NEUROPHYSIOLOGICAL AND NEUROPHARMACOLOGICAL EFFECTS OF ASCORBIC ACID ON BEHAVIOUR

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DECLARATION

This is to certify that I carried out the research in this thesis in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of Prof. C.O.N. Wambebe and Prof. M.A. Ali.

Where use was made of the published work of other investigators, this is acknowledged and the source of information duly referenced. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.



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DEDICATION

To my students at the Ahmadu Bello University, for their keen interest in the physiology of Central Nervous System, and to Neuroscientists in all the Nigerian Universities.

CERTIFICATION

This thesis entitled "Neurophysiological and Neuropharmacological effects of ascorbic acid on behaviour" by Ebelechukwu Boniface Ezenwanne meets the requirements governing the degree of Doctor of Philosophy of Ahmadu Bello University and it is approved for its contribution to scientific knowledge and literary presentation.

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PUBLICATIONS

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2. Ezenwanne, E.B. and Anuka, J.A. (1992). The pattern of gross behavioural activities in acute administration of varying doses of ascorbic acid in rats. Nig. J. of Neuroscience. P. 47 - 52.

3. Anuka, J.A., Wambebe, C., Sokomba, E. and Ezenwanne, E.B. (1991). Thermoregulatory effect of Histamine in young chicks. West African Journal of Pharmacology. (In Press).

4. Ezenwanne, E.B. (1992). The effects of Vitamin C on pentobarbitone-induced sleep in rats. Nig. J. of Neuroscience. P. 141 - 146.

5. Ezenwanne, E.B. and Adeoshun, I.O. The effect of posture and pulmonary stretch on Sinus Arrhythmia. Abstract In: *Proceedings of the Nig. J. of* Physioloigical Sciences, 1986.

6. Adeoshun, I.O. and Ezenwanne, E.B. Effects of kolanut on pulmonary functions and heart rate in man. Presented at the 14th Annual Scientific conference of the West African Society of Pharmacology (WASP), at Ahmadu Bello University, Zaria, 10 - 12th April, 1985.

7. Ezenwanne, E.B. The EEG and EMG effects of intracerebroventricularly administered ascorbic acid in rat. Presented at the 2nd Annual Scientific Conference of Nig. Society of Neuroscientists. 4 - 5th. Dec., 1991.

8. Anuka, J.A., Ezenwanne, E.B. and Hussaini, I.M. Preliminary investigation of some central effects of 3-Benzylidine Amino oxypropionic acid (3-BOPA). In: Proceedings of the 2nd National Scientific Conference of the National Association of Academic Pharmacists (NAAP). Ahmadu Bello University, July, 1992. (In Press).

ABSTRACT

Healthy adult albino rats (Wistar strain, inbred) were used to study the neurophysiological and neuropharmacological factors in the effects of ascorbic acid on gross behaviour.

Varying doses of ascorbic acid, ranging from 5 to 320mg/kg, were administered separately to rats (i/p) to monitor any changes in gross behaviour. Behavioural excitatory effects were noticed at low doses of ascorbic acid (5 - 80mg/kg), while at higher doses (160 - 320mg.kg) the rats were sedated. The peak effect in the observed behavioural excitatory effects of ascorbic acid was noticed at 80mg/kg dose. Two doses of ascorbic acid (10 and 80mg/kg, i/p) were separately administered chronically to rats for 30 days to study gross behavioural patterns during the period of the administration. In the lower dose (10mg/kg), behavioural excitatory effects were consistently observed. However, following the withdrawal of administration, depression in gross behaviour was noticed in these rats. In the 80mg/kg dose treated rats, behavioural depression, which followed after an initial

excitatory effect, was observed throughout the period of the chronic administration. These results indicate that ascorbic acid may have both central nervous system activation and sedative properties. It was assumed that these effects may have been produced through mechanisms linked with the neurotransmitter systems that traditionally regulate various forms of behaviour in the brain.

Ascorbic acid (80 - 300mg/kg, i/p) antagonized pentobarbitone-induced sleep in rats. The duration of the induced sleep was effectively shortened while also the onset of the sleep was significantly delayed at the 80mg/kg dose of ascorbic acid.

Some electrophysiological studies were undertaken in rats in various treatments with ascorbic acid. The EEG and EMG did not reveal any obvious changes 10min. after the i/p administration of 80mg/kg dose of ascorbic acid. At 25min. however, there was desynchronization of the frontal cortex and optic cortex which was associated with slight increase in amplitudes of the EMG activity. These changes in the EEG and EMG were no longer noticed at about 60min. later. Ten minutes after the i/p

administration of 300mg/kg dose of ascorbic acid, there was no noticeable change in the EEG and EMG. However, at 25min., desynchronization of the EEG of the frontal cortex and optic cortex associated with increased EMG activity was observed and these changes persisted for up to 60min. after.

EEG studies in chronic administration of ascorbic acid (80mg/kg), i/p) revealed definite desynchronization associated with mildly increased EMG activity 1-3 days at the start of the administration. However, these changes were no longer observed in the subsequent period during the chronic administration. The EEG of the frontal cortex, optic cortex and brain stem reticular formation were desynchronized 10 min. after intracerebroventricular administration of 10 micrograms of ascorbic acid in the rat. At 25 min., the EEG of the optic cortex then showed high amplitudes and irregular spikes. On the other hand, no appreciable changes in the EEG was observed 10min. after the intracerebroventricular administration of 40 micrograms dose of ascorbic acid. However, at 25 min. the EEG of the three cortical regions - the frontal cortex, optic cortex and brain stem reticular formation, were

desynchronized.

Intracerebroventricularly administered ascorbic acid apparently had no noticeable effects on EMG activity. These effects on the EEG further indicate that ascorbic acid probably has definite and definable modulating influences on some systems in the CNS.

Ascorbic acid modified the normal sleep of rat. The sleeping period was significantly shortened in the administered ascorbic acid (80 and 320mg/kg, i/p). On the other hand, DOPS (10mg/kg, i/p) promoted normal sleep in rats. The effect of ascorbic acid on the normal sleep of rat was further examined using observational EEG techniques. The EEG studies revealed prominent sleep presence in the rat after i/p administration of DOPS (10mg/kg). Sleep was also prominently detected in the EEG of the rat after the pretreatment of 320mg/kg dose of ascorbic acid with DOPS (10mg/kg, i/p). However, after the DOPS-pretreatment of 80mg/kg dose of ascorbic acid as well as during chronic administration of 80mg/kg dose of ascorbic acid, normal sleep was not detected in the EEG of the rat. These results indicate that ascorbic acid probably has

modulating influences on sleep mechanism in the brain. Specifically, the major import of these results was the clear indication that, at least, some of the neurotransmitter systems in the central regulatory mechanism of sleep, notably, the noradrenergic system in particular, must be linked with the mechanism of action of ascorbic acid for the observed behavioural effects.

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LIST OF ABBREVIATIONS

Abbreviation	Full Meaning
ACh	Acetylcholine
ACTH	Adrenocorticotropic hormone
AsA	Ascorbic acid
BDH	British Drug Houses
BSRF	Brain stem reticular Formation
CNS	Central nervous system
Cm	Centimetre
Cont.	Control
CSF	Cerebrospinal fluid
DA	Dopamine (Dihydroxyphenylethy-lamine)
L-DOPA	Dihydroxyphenylalanine
DOPS	3,4-Dihydroxyphenyl-serine
EEG	Electroencephalogram
EMG	Electromyogram
FC	Frontal cortex
Fig.	Figure
GABA	Gamma-aminobutyric acid
g	Gram
5-HT	5-Hydroxytryptamine (Serotonin)
ICV	Intracerebroventricular

IH	Incertohypothalamic system
i/p	Intraperitoneal
kg	Kilogramme
LAAD	L-aromatic acid decarboxylase
MC	Mesocortical system
mg	Milligramme
min.	Minute
ml	Millilitre
mm	Millimitre
MVT	Movement
NE	Norephinephrine
NREM	Non-Rapid-Eye movement
NS	Nigrostriatal system
Oo	Control
OC	Optic cortex
PNMT	Phenylethanolamine N-methyl transferase
POA	Preoptic area
PV	Periventricular system
RAS	Reticular activating system
REM	Rapid-Eye movement
SAL	Normal saline
Sec	Second
SEM	Standard error of the mean

TI	Tuberoinfundibular system
uV	Micro volt
Wt	Weight

CHAPTER ONE

1 REVIEW OF LITERATURE

1.1 GENERAL INTRODUCTION

There is great interest in the physiology of behaviour, especially in relation to the neurotransmitter systems in the central nervous system (CNS). For example, neuroscientists have known for some time that a sudden change in behaviour was always coincident with a change in the concentration of some transmitter substance in the brain. On the other hand, a sudden uneven distribution of a transmitter substance in the CNS was always accompanied by a modification of the physiological processes in that part of the brain. Such observations have always aroused great curiosity in the nature and properties of the transmitter substance involved as well as the characteristics of the transmission process.

One of the long term objectives and major reasons for the increasing interest in the central neurotransmitter systems is the quest for greater understanding of the mechanism underlying the

actions of drugs that modify behaviour, especially the agents that produce various forms of behavioural aberrations such as hallucinations, delusions, changes in mood and other manifestations of psychosis. For example, it has been noted that drugs that modify human behaviour (e.g. psychotomimetic agents and tranquilizers) appear to achieve this by modulating transmission across synapses in the brain (Ganong, 1987). Therefore, the fact that transmission at synapses in the nervous system is chemical in nature is of great physiological and pharmacological importance.

1.2 SYNOPSIS OF THE MAJOR NEUROTRANSMITTER SYSTEMS IN THE CENTRAL NERVOUS SYSTEM.

1.2.1 GENERAL

The nature of most chemical mediation at many sites in the CNS is not understood. In addition, the number of identified synaptic mediators are increasing and so are the locations in which given substances are strongly suspected to be transmitters. Among the neurotransmitters that

have been studied, Acetylcholine (ACH), Dopamine (DA), Norepinephrine (NE) and Gamma-aminobutyric acid (GABA) have been more prominently investigated. Ganong (1987) documented the major neurotransmitter systems in the central nervous system and identified the pathways.

1.2.2 ACETYLCHOLINE (ACh).

The development of antibodies specific for choline acetyltransferase has permitted mapping of cholinergic pathways in the brain by immunocytochemical techniques. Acetylcholine has been shown to be distributed throughout the CNS with high concentrations in the cerebral cortex, thalamus, various nuclei in the basal forebrain, hippocampus and related areas. It has also been specified that cholinergic neurons are not found everywhere in the brain, but the ascending reticular activating system consists mainly of cholinergic neurons (Burn, 1975). This specification is supported by the evidence that anticholinesterases cause arousal, as shown by

fast waves and asynchrony in the EEG. Atropine abolishes the effect and causes slow waves. ACh is generally accepted as an excitatory neurotransmitter in the CNS. One of the notable excitatory roles of ACh is in the basal ganglia where its importance lies with the production of Parkinson's disease. There is overwhelming evidence that enhanced ACh produces convulsions. However, there are reports that ACh has some inhibitory roles in the cerebral cortex (Krynjeric and Philips, 1963). Celesia and Jasper (1966) also noted that ACh concentration is decreased during convulsive activity induced by electroshock. The hydrolysis of ACh at cholinergic receptors during periods of excitation was also reported by the same workers. ACh is thus, linked both directly and indirectly to a variety of brain functions. Same workers demonstrated that injection of ACh derivatives into the hypothalamus and in other parts of the limbic system induces drinking behaviour in the experimental animal.

The distribution of choline acetyltransferase and acetylcholinesterase parallels that of acetylcholine throughout the CNS. Pseudocholinesterase is also found in many parts of the brain.

1.2.3 NOREPINEPHRINE (NE)

Fluorescence microscopy revealed that *norepinephrine is present in neurons* that have origin in the cells of the midbrain, and many of the neurons terminate in the hypothalamus.

Distribution studies revealed that the hypothalamus has the highest content of norepinephrine and that concentration is slightly lower in the midbrain, medulla and other brain *regions*.

It has been speculated that mood may be related to the amount of free norepinephrine available at synapses in the brain. In this consideration, depression result when too little norepinephrine is available, while drugs such as

monoamine oxidase inhibitors and amphetamine which increase free norepinephrine, elevate mood.

1.2.4 EPINEPHRINE

It is known that a system of phenylethanolamine-N-methyltransferase (PNMT) - containing neurons with cell bodies in the medulla and projecting to the hypothalamus, secrete epinephrine. There are also epinephrine secreting neurons which are known to project to the thalamus, periaqueductal grey and to the spinal cord.

The function of epinephrine in the CNS is uncertain. Epinephrine has been less studied in the brain than the other catecholamines, and its role, if any, in the brain function is not clear.

1.2.5 DOPAMINE (DA)

Accumulated evidence, many of which are histochemical, biochemical and pharmacological from the work of many researchers support the

existence of seperate dopamine (DA) secreting neurons in the CNS (Carlsson, 1959; Anden et al., 1964). It was noted that dopamine was present in high concentration in the corpus striatum (Burn, 1975) and in the substantia nigra (Bertler, Carlsson and Rosengren, 1958).

Dopaminergic neurons have their cell bodies in the midbrain from where they project to areas including olfactory tubercle, nucleus accumbens, related limbic areas and the frontal, cingulate, entorhinal and perirhinal cortices and all of these projections together form the MESOCORTICAL SYSTEM of the dopaminergic neurons. A separate intra-hypothalamic system of dopaminergic neurons project from cell bodies in the arcuate nucleus to the external layers of the median eminence of the hypothalamus forming the TUBEROINFUNDIBULAR SYSTEM. There is the INCERTOHYPOTHALAMIC SYSTEM made up of short dopaminergic neurons around the third and fourth ventricles. Some dopaminergic neurons also project from the arcuate nucleus to the intermediate and posterior lobes of the

pituitary, forming the TUBEROHYPOPHYSEAL SYSTEM. Fig. 1.2.5.1 illustrates these dopaminergic systems.

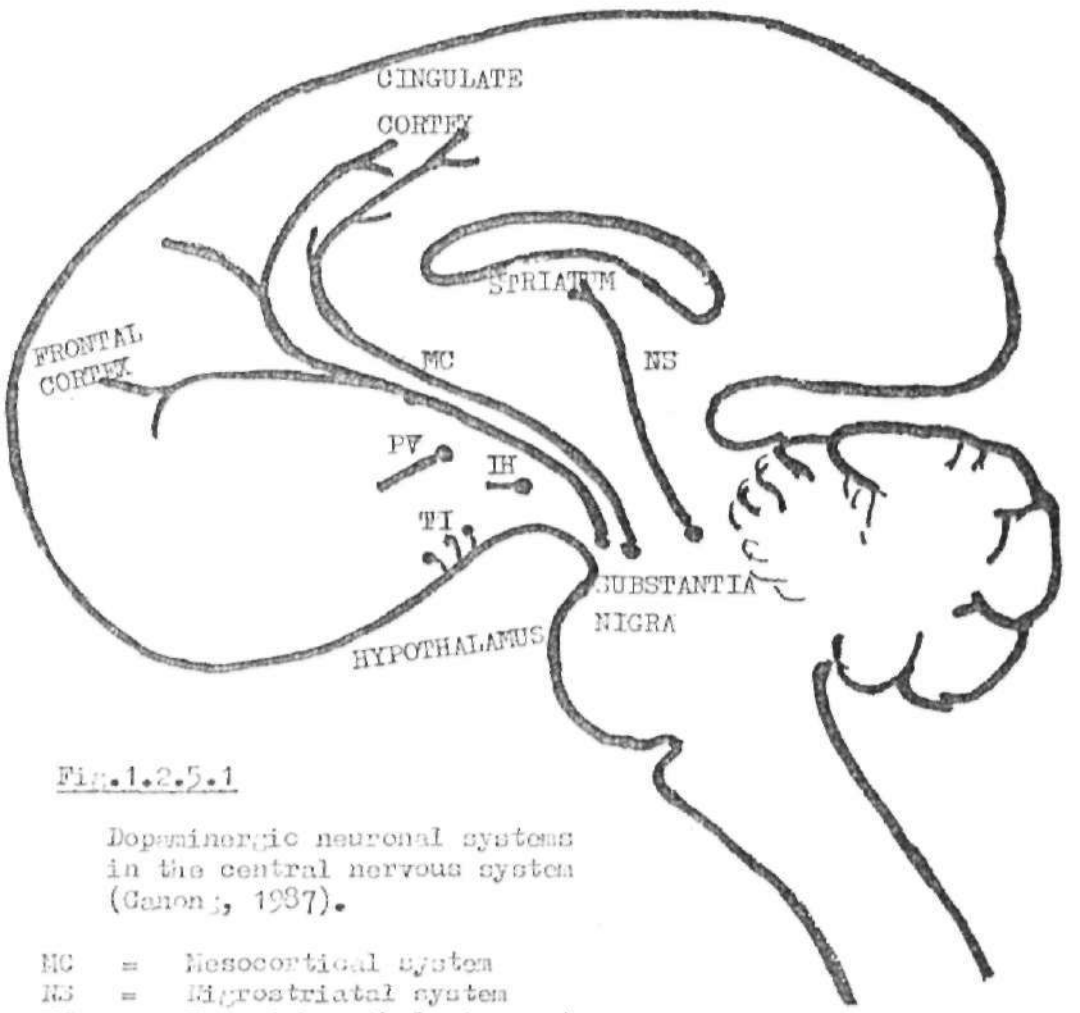


Fig.1.2.5.1

Dopaminergic neuronal systems in the central nervous system (Ganong, 1987).

MC = Mesocortical system
NS = Nigrostriatal system
IH = Incertohypothalamic system
TI = Tuberoinfundibular system
PV = Periventricular system

The nigrostriatal system of the dopaminergic neurons are believed to be related to motor functions since it has been observed that degeneration of this system results into Parkinson's disease - a motor disorder. In 1960, Ehringer and Hornykiewicz observed a decreased dopamine content in the corpus striatum of patients with Parkinson's disease. These observations offered enough reasons to believe that dopamine in the striatum has physiological functions, and in 1966, Hornykiewicz implied a patho-physiological role for brain dopamine in psychomotor function.

From studies carried out in normal humans, it was observed that there is a steady loss of dopamine receptors in the basal ganglia with age and that the loss is greater in men than in women.

1.2.6 GAMMA - AMINOBUTYRIC ACID (GABA)

GABA is a neutral amino acid and is found to be distributed throughout the CNS. GABA was found

to be an inhibitory transmitter at the crustacean neuromuscular junction while glutamate is excitatory at the site (Burn 1975). This worker believed that the actions of GABA and glutamate at the crustacean neuromuscular junction take place in the mammalian central nervous system, where GABA appears to be the major postsynaptic inhibitory transmitter in the brain and is probably also the presynaptic inhibitory transmitter in the cuneate nucleus and in the spinal cord.

1.2.7 SEROTONIN (5 - Hydroxytryptamine).

It has been noted that the serotonin - secreting neurons have their cell bodies in the midline raphe nucleus of the brain stem and they project to the hypothalamus, the limbic system, the neocortex and the spinal cord. There is also a prominent serotonergic innervation of the suprachiasmatic nuclei of the hypothalamus.

A possible correlation between behaviour and variations in brain serotonin content is still

being examined. However, the relation of serotonin to mental functions remains uncertain.

1.3 *THE NEUROPHYSIOLOGIC BASIS OF BEHAVIOUR*

1.3.1 BACKGROUND THEORY OF VARIOUS STRUCTURES IN THE BRAIN AND THEIR BEHAVIOURAL ROLES.

In considering the behavioural functions of the brain, it is important to note that behaviour is a function of the entire nervous system. All parts of the nervous system are involved. Therefore behaviour is not a function performed by specific parts of the brain.

The two most common methods for determining the behavioural roles of various structures of the brain have been to study the effect of destroying or stimulating specific brain areas and observing the effects on behaviour.

The limbic system, a group of deep structures are of primary consideration in certain behaviours. Thus, while learning, memory, judgement, language and related processes are

grouped into what is normally regarded as the intellectual functions (or higher functions) of the central nervous system (and this involves a large part of the neocortex), those instinctual patterns of behaviour, subconcious motor and sensory drives, intrinsic feelings of pleasure or pain and other related behaviours are performed mainly by the structures of the limbic system while the cerebral hemispheres also play a part. In addition to playing a role in olfaction, the limbic system is also concerned with motivation, control of sexual behaviours, expressions of rage and fear etc. These later behaviours essentially form the circuit for the maintainance of homeostatic equilibriums.

Behaviour is a complex reaction of higher function with the environment and is characterised by two phenomena - PLASTICITY, which is the ability to acquire new modes of responding and ENERGETICS, which is variability in the intensity of responding.

Neurophysiologists, in the past lumped the entire limbic system together as a unit, that controls the different emotional and behavioural patterns of the nervous system. However, with increasing knowledge of neurophysiology it has become known that different areas of the system perform specific functions. Thus, it is now known that Amygdala regulates anger and aggression. Stimulation of amygdala through implanted electrodes in unanesthetized cats and monkies elicit responses clearly related to affective attack and defence. Amygdalectomy removes fear and rage and animal would become exceedingly docile. The hippocampus is concerned with memory, while the septum controls pleasurable emotions. The frontal lobe together with other limbic structures affect the hypothalamic center for appetitive behaviour, anger and sexual drives. It appears that the later are some of the autonomic functions belonging to the many behaviours available to the animal in the economy of constant internal environment.

1.3.2 THEORETICAL CONCEPTS IN THE PHYSIOLOGY OF BEHAVIOUR.

Several theories have been put forward in the quest to explain behaviour and most of these have now become basic concepts in the study of behaviour. Stellar (1954) primarily attempted to address the neurophysiologic basis of spontaneous behaviour and proposed that the amount of spontaneous behaviour is a direct function of the level of activity in the excitatory centres in the hypothalamus. These hypothalamic centres were believed to receive inputs from internal and external environments, cortical and thalamic centres and related inhibitory centres for the integration of various negative inputs, to depress the activity of the excitatory centre. The algebraic sum of these inputs, he explained, determine the output along the final common pathway for behaviour. This proposal was to become the model on which the theory of behavioural centres was based and developed to explain all types of behaviours.

Morgan (1965) postulated the first theory of behavioural centres based on Stellar's (1954) model. According to Morgan (1965), there are excitatory and inhibitory centres throughout the brain and they all control the pattern of a response; the magnitude of a response was thought to be dependent on the balance between the excitatory and inhibitory influences. Another theory emphasized the outcome of a pattern of activity in the network of neural units (Morgane, 1964). In such neural arrangement, Morgane (1964) explained, the final output does not depend on a single nucleus, but that there are multiple pathways which by interaction, their inputs are integrated to produce specific behavioural patterns, hence, the neural network offers considerable resistance to disruption by individual pathways or units in the brain, and malfunction or damage to any unit or pathway would not necessarily impair function. Morgane's (1964) theory was rooted in the ideas of Flourens (1824). According to Flourens (1824), despite the fact

that specific brain regions have certain functions and properties, there was unity in the CNS, so that loss of one unit's function may reduce the energy of the whole, but would not necessarily abolish the overall function or the output of the system. This theory implies that there is a specific function to each anatomical nucleus in the sense that it enables integration of information in the system, but also there is a joint function in the output of the network.

As a consequence of these concepts of functional control of behaviour by centres in the brain, theories of biochemical basis of behaviour followed with similar views. For example, drugs were believed to modify behaviour by changing the levels of excitation and inhibition of specific sites in the brain. Duffy (1962) stated that chlorpromazine acts on the hypothalamus to reduce its level of activation while amphetamine activated it. The basic assumption underlying these studies is that the levels of neural activity at any given site may be directly or

indirectly related to the concentration of chemical agents within the locality. In particular, research has focussed on presumed CNS transmitter substance which may explain drug-induced changes in behaviour. The idea of drugs modifying levels of activity in neural centres was extended to the biochemical studies of the brain and extensive work has been done to determine the levels of neurochemical substances at specific sites with the attempts to correlate them with definite behavioural patterns.

1.4 THE BIOCHEMICAL BASIS OF BEHAVIOUR.

1.4.1 BIOGENIC AMINES IN REGULATION OF BEHAVIOUR.

Extensive work in the past has focussed on the possible roles of the biogenic amines in behaviour. The present knowledge of the involvement of catecholamines in the regulation of behaviour began to emerge through several studies. The biogenic amines, as putative neurotransmitters, have already been known to play important roles in the regulation of central

nervous system functions (Hornykiewicz, 1966; Chase and Murphy, 1973). It has been noted, for example, that neurological disturbances caused by brain damage due to injury or exposure to toxic substances are often accompanied by changes in the endogneous levels of biogenic amines. The levels of these brain amines have been determined in various brain regions in rats (Langlais, Huang and Gabay, 1975).

The principal catecholamines found in the body are norepinephrine, epinephrine and dopamine and all three are present in the brain. Cannon (1915) first noted that the hormone of the adrenal medulla was involved in mammalian resistance to stress. With the identification of norepinephrine and epinephrine in the brain, it was considered therefore that they too might actually be regulating responses such as fear, anger or rage, mood, hunger, etc., within the central nervous system.

It was initially believed that dopamine was

present in the nervous system only as the intermediate precussor in the biosynthesis of norepinephrine, and this was also considered to be its primary function in the brain. Later, evidence accumulated which indicated that dopamine was actually, on its own right, a neurotransmitter. Its neurotransmitter role has been prominently studied, especially in the striatum, median eminence, hypothalamus, the limbic lobe and neocortex. The first suggestion that dopamine may have important physiological function came after the work of Blaschko (1957) and those of Carlsson, Lindqvist and Magnosson (1957). In 1967, Goldstein, Anagnoste, Owen and Batista showed that certain brain lesions produce a greater loss of caudate dopamine than norepinephrine. This discovery was to confirm the existence of separate dopaminergic and noradrenergic neuronal systems in the brain since the lesion appears to primarily affect depamine. Since then, a number of reports have accumulated which indicated different functions for

norepinephrine and dopamine in the physiology of behaviour.

In 1948, Leimdofer produced a deep, but normal sleep in man by intracisternal injection of norepinephrine. In 1954, Feldberg and Sherwood reported producing similar effects in cats through an intraventricular route of administration. Sedation has also been observed in various species of experimental animals after administration of norepinephrine into the brain. Godwin, Murphy, Brody and Runney (1970) showed that dihydroxyphenylalanine (DOPA), a precursor of norepinephrine biosynthesis, elicits both behavioural and locomotor activation accompanied by arousal pattern of the EEG when administered in large doses. In an earlier study, Van Rossum (1968) showed that cocaine-induced behavioural excitation which had been antagonized by a pretreatment with reserpine was restored following DOPA administration. These reports appeared to confirm an earlier observation that dopamine, and not norepinephrine, is the principal catecholamine

subserving the arousal functions in the brain (Everett and Wiegand, 1962). Secondly, the reports were further supported by the work of Crevelling, Daly, Tokuama and Witkop (1968) who used dihydroxyphenylserine (DOPS) to demonstrate that norepinephrine does not reverse reserpine-induced sedation.

Many experimental observations and reports finally led to the agreement that Dopamine and Norepinephrine mediate different behavioural roles. Norepinephrine was associated with gross spontaneous behavioural activity while Dopamine was associated with stereotyped behaviour (Randrup and Jonas, 1967). Other workers who worked seperately, also independently demonstrated that direct injection of dopamine into the corpus striatum induced gnawing behaviour in the experimental animal (Ernst, 1967); Randrup and Munkvad, 1967).

1.5 *THE NEUROPHYSIOLOGIC BASIS OF SLEEP AND* WAKEFULNESS.

1.5.1 SLEEP AND WAKEFULNESS.

Sleep is a behavioural state, and can be defined as the product of activities in the neural systems in the phylogenetically old reticular core of the brain. A fact that has now been fully established is that the preoptic area (POA) in the hypothalamus is deeply involved in the central regulatory mechanisms of sleep. It was shown that stimulation of this area produces cortical electroencephalogram (EEG) synchronization and leads to sleep. On the other hand, lesions of the preoptic area suppressed sleep (Nauta, 1946). Chemical stimulation experiments also confirmed the importance of the preoptic area in sleep mechanism; thus, introduction of various sleep inducing substances into the preoptic area through microinjection was shown to produce sleep in the experimental animal, while a similar introduction of acetylcholine

into the area elicited wakefulness. Similar to the preoptic area, workers have also found one other "sleep centre" which appears to lie in the thalamus itself. Stimulation of this area produces sleep in concious unrestrained animals.

The posterior hypothalamic area was also linked with sleep regulation. Nauta (1946) showed that lesions of the posterior hypothalamic area disturbed wakefulness, while it was also shown that high frequency stimulation of the area induces persistent EEG desynchronization, an indication of arousal of the cerebral cortex for alertness and wakefulness. However, whether posterior hypothalamus per se is responsible for waking or stimulation of the fibers travelling through it is still questionable and unsettled. Hess (1955) applied the term `dynamogenic zone' to posterior hypothalamus when he first noted that its stimulation elicits an active display of autonomic and somatic behaviours accompanied by EEG desynchronization. Therefore, the posterior hypothalamus was earlier refered to as the

"waking" area in the brain. Other regions known to similarly produce wakefulness upon stimulation include the lateral hypothalamic area, the mammilary bodies including the mammilothalamic tract. It is suspected that the mammilary bodies may be of greater importance than the lateral hypothalamic area in producing alertness or wakefulness.

Considerable evidence suggest that the neural systems concerned with sleep and waking states are in the brain stem, and are associated with specific neuro-humours. Since the classical hypnotoxin experiments of Legendre and Pieron (1910) the existence of some sleep-regulating substances have been proposed and a number of endogenous factors have actually been listed (Inoue, 1985). Most of the endogenous substances listed were prostaglandins. Other substances also mentioned to have regulatory influences on sleep were diclofenac sodium and indomethacin (both of which are prostaglandin synthesis inhibitors), aspirin, melatonine and uridine. It is thus

believed that sleep is regulated by multiple endogenous factors present in the cerebrospinal fluid. These humoral factors are believed to be functionally linked such that sleep regulation involves a complicated mutual interference mechanism among them.

The reticular formation lies in the medulla and pons, extending slightly into the midbrain. From present knowledge, activity in the so-called medullary "sleep zones" lead to production of synchrony and sleep, and it is known that such activity is transmitted to the thalamus via the ascending reticular system.

An awake human responds to stimuli because the Reticular Activating System (RAS) is in contact with the environment to alert the cortex in maintaining wakefulness by bursts of electrical activity.

The reticular formation has extensive interconnections with the sensory, motor and integrative nuclei throughout the brain stem and receives collateral inputs from a variety of these pathways. Activity in the reticular activating

system produces a widespread activation of the cerebral cortex resulting into arousal and heightened states of attentiveness. The greater the stimulation of the reticular activating system, the more alert and attentive the individual will be to the incoming sensory information. After a prolonged period of activity, the reticular formation becomes less responsive to stimulation. Evidence is accumulating that regulation of the awake/sleep cycle involves an interplay between the brain stem nuclei. When the reticular activating system becomes inactive, so will the cerebral cortex in what is then sleep. The state of sleep will be terminated by any stimulus sufficient to activate the reticular activating system.

1.5.2 NEUROTRANSMITTERS IN SLEEP.

1.5.2.1 NON-RAPID-EYE MOVEMENT SLEEP (NREM).

The non-rapid-eye movement sleep (or slow-wave sleep) is the first stage upon falling asleep. NREM sleep is thought to be produced by the absence of desynchronizing activity in the

ascending reticular system. The synchronizing activity of the thalamus is believed to be augmented by the ascending activity from the pons and hindbrain in the production of this first stage of sleep.

It has been noted that serotonin is involved in the production of NREM sleep; the discharge of the serotonin secreting neurons was linked with the onset of NREM sleep. This observation was also further supported by the report that drugs that deplete brain serotonin such as parachlorophyenylalanine caused insomnia in cats while administration of 5-hydroxytryptophan which restores the serotonin content of the brain, abolished the effect (Ganong, 1987).

1.5.2.2 *RAPID-EYE-MOVEMENT SLEEP (REM).*

The mechanism which triggers rapid-eye movement sleep (or paradoxical sleep) is believed to be in the pontine reticular formation, but no single transmitter is yet known to be responsible for its production. It is not yet certain what initiates the discharge in the pons that heralds

the onset of REM sleep, but it is thought that the locus coeruleus and the norepinephrine-secreting neurons emanating from it play important role in the phenomenon.

In the effort to explain the production of REM sleep, Begley (1989) suggested the existence of two kinds of neuronal systems that control sleep in the brain stem, each using a different chemical to communicate. One of the neuronal systems use acetylcholine and they are "On" during REM sleep, while the other system uses norepinephrine and serotonin and they are "Off" when the acetylcholine neurons turn on. Begley (1989) explained that acetycholine neurons send rapid bursts of electrical signals to the cortex, the seat of higher thought and vision, and suggested that acetylcholine may be the very stuff that REM sleep is made of. There has also been some evidence noted from the findings of other workers which seem to support these claims. It has been noted that when drugs that mimic acetylcholine were injected into the brain stems of cats, they were seen to undergo REM sleep; and

in humans, brain stem lesions that deplete the forebrain of norepinephrine, suppressed REM sleep, while drugs that increase the availability of acetylcholine in the brain, hastened the onset of REM sleep and dreams. From these accounts, it could be seen that the neurotransmitter agent involved in the production of REM sleep is still an unresolved issue as no particular transmitter has been earmarked with certainty. However, most workers believe that the serotonin mechanism for NREM sleep and whatever mechanism that may be involved in the REM sleep must be functionally linked. Thus, it is believed that in NREM sleep, it is the rate of turnover of serotonin or of its catabolic products that is monitored by a clock-like mechanism in the brain such that provides the signal for the transition from one sleep stage to another.

