this and other experiments suggest that these processes can be carried out successfully during waking if the need arises. Under conditions of REM deprivation or total sleep deprivation, the CNS could be operating under conditions of "overload" by carrying on these functions in addition to processing sensory input. It would be informative to compare animals requiring a greater need for REM sleep as evidenced by the number of awakenings and the post deprivation REM sleep to total sleep time ratio, with animals showing a lesser REM sleep need.

LECONTE & BLOCH (1970) found that REM deprivation for two days after training produced a marked RD with an incompletely learned shuttlebox avoidance task. PEARLMAN & GREENBERG (1973) replicated this observation using one day of REM deprivation followed by a recovery day. The recovery period allowed non-specific effects, eg. stress, fatigue and confinement, to subside before retention testing. The deficit could not be ascribed to impaired learning ability, since learning following retention testing was found to be normal. LECONTE & HENNEVIN (1971) recorded EEG following the avoidance procedure and found that the animals usually fell asleep rapidly, showing increased REM during the first three hours/

following training. HENNEVIN et al (1971) reported that this increase was specifically related to acquisition of the response. When a performance plateau, following commencement of training, was reached, the increase in % REM sleep, no longer occurred. These findings suggested a critical period for stage REM effects on consolidation in this situation. SMITH et al (1974) found that mice showed a prolonged increase in REM sleep preceding correct maze performance, and suggest two sleep mechanisms for learning. One is concerned with long-term REM sleep and the other with a short-term increase evident in the first half hour of sleep after the training session. FISHBEIN et al (1974) also showed evidence that learning induced a protracted augmentation of REM sleep, an effect which lasted for at least 24 hours.

PEARLMAN & GREENBERG (1973) investigated the effect of REM deprivation in just the few hours following learning. Since only a brief period of REM deprivation was involved, the animals had ample time to recover before retention testing. It was found that REM deprivation for two hours following training caused a marked RD. Thus the deficit which can be demonstrated one to two hours following training - Kamin effect - (BRUSH, 1970) might result from the disruption of the REM consolidation mechanism by the retention effect. Again, the complexity of the shuttlebox task might contribute to the vulnerability to REM deprivation (BOLLES, 1970), whereas the simpler conditioned avoidance as used by JOY & PRINTZ is unaffected.

The acquisition of avoidance conditioning can be modified by changes in the environment prior to training (SAGALES & DOMINO, 1973). Both stress and REM sleep deprivation had no effect on acquisition and short-term retention, but did impair long-term retention of the conditioned behaviour. Moderate environmental stress also produced significant behavioural changes. Total brain ACh, under altered environmental conditions, also changed significantly.

REM sleep deprivation also tends to produce a generalised enhanced drive state (DEMENT et al, 1967). This was supported when it was shown that REM deprivation had a significant inverse effect on latency but not accuracy in a T maze (HICKS & PAULUS, 1973 ; HICKS et al, 1973).

The involvement of REM sleep in memory and learning has been studied almost exclusively by means of shock motivated behaviour. HOLDSTOCK & VERSCHOOR (1973) found a complete absence of effect of REM deprivation on retention of food motivated behaviour in a T maze, in agreement with the JOY & PRINTZ and ALBERT studies. Thus again there is a suggestion that the kind of task is an important variable in investigating the amnesic effect of REM deprivation. Although results point to an interactive effect between REM deprivation and the nature of the task, STERN (1971a) reported acquisition impairments which were not task specific, and PEARLMAN (1970) found RDs in a complex maze.

(4) EXPERIMENTS USING SELECTIVE SLEEP DEPRIVATION II: STUDIES WITH HUMANS

(a) The relationship between sleep and memory.

JENKINS & DALLENBACH (1924) conducted the first experiments investigating the relationship between memory and sleep and wakefulness in man. Nonsense syllable lists were first learned and recalled later. In the waking condition, lists were learned to criterion in the morning and in the sleep condition the lists were learned at night. In both conditions, four different time intervals between learning and recall were used, ie. one, two, four or eight hours. In the waking condition there was an initial sharp decrement in recall followed by a slower decline. In contrast, results from the sleeping condition showed a slight drop, followed after the two hour interval by very little forgetting. At each interval there was a difference between the amount of forgetting in the sleeping compared to the awake state. Thus JENKINS & DALLENBACH concluded that forgetting is not so much a matter of the decay of old material, as interference, inhibition or obliteration of the old by the new. Although this study indicated that RI might be occurring while awake, it is by no means proof that other forgetting processes are absent. Since there is impairment in the first two hours of sleep, some positive process in sleep occurring after the first two hours might actively consolidate the memories. This is a function that has been specifically proposed for REM sleep. Sleep does not always occur immediately on going to bed so this might be a period of interpolated, interfering metal activity. There are /

also many methodological flaws in the experiment:-

(i) learning was always terminated when subjects could no longer recall all the items, and thus the free reproduction test was not administered until subjects believed they could successfully recall all items. Consequently different subjects underwent different degrees of overlearning.

(ii) diurnal variations in learning and recall ability could account for the differences.

(iii) no statistical tests were applied and thus only trends could be reported.

VAN ORMER (1932) required subjects to learn three lists of nonsense syllables to criterion by serial anticipation from a memory drum. After one, two, four or eight hours of either sleep or wakefulness, subjects relearned the lists to criterion. Savings scores were calculated with a correction factor for independently established diurnal variations in learning ability. Results showed statistically significant superior performance by subjects having slept, only after an OL to RL interval of four or eight hours. Almost no differences were seen after one hour, and after two hours a trend in favour of sleeping subjects was found. No further forgetting appeared after the first hour of sleep.

McGAUGH & HOSTETTER (1961) demonstrated that the difference between retention after sleeping or waking intervals was not shown if sleep occurred between eight and sixteen hours following OL.

NEWMAN (1939) tested subjects for retention of a one paragraph meaningful story. Sleeping subjects were found to be less likely to forget non-essential material, but did not show superior retention for the essential elements of the story.

EKSTRAND (1967) investigated the effect of sleep on the recall of a single list, and investigated the relationship of sleep to RI and PI, introduced prior to eight hours of ensuing sleep or wakefulness. Subjects learned either one or two lists of paired associates to criterion and the lists were recalled following the sleep or wakefulness interval. Subjects learning the two lists, recalled either the first (RI condition) or the second (PI condition). For each list, four successive recall tests, without feedback were given. Sleep following the learning of a single list significantly reduced the amount of forgetting. This difference appeared in the first two of the four recall tests. Recall of the first of the two lists was significantly enhanced, RI was reduced, by the intervention of eight hours of sleep. This may have been due to the spontaneous recovery of extinguished first list responses, but this is not a strong or reliable phenomenon and is therefore unlikely. Sleep did not seem to enhance the recall of second list responses and EKSTRAND does not report statistical analysis of the effect of sleep on PI.

The finding that forgetting takes place at a rapid rate during the first one or two hours of sleep could be due to:- /

(a) forgetting occuring during the length of time taken to fall asleep.

(b) the first REM period or the first one or two hours of sleep somehow stop forgetting by inducing consolidation.

(c) two memory stores are involved and information is lost from the STM store during sleep and wakefulness, but from the LTM store only during wakefulness. This does not imply that transfer from STM to LTM store takes place more efficiently during sleep but presupposes that material in the LTM store is less susceptible to weakening during sleep.

WILKINSON (1964) orally presented subjects, following 60 hours of sleep loss, with lists of numbers to be learned to a criterion of two faultless anticipations. Errors preceding the attainment of criterion rather than the number of trials to reach the criterion were measured and this was not influenced by the sleep loss.

WILLIAMS & WILLIAMS (1966) orally presented subjects with lists of monosyllabic words and short term retention of these was compared following one or two nights of sleep deprivation with controls. Control groups were supplied by the summed average of the retention exhibited by the same subjects on the day before the first night of sleep loss and the day following the first night of recovery sleep. Short term retention was impaired by one night of sleep loss and even more so after the two nights. Poor sleepers showed performance inferior to controls after 32 hours of sleep deprivation whereas normal sleepers showed impaired performance only following 56 hours of deprivation.

Impairment of long-term retention was also found following sleep loss (WILLIAMS et al, 1966). Subjects were given lists of information items every day, over an eleven day period of baseline sleep, sleep deprivation, and recovery sleep. Controls were not sleep-deprived. Feedback was provided after the first recall test given ten minutes following OL but not after the second test. 24 hours after OL, the third recall test was given. An index of the forgetting occurring over the 24 hour period was calculated by subtracting the absolute number of correct items on the third recall test from the scores obtained on the second test. Sleep-deprived subjects showed more forgetting than their matched controls when OL followed 27, 51 or 75 hours of sleep loss. The two groups did not differ in retention performance when learning occurred prior to the first night of sleep loss or after the first night of recovery sleep.

It seems therefore that it is necessary for learning to be both preceded and followed by sleep loss if a 24 hour RD is to occur. These findings may be results of failure in storage or retreival. Failure in storage could reflect difficulties at the STM or the LTM levels. A second study by WILLIAMS et al, failed to control for the amount of OL. Subjects were allowed ten seconds to examine 25 different pictures, EEG and visual observations ensured that lapsing did not occur during the ten second period. 24 hours later, subjects were asked to choose the 25 pictures from a much larger array on the following occasions:-

(a) two days prior to sleep loss.

(b) on the day preceeding the night of sleep loss.

(c) on the day following the night of sleep loss.

(d) on the day following the night of recovery sleep.

The 24 hour recognition scores from the first and fourth occasions were combined to form a control baseline estimate of retention performance. Subjects did not show an RD when learning preceded recognition testing following a single night of sleep loss. Subjects learning following the night of sleep loss and given RT following the night of recovery sleep showed a significant RD. These results indicate that the night of sleep loss following learning does not impair retention when learning occurs five and a half to eight and a half hours prior to the time of the normal onset of sleep. Thus sleep loss causes impairment of ordered material shown to be registered in immediate memory. Sleep loss therefore appears to interfere with the transfer of material from immediate memory to the STM store. WILLIAMS et al (1959) also suggest that sleep loss interferes with transfer from STM to LTM.

PORTNOFF et al (1966) claimed that NREM sleep interferes with memory consolidation. They awakened subjects from NREM sleep by the presentation of a word which subjects repeated to ensure stimulus registration. On some occasions subjects were required to perform a motor task before going back to sleep. Each morning retention was assessed by a free recall task followed by a recognition task. Words whose presentation was not followed by a motor/

task were recalled less well on both tests. The consolidation theory provides an alternative explanation of these results. Thus if memory is dependent on the occurrence of a fixation process, which continues for some time following the cessation of perception, this might be more effective during the waking state than during NREM sleep. Subjects would be in a different state of arousal following perception of the stimulus words in the two conditions. Results of studies by PARE (1961) suggested that the degree of consolidation may be directly related to the level of arousal during the consolidation period.

SAMPSON (1966) studied the effects of sleep deprivation and REM deprivation on human immediate memory. Prior to undergoing three nights of sleep curtailment to two and a half hours, or REM interruption, baseline measures of forward and backward digit span performance were established. Subjects then received three treatment nights including a control of uninterrupted sleep in counterbalanced order. Neither of the manipulations resulted in performance decrements.

(b) The effect of REM deprivation on memory and learning.

Different approaches to the effects of REM deprivation on memory and learning have been carried out:-

(i) the effects of REM deprivation on memory and learning following the deprivation.

(ii) the effects of learning on the ensuing sleep patterns, in particular the percentage, duration and intensity of REM sleep.

(iii) subjects can be compared for learning ability over the first or second halves of the night. The rationale behind this is that the first half of the night is high in SWS and low in REM %, whereas the second half of the night contains almost no stage 3 + 4 but is high in REM %.

If memory consolidation takes place during REM sleep, then REM deprivation between learning and recall should interfere with memory. With respect to learning, it has also been suggested that REM sleep provides a necessary precondition (meta programming) for subsequent wakeful acquisition. REM deprivation should therefore interfere with subsequent learning of novel tasks.

FELDMAN & DEMENT (1968) reported an LTM decrement in retention of a learned task after one night of REM deprivation. Two possible relationships between REM sleep and memory consolidation were investigated by FELDMAN (1969).

(i) Memory consolidation takes place or is initiated during REM sleep.

(ii) REM sleep may act to prepare the nervous system for consolidation of information learned the following day.

Though his results were statistically significant, they were often contradictory. REM deprivation may lead to impaired or facilitated post deprivation learning. Savings in material learned following the institution of the experimental or control procedures may be either impaired or facilitated. On some occasions, REM deprivation did not affect post-deprivation learning or retention of material learned following deprivation. This seemed to depend on the/

type of material presented, and the type of learning procedure used. REM deprivation did not influence retention of material learned prior to the deprivation.

GREENBERG et al (1970) studied subjects who were REM deprived for three nights. No differences were found in the learning of the word lists after these three nights. ADELMAN & HARTMANN (1968) achieved an almost total absence of REM sleep by the administration of 100 mg. amytriptyline and reported a decrement in STM for nonsense syllables.

CHERNIK (1972) carried out a study in which sixteen subjects were REM deprived for two consecutive nights after having learned a list of paired associate adjectives. Yoked controls were awakened the same number of times but not from REM sleep. Retention of half of the list was tested after the deprivation nights, and retention of the other half was tested the morning after the recovery night. A second verbal learning task, a series of trigrams, was learned on deprivation day and recalled on the day after a night of recovery sleep. Mood was assessed using Clyde & McNair Mood Scales, and perceptual motor performance and a cancellation test were also assessed, before and after the deprivation and after recovery. There were no significant differences between experimental and control subjects on any of the RTs, learning, mood or performance. REM sleep was reduced to 2.8% and 3.6% of the total sleep on the first and second deprivation nights compared to 20% and 23% for the controls.

MUZIO et al, (1971) found no significant differences in recall, between conditions of REM deprivation or sleep interruption. There was however a significant reduction in the performance of both these groups when compared with performance of subjects allowed uninterrupted sleep. The finding that for two out of three post sleep recall tests both the REM deprived and the sleep interrupted groups had significantly reduced scores, did not support the hypothesis that REM sleep provides necessary conditions for learning. These authors conclude that any interference with normal sleep will result in RD.

Results of experiments by PORTNOFF et al (1966); BAEKELAND & LASKY (1968) and KOUKKOU & LEHMANN (1968), report that waking rather than sleeping following new learning promotes better retention. Thus one interpretation is that consolidation is favoured by a certain level of cortical arousal. Arousal could therefore be greater during waking and REM sleep than NREM. STONES (1973) also investigated the role of arousal on the memory process. His experimental design incorporated a measure of immediate retreival, and of delayed retreival when learning was either followed by, or not followed by, a period of NREM sleep. Subjects attempted free recall of verbal material immediately after, and after a 20 minute delay, on two consecutive nights. They were instructed to verbalise their thoughts as completely as possible throughout list presentation. In a balanced design learning was preceded by either wakefulness or 30/

minutes sleep. Delayed memory and percentage forgetting were found to be impaired after the sleep condition, as were rehearsal and recoding. A measure of rehearsal and recoding was found to be related to the delayed recall and forgetting but not to immediate recall. Thus the memory loss after sleep could, in part, be attributed to a failure in rehearsal and recoding at the time of learning.

YAROUSH et al (1971) investigated the effect of the first four hours (high NREM, low REM) and the second four hours (low NREM, high REM) of sleep on memory. Three groups of subjects learned paired associate word lists and were tested for recall after the four hour retention interval. The third group (awake) learned in the daytime. The results showed that the first half night condition was consistently superior on several measures of recall. The second half night and the awake condition did not differ significantly from each other. However, artifacts due to the time at which the tests were given and the retention measured in the different groups could also explain the results (BARRETT & EKSTRAND, 1972). This is particularly true in view of the effects of circadian and other biological rhythms (LUCE, 1970) on a variety of behaviours. Forgetting over intervals of sleep or wakefulness, where intervals for both conditions occurred at the same time of day were compared by BARRETT & EKSTRAND. One sleep condition with high stage 4 and low REM was compared with another containing high REM and low stage 4, both run at the same time of day. Retention of paired associates in/

these groups, as well as one group which had remained awake, were compared. The retention interval was from 2.50 till 6.50 am. Memory over the sleep intervals was found to be superior to awake, and retention over the high stage 4 interval was superior to the high REM interval. Results differ from the YAROUSH study in that memory over the second half of the night, while inferior to the first half, is superior to the awake condition. Their data on sleep stages suggest that either stage 4 facilitates memory or that REM sleep inhibits it, or both. REM sleep could be acting in the manner of an interpolated interfering experience.

HOCKEY et al (1972) attempted to reduce the confounding variables by comparing retention during wakeful and sleep periods for two sets of time intervals. Subjects were assigned to one of four conditions:-

(a)	night sleep	11.00 pm to 04.00 am
(b)	night awake	11.00 pm to 04.00 am
(c)	morning sleep	06.30 am to 11.30 am
(d)	morning awake	06.30 am to 11.30 am

Night conditions yielded less percentage loss than morning conditions. Night sleep percentage loss was less than night awake or morning awake percentage loss. There was no difference between morning sleep and morning awake percentage loss. The authors concluded that time of day is more important than sleep versus wakefulness and that a simple wake interference hypothesis is inadequate. Perhaps night sleep with its high predominance of NREM, unlike morning sleep,/

predominantly REM and stage 2, preserves information via consolidation. Thus sleep per se does not seem to be crucial to the reduced forgetting found at night, and is of no benefit at all if taken in the morning. In the light of the BARRETT & EKSTRAND study, it is only in the absence of prolonged REM sleep that sleep is more beneficial than wakefulness for retention of certain kinds of material. REM sleep appears to interfere with retention in the same way that waking does. Two more experiments by FOWLER et al (1972) also demonstrated that memory over an interval containing relatively high amounts of REM sleep was inferior to memory over an interval containing relatively high amounts of stage 4 sleep. These authors believe that, for humans at least, REM sleep does not facilitate memory consolidation and that stage 4 might be beneficial.

A study by GREISER et al (1972) was designed to investigate the adaptive function of REM sleep by examining the differential effects of REM sleep or REM deprivation on the recall of threatening and non-threatening material. They found that subjects who slept recalled neutral material better than those who did not sleep, and subjects being allowed REM sleep recalled threatening material better than those who were REM deprived. Their results also indicated that NREM sleep facilitated the recall of non-emotional material, whilst REM sleep dealt with material containing affective components.

The question of the type of material dealt with in REM sleep was also investigated by CARTWRIGHT et al (1973)./

On an immediate recall trial, "social interaction" adjectives e.g. dominant, and "social emotional" words e.g. warm, were equally high in recall value for control and REM deprived subjects. In the morning however, (delayed recall) these words were increased in frequency of recall for the REM sleep group, and reduced in frequency for the REM deprived group. The REM deprived group showed a rise in recall for individualistic words. CARTWRIGHT believes that REM sleep may therefore function for "reality interaction", which when restricted leaves subjects, "internally oriented".

STONES (unpublished) attempted to investigate aspects of the relationship between sleep and memory retention with the emphasis on the possible differential contributions within NREM sleep. No attempt was made to deprive subjects of NREM sleep, but a correlation was performed between percentage sleep stages and percentage retention. Retention intervals of different duration and different proportions of sleep and wakefulness were employed. He found a significant average correlation between the percentage retention and the percentage of stages 3 + 4 sleep, which he believed provides evidence for the facilitation of memory retention associated with stages 3 + 4 sleep relative to stage 2.

Perhaps the only experiments which provided evidence for a positive role of REM sleep in the consolidation of memories in humans were those of EMPSON & CLARKE (1970). Their theory is that REM sleep is a positive /

process of consolidation and does not merely protect the memory trace from new input as the interference theory of forgetting would predict. They believe that REM sleep might either allow "setting" of the trace or might allow for active updating and reorganising of the trace. If NREM deprived subjects showed better retention than the REM deprived subjects, then this would support the "positive action" theories rather than the "interference" theories. Also if the amount of organisational distortion was found to be greater in REM deprived subjects, then this would support the "reprogramming" as opposed to the "setting" theories.

Three sorts of material were learned and then recalled by subjects the following morning:-

(a) a list of nouns,

(b) sentences which were syntactically correct but meaningless,

(c) a prose passage.

The REM deprived group recalled less of all three types of material but this effect was only statistically significant in the case of the prose passage. The effect could be seen both in the number of words recalled and in the accuracy of recall. Measurements of restructuring of the word lists were both in the reprogramming direction. EMPSON & CLARKE concluded that REM sleep aids the recall of meaningful material, but not noun lists or meaningless sentences. Sentences described, as "meaningful" were/

those of correct English semantic and syntactical structure which made sense when read.

(5) DISCUSSION OF EXPERIMENTS INVOLVING SELECTIVE SLEEP DEPRIVATION.

Although most of the animal studies employing selective sleep deprivation have shown that REM sleep facilitates the consolidation of memories of certain types of learning task, most of the studies using humans have found that either there is no difference between REM deprived or NREM deprived retention scores or that those being REM deprived perform better than those being deprived of SWS. Thus human memory with its unique verbal aspects seems to be qualitatively differently affected by sleep stages compared with animal memory. GESCHWIND (1965) has pointed out that animal memory always involves cortical-limbic neuronal interconnections whereas human memory may involve wither cortical-cortical neuronal interconnections or cortical-limbic interconnections. Human cognitive learning and memory might involve only cortical-cortical interconnections and since the results of animal studies of REM deprivation frequently show a REM deprivation RD, GREENBERG (1970) suggests that REM sleep deals with cortical-limbic interconnections only. One of the ways in which this might be investigated would be to study patients who have lesions of cortical-limbic circuits such as post alcoholic Korsakoffs patients who have lesions of the mamilliary bodies disrupting the hippocampal cortical pathways. Such patients also have memory disturbances. GREENBERG et al (1968) found that/

the percentage of REM sleep in patients with recent Korsakoffs, was as high as 30% of the total sleep time, as opposed to patients with other chronic disabilities who had an average of about 20% REM. The high amount of REM sleep seen in patients with recent Korsakoffs could also, however, be a result of a alcohol withdrawal. The lesions producing the memory impairment might possibly also impair the normal functioning of the REM sleep mechanism.

Because animal learning involves cortical-limbic associations, it could be argued that animal learning would be more susceptible to REM deprivation impairments. Studies by STERN (1971a; 1971b); FISHBEIN (1970; 1971) and also by GREENBERG & PERLMAN (1973) confirm the role of REM sleep in the learning experience of animals.

The kind of memory storage involved with human REM sleep might also only involve cortical-limbic pathways and this would be concerned with emotionally meaningful memories. There have been several studies with humans which suggest that REM sleep serves an adaptive function involving memory for affectively loaded material (CLEMES & DEMENT, 1967; GREENBERG et al, 1969; 1970; 1972a; 1972b; WHITKIN, 1969).

GREENBERG (1970) observed that a dream consists of perceptual events and ROFFWARG et al (1962; 1966) reported correlations between eye movements and visual imagery in dreams. However this correlation has been/

recently refuted (JACOBS et al, 1971; MOSKOVITZ & BERGER, 1969; KOULACK, 1972). Blind patients who once possessed sight show a fading of visual imagery in their dreams over time (BERGER et al, 1962). Dreaming may thus have a connection with present day experiences rather than historical events. This also lends support to hypotheses for REM sleep which are connected with problem solving or "coping" capacities and mechanisms. GREENBERG believes that dreams are associated with perceptions experienced by subjects during waking but these are not exact replicas of the waking experience. Dreaming may serve to transfer these experiences from perceptual areas or STM to stable storage. Thus the perceptual areas are "cleared" so that they may be used again the following day. REM deprivation might then cause difficulty in learning new material or recalling recently perceived material, since the perceptual areas would be "saturated". In experiments by GREENBERG et al (1971) REM deprivation did not show any clear effects on memory of word association lists or nonsense syllables and from this and many other experiments it seems that REM sleep is not involved in purely cognitive memory.

Material recalled in dreams is often of an emotionally meaningful kind and it could be only this material which is dealt with in REM sleep. Additional support comes from the finding that there is hippocampal activation during REM sleep in cats, similar to that seen when the cat is awake and excited (PARMEGGIANI & ZANNOCCO, 1963 ; PASSOUANT & CADHILLAC, 1962). Hippocampal theta/

activity has also been related to learning and memory processes in animals (MEISSNER, 1966).

Studies by WHITMAN et al (1962) showed that the experimental situation is often represented in the dreams of laboratory subjects. WITKIN & LEWIS (1965) showed emotionally-charged films to subjects before going to sleep and found that material from the films was often incorporated into dreams. BREGER (1967) found that presurgical patients and patients in group therapy incorporated some stressful features of their experiences into dreams. These studies all show that emotional daytime experiences may be incorporated into dreams. GREENBERG et al (1968) showed subjects a stressful film at bed time and again on awakening the following morning. Previous studies have shown that there is considerable adaptation in terms of lessened anxiety under normal conditions on second viewing. REM deprived subjects, however, were shown by GREENBERG to adapt less than control groups. GREENBERG proposed that present in every day life are experiences, meaningful both in the context of the present moment and in terms of a reminder of earlier life experiences. Thus dreams might form experiences of the day into part of a memory "schema" in association with the earlier memories. These new experiences and the emotions which they involve would be dealt with in the same manner as the earlier experiences, and the same kind of defence mechanisms would be used which would integrate the behaviour of the organism. To determine the kind of psychological changes taking place/

with REM deprivation GREENBERG et al (1970) and FISS et al (1968) tested subjects under baseline conditions, REM deprivation and control awakenings. The greatest changes took place after REM deprivation, and the typical defences seen on baseline were lost. Material which had been well defended against in baseline appeared in a much more open fashion. Thus under conditions of REM deprivation, conflictual material is much less repressed.

As a corollory, this could be an explanation as to why so much of dream content is forgotten. If a function of REM sleep is to restore defences against "conflictual" material, then when this task is accomplished, the material is no longer accessible to conciousness. Only when REM sleep is interrupted or when the task cannot be completed because the conflict is overwhelming, or when defences are much reduced such as in illness or therapy, can the dreams be remembered. A counter argument would be that in conditions of contentment or serenity dreaming does not stop. Another possible corollary is that dreams per se may serve the purpose of bringing new perceptions or new experiences into contact with old memories, thus leading to modified patterns of information storage which can then allow for growth or change. It has been suggested that the addition of new information can lead to a higher percentage of REM sleep (DEWAN, 1969; ZIMMERMAN et al, 1972).

The possible role of sleep stages other than REM in memory consolidation has been little investigated./

Although as previously mentioned EKSTRAND et al compared REM deprived with stage 4 deprived and with a pseudo deprived group of controls, no significant effects on memory retention were found. However, the group deprived of stage 4 sleep still experienced a substantial amount (about 50% of normal) of stage 4. It is possible that the amount of delta activity contained in the sleep of the stage 4 deprived group was considerably in excess of 50% of the delta activity present in the EEG of the other groups. Thus the EKSTRAND et al study cannot be regarded as a crucial test of the hypothesis that NREM sleep stages contribute differentially to memory consolidation.

Similarly, the YAROUSH et al study indicated that memory retention was better over the first than the second half of the night. However, as discussed earlier, there were also many other intervening variables.

In the current literature there are contesting theories attempting to account for facilitation of memory storage. It has been suggested that the degree of interference during the retention interval is inversely related to the amount retained. Results of the previously discussed study by STONES could indicate that intereference was greater in stage 2 than during stages 3 + 4. Consolidation might also be greater during certain sleep stages (which would be 3 + 4 in terms of the STONES study). There is considerable evidence that consolidation does take place during sleep and a consolidation hypothesis does seem plausible. The greater/

loss over the later rather than earlier intervals of the night may be viewed in the light of a lower proportion of sleep stages 3 + 4 associated with the later intervals. It is also possible however, that circadian effects or a lower level of learning may have contributed. Although learning was preceded by sleep under all experimental conditions, the duration of prior sleep was greater for the later learning experiences. It has also been shown that prior sleep has an adverse effect on rehearsal and recoding (STONES, 1973).

CHAPTER VIII.

DISTORTED VISUAL INPUT AND SLEEP

(1) BACKGROUND

As has been already pointed out (see Chapter VII) it was hypothesised by OSWALD (1969) that REM sleep was a time for non specific synthesis within cerebral neurones. During sleep, at a time of minimum sensory input to the cortex, material from STM stores could be commited to LTM via the biochemical process of consolidation. I have speculated that this biochemical process could take the form of new anatomical connections being manufactured between cortical neurones and this of course would involve protein synthesis.

The hypothesis was that if human subjects were given a learning task which was very much greater than the amount of learning carried out during a normal day, then this would result in an increase in the percentage of REM sleep on the nights following the days where they had engaged in the increased learning.

A direct relationship between the amount of learning and the percentage of REM sleep was also predicted by DEWAN in his programming hypothesis (see Chapter VII). Although his theory did not contain reference to protein synthesis, he predicted that visual field inversion and distortion should result in increased REM sleep time, since it is a massive learning task and since subjects who exercised willed motor activity have been shown by KOHLER (1964) to be capable of adaptation to the distortion. Thus by inference some kind of memory storage process must have taken place.

A corollary to the theory that massive learning should increase REM sleep is that sensory deprivation should decrease the percentage, on the nights following the deprivation experience.

The appearance of DEWAN'S programming hypothesis gave rise to a number of experiments involving the use of distorted visual feedback.

ZIMMERMAN et al (1970) ran a total of eighteen subjects. The first group contained eight subjects, four of which (S1 to S4) experienced "moderate distortion" of the visual field. Objects were displaced horizontally from left to right but remained the right way up (visual field was 29 x 29 degrees). Subjects S4 to S8 experienced vertical inversion "extreme distortion" of the visual field with no left right reversal (visual field reduction was the same as above).

Subjects wore the distorting lenses for either one half day, one day, two days or three days. All subjects were instructed to carry out motor activity.

The night recording sequence was:

Throwaway: one night.

Pre-experimental baselines: two nights.

Experimental: one, two or three nights, consecutively.

Post-experimental baselines: two nights, at least one week subsequent to the experimental nights.

Results (see Table 2) showed that the group of subjects who had experienced horizontal displacement of the visual field, demonstrated no difference in the percentage of REM sleep across baseline or experimental conditions.

Table 2.

THE EFFECTS OF HORIZONTAL VISUAL DISTORTION "MODERATE" ON TOTAL SLEEP TIME AND PERCENTAGE OF REM SLEEP (from ZIMMERMAN et al, 1970).

	Time of experiencing distortion.	Baseline nights			Experimental nights		
		no. of nights	sleep time (mean)	REM %	no. of nights	sleep time (mean)	REM %
S1	½ day	4	454	24.7	1	461	18.7
S2	1 day	4	409	17.4	1	459	15.7
S3	2 days	4	464	29.8	2	446	25.7
S4	3 days	4	437	21.3	3	423	24.4
Moderate distortion (Group mean)			441	23.3		447	21.6

Table 3.

THE EFFECTS OF VERTICAL VISUAL DISTORTION "EXTREME" ON TOTAL SLEEP TIME AND PERCENTAGE OF REM SLEEP (from ZIMMERMAN et al, 1970).

	Time of experiemcing distortion.	Baseline nights			Experimental nights		
		no. of nights	sleep time (mean)	REM %	no. of nights	sleep time (mean)	REM %
S5	½ day	4	391	23.8	1	467	28.1
S6	1 day	4	470	22.5	1	447	29.1
S7	1 day	4	386	19.3	1	468	28.2
S8	3 days	4	473	23.4	3	472	27.5
S9	5x½ days	4	468	18.2	5	467	21.1

In the group of four subjects experiencing vertical inversion of the visual field, each of these showed a clear increase in the percentage of REM sleep (see Table 3).

On the basis of these results ZIMMERMAN et al hypothesised that the extremeness of the visual distortion might be an important variable in producing the increased REM sleep. Therefore they ran 10 additional subjects each of whom underwent "extreme alteration" of the visual field.

One subject S9 wore the upside down inverting spectacles for 5 x $\frac{1}{2}$ days on five consecutive days for eight hours a day.

S10 to S13 did not wear prisms fitted into goggles but instead wore a lightweight metal headband with a visor-like reflecting mirror attached at the front at the level of the subjects eyebrows. With this device the subjects looked through a narrow slit where their visual environment could be seen as an inverted image approximately 180 degrees in width.

S14 to S18 observed the visual field rotated through 90 degrees, a rotation which produced an extreme alteration from the normal visual environment.

The sequence of nights was:

Throwaway: one night.

Pre-experimental baselines: two nights.

Experimental: number of nights contingent upon the number of days that subjects wore the distorting lenses.

Post-experimental baselines: two nights recorded at least one week subsequent to the experimental nights.

The results are shown in Tables 4 and 5.

The reason for employing different "extreme" types of visual distortion was to see whether the effect was generalised or specific. Each of the subjects who experienced the visual distortion of the "extreme" type showed an increase in the percentage of REM sleep on nights following the experimental days when compared with baseline. This is in contrast to the "moderate" inversion where there were essentially no differences between the experimental and baseline nights.

Their results also showed that the latency to the first REM period was shorter on the experimental nights for 13 of the 14 subjects experiencing "extreme" distortion. Subjects experiencing "moderate" distortion also had shorter REM onset latencies on their experimental nights.

The most striking effect found by ZIMMERMAN et al was the sharp increase in phasic activity as estimated by counting the number of epochs of REM sleep periods containing eye movements. Total phasic activity was estimated by scoring the number of 4-second epochs of REM sleep containing criterion-sized eye movements.

Table 6 shows that all but one of the subjects experiencing "extreme" distortion displayed increased phasic activity on the experimental nights, two subjects in the "moderate" distortion group also showed phasic activity increases.

These results of ZIMMERMAN et al appeared to demonstrate that REM sleep can be substantially/

Table 4.

THE EFFECTS OF MIRROR, VERTICAL, VISUAL INVERSION "EXTREME" ON TOTAL SLEEP TIME AND PERCENTAGE REM SLEEP (from ZIMMERMAN et al, 1970).

	Time of experiencing distortion.	Baseline nights			Experimental nights		
		no. of nights	sleep time (mean)	REM %	no. of nights	sleep time (mean)	REM %
S 10	3 days	4	454	13.7	3	456	15.6
S 11	3 days	4	470	21.2	3	475	24.5
S 12	3 days	4	454	19.9	3	461	27.8
S 13	3 days	4	470	18.9	3	468	21.0

Table 5.

THE EFFECTS OF 90 DEGREE VISUAL ROTATION "EXTREME" ON TOTAL SLEEP TIME AND PERCENTAGE REM SLEEP (from ZIMMERMAN et al, 1970).

	Time of experiencing distortion	Baseline nights			Experimental nights		
		no. of nights	sleep time (mean)	REM %	no. of nights	sleep time (mean)	REM %
S 14	½+1 day	4	451	15.1	2	469	24.0
S 15	2 days	4	456	29.6	2	446	37.5
S 16	3 days	2	467	16.5	3	463	25.1
S 17	3 days	4	460	20.5	3	418	21.8
S 18	3 days	4	439	22.5	3	450	28.3
Extreme distortion (Group mean)			451	20.0		461	25.7

Table 6.

PHASIC ACTIVITY AFTER EXPERIENCING VISUAL FIELD DISTORTION (from ZIMMERMAN et al, 1970).

