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The Relationship of Central Monoamines to Drug-Induced Sleep in the Rat, Examined on a 24-Hour Basis

Samuel Gene Speciale
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The Relationship of Central Monoamines to Drug-Induced
Sleep in the Rat, Examined on a 24-Hour Basis

by

Samuel Gene Speciale, Jr.

A Dissertation Submitted to the Faculty of the Graduate
School of Loyola University in Partial Fulfillment of
the Requirements for the Degree of Doctor of Philosophy

November, 1973

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DEDICATION

This dissertation is dedicated to my parents, family and friends, who have made this experience easier.

VITA

Samuel Gene Speciale, Jr. is the son of Samuel G. Speciale and Beatrice M. Speciale of Rochester, New York. He was born April 22, 1944 in Highland Park, Illinois.

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Mr. Speciale's publications include the following:

Walker, C.A., S.G. Speciale, Jr. and A.H. Friedman. Circadian changes in pentobarbital-induced sleeping time and their relationship to brain biogenic amine levels in the rat. The Pharmacologist 10: 160, 1968.

Speciale, S.G., Jr. and A.H. Friedman. Effects of altered rat brain biogenic amine levels on pentobarbital-induced sleep. The Pharmacologist 12: 198, 1970.

Speciale, S.G., Jr. and A.H. Friedman. Melatonin potentiation of pentobarbital-induced sleep as a function of time of day. Fed. Proc. 30: 389, 1971.

Walker, C.A., S.G. Speciale, Jr. and A.H. Friedman. The influence of drug treatment on norepinephrine levels and ultrastructure of the rat hypothalamus and caudate nucleus during a programmed light-dark cycle. Neuro-pharmacology 10: 325-334, 1971.

Speciale, S.G., Jr. and A.H. Friedman. Alteration in pharmacologically-induced sleep as a function of drug treatment and time of administration. The Pharmacologist 13: 305, 1971.

Speciale, S.G., Jr. and A.H. Friedman. Does histamine play a role in sleep in rats by pentobarbital sodium or γ -butyrolactone? Fifth International Congress on Pharmacology, 1972.

Table of Contents

I. Introduction	1
A. The problem	1
B. Terminology	2
C. Practical considerations	3
II. Review of the literature	5
A. Parameters affecting biological rhythms	5
1. Sex	5
2. Species	6
3. Age	6
4. Population	7
5. Illumination	7
6. Temperature	9
7. Barometric pressure	9
8. Feeding habits	9
9. Miscellany	10
B. Physiological parameters affecting biological rhythms	11
1. Motor activity	11
2. Body temperature	11
3. Blood composition	12
4. Urinary excretion	13
5. Hormones of blood and urine	14
6. Rhythms in constituents of carbohydrate, lipid and protein metabolism	17
7. Enzyme systems	19
8. CNS amines	21
a. 5HT	21
b. NE	22
c. DA	23
d. ACh	23
e. Miscellany	23
C. Circadian rhythms in drug response	24
1. CNS depressants	24
2. CNS stimulants	25
3. Allergic response	26

4. Drugs altering monoamine levels	27
5. Analgetics	28
6. Miscellany	28
D. CNS amines	29
1. Serotonin	29
a. Metabolism	29
b. Physiological effects	32
1) Serotonin	32
2) Metabolites	33
3) Precursors	35
4) Blockade of synthesis or storage	37
a) p-chlorophenylalanine	37
b) reserpine	39
c) MAO inhibition	41
c. Pineal gland	41
1) Melatonin	42
a) localization	42
b) pharmacological effects	44
2) Anatomy	47
3) Other pineal amines	51
2. Catecholamines	53
a. Metabolism	53
b. Biosynthesis	56
c. Degradation	57
d. MAO inhibition	58
e. Pharmacological effects of CAs in the CNS	59
f. Inhibition of synthesis	61
3. Histamine	63
a. Localization	63
b. Synthesis	66
c. Physiological effects	67
1) Histamine and related substances	67
2) Modification of CNS histamine by drugs	68
4. Acetylcholine	71

a.	Formation and localization	71
b.	Pharmacological effects	72
E.	Sleep-wakefulness phenomena	76
1.	Sleep phenomenology	76
2.	Sleep deprivation	79
a.	PS deprivation	79
b.	Neurochemical alterations	80
3.	Drugs acting on sleep stages	81
a.	Reserpine	81
b.	MAO inhibitors	82
c.	Alpha-methyl-p-tyrosine	82
d.	p-Chlorophenylalanine	82
e.	Gamma-butyrolactone	83
4.	Localization of "sleep centers"	83
a.	Lesion experiments	84
b.	Stimulation experiments	87
c.	Humoral sleep factors	91
1)	Cerebrospinal fluid	91
2)	Plasma	92
3)	Neurohumors	94
F.	Barbiturates	98
1.	Distribution	98
a.	Physiochemical factors	99
b.	Physiological factors	100
2.	Termination of activity and metabolism	101
3.	Central effects	104
a.	EEG	105
b.	CNS excitability	105
c.	Analgesia	106
d.	Respiration	106
4.	Peripheral effects	107
a.	Cardiovascular	107
b.	Gastrointestinal	108
c.	Urogenital	108

5. Mode and sites of action	108
a. Biochemical effects	109
b. Neurophysiological effects	109
c. Reticular activating system	111
G. Gamma-butyrolcatone	113
1. Sedative actions	113
2. Active form	115
3. GABA in the CNS and Relationship to GBL	117
4. Involvement of other amines with GBL	120
5. Mechanism of action	121
H. Alterations of Drug-induced Sleep	123
1. Physiological effects	123
a. Temperature	124
b. Diet	125
c. Sex	126
d. Hormones	127
e. Pathology	127
2. Pharmacological effects	128
III. Materials and methods	136
A. Experimental animals	136
B. Animal housing conditions	136
C. Experimental procedures	137
1. Measurement of drug-induced sleep	137
2. Pretreatment drug dosages	138
3. Brain dissection	142
4. Amine analysis	144
5. Fluorometry utilizing the Aminco-Bowman Spectrophotofluorometer	151
6. Rectal temperature	152
7. Statistical methods	153
D. Drugs and chemicals (see Appendix A)	286