1.6 *ASCORBIC ACID.*

Ascorbic acid was discovered as a substance with the chemical formula $C_6H_8O_6$ from the adrenal

cortex of guinea - pig by Szent-Gyorgi and Haworty (1933). The substance was later found to cure scurvy and was subsequently given the name Ascorbic Acid.

Ascorbic acid is now known to be a water soluble vitamin (Vitamin C). Since its identification, numerous, mainly chemical investigations have been carried out on it. However, in spite of several investigative studies, the mechanism underlying the actions of ascorbic acid in subserving its many attributed effects is not yet known.

A variety of functions were first suggested by Baker (1967). A deficiency of ascorbic acid in man, guinea-pig and monkey has been found to lead to conditions like scurvy, intramuscular and subcutaneous haemorrhage, tenderness in joints, general weakness and weakening of the connective tissues of the skin, tendons and walls of blood vessels; lethergy, loss of appetite, anaemia and slow healing of wounds. Thus, there has been strong suggestions that the daily taking of large

doses of vitamin C may decrease the likelihood of contracting a wide range of human disorders.

A lot of work has been done on the sources of this vitamin. From all the animals studied so far, it was shown that ascorbic acid is not synthesized anywhere in the brain (Vitler, Sebrell and Harris, 1967). Of all the animals studied so far, man and other primates are dependent on food sources for the vitamin (Roy and Guha, 1958). Most people, even primitive people in many parts of the world know of the necessity for fresh foods, particularly from plant sources (especially fruits and vegetables). The recommended daily dose of vitamin C is 75 mg for an adult male and 70 mg for a female (Food and Nutrition Board of U.S. National Research Council). Various popular books (Pauling, 1970; Webstar, 1971) even recommended doses well in excess of these figures.

The nature of the reactions which ascorbic acid undergoes in the body has been ascertained. Thus, it is now known that when utilized, ascorbic acid is oxidized to dehydroascorbic acid which is

rapidly reduced again, regenerating ascorbic acid; and when dehydroascorbic acid is oxidized, 2, 3 - Diketogulonic acid is formed (Martin and Mecca, 1961; Schneider and Staudinger, 1964; Dayton, Snell and Perel, 1966; Hammarstrom, 1966; Heath and Fiddick, 1966). The reaction is illustrated in Fig. 1.6.1. It has been suggested that dehydroascorbic acid is the form for transport of ascorbic acid across cellular barriers (Patterson and Mastin 1951; Martin, 1961; Martin and Mecca, 1961).

Both ascorbic acid and dehydroascorbic acid are said to have affinity for the sites of catecholamine synthesis in the CNS (Sjostrand, 1970; Subramanian, 1977), and that injected ascorbic acid and dehydroascorbic acid are both distributed fairly equally in the body (Hammarston, 1966).

It is now known that ascorbic acid is highly concentrated in the brain (Shimizu, Matsunami and Onishi, 1960; Subramanian, 1977; Kuo, Hata, Yeshida, Yamatodani and Wada, 1979; Lelland et

al., 1979), and that it is preferentially distributed in the brain (Subramanian, 1977). Subramanian (1977) clearly mapped out the regional variation in the distribution of ascorbic acid in the brain. He reported that in magnitude of the content in various regions, the order is hypothalamus, hippocampus, cerebral cortex, cerebellum, striatum, medulla and pons, and the midbrain, respectively. It was also observed that there is regional differences with regards to the active transport of ascorbic acid in the brain, with the hypothalamus being able to accumulate more than any other region and that the rate of accumulation roughly parallels the content in that region.

1.6.1 PROFILE OF BRAIN ASCORBIC ACID.

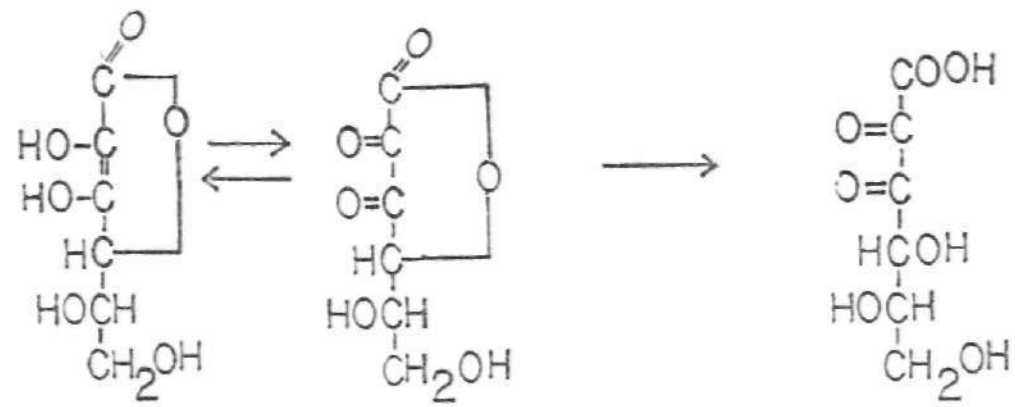
The transport of ascorbic acid from body fluids into the brain, has been studied. It has been noted that ascorbic acid has a blood-brain barrier, being first transported to the cerebrospinal fluid (CSF) by a saturable transport. Transport is said to be stereospecific

and the probable locus of transfer from blood to the CSF appears to be the choroid plexus (Spector and Lorenzo, 1974). From the CSF ascorbic acid appears to diffuse in the extracellular compartment, and then actively transported into the intracellular compartment (Spector and Lorenzo, 1974).

Existing knowledge of the pharmacological properties of ascorbic acid in the CNS is still very hazy. In view of the prominence and preferential distribution in the CNS and presumably assuming the homogeneity of ascorbic acid, it is strongly believed that it may be involved in some central physiological functions.

A deficiency of ascorbic acid has been reported to cause symptoms of depression in man and impairment of nervous activity (Lyhs, 1961), while high amounts of ascorbic acid may cause insomnia, irritability and anxiety (Glatzel, 1966; Pitt and Pollit, 1971). Ascorbic acid has also been implicated in the pathophysiology of anxiety

Fig. 1.6.1. The reactions of ascorbic acid in the body.



Ascorbic acid Dehydroascorbic acid 2,3-Diketogulonic acid

(Slater, 1962) and of Schizophrenia (Pitt and Pollit, 1971). Inspite of all these reports and the increasing evidence of the effects in the central and peripheral nervous system, there is still no clear understanding of the mechanism of action or the actual physiological role of ascorbic acid in the brain. Pfeiffer, Goldstein, Murphree and Nicols (1968) showed that 1 - 3g ascorbic acid depressed the electroencephalogram (EEG) in man, while Benton (1981) on the other hand, observed that ascorbic acid (1 - 2g orally) reduced complex reaction time, while psychomotor co-ordination was disrupted in man. In some recent experiments with rats, it was observed that ascorbic acid desynchronized the EEG of the frontal and optic cortices, while it dose-dependently activated their gross behaviour (Wambebe and Sokomba, 1986). Similarly, Erube (1982) reported gross behavioural and EEG activation in young chicks after treatment with ascorbic acid.

Parkinsonism is a dopamine deficiency disease

which is known to result from a decrease or an injury to the dopaminergic neural system in the basal ganglia. It was reported in levodopa therapy, that a combination of levodopa and ascorbic acid was highly rewarding in patients with Parkinson's disease (Sacks and Simpson, 1975). These workers found that when the dose of ascorbic acid was increased while reducing that of levodopa, there was a faster improvement and the side effects were minimal.

These reports clearly indicate that ascorbic acid may play a fundamental physiological role in the normal functioning of the brain and the CNS in general.

In another report, an interruption of the intake of ascorbic acid resulted in acute onset of migraine in patients who have had this attack chronically (Bali and Callaway, 1978). There are strong suggestions that ascorbic acid may work by close links with neurotransmitters and the synaptic transmission processes in the CNS, but no

definite conclusions have been drawn yet. Many workers have identical opinions that it would be difficult to suggest that a substance not synthesized in the brain but exhibits several prominent unexplained characteristics of distribution and retention, is devoid of any important function in the brain.

1.6.2 STUDIES OF THE POSSIBLE INVOLVEMENT OF ASCORBIC ACID IN CENTRAL NEUROTRANSMITTER SYSTEMS.

Various neurotransmitter systems in the CNS have been implicated in the possible mechanism of action of ascorbic acid. In addition, ascorbic acid has been linked either directly or indirectly with the metabolism of almost all the important putative neurotransmitters in the CNS (Subramanian, 1977). Among the neurotransmitter systems postulated for the mechanism of action of ascorbic acid include:

1. Inhibition of cholinesterase enzyme and release of acetylcholine (Sjostrand, 19670;

Kuo et al., 1979),

2. Release of norepinephrine from its storage sites (Sjostrand, 1970; Kuo et al., 1979), and

3. Modulation of dopaminergic activity (Tolbert, Thomas, Middaugh and Zem, 1979). Tolbert et al. (1979) reported of the specific neurochemical, physiological and behavioural alterations in dopaminergic systems produced by ascorbic acid.

Obviously, their focus of attention was on the stereotyped behaviour which is believed to be mediated by dopaminergic neural system. Many workers also believe that ascorbic acid play an indirect role in the hydroxylation of tryptophan which is the rate limiting step in the synthesis of 5-hydroxytryptamine (Serotonin).

In the metabolism of catecholamines, it has been observed that ascorbic acid may act as a cofactor in the beta hydroxylation of dopamine by Dopamine - B - hydroxylase which is a rate

limiting step in the synthesis of norepinephrine, and it is thought to be indirectly involved as a mediator in tyrosine hydroxylase having a control in the synthesis of L-dihydroxyphenylalanine (DOPA). Apart from this, it has also been mentioned that ascorbic acid appears to be a competitive inhibitor in the methylation of norepinephrine to normetanephrine which could play a role in the regulation of catecholamine catabolism.

1.6.3 EXAMINING POSSIBLE ROLE OF VITAMIN C IN MENTAL STATES.

Ascorbic acid has been implicated in the pathophysiology of depression, schizophrenia, stress and anxiety. Depression may be seen as a feeling of gloom which may be due to a failure in an endeavour or a disappointment in expectation or hope. Depression can even be induced by administration of some agents. Depression has many phases: In reactive depression, the patient does not lose touch with reality and is usually

due to stress. In severe depression which is a psychotic state, there is usually a predisposition to it in the person's make-up; he then loses touch with reality. In all types of depression, even in the depression that occasionally occur after menopause, there is a risk of suicide. Depression was obtained when a part of the brain was blocked by 25% potassium chloride which caused a decrease in ascorbic acid level as compared to other parts of the brain that were not blocked (Lyhs and Rvidiger, 1963). In another development, ascorbic acid has been found to relieve depression syndrome associated with adrenocorticotropic hormone (ACTH). Cocchi, Silenzi, Calabri and Salvi (1980), reported successfully treating depression in ACTH therapy as well as patients suffering from idiopathic depression, with ascorbic acid.

The subject of the possible involvement of ascorbic acid in schizophrenia has been one of controversy. Schizophrenia is the generic term used for a group of disorders characterized by a progressive loss of emotional stability,

judgement and contact with reality. In 1971, Pitt and Pollitt reported that schizophrenics have high amounts of ascorbic acid in the brain when compared to controls in the same ward receiving the same food. Some workers also found that schizophrenics required a higher amount of ascorbic acid than controls. Binette, Ferron and Granel (1965) had earlier reported that contrary to previous reports, low levels of ascorbic acid was not characteristic of the cerebrospinal fluid and blood of schizophrenics whether or not they were receiving tranquilizers. However, these reports clearly indicate that the role (if any) of ascorbic acid in the aetiology and management of schizophrenia is still far from clear.

Kubala and Katz (1966) found that administration of ascorbic acid to children increased their intelligence quotient by 5 per cent compared to controls not receiving the treatment. Also human subjects receiving high amounts of ascorbic acid were reported to have shown great alertness (Masek, Hruba and Novakova,

1976). These reports have lead to the belief that ascorbic acid might improve mental functions.

1.7 THE OBJECTIVES OF THIS STUDY

1.7.1 EXPECTATIONS.

Presently in the society, there is this problem of increasing use of drugs by people to modify mood or behaviour to suit an occasion or for some other purpose. This is usually done either individually or in groups. The practice has been referred to as drug abuse. While the government organizes various social enlightenment programmes to dissuade people from engaging in this dangerous habit, what has been of primary concern to biomedical research is understanding the mechanisms by which such drugs modify behaviour or mood. There is no doubt that it will be of immense benefit to mankind if through such knowledge, measures could be developed to counter the effects of the drugs in the brain. This project is intended to study the neurophysiological factors underlying any

behavioural effects of ascorbic acid, with the aim of ascertaining the mechanism of action by which this is achieved in the CNS. In the light of this, expectation is that the data obtained from this study would shed more light into the actions of these central nervous system drugs in general, and this is the justification and long term objectives of this project.

1.7.2 A SURVEY OF CENTRAL PROCESSES.

In earlier reports, the catecholamines were associated with various roles in regulation of behaviour. Serotonin, norepinephrine and acetylcholine were also variously linked with sleep regulation. Among the catecholamines, norepinephrine was associated with gross spontaneous behavioural activity in addition to its supposed involvement in sleep. Norepinephrine is also thought to mediate sedation in the brain (Leimdofer, 1948; Feldberg and Sherwood, 1954). Dopamine was associated with stereotyped behaviour (Randrup and Jonas, 1967) in addition to its

supposed arousal functions in the brain (Everett and Wiegand, 1962), while DOPA was linked with locomotor and other behavioural activation (Bunney, 1970).

There are increasing reports on the behavioural or central nervous system effects of ascorbic acid. It is the consideration of this study that such effects could be achieved by some mechanisms either physiologically, pharmacologically or both. This may thus, explain the usually obvious assumption that a change in behavioural output of a normal animal must result from alteration in the central nervous system activity. In a variety of such applications, stereotyped behaviour is widely used as an index of dopaminergic stimulation (Costal, Naylor and Piner, 1974). In this regard therefore, the magnitude of change in stereotyped behaviour could be indicative of an involvement with dopaminergic neural systems and transmission. The objective of this project is to study the physiological processes and factors underlying any behavioural

changes manifested in the course of ascorbic acid treatment in experimental animals. The study shall compute all behavioural activities seen during the various ascorbic acid treatments with the hope that the results when analysed may offer the starting point for further investigations to ascertain what neural system or systems that may be involved in the central mechanism of action of ascorbic acid.

One way it may be possible to study the neural processes involved in the mechanism of arousal in the brain is by examining sleep. The arousal mechanisms of the CNS is implicit in sleep physiology. In this study, it is intended to produce sleep using a short acting barbiturate. Different doses of ascorbic acid shall be used to examine pentobarbitone-induced sleep in the rats. Thus, the possible role of the various neurotransmitters in the reported arousal effects of ascorbic acid can be assessed or evaluated. The second aspect of this would be to evaluate possible links between ascorbic acid - induced

changes and the system that traditionally mediate the arousal functions of the brain for alertness and maintenance of wakefulness (e.g. the cholinergic neural system which forms the reticular activating system).

The prominence of ascorbic acid in the brain as found by workers (Lyhs and Rvidiger, 1963; Sjostrand, 1970) has offered enough indication that it may be involved in some central physiological functions. Electrical activities of the brain has often been observed by EEG and has also been employed to monitor neurotransmitters in the brain especially in animals that are in resting state or sleep. One of the objectives of this study therefore is to employ this technique for an over-all assessment of possible states of arousal or sedation during different treatments with ascorbic acid. It may be possible to quantify and evaluate dose-response relationships to examine the degree of an observed EEG response and correlate these with other results.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS.

Adult albino rats (Wistar strain, inbred) obtained from the animal house of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, were used in this study. Animals were aged between 7 - 9 months and weighing between 200 - 295 g.

2.2 DRUG SOLUTIONS

2.2.1 PREPARATION OF DRUG SOLUTIONS

(a) Sodium pentobarbitone. BP, 60mg/ml

(Abbott laboratories Ltd., Watford, England).

Volume of the stock solution required to deliver the appropriate dose to the animal was determined from the relationship:

$$\text{Volume (ml)} = D \times W \times \frac{1}{60}$$

Where, D = The required dose in mg/kg

W = The weight of the animal in kilograms

60 = The concentration of the stock solution (Sodium pentobarbitone, mg/ml).

(b) Ascorbic acid (BDH Chemicals Ltd., Poole, England).

The ascorbic acid used in this study was obtained commercially in pure crystalline form. Weighed quantities of the drug were dissolved in appriopriate volumes of saline. The doses of ascorbic acid used in this study were calculated from the stock solutions which were prepared as given in appendix 3.

(c) Preparation of ascorbic acid solution for intracerebroventricular (ICV) administration.

The stock solutions for the doses of ascorbic acid used in the ICV administration were prepared, employing the procedure given in appendix 3.

2.2.2 STORAGE AND PRESERVATION OF DRUG SOLUTIONS.

A fresh drug solution was prepared for each day's experiment in order to ensure that the stability of the drug used was maintained. In the

course of each day's experiments, care was taken to keep each drug solution in a cool place and inside air-tight amber-coloured containers and away from light.

2.3 ROUTES OF ADMINISTRATION.

Ascorbic acid and sodium pentobarbitone were administered intraperitoneally (i/p). However intracerebroventricular administration of ascorbic acid was employed for some EEG studies.

2.4 CONTROLS.

The control animals were administered with the solvent vehicle (ie. physiological saline) used in preparing the ascorbic acid solutions. The volume of physiological saline administered to the control animal were determined as 0.2% of its body weight in grams. For the control recordings in the ICV experiments, volume of physiological saline administered by the intracerebroventricular route was 10 microlitres, being the same volume as used for delivering the required ascorbic acid doses in the ICV administration.

In the control experiments for sleep, the animals were treated with sodium pentobarbitone only. Pentobarbitone doses were determined as described in section 2.2.1 (a).

2.5 PREPARATION OF MATERIALS AND CALIBRATION OF EQUIPMENTS FOR EEG STUDIES.

2.5.1 IMPLANTATION OF ELECTROCORTICAL AND ELECTROMYOGRAM ELECTRODES.

The rats used were anaesthetised with sodium pentobarbitone (40 mg/kg) intraperitoneally, before they were implanted with electrodes made from the pointed ends of stainless steel insect pins No. 00 according to the methods of Spooner (1965). Stainless steel screws (obtained from Grass) were mainly used to prepare the monopolar electrodes as were employed by Wambebe and Sokomba (1986). After sufficient depth of anaesthesia, the rats were held firmly with ear plugs in a stereotaxic apparatus. The sagittal sutures were exposed by cutting and removing the skin over the skull with a sterile surgical blade. The brain

sites implanted were frontal cortex (FC), optic cortex (OC), and brain stem reticular formation (BSRF) with the help of stereotaxic instrument using the co-ordinates as given below. The monopolar electrodes were drilled in place and secured chronically to the skull overlying the brain site. The bipolar electrodes for the brain stem reticular formation were implanted by lowering the electrode to the appropriate depth from the top of the skull. The monopolar reference electrodes were drilled in place at the upper surface of the mouth. The electrodes were then held firmly in place with dental cement and acrylic fluid.

The electromyogram (EMG) electrodes were made from stainless steel insect pins No. 00, bent at the ends to form hooks. The electrodes were positioned in the proximity of the head and inserted in the dorso-lateral aspect of the neck. The electrodes were then secured chronically to the neck with dental cement and acrylic fluid.

The stereotaxic co-ordinates for the

implantation of the brain sites in the rat were:

FC:	anterior-posterior	=	+ 1.9mm
	lateral	=	+ 1.5mm
OC:	Anterior-posterior	=	- 9.2mm
	lateral	=	2.8mm
BSRF:	anterior-posterior	=	- 3.8mm
	lateral	=	+ 2.0mm
	vertical	=	- 8.2mm

These brain sites were located with reference to the bregma, using the stereotaxic co-ordinates.

2.5.2 ARRANGEMENT OF ELECTRODES IN THE MALE CONNECTOR.

The electrodes of the implanted rat were attached to the male connector in the order as given below:-

ELECTRODE IMPLANTED	POSITION IN MALE CONNECTOR
Reference (monopolar)	- No. 1
Frontal cortex (monopolar)	- No. 2
Optic cortex (monopolar)	- No. 3
RSRF (Bipolar)	- Nos. 4 & 5
Two EMG electrodes	- Nos. 6 & 7

After the implantation process has been completed, the animals were kept warm with an anglepoised lamp and allowed 24 hours for full recovery and for the implanted electrodes to stabilize before the recording of the EEG.

2.6 RECORDING OF THE ELECTROENCEPHALOGRAM (EEG) AND ELECTROMYOGRAM (EMG).

The implanted animals were put inside a screened cage in a quiet room and were allowed one hour to acclimatize to the environment. The Grass Polygraph machine, Model 79D (Grass Medical Instruments, Quincy Mass, U.S.A.) was used to record both the EEG and EMG. Before the implanted animals were connected to the polygraph machine,

it was calibrated for sensitivity, voltage and peak frequency and was also standardized for slow and fast paper speed recording.

The male connector carrying the electrodes of the implanted animal was then hooked into the female connector. In its channels for the monitoring of incoming electrophysiological signals, the polygraph machine then monitors the EEG and EMG of the different brain sites in the rat as represented below:-

Grass Electrode Selector Panel	Electrophysiological Signal Monitored
1-2	FC
1-3	OC
4-5	BSRF
6-7	EMG

2.6.1 RECORDING.

Animals were, first, administered physilogical saline and a control recording was made for one hour. They were then administered

with the appropriate dose of ascorbic acid and recording of the EEG and EMG was repeated for another one hour.

The chart speed during normal recording was 2.5mm per second. However, for every, 10 minutes during the normal recording, the speed was changed to 25mm per second to facilitate analysis of voltage and peak frequency.

2.7 THE ELECTROPHYSIOLOGICAL STUDIES

2.7.1 EEG STUDIES IN ACUTE ADMINISTRATION OF ASCORBIC ACID.

Ascorbic acid doses used in this study were 80 and 300mg/kg. Animals were implanted with the electrocortical and electromyogram electrodes as described in section 2.5.1. The EEG and the EMG were recorded, first, after administration of physiological saline for the control, and after the administration of the appropriate dose of ascorbic acid applying the procedures described under section 2.6.1.

2.8 TECHNIQUES FOR THE INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION IN EEG STUDIES.

2.8.1 PREPARATION OF GUIDE CANNULAE.

Guide cannulae for intracerebroventricular administration were constructed from 0.61mm diameter intramedic polyethylene tubing. With the aid of flame from bunsen burner, a bulb was blown in the middle of a piece of tubing. The length of the end of the tubing to be inserted into the ventricle was 4mm from the bulb, while the length from the other end of the bulb on top of the skull through which injection would be made was 1cm. The dead volume of the cannulae was approximately 2 microlitres. Normal saline was passed through the guide cannula before insertion into the skull to ensure that it was not blocked.

2.8.2 SURGERY OF THE INTRACEREBROVENTRICULAR CANNULAE IMPLANTATION.

The rats were anaesthetized with pentobarbitone sodium (dose, 40 mg/kg ip). The

skin over the skull was cut open with a sterile surgical blade to expose coronal and sagital sutures. The guide cannula was positioned 1mm posterior to bregma and with the help of a hand drill, it is lowered to a depth of 4mm from the top of the skull into the left ventricle. In all animals, some CSF appeared in the guide cannulae after insertion. This was an indication of correct placement into the ventricle as no CSF would have appeared if the insertion was into tissues. The guide cannulae was then firmly secured in place with dental cement and acrylic fluid along with the electrocortical and electromyogram electrodes which were also implanted during the surgery. Injections were made from a 10 microlitre Hamilton syringe over a period of 5 sec. with the needle remaining in position for a further 5 sec. before being withdrawn. The total injection volume was approximately 8 microlitres.

2.8.3 ASCORBIC ACID DOSES IN THE ICV ROUTE.

Two doses of ascorbic acid (10 and 40 micrograms) were used to study the EEG effects following administration by the ICV route.

2.9 SLEEP STUDIES.

2.9.1 GENERAL PREPARATION AND TREATMENT OF TEST ANIMALS.

Adult male albino rats weighing between 200-290g were used.

The sleep experiments were carried out in a well ventilated room, and in a quiet surrounding. Ambient temperature of about $24\pm2^{o}c$ was maintained throughout the experiments, and experimenting period was restricted to between 0800 hrs-1800 hrs each time so as to avoid the effect of circadian variation in brain levels on behaviour.

After they are removed from the animal house, the rats were allowed one hour to acclimatize to the new environment before the

experiments were started. Rats were weighed in order to determine drug treatment doses. Ascorbic acid doses employed in the sleep experments were 5, 10, 20, 40, 80, 200, and 300mg/kg of body weight respectivley, and the pentobarbitone dose was 20mg/kg in each experiment. Ascorbic acid were first adminstered before pentobarbitone after the appropriate time interval. In the controls, animals were given only pentobarbitone with the elimination of ascorbic acid treatment. The sleep experiments were designed to investigate;

(a) the effect of ascorbic acid on pentobarbitone-induced sleep, and

(b) the pattern of sleep in different pretreatment times with a fixed intraperitoneal dose of ascorbic acid (80mg/kg).

The pre-treatment times investigated were 5,10, 15,20,25,30,35,40,45,50,55 and 60 min., respectively.

The rats were regarded as asleep when their righting reflexes were lost. Experiment using

each ascorbic acid dose was repeated five times; similarly each pre-treatment time was investigated five times. A fresh animal was used each time of experiment, and each animal was observed separately at a time for sleep. Animals that did not sleep within a 30 minute period of observation were taken as not asleep. The onset and duration of sleep were timed using a stop clock. All the test experiments were carried out concurrently with the controls.

2.10 GROSS BEHAVIOURAL STUDIES.

2.10.1 BEHAVIOURAL TESTING IN ACUTE ADMINISTRATION OF ASCORBIC ACID.

Two rats of comparable size, weight and age and also of the same sex were kept in a transparent perspex cage of dimensions 25cm x 25cm x 15cm; each pair of rats were observed at a time. The experiments were performed in a quiet room free from disturbances and with an ambient temperature of 24 $\pm$ 2^{O}C. On removal from the animal house, the animals were first weighed and

allowed two hours to acclimatize to the new environment before any experiments. All the behavioural experiments were restricted to the same seasonal period and between 0900 hrs - 1800 hrs each day so as to avoid behavioural changes resulting from seasonal and circadian variation.

The control and test groups were observed simultaneously, and observation lasted for one hour. The control groups were given physiological saline, while the test groups were administered with the appropriate doses of ascorbic acid. Technical assistance was employed in the observations and recording of behavioural activities, but care was taken to ensure that the technical assistants were not aware of the type of treatments given to the animals. The doses of ascorbic acid used for the experiments were 5,10,20,40,80,160 and 320 mg/kg of body weight respectively and experiment with each dose was repeated five times using fresh pair of animals each time. Each behavioural activity observed were registered using hand tally counters

and a recording chart designed for this study. The behavioural parameters monitored were climbing, scratching, feeding, sniffing, rearing, grooming, stereotyped movements, aggression and locomotor activity.

2.10.2 BEHAVIOURAL TESTING IN CHRONIC ADMINISTRATION OF ASCORBIC ACID.

The behavioural parameters monitored in this study were the same as in section 2.10.1. Two doses of ascorbic acid (10 and 80 mg/kg) were separately administered to different groups of animals. Administration was done daily and at the same period in the day, for 30 days. A pair of male rats of comparable size, weight and age were kept in a transparent perspex cage of dimensions 25cm x 25cm x 15cm and received identical experimental treatment. The chronic administration experiment was repeated three times in the following procedural arrangement:-

In each Experimental Group (6 animals),

(1) A pair of animals received 10mg/kg dose for 30 days;
(2) A pair of animals were placed on 80mg/kg dose for 30 days; and
(3) A pair of animals received physiological saline (0.2% their body weight in grams) for 30 days.

Thus, altogether, 3 Experimental groups were used to investigate behaviour in chronic administration of ascorbic acid.

With the help of technical assistants, all the animals were always observed at the same time. Behavioural activities were measured every 3 days using hand tally counters and a specially designed recording chart to record all behavioural activities for one hour. Thus, a total of 90 days were used to study the behavioural pattern in chronic administration of ascorbic acid.

2.11 STATISTICAL ANALYSIS.

All data obtained were compared by the

student's t-test regarding $p<0.05$ as being statisically significant.

2.12 STUDIES ON THE NORMAL (NATURAL) SLEEP OF RAT -- EXPERIMENTAL PROCEDURES FOR CONFIRMATORY TESTS.

2.12.1 THE CONTROLS.

Healthy adult rats were kept in a well ventilated room in a quiet surrounding. The animals were allowed a few days to acclimatize to the environment, and were, each, kept in a separate cage. A rat was administered with normal saline (0.4ml.,i/p) as soon as it was noticed to be reclining for sleep. The rat was then observed and monitored for normal sleep only if it resumed sleep in less than 25 min. after it has been administered with saline. However, if the rat eventually did not resume sleep after the adminstration of saline, the experiment in respect of that rat is cancelled. To determine whether or not a reclining rat is asleep, a little noise was made close to it along with touching it very lightly using cotton wool; an awake animal usually

reacted to this disturbance. For each animal, a routine injection with saline was practiced on it for a few days so as to make the animal get used to being injected before the start of the actual experiment. Once sleep was confirmed in the animal, the duration of the normal sleep was timed using a stop clock.

After the timing of sleep, the animal was still monitored to note the number of times it may sleep again in the accompanying period of two hours. The experiment was repeated ten times using a fresh animal each time, and they were all performed between 1100 hours-1500 hours each day.

2.12.2 INVESTIGATING THE NORMAL SLEEP OF RAT AFTER DOSING WITH ASCORBIC ACID.

The doses of ascorbic acid used in the experiments were 5,80 and 320 mg/kg of body weight, respectively, and administration was done intraperitoneally. The choice of these doses, were based on earlier observations in the rat from previous experiments with ascorbic acid. The rat was observed for normal sleep after it has been

administered with the appropriate dose of ascorbic acid. The procedures for the observation and monitoring of normal sleep in the rat was done as in the controls and the experiments were restricted to between 1100 hours - 1500 hours in the day times only. Experiment with each dose of ascorbic acid was repeated ten times using a fresh animal each time.

2.12.3 INVESTIGATING THE NORMAL SLEEP OF RAT AFTER DOSING WITH 3, 4-DIHYDROXYPHENYL-SERINE (DOPS).

The dose of DOPS used in this study was 10mg/kg of body weight and was administered intraperitoneally. The rat for the experiment was prepared as in the controls; it was observed and monitored for normal sleep after receiving a single dose of DOPS. The experiments were done in the day times only and were restricted to between 1100 hours - 1500 hours.

DOPS (10 mg/kg, i/p) was also used to pretreat two doses of ascorbic acid (80 and 320 mg/kg, i/p) in the rat, and each of the doses was

separately and independently pretreated. The pretreatment time with DOPS prior to the administration of ascorbic acid was 15 minutes. Experiment for each dose was repeated ten times and each time, a fresh animal was used.

2.12.4 EEG INVESTIGATION OF THE NORMAL SLEEP OF RAT AFTER DOSING WITH ASCORBIC ACID, DOPS AND DOPS-PRETREATED ASCORBIC ACID.

Rats were implanted with electrocortical and electromyogram electrodes as described in Section 2.5.1 and 2.5.2, while the recording of the EEG and EMG was carried out as in Section 2.6.

The implanted rat was, first, left to stay in the cage in the recording room for about two days with normal feeding and this was to allow the animal get used to the environment before the start of the test experiments.

The dose of DOPS used in this study was 10 mg/kg and those of ascorbic acid were 80 and 320 mg/kg, respectively. Administration was done

intraperitoneally. At the specified doses, each drug was administered to an implanted rat at exactly 1300 hours in the afternoon when the experiment is started. Recording of the EEG and EMG were carried out steadily without breaking for 2 hours immediately after administration. The two doses of ascorbic acid (80 and 320 mg/kg) were separately administered chronically to different implanted rats for three days and each day, the procedure for the EEG recording was carried out. A clear synchronizing pattern of the EEG, when observed during the test period, was indicative of sleep in the animal in addition to the usual methods for confirming if animal was asleep.

The choice of these doses (80 and 320mg/kg) was based on observations from previous experiments. At these doses, the observed dual behavioural effects of ascorbic acid - Activation and Sedation - were most pronounced; the peak excitatory effects were noticed at 80mg/kg dose, while sedative effects were most significant at 320mg/kg.

CHAPTER THREE

3.0 THE PATTERN OF GROSS BEHAVIOURAL ACTIVITIES IN ACUTE ADMINISTRATION OF ASCORBIC ACID.

3.1 SUMMARY

3.1.1 The effect of ascorbic acid on gross behaviour was studied in rats.

3.1.2 Different doses of ascorbic acid were administered to the animals to monitor various behavioural activities.

3.1.3 Changes in gross behavioural activities were observed.

3.1.4 Behavioural excitation was noticed at low doses of ascorbic acid (5-80 mg/kg i/p), while the animals were behaviourally sedated at higher doses (160-320 mg/kg i/p).

3.1.5 The peak effect of the behavioural activation was noticed at 80 mg/kg dose of ascorbic acid.

3.1.6 Aggressive behaviour was not seen during any of the test experiments.

3.1.7 The results indicate that ascorbic acid may have both behavioural excitatory and

sedative effects and it was assumed that these effects in the brain may have been produced through mechanisms linked to neurotransmitter systems that traditionally regulate various forms of behaviour.

