CHANGES IN SLEEP PARAMETERS AND AMINERGIC FUNCTIONING IN WALKER-WALKER AND SPONTANEOUSLY HYPERTENSIVE RATS

by

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ABSTRACT

The importance of brain monoaminergic systems in mediating sleep-waking behaviour is attested by an impressive array of research evidence, although the specific relationships which have been proposed remain highly controversial. Confusion in this area stems from the problem that the biogenic amines do not cross the blood-brain barrier, thus most research has involved relatively gross biochemical or anatomical manipulations.

The present study was undertaken using two strains of rat in which preliminary evidence suggested the presence of naturally-occurring abnormalities in peripheral and central monoaminergic functioning. Investigations of the Walker-Walker strain of rat revealed significant changes in the composition and duration of the sleep-waking cycle manifested by reductions in deep wave sleep (SWS) and paradoxical sleep (PS) which were compensated by increases in shallow SWS and waking states. Spectral analysis of the EEG using fast-Fourier transform demonstrated significant group differences during deep SWS. Walker-Walker rats showed reductions in power over a wide range of frequencies (1.56 - 25.0 H_z) in comparison to controls. Further analysis revealed that the deep SWS of the Walker-Walker strain was similar to the shallow SWS of the control strain. Although profound reductions in levels of blood platelet serotonin were observed in Walker-Walker rats,

whole brain levels of serotonin showed slight but non-significant reductions. The findings suggested that the abnormalities in peripheral serotonergic functioning in the Walker-Walker strain may reflect disordered metabolism of this monoamine within the central nervous system, and that normal serotonergic functioning may be necessary for the patterns of SWS and PS to become fully manifest. It was further suggested that low levels of serotonin storage or storage capacity may be a common denominator between pigmentary dilution, docility, changes in sleep parameters and hemorrhagic tendency in the Walker-Walker strain.

Investigations of the spontaneously hypertensive rat (SHR) demonstrated significant reductions in deep SWS and PS in comparison to normotensive controls (WKy). These reductions were accompanied by slight but nonsignificant increases in shallow SWS and waking states. Systolic blood pressure level showed a significant inverse relationship to total duration of deep SWS. Computerized spectral analysis of the EEG showed significant reductions in power in frequency bands between 3.61 - 25.0 H_z during awake and all sleep states in the SHR. These reductions were greatest in hypertensive rats receiving 0.9% saline instead of tap water for drinking. The findings of increased EEG activation during the waking state and increased EEG desynchrony during SWS suggested intrinsic differences in arousability in the SHR. The results were discussed with reference to

reports of neurochemical differences in the SHR. It was concluded that the findings are best explained in terms of increased arousability of the SHR, probably as a result of central factors.

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This thesis is dedicated to one who was a source of constant encouragement and inspiration throughout my graduate career

TO MY FATHER

FRANCIS (FRANK) McCLINTOCK

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CHAPTER 1

INTRODUCTION

In recent years considerable attention has focussed on the functional role of the monoamines (dopamine, norepinephrine, epinephrine and serotonin) in the CNS. These substances are the subject of intensive interest and research primarily because of their putative role in brain function. Accumulating evidence from over two decades of research now suggests that these substances function as synaptic neurotransmitters, since they fulfill many of the previously established criteria for neurochemical transmission (McLennan, 1967).

Considerable information is presently available concerning the anatomical distribution of monoaminergic systems in the CNS, through the now classic histochemical fluorescent studies of Dahlstrom and Fuxe (1964). Most serotonin-containing neurons have been identified within the midline raphe nuclei of the medulla, pons and lower midbrain (Anden, Dahlstrom, Fuxe and Larsson, 1965; Dahlstrom and Fuxe, 1964). Noradrenergic cell bodies have been identified in the medulla, pons and diencephalon, in cell formations which are lateral to the midline (Anden Dahlstrom, Fuxe, Olson and Ungerstadt, 1966; Fuxe, 1965) with highest concentrations in the locus coeruleus.

Despite the relative wealth of neuroanatomical information on monoaminergic systems in the CNS, the functional role of these monoamines in behaviour is less well

understood. The anatomical data appear to provide some support for the existence of a functional interaction between serotonergic and noradrenergic systems in the brain. This concept of the existence of two central opposing systems regulating brain function was proposed in the 1950's by Brodie and Shore (1957). Their now classic hypothesis proposed that the monoamines serotonin and norepinephrine mediate physiologically antagonistic systems which regulate brain functioning. This hypothesis, based pharmacological evidence, and on Hess' (1954) concept of ergotropic and trophotropic subcortical divisions coordinating autonomic, somatic and psychological functioning, proposed that norepinephrine (NE) may mediate sympathetic responses to produce arousal. On the other hand trophotropic responses, which are considered primarily restorative in nature, were presumably mediated by serotonin (5-Hydroxytryptamine) (5-HT).

Due to considerable metholological advances in the neurosciences this hypothesis has recently been challenged. Psychopharmacological studies using parachlorophenylalanine (PCPA) - a substance which blocks serotonin synthesis (Koe and Weissman, 1966) have cast doubt on the concept that serotonin is the mediator for a central parasympathetic (trophotropic) system. However, a variety of research evidence does suggest a mutual involvement of serotonin and norepinephrine in a variety of behaviours. Evidence now suggests that NE and 5-HT exert antagonistic

effects on body temperature regulation (Feldberg and Myers, 1964) sexual behaviour (Gessa and Tagliamonte, 1974), goal directed behaviour (Stein and Wise, 1968) and sleep (Jouvet, 1969).

The Jouvet (1969) neurochemical model of sleep is basically an extensive modification of the Brodie-Shore hypothesis. According to this model, serotonin and norepinephrine in the brain function antagonistically to regulate the cortical synchronizing and desynchronizing mechanisms which operate during sleep and waking.

Since the present research is primarily concerned with the neurochemistry of sleep, Jouvet's hypothesis and the variety of research evidence on which it is based will be presented in some detail. However, before discussing the biochemical and pharmacological data, it seems appropriate to first consider some aspects of sleep in general, and the dichotomy of sleep in particular.

Electrophysiological and Behavioural Correlates of Sleep

Since the classic work of Aserinsky and Kleitman (1955) and Dement and Kleitman (1957) demonstrating cyclic changes in electrophysiological and behavioural activity during sleep, earlier misconceptions of sleep as a unitary state of rest accompanied by slow cortical activity necessarily became abandoned. Evidence that the mammalian sleeping brain successively passes through specific qualitatively different states became evident from polygraphic studies.

These cyclic changes were observed in a variety of mammalian species (Allison and Cicchetti, 1976; Jouvet, 1962) including rats (Roldan, Weiss and Fifkova, 1963; Swisher, 1962; Van Twyer, 1969). Research has demonstrated that mammalian sleep is composed of at least two qualitatively distinct states which are considered to be mediated by different neural systems (Jouvet, 1962) and by different neurochemical transmitters (Jouvet, 1967). These two states have been labelled telencephalic or slow wave sleep (SWS) and rhombencephalic, fast-wave, or paradoxical sleep (PS) also referred to as rapid eye movement sleep (REM) (Jouvet, 1961; 1967).

Most mammals show periodic shifts from SWS to PS, and these states are easily recognizable by polygraphic techniques using electrodes permanently implanted in the organism's brain. During slow wave sleep the laboratory rat adopts a posture characteristic of sleep with eyes semi-closed. Although muscle activity gradually decreases to low levels, a degree of postural tonus remains in some muscle groups, including the neck muscles (Jouvet, 1969). The EEG during this stage of sleep is characterized by high voltage slow waves.

With the onset of paradoxical sleep (or REM sleep) a fast low voltage cortical activity similar to that of waking occurs in conjunction with complete atonia of the neck muscles. These changes in tonic activity are

accompanied by periodic rapid eye movements, muscle twitches, vibrissae and marked autonomic variability. These phasic changes appear to be associated with the occurrence of intermittent bursts of large fast waves, termed ponto-geniculo-occipital (PGO) spikes (Jouvet, 1969).

The presence of PGO spikes in the lateral geniculate (LGN) and visual cortex of the cat during PS has been well documented, but researchers have reported their absence in the LGN of the rat (Stern, Forbes and Morgane, (1974). However, recent investigators (Farber, Marks, Barwise and Roffwarg, 1976) have reported a pontine sharp wave which, it its electrophysiological characteristics and distribution, appears to correspond to the pontine PGO wave of the cat. This evidence now appears to suggest that the brainstem mechanisms responsible for PGO spikes in the cat are also present in the rat (Reiner and Morrison, 1976). The major electrophysiological and behavioural correlates of sleep in the rat are summarized in Table 1.

The Ontogeny of Sleep-Wakefulness Behaviour

The ontogeny of sleep-wakefulness behaviour has been extensively studied in humans (Kleitman and Engelmann, 1953; Parmelee, Wenner and Schultz, 1964; Parmelee, Schulte, Akiyama, Wenner, Schultz and Stern, 1968; Petre-Quadens, 1967) and other mammalian species (Jouvet-Mounier, Astic and Lacote, 1970; Shimizu and Himwich, 1968). These investigations have shown that slow wave sleep (or quiet sleep)

Table 1. Electrophysiological and Behavioural Correlates of Sleep in the Rat

	Awake	Slow wave sleep (SWS)	Paradoxical sleep (REM)
Cortical Activity	low voltage fast waves	spindles and high voltage slow waves	low voltage fast waves
Hippocampal Activity	rhythmic theta waves	irregular high voltage slow waves	regular theta waves
E.M.G.	high amplitude	low amplitude	isoelectric with twitches
Eye Movement	present	none	biphasic bursts
Behaviour	sitting up or lying head up eyes open	sitting or lying eyes open or semi-closed	complete atonia lying flat or curled up twitching of extremities, neck muscles, vibrissae irregular respiration
Duration/Total Sleep Time		70-80%	20-30%

gradually increases in amount as the organism matures. This increase appears to be primarily a function of maturation of the nervous system and not environmental experience, since the newborns of species whose central nervous system is well developed at birth (eg the guinea pig) have alternating periods of slow wave sleep and paradoxical sleep which resemble the adult pattern in form and amount (Jouvet-Mounier et al, 1968).

In the adult laboratory rat, slow wave sleep generally occupies between 80 - 90% of total sleep time with PS occupying the remaining 10 - 20%, and stability in the rat's sleep EEG over the major portion of the life span has been documented (Rosenberg, Zepelin and Rechtschaffen, 1976).

Possible Monoaminergic Mechanisms Involved in Sleep - the Jouvet Hypothesis

In its simplest form, the Jouvet (1969) neurochemical model of sleep proposes that the normal adult mammalian brain undergoes cyclical changes from waking, to slow wave sleep, and finally to paradoxical sleep in a specific sequence: transitions from waking to SWS to PS are reversible, however, the final step from PS to waking is never reversed.

At the core of Jouvet's monoaminergic theory is the view that SWS depends upon the release of serotonin from neurons located primarily in the Raphe nuclei of the brain stem, whereas the induction of PS depends on the action of a deaminated metabolite of serotonin in conjunction with the

release of noradrenaline from the locus coeruleus. During the transition from SWS to PS, monoamine oxidase (MAO) is hypothesized to play a major role. Jouvet postulates that the mechanism responsible for SWS "primes" and triggers PS, thus the first step of PS appears to be MAO dependent. However, noradrenergic mechanisms and cholinergic mechanisms operate in conjunction with one another to produce the tonic aspects of PS.

Although a convincing body of research evidence does provide support for the view that serotonin and norepinephrine are important for the induction and maintenance of SWS and PS, the specific relationships proposed by Jouvet remain highly controversial. Relationships which are the direct opposite of those postulated by Jouvet have also been proposed (Wyatt, Engleman, Sjoerdsma and Snyder, 1970), although the evidence on which they are based is equally equivocal (Williams, Holloway and Griffiths, 1973).

Relation of Chemical Systems and Sleep: Methodological Problems

Undoubtedly confusion in this area stems primarily from a major intrinsic methodological problem in studies of this nature - a problem which is intrinsic to the determination of the physiological role of the monoamines in regulating various behaviours, namely that serotonin and norepinephrine do not cross the blood-brain barrier. Manipulations of brain levels of these amines have thus been

necessarily indirect, involving either the administration of metabolic precursor substances, or drugs which alter the uptake, storage or metabolism of serotonin and/or norepinephrine. However, the action of administered pharmacological agents is not usually specific, in fact agents such as para-chlorophenylalamine (P-CPA) and alpha-methyl-paratyrosine (α-MPT), which were previously considered to have selective effects, have now been found to induce additional neurochemical changes (Lane, Smith, Shea and McBride, (1976). Table 2 summarizes some of the strategies used to alter levels of functional 5-HT in the brain, and the concomitant problems. To facilitate further discussion, the metabolic pathways of serotonin (5-hydroxytryptamine) and the catecholamines are illustrated in Figures 1 and 2.

Thus, as Weissman (1973) as noted, there are no completely specific known means to either elevate or diminish 5-HT receptor activity. Each of the strategies used to increase or decrease functional levels of 5-HT in the brain (Table 2) has certain limitations. Lack of specificity to varying degrees is found with the usual known methods of depleting brain 5-HT levels. Reserpine which was first shown by Pletcher, Shore and Brodie (1955) to reduce levels of endogenous 5-HT was subsequently found to deplete central levels of norepinephrine (Carlsson, Rosengren, Bertler and Nilsson, 1957).

PCPA, an inhibitor of the enzyme tryptophan hydroxylase, blocks the synthesis of 5-HT. However, it slightly

Table 2. Methods used to Alter Levels of Functional 5-HT in Brain

	Method	Effect on 5-HT	Problems
1.	5-HT releasers: reserpine	↓	↓catecholamines
2.	Inhibition of 5-HT synthesis: PCPA	↓	"experimental PKU"
3.	Lesions to serotonergic systems: raphe destruction	↓	damage to other tracts
4.	5-HT receptor blockade: LSD	turnover↓	other blocking effects
5.	Tryptophan deficient diet	↓	nutritional deficits
6.	5-HT precursors: 5-HTP, tryptophan	↑	metabolism via non 5-HT pathways
7.	MAO inhibitors	↑	interactions with catechol - and other amines
8.	Low/high endogenous levels: genetic inbred strains carcinoid syndrome	↓/↑	other genetic-linked traits or factors which may co—vary

FIGURE 1

Major metabolic pathway in the formation of serotonin (5-hydroxytryptamine).

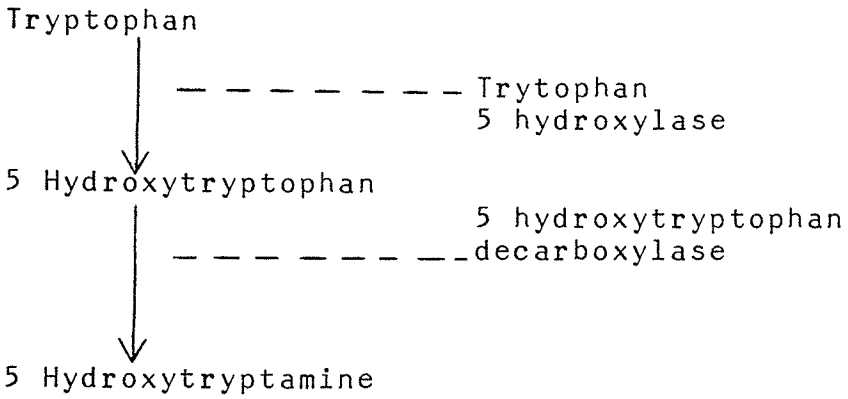
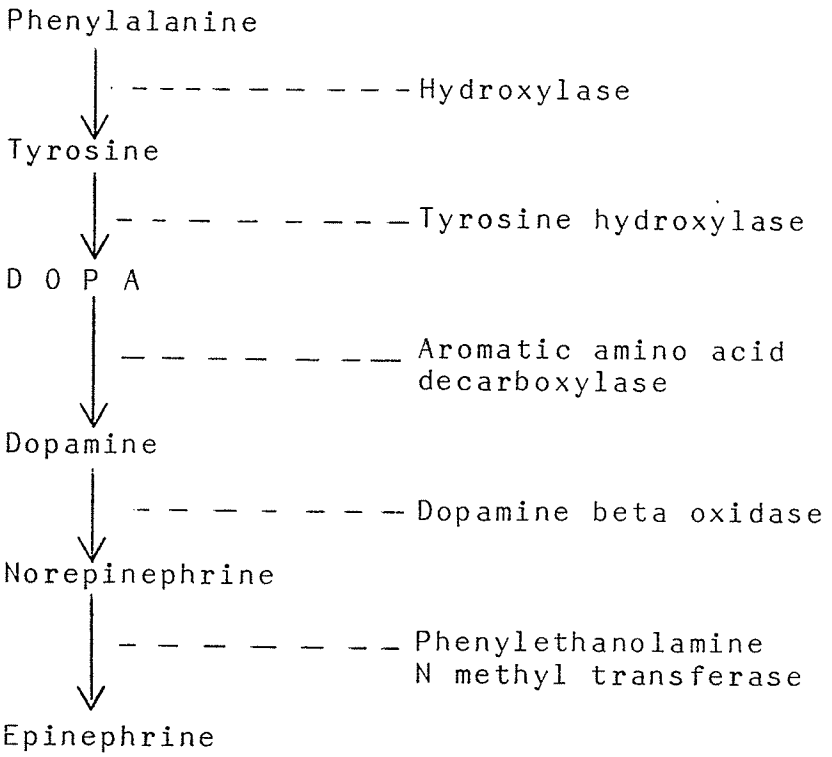

Tryptophan
Trytophan
5 hydroxylase
5 Hydroxytryptophan
5 hydroxytryptophan
decarboxylase
5 Hydroxytryptamine

FIGURE 2

Metabolic pathway in the formation of catecholamines.


Phenylalanine
Hydroxylase
Tyrosine
Tyrosine hydroxylase
D O P A
Aromatic amino acid decarboxylase
Dopamine
Dopamine beta oxidase
Norepinephrine
Phenylethanolamine N methyl transferase
Epinephrine

but significantly depletes the brain of catecholamines for a few hours after its administration (Koe and Weissman, 1966). Furthermore, Lipton, Gordon, Guroff and Undenfriend (1957) reported that PCPA causes a build up of phenylalanine and phenylalanine metabolites in the blood, thus as Resnick (1972) has noted, in essence, PCPA treatment in animals results in a biochemical state of phenylketonuria ("experimental PKU").

Comparable difficulties in lack of specificity are also found with methods of increasing functional 5-HT. Administration of the precursor substances tryptophan or 5-HTP, in addition to elevating brain 5-HT levels, leads to the metabolism of substrates (tryptophan) through other pathways, to a non-physiological distribution of 5-HT (eg after 5-HTP) in brain, and also to peripheral effects. Monoamine oxidase inhibitors (MAOI), although they do elevate levels of brain 5-HT, also exert a variety of other physiological effects (Goodman and Gilman, 1970).

As a result of these problems, it is difficult to draw definite conclusions from some of the experimental literature concerning the complementary role of serotonergic and catecholaminergic systems in NREM and REM sleep. Although the evidence to date strongly indicates an involvement of the monoamines in sleep activity, the specific relationships which have been proposed remain less clear.

The Jouvet hypothesis of sleep is based upon extensive converging neurophysiological and neuropharmacological

studies conducted primarily with cats. These studies and others will be subsequently presented in greater detail. However, a preliminary overview of this evidence indicates that the manipulation of brain levels of serotonin, either by pharmacological agents (eg PCPA), or by destruction of serotonergic centers in the brain (the raphe nuclei), cause reductions in slow wave sleep. On the other hand, reductions in brain levels of noradrenaline (eg by α - MPT) or by destruction of noradrenergic centers (the locus coeruleus) cause reductions in PS. These findings constitute the main body of evidence in support of Jouvet's hypothesis.

Review of the Literature

(a) Serotonergic Control of SWS: Supporting Evidence

As previously discussed, since serotonin does not cross the blood-brain barrier, most methods used to define its role in mediating sleep have been indirect. However, in organisms such as chickens whose blood-brain barrier is permeable, administration of 5-HT has been found to induce cortical synchronization and sleep (Spooner and Winters, 1965). Consistent with these findings are reports of abrupt and prolonged EEG synchrony in cats, following the administration of serotonin either intraventricularly (Koella and Czicman, 1966), intraarterially (Roth, Walton, and Yamamoto, 1970) or intravenously (Bradley, 1958).

Attempts to increase brain levels of 5-HT by per-

ipheral administration of its precursor 5-hydroxytryptophan (5-HTP) have resulted in potentiation of SWS and a temporary suppression of PS in the cat (Glassen and Mantegazzini, 1960; Jouvet, 1969). Since the duration of SWS increase in these studies was paralleled by the increase of 5-HT from exogenous 5-HTP, it is a reasonable assumption that the SWS increase was the result of an increase in endogenous 5-HT levels. However, Jouvet (1967) has cautioned that 5-HTP may act directly upon systems other than the serotonergic neurons, furthermore, 5-HTP may also have some inhibitory effect upon DOPA decarboxylase (McGeer, McGeer and Wada, 1963) thereby decreasing levels of brain catecholamines. Similar increases in SWS have also been reported following the administration of L-tryptophan in rats (Hartmann, 1967) cats (Jouvet, 1971b) and humans (Hartmann, 1967b; Griffiths, Lester, Coulter and Williams, 1972; Wyatt, Engleman, Kupfer, Fram, Sjoerdsma and Snyder, 1970). However, as was previously discussed under methodological problems, alterations in sleep produced by L-tryptophan may be associated with metabolic routes other than the brain serotonergic system.

Pharmacologically – induced reductions in cerebral 5-HT levels by reserpine have been shown to suppress both SWS and the tonic components of PS while also triggering long lasting PGO activity. Further attemps to restore 5-HT levels following reserpine by administration of its precursor 5-HTP, have demonstrated an immediate reappearance of the

EEG patern typical of SWS. On the other hand, administration of DOPA, which restores brain catecholamine levels, resulted in the reappearance of the tonic aspects of PS (Jouvet 1967).

One of the most widely studied drugs in relation to its effects on sleep is PCPA. As previously noted, PCPA decreases 5-HT concentrations in brain by inhibiting tryptophan hydroxylase - the rate-limiting enzymatic step in 5-HT synthesis (shown in Figure 1). Single high dosages of PCPA produce no alterations in behaviour or EEG recording during the first 14 - 18 hours. Thus the drug in itself appears to have no direct pharmacological action upon the brain. Following this period an abrupt decrease in both SWS and PS occurs. This prolonged state of behavioural and EEG wakefulness has been reported in a variety of species (Florio, Scotti, de Carolis and Longo, 1968; Koella, Feldstein and Czicman, 1968; Mouret, Bobillier and Jouvet, 1968; Torda, 1967). In rats, marked decreases in SWS were found to be correlated temporally with the reductions of 5-HT levels in the brain (Florio et al, 1968; Mouret et al, 1968) with SWS returning to normal levels as the brain 5-HT levels returned to baseline values (Mouret et al, 1968). These inhibiting effects of PCPA on sleep were rapidly reversed following the administration of 5-HTP when the PCPA insommia had reached its maximum (Koella et al, 1968; Mouret et al, 1968), and qualitatively normal sleep patterns returned for 6 - 8 hours (Jouvet, 1969).

The increase in waking behaviours following PCPA

is accompanied by the appearance of almost permanent PGO spike activity which persists until the sleep patterns return to normal. This mechanism of PGO activity during waking following PCPA is now the topic of considerable debate and will be subsequently discussed when considering the contradictory evidence for a serotonergic control of SWS.

A more direct line of investigation based on the histochemical fluorescent studies developed by Falk, Hillarp, Thieme and Torp (1962) defining a detailed regional anatomy of the chemical localization of monoamines in the CNS, was undertaken by Jouvet and associates (Jouvet, 1969; 1972). On the basis of the Dahlstrom et al findings, Jouvet selectively altered brain levels of serotonin in cats by surgical ablation of serotonergic cells of the raphe nuclei. Such manipulations induced severe and long lasting insomnia with significant correlations between the degree of raphe destruction, its associated reduction in brain 5-HT, and degree of insomnia. These effects could not be reversed by administration of 5-HTP, since the precursor could not be metabolized by the degenerated 5-HT terminals.

Similar findings of persistent behavioural arousal with high frequency low amplitude EEG activity were also reported in rats following lesions of the raphe nuclei (Kostowski, Giacalone, Garattini and Valzelli, 1968) and following a midsaggital split of the brain stem which destroys most the raphe neurons (Michel and Roffwarg, 1967).

The studies previously reviewed provide strong evidence to support Jouvet's hypothesis that 5-HT is primarily involved in initiating and maintaining SWS. However, other findings which will now be reviewed, have caused researchers to question the unique importance of 5-HT in SWS.

(b) Serotonergic Control of SWS: Contradictory Evidence

The most serious criticisms directed against a serotonergic control of SWS are based on studies documenting the effects of chronic administration of PCPA. Various researchers (Dement, Mitler and Henrksen, 1972: Henriksen and Dement, 1972) have shown that in animals given repeated daily doses of PCPA, total insomnia was only transient. SWS and PS gradually returned to normal values (up to 70% of baseline) despite continued reductions in brain 5-HT levels of close to 90%.

Although these effects may be explained in terms of post-synaptic supersensitivity to 5-HT induced by prolonged 5-HT depletion, they are, nevertheless, difficult to reconcile with Jouvet's clear assertion that serotonin initiates and maintains SWS.

Furthermore, other researchers (Rechtschaffen, Lovell, Freedman, Whitehead and Aldrich, 1973) have reported no significant changes in the sleep patterns of rats following repeated administrations of various doses of PCPA, although this conclusion was reached prior to their recent interest in the dichotomy of NREM sleep.

In general, the effects of PCPA are not consistent across species. In humans treated with PCPA for medical reasons, Wyatt (1972) observed little change in SWS but marked reductions in PS. These reductions in PS were reversed following the administration of 5-HTP - the direct opposite of what one would predict on the basis of Jouvet's proposals.

Additional problems with Jouvet's postulated serotonergic control of SWS are evident from studies using reserpine-induced depletions of monoamines.

The reserpine-induced PGO waves which are believed to be identical to spontaneous PGO waves (Brooks and Gershorn, 1971) have been found to occur throughout the period of elimination of PS (Delorme, Jeannerod and Jouvet, 1965). Dement (1969) has noted that these long periods of PS suppression induced by reserpine were not followed by compensatory rebounds and has advanced the hypothesis that serotonin functions to prevent phasic events, (rapid eye movements, PGO waves) which occur during PS, from breaking through to SWS and waking states (Dement, Zarcone, Ferguson, Cohen, Pivak and Barchas, 1968).

On the basis of these studies with reserpine and those documenting the effects of repeated PCPA administration, there does appear to be evidence for the view that brain 5-HT tonically suppresses the PGO wave as Dement et al (1968), and Dement, Henriksen, Jacobs and Mitler, (1973) have suggested.

Investigations of the unit activity of 5-HT containing neurons (McGinty, Harper and Fairbanks, 1973) have demonstrated a reduction of raphe unit firing in SWS. This implies that the release of 5-HT is reduced during SWS and appears to be inconsistent with Jouvet's theory that 5-HT is directly involved in the maintenance of SWS. These findings have been extensively cited by researchers as providing serious problems for Jouvet. Although the relationship between single unit activity and levels of neurotransmitters is still not fully understood and remains somewhat speculative, these findings tend to suggest that serotonin may be diminished rather than enhanced during SWS - the opposite of what Jouvet proposes. Consistent with these findings are reports that electrical stimulation of the raphe of cats caused no increase in SWS, although some suppression of the phasic components of PS occurred (Jacobs, Asher and Dement, 1973).