IV. Results	155
A. Duration of hypnotic sleep on a 24-hour basis	155
1. Pentobarbital	155
2. Gamma-butyrolactone	158
B. Effects of drug pretreatment on PB-sleep duration	158
1. Drugs affecting the NE and DA systems	158
a. DOPA-pargyline	158
b. AMPT	165
2. Drugs affecting the 5HT system	165
a. pCPA	165
b. TP	165
c. 5HTP	172
d. Melatonin	172
e. Compounds structurally-related to 5HT	173
3. Drugs affecting the histamine system	173
a. L-histidine	173
b. Decaborane	181
c. Diphenhydramine	182
4. Drugs affecting the cholinergic system	182
C. Effects of histidine pretreatment on GBL-sleep duration	185
D. CNS amines at $t \frac{1}{2}$ following pretreatment and PB regimens	185
1. Time-course for amine levels in controls	185
2. Drugs affecting the NE and DA systems	192
a. DOPA-pargyline	192
b. AMPT	197
3. Drugs affecting the 5HT system	202
a. pCPA	202
b. TP	207
c. Melatonin	212
4. Drugs affecting the histamine system	212
a. L-Histidine	212
b. Decaborane	212

c. Diphenhydramine	226
5. GBL	226
E. The effect on rectal temperature of PB alone and in combination with drug pretreatments	235
1. Untreated animals	235
2. Treated animals	235
V. General Discussion	248
A. Introduction	248
B. Drugs affecting the NE and DA systems	249
1. DOPA-pargyline	249
2. AMPT	252
C. Drugs affecting the 5HT system	256
1. pCPA	256
2. TP	259
3. Melatonin	261
4. 5HTP and 5HT-related compounds	264
D. Drugs affecting the histamine system	265
1. Histidine	265
2. Decaborane	268
3. Diphenhydramine	270
E. Gamma-butyrolactone	273
F. Anticholinesterases	275
VI. Summary	276
VII. Appendix	286
A. Drugs, chemicals and equipment	286
B. Analyses of amine patterns	289
C. Additional amine studies	298
VIII. Bibliography	302

List of Abbreviations

AAADC	L-aromatic amino acid decarboxylase
ACh	acetylcholine
AChE	acetylcholinesterase
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
alc. DH	alcohol dehydrogenase
ald. DH	aldehyde dehydrogenase
AMMT	alpha-methyl-m-tyrosine
AMPT	alpha-methyl-p-tyrosine
AOAA	amino-oxyacetic acid
ARAS	ascending reticular activating system
ATP	adenosine triphosphate
BBB	blood brain barrier
CA(s)	catecholamine(s)
ChAc	choline acetylase
CoA	coenzyme A
COMT	catechol-O-methyl transferase
CN	caudate nucleus
CNS	central nervous system
CRF	corticotrophin releasing factor
CST	central standard time
CV	cardiovascular

DA	dopamine
DAMP	dibutyryl adenosine 3',5'-monophosphate
DB	decaborane
DET	N,N -diethyltryptamine
DH	dopamine -hydroxylase
DMT	N,N -dimethyltryptamine
DOPA	3,4-dihydroxyphenylalanine
DOPAC	dihydroxyphenylacetic acid
DPH	diphenhydramine
DPN	diphoscopyridine nucleotide
EDTA	ethylene diamine tetraacetic acid
EEG	electroencephalograph
EMP	electromotor potential
Epi	epinephrine(adrenaline)
EPSP	excitatory postsynaptic potential
FFA	free fatty acid
GA	glutamic acid
GABA	gamma-aminobutyric acid (4-aminobutyric acid)
GAD	glutamic acid decarboxylase
GAH	glutamic acid hydrazide
GBL	gamma-butyrolactone
GHB	gamma-hydroxybutyric acid
GI	gastrointestinal

H	histamine
HB	hexobarbital
HBO	hexobarbital oxidase
Hd	histidine
HdDC	histidine decarboxylase
HGH	human growth hormone
HIOMT	hydroxyindole-0-methyl transferase
HMT	histamine methyl transferase
HVA	homovanillic acid
IA(s)	indoleamine(s)
LAOT	inferior accessory optic tract
IM	intramuscular
IP	intraperitoneal
IPSP	inhibitory postsynaptic potential
IRI	immunoreactive insulin
IV	intravenous
lactate DH	lactate dehydrogenase
LGB	lateral geniculate body (nucleus)
LH	luteotropic hormone
LSD	lysergic acid diethylamide
M	melatonin
MAO	monoamine oxidase
MAOI(s)	monoamine oxidase inhibitor(s)
MB	midbrain

MES	minimal electroshock seizure
MET	metanephrine
MFB	median forebrain bundle
NE	norepinephrine (noradrenaline)
nm	nanometers
NM	normetanephrine
NREM	non-rapid eye movement (SWS)
PB	pentobarbital
PBI	protein bound iodine
pCPA	p-chlorophenylalanine
PGO	ponto-geniculate-occipital
PMT	phenylethanolamine-N-methyl transferase
POT	primary optic tract
PS	paradoxical sleep (REM)
R	reserpine
RAS	reticular activating system
REM	rapid eye movement (PS)
RF	reticular formation
SC	subcutaneous
SCG	superior cervical ganglion
S-W	sleep-wakefulness
SWS	slow wave sleep (NREM)