3.2 INTRODUCTION

When the behaviour output of a normal animal changes in magnitude, it is generally taken that the change must have resulted from alterations of central nervous system activity. Ruch and Pattern (1966) noted that such alteration in central nervous system activity could be influenced by humoral mechanism or by intrinsic activity. Chemical agents modify levels of neural activity in the brain to induce behavioural changes. Much information is available on the interaction of biogenic amines with a wide variety of chemical and endogenous substances elsewhere in the body except in the brain where very little is known. For example, calcium ions have been shown to influence the release of acetylcholine at the nerve terminal (Miledi, 1973). Much work has been

done on the cellular and subcellular distribution of the biogenic amines, their enzymatic formation and catabolism peripherally. However, little is known about these amines in the brain, especially with regard to their postulated behavioural modulatory roles. Vogel, Orfel and Century (1969) noted that dopamine, norepinephrine and serotonin for ecxample, were important modulators of mood and behaviour.

It is possible that substances which have pharmaco-behavioural properties in the central nervous system operate through interaction with brain amines which are currently thought to act as modulators of mood and behaviour. Serotonin (5-HT) for example, is believed to regulate locomotor activity and consumatory behaviour (Salles and Salles, 1979), while dopamine is believed to mediate stereotyped behaviour (Costall et al., 1974; Tolbert et al., 1979).

Ascorbic acid may be analogous to amphetamine judging from the several reports of the symptoms such as anxiety, irritability, depression,

impairment of nervous activity etc., which have been observed either due to high levels or deficiency of the vitamin (Lyhs, 1961; Slater, 1962; Pitt and Pollit, 1971). Amphetamine is thought to exert its actions mainly on the noradrenergic and dopaminergic neural systems to induce central effects (Trulson and Jacobs, 1979). Thus a probable central nervous system activity attributable to ascorbic acid may be viewed against this background. The aim of the present study is to ascertain, first, if injected ascorbic acid would cause any behavioural effect in the rat; and if so, secondly, to determine the pattern of behavioural activities manifested at different ascorbic acid doses and to compare these with previous reports. Since various brain amines have been postulated to either directly or indirectly regulate various forms of behaviour, it is hoped that the data obtained from this study would serve for a screening of various neurotransmitter systems in the central nervous system to ascertain the neural mechanism (if any)

involved in the actions of ascorbic acid.

3.3 MATERIALS AND METHODS

Refer to Section 2.10.1

3.4 RESULTS

3.4.1 EFFECT OF ACUTE ADMINISTRATION OF ASCORBIC ACID ON GROSS BEHAVIOUR.

The pattern of gross behavioural activities were studied in acute administration of different doses of ascorbic acid (i/p). Definite changes in gross behaviour were observed.

Low to moderate doses of ascorbic acid (5-80 mg/kg) induced behavioural excitation with attendant increases in gross activities in various behavioural parameters, while high doses (160 - 320 mg/kg) produced behavioural sedation in the animals (Tables 3.2; 3.3 F & G, Appendix I; Figs. 3.0.1 and 3.0.2).

The excitatory effects were most significant at 80 mg/kg dose (Tables 3.1, 3.2 and 3.3 E, Appendix I; Fig. 3.0.1). The magnitude of change

in activities were most notable and significant in the scratching and locomotor activities in all test doses (Table 3.1; Figs. 3.0.1 & 3.0.2). Stereotyped behaviour was increased at higher dose (Table 3.2).

Aggressive behaviour was not seen throughout the experiments (Tables 3.1; 3.3 A - H, Appendix I).

TABLE 3.1

Mean values of gross behavioural activities in acute administration of various doses of ascorbic acid (i/p). n = 5 per experiment

Behavioural Parameters	Doses (mg/kg) CONTROL	5	10	20	40	80	160	320
- CLIMBING	9.8 ± 1.15	11.2 ± 0.96	10.6 ± 2.58	10 ± 2.70	10 ± 1.14	12.6 ± 2.18	4 ± 0.54	0.8 ± 0.48
- SCRATCHING	39 ± 2.36	0.8 ± 3.16	47 ± 1.30	47.2 ± 2.28	55.6 ± 1.88	71 ± 5.11	23 ± 7.44	21 ± 1.61
- FEEDING	0.2 ± 0.20	0	2 ± 1.22	2 ± 0.54	3.6 ± 2.06	1 ± 0.63	0.4 ± 0.39	0
- SNIFFING	5.6 ± 0.50	7.8 ± 0.66	10 ± 0.99	11.4 ± 1.30	14.4 ± 1.07	15.6 ± 1.93	7 ± 0.70	0.8 ± 0.48
- REARING	1.0 ± 0.63	1.8 ± 1.11	5.4 ± 1.16	4.8 ± 0.73	3.8 ± 0.86	8.2 ± 1.85	1 ± 0.99	0
- GROOMING	8.8 ± 0.96	11.0 ± 0.94	10.4 ± 1.32	14.8 ± 1.93	22.8 ± 3.81	25.2 ± 3.24	8.2 ± 0.86	5.8 ± 0.73
- STEREOTYPED MVT.	2.0 ± 0.31	2.8 ± 1.51	2.8 ± 0.58	4.6 ± 0.81	5.2 ± 1.11	6.8 ± 1.24	5.8 ± 0.9	76 ± 0.59
- AGGRESSION	0	0	0	0	0	0	0	0
- LOCOMOTOR ACTIVITY	11.8 ± 1.01	13.8 ± 3.07	29.4 ± 2.52	18.6 ± 2.35	21.6 ± 2.42	22.4 ± 1.16	13.6 ± 1.0	15.8 ± 1.31

TABLE 3.2

The Difference in the Test and Control Mean Values of Activities in Various Behavioural Parameters

Dose (mg/kg)	Climbing	Scratching	Sniffing	Rearing	Grooming	Stereotyped mvt.	Locomotor Activity
5	1.4	1.8	2.2	0.2	2.2	0.8	2.0
10	0.8	8.0	4.4	4.4	1.6	0.8	17.6
20	0.2	8.2	5.8	3.8	6.0	2.6	6.8
40	0.2	16.6	8.8	2.8	14.0	3.2	3.8
80	2.8	32.0	15.6	7.2	16.4	4.8	10.6
160	-5.8	-16.0	1.4	0	-0.6	3.8	1.8
320	-9.0	-18.0	- 1.4	-1.0	-3.0	5.6	4.0

Values are Test mean - Control mean.
Negative values indicate decrease in activity compared to control.

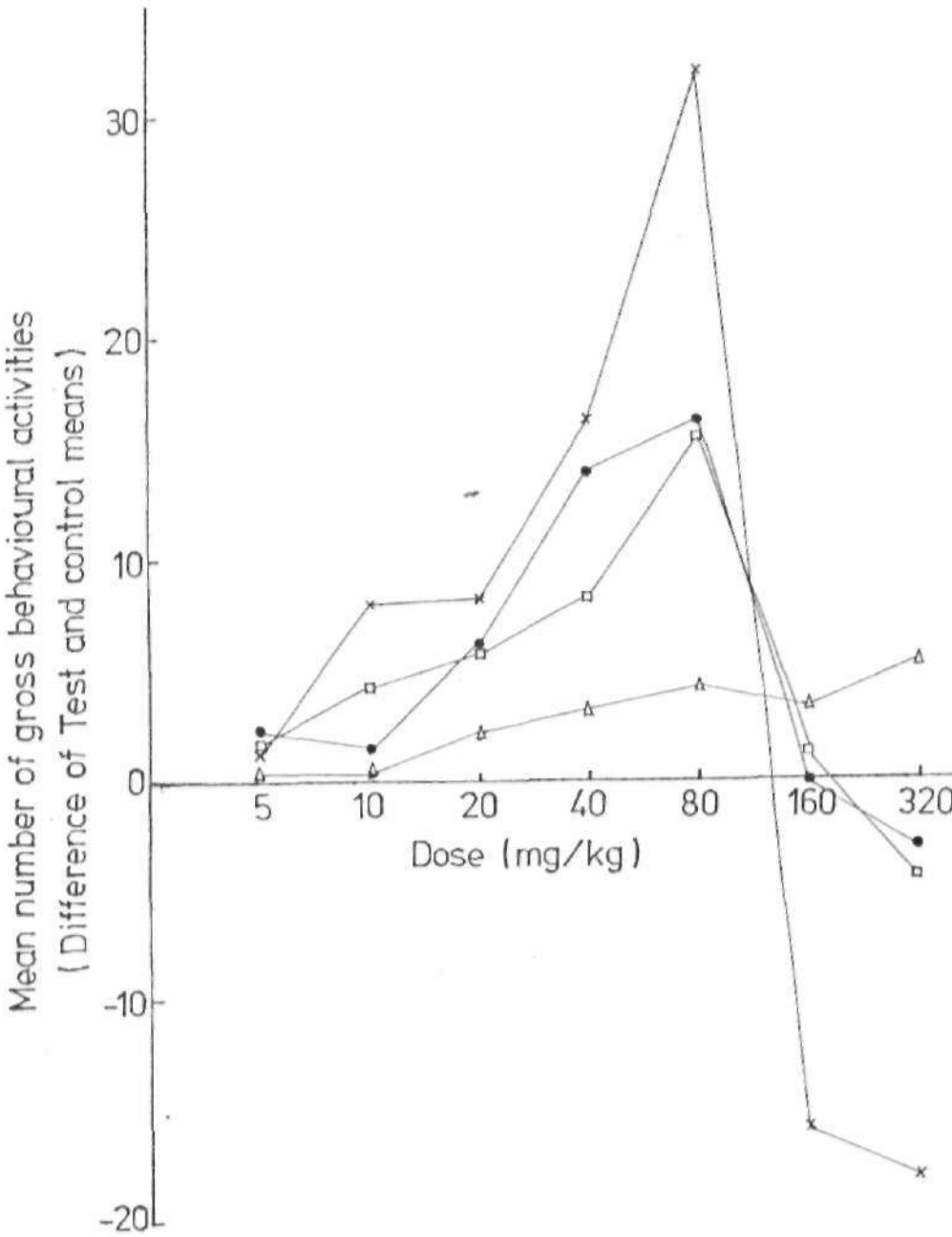


Fig. 3.0.1

Difference of Test and Control means were computed for Stereotyped movement (Δ——Δ), Scratching (X——X), Grooming (●——●) and Sniffing (□——□) activities at various ascorbic acid doses. Negative values are indicative of behavioural sedation.

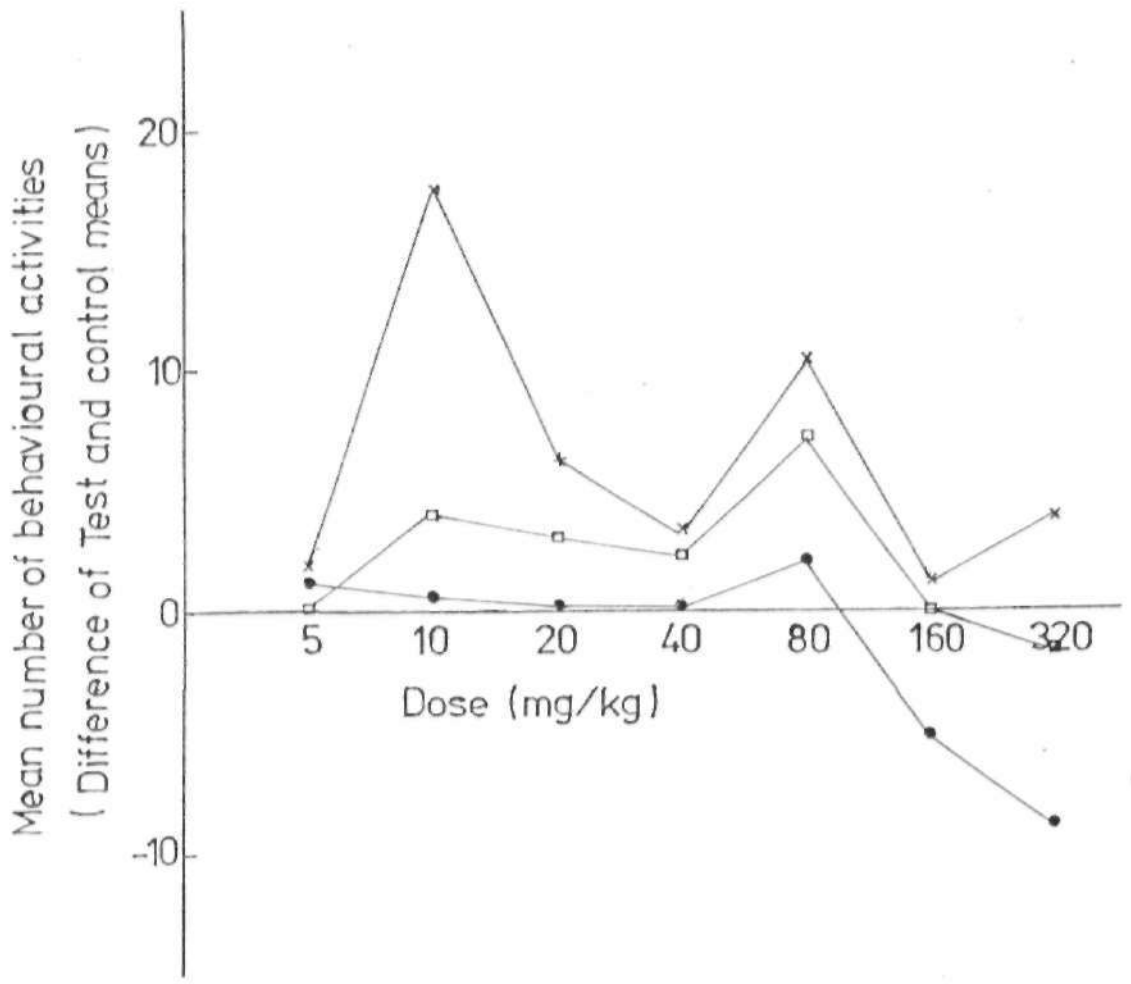


Fig. 3.0.2

Difference of Test and Control means were computed for Locomotor (X——X), Climbing (●——●) and Rearing (□——□) activities at avarious ascorbic acid doses. Negative values are indicative of behavioural sedation.

3.5 DISCUSSION

The present data support the suggestion that ascorbic acid probably has some clearly definable roles in the central nervous system and hence behaviour. In this study, ascorbic acid produced significant behavioural changes dose-dependently, with peak excitatory effects at 80 mg/kg (see Fig. 3.0.1). Low doses (5-80 mg/kg) had behavioural arousal effects, while in high doses (160-320 mg/kg) there was significant sedative effects in behavioural activity. A similar observation had been made in young chicks where low ascorbic acid doses were reported to induce excitatory effects in the chicks, while high doses sedated them (Erube, 1982). However, Wambebe and Sokomba (1986) reported observing only excitatory effects in rats with ascorbic acid (50 - 200 mg/kg i/p).

The present observations essentially disagree with the report of Wambebe and Sokomba (1986). The discrepancy may have been due to species variation in the animals used. On the other hand, it is possible that the discrepancy was due to

differences in the dose level of ascorbic acid used by the different workers; in this study, doses as much as 320mg/kg were used and at which the sedative effects on gross behaviour were generally observed

The behavioural regulatory roles of the central amines have been extensively reported (Blaschka, 1957; Everett and Wiegand, 1962; Randrup and Jonas, 1967; Van Rossum, 1968; Godwin et al, 1970). Present results indicate that stereotyped movements increased markedly when the doses were high, eg. at 320 mg/kg (Table 3.2, Fig. 3.0.1) Meanwhile at this level of dose also, there seemed to be depression in most of other behavioural activities (Figs. 3.0.1 & 3.0.2). It is now generally accepted that dopamine mediates stereotyped behaviour (Randrup and Jonas, 1967; Mignot and Savini, 1981). Furthermore, it has been noted that stimulation of central dopamine receptors increase locomotor activity (Van Rossum and Hurkmans, 1964). In the present results, locomotion was the only activity slightly present at 320 mg/kg dose (Fig. 3.0.2). In the light of

the present data, it seems suggestive therefore that the central actions of ascorbic acid might be linked with dopaminergic mechanism. These results also raise the question that mode of action could either be pharmacological or physiological, possibly, as a function of the amount of ascorbic acid available at site in the brain. Earlier workers have also noted that dopaminergic mechanism may contribute indirectly or directly to the observed behavioural effects of ascorbic acid (Wambebe and Sokomba, 1986).

Dihydroxyphenylalanine (DOPA) and Dopamine (intermediate precursors of norepinephrine biosynthesis) are both believed to mediate the arousal functions in the central nervous system (Everett and Wiegand, 1962; Godwin et al., 1970), while norepinephrine is thought to mediate sedation (Leimdofer, 1948; Feldberg and Sherwood, 1954). Also, it has been noted that serotonin regulates locomotor and consumatory behaviour (Salles and Salles, 1979). Considering the present results, it is possible that the noradrenergic neural mechanism may readily explain

the observed dual behavioural effects of ascorbic acid in this study. Therefore it is possible that in the noradrenergic neural system, Dopa and Dopamine were utilized by ascorbic acid in inducing the excitatory effects, while the observed sedation was produced via norepinephrine mediation. A group of workers have noted that low doses of ascorbic acid enhanced release of norepinephrine from synaptic vesicles in rat brain (Kuo et al., 1979), and the involvement of ascorbic acid in the metabolism of norepinephrine at the noradrenergic nerve terminals was earlier reported (Subramanian, 1977). Thus, it is possible that ascorbic acid may interfere physiologically or pharmacologically at the noradrenergic neural processes to produce the observed behaviourally opposing effects - excitation and sedation.

CHAPTER FOUR

4.0 THE EFFECT OF CHRONIC ADMINISTRATION OF ASCORBIC ACID ON GROSS BEHAVIOUR

4.1 SUMMARY

4.1.1 Adult male rats were used to study behavioural patterns in chronic administration of ascorbic acid.

4.1.2 Two doses (10 and 80 mg/kg i/p) of ascorbic acid were each, separately administered to different sets of animals for 30 days.

4.1.3 Behavioural excitatory effects was observed in the lower ascorbic acid dose (10 mg/kg i/p), and this was consistently present throughout the period of the chronic administration.

4.1.4 Sedative effects was observed in the 80 mg/kg dose treated rats after an initial behavioural excitation; the observed depression in gross behavioural activities featured consistently throughout the chronic administration.

4.1.5 Following the withdrawal of the chronic administration, depression in gross behaviour was noticed in the 10 mg/kg treated rats.

4.1.6 Aggressive behaviour was not seen in any of the chronic administration experiments.

4.1.7 There was no appreciable change in body weight in the rats both during and after the chronic treatments with ascorbic acid.

4.1.8 It was concluded that ascorbic acid is probably, a centrally active substance.

4.1.9 It was assumed that the noradrenergic neural mechanism may be the genesis of the physiologic modulation of behaviour of ascorbic acid.

4.2 INTRODUCTION

Tolbert, Thomas, Lawrence, Middaugh and Zemp (1979) studied the possible effects of ascorbic acid on the central dopaminergic systems in the

mice, and observed that pharmacological doses of ascorbic acid (i/p) produced significant elevation of brain ascorbate concentration which blocked the amphetamine - induced increase in stereotyped behaviour. From this report, it can be deduced that there was a kind of mutual interference between ascorbic acid and amphetamine at sites in the behavioural systems in the brain, hence the blockade. The other important import of this report is that which by implication, associated or specified that dopaminergic systems may be linked with the mechanism of action of ascorbic acid in the central nervous system.

A lot of work have been expended on the brain momoamines in the quest to ascertain the nature or the physiology underlying their interactions with centrally active agents or drugs, but very scanty progress has been made in this direction. A concept in biochemical basis of behaviour is that drugs modify behaviour by altering levels of neural activity at specific sites in the brain. This concept could also be taken to imply the

modifying of levels of neural transmission processes at specific sites. A number of publications (Steinberg, 1964; Chen, 1964; Jacobsen, 1964) highlighted the problems in the study of psychoactive agents, one of which, they observed, was the inability to correlate brain levels of a substance with its behavioural effects (ie. effects on behavioural systems in the brain). The present project was designed to study the pattern of behaviour in adult rats in chronic administration of two separate doses of ascorbic acid - 10 mg/kg and 80 mg/kg i/p, respectively. As was noted from the work of Tolbert et al. (1979), expectation from this study is that different brain levels of ascorbic acid would be produced in the course of administering the different doses; in other words, the higher dose (80 mg/kg), is expected to produce higher brain level of ascorbic acid during the chronic administration.

The objective of this study therefore, was to measure gross behavioural activities in the rats

both during and after chronic treatment with ascorbic acid. Such data will be used for comparative analysis with the acute effects.

4.3 MATERIALS AND METHOD

Refer to Section 2.10.2.

4.4 RESULTS

4.4.1 Gross Behavioural pattern during chronic administration of ascorbic acid.

4.4.1.1 The 80 mg/kg dose treated.

Hyperactivity, denoting behavioural excitation was noticed at the initial administration, the first day ($P<.05$); Table 4.4.1).

From the 3rd day, animals were sedated ($P<.05$); these were noted as marked decrease in gross behavioural activities, denoting depression in gross activities (Table 4.4.1; Fig. 4.0.1).

Depression in gross activities persistently

featured in the 80 mg/kg treated rats throughout the chronic administration (Fig. 4.0.1). This was significant, particularly on the 9th (P<.01), 12th (P<.01), 21st (P<.02), 24th (P <.02) and 30th (P <.05) days, respectively (Table 4.4.1).

4.4.1.2 The 10 mg/kg dose treated rats

No significant behavioural changes were noticed in the first three days of the chronic administration.

Hyperactivity was noticed from the 6th day of the administration and as compared to the control, the observed behavioural excitation was significant on the 15th (P <.05), 18th (P <.01), 24th (P <.01) and 30th (P <.01) days, respectively (Table 4.4.1).

4.4.2 Pattern of gross behaviour after withdrawal of chronic administration of ascorbic acid.

The animals treated with the 10 mg/kg dose, were behaviourally depressed following the withdrawal of administration; significant decrease

(P <.01) in gross activities were observed on the third post-administration day (Table 4.4.2). The observed depression in gross behavioural activities consistently featured in the 10 mg/kg dose treated rats throughout the post-administration period, as was significant on the 9th (P <.01) and 12th (P <.02) days, respectively (Table 4.4.2; Fig. 4.0.2).

Depression in behavioural activities persisted in the 80 mg/kg treated animals up to the third day after the withdrawal of administration (P <.001); Table 4.4.2, Fig. 4.0.2).

Aggressive behaviour was not seen after the withdrawal of chronic administration.

4.4.3 The pattern of body weight during and after the chronic administration of ascorbic acid.

There was no definite or appreciable change in body weight of the rats during

and after the chronic administration of ascorbic acid in both the 10 and 80 mg/kg treated groups (Table 4.4.1.2).

TABLE 4.4.1:

Gross behavioural activities were measured in chronic administration of ascorbic acid in rats (i/p). Period of administration: 30 days in each group

	Days 1			3			6			9			12		
DOSE (mg/kg)	Cont.	10	80	Cont.	10	80	Cont.	10	80	Cont	10	80	Cont.	10	80
GROSS 1st Group	141	124	199	153	135	86	177	260	154	122	159	61	93	163	20
ACTI- 2nd Group	98	162	206	99	109	64	156	172	97	164	177	42	106	92	49
VITIES 3rd Group	151	111	260	121	89	68	161	216	116	142	191	54	87	151	37
MEAN TOTAL GROSS ACTIVITIES	130	132	221*	124.3	111	72*	164.6	216	122.3	142.6	175.6	52.3***	95.3	135.3	35.3***
±SEM	16.2	15.3	19.2	15.6	13.3	6.7	6.3	25.4	16.7	12.1	9.2	5.5	5.6	21.9	8.4

Number of activities. Each figure is the count per 60min. each day.
*P <0.05, ***P <0.01 compared to physiological saline control, Student's t - test.

TABLE 4.4.1 Cont'd.

Days 15 - 30

	15			18			21			24			27			30		
DOSE (mg/kg)	Cont.	10	80	Cont.	10	80	Cont.	10	80	Cont	10	80	Cont.	10	80	Cont.	10	80
GROSS 1st Group	73	134	65	89	187	63	123	118	66	85	195	19	59	123	27	101	139	61
ACTI- 2nd Group	56	101	48	58	172	39	94	167	24	61	162	44	68	66	60	70	174	37
VITIES 3rd Group	58	93	53	68	156	51	105	159	39	78	149	32	87	79	39	83	159	48
MEAN TOTAL GROSS		*			***				**		***	**					***	*
ACTIVITIES	62.3	109.3	55.3	71.6	171.6	51	107.3	148	43	74.6	168.6	31.6	71.3	89.3	42	84.6	157.3	48.6
±SEM	5.3	2.97	5.05	9.1	8.9	6.9	8.4	15.1	12.3	7.1	13.7	7.2	8.2	17.2	9.6	8.9	10.1	6.9

Number of activities. Each figure is the count per 60min. each day.
*P <0.05, **P <0.02, ***P< 0.01 compared to physiological saline control, Student's t - test.

TABLE 4.4.2

The pattern of gross behavioural activities after withdrawal of chronic administration of ascorbic acid

Post administration days:-

	Days 3			6			9			12			15		
DOSE (mg/kg)	Cont.	10	80	Cont.	10	80	Cont.	10	80	Cont	10	80	Cont.	10	80
GROSS 1st Group	157	49	45	54	33	95	64	16	148	133	95	67	121	103	73
ACTI- 2nd Group	122	68	61	78	80	46	96	32	96	161	64	102	156	142	99
VITIES 3RD Group	135	50	70	63	54	69	78	22	110	155	111	79	129	114	76
MEAN TOTAL GROSS		***	***					***			**				**
ACTIVITIES	138	55.6	58.6	65	55.6	70	79.3	23.3	118	149.6	90	82.6	135.3	119	82.6
±SEM	10.2	6.1	7.3	7.0	13.6	14.1	9.2	4.6	15.5	8.5	13.8	10.2	10.6	11.6	8.2

Number of activities. Each figure is the count per 60min. each day.
**P < 0.02, ***P < 0.01 compared to physiological saline control, Student's t - test.

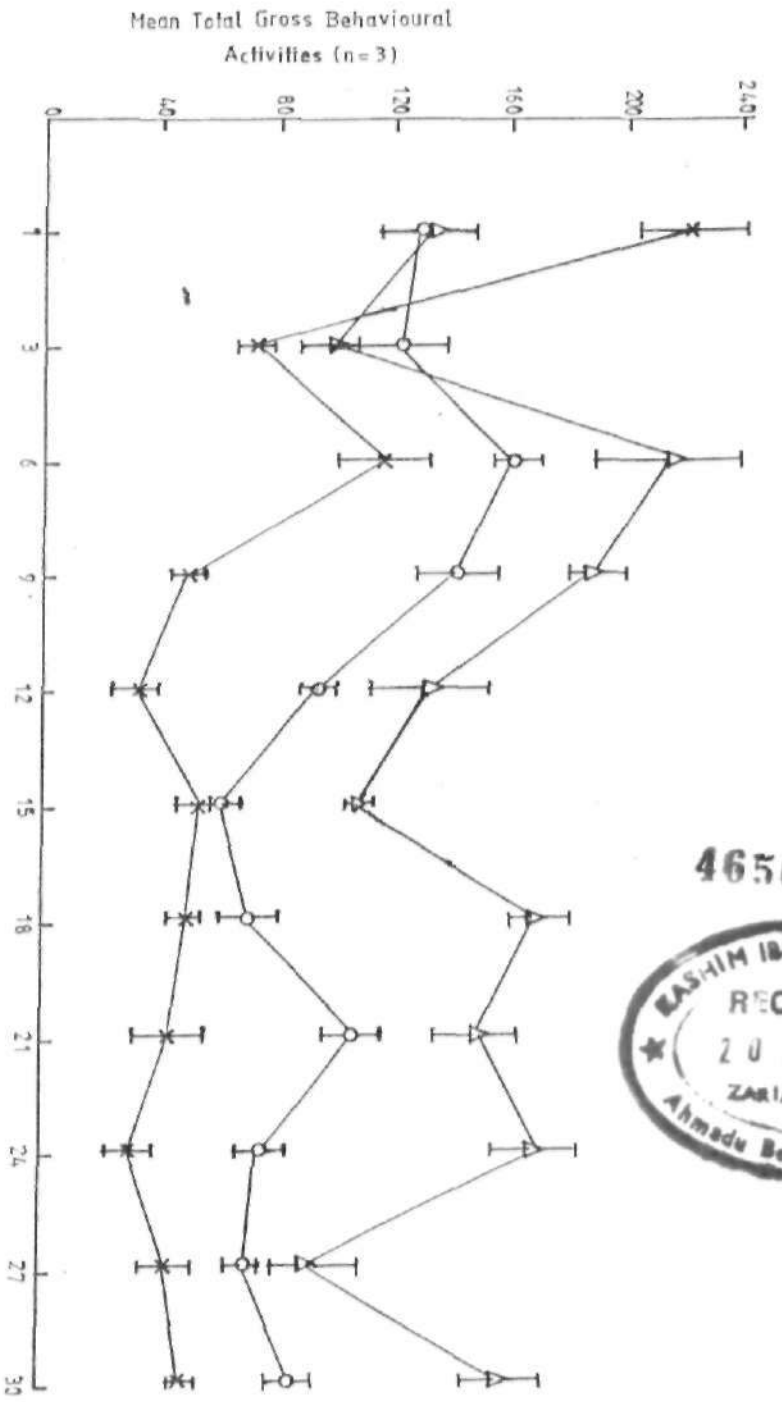
TABLE 4.4.1.2

Body weight in the rats were determined every 3 days during and after the chronic administration of ascorbic acid (i/p).

WT. (gm)

During Administration (30 days)	80mg/kg TREATED		10mg/kg TREATED		CONTROL	
Day	Paird	Group	Paird	Group	Paird	Group
1	237	236	205	198	206	235
3	237	238	204	198	204	234
6	237	238	206	200	207	240
9	235	228	206	204	209	240
12	233	238	204	207	203	241
15	234	237	207	207	207	240
18	232	237	208	204	207	239
21	232	240	203	210	214	239
24	234	240	204	205	210	242
27	233	239	204	203	210	244
30	235	236	200	207	206	238
POST ADMINISTRATION (15 days)						
3	234	239	202	210	207	240
6	236	238	206	208	210	239
9	240	240	210	210	208	239
12	242	240	210	205	210	242
15	239	238	206	206	209	240

Averaged values, n = 3



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Table 4.C.1

Effect of gross behavioural activities during chronic administration of ascorbic acid in rats. Duration: 30 days. Vertical bars represent standard deviations. (▲——▲) = Group on 10mg/kg dose; (x——x) = Group on 80mg/kg dose; (o——o) = Control.

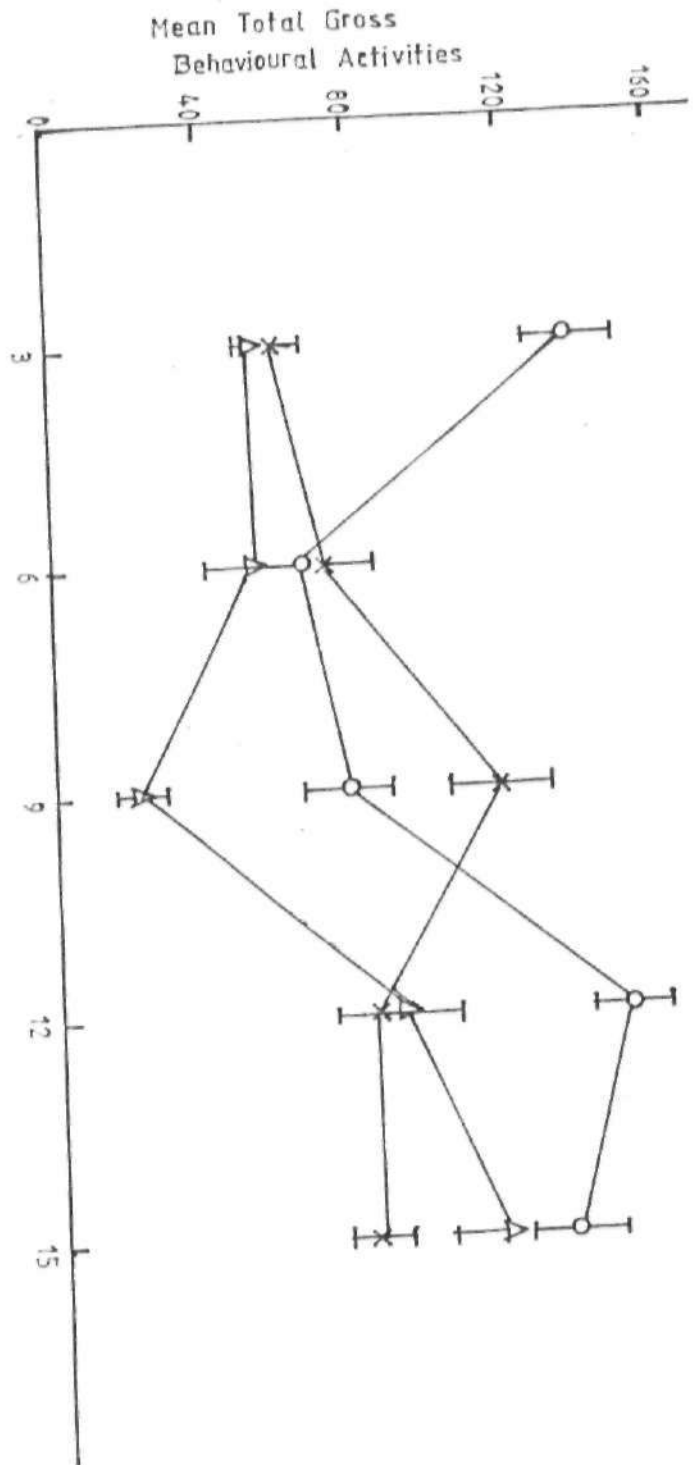


Fig. 4.0.2

Pattern of gross behavioural activities after withdrawal of chronic administration of ascorbic acid in rats. Vertical bars represent standard deviations. (Δ—Δ) = 10mg/kg treated group; (▽—▽) = 80mg/kg treated group; (O—O) = Control.