Another problem relates to the data obtained from lesions made to the raphe system. As Drucker-Colin (1976) has pointed out, the lesions described by Jouvet (1969) as producing insomnia include tegmental areas which are known to contain large amounts of cholinesterase (Shute and Lewis, 1967). Thus it is entirely possible that Jouvet's findings may be partially attributable to damage produced to an ascending cholinergic system.

The findings previously discussed cast some doubt on the specific relationships proposed by Jouvet. However, in general the bulk of the evidence does appear to implicate serotonin in SWS.

(c) Serotonin or Serotonergic Metabolite - the PS Trigger?

The majority of available evidence points to 5-HT metabolites having a role in triggering the PS state. Monoamine oxidase inhibitors (MAOIs) which prevent the catabolism of both brain NE and 5-HT have been found to cause marked reductions or occasionally a complete suppression in PS and PGO spike activity (Jouvet, 1969). These observations suggest that monoamine oxidase (MAO) is necessary for the transition from SWS to PS. Among the deaminated metabolites of 5-HT, 5-hydroxy-indolacetaldehyde has been implicated (Jouvet, 1972) since this substance appears to have some central hypogenic effect on chickens (Sabelli, Giardina, Alivisatos, Seth and Unger, 1969).

The concept that the serotonergic system plays a role in the priming of PS is also based on observations that SWS appears to be a normal prerequisite for PS. Jouvet (1972) has noted that no PS can occur under a minimum amount of SWS which is 16% of the recording time. The brain regions which have been implicated in the priming of PS have been those of the caudal raphe (Jouvet, 1972).

(d) Catecholaminergic Control of PS: Supporting Evidence

Evidence implicating norepinephrine in PS is

based on experiments demonstrating that drugs which reduce brain levels of NE also cause a suppression in PS. Alpha-methylparatyrosine (∝-MPT), which inhibits NE by blocking tyrosine hydroxylase (Spector, Sjoerdsma and Undenfriend, 1965) caused a marked and selective reduction in PS with increased SWS (Fujimori and Himwich, 1971; Weizman, McGregor, Moore and Jacoby, 1969; Torda, 1968). These effects were shown to be reversed by intracerebral injections of NE in the rat (Torda, 1968, 1969). Similar selective reductions in PS were found following the administration of disulfiram - a substance which impairs NE synthesis by inhibiting dopamine B-hydroxylase (Froment and Duzan-Peyrethon, 1969).

The described reductions in PS following depletion of brain NE are consistent with Jouvet's (1969) observations that bilateral lesions of the locus coeruleus - a nucleus rich in NE-containing neurons, is followed by a total or sub-total disappearance of both the phasic and tonic events of PS. In these studies there appeared to be a three way correlation between the extent of the lesion, the decrease in NE present in basal forebrain and telencephalon, and the reduction in PS. Thus, the greater the extent of tissue destruction, the greater the reduction in brain NE and the suppression in PS. This same suppression of PS has also been observed following specific depletions of NE by micro-injections of 6-hydroxydopamine (Laguzzi, Pettijean, Pujol and Jouvet, 1972; Zolovick, Stein, Jalowiec, Panksepp and Morgane, 1973).

A variety of pharmacological agents have been used to study the effects of increased catecholamine activity on human sleep. These include studies of L-dopa, the biological precursor of dopamine and NE (Wyatt, 1972) cocaine (Post, Gillin Wyatt and Goodwin, 1974) and clonidine (Autret, Minz, Beillevair, Cathala, and Castaigne, 1976). In all of these studies reductions in PS duration were found.

Other studies have shown that the intracisternal or intraventricular administration of NE induces sleep or sedation (Mandell, Spooner and Brunet, 1969). Consistent with these reports are findings that the injection of dihydroxyphenylserine, a precursor of noradrenaline which passes freely through the blood-brain barrier, caused increases in both PS and SWS in rats (Havlicek and Sklenovsky, 1967).

Further evidence for a catecholaminergic regulation of PS is provided by findings of increased PS in young chickens (which have no blood-brain barrier) following injections of norepinephrine (Clarenback and Cramer, 1972). In addition, rats which have been deprived of PS for several days show increased turnover of cerebral NE during the well documented rebound which follows PS deprivation (Pujol, Mouret, Jouvet, and Glowinski, 1968).

(e) Catecholaminergic Control of PS: Contradictory Evidence

Despite the convincing body of evidence implicating norepinephrine in the control of PS, there does exist a variety

of studies which fail to support the findings previously discussed.

Several researchers (Branchey and Kissin, 1970; Marantz and Rechtschaffen, 1967) have been unable to observe changes in PS following depletion of NE by α-MPT. In rats, α-MPT produced no changes in sleep parameters, and in addition did not prevent PS rebound following PS deprivation (Marantz, Rechtschaffen, Levell and Whitehead, 1968). Other investigators (Branchey and Kissin, 1970) have found increases in SWS, not PS, following α-MPT. To complicate matters even further, Hartmann, Bridwell and Schildkraut, found that in rats, α-MPT given intraperitoneally caused reductions in PS while oral α-MPT increased it. However, Dement et al (1972) noted that α-MPT is nephrotoxic, and it is difficult to achieve total depletion in brain levels of NE although NE turnover may be stopped. Thus perhaps some of the problems of inconsistent research findings may be attributable to these factors.

Further problems are based on findings of only slight alterations in PS following lesions to the locus coeruleus (Henley and Morrison, 1974), while others (Jalowiec, Morgane, Stern, Zolov and Panksepp, 1973) have found that these lesions caused no changes in sleep parameters.

Other objections to Jouvet's claim for a role of NE in PS are based on findings that the intracerebral administration of NE induced prolonged electrocortical and

behavioural arousal and suppressed PS (Cordeau, de Champlain and Jacks, 1971; Torda, 1968).

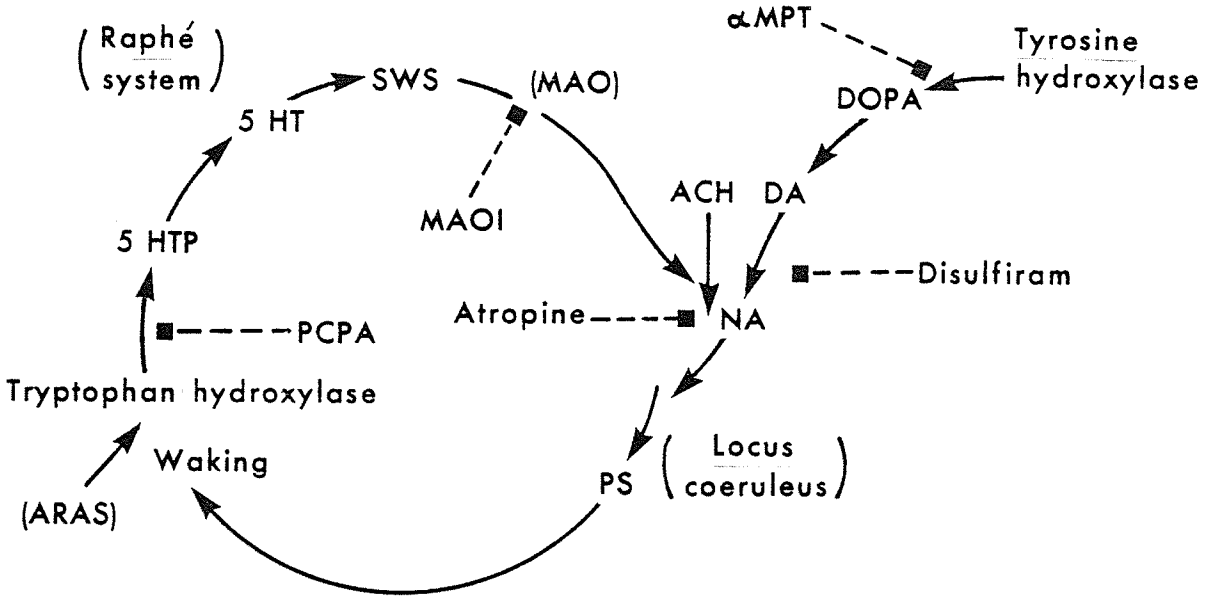
From the studies previously reviewed, one finds that despite the convincing confluence of evidence which supports Jouvet's neurohumoral theory, there also exists contradictory evidence, or alternative explanations. Although the bulk of the evidence does tend to support the view that the induction and maintenance of SWS is regulated by serotonin levels in the brain, whereas PS depends on brain NE, the findings also suggest that Jouvet's account of the role of these monoamines in sleep processes may be somewhat oversimplified. A summary of the possible monoaminergic mechanisms involved in the regulation of the sleep-waking cycle postulated by Jouvet (1969) and the actions of various pharmacological agents which may block certain steps of the cycle is presented in Figure 3.

Statement of the Purpose of the Study

The purpose of this study was thus to investigate sleep parameters and monoaminergic functioning in two strains of rat which were believed to have abnormal peripheral and central levels of monoamines. These two strains of rat were: 1) The Walker-Walker or Fawn-hooded rat - a recently discovered mutant strain of rat in which preliminary investigations by the present author revealed profound reductions in peripheral levels of serotonin. On the basis of the

FIGURE 3

Schematic diagram representing the possible monoaminergic mechanisms involved in the two states of sleep postulated by Jouvet (1969). The actions of several drugs which block certain steps of the cycle are represented.


Raphé system
SWS
(MAO)
αMPT
Tyrosine hydroxylase
5 HT
DOPA
MAOI
ACH
DA
5 HTP
Disulfiram
Atropine
NA
PCPA
Tryptophan hydroxylase
Waking
Locus coeruleus
PS
(ARAS)

literature which will subsequently be reviewed in detail, it was believed that the abnormal levels of serotonin found peripherally in this strain of rat would also be manifested centrally.

2) The spontaneously hypertensive strain of rat, described by Japanese investigators (Okamoto and Aoki, 1973). This strain of rat has been recently described as having low brainstem levels of norepinephrine (Yamori, Lovenberg and Sjoerdsma, 1970) and decreased rates of norepinephrine turnover (Louis, Kraus, Kopin and Sjoerdsma, 1970).

On the basis of the extensive research evidence concerning the role of the monoamines in sleep, it was predicted that each of these strains of rat would show significant alterations in sleep parameters in comparison to control strains. The rationale underlying the selection of the most appropriate controls for each of these experiments will be later discussed.

On the basis of Jouvet's speculations concerning the role of serotonin in SWS and norepinephrine in PS, it was hypothesized that the Walker-Walker strain would show significant reductions in SWS in comparison to controls. Animals of the SHR strain were hypothesized to show alterations in PS in comparison to controls.

The use of inbred genetic strains of rats in order to clarify neurochemical differences and their relationship to well established behavioural differences is a particularly

useful research strategy. This is because the differences observed occur without introducing non-physiological conditions which are usually unavoidable in the production of neurochemical and behavioural changes with other methods, as was previously discussed.

A review of the literature and the logic underlying each of the experiments will be discussed in greater detail.

CHAPTER 2

SLEEP PARAMETERS, PERIPHERAL AND CENTRAL MONOAMINERGIC FUNCTIONING IN THE WALKER-WALKER STRAIN OF RAT

The Walker-Walker of Fawn-hooded (F-H) rat is a recently discovered strain derived from both Wistar and Long-Evans ancestors. This strain of rat is characterized by its distinctive beige coat colour, and by a hereditary hemorrhagic diathesis. Fawn-hooded animals have episodes of spontaneous bleeding as well as severe blood loss on trauma.

Preliminary investigations by the present author have established that F-H rats have a defect in hemostasis similar to that described by Raymond and Dodds (1975) and by Tschopp and Zucker (1972). These animals have a normal blood coagulation cascade, normal platelet number and grossly abnormal bleeding times (in excess of 30 minutes). In addition to abnormalities in platelet aggregation with thrombin and collagen, blood platelet serotonin levels of F-H animals were found to be markedly reduced in comparison to Long-Evans controls. (The profound reductions in blood platelet serotonin levels will be later presented in the Results Section.)

In addition to these physiological differences, some researchers have commented that the behaviour of these rats is markedly different from that of other strains (Tobach, Bellin and Das, unpublished). Fawn-hooded rats are observed to be unusually docile and less excitable than Wistar or Long-Evans strains, although to date these behavioural

differences have not been documented.

These observations and the preliminary data showing considerable reductions in blood platelets suggested that such findings may have important implications, since recently some investigators (Page, 1968; Pletscher, 1968; Sneddon, 1973) have proposed that the blood platelet may serve as a model for the serotonergic neuron. This assumption is based on evidence that blood platelets and neurons both possess similar mechanisms for the accumulation of serotonin, storage of the amine in electron-dense subcellular organelles, and for formation of deaminated metabolites -5 hydroxyindoleacetic acid and 5-hydroxytryptophol (Pletscher, 1968). In an attempt to correlate the similarities in biochemical and pharmacological characteristics of blood platelets and presynaptic nerve terminals, Sneddon (1973) concluded that uptake, storage, and transport processes for 5-HT are very similar, if not identical in platelets and synaptosomes. Some of the major similarities between the two are presented in Table 3.

Accumulating research evidence has indicated abnormalities in the pharmacodynamics of platelet serotonin in some conditions characterized by disturbed brain functioning. Abnormalities in platelet serotonergic function has been reported in conditions such as autism (Boullin, Coleman and O'Brien, 1970) and some affective disorders (Coppen, Turner, Rowsell and Padgham, 1976). Some of this

Table 3. Structural and Functional Similarities Between Blood Platelets and Synaptosomes

	Platelets	Synaptosomes
Limiting membrane	+	+
Mitochondria	+	+
Monoamine oxidase	+	+
Active transport mechanism for 5-HT	+	+
Synthesis of biogenic amines	-	+
Amines stored in subcellular granules	+	+
Size of storage granules	50-250nm	50-300nm
Granule membrane	+	+
Granules discharged by appropriate stimulation (thrombin/nerve stimulation)	+	+
Stored amine released by:		
Reserpine	+	+
Sympathominetics	+	+

evidence will now be reviewed in order to provide further justification for the view that blood platelet serotonergic functioning may serve as a model for central serotonergic functioning.

Evidence Supporting the Use of the Blood Platelet as a Model for Serotonergic Neuronal Functioning

(a) Down's Syndrome

In patients with Down's Syndrome, platelet serotonin levels have been found to be significantly lower than in age-matched controls (Boullin and O'Brien, 1971 ; Lott, Chase and Murphy, 1972; Rosner, Ong, Paine and Mahanad, 1965; Tu and Zellweger, 1965). Further investigations attributed the low levels of serotonin to abnormal uptake mechanisms associated with deficient storage of 5-HT and reduced levels of ATP (Boullin and O'Brien, 1971), and these findings led to the suggestion that the reduced platelet serotonin levels in Down's syndrome patients may reflect disordered metabolism of this monoamine within the central nervous system. This hypothesis was examined in a recent study of monoaminergic function in platelets and concentrations of 5-HIAA (the principal serotonin catabolite in cerebrospinal fluid (Lott, Murphy and Chase, 1972). Although platelet serotonin levels were found to be reduced in Down's syndrome patients, steady state levels of 5-HIAA in CSF did not differ from those of age-matched controls.

Such findngs suggest some need for caution in the

extrapolation of data from the platelet to the neuronal level. However, the usefulness of 5-HIAA concentrations in CSF as an index of central functional activity of 5-HT remains somewhat controversial (Greene and Grahame-Smith, 1976). Thus, the criticisms based on Lott et al (1972) findings may not be as serious as they were initially assumed to be.

(b) Autism

Studies of platelet biochemistry of autistic children have revealed some discrepancies, mainly because of problems involved in the selection of the diagnostic criteria for autism. Although elevated blood 5-HT levels have been reported, (Schain and Freedman, 1961), the validity of these findings have been questioned by other researchers (Ritvo, Yuwiler, Geller, Ornitz, Saeger and Plotkin, 1970).

Studies of platelet serotonin uptake and release have demonstrated that platelets of autistics show slightly increased uptake, but decreased ability to retain the accumulated serotonin in comparison to age-matched controls (Boullin, Coleman, O'Brien and Rimland, 1971; Boullin, Coleman and O'Brien, 1970), and clinical predictions on the basis of platelet 5-HT efflux have been made (Boullin et al, 1971).

(c) Affective Disorders

Although a number of researchers have proposed

that alterations in brain serotonin levels are associated with affective disorders (Ashcroft, Crawford, Eccleston, Sharman, MacDougall, Stanton and Binnis, 1966; Dewhurst, 1968), other hypotheses have also been advanced (Bunney and Davis, 1965; Schildkraut, 1965). However, the 5-HT hypothesis of affective disorders still remains equally tenable (Sneddon, 1973) and many investigations suggest a reduction in central 5-HT synthesis in depressive illness (Ashcroft et al 1966; Coppen, 1968; Coppen, Prange, Whybrow and Noguera, 1972; Shaw, Camps and Eccleston, 1967).

Peripheral abnormalities in serotonin metabolism in severe depression have also been reported (Coppen, Shaw, Malleson, Eccleston and Gundy, 1965). Reduced whole blood 5-HT levels have been reported in severe endogenous depressives, with values returning to normal following clinical recovery (Coppen, Turner, Rowsell and Padgham, 1976). Since whole blood estimates of 5-HT are essentially measures of platelet 5-HT, the reduced 5-HT content in the blood of depressives reflects a reduction in platelet levels of this amine. These findings are supported and extended by a recent study reporting reduced uptake of 5-HT in platelets of endogenous depressives (Hallstrom, Rees, Pare, Trenchard, and Turner, 1976).

A series of recent studies have suggested that platelet monoamine oxidase activity may provide a biochemical correlate of schizophrenia (Murphy and Wyatt, 1972; Wyatt,

Murphy, Belmaker, Cohen, Donnelly and Pollin, 1973) and vulnerability to psychiatric disorders (Buchsbaum, Coursey and Murphy, 1976). Monoamine oxidase (MAO) plays an important role in biogenic amine metabolism ie. it catalyses one of the major pathways for the metabolism of serotonin and the catecholamines. Inhibition of this enzyme causes increased 5-HT levels in both brain (Bevan-Jones, Pare, Nicholson, Price and Stacey, 1972) and platelets (Marshall, Stirling, Tait and Todrick, 1960).

Marked reductions in platelet MAO activity have recently been reported in schizophenics in comparison to age-matched controls (Murphy and Weiss, 1972) and the possibility that similar reductions in brain MAO may exist remains an interesting speculation. Although brain MAO has been described as having four isoenzyme forms, the platelet enzyme is homogenous (Collins, Sandler, Williams and Youdim, 1970) and is believed to most resemble the brain hypothalamic form (Murphy and Weiss, 1972). These reports suggest that a partial overlap in characteristics of platelet and brain MAO may exist, and this concept has been advanced and extended (Robinson, Davis, Nies, Ravaris and Sylvester, 1971).

One of the major criticisms directed against the use of peripheral measures, such as levels of platelet 5-HT, is that there are no a priori reasons for equating central events with those occurring peripherally. However, the

opposite may also be argued, ie that abnormalities in platelet 5-HT may be a reflection of widespread abnormalities in serotonin metabolism, and may reflect changes in 5-HT containing neurons within the CNS. Thus it is believed that the platelet may justifiably be used as a model of the serotonergic neuron, although caution in extrapolating findings from one to the other is advised (Sneddon, 1973).

As was previously noted, preliminary research revealed marked reductions in blood platelet levels of 5-HT of F-H rats in comparison to Long-Evans controls. These findings, in conjunction with reports of behavioural differences, raised the question of whether the abnormalities observed in the platelets of F-H rats might reflect abnormalities of a more central nature. Of particular relevance to this question are a series of recent reports documenting significant differences in plasma serotonin levels and active avoidance learning in inbred strains of mice (Eleftheriou and Bailey, 1972; Oliverio, Castellano, Ebel and Mandel, 1974; Oliverio, Eleftheriou, and Bailey, 1973). These authors reported that plasma serotonin and avoidance learning were controlled by a single major gene, suggesting a relationship between plasma serotonin and shock-avoidance behaviour. Closer examination of the data revealed an inverse relationship between plasma serotonin levels and avoidance performance - animals with reduced plasma 5-HT performed significantly

better in shock-avoidance (Eleftheriou and Bailey, 1972). Although no explanation for the observed relationship was provided, one might hypothesize that the low levels of 5-HT in platelets were also manifested centrally. Further support for this interpretation is provided by the now well established relationship between brain serotonin levels and sensitivity to pain (Harvey and Lints, 1965; Tenen, 1967).

On the basis of the studies reviewed, and on observations of the behavioural differences of Fawn-hooded rats, it was hypothesized that the abnormalities in platelet serotonergic functioning of these animals would reflect changes in central serotonergic functioning. It was thus hypothesized that F-H rats would show significant reductions in brain levels of serotonin in comparison to Long-Evans controls. On the basis of the research evidence concering the importance of serotonin in SWS, it was further hypothesized that F-H rats would show significant reductions in SWS in comparison to the control strain.

Method

I. Investigation of sleep parameters

Subjects

Six Fawn-hooded Walker-Walker and 6 black-hooded Long-Evans male rats, approximately 3 months of age, ranging in weight from 250 - 350 gms were used. Animals were raised under identical environmental conditions (12 hr light/dark

cycle, 18.00 hr to 6.00 hr) and were housed in group cages (3 rats/cage) until the time of the experiment.

Apparatus

Electrocotrical activity was recorded from a GRASS Model 78 polygraph. Muscle movement was monitored by a piezoelectric system (Havlicek and Sklenovsky, 1967) which allows the finest vibrations of the recording cages to be detected.

Recording cages of transparent plexiglass measuring 21 x 24 x 45 cm were used. Animals were housed individually in these cages following electrode implantation and during the recording session. Thus, problems of adjustment to novel environmental conditions during the experiment were strictly controlled.

Procedure

Animals were anaesthetized with nembutal (40 mg/Kg intraperitoneally) and 4 platinum epidural electrodes were implanted over the somatosensory cortex. To permit comparison of EEG spectral analysis across animals, electrodes were implanted in a standardized manner and location using skull landmarks. The electrodes, soldered to a micro-connector, were fastened to the skull by acrylic cement. Excessive cutaneous bleeding, particularly with the Fawn-hooded rats, was controlled by electrocautery and application of pads of gelfoam (Upjohn) soaked in topical Thrombin (Parke-Davis). During the period of recovery, as well as

during the experiment, animals were housed individually.

Following a recovery period of 10 days, the animals were habituated to the experimental environment (5 consecutive 6 hour sessions). During the final adaptation session, the male half of the microconnector was connected to the animal's head, in order to allow for habituation to the recording wires.

Animals were run in pairs, with each pair consisting of one animal from each of control and experimental groups. Individual cages were placed side by side, but far enough apart so that the behaviour of one animal did not influence the state of the other. Thus recordings of each pair were done simultaneously over the same period of time.

Experiments were conducted between 9:00 and 18:00 hr in a sound proof electrically shielded room of constant temperature ($23^{o}C \pm 2^{o}C$) with a one way mirror. Polygraphic recordings were taken over a 6 hour period with recording wires connected to the apparatus in such a way as to permit free unrestrained movement of the animal. Throughout the recording session food and water were available and libitum. During recording the animals were observed through the one-way mirror and the behavioural state directly noted on the EEG record. This provided a guide for interpretation of the EEG record and allowed for accurate assessment of the wake-sleep state of the animal during periods when the behavioural state was not directly observable.

On the basis of behavioural and individual polygraphic records three basic states were differentiated - awake, slow wave sleep, and paradoxical (REM) sleep, according to criteria previously established (Havlicek) and Sklenowsky, 1967). Slow wave sleep was further broken down into deep-slow wave sleep, and shallow-slow wave sleep (wave amplitude approximately half that of deep-slow wave sleep). Similar subdivision of slow wave sleep into light slow wave sleep and deep slow wave sleep has been independently adopted for rat (Rosenberg, Bergmann and Rechtschaffen, 1976) and cat NREM sleep (Ursin, 1968) and has been found to be a functionally meaningful dichotomy in terms of diurnal cycle and differential effects of PCPA.

The data obtained were scored visually and classified into the previously defined four categories. Transient shifts in state of less than 20 seconds duration were scored as the state which predominated throughout that segment of the record. The author was checked on previously scored data and found to be more than 90% reliable ($r = 0.92$).

Mean durations of each state (awake, shallow-slow wave sleep, deep-slow wave sleep and paradoxical sleep) were obtained for each 6 hour session total and for each hour of the recording session. These data were statistically evaluated by a 2 x 6 repeated measures analysis of variance.

Computer processing of the EEG for fast-Fourier spectral analysis

During the session, segments of the recording were stored on magnetic tape for subsequent processing by computer

for fast-Fourier spectral analysis (described in detail by Havlicek, Childiaeva and Chernick, 1975).

Polygraphic recordings of EEG (2 channels) and muscle activity were divided into 11 second epochs by a "trigger pulse" which was automatically recorded on a separate channel and used for both the quantification of sleep/wake state durations, and for triggering the computer during the fast-Fourier transform of the EEG.

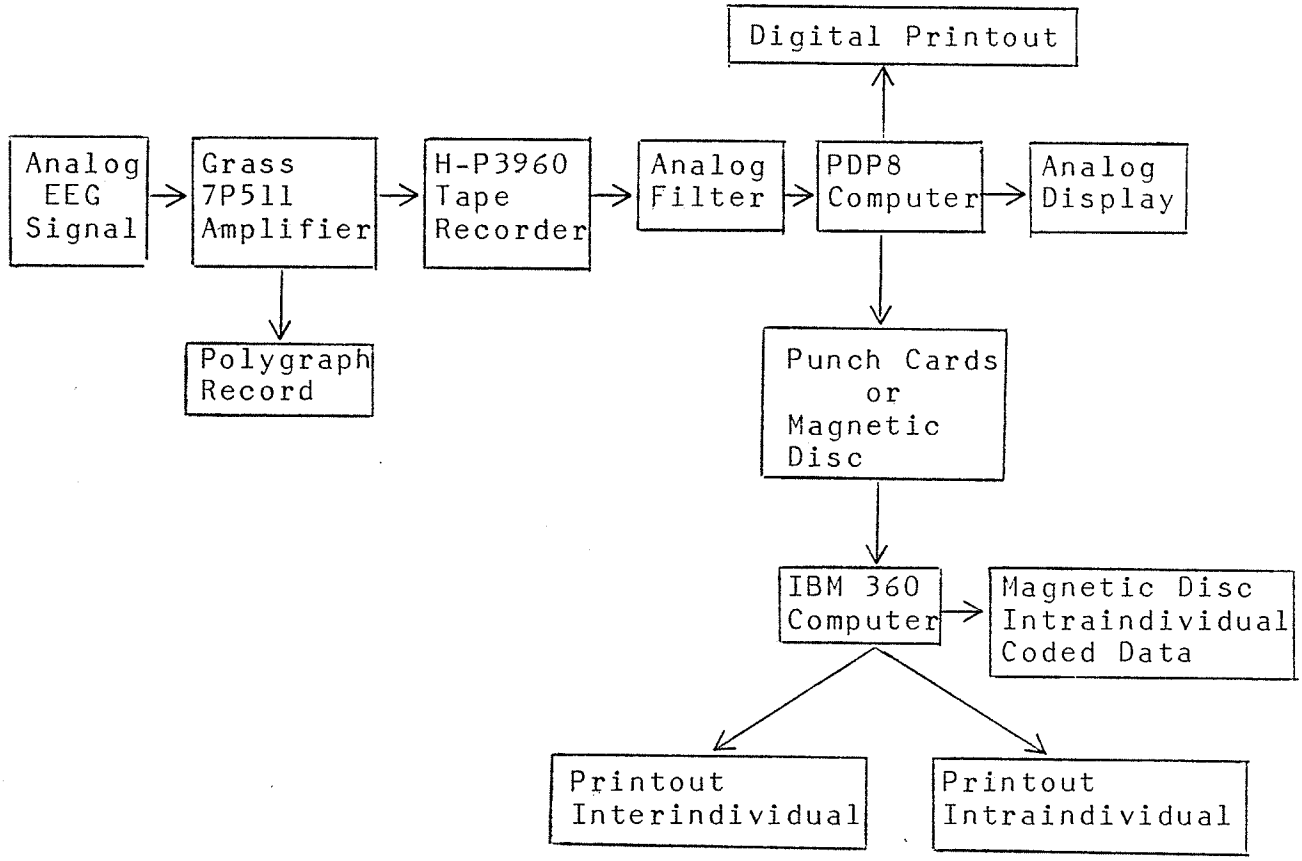
The EEG signal and trigger pulse were recorded using a Hewlett-Packard 3960 Instrumental FM tape recorder for subsequent processing. The fast-Fourier transform (FFT) was performed off-line using a PDP-8/E computer (described by Havlicek, Childiaeva and Chernick, 1975).