T4	thyroxine
TBG	thyroid binding globulin
TH	tyrosine hydroxylase
TP	tryptophan
TPH	tryptophan-5-hydroxylase
TSH	thyroid stimulating hormone
TT	tyrosine transaminase
VMA	vanillimandelic acid (3-methoxy 4-hydroxymandelic acid)
4DAB	4-dimethyl aminoazobenzene
5HI(s)	5-hydroxyindole(s)
5HIAA	5-hydroxyindoleacetic acid
5HIAc	5-hydroxyindoleacetaldehyde
5HT	5-hydroxytryptamine (serotonin)
5HTP	5-hydroxytryptophan
5HTPD	5-hydroxytryptophan decarboxylase
5HTPL	5-hydroxytryptophol
5MIAA	5-methoxyindoleacetic acid
5MT	5-methoxytryptamine
5MTPL	5-methoxytryptophol
17OCHS	17-hydroxycorticosteroid

List of Tables

Table 1	The duration of pentobarbital-induced sleep	156
Table 2	The duration of GBL-induced sleep	159
Table 3	The duration of pentobarbital-sleep after pretreatment with drugs affecting the NE and DA systems	161
Table 4	The duration of pentobarbital-sleep after pretreatment with drugs affecting the 5HT system	166
Table 5	The effects of compounds structurally-related to 5HT on pentobarbital-induced sleep in mice	174
Table 6	The duration of pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system	175
Table 7	The duration of pentobarbital-induced sleep after pretreatment with two cholinesterase inhibitors, in mice	183
Table 8	The duration of gamma-butyrolactone-sleep after pretreatment with histidine	186
Table 9	CNS concentrations of NE, DA and 5HT after DOPA-pargyline pretreatment with PB, on a 24-hour basis	195
Table 10	CNS concentrations of NE, DA and 5HT after AMPT pretreatment with PB, on a 24-hour basis	198
Table 11	CNS concentrations of NE, DA and 5HT after pCPA pretreatment with PB, on a 24-hour basis	203
Table 12	CNS concentrations of NE, DA and 5HT after TP pretreatment with PB, on a 24-hour basis	208
Table 13	CNS concentrations of NE, DA and 5HT after M25 pretreatment with PB, on a 24-hour basis	213
Table 14	CNS concentrations of NE, DA and 5HT after Hd500 pretreatment with PB, on a 24-hour basis	217
Table 15	CNS concentrations of NE, DA and 5HT after DB pretreatment with PB, on a 24-hour basis	221
Table 16	CNS concentrations of NE, DA and 5HT after DPH pretreatment with PB, on a 24-hour basis	227

Table 17	CNS concentrations of NE, DA and 5HT after GBL, on a 24-hour basis	231
Table 18	The 24-hour pattern of rectal temperature in untreated rats	236
Table 19	The time-course of PB hypothermia in controls and treated rats, 0600 hours	239
Table 20	The time-course of PB hypothermia in controls and treated rats, 1200 hours	242
Table 21	The time-course of PB hypothermia in controls and treated rats, 1800 hours	244
Table 22	The time-course of PB hypothermia in controls and treated rats, 2400 hours	246
Table 23	CNS concentrations of NE, DA and 5HT at the time of awakening from PB (50mg/kg, IP)	299

List of Figures

Figure 1	Indoleamine metabolism	30
Figure 2	Catecholamine metabolism	54
Figure 3	Formation and metabolism of histamine	64
Figure 4	GBL-GABA pathways	114
Figure 5	Chemical structures	141
Figure 6	Brain dissection	143
Figure 7	Amine analysis flowsheet	147
Figure 8	Pentobarbital-induced sleep	157
Figure 9	GBL-induced sleep	160
Figure 10	Pentobarbital-induced sleep after pretreatment with drugs affecting the NE and DA systems, 0600-1200 hours	162
Figure 11	Pentobarbital-induced sleep after pretreatment with drugs affecting the NE and DA systems, 1800-2400 hours	163
Figure 12	Circadian pattern of pentobarbital-induced sleep after pretreatment with drugs affecting the NE and DA systems	164
Figure 13	Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 0600 hours	167
Figure 14	Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 1200 hours	168
Figure 15	Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 1800 hours	169
Figure 16	Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 2400 hours	170
Figure 17	Circadian pattern of pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system	171
Figure 18	Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 0600 hours	176
Figure 19	Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 1200 hours	177

Figure 20	Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 1800 hours	178
Figure 21	Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 2400 hours	179
Figure 22	Circadian pattern of pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system	180
Figure 23	Pentobarbital-induced sleep in mice after pretreatment with two anticholinesterases	184
Figure 24	The time-course of CNS amine concentrations of CONTROLS--0600 hours	187
Figure 25	The time-course of CNS amine concentrations of CONTROLS--1200 hours	188
Figure 26	The time-course of CNS amine concentrations of CONTROLS--1800 hours	189
Figure 27	The time-course of CNS amine concentrations of CONTROLS--2400 hours	190
Figure 28	The effects of DOPA-pargyline pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROLS	195
Figure 29	The effects of DOPA-pargyline pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--TREATED	196
Figure 30	The effects of AMPT pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROL	200
Figure 31	The effects of AMPT pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT-- TREATED	201
Figure 32	The effects of pCPA pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROL	205
Figure 33	The effects of pCPA pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--TREATED	206
Figure 34	The effects of TP pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROL	210
Figure 35	The effects of TP pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--TREATED	211
Figure 36	The effects of M25 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROL	215

Figure 37	The effects of M25 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--TREATED	216
Figure 38	The effects of Hd500 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROL	219
Figure 39	The effects of Hd500 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--TREATED	220
Figure 40	The effects of DB pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROL	223
Figure 41	The effects of DB pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--TREATED	224
Figure 42	The effects of DPH pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--CONTROL	229
Figure 43	The effects of DPH pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT--TREATED	230
Figure 44	The NE, DA and 5HT concentrations in the CNS for GBL--CONTROL animals	233
Figure 45	The NE, DA and 5HT concentrations in the CNS for GBL--TREATED animals	234
Figure 46	Rectal temperature (°C.) in untreated rats	237
Figure 47	Rectal temperature in CONTROLS and drug pretreated groups, with pentobarbital--0600 hours	238
Figure 48	Rectal temperature in CONTROLS and drug pretreated groups, with pentobarbital--1200 hours	241
Figure 49	Rectal temperature in CONTROLS and drug pretreated groups, with pentobarbital--1800 hours	243
Figure 50	Rectal temperature in CONTROLS and drug pretreated groups, with pentobarbital--2400 hours	245
Figure 51	The effects of pentobarbital on the CNS concentrations of NE, DA and 5HT in rats, at the time of awakening	300