4.5 DISCUSSION

It is obvious from the present data, that marked behavioural changes were present in the course of chronic administration of ascorbic acid. Depression in gross behavioural activities was observed in the 80 mg/kg treated animals and this has similarly been noticed during acute test administration of large doses of ascorbic acid (Chapter 3, Section 3.4.1). Tolbert, Thomas, Lawrence, Middaugh and Zemp (1979) reported that acute administration of large doses of ascorbic acid (i/p) produced significant increase in brain ascorbate concentrations. Considering the biochemical basis of behaviour, it is believed that the level of neural activity at any site in the CNS, is directly or indirectly related to the concentration of the chemical agent within the locality, thus, to explain drug-induced changes in behaviour. From the present study, it is likely that at the 80 mg/kg dose, higher brain level or concentration of ascorbic acid was produced in the course of the chronic administration, than at the

10 mg/kg dose. It could be that as a result of the level of ascorbic acid in the brain, physiological or pharmacological processes may be induced that result into either activation of the CNS or sedation; thus, the production of a pattern of behavioural output.

In contrast to sedation observed with the 80 mg/kg treated group, behavioural excitatory effects was seen in the 10 mg/kg treated rats. It is possible that excitation on the other one hand, or sedation, were separate behavioural effects or responses elicited in the behavioural systems as a pattern of behavioural output resulting from the brain level or the concentration of ascorbic acid.

Norepinephrine is thought to mediate sedation in the brain (Leimdofer, 1948; Feldberg and Sherwood, 1954), while Dopa and Dopamine (intermediate precursors of norepinephrine biosynthesis) are believed to mediate excitation (Everett and Wiegand, 1962; Godwin et al;, 1970). It was also reported that ascorbic acid may be involved in the metabolism of norepinephrine at

the noradrenergic terminals (Subramanian, 1977). In the light of these and similar reports, and since the noradrenergic systems were also implicated in the possible mechanism of action of ascorbic acid (Sjostrand, 1970; Kuo et al., 1979), the possibility that ascorbic acid may interfere physiologically or pharmacologically with the noradrenergic neural processes to produce the dual behavioural effects - excitation and sedation - is hereby suggested in this study, and this is a likely tenable hypothesis.

Serotonergic system is postulated to regulate locomotor activity (Salles and Salles, 1979). Michael, Trulson, Barry and Jacobs (1979) reported a fall in brain serotonin and 5-hydroxy-indoleacetic acid levels during chronic administration of amphetamine. This implied that chronic amphetamine treatment decreased central serotonergic transmission. Blumgberg and Co-workers (1976) noted that the limbic noradrenaline stimulated cyclic AMP was altered 1-2 days after the withdrawal of chronic amphetamine treatment in

rats. Kebabian, Petzhold and Greengard (1972) had previously reported an increase in striatal dopamine stimulated adenylate cyclase 1-2 days following withdrawal of chronic amphetamine treatment in rats. All these reports seem to indicate the possible roles or contribution of serotonergic system, the limbic noradrenergic or the striatal dopaminergic mechanism in the central effects of amphetamine. From the results of this study, the animals were behaviourally depressed 1-2 days following the withdrawal of chronic administration of ascorbic acid in the 10 mg/kg treated group (Table 4.4.2). Depression was reported in rats following withdrawal from chronic treatment of both amphetamine and bupropion to which equal weights of ascorbic acid had been added (Hartley, Miller and Webster, 1981). Other workers also found depression in both rats and man following withdrawal from chronic treatment with amphetamine (Schildkraut, Watson, Draskoczy and Hartmann, 1971; Tonge, 1974).

The observations in this study suggest close

similarity with the reports of the amphetamine-treated rats by previous workers. Amphetamine has been noted to exert its actions mainly on the noradrenergic and dopaminergic systems in the brain to induce its effects (Trulson et al., 1979). There is however no scientific evidence to support that ascorbic acid and amphetamine would have exactly identical actions in the central nervous system. Nonetheless on the basis of the present study, it is suggestive that the brain monoamines - Serotonin, Noradrenaline and Dopamine - are possibly linked to the central mechanism of action of ascorbic acid, if it is presumed that ascorbic acid and amphetamine have identical actions in the CNS.

In this project, adult animals were mainly used. From Table 4.4.1.2, it is clear that there was no notable or definite body weight change both during and after the chronic treatments with ascorbic acid. This observation appears to lend further support to the speculation that ascorbic acid might be principally, a centrally active substance with few known peripheral effects.

5.0 EFFECT OF ASCORBIC ACID ON PENTOBARBITONE-INDUCED SLEEP IN RATS.

5.1 SUMMARY

5.1.1 Adult rats were used to study the effect of ascorbic acid on pentobarbitone-induced sleep, and to investigate the pattern of the induced sleep in different pretreatment times with ascorbic acid (i/p).

5.1.2 Pentobarbitone-induced sleep was antagonised by ascorbic acid in a dose-dependent manner.

5.1.3 The duration of the sleep tended to be shortened with increasing dose of ascorbic acid.

5.1.4 The dose of ascorbic acid had no significant influence on the onset of pentobarbitone-induced sleep.

5.1.5 It is possible that ascorbic acid exercises opposing actions on sleep mechanism in the central nervous system.

5.1.6 20 - 25 minutes may be the time it takes for the parenterally administered ascorbic acid to reach the sites in the brain.

5.1.7 Putative neurotransmitters postulated to regulate sleep in the brain, are possibly involved in the central mechanism of action of ascorbic acid.

5.2 INTRODUCTION

The subject of the arousal mechanisms of the central nervous system is implicit in the physiology of sleep. Sleep is a behavioural state. The phenomenon may be said to be the product of activities in the neural systems located in the phylogenetically old reticular core of the brain. This explanation, however, may also mean an over-simplification of the subject of sleep.

It has been generally agreed that the neural systems principally concerned with sleep and waking states, are in the brain stem. Thus, while

Norepinephrine (Ganong, 1987; Begley 1989). Dopamine has also been implicated in sleep mechanism. Holtz et al. (1957) reported that L - dopa, a precursor of dopamine, abolished hexobarbitone - induced sleep in mice. Similarly, Osuide and Wambebe (1980) have reported that increased central dopamine receptor activity may be involved in both behavioural alertness and waking process from pentobarbitone sleep in chicks.

Pentobarbitone is a short acting barbiturate, and was used in this study. Barbiturates are widely used in anaesthesia especially in animals (Tavernor, 1976; Harvey, 1985). In spite of several studies the mechanism by which drugs produce general anaesthesia remains a mystery. What is known presently, is that many of the neurons in the brain are hyperpolarized during anaesthesia, and this raises speculation that general anaesthetics may produce sleep by depressing conduction in the reticular activating system. It is presently believed that the

pontomesencephalic reticular formation to the thalamus, subserve the arousal functions of the ascending reticular system.

Alcohol is known to be a depressant of the CNS. The effects of alcohol on the CNS are proportional to its concentration in the blood. The mechanism by which alcohol produces sleep has been a subject that attracted much attention in the past. It is now known that in producing sleep, alcohol (as well as the barbiturates) exerts its depressant effects on the CNS by dissolving in lipid membranes, thereby perturbing the function of ion channels and other proteins embedded therein. In this same mechanism for sleep production, attention was also drawn to the capacity of alcohol (and the anesthetic barbiturates) to augment GABA-mediated synaptic inhibition as well as fluxes of chloride. The objectives of the present study are two folds - First, the project was designed to investigate the effect of ascorbic acid on sleep in normal and healthy adult rats using a short acting barbiturate, with the aim of establishing

the neurotransmitter system or systems that may be involved in the central mechanism of action of ascorbic acid and secondly, to study the pattern of pentobarbitone - induced sleep in different pretreatment times with a fixed intraperitoneal dose of ascorbic acid with the aim of using the results to ascertain the time it may take for parenterally administered ascorbic acid to reach the sites in the brain for any observed effects.

5.3 MATERIALS AND METHODS

Refer to Section 2.9.1

5.4 RESULTS

5.4.1 The pattern of pentobarbitone-induced sleep in different doses of ascorbic acid (i/p).

Ascorbic acid dose-dependently modified pentobarbitone-induced sleep in rats; the duration of the induced sleep was significantly decreased as ascorbic acid dose increased from 40 to 300 mg/kg (Table 5.5.1). The decrease in the duration of the induced sleep was most significant at 80 mg/kg ($P < .001$; Table 5.5.1; Fig. 5.5.1.1).

Similarly, the time lapse between administration and the onset of sleep in the ascorbic acid-pretreated pentobarbitone-induced sleep was significantly delayed at 80 mg/kg ascorbic acid dose ($P < .05$, Table 5.5.1).

5.4.2 The pattern of pentobarbitone-induced sleep in different pretreatment times with a fixed intraperitoneal dose of ascorbic acid (80 mg/kg).

The duration of the sleep in the ascorbic acid - pretreated pentobarbitone-induced sleep was significantly reduced when not more than 25 minutes was the time of pretreatment of ascorbic acid (Table 5.5.2; Fig. 5.5.0.1); the peak effect was seen in the pretreatment times of 20 and 25 minutes prior to pentobarbitone administration ($P < 01$; Table 5.5.2).

The onset of the pentobarbitone - induced sleep was significantly delayed ($P < .05$) when 25 minutes was the pretreatment time with ascorbic acid (Table 5.5.2). On the other hand, the onset

of the induced sleep appeared to be mildly delayed when the pretreatment times with ascorbic acid was more than 30 minutes (Table 5.5.2).

The duration of the induced-sleep was not appreciably reduced when the pretreatment time with ascorbic acid prior to pentobarbitone administration was more than 30 minutes (Table 5.5.2); the test animals tended to sleep nearly as long as those of the control group (Fig. 5.5.0.1).

TABLE 5.5.1: Effect of Ascorbic acid on pentobarbitone - induced sleep in rats

DRUGS (Dose: mg/kg i/p)		SLEEP PATTERN (min)			
		Onset of Sleep		Duration of Sleep	
Ascorbic Acid	Pentobarbitone	Mean	± SEM	Mean	± SEM
CONTROL	20	5.24	0.37	96.05	6.99
5	20	3.20	0.25	96.82	9.17
10	20	6.23	2.09	85.82	7.18
20	20	5.79	1.56	88.47	6.00
40	20	6.02	0.71	83.86	9.47
80	20	7.04*	0.60	41.50****	2.00
200	20	3.52	0.52	54.12***	5.00
300	20	3.22	0.31	56.00***	8.92

n = 5 for each experiment

Pretreatment time with ascorbic acid in each experiment was 25 min.
*P < 0.05, ***P < 0.01, ****P < 0.001
*, *** and **** are significantly different from pentobarbitone control at the indicated P levels, Student's t - test.

TABLE 5.5.2: The pattern of sleep in different pretreatment times (min.) with Ascorbic acid on pentobarbitone-induced sleep in adult rats

DRUGS (Dose: mg/kg i/p)			SLEEP PATTERN (min)			
			Onset of Sleep		Duration of Sleep	
Ascorbic Acid	Pentobarbitone	PRETREATMENT TIME (min)	Mean	± SEM	Mean	± SEM
CONTROL	20	-	3.31	0.31	83.56	2.46
80	20	5	2.96	0.20	66.35*	5.57
80	20	10	3.85	0.41	67.17*	5.81
80	20	15	3.71	0.38	66.92*	5.52
80	20	20	6.30	2.16	58.03***	5.18
80	20	25	8.40*	2.24	56.32***	6.36
80	20	30	4.87	0.44	69.32*	5.01
80	20	35	5.53	0.43	72.26	5.94
80	20	40	3.64	0.15	70.39	5.83
80	20	45	4.64	0.63	73.31	4.31
80	20	50	6.51	2.05	75.08	3.94
80	20	55	4.08	0.59	71.04	5.44
80	20	60	5.10	1.08	78.70	3.23

n = 5 per experiment. *P < 0.05, ***P < 0.01
* and *** are significantly different from pentobarbitone control at the indicated P levels, Student's t - test.

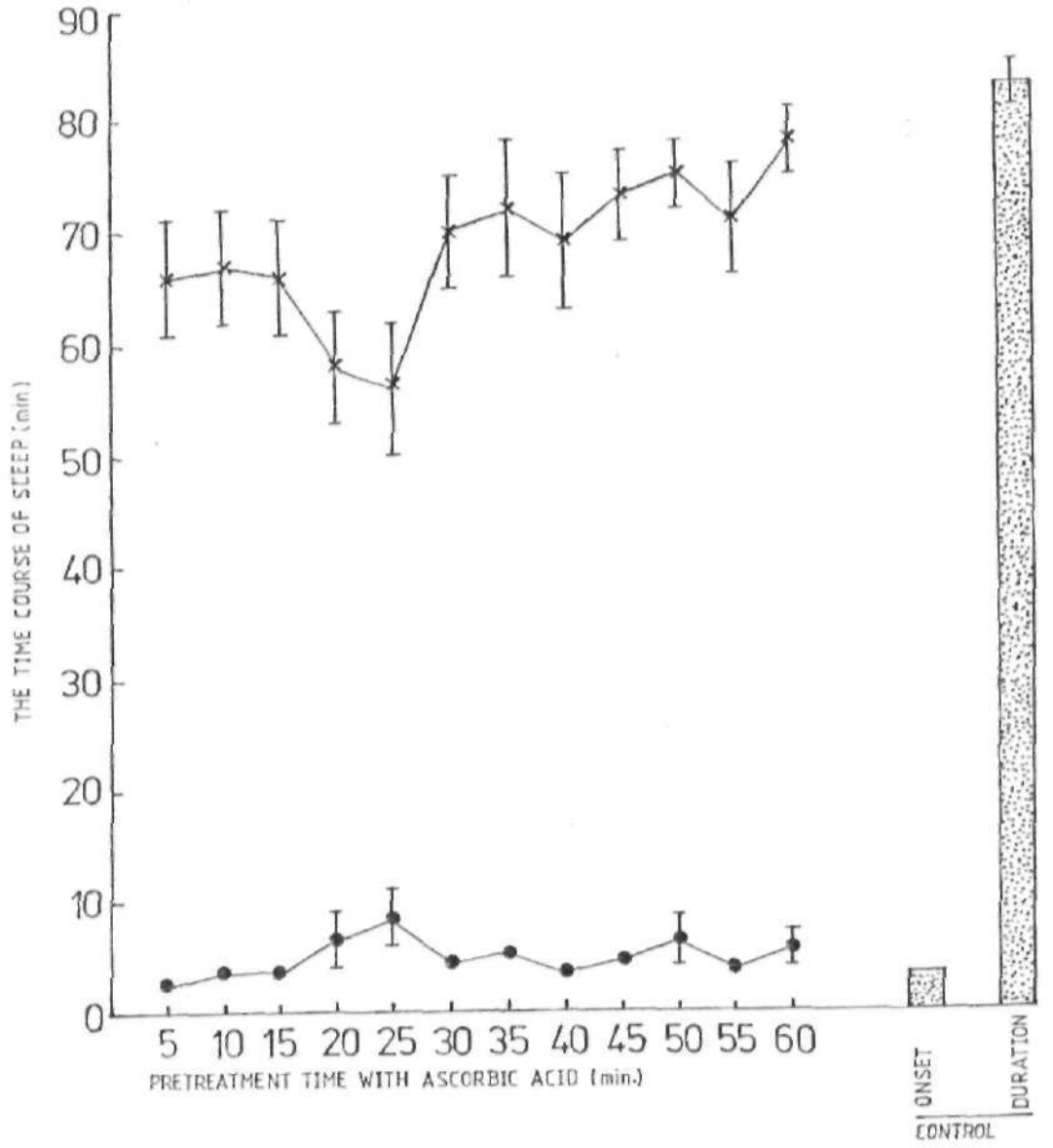


Fig. 5.5.0.1

The pattern of pentobarbitone-induced sleep in rats in different times (min.) of pretreatment with ascorbic acid. Vertical bars represent standard deviations.
●—●—● = Onset of sleep
X—X—X = Duration of sleep
Mean values, n = 5

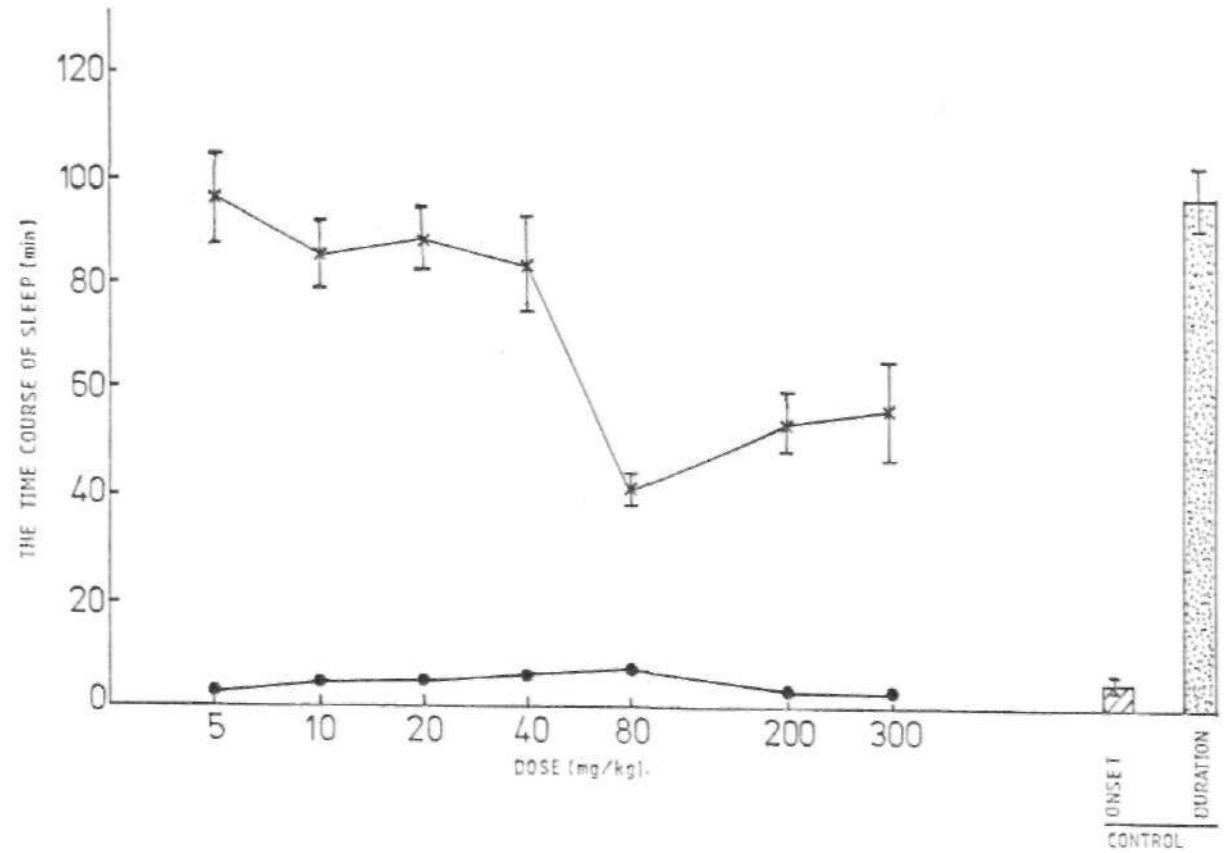


Fig. 5.5.1.1

The pattern of ascorbic acid-pretreated pentobarbitone-induced sleep in rats in different doses of ascorbic acid (i/p). Vertical bars represent standard deviations.

●—●—● = Onset of sleep
X—X—X = Duration of sleep
Mean values, n = 5

5.5 DISCUSSION

As shown in Table 5.5.1, doses of ascorbic acid of over 40 mg/kg significantly modified pentobarbitone - induced sleep. This was seen as shortening of the duration of the induced sleep. It is noted that the observed effect was most pronounced at 80 mg/kg dose where in addition, the onset of sleep was significantly delayed ($P < 0.05$, Table 5.5.1). These observations indicate that ascorbic acid may have modulating activity on the neural processes of the sleep-regulating systems in the CNS. The nature of the action could be described as antagonizing effects, hence the tendency to shorten the sleep duration and delay the onset (Table 5.5.1; Fig. 5.5.0.1). These results are therefore further indication that ascorbic acid is a centrally active substance with definite CNS activation properties.

Various substances, for example, the amino acids, L-glutamic and L - aspartic acids have been known to possess excitatory effects in mammalian CNS (Curtis, Phillis and Watkins, 1959; Johnson,

1972), although the mechanism by which these effects are achieved is not yet known. Some substances are believed to produce arousal of the brain by lowering the threshold of reticular neurones in the brain stem as was recently attributed to epinephrine and norepinephrine (Ganong, 1987). It is possible that such might be the nature of the mechanism employed by ascorbic acid to suppress sleep or in inducing CNS alerting effects. It is also possible that ascorbic acid establishes direct interacting links with putative neurotransmitter systems that traditionally regulate sleep in the brain. Presently, serotonin and noradrenaline are believed to mediate sleep production (Ganong, 1987). Acetylcholine was also reported to play a regulatory role in sleep (Begley, 1989) as well as endogenous brain dopamine which is also believed to be involved in the mechanism of sleep. Wambebe and Osuide (1983) suggested that endogenous dopamine may be involved in the waking process from sleep in chicks and rats. Similarly, Lidbrink (1974) showed that

behavioural waking in the rat was suppressed by destruction of the ascending nigroneostriatal dopamine system.

The hypothalamus, a core structure in the limbic system, is known to be deeply involved in the central regulatory mechanisms of sleep, and considerable evidence exist to show that the neural systems concerned with sleep and waking states are mostly in the brain stem. From the results of this study however, it may be concluded that the physiological mechanisms of sleep (or sedation) in the brain, involve neural processes that are probably sensitive to administered ascorbic acid. Thus, the possible involvement of putative sleep-regulating neurotransmitter systems in the central mechanism of action of ascorbic acid can not be ignored.

The observed effects on the induced sleep was more pronounced when 20 - 25 minutes was the pretreatment time with ascorbic acid prior to pentobarbitone administration (Table 5.5.2; See also Fig. 5.5.0.1). It is therefore suggested

that 20 - 25 minutes represent the average time it takes for the parenterally administered ascorbic acid to reach the sites in the brain for the observed effects.

CHAPTER SIX

6.0 ELECTROPHYSIOLOGICAL STUDIES - THE EFFECTS OF ASCORBIC ACID ON THE EEG AND EMG OF RAT.

6.1 SUMMARY

6.1.1 Adult rats were used to study the EEG and EMG effects of ascorbic acid.

6.1.2 EEG and EMG patterns were studied in the acutely, chronically and in intracerebroventricularly administered ascorbic acid.

6.1.3 Activation of the EEG and EMG was mainly observed in acute administration of ascorbic acid (80 and 300 mg/kg i/p).

6.1.4 During chronic administration of ascorbic acid (80 mg/kg i/p), EEG and EMG activation was noticed 1 - 3 days of the commencement of the administration; whereas from the 6th day, synchronization of the EEG was generally observed.

6.1.5 The EEG effect of intracerebroventricularly (icv) administered ascorbic acid was noticed as both desynchronization and synchronization of the various cortical regions, with mild activation of the EMG.

6.1.6 The EEG and EMG activation noticed in acute administration was regarded as consistent with the observed behavioural excitatory effects of ascorbic acid.

6.1.7 The EEG and EMG activation was also noted as consistent with the observed antagonizing effects on barbiturate sleep.

6.1.8 The desynchronization and synchronization of the EEG produced by ascorbic acid in the various cortical regions during the chronic and in the ICV administrations were noted as indicative of behavioural excitatory and sedative properties of ascorbic acid.

6.1.9 It is concluded that ascorbic acid probably has modulating activities in the neural systems that subserve arousal on the one hand, and in those that mediate sedation in the brain; specifically, it was assumed that the noradrenergic mechanism might be the site of action of ascorbic acid for the physiologic modulation of behaviour.

6.2 INTRODUCTION

The electroencephalogram (EEG) is a record of the electrical activity of the brain, while the electromyogram (EMG) is a record of the action potentials in skeletal muscles (ie. a record of the electrical changes in skeletal muscles). Activation of skeletal muscle is via its motor nerve, hence, the structural basis of electromyography is the motor unit. Some workers observed that there is little (if any) spontaneous activity in skeletal muscles. Electrical activity in the skeletal muscle is largely influenced from higher centers; hence, the EMG could be regarded as being indirectly a record of activity in the

higher centres.

Motor neurons, the final common pathway to skeletal muscles, are constantly being bombarded by impulses from higher centers and several pathways namely; medullary, pons, midbrain, subcortical including several segmental and intersegmental afferents. It is the algebraic summation of these influences (som excitatory, others inhibitory) that finally give rise to tension in skeletal muslces (ie. muscle tone), and this may be evaluated as EMG. Muscle tone depend ultimately on the pattern and rate of discharge along the spinal neurons and cranial equivalent. In general, factors influencing muscle tone include changes in the connective tissue coverings, changes in muscle proteins, changes in neuromuscular junction, changes in reflex arc to skeletal muscles. Other factors include segmental and intersegmental mechanisms, supra-segmental mechanisms, cortical projection systems (Indirect extrapyramidal tracts and Direct pyramidal tracts) and Psychic influences which is also another factor.

The EEG has been a tool for diagnosis of neurological conditions, the study of which has advanced the general knowledge of brain functions.

The EEG is employed to study brain changes related to functions and as such, has been of immense diagnostic value in monitoring or measuring drug-induced changes in brain function. It is the aim of the present study to examine the EEG and EMG in the rat following administration of ascorbic acid.

6.3 MATERIALS AND METHODS

Refer to Sections 2.5, 2.6, 2.7 and 2.8

6.4 RESULTS

6.4.1 The EEG and EMG effects of ascorbic acid in acute administration in the rat.

6.4.1.1 80 mg/kg dose (i/p)

There was no appreciable change in the EEG and EMG 10 minutes after administration (Fig. 6.0.1a). However, at about 25 minutes, there was

obvious decrease in the amplitudes of the EEG of the frontal cortex and optic cortex, while the EMG activity was slightly activated (Fig. 6.0.1b). When it was 60 minutes after administration, no observable changes in the EEG of the various cortical regions was present; the EEG became similar to those of control. However, the EMG still showed activation at this period (Fig. 6.0.1c).

6.4.1.2 300 mg/kg dose (i/p).

There was no appreciable change in the EEG and EMG of the various cortical regions 10 minutes after administration (Fig. 6.0.2a). At 25 minutes after administration, decrease in the amplitudes of the EEG of the frontal cortex and optic cortex was noticed, while the EMG activity was slightly activated (Fig. 6.0.2b). At 60 minutes after administration, the EEG of the frontal cortex and optic cortex still showed decrease in the amplitudes of the waves; meanwhile, the activation of the EMG activity was more prominent (Fig. 6.0.2c).

6.4.2 The EEG and EMG effects of chronic administration of ascorbic acid (80 mg/kg i/p) in the rat.

The EEG of the frontal cortex and optic cortex showed decrease in amplitudes 1 - 3 days of the commencement of the chronic administration, while the activity of the EMG was apparently activated (Fig. 6.0.3 B,C).

On the sixth day of the administration, the EEG of the frontal cortex and optic cortex became essentially similar to those of control, while the EMG remained mildly activated (Fig. 6.0.3 D).

From the 9th to the 12th day of the chronic administration, the EEG of the frontal cortex and optic cortex were apparently similar to those of control; meanwhile, the EMG activity remained mildly activated (Fig. 6.0.3 E,F). The EEG of the brain stem reticular formation featured with undeterminable pattern throughout the chronic administration (Fig. 6.0.3 A-F).

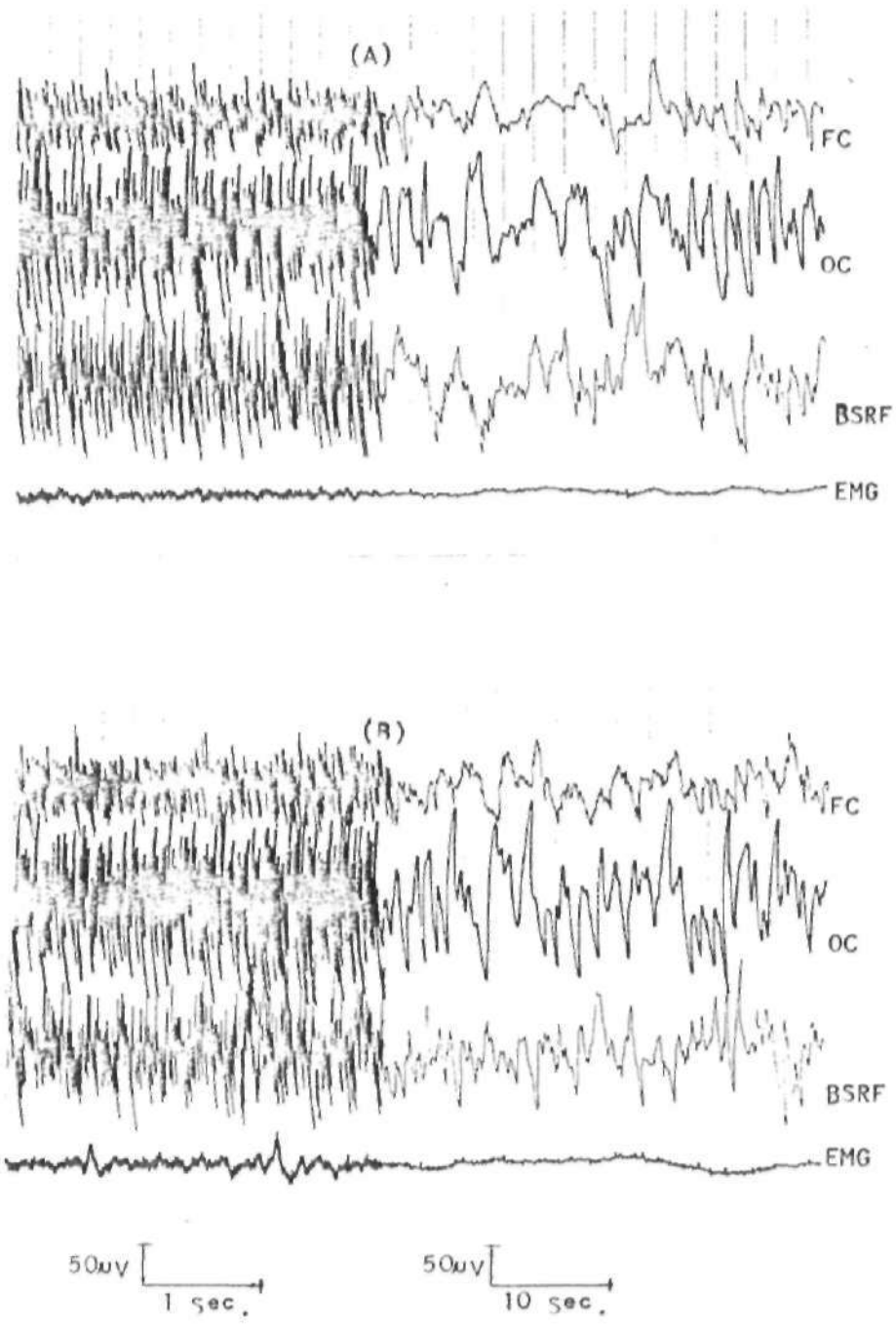


Fig. 6.0.1(a)

Effect of ascorbic acid (80mg/kg i/p) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (B) = 10 min. after injection.