Rothman's (1968) version of the FFT was used and the EEG spectrum for each 10.24 second epoch was obtained. EEG spectral data were integrated into the usual clinical frequency bands delta 1, delta 2, theta 1, theta 2, alpha 1, alpha 2, beta 1, beta 2 and an integrated EEG "Sum" (as described in detail by Havlicek et al, 1975). This system allows for non-stop analysis of sequential epochs of two channels of EEG. The processing of the EEG is schematized in Figure 4. Since EEG filters were used which affect the Delta 1 analysis, the delta 1 band was not reported.

Intra and interindividual statistical comparisons were performed by computer using analysis of variance and Duncan's multiple range test.

FIGURE 4

Block diagram of the computer processing of the EEG.
Reproduced with permission Havlicek et al (1975).


Digital Printout
Analog EEG Signal
Grass 7P511 Amplifier
H-P3960 Tape Recorder
Analog Filter
PDP8 Computer
Analog Display
Polygraph Record
Punch Cards or Magnetic Disc
IBM 360 Computer
Magnetic Disc Intraindividual Coded Data
Printout Interindividual
Printout Intraindividual

II. Measurement of brain monoamines

Procedure

Rats were killed by decapitation with a minimum of prior stress. Brains were quickly excised, washed in hypertonic saline solution and frozen at -40°C until required for assay. Since daily rhythms of biogenic amines have been demonstrated in whole rat brain (Asano, 1971) and some brain regions (Quay, 1968), all animals were sacrificed at approximately the same time of day.

Whole brain monoamine levels were measured by the method of Shellenberger and Gordon (1971) using internal and external standards for maximal accuracy.

Details of the assay procedure are described in Appendix 1.

III. Measurement of blood platelet serotonin levels

Procedure

Levels of serotonin in blood platelets were measured with a highly sensitive procedure, which employs the reaction product of 5-HT and orthophthalaldehyde, (OPT) using a modification of the Drummond and Gordon (1974) procedure.

Blood was drawn by cardiac puncture, collected into plastic tubes containing 1/10 vol. sodium citrate (0.1 Molar) and carefully mixed. Platelet rich plasma was obtained by centrifugation at 1000 rpm for 10 minutes. The platelets were washed with ice-cold EDTA saline solution

(0.4% W/V in isotonic saline) then platelets were sedimented by centrifugation (10.000 rpm for 5 minutes).

The platelet pellet was lysed with distilled water and proteins in the lysed pellet were precipitated with trichloracetic acid (6 Molar). Proteins were removed by centrifugation and the supernatant was then reacted with OPT/HCl solution (1 part orthophthalaldehyde (0.05T) in absolute alcohol to 10 parts of 8NHCl). The mixture was heated at 100°C for 10 minutes, cooled on ice then washed with chloroform. Fluorescence in samples of the aqueous phase was measured in an Aminco-Bowman spectrophotofluorometer at 370 nm excitation, 470 nm emission.

Standard curves were prepared by similarly treating solutions containing known amounts of serotonin creatinine sulfate (Sigma). Tissue blanks were prepared by adding potassium ferricyanide (0.2%) to aliquots of the supernatant of each sample following protein precipitation. These tissue blanks were carried throughout the described procedure and each value subtracted from each sample reading.

Values were expressed in nanamoles/10^8 platelets, and differences between groups statistically evaluated by the Student's t-test.

Results

Fawn-hooded rats showed significant changes in all wake-sleep parameters in comparison to the Long-Evans control strain. Marked reductions in deep slow wave sleep

($F = 8.28$, df: 1,55, $p < 0.01$) and paradoxical sleep ($F = 44.03$, df: 1,55, $p < 0.01$) were observed in the Fawn-hooded group. These animals spent significantly more time awake ($F = 2.46$, df: 1,55, $p < 0.05$) and in shallow slow wave sleep ($F = 30.73$, df: 1,55, $p < 0.01$) than the Long-Evans control strain. The observed reductions in deep slow wave sleep and paradoxical sleep (PS) were thus compensated by prolongations of shallow slow wave sleep and awake states throughout the recording session. Fawn-hooded animals showed proportionately greater oscillations of shallow SWS and wakefulness, particularly during the first 4 hours of recording. Thus, in general they did not tend to progress into a deeper SWS or PS to the same extent as the Long-Evans strain. This trend is illustrated in Figure 5. Overall session totals (illustrated in Figure 6) indicated that the most striking difference between groups was the consistent reduction in PS in the fawn-hooded rats. When expressed as percentages of total recording time, fawn-hooded rats on average spent 9.1% of the total recording time in PS in comparison to 16.5% observed in the Long-Evans strain. These data are presented in Table 4.

The overall reductions in PS observed in fawn-hooded animals were directly attributable to a reduction in the frequency of PS episodes, and to a reduction in the mean duration of PS episodes. Thus, not only did fawn-hooded

FIGURE 5

Hourly mean durations of the various components of the sleep-wake cycle of Fawn-Hooded and Black-Hooded Long-Evans rats.

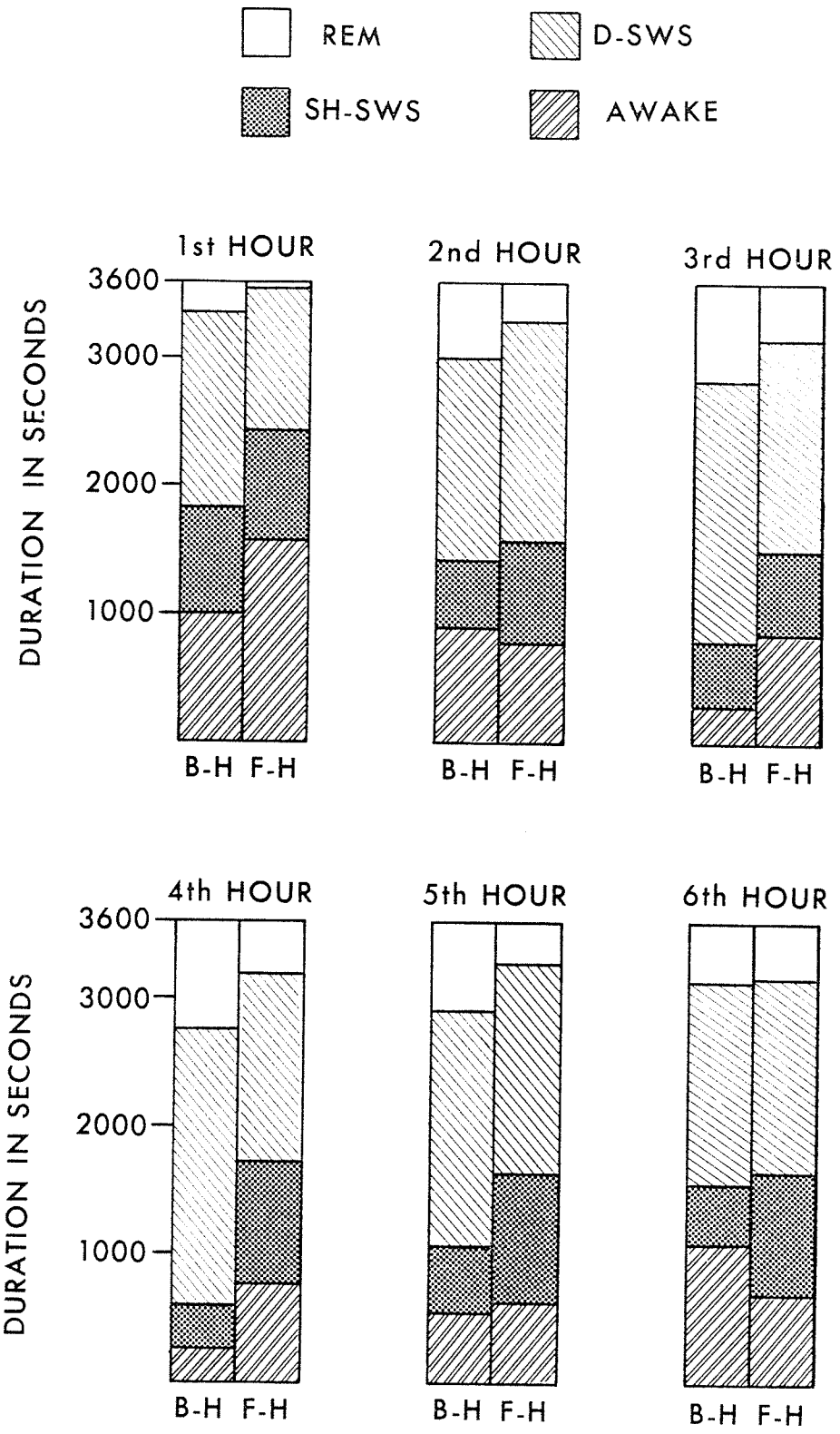

REM
D-SWS
SH-SWS
AWAKE
1st HOUR
2nd HOUR
3rd HOUR
4th HOUR
5th HOUR
6th HOUR
DURATION IN SECONDS
3600
3000
2000
1000
B-H
F-H

FIGURE 6

Total mean durations of the various components of the sleep-waking cycle of Fawn-Hooded and Black-Hooded Long-Evans rats/ 6 hour recording session.

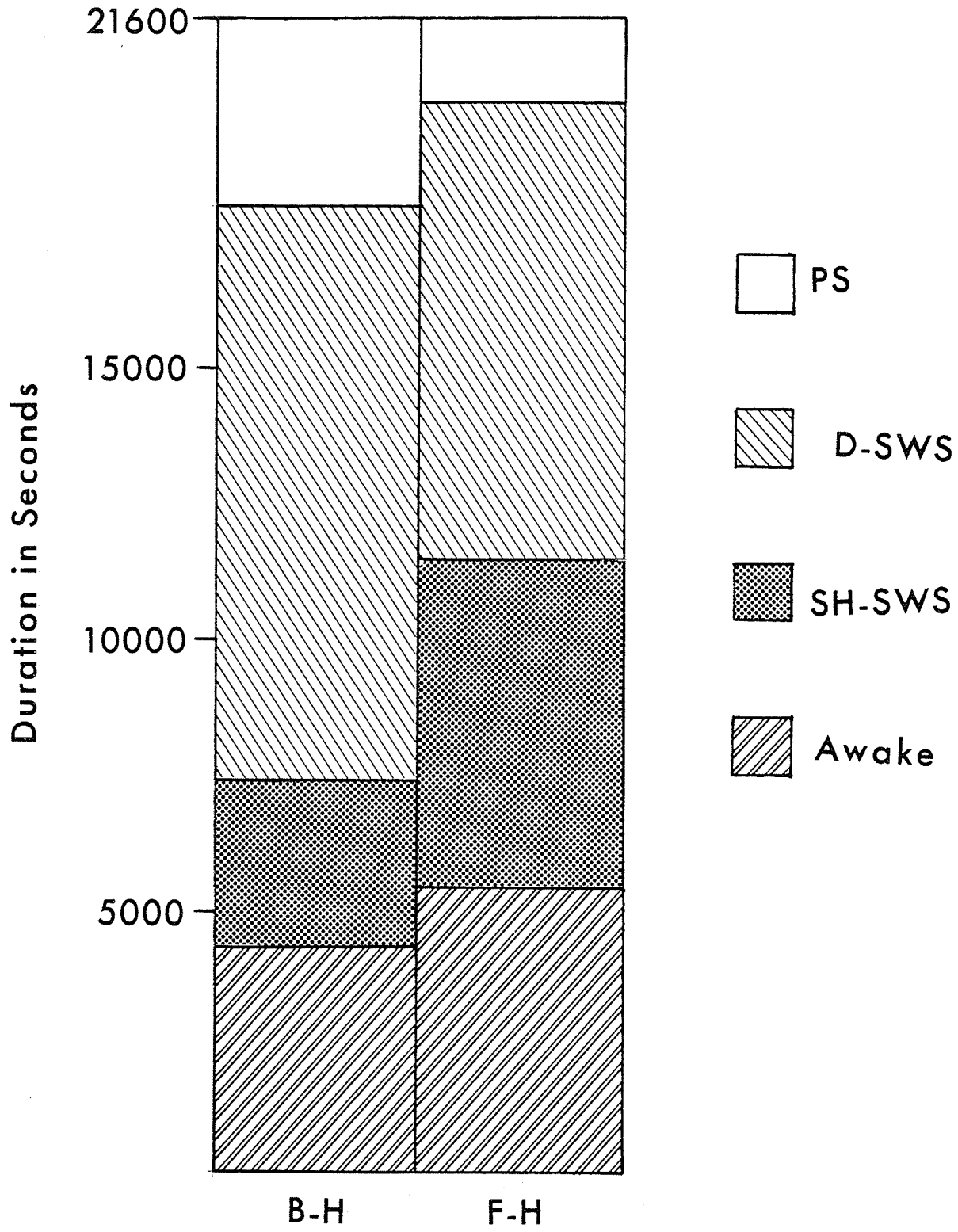

21600
15000
10000
5000
Duration in Seconds
B-H
F-H
PS
D-SWS
SH-SWS
Awake

Table 4. Mean Amounts of Paradoxical Sleep (PS) Shallow Slow Wave Sleep (SH-SW), Deep Slow Wave Sleep (D-SWS) and Waking (W) Expressed as Percentages of Total Recording Time in Fawn-Hooded (F-H) and Black-Hooded Long-Evans (L-E) Control Rats

	L-E	F-H
W	± 19.828 6.259	± 24.43 8.621
SH-SWS	± 14.035 5.406	± 24.295 6.657
D-SWS	± 49.56 6.811	± 42.147 10.305
PS	± 16.535 2.76	± 9.115 1.369

rats exhibit PS episodes less often than controls, but in addition the average length of their PS episodes were considerably shorter - with average PS durations of 118 sec in comparison to the control group average of 141 sec. These data are presented in Table 5.

Absolute frequencies of PS durations for each pair of rats are presented in Figures 7 through 12, again clearly indicating the shorter durations and less frequent occurrence of PS episodes in the fawn-hooded animals. The cumulative distributions of PS durations of fawn-hooded and control groups were compared using the Kolmogorov-Smirnov 2 sample test. However, the group differences just failed to reach the 0.05 level of significance (X^2 =5.8, df: 2, $p < 0.1$) (Critical X^2 Value = 5.9). The cumulative frequency distributions for experimental and control groups are illustrated in Figure 13.

Spectral analysis of the EEG using fast-Fourier transform revealed significant group differences during shallow SWS which became more pronounced during deep SWS. During shallow SWS fawn-hooded rats showed significantly lower power in theta 2, alpha 1 (5.66 - 9.47 H_z) and beta 2 (17.58 - 25.0 H_z) frequency bands in comparison to controls. Significant quantitative differences between groups during deep SWS were observed for all frequency bands with the single exception of the alpha 2 band. Fawn-hooded rats showed marked reductions in power in delta 2 (1.56 - 3.51 H_z),

theta 1 (3.61 - 5.57 H_z), theta 2 (5.66 - 7.52 H_z), alpha 1 (7.62 - 9.47 H_z) beta 1 (12.6 - 17.47 H_z) beta 2 (17.58 - 25.0 H_z) bands and an integrated EEG "Sum" (1.56 - 25.0 H_z) in comparison to L-E controls. These differences are illustrated in Figures 14 and 15. No such differences in spectral characteristics were evident during PS or waking states. F-H rats thus showed a less pronounced synchronization, or deactivation than L-E controls in that the frequency spectrum of the EEG during SWS was closer to that of the arousal state.

Comparison of the frequency spectrum of deep SWS of F-H rats with the shallow SWS of control animals revealed almost identical wave patterns. Thus the deep SWS of F-H rats closely resembled the shallow SWS of the L-E controls (illustrated in Figure 16).

These similarities were also recognizable by visual inspection of the EEG recording. Representative samples of the EEG for a pair of animals both in deep SWS (presented in Figure 17) demonstrated that the amplitude of the wave form of the F-H animal was considerably less than its matched control. This trend was observed in all pairs of animals without exception. Similarities in wave amplitude during the deep SWS of F-H animals and the shallow SWS of controls are illustrated in Figure 18.

Levels of blood platelet serotonin showed profound reductions in F-H rats in comparison to L-E controls. These

Table 5. Mean and Standard Deviations of PS Episodes (in seconds) for Pairs of Fawn-Hooded (F-H) and Long-Evans (L-E) Rats

	F-H	L-E (Controls)
Pair # 1	94.05 ± 11.0	128.7 ± 13.5
Pair # 2	166.21 ± 21.1	123.8 ± 15.8
Pair # 3	77.7 ± 9.8	143.2 ± 14.7
Pair # 4	116.3 ± 13.7	149.9 ± 13.0
Pair # 5	150 ± 24.5	170.0 ± 25.6
Pair # 6	142.7 ± 22.9	147.7 ± 16.0
Group Mean	118.06 ± 7.1	141.3 ± 6.9

FIGURE 7

Frequency distribution of durations of PS episodes for Pair #1.

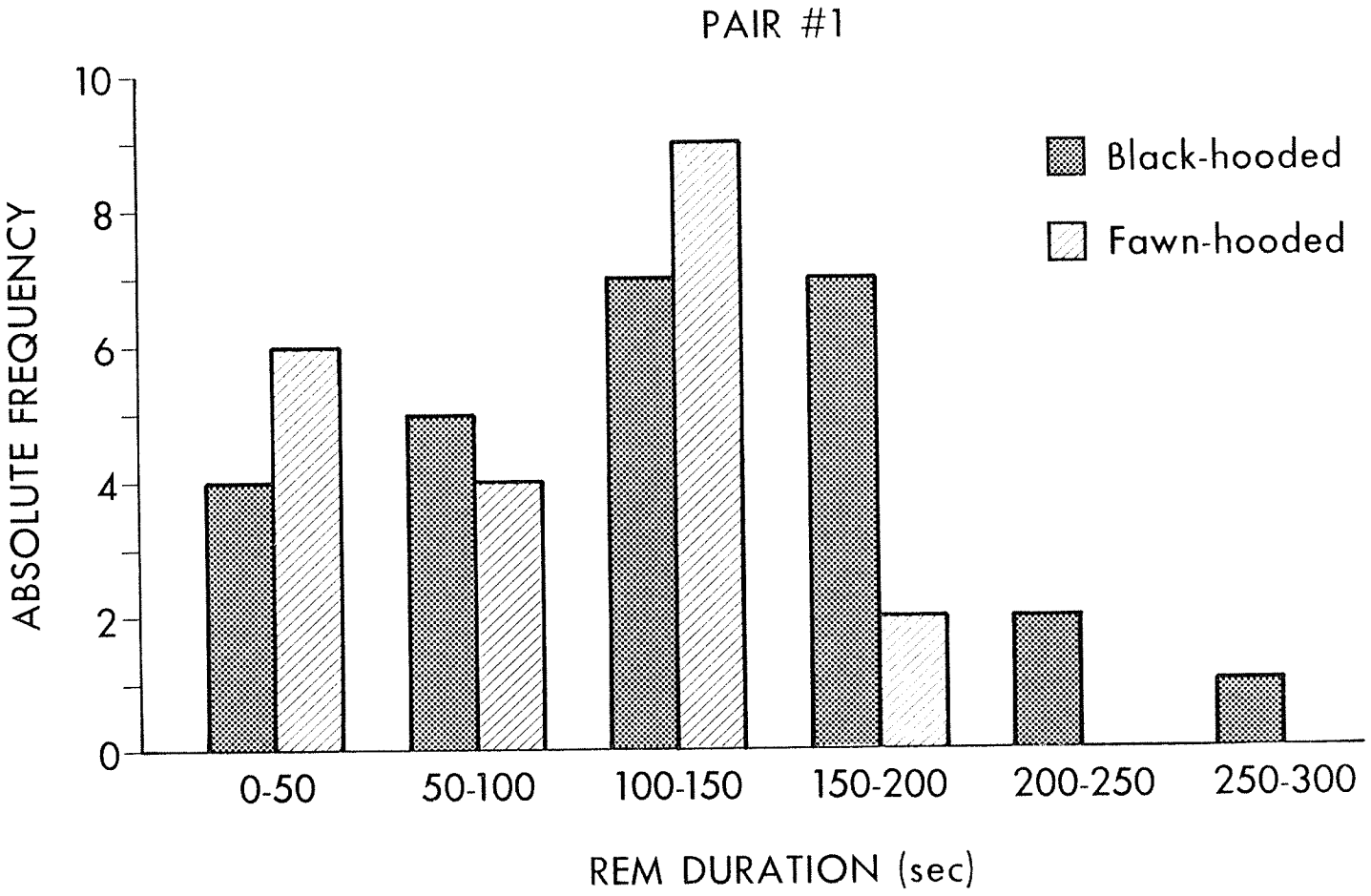

PAIR #1
ABSOLUTE FREQUENCY
10
8
6
4
2
0
Black-hooded
Fawn-hooded
0-50
50-100
100-150
150-200
200-250
250-300
REM DURATION (sec)

FIGURE 8

Frequency distribution of the durations of PS episodes for Pair #2.

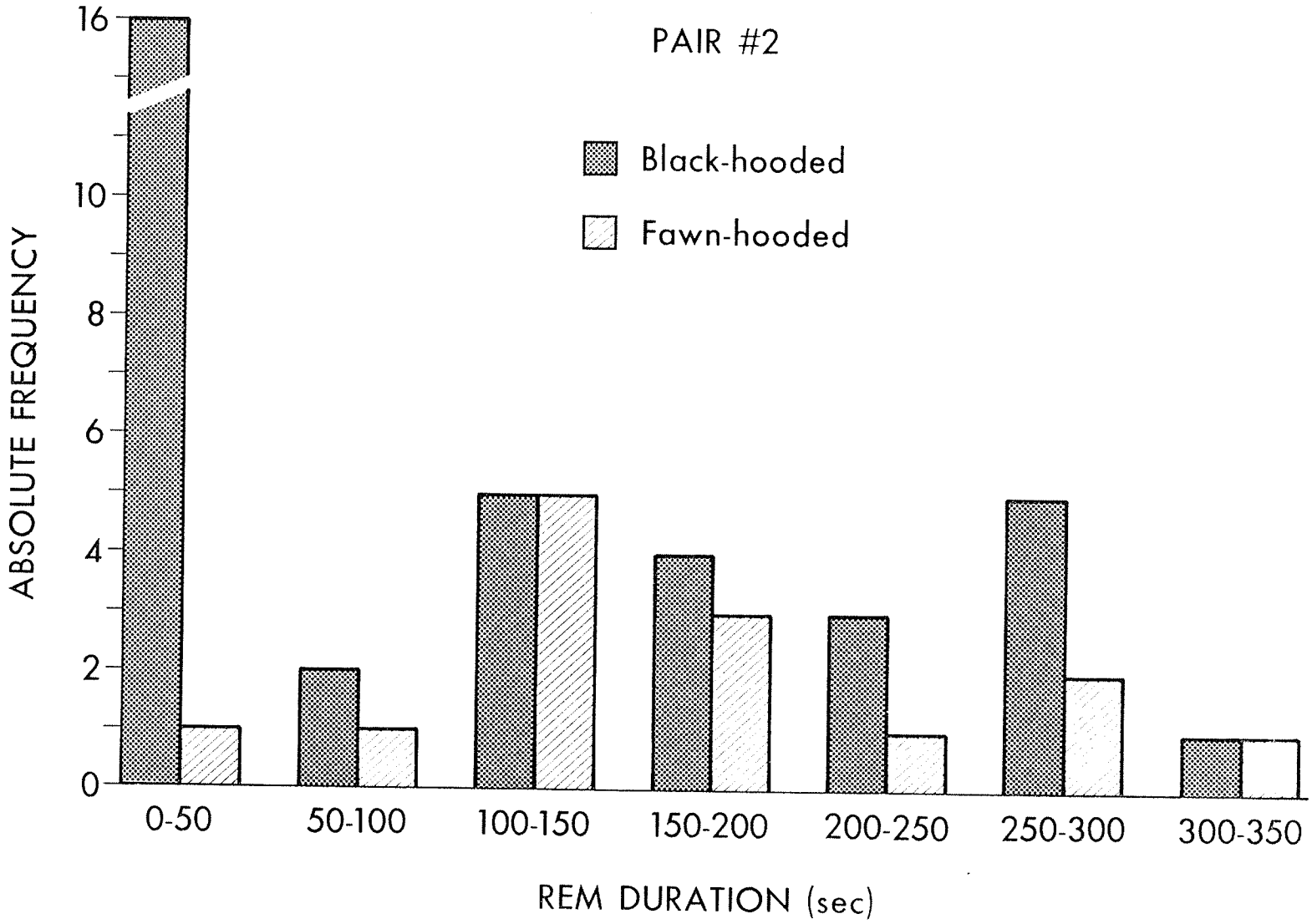

PAIR #2
Black-hooded
Fawn-hooded
ABSOLUTE FREQUENCY
16
10
8
6
4
2
0
0-50
50-100
100-150
150-200
200-250
250-300
300-350
REM DURATION (sec)

FIGURE 9

Frequency distribution of the durations of PS episodes for Pair #3.