I. INTRODUCTION

A. The problem

This investigation involves an examination in rats, of the effects on the duration of drug-induced sleep or hypnosis resulting from the pretreatment with pharmacological agents that affect one or more neuroamine systems. The primary index of the duration of sleep used is the loss and recovery of the righting reflex. The excitatory and depressant activity of monoamines in the CNS have been revealed by studies involving their direct application, systemic injection and pharmacological alteration or, inferentially, from studies demonstrating diurnal or circadian fluctuations (vide infra). In as much as Friedman and Walker (1969), among others, have shown that the duration of pentobarbital hypnosis varies with the time of day, and that levels of monoamines in several CNS areas also follow a circadian pattern (vide infra), it was of interest to extend these studies and examine the effects that pretreatment with drugs influencing the monoamine systems might have on CNS monoamine levels and sleep duration. Such an approach seeks to determine a possible interrelationship between central amines and drug-induced sleep. Because the exact mechanism of sleep produced by hypnotics is unclear, experiments were designed to examine the nature of the involvement of monoamines in the phenomenon. Furthermore, the efficacy of the modulating drugs used for these pretreatments has not heretofore been examined on a 24-hour basis.

The circadian pattern for hypnotic sleep duration is inversely related to metabolizing enzyme activity (vide infra). Nair and Casper (1969) sug-

gested that there might be a central component for the control of the enzyme activity, and in turn, the duration of hypnosis. By utilizing appropriate dosages of the pretreatment drugs that significantly alter amine levels (according to published reports), the effect of changes in the levels of CNS monoamines on the duration of action of hypnotics might be demonstrated. The presence or absence of specific monoamines, as well as their relative levels might serve to augment or limit the hypnotic effect. Daily fluctuations of these amines could change the sensitivity of the CNS and its possible regulation of drug metabolizing systems, thereby resulting in an altered effect of the hypnotic.

B. Terminology.

The present study examines periodic alterations in 1) the duration of drug-induced sleep and 2) central monoamine levels, and possible interrelationships. The review of the literature summarizes studies on states of normal sleep and the effects of drugs on normal sleep. Furthermore, it examines the nature of the formation of specific monoamines, their effects on sleep and the pharmacology of drugs utilized to induce sleep. The stages of normal sleep and drugs which affect these stages are discussed. It is understood that drug-induced sleep differs markedly from normal sleep. Briefly, the stages of sleep can be classified as paradoxical sleep (PS or REM, rapid eye movement) or slow wave sleep (SWS or non-REM, NREM). The monoamines to be considered in these phenomena include; norepinephrine (NE), dopamine(DA), epinephrine (Epi), serotonin (5HT), histamine (H) and acetylcholine (ACh).

The term "circadian", coined by Halberg, is derived from the Latin, circa diem, which means "about one day". It refers to rhythms that have a frequency of about one cycle per day, or a period of 20-28 hours. The term "diurnal" has been used by some workers to describe rhythms that have been measured on a 24-hour basis, although it also has a secondary meaning of "daily", as opposed to nocturnal, or nightly. Wurtman (1967) criticized the ambiguous use of the term "circadian", which he suggested should be restricted to free-running rhythms. The problem arises in studies in which the animal must be sacrificed to determine the aspect under study and can only be measured once (eg. toxicity, brain amines, etc.) as opposed to rhythms which can be continuously measured (eg. temperature, urine or blood contents, etc.). In this study the terms circadian, 24-hour, diurnal or daily rhythms are used interchangeably. To establish whether or not rhythms are free-running requires examination for several cycles, as well as their insensitivity to exogenous cues.

Halberg also proposed terms to refer to non-circadian rhythms. Ultradian rhythms have a frequency greater than one cycle per day, or a period between one and 19.9 hours. Infradian refers to a rhythm with a frequency of less than one cycle per day, or a period greater than 28.5 hours. Exogenous rhythms are those which can be altered by changes in environmental parameters, while endogenous rhythms (free-running) appear to be under internal control, as they persist under an unchanging environment, such as constant light or constant darkness.

C. Practical considerations

The present study concerns itself only with the effects on drug-induced

sleep and not with the effects of drugs on normal sleep. Effects of these drugs on normal sleep and drug-induced sleep are cited with regard to possible relationships to the results of this study.

In studies of circadian rhythms of physiological and pharmacological phenomena, an investigator concerns himself with the problem of frequency of sampling. Some investigators are content to show differences between the and dark periods, while others take measurements every hour. As in previous studies from this laboratory, data is obtained every six hours (0600, 1200, 1800 and 2400 hours), giving two points in the light and two in the dark. Ordinarily this is sufficient to show differences between light and dark phases and demonstrate the basic 24-hour pattern. Pretreatment and sacrifice times are bracketed around these test times, so that the average time occurs at the six-intervals.

II. REVIEW OF THE LITERATURE

A. Parameters affecting biological rhythms.

The importance of biological rhythms in physiological and pharmacological phenomena is becoming better appreciated. Biological rhythms (growth, motor activity, feeding, etc.) of plants and submammalian species (protozoa, coelenterates, mollusks, insects and birds) have been demonstrated (cf. Bunning, 1967; von Mayersbach, 1967; Walker, 1969). This review however, will be confined to studies of rhythms in mammals, primarily mice and rats. It will consider parameters affecting biological rhythms and studies demonstrating physiological and pharmacological rhythms.