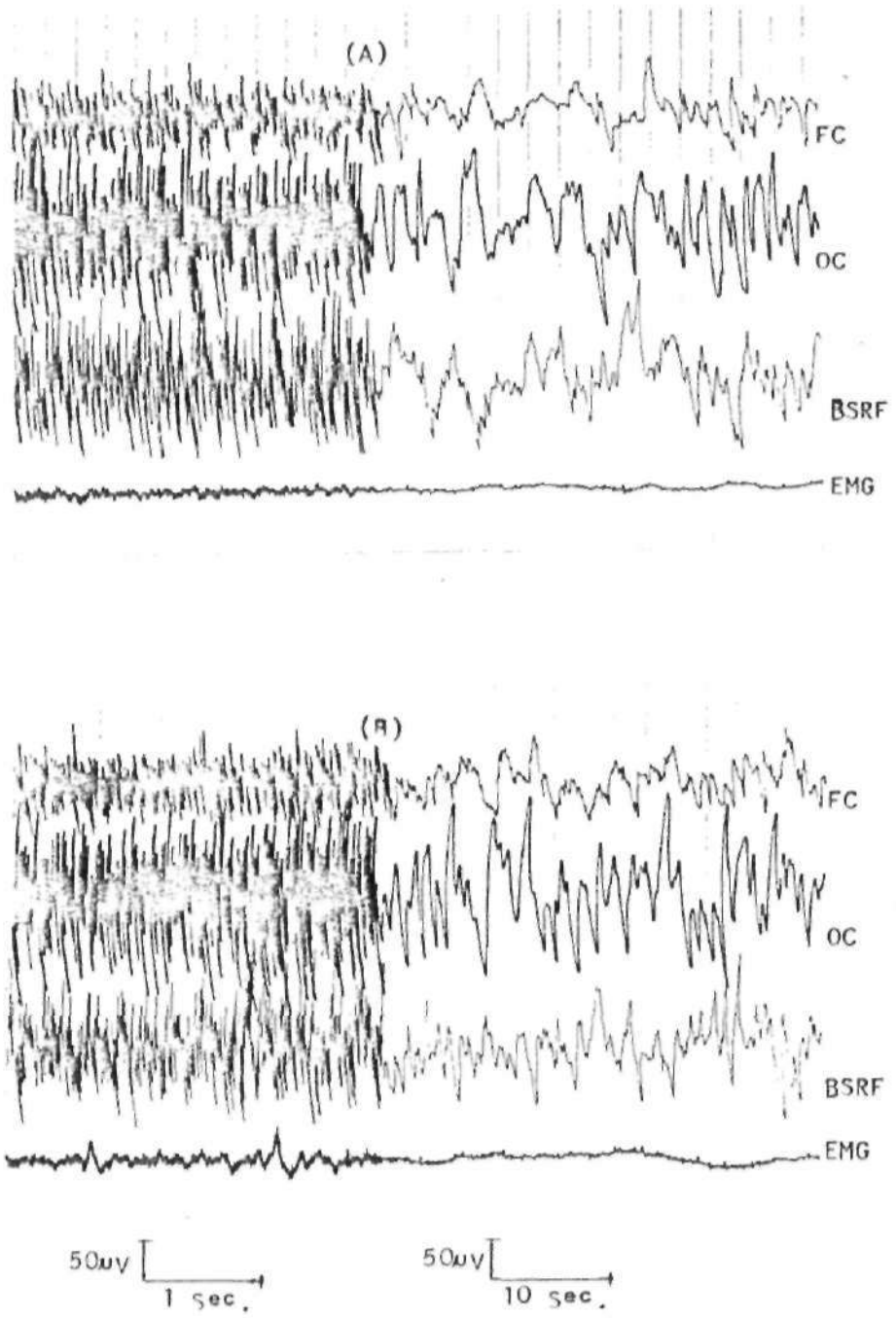


Fig. 6.0.1(a)

Effect of ascorbic acid (80mg/kg i/p) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (B) = 10 min. after injection.

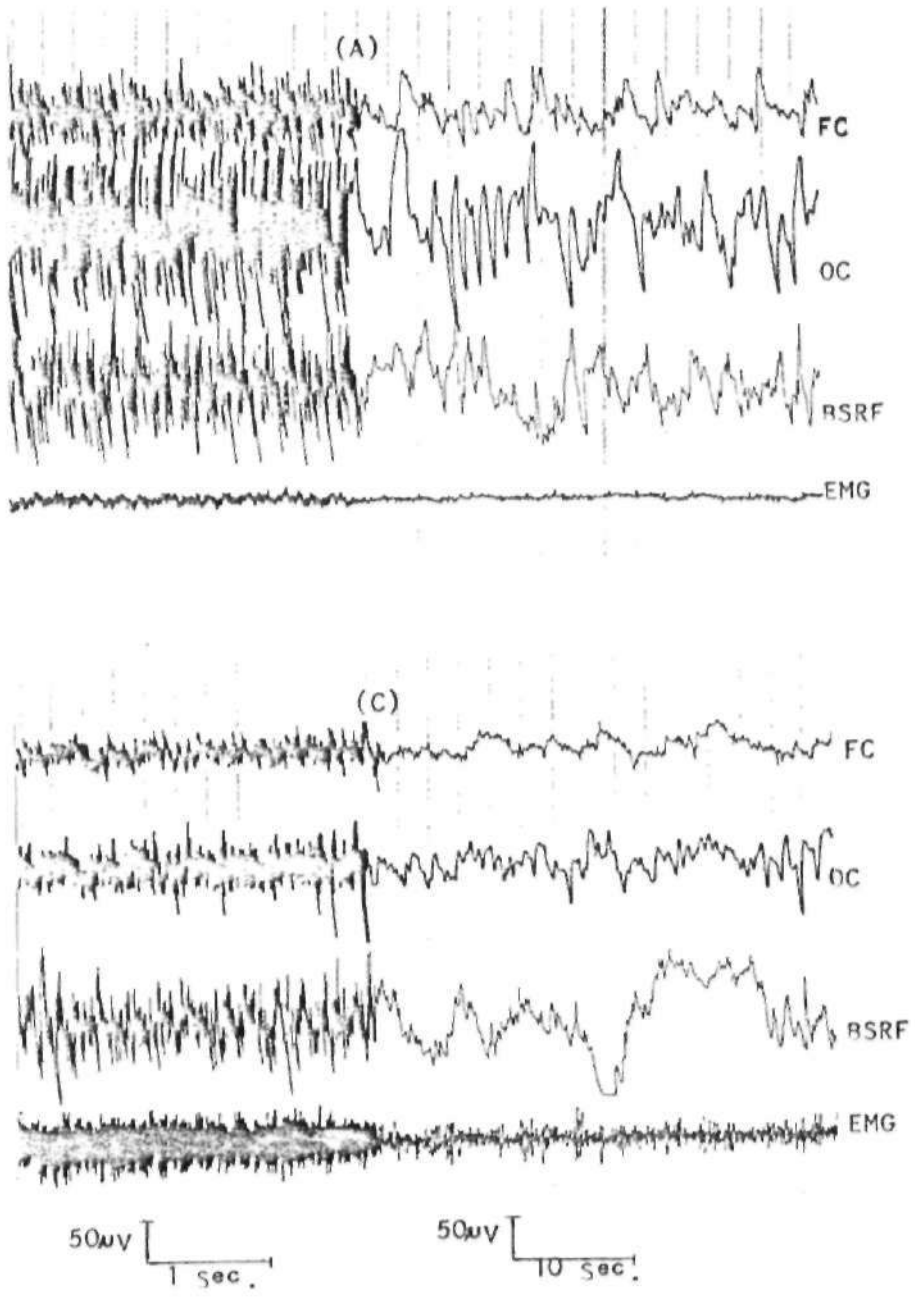


Fig. 6.0.1(b)

Effect of ascorbic acid (80mg/kg i/p) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (C) = 25 min. after injection.

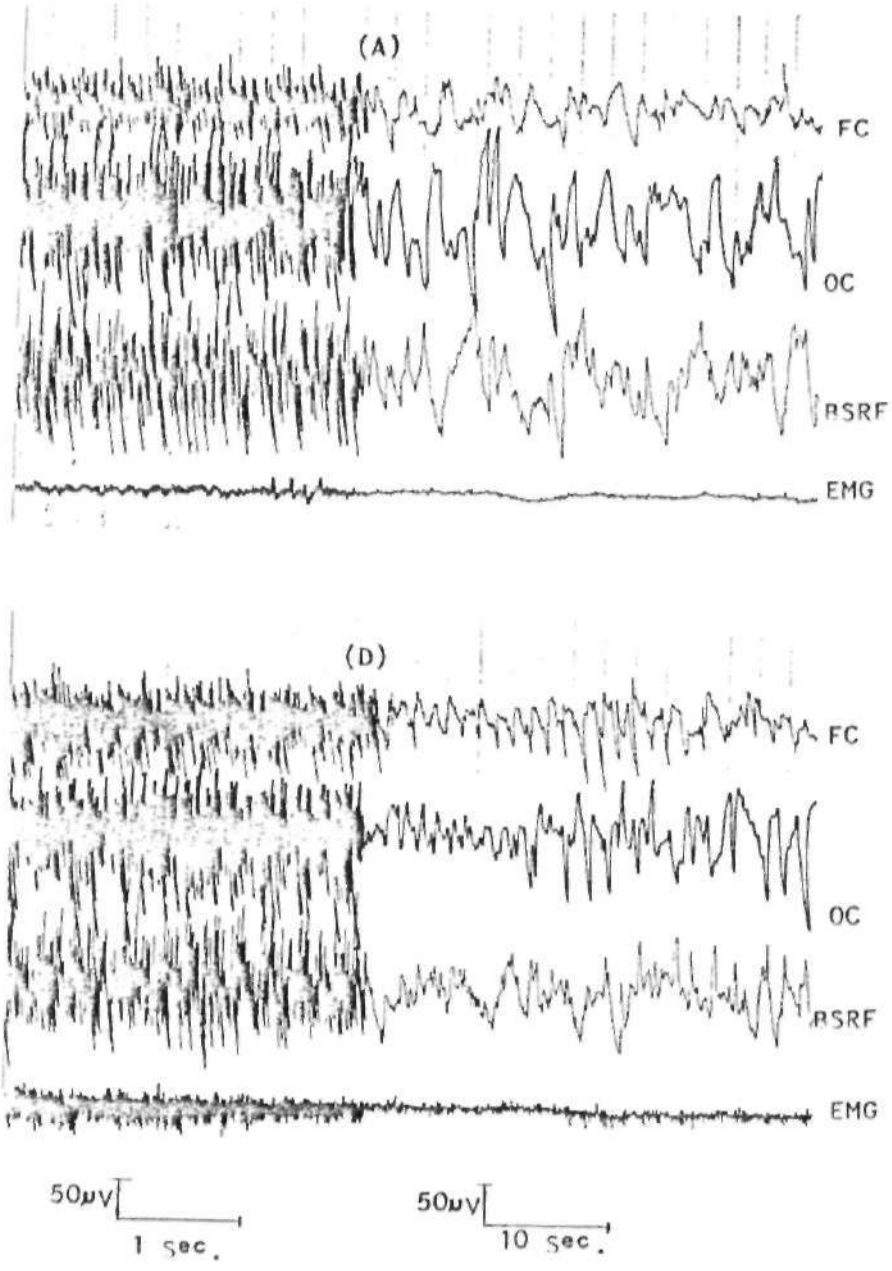


Fig. 6.0.1(c)

Effect of ascorbic acid (80mg/kg/p) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (D) = 60 min. after injection.

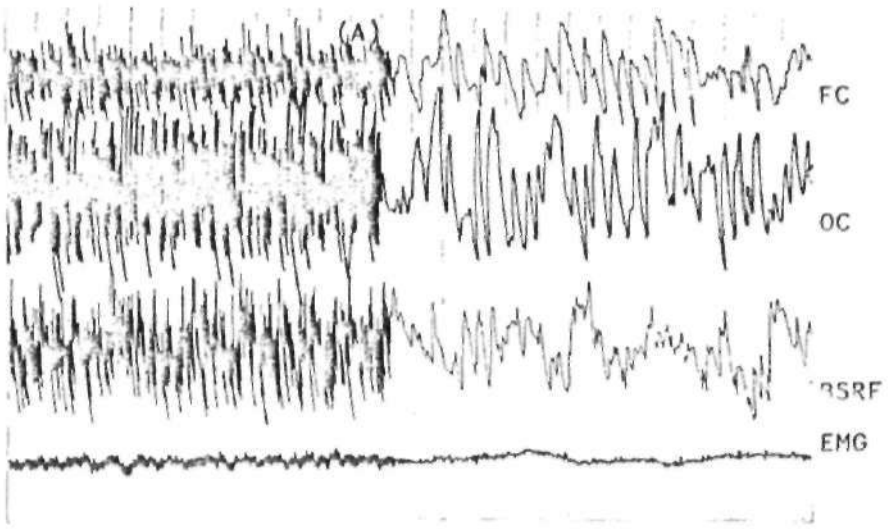


Fig. 6.0.2(a)

Effect of ascorbic acid (300 mg/kg i/p) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (B) = 10 min. after injection.

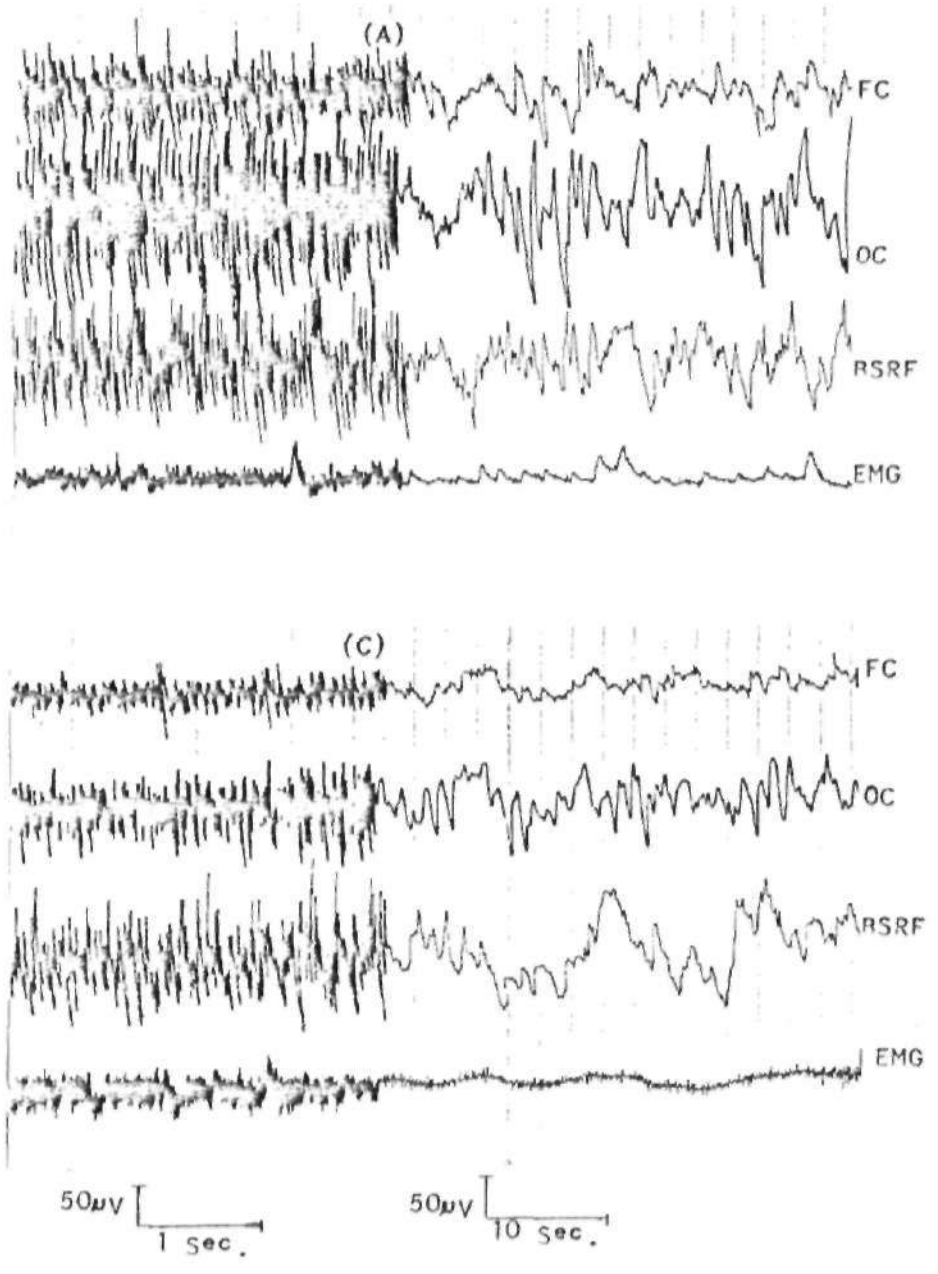


Fig. 6.0.2(b)

Effect of ascorbic acid (300mg/kg i/p) on the ECG and EMG of rat. Upper panel (A) = Control; Lower panel (C) = 25 min. after injection.

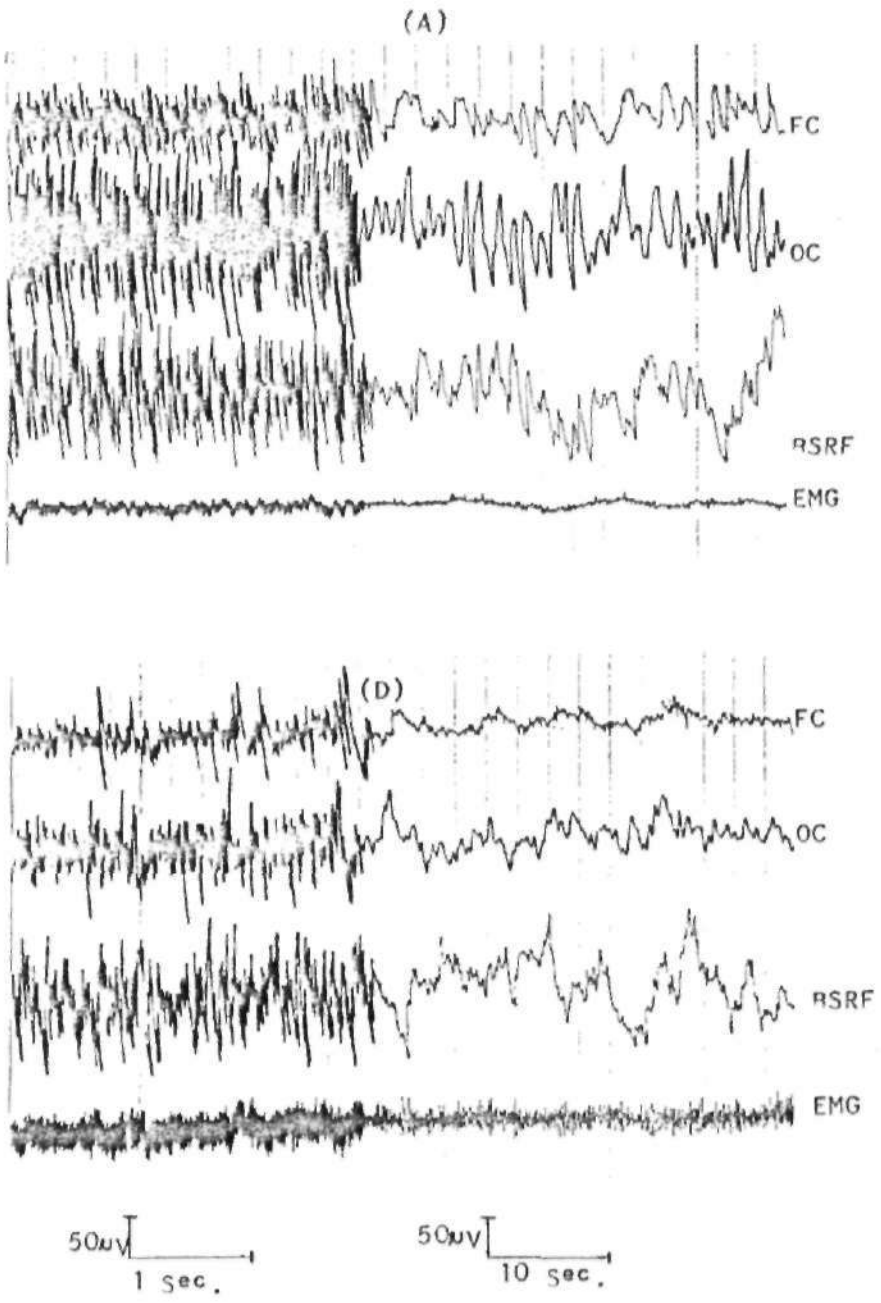
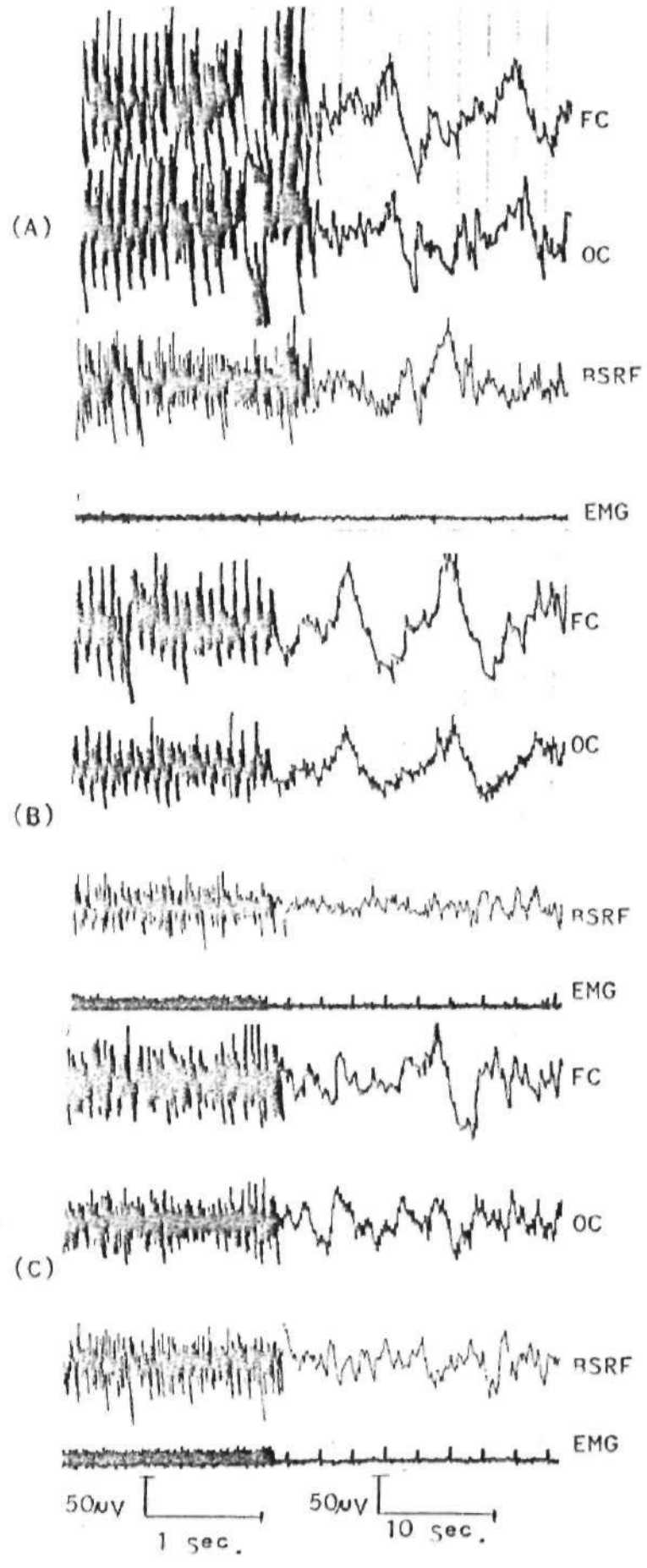
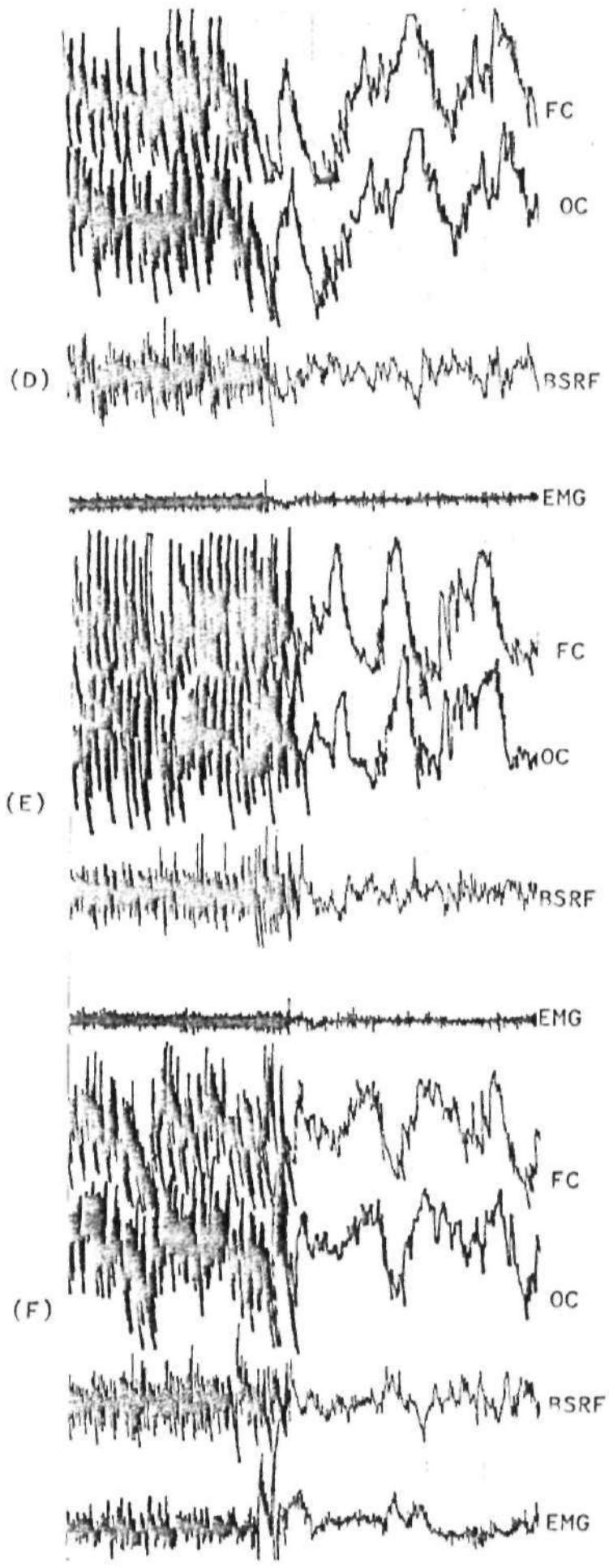


Fig. 6.0.2(c)

Effect of ascorbic acid (300mg/kg i/p) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (B) = 60 min. after injection.


(A)
FC
OC
RSRF
EMG
(B)
FC
OC
RSRF
EMG
(C)
OC
RSRF
EMG
50uV
1 sec.
50uV
10 sec.


(D)
FC
OC
BSRF
EMG
(E)
FC
OC
BSRF
EMG
(F)
FC
OC
BSRF
EMG

6.4.3 The EEG and EMG effects of intracerebroventricularly (ICV) administered ascorbic acid in rat.

6.4.3.1 In the ICV administration of 10 micrograms dose of ascorbic acid.

Ten minutes after the administration, the EEG of the frontal cortex, optic cortex and brain stem reticular formation showed marked decrease in waves amplitudes, while the EMG activity was apparently not changed (Fig. 6.0.4a). At 25 minutes after administration, the decrease in amplitudes was still observed in the EEG of the frontal cortex and brain stem reticular formation, while irregular spikes and high amplitudes was noticed in the EEG of the optic cortex (Fig. 6.0.4b).

At 60 minutes after the ICV administration, the EEG of the frontal cortex, optic cortex and brain stem reticular formation became essentially similar to control, while the EMG activity remained fairly unchanged (Fig. 6.0.4c).

6.4.3.2 In the ICV administration of 40 micrograms dose of ascorbic acid.

At 10 minutes after administration, the frontal cortex showed slight decrease in the amplitudes of the EEG waves; the EEG of the optic cortex was not readily determinable at this stage, while the EEG of the brain stem reticular formation and the EMG activity were not changed appreciably (Fig. 6.0.5a).

At 25 minutes after administration, the EEG of the three cortical regions - frontal cortex, optic cortex and brain stem reticular formation, showed decrease in amplitudes, while the EMG activity was not appreciably changed (Fig. 6.0.5b). When it was 60 minutes after the administration, the EEG of the three cortical regions became essentially similar to those of control, while the EMG activity was still not appreciably changed at this time (Fig. 6.0.5c).

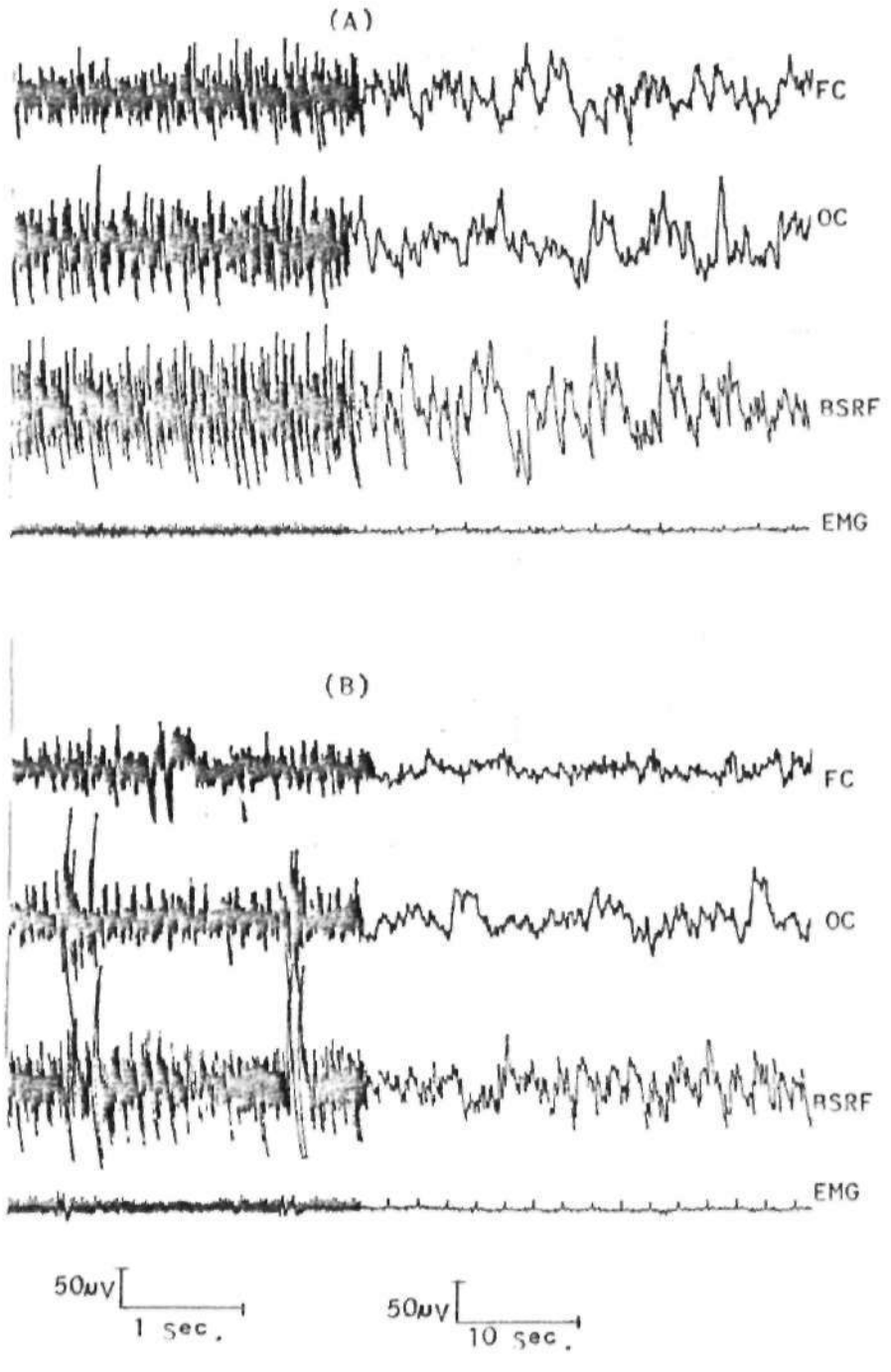


Fig. 6.0.4(a)

Effect of intracerebroventricularly (ICV) administered ascorbic acid (10 micrograms) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (B) = 10 min. after injection.

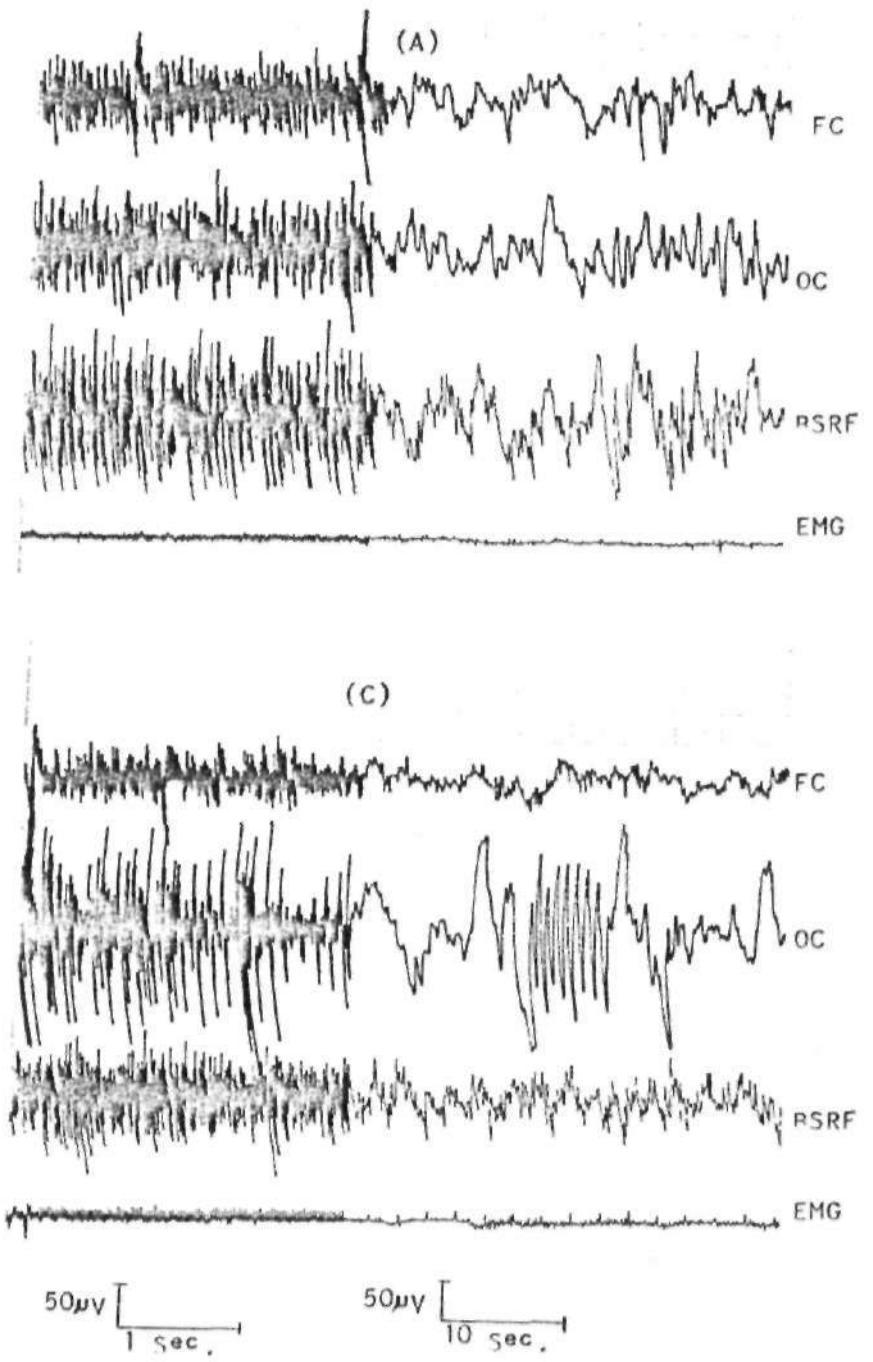


Fig. 6.0.4(b)

Effect of intracerebroventricularly (ICV) administered ascorbic acid (10 micrograms) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (C) = 25 min.after injection.