	Total phasic activity		
	Baseline nights (mean)	Experimental nights (mean)	% Increase.
Horizontal displacement (moderate distortion)			
S1	195	202	3.6
S2	116	172	48.3
S3	513	491	- 4.3
S4	426	617	44.8
Mean increase (moderate distortion)			23.1
Vertical inversion (extreme distortion)			
S5	260	406	56.1
S6	232	177	-23.7
S7	199	459	130.7
S8	296	529	78.7
S9	168	361	23.1
Mirror, vertical inversion (extreme distortion)			
S 10	125	165	32.0
S 11	297	444	49.5
S 12	350	662	89.1
S 13	241	298	23.7
90 degree rotation (extreme distortion)			
S 14	474	574	21.1
S 15	425	479	12.7
S 16	311	531	70.7
S 17	483	683	41.4
S 18	357	507	42.0
Mean increase (extreme distortion)			52.8

increased when subjects are exposed to certain types of distorted vision. The data indicated also that phasic acitivity increased more dramatically than percent REM sleep. These authors therefore concluded that the percentage of REM sleep is a relatively gross measure, phasic activity being a much more sensitive response to the stimulus events of the preceding day. In the group experiencing "moderate visual distortion", although there was no increase in the REM, two of the four did show an increase in the amount of phasic activity and all of the subjects in this group showed a decrease in the latency to the first REM period.

The authors drew the conclusion from this experiment that in seeking to determine relationships between waking experiences and REM sleep, observations of phasic activity would probably be more fruitful than simple measures of percentage REM sleep. They believed that REM sleep is in some way connected with the processing of sensory input, in particular novel sensory input, and is thus related to perceptual learning.

The authors admitted that certain other interpretations of the data were possible; and that increased REM sleep might be a result of:-

(a) stress. This is unlikely since GIBSON & BROUGHTON (1969) have shown that stress acts to decrease REM sleep rather than increase it.

(b) looking through a relatively narrow field. However, the four subjects who experienced the mirror vertical inversion in which the visual field was 180 degrees showed both an increase in the REM % and in phasic activity.

(c) any sustained change in visual stimulation.
(d) altered stimulation to the vestibular nuclei. POMPEIANO & MORRISON (1965) have shown using cats that the vestibular nuclei are essential for REM sleep, especially its phasic components.
(e) consequence of reticular formation stimulation resulting from the distorted vision experience. FREDRICKSON & HOBSON (1969) have reported increased REM sleep also in cats following sustained electrical stimulation in the reticular formation.

I would criticise this work further and point out that in all cases only one laboratory adaptation night was used. It is well known that the first two nights in the laboratory are often unusual, having more stage 1 and time awake and less stage REM (AGNEW et al, 1966; HARTMANN, 1970). Thus if one of the baseline nights was low in stage REM then this would lower the mean of the % REM on baseline nights, resulting in a spurious increase in REM sleep. Looking at the data of ZIMMERMAN et al, this does indeed seem to be the case. It is usually reckoned that the normal % REM sleep in seven and a half hours is between 20 and 25%. In the group experiencing the extreme distortion, seven out of the 14 subjects had less than 20% REM. Subject S10 had only 13.7% on baseline nights which is definitely not characteristic of a nights sleep, and S14 had 15.1%, again very low. When the experimental nights are considered, eight of the 14 have higher than average % REM. I would suggest/

that if another throwaway night had been used for each subject, the increases would not have been seen. Another consideration is that if sleep was disturbed or abnormal on baseline nights then there was likely to be a rebound increase in % REM resulting from the REM suppression during the low % REM night. If this rebound occurred on the experimental night, it could account for the differences found. Unfortunately I do not have the details of their individual night REM %. I also consider that the use of only two pre-experimental baseline nights is sufficient to allow the conclusion that a stable baseline has been reached. The same argument can be applied to the experimental nights. In two cases only one experimental night was used and the maximum was three. It cannot be said therefore with such a small group of subjects that the difference was not due to artifact.

When calculating the increase in REM % on the experimental nights, they compared the experimental night with the mean of the sum of pre- and post-experimental nights. The post experimental nights may, however contain an after effect i.e. REM % may be low on the post experimental nights. The percentage change resulting from this procedure might therefore be artificially very high.

Both of the 90 degree tilt subjects who showed the largest increases in % REM had baseline nights which are lower than the normal range i.e. they were only 15.1% and 16.5%. The % REM on the experimental nights of these two subjects was within the normal range i.e. 24.0% and 25.1%. One of these subjects had only two baseline/

nights and objections to this have already been pointed out. Only one subject showed a REM % on his experimental nights which was abnormally high i.e. 29.6%. The % REM for individual nights is not shown and so it is not known what kind of distribution in percentages was obtained, in any one set of conditions.

It is interesting to note that with the "horizontal displacement" subjects, the REM % decreased rather than increased. This is an inconsistency as presumably new learning still had to take place.

Another criticism is based on the fact that no statistical tests were employed in analysing the data and it is my experience that in experiments of this kind with human subjects the individual variation is rather large, especially when measuring such parameters as eye movement density. Thus even if there are differences, they may not be statistically significant.

A study using a different type of visual distortion was carried out by BOWE-ANDERS et al (1974). Their study was designed to examine the effects of long-term perceptual alterations on colour and illumination, which these authors have shown to be associated with changes in dream content, on physiological parameters of sleep (ROFFWARG et al, 1971).

Three subjects were used and each subject spent one adaptation night in the laboratory followed by an interval of seven nights. Four or five baseline nights were then recorded for each subject which were then immediately followed by four or five experimental days in/

which the filtering lenses were worn. These filtering lenses blocked all wavelengths of light shorter than 605 μ, allowing the passage only of the red/orange band. Thus colour was perceived exclusively in the red/orange spectrum. Illumination was reduced by 89%.

Nightly testing of the subjects indicated a uniform inability to differentiate between hues, and no progressive physiological adaptation in terms of colour perception took place.

Results showed that the percentage of REM sleep remained relatively constant in every subject throughout experimental and baseline conditions, as shown in Table 7.

The slight decrease in the latency to the first REM period, as well as the increase in REM intensity (see Table 8), failed to achieve statistical significance using the Freidman two way analysis of variance. In addition to the analysis of variance, these authors performed a power analysis (COHEN, 1969) to determine whether the failure to achieve statistical significance between baseline and experimental conditions resulted from the small numbers of subjects in the study rather than from the lack of experimental effect. In order to achieve statistically significant differences between experimental and baseline conditions at $p < 0.05$, an n of 20 would have been required for REM latency, 130 for REM intensity, 400 for REM time and more than 3000 for REM %.

Table 7.

THE EFFECTS OF ALTERATION IN COLOUR AND ILLUMINATION ON TOTAL SLEEP TIME AND PERCENTAGE OF REM SLEEP (from BOWE-ANDERS et al, 1974).

	Baseline nights			Experimental nights		
	no. of nights	sleep time (mean)	REM %	no. of nights	sleep time (mean)	REM %
S1	5	490.5	23.6	4	495.5	23.8
S2	5	475.9	21.6	3	641.5	21.0
S3	4	405.3	21.8	5	419.3	22.2
Group mean		457.2	22.3		458.8	22.3

Table 8.

THE EFFECTS OF ALTERATION IN COLOUR AND ILLUMINATION ON PHASIC ACTIVITY AND REM SLEEP LATENCY (from BOWE- ANDERS et al, 1974).

	Baseline nights		Experimental nights	
	REM intensity	REM latency	REM intensity	REM latency
S1	1.33	67.6	1.36	66.6
S2	0.72	82.4	1.00	78.3
S3	1.42	65.6	1.46	56.3
Group mean	1.16	71.9	1.27	67.1

It should also be noted here that none of the nights recorded under experimental or baseline conditions had percentages of REM sleep which fell outside the range that is considered normal, and thus the study differed in this important aspect from that of ZIMMERMAN et al. The authors point out that no physiological adaptation in terms of colour perception took place for these subjects though they do not give details of the method of assessment. This could be interpreted to mean that no learning process took place. Since the hypothesis undergoing investigation is that increased learning results in increased REM percentage, it was not surprising that there were no observed increases. The authors conclude that although subjects were functioning for four or five days under conditions of unabated colour distortion, lighting reduction, restriction of the range of peripheral vision, and a sense of psychological isolation, there were no demonstratable significant changes with regard to alterations in REM stage latency, time, proportion or intensity between baseline and goggle conditions. It should also be noted that the authors examined other sleep parameters and found no changes there either.

ZIMMERMAN & STOYVA (1971) reported to the APSS a study designed to answer the following questions:

(a) do distorted vision subjects who engage in active relearning of the visual motor skills show greater increases in REM sleep than do subjects who are relatively passive?

(b) Does REM sleep remain above baseline levels while subjects are readapting to normal vision, after having worn distorting glasses for one or more days?

Each of their nine subjects served under both an active and a passive learning condition. One the day of the active learning condition, subjects engaged in a great deal of visual motor activity, while wearing the glasses. The following day they returned to normal vision and this was a readaptation day. Sleep was recorded on the nights following both these days. After an interval of at least one week, subjects experienced another day of distorted visual input during which they were relatively passive and engaged only in minimal visual activity. This was followed by another readaptation day. Again, sleep was recorded after both days. In addition each subject had two laboratory adaptation (throwaway) nights; one pre-experimental baseline night; and one post experimental baseline night, the latter being recorded at least one week after the subjects' final return to normal vision. These authors do not state whether their records were scored blind.

The hypothesis that percent REM sleep would be increased to a greater degree in subjects undertaking active avoidance learning was not supported and six out of the nine subjects showed higher levels of REM sleep on the night following the passive visual conditions.

There was however, a clear readaptation effect. Each of the nine subjects showed higher levels of REM/

sleep on the two readaptation nights than on the two baseline nights, this was statistically significant at $p < 0.01$. This readaptation effect however, could also be interpreted to mean that subjects were better adapted to the laboratory conditions. The mean level of REM sleep was 19.1% on baseline nights and REM sleep on readaptation nights increased to a mean of 25.7%. In addition six subjects showed decreased REM latencies.

This study confirmed the earlier findings by these authors in that the percentage REM sleep on experimental nights increased above baseline levels. This increase was significant at $p < 0.01$. Experimental nights also showed significantly reduced latencies to the first REM period.

A further study was carried out by these authors to attempt to extend their findings. This time cats with chronically implanted electrodes were used. Cats wore a lightweight frame which positioned a vision-inverting lens system in frontof each eye. This condition was then followed by a period of at least five days with the cats wearing the same basic frame with the lenses removed to control for the decrease in visual field (tunnel vision) which accompanied the period of visual inversion. Cat C1 had the tunnel vision control, preceding the period of inverted vision.

Results were as follows:

Cat C1. The mean of ten inverted vision experiments was 4.41% lower than the mean of the ten baselines. Mean of the ten tunnel vision controls was 15.6% higher than baseline.

Cat C2. Mean of the ten inverted vision experimental recordings was 7.0% lower than the mean of the baselines, but the mean of five tunnel vision controls was 12.4% higher than baseline.

Cat C3. Mean of the ten inverted vision experimental recordings was 19.6% higher than baseline, and the mean of nine tunnel vision controls was 19.1% higher than baseline.

The tunnel vision control condition of all three cats was higher than in the baseline condition when the absolute amount of REM, in terms of minutes per day, was measured. For the cats C1 and C3, the mean number of minutes of REM in the experimental condition was greater than for baseline.

However, none of the three cats had a statistically significant increase in the percentage of REM sleep during the inverted vision period. It should be noted, however, that the study suffers from a design fault in that the tunnel vision period always followed the period of visual distortion. A stress effect should also be considered since the tunnel vision was probably less frightening for the cats than visual inversion.

Since any observed increase was matched by the tunnel vision period, the authors suggested that a homeostatic mechanism may have been operating to increase the percentage of REM sleep and in particular the number of rapid eye movements. They suggested that if it could be further ascertained that the tunnel vision condition suppresses eye movements in the waking/

state, then these findings would lend support to the binocular co-ordination theory of REM sleep advanced by BERGER (see Chapter III).

SCOTT (1971) reported that there are a number of environmental conditions which have been tested for their effects on REM time. These include one half, one or two days of isolation; exercise; no talking; and isolation of two subjects together. In every condition there is an enhanced REM time which was in every case significant at $p < 0.02$. At the same time REM onset latency was decreased and furthermore, there was an increase in body movement activity during the nights following the experimental days. Although the experimental nights were significantly different from baselines, the nights following the experimental nights were not significantly different.

There were 29 experimental nights with REM % greater than the median for the baseline nights out of a possible 42, and SCOTT points out here that 13 would have been expected by chance. However the 13 nights which were not above the median were not randomly distributed (as would be expected by chance). All but two of these nights below the median were located either at the beginning or the end of a subject's sequence of nights. This would seem to indicate some complex adaptation phenomena during the experiment. The remaining two nights below the median were reported by the subject as being particularly stressful. This author did not report details of experimental design or individual results.

These findings are not consistent with the social isolation hypothesis, but they are consistent with the programming hypothesis of DEWAN. Exposure to the experimental situation would require reprogramming and hence, REM enhancement. Stress may have either prevented or retarded the reprogramming. On the other hand, subjects who do not show any effects may have learned to be so adaptable that reprogramming is minimal. POTTER & HERON (1972) investigated sleep patterns during long-term perceptual deprivation and found that subjects slept excessively long hours in the beginning, during the first 24 hours about four hours of wakefulness was exchanged with stage 2 sleep. However, total sleep time as well as sleep stage distribution returned to normal levels by the fourth day. In particular neither the amount of REM time nor stage 4 showed any sustained effect of the deprivation procedure. Only REM density changed systematically, the longer the deprivation lasted the more eye movements per minute were recorded.

Thus it can be seen that some of the data supporting the hypothesis that distorted visual feedback increases REM sleep and also the corollary that sensory deprivation or isolation should decrease REM sleep are based on unsound experimental procedure or at best lend themselves to a large variety of different interpretation. The experiment of BOWE-ANDERS et al, which I consider to contain the most exacting design and data analysis, did not support the hypothesis. However, since their experimental conditions were not particularly "extreme" and since they were unable/

to demonstrate that any adaptation to the experimental conditions was taking place, the relationship between distorted vision and REM sleep still remains unresolved.

In my own experiment (to be described next) I used spectacles which rotated the visual field through 90 degrees and reversed it from left to right. This was described by ZIMMERMAN et al to be the "most extreme" type of visual distortion. Having worn these spectacles for a short while myself, I endorse that they provide visual conditions of great difficulty. When viewed through the spectacles, people appeared as if they were walking on walls, jumping over doors and going in the reverse direction to their actual path of traverse.

(2) EXPERIMENT TO DEMONSTRATE THE EFFECTS OF DISTORTED VISUAL INPUT ON SLEEP.

(a) Materials

Plastic spectacle frames were used and these were specially made by BUTTERWORTHS, Edinburgh. These frames held the inverting prisms which were very heavy. The frames had opaque sides in order to eliminate peripheral and sideways vision and had tubes on the front in order to hold the two dove prisms which could be slotted in when required. Plain glass could also be introduced into the tubes instead of the prisms and this was necessary for the tunnel vision controls. The tubes were at an angle of 45 degrees to the vertical axis which, when the prisms were in place, gave the required rotation of 90 degrees in an anticlockwise direction. The visual field when viewed through the tubes was reduced to 26 degrees/

The heavy spectacles were kept in place on the subjects' heads by means of an anaesthetists' facemask headband. The frames were prevented from marking the subjects faces or causing any unnecessary discomfort by carefully padding with foam rubber. The design of the dove prisms is shown in Fig. 11 and the dimensions are also indicated.

Technique for recording sleep.

Silver-silver chloride surface disc electrodes filled with Cambridge electrode jelly were used throughout for recording sleep. Nine electrodes were employed for each recording. Seven of these were attached to the faces of subjects for recording EOG and EMG. EEG was recorded from two electrodes attached to the scalp. Electrodes were attached to the face by means of micropore tape covered by zinc oxide plaster tape and scalp electrodes were attached using collodion glue.

Four electrodes placed on the face were used to record EOG, two were used for EMG and an earth electrode was placed in the middle of the forehead. For EOG recording, two electrodes were placed approximately supra orbitally, and two were placed approximately one centimeter below and slightly lateral to the outer canthus of each eye. Bipolar recordings were obtained by connections recorded from the upper electrode from one eye and the lower electrode from the contralateral eye. Thus a summation of horizontal and vertical eye movements was obtained as well as oblique eye movements.

Submental EMG electrodes were placed on the bony ridge beneath the chin./

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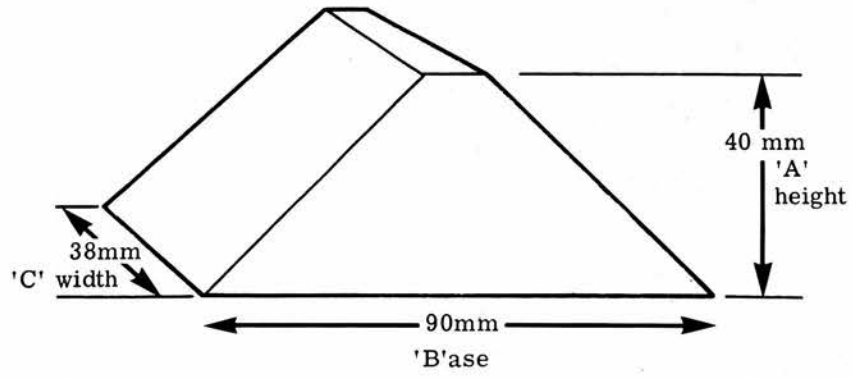

Fig. 11.

DISTORTED VISUAL INPUT AND SLEEP EXPERIMENT.

Details of prism lenses as used in the spectacles.

The scalp EEG electrodes were both placed in the mid line of the head, one towards the front of the head, in line from ear lobe to ear lobe and the other, just behind the crown of the head. The front corresponds to a position between F2 and C2 and the rear one corresponds to a position between the P2 and O2 positions of the international 10/20 system. Electrode placements are illustrated in Fig. 12.

Paper speed was 15mm. per second.

Settings for polygraphic recordings were :-

	EOG	EEG	EMG
Time constant	0.3	0.3	0.03
Filters	3.0	3.0	0.0
Gains	4.5	5.0	6.0

The subjects were three healthy, young, male, medical student, paid volunteers. They did not normally wear spectacles. They took part in the experiment simultaneously and were permitted to communicate with one another.

(b) Procedure.

Subjects reported to the laboratory, and during the subsequent 20 days they resided in the University of Edinburgh, Department of Psychiatry. The first two days were for laboratory adaptation and the EEG recordings obtained on the nights following these first two days were discarded for the purposes of data analysis. The pre-experimental period consisted of six days, the first to the sixth of the study, with corresponding nights of recorded sleep. During this pre-experimental period, subjects wore the spectacle frames but instead of carrying the prisms, the frames contained only plain glass of the same weight

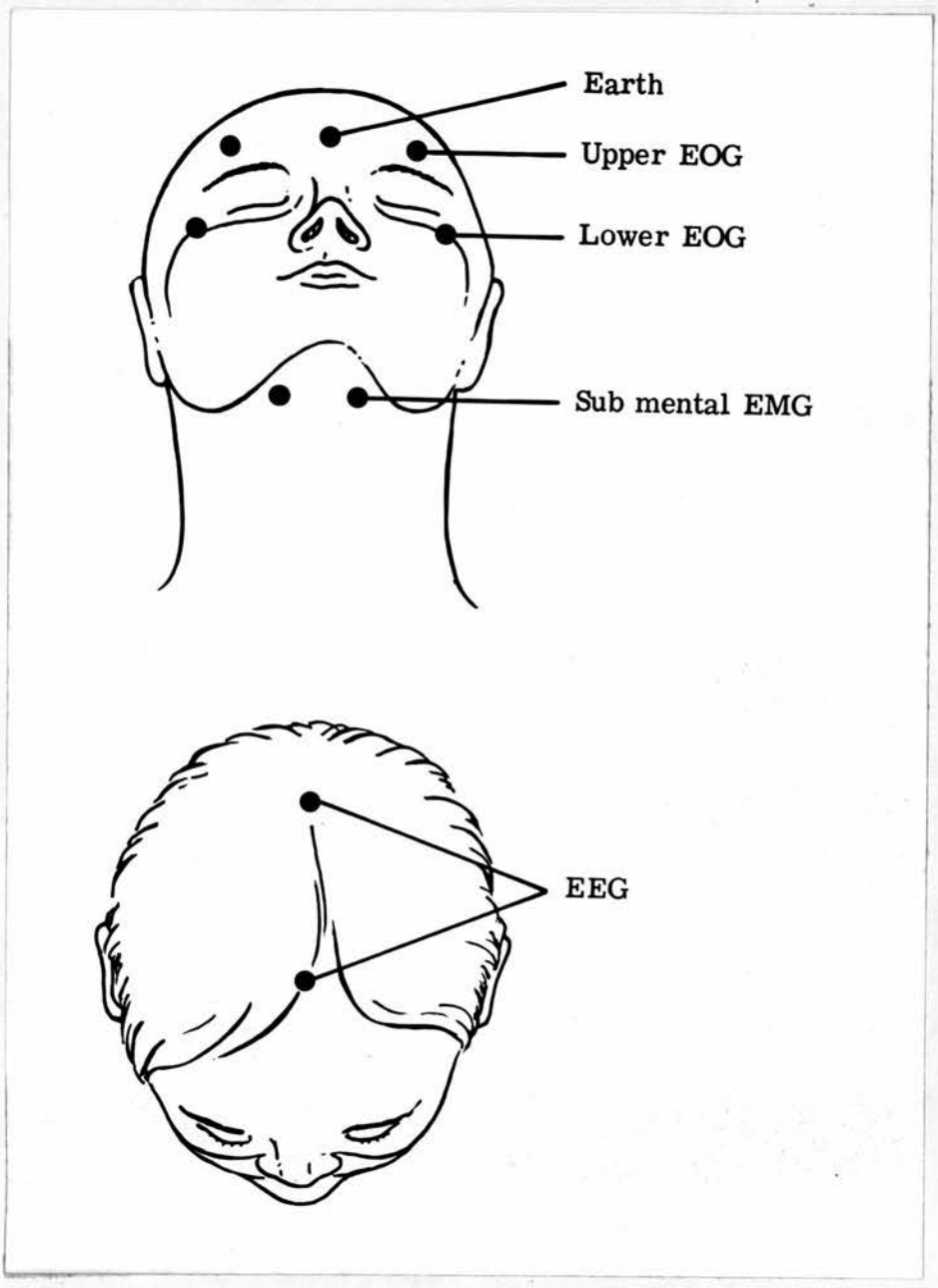

Fig. 12.

ELECTRODE PLACEMENTS USED FOR RECORDING A NIGHT OF SLEEP.

Upper diagram shows EOG and EMG electrode placements.

Lower diagram shows EEG electrode placements.

as the prisms. This constituted a control for the reduction in size of the visual field and for the wearing of the heavy spectacles and thus the subjects saw the visual environment as it normally is but the field of vision was greatly reduced as mentioned above.

The experimental period was the seventh to the twelfth of the study with corresponding nights of recorded sleep. During this period subjects wore the spectacles containing the distorting lenses during the time they were awake.

During the post-experimental period, the thirteenth to eighteenth of the study, subjects did not wear any spectacles at all but continued to reside in the department and carried out the same activities, as far as possible, as those engaged in during the prism period.

The spectacles were worn from 08.00 hours i.e. they were put on immediately after arising in the mornings until 22.30 hours i.e. bed time. Approximately one hour in the morning and one in the afternoon was allowed without the spectacles so that subjects could leave the confines of the department and take some exercise. Subjects were instructed to maintain the level of exercise to which they were used and to keep this level as constant as possible throughout the eighteen day period of the study. This was specially controlled as it has been reported that changes in the amount of exercise can result in changes in the amount of stages 3 + 4 sleep (see Chapter I).

During the remainder of the day subjects occupied themselves with their normal leisure time activities, reading; writing letters; watching television; listening to music; conversing and playing cards. Whilst wearing the prism lenses, subjects experienced a great deal of difficulty in moving around especially at first and were often helped and guided.

Adaptation tests.

During the period of wearing the prisms subjects were required to carry out 2 tests, once a day in the afternoon. These tests were for the purpose of showing that adaptation to wearing the prisms was taking place ie. would show as improvement on the tests.

Reading test.

This consisted of four lists of 30, seven-letter words chosen from a sequence in MILLER (1966). Examples of these lists can be seen in Figs 13-16. The different lists contained transformations of words as follows. (This test was suggested by Dr. N.J. WADE).

(1) letters normal and letter order normal.

(2) letters reversed from left to right but letter order normal.

(3) letters upside down but letter order normal.

(4) letters upside down and letters reversed from left to right but letter order normal.

No two lists contained the same words. The starting point of each list was marked with an asterisk to guide the disoriented subjects, and the sets of words were different each day. Each subject read one list of each/

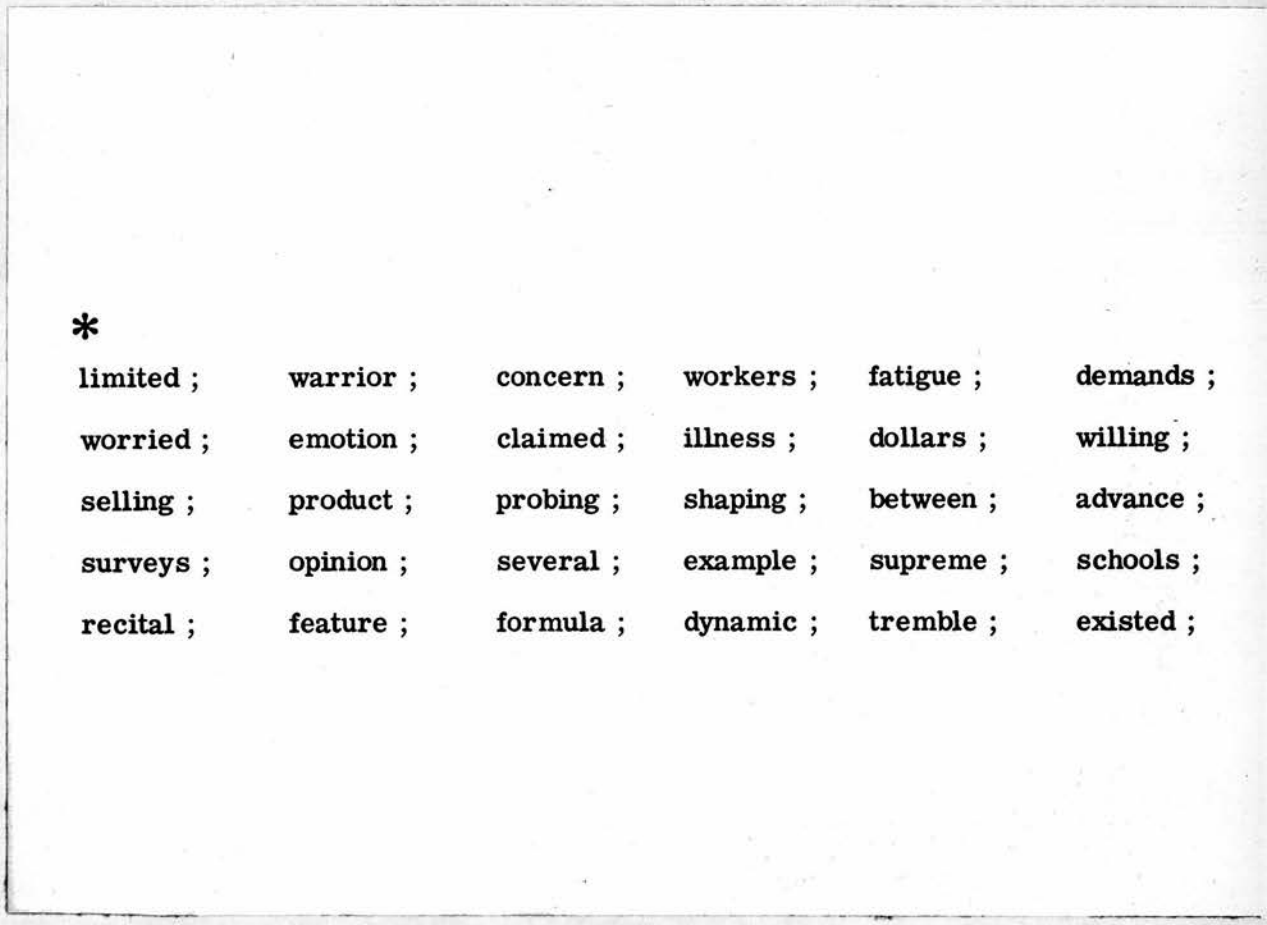

Fig. 13.

DISTORTED VISUAL INPUT AND SLEEP: READING TEST.

Examples of the transformation of words reading test.

Transformation 1.

*

; setaler ; secruos ; qossesb ; ttmetta ; sdnapxe ; equlcni ;

; ydilob ; tcepsus ; sworran ; egralne ; sdrawot ; erutpac ;

; lacinyc ; ylraelc ; stnemom ; elcihev ; skcatta ; sesiarp ;

; seeruoc ; sweiver ; segdirb ; srehtom ; srevyarp ; snosaer ;

. dealing ; sdrocer ; elcarim ; brotcod ; obecalp ; deirrac ;

Fig. 14.

DISTORTED VISUAL INPUT AND SLEEP: READING TEST.

Examples of transformation of words reading test.

Transformation 2.

*

privacy :	claimed :	assault :	century :	extreme :	elderly .
consist :	grasped :	sixteen :	targets :	crumble :	because :
numbers :	exactly :	squared :	rhythms :	experts :	maximum :
aspects :	herself :	similar :	imposes :	triplet :	prefers :
violent :	towards :	outcome :	critics :	choices :	reflect :

Fig. 15.

DISTORTED VISUAL INPUT AND SLEEP: READING TEST.

Examples of transformation of words reading test.

Transformation 3.

*

eslupmi ;	tibahni ;	gnilkni ;	tcefrep ;	ytsejam ;	ssentaf ;
lacigam ;	ysatnaf ;	derethe ;	stegdag ;	retsalp ;	dereVoc ;
nraeler ;	esreVer ;	yleSool ;	yldilos ;	gnizama ;	gnittif ;
sereHps ;	detacol ;	snoiger ;	ylhguor ;	stcerid ;	themmoc ;
erehwon ;	sdnuorg ;	laitaps ;	yllacol ;	deliops ;	seldnah ;

Fig. 16.

DISTORTED VISUAL INPUT AND SLEEP: READING TEST.

Examples of transformation of words reading test.

Transformation 4.

of the four transformations on the last day of the pre-experimental period and on each day of the experimental period. Each of the attempts was tape-recorded and the number of words correctly read per minute, of each list was noted, as was the total number of words read by each subject over all the four transformations. This summed measure of disability was used rather than the number of words for each transformation since some of the transformations were easier than others.

Manual dexterity test.

Subjects were confronted with four differently coloured pots, each with a narrow bore tube projecting vertically from the top of the pot. The tubes were just wide enough to allow a small pellet (of the type used in airguns) to pass down the tube and into the pot. The subjects also had differently coloured trays containing pellets of the same colour as the tray and as the pot. The pots and trays of pellets were arranged as follows:

Pots	Green	Yellow	Blue	Red
Trays containing Pellets:	Blue	Red	Green	Yellow

Moving in sequence along the line of trays from left to right, subjects had to put one pellet at a time into the correspondingly coloured pot. Subjects were instructed to work as quickly and accurately as possible and the numbers of pellets which subjects successfully put into the pots in two minutes was counted. Subjects were allowed unlimited spaced practice at this task in/

in the period prior to wearing the prisms until a plateau in performance was reached. They then performed the task once per day whilst wearing the prisms.

Sleep.

Sleep recordings of EOG, EEG and EMG were carried out nightly throughout the period of study. The records were scored by OSWALD into the stages proposed by RECHTSHAFFEN & KALES (1968). Lights out was from 22.30 until 07.30 hours.

Eye movement profusion was measured by selecting a sample of sleep records, and from these, the middle ten minutes of REM sleep which occurred at a time following the first three hours of sleep was chosen for counting the profusion. Care was taken to ensure that there was at least five minutes of REM sleep immediately prior to the section chosen for the eye movement count. Two-second epochs of the selected period were scored for the presence or absence of eye movements. The sleep records selected were as follows : the last (sixth) night of the pre-experimental period; the first night of the experimental period; the last (sixth) night of the experimental period; the first night of the post-experimental period and the last (sixth) night of the post-experimental period.

(c) Results.

Sleep.

The results are tabulated in Tables 9-16 and some of the results are shown graphically in Figs. 20-24. It should be pointed out that all measured parameters of sleep fell within normal limits during the eighteen

days of the study. The only exception was that on one night, the fifth post-experimental night, one subject had 36.5% REM sleep. As this was a single abnormal value among the 54, we attributed it to chance.

Total sleep time for each subject night is shown in Table 9 together with the mean value for each subject under each condition. The percentage of REM sleep for each subject is shown in Fig. 20, and the mean percentage of REM sleep for each subject under each condition is shown in Table 12. None of the subjects showed a change in the percentage of REM sleep which bore a relation to the change in visual environment.

The time spent in REM sleep in the first two hours of sleep did not show a consistent difference during the experimental period (see Table 13 and Fig. 24).

The proportion of the nights sleep spent in stages 3 + 4 was not altered consistently (see Table 14 and Fig. 23).

In each subject the number of shifts to stage 1 or awake during the first three and the first six hours of sleep was greater during the experimental period when compared with the pre-experimental period (see Table 15 and Figs. 21 and 22). The Freidman two way analysis of varince indicated, however, that this difference was not significant.

During the experimental period the latencies to the first REM period were smaller in two out of the three subjects than in either the pre-experimental period or

Table 9.

DISTORTED VISUAL INPUT: TOTAL SLEEP TIME.

	subjects	H	P	R
		Time (minutes)		
Pre-experimental nights	1	421	424	418
	2	444	434	429
	3	446	430	457
	4	423	415	418
	5	453	448	453
	6	447	433	437
Pre-experimental nights (mean)		439	431	436
Experimental nights	1	470	466	489
	2	403	437	456
	3	439	460	454
	4	460	462	477
	5	485	456	473
	6	455	450	444
Experimental nights (mean)		452	455	466
Post-experimental nights	1	495	476	477
	2	455	466	462
	3	465	451	455
	4	487	476	474
	5	274	433	475
	6	433	447	430
Post-experimental nights (mean)		433	458	462

Table 10.

DISTORTED VISUAL INPUT: REM ONSET LATENCY.

	subjects	H	P	R
		Time (minutes)		
Pre-experimental nights	1	91	146	61
	2	73	124	83
	3	79	80	59
	4	95	61	55
	5	71	67	50
	6	60	72	72
Pre-experimental nights (mean)		78	93	63
Experimental nights	1	64	72	5
	2	76	67	58
	3	125	146	55
	4	61	58	58
	5	78	59	67
	6	110	61	75
Experimental nights (mean)		87	72	53
Post-experimental nights	1	67	60	48
	2	90	73	49
	3	49	59	62
	4	103	94	51
	5	0	66	58
	6	160	85	64
Post-experimental nights (mean)		.78	73	55

Table 11.

DISTORTED VISUAL INPUT: A SAMPLE OF EYE MOVEMENT COUNTS.

	subjects	H	P	R
		number		
Pre-experimental night	1	151	139	69
Experimental night	1	128	143	182
Experimental night	6	137	192	93
Experimental nights (mean)		133	118	138
Post-experimental night	1	33	136	154
Post-experimental night	6	119	161	126
Post experimental nights (mean)		76	149	140

Table 12.

DISTORTED VISUAL INPUT: MEAN PERCENTAGE REM SLEEP DURING THE WHOLE NIGHT SLEEP.

subjects	H	P	R
	REM %		
Pre-experimental nights	25.5	26.8	24.1
Experimental nights	25.2	27.8	22.3
Post-experimental nights	25.0	28.0	22.8

Table 13.