1. Sex

Sex hormones (vide infra) alter the ongoing biorhythms and result in differences in male and female rhythms. Slonaker (1912) demonstrated that motor activity increases progressively and is maximal at the peak of estrus. Colvin et al. (1968) attributed variations in the sleep-wakefulness (S-W) cycle to the superimposition of the estrous cycle. EEG monitoring of chronically-implanted female rats revealed that the pre-estrus rat has PS in the afternoon, followed by an alert pattern in the evening. The day of vaginal cornification is marked by a higher percentage of PS, after a night in which the animal is alert and PS is absent. The hormonal changes of proestrus appears to increase alertness, which is compensated for on the following night.

Dray et al. (1965) reported a diurnal rhythm of testosterone levels in man, which peaked at 0800-0900 hours and reached a trough at midnight.

This variation appeared to be due to a decrease in its production, rather than an alteration in metabolism. (Women had too little testosterone to detect a circadian rhythm.) Baird and Guevera (1969) found no differences in the mean plasma estrone or estradiol content of six non-pregnant women and five men, sampled at 0800 or 1700 hours. However, Tulchinsky and Korenman (1970) reported that plasma estrone levels in women and men were three times as high at 0800-0900 hours than at 2400 or 0400 hours. The differences between circulating sex hormones in male and female influence the basic biorhythm, as well as the response to drugs (vide infra).

2. Species

The differences between species has been examined most extensively in rodents, which are nocturnal. The ability to adapt to altered photoperiods differs among species. Phase-shifting leads to a resynchronization of the biorhythm in two days in spiders (Aschoff, 1963), while about three weeks are needed for mice and rats to readapt (Hanin et al., 1970).

3. Age

Differences in physiological and pharmacological response of the immature and adult animal have been reported. These include the development of liver metabolizing enzymes, brain biogenic amines (vide infra) and the S-W cycle. The newborn of most higher species start with very short S-W cycles which merge, then prolong into the 24-hour adult pattern (cf. Ellington, 1972). Enzyme development influences metabolism of drugs and/or endogenous substances. The changes in sleep cycle and brain amines are probably inter-related, both reflecting the maturation of the CNS. Shaywitz et al. (1971) were unable to correlate growth hormone and sleep state in newborns, although

levels were elevated for the first 2-3 days after birth and became normal by day 4 (cf. Mills, 1966). In the adult, growth hormone release is related to SWS (vide infra).

4. Population

Quay et al. (1969) examined the effect of isolation on the anatomy of the CNS. Isolated rats have greater body and pineal weights and a higher ratio of pineal to brain weight, than do grouped animals. Grouped animals exhibited higher mean cerebral cortical weight than isolated rats. The ratio of cortical acetylcholinesterase to cholinesterase decreased in grouped rats, when more than two rats were maintained per cage. There were no differences in pineal 5HT levels or pineal HIOMT activity between grouped and isolated rats. Amphetamine toxicity in aggregated mice has been shown by several investigators to be greater than for isolated mice. Walker (1972) reported that amphetamine was most toxic during the dark (active) phase for isolated or aggregated mice.

5. Illumination

The influence of light on biorhythms can be considered with regard to photoperiod (relative duration of light-dark periods), intensity and spectrum of the light source. This includes not only circadian periods, but longer periods, such as monthly (lunar) and seasonal (solar). The physiological transducer for these effects involves the eye-pineal axis (cf. pineal gland, under 5HT section) and possible extraretinal receptors, which respond to direct penetration of light through the skull (Lisk and Kannwischer, 1964).

Biorhythms are most easily synchronized by light-dark photoperiods, usually of a 12:12 or 14:10 hours relationship (cf. Aschoff, 1969). The exact

ratio requirement of light and dark is probably species specific. A number of studies have examined the time required for animals to adapt to 1) a specific photoperiod, 2) reversal of the photoperiod and 3) constant light, constant darkness or a new photoperiod. Some rhythms are resistant to photoperiod changes and require days or weeks to readapt fully. Others do not readapt, i.e. free run, and are not controlled by light, but by some other environmental factor or the rhythm might fade out. Von Mayersbach (1967) points to difficulties involved in such experiments. He was never able to duplicate values 24 hours apart, indicating that certain cycles might be greater or lesser than 24 hours or that in the process of removing animals those to be used subsequently are disturbed.

Reiter (1969) reported that the levels of light found in most artificially-lit animal rooms (10-150 footcandles) are sufficient to have an inhibitory action on the pineal, and probably affect other systems. Harker (1964) stated that light intensity has relatively little effect on period length, but changes in light intensity determine phase setting.

Marshall and Bowden (1936) demonstrated the effects of wavelength of light on the timing of the sexual cycle of the ferret. Estrus begins earlier in animals exposed to ultraviolet light (3650 \AA). Infrared light has no effect. Luce-Clausen and Brown (1939) showed a delayed onset of estrus in rats maintained in darkness but a normal onset for animals exposed to visible or infrared illumination. Spaulding et al. (1969) studied the effects of spectral components of light on motor activity in mice. The lowest activity occurred in mice exposed to blue or green light, or daylight; intermediate activity occurred under yellow, while the highest activity was in red light

or darkness. Wurtman and Weisel (1969) found that rats maintained under Vita-Lite, which closely (93%) approximate the daylight spectrum, have larger gonads and smaller spleens than those under conventional cool-white fluorescent lights. Cool-white lights are deficient in the near-UV wavelengths and have greater amounts of the yellow and orange wavelengths.

6. Temperature

The effects of ambient temperature on biorhythms appear to be minimal within a 2-6°C. range from normal environmental temperature, to which the mammalian thermoregulatory system can adjust. Body temperature is a balance between heat production and heat loss. Heat production is high during the day in man, due to muscular activity, but even subjects at rest and fasting exhibit a diurnal temperature rhythm. Halberg (1959) found that artificial alterations of body temperature have no effect on the activity rhythm (cf. Sweeney and Hastings, 1960).

7. Barometric pressure

Harker (1958) described barometric pressure as a weak synchronizer, i.e. although it is incapable of controlling a biorhythm, it might have an influence on an existing rhythm. Hayden and Lindberg (1969) entrained body temperature rhythm in mice by fluctuations in ambient barometric pressure, under constant ambient temperature and darkness.