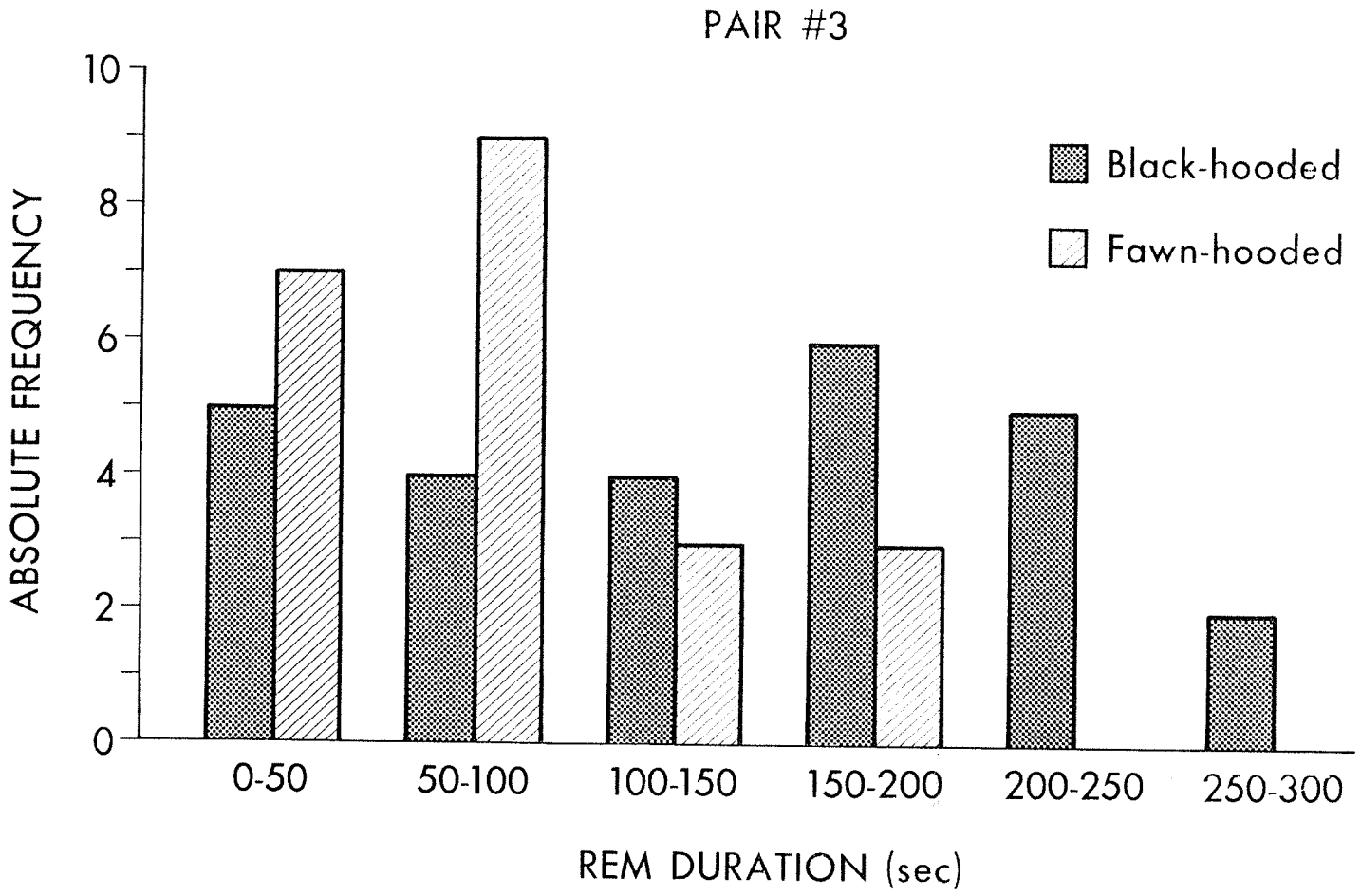

PAIR #3
ABSOLUTE FREQUENCY
10
8
6
4
2
0
Black-hooded
Fawn-hooded
0-50
50-100
100-150
150-200
200-250
250-300
REM DURATION (sec)

FIGURE 10

Frequency distribution of the durations of PS episodes for Pair #4.

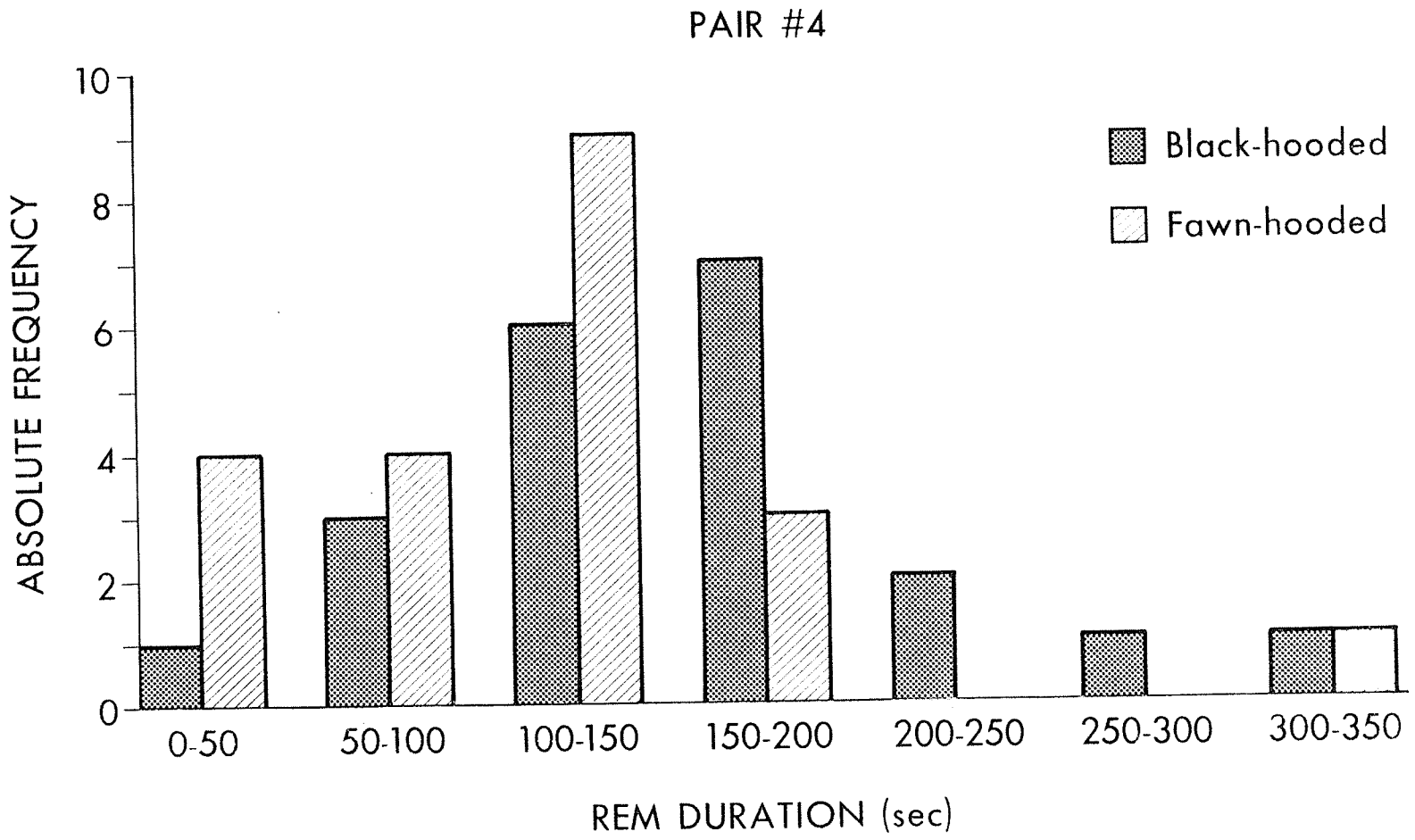

PAIR #4
ABSOLUTE FREQUENCY
10
8
6
4
2
0
Black-hooded
Fawn-hooded
0-50
50-100
100-150
150-200
200-250
250-300
300-350
REM DURATION (sec)

FIGURE 11

Frequency distribution of the durations of PS episodes for Pair #5.

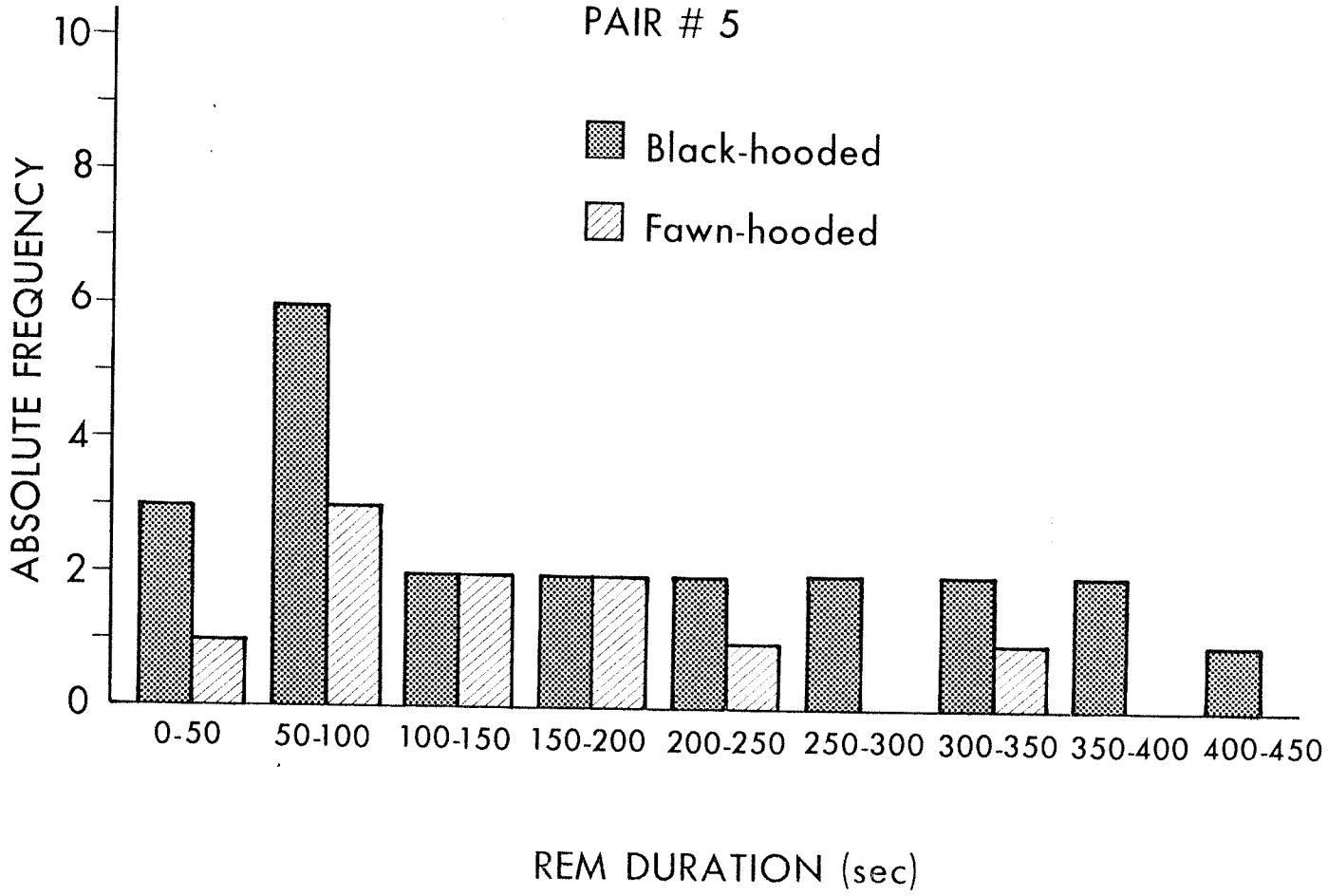

PAIR # 5
Black-hooded
Fawn-hooded
ABSOLUTE FREQUENCY
10
8
6
4
2
0
0-50
50-100
100-150
150-200
200-250
250-300
300-350
350-400
400-450
REM DURATION (sec)

FIGURE 12

Frequency distribution of the durations of PS episodes for Pair #6.

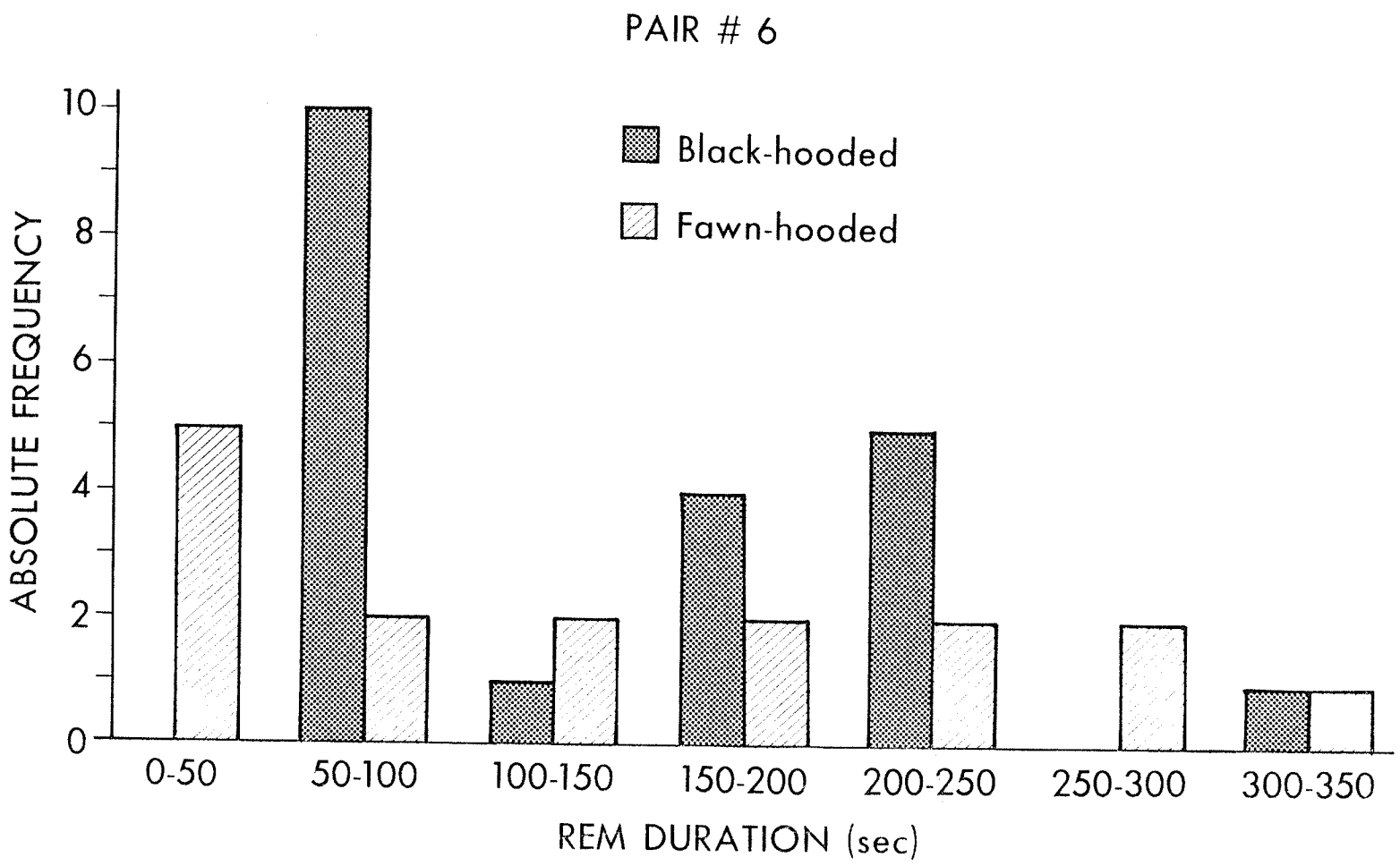

PAIR # 6
Black-hooded
Fawn-hooded
ABSOLUTE FREQUENCY
10
8
6
4
2
0
0-50
50-100
100-150
150-200
200-250
250-300
300-350
REM DURATION (sec)

FIGURE 13

Relative frequency of the durations of PS episodes for Fawn-hooded and Long-Evans Rats.

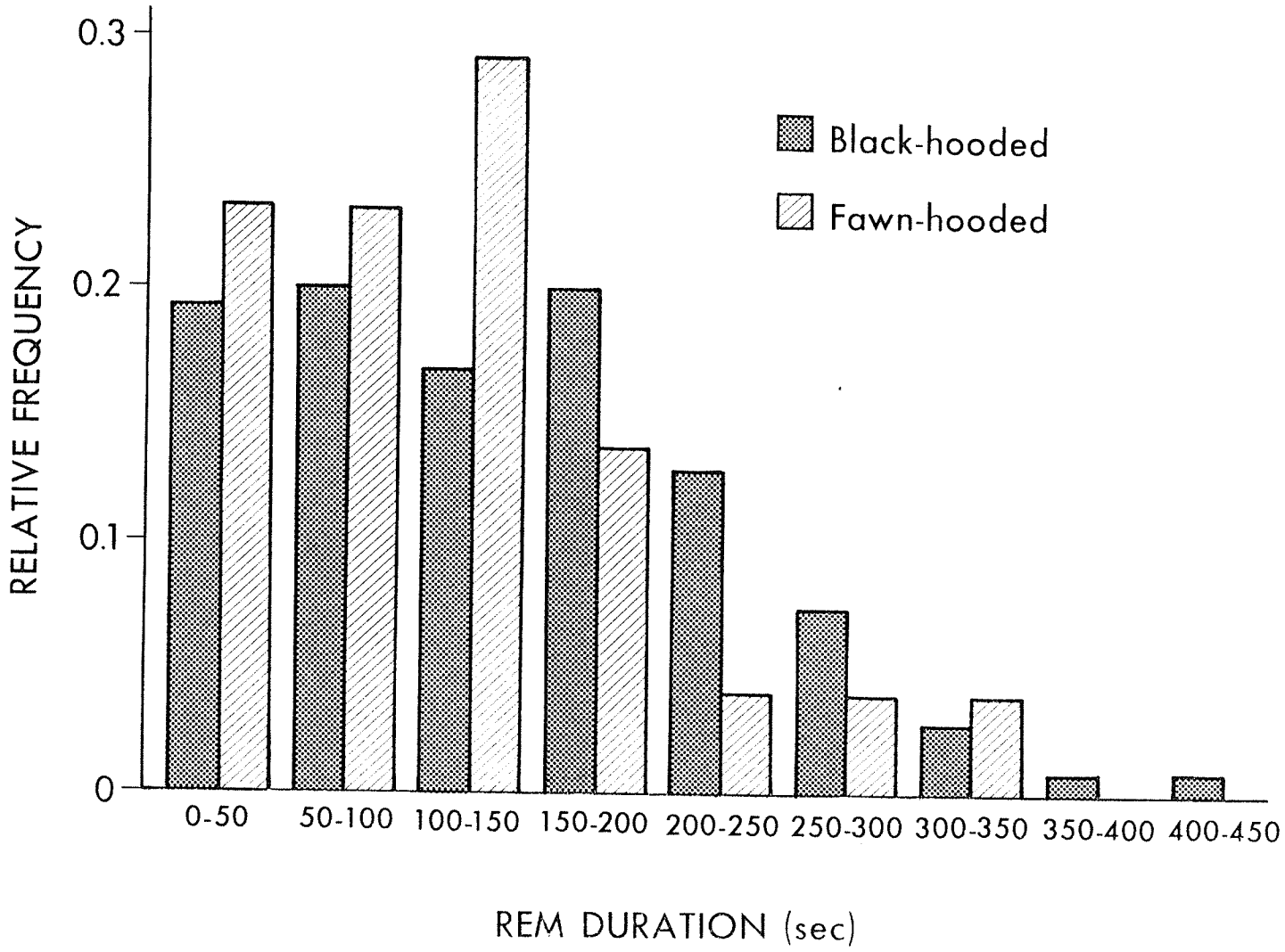

0.3
0.2
0.1
0
RELATIVE FREQUENCY
Black-hooded
Fawn-hooded
0-50
50-100
100-150
150-200
200-250
250-300
300-350
350-400
400-450
REM DURATION (sec)

FIGURE 14

Frequency spectrum of the EEG in the group of F-H rats and control L-E rats during shallow slow wave sleep.

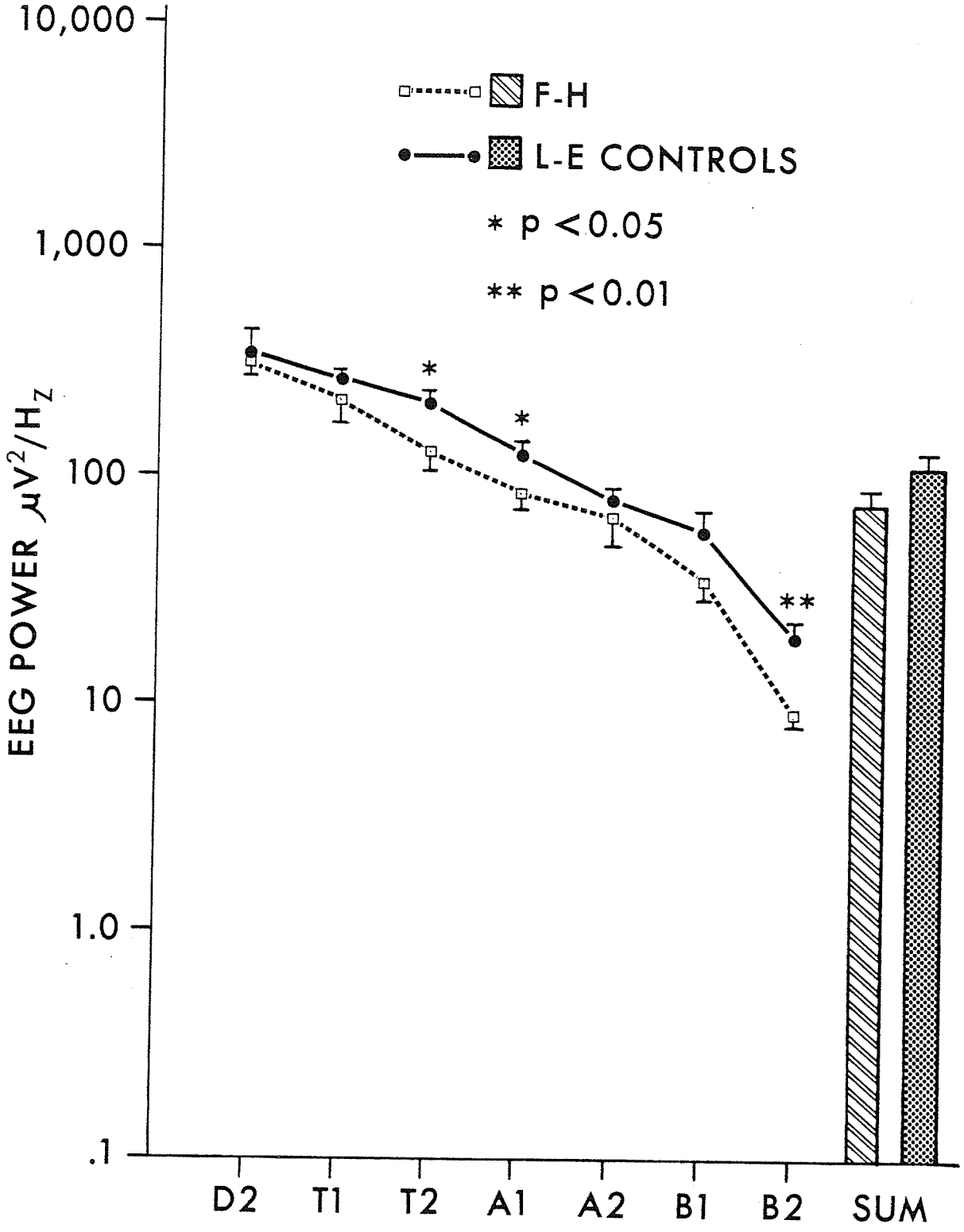

10,000
1,000
100
10
1.0
.1
EEG POWER μV²/Hz
F-H
L-E CONTROLS
* p < 0.05
** p < 0.01
*
*
**
D2
T1
T2
A1
A2
B1
B2
SUM

FIGURE 15

Frequency spectrum of the EEG in the group of F-H rats and control L-E rats during deep slow wave sleep.

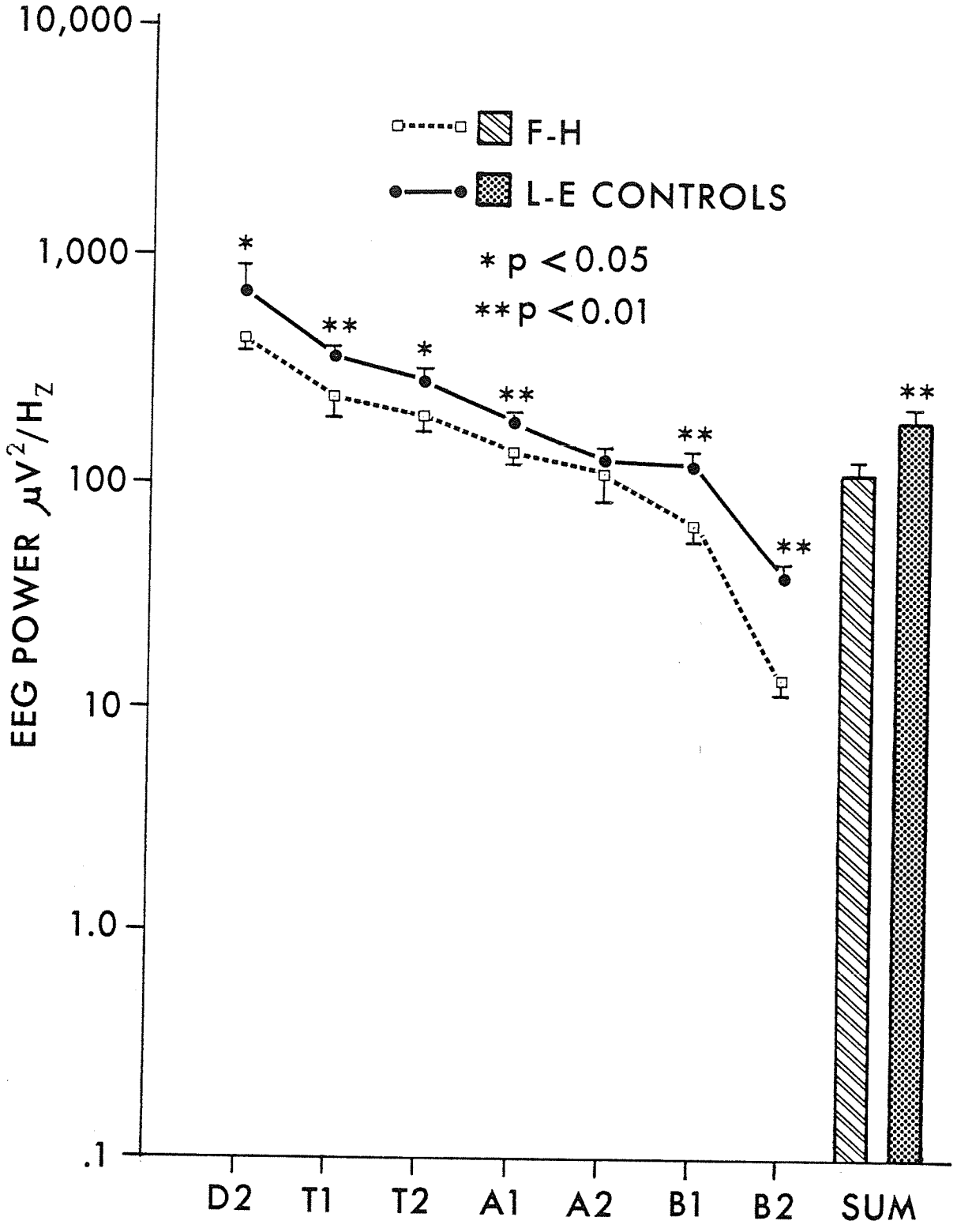

10,000
1,000
100
10
1.0
.1
EEG POWER μV²/Hz
F-H
L-E CONTROLS
* p < 0.05
** p < 0.01
*
**
*
**
**
**
**
D2
T1
T2
A1
A2
B1
B2
SUM

FIGURE 16

Similarities in the spectral analysis of F-H rats during deep SWS and control L-E rats during shallow SWS.