DISTORTED VISUAL INPUT: MEAN TIME IN REM SLEEP IN THE FIRST TWO HOURS OF SLEEP.

subjects	H	P	R
	time (minutes)		
Pre-experimental nights	11.1	3.7	20.8
Experimental nights	15.5	3.4	18.4
Post-experimental nights	14.9	7.3	19.2

Table 14.

DISTORTED VISUAL INPUT: MEAN PERCENTAGE STAGES 3 + 4 DURING THE WHOLE NIGHT SLEEP.

subjects	H	P	R
	Stages 3 + 4 %		
Pre-experimental nights	24.9	23.8	30.9
Experimental nights	20.2	24.4	27.1
Post-experimental nights	22.1	23.5	29.4

Table 15.

DISTORTED VISUAL INPUT: MEAN NUMBER OF SHIFTS TO STAGE 1 AND AWAKE IN THE FIRST THREE AND FIRST SIX HOURS OF SLEEP.

subjects	H		P		R	
	Mean number of shifts					
	1st 3 hrs	1st 6 hrs	1st 3 hrs	1st 6 hrs	1st 3 hrs	1st 6 hrs
Pre-experimental nights	14	28	8	18	6	18
Experimental nights	21	39	12	22	8	21
Post-experimental nights	16	35	6	18	6	19

Table 16.

DISTORTED VISUAL INPUT: MEAN REM ONSET LATENCY.

subjects	H	P	R
	Time (minutes)		
Pre-experimental nights	78	93	63
Experimental nights	87	72	53
Post experimental nights	78	73	55

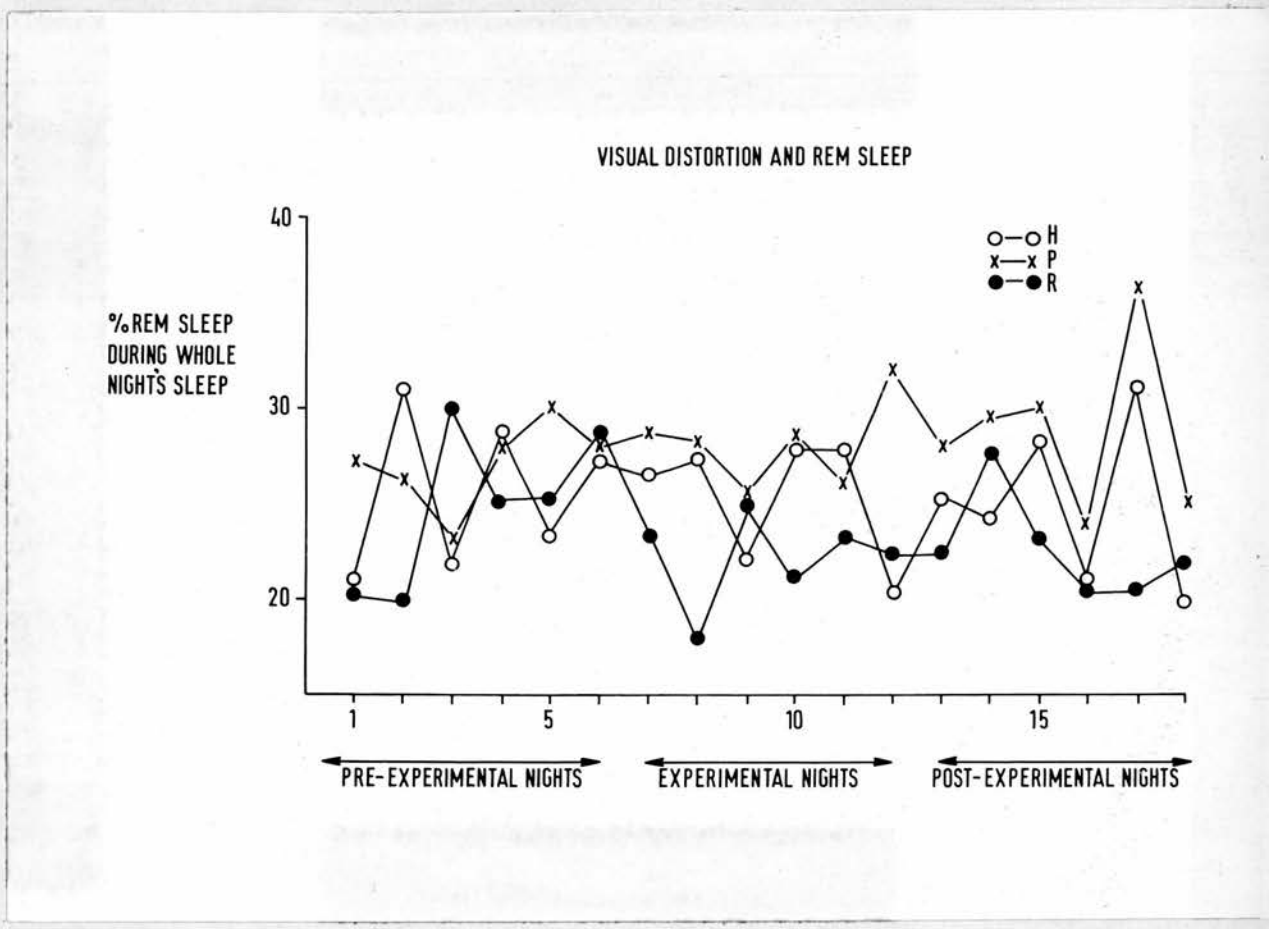

Fig. 20.

VISUAL DISTORTION AND REM SLEEP.

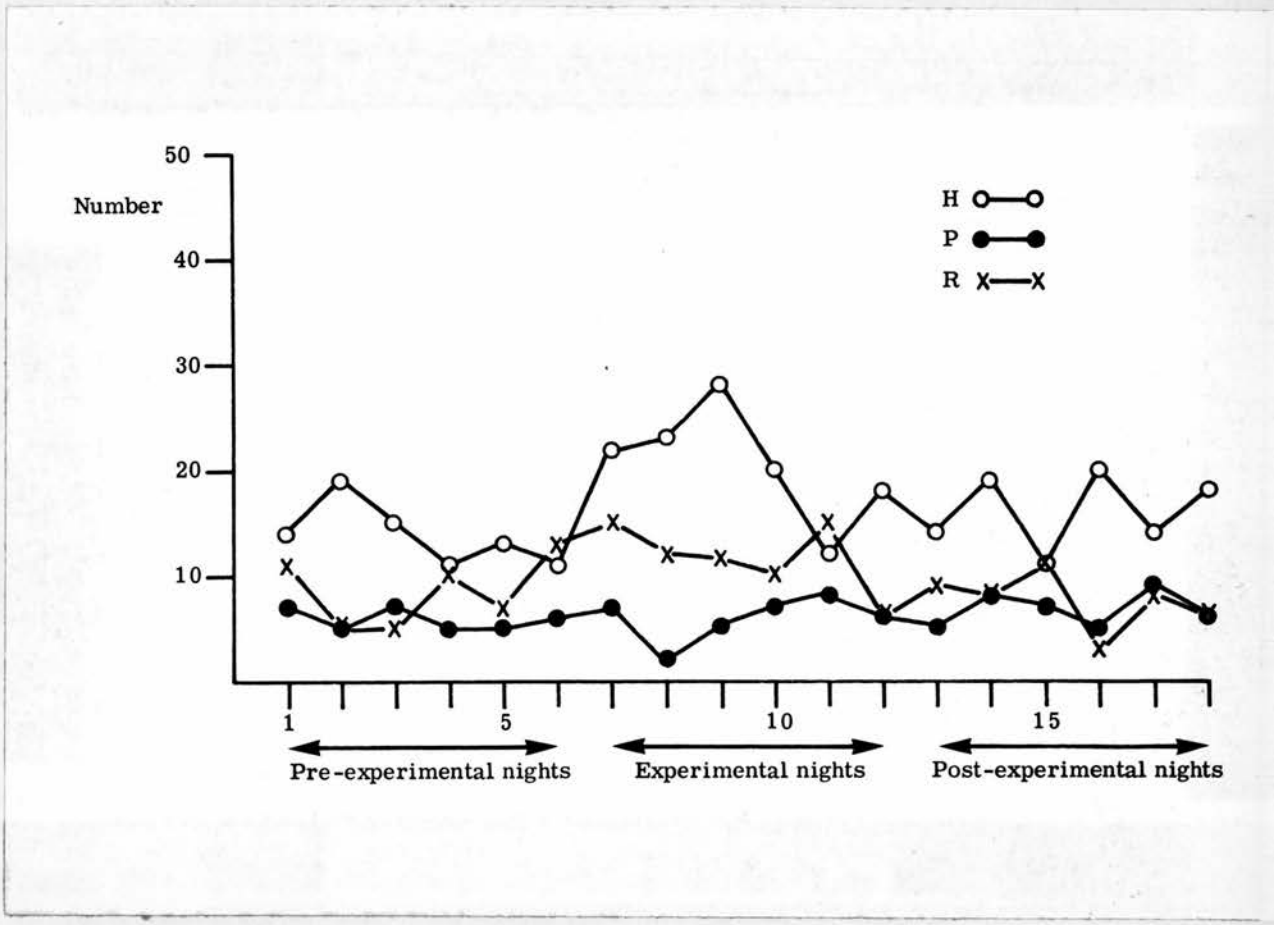

Fig. 21.

DISTORTED VISUAL INPUT AND SLEEP.

Number of shifts to Stage 1 and awake in the first three hours of sleep.

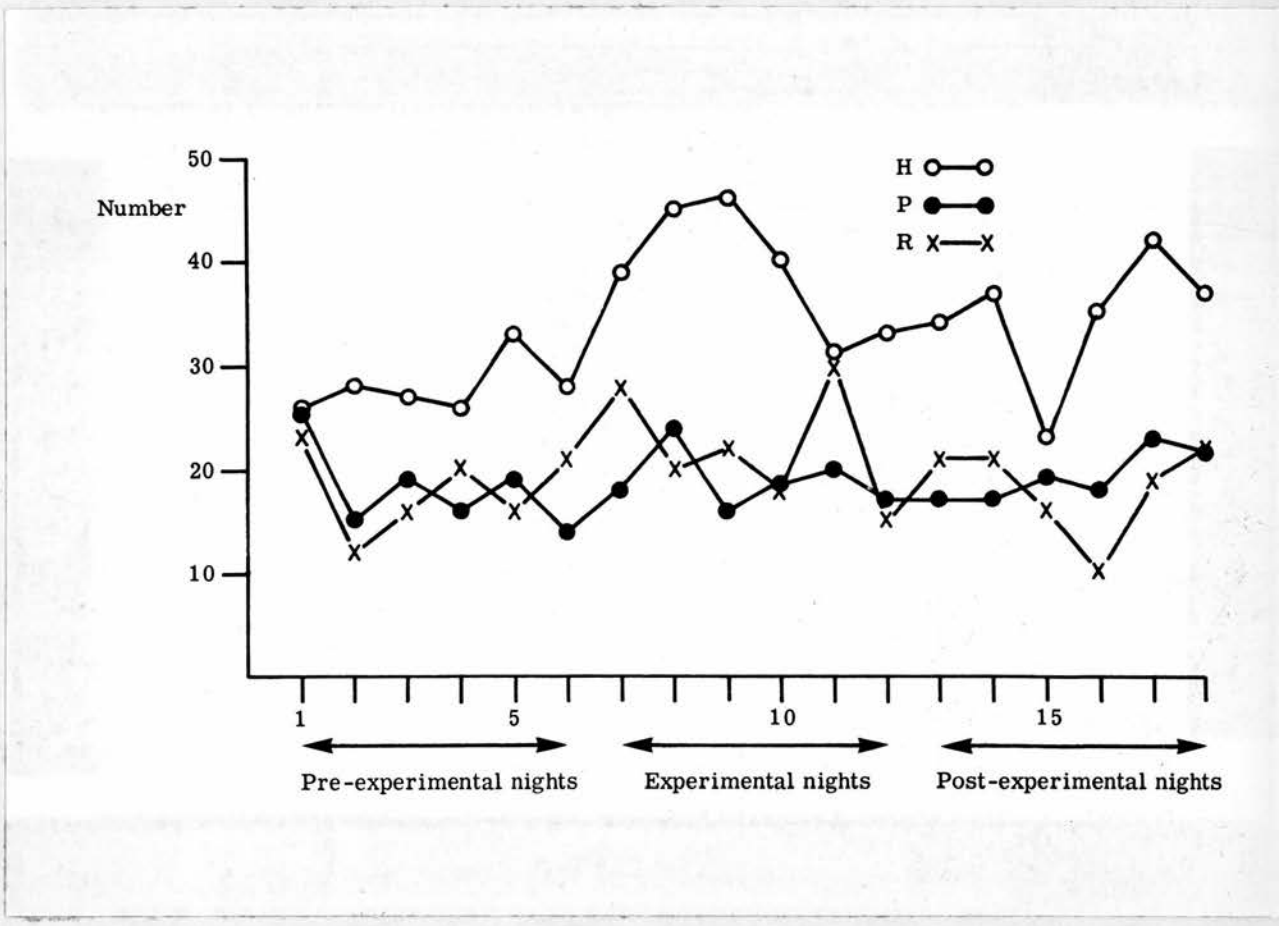

Fig. 22.

DISTORTED VISUAL INPUT AND SLEEP.

Number of shifts to Stage 1 and awake in the first six hours of sleep.

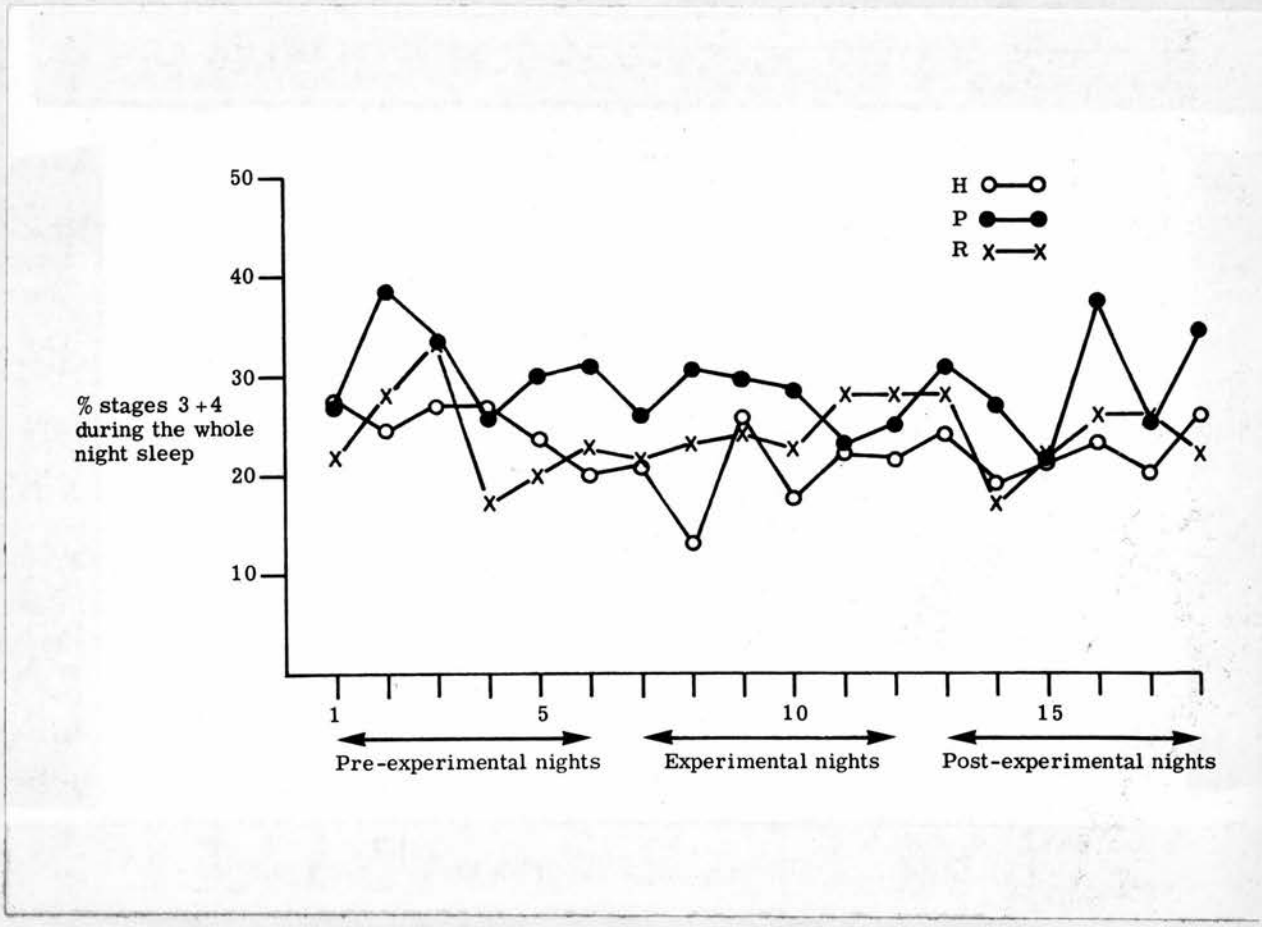

Fig. 23.

DISTORTED VISUAL INPUT AND SLEEP.

Percentage of Stages 3 + 4 sleep during the whole night sleep.

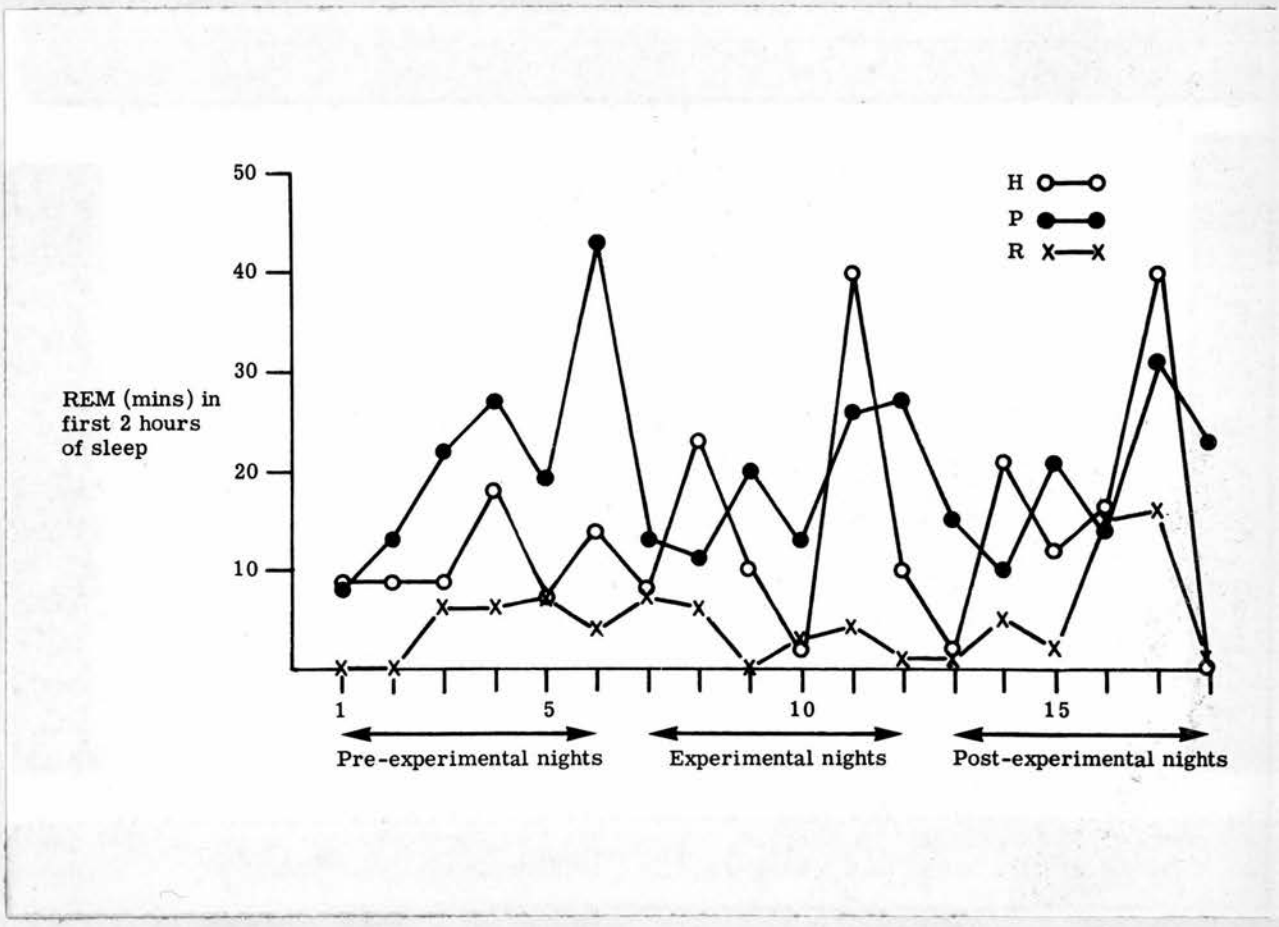

Fig. 24.

DISTORTED VISUAL INPUT AND SLEEP.

Time spent in REM sleep in the first two hours of sleep.

the post experimental period (see Table 16) but these changes were small and were found not to be statistically significant.

The Freidman two way analysis of variance also demonstrated that the experimental manipulations had no significant effects on the number of epochs containing eye movements. The number of epochs containing eye movements for each subject under each condition is tabulated in Table 11.

Performance tests.

Reading test.

A gradual increase in the number of words correctly read each day occurred in all three subjects throughout the experimental period (see Fig. 17). However no subject achieved as high a score in the experimental period as had been attained when tested on the last day of the pre-experimental period.

Manual dexterity test.

In the experimental period, an increase in the number of pellets correctly placed by the subjects, was seen throughout the period (shown in Fig. 18). This increase could not be attributed to the known practice effect of this task since a plateau in performance had previously been reached in the practice trials carried out in the pre-experimental period (see Fig. 19).

These two tests indicated that subjects were indeed learning to cope, to a certain extent, with their altered visual environments and adaptation was occurring.

Subjective.

Subjects reported that the restricted field of vision caused by wearing the spectacle frames containing

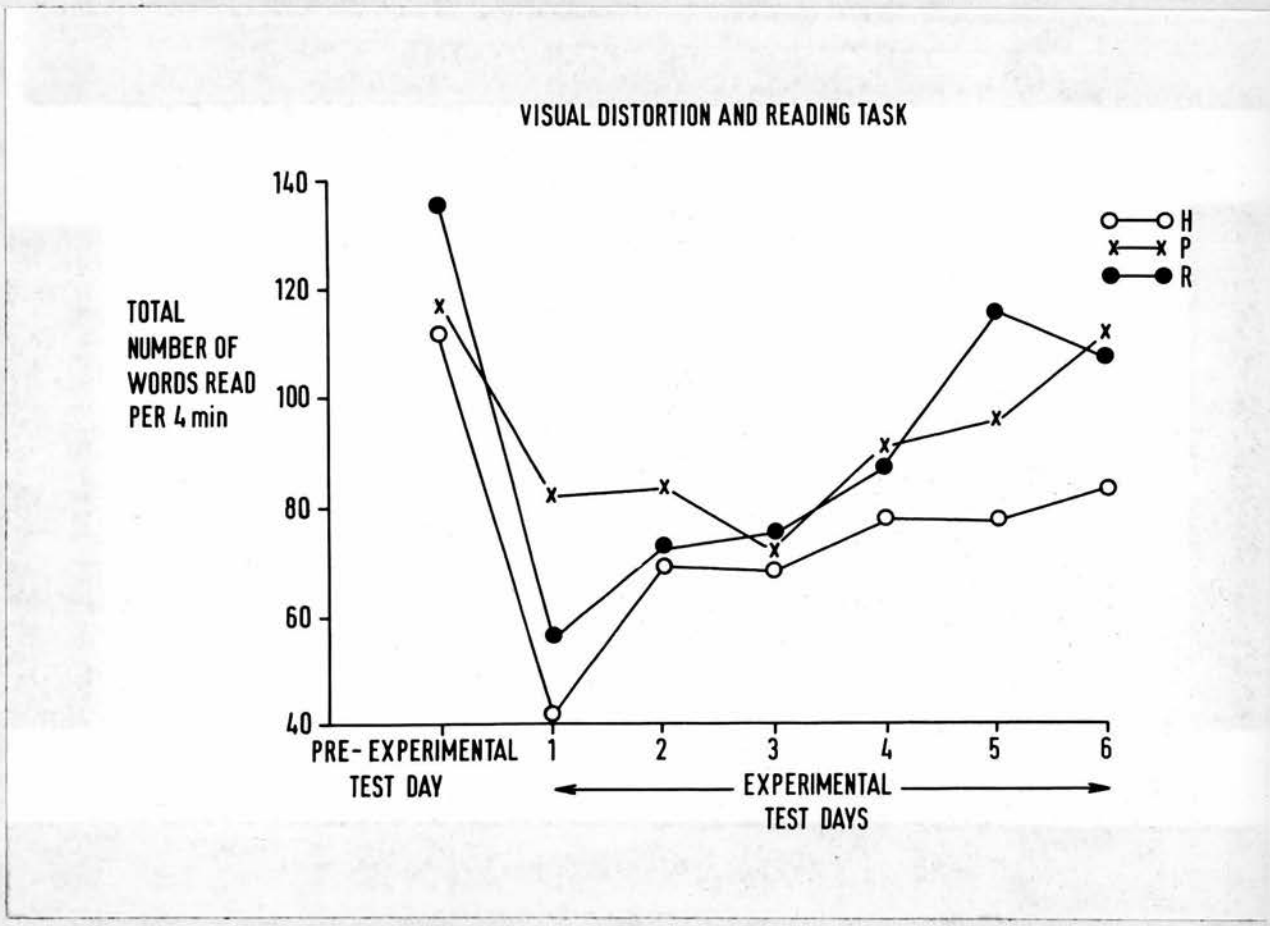

Fig. 17.

VISUAL DISTORTION AND READING TEST: RESULTS.

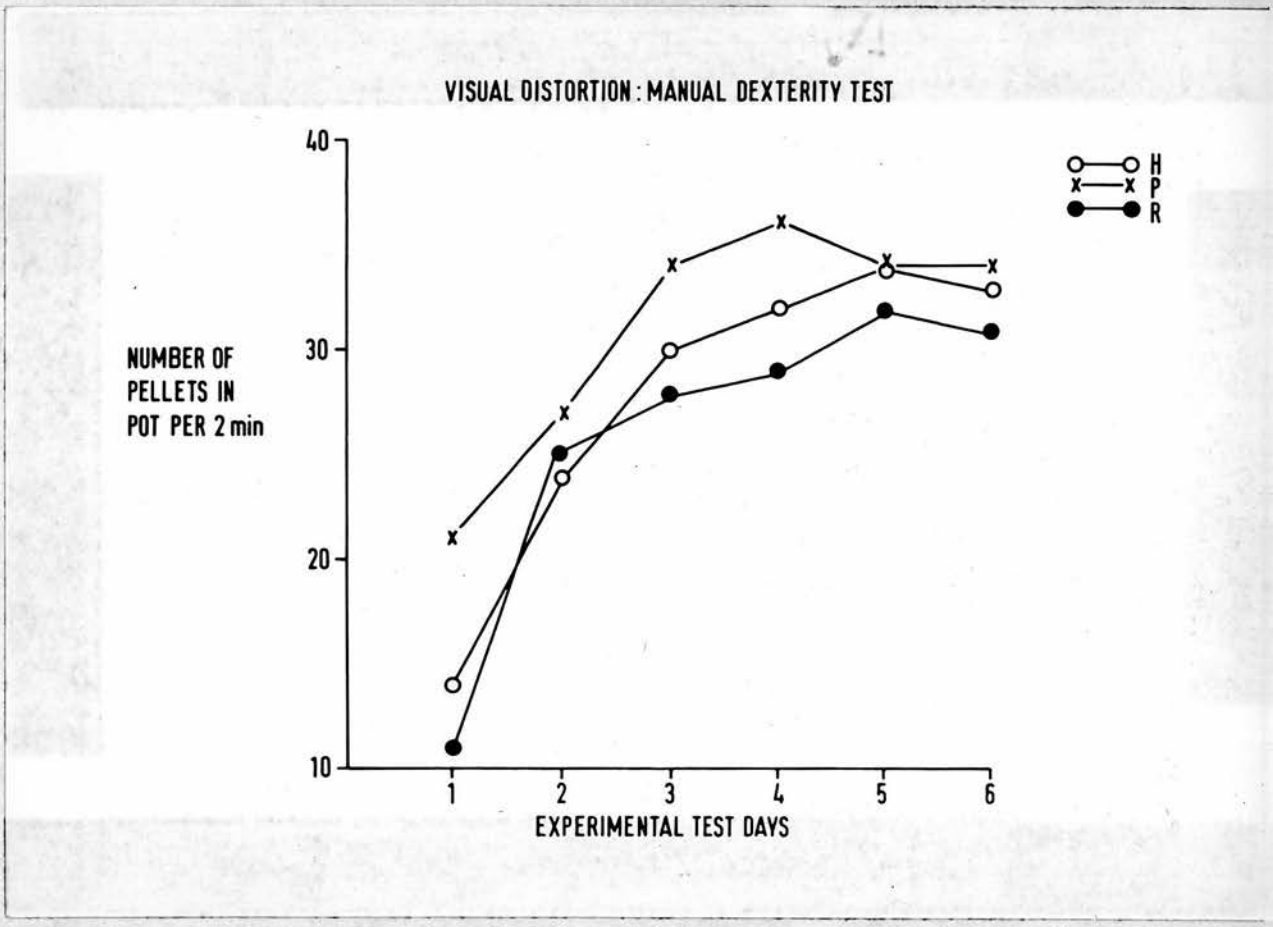

Fig. 18.

VISUAL DISTORTION AND MANUAL DEXTERITY TEST, RESULTS.

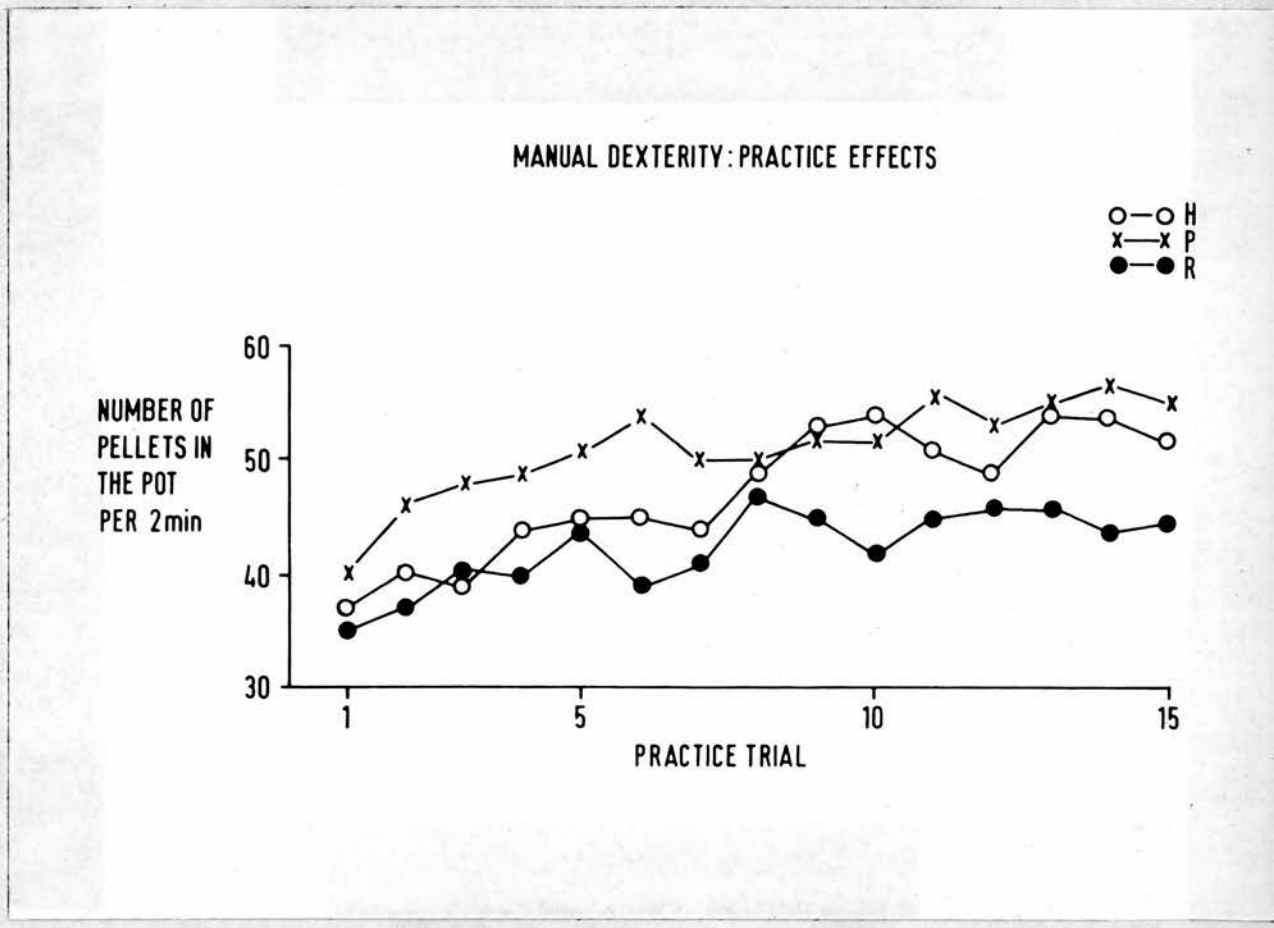

Fig. 19.

MANUAL DEXTERITY TEST: PRACTICE EFFECTS.

plane glass required them to adopt an increased scanning technique for their visual activity. They tended to move their heads from left to right a great deal when moving about, to check for obstructions and kept looking downwards at their feet. They often used touch to help them, and felt with their feet for changes in ground level.

On the first day two subjects vomited about two hours after putting on the spectacles and complained of nausea for the rest of the day. On the first two days of wearing the distorting prisms subjects were tense and obviously anxious. They were able to do very little for themselves. Tasks such as operating a knife and fork to get food into their mouths were very difficult. They encountered many difficulties in every day activities such as getting through doors, turning corners or sitting down into chairs. By the third day there was an improvement in the way they were able to get around and they were fairly accurate in playing cards, operating a knife and fork or replacing a cup onto a saucer.

(d) Discussion

The performance tests confirmed that learning was taking place throughout the six day prism-wearing experimental period. However there was no change in the percentage of REM sleep during the experimental period compared with the pre and post experimental periods. Neither was there any signficant change in the eye movement profusion. Thus we were not able to confirm the report of ZIMMERMAN et al (1970). As has been previously pointed out, they used only one throwaway night which meant that their baseline values might have been spuriously low, both in the/

duration of REM sleep and in the profusion of eye movements.

BAEKELAND & LASKY (1966) reported that increased physical exercise during the day resulted in an increased percentage of stages 3 + 4 at night. Even though our subjects took a reduced amount of exercise relative to their reported norm, there was no significant alteration in these sleep parameters.

The amount of REM sleep in the first two hours of sleep and the delay to the first REM period are sensitive indices of altered REM sleep mechanisms (OSWALD, 1968), yet neither of these parameters was altered in our study even sufficiently to suggest that distorted vision was having a slight effect on REM sleep. The records were scored by OSWALD and although were not scored blind, he had every reason to hope that the hypothesis would be confirmed and so any bias present would tend to support the hypothesis which would confirm writings of his own (1969), in which he had drawn upon the claims of ZIMMERMAN et al to support his own theories.