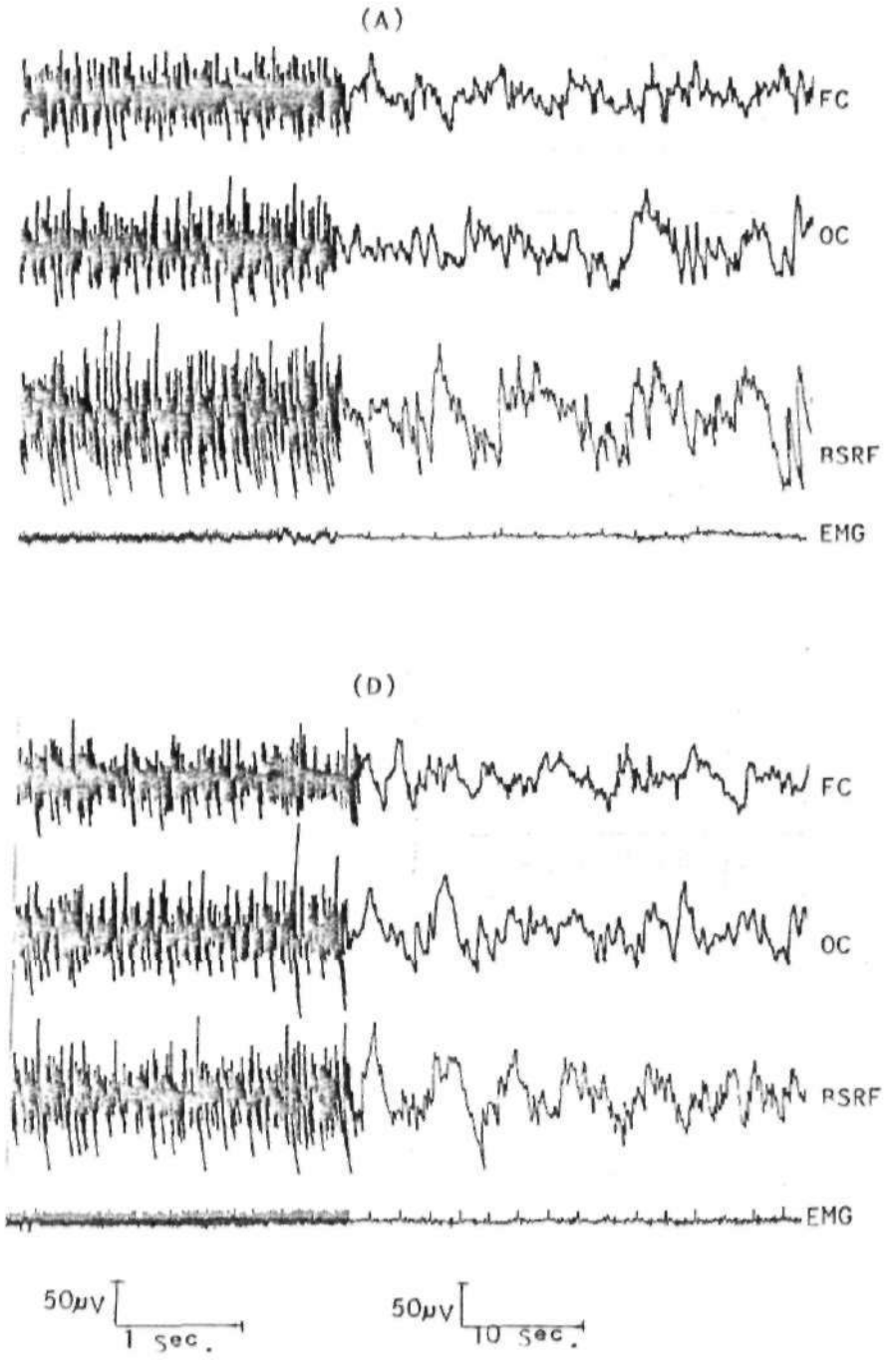


Fig. 6.0.4(c)

Effect of intracerebroventricularly (ICV) administered ascorbic acid (10 micrograms) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (D) = 60 min. after injection.

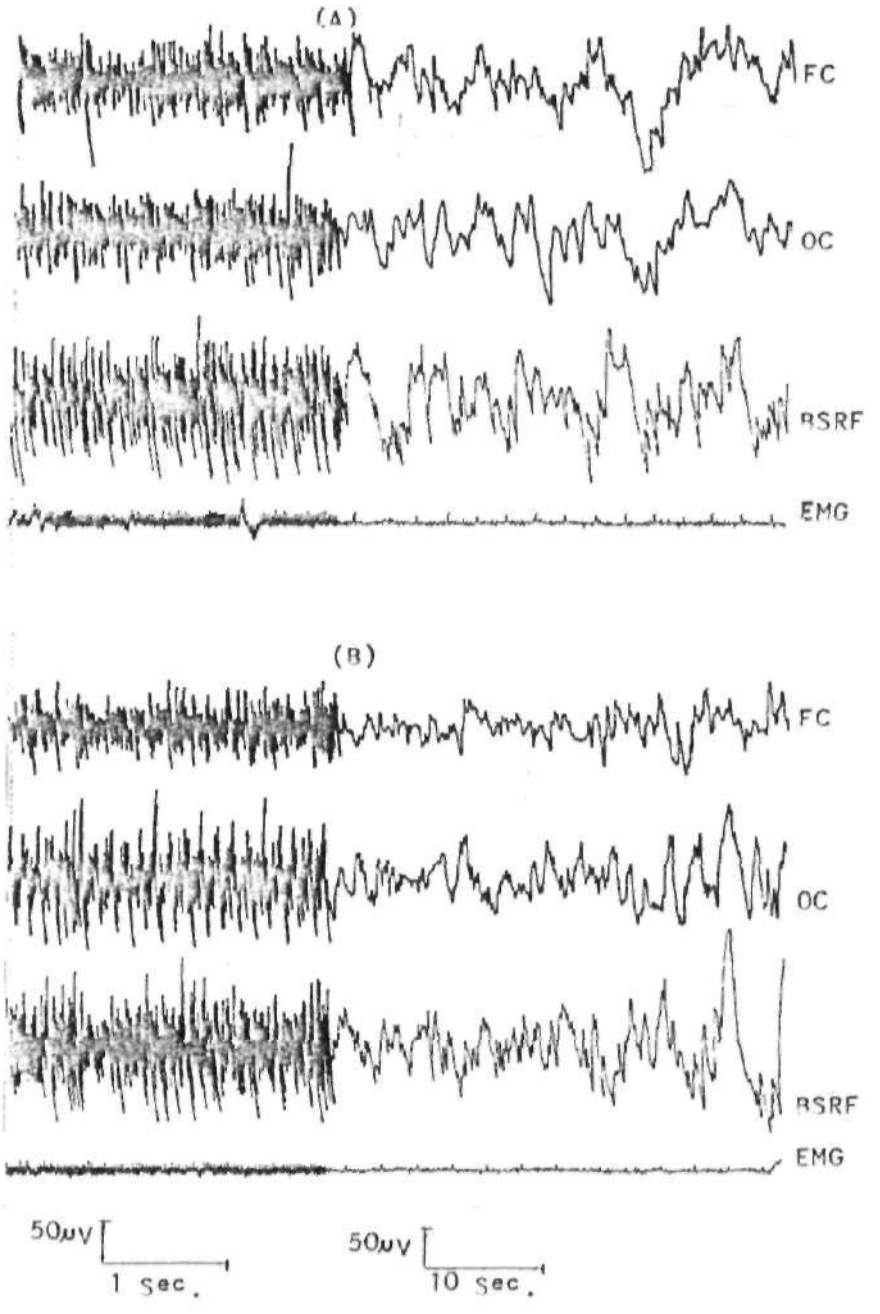


Fig. 6.0.5(a)

Effect of intracerebroventricularly (ICV) administered ascorbic acid (40 micrograms) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (B) = 10 min. after injection.

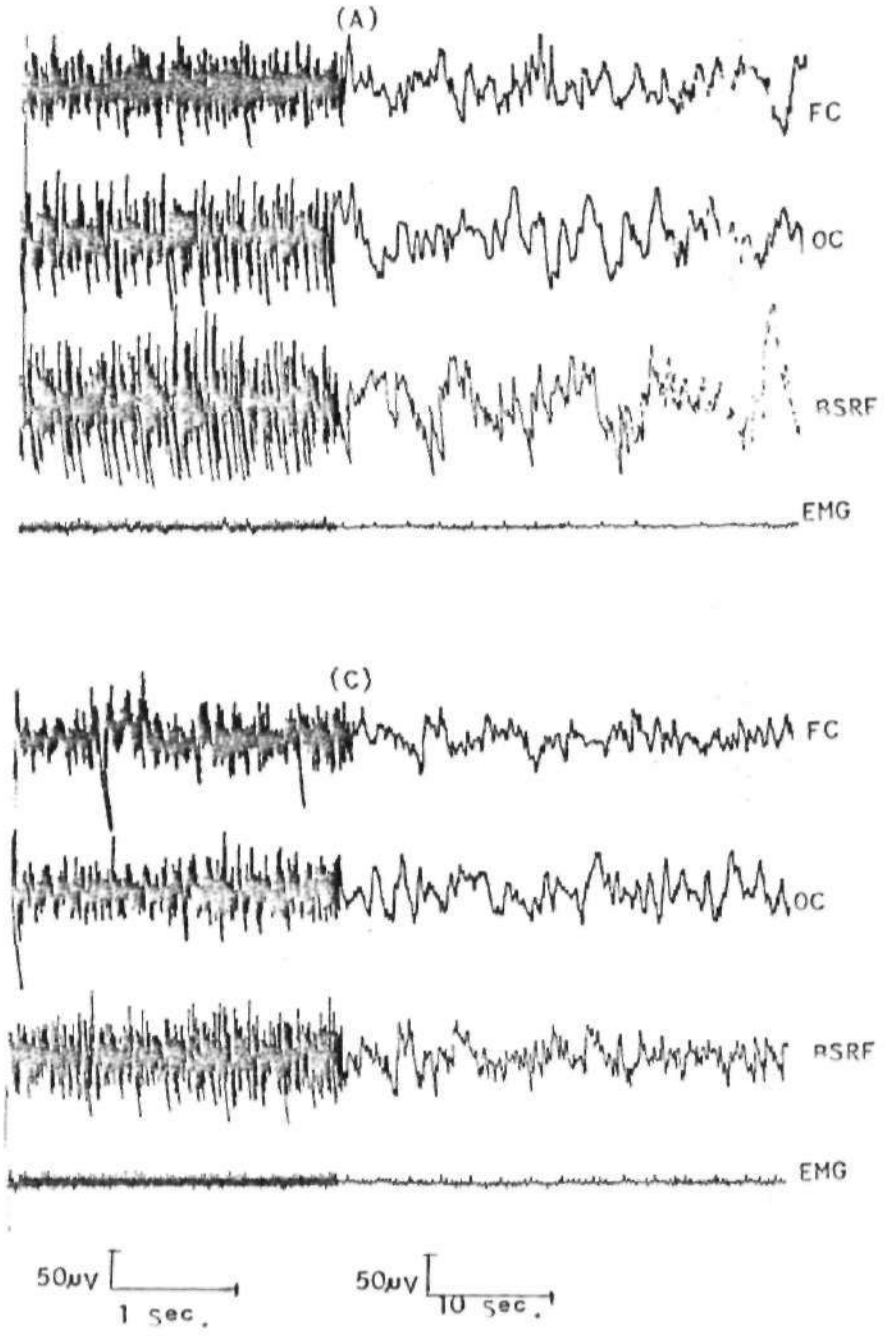


Fig. 6.0.5(b)

Effect of intracerebroventricularly (ICV) administered ascorbic acid (40 micrograms) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (C) = 25 min. after injection.

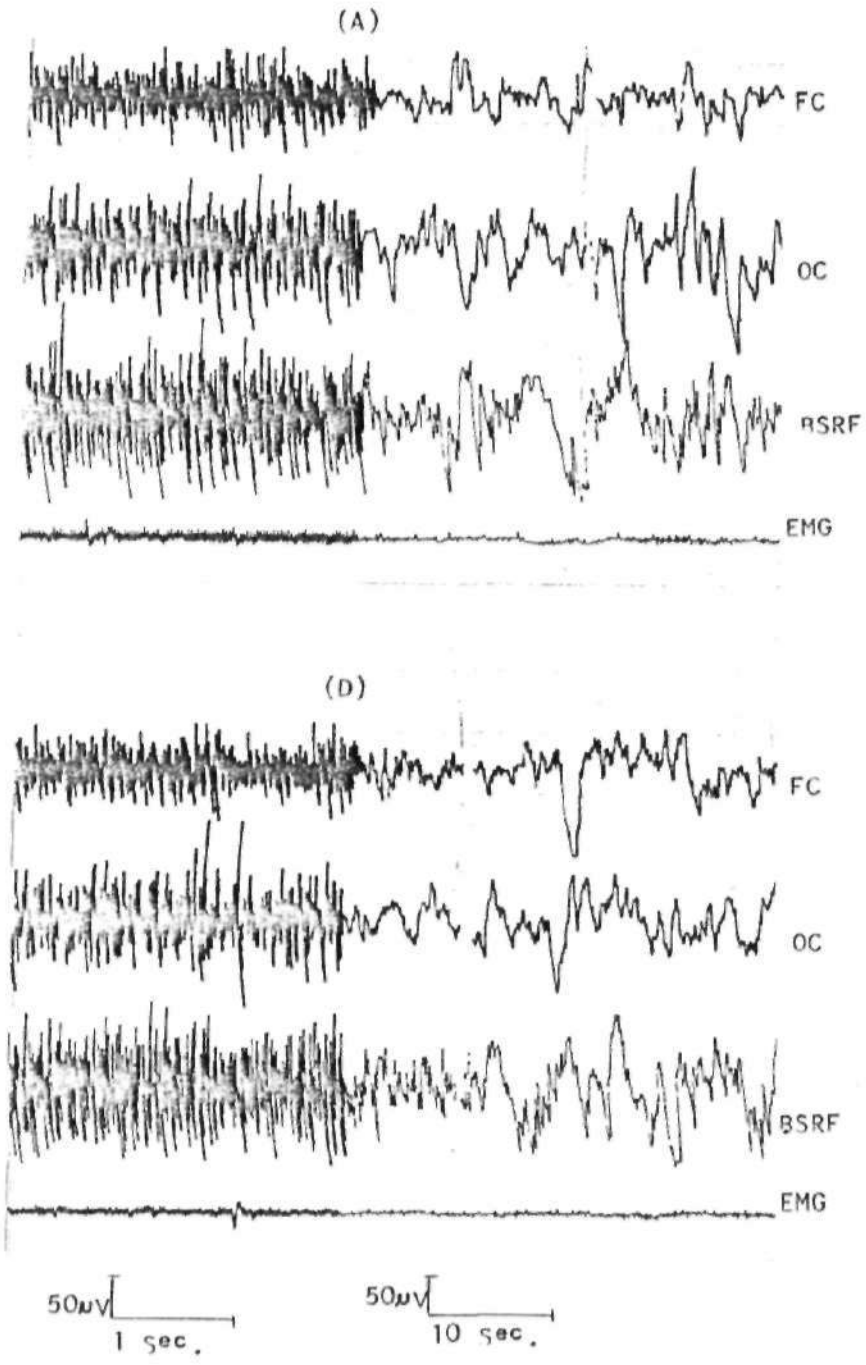


Fig. 6.0.5(c)

Effect of intracerebroventricularly (ICV) administered ascorbic acid (40 micrograms) on the EEG and EMG of rat. Upper panel (A) = Control; Lower panel (D) = 60 min. after injection.

6.5 DISCUSSION

The results of this study indicate that ascorbic acid may have modulating actions on some neural processes in the brain, hence the EEG and EMG changes produced by it. In the acute intraperitioneal administration of both 80 and 300 mg/kg doses, decrease in amplitudes was noticed in the EEG of the frontal cortex and optic cortex, while the EMG activity was also increased. Thus, these results confirm earlier reports of Wambebe and Sokomba (1986).

A decrease in amplitude of EEG waves is indicative of increased frequency (ie. desynchronization), which is thus indicative of central nervous system activation. Activation of the CNS may be viewed against the background of activity in the brain stem, the ascending reticular activating system and the thalamus. It is therefore possible that ascorbic acid may modify levels of activity in the neural systems that traditionally mediate arousal in the brain. An EEG desynchronization is expected to be

accompanied by increased EMG activity as was also noticed in some of the results in this study. The activation of the EEG and EMG by ascorbic acid as noticed in the acute administration in this study, is therefore consistent with the observed behavioural excitatory effects of ascorbic acid. It is also consistent with the observed antagonistic effects on barbiturate sleep noticed in previous studies (Chapter 5). Therefore, the present data provide additional evidence of a possible excitatory role of ascorbic acid in the CNS. Increased EMG activity in a behaviourally awake animal is normally associated with behavioural arousal. Thus, the effect of ascorbic acid on the EMG as noticed in this study, also appears to suggest possible central arousal role of ascorbic acid and may be attributed to a suppression or a decrease in the effect of the inhibitory pathways in the central nervous system.

The data on the EEG in the chronic as well as in the intracerebroventricular administration of ascorbic acid appear to support the notion that

ascorbic acid possesses both CNS activation and sedative qualities: A decrease in the amplitude of the EEG of the frontal cortex and optic cortex was noticed 1 - 3 days of the commencement of the chronic administration. But from the 6th day, increase in the amplitudes of the EEG of these cortical regions were generally observed, while the EMG activity showed mild activation (Fig. 6.0.3 A - F). It was also observed that the EEG effect following the ICV administration of ascorbic acid manifested as desynchronization on the one hand, and synchronization on the other, even in the various cortical regions; these EEG effects however, was not in any consistent or definite pattern (Figs. 6.0.4a, b.c; 6.0.5 a,b,c). These EEG changes produced by ascorbic acid in the chronic and in the ICV administration are also concluded as consistent with the observed behavioural excitation and sedative effects of ascorbic acid.

The EEG and EMG effects of ascorbic acid have

also been studied in young chicks. Erube (1982) reported EEG and EMG activation following low doses of ascorbic acid (i/p), while high doses descreased the EMG activity and synchronized the EEG of the hyperstriatum, optic tectum and pontine reticular formation in the chicks. There were also reports that large doses of ascorbic acid (orally) reduced complex reaction time and deactivated the EEG in humans (Pfeiffer et al., 1968; Benton, 1981). In this study, the results appear to generally agree with previous observations and this is seen as further indication that ascorbic acid may have both CNS excitatory and sedative effects and it is possible that these effects may be mediated via the noradrenergic system. However, due to limitations inherent in the study of drug actions and in a study of this nature, the noradrenergic mechanism may not be the only significant site in the behavioural excitation and sedative effects of ascorbic acid as suggested in this study. Nonetheless, the present data, at least, raised

the consideration that the mode of action of ascorbic acid might be linked to more than one central regulatory systems.

CHAPTER SEVEN

7.0 THE EFFECTS OF ASCORBIC ACID ON THE NORMAL SLEEP OF RAT.

7.1 SUMMARY

7.1.1 The possible effects of ascorbic acid on the normal sleep of rat was investigated using healthy adult rats.

7.1.2 There was definite reduction in the duration of the normal sleep after the rats were given ascorbic acid (80 and 320 mg/kg, i/p).

7.1.3 After the rats were treated with DOPS (10 mg/kg, i/p), there was significant increase in sleeping period of the normal sleep.

7.1.4 After the rats were pretreated with DOPS (10 mg/kg, i/p), the tendency for decrease in the sleeping period was again noticed at the 80 and 320 mg/kg doses of ascorbic acid, respectively.

7.1.5 On chronically administering 320 mg/kg dose of ascorbic acid, deactivation of the EEG, indicative of sleep in the rat, was noticed

on the third consecutive administration; on the other hand, sleep was not noticed in the EEG throughout the test period when 80 mg/kg dose was similarly chronically administered for three days.

7.1.6 After the rat was treated with DOPS (10 mg/kg, i/p) in acute administration, normal sleep was readily observed in the EEG during the test period; similarly, sleep was observed after the rat was pretreated with DOPS (10mg/kg, i/p), prior to 320mg/kg dose of ascorbic acid.

7.1.7 Sleep was not noticed in the EEG of the rat, after the pretreatment of 80 mg/kg dose of ascorbic acid with DOPS (10 mg/kg, i/p).

7.1.8 Sleeping frequency of the normal sleep was not influenced in both ascorbic acid and DOPS treatments.

7.1.9 The results suggest that neurotransmitter systems that regulate sleep might be linked

to the mechanism of ascorbic acid for the observed effects.

7.2 INTRODUCTION

In previous chapters it was reported that low doses (10 - 80 mg/kg) of intraperitoneally administered ascorbic acid induced excitation while sedative effects were clearly noticed in large doses (chapters 3 and 4). It was also noticed that barbiturate sleep was effectively modified by ascorbic acid, and definite changes of activation and synchronization of the EEG and EMG of rat were variously observed in acutely, chronically and intracerebroventricularly administered ascorbic acid.

In the light of these and other observations, the possible involvement of some neurotransmitter systems in the mechanism of action of ascorbic acid in the brain was suggested. In most of the preceeding experiments, the noradrenergic system was noted as possible site of action of ascorbic acid and this suggestion was informed mainly from

the observation that both behavioural excitatory and sedative effects were consistently observed during experiments with ascorbic acid.

Several studies on behavioural neurochemistry in the past have led many neuroscientists to suggest that the monoamine transmitters in the brain are related to behavioural responses. Sleep is a behavioural state in the animal, and the literature is clear on the involvement of the neurotransmitter systems - the noradrenergic, the serotonergic and the cholinergic - in sleep regulation. For example, it was shown that selective depletion of brain serotonin, which can be achieved by administering p-chlorophenylalanine, a compound that blocks the conversion of tryptophan to 5-hydroxytryptophan (The rate limiting step in serotonin biosynthesis) produces prolonged wakefulness in the animal, and it is also known that sleep is suppressed by brain stem lesions that deplete the cortex of norepinephrine (Ganong, 1987). The present study was designed to see if ascorbic acid would modify

the natural sleep of rat. At the end of this study therefore, it might be possible to establish the neural mechanism in the brain that is the basis of the observed behavioural effects of ascorbic acid.

7.3 MATERIALS AND METHODS

Refer to Section 2.12

7.4 RESULTS

7.4.1 The influence of ascorbic acid on the normal sleep of rat.

The rats slept less after receiving ascorbic acid (i/p); the duration of the normal sleep was significantly reduced at 80 mg/kg ($P < 0.01$) and at 320 mg/kg ($P < 0.02$), respectively (Table 7.4; Fig. 7.4.0.1).

After the rats were given DOPS (10 mg/kg, i/p), they slept more and the duration of the normal sleep was significantly increased ($P < 0.01$, Table 7.4). However, rats pretreated with DOPS (10 mg/kg) prior to the administration of ascorbic acid (80 and 320 mg/kg, respectively) showed the tendency to sleep less and the duration

of the normal sleep was significantly reduced at the 320 mg/kg of ascorbic acid ($P < 0.05$, Table 7.4; Fig. 7.4.0.2).

In the test period after administration, the EEG of the frontal cortex and brain stem reticular formation showed deactivation, indicative of sleep in the rat on the third consecutive time of chronic administration of 320 mg/kg dose of ascorbic acid (Fig. 7.4.0.4D). On similar chronic administration of 80 mg/kg of ascorbic acid for three days, the EEG of the rat remained activated throughout the test periods as sleep was not observed (Fig. 7.4.0.3 A-D).

During the test period after acute administration of DOPS (10 mg/kg, i/p) in the rat, there was deactivation of the EEG, indicative of sleep (Fig. 7.4.0.5. B). After the pretreatment of ascorbic acid (320 mg/kg, i/p) with DOPS (10 mg/kg, i/p), the EEG of the frontal cortex and brain stem reticular formation showed deactivation, indicative of sleep in the rat during the test period (Fig. 7.4.0.7 B,C).

However, when 80 mg/kg of ascorbic acid was similarly pretreated with DOPS (10 mg/kg, i/p) sleep was not detected in the EEG of the rat throughout the test period (Fig 7.4.0.6 B,C).

The frequency of sleep in the rat remained fairly unaffected at an average of twice in two hours in all the different test experiments (Table 7.4).

TABLE 7.4

DRUG	DOSE (mg/kg, i/p)	DURATION OF NORMAL SLEEP IN DIFFERENT RATS (Min.)										MEAN ± SEM	MEAN TOTAL SLEEP OCCURENCE IN 2 HRS. (n = 10)
CONTROL	-	44	34	47	42	51	40	34	39	33	48	41.2±2.00	2
ASCORBIC ACID	5	38	41	32	46	55	49	33	48	47	44	43.3±2.31	2
	80	26	31	38	27	18	23	35	42	30	23	29.3±2.34 ***	2
	320	38	41	28	37	33	26	28	44	36	30	34.1±1.91 **	1
DOPS	10	61	70	51	44	53	40	50	52	48	50	51.9±2.66 ***	2
DOPS-PRETREATED ASCORBIC ACID	80	42	37	31	40	35	44	28	45	32	51	38.5±2.27	2
	320	28	41	37	24	30	46	26	43	18	33	32.6±2.86 *	1

The duration (min.) of normal sleep was computed after intraperitoneal administration of the various drugs in the rat at the specified doses. * $P < 0.05$), **$P < 0.02$ and ***$P < 0.01$ are significantly different from the saline control at the indicated P levels, Student's t - test.

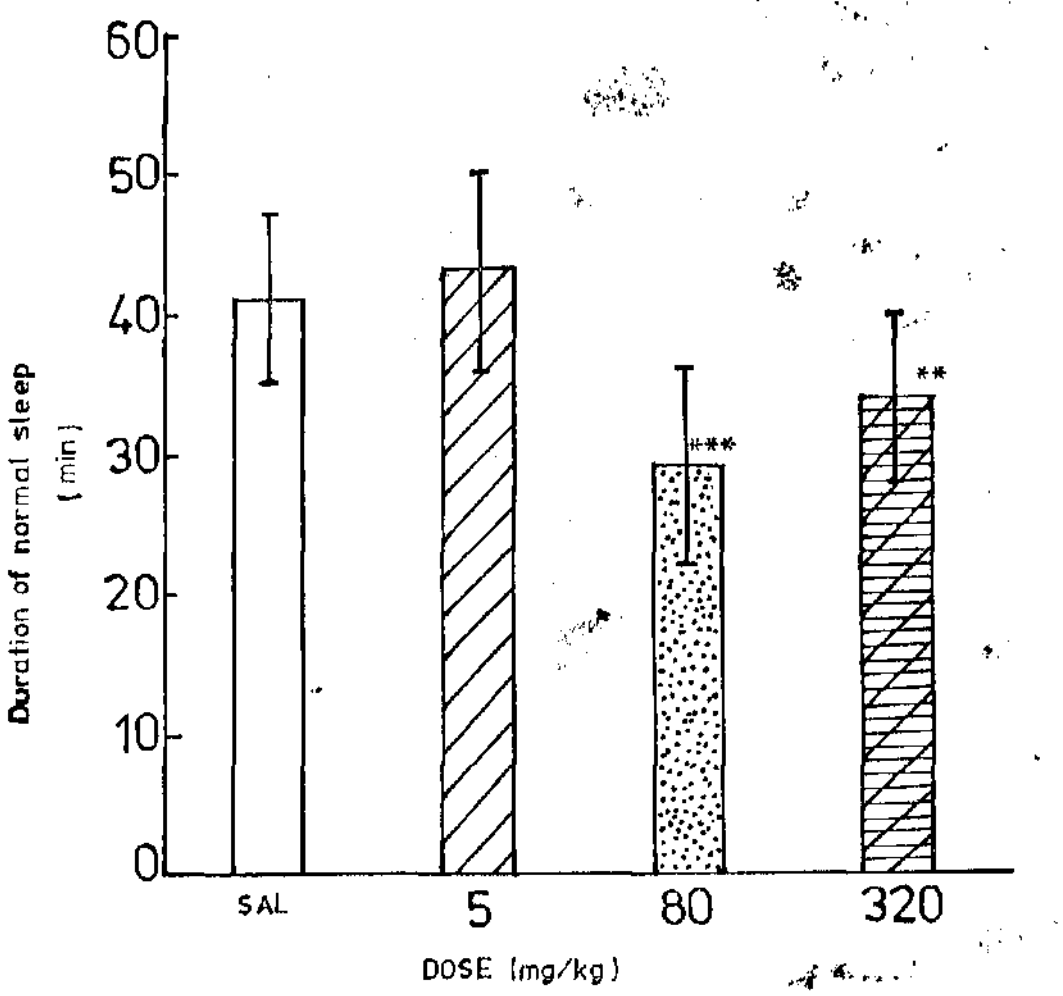


Fig. 7.4.0.1

The effect of ascorbic acid on the normal sleep of rat.
Vertical bars represent standard deviations.
** $p < 0.02$, ***$p < 0.01$ t - test.
(n = 10 per histogram).