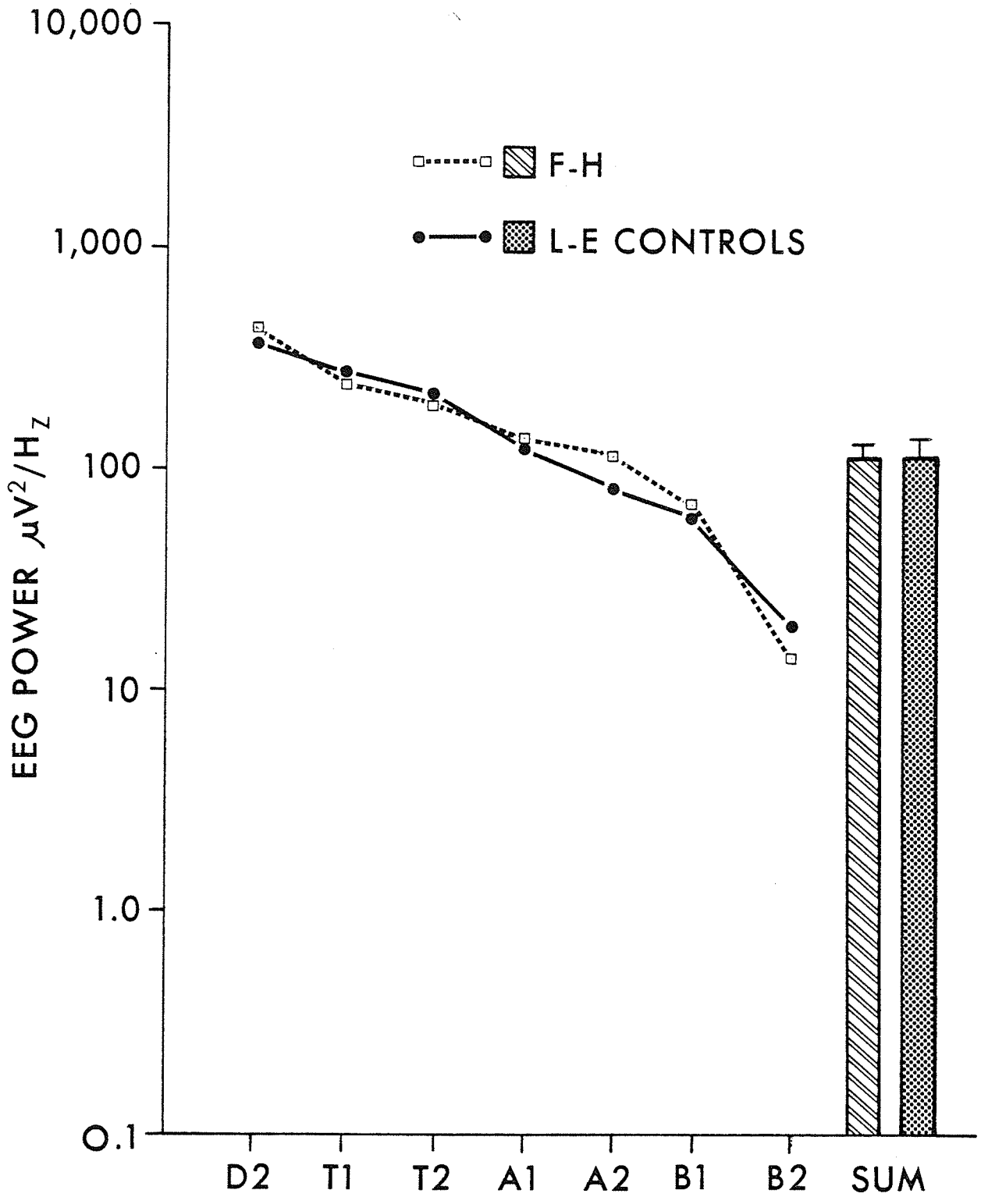

10,000
1,000
100
10
1.0
0.1
EEG POWER μV²/Hz
F-H
L-E CONTROLS
D2
T1
T2
A1
A2
B1
B2
SUM

FIGURE 17

Typical polygraphic recording during deep slow wave sleep in one F-H rat and one L-E control rat.

1,2 - EEG tracing L-E control
3 - Muscle activity L-E control
4,5 - EEG tracing F-H rat
6 - Muscle activity F-H rat

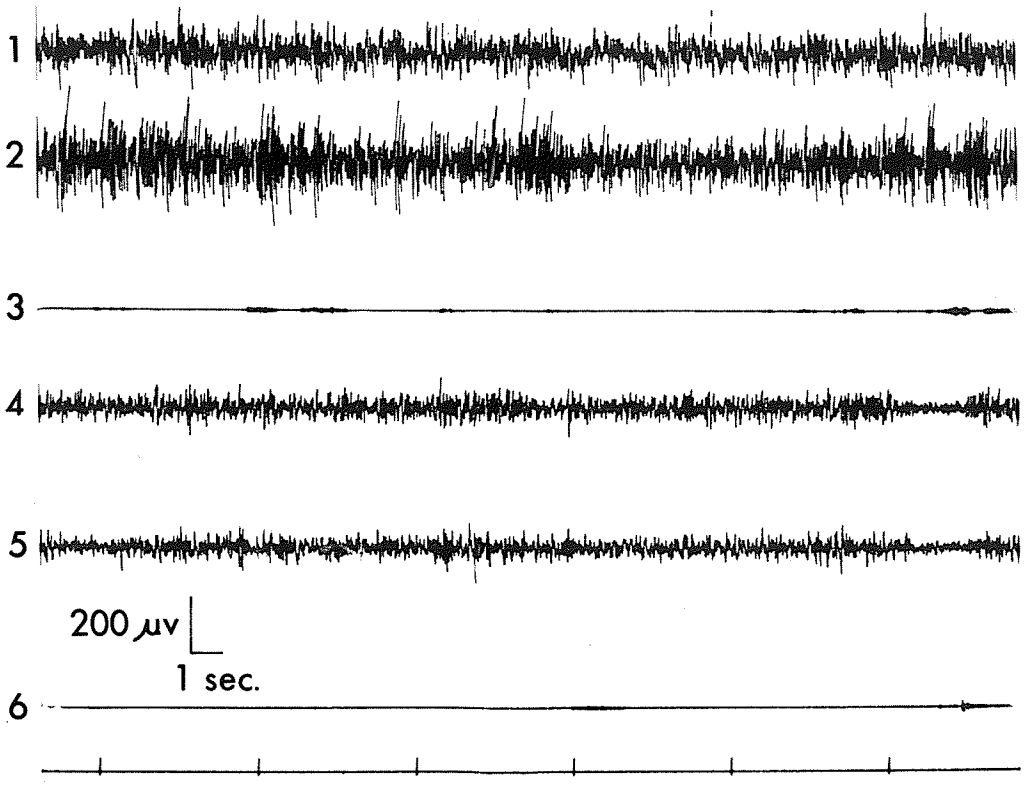

1
2
3
4
5
200 μv
1 sec.
6

FIGURE 18

Polygraphic recording in one F-H rat during deep slow wave sleep and one L-E control rat during shallow slow wave sleep.

1,2 - Electrocortical activity L-E control
3 - Muscle activity L-E control
4,5 - Electrocortical activity F-H rat
6 - Muscle activity F-H rat

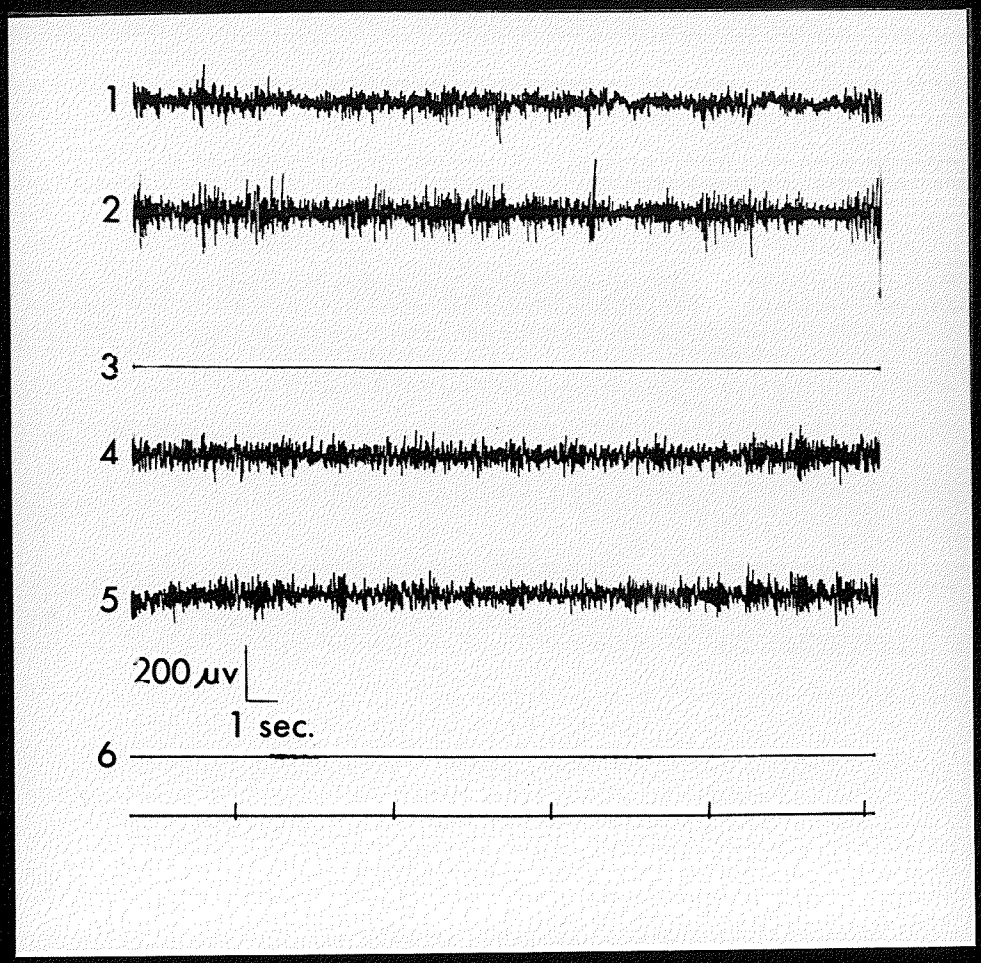
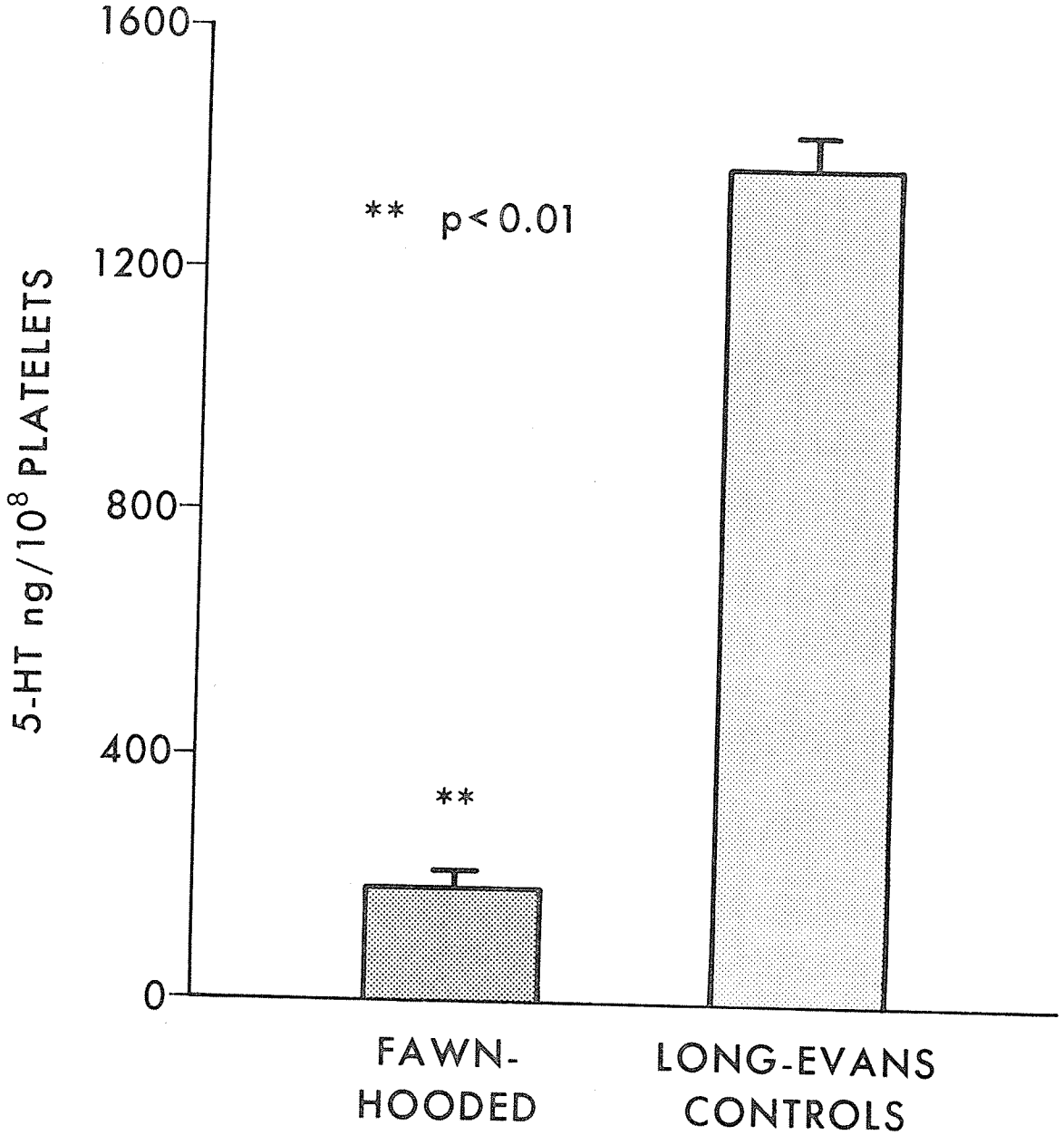

1
2
3
4
5
200 μv
1 sec.
6

FIGURE 19

Marked reductions in mean levels of blood platelet serotonin of F-H rats in comparison to L-E controls.


1600
1200
800
400
0
5-HT ng/10^8 PLATELETS
** p< 0.01
**
FAWN-
HOODED
LONG-EVANS
CONTROLS

differences were highly significant ($t = 11.64$, df: 10, $p < 0.01$) with average values of the F-H group close to 7 times lower than those of the L-E control strain. These differences are shown in Figure 19.

Whole brain levels of serotonin, norepinephrine and dopamine are presented in Table 6. In the F-H group there was a consistent overall reduction in brain serotonin and a slight increase in norepinephrine. However these differences did not reach significance. Thus, the profound reductions observed in platelet 5-HT were not manifested to a similar degree within the CNS.

The main findings of the present experiment were the significant changes in the composition and duration of the main states of the Fawn-hooded animals which form the basis of the sleep-waking cycle. The combination of a reduction in PS and deep SWS in conjunction with a significant increase in the duration of shallow SWS and waking states of F-H rats in comparison to the L-E control rats was consistently observed for each pair of animals without exception.

The results clearly revealed both qualitative and quantitative differences in the EEG patterns during deep SWS. The frequency spectrum of deep SWS of F-H rats corresponded to the spectral characteristics of the shallow SWS of the L-E strain. These differences were also recognizable by visual analysis. No such differences in

Table 6. Brain Levels of Monoamines (ug/g tissue) for Control and Experimental Groups. Values Shown are Means (8 animals) ± Standard Error of the Mean.

	5-HT	NE	DA
Fawn-Hooded	374.88 ± 16.15	300.25 ± 10.89	624.99 ± 46.02
Long-Evans Controls	403.36 ± 20.74	283.71 ± 11.49	673.75 ± 34.23

spectral analysis were evident during waking or PS.

Although peripheral levels of serotonin were markedly reduced in the F-H group, brain levels of 5-HT in these animals showed a consistent but nonsignificant reduction in conjunction with nonsignificant increases in brain NA, in comparison to the L-E control strain (Table 6).

Discussion

The findings of the present experiment appear to provide further support for the hypothesis that serotonin is involved in the induction and maintenance of slow wave sleep. This is suggested by observations that the SWS of the F-H group was markedly different in nature from the SWS of L-E controls.

The slow wave sleep of F-H animals tended to be of a more superficial nature, evidenced by the findings that in these animals the duration of deep SWS was markedly reduced in comparison to the controls. This reduction in deep SWS was compensated to an extent by prolongations in the duration of shallow SWS. Thus, in general the F-H rats did not progress into stages of deeper SWS to the same degree as did the L-E control group.

Since SWS normally appears to be a prerequisite for PS, observations that F-H animals manifested significant reductions in the duration of PS in conjunction with the deep SWS reductions is not surprising. These

results are consistent with evidence from lesion and pharmacological studies indicating that procedures which affect levels of serotonin in the brain will also eventually affect PS (Jouvet, 1972).

In the present experiment, F-H rats did not progress into the more advanced stages of sleep to the same degree as did the control rats. As a result, these F-H animals demonstrated a suppression in PS. Episodes of PS occurred less frequently in these animals, and in addition the average length of episodes of PS was considerably reduced when compared to the L-E control strain.

Thus, the sleep-waking cycles of F-H rats showed distinct quantitative differences in the average durations of each of the states of sleep. These findings of quantitative differences in the EEG during sleep are complemented by the results of the spectral analysis demonstrating qualitative differences in the EEG during slow wave sleep. Computerized spectral analysis of the EEG during deep slow wave sleep revealed a less pronounced synchronization in F-H rats in comparison to L-E rats. Thus, the EEG pattern of these F-H animals during deep SWS was relatively less deactivated than the EEG pattern of the L-E control strain. When the averaged power spectra of these animals was further analyzed, it became evident that the deep SWS of the F-H animals corresponded to the shallow SWS of the control strain.

These differences in power spectra characteristics were observed only during slow wave sleep. No such differences were detected either during PS or the waking state.

Also of considerable interest were the observations of overall reductions in total sleep time of the F-H group. These animals spent significantly more time in the waking state in comparison to the L-E strain. These findings were unexpected on the basis of observations of increased docility and reduced emotionality in these animals, implying a functional alteration in the organization of the sleep-wake cycle rather than changes secondary to increased arousability.

The profound reductions in endogenous 5-HT levels observed in the blood platelets of the F-H group did not appear to be manifested to the same degree in 5-HT levels of whole brain. However, serotonin is unevenly distributed in the brain (Hillarp, Fuxe and Dahlstrom, 1966) thus measurements of 5-HT based on the analysis of whole brain may not reflect changes of this amine in specific brain regions. Furthermore, since blood platelets contain extremely high concentrations of serotonin (in comparison to brain) it is possible that differences between groups may be considerably amplified in measurements of platelet 5-HT. Although consistent reductions were observed in brain levels of 5-HT in the F-H group, the failure of these differences to reach statistical significance may

be partially attributable to the relatively small sample size. Perhaps with larger sample size and reduced intra-group variability, significant differences may have been detectible.

Furthermore, it has long been recognized that an understanding of the functional state of serotonin in the brain requires knowledge of both its endogenous level and its turnover rate. The attempt to relate a specific behaviour, such as slow wave sleep, to endogenous levels of brain 5-HT is compounded because it appears that there are two stores of the transmitter in the terminals of serotonergic neurons -- one large, mainly non-releasable storage pool, and a small labile or functional pool. This small functional pool which is preferentially released by nerve stimulation or some pharmacological agents, consists mainly of newly synthesized serotonin, and may represent only a small percent of the total stores of brain 5-HT. This functional pool is reflected in studies of the turnover rate of serotonin.

In the present study, an important consideration would be whether or not there is a change in the functional pool of serotonin. Thus determination of the turnover of serotonin would provide more informative and perhaps more conclusive data concerning the functional state of serotonin in the fawn-hooded

strain of rat.

The possibility that low levels of 5-HT in the platelets of F-H animals may reflect disordered indoleamine transport or metabolism within the central nervous system is suggested by recent evidence that blood platelets have certain structural and functional similarities to amine-containing neurons (Paasonen, 1971; Pletscher, 1968; Sneddon, 1973). It appears highly unlikely that platelet concentrations of serotonin reflect the level of this amine in the brain as was originally proposed by Udenfriend (1957), particularly in light of recent investigations (Fernstrom and Wurtman, 1971) demonstrating the importance of tryptophan availability in the modulation of serotonin synthesis in the CNS. However, it is suggested that the 5-HT abnormalities observed in the platelets of F-H animals may reflect as yet undetermined abnormalities in metabolism of this monoamine within the CNS.

This is supported by the present findings of alterations in the sleep-waking cycle of the F-H strain. These animals manifested both qualitative and quantitative differences in the EEG patterns during the states of sleep; and of particular interest were the findings that the F-H group did not progress into stages of deep SWS to the same extent as the controls.

Since the study of the states of sleep may provide an index for examining the functioning of the central nervous system (Valtax, Bugat and Jouvet, 1972) it may be concluded that the F-H strain show some alterations in CNS functioning. Furthermore, on the basis of the extensive literature linking serotonin with SWS, it seems entirely possible that these alterations in CNS functioning in the F-H strain involve serotonergic mechanisms.

Additional support for this conclusion is provided by observations by the present author, and by other investigators (Tobach, unpublished) that the behaviour of F-H animals is markedly different from that of other strains. These F-H rats are unusually docile, less aggressive and less excitable than either the L-E or Wistar strains.

Observations by the present author tend to suggest that the beige coat colouring of the F-H strain appears to be inherited as an autosomal recessive trait. The principal phenotypic manifestations of these rats include coat colour dilution (not albinism per se), and a congenital hemorrhagic diathesis which resembles that of platelet storage pool defect in humans (Holmsen and Weiss, 1970), particularly the variant which includes

oculocutaneous albinism - commonly referred to as the Hermansky-Pudlak Syndrome (White, Edson and Desnick, 1971). This syndrome of abnormalities observed in the F-H strain is very similar to that reported in beige mice (Holland, 1976) and some mutant strains of mink (Padgett, Leader, Gorham and O'Mary, 1964). It is of particular interest to note that beige mice have been observed to have a disorder in platelet functioning which is characterized by very low levels of serotonin, impaired uptake and storage of serotonin (Holland, 1976). These abnormalities were observed by the present author in fawn-hooded rats. However no behavioural studies with these beige mice have been reported.

The possibility that the low levels of 5-HT storage or storage capacity may be a common denominator between light coat colour, docile behaviour, changes in sleep parameters and bleeding tendency is suggested by a variety of evidence. Release of melanocyte stimulating hormone (MSH) by various stimuli has been shown to be mediated by the activation of 5-HT containing neurons, and this release can be blocked by PCPA or methylsergide (Taliesnik, Celis and Tomatis, 1974).

The pigmentation of skin and hair originates from the neural crest embryologically. Knowledge of the nature of pigment formation and the genetic influences exerted on it may thus lead to a better understanding of

basic metabolic variations of which coat colour may be only a factor. The prominent role of aromatic amino acids as precursors both to pigment (Melanin) and to many crucial hormones (catecholamines) suggests that a gene which results in a defect in pigmentation may simultaneously cause other abnormalities, both metabolic and neurochemical.

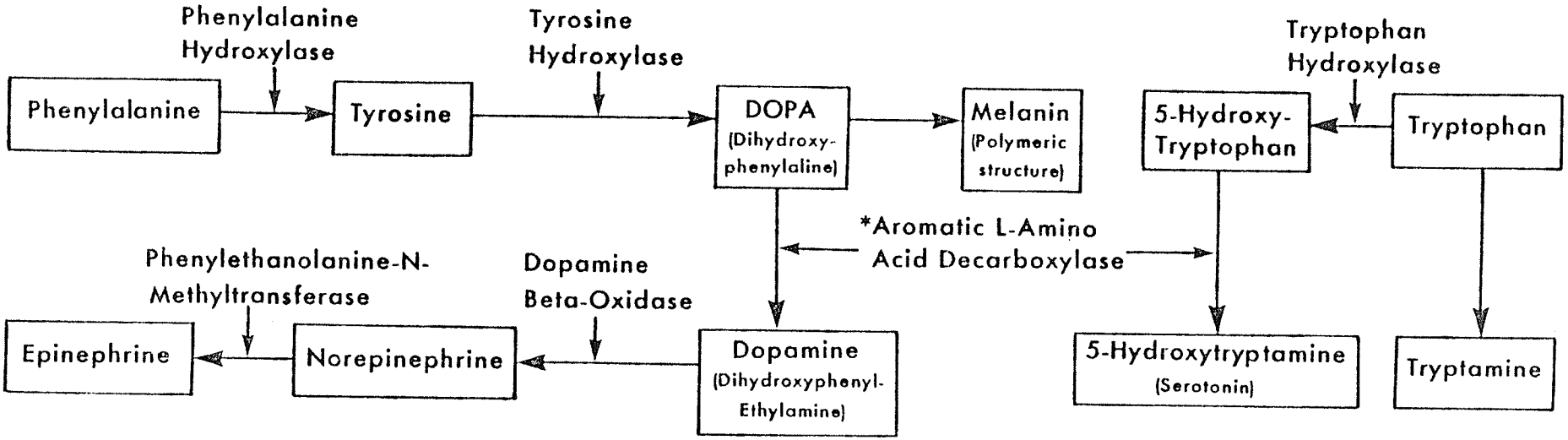
Melanin is distributed in the body in the skin, hair, retina of the eye, and in neurons in certain areas of the brain. These areas include the substantia nigra of the midbrain, and the locus coeruleus situated in the floor of the fourth ventricle. Melanin, or pigment is derived from tyrosine by way of 3,4 dihydroxyphenylalanine, (DOPA) and its oxidation product 3,4 dioxyphenylalanine (dopaquinone) which progresses to further melanin precursors and to melanin (Harper, 1963). With respect to the metabolism of phenylalanine, it is of interest that tyrosine is also a direct precursor of norepinephrine and epinephrine. These interrelationships are schematized in Figure 20.

Since tyrosine is the usual intermediate precursor to pigment in both humans and animals, any inhibitor of tyrosine synthesis, if severe enough, would be expected to cause changes in the amount or type of pigment formed. A reduction in phenylalanine hydroxylase activity has been reported in animals of diluted pigmentation (Coleman, 1960)

FIGURE 20

Interrelationships between the biosynthetic pathways of catecholamines, serotonin and melanin.

*Note that the same enzyme is believed to catalyze the decarboxylation of 5-hydroxytryptophan and DOPA. However, recent evidence (Dakshinimurti, Hercl, Leblancq & Havlick, 1976) suggests that 5H-TP decarboxylase is most likely different from DOPA-decarboxylase.


Phenylalanine Hydroxylase
Tyrosine Hydroxylase
Tryptophan Hydroxylase
Phenylalanine
Tyrosine
DOPA (Dihydroxy-phenylaline)
Melanin (Polymeric structure)
5-Hydroxy-Tryptophan
Tryptophan
*Aromatic L-Amino Acid Decarboxylase
Phenylethanolanine-N-Methyltransferase
Dopamine Beta-Oxidase
Epinephrine
Norepinephrine
Dopamine (Dihydroxyphenyl-Ethylamine)
5-Hydroxytryptamine (Serotonin)
Tryptamine

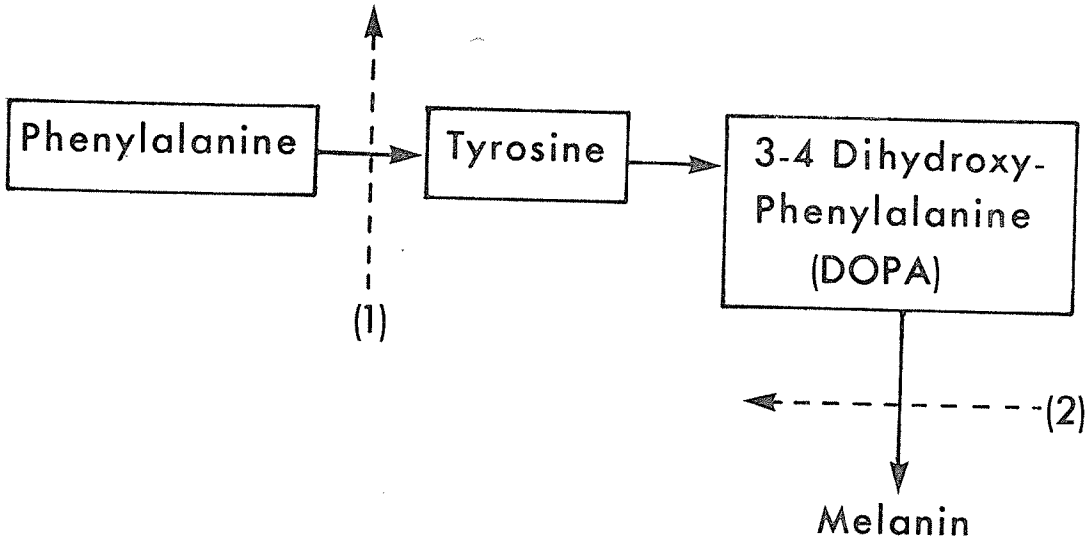
thereby suggesting biochemical defect for this diluted pigmentation.