8. Feeding habits

Feeding may be involved as a phasing factor of other biorhythms but it is difficult to devise methods of testing its role. If food is present only at one time during the day, animals will feed then, but if present continually rodents will eat during the dark phase. Honova et al. (1968) showed

a rhythm of rat liver tyrosine transaminase, which they stated might be controlled by the feeding pattern in rats and man. Studies of diurnal fluctuations of plasma amino acids (Wurtman et al., 1967b, 1968b), of course, must consider the effect of dietary intake and its amino acid content.

9. Miscellany

Other factors which might subtly influence ongoing circadian rhythms include sound, odors, lunar or seasonal variations, magnetism and electrical fields. Sound can interrupt sleep and activity rhythms, depending upon intensity and duration. Halberg et al. (1954) reported that the activity of normal mice affected blind mice. After exposure of rats to the pungent odor of isonitrile for six hours, Miline et al. (1963) noted atrophic alterations of pineal parenchymal cells (which might also be due to a direct effect). Taste, smell and hearing rhythms in man (Henkin et al., 1963; Henkin, 1968), are influenced by adrenal hormones.

The influence of the moon and sun on living things is an old observation that is coming under scientific scrutiny. Rhythms related to the lunar cycle have been demonstrated for lower aquatic species (cf. Harker, 1964; Bunning, 1967). Brown et al. (1970) have demonstrated that living systems are able to distinguish among strengths and directions of magnetic, electrostatic and gamma radiation. Radiation is probably involved in the seasonal variations (well-appreciated for plants) and cycles of long duration such as the eleven-year sunspot cycles (Brown et al., 1958). Wever (1971) showed that a 10.0 Hz alternating electrical field can affect human circadian rhythms (activity and rectal temperature).

B. Physiological parameters

1. Motor activity

Circadian rhythms in motor activity with a peak in the dark have been demonstrated in the mouse (Saelens et al., 1968) and the rat (Quay, 1970). Holmquest et al. (1966) studied the relationship between motor activity and body weight and the adrenals, in rats. After 40 days of random illumination, the activity rhythms were lost without affecting the steady rise of body weight or endocrine organ weight. Quay (1970) reported that normal and pinealectomized rats could reverse their activity rhythms after photoperiod reversal. Curtis (1937) demonstrated diurnal activity rhythms for sheep and pigs, which was maximal during the day. Lindsley et al. (1964) initiated a circadian activity rhythm in young monkeys that had been maintained in darkness for three years. This rhythm required 3-5 weeks to stabilize after the initiation of the standard photoperiod. Winget et al. (1969) found a correlation between diurnal motor activity and body temperature in the monkey. Rüter et al. (1967) reported that rats under constant light were less active and rats under constant dark were more active, than rats maintained under a normal light-dark photoperiod.

2. Body temperature

Many studies have shown that the diurnal pattern of body temperature fluctuates in phase with the activity rhythm (Brahmachary, 1967; Friedman and Walker, 1968; cf. Kleitman, 1963). Julku et al. (1970) showed that mice exposed to a cold environment had better thermoregulatory ability at 1600 hours than at 0500 hours. Smith (1969) studied human rectal tempera-

ture and thermoregulation (as measured by core-periphery heat conductance) and found that they generally peaked in the light phase.

3. Blood composition

Halberg et al. (1954) reported circadian variation of eosinophils in rats. Even blind mice exhibit a circadian rhythm in eosinophils, lymphocytes and other white cells, as well as adrenal cortical hormones (Haus et al., 1967). After adrenalectomy or under constant light, the rhythms disappeared. Pauly and Scheving (1965) showed similar rhythms for neutrophils, eosinophils and lymphocytes, with peaks at the onset of inactivity, when the light period began. Under continuous darkness, the rhythms persisted but became dissociated with continuous light. In pregnant women, total counts of leukocytes, segmented neutrophils and stab neutrophils was maximum during the day and fell to a minimum in the early morning, while eosinophils and lymphocytes showed a reverse pattern (Luce, 1970). Diurnal rhythms for circulating eosinophils have also been demonstrated for the monkey (Migeon et al., 1955) and man (Donato and Strumia, 1952; Halberg et al., 1951). Scheving and Pauly (1967) demonstrated that the diurnal rhythm for blood clotting time in rats was longest at 0130 hours. Scheving et al. (1968c) also found diurnal rhythm in the levels of several rat blood proteins, such as mucoproteins, albumin and several globulins (including gamma-globulin), which peak at the end of the phase. Buckell and Elliott (1959) reported a diurnal pattern of plasma fibrinolytic activity. In humans, blood pH rises over the day, then decreases during the night, with the onset of sleep (Cullen and Earle, 1929).

Bonilla and Stringham (1968) were unable to demonstrate a diurnal rhythm for serum Ca^{++} in mice, but this might be due to stress or interruption of the

rhythm, since each animal was sampled four times during the day by orbital venous plexus puncture. Bahorsky and Bernardis (1967) observed that normal rats had a diurnal rhythm in serum Na^+ and K^+ levels, peaking in the early dark phase, but found no rhythm in serum phosphorus or urea nitrogen. Fasted rats maintained the serum Na^+ rhythm, but lost the serum K^+ rhythm.

4. Urinary excretion

Norn (1929) demonstrated a circadian rhythm for urinary Na^+ , K^+ and Cl^- in man, which peaked during the day and troughed at night. Min et al. (1966) examined electrolyte excretion in normal and fasted subjects. In normal subjects, with an evenly spaced diet and minimum activity, a diurnal rhythm for Mg^{++} and Ca^{++} excretion was observed, which peaked in the early morning. The pattern for phosphorus excretion was reversed. Na^+ and K^+ excretion peaked in the late morning. The electrolyte patterns observed in fasting, obese patients were almost identical to those of normals, except the K^+ and phosphorus rhythms were absent. Fiorica et al. (1968) found similar patterns for human urinary Mg^{++} and Ca^{++} excretion. During and following exercise their excretion was decreased, suggesting that the activity cycle might cause their rhythm. Cohn et al. (1970) reported that rats fed ad libitum exhibit diurnal rhythms in the rates of food consumption and urinary excretion of Na^+ , K^+ , Cl^- , inorganic phosphorus and creatinine, with peak values during the dark. If the rats are fed 1/24 of their daily food allotment hourly, the excretion rhythms are either extinguished or markedly decreased, suggesting that the food or feeding might be a synchronizer. Acid and ammonium excretion is high at night and decreases in the morning, and is not dependent on whether the subject is fed or fasting or the night is spent awake and

recumbent, or active (cf. Mills, 1966).