The results of our study therefore lend no support to the hypothesis that REM sleep relates to learning and brain protein synthesis. However the results should not be taken to provide active evidence against such a hypothesised relationship since if massive new learning were able to provoke greater than normal synthesis of protein giving rise to increased REM sleep, the degree of the increase would be very small in relation to the normal/

rate of synthesis arising out of the learning situations which must inevitably occur on ordinary days. Also there must be a considerable turnover of cerebral proteins which would be required for the ordinary turnover of cellular components. This bioligical circumstance is one which could be overlooked if theories were cast merely on computer programming analogies. Another way of looking at the process could be that the reason why REM percentage is so constant is that there has evolved a set length of time during sleep which is given over to dealing with new information so that if there is more to process in any one day owing to greater amount of learning having taken place, then it might be that only the most important aspects are processed. These aspects would be governed by the personality and life-style of the subject.

CHAPTER IX.

REM SLEEP AND MEMORY: A PILOT EXPERIMENT.

(1) REM DEPRIVATION OR REM SLEEP: EFFECTS ON MEMORY.

(a) Hypothesis.

One of the ways of investigating the relationship between REM sleep and memory is to deprive subjects of REM sleep and then note the effects on remembering (see Chapter VII). One such REM deprivation study with human subjects was performed by EMPSON & CLARK (1970) who found that memory for meaningful verbal material was impaired whereas memory for other types of material such as lists of nouns or nonsense syllables remained unaffected.

Following from the results of this experiment, I hypothesised that previously learned material should be remembered better by a group of subjects awakened after ten minutes of REM sleep than if they were awakened at the onset of the REM period, since the latter procedure would be REM deprivation. The first REM period of the night is often very short and is sometimes missed altogether, so the second REM period was chosen for the investigation. One factor unaccounted for by using only two experimental groups was that the degree of arousal might be greater in the group having the ten minutes of REM sleep and therefore a third group was also used as will be described in the next section.

(b) Procedure.

Subjects were paid student volunteers. Each subject underwent two adaptation night and a testing night. Adaptation nights were used since it had been found that the first REM period was sometimes missed if subjects were not properly adapted. Subjects served only once in the experiment.

The three nights for each subject were not consecutive, either one or two nights were interspersed between the nights of laboratory attendance. Two subjects were run per recording session: one undergoing one of the adaptation nights and the other undergoing the testing night. Thus the two night intervening interval could be either between the first and second adaptation nights or between the second adaptation night and the testing night. However the between-nights interval was counterbalanced across subjects.

Subjects reported to the laboratory at 22.30 hours and had the recording electrodes attached as explained in Chapter VIII. They were instructed on the first night that they would be awakened twice during the night and asked to get up to perform some short tasks but were given no other instructions or information. They were then allowed to get into bed and go to sleep.

Subjects undergoing adaptation nights were awakened twice during the night once at approximately one and half hours after falling asleep and the second time at roughly three hours after sleep onset. It was attempted as far as possible to make these awakenings at the same time as they would be made on the testing night and to make the awakenings out of the same sleep stages on the adaptation nights as they would be on the testing nights. However the times of the adaptation awakenings were not absolutely critical. One adaptation nights, once awake subjects were unplugged from the headboxes in the bedrooms and asked to come through to an adjoining room where they performed for ten minutes at a digit symbol substitution test. These awakenings on/

the adaptation nights were solely for the purpose of getting the subjects used to being awakened during the night and it was also hoped that once they were adapted and familiar with the idea, they would fall asleep more quickly after awakening on the testing night than if they were not adapted.

The times of the awakenings and the procedure at each awakening for the testing night were as follows:

(1) At the onset of the first REM period.

Subjects were asked to get up out of bed and go into the testing room where they learned the material to an appointed criterion. Subjects were asked to learn the material at this time rather than before going to bed since it is hypothesised that forgetting could take place during the time it takes to fall asleep after learning. By waking at the onset of the first REM period ie. after an initial period of sleep, it was hoped that subjects would fall asleep very much more quickly than at initial retiring. Furthermore it was hoped that by keeping subjects awake during the time when they would normally be having their first REM period, they would not go back into REM sleep on returning to bed but would continue with their normal sleep cycle. At the onset of the second REM period, therefore, subjects would not have had any previous REM sleep.

Subjects were previously assigned to one of three groups. Each of these three groups had the second awakening at a different time. Thus the second awakening was at one of the following times and the procedure at each awakening is also described: /

(2) At the onset of the second REM period.

Subjects were asked to get up a second time and to relearn the same material to the same criterion. They then learned a second set of equivalent material to the same criterion also.

(3) After ten minutes of REM sleep in the second REM period. The task was the same as (2) above.

(4) At the onset of the second REM period.

This time subjects were kept awake for ten minutes before performing the relearning and learning task of the second awakening. During the ten minutes they were kept awake they were occupied on the digit symbol substitution task. This procedure was used as a control for the difference in level of arousal which could be postulated between the beginning and end of the REM periods. Subjects then performed the relearning and learning task as in (2) above.

After completing awakening (1) and one of either (2), (3) or (4) above, subjects were allowed to sleep undisturbed for the remainder of the night.

Thus in summary the awakenings for the three groups were as follows:

Group (a) undergoing awakenings (1) + (2)

Group (b) undergoing awakenings (1) + (3)

Group (c) undergoing awakenings (1) + (4)

4 subjects were used in each group.

Subjects' sleep was monitored using EOG, EEG and sub mental EMG up until the time of the second awakening but not thereafter. Criteria for the onset of REM periods/

was as in the RECHTSHAFFEN & KALES (1968) scoring manual, and subjects were awakened as soon as any two of the following criteria for REM sleep were present:- low EMG, low voltage non-spindling EEG, eye movements. Subjects were awakened by having their names called through a speaker mounted on the headboard of their beds.

The tasks.

The material to be learned and relearned consisted of two prose passages chosen from the editorials of the Scotsman newspaper of 1962. Each passage consisted of a complete sentence of 24 words in length (see Appendix). These were learned by the process of serial anticipation from a memory drum. Words were presented serially one at a time and since only one word was exposed at a time, this word served both as a cue to the following item and gave feedback by serving to correct or confirm the subject's anticipations. When the material was shown for the first time, subjects were asked to repeat each word out loud as it was shown, to ensure registration of the item. On subsequent trials, subjects were asked to anticipate the word following the one shown.

The interval between each word was four seconds and the interval between each sentence trial was 12 senconds. Because each item in the sentence was both a stimulus and a response item, an initial word was needed to instruct the subject to begin. This initial word was "start" which preceded the beginning of each sentence.

The criterion of mastery was one faultless anticipation./

Following the interval between the two awakenings, subjects relearned the same material as at the first awakening to the same criterion. The number of trials required for the relearning could then be compared with the number of trials to learn the material originally. If any of the material was retained, a saving in the number of trials to relearn the material would be found.

The relearning number of trials was compared with the number of trials to learn a second, different but equally difficult sentence. The second sentence was learned immediately following relearning of the first sentence. If this second sentence were not used, groups would be relearning under different conditions i.e. a condition of REM deprivation as opposed to REM sleep. Possible differences in relearning performance between the groups could then be explained as a state dependent effect. If it could be demonstrated that there was no difference in learning ability between the groups, as would be shown by learning the second sentence at almost the same time as the relearning, differences in savings percent would be a result of the REM deprivation and not because there were differences in learning ability.

Savings was calculated according to the formula :-

$$\text{Savings } \% = \frac{OL - RL}{OL} \times 100$$

where :- OL is the number of trials for learning the second sentence.

RL is the number of trials for relearning the first sentence.

(c) Results.

Table 17 shows the individual number of trials to criterion and the means for learning the first and second sentences and for relearning the first sentence for the three groups. Table 18 shows the mean savings percent. Though not part of the original experimental design, savings was also calculated as a percentage of the first sentence (shown also in Table 18).

It can be seen that when the calculation involved the second sentence, the group which exhibited the highest savings percent i.e. remembered the first sentence best of all, was group C, the group which had no REM sleep but had been kept awake for ten minutes prior to the relearning task. The group which performed the worst, group A, was the one experiencing REM deprivation. When savings was calculated as a percentage of the first sentence, the group kept awake for ten minutes again performed the best. With this calculation worst performance was by group B, the group which had the ten minutes of REM sleep.

EMPSON & STONES (unpubl. 1971) found that the effects of REM deprivation were most profound in the first half of the sentence, so savings was also calculated using only the first half of the sentence. The results are shown in Tables 19 and 20. The group remembering the most was again the one which had been deprived of REM sleep but kept awake for ten minutes, when the calculation was as a percentage of the second sentence. The REM sleep

Table 17.

REM SLEEP DEPRIVATION AND MEMORY: TRIALS TO CRITERION.

	sentence 1 (learn)	sentence 2 (learn)	sentence 1 (relearn)
Group A	5	3	2
REM 0	7	4	2
	16	9	4
	10	6	6
Mean	9.5	5.5	3.5
Group B	10	11	6
REM 10	4	3	2
	10	5	3
	20	12	7
Mean	11	7.75	4.5
Group C	11	4	4
REM 0 +	7	3	3
AWAKE 10	8	4	2
	12	10	2
Mean	9.5	5.25	7.25

Table 18.

REM SLEEP DEPRIVATION AND MEMORY: SAVINGS PERCENT.

	Savings % (of sentence 2)	Savings % (of sentence 1)
Group A	33.0	60.0
	50.0	71.4
	55.6	75.0
	0	40.0
Mean	34.6	61.6
Group B	45.5	40.0
	33.3	50.0
	40.0	70.0
	41.7	65.0
Mean	40.1	56.2
Group C	63.6	63.6
	57.1	57.1
	50.0	75.0
	80.0	83.3
Mean	62.7	69.75

Table 19.

REM SLEEP DEPRIVATION AND MEMORY: TRIALS TO CRITERION FOR THE FIRST HALF OF SENTENCE.

	sentence 1 (learn)	sentence 2 (learn)	sentence 1 (relearn)
Group A	5	3	2
REM 0	5	2	2
	13	9	4
	8	5	5
Mean	7.75	4.75	3.25
Group B	10	9	6
REM 10	3	2	2
	10	5	3
	16	8	7
Mean	9.75	6	4.5
Group C	5	3	2
REM 0 +	8	3	2
AWAKE 10	9	4	4
	10	5	2
Mean	8	3.75	2.75

Table 20.

REM SLEEP DEPRIVATION AND MEMORY: SAVINGS PERCENT FOR THE FIRST HALF OF SENTENCE.

	Savings % (of sentence 2)	Savings % (of sentence 1)
Group A	33.3	60.0
	0	60.0
	55.6	9.0
	0	60.0
Mean	22.25	47.25
Group B	33.3	40.0
	0	33.3
	40.0	70.0
	12.5	56.3
Mean	21.45	49.9
Group C	0	55.6
	33.3	40.0
	33.3	75.0
	60.0	80.0
Mean	31.65	62.65

Table 21.

REM SLEEP DEPRIVATION AND MEMORY: NUMBER OF ERRORS IN THE FIRST LEARNING OR RELEARNING TRIAL OF EACH RUN.

Number of errors.

	sentence 1 (learn)	sentence 2 (learn)	sentence 1 (relearn)
Group A REM 0	24	12	7
	29	15	5
	30	22	13
	24	17	4
Mean	26.75	16.5	7.25
Group B REM 10	15	10	2
	15	20	5
	31	13	2
	32	29	18
Mean	23.25	18	6.75
Group C REM 0 + AWAKE 10	29	21	16
	15	8	5
	15	13	1
	25	17	4
Mean	22.00	14.75	6.5

Table 22.

REM SLEEP DEPRIVATION AND MEMORY : ERRORS IN THE FIRST RELEARNING TRIAL COMPARED WITH THE FIRST LEARNING TRIAL OF THE SECOND AND THE FIRST SENTENCES.

	Relearning errors compared with sentence 2	Relearning errors compared with sentence 1
Group A	0.58	0.29
	0.33	0.17
	0.59	0.43
	0.23	0.17
Mean	0.43	0.27
Group B	0.25	0.33
	0.20	0.13
	0.15	0.07
	0.62	0.56
Mean	0.30	0.27
Group C	0.76	0.55
	0.56	0.33
	0.08	0.07
	0.24	0.16
Mean	0.40	0.28

group remembered least. When the first sentence only was considered, the group remembering the most was again the one which had been kept awake for the ten minutes, and the group performing worst (group B) was the one which had REM sleep.

The only other calculation carried out at this point was a comparison of the number of errors made in the first relearning trial of the first sentence. This was expressed as a proportion of the number of errors in the first learning trial of the second and the first sentence. As far as the absolute number of mistakes in the first relearning trial was concerned, the group making the least number of mistakes was group A (REM deprived) and group C made the least number of mistakes. These results are shown in Table 21. The differences between the groups were very slight. Table 22 shows the calculations comparing the number of errors in the first relearning trial with the first learning trial of the first or second sentences. The group which made proportionally fewer errors was group B, when the comparison was made with the second sentence. No differences between the groups were found when the calculation was carried out using the first learning trial of the first sentence.

The reason why no statistical analysis was carried out on the results of this experiment was because of one very obvious defect in this experimental design, which was seen only in retrospect. If the number of trials to criterion for learning the first and second sentences

are examined (Table 17), it will be seen that all subjects except one (in group B) learned the second sentence more easily than the first i.e. fewer trials to criterion were taken with the second sentence. Even though the second sentence might have been easier to learn, there also does seem to be a practice effect. Subjects were therefore learning how to learn by this method. Since this effect would probably also be involved in the relearning, the experiment was abandonned and a new design considered.

There was also a second design fault which was seen only after the completion of the major experiment of this thesis, and it is that the sentences were not presented to subjects in counterbalanced order.

CHAPTER X.

EXPERIMENT TO INVESTIGATE THE EFFECTS OF REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION ON MEMORY.

(a) Aims of the experiment and experimental design.

The pilot study indicated an obvious design fault in those experiments. This major factor which emerged from the pilot study was that subjects progressively gained familiarity with the learning task and therefore always performed better in the second awakening compared with the first. It was therefore felt important that subjects be allowed some practice at the learning task, prior to the experimental night. This was achieved by making the learning task part of the procedure on adaptation nights. To overcome the practice effects, sentences of the same length but of different content were incorporated into the adaptation nights. On adaptation nights however, subjects did not relearn the first sentence.

Some other alterations were made in the main study. On some of the measured responses in the pilot study, subjects who relearned most easily were those who had been kept awake for the ten minutes prior to commencing the learnimg procedure for the second time. One possible reason therefore for the better performance was that subjects were more wide awake at this time and more highly aroused and motivated. Critical flicker fusion, a measure of general cortical arousal (VENABLES, 1963) was included at the awakening prior to the learning procedure. This served as a check for the level of arousal. Also, all subjects were kept awake for ten minutes before the learning,

and during this time they were occupied on a card sorting task. This was predominantly a motor task and was effective in keeping the subjects from falling asleep again. The cognitive aspects of this task, it was felt were sufficiently different from the learning task to keep interference at a minimum. As some subjects were awakened at the beginning of the REM period and some were awakened after ten minutes of REM sleep the critical flicker fusion would indicate whether there were any major differences between the groups in terms of level of arousal at the time of testing.

Another difference in the main study was that a fourth night was added which followed after the experimental testing night. On this night, subjects were given the antibiotic, doxycycline (trade name, Vibramycin). Subjects took 200 mg. of this antibiotic, which is believed to act to some extent as a cerebral pritein biosynthesis inhibitor (CLENDENNING, 1965; WEINSTEIN, 1972). It has been found in studies using animals that the administration of protein synthesis inhibitors such as puromycin, CXM and AXM, resulted in an impairment of memory for some previously learned behaviours (see Chapter VI). Thus the administration of doxycycline to subjects in my experiment was a very tentative exploration of the possible effects of protein synthesis inhibition of human memory.

The awakening procedures were also different in the main study compared with the pilot. I decided to use

two groups of subjects and to give each group three awakenings. Details of the awakening procedures will be given below. The reason for this change was the pilot study finding that the group REM deprived in the second REM period tended to go into the third REM period sooner than the group allowed REM sleep in the second REM period. Consequently, the REM deprived group not only had less REM sleep but also had less sleep. It was hoped that by lenghthening the time between original learning and relearning, total sleep time would be on average the same for the two groups. As an additional precaution to try to keep total sleep time the same for the two groups, they were awakened for the third time not necessarily at the onset of the third REM period but at one hour of sleep following the second awakening. If the onset of the third REM period occurred before this one hour of further sleep had elapsed, then subjects were awakened at that time. Otherwise the third awakening was always from stage 2.

Predictions.

The experiment was designed to show any possible effects of REM deprivation and protein synthesis inhibition on memory. Two groups of subjects were used, those being allowed 15 minutes of REM sleep in the second REM period (REM 15 group) and those being REM deprived (REM 0 group). Every subject took doxycycline on the final night giving rise to two further groups : REM 15 plus doxycycline (REM 15 + D) and REM deprived plus doxycycline (REM 0 + D).

The following predictions were made :-

(1) If both REM sleep and cerebral protein biosynthesis are important in stable memory formation then

(i) the REM 15 group would show superior savings.

(ii) the REM 0 + D group would show the lowest savings percent.

(iii) no clear predictions could be made concerning differences between the REM 15 + D and the REM 0 groups, but these should both show savings scores lower than the REM 15 group but not as low as the REM 0 + D group.

(2) If REM sleep but not cerebral protein synthesis, or if the doxycycline is ineffective in inhibiting the protein synthesis, is the important factor in stable memory formation the performance by the REM 15 and REM 15 + D groups should be equivalent and should be superior to the REM 0 and REM 0 + D groups.

(3) If cerebral protein synthesis but not REM sleep is most important in stable memory formation the performance by the REM 15 and REM 0 groups should be equivalent and superior to that of the REM 15 + D and REM 0 + D groups.

(4) If neither REM sleep or cerebral protein biosynthesis are important factors then there should be no consistant differences between any of the four groups.

(c) Materials and methods.

Each subject undertook to spend four nights in the laboratory. The first two nights were for laboratory adaptation, the third night was the experimental night and on the fourth night subjects were given 200 mg. of the anti-

biotic, doxycycline. The antibiotic administration night had to follow the experimental night in case there were any rebound withdrawal effects of the antibiotic on sleep (OSWALD, 1970). For reasons of economy of subjects, it was decided to use each subject twice i.e. on the experimental and doxycycline nights. Since each subject needed two adaptation nights, to use a different set of subjects for the doxycycline could have meant a large extra number of subject nights. This procedure somewhat limited the experimental design.

Subjects were paid female student volunteers aged between 18 and 26. Thirty subjects were used, fifteen in the REM 15 group and fifteen in the REM 0 group. The subjects used were all female and HARTMANN (1966) showed that there was an increase in REMS pressure in the late progestational stage of the menstrual cycle. This late part of the cycle was found in HARTMANN'S study to be associated with significantly more REM time particularly in those women with symptoms of premenstrual tension. Data was therefore collected from my subjects regarding the stage of their menstrual cycle on experimental and doxycycline nights. Information was also collected as to whether or not each subject took a contraceptive pill.

Learning and recall.

The material was learned by a method of serial anticipation from a memory drum. The material to be learned consisted of eight sentences (a separate one on each testing occasion). Each sentence was 40 words in length, and sentences were selected from editorials of the Scotsman

newspaper during the year 1962. Newspaper editorials were chosen as it was hoped that the content of the sentences would then be of roughly the same interest value to all the subjects. Sentences were matched for difficulty by selecting the four to be used on experimental and doxycycline nights from a much larger array of sentences. These four were the most similar in the mean number of trials to reach the criterion of one faultless anticipation, when tested informally with a group of subjects other than those used in the experiment. Application of the Kruskal Wallis one way ANOVAR (SEIGAL, 1956) indicated that there were no significant differences in the number of trials to criterion for learning these four.

Words of the sentence were presented serially from the memory drum in precisely the same manner as in the pilot study (see Chapter V111). Intervals between presentation of words were three seconds and the interval between trials was nine seconds. The criterion of mastery was one perfect anticipation. Each subject initially repeated each word as it was shown and on subsequent trials anticipated the word following the one shown until the criterion of mastery was attained.

Relearning procedures were identical to learning procedures except that the initial trial, where subjects repeated the words was omitted. The number of trials required for the relearning was then compared with the number of trials required to learn an equivalent sentence at a similar time to the relearning. It is well known that there are peaks and troughs in performance levels at different times of the

day (COLQUHOUN, 1971) and it is thought that this would also be true at different times of the night. Thus on the relearning session, the subjects learned a new sentence and then relearned the original one. The fact that the new sentence would interfere with memory of the original one was thought to be an unimportant factor since it would be the same for all subjects. The memory drum was manufactured by Forth Instuments Ltd., Edinburgh, Model No. S.D.1. Instuctions given to subjects are set out in the Appendix.

Card Sorting Decision Time Task.

This task was designed by CROSSMAN (1953) and has been used extensively e.g. MALPAS et al (1970). The task consists of a board (shown in Fig. 25) divided into eight sections. Each section displayed a playing card with catagories numbered from 1 to 8 (as shown), The subject sat facing the board and immediately in front of him was a recess into which the packs of playing cards, face downwards, could be placed. The task was to sort the cards into the appropriate catagory which he did by picking up the cards one at a time, looking at them and then placing them in the pile corresponding to the face value of the card. Subjects were instucted to use only the preferred hand and to work as quickly and accurately as possible. Subjects had to carry out a number of trials and the cards in the subject's recess were numbered differently on different occasions. There were four different packs of cards.

Pack 1 contained only the numbers 1 and 2, i.e. 16 of each. These were mixed up and constituted two catagories of choice.

Pack 2 contained the numbers 5,6,7 and 8, eight of each. These were mixed, and gave four catagories of choice.

Pack 3 contained all eight catagories, four of each one. Pack 4 contained all blank cards.

Packs 1, 2, 3 and 4 were sorted into the corresponding catagory according to the face value of the card and the time taken to sort all 32 cards in each pack was recorded per trial. After each trial, cards were shuffled and the next pack was presented to the subject, face downward. Instructions to the subject are shown in the Appendix. For Pack 4, the procedure was different, subjects had to pick up the cards one at a time and place them face upwards in sequence in either the two, four or eight catagories of choice. This procedure enabled the time actually taken to make the requisite movements, to be measured. It takes longer to sort cards into eight piles than into two. This will be called, movement time. If the movement time was then subtracted from the total time of each catagory, then the time taken to make the decision can be isolated.

Thus :- Decision time per catagory = Total time - movement time (for that catagory)

Subjects carried out each trial in duplicate in counterbalanced order as shown below :-

Trial	1	Total time	2 catagories
"	2	" "	4 "
"	3	" "	8 "
"	4	Movement time	2 "
"	5	" "	4 "
"	6	" "	8 "
"	7	" "	8 "
"	8	" "	4 "
"	9	" "	2 "
"	10	Total time	8 "
"	11	" "	4 "
"	12	" "	2 "

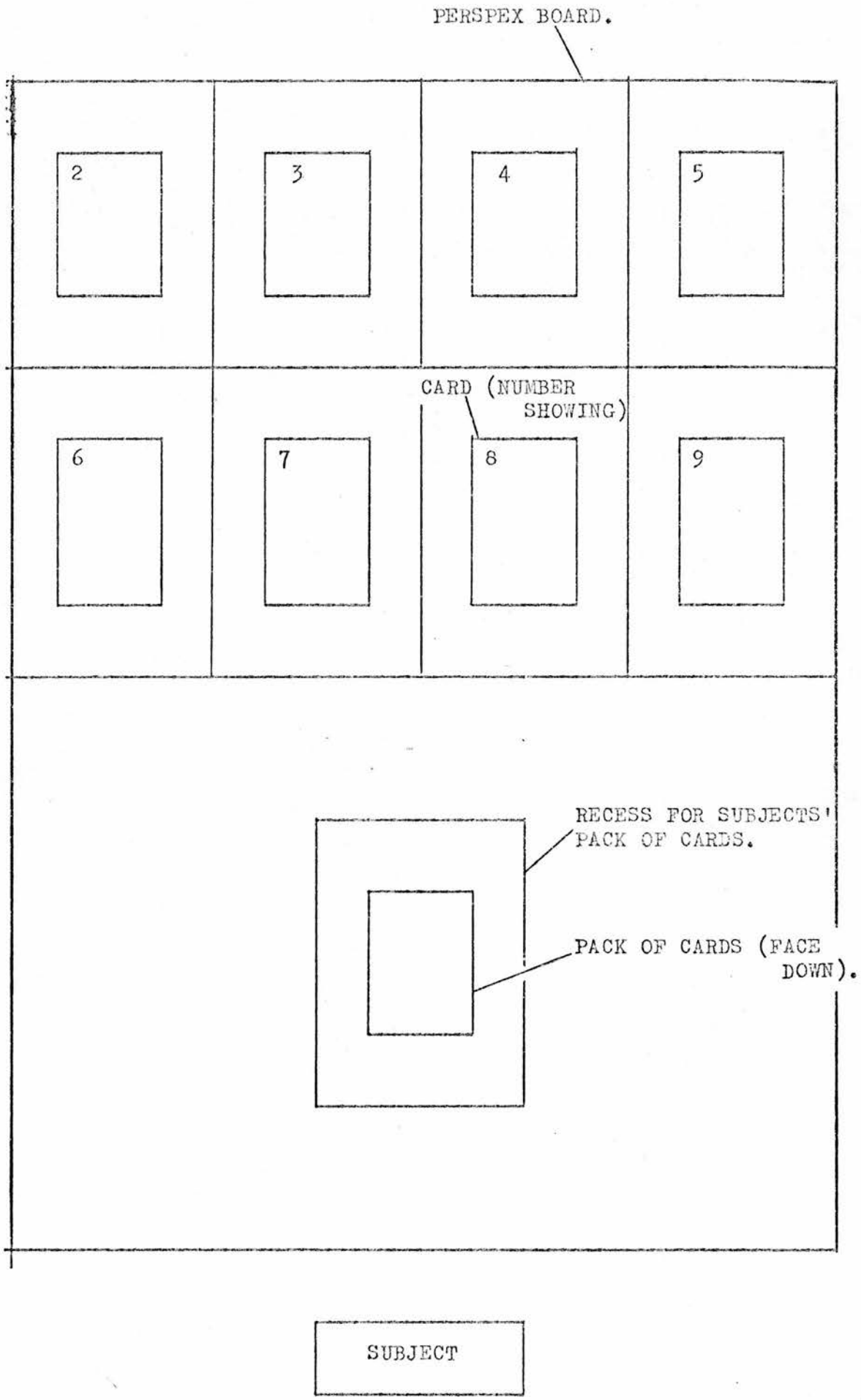

Fig. 25.

DIAGRAM OF BOARD FOR CARD SORTING.

Since this task was utilised purely for the purposes of keeping the subjects awake for 15 minutes, the results were not analysed. The task was useful for this purpose since it required the subject to make some mental effort but was dissimilar enough from the verbal learning task for minimal interference. Subjects were also required to make physical movements in sorting the cards. They were off course unaware that the results were not going to be examined, and since each trial was timed, the task caused subjects to maintain a high level of arousal. Subjects were also asked to correct any mistakes of which they were aware and the time taken to correct the errors was included in the total time for each trial.

Critical flicker fusion

Flicker fusion frequency was measured with an SLE clinical photic stimulator and a lamp of seven and a half inches diameter with a ground glass front. The dial on the stimulator gave readings from 20-100 cycles per second. The dial was turned slowly from the lower to the upper limit, or from the upper to the lower limit, four times in each direction, in alternating directions. When the rate of flicker was increased subjects were asked to say "now" at the first instant when they no longer saw the light as flickering but saw it as one continuous (fused) light . When the trial was begun with the light in the fused condition, subjects were asked to say "now" on first perceiving the light as flickering. The mean of the four trials in each of the two conditions was taken and CFF was then calculated according to the formula :-

$$CFF = \frac{\text{Increasing c/s - decreasing c/s}}{2}$$

(c) Procedure on each night.

(i) Adaptation nights.

Only one subject was recorded per night in the laboratory. Subjects arrived at about 22.30 hours and were wired up with the recording electrodes. The method of electrode attachment and recording procedure was identical with that described in Chapter VIII. A digram of the sequence of events is shown in Fig. 26.

The subject was allowed to go to bed, the electrodes were plugged into the head box and the lights were turned out at approximately 23.30 hours. Two channels of EOG, one channel of EEG and one of EMG was maintained using an Alvar Polaron Reega Mini 7 polygraphic recorder. Subjects then went to sleep and were allowed to remain sleeping until the onset of the first REM period. At this point the subject was awakened by the experimenter calling the subject's name from a microphone in the experimenter's room. The subject heard his name through a loudspeaker in his own room. Subjects were asked to respond to the calling of the name by saying something so that the experimenter could be sure the subject was awake. At the same time as the call up, the light in the subject's room was turned on by a switch external to the bed room. The experimenter then went into the subject's room, unplugged the electrodes and the subject was then required to get up out of bed and go into an adjoining room for the first testing session.

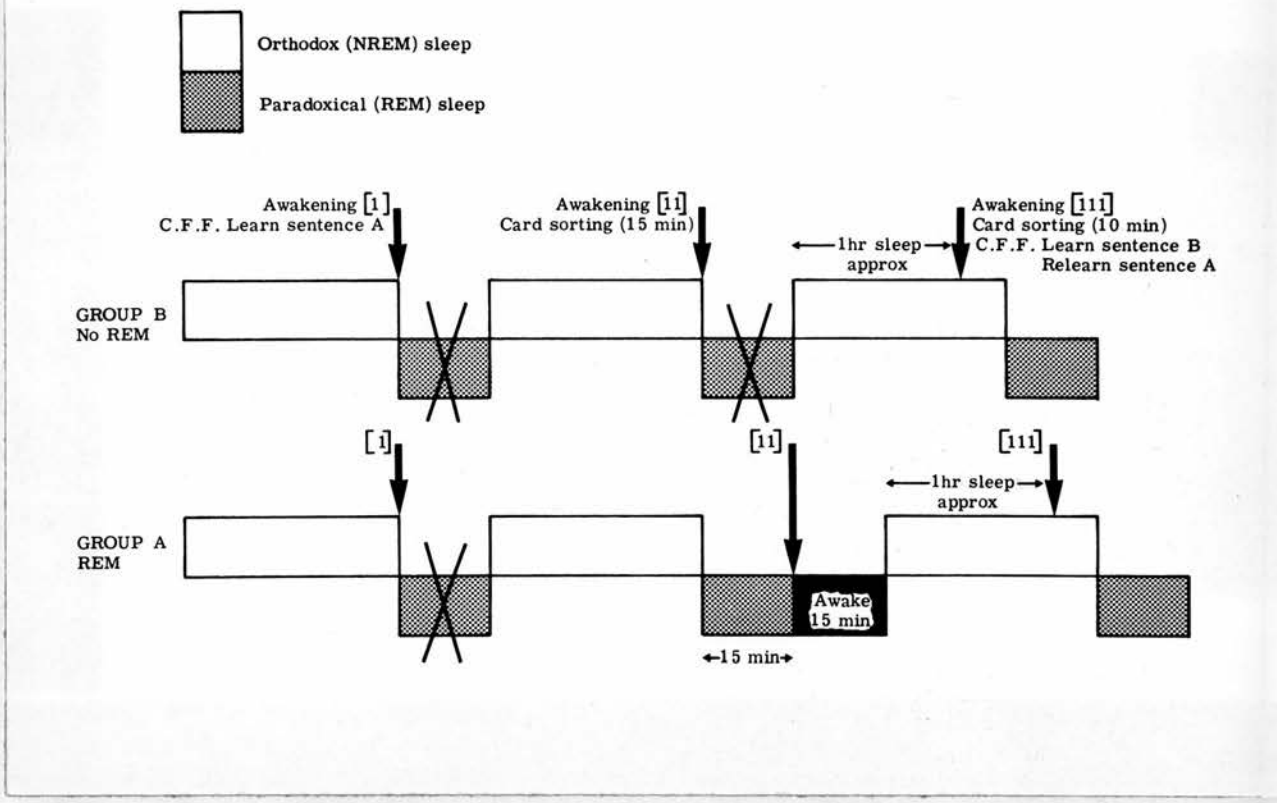

Fig. 26.

MEMORY AND REM SLEEP.

Diagram of times of awakening and procedure at each awakening.

At the first awakening, critical flicker fusion was first carried out and this was subsequently compared with the readings at the third awakening to check if there were any differences in level of arousal between these two awakenings. Subjects then learned the first sentence to the required criterion and then were allowed to return to bed. The electrodes were replugged into the head boxes and subjects then slept again.

For the second awakening subjects in the REM 0 group were allowed to sleep until the onset of the second REM period and subjects in the REM 15 group were awakened after 15 minutes of REM sleep in the second REM period. They were awakened and again got out of bed and performed the card sorting task to keep them awake for 15 minutes. This task was not scoredand was purely to make sure that subjects were completely awake and so that they did not go back into REM sleep again on being allowed to return to bed. Subjects did not know the true nature of this task otherwise their motivation might have been impaired and consequently their degree of arousal lower.

Subjects then returned to bed and recording recommenced. They were then allowed to sleep again until either the onset of the third REM period or for one further hour, whichever occurred first. This was to attempt to keep the TST constant for the two groups, as mentioned at the beginning of this chapter. Subjects who had been REM deprived would tend to go into REM sleep sooner than those who had the 15 minutes of REM sleep. Subjects not awakened at the

onset of the third REM period were always awakened from stage 2 sleep. At the third awakening, subjects first of all carried out the card sorting task for ten minutes. It had been found in the pilot experiment, that subjects usually performed less well at the later awakenings and this was thought to be because they were more sleepy, since it was later on in the night. The initial card sorting task was therefore introduced at this awakening to make sure that subjects were completely awake before commencing the memory tasks. On completion of the card sorting, CFF was then measured in the same manner as previously described and it was hoped that there would be no differences in CFF for each subject between the first and the third awakenings. Subjects then learned a second sentences, equated for difficulty with the first, to the same criterion.

Subjects then returned to bed, and were allowed to sleep undisturbed for the remainder of the night. Recording was terminated at this point.

(ii) Testing night.

Procedure was exactly the same as on the adaptation nights, except that at the third awakening, after the subjects had learned the second sentence they then had to relearn the first sentence, omitting the first repeat trial, to the same criterion of mastery.

(iii) Doxycycline night.

About 15 minutes prior to lights out, subjects were given the 200 mg. of doxycycline (D). They were not told that they would have to relearn the first sentence in a similar

way as they did on the testing night as this might have led to rehearsal of the first sentence. On nights other than the doxycycline night, subjects were given matched placebo capsules.

(d) Results.

After the two adaptation nights it was found that all subjects fell asleep quite quickly and easily after each awakening, and only two subjects had to be discarded from the experiment because they took too long to fall asleep again. One other subject was discarded on the doxycycline night after the first awakening because she felt too ill and nauseated to tolerate being woken up twice more during the night. Some other subjects reported feeling slightly unwell after taking doxycycline but not too unwell to continue to participate in the experiment. They usually only reported having felt unwell in retrospect on the following morning, but not at the time of testing. Subjects feeling unwell were found in both the REM 15 and REM 0 groups.