Pigmentary dilution (of skin, hair and eye colour) is frequently observed in human individuals with phenylketonuria (Folling, Mohr and Ruud, 1945). In phenylketonuria (PKU) there is a single metabolic block in the conversion of phenylalanine to tyrosine. This abnormality has been described by Knox and Hsia (1957) and a schema of the pathogenesis of diluted pigmentation (hypomelanosis) is presented in Figure 21. Usually PKU, if untreated, is accompanied by severe mental deficits. Although a biochemical basis for the profound impairment of mental functioning which occurs in PKU has not yet been discovered, reference has been made to the possibility of a serotonin deficiency as a factor affecting cerebral function (Harper, 1963). Low levels of serotonin have been reported in the blood of individuals with PKU, in conjunction with diminished excretion of 5-hydroxyindoleacetic acid. These values return to normal levels when PKU subjects are given low phenylalanine diets (Harper, 1963).

An animal model of this abnormality has been described in mice of diluted pigmentation (Coleman, 1960). These animals are described as extremely nervous and particularly susceptible to audiogenic seizures possibly as a result of reduced brain levels of 5-HT, NE or GABA. However no other behavioural studies have been reported

FIGURE 21

Disturbances of phenylalanine metabolism
associated with disorders of pigmentation.



(1) Phenylketonuria

(2) Albinism

in this strain of mice, nor was there any mention of bleeding tendencies in these animals.

Other examples of diluted pigmentation have also been observed in the Chediak-Higashi syndrome - a rare autosomal recessive trait characterized by abnormal platelet function and pigmentary dilution which affects eye, skin and hair to varying degrees, ranging from partial albinism in individuals of light skinned races to a reddish brown pigmentation observed in Mediterranean and Oriental races (Stegmaier and Schneider, 1965). This syndrome has also been described in the Aleutian mink, cats, cattle (Bell, Camacho, Meyers and Padgett, 1975) and beige mice (Holland,1976) on the basis of similarities to the human manifestations.

Results of the present experiment suggest that the F-H strain of rat bears certain similarites to the models previously described. Some of these similarities include diluted pigmentation, and hemorrhagic tendency due to abnormalities in peripheral aminergic functioning. If the F-H strain of rat indeed belongs to the same category of abnormalities as previously described, the present findings of abnormalities in serotonergic mechanisms controlling sleep in these animals is of considerable clinical and experimental significance.

It is of particular interest to note that one important area in which melanin is distributed is the

locus coeruleus (Bloom, 1968). This same area is crucial to the staging of sleep, particularly to the staging of PS. One might assume that abnormalities of pigmentation observed in the F-H strain may also exist in the locus coeruleus of the brain of these animals. However, the degree to which these differences may play a role in sleep staging remains somewhat speculative at this point.

The present findings of changes in sleep parameters of F-H rats also suggest that there are some abnormalities in central serotonergic functioning in these animals, manifested by qualitative and quantitative changes in the EEG patterns of sleep. It is also suggested by the present author, that the abnormalities observed in peripheral aminergic functioning, in the F-H strain of rat, may be a reflection of a widespread malfunction of indolealkylamine metabolism and that the plaletet abnormalites may indeed reflect changes in central serotonergic mechanisms in these rats. It is further suggested that the presence of normal serotonergic functioning may be a necessary condition for the patterns of sleep, both SWS and PS to become fully manifest.

It has been known for the past twenty years that brain amine neurotransmitters such as serotonin and the catecholamines play a key role in brain functioning and in the control of emotion, perception, motivation and learning. Disturbances in these amines have been

linked to the pathogenesis of various disorders and there is a growing base of information about how these amines may be manipulated. Findings from the present experiment clearly suggest that the F-H strain of rat may provide a useful experimental model for studies in these areas in the future.

CHAPTER 3

INVESTIGATION OF SLEEP PARAMETERS IN SPONTANEOUSLY HYPERTENSIVE RATS

In general it appears that alterations to central serotonergic mechanisms produce more dramatic and severe effects on sleep than do alterations of adrenergic mechanisms. However, from the extensive research literature on the neurochemistry of sleep, it is clearly evident that central adrenergic mechanisms play an important role in PS, particularly in the maintenance of the tonic components of PS (Jouvet, 1969).

Alterations in central noradrenergic mechanisms have recently been reported in spontaneously hypertensive rats (Yamori, Lovenberg and Sjoerdsma, 1970; Yamori, Ooshima, and Okamoto, 1973). However, the sleep-waking cycles of these animals has not been documented. Although numerous studies have investigated cardiovascular effects of SWS and PS in normotensive and hypertensive animals (Zanchetti, 1972; Zanchetti, Baccelli, Guazzi and Mancia, 1973; Zanchetti, Guezzi and Baccelli, 1967) and humans (Bristow, Honour, Pickering and Sleight, 1969; Richardson, Vetrovec and Williamson, 1973), no studies have been directed towards the effects of hypertension on sleep parameters. Furthermore, since the spontaneously hypertensive strain of rat has been described as having abnormalities in central noradrenergic functioning, the study of the sleep-waking cycle of these animals represents a worthwhile undertaking, particularly

with respect to the important role of central noradrenergic mechanisms in PS postulated by Jouvet.

Characteristics of Spontaneously Hypertensive Rats

The spontaneously hypertensive strain of rat (SHR) established as an inbred strain by Okamoto and his associates at Kyoto University, Japan, in 1962, has been the subject of extensive study during the past decade. This is primarly because in many important respects the progression and characteristics of hypertension in the SHR closely mimics those of essential hypertension in humans (Hallback and Weiss, 1977). Furthermore, the pathological changes which are commonly associated with sustained hypertension in humans (vascular disease, myocardial infarction, cerebral hemorrhage) have also been observed in the SHR (Okamoto, Aoki, Nosaka and Fukushima, 1964).

Hypertension in the SHR is multi-factionally inherited via a small number of major genes (Tanase, Suzuki, Ooshima, Yamori and Okamoto, 1970) and is modifiable by environmental factors such as stress (Yamori, Matsumoto, Yamabe and Okamoto, 1969). The primary initiating mechanisms for hypertension in these animals are still not completely understood. However, the findings indicate that central neurogenic factors are of major importance as initiating mechanisms, although non-neurogenic vascular mechanisms are subsequently involved (Yamori, 1976).

Experiments by various researchers have indicated a dysfunction of central vasomotor centers in the SHR (Okamoto, 1969) and there now appears to be little doubt that neurogenic influences contribute to the hypertension of the SHR. Studies by Okamoto and his associates (Okamoto, 1969; 1972) have shown that efferent nervous and hormonal control of the cardiovascular system is different in SHR and normal control rats. This neurogenic sympatho-adrenal abnormality appears to be central rather than peripheral in origin.

Other evidence for a central origin of altered neurohumoral control is provided by a series of studies by Hallback and her co-workers. These investigators have found that when exposed to brief episodes of sensory stimulation, the SHR exhibit exaggerated heart rate and blood pressure elevations in comparison to control rats (Hallback, 1975). Tachycardia responses to these sensory stimuli were not only more intense in the SHR, but were also more consistently elicited than in normal controls. Normotensive and renal hypertensive rats occasionally responded to stressful sensory stimuli with an autonomic pattern which included vagal activation, whereas the SHR appeared to engage preferentially in sympathetic patterns of response (sympathetic excitation combined with vagal suppression). This response tendency was believed to represent a genetically determined primary disturbance rather than a secondary phenomenon, since it was also

observed in young SHR (11 weeks) during the developing phase of hypertension, but was not observed in age-matched genetically normotensive rats with renal hypertension.

The hyperreactivity of SHR to environmental stimuli did not appear to be attributable to a generalized hyper-reactivity involving all autonomic efferents but rather was confined to sympathetic outflow. This is supported by findings that direct vagal stimulation caused equal decrements in heart rate in both the SHR and normal control rats (Hallback, 1976). Furthermore, sympathetic stimulation using isoprophylnorepinephrine caused increased heart rate responses in SHR and control rats of approximately equal magnitude. These findings led Hallback (1976) to conclude that the heart rate response to vagal and sympathetic excitation is not attributable to abnormal responsiveness at the peripheral neuroeffector site, but instead is at the level of the CNS, involving the intensity of the centrally induced neural discharge.

Further evidence that the hyperreactivity of the SHR to environmental stimuli is not a reflection of a generalized hyperreactivity is provided by findings that prolonged immobilization, a procedure known to induce vagally mediated gastric ulceration in rats (Brodie, 1968), results in the same incidence of ulceration in SHR and normal control rats (Hallback, Magnusson and Weiss, 1974). Thus, centrally mediated autonomic responses to stress

affected vagal control of the gastrointestinal tract to the same degree in SHR and normotensive control strains.

These findings are consistent with behavioural observations of altered behaviour in the SHR. Folkow (1974) described the Okamoto strain of hypertensive rats as "choleric" in temperament, excitable from early ages onward, resistant and aggressive to handling. Studies from other laboratories have also reported that the SHR are more aggressive than normotensive Wistar control strains (Hallback and Weiss, 1977; Shimamoto and Nagaoka, 1972). In this regard, it is interesting to note that similar kinds of behaviours have been observed to accompany blood pressure elevation in experimental animals following the chronic stress of Sidman avoidance schedules (Forsythe, 1969; Forsythe and Harris, 1970).

The importance of central noradrenergic mechanisms in the regulation of arterial blood pressure has become increasingly apparent in recent years. Experiments have demonstrated a relationship between pathological elevations of blood pressure and changes in central catecholamine metabolism. In the SHR, endogenous norepinephrine levels, and the activity of enzymes associated with norepinephrine synthesis are reduced in the brainstem and hypothalamus (Yamori, Lovenberg and Sjoerdsma, 1970; Yamori, 1970) in comparison to normotensive Wistar controls.

It was subsequently demonstrated (Louis, Kraus, Kopin and Sjoerdsma, 1970; Yamabe, DeJong and Lovenberg,

1973) that the catecholamine level, turnover rate of noradrenaline and in-vivo synthesis rate were lower in SHR than in normotensive Wistar rat strains. However, the choice of controls for studies of this type has remained a problem. Initially rats of normotensive Wistar strains were generally used as controls. Comparisons based on these controls clearly demonstrated lower noradrenaline levels, decreased activity of enzymes involved in norepinephrine synthesis and decreased noradrenaline synthesis in the brain stem of SHR (Louis et al, 1970). However, these findings were not corroborated in subsequent studies in which rats of the genetically related normotensive Wistar-Kyoto strain were used as controls (Lovenberg, Yamabe, DeJong and Hansen, 1973; Sjoerdsma, 1971; Yamabe et al, 1973).

From a genetic point of view, the Wistar-Kyoto rat (the normotensive parent strain of the SHR) is probably the most appropriate control according to the Okamoto group of researchers (Yamabe et al, 1973).

Such problems seriously limit any definite assertions concerning central adrenergic functioning in the SHR. Difficulties have arisen not only as a result of selection of inappropriate control groups, but also as a result of selection of animals during the various stages of hypertension. Since hypertension is an age-dependent disorder with severity varying as a function of time (Albrecht, Vizek and

Krecek, 1972) attempts to compare data obtained during the prehypertensive stage with data from subsequent stages of the disorder causes confusion. Thus, apparent inconsistencies in literature reports of central norepinephrine levels and metabolism may be attributed to these factors. Although some researchers (Takaori, Tanaka and Okamoto, 1972) have reported slight reductions in NE in the brain stem, and significantly increased levels of brain serotonin in mature SHR relative to normotensive Wistar-Kyoto controls, these changes have not been observed in younger animals (Nakamura, Gerold and Theonen, 1972).

The interaction of environmental factors with a particular genetic predisposition to hypertension is an issue of major importance to investigators studying the mechanisms of chronic hypertensive disease. Of considerable interest is the role of dietary factors, in particular, the role of excessive salt intake. Evidence from epidemiological studies of various populations has established a close correlation between the amount of dietary salt intake and incidence of hypertension in these populations (Dahl and Love, 1957).

Animal experiments have demonstrated that beginning early in life, the administration of excess salt to susceptible rat strains for considerable periods of time can induce hypertension (Dahl, Heine and Tassinari, 1962). Although the mechanism by which salt intake promotes hyper-

tension are not fully understood, excess sodium is believed to be a crucial factor in some forms of congenital hypertension.

In the present experiment sleep patterns of mature SHR were examined and compared with those of age-matched normotensive Wistar-Kyoto controls. To induce further increases in arterial blood pressure, some animals of SHR and control groups were placed on a high sodium intake by substituting 0.9% saline for their drinking water. An attempt was then made to relate the findings to existing literature reports of abnormalities in central noradrenergic functioning in the SHR strain.

Method

Subjects

Twelve male spontaneously hypertensive rats (SHR) and twelve age-matched normotensive rats of the Wistar-Kyoto (WKy) strain were used. Both the SHR and WKy strains were obtained from original colonies of rats at Kyoto University, Japan (Okamoto and Aoki, 1963) and were maintained as inbred colonies by successive matings at the animal care facilities of the Department of Pharmacology, University of Manitoba.

Animals were raised under identical environmental conditions (12 hr light/dark cycle, 18.00 hr to 6.00 hr) and were housed in group cages (3 rats/cage). Until the age of 3 months all animals received standard laboratory

rat chow (Ralston) and tap water ad lib.

At three months of age, animals were randomly assigned to either of 2 groups. The first of these groups continued to receive tap water, the second group received 0.9% saline (NaCl) for drinking. Animals were maintained on either tap water or saline until the time of the experiment.

Procedure

Sleep studies were conducted on healthy mature male rats at the age of 6 months. Electrode implantation and procedural details were the same as previously described in Experiment 1. For the sleep parameters, data were statistically evaluated by analysis of variance (2X2 factorial).

Following completion of the sleep studies, systolic blood pressure was measured using the tail-cuff plethysmographic method with the rat under light ether anaesthesia, placed on a heating pad to maintain body temperature at 36 - 37^{o}C. The average of 3 recordings of the blood pressure taken at appoximately 30 second intervals was used as the mean blood pressure value. Variability among the recordings on a given rat was less than 5%.

Blood pressure data were statistically evaluated by a 2X2 factorial analysis of variance, and a correlation matrix among sleep parameters and blood pressure was performed.

Results

Figure 22 summarizes the mean systolic blood pressure levels for SHR and WKy groups. Analysis of variance (2X2 factorial) demonstrated that SHR had significantly higher blood pressures than age-matched WKy controls, $F = 26.68$, df: 1,18, $p < 0.01$. However, contrary to prediction, animals maintained on 0.9% saline did not show significant blood pressure elevation in comparison to animals receiving tap water, nor was there a significant interaction between genetic strain X diet as was predicted (Table 7).

A significant negative correlation was found between blood pressure and total duration of deep slow wave sleep, $t = -2.84$, df: 19, $p < 0.05$. Thus, animals with higher systolic blood pressures spent less time in deep slow wave sleep in comparison to animals with lower blood pressure. This inverse relationship between deep SWS and systolic blood pressure is illustrated in a scatterplot of the two variables in Figure 23. Blood pressure also showed a negative but non significant linear relation to total duration of PS. The correlation matrix between blood pressure and sleep-wake variables is presented in Table 8. Total mean durations of various components of the sleep-waking cycle of SHR and WKy rats maintained on either water or 0.9% saline for drinking are presented in Figure 24.

FIGURE 22

Mean systolic blood pressure of SHR and WKy control rats loaded with 0.9% NaCl in the drinking water or without NaCl.

Vertical lines indicate Standard error.

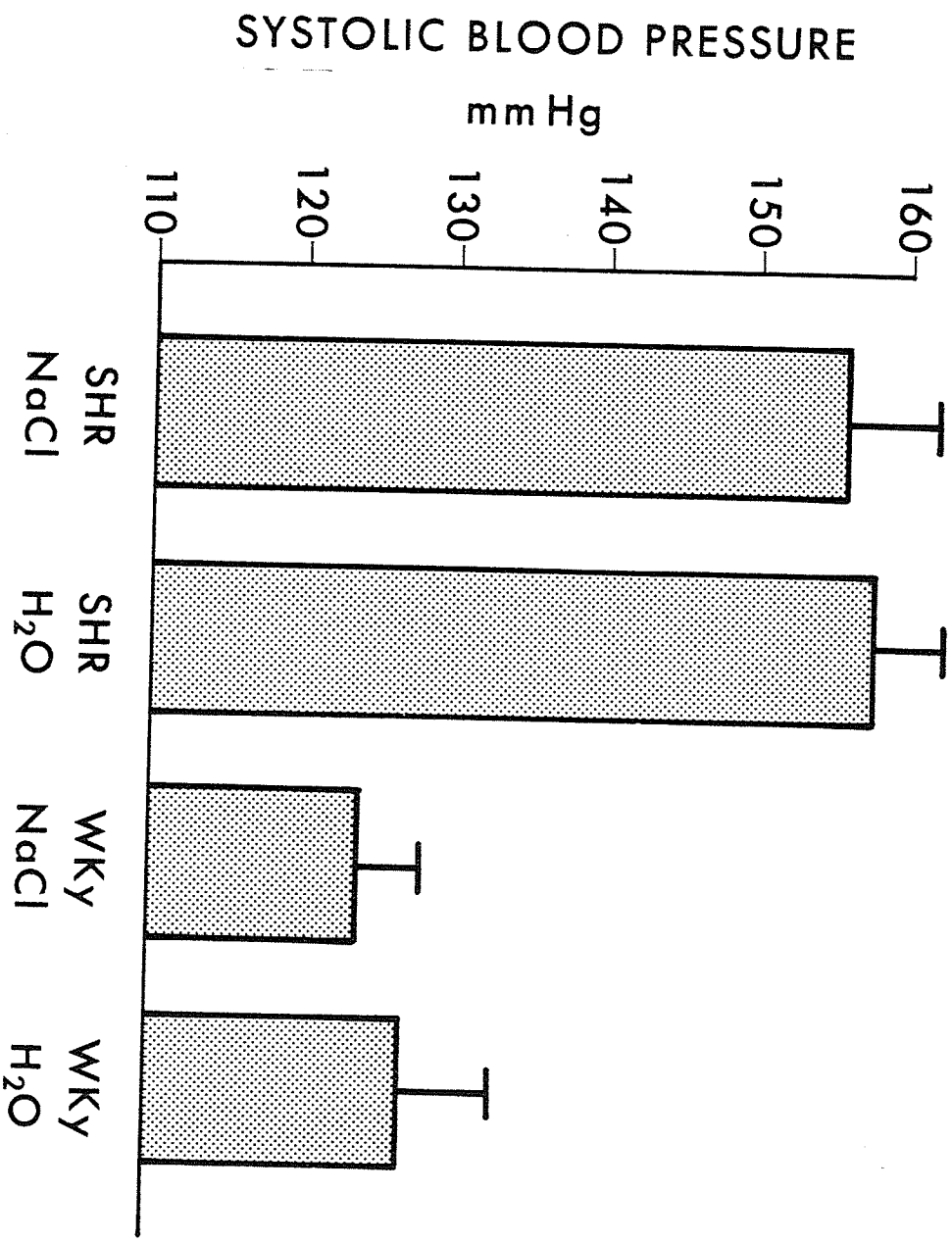

SYSTOLIC BLOOD PRESSURE
mm Hg
110
120
130
140
150
160
SHR NaCl
SHR H2O
WKy NaCl
WKy H2O

Table 7. Analysis of Variance: Systolic Blood Pressure

Source	df	MS	F
Diet	1	4.41	0.12
Genetic Strain	1	996.454	26.68**
Diet X Strain Interaction	1	0.588	0.02
Error	18	37.35	

** $p < 0.001$

Table 8. Correlation Matrix Between Blood Pressure and Wake-Sleep Parameters

	1	2	3	4	5
1	1.0				
2	.22	1.0			
3	.31	- .55	1.0		
4	- .54*	- .40	- .50	1.0	
5	- .27	- .24	- .29	.26	1.0

Variable 1 Systolic Blood Pressure
Variable 2 Total % Awake
Variable 3 Total % Shallow SWS
Variable 4 Total % Deep SWS
Variable 5 Total % PS

* $p < 0.05$

FIGURE 23

Relationship between systolic blood pressure level and total amount of deep slow wave sleep in SHR and WKy rats.

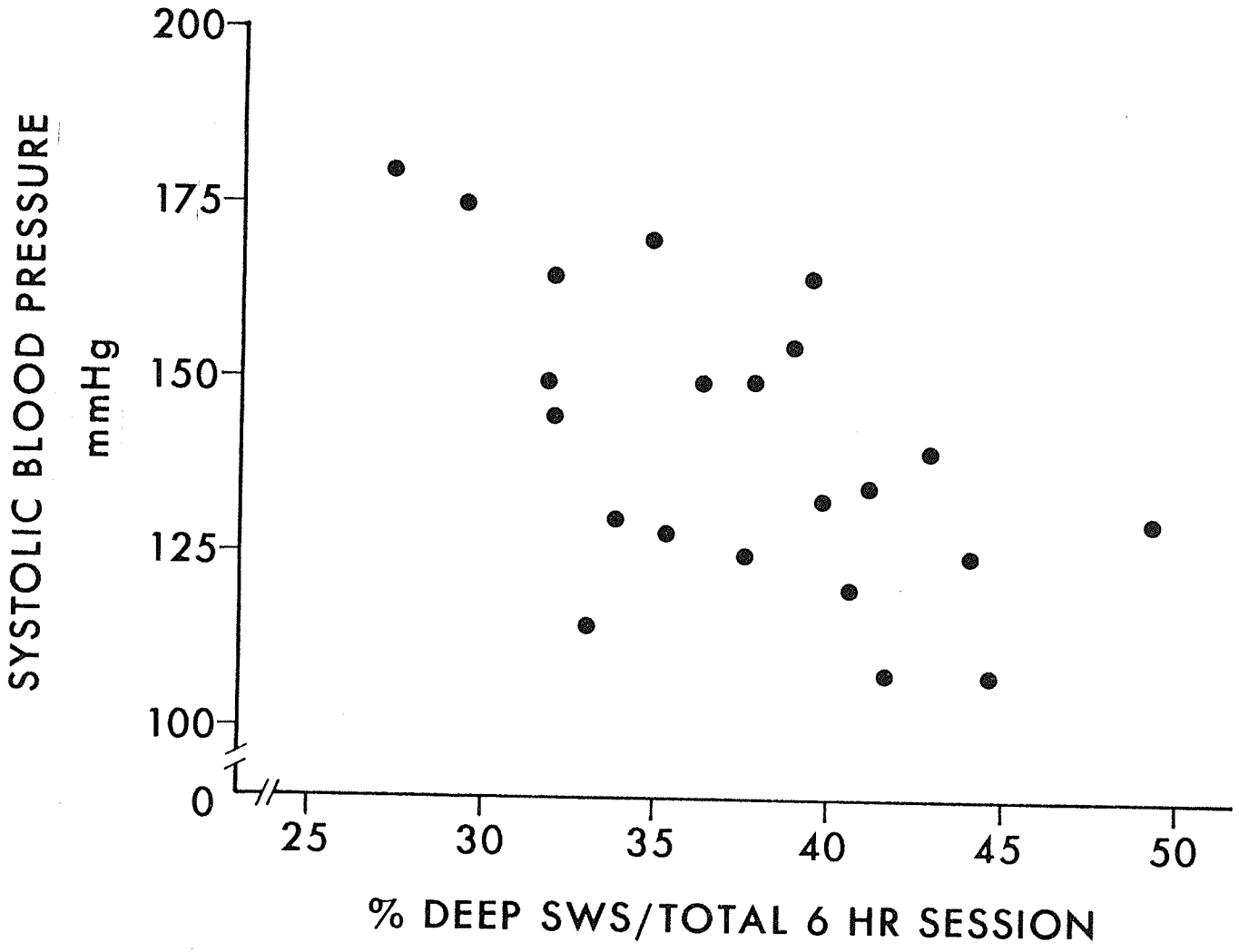
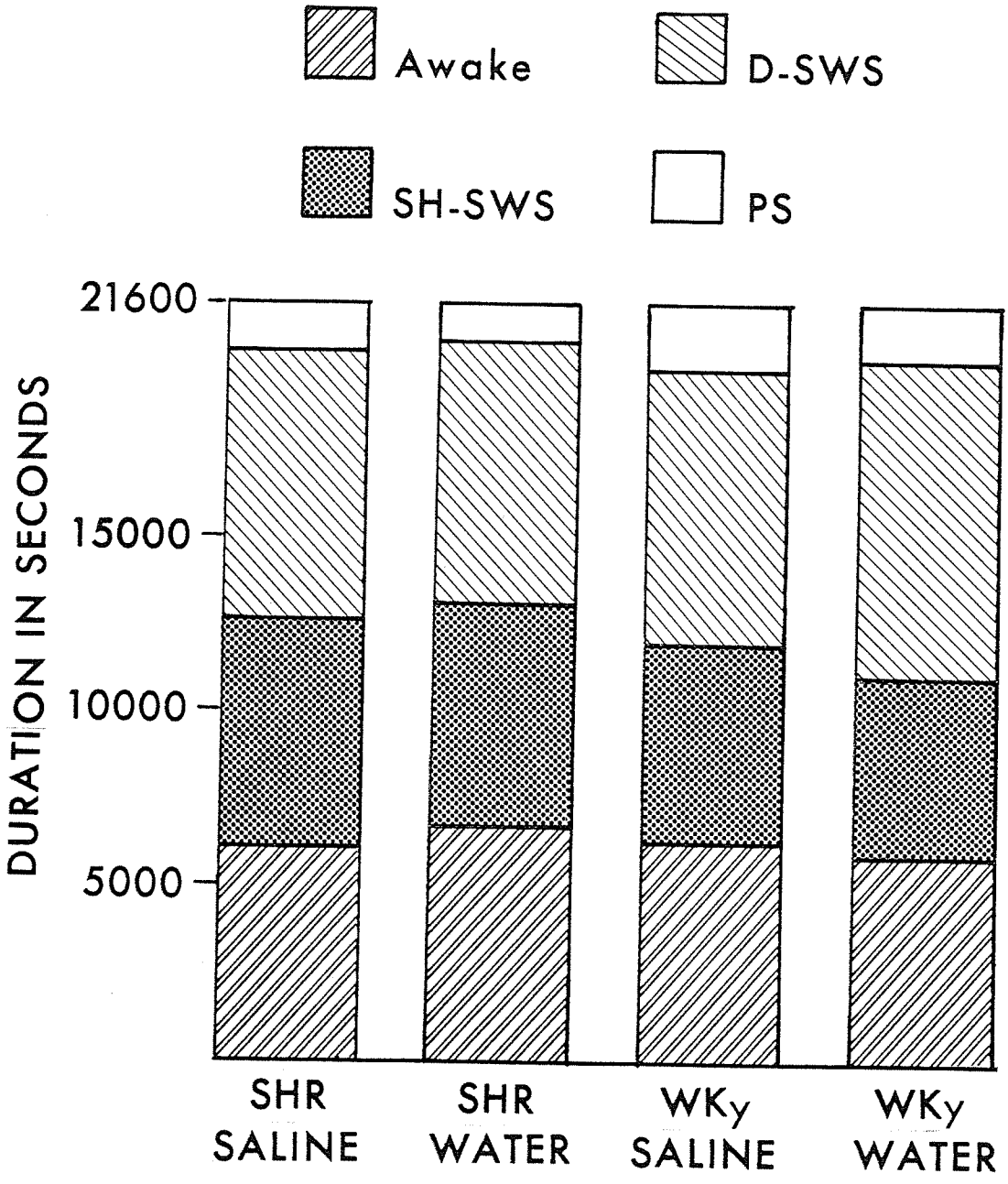

SYSTOLIC BLOOD PRESSURE
mmHg
200
175
150
125
100
0
25
30
35
40
45
50
% DEEP SWS/TOTAL 6 HR SESSION

FIGURE 24

Total mean durations of various components of the sleep-waking cycle of SHR and age-matched WKy control rats maintained on either 0.9% saline or water for drinking.