5. Hormones in blood and urine

The rate of urine flow is the result of several potentially independent influences, of which the rate of liberation of posterior pituitary antidiuretic hormone (ADH) and the total solute excretion are probably the most important. Zsoter and Sebok (1955) reported a diurnal pattern for human serum ADH levels, which peaks during the night, and helps to explain the decreased urinary output while asleep.

Venning et al. (1956) reported that human urinary aldosterone excretion was greater during the day than the night. The phenomena was not affected by changes in sodium intake but was abolished if subjects remained horizontal (Muller et al., 1958). However, plasma aldosterone has a circadian pattern, even in subjects continuously horizontal (Michelakis and Horton, 1970). An identical pattern for human plasma renin has been shown (Gordon et al., 1966), suggesting that the aldosterone rhythm might be driven by renin secretion.

Circadian variation in the urine and plasma levels of 17-hydroxycorticosteroids (17OHCS) have been demonstrated in humans and many animal species (cf. Nichols and Tyler, 1967; Krieger, 1970). In humans, plasma 17OHCS levels peak in the early morning, just prior to awakening, while in the rat, the peak occurs in the early evening. Plasma adrenocorticotrophic hormone (ACTH) exhibits a similar rhythm. Urinary 17OHCS excretion follows a similar circadian pattern, but the cycle lags about two hours behind plasma 17OHCS. 17OHCS rhythms, in man and rodents, can be reversed within eight days by reversal of the photoperiod. A child has no diurnal 17OHCS cycle but develops one between 3-13 years of age. The infant monkey, however, exhibits

a circadian 17OHCS pattern in the first week of life (Bowman et al., 1970). The plasma 17OHCS rhythm is absent in Cushing's disease (Doe et al., 1960), in which pituitary ACTH formation is increased. The response of humans to ACTH administration is decreased at night, suggesting a rhythm for adrenal sensitivity (Perkoff et al., 1959). The control of the 17OHCS rhythm might be due to changes in corticotrophin-releasing factor (CRF) or the sensitivity of anterior pituitary neurosecretory cells to CRF-induced ACTH release (Clayton et al., 1963). Krieger et al. (1968) demonstrated that pretreatment of cats with atropine and thiamylal just prior to the normal rise of plasma 17OHCS, blocks the rise. Krieger and Rizzo (1969) found that any alteration of CNS 5HT levels blocked the daily rise of plasma 17OHCS, but alteration of central NE with depletors or blockers had no effect on the rhythm.

Hellman et al. (1969) reported that human cortisol content exhibited a diurnal pattern. It was not smooth, but exhibited a series of steep peaks, due to about eight episodic bursts of cortisol per day. A circadian rhythm for mouse serum and adrenal gland corticosterone peaked at 1600 hours and troughed at 0400 hours (Reinberg and Halberg, 1971). In serum and in the adrenal, the greatest corticosterone response to saline occurred 4-8 hours before the normal circadian corticosterone peak, while the greatest response to ACTH was found eight hours after the peak. Ungar and Halberg (1962) and Andrews (1971) reported an in vitro circadian response of mouse and hamster adrenals to ACTH. Hodges and Mitchley (1970) found that circadian and stress-induced changes in plasma corticosterone levels in rats were abolished by betamethasone in the drinking water. Adrenal sensitivity to ACTH was unimpaired. The normal plasma corticosterone rhythm returned within one day of

drug withdrawal, but the stress response was normal only after three days. Suprachiasmatic nuclear lesions and a caudal frontal cut abolished the adrenal corticosterone rhythm, while blinding shifted the rhythm (Moore and Eichler, 1972).

DeGroot (1967) found that the ovaries of prolactin-treated (pseudo-pregnant) rats contained the lowest ascorbate levels and highest progesterone concentrations at 1600 hours. The latter response is thought to be due to luteotropic hormone (LH) release, since hypophysectomy prevented the progesterone rise. Serum prolactin of lactating cows exhibits a circadian fluctuation, although individual patterns show sporadic release (Koprowski et al., 1972). Clark and Zarrow (1967) found a diurnal rhythm in ovarian cholesterol content in the mature, cycling rat, which appears to be under the control of a daily release of LH. Rats maintained in constant light showed an earlier onset of this diurnal ovarian cholesterol pattern, while the pattern was absent in androgenized (sterilized) animals (Zarrow et al., 1969). Pronounced rhythms of plasma immunoreactive FSH in both sexes have been reported (cf. Curtis, 1972). They peak at 0400-0900 hours.

Auerbach (1963) found a diurnal pattern in plasma protein bound iodine (PBI) in humans, which was maximum at 1230 hours and minimum at 0730 hours. Bushler et al. (1968) reported similar results and also found PBI in phase with plasma thyroxine (T₄), hematocrit, plasma proteins and plasma thyroid binding globulin (TBG). The patterns were reversed by reversing the sleep schedule, but not by dexamethasone treatment or rescheduling meals. The rate of disappearance of thyroid hormone from blood is cyclic in humans, calves and rats, being maximum at the time of their greatest activity (cf. Curtis, 1972).

Diurnal variation has been reported in plasma PBI of rabbits, of thyroid ^{131}I content in cats and monkeys after tracer doses of ^{131}I , plasma thyroid stimulating hormone (TSH) in humans and rats and rat pituitary TSH (cf. Curtis, 1972).