Non parametric statistics were used throughout to analyse the data. Out of the four groups :- REM 15; REM 0; REM 15 + D and REM 0 + D, subjects in the REM 15 and REM 15 + D groups were the same while subjects in the REM 0 and REM 0 + D groups were the same but were different from subjects in the REM 15 groups. The Wilcoxon matched-pairs signed-ranks test (see SEIGAL, 1956) was used to compare performance in groups where subjects were the same i.e. comparing the REM 15 and REM 15 + D groups or the REM 0 and REM 0 + D groups. To compare groups containing different subjects, the Mann Witney U test was used.

Memory.

The number of trials to criterion for the learning of the second sentence and relearning the first sentence are shown in Table 23 for the whole sentence, Table 24 for the first half of the sentence, Table 25 for the second half of the sentence and summarised in Table 32. Individual savings scores were calculated from the number of trials to criterion according to the formula :-

$$\text{Savings } \% = \frac{OL - RL}{OL} \times 100$$

where:- OL is the number of trials to criterion for learning the second sentence.

RL is the number of trials to criterion for relearning the first sentence.

The means and standard deviations of the savings scores for each group were then calculated and it should be noted that in all cases, standard deviations are large when compared with the means. Thus there was a great deal of variation amongst individual performances in each of the groups.

The number of trials to criterion to learn the second sentence, the number of trials to relearn the first sentence and the savings scores calculated from these for individual subjects for the whole sentence are shown in Table 23. Means and standard deviations for each group are also shown. The sentences were then divided into two halves, solely on the number of words, and savings scores were calculated for each half of the sentences. Table 24 shows the number of trials to criterion for learning the first half of second sentences, relearning the first half of first sentences

Table 23.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES.

	REM 15 group			REM 15 + D group		
	second sentence (learn)	first sentence (relearn)	savings %	second sentence (learn)	first sentence (relearn)	savings %
	9	3	66.7	7	3	57.1
	15	11	26.7	15	6	60.0
	3	2	33.3	4	2	50.0
	14	5	64.3	10	5	50.0
	15	5	66.7	-	-	-
	6	2	66.7	6	3	50.0
	10	5	50.0	11	3	72.7
	3	2	33.3	5	4	20.0
	6	2	66.7	5	2	60.0
	6	4	33.3	4	2	50.0
	8	3	62.5	6	6	0.0
	7	6	14.3	6	2	66.7
	11	6	45.5	8	3	62.5
	8	2	75.0	4	3	25.0
Mean	8.6	4.3	48.7	6.9	3.5	45.8
s.d.	3.8	2.6	18.9	3.2	1.5	20.9
	REM 0 group			REM 0 + D group		
	3	2	33.3	2	3	-50.0
	6	7	-16.7	9	8	11.1
	5	4	20.0	4	7	-75.0
	11	7	36.4	8	10	-25.0
	6	2	66.7	5	3	40.0
	5	3	40.0	4	2	50.0
	8	4	50.0	6	5	16.7
	6	3	50.0	8	3	62.5
	6	3	50.0	5	3	40.0
	8	4	50.0	7	7	0.0
	8	3	62.5	8	3	62.5
	4	2	50.0	4	4	0.0
	5	3	40.0	4	3	25.0
	4	3	25.0	3	2	33.3
	15	7	53.3	13	7	46.2
Mean	6.7	3.8	40.7	6.0	4.7	15.8
s.d.	3.1	1.8	14.1	2.9	2.5	39.0

Table 24.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES FOR THE FIRST HALF OF SENTENCE.

second sentence (learn)	first sentence (relearn)	savings %	second sentence (learn)	first sentence (relearn)	savings %
REM 15 group			REM 15 + D group		
6	3	50.0	7	3	57.1
15	7	53.3	13	2	84.6
3	2	33.3	4	2	50.0
13	5	61.5	2	3	-33.3
11	5	54.6	-	-	-
5	2	60.0	3	3	0.0
9	5	44.4	9	3	66.7
2	2	0.0	3	2	33.3
5	2	60.0	2	1	50.0
8	4	50.0	5	5	0.0
4	4	0.0	4	2	50.0
6	3	50.0	3	3	0.0
6	5	16.7	4	2	50.0
5	6	-20.0	7	2	71.4
4	2	50.0	2	2	0.0
Mean 6.8	3.8	37.6	4.9	2.5	34.3
s.d. 3.7	1.7	25.0	3.2	0.9	33.5
REM 0 group			REM 0 + D group		
3	2	50.0	2	2	0.0
5	2	-40.0	7	4	42.9
5	3	40.0	4	4	0.0
8	7	12.5	7	5	28.6
4	2	50.0	4	2	50.0
3	2	33.3	2	1	50.0
7	3	57.1	4	4	0.0
6	2	66.7	8	3	62.5
6	2	66.7	4	2	50.0
8	4	50.0	7	7	0.0
8	3	62.5	5	2	60.0
4	2	50.0	2	3	-50.0
2	1	50.0	4	3	25.0
1	3	-200.0	2	2	0.0
11	7	36.4	7	5	28.6
Mean 5.4	3.3	25.7	4.6	3.3	23.2
s.d. 2.7	2.0	65.2	2.1	1.6	30.1

Table 25.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES FOR THE SECOND HALF OF SENTENCES.

second sentence (learn)	first sentence (relearn)	savings %	second sentence (learn)	first sentence (relearn)	savings %
REM 15 group			REM 15 + D group		
9	3	66.7	7	3	57.1
15	9	40.0	15	6	60.0
3	1	66.7	3	2	33.3
14	5	64.3	10	5	50.0
15	5	66.7	-	-	-
6	2	66.7	6	2	66.7
8	4	50.0	10	3	70.0
3	2	33.3	5	4	20.0
6	1	83.3	5	2	60.0
6	6	0.0	6	4	33.3
5	3	40.0	4	2	50.0
7	3	57.1	6	4	33.3
5	6	-20.0	6	2	66.7
11	4	63.3	8	3	62.5
6	2	66.7	4	3	25.0
Mean 7.9	3.7	49.7	6.8	3.2	49.1
s.d. 4.0	2.2	26.9	3.1	1.3	16.3
REM 0 group			REM 0 + D group		
3	2	33.3	2	3	-50.0
6	4	33.3	8	8	0.0
5	4	20.0	4	7	-75.0
11	6	45.5	18	10	44.4
6	2	66.7	5	3	40.0
5	1	80.0	4	2	50.0
8	4	50.0	6	5	16.7
6	3	50.0	4	3	25.0
5	3	40.0	5	3	40.0
8	4	50.0	6	5	16.7
6	2	66.7	8	3	62.5
4	2	50.0	4	4	0.0
5	3	40.0	4	1	75.0
4	3	25.0	3	2	33.3
15	5	66.7	13	7	46.2
Mean 6.5	3.2	47.8	6.3	4.4	21.7
s.d. 3.1	1.3	16.3	4.2	2.6	38.9

Table 26.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION:

MEAN SAVINGS PERCENT.

	REM 15 group		REM 0 group		REM 15 + D group		REM 0 + D group	
	M	s.d.	M	s.d.	M	s.d.	M	s.d.
whole sentence	48.7	18.9	40.7	14.1	45.8	20.9	15.8	39.0
first half of sentence	37.6	25.0	25.7	65.2	34.3	33.5	23.2	30.1
second half of sentence	49.7	26.9	47.6	16.3	49.1	16.3	21.7	38.9

and savings scores for each individual subject. Means and standard deviations for each group are calculated. Table 25 shows the same calculations as Table 24, but for the second half of the sentences. Table 32 summarises the means and standard deviations for each group for the whole sentence and the first and second halves of the sentences. Reference to Table 26 reveals that the REM 15 group showed the highest savings score, when the whole sentence was considered. It had been hypothesised that this group would perform the best. Both the REM 15 groups did better than the REM 0 groups, and the group performing worst of all was the REM 0 + D. These results were in accordance with the predictions made assuming that both REM sleep and cerebral protein biosynthesis were important for stable memory formation. There was a considerable difference in the REM 0 + D group when compared with the other three. When individual performances of subjects in this group were examined by eye (see Tables 23, 24 and 25) it was found that there was a lot of difference in individual performances and this latter group showed a larger standard deviation of the savings scores than any of the other groups. When the mean savings from the first half of sentences was examined, the REM 15 group again saved the most and both the REM 15 and REM 15 + D saved more than the REM 0 and REM 0 + D groups. Performances of the REM 15 and REM 15 + D groups were similar to each other as was performance of the REM 0 and REM 0 + D groups. The REM 0 + D group showed the worst performance. The REM 0 + D group performed much worse than the other three groups when the second half of sentences

was considered. The REM 15, REM 15 + D and REM 0 groups all showed similar savings scores.

The number of trials to criterion for learning the first and second sentences for the experimental and doxycycline nights for the four groups, with means and standard deviations is shown in Table 27 for the whole sentence, Table 28 for the first half of the sentence and Table 29 for the second half of the sentence. Reference to Table 30 shows that for the whole sentence, the REM 0 + D group learned the second sentence significantly more easily than the first, and the REM 15 group took significantly more trials to criterion to learn the second sentence than any of the other groups. Therefore either the sentences were not sufficiently equated for difficulty or the subjects were not equated for learning ability across the four groups. Either or both of these effects could contribute to the differences in savings percent between the four groups.

Reference to Table 31 shows that where the whole sentence was concerned, the REM 0 + D group saved significantly less than the REM 0 or the REM 15 group but not significantly less than the REM 15 + D group. The savings percent of the REM 15 and REM 0 groups were not significantly different from each other so hypotheses (i)and (ii) of the experiment were not supported. Possible reasons for this will be discussed later. Although the performance of these groups was not significantly different, they were in the predicted direction. The REM 15 group performed the best and the REM 0 + D group performed worst with the REM 15 + D groupd showing performance between the

Table 27.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF TRIALS TO CRITERION FOR LEARNING THE FIRST AND SECOND SENTENCES.

first sentence	second sentence	first sentence	second sentence
REM 15 group		REM 15 + D group	
7	9	4	7
12	15	11	15
4	3	4	4
8	14	27	10
10	15	-	-
6	6	9	6
9	10	10	11
4	3	6	5
4	6	4	5
10	8	11	6
4	6	6	4
10	8	7	6
8	7	4	6
9	11	8	8
5	8	5	4
Mean 7.33	8.60	8.29	6.93
s.d. 2.60	3.70	5.76	3.04
REM 0 group		REM 0 + D group	
5	3	5	2
8	6	12	9
11	5	13	4
11	11	12	8
5	6	4	5
4	5	5	4
7	8	6	6
7	6	9	8
5	6	6	5
7	8	7	7
6	8	5	8
5	4	5	4
3	5	4	4
4	4	3	3
11	15	16	13
Mean 6.60	6.67	7.47	6.00
s.d. 2.56	2.96	3.83	2.76

Table 28.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION:

NUMBER OF TRIALS TO CRITERION FOR THE FIRST HALF OF SENTENCES.

first sentence	second sentence	first sentence	second sentence
REM 15 group		REM 15 + D group	
7	6	4	7
11	15	6	13
3	3	3	4
6	13	7	2
10	11	-	-
6	5	3	3
7	9	6	9
1	2	3	3
4	5	3	2
10	8	4	5
4	4	4	4
8	6	3	3
8	6	3	4
9	5	8	7
4	4	3	2
Mean 6.53	6.80	4.29	4.86
s.d. 2.80	6.28	1.67	3.04
REM 0 group		REM 0 + D group	
4	3	3	2
8	5	6	7
8	5	8	4
11	8	7	7
4	4	3	4
4	3	1	2
7	7	5	4
5	6	6	8
4	6	4	4
7	8	7	7
5	8	5	5
4	4	2	2
2	2	4	4
1	1	3	2
11	11	10	7
Mean 5.67	5.40	4.93	4.60
s.d. 2.84	2.60	2.35	2.06

Table 29.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION:

NUMBER OF TRIALS TO CRITERION FOR THE SECOND HALF OF SENTENCES.

first sentence	second sentence	first sentence	second sentence
REM 15 group		REM 15 + D group	
6	9	4	7
12	15	11	15
4	3	4	3
8	14	20	10
9	15	-	-
5	6	9	6
9	8	9	10
4	3	6	5
3	6	4	5
10	6	10	6
4	5	6	4
10	7	7	6
7	5	4	6
8	11	6	8
5	6	5	4
Mean 6.93	7.93	7.50	6.79
s.d. 2.65	3.91	4.15	3.03
REM 0 group		REM 0 + D group	
5	3	5	2
7	6	12	8
11	5	11	4
8	11	12	18
5	6	4	5
4	5	5	4
7	8	6	6
6	6	9	4
5	5	6	5
7	8	6	6
3	6	5	8
5	4	5	4
3	5	4	4
3	4	2	3
8	15	16	13
Mean 5.80	6.47	7.20	6.27
s.d. 2.17	2.96	3.76	4.06

Table 30.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: SIGNIFICANT DIFFERENCES BETWEEN GROUPS FOR LEARNING THE FIRST AND SECOND SENTENCES (CALCULATED FROM THE NUMBER OF TRIALS TO CRITERION).

COMPARING FIRST AND SECOND SENTENCES (Wilcoxon matched-pairs signed-ranks test).

REM 15 group	N/S
REM 15 + D group	N/S
REM 0 group	N/S
REM 0 + D group	second < first sentence, significant at $\alpha < 0.02$, two-tailed.

COMPARING REM 15 & REM 15 + D GROUPS (Wilcoxon matched-pairs signed-ranks test).

first sentence	N/S
second sentence	REM 15 + D < REM 15, significant at $\alpha < 0.02$, two-tailed.

COMPARING REM 0 & REM 0 + D GROUPS (Wilcoxon matched-pairs signed-ranks test).

first sentence	N/S
second sentence	N/S

COMPARING REM 15 & REM 0 GROUPS (Mann-Whitney U test).

first sentence	N/S
second sentence	REM 0 < REM 15, significant at $p < 0.1$, two-tailed.

COMPARING REM 15 + D & REM 0 + D GROUPS (Mann-Whitney U test).

first sentence	N/S
second sentence	N/S

COMPARING REM 15 &REM 0 + D GROUPS (Mann-Whitney U test).

first sentence	N/S
second sentence	REM 0 + D < REM 15, significant at $p < 0.01$, two-tailed.

COMPARING REM 15 + D & REM 0 GROUPS (Mann-Whitney U test).

first sentence	N/S
second sentence	N/S

Table 31.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION:

SUMMARY OF SIGNIFICANT DIFFERENCES IN SAVINGS PERCENT.

WHOLE SENTENCE.

Wilcoxon matched-pairs signed-ranks test comparing :-

(a) REM 15 & REM 15 + D groups	N/S
(b) REM 0 & REM 0 + D groups	REM 0 + D < REM 0, significant at $\alpha < 0.01$, one-tailed.

Mann-Whitney U test comparing :-

(a) REM 15 & REM 0 groups	N/S
(b) REM 15 + D & REM 0 + D groups	N/S
(c) REM 15 & REM 0 + D groups	REM 0 + D < REM 15, significant at $p < 0.01$, one-tailed.
(d) REM 15 + D & REM 0 groups	N/S

FIRST HALF OF SENTENCES.

Wilcoxon matched-pairs signed-ranks test comparing :-

(a) REM 15 & REM 15 + D groups	N/S
(b) REM 0 & REM 0 + D groups	N/S

Mann- Whitney U test comparing :-

(a) REM 15 & REM 0 groups	N/S
(b) REM 15 + D & REM 0 + D groups	N/S
(c) REM 15 & REM 0 + D groups	N/S
(d) REM 15 + D & REM 0 groups	N/S

SECOND HALF OF SENTENCES.

Wilcoxon matched-pairs signed-ranks test comparing :-

(a) REM 15 & REM 15 + D groups	N/S
(b) REM 0 & REM 0 + D groups	REM 0 + D < REM 0, significant at $\alpha < 0.005$, one-tailed.

Mann-Whitney U test comparing :-

(a) REM 15 & REM 0 groups	N/S
(b) REM 15 + D & REM 0 + D groups	REM 0 + D < REM 15 + D, significant at $p < 0.025$, one-tailed.
(c) REM 15 & REM 0 + D groups	REM 0 + D < REM 15, significant at $p < 0.01$, one-tailed.
(d) REM 15 + D & REM 0 groups	N/S

Table 32.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION:

MEAN NUMBER OF TRIALS TO CRITERION.

	REM 15		REM 0		REM 15 + D		REM 0 + D	
	M	s.d.	M	s.d.	M	s.d.	M	s.d.
Whole sentence								
sentence 1 (learn)	7.33	2.60	6.60	2.64	8.29	5.98	7.47	3.96
sentence 2 (learn)	8.60	3.83	6.67	3.06	6.93	3.15	6.00	2.85
sentence 1 (relearn)	4.27	2.46	3.80	1.78	3.50	1.45	4.67	2.85
First half of sentence								
sentence 1 (learn)	6.53	2.90	5.67	2.94	4.25	1.77	4.93	2.43
sentence 2 (learn)	6.80	3.73	5.40	2.69	4.86	3.16	4.60	2.13
sentence 1 (relearn)	3.85	1.68	3.33	2.02	2.50	0.94	3.27	1.58
Second half of sentence.								
sentence 1 (learn)	6.93	2.74	5.80	2.24	7.50	4.31	7.20	3.90
sentence 2 (learn)	7.93	4.04	6.47	3.07	6.79	3.14	6.27	4.20
sentence 1 (relearn)	3.73	2.19	3.20	1.32	3.08	1.19	4.40	2.56

two extremes. The REM 15 group showed better performance than the REM 15 + D group and the REM 0 was better than the REM 0 + D. The REM 15 group was better than the REM 0 and the REM 15 + D was better than the REM 0 + D. The REM 15 + D showed better performance than the REM 0, possibly showing REM sleep was a more powerful influence on stable memory formation than cerebral protein biosynthesis under the conditions of this experiment.

When the sentence was split into two halves for purposes of analysis significant differences again appeared but only where the second half of sentences was considered (see Table 31). In the second half of sentences, the REM 0 + D group performed significantly worse than any of the other three groups and performance of the other three groups were not significantly different from each other. The non significant differences in the first and second halves of the sentences reflected the pattern of the whole sentence.

The number of errors in the first learning and relearning trial for the first learning and relearning trial for the first and second sentences were then examined, since it is possible that this analysis might be more sensitive than the number of trials to criterion. Table 33 shows the mean number of errors with standard deviations for the four groups for learning the first and second sentences and for relearning the first sentence. The number of errors made in the first relearning trial of the first sentence was expressed as a proportion of the number of errors in the first learning trial of the second sentence

Table 33.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: MEAN NUMBER OF ERRORS IN THE FIRST LEARNING OR RELEARNING TRIAL.

	REM 15		REM 0		REM 15 + D		REM 0 + D	
	M	s.d.	M	s.d.	M	s.d.	M	s.d.
Whole sentence								
sentence 1 (learn)	15.93	7.41	17.40	8.47	18.43	6.39	15.77	6.25
sentence 2 (learn)	20.20	7.16	18.07	7.75	16.64	5.88	16.80	6.53
sentence 1 (relearn)	15.53	9.03	15.07	9.54	11.21	6.20	11.53	7.02
First half of sentence								
sentence 1 (learn)	7.00	3.72	6.40	4.82	6.29	3.41	4.93	3.22
sentence 2 (learn)	8.47	4.70	9.13	4.05	5.43	2.38	5.33	3.22
sentence 1 (relearn)	8.80	4.31	8.53	4.75	4.93	3.83	5.20	3.45
Second half of sentence								
sentence 1 (learn)	8.93	4.68	11.00	4.05	12.29	3.71	11.73	4.61
sentence 2 (learn)	11.73	3.68	8.93	2.66	11.14	4.06	11.60	4.48
sentence 1 (relearn)	6.60	5.38	6.53	5.21	6.29	3.34	6.40	4.36

and the means and standard deviations resulting from this procedure are shown in Table 34, for the whole sentence and for the first and second halves of sentences. Individual results are shown in Table 35 for the whole sentence, in Table 36 for the first half of the sentence and in Table 37 for the second half of the sentence. Examination of the means resulting from this procedure, by eye, shows that as far as the whole sentence was concerned, both the REM 0 groups made a larger proportion of mistakes in the first relearning trial of the first sentence than the REM 15 groups, the REM 15 + D groups making the smallest proportion of mistakes and the REM 0 group making the largest. The REM 15 group made the greatest number of mistakes proportionally in the first half of the sentence and the smallest proportion of mistakes in the second half of the sentence, out of the four groups. The REM 0 group showed the smallest proportion of mistakes in the first half of the sentence and the greatest proportion in the second half of the sentence. All groups made a larger proportion of mistakes in the first half of the sentence than in the second. In all groups, the proportion of mistakes made in the first relearning trial was greater than the whole sentence in the first half of the sentence and less than the complete sentence in the second half of the sentence. No very clear cut effect emerged as a result of this analytical procedure.

Graphs were also plotted of the mean number of errors per trial for each of the four groups (see Figs. 27, 28 and 29) and the number of errors was also sub-divided into errors

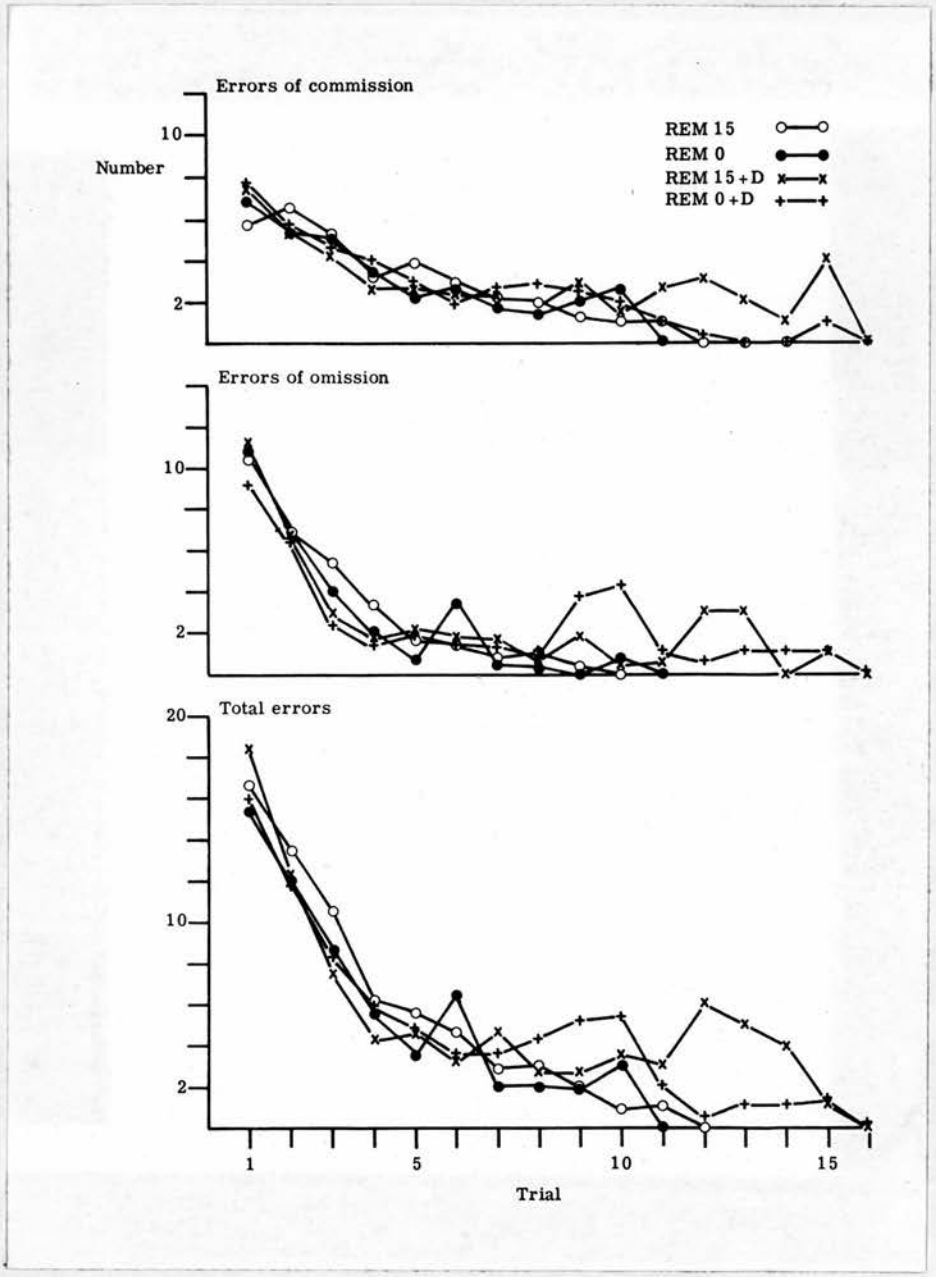

Fig. 27.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION.

Errors in the first trial of each run: first sentence (learn).

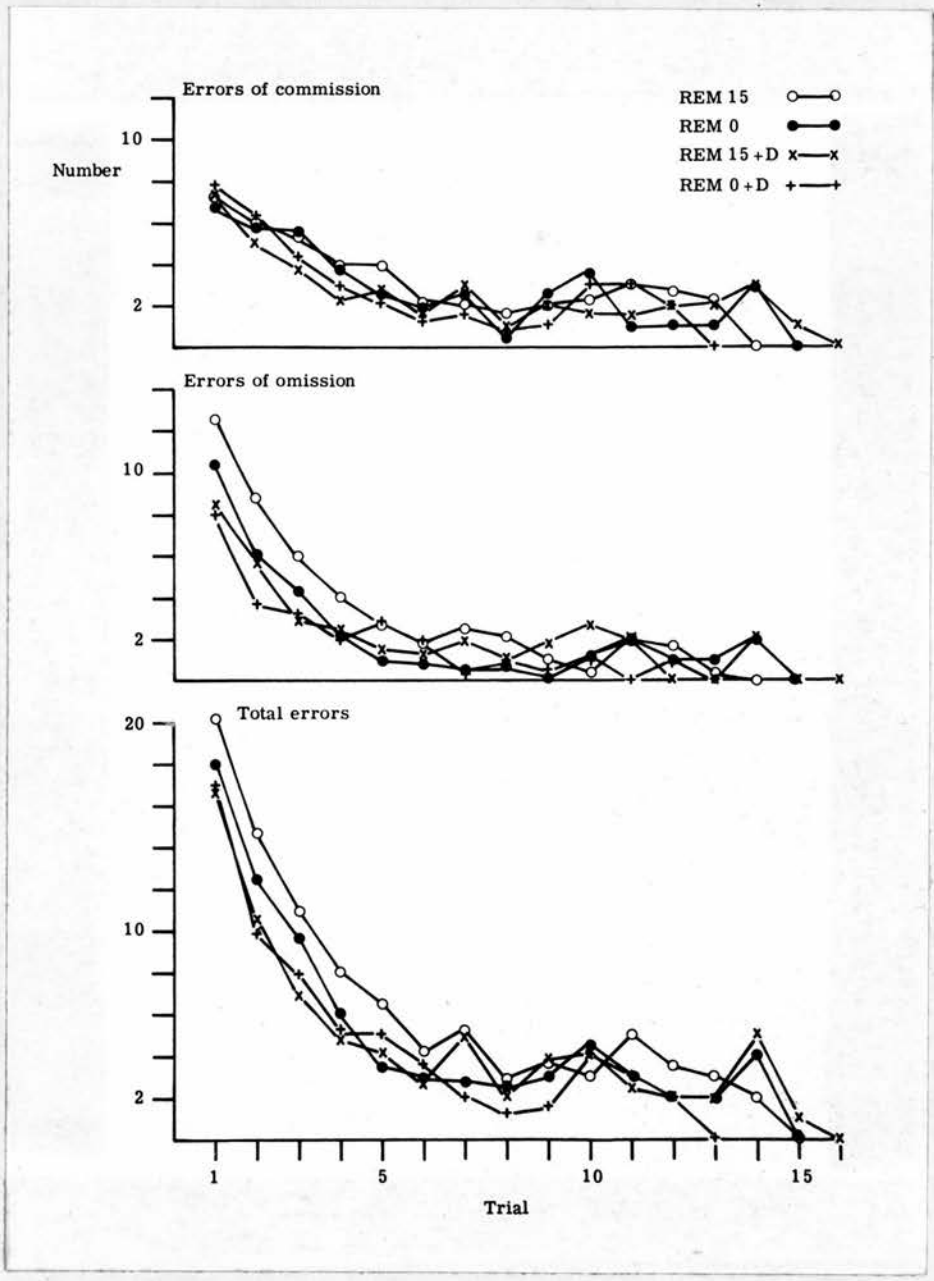

Fig. 28.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION.

Errors in the first trial of each run: second sentence (learn).

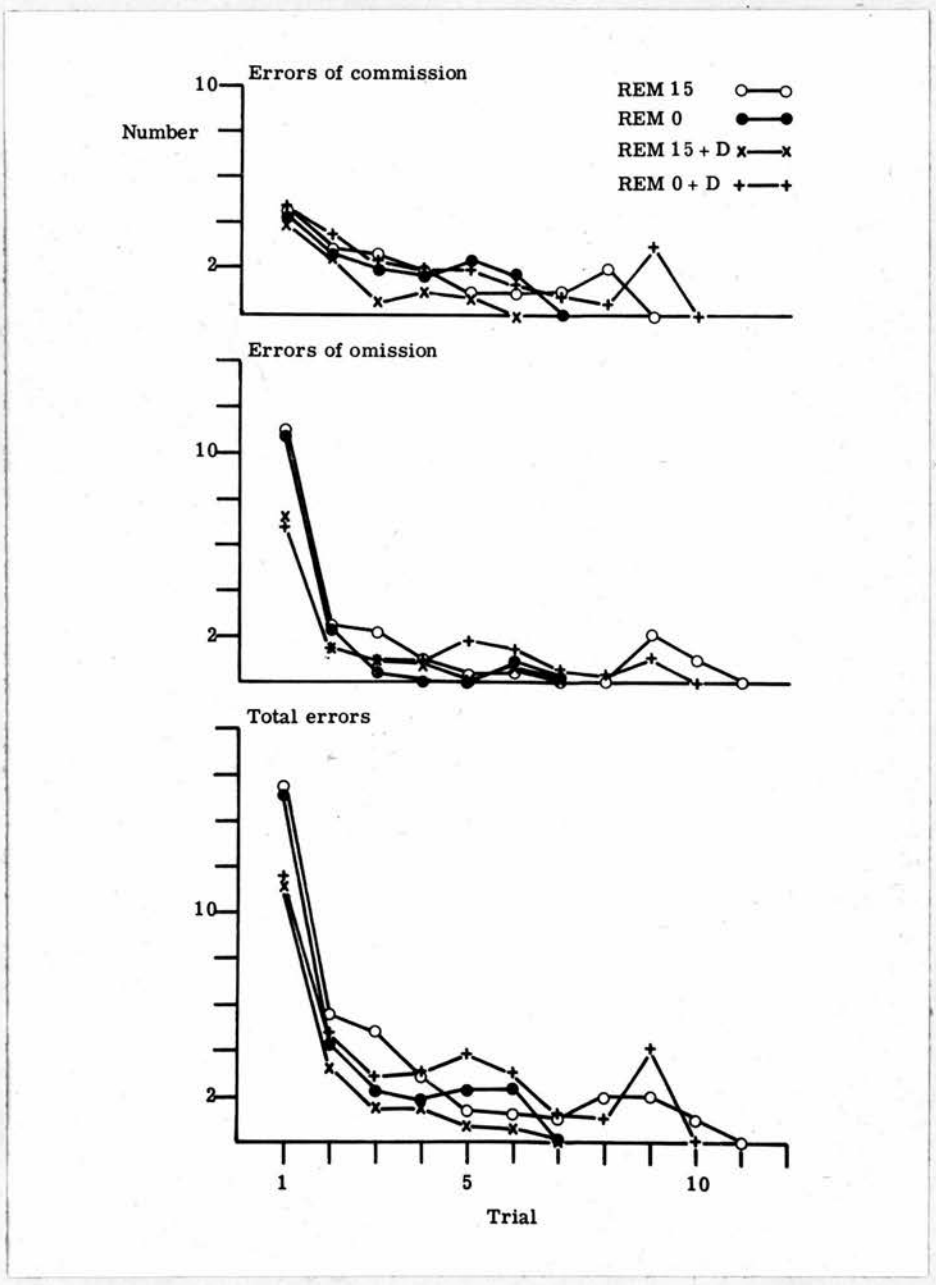

Fig. 29.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION.

Errors in the first trial of each run: first sentence (relearn).

Table 34.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: MEAN ERRORS IN THE FIRST RELEARNING TRIAL COMPARED WITH THE FIRST LEARNING TRIAL OF THE SECOND SENTENCE.