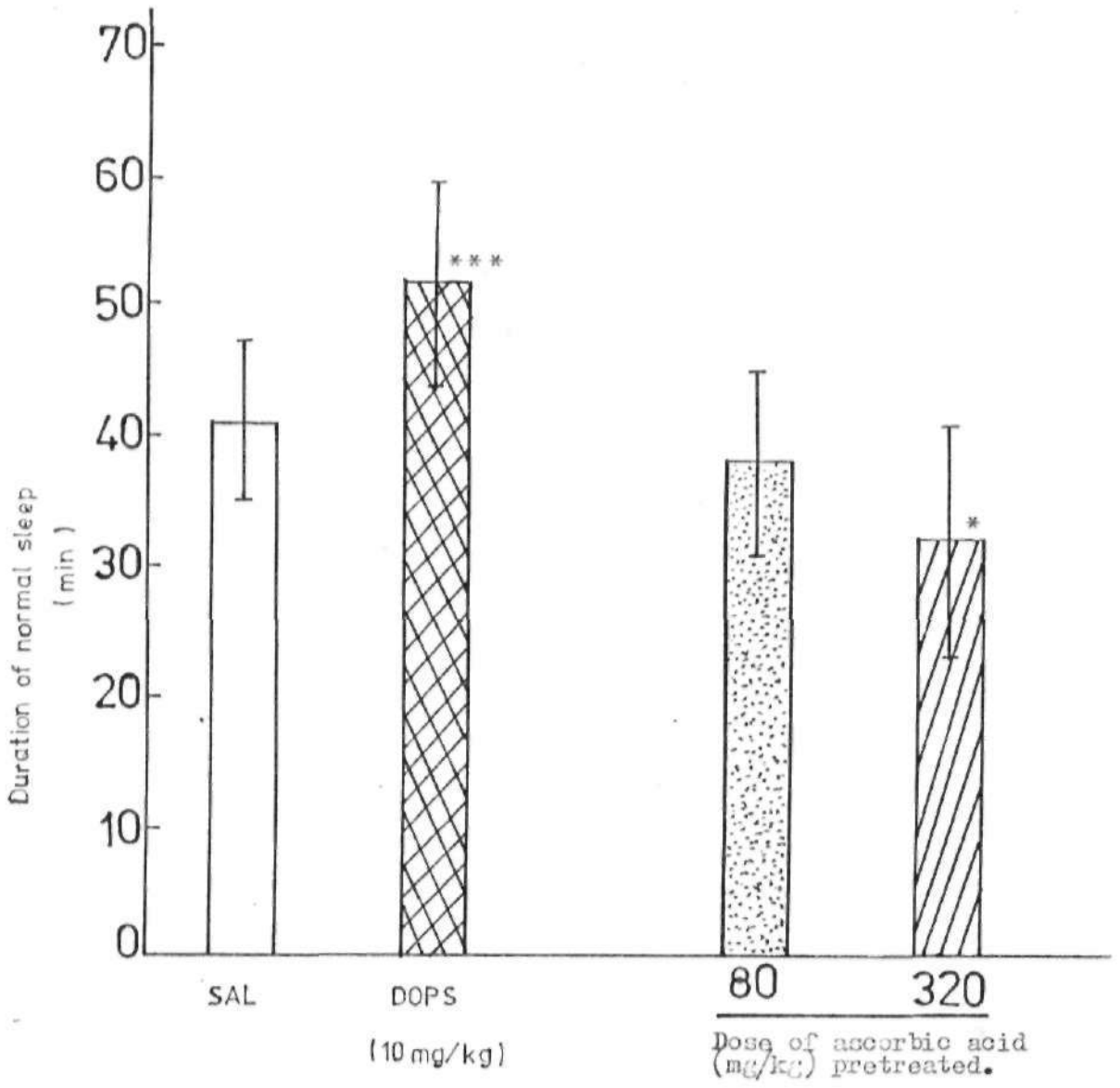
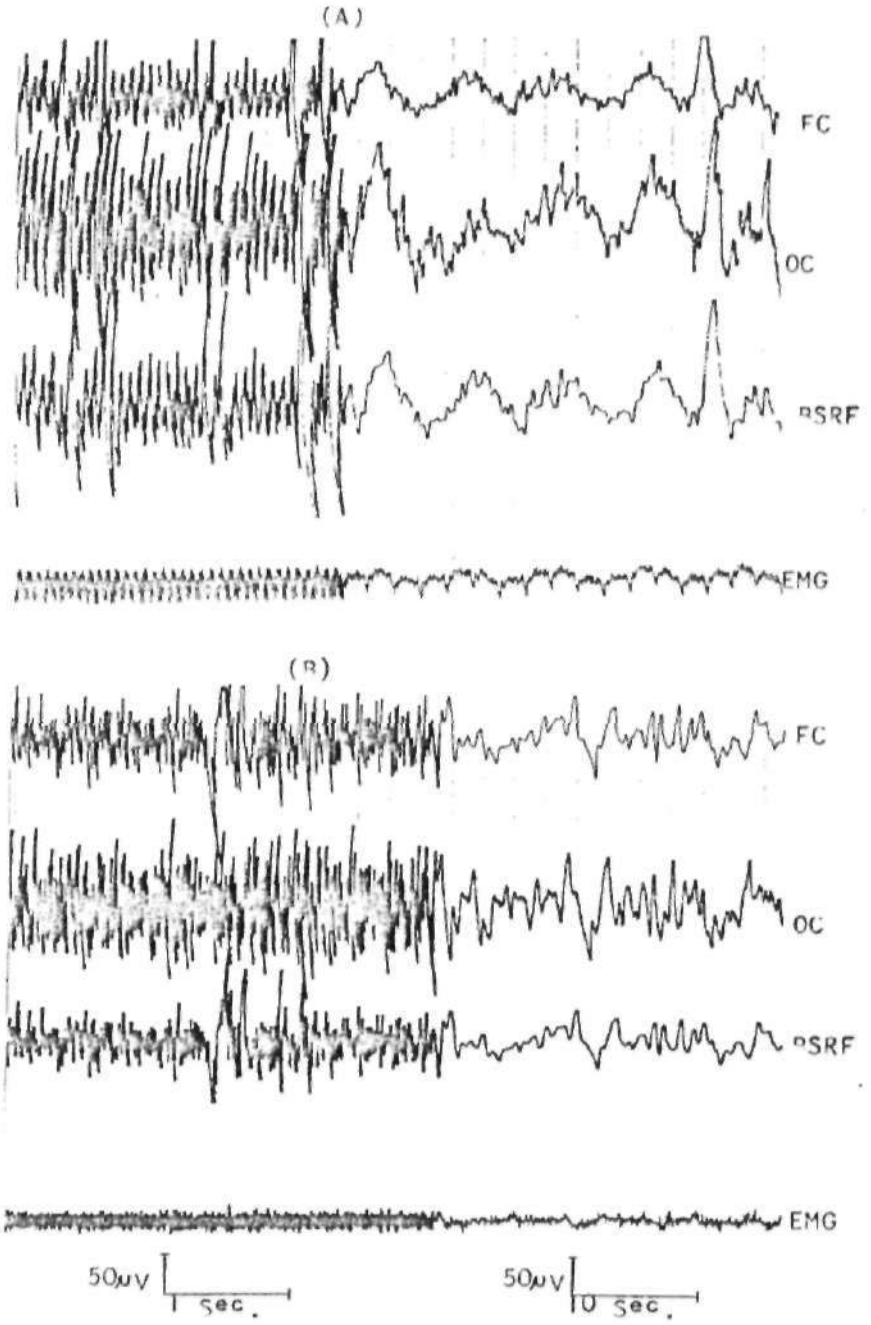
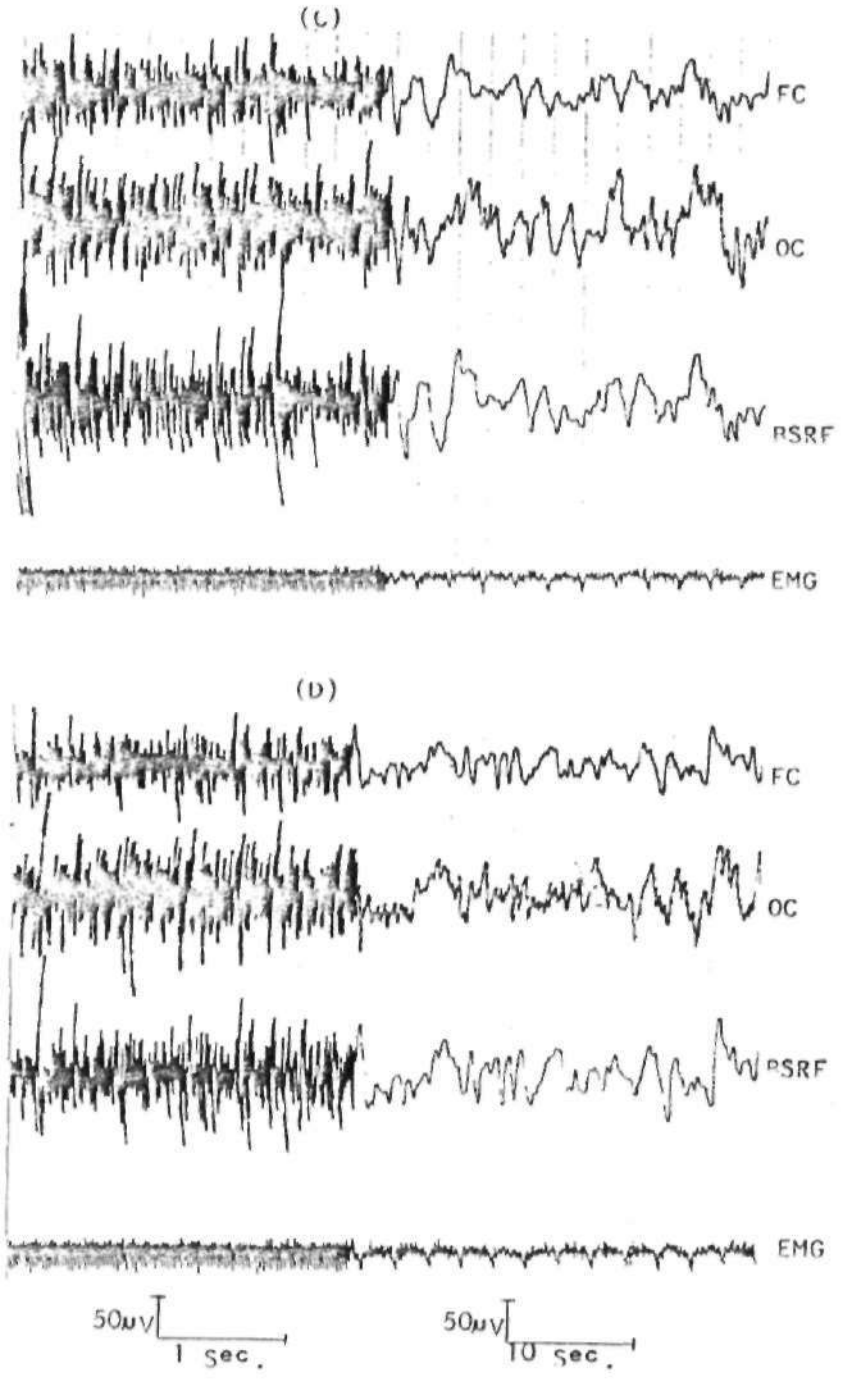
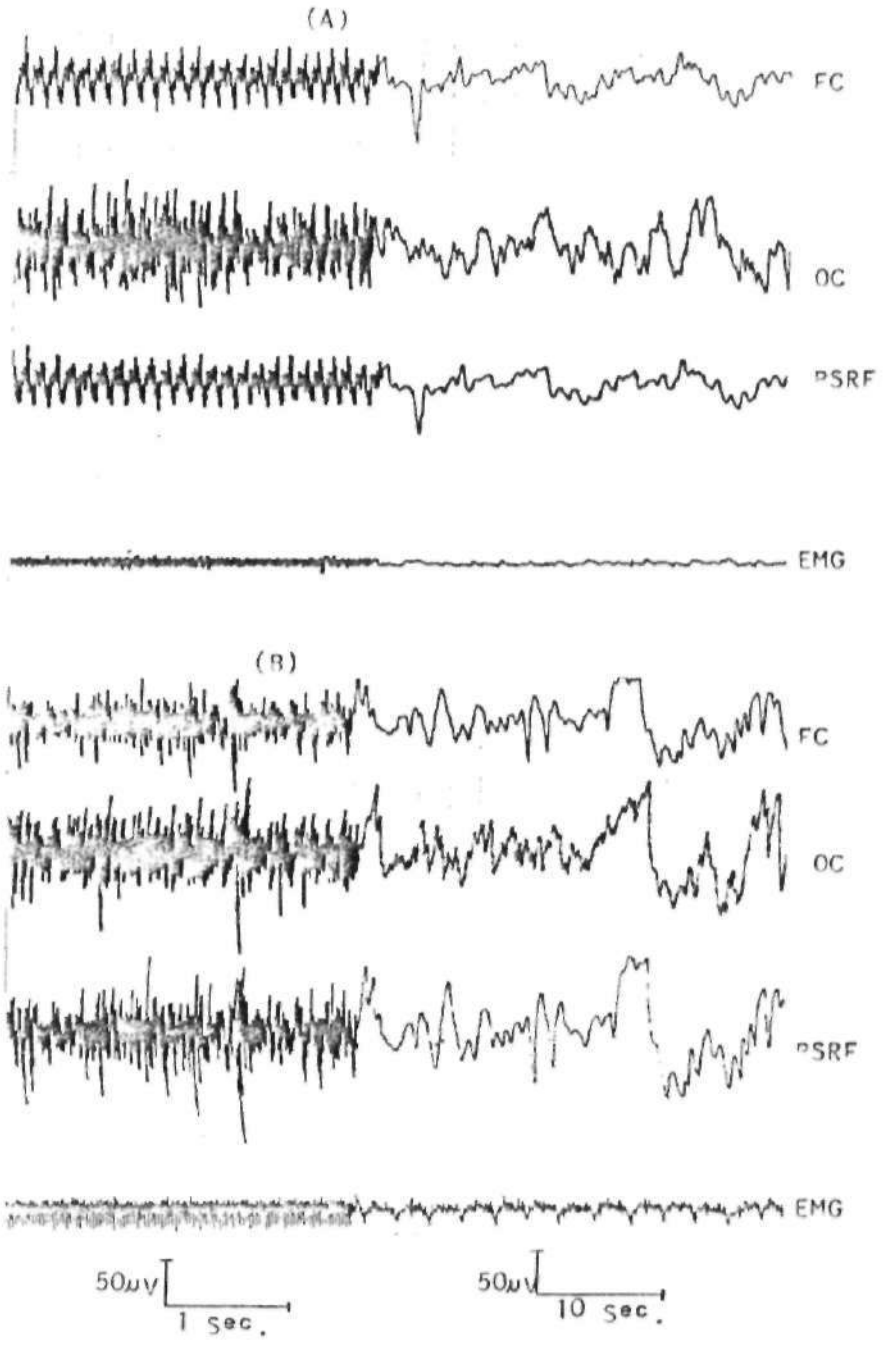


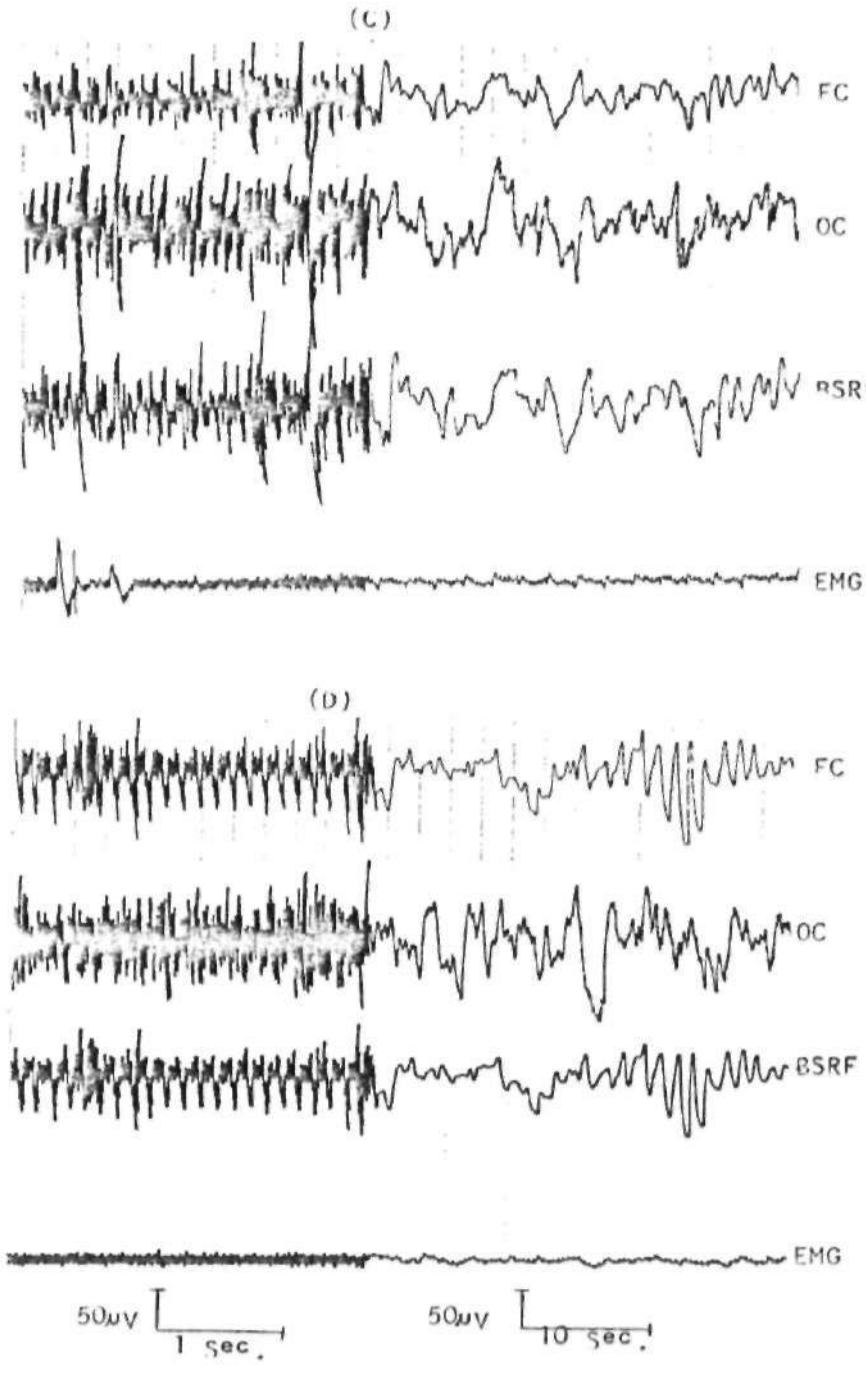
Fig. 7.4.0.2

The effect of DOPS on the normal sleep of rat and the sleep pattern after pretreating ascorbic acid with DOPS (10 mg/kg, i/p). Vertical bars represent standard deviations. *$p < 0.05$, ***$p < 0.01$ t - test. (n = 10 per histogram).


(A)
FC
OC
PSRF
EMG
(B)
FC
OC
PSRF
EMG
50μV
1 sec.
50μV
10 sec.


(C)
FC
OC
RSRF
EMG
(D)
FC
OC
RSRF
EMG
50μv
1 sec.
50μv
10 sec.


(A)
FC
OC
PSRF
EMG
(B)
FC
OC
PSRF
EMG
50μV
1 sec.
50μV
10 sec.


(C)
FC
OC
RSRF
EMG
(D)
FC
OC
RSRF
EMG
50μV
1 sec.
50μV
10 sec.

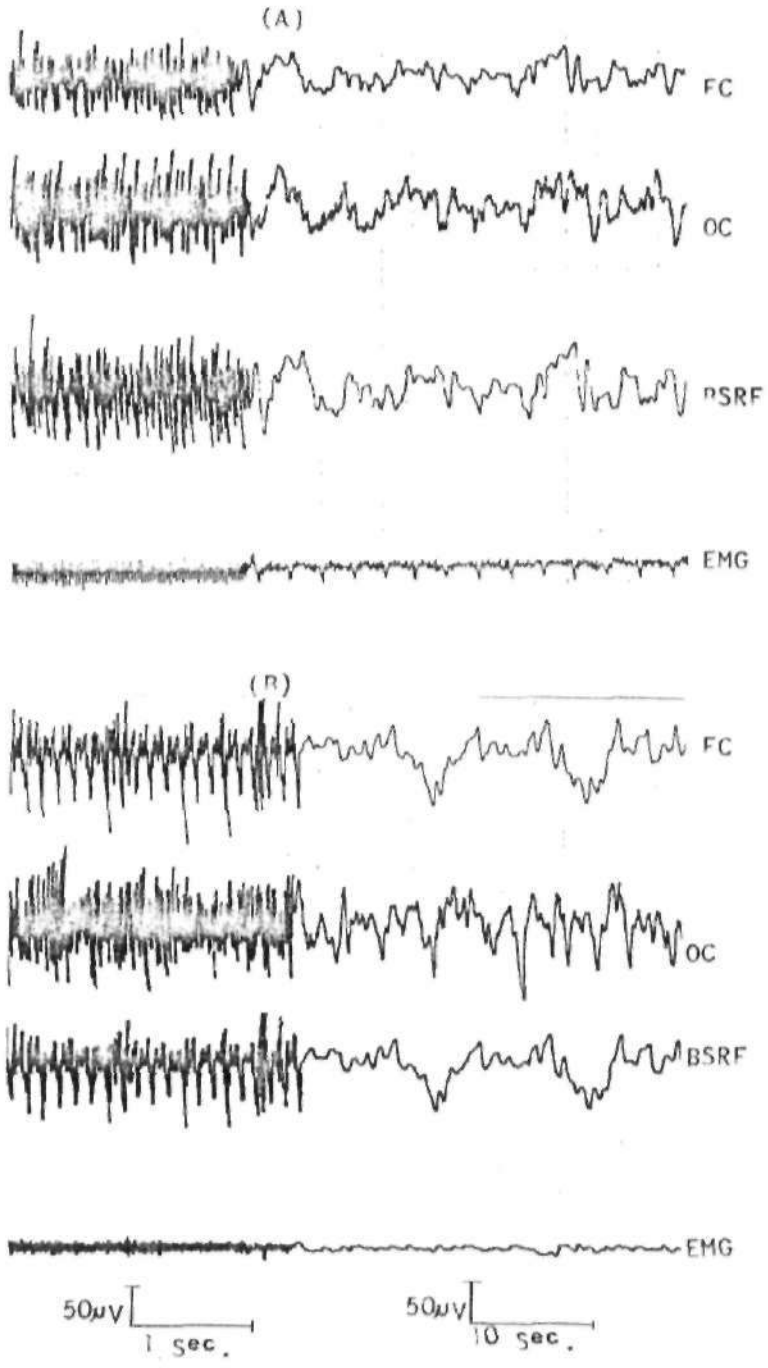
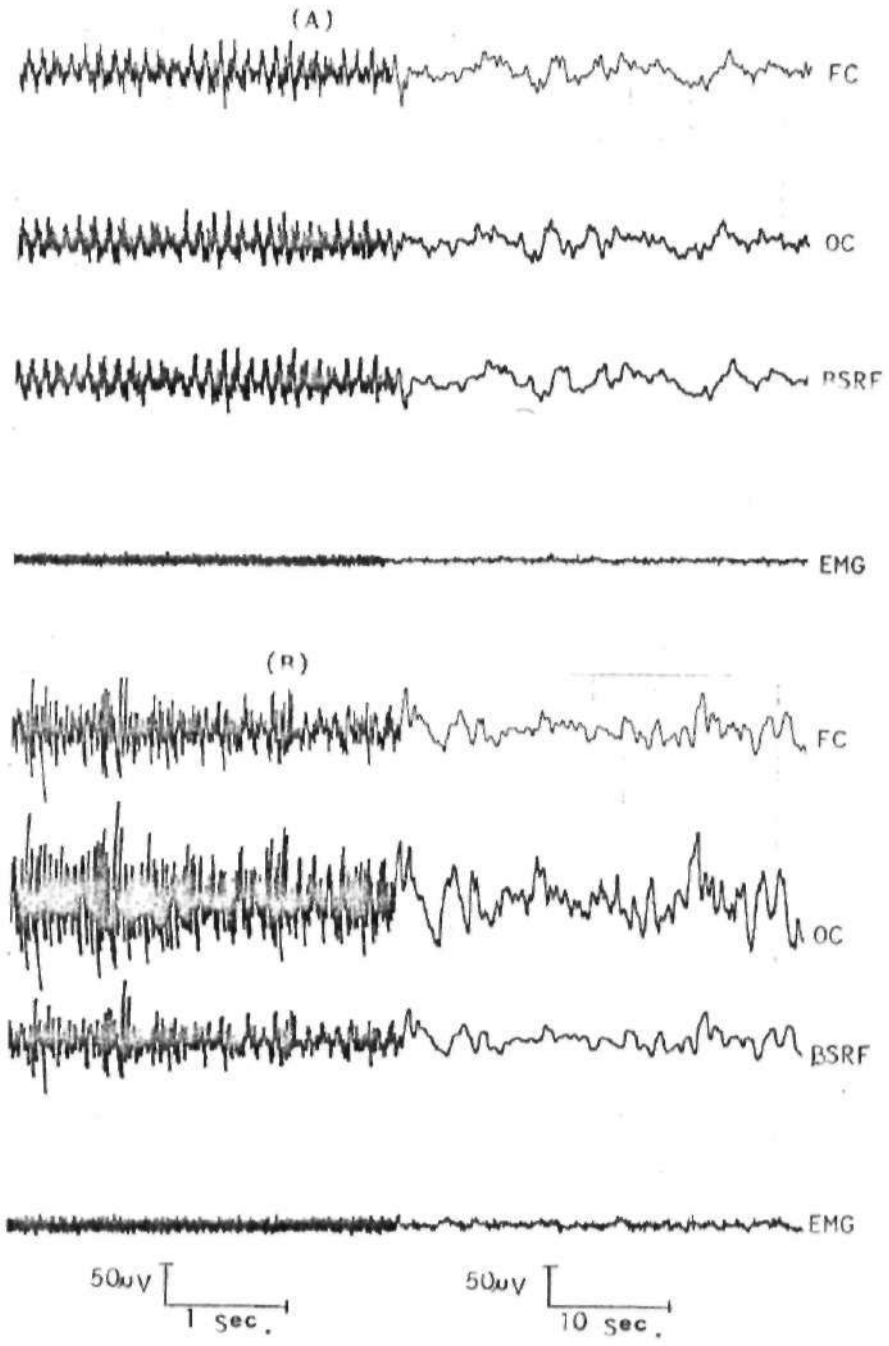
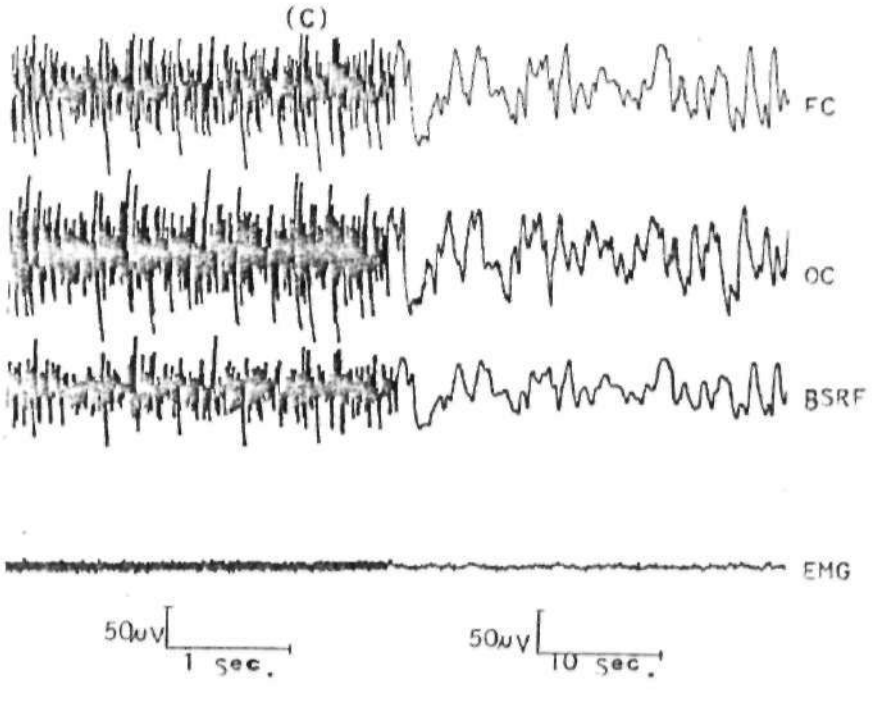
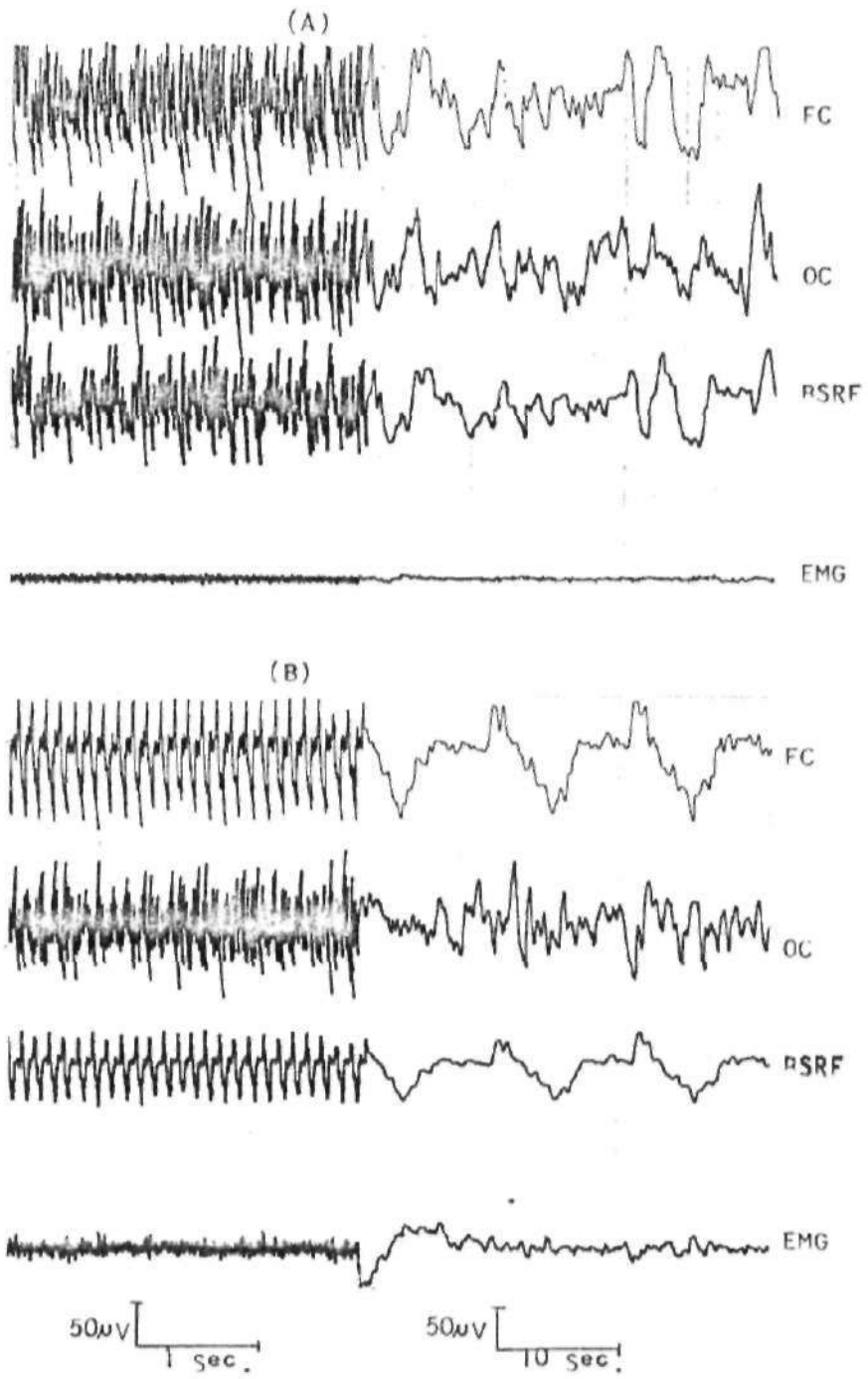


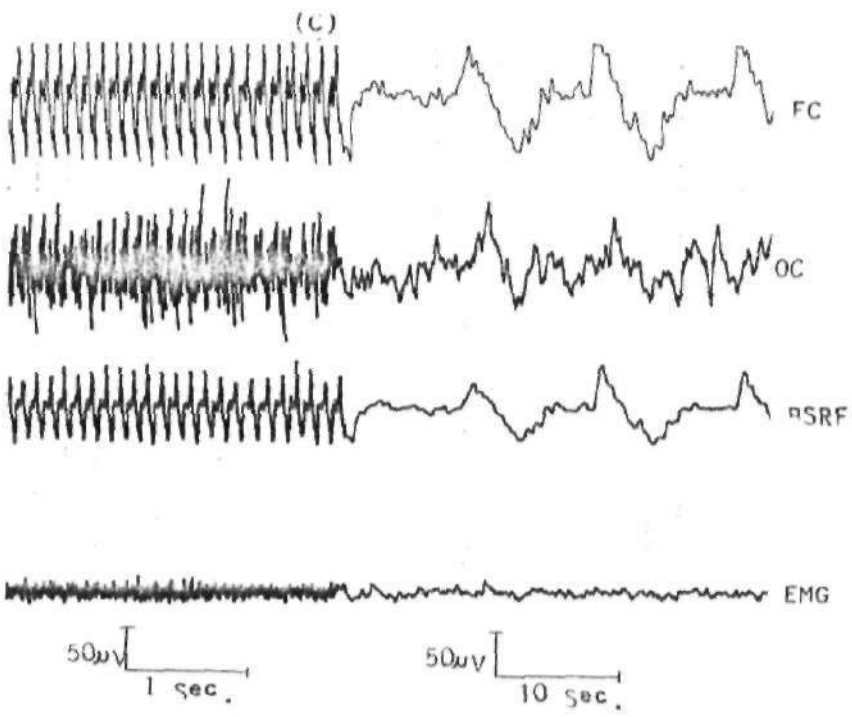
Fig. 7.4.0.5

EEG investigation of the normal sleep of rat after dosing with DOPS (10 mg/kg, i/p).
A = Control; the EEG and EMG of the normal sleep. B = The EEG and EMG during the experimental period after administration.


(A)
FC
OC
PSRF
EMG
(B)
FC
OC
BSRF
EMG
50uV
1 sec.
50uV
10 sec.


(C)
FC
OC
BSRF
EMG
50μV
1 sec.
50μV
10 sec.


(A)
FC
OC
RSRF
EMG
(B)
FC
OC
RSRF
EMG
50μV
1 sec.
50μV
10 sec.


(c)
FC
OC
RSRF
EMG
50μV
1 sec.
50μV
10 sec.

7.5 DISCUSSION

It is obvious from the results of this study, that ascorbic acid influenced the normal sleep of rat. The animals were sleeping less after they received ascorbic acid, and this showed as significant shortening in the sleeping period at both the 80 mg/kg ($P < 0.01$) and 320 mg/kg ($P < .0.02$) doses, respectively (Table 7.4). On the contrary, DOPS (10 mg/kg, i/p) had the effect of promoting sleep, thus the animals slept more following the administration ($P < 0.01$, Table 7.4). To a large extent, these results were consistent with the observations in the EEG after similar test treatments in the rats. Thus, it was observed that after receiving DOPS (10 mg/kg, i/p), sleep was readily detected in the EEG of the rat during the test period (Fig. 7.4.0.5), and the absence of sleep was obvious throughout the period of chronic administration of 80 mg/kg of ascorbic acid for three days (Fig. 7.4.0.3 A - D). From these results, it can be suggested that DOPS and ascorbic acid induced opposing influences on the

mechanism of sleep in the brain. Sokomba (1980) similarly observed that chicks were sedated after they were administered with DOPS (5 - 10 mg/kg, i/p), and that d-amphetamine effect was inhibited after pretreating chicks with DOPS (5 - 10 mg/kg, i/p).

DOPS has always been regarded as a catecholaminergic agent. Harvey, Bader and Nagarkatti (1990) noted that DOPS is a metabolic precursor of norepinephrine. DOPS is known as an agent that elevates the concentration of norepinephrine in the body; most workers believe that DOPS is rapidly metabolized directly into norepinephrine in the body by LAAD. In this study, it is strongly believed that the observed effect of DOPS on sleep may have been via increased noradrenergic activity.