Awake
D-SWS
SH-SWS
PS
21600
15000
10000
5000
DURATION IN SECONDS
SHR SALINE
SHR WATER
WKy SALINE
WKy WATER

Analysis of variance (2X2 factorial) for each of the sleep-wake parameters demonstrated significant reductions in deep SWS in hypertensive rats in comparison to normotensive WKy controls, $F = 5.27$, df: 1, 19, $p < 0.05$. However, the addition of excess dietary salt caused no significant changes in the amount of time spent in deep SWS, nor was there a significant interaction between diet X genetic strain. A summary table for the analysis of variance of deep SWS duration is presented in Table 9.

Hypertensive rats also demonstrated significant reductions in PS, $F = 4.5$, df: 1, 19, $p < 0.05$, with a total mean PS duration of 1182 seconds in comparison to the mean duration of 1530 seconds of the WKy controls. The reductions in deep SWS and PS of the SHR are illustrated in Figure 25. These data are then expressed as percentages of total recording time in Table 10. An unexpected finding was the observation that animals receiving 0.9% saline in the drinking water spent significantly more time in PS than those without saline, $F = 7.09$, df: 1, 19, $p < 0.05$.

Rats of the hypertensive strain consistently spent less time in PS than normotensive WKy controls throughout each consecutive hour of recording time. However, the differences between groups gradually increased, particularly during the latter half of the recording session. This trend is illustrated in Figures 26 and 27.

The reductions in PS observed in the SHR were

FIGURE 25

Total mean durations of the various components of the sleep-waking cycle of SHR and WKy rats irrespective of diet.

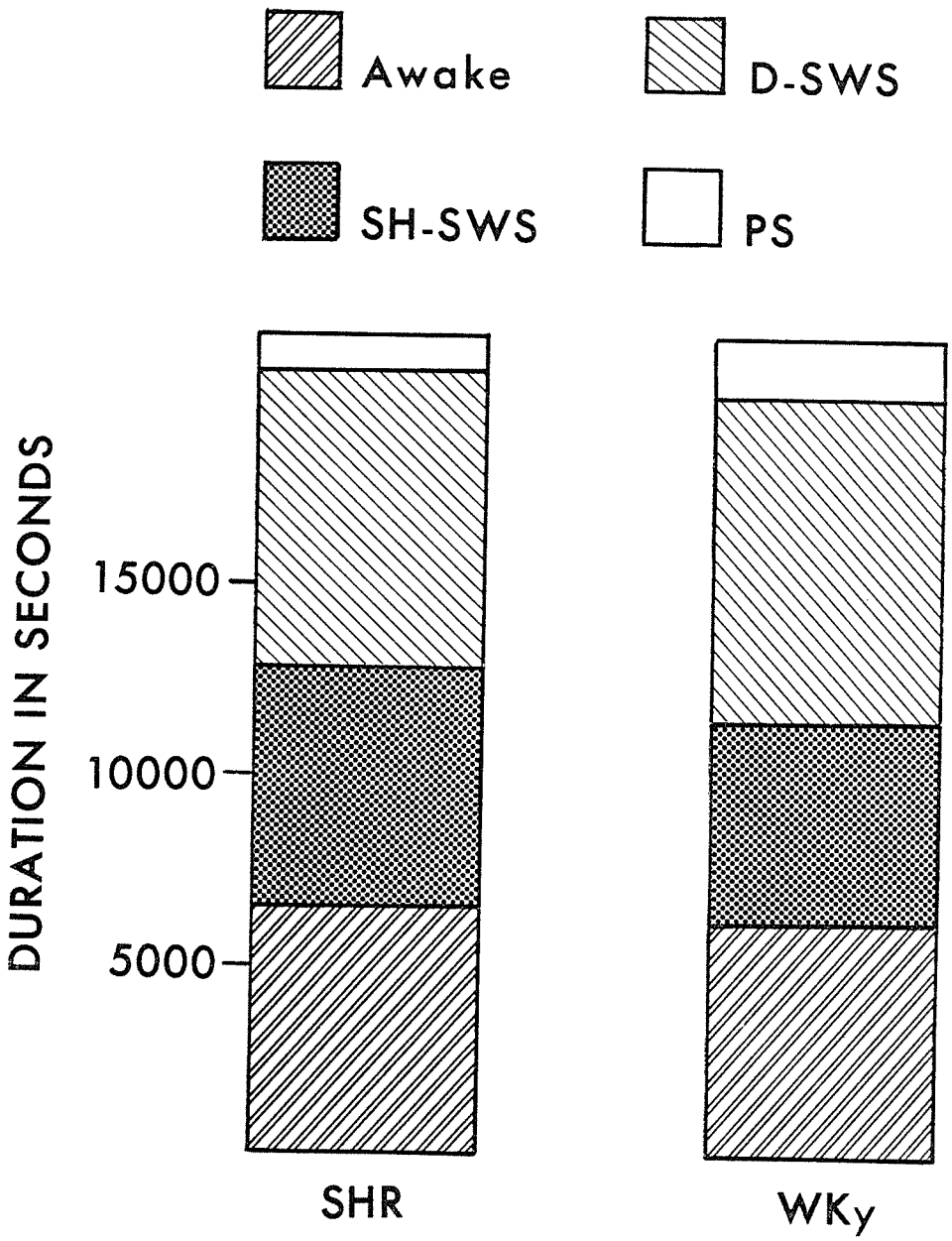

Awake
D-SWS
SH-SWS
PS
DURATION IN SECONDS
15000
10000
5000
SHR
WKy

Table 9 . Summary of Analysis of Variance of Deep Slow Wave Sleep

Variable	df	MS	F
Diet	1	509534.2	3.01
Genetic Strain	1	891733.9	5.27*
Diet X Strain Interaction	1	496578.6	2.94
Error	19	966512	

* $p < 0.05$

Table 10. Mean Amounts of Waking (W), Shallow Slow Wave Sleep (SH-SWS), Deep Slow Wave Sleep (D-SWS) and Paradoxical Sleep (PS) Expressed as Percentages of Total Recording Time in SHR and Normotensive WKy Control Rats

	SHR		WKy	
	Saline	Water	Saline	Water
W	± 28.3 3.68	± 31.3 3.19	± 29.2 2.2	± 28.4 2.0
SH-SWS	± 30.4 3.34	± 29.10 3.13	± 26.6 2.18	± 22.9 1.89
D-SWS	± 34.9 1.87	± 35.0 1.7	± 36.0 1.73	± 42.6 1.7
PS	± 6.4 0.23	± 4.6 0.56	± 8.2 1.0	± 6.1 0.72

FIGURE 26

Mean durations of time spent in awake, shallow slow wave sleep, deep slow wave sleep and paradoxical sleep states for SHR and WKy rats for the first three hours of the recording session.

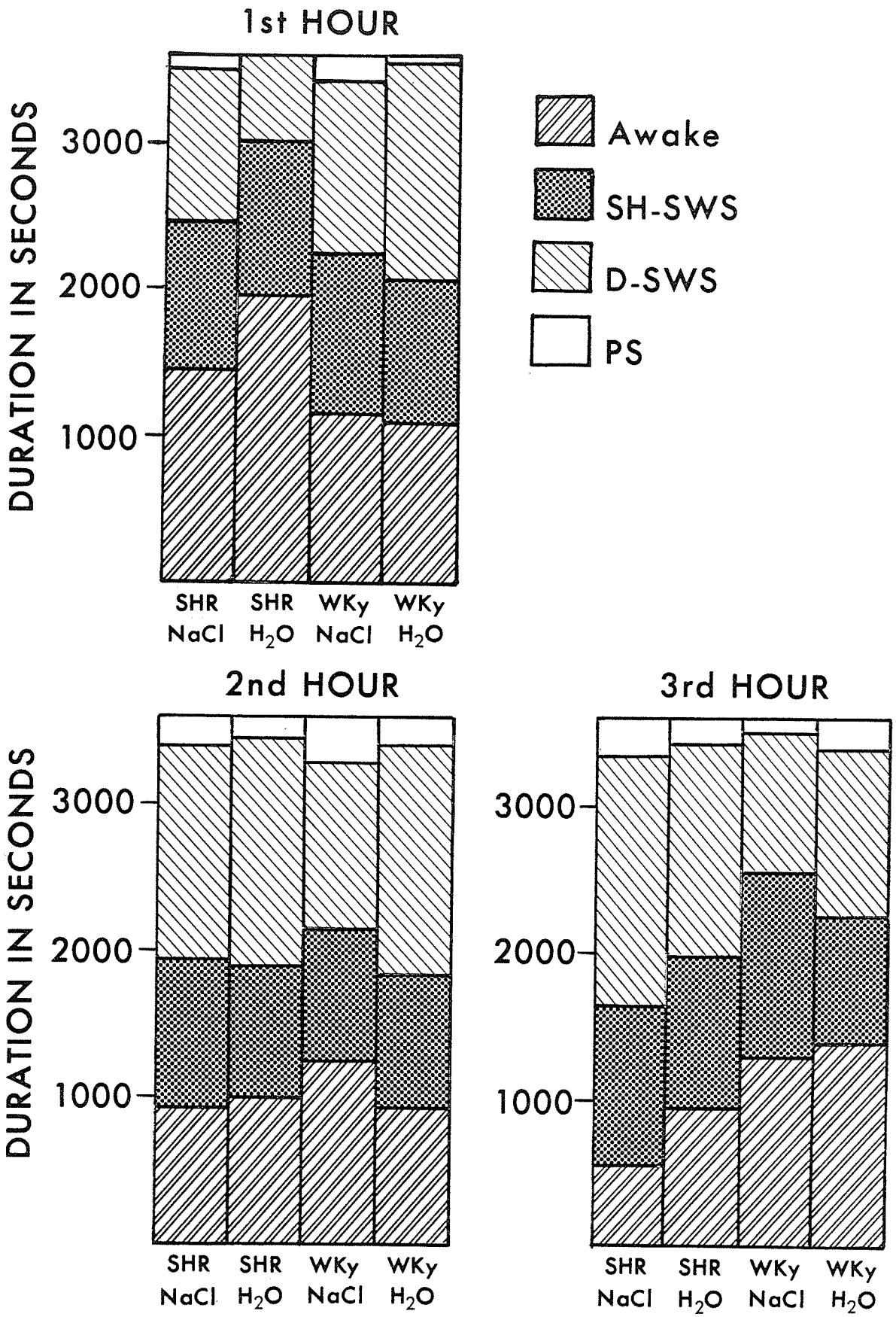
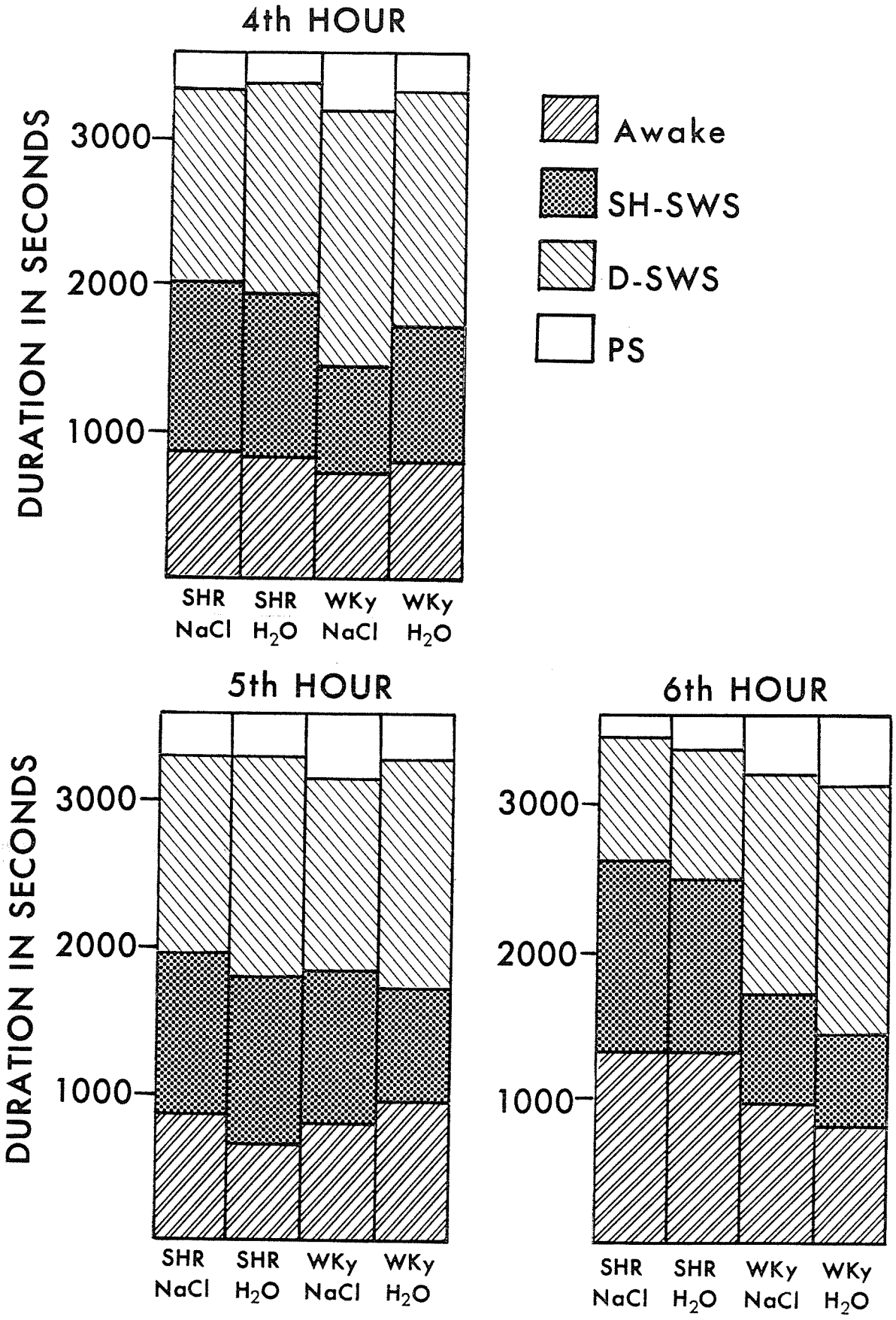

1st HOUR
2nd HOUR
3rd HOUR
DURATION IN SECONDS
3000
2000
1000
Awake
SH-SWS
D-SWS
PS
SHR NaCl
SHR H_2O
WKy NaCl
WKy H_2O

FIGURE 27

Mean durations of time spent in awake, shallow slow wave sleep, deep slow wave sleep and paradoxical sleep states for SHR and WKy rats during the last half of the recording session.


4th HOUR
DURATION IN SECONDS
3000
2000
1000
Awake
SH-SWS
D-SWS
PS
SHR NaCl
SHR H_2O
WKy NaCl
WKy H_2O
5th HOUR
DURATION IN SECONDS
3000
2000
1000
SHR NaCl
SHR H_2O
WKy NaCl
WKy H_2O
6th HOUR
3000
2000
1000
SHR NaCl
SHR H_2O
WKy NaCl
WKy H_2O

attributable to a reduction in the frequency of occurrence of PS episodes rather than to a reduction in the duration of PS episodes. In fact, the average duration of PS episodes in the SHR was slightly longer ($\bar{X}$ = 115 seconds) in comparison to the WKy control group average of 99 seconds. These data are presented in Table 11 . Analysis of variance (2X2 factorial) of the frequency spectra during SH-SWS, D-SWS, PS and awake states demonstrated significant group differences for each of these states in theta 1 (3.61 - 5.57 H_z) theta 2 (5.66 - 7.52 H_z) alpha 1 (7.62 - 9.47 H_z) alpha 2 (9.57 - 12.50 H_z), beta 1 (12.60 - 17.48 H_z), beta 2 (17.58 - 25.0 H_z) and an integrated EEG "Sum" (1.56 - 25.0 H_z). Significant main effects for strain and diet were demonstrated for these frequency bands during awake and all three sleep states. A summary of the F ratios for each of these analysis is presented in Table 12.

SHR receiving saline for drinking showed significant lower power in all bands of the frequency spectrum, during awake and sleep states, except for the theta 1 and beta 2 bands during deep SWS. Normotensive WKy controls maintained on saline for the same period of time showed reduced power for most bands and integrated EEG Sum, in comparison to WKy controls receiving no saline. This tendency was also observed during SH-SWS, D-SWS and PS.

Under all sleep-wake conditions, rats of the

Table 11. Mean Durations of PS Episodes (in seconds) in SHR and WKy rats maintained on either 0.9% NaCl or Tap Water for Drinking

	SHR		WKy	
Rat #	Saline	Water	Saline	Water
1	126.5	126.5	104.9	74.7
2	117.8	108.6	132.5	88.0
3	115.5	118	118.5	71.5
4	92.9	98.6	110.8	69.9
5	74.9	122	90.6	83.3
6	-	161	122.3	120.3
Overall Means	105.5	122.4	113.2	84.6
	114.7		98.9	

Table 12. F Ratios of Analysis of Variance (2X2 factorial) of EEG Power of Delta 2, Theta 1, Theta 2, Alpha 1, Alpha 2, Beta 1, Beta 2 Frequency Bands and Integrated EEG "Sum" During Awake, Shallow SWS, Deep SWS and PS States

		Awake	SH-SWS	D-SWS	PS
Delta 2	Diet	5.32*	2.93	1.72	7.29*
	Strain	0.17	0.14	0.09	5.92*
Theta 1	Diet	7.29*	9.06**	7.27*	0.85
	Strain	5.99*	9.66**	1.33	11.46**
Theta 2	Diet	5.48*	7.52*	10.67**	13.0**
	Strain	9.96**	15.77**	5.90*	25.51**
Alpha 1	Diet	9.76**	9.54**	9.05**	11.03**
	Strain	8.82**	8.80**	6.72*	13.75**
Alpha 2	Diet	9.32**	6.47*	9.41**	18.53**
	Strain	16.19**	15.18**	5.25*	12.04**
Beta 1	Diet	7.06*	5.53*	9.90**	12.98**
	Strain	8.12*	8.71**	1.82	7.26
Beta 2	Diet	6.01*	10.93**	18.24**	11.64*
	Strain	6.32*	11.31**	6.81*	5.19*
EEG Sum	Diet	9.98**	8.2*	5.65*	16.72**
	Strain	5.85*	4.97*	4.8*	12.7**

* $p < 0.05$

** $p < 0.01$

hypertensive strain that were maintained on saline showed the lowest power in all frequency bands, including integrated EEG Sum, whereas the WKy controls maintained on water had the highest power for all frequencies, including integrated EEG Sum. Although some overlap between the frequency spectral characteristics of hypertensive rats maintained on water and WKy controls maintained on saline was observed, in general the SHR irrespective of diet tended to show a reduction in power in comparison to WKy controls particularly in the higher frequencies throughout awake and all sleep states. These differences are illustrated in Figures 28 - 31.

The most important findings of the present experiment were the significant reductions in deep SWS and PS of the SHR in comparison to normotensive WKy controls. This combination of reduced PS and deep SWS of the SHR was accompanied by slight but nonsignificant increases in shallow SWS and waking states. Differences in PS between hypertensive and WKy control groups became gradually more pronounced towards the latter half of the recording session.

Correlations between systolic blood pressure and total duration of each of the components of the sleep-wake cycle revealed a significant inverse relationship between systolic blood pressure level and total amount of time spent in deep SWS.

Comparison of the frequency spectrum of the EEG

FIGURE 28

Frequency spectrum of the EEG in SHR and normotensive WKy rats during the awake state. Frequency bands are indicated on the horizontal scale : D_2 = 1.56 - 3.51 H_z, T_1 = 3.61 - 5.57 H_z, T_2 = 5.66 - 7.52 H_z, A_1 = 7.62 - 9.47 H_z, A_2 = 9.57 - 12.5 H_z, B_1 = 12.6 - 17.48 H_z, B_2 = 17.58 - 25.0 Hz. SUM = 1.56 - 25 H_z.
Vertical lines indicate standard errors.

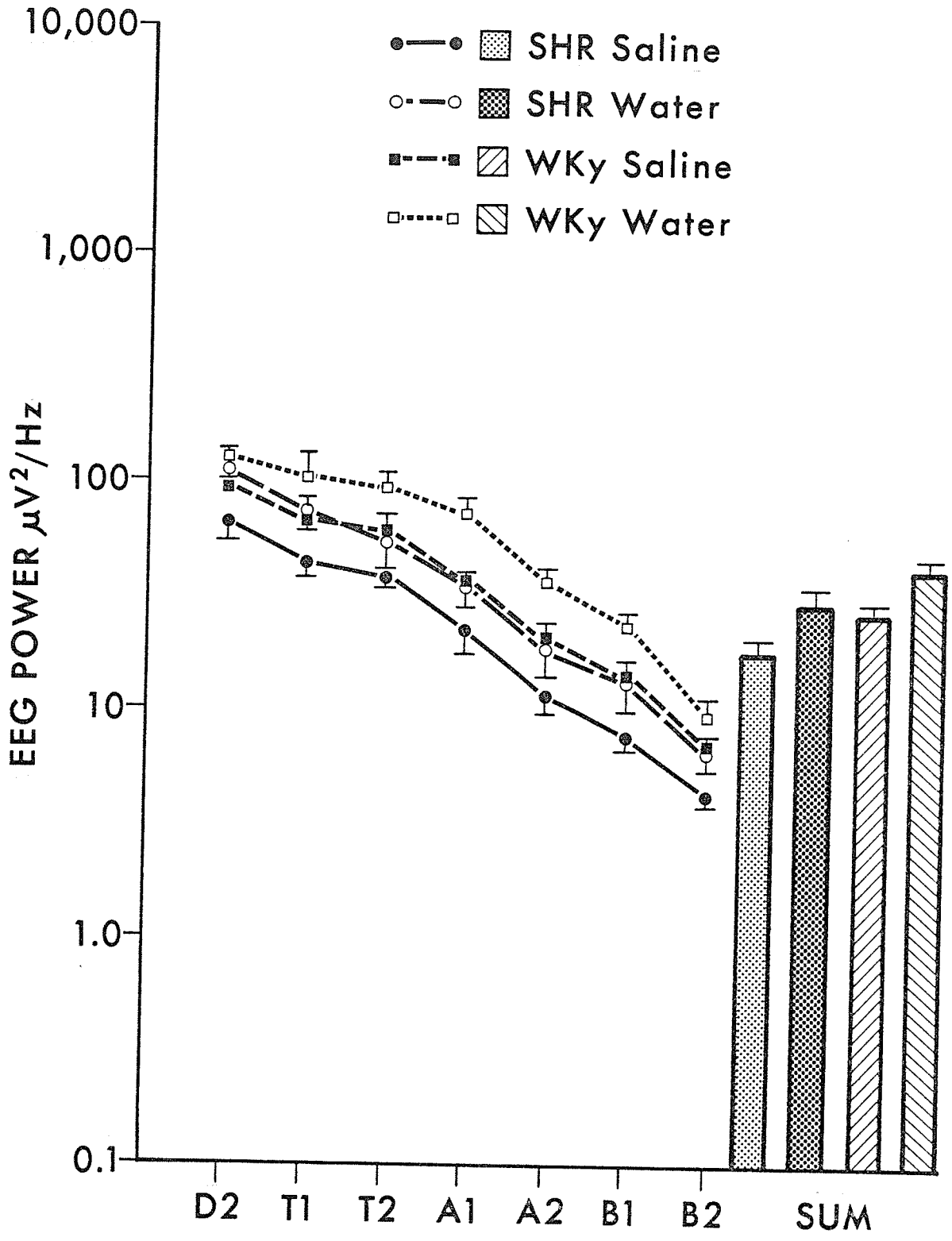

10,000
1,000
100
10
1.0
0.1
EEG POWER μV²/Hz
SHR Saline
SHR Water
WKy Saline
WKy Water
D2
T1
T2
A1
A2
B1
B2
SUM

FIGURE 29

Frequency spectrum of the EEG in SHR and normotensive WKy rats during shallow slow wave sleep. Frequency bands are indicated on the horizontal scale: D_2 = 1.56 - 3.51 H_z, T_1 = 3.61 - 5.57 H_z, T_2 = 5.66 - 7.52 H_z, A_1 = 7.62 - 9.47 H_z, A_2 = 9.57 - 12.5 H_z, B_1 = 12.6 - 17.48 H_z, B_2 = 17.58 - 25.0 H_z. SUM = 1.56 - 25 H_z.
Vertical lines indicate standard errors.

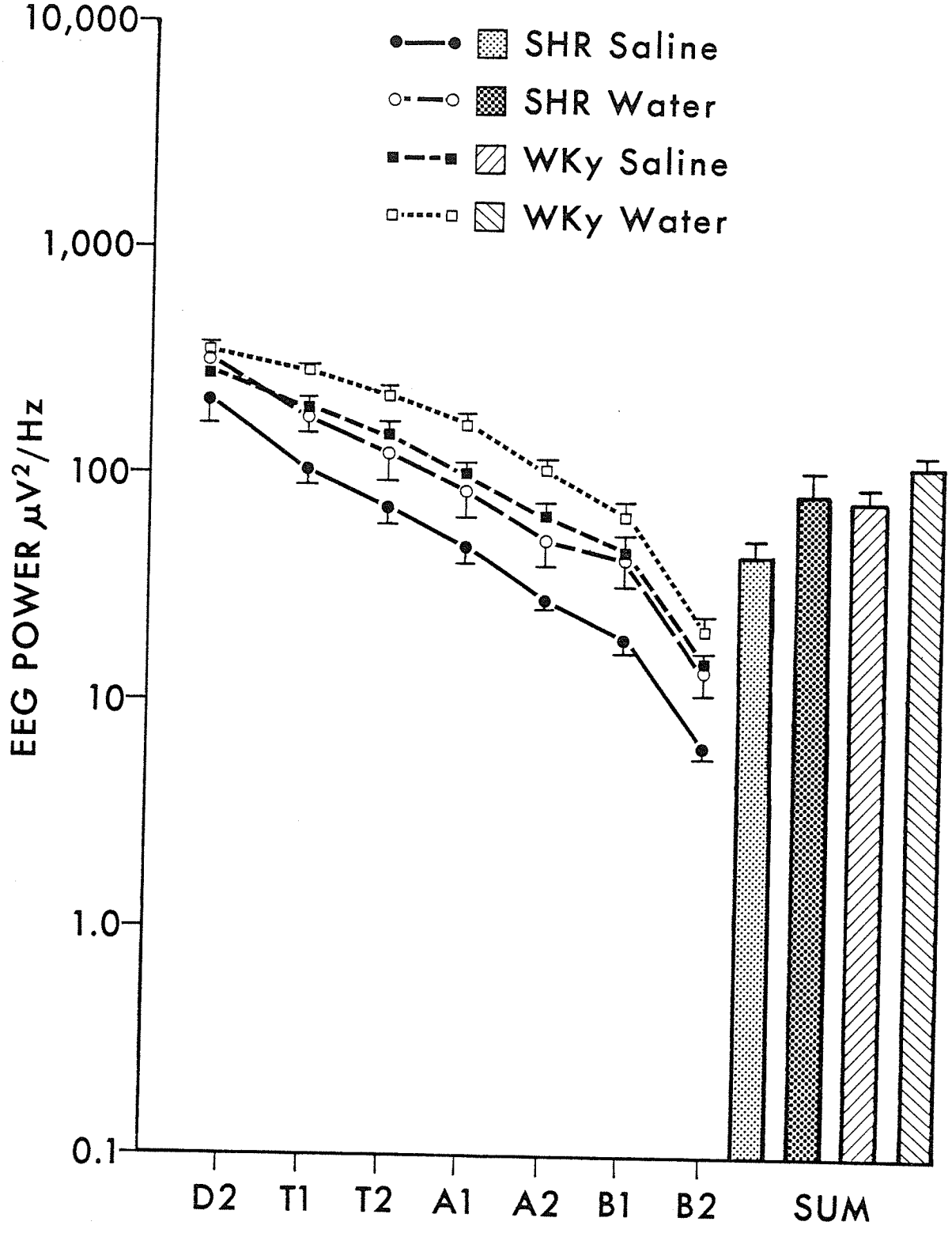

10,000
1,000
100
10
1.0
0.1
EEG POWER μV²/Hz
SHR Saline
SHR Water
WKy Saline
WKy Water
D2
T1
T2
A1
A2
B1
B2
SUM

FIGURE 30

Frequency spectrum of the EEG in SHR and normotensive WKy rats during deep slow wave sleep. Frequency bands are indicated on the horizontal scale: D_2 = 1.56 - 3.51 H_z, T_1 = 3.61 - 5.57 H_z, T_2 = 5.66 - 7.52 H_z, A_1 = 7.62 - 9.47 H_z, A_2 = 9.57 - 12.5 H_z, B_1 = 12.6 - 17.48 H_z, B_2 = 17.58 - 25.0 H_z. SUM = 1.56 - 25 H_z.
Vertical lines indicate standard errors.