	REM 15	REM 15 + D	REM 0	REM 0 + D
Whole sentence				
Mean	0.75	0.70	0.85	0.76
s.d.	0.33	0.39	0.45	0.36
First half of sentence				
Mean	1.86	1.21	1.20	1.32
s.d.	3.02	1.44	1.12	1.29
Second half of sentence				
Mean	0.50	0.65	0.73	0.56
s.d.	0.33	0.43	0.55	0.56

Table 35.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF ERRORS IN THE FIRST RELEARNING TRIAL COMPARED WITH THE FIRST LEARNING TRIAL OF THE SECOND SENTENCE.

sentence E (relearn)	sentence F (learn)	$\frac{E}{F}$	sentence G (relearn)	sentence H (learn)	$\frac{G}{H}$
REM 15 group			REM 15 + D group		
13	23	0.56	9	14	0.64
25	32	0.78	18	25	0.72
4	8	0.50	8	5	1.60
27	26	0.97	23	23	1.00
19	26	0.73	6	12	0.50
6	16	0.38	12	17	0.71
11	26	0.42	8	17	0.47
6	7	0.86	3	16	0.19
3	19	0.16	20	15	1.33
24	27	0.89	11	11	1.00
13	15	0.87	18	28	0.64
17	24	0.71	4	18	0.22
30	19	1.58	7	18	0.39
25	21	1.19	10	14	0.71
10	14	0.71	-	-	-
Mean 15.53	20.20	0.75	11.15	16.64	0.70
s.d. 8.72	6.92	0.33	6.20	5.66	0.39
REM 0 group			REM 0 + D group		
7	10	0.70	8	6	1.33
40	20	2.00	18	31	0.58
14	22	0.64	13	13	1.00
24	19	1.26	20	24	0.83
14	13	1.08	5	11	0.46
5	15	0.33	5	12	0.42
15	27	0.57	19	21	0.91
7	24	0.29	10	20	0.50
16	27	0.59	15	20	0.75
17	16	1.06	21	18	1.67
27	19	1.42	6	17	0.35
11	19	0.58	8	15	0.53
4	11	0.36	1	16	0.06
8	8	1.00	3	7	0.43
17	21	0.81	21	21	1.00
Mean 15.07	18.07	0.85	11.53	16.80	0.76
s.d. 9.22	5.66	0.45	6.78	6.31	0.36

Table 36.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF ERRORS IN THE FIRST RELEARNING TRIAL COMPARED WITH THE FIRST LEARNING TRIAL OF THE SECOND SENTENCE. FIRST HALF OF SENTENCES.

sentence E (relearn)	sentence F (learn)	E/F	sentence G (relearn)	sentence H (learn)	G/H
REM 15 group			REM 15 + D group		
8	10	0.80	5	5	1.00
14	14	1.00	6	8	0.75
4	2	2.00	4	3	1.33
13	12	1.08	14	7	2.00
12	14	0.86	3	4	0.75
4	6	0.67	4	7	0.57
7	13	0.54	2	5	0.40
5	3	1.67	0	5	0.00
3	8	0.38	12	6	2.00
12	16	0.75	6	1	6.00
10	6	1.67	5	11	0.45
11	10	1.10	2	5	0.40
13	1	13.00	2	4	0.50
14	7	2.00	4	5	0.80
2	5	0.40	-	-	-
Mean 8.80	8.47	1.86	4.93	5.43	1.21
s.d. 4.31	4.70	3.02	3.83	2.38	1.44
REM 0 group			REM 0 + D group		
5	6	0.83	3	5	1.00
20	10	2.00	6	13	0.46
10	12	0.83	8	3	2.69
12	9	1.33	7	9	0.77
6	7	0.86	3	4	0.75
5	10	0.50	0	2	0.00
9	14	0.64	10	8	1.25
4	13	0.31	4	6	0.67
8	16	0.50	5	7	0.71
8	10	0.80	9	3	3.00
15	8	1.87	3	4	0.75
9	9	1.00	5	1	5.00
1	2	0.50	1	8	0.13
5	1	5.00	2	3	0.67
11	10	1.10	12	6	2.00
Mean 8.53	9.13	1.20	5.20	5.33	1.32
s.d. 4.75	4.05	1.12	3.45	3.33	1.29

Table 37.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF ERRORS IN THE FIRST RELEARNING TRIAL COMPARED WITH THE FIRST LEARNING TRIAL OF THE SECOND SENTENCE. SECOND HALF OF SENTENCES.

sentence E (relearn)	sentence F (learn)	E/F	sentence G (relearn)	sentence H (learn)	G/H
REM 15 group			REM 15 + D group		
5	13	0.39	4	9	0.44
12	18	0.67	12	17	0.71
0	6	0.00	4	2	2.00
14	14	1.00	9	16	0.56
7	12	0.58	3	8	0.38
2	10	0.20	8	10	0.80
4	13	0.31	6	12	0.55
1	4	0.25	3	11	0.27
0	11	0.00	8	9	0.89
12	11	1.09	5	10	0.50
3	9	0.33	13	15	0.76
6	14	0.43	2	13	0.15
17	18	0.94	5	14	0.36
11	14	0.79	6	9	0.67
5	9	0.56	-	-	-
Mean 6.60	11.73	0.50	6.29	11.24	0.65
s.d. 5.38	3.86	0.33	3.34	4.06	0.43
REM 0 group			REM 0 + D group		
2	4	0.50	5	3	1.67
20	10	2.00	12	19	0.63
4	10	0.40	5	10	0.50
12	10	1.20	14	15	0.93
8	6	1.33	2	7	0.29
0	5	0.00	5	10	0.50
6	13	0.46	9	14	0.64
6	11	0.27	6	14	0.43
8	11	0.73	10	13	0.77
9	6	1.50	12	15	0.80
12	11	1.09	3	13	0.23
2	10	0.20	3	14	0.21
3	9	0.33	0	8	0.00
3	7	0.43	1	4	0.25
6	11	0.55	9	15	0.60
Mean 6.53	8.93	0.73	6.40	11.60	0.56
s.d. 5.21	2.66	0.55	4.36	4.48	0.56

of ommission and commission, but no clear cut effects emerged.

Thus it can be seen that there were very few statistically significant effects regarding savings as measured by the number of trials to criterion and the proportion of errors made. The significant effects which did emerge mostly involved the REM 0 + D group showing worse performance than the other groups, under the conditions of this experiment.

Sleep.

Sleep parameters were measured for two separate intervals and these were :-

(i) The interval prior to the original learning from the beginning of recording, i.e. from lights out until the first awakening.

(ii) The interval between the original learning and the relearning, this included the second awakening and was measured from lights out after the first awakening until the third awakening.

Considering the first interval, Table 38 shows that the total time in bed prior to the first awakening was shortest in the REM 15 group and longest in the REM 15 + D group, however there were no significant differences between the groups as analysed using the Mann Witney U test and the Wilcoxon matched-pairs signed-ranks tests (see Tables 39a and 39b).

There were no significant differences in total sleep time and sleep onset latency (Tables 39a and 39b) Table 38 shows the means and standard deviations for the four groups.

Both the time spant in the different sleep stages and the time spent in each stage as a percentage of the total

Table 38.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION:

SLEEP STAGE DATA FOR INTERVAL PRIOR TO ORIGINAL LEARNING.

	REM 15		REM 15 + D		REM 0		REM 0 + D	
	M	s.d.	M	s.d.	M	s.d.	M	s.d.
Time in bed (min)	88.2	15.6	109.4	36.0	105.7	31.3	101.6	26.9
Total sleep time (min)	67.9	13.6	83.7	32.1	77.4	25.1	71.7	18.9
Latency to sleep onset (min)	21.5	16.6	25.2	10.0	28.3	23.7	30.0	25.3
Time in stage 1 (min)	2.9	3.8	5.8	7.7	8.2	8.8	4.1	4.8
Stage 1 (% of total sleep time)	4.5	6.1	5.6	5.7	11.3	12,7	4.3	4.5
Time in stage 2 (min)	17.7	4.9	27.2	21.9	24.5	12.8	25.2	15.4
Stage 2 (% of total sleep time)	27.4	9.0	30.4	12.0	31.0	10.7	32.4	13.7
Time in stages 3+4 (min)	46.3	15.1	47.7	10.8	45.0	19.6	38.1	16.0
Stages 3+4 (% of total sleep time)	66.1	14.1	59.9	16.4	57.2	17.1	54.9	22.6
Time in stage REM (min)	0.8	1.9	0.9	2.1	0.2	0.2	0.2	0.3
Stage REM (% of total sleep time)	1.1	2.2	1.1	2.3	0.3	0.4	0.4	0.4

Table 39a.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: STATISTICAL ANALYSIS OF SLEEP STAGE DATA FOR THE INTERVAL PRIOR TO ORIGINAL LEARNING.

	Mann-Whitney U test comparing REM 15 & REM 0	Mann-Whitney U test comparing REM 15 + D & REM 0 + D	Wilcoxon matched-pairs signed-ranks test comparing REM 15 & REM 15 + D
Time in bed (min)	N/S	N/S	N/S
Total sleep time (min)	N/S	N/S	N/S
Latency to sleep onset (min)	N/S	N/S	N/S
Time in stage 1 (min)	N/S	N/S	$p < 0.05$ (two tailed)
Stage 1 (% of total sleep time)	N/S	$\alpha < 0.1$ (two tailed)	$p < 0.05$ (two tailed)
Time in stage 2 (min)	N/S	N/S	$p < 0.05$ (two tailed)
Stage 2 (% of total sleep time)	N/S	N/S	N/S
Time in stages 3+4 (min)	N/S	N/S	N/S
Stages 3+4 (% of total sleep time)	N/S	N/S	N/S
Time in stage REM (min)	N/S	N/S	N/S
Stage REM (%of total sleep time)	N/S	N/S	N/S

Table 39b.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: STATISTICAL ANALYSIS OF SLEEP STAGE DATA FOR THE INTERVAL PRIOR TO ORIGINAL LEARNING (continued).

	Wilcoxon matched-pairs signed-ranks test comparing REM 0 & REM 0 + D	Mann-Whitney U test comparing REM 15 & REM 0 + D	Mann- Whitney U test comparing REM 0 & REM 15 + D
Time in bed (min)	N/S	N/S	N/S
Total sleep time (min)	N/S	N/S	N/S
Latency to sleep onset (min)	N/S	N/S	N/S
Time in stage 1 (min)	N/S	N/S	N/S
Stage 1 (% of total sleep time)	N/S	N/S	N/S
Time in stage 2 (min)	N/S	N/S	N/S
Stage 2 (% of total sleep time)	N/S	N/S	N/S
Time in stages 3+4 (min)	$\bar{p} < 0.05$ (two tailed)	N/S	N/S
Stages 3+4 (% of total sleep time)	N/S	N/S	N/S
Time in stage REM (min)	N/S	N/S	N/S
Stage REM (% of total sleep time)	N/S	N/S	N/S

was calculated for the four groups. The significant effects were as follows : The REM 15 group spent significantly less time ($p < 0.05$, two tailed)in stage 1 than the REM 15 + D group. When time in stage 1 was expressed as a percentage of total sleep time the REM 15 + D group spent significantly more time in stage 1 than the REM 15 group ($p < 0.05$, two tailed) or the REM 0 + D group ($\alpha < 0.1$, two tailed). The REM 15 group spent significantly less time in stage 2 when compared with the REM 15 + D ($p < 0.05$, two tailed). The REM 0 + D group spent significantly less time in stages 3 + 4 than the REM 0 group ($p < 0.05$, two tailed)

When the interval between learning and relearning was considered, many significant effects emerged. Table 40 shows the means and standard deviations for all the parameters measured at this interval and Tables 41a and 41b give the results of the statistical tests used. There were no significant effects when the total length of time between the original learning and the relearning was measured. When total sleep time was examined, the REM 15 group had significantly more sleep than the REM 0 group ($\alpha < 0.002$, two tailed); the REM 15 + D group had significantly more sleep than the REM 0 + D group ($\alpha < 0.002$, two tailed); the REM 15 group had significantly more sleep than the REM 0 + D group ($\alpha < 0.002$, two tailed) and the REM 0 group had significantly less sleep than the REM 15 + D group ($\alpha < 0.002$, two tailed). Sleep onset latency following the first awakening also produced significant differences between the groups as follows : The REM 0 group took significantly longer to fall asleep than the REM 15 group ($\alpha < 0.1$, two tailed); the REM 0 + D

Table 40.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: SLEEP STAGE DATA FOR INTERVAL BETWEEN ORIGINAL LEARNING AND RELEARNING.

	REM 15		REM 0		REM 15 + D		REM 0 + D	
	M	s.d.	M	s.d.	M	s.d.	M	s.d.
Total time (min)	175.9	37.6	161.1	36.8	151.4	28.9	175.4	54.4
Total sleep time (min)	124.3	27.8	86-1	26.4	122.6	20.3	89.2	35.5
Time awake (min)	51.6	15.0	75.0	26.8	58.8	18.8	92.5	51.0
Time awake (% of total sleep time)	29.3	4.9	46.7	12.8	32.2	7.9	50.8	18.3
Time in stage 1 (min)	5.0	4.0	3.7	2.7	8.5	10.2	8.8	18.4
Stage 1 (% of total sleep time)	2.8	1.9	2.2	1.5	4.6	5.2	3.8	7.2
Time in stage 2 (min)	63.8	16.9	58.8	22.7	59.5	16.6	52.6	27.1
Stage 2 (% of total sleep time)	36.7	7.4	36.6	12.0	32.7	7.2	30.8	14.9
Time in stages 3+4 (min)	39.5	13.6	23.1	11.9	40.0	16.0	21.0	12.6
Stages 3+4 (% of total sleep time)	22.5	6.7	14.4	6.2	22.2	9.3	13.7	10.1
Time in stage REM (min)	16.0	8.6	0.6	0.6	14.9	6.3	0.8	0.8
Stage REM (% of total sleep time)	8.9	2.6	0.4	0.4	8.4	3.6	0.6	0.6

Table 41a.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: STATISTICAL ANALYSIS OF SLEEP STAGE DATA FOR INTERVAL BETWEEN ORIGINAL LEARNING AND RELEARNING.

	Mann-Whitney U test comparing REM 15 & REM 0	Mann-Whitney U test comparing REM 15 + D & REM 0 + D	Wilcoxon matched-pairs signed-ranks test comparing REM 15 and REM 15 + D.
Total time interval (min).	N/S	N/S	N/S
Total sleep time (min).	$\alpha < 0.002$ (two tailed)	$\alpha < 0.002$ (two tailed)	N/S
Sleep onset latency after first awakening (min).	$\alpha < 0.1$ (two tailed)	N/S	N/S
Time awake including second awakening (min).	$\alpha < 0.02$ (two tailed)	$\alpha < 0.05$ (two tailed)	N/S
Time awake including second awakening (% of total time).	$\alpha < 0.002$ (two tailed)	$\alpha < 0.02$ (two tailed)	N/S
Time in stage 1 (min).	N/S	N/S	N/S
Stage 1 (% of total sleep time).	N/S	N/S	N/S
Time in stage 2 (min)	N/S	N/S	N/S
Stage 2 (% of total sleep time)	N/S	N/S	N/S
Time in stages 3+4 (min).	$\alpha < 0.002$ (two tailed)	$\alpha < 0.02$ (two tailed)	N/S
Stages 3+4 (% of total sleep time).	$\alpha < 0.002$ (two tailed)	$\alpha < 0.05$ (two tailed)	N/S
Time in stage REM (min).	$\alpha < 0.001$ (one tailed)	$\alpha < 0.001$ (one tailed)	N/S
Stage REM (% of total sleep time).	$\alpha < 0.001$ (one tailed)	$\alpha < 0.001$ (one tailed)	N/S

Table 41b.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: STATISTICAL ANALYSIS OF SLEEP STAGE DATA FOR INTERVAL BETWEEN ORIGINAL LEARNING AND RELEARNING.

	Wilcoxon matched-pairs signed-ranks test comparing REM 0 & REM 0 + D	Mann-Whitney U test comparing REM 15 & REM 0 + D	Mann-Whitney U test comparing REM 15 + D & REM 0
Total time interval (min).	N/S	N/S	N/S
Total sleep time (min).	N/S	$\alpha < 0.002$ (two tailed)	$\alpha < 0.002$ (two tailed)
Sleep onset latency after first awakening (min).	N/S	$\alpha < 0.1$ (two tailed)	N/S
Time awake including second awakening (min).	N/S	$\alpha < 0.05$ (two tailed)	N/S
Time awake including second awakening (% of total time).	N/S	$\alpha < 0.002$ (two tailed)	$\alpha < 0.02$ (two tailed)
Time in stage 1 (min).	N/S	N/S	N/S
Stage 1 (% of total sleep time).	N/S	N/S	N/S
Time in stage 2 (min).	N/S	N/S	N/S
Stage 2 (% of total sleep time).	N/S	N/S	N/S
Time in stages 3+4 (min).	N/S	$\alpha < 0.002$ (two tailed)	$\alpha < 0.02$ (two tailed)
Stages 3+4 (% of total sleep time).	N/S	$\alpha < 0.05$ (two tailed)	$\alpha < 0.05$ (two tailed)
Time in stage REM (min).	N/S	$\alpha < 0.001$ (one tailed)	$\alpha < 0.001$ (one tailed)
Stage REM (% of total sleep time).	N/S	$\alpha < 0.001$ (one tailed)	$\alpha < 0.001$ (one tailed)

group took significantly longer to fall asleep than the REM 15 group ($\alpha < 0.1$, two tailed). There were also significant differences in time spent awake. The REM 15 group spent less time awake than either the REM 0 group ($\alpha < 0.02$, two tailed) or the REM 0 + D group ($\alpha < 0.05$, two tailed). The REM 15 + D group also spent less time awake than the REM 0 + D group ($\alpha < 0.05$, two-tailed). When the time awake was calculated as a percentage of the total sleep time the REM 15 group had a smaller percentage of time awake than the REM 0 group ($\alpha < 0.002$, two tailed) or the REM 0 + D group ($\alpha < 0.002$, two tailed) and the REM 15 + D group had a lower percentage of time awake than the REM 0 group ($\alpha < 0.02$, two tailed), or the REM 0+D group ($\alpha < 0.02$ two tailed)

Neither the length of time spent in stage 1 nor the percentage of total sleep time spent in stage 1 were significantly different in any of the four groups and a similar lack of differences was found when the length of time spent in stage 2 or the percentage of total sleep time spent in stage 2 were examined.

Stage REM was the sleep stage which had been experimentally manipulated so that these differences showed up as very highly significant. Both the REM 15 groups were significantly higher in REM time and REM percentage than the REM 0 groups ($\alpha < 0.001$, one tailed) in all cases. REM time and REM percentage did not differ significantly between the REM 15 and REM 15 + D groups or the REM 0 group when compared with the REM 0 + D group.

Significant differences were however found in the length of time and percentage of total sleep time spent

in SWS, stages 3 + 4. The REM 15 group spent significantly more time in stages 3 + 4 sleep than the REM 0 group ($\alpha <$ 0.002, two tailed) or the REM 0 + D group ($\alpha <$ 0.002, two tailed) and the REM 15 + D group spent more time in these sleep stages than either the REM 0 group ($\alpha <$ 0.02, two tailed) or the REM 0 + D group ($\alpha <$ 0.02, two tailed). When stages 3 + 4 were expressed as a percentage of the total sleep time, the REM 15 group spent significantly higher percentages of time in these sleep stages than either the REM 0 group ($\alpha <$ 0.002, two tailed) or the REM 0 + D group ($\alpha <$ 0.05, two tailed). The REM 15 + D group had a higher percentage of stages 3 + 4 sleep than the REM 0 group ($\alpha <$ 0.05, two tailed) or the REM 0 + D group ($\alpha <$ 0.05, two tailed).

The Wilcoxon matched-pairs signed-ranks test and the Mann Witney U test were used to compare pairs of groups on the time spent awake in the first and second awakenings. The comparisons made were: REM 15 with REM 0; REM 15 + D with REM 0 + D; REM 15 with REM 15 + D; REM 0 with REM 0 + D; REM 15 + D with REM 0 and REM 15 with REM 0 + D. No significant differences were found in any of the comparisons made.

Mean critical flicker fusion was always higher on the third compared with the first awakening. Reference to Tables 42 and 43 will show that these differences were small and statistical analyses were not carried out. It was considered that these differences were small enough to allow the interpretation that there was no difference in level of arousal at the time of relearning between the

Table 42.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: CRITICAL FLICKER FUSION ON EXPERIMENTAL NIGHTS.

First awakening			Third awakening		
Inc.c/s (mean)	Dec.c/s (mean)	$\frac{\text{Inc.+Dec.}}{2}$	Inc.c/s (mean)	Dec.c/s (mean)	$\frac{\text{Inc.+ Dec.}}{2}$
REM 15 group					
34.5	28.0	31.3	33.8	30.8	32.3
34.2	37.0	35.6	34.3	32.8	33.6
31.0	29.0	30.0	32.5	29.3	30.9
39.3	60.0	49.7	35.0	66.0	50.5
39.0	31.0	35.0	40.3	40.5	40.4
32.3	31.0	31.7	33.3	31.3	32.3
27.9	64.0	46.0	28.1	67.8	48.0
37.3	41.3	39.3	39.5	53.0	45.8
38.3	62.8	50.6	37.8	71.0	54.4
30.3	33.0	31.8	30.0	29.5	29.8
36.0	50.3	42.3	37.0	71.6	54.3
34.5	39.3	36.9	40.3	54.4	42.7
36.6	76.4	56.5	37.8	75.3	56.6
32.9	53.1	43.0	27.4	51.5	39.5
29.8	30.0	29.9	30.9	30.9	30.9
Mean		39.4			41.8
REM 0 group					
46.8	29.0	37.9	38.5	36.5	37.4
35.0	40.0	37.5	35.5	36.0	35.8
28.0	43.0	35.5	27.5	50.0	38.8
35.8	36.3	36.1	36.8	39.5	38.2
38.0	27.0	32.5	36.8	27.0	31.9
34.8	47.8	41.3	35.0	58.0	46.5
35.0	42.3	38.5	37.8	49.0	43.3
26.8	35.3	31.6	28.3	30.0	29.1
56.8	66.0	60.9	56.o	49.5	52.8
38.5	54.8	41.7	37.5	55.8	46.7
39.5	53.8	46.7	37.5	53.0	45.2
33.4	67.0	50.2	31.5	77.8	54.7
55.6	81.8	68.7	56.6	82.9	69.8
42.8	43.6	43.2	40.1	58.6	49.9
35.8	38.0	36.9	34.6	37.8	36.2
Mean		42.6			43.7

Table 42.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: CRITICAL FLICKER FUSION ON DOXYCYCLINE NIGHTS.

First awakening			Third awakening		
Inc.c/s (mean)	Dec.c/s (mean)	Inc.+ Dec. / 2	Inc.c/s (mean)	Dec.c/s (mean)	Inc.+ Dec. / 2
REM 15 group					
32.0	28.0	30.0	33.3	29.3	31.3
34.0	32.5	33.3	35.3	39.5	37.4
33.0	28.0	31.5	31.8	28.5	30.1
50.0	67.0	58.5	34.8	64.5	49.7
34.0	31.0	32.5	34.3	35.3	34.8
27.3	54.0	40.8	26.8	55.0	40.9
37.3	41.3	39.3	40.0	53.0	46.5
35.5	55.3	45.4	36.0	63.3	49.7
30.3	29.3	29.8	29.8	29.8	29.8
33,8	41.8	37.8	33.5	61.8	47.7
36.4	47.0	41.7	38.4	41.3	39.9
36.5	55.8	46.2	37.0	77.1	57.1
31.5	58.1	44.8	30.6	65.3	47.9
31.0	30.6	30.8	32.0	30.9	31.5
Mean		38.7			40.9
REM 0 group					
38.0	34.3	36.2	40.0	57.8	48.9
37.8	32.3	35.1	37.0	44.5	40.8
27.0	44.0	35.5	27.8	48.3	38.1
36.5	30.5	33.5	36.0	37.8	36.9
35.8	27.8	29.5	35.0	29.5	32.5
35.5	46.2	37.8	35.8	62.8	49.3
38.8	45.3	42.1	39.0	57.5	48.3
28.1	33.3	30.7	27.5	30.0	28.9
50.8	61.7	56.2	53.8	60.8	57.3
39.8	43.8	44.1	40.3	56.3	48.9
38.3	53.5	45.9	39.0	50.8	44.9
31.0	71.5	51.3	32.0	75.5	53.8
51.5	84.6	68.1	57.1	85.5	71.5
41.8	32.9	37.4	41.1	56.3	48.7
35.4	32.5	34.0	34.9	41.3	38.1
Mean		41.7			45.7

groups at the time of the relearning although it should be noted that the REM 0 + D group on the third awakening did show the highest CFF rating. This means that they saw the flickering light fuse slightly later than other groups and therefore the level of arousal in this group might be slightly lower.

Menstrual cycle data.

In the REM 15 group, 7 out of the 15 subjects were taking a contraceptive pill and in the REM 0 group 8 subjects were taking such a pill. In the REM 15 group four subjects had irregular menstrual cycle periods, two subjects having a long cycle and one subject having a short cycle time. In the REM 0 group, two subjects had menstrual periods of long cycles. The phases of the menstrual cycles of the four groups was as follows :-

	Menses	Pre-ovulatory stage	Progestational stage
REM 15	4 subjects	8 subjects	8 subjects
REM 15 + D	5 "	4 "	6 "
REM 0	1 "	8 "	6 "
REM 0 + D	0 "	4 "	11 "

It can be seen that the REM 0 + D group, which performed the worst with regard to savings percent on the memory tasks was the group which had the highest proportion of subjects in the progestational stage and it should be remembered that it had been shown by HARTMANN (1966) that there is increased REM pressure and REM time at this period. Since this group was REM deprived, it is possible that they might have experienced more stress by the REM than other groups which would contribute to the poorer

performance by this group. I should like to stress that this menstrual cycle data was only collected in case there were any highly significant differences between the groups to insure that any significant effects could not be explained by factors other than REM sleep or doxycycline administration. For these same reasons the 16 PF personality inventory was also administered to all subjects to insure that there were no major personality differences between subjects in the REM 15 or the REM 0 groups but differences between the REM 15 and REM 0 groups on the memory task was not considered sufficiently different to warrant the amount of work involved in analysing the data from the personality inventory.

(e) Summary and discussion of results.

The major differences emerging when sleep parameters were examined were found in the interval between original learning and relearning. Although there were no significant differences between the groups in total time between learning and relearning, groups did differ in the time spent asleep, awake and in SWS. The main differences were found according to whether subjects were REM deprived or had REM sleep. The REM 15 group spent more time asleep than REM 0 groups, thus the percentage of total time spent awake and the length of time spent awake was correspondingly less in the REM 15 group. The amount of time spent in stage REM, which was the experimentally manipulated variable was indeed significantly greater in REM 15 than REM 0 groups and this was also true when stage REM was considered as a percentage of the total sleep time. The

differences found in SWS were rather surprising, both REM 15 groups having significantly more SWS than the REM 0 groups. Thus it can also be said that depriving subjects of REM sleep also made them more wakeful and resulted in less time being spent in SWS.

Even though these significantly different effects on sleep stages were found as a result of REM deprivation, significant effects on memory for the material used were not found when performance of the REM 0 group was compared with performance by the REM 15 group. It was only when the REM deprivation procedures were combined with doxycycline administration that conditiond became sufficiently difficult to impair the memory mechanism. It should also be remembered that the REM 0 + D group also contained a greater number of subjects in the progestational phase of the menstrual cycle.

A possible explanation of the poorest performance by the REM 0 + D group was that the second sentence was easier for them to learn than the second sentence for any of the other groups. The decrease in savings percent could therefore be a result not of the increase in the number of trials to relearn the first sentence, but a decrease in the number of trials to learn the second sentence. The Wilcoxon matched-pairs signed-ranks test did demonstrate that the REM 0 + D group found the second sentence significantly easier to learn than the first sentence, and that the REM 0 + D group found the second sentence significantly easier to learn than the REM 15 group. Of all four groups, the REM 0 + D group did exhibit the lowest

number of trials to criterion for learning the second sentence. It could therefore be argued that the significant decrement in savings percent shown by this group could be due to a combination of the effect of increased difficulty in relearning the first sentence plus a less difficult second sentence. However the REM 15 + D group and the REM 0 group also found the second sentence easier to learn than the REM 15 group but this did not result in a significant difference in savings percent between these groups.

The problem was further investigated by calculating savings as a percentage of the first sentence. This was not part of the original design of the experiment, but was intended to find out whether the REM 0 + D group would still show a significant difference in relearning performance when the less difficult second sentence was not included in the calculation. The savings percent calculated as a percentage of the first sentence for the four groups for the whole sentence is shown in Table 44. It can be seen that the poorest savings percentage was again shown by the REM 0 + D group. Tables 45 and 46 show the calculations for the first and second halves of the sentences, and on all occasions the REM 0 + D group exhibit the lowest savings scores. The means and standard deviationd for the savings scores when calculated as a percentage of the first sentence are summarised in Table 47.

Statistical analyses were also carried out on these calculations, the same statistical tests being used as when savings was calculated as a percentage of the second

Table 44.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES CALCULATED AS A PERCENTAGE OF THE FIRST SENTENCE.

first sentence (learn)	first sentence (relearn)	savings %	first sentence (learn)	first sentence (relearn)	savings %
REM 15 group			REM 15 + D group		
7	3	57.1	4	3	25.0
12	11	8.3	11	6	45.5
4	2	50.0	4	2	50.0
8	5	37.5	27	5	81.5
10	5	50.0	-	-	-
6	2	66.7	9	3	66.7
9	5	44.5	10	3	70.0
4	2	50.0	6	4	33.3
4	2	50.0	4	2	50.0
10	6	40.0	11	5	54.6
4	4	0.0	6	2	40.0
10	3	70.0	7	6	14.3
8	6	25.0	4	2	50.0
9	6	33.3	8	3	62.5
5	3	40.0	5	3	40.0
Mean 7.3	4.3	41.5	8.3	3.5	48.8
s.d. 2.6	2.4	18.6	5.8	1.4	17.4
REM 0 group			REM 0 + D group		
5	2	60.0	5	3	40.0
8	7	12.5	12	8	33.3
11	4	63.6	13	7	46.2
11	7	36.4	12	10	16.7
5	2	60.0	4	3	25.0
4	3	25.0	5	2	60.0
7	4	42.9	6	5	16.7
7	3	57.1	9	3	66.7
5	3	40.0	6	3	50.0
7	4	42.9	7	7	0
6	3	50.0	5	3	40.0
5	2	60.0	5	4	20.0
3	3	0	4	3	25.0
4	3	25.0	3	2	33.3
11	7	36.4	16	7	56.3
Mean 6.2	3.8	40.8	7.5	4.7	35.2
s.d. 2.8	1.7	18.2	3.9	2.4	17.9

Table 45.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES CALCULATED AS A PERCENTAGE OF THE FIRST SENTENCE. FIRST HALF OF SENTENCES.

first sentence (learn)	first sentence (relearn)	savings %	first sentence (learn)	first sentence (relearn)	savings %
REM 15 group			REM 15 + D group		
7	3	57.1	4	3	25.0
11	7	36.4	6	2	66.7
3	2	33.3	3	2	33.3
6	5	16.7	7	3	57.1
10	5	50.0	-	-	-
6	2	66.7	3	3	0
7	5	28.6	6	3	50.0
1	2	-100.0	3	2	33.3
4	2	50.0	3	1	66.7
10	4	60.0	4	5	-25.0
4	4	0	4	2	50.0
8	3	62.5	3	3	0
8	5	37.5	3	2	33.3
9	6	33.3	8	2	75.0
4	2	50.0	3	2	33.3
Mean 6.5	3.8	32.1	4.3	2.5	35.6
s.d. 2.8	1.6	39.4	1.7	0.9	27.6
REM 0 group			REM 0 + D group		
4	2	50.0	3	2	33.3
8	7	12.5	6	4	33.3
8	3	62.5	8	4	50.0
11	7	36.4	7	5	28.6
4	2	50.0	3	2	33.3
4	2	50.0	1	1	0
7	3	57.1	5	4	20.0
5	2	60.0	6	3	50.0
4	2	50.0	4	2	50.0
7	4	42.9	7	7	0
5	3	40.0	5	2	60.0
4	2	50.0	2	3	-50.0
2	1	50.0	4	3	25.0
1	3	-200.0	3	2	33.3
11	7	36.4	10	5	50.0
Mean 5.7	3.3	29.9	4.9	3.3	27.8
s.d. 2.8	2.0	62.5	2.4	1.5	26.8

Table 46.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES CALCULATED AS A PERCENTAGE OF THE FIRST SENTENCE. SECOND HALF OF SENTENCES.

first sentence (learn)	first sentence (relearn)	savings %	first sentence (learn)	first sentence (relearn)	savings %
REM 15 group			REM 15 + D group		
6	3	50.0	4	3	25.0
12	9	25.0	11	6	45.5
4	1	75.0	4	2	50.0
8	5	37.5	20	5	75.0
9	5	44.4	-	-	-
5	2	60.0	9	2	77.8
9	4	55.6	9	3	66.7
4	2	50.0	6	4	33.3
3	1	66.7	4	2	50.0
10	6	40.0	10	4	60.0
4	3	25.0	6	2	66.7
10	3	70.0	7	4	42.9
7	6	14.3	4	2	50.0
8	4	50.0	6	3	50.0
5	2	60.0	5	3	40.0
Mean 6.9	3.7	48.2	7.5	3.2	52.4
s.d. 2.7	2.1	16.9	4.2	1.2	14.8
REM 0 group			REM 0 + D group		
5	2	60.0	5	3	40.0
7	4	42.9	12	8	25.0
11	4	36.4	11	7	36.4
8	6	25.0	12	10	16.7
5	2	60.0	4	3	25.0
4	1	75.0	5	2	60.0
7	4	42.9	6	5	16.7
6	3	50.0	9	3	66.7
5	3	40.0	6	3	50.0
7	4	42.9	6	5	16.7
3	2	33.3	5	3	40.0
5	2	60.0	5	4	20.0
3	3	0	4	1	75.0
3	3	0	2	2	0
8	5	37.5	16	7	56.2
Mean 5.5	3.2	40.4	7.2	4.4	36.3
s.d. 2.3	1.3	20.1	3.8	2.5	21.0

Table 47.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: MEAN SAVINGS PERCENT CALCULATED AS A PERCENTAGE OF THE FIRST SENTENCE.

	REM 15 group		REM 0 group		REM 15 + D group		REM 0 + D group	
	M	s.d.	M	s.d.	M	s.d.	M	s.d.
whole sentence	41.5	18.6	40.8	18.2	48.8	17.4	35.2	17.9
first half of sentence	32.1	39.4	29.9	62.5	35.6	27.6	27.8	26.8
second half of sentence	48.2	16.9	40.0	20.1	52.4	14.8	36.3	21.0

sentence. The significant effects are shown in Table 48. Considering the whole sentence, the only statistically significant effect found was that the REM 0 + D group saved less than the REM 15 + D ($p < 0.01$, two tailed). In the second half of the sentence the REM 0 + D group again saved significantly less than the REM 15 + D group ($p < 0.10$, two tailed) but no significant differences were found between the groups when the first half of the sentences were examined.

Reference to Table 30 shows that for the REM 0 + D group, the first sentence was not significantly easier for them to learn than any other group. Since the poorest performance was always exhibited by the REM 0 + D group this cannot be explained solely in terms of this group having the easiest to learn second sentence.

Although subjects sometimes complained of feeling unwell or more drowsy on the doxycycline night, it cannot be argued that they were feeling too unwell on the doxycycline night to concentrate or had final night fatigue since they required fewer trials to learn the second sentence on the doxycycline night compared with the experimental night. Moreover, had they benefitted from their experiences on the experimental night, and engaged in rehearsal of the first sentence on the doxycycline night, they should have been able to relearn more quickly on the doxycycline nights (STONES, 1973).

A disadvantage in the experimental design was that the doxycycline night always followed the experimental night, as has been pointed out earlier and was for reasons of subject-night economy. Had the experimental and

Table 48.

REM SLEEP DEPRIVATION AND PROTEIN SYNTHESIS INHIBITION: SIGNIFICANT DIFFERENCES IN SAVINGS PERCENT CALCULATED AS A PERCENTAGE OF THE FIRST SENTENCE.

WHOLE SENTENCE.

Wilcoxon matched-pairs signed-ranks test comparing :-

(a) REM 15 & REM 15 + D groups	N/S
(b) REM 0 & REM 0 + D groups	N/S

Mann-Whitney U test comparing :-

(a) REM 15 & REM 0 groups	N/S
(b) REM 15 + D & REM 0 + D groups	REM 0 + D $<$ REM 15 + D, significant at $p < 0.01$, two tailed.
(c) REM 15 & REM 0 + D groups	N/S
(d) REM 15 + D & REM 0 groups	N/S

FIRST HALF OF SENTENCES.

Wilcoxon matched-pairs signed-ranks test comparing :-

(a) REM 15 & REM 15 + D groups	N/S
(b) REM 0 & REM 0 + D groups	N/S

Mann-Whitney U test comparing :-

(a) REM 15 & REM 0 groups	N/S
(b) REM 15 + D & REM 0 + D groups	N/S
(c) REM 15 & REM 0 + D groups	N/S
(d) REM 0 & REM 15 + D groups	N/S

SECOND HALF OF SENTENCES.

Wilcoxon matched-pairs signed-ranks test comparing :-

(a) REM 15 & REM 15 + D groups	N/S
(b) REM 15 + D & REM 0 + D groups	N/S

Mann-Whitney U test comparing :-

(a) REM 15 & REM 0 groups	N/S
(b) REM 15 + D & REM 0 + D groups	REM 0 + D $<$ REM 15 + D, significant at $p < 0.1$, two tailed.
(c) REM 15 & REM 0 + D groups	N/S
(d) REM 15 + D & REM 0 groups	N/S

doxycycline nights been counterbalanced then a longer period would have been required between the two nights to ensure that when the doxycycline night preceded the experimental night, the effects of doxycycline had completely worn off before the experimental night. It might then have been necessary to intersperse one further night between the two, in order to maintain the degree of adaptation. Since the experimental night always did come first, any mental set acquired by subjects on this night, such as an expectation to have to relearn the first sentence, they would be able to apply this on the doxycycline night. However, as has been pointed out, this would have resulted in improved performance, whereas performance was worse. Had experimental and doxycycline nights been counterbalanced, it is possible that a more highly significant decrement in relearning performance might have been found.