It is particularly noteworthy that after pretreating 320 mg/kg dose of ascorbic acid with DOPS (10 mg/kg, i/p), sleep was still observed in the rat as detected in the EEG during the test period (Fig. 7.4.0.7). This result was surprising

and unexpected; viewed from earlier observations with ascorbic acid in this study, expectation was that the sleep promoting effects of DOPS would become antagonized by ascorbic acid. Or put in another way, it was expected that the DOPS - promoted sleep in the rat would be blocked following the administration of ascorbic acid, particularly, at such high dose level of ascorbic acid. However, it has been consistently noticed from previous experiments that rats were behaviourally sedated with large doses of ascorbic acid (Chapters 3 & 4). Sedation, in effect, is akin to sleep in the animal. Thus, it may seem feasible with the present observations, to assume that sedative effects usually induced at high dose levels of ascorbic acid was, in this case, actually facilitatory to DOPS in the sleep-promoting influences of the later. It will however, be admitted that there are lots of limitations in a study of this nature. Therefore a straight-forward explanation such as the above, to the present observations may be far from reliable and untenable if based solely on the

available data from this study.

Much work have been done on the neurotransmitter systems that are concerned with sleep regulation in the brain. Martini (1989) noted that the mesencephalic portion of RAS appears to be the center of the awake/sleep cycles, and observed that norepinephrine was associated with the initiation and maintenance of RAS activity and sleep. The same worker (Martini, 1989) also reported that the serotonergic system promotes sleep by depressing RAS activity. The cholinergic system was also implicated in sleep regulation (Begley, 1989). In this study, the following were to be noted:

1. The normal sleep of rat was modified in the administered ascorbic acid.
2. It appears suggestive that dose was also an important factor in the pattern of the modifying influence on sleep.

Considering the results of this study and the various observations, it is strongly suggestive

that, at least, some of the putative neurotransmitter systems known in sleep regulation must be linked with the mechanism of action of ascorbic acid for the observed effects. Among these neurotransmitter systems, the monoamine transmitters, notably, the noradrenergic system may be of greater significance and may be assumed likely to be the central effector site of ascorbic acid.

CHAPTER EIGHT

8.0 GENERAL DISCUSSION AND CONCLUSION

The results from this project have shown that administered ascorbic acid (i/p) was accompanied by marked behavioural and EEG changes, and this could have been as a result of activation of some physiological processes by some mechanisms in the central nervous system. Lyhs and Tegeler (1963) noted that when the concentration of ascorbic acid in the brain of guinea-pigs was increased by injection of dehydroascorbic acid, a rise in the higher nervous system activity lasting for several days was observed. In 1970, Sjostrand reported that the arousal effects observed in anaesthetized animals after they were injected with dehydroascorbic acid, became inhibited after decerebration or after spinal cord section. This report offered further proof that the observed effects of ascorbic acid was definitely of central origin. Dehydroascorbic acid is known to be rapidly reduced to ascorbic acid in the body and then retained in the nervous tissues (Patterson

and Mastin, 1951; Hammarstron, 1966; Heath and Fiddick, 1966). Within this period of increasing research to ascertain the mechanism underlying the possible involvement of ascorbic acid in the function of the CNS, many reports by other workers agree with Gellhorn (1943) observation that the behavioural patterns observed after the administration of dehydroascorbic acid in the animal were similar to those evoked by stimulation of certain sympathetic centers in the brain. In his observation, Gellhron (1943) was actually making reference to sham rage which is characterised by peripheral signs of sympathetic hyperactivity and aggressiveness. Gellhorn (1943) also reported that the behavioural patterns he observed was associated with a lowering of the brain noradrenaline content. Also among the earlier workers were Crandon, Lind and Dill (1940) who reported that in experimental ascorbic acid deficiency in man, mental and emotional changes were observed. It was therefore speculated that ascorbic acid may have properties or actions

similar to d-amphetamine in the brain and this speculation intensified after the work of Trulson et al. (1979), who reported that d-amphetamine exerts its actions mainly on the noradrenergic and dopaminergic systems in the brain to induce its effects. However in spite of several reports and observations by workers, the problem of ascertaining physiological data to support the possibility that ascorbic acid might act like d-amphetamine in the brain remained unresolved.

Data accumulated in this project indicate that excitatory and sedative effects were differentially induced by ascorbic acid and was seen to be related to the dose of the substance. It is tempting to argue that the dual behavioural effects produced by ascorbic acid were probably due to activation of different mechanisms in the brain as a consequence of the level of ascorbic acid reaching the brain. In agreement with such a point of view might be the work of Tolbert et al. (1979) who first noted that large doses of ascorbic acid (i/p) induced significant elevation

of brain ascorbic acid in rats. If a suggestion that different mechanisms may have been activated in the brain to induce the behavioural excitatory and sedative effects would be adopted, there then might also be the need to re-examine some of the systems earlier proposed by workers as possible mechanisms of ascorbic acid, namely, modulation of dopaminergic activity (Tolbert et al., 1979), release of norepinephrine from the storage sites (Sjostrand, 1970; Kuo et al., 1979) and inhibition of cholinesterase enzyme and release of ACh (Sjostrand, 1970; Kuo et al., 1979). It would however be recalled that in these postulations, most of these workers were making reference to excitatory effects which was what they claimed to have primarily observed with ascorbic acid.

Decrease in wave amplitudes of the EEG implied higher frequency, indicating activation of the CNS at the commencement of chronic administration of ascorbic acid. Later in the course of the chronic administration, increase in wave amplitudes of the EEG was noticed, and which

denoted deactivation or decrease in CNS activity (sedation). Also, activation and deactivation of the EEG were variously observed in ICV administration. These EEG observations were consistent with and, thus, confirmatory to the behavioural excitatory and sedative effects of ascorbic acid seen in the acute administration in this project.

The normal sleep of rat was modified in doses of the administered ascorbic acid. This result was noted as important finding that greatly decreased the likelihood of further speculations. The result was a clue which made certain the strong notion that some of the putative neurotransmitter systems that regulate sleep must be linked to the mechanism of action of ascorbic acid in the brain. The possibility of involvement of the sleep-regulating neurotransmitter systems in the mechanism of action of ascorbic acid appears even more probable when considered against the background of the fact that the central mechanisms of arousal of the cortex are in effect,

product of neural processes implicit in sleep. With the exception of ACh, most of the neurotransmitter systems that regulate sleep in the brain are monoamine transmitters, namely, noradrenaline, dopamine and serotonin. It is however, pertinent to know if manipulation of the monoamine transmitters could have been the only effective way to modify mood or behaviour by a psychotomimetic agent?

Izquierdo, Jofre and Acevedo (1968) reported that ascorbic acid injected intraperitoneally into rats caused an increase in the endogenous noradrenaline level and a decrease in the dopamine level in various parts of the brain. Sjostrand (1970) also showed that injected dehydroascorbic acid was followed by a release of brain noradrenaline. During those days of increasing research to ascertain the nature of the interaction of ascorbic acid at the noradrenergic terminal, it became generally accepted that ascorbic acid might play a physiological role in the storage mechanism of noradrenaline in

granules. In the light of the increasing evidence and reports implicating the noradrenergic system in the possible mechanism of ascorbic acid, in the light of the results of this study and available data, the noradrenergic neural system appeared to present the most tenable and the most reliable physiological candidate as site of action of ascorbic acid in the brain for the observed behavioural effects. This assertion also stems from the fact that among the neurotransmitter systems that regulate sleep, the noradrenergic system possesses the neural mechanism for dual mediative roles in the brain (ie. CNS arousal and sedation functions). It is possible that the neural mechanism in the noradrenergic system could have been effectively utilized by ascorbic acid to subserve or to produce the behavioural excitatory and sedative effects. Furthermore, it is possible to consider that the stage in the neural processes of the noradrenergic mechanism that becomes the site of action of ascorbic acid would be dependent upon the concentration or level of ascorbic acid

in the brain as given by the dose. In this regard, the action of ascorbic acid could be at the level of the intermediate precursors (Dopa and Dopamine) to subserve CNS arousal; on the other hand, the action could be at the synthesized end product (Norepinephrine) to produce sedation (See Chapter 3). However, the possibility of involvement of other systems, mostly, the dopaminergic, the cholinergic or the serotonergic systems, in the mechanism of action of ascorbic acid cannot be completely ruled out on the basis of the present data. For example, in the cholinergic system, although it has been generally accepted as an excitatory transmitter in the CNS, there has been reports of some inhibitory roles of ACh in the cerebral cortex (Krynjeric and Philips, 1963). It will be the objectives of further research to determine for example, the possible contributions of the cholinergic and/or the serotonergic systems in the mechanism of action of ascorbic acid for the observed effects. Nonetheless, on the strength of the results of

this project and available data, it is concluded that the noradrenergic neural mechanism may have been the central effector site of action in the physiologic modulation of behaviour of ascorbic acid.

REFERENCES

Anden, N.E., Carlsson, A. Dahlstrom, A., Fuxe, K., Hillarp, N.A. and Larson, K. (1964). Demonstration and Mapping out of nigro-neostriatal dopamine neuron. Life Sciences 3: 523 - 530.

Baker, E.M. (1967). Vitamin C requirements in stress. American Journal of Clinical Nutrition 20: 583 - 590.

Bali, L. and Callaway, E. (1978). Vitamin C and Migraine: a case report (Letter). New English Journal of Medicine. 299 (7): 364.

Begley, S. (1989). The stuff that dreams are made of. Special report. In: Newsweek International Magazine. August 14 P. 37 - 46.

Benton, D. (1981). The influence of Large doses of Vitamin C on psychological functioning. Psychopharmacology 75: 98 - 99.

Bertler, A., Carlsson, A. and Rosengren E. (1956). Release by reserpine of catecholamines from rabbits hearts. Naturwissenschaften 43: 521.

Betler, A., Carlsson, A. and Rosengren E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. Acta physiologica Scandinavica 44: 273 - 292.

Binette, Y., Feron, L.B. and Granel, G.G. (1965). Vitamin C in Schizophrenia. Union Med. Canada, 94 (10): 1272 - 5.

Blaschko, H. (1957). Metabolism and Storage of biogenic amines. Experientia. 13: 9 - 12.

Blumgberg, J.B., Vetulain, J., Stamwarz, R.J. and Sulser, F. (1976). The noradrenergic c AMP generating system in limbic forebrain. Pharmacological characterization and the possible role of limbic noradrenergic mechanisms in the mode of action of antipsychotics. European Journal of Pharmacology 37: 357 - 366.

Burn, J.H. (1975). Transmitters in the brain. In: The Autonomic nervous system for students of physiology and pharmacology 5th Ed. Blackwell, Oxford P. 71.

Cannon, W.B. (1915). Bodily changes in pain and hunger, fear and rage. D. Appleton and Co. New York.

Carlsson, A. (1959). The occurence, distribution and physiological role of catecholamines in the nervous system. Pharmacological Reviews 11: 490 - 493.

Carlsson, A., Lindqvist, N. and Magnusson, T. (1957). 3, 4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature (Lond.), 180: 1200.

Celesia, G.G. and Jasper, H.H. (1966). Acetylcholine release from cerebral cortex in relation to state of activation. Neurology (Minneap) 16: 1053.

Chase, T.N. and Murphy, D.L. (1973). Serotonin and central nervous system function. Annual Review of Pharmacology 13: 181 - 197.

Chem, G. (1964). Antidepressive, analeptics and apetite suppressants. In Evaluation of Drug Activities. Pharmacometrics. Laurence, D.R. and Bachrach, A.L. (eds) Academic Press N.Y. 1: 239 - 260.

Cocchi, P., Silenzi, M., Calabi, G., and Salvi, G. (1980). Antidepressant effect of Vitamin C. Paediatrics. 65 (4): 862 - 863.

Costall, B., Naylor, R.J., and Pinder, R.M. (1974). Design of agents for stimulation of neostriatal dopaminergic mechanisms. Journal Pharmacy and Pharmacology 26: 753 - 762.

Cradon, J.H., Lund, C.C. and Dill, D.B. (1940). Experimental human scurvy. New English Journal of Medicine. 223: 353 - 369.

Curtis, D.R., Phillis, J.W. and Watkins J.C. (1959). Chemical excitation of spinal neurons. Nature (Lond.) 183: 611 - 612.

Dayton, P.G., Snell, M.M. and Perel, J.M. (1966) Ascorbic and dehydroascorbic acids in guinea pigs and rats. Journal of Nutrition. 88: 338 - 344.

Duffy, E. (1962). Action and behaviour. New York. Wiley.

Ehringer, H., and Hornykiewicz, O. (1960). Verteilung Von. Noradrenaline and dopamine (3-hydroxytyramin) im Gehirn des meschen und ihr verhalten bien Erkrankungen des extra pyramidalen systems. Klin. Wscher 38: 1236 - 1239.

Ernst, A.M. (1967). Mode of action of apomorphine and dex-amphetamine in gnawing compulsion in rats. Psychopharmacologia (Berlin). 10: 316 - 323.

Erube, A.J. (1982). Some behavioural and EEG effects of ascorbic acid in young chicks. B.Sc. (Pharmacy) degree project. Faculty of Pharmaceutical Sciences. Ahmadu Bello University.

Everett, G.M. and Wiegand, R.G. (1962). Central amines and behavioural states. A critique and new data. In "Proceedings of the first international pharmacological meeting." Borje Uvnas (ED). Pergamon Press. New York. P. 85 - 95.

Feldberg, W. and Sherwood, S.L. (1954). Behaviour of cats after itraventricular injections of eserine and DFP. Journal of Physiology 125: 488 - 500.

Ganong, W.F. (1987). Arousal mechanisms, sleep and electrical activity of the brain. Review of Medical Physiology. 13th Edition. Prentice-Hall International, Inc. P. 156 - 165.

Gellhorn, E. (1943). Autonomic regulations. Interscience Publ. Inc. New York.

Glatzel, H. (1966). Gibt et eine C-Hypervitaminose? Medical Klin. 61: 665 - 667.

Godwin, S.K., Murphy, D.L., Brody, H.K.H. and Bunney, W.E. (1970). L-Dopa, Catecholamines and behaviour. A clinical and biochemical study in depressed patients. Biol. Psychiat. 2: 341 - 366.

Goldstein, M., Anagnoste, B., Owen, W.S. and Battista, A.F. (1967). Effects of ventromedial tegmental lesions on the disposition of dopamine in the caudate nucleus. Brain Research 4: 298 - 300.

Hammarstrom, L. (1966). Autoradiographic studies on the distribution of carbon 14 - labl1ed ascorbic acid and dehydroascorbic acid. Acta Physiological Scandinavica 70 Suppl. P. 289.

Hartley, J.E., Miller, A.A,. and Webster R.A. (1981). Behavioural and Biochemical changes following withdrawal of chronic dexamphetamine or bupropion administration in rats. British Journal of Pharmacology 73: 268.

Harvey, S.C. (1985). Hypnotics and Sedatives. In: The Pharmacological Basis of Therapeutics. 7th Ed. Gilman, A.G., Goodman, L.S., Rall, T.W., Murad, F. (eds). MacMillan Co. Inc. New York. P.391.

Harvey, D., Bader, A. and Nagarkatti, J. (eds.). Catalog handbook of fine chemicals. Aldrich Chemical Company Inc. 1990. Milwaukee, Wisconsin U.S.A. P. 663.

Heath, H. and Fiddick, R. (1966). The active transport of ascorbic acid by the rat retina. Experimental Eye Research 5: 156 - 163.

Hess, W.R. (1955). Experimental data on hypothalamic mechanism of emotion. Archives of Neurology and Psychiatry 73: 127 - 129.

Holtz, P., Balzer, H., Westermann, E., and Wezler, E. (1957). Beeinflussung der Evipannarkose durch Reserpine, iproniazid and biogene amine. Archiv fur Experimentalle Pathologie and Pharmakologie, 231: 333 - 336.

Hornykiewicz, O. (1966). Dopamine (3, hydroxytryptamine) and brain function. Pharmacological Reviews 18: 925 - 964.

Inokuchi, A. and Oomura, Y. (1986). Effects of sleep-promoting substance on rat hypothalamic neuron activity. Brain research bulleLin 16: 429 - 433.

Inoue, S. (1985). Endogenous sleep substances and sleep regulation. Onoue, S., and Borbely, A.A. (editors). Academic Societies Press. Tokyo/VNU Science Press BV, Utrecht. P. 3 - 12.

Izquierdo, J.A. Jofre, I.J. and Acevedo, C. (1968). The effect of ascorbic acid on the cerebral and adrenal catecholamine content in the male rat. Journal of Pharmacy and Pharmacology. 20: 210 - 214.

Jacobsen, E. (1964). Tranquilizers and Sedatives. In: Evaluation of Drug activities. Pharmacometrics. Laurence, D.R. and Bachrach, A.L. (editors). Academic press New York. 1: 213 - 237.

Johnson, J.L. (1972). Glutamic acid as a synaptic transmitter in the nervous system. Brain Research. 37: 1 - 19.

Kebabian, J.W., Petzhold, G.L. and Greengard, P. (1972). Dopamine - Sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity of the dopamine receptor. Proc. National Academy of Sciences U.S.A. 69: 2145 - 2149.

Krynjeric, K. and Phillips, J.W. (1963). Pharmacological Properties of acetylcholine-sensitive cells in the cerebral cortex. Journal of Physiology 166: 328 - 350.

Kubala, A.L. and Katz, M.M. (1966). Nutritional factors in Psychological test behaviour. Journal of Genetic Psychology. 96: 343.

Kuo, C.H., Hata, F., Yoshida, H., Yamatodani, A. and Wada, H. (1979). Effect of ascorbic acid on release of acetylcholine from synaptic vesicles prepared from different species of animals and release of noradrenaline from synaptic vesicles of rat brain. Life Sciences 24: 911 - 916.

Langlais, P.J., Huang, P.C. and Gabay, S. (1975). Regional Neurochemical studies on the effect of B, B'-iminodipropionitrile (IDPN) in the rat. Journal of Neuroscience Research. 1: 419 - 435.

Legendre, R. and Pieron, H. (1910). Le probleme des facteurs du sommeil. Resultats d' injections vasculaires et intracerebrales de liquides insomniques. C.R. Soc. Biologie (Paris) 68: 1077 - 1079.

Leimdofer, A. (1948). A pulmonary cardiovascular reflex in man. Federation Proc. 6: 151.

Lelland, C.T., Thomas, T.N., Middaugh, L.D., and Zemp, J.W. (1979). Effect of ascorbic acid on neurochemical, behavioural and physiological systems mediated by catecholamines. Life Sciences 25: 2189 - 2195.

Lidbrink, P. (1974). The effect of lesions of ascending noradrenaline pathways on sleep and waking in the rat. Brain Research. 74: 19 - 40.

Lyhs, L. (1961). Influence of ascorbic acid deficiency on the functions of the CNS of the guinea-pigs. Monatsscher Viterinarmed. 16: 131 - 134.

Lyhs, L. and Rvidiger, W. (1963). Functional decortication and content of ascorbic acid in the cerebrum of rats. Acta Biologie et. Medicine Germanica 10: 311 - 316.

Lyhs, L. and Tegeler, G. (1963). Uber den Einfluss der Dehydroaskorbin saure auf die bedingtreflektorische Tafigkeit bei Meerschweinchen. Acta biologie et. medicine Germanica 11: 182 - 188.

Martin, G.R. (1961). Studies on the tissue distribution of ascorbic acid. Annual New York Academy of Science 92: 141 - 147.

Martin, G.R. and Mecca, C.E. (1961). Studies on the distribution of L-ascorbic acid in the rat. Archs Biochemistry and Biophysics. 93: 110 - 114.

Martini, F. (1989). Information processing and higher-order functions. In: Fundamentals of Anatomy and Physiology. 1st Ed. Prentice-Hall Inc. New Jersey P. 391 - 402.

Masek, J., Hruba, F., and Novakova (1976). Vitamin C and vigilance. Review of Szech. Medicine 22: (4): 209 - 15.

Mignot, G. and Savini, E.C. (1981). Dopaminergic stereotyped and turning behaviour in the rabbit. British Journal of Pharmacology 73: 270.

Miledi, R. (1973). Transmitter release induced by injection of calcium ions into nerve terminals. Proc. R. Society of London. B 183: 421 - 425.

Morgan, C.T. (1965). Physiological Psychology. New York: McGraw-Hill.

Morgane, P. (1964). Limbic-hypothalamic-midbrain interaction in thirst and thirst-motivated behaviour. In: "Thirst". M.J. Wayner (editor). Pergamon. London, P 94 - 126.

Nauta, W.J.H. (1946). Hypothalamic regulation of sleep in rats. An experimental study. Journal of Neurophysiology 9: 285 - 316.

Osuide, G. and Wambebe, C. (1980). Antagonism of Pentobarbitone sleep by dopamine, Levodopa and apomorphine in chicks. Clinical and Experimental pharmacology and physiology 1: 237 - 248.

Patterson, J.W. and Mastin, D.W. (1951). Some effects of dehydroascorbic acid on the central nervous system. American Journal of Physiology 167: 119 - 126.

Pauling, L. (1970). Vitamin C and the common cold. Freeman, W.H. (editor). San Fransisco.

Pfeiffer, C.C., Goldstein, L., Murphree H.B., and Nicols, R.C. (1968). A critical survey of possible biochemical stimulants. In: Efron, D.H. (ed) Psychopharmacology - a review of progress. Department of Health, Education and Welfare, Washington D.C.

Pitt, B. and Pollit, N. (1971). Ascorbic acid and chronic schizophrenia. British Journal of Psychiatry. 118: 227 - 228.

Pitt, B. (1974). Vitamin C and Schizophrenia (Letter). Lancet. 2: 1153 - 1154.

Randrup, A. and Jonas, W.J. (1967). Brain dopamine and the amphetamine-reserpine interaction. Journal of Pharmacy and Pharmacology 19: 483 - 484.

Randrup, A. and Munkvad, I. (1967). Stereotyped activities produced by amphetamine in several animal species and man. Psychopharmacologia. 11: 300-310.

Roy, R.N. and Guha, B.C. (1958). Species difference in regard to the biosynthesis of ascorbic acid. Nature (Lond.). 182: 319-320.

Ruch, T.C. and Patton, H.D. (1966). Mechanisms altering intensity and direction of behaviour. In: Physiology and Biophysics. 19th Ed. W.B. Saunders Co. (Lond). P. 496.

Ruch, T.C. and Patton, H.D. (1966). Prefrontal areas, Homotypical cortex - The Association Areas. In: Physiology and Biophysics. 19th Ed. W.B. Saunders Co. (Lond). P. 465 - 479.

Sacks, W. and Simpson, G.M. (1975). Ascorbic acid in levodopa therapy (letter). Lancet 1: 527.

Salles, S.S.M. and Salles, K.S. (1979). Behavioural effect of selective and non-selective lesions of median raphe nucleus in the rat. Japanese Journal of Physiology 30(1): 105 - 114.

Sears, M.L. (1969). Vitamin C as a requirement for the storage of norepinephrine by the iris. Biochemical Pharmacology 18: 253 - 256.

Schildkraut, J.J., Watson, R., Draskoczy, P.R. and Hartmann, E. (1971). Amphetamine withdrawal: Depression and M.H.P.G. exertion. Lancet, 11: 485 - 486.

Schneider, W. and Staudinger, H. (1964). Zum Wirkungsmechanismus von Vitamin C. Klin. Wschr. 42: 879 - 884.

Shimizu, N., Matsunami, T. and Oshini, S. (1960). Histochemical Demonstration of Ascorbic Acid in the Locus coeruleus of the Mammalian Brain. Nature 186: 479 - 480.

Sjostrand, S.T. (1970). Pharmacological properties of dehydroascorbic acid: Effects on the central and peripheral nervous system in experimental animals. Acta Physiologica Scandinavica Supplementum 356: 1.

Slater, G. (1962). Adrenal ascorbic acid and plasma adrenocarticoid response to fasting in young rats. Endocrinology 70: 18 - 23.

Sokomba, E.N. (1980). Some studies on drug-induced behaviour in young chicks (Ph.D. Thesis in Pharmacology). Ahmadu Bello University. P. 114 - 152.

Spector, R. and Lorenzo, A.V. (1974). Specificity of ascorbic acid transport system of the central nervous system. American Journal of Physiology 226 (6): 1468 - 1473.

Spooner, C.E. (1965). Preparation and implantation of brain electrodes in the young chick. Electroencephalography and Clinical Neurophysiology 18: 419 - 421.

Steinberg, H. (1964). Editor: Animal Behaviour and Drug Action. J. and A. Churchill, Ltd. London.

Stellar, E. (1954). The Physiology of motivation. Psychological Reviews 61: 5 - 22.

Subramanian, N. (1977). On the brain ascorbic acid and its importance in metabolism of biogenic amines. Life Sciences. 20 (9): 1479 - 1484

Szent-Gyorgyi, A. and Haworth, W.N. (1933). `Hexuronic acid' (ascorbic acid) as the antiscorbutic factor. Nature (Lond). 131: 24.

Tavernor, W.D. (1976). Anaesthesia. In: The UFAW Handbook on the Care and Management of laboratory Animals. 5th Edition UFAW Churchill and Livingstone, N.Y. (editors) P. 150 - 159.

Tolbert, L.C., Thomas, T.N. Middaugh, L.D. and Zemp, J.W. (1979). Effect of Ascorbic acid on Neurochemical, Behavioural and Physiological systems mediated by catecholamines. Life Sciences 25: 2189 - 2195.

Tonge, S.R. (1974). Noradrenaline and 5-hydroxytryptamine metabolism in six areas of rat brain during post amphetamine depression. Psychopharmacologia (Berl). 38: 181 - 186.

Trulson, M.E. and Jacobs, B.L. (1979). Chronic amphetamine administration decreases brain tryptophan hydroxylase activity in cats. Life Sciences 26: 329 - 335.

Van Rossum, J.M. (1968). Significance of dopamine receptor blockade for the action of neuroleptics. Excerpta. Medicine Internat. Congr. P. 129.

Van Rossum, J.M. and Hurkmans, J.A. (1964). Mechanism of action of psychomotor stimulant drugs. Significance of dopamine in locomotor stimulant action. International Journal of Neuropharmacology 3: 227 - 239.

Vitler, R.W., Sebrell, W.H. and Harris, R.S. (1967). The Vitamins Ed. In: Academic Press, New York 1: 487.

Vogel, W.H., Orfei, V. and Century, B. (1969). Activities of enzymes, involved in the formation and destruction of Biogenic amines in various areas of human brain. Journal of Pharmacology 165(2): 196 - 203.

Wambebe, C. and Osuide, G. (1983). Influence of FLA-63 on pentobarbitone sleep in chicks and rats. Clinical and experimental pharmacology and physiology. 10: 555 - 566.

Wambebe, C. and Sokomba, E. (1986). Some behavioural and EEG effects of ascorbic acid in rats. Psychopharmacology. 89: 167 - 170.

Webster, J. (1971). Vitamin C. The protective vitamin. Award Book, New York.

APPENDICES

Appendix 1

TABLE 3.3: Gross Behavioural activities were measured in acute administration of different doses of ascorbic acid (A-G)

Behavioural Parameters	A: 5 mg/kg i/p Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM	B: 10mg/kg i/p Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM
CLIMBING	10	13	9	10	14	56	11.2	0.96	3	6	13	15	16	53	10.6	2.58
SCRATCHING	37	49	39	32	47	204	40.8	3.16	48	50	43	45	49	235	*** 47.0	1.30
FEEDING	0	0	0	0	0	0	0	0	0	0	0	5	5	10	2.0	1.22
SNIFFING	8	10	8	6	7	39	7.8	0.66	10	9	7	11	13	50	*** 10.0	0.99
REARING	0	4	0	5	0	9	1.8	1.11	4	6	2	6	9	27	*** 5.4	1.16
GROOMING	13	13	10	11	8	55	11.0	0.94	6	11	13	9	13	52	10.4	1.32
STEREOTYPED MVT.	0	5	5	4	0	14	2.8	1.15	1	4	2	3	4	14	2.8	0.58
AGGRESSION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LOCOMOTOR ACTIVITY	10	26	10	11	12	69	13.8	3.07	30	20	32	30	35	147	*** 29.4	2.52

Number of activities. Count per 60min. in 5 hours (1 - v).
* P < .05, ** P < .02, *** P < .01 compared to physiological saline control; Student's t-test. n = 5 per experiment.

	C: 20 mg/kg i/p								D: 40 mg/kg i/p							
Behavioural Parameters	Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM	Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM
CLIMBING	14	13	16	3	4	50	10.0	2.70	8	13	10	12	7	50	10.0	1.14
SCRATCHING	47	55	45	48	41	236	** 47.2	2.28	62	57	51	53	55	278	** 55.6	1.88
FEEDING	4	2	2	1	1	10	2.0	0.54	2	0	5	11	0	18	3.6	2.06
SNIFFING	12	5	11	13	16	57	** 11.4	1.80	15	13	16	17	11	72	*** 14.4	1.07
REARING	5	6	6	5	2	24	*** 4.8	0.73	5	1	4	6	3	19	* 3.8	0.86
GROOMING	18	15	10	20	11	74	** 14.8	1.93	20	29	18	34	13	114	*** 22.8	3.81
STEREOTYPED MVT.	2	5	4	7	5	23	** 4.6	0.81	5	3	3	9	6	26	* 5.2	1.11
AGGRESSION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LOCOMOTOR ACTIVITY	24	10	20	18	21	93	* 18.6	2.35	17	15	24	28	24	108	*** 21.6	2.12

Number of activities. Count per 60min. in 5 hours (1 - v).
* P < .05, ** P < .02, *** P < .01 compared to physiological saline control;
Student's t-test. n = 5 per experiment.

	E: 80 mg/kg i/p								F: 160 mg/kg i/p							
Behavioural Parameters	Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM	Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM
CLIMBING	20	12	7	10	14	63	12.6	2.18	6	4	3	3	4	20	*** 4.0	0.54
SCRATCHING	68	59	70	90	68	355	*** 71	5.11	25	14	34	19	23	115	*** 23.0	7.44
FEEDING	3	0	0	0	2	5	1.0	0.63	2	0	0	0	0	2	0.4	0.39
SNIFFING	20	14	10	14	20	78	*** 15.6	1.93	7	5	9	8	6	35	7.0	0.70
REARING	14	8	6	3	10	41	*** 8.2	1.85	0	0	0	5	0	5	1.0	0.99
GROOMING	23	30	34	15	24	126	*** 25.2	3.24	11	9	6	8	7	41	8.2	0.86
STEREOTYPED MVT.	8	7	2	8	9	34	*** 6.8	1.24	8	7	4	6	4	29	* 5.8	0.79
AGGRESSION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LOCOMOTOR ACTIVITY	20	26	22	20	24	112	*** 22.4	1.16	18	13	10	11	16	68	13.6	1.50

Number of activities. Count per 60min. in 5 hours (1 - v).
* P < .05, ** P < .02, *** P < .01 compared to physiological saline control;
Student's t-test. n = 5 per experiment.

Behavioural Parameters	G: 320 mg/kg i/p Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM	H: CONTROL Physiological saline i/p Activities 1	11	111	1v	v	TOTAL	MEAN	± SEM
CLIMBING	2	2	0	0	0	4	*** 0.8	0.48	9	10	7	9	14	49	9.8	1.15
SCRATCHING	25	20	24	20	16	105	*** 21.0	1.61	40	30	44	41	40	195	39.0	2.36
FEEDING	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0.2	0.20
SNIFFING	2	0	2	0	0	4	*** 0.8	0.48	7	6	4	5	6	28	5.6	0.50
REARING	0	0	0	0	0	0	0	0	3	0	0	0	2	5	1.0	0.63
GROOMING	8	7	5	4	5	29	*** 5.8	0.73	10	6	10	7	11	44	8.8	0.96
STEREOTYPED MVT.	7	9	9	7	6	38	*** 7.6	0.59	3	2	2	1	2	10	2.0	0.31
AGGRESSION	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LOCOMOTOR ACTIVITY	20	13	16	17	13	79	15.8	1.31	12	9	10	14	14	59	11.8	1.01

Number of activities. Count per 60min. in 5 hours (1 - v).
* P < .05, ** P < .02, *** P < .01 compared to physiological saline control;
Student's t-test. n = 5 per experiment.

Appendix 2

A. STATISTICS

A.1 SHARP EL-506P Scientific calculator was used for the statistical calculations.

A.2 Formula for Standard Error of Mean (SEM),

$$SEM = \frac{S}{\sqrt{n}}$$

S = Standard deviation

n = Size of the sample

ie. when 10 microlitres is drawn out of this stock solution, it will contain 20 micrograms of the drug.

Appendix 4

Implantation of the electrocortical and electromyogram electrodes in the skull of rat.

Locating the FC, OC and BSRF using the Standard Co-ordinates:

Example:

With reference to Bregma, let the readings at zero in stereotaxic machine be: AP = 58.9 mm

L = 19.7 mm

V = 22.1 mm

Thus,

	ON THE SKULL OF RAT: CO-ORDINATES
FC: AP = 58.9 + 1.9 mm	60.8 mm
L = 19.7 + 1.5 mm	21.2 mm

OC: AP = 58.9 - 9.2 mm 49.7 mm

L = 19.7 - 2.8 mm 16.9 mm

BSRF: AP = 58.9 - 3.8 mm 55.1 mm

L = 19.7 + 2.0 mm 21.7 mm

V = 22.1 - 8.2 mm 13.9 mm

For the Standard (Stereotaxic) co-ordinates for implantation in rat, see Chapter 2, sub Section 2.5.1.