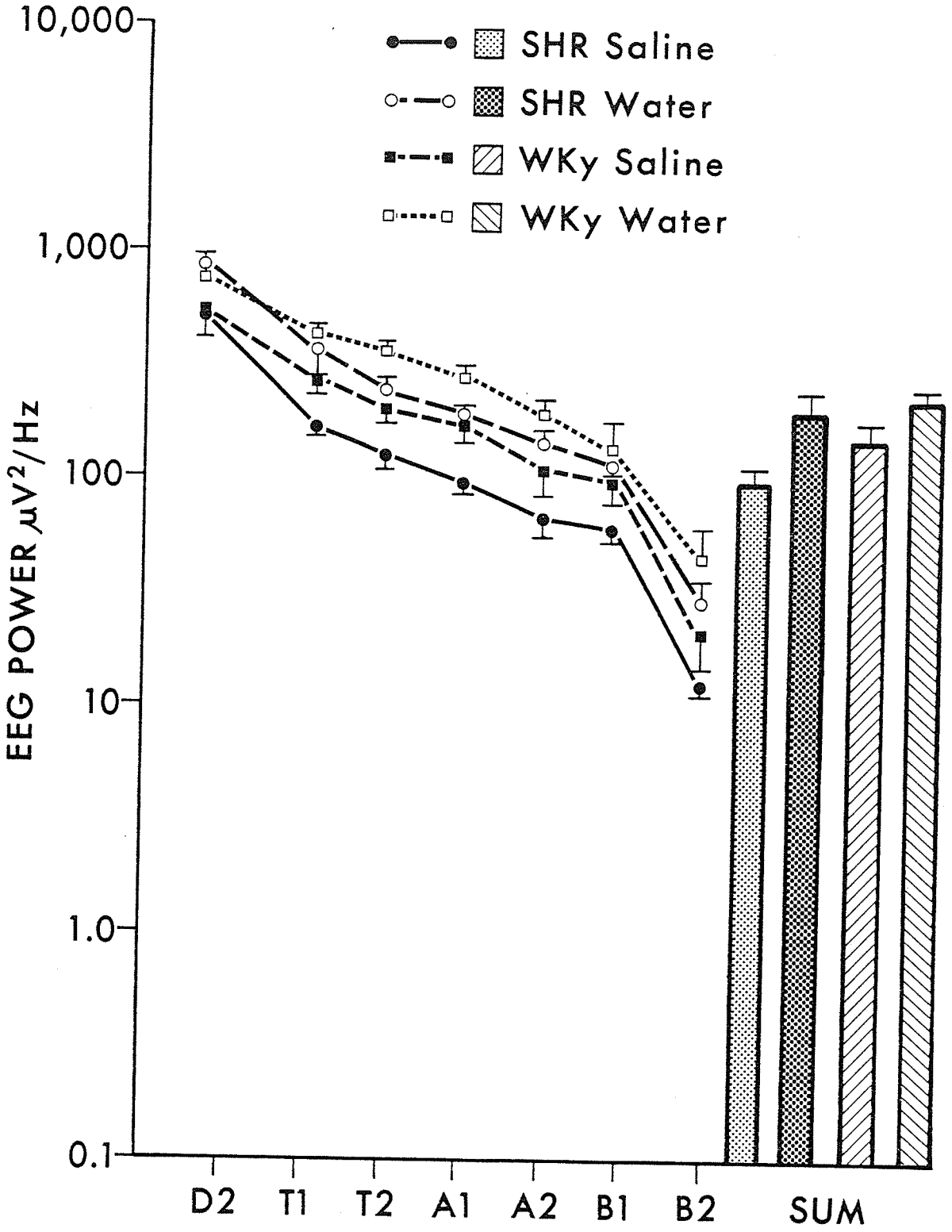
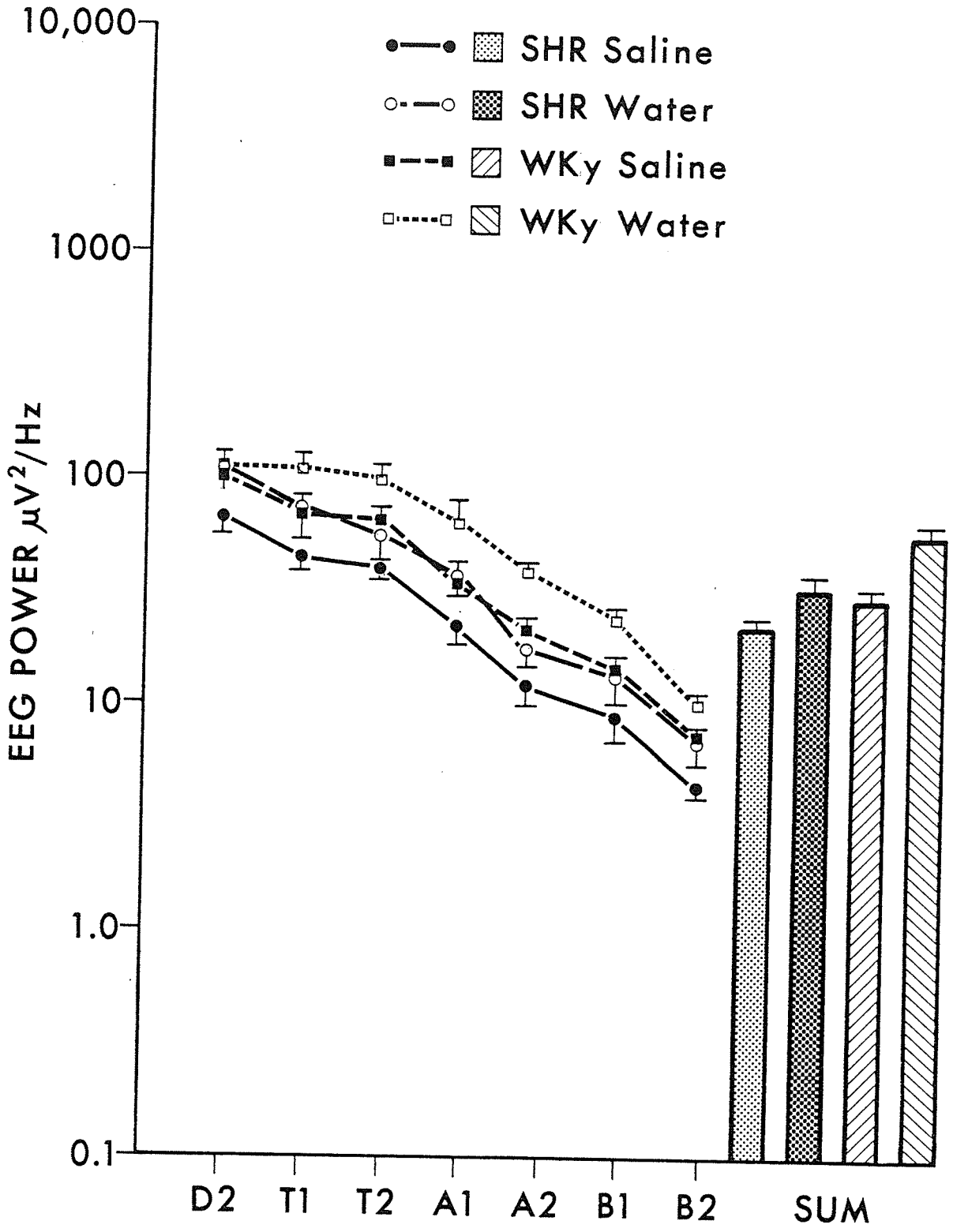

10,000
1,000
100
10
1.0
0.1
EEG POWER μV²/Hz
SHR Saline
SHR Water
WKy Saline
WKy Water
D2
T1
T2
A1
A2
B1
B2
SUM

FIGURE 31

Frequency spectrum of the EEG in SHR and normotensive WKy rats during Paradoxical sleep. Frequency bands are indicated on the horizontal scale: D_2 = 1.56 - 3.51 H_z, T_1 = 3.61 - 5.57 H_z, T_2 = 5.66 - 7.52 H_z, A_1 = 7.62 - 9.47 H_z, A_2 = 9.57 - 12.5 H_z, B_1 = 12.6 - 17.48 H_z, B_2 = 17.58 - 25.0 H_z. SUM = 1.56 - 25 H_z.
Vertical lines indicate standard errors.


10,000
1000
100
10
1.0
0.1
EEG POWER μV²/Hz
SHR Saline
SHR Water
WKy Saline
WKy Water
D2
T1
T2
A1
A2
B1
B2
SUM

in SHR and WKy controls receiving either water or 0.9% saline for drinking revealed significant differences in all frequency bands, with the single exception of delta 1 and delta 2 bands, under the awake and all sleep states.

Discussion

Although previous research studies have investigated relationships between sleep and hypertension in both humans (Bristow et al, 1969; Richardson et al, 1973) and experimental animals (Junquiera and Krieger, 1976) these investigations have been primarily directed towards the effects of sleep on blood pressure. The study of these cardiovascular changes during sleep provided a useful method for studying the contribution of neural influences in blood pressure control. Thus, the cardiovascular concomitants of both SWS and PS are now well documented. However, there are to date no studies concerning the effects of hypertension on sleep cycle parameters.

The present findings of significant reductions in deep SWS and PS in the SHR in comparison to age-matched controls of the normotensive WKy strain are of particular interest for two important reasons. (1) because in many important respects this type of inherited hypertension in the SHR closely resembles that of human essential hypertension. (2) because of the possible relationship

between these changes in sleep parameters and previous reports of alterations in central noradrenergic functioning in the SHR (Yamori et al, 1970, 1973). The latter issue will be subsequently discussed in some detail following a discussion of the main findings of this study.

The present experiment demonstrated that the SHR exhibited reductions in deep SWS and PS which were compensated by slight, but nonsignificant increases in shallow SWS and awake states. These differences were observed irrespective of dietary status.

It is interesting to note that the addition of excess dietary salt by substituting 0.9% saline solution for drinking water caused no significant changes in sleep state durations nor in blood pressure levels in the hypertensive strain of rat. Since it is generally the case that blood pressure raising stimuli (such as excess sodium intake) superimposed on high blood pressure of genetic origin may lead to further increases in blood pressure level (Dahl, 1972), the present results suggest that sodium balance may not play a critical role in the hypertension of the SHR. These findings are consistent with observations by others (Baer, Knowlton, Kirshman and Laragh, 1973) that chronic sodium depletion does not restore the blood pressure of the SHR to normal levels, nor does it prevent the progressive rise in blood pressure

associated with aging in this strain of animals.

Of particular interest were the present findings demonstrating a significant inverse relationship between blood pressure level and amount of deep SWS. Thus, greater reductions in deep SWS were accompanied by greater systolic blood pressure elevation. This may be due to increased sympathetic influence among animals with increased blood pressure levels, presumably activated as a result of central mechanisms.

The differences in PS between SHR and WKy groups became gradually more pronounced as the recording session progressed. The single exception to this trend occurred during the third hour of the session in which WKy rats in the 0.9% saline condition showed an average reduction in PS in comparison to the SHR groups. However, these values were markedly influenced by the prolonged wakefulness of a single animal in the WKy saline group which subsequently demonstrated borderline hypertension. Prolonged periods of wakefulness and activity, characterized by excessive rearing and exploratory behaviours continued throughout the entire six hour session in the SHR group in comparison to the WKy controls.

These observations are consistent with previous behavioural studies demonstrating increased locomotor activity in open field and activity wheels in the SHR in comparison to Wistar controls (Pappas, Peters, Saari,

Solerian and Minch, 1974); although these findings were not corroborated by others (Rosencrans and Adams, 1976).

Spectral analysis of the EEG of SHR and WKy rats demonstrated significant changes in EEG power in almost all frequency bands during awake and all sleep states. Hypertensive animals, irrespective of diet, had significant reductions in EEG power in most frequency bands during all vigilance states. These reductions were greatest in animals maintained on 0.9% saline, thus revealing differences in tonic EEG activity which were not detectible by simple visual analysis.

Hypertensive rats in general showed increased EEG desynchrony in comparison to normotensive WKy controls. This was evidenced by reductions in power in all bands between 3.61 - 25.0 H_z during awake and all sleep states. This desynchrony was even more pronounced in animals receiving 0.9% saline instead of tap water for drinking. The observed reductions in EEG power in most frequency bands in the SHR demonstrate that these rats have higher arousability than the control strain. These SHR animals are thus more aroused than the WKy animals during the waking state - evidenced by findings of reduced power in all frequency bands between theta 1 through beta 2 including integrated EEG "Sum". This increased arousability also extended through the stages of sleep, evidenced by findings of reductions in power during

SWS and PS.

The present findings of increased arousability (or increased excitability) in the SHR tend to suggest that these animals have greater levels of sympathetic activation which is possibly a reflection of altered neurohormonal balance of cerebral origin. This is also supported by the previously described observations of excessive rearing behaviour of the SHR during the recording sessions, since rearing behaviour has been correlated with the degree of cortical excitment (Holland and Gupta, 1966).

A relationship between the present observations of alterations in sleep parameters in the SHR and changes in central noradrenergic mechanisms is suggested by studies indicating reductions in levels of NE in the lower brainstem and hypothalamus (Yamori, 1970), decreased activity of enzymes associated with NE synthesis, (Yamori et al, 1970) in the SHR in comparison to normotensive Wistar controls. Results of the present study showed that the greatest changes in the sleep parameters of the SHR were the reductions in PS. These findings would appear to be consistent with the reports of reductions in central noradrenergic activity, particularly with respect to the involvement of NE in PS postulated by Jouvet.

However, a potential problem in studies using

genetically homologous rats, such as the SHR, is the occurrence of "passenger traits". These traits may in fact be unrelated to hypertension per se, existing simply as traits which covary. Thus one important difficulty in studying neurochemical alterations in the SHR is the use of appropriate controls for comparisons.

Subsequent observations using Wistar-Kyoto rats, the normotensive parent strain of the SHR, as controls, indicated that some of the previously reported abnormalities in central noradrenergic functioning become less significant (Yamabe, deJong and Loverberg, 1973). Endogenous NE in the brainstem of SHR appeared to be not significantly different from WKy controls (Yamori, Ooshima and Okamoto, 1972). Thus, previously reported differences in noradrenergic functioning in the SHR may have involved genetic factors in the closed colony of rats. Subsequent investigations (Osumi, Tanaka and Takaori, 1974) revealed that levels of adrenaline and serotonin in the brain of the WKy are lower than those of other age-matched Wistar strains.

On the basis of these reports, current investigations of the SHR have used the normotensive WKy, from which ancestors of the SHR were derived, for comparison. These animals appear to have some hypertensive traits and occasional occurrence of mild hypertension is noted (Yamori et al, 1973). However, Sjoerdsma (1972) has indicated that, for precisely these reasons, the normotensive WKy strain may <u>not</u> be a

totally suitable control group in studies of hypertension.

Preliminary investigations conducted in the present laboratory demonstrated that levels of NE and dopamine in the forebrain and hindbrain of the SHR were similar to levels observed in other strains of rat, although admittedly, differences in regional levels would not have been detected by these measurements. Recent studies by investigators using sensitive radioimmunoassay of punched regions of the brain have demonstrated elevated NE levels in most noradrenergic cell body regions of the brainstem in mature SHR in comparison to WKy controls. The only exception was in the locus coeruleus where no differences between the two strains were demonstrated (Versteeg, Vander Gugten, Wiznen, Smeets, and deJong, 1977).

There is a relative paucity of information concerning serotonergic mechanisms in the SHR. Although some have reported significant increases in the whole brain of mature SHR in comparison to normotensive WKy controls (Takor, Tanaka and Okamoto, 1974) no differences were subsequently found in brainstem or telencephalon (Osumi, Tanaka and Takaori, 1974).

On the basis of this confusing literature, it is somewhat speculative to attribute the present observations of alterations in sleep parameters to changes in central noradrenergic functioning. From the Versteeg et al (1977) findings it appears that concentrations of NE in the locus

coeruleus are similar in both the SHR and WKy groups. However, these findings do not preclude the possibility of changes in brainstem NE metabolism. Generally speaking NE turnover is a better index of NE metabolism than endogenous NE levels, since the same endogenous level can be maintained with rates of synthesis and degradation which may be different. In this regard, it is of interest to note that some researchers have in fact reported differences in brainstem NE turnover (Yamori et al, 1972) although the animals used by these investigators were of a younger age than those used in the present experiment (3 months and 6 months respectively). It is also of interest to note that enzyme activity related to cholinergic mechanisms (ie acteylcholinesterase activity) has been reported to be increased in the brainstem of the SHR in comparison to three normotensive control strains (Yamori et al, 1972).

On the basis of the evidence it is difficult to attribute the present observations of changes in sleep parameters in the SHR to changes in brainstem noradrenergic functioning per se. However, it does appear likely that metabolic deviations in the central nervous system of the SHR tend to increase sympathetic outflow intrinsically, and in response to environmental stimuli. In the present study, despite the repeated lengthy periods of adaptation, the SHR did not appear to habituate as readily to the experimental situation. Thus the recording session may

have activated stress reactions in these animals via the hypothalamo-adrenomedullary system with concomitant increases in sympathetic activity.

The findings of the present study thus appear to be best explained in terms of increased arousability of the SHR, probably as a result of a central hyperreactivity. This increased arousability was clearly evident from the EEG spectral analysis and by behavioural observations of increased excitability and overactivity. As a result these animals demonstrated significant reductions in deep SWS and PS in comparison to normotensive controls.

Since the SHR is generally considered to be a model of human essential hypertension, the present findings of changes in sleep parameters in genetically hypertensive rats may have important implications.

Although the findings of both experiments 1 and 2 appear to provide support for the Jouvet neurochemical model of sleep, possible alternative interpretations must also be considered.

In both experiments, attempts were made to correlate spontaneous changes in internal brain chemistry with changes in sleep parameters. This approach, however, leaves unanswered the basic question of cause and effect, which can only be studied by experimental manipulation.

In the case of the Jouvet sleep hypothesis, evidence from the present study is generally consistent with other research evidence, and tends to suggest that 5-HT and the relationship between 5-HT and NA is somehow involved in regulating sleep behaviours. However, the neurochemical mechanisms involved are probably much more complex than Jouvet suggests.

CHAPTER 4

SUMMARY AND CONCLUSIONS

The present study investigated the relationship between sleep cycle parameters and aspects of monoaminergic functioning in two strains of rat in which preliminary evidence suggested the presence of abnormalities in peripheral and/or central levels of biogenic amines. The two strains of rat studied were:

(1) The Walker-Walker fawn-hooded rat - a recently discovered mutant strain characterized by its distinctive beige coat colour, a hereditary hemorrhagic diathesis resembling platelet storage pool disease in humans, and marked behavioural docility.

(2) The spontaneously hypertensive strain, developed by Japanese investigators (Okamoto and Aoki, 1963) as a model of human essential hypertension and described as having abnormalities in central noradrenergic functioning.

Investigations of the Walker-Walker strain revealed significant changes in the composition and duration of the sleep-waking cycle. F-H rats showed reductions in deep SWS and PS which were compensated by increases in shallow SWS and awake states. The SWS of these animals was of a more superficial nature, thus they did not progress into stages of deep SWS or PS to the same degree as the L-E controls.

Spectral analysis of the EEG using fast-Fourier

transform showed a less pronounced synchronization in F-H animals during deep SWS. Further analysis demonstrated that the deep SWS of the F-H strain corresponded to the shallow SWS of the L-E control strain.

Although profound reductions in levels of blood platelet serotonin were observed in F-H animals, whole brain 5-HT levels showed slight but nonsignificant reductions.

The findings suggested the following points:
(1) That the abnormalities in peripheral aminergic functioning in the F-H strain of rat may reflect disordered metabolism of this monoamine within the CNS.
(2) That the presence of normal serotonergic functioning may be necessary for the patterns of SWS and PS to become fully manifest.
(3) That the low levels of 5-HT storage or storage capacity may be a common denominator between pigmentary dilution, docile behaviour, changes in sleep parameters and hemorrhagic tendency in the F-H strain of rat.

Investigations of the spontaneously hypertensive strain of rat (SHR) demonstrated significant reductions in deep SWS and PS in comparison to normotensive Wistar-Kyoto (WKy) controls. These reductions in deep SWS and PS were accompanied by slight but nonsignificant increases in shallow SWS and waking states. Differences in PS

between SHR and WKy controls became more pronounced as the session progressed.

A significant negative correlation between systolic blood pressure level and total duration of deep SWS was demonstrated. Thus, the greater the degree of hypertension, the less time was spent in deep SWS.

Computerized spectral analysis of the EEG showed significant reductions in power in the SHR in frequency bands between 3.61 - 25.0 H_z during awake and all sleep states. These reductions were greatest in hypertensive animals receiving 0.9% saline instead of tap water for drinking. These findings of increased activation during the waking state and increased desynchrony during SWS suggested intrinsic differences in levels of arousability in the SHR.

The results were discussed in terms of their relationship to reports of neurochemical differences in the SHR. It was concluded that the present observations are best explained in terms of increased arousability of the SHR, probably as a result of central factors.

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APPENDIX

Assay Procedure for Noradrenaline and 5-Hydroxytryptamine

Tissue samples were obtained by dissecting out the brain, rinsing in hypertonic solution, drying off excess solution on a paper towel and freezing in liquid nitrogen. Immediately prior to analysis the frozen brains were individually weighed on a Sartorius balance to the fourth decimal place.

Extraction Procedure

Weighed samples were placed in a homogenizer and 3 ml ice-cold perchloric acid (0.4N) was added for samples 1 gm (6 ml perchloric acid for samples 1 - 2 gms). Samples were homogenized until the suspension was smooth.

The homogenate was transferred into plastic centrifuge tubes and allowed to stand for 10 minutes in a bucket of crushed ice. The mixture was centrifuged (20,000 g for 20 mins.) then the supernatant was transferred into labelled beakers and stored in the refrigerator.

2.5 ml perchloric acid (0.4N) was then added to the pellet in each centrifuge tube (or 5.5 ml for 1 - 2 gm samples). The pellet was then scraped from the bottom of the centrifuge tube with a glass rod, re-homogenized and centrifuged 10 - 15 minutes at 20,000 g as before. The resulting supernatant was added to the previous volume from the same sample. The pH was then adjusted to 2.7 (for 1 gm sample or 3 - 4 for 1 - 2 gm sample) with

Tricine solution and left overnight in the refrigerator.

Noradrenaline Assay

0.3 gms aluminum oxide was added to empty centrifuge tubes. The pH of the supernatants was then raised to exactly 7.95 using Tricine solution. These pH adjusted samples were then added to the preweighed aluminum oxide in the centrifuge tubes. The tubes were capped, rotated for 20 - 25 minutes, then centrifuged at 5,000 - 10,000 rpm for 5 minutes. The supernatant was then drawn off and saved for estimation of 5-HT by immediate refrigeration.

The aluminum oxide in the bottom of the centrifuge tubes was rinsed twice using a hard stream of deionized water, each time pipetting off the top liquid with an aspirator. Following the last wash, the sample was centrifuged at 5,000 rpm for 5 minutes.

To the aluminum oxide absorbed material exactly 4 ml perchloric acid (0.05N) was added. The tubes were then rotated 20 - 25 minutes. During this procedure internal standards of noradrenaline were prepared and carried throughout the described absorption.

For each sample, 3 tubes were prepared (A, B - parallel samples and C - blank) containing 1.5 ml phosphate EDTA buffer pH 7.0. To tubes A and B, 1 ml of sample was added and mixed. External standards were prepared at this point by adding 1 ml of standard solution of known

concentration to 1.5 ml phosphate buffer.

Using a stop watch, 0.2 ml iodine reagent was added and mixed immediately. After 2 minutes, 0.5 ml alkaline solution was added and the solution mixed again. After 2 minutes 0.4 ml glacial acetic acid was added and mixed immediately.

To the blanks ("C" tubes) the following were added and immediately mixed:

0.5 ml alkaline solution (mix)

0.2 ml iodine reagent (mix)

0.4 ml glacial acetic acid (mix)

The tubes were placed in 100°C oven for exactly 5 minutes, then cooled on ice for 1 hour. Fluorescence was read at 385 nm excitation, 485 nm emission.

5-Hydroxytryptamine Assay

To each sample 5 gms of solid NaCl was added. The solution was mixed for 10 minutes using an automatic shaker. 20 ml n-Heptanol was then added, mixed, then 0.8 ml K_2CO_3 (75%) was added and the solution immediately shaken by hand. The samples were then centrifuged at 5,000 rpm for 10 minutes. The layer of n-Heptanol was then drawn off and 10 ml borate buffer added. The solution was mixed 10 minutes, then centrifuged for 10 minutes at 5,000 rpm.

After centrifugation, 15 ml of the heptanol

layer was added to the phosphate buffer, taking care to avoid contamination with borate buffer. The solution was mixed 10 minutes then centrifuged at 5,000 rpm for 5 - 10 minutes.

The contents were poured into separatory funnels and the phosphate layer (lower layer) containing the 5-HT was drawn off. The tubes were capped and stored overnight.

The next day, 1 ml samples were measured into appropriately labelled tubes. 0.1 ml freshly prepared ninhydrin solution was added and stirred immediately. The tubes were then baked at 100°C for 25 minutes, cooled at room temperature and fluorescence read (383 nm excitation, 486 nm emission).

After reading the samples, 1 ml of fresh alkaline sulfite solution (2.5%) was added to each tube and the solution mixed. Tubes were allowed to stand for 20 minutes at room temperature, then fluorescence read at the same excitation and emission settings.

Preparation of solutions used in the above procedure are described in detail by Shellenberger and Gordon (1971).