The omission of a procedure whereby different sentences were balanced across conditions was also a serious fault in the design of the experiment. This was realised only in retrospect but was however rectified in the daytime experiments to be described in the chapter to follow. Even though the sentences were informally equated for difficulty prior to the start of the experiment, it can be seen that there were significant differences in ease of learning the sentences. This was particularly shown by the REM O + D group such that combined with the differences in learning ability between groups, the REM O + D group being the poorest learners, might offer a sufficient explanation of memory impairment of this group. However there

were no significant differences among the groups for learning the first sentence so differences in learning ability among the groups cannot be considered as a very important factor. Also, if the second sentence really was easier to learn on the doxycycline night, then it sould also have been significantly easier for the REM 15 + D group compared to the REM 15 group.

Investigation of sentence learning i.e. connected discourse is handicapped by two main problems (DEESE, 1961).

(i) the problem of scoring achievement in learning and retention. This problem is chiefly one of finding suitable units in terms of which retention can be scored.

(ii) the concept of the structure of connected discorse.

Because of these problems, I selected the learning technique of serial anticipation from a memory drum primarily for the ease and neatness of scoring the results. An alternative would have been to allow the subjects a finite amount of time to read and attempt to learn the sentences and then assess retention by means of free recall at the required time of testing. With this method however, there is no control for the original amount of learning. If original learning were assessed at the time, then this would constitute an extra rehearsal of the sentence and would tend to bias results in favour of those subjects who were good learners. In addition if mistakes were made in this assessment trial then these might tend to be remembered rather than the correct sentence.

When designing my experiment, I considered only

the semantic and not the linguistic aspects of sentence memory but there is the additional problem of what aspects of a complex sentence are remembered. Since linguistic structure of the sentences was not identical this could also contribute to differences in ease of learning or remembering the sentences. Since syntactical structure does seem to have psychological reality (LESLIE, 1974) then such a factor should be considered in memory of sentences at least under conditions of verbatim recall.

In Chapter VII, the possibility was pointed out that it might only be a particular type of memory that is consolidated during REM sleep i.e. material that was emotionally meaningful to the subjects and which involved cortical-limbic neuronal interconnections. The material used in my experiments was of a type very different to this since it was chosen in the first place to be meaningful i.e. not nonsense, but rather neutral in content. This was so that it had roughly the same interest value for all subjects, otherwise subjects being most interested in the content of any one sentence would have tended to remember it better, regardless of treatment condition. Because of the nature of the material and the learning procedure selected, subjects used a rote learning method. This in itself is a rather tedious and uninteresting method of learning for people of high IQ level. Thus there would be very little emotional meaning attached by subjects to the material and it might therefore not be especially consolidated during REM sleep.

The task took only a short time to complete and was

certainly not difficult for the subjects. If information is stored in a labile manner prior to transferrence to stable memory, then it must take its place amongst all the other information in the labile store awaiting consolidation. The material learned for this experiment would probably occupy only a minute proportion of the total amount accumulated throughout the day and thus might not be preferrably consolidated even on those subjects allowed REM sleep. If the mechanism of protein synthesis is the one which operates especially during REM sleep to consolidate memory then it seems logical to ask why the REM 0 group remembered any of the material at all, since they had no REM. Three possible explanations, which are by no means mutually exclusive, are :- firstly that the memory seen at testing is still labile memory, the interval between OL and RL being insufficient to allow consolidation to occur in full either in the REM 15 or REM 0 groups. Secondly, consolidation via protein synthesis takes place continuously and the process occurred to a similar extent under both conditions especially since the material might be of a type not requiring the presence of REM sleep for consolidation. The third explanation is that cerebral protein synthesis is not confined to REM sleep but might be increased in REM sleep.

SWS and TST were reduced when subjects were deprived of REM sleep, and so varying these sleep parameters did not seem to affect memory either. SWS was however not totally abolished as was REM, and it would be interesting to discover what effects total SWS deprivation might have

under these conditions.

Subjects were awakened on three occasions during the night and asked to perform the task at a time when they would normally be sleeping. This might be experienced as stressful by all experimental groups and performance by subjects being allowed REM sleep might be depressed to the level of those being deprived of REM, thus masking any improvements in performance as a result of being allowed REM sleep. One way of excluding this problem would be to REM deprive one group of subjects throughout the whole night and test retention on the following morning. A control group could be allowed REM sleep and could be woken up the same number of times as the REM deprived group but from sleep stages other than REM. The relearning and learning of a second sentence would then be carried out at a time when all subjects would be alert.

Doxycycline, when administered without REM deprivation also failed to reveal memory impairments. No data was collected from subjects regarding what they ate prior to coming to the laboratory, and it is known that absorption of tetracyclines from the gastro-intestinal tract is hindered by the presence of such foods as milk products. I have no information as to whether the particular tetracycline used crosses the blood brain barrier but other tetracyclines do and reach the CNS. They are used clinically to treat meningitis (WEINSTEIN, 1965). The possibility must be considered that it does not cross the blood brain barrier, or if it does in only very small quantities, then with the dose administered, this might

not be sufficient on its own to inhibit protein synthesis to the degree required to arrest memory formation. In the animal studies where protein synthesis inhibitors were used, large doses were given, intracerebrally or subcutaneously and these inhibited protein synthesis by more than 90% for several hours. This type of experiment would off course be impossibe with human subjects. The degree of inhibition caused by doxycycline might have been too low to appreciably affect the memory mechanism for a task which was relatively easy for subjects.

Significant retention deficits were found when the two treatments were combined. It is possible that the administration of doxycycline somehow made subjects more sensitive to REM deprivation. Reference to animal studies does not provide any further information since I know of none up until the time of writing where these two treatment conditions have been combined, though as previously described, either treatment is sufficient to impair animal memory.

Thus when doxycycline is given without REM deprivation there might be a slight but insignificant effect on memory. Reference to Table 26 will show that the REM 15 + D group did save slightly less than the REM 0 group. REM deprivation procedures on their own might have no significant detrimental effects on memory because consolidation is able to take place to a sufficient degree in stages of sleep other than REM, or more likely during wakefulness perhaps in the time it takes to fall asleep and during the awakening procedures. Reference to Table 26

shows that the REM 0 group did in fact save less than the REM 15 group though this difference was not significant. It is also possible that other mechanisms or pathways of protein biosynthesis might be used when the main pathway is blocked, since the concept of redundancy in the CNS is well known. For example it could be that there is a pool of previously synthesised protein that can be used for the short time that the inhibition lasts. Thus only when the two treatments are combined which may block all effective protein synthesis and utilisation, is consolidation of this type of material significantly impaired.

Chapter XI.

DOXYCYCLINE AND MEMORY: DAYTIME STUDIES.

In the previous chapter it was shown that when doxycycline was administered to subjects in conjunction with REM deprivation there were significant impairments in memory when compared with a group of subjects who were REM deprived or who were allowed some REM sleep. When doxycycline was administered in conjunction with REM sleep, it had no significant effect on memory. One possible explanation for this was that subjects were being tested at a time when they would normally be sleeping and therefore performance in all groups was depressed as a result of a non specific stress effect and this would mask the effects of doxycycline administration.

There is a well known circadian effect on performance (MILLS, 1973; LUCE, 1971). KLEITMAN, (1963) has presented graphs of a number of test scores which, over a working day follow a time course very similar to that of body temperature. This parallel has been observed with an adding test (LOVELAND & WILLIAMS, 1963) as well as with simple reaction time (KLEIN et al, 1968) and with speed of manipulation (SCHUBERT, 1969). Body temperature reaches a high point during the day and is at its lowest in late sleep (LUCE, 1971). KLEIN et al (1968) showed that mental performances were at their peak between 2 and 4 p.m. and poorest performances occurred between 2 and 4 a.m. Since subjects in the night time study would be performing the relearning at a time when their general level of performance would be at its lowest, the arguement put foreward

in the above paragraph seems valid. In order to investigate this possibility and to discover if doxycycline has any effect on memory unrelated to sleep or to REM sleep, two further studies were carried out with doxycycline. These both investigated the administration of the drug during the day.

The procedure for learning and relearning was identical to the night time studies but instead of a period of sleep between learning and relearning, a period of wakefulness was interpolated. The time span of the interpolated wakefulness was three and a half hours which was roughly equivalent to the mean time between learning and relearning in the night time study. It was hypothesised than subjects receiving doxycycline would show a smaller savings score than those receiving placebo.

(1) FIRST EXPERIMENT.

(a) Method.

Four subjects were used in this experiment which was used as a pilot experiment to a larger study to be carried out subsequently. The subjects were female students aged between 20 and 22 and were different from the subjects used in the night time study.

The experiment was carried out on three successive days, each subject having one adaptation day and two experimental testing days, on one of which they received doxycycline. The adaptation day was again merely to accustom subjects to conditions in the laboratory and put them at their ease, to familiarise them with the serial anticipation method of learning and to overcome

practice effects seen with this type of learning task. The two adaptation sentences were the same as the two used on the second adaptation night of the night time study, and again performance results were not analysed. The adaptation sentences were of the required length but were not matched for difficulty.

On the adaptation day, subjects reported for the experiment and learned the first sentence to the criterion of one correct anticipation. They then went away for three and a half hours. No attempt was made to control the activities of subjects during this period. On returning to the laboratory after the appropriate interval, they learned the second sentence to the same criterion.

On the second and third days of the experiment subjects were asked to take two capsules which they were told contained an antibiotic. The capsules were taken just prior to the original learning. On one of the days the capsules contained placebo and on the other, they contained the doxycycline. The dose of doxycycline was 200 mg. i.e. the same dose that had been used in the night time study. Two of the four subjects received placebo on the second day of the experiment and the other two received active drug. On the third day the order was reversed, those having received placebo on the second day received active drug on the third day and vice versa. Subjects were told that they would be receiving antibiotic on both days and therefore did not know that on one day it was placebo. The experimenter however was not blind and knew whether each subject received drug

or placebo.

The four sentences A, B, C and D used on the second and third days of the experiment were the same as had been used on the experimental and doxycycline nights of the night time study. These were all prior matched for difficulty but whereas in the night time study they were always learned in the same order i.e. on experimental nights subjects learned sentence A first and then sentence B, and on doxycycline nights they learned C first and then D, in this daytime study, the sentences were presented in a different counterbalanced order for each subject.

Subjects reported for experimental testing sessions at half hourly intervals as follows :-

	day 2	day 3
Subject 1	09.30 hours	10.00 hours
" 2	10.00 "	09.30 "
" 3	10.30 "	11.00 "
" 4	11.00 "	10.30 "

Each subject reported for the second session exactly three and a half hours after the commencement of the first session. At the second session subjects learned a second sentence to the prescribed criterion and then relearned the sentence from the first session, also to the same criterion. The order of drug administration or placebo and the order of presentation of the sentences for each subject is shown in Table 49.

The amount of material remembered was measured by the calculation of a savings score in the same manner as in the night time study. This method compared the

Table 49.

DOXYCYCLINE DAYTIME STUDY I: ORDER OF SENTENCE PRESENTATION AND DOXYCYCLINE ADMINISTRATION.

Subject 1 Doxycycline	Subject 2 Doxycycline
sentence A (learn) " B " " A (relearn)	sentence C (learn) " D " " C (relearn)
Subject 2 Placebo	Subject 1 Placebo
sentence B (learn) " A " " B (relearn)	sentence D (learn) " C " " D (relearn)
Subject 3 Placebo	Subject 4 Placebo
sentence A (learn) " B " " A (relearn)	sentence C (learn) " D " " C (relearn)
Subject 4 Doxycycline	Subject 3 Doxycycline
sentence B (learn) " A " " B (relearn)	sentence D (learn) " C " " D (relearn)

the relearning of material learned in the first session with the learning of equivalent material at the second session. Thus the number of trials to attain criterion for relearning the first sentence was expressed as a percentage of the number of trials to criterion taken to learn the second sentence.

In addition to the four subjects used in this experiment, two subjects were run prior to the start of the experiment to see if there would be any problems in experimental procedure. The sample size of only four subjects was too small to be amenable to any statistical analysis of the results. Inclusion of the two subjects run prior to the start of the experiment permitted the computation of the non parametric sign test (see SIEGAL, 1956) which is applicable to test for differences between two related samples. Since no changes were made in the experimental procedure with the four subjects as a result of the two subjects run first, it was felt legitimate to add these results.

(b) Results.

The number of trials to criterion for learning the first and the second sentences and for relearning the first sentence for the six subjects is shown in Table 50. Savings percent calculated as a percentage of the second sentence and the first sentence is shown also in Table 50.

When savings was calculated as a percentage of the second sentence mean savings percent was 29,7 s.d. 23.2 in the doxycycline condition compared with 47.5 s.d. 14.0 in the placebo condition. When the non parametric sign

Table 50.

DOXYCYCLINE DAYTIME STUDY I: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES.

	first sentence (learn)	second sentence (learn)	first sentence (relearn)	savings as a percentage of second sentence	savings as a percentage of first sentence
Placebo					
	6	6	3	50.0	50.0
	5	5	3	40.0	40.0
	5	5	3	40.0	40.0
	7	5	3	40.0	57.1
	9	8	5	37.5	44.4
	5	9	2	77.8	60.0
Mean	6.2	6.3	3.2	47.5	48.6
s.d.	1.5	1.6	0.9	14.0	7.9
Doxycycline					
	7	3	2	33.3	71.4
	4	4	1	75.0	75.0
	6	5	4	20.0	33.3
	6	5	5	0.0	16.7
	13	9	6	33.3	53.9
	4	6	5	16.7	-25.0
Mean	6.7	5.3	3.8	29.7	37.6
s.d.	3.0	1.9	1.8	23.2	34.6

test was used to compare these two conditions the savings percent of the doxycycline group was found to be significantly lower than in the placebo condition ($p < 0.109$). Significant differences between the two conditions were not found when savings was calculated as a percentage of the first sentence although performance by the doxycycline group was again lower than the placebo group.

(c) Discussion.

Differences in retention between the two conditions was significant when savings was calculated as a percentage of the second sentence albeit with such a small number of subjects. Five out of the six subjects obtained a lower score in the doxycycline condition. When savings was calculated as a percentage of the first sentence only three out of the six subjects obtained a lower score in the doxycycline condition and although the mean percentage savings was lower in the doxycycline group, this was not statistically significant.

It cannot however be assured that doxycycline had a direct effect on memory since three of the subjects complained of feelings of nausea after taking doxycycline and these three actually vomited, one subject only half an hour after taking the antibiotic and the other two between one and a half and three hours after the antibiotic. In the case of this first subject it cannot be assumed that the antibiotic had been absorbed and assimilated. The effects on memory of the previously learned material may be attributable to the non specific effects of illness and nausea. It should however be noted that

the effects of nausea and illness do not appear to have adversely affected the number of trials to learn the second sentence. Subjects who had vomited following one and a half hours after doxycycline ingestion took the same number of trials or less to reach the criterion for learning the second sentence in the doxycycline compared to the placebo condition.

(2) SECOND EXPERIMENT.

The first daytime experiment contained only a small number of subjects, since a significant decrement in performance on the memory task was found when savings was calculated as a percentage of the second sentence, it was decided to run a further 12 subjects under the same experimental conditions as the first daytime study. The design of this study was similar to that of the previous daytime study . Since the procedure was similar it will not be described again. The only differences were that subjects were asked to take the capsules one hour prior to reporting to the laboratory for the first session of the day. They were also asked to take a light breakfast on those days. By this procedure it was hoped that the nausea experienced by subjects in the first experiment would be avoided and that absorption of the antibiotic from the gastro-intestinal tract would be similar for all subjects. The other difference was that the allocation of the capsules was not carried out by the experimenter, so that the experimenter was effectively blind, not knowing on which day subjects received antibiotic. This made the scoring of the retention trials less biased.

Results.

The number of trials to criterion and the savings percent was calculated for each subject by the same manner as previously described and the results are shown in Table 51.

Examination of this table shows that in only five out of the 12 subjects was retention actually worse in the doxycycline condition compared with placebo and in two subjects performance was the same in the two conditions. The other five subjects actually remembered more when they had received doxycycline. Mean savings percent was 52.1 s.d. 18.0 in the doxycycline condition compared with a mean savings of 32.4 s.d. 45.7 when savings was calculated as a percentage of the second sentence. When savings was calculated as a percentage of the first sentence, the doxycycline group also saved more 50.9 s.d. 19.9 than the placebo condition 49.8 s.d. 26.1. These differences were small and statistical tests were not carried out when the calculation involved the first sentence.

The argument proposed in the night time study that there were differences in ease of learning between the first and second sentences cannot be used here since the sentences were counterbalanced across the subjects. The Wilcoxon matched-pairs signed-ranks test showed that there were no significant differences between the groups for learning the first or the second sentences or for relearning the first sentence calculated from the number of trials to criterion. This statistical test also showed no statistically significant effects between the groups

Table 51.

DOXYCYCLINE DAYTIME STUDY II: NUMBER OF TRIALS TO CRITERION AND SAVINGS SCORES.

	first sentence (learn)	second sentence (learn)	first sentence (relearn)	savings as a percentage of second sentence	savings as a percentage of first sentence
Placebo					
	3	2	1	50.0	66.7
	11	8	5	37.5	54.6
	10	7	2	71.4	80.0
	5	3	6	-100.0	-20.0
	5	6	3	50.0	40.0
	8	7	4	42.9	50.0
	7	9	2	77.8	71.4
	9	7	4	42.9	55.6
	14	7	3	57.1	78.6
	8	8	6	25.0	25.0
	11	10	6	40.0	45.5
	6	6	3	50.0	50.0
Mean	8.1	6.7	3.8	32.4	49.8
s.d.	3.0	2.2	1.6	45.7	26.1
Doxycycline					
	3	2	1	50.0	33.3
	12	16	4	75.0	66.7
	6	6	3	50.0	50.0
	9	6	4	33.3	55.6
	4	9	3	66.7	25.0
	11	4	3	25.0	72.7
	19	8	3	62.5	84.2
	9	13	4	62.9	55.6
	8	10	3	70.0	62.5
	6	7	5	28.6	16.7
	7	7	5	28.6	28.6
	5	6	2	66.7	60.0
Mean	8.3	7.8	3.3	52.1	50.9
s.d.	4.2	3.7	1.1	18.0	19.9

with savings calculated as a percentage of the second sentence.

Conclusions and discussion of the two daytime studies.

It would seem from the data analysis of the second daytime experiment that there were no significant differences in savings scores between the two conditions. Any non-significant effects found were in a direction opposite to that predicted. Thus the significant decrease seen with doxycycline in the first daytime experiment might have been a chance effect since only six subjects were used. None of the subjects in the second daytime experiment vomited or even complained of feeling unwell and this could have been a result of the instruction to partake of a light breakfast. It could also be a chance effect that subjects feeling unwell in the first daytime study also reacted badly to the doxycycline. Only one subject out of the 30 run in the night time studies complained of feeling too unwell to continue with the experiment on her doxycycline night. Others who felt unwell after doxycycline did not complain of feeling incapacitated in terms of the learning task or ability to concentrate. Subjects were instructed not to discuss the experiment amongst them selves and therefore were unlikely to have a preconceived idea that they might experience nausea.

To conclude that the antibiotic doxycycline had no effect on memory, it was felt would be premature from the results of the second daytime study. A number of factors such as pharmacolocical action of the antibiotic as well

as individual differences amongst subjects with regard to the rate of absorption, sensitivity to its effect and possible masking effect due to the experimental design, might have combined to prevent the emergence of any effects. Individuals would probably show variation in rates of absorption and excretion of the antibiotic. Also there was probably variation in interpretation of what constituted a light breakfast which would further affect individual rates of absorption of the doxycycline.

Doxycycline has pharmacological actions similar to tetracycline which reaches a peak in blood levels two to four hours after ingestion of a single dose (SHILS, 1962). The average learning time for the first morning session of the task was about 15 minutes and peak blood level of the drug would occur between 45 minutes and two hours 45 minutes after the end of the initial learning of the sentence. Experiments have shown (see Chapter II) that the greatest amount of forgetting takes place within three hours after initial learning so that consolidation should have begun in my experiment in the interval between learning and relearning. It is not however known whether the biochemical processes involved in stable memory formation begin with the reception of stimuli or whether the three hours is the time taken for labile memory to decay, the biochemical processes becoming active only after this decay.

As previously mentioned, it is not known exactly whether doxycycline crosses the blood brain barrier. If it does not, then it would not be acting as a cerebral

protein biosynthesis inhibitor in my experiments. However since the molecule is of similar configuration to other tetracyclines, it is assumed that the fate, excretion and absorption of doxycycline is similar to these. After a single oral dose, peak levels of tetracycline are attained within two to four hours and persist for six hours or longer. Detectable concentrations in the plasma are sometimes observed even after 24 to 30 hours. The administration of 250 mgms. of tetracycline every six hours produces plasma concentrations of one to three µgms./ml. after the second dose and these levels are maintained during continued treatment. If the dose is increased to 500 mgms. every six hours, plasma levels of three to five µgms./ml.are attained. When one gram of the drug is given every six hours, the plasma concentration is usually higher than five µgms./ml. (WEINSTEIN, 1965).

The distribution of tetracyclines has a relatively higher volume than that of the body water indicating sequesteration in some tissues. Tetracyclines are bound to the plasma proteins in varying degrees (KUNIN, 1962). All tetracyclines are removed from the blood by the liver where they are concentrated and excreted by way of the bile into the intestine, from where they are absorbed. Biliary levels of these agents average at least five to ten times higher than the simultaneous plasma concentrations.

Spinal fluid levels of chlorotetracycline average about 25% of those in the plasma. Spinal fluid levels vary considerably regardless of dose. Inflammation of the meninges is not a prequisite for the passage of tetra-

cyclines into the cerebrospinal fluid and duration of treatment is probably a major determinant. The injection of 0.5 grams of tetracycline itself intravenously, leads to a gradual appearance of the drug in the spinal fluid over a period of six hours and the levels attained range from 0.5 to six µgrm./ml. One gram of oxytetracycline given intravenously every 12 hours produces a spinal fluid concentration of about four µgms./ml. Dimethylchlorotetracycline appears in the spinal fluid in only small amounts and the levels of this tetracycline are only two to five percent of the levels in plasma after oral administration of 0.5 to one gram. Oral administration of tetracycline itself also yields very low spinal fluid levels (WEINSTEIN, 1965).

As well as the inhibition of cerebral protein synthesis it is probable that non-cerebral protein synthesis is also to a degree inhibited by the tetracycline administration. Although this presumably is not concerned with learning it might possibly contribute to a non specific effect which in turn could produce the memory impairment.

All tetracyclines are adequately but incompletely absorbed from the gastro-intestinal tract. Absorption is however impaired by the presence of milk or milk products in the intestine. A wide variety of plasma levels of antimicrobial activity has been found between different individuals and this may be related th the irregularity of absorption of tetracyclines (WEINSTEIN, 1965). No control for the type of food eaten prior to doxycycline intake was carried out with my experimental subjects.

In the first daytime experiment, some of the subjects vomited, presumably after taking the antibiotic on an empty stomach. With this in mind subjects in the second experiment were asked to take a light breakfast. Non of the subjects vomited in this experiment although one reported feeling slightly unwell and another spontaneously commented that learning was a real effort on the doxycycline day. Of the twelve subjects in the second daytime experiment, seven of them guessed correctly as to which day they had received the antibiotic so presumably they felt some effect from it. In view of the fact that some subjects might have exerted a greater effort on the antibiotic day, it is possible that the apparent failure of the antibiotic to impair memory might be attributable to a degree of overlearning. Such motivational factors as increased effort in an attempt to overcome feelings of illness could mask the detrimental effects of the antibiotic on relearning.

Furthermore the experimental design may have encouraged retroactive inhibition. The second session relearning of the first sentence always followed the learning of the second sentence and may have detrimentally affected relearning, thus lowering the savings score. Although this would affect antibiotic and placebo conditions equally, it may have been sufficient to mask any small differences between antibiotic and placebo days.

It seem likely therefore that factors related to pharmacological actions of the antibiotic and individual differences among subjects with regard to these features

together with motivational differences amongst subjects and possible masking effects of the experimental design make impossible any firm conclusions regarding the effects of doxycycline on memory. The same arguements which were applied to the results from the night time study can also be applied in these daytime studies.

In the night time studies, the effects of doxycycline without REM sleep deprivation did not significantly impair memory and therefore results found with the daytime studies agree with the night studies. The significant memory decrement as a result of doxycycline therefore seems to be found only when the antibiotic is given in conjuction with REM sleep deprivation.

Chapter XII.

SLEEP, CEREBRAL PROTEIN BIOSYNTHESIS AND STABLE MEMORY FORMATION.

(1) A HYPOTHESIS AND A MODEL FOR THE FUNCTION OF REM SLEEP.

Sleep forms part of the 24 hour sleep waking cycle in humans. Infants not yet conditioned by social environments, subjects in sensory deprivation experiments where there are no cues as to the time of day, and animals reared in conditions of darkness, still exhibit behavioural and physiological changes with surprising regularity. Sleep also occurs regularly. The structure of the "switching mechanism" which allows this to occur is as yet unknown, but I have shown evidence (see Chapter IV) that circadian cycles in bacteria and lower animals are brought about by the synthesis of protein. If protein synthesis is inhibited, then the cycle is abolished. I suggest that the appearance of REM sleep is also brought about by the synthesis of protein which occurs via genetically determined DNA, bearing in mind that enzymes are protein in nature and are critical in the production of hormones and releasing factors.

It is interesting to note that some behavioural changes may also occur in a cyclical manner during waking. OSWALD et al (1970) have demonstrated a cyclical pattern of oral events in waking life in humans which can be related to the cycle of events that follow the periodicity in sleep.

Waking relaxed brain activity and the brain activity in REM sleep share many characteristics in common and

are in many respects more similar to each other than REM sleep is to SWS.(see Chapter I). If memory consolidation takes place during waking, then I see no reason to suppose that it cannot also take place in REM sleep. The type of memories consolidated during REM sleep may be qualitatively different from those consolidated during waking and there are also qualitative differences in gross brain activity, as measured by EEG recording, between these two states. Some animal experiments where REM deprivation has been used have resulted in memory impairments whereas human experiments have for the most part failed to show this result.

It has been suggested (see Chapter VII) that memories consolidated during REM sleep are especially those involving cortical-limbic neuronal interconnections and this may account for the fact that animal memory is more susceptible to interference by REM deprivation. Human memory, on the other hand, involves not only the cortical-limbic interconnections but also cortical-cortical ones. The type of memory involving cortical-limbic interconnections would be related to emotionally meaningful material or memories which are in some way emotionally "tagged", involving processes of adaptation and ability to "cope" (see Chapter VII). OSWALD et al (1970) have stated that the changes of a physiological nature which accompany REM sleep e.g. penile erections, irregularity of respiration and heart rate,appear to be characteristic of behaviour governed more by emotion than by reason. There have been a number of experiments (see Chapter VII) which

have shown that it is just this type of material which is sensitive to the effects of REM deprivation, whereas purely cognitive material such as that used in my experiments may not be. Much of this data was only published after my experiments had been completed.

Human memory is unique in its verbal nature. Humans in their waking lives also have to respond to very many different types of stimuli, ranging from internal ones which may be autonomically generated, through external stimuli generated from the environment, to purely cognitive stimuli such as thought. Among these, a distinction can also be made between stimuli that have been previously experienced and are therefore "familiar" to stimuli never before encountered which are "novel". Familiar stimuli may correspond to those already represented in a stable form in the CNS.

Considering the way the brain is constructed from neuronal and glial cells and axons with dendritic connections via synapses between neurons, familiar stimuli may be retrieved from information already stored. This could be achieved by firing patterns in neuronal circuits, the first response to a stimulus being the firing of specific neurons within the brain in specific patterns corresponding to the stored information. These could take the form of synaptic facilitation in the case of familiar stimuli. Novel stimuli would have to be laid down in a stable manner so that they may give rise to specific neuronal firing when encountered on future occasions.

Stimuli previously unrepresented in the CNS may be

may be rejected at the level of perceptual stores or filters since they may be of no interest or utility value to the organism. Others may require storage. The attention paid to the novel stimuli may be the determining factor as to whether or not they are stored in a stable manner. During REM sleep, reception of stimuli from the external environment is decreased compared to waking and if the theory of CRAIK & LOCKHART (see Chapter II) is considered, then REM sleep could be a time during which novel stimuli with utility and emotional significance, could be processed with minimal interference. The iconic and labile stores could also be "cleared' at this time, for use on future occasions. I would also suggest that the material undergoing consolidation is that which has not been processed during the preceding period of wakefulness and is present in a labile form.

Reference to Chapter II will show that there have been a number of biochemical, electrophysiological and neuroanatomical theories proposed to account for the process whereby labile memory is transformed to the stable form. Two alternatives are apparent. One proposes that specific macromolecules are the site of memory storage whereas the other considers that changes in synaptic connections and the neuronal patterns thus formed, is the important factor. The initial changes occurring as a result of sensory input have been hypothesised by HYDÉN (1973) and these are regarded as the basis of labile memory. If the necessary structural and functional changes are accomplished by the synthetic processes, the memory trace

becomes very stable and resistant. A working hypothesis of this point of view has been put foreward by MATTHIES (1973) and is shown schematically in Fig. 30.

The induction of protein synthesis and the subsequent formation and incorporation of macromolecules takes some length of time and the latency in development of stable memory can be observed in some behavioural experiments. From results of experiments other than with ECS, since ECS is postulated to affect only the labile form of memory, it seems that the duration of the consolidation phase lasts for a matter of hours following acquisition. Memories to be consolidated have to be retained during this period and two neuronal mechanisms possibly exist :-

(i) nuclear regulation characterised by synthetic processes which occur during consolidation.

(ii) synaptosomal regulation characterised by conformational changes leading to altered synaptic effectiveness.

MATTHIES (1973) calls this intermediate memory. He also postulated the existence of a messenger metabolite which may induce both the synaptosomal and the nuclear regulation. There is no reason to suppose that this role could not equally well be served by one of the RNA species or a protein or polypeptide intermediate in the synthetic chain of the new structural protein.

If protein is required for the REM phase laying down of memories, this idea can be derived directly from the hypothesis of OSWALD (1969; 1970) mentioned in Chapter VII. Evidence in support of the hypothesis comes from

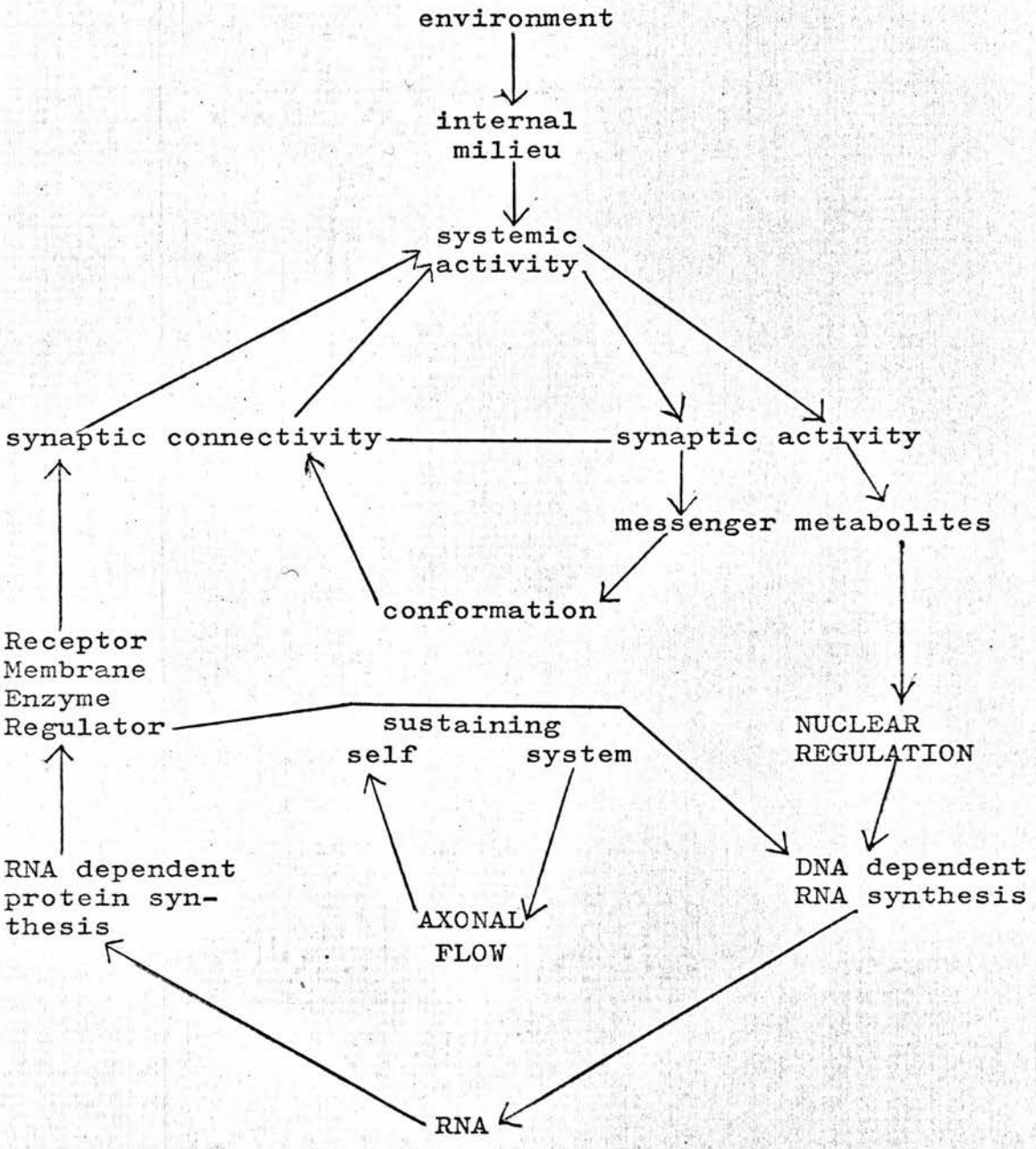

Fig. 30.

SCHEMATIC REPRESENTATION OF THE INTRANEURONAL REGULATION OF INTERNEURONAL SYNAPTIC CONNECTIVITY.

From:- MATTHIES, H. In "Memory and the Transfer of Information." Zippel, H. (Ed.). New York: Plenum Press. (1973).

experiments of BRODSKII et al (1973). These authors measured the "intensity" of protein synthesis in brain tissue which was isolated in either SWS or REM sleep or wakefulness, from the supraoptic and red nuclei of rat brain. REM sleep preceding the isolation of brain tissue and its incubation with the amino acid 4,5-H^3 leucine, gave a protein radioactivity twice that of brain tissue isolated during NREM sleep under the same conditions of tissue incubation in vitro. The result obtained from REM sleep was the same as that obtained from the waking brain. Changes in the intracellular reserves of amino acids and the permeability of cells for endogenous precursors are not known, and the possibility that protein breakdown in NREM and REM sleep is different, also remains. Therefore measurement of the radio activity of H^3leucine in proteins may not give a strictly accurate idea of the intensity of protein synthesis. Nevertheless the changes do seem to bear some relation to change in the intensity of protein synthesis in different phases of sleep. KOGAN (1974) reported a study of the level of protein synthesis in brain slices biopsied in SWS and REM sleep. The specific radioactivity of 4,5,-H^3 leucine was 50% lower and the mean protein content in large pyramidal cells was 28.9% lower in SWS compared to wakefulness. In brain tissue biopsied during REM sleep however, specific radio activity of the labelled leucine was found to be 7% higher than in wakefulness and the mean protein content was 3% higher.

The theory of HARTMANN (1973) whereby proteins synthesised during the anabolic phase of SWS could be

utilised during REM sleep, also supports some of the data satisfactorily. HARTMANN'S proposal takes into account the two states of sleep and relates them in a functional and temporal manner. It also takes into account the fact that the anabolic ohase precedes the utilisation phase in every sleep cycle and that the amount of SWS is greater compared to REM sleep in the early part of the night and vice versa in the later part of the night.

It has been shown that GH secretion occurs concomitant to SWS (see Chapter I) and GH does increase the synthesis of RNA and protein. There is also evidence to show that GH induces REM sleep in the cat (STERN & MORGANE, 1974). These results are one of the most direct demonstrations of the elevation of REM time with a drug which enhances protein synthesis.

The manufacture of new neuronal interconnections for the formation of stable memory during REM sleep is unlikely to be the only process occurring during REM sleep. The REM state is probably not a single homogenious state and gives rise to many different theories to try to ascertain the functions of REM sleep (see Chapter VII). Dreaming could also be a result following from the formation of new neuronal interconnections. One suggestion why dreams are sometimes so bizarre and fantastic in content could be that the "conscious effort of will" which directs thought and therefore neural pathways during waking, is absent during REM sleep. That dreaming is to sleep as thinking is to wakefulness is not an unasonable idea. During waking, however, thinking is often in response

to internal or external stimuli, whereas in REM sleep, the stimuli which direct the dream may be already present in the stable store and their release could be related to the increased cortical excitability seen in REM sleep. Further electrical activity could then be elicited as the growth due to neuronal interconnection takes place.

It is possible that the eye movements seen during REM sleep are an attempt to track the perceptual events of the dream and may correlate with dream intensity (FIRTH, 1973). BERGER'S (1969) hypothesis indicates a function of REM sleep is to exercise the eye muscles and maintain neural pathways involved in vision and binocular depth perception.

All the above activities can be functionally related and I do not see the theoretical necessity to separate them. Thus protein synthesised in the previous SWS period could be utilised during REM sleep to maintain the neural pathways involved in vision. Other events which have come to be confined within REM periods such as penile erections, muscle twitches, irregular breathing and heart rate, I suggest are due to the fact that it is the cortical-limbic pathways which are being especially formed during REM sleep.

The major evidence linking REM sleep, cerebral biosynthesis of protein and stable memory formation is indicated in Fig. 31.

(2) PROPOSALS FOR FUTURE RESEARCH.

Reference to the following section where the results are discussed in an overall manner, will show that a

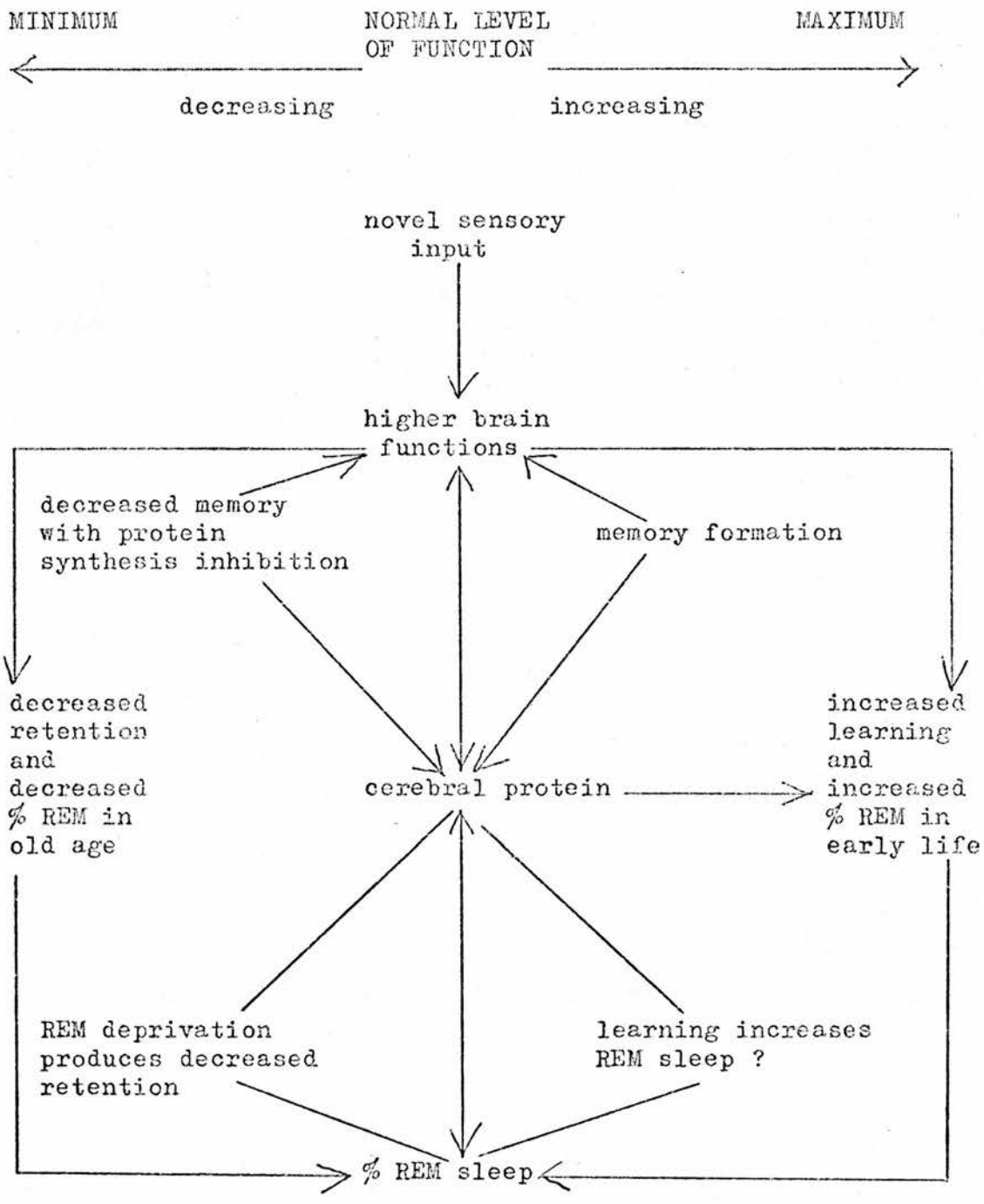

Fig 31.

MAJOR EVIDENCE LINKING REM SLEEP, CEREBRAL PROTEIN BIOSYNTHESIS AND MEMORY FORMATION.

number of alternative explanations are offered accounting for results of the experiments undertaken in this Thesis. I now propose to suggest other experiments which could be devised using human subjects which would further elucidate the relationship between REM sleep, cerebral protein biosynthesis and stable memory formation. These experiments will be discussed under the headings :

(a) Protein synthesis inhibition

(b) REM sleep deprivation

(c) Memory.

(a) Since antibiotics have been shown to inhibit cerebral protein synthesis in animals, it is of importance to show that any antibiotic used with humans actually does come into contact with cerebral neurons. It is likely that the fate of the antibiotic once administered would be similar in animals and humans. Thus to investigate the fate of doxycycline, animals could be used and the antibiotic administered in equivalent dose. If this antibiotic were tagged with a radio active label, at various intervals following administration, animals could be sacrificed, the braines sectioned and then autoradiographs could be carried out to see how much of the label penetrated the CSF and to which areas it was confined. An alternative and cruder method would be to homogenise whole brains thus obtaining a measure of complete brain incorporation of the label. Experiments of this nature would give information of the absorption of the antibiotic and also on the time course of the incorporation.

Once in the brain, there is still the problem of

the effectiveness of cerebral protein synthesis inhibition, and also the extent to which synthesis is inhibited. This is presumably possible to assay, since research by BARONDES and co-workers does quote figures for the degree and time course of the inhibition.

There is evidence that tetracycline itself enters the brain therefore it might be advantageous to repeat such experiemnts as I carried out but with tetracycline instead of doxycycline. There is also evidence that tetracycline stays in the body for at least two days using a 500 mg. dose. It seems feasible to give a dose as high as this to humans. It also seems that a higher percentage of tetracycline can be found in the CSF if an intravenous route of administration is used. If the antibiotic is administered orally, there does seem to be a difference in absorption depending on whether or not subjects have food in their alimentary systems prior to antibiotic intake and what type of food this is. Therefore careful control of diet prior to the administration seems important.

There are in addition, other antibiotics which have an effect on protein synthesis and an investigation of the effects of some of these might indicate whether the effects are of a general type. A different antibiotic might enter the CSF more readily than doxycycline and be more efficient in inhibiting protein synthesis.

Another approach would be that instead of inhibiting the synthesis attempts could be made to try to augment it and observe the effects on both sleep and memory. One way of attempting this would be to feed

subjects a diet high in amino acid and protein precursors. Vitamin B_{12} has been associated with greater learning ability in rats (ENESCO, 1968). To feed human subjects a diet low in these precursors for any length of time might be dangerous (RUNCIE & THOMPSON, 1970) but advantage could be taken of people who eat diets naturally low in such substances such as strict vegetarians (vegans) or fruitarians. The sleep and learning and memory capacities of these could then be compared with a group who eat a diet very rich in protein precursors. MacFADYN et al (1973) showed that starvation for four days caused a marked increase in SWS and a fall in REM sleep. It would be interesting to observe how subjects undergoing this type of regimen would perform on memory tasks such as those used in my experiments.

Whatever antibiotic was being used, it would be important to investigate the effects of that antibiotic also on sleep. Earlier in this thesis I indicated that some antibiotics cause an augmentation of REM sleep.

Since I have postulated that REM sleep might be a time when previously synthesised protein is utilised, it would be interesting to investigate the effects of inhibition of axonal transport of protein and the effects on memory.

(b) A period of between three and five hours has been postulated as the length of time required for the formation of stable memory. The period between OL and RL in my experiment was in the region of three and a half hours. This might have been too short for the full expression

of stable memory and it might be advantageous to use a longer period of REM sleep deprivation. Since the hypothesis indicates that GH secretion stimulates protein synthesis and REM sleep occurs predominantly in the last half of the night it might be better to carry out the REM deprivation procedures over the last rather than the first half of the night. This is however more difficult since a "REM pressure" builds up and subjects tend to want to go into REM sleep with greater frequency. An increase in the frequency of awakenings is required and subjects tend to obtain a very disturbed night of sleep.

It might be possible to disturb sleep less if a slightly different method of awakening were used. Instead of being required to get up out of bed but were merely made to shift from the REM state a less highly arousing stimulus then it is possible that their sleep would be disturbed to a lesser degree. However, it is unlikely to be such an effective method of depriving subjects of REM sleep, certainly in the later half of the night there would be increasing attempts to regain the REM state. Since the non-REM deprived group would need to be awakened the same number of times, the awakening procedures might interfere with sleep to such an extent that it would interfere with memory equally in the two groups, thus masking any memory impairment effect solely due to REM deprivation. It is difficult to predict the outcome of such manipulations.

Subjects could also be deprived of SWS and the effects on memory observed. I would predict that if memory

formation depends on proteins synthesised in SWS or if protein synthesis is dependent upon the production of GH in SWS to stimulate protein synthesis, SWS deprivation should be more effective than REM sleep deprivation in producing memory impairments. It would be necessary to show that GH secretion was blocked along with SWS deprivation.

Another prediction from the model which could be experimentally verified relates to dreaming. If the mental content of REM periods represents the "spread" of the neural trace in the cortex, then I would predict that subjects dreaming about their learning experience would remember the experience better than those not dreaming about it or dreaming about an unrelated subject. This would be relatively easy to test by collecting dream reports from subjects when they were awakened.

(c) From the animal experiments and from a few of the human experiments so far carried out to investigate the effects of REM sleep deprivation on memory, there is a consistant relationship between the type of material to be learned and remembered and the effect that REM deprivation has. Considering the human experiments, it seems that purely cognitive material such as nonsense syllables, adjective check lists and lists of digits are unaffected by REM deprivation. There is one report that meaningful material is remembered better after REM sleep than after deprivation of this stage. The word "meaningful" is ambiguous. EMPSON & CLARK used the term to discribe material in the form of sentences rather than lists, and authors

such as CARTWRIGHT and GREENBERG have used it to describe material which has personal significance, rather than uninteresting material which would be meaningful by EMPSON & CLARK'S criteria. The material I used would be included under the first definition of the word "meaningful" but it was not of great personal significance to subjects. CARTWRIGHT (1973) reported to the Association for the Psychophysiological Study of Sleep results of an experiment where subjects sorted lists of adjectives either in a way that described themselves or in a way which described a person they would like to be. In this study, the REM deprived group recalled significantly fewer words in total. When the types of adjectives recalled by the two groups was analysed it was found that "social interaction" words were recalled less frequently by the REM-deprived group and this group also recalled more "individualistic" words. No comparisons were made using adjectives which were not personally significant, but the results do indicate the possibility of a particular type of memory being especially consolidated during REM sleep. It would therefore be interesting to a similar method to compare recall of personally significant adjectives with other, neutral ones.

The method of learning also causes a problem when it comes to interpretation of performance. Since there are many difficulties in scoring what is remembered from a complex sentence (see Chapter X), the method of serial anticipation was selected for use in my experiments. Errors were either those of omission or commission. When

errors of commission were made, these were scored as wrong regardless of whatever word was proposed e.g. there is a difference between using a word which bears a resemblence to the correct one and one which bears no resemblence. This method therefore has an important disadvantage if meaning is an important criterion. An alternative method would be to present subjects with a meaningful paragraph or sentence, allow them to read it, and then assess memory after the appropriate interval. Difficulties in scoring results are even greater under these conditions. However, a complex battery of analytical procedures, including semantic and linguistic syntactical analysis of the rembered material might render this method more suitable for assessing memory for personally significant data.

Thus a large number of experiments need to be carried out, before statements can be made with any reliability regarding the relationship between REM sleep, cerebral protein synthesis and memory can be made. One of the criteria of a useful hypothesis is that it will generate further experiments to test it. Not least among the methodological problems in investigating memory is the task of finding a method with which to satisfactorily study learning and memory. None of the methods currently in use can be truly translated into everyday learning experiences in man. As far as tequniques of REM deprivation are concerned, there is little evidence to support this state leads to consistent change in any waking activity, some subjects report feeling slowed down after REM deprivation compared with their normal activity while others report

feeling activated (CARTWRIGHT & RATZEL, 1972). Thus REM sleep deprivation seems to be equivalent in some aspects to a mild stress situation, which mobilises some subjects to perform better, whilst impairing performance in others. Thus personality factors in relation to REM deprivation should also be taken into account.

(3) OVERALL DISCUSSION

The relationship between REM sleep, protein synthesis and stable memory can be investigated in terms of different pairs of relationships within the postulated whole relationship. The relationship between REM sleep and memory can be examined without the need to infer the underlying neurochemical and physical pathways and mechanisms. Two aspects of this relationship considered in the Thesis are :-

(i) the effects of REM sleep deprivation on learning following the deprivation

(ii) the effects of REM sleep deprivation on memory of material learned prior to the deprivation.

The relationship between protein synthesis and memory may then be examined separately, as may the relationship between protein synthesis and REM sleep.

A discussion of the results of the individual experiments has already been discussed at the ends of the chapters dealing with each experiment and it now remains for the results to be discussed in an overall manner. This is a difficult task in this Thesis since many of the predicted effects did not occur. The discussion therefore appears to take the form of providing a series of excuses to explain

the reasons why I did not confirm what I had predicted. However, the lack of positive results do not necessarily indicate that the predictions are incorrect but that they have not been supported by this experimental data under the conditions of the experiments.

The Distorted Visual Input experiment was designed to investigate one aspect of the overall relationship between REM sleep, cerebral protein biosynthesis and stable memory i.e. dealing with the relationship between REM sleep and learning. These experiments were carefully executed and well controlled and no changes were seen in REM sleep as result of this massive learning task. Neither were there any significant changes in phasic activity as measured by comparing numbers of epochs containing eye movements in the experimental and control conditions. Nor were there any significant changes, for that matter, in any of the other sleep parameters. This was disappointing since ZIMMERMAN et al had claimed that increased learning did produce increases in REM percent and phasic activity. However on close scrutiny of their data, which is discussed earlier, many of their positive findings could be traced to inconsistencies in experimental methods and misinterpretation of the findings.

If this lack of effect is examined in the light of my proposed model, an explaination does appear. It seems to me that if protein synthesis occurring during sleep, be it during REM sleep as proposed by OSWALD or during SWS as proposed by HARTMANN, it is in some qualitative or qhantitative way different from that in wakefulness,

then this synthesis would be part of a cycle of synthesis and utilisation under genetic control. If this protein is synthesised during SWS, then it could be utilised during REM for the consolidation of all learning and not only that specific to the experimental learning task. Thus the amount of protein produced is stable and pre-programmed and behavioural manipulations might not increase the amount of protein synthesised or the percentage of REM sleep. It is interesting to note that although depression of the percentage of REM sleep is fairly easy to produce, both by behavioural procedures e.g. first night effects, stress and almost all drugs, it is very difficult to increase REM percentage. There are no known behavioural manipulations which in humans will increase REM percentage apart from prior suppression giving rise to a rebound increase on nights following the suppression. The same is true for most drugs. These mainly decrease REM percentage on administration resulting in the well known REM rebound on withdrawal. However, there are exceptions. Resrpine increases the percentage of REM sleep (HARTMANN, 1966) as does thymoxamine, an alpha adrenergic blocking agent (ADAM et al, 1974).

The occurrance of the REM rebound was one of the main considerations giving rise to OSWALD'S REM sleep-protein synthesis hypothesis. When brain cells are in some way injured by REM sleep deprivation or are "poisened" by drugs, then protein is necessary for repair processes, and repair is accompanied by rebound increases in REM sleep. This hypothesis need not be negated by postulating protein

utilisation rather than protein synthesis during REM sleep. Utilisation could be blocked by REM deprivation or suppression, and repair facilitated by a longer period of utilisation during the REM rebound.

A relationship between REM sleep and protein synthesis is suggested since periods of elevated protein synthesis are often accompanied by periods of elevated REM sleep. This positive correlation appears during early life (JOHNSON & SELLINGER, 1971; MILLER, 1969; ROFFWARG et al; 1966), during recovery from drug abuse (JARLSTEDT, 1972; KHAZAN & COLASTANTI, 1972; OSWALD, 1969), during recovery following inhibition of protein synthesis with puromycin and cycloheximide (STERN et al, 1972) and perhaps following learning in animals. Although human studies have mainly failed to demonstrate increases in REM sleep as a result of learning, animal studies have claimed to do so. LUCERO (1970) trained rats in a labyrinth-like maze for one and a half to two hours and demonstrated an augmentation in REM sleep in three recorded hours immediately consecutive to learning. LECONTE et al (1973a; 1973b); HENNEVIN et al (1971) and SMITH et al (1972) have all reported increases in REM during the immediate hours following learning and FISHBEIN et al, (1974) found that learning produced an elevation of REM time which lasted for at least 24 hours. Other positive findings have been reported by GAITO & BONNETT (1971); GLASSMAN & WILSON (1970) and HYDÉN & LANGE (1968). It is possible that REM sleep plays a role in the maintainance of only certain types of protein in the CNS since there

is no marked overall reduction in total protein synthesis in the brain following 54 hours of REM sleep deprivation in the rat (BOBILLIER et al, 1971). In other experiments by these authors, however, incorporation of AAs into protein was impaired during REM sleep deprivation. In these experiments, the type of experimental design and the treatment conditions both have a significant effect (REICH et al, 1972). There is no data indicating the temporal relationship between sleep and protein synthesis.

The highest percentage of REM sleep occurs in early life, but it is at its lowest during senility (FEINBERG, 1969). A decreased memory for recent events and a decreased ability to learn and to remember information also accompanies senility. This can be explained in terms of normal physiological processes of growth and aging. The few months of life are probably a time when very great amounts of acquisition, growth and stress occur and there is probably a need to form the greatest number of neural pathways. The brain also increases in weight. Once mature growth ceases and the brain then has to be maintained and repaired if injury occurs. Thus protein synthesis could be greatest during early life to make possible stable storage of a very large number of memories. I have suggested that the rate of protein synthesis is genetically determined, it would decline from birth to maturity, remain steady during adulthood and then decline further during old age and senility. This type of exponential change is seen in a great many biological systems. Since the subjects in my experiments were young adults I suggest

that they would be exhibiting a stable amount of protein synthesised per 24 hours.

Anecdotal reports from subjects wearing the prism lenses suggested that they found the experience rather stressful. As has already been pointed out, stress will tend to lower the percentage of REM sleep on nights following stressful daytime experiences. If the evidence from animal studies where increased learning resulted in increased REM sleep is a real effect, then it is possible that an increase in REM percentage might have been seen in my experiment had it not been masked by the stress effect tending to lower the REM percent. The normal percentage of REM sleep seen on experimental nights might have been a result of learning tending to increase the REM percentage and stress counteracting this effect. If this were the case, there might have been a rebound in the post experimental period, but no rebound actually occurred. No dream reports were collected from subjects whilst wearing the prisms. If, as suggested by my model, dream content might result of specific interneuronal connections being synthesised for stable memory formation, then I predict that subjects dreaming about their learning experience would adapt more rapidly to the distorted visual input. Improvements in adaptation would be seen in a more rapid attainment of plateaux in the performance tasks used to demonstrate adaptation.

The observation that in man, REM sleep occurs predominantly during the later part of the night could be accounted for if REM sleep does control certain aspects

of cerebral protein biosynthesis. During the first three to four hours in a normal night of sleep SWS predominates (HARTMANN, 1967; SNYDER, 1971). It is primarily in SWS that GH is released. GH exerts a powerful and widespread stimulant effect on cell growth and the larger proportion of REM sleep in the second half of the night would occur following GH secretion. An increase in CNS protein synthesis could then result. A growth hormone-REM sleep relationship may also account for the positive correlation between the length of the SWS period and the length of the subsequent REM episode (HARTMANN, 1966). Evidence in favour of this relationship was found by STERN & MORGANE, (1974) where administration of GH induced REM sleep in cats. The effect was quite marked, reaching the peak amount of REM in the third hour following GH administration. This finding has not been confirmed in man.

The relationship between memory and protein synthesis has been examined very thoroughly in animals. Experiments using protein synthesis inhibitors have already been described and I think the overall results point to the fact that stable memory formation does seem to depend on the synthesis and utilisation of protein. This seems to be a very plausible mechanism for man also, but the problem is much more difficult to investigate in man. I do not know of any studies to date where the relationship between protein synthesis and memory has been investigated in man let alone the relationship between protein synthesis and REM sleep. In an attempt to begin exploration of this relationship, I administered the antibiotic,

doxycycline. JOHNSON et al (1946) first mentioned that antibiotics have an effect on the CNS. The reasons for the choice of this particular compound stemmed from unpublished work of OSWALD where increases in REM percentage resulting from antibiotic administration were found in humans. Elevation of REM sleep following antibiotic administration in rats using cycloheximide and puromycin has been observed by STERN et el (1972).

Doxycycline is one of the tetracycline group of antibiotics. The mode of interference with protein synthesis is probably through interference with the transfer of AAs from sRNA to the polypeptide on the ribosome (FEINGOLD, 1963). Mammalian protein synthesis does seem to be affected by tetracyclines (SHILS, 1963; WEINSTEIN, 1965), although only with higher doses and to a much lesser degree than bacterial protein synthesis (CLENDENNING, 1965). The dose levels which constitute "higher" are not mentioned. Work with cell free systems has shown that the incorporation of AAs was inhibited at the stage of the binding of the aminoacyl t RNA to the ribosome. The clinical recommended dose of doxycycline is 100 mgms. every 12 hours. Our single dose of 200 mgms. was therefore higher. Tetracycline itself is readily absorbed, the peak blood level is reached within two to four hours after single oral doses and effective serum levels are maintained for at least six hours. In the absence of infection, tetracycline itself passes into the CSF more effectively than other tetracyclines and the distribution of tetracyclines once absorbed is discussed in the previous chapter. Tetra-

cyclines are found in the CSF following oral medication and the concentration is about 25% of that in the plasma. A higher concentration can be attained after intravenous injection. There is no data indicating the degree to which doxycycline enters the brain but it is used clinically to treat meningitis and therfore probably reaches central neurons. Tetracycline persists in the body and detectible amounts are present in the urine two days after a single dose of 500 mg. Other antibiotics known to affect protein synthesis in bacteria and yeasts but not necessarily in man, include erythromycin and lincomycin. Chloramphenicol affects human protein synthesis (HASH, 1972) but is too toxic to use in this type of experiment.

With my experimental subjects, administration of doxycycline alone failed to produce statistically significant memory impairments. There may however be an insufficient concentration of the antibiotic at the time of memory testing. However, as outlined in the model, it is possible that protein required for stable memory formation is synthesised prior to the peak level of antibiotic in the CNS. Doxycycline would therefore not interfere with the utilisation of this protein in the group of subjects allowed REM sleep. In order to investigate the utilisation of previously synthesised protein during REM sleep it would be necessary to use a compound which in some way inhibits cerebral axonal protein transport. Such a compound has been used by CRONLY-DILLON (see Chapter II) with rats and I do not know whether this would be a reasonable compound to use with humans.

Inhibition of cerebral protein synthesis for a longer period than that used in my experiments might also provide useful data for clarifying the relationship.

An alternative view of the relationship between REM sleep and protein synthesis is held by STERN & MORGANE (1974) and HARTMANN (1973). They hypothesise that REM sleep plays a role in maintaining the functioning of catecholamine containing neurons within the CNS. The main lines of evidence supporting this view are :-

(a) following REM deprivation the responsiveness of catecholamine systems is depressed.

(b) administration of drugs which enhance catecholamine activity can reverse some of the behavioural deficits seen after REM deprivation.

(c) acute administration of pharmacological agents which depress catecholamine activity e.g. AMPT and reserpine produce a compensatory increase in REM time, whereas increasing catecholamine availability at the synapse by imipramine, MAOIs and ECS, decreases REM sleep.

However, the enzymes which control catecholamine synthesis are a product of protein synthesis and therfore decreases in protein synthesis would be expected to disrupt catecholamine functioning. Changes in cerebral protein synthesis would produce parallel changes in brain catecholamines. ROBERTS et al (1970) found that learning impairments in mice produced by the protein synthesis inhibitor puromycin, were reversed by administration of catecholamine potentiating drugs. These correlated findings suggest that REM deprivation might impair protein

synthesis with consequent disruption of catecholamine functioning.

The remaining aspect of the total relationship investigated in this Thesis was the influence of REM deprivation on memory for material learned prior to the deprivation. It was found that REM deprived subjects did not exhibit statistically significant memory impairments although savings percent was lower in this group. If either protein synthesis or utilisation is occurring during REM sleep then it is difficult to see why the REM deprivation did not produce the significant memory impairments. There is some experimental data indicating that in humans, it might be only material of an emotionally significant nature from the formation of cortical limbic neuronal interconnections which is consolidated especially during REM sleep. Purely cognitive material, laid down via cortical-cortical interconnections might be consolidated during waking. Subjects did spend some time awake between learning and relearning. This included the second awakening and also the length of time taken to fall asleep again after the first and second awakenings. This might provide a sufficient period for consolidation since this period spent awake would occur within the prescribed period hypothesised for stable memory formation. Alternatively, since the time course of consolidation is uncertain, memory seen at the time of relearning might be labile memory and therfore unaffected by REM sleep deprivation. A similar relearning performance by both groups would result. In none of the previous studies

with human subjects has REM deprivation been shown satisfactorily to disrupt memory. As with the animal studies it seems that the nature of the task used is one of the critical variables. Whether sleep deprivation impairments of newly formed memory are actually due to protein synthesis disruption has never been demonstrated directly in man.

When the antibiotic doxycycline, postulated to be acting as a cerebral protein biosynthesis inhibitor, was administered, in combination with REM sleep deprivation then a significant decrement in performance on the task was found. Performance by the REM 0 + D group was significantly worse than either the REM 15 or the REM 0 groups. When neither of the two conditions on its own produced the decrement, the question remains as to how, when the two treatments are combined, such a marked effect is found. It could be merely that the combination of treatments caused subjects to feel particularly unwell, thereby especially lowering their performance. There were however no anecdotal reports from subjects which suggested that they felt worse in the REM 0 + D condition than in the REM 15 + D condition. I suggest that in accordance with my proposed model, protein synthesis would be partially blocked during doxycycline administration. This blockage would be insufficient on its own to show a significant impairment. With the additional interference of protein utilisation, by depriving subjects of REM sleep, these combined treatments caused a sufficient disruption of the memory consolidation process to reveal a decrement in

performance.It must also be remembered that the techniques of REM derivation also caused subjects to have less stages 3 + 4 sleep and less total sleep. If protein synthesis in the SWS periods preceding the REM periods is in some way qualitatively or quantitatively important for memory consolidation, and if SWS is curtailed, then an effect on protein synthesis would also be found.

If protein synthesis is one of the major functions of REM sleep, then another question remaining is why REM sleep is not seen to the same extent in creatures other than mammals. Non mammalian organisms presumably still have to assimilate information concerning their environments. Electrophysiological features resembling REM sleep have not been seen in most fish although rapid eye movements have been reported in the Bermuda Reef fish (TAUBER & WEITZMAN, 1969). REM sleep has not been seen in amphibia or reptiles (TAUBER, 1974) but it has been seen in some birds (TRADARDI, 1966; VAN TWYVER & ALLISON, 1972; WALKER & BERGER, 1972). Such creatures, however, might not need such an extensive memory store since they tend to be less flexible in their responses than mammals. However, if it is postulated, as in the model, that proteins are not actually synthesised only during REM sleep, but are utilised at this time to form stable memories, there might not be a need for REM sleep in creatures other than mammals. In these, memories might be consolidated during wakefulness from protein synthesised during sleep. REM sleep might be necessary in mammals because of the larger variety of responses

to environmental stimuli required by these animals during waking. A period empty of environmental sensory input would be advantageous for memory consolidation.

It can be seen from the above discussion that REM sleep need not be considered as a single homogenious state. A remaining problem is of the nature of the primary indicator of the need for REM sleep which gives rise to REM sleep pressure and results in the REM rebound following REM suppression. If it is postulated that REM sleep is a period when previously synthesised protein is utilised, then many of the processed postulated to take place during REM sleep could be accounted for, These would include stable memory formation, catecholamine synthesis and the maintainance of pathways involved in vision. The hypothesis is also consistant with the changes in REM percentage seen in early life, old age and senility and in mental defect.

In the night time studies, the experimental results did not support the findings of EMPSON & CLARK upon whose experiment I based my own. Although significant memory impairments were found when REM deprivation and doxycycline were administered together, REM deprivation on its own produced impairments in the predicted direction but these were not significant.

The Thesis, however, indicates a new method for investigating one of the functions of REM sleep in man. It also combines previously used approaches, mainly from animal studies, to demonstrate relationships between REM sleep, memory and cerebral protein biosynthesis and many proposals for future experiments are indicated.

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APPENDIX 1.

Instructions for learning sentences.

This is a learning test. You will be shown a sentence 40 words in length. The words will be shown to you one at a time, in sequence, from the window of the rotating drum in front of you. On the first occasion when you see the words, you should repeat each word out load as it is shown to you. On subsequent occasions you should anticipate out loud the word following the one shown. The word "start" at the beginning is just to tell you when to begin and should not be repeated. The sentence will be shown to you a number of times until you have anticipated every word correctly.

Instructions for relearning sentences.

Please think about the sentence you learned earlier tonight (today). I am going to show it to you again and you should anticipate in exactly the same manner as you did before but without repeating the words out loud on the first trial. So just go straight into the anticipation trials. Again the sentence will be shown to you until you have anticipated every word correctly.

Instructions for card sorting task.

You have in front of you, a board with the numbers: 2, 3, 4, 5, 6, 7, 8 and 9 on it. On your right hand side you will see four packs of cards, face downwards. Please pick up the first pack of cards and place it, still face downwards, into the recess on the board in front of you. This pack of cards will have only the numbers two and three in it. When I tell you, using only your prefferred hand, I want you to turn up the cards one at a time and place each one into the appropriately numbered recess on the board. In other words, if there is a number two on the turned up card, then place it in the recess on the board which shows the number two. This test will be timed, so please work as quickly and accurately as you can and if you make a mistake then please correct it.

Similar instructions were given for the four and eight catagories of choice and for movement time the instructions were as follows :-

The final pack of cards contains only picture cards. Please turn these up one at a time in the same manner as you have been doing with the numbered cards, but this time place them alternately into the number two and three slots on the board. This is to discover the length of time it takes for you to make the movement, so please work as quickly and accurately as possible once again.

These instructions were repeated for the four and eight catagories of choice.

Instructions for CFF.

Please look at the lamp in front of you. When I turn it on, you will see a series of equally spaced discrete flashes or flickers. As I increase the rate of flickering, you will no longer

see it flickering or flashing, but will see it as one continuous light. Also, as I begin to decrease the flicker rate, you will see it start to flicker again. First of all I shall start it flickering slowly and I want you to say "NOW" as soon as you no longer see it flicker but it becomes one fused light. Then I shall start it as a fused light and as I decrease the flicker rate I want you to say "NOW" as soon as you see it begin to flicker. I shall repeat this four times in either direction.

These instructions were accompanied by a demonstration of the CFF apparatus.

APPENDIX 2.

The Sentences.

Pilot Experiment: Sentence 1.

If pensions were made completely transferrable this cost would disappear and employers would be more willing to consider applicants from outside their own service rather than to promote within it someone who might be quite unsuitable.

Pilot Experiment: Sentence 2.

What I have also gained is some imaginative appreciation of our inheritence in stone and of the richness added to contemporary communities by the blending in of some buildings which represent workmanship of an earlier age.

Adaptation Night 1: Sentence A.

We have all encountered the salesgirl who chatters unconcernedly to her fellow assistants while we stand politely waiting for her to deign to notice us but she is there to serve so why shouldn't we insist that she does so.

Adaptation Night 1: Sentence B.

This traditional method of heating can be discribed only as the filthy habit of a filthy nation and it is not tolerated in many countries which have really cold climates and where the science of home heating is better understood.

Adaptation Night 2: Sentence C.

Suburban travellers covering a wide section of the community are worried about this problem and my own concern is primarily for the school children who are already faced with long and arduous journeys by bus services which are barely adequate.

Adaptation Night 2: Sentence D.

Police dogs can be trained to rescue drowning people and instead of running down to the water and striking out they are taught to take a flying leap thus saving several uneccesary swimming strokes and many valuable seconds of time.

Experimental Night: Sentence E.

A balance should be struck between the national good and the treatment of state employees without surrendering these employees to the possible tyranny of unfettered state control or leaving them free to hold the rest of the country to ransom.

Experimental Night: Sentence F.

The great amount of detail to be assimilated and recalled selectively according to the question set has compelled examiners to modify the difficulties of the examination by sometimes including the type of question which requires little thought and less judgement.

Doxycycline Night: Sentence G.

It is not as certain as your article yesterday implied that sufficient thought has gone into the additional problem of safeguarding the public from increased powers acquired by those employed in the monopolistic works and services so essential to modern living.

Doxycycline Night: Sentence H.

One of the main reasons for studying history is for pupils to learn to analyse historical situations and thus demonstrate their ability to elucidate an idea and select facts on which it is based whilst discarding others which appear irrelevant.

Fig. 32.

"PERFORCE TO DREAM ?"