Von Euler et al. (1955) reported a diurnal variation for free and conjugated urinary catecholamines in humans. Minimum excretion rates of free Epi and NE were at night, while the maximum rates, about three times the minimum, occurred at 0800-1100 hours. Catecholamine excretion is affected by emotion, exertion, and posture, but its rhythm persists, with reduced amplitude, during bed rest, sleep deprivation or constant darkness (cf. Curtis, 1972). Scheving et al. (1968b) reported that rat adrenal epinephrine fluctuates daily, with maximum concentrations occurring during the light phase.

Fasting diabetics have a diurnal pattern for blood sugar, which peaks at about 0800 hours. Since a diurnal pattern of immunoreactive insulin (IRI) has not been demonstrated in fasting diabetics, the blood glucose pattern might be due to insulin resistance, which leaves the glucose-mobilizing effects of the ACTH-cortisol cycle unopposed (Faiman and Moorhouse, 1967). In normal subjects, plasma IRI exhibits peaks associated with meals and falls during the night, so it is uncertain if there is a circadian pattern.

Plasma growth hormone peaks at almost any time of day, but the largest and most consistent peaks occur at the onset of the first period of SWS (vide infra).

6. Rhythms in constituents of carbohydrate, lipid and protein metabolism.

Circadian variations in blood glucose have been demonstrated in

the rat (Pauly and Scheving, 1967; Friedman and Walker, 1969), mouse (Dziekanouska and Andrzej, 1961), chicken (Twiest and Smith, 1970) and cow (Allcroft, 1933). Maximum levels occur during the period of maximum activity. Friedman and Walker (1969) found a biphasic pattern for blood glucose, with peaks at the end of the light and dark periods. Bahorsky and Bernardis (1967) obtained similar results in rats, and that were abolished by fasting. Normal subjects exhibit diurnal variation in the glucose tolerance test, while hyperglycemic patients do not (Jarrett and Keen, 1969).

Agren et al. (1931) reported a circadian pattern for liver glycogen in the rat and rabbit. These rhythms were abolished by adrenalectomy. Halberg et al. (1960) found a similar liver glycogen rhythm in the mouse, which persisted after food deprivation, even to the point of death of the animal. Sollberger (1964) reported circadian variations in liver glycogen levels in several species, which generally peaked during the period of greatest motor activity. Fuller and Diller (1970) found that the hepatic liver glycogen rhythm in rats fed ad libitum peaks at 0500-0800 hours. In rats fed a single meal from 0800-1200 hours, hepatic glycogen levels exhibited a prolonged peak from 1200-2000 hours. They also found that the circadian pattern for plasma free fatty acids was inversely related to liver glycogen content.

Feigen et al. (1968) demonstrated in humans, that blood amino acids levels (except citrulline) exhibit a diurnal rhythm, which peak between 1200-2000 hours and trough between 0400-0800 hours. Increases and decreases in total protein content of an isocaloric diet or exercise had no effect on the pattern. Reversal of the S-W cycle resulted in a rapid reversal in the amino acid pattern. Wurtman et al. (1968b) obtained similar results. Coburn et al.

(1968) reported a circadian rhythm for plasma tyrosine in rats, but not for plasma phenylalanine, which was controlled by food intake. A circadian rhythm for plasma tyrosine occurred in human phenylketonuric subjects (who lack phenylalanine hydroxylase), suggesting that dietary absorption or liver metabolism might be controlling the rhythm. Tewksbury and Lohrenz (1970) found that urinary amino acid levels exhibit a rhythm similar to those of whole blood, suggesting that the basic amino acid rhythm is normally present and is augmented by food intake.

7. Enzyme systems

Wurtman and Axelrod (1967) reported that liver tyrosine transaminase (TT; EC 2.6.1.5) in rats peaked shortly after the onset of darkness and was low during the day. Adrenalectomy, hypophysectomy, thyroidectomy, pancreatectomy, constant darkness or fasting did not alter the pattern. Transection of the spinal cord suppressed the rhythm at peak levels, while constant light decreased levels. Black and Axelrod (1968) showed that the enzyme activity was reduced by increasing NE, in vitro and in vivo, while depleting NE increased enzyme activity. Honova et al. (1968) reported that in the rat, the enzyme rhythm appeared within 48 hours after birth, but was 180° out of phase with the adult pattern, which develops 21-23 days after birth. Brain TT activity does not fluctuate, indicating that separate control mechanisms exist for central and peripheral TT activity (cf. Fuller, 1970).

Radzialowski and Bousquet (1968) demonstrated a circadian pattern in rats, for hexobarbital oxidase (HBO), aminopyrine N-demethylase, p-nitro-anisole O-demethylase, 4-dimethylaminoazobenzene reductase (4DAB) activities, all peaking at 0200 hours. Adrenalectomy abolished all but the 4DAB pattern.

The peak of diurnal rat plasma corticosterone levels occurs at the time of minimal enzyme activities (1400 hours). Exogenous corticosterone abolished the diurnal aminopyrine and *p*-nitroanisol enzyme activities, suggesting that the adrenal might be involved in regulation of hepatic drug-metabolizing enzymes. Jori et al. (1971) found a similar circadian pattern for the liver enzymes metabolizing hexobarbital, imipramine, *p*-nitroanisol and aminopyrine, which could be reversed by photoperiod reversal. Nair and Casper (1969) found a circadian rhythm for HBO and O-demethylase activities in the rat liver, which were maximal midway through the dark phase. The daily pattern for HBO activity correlated inversely with the circadian pattern of HB sleeping time; ie., sleep was longest when HBO activity was minimal. Continuous light or darkness abolished the enzyme rhythm, although the activity was significantly higher in animals subjected to constant dark. Chedid and Nair (1972) demonstrated electron microscopically a circadian pattern for the relative amounts of hepatic endoplasmic reticulum in the rat. Roberts et al. (1970) reported a circadian variation for HBO activity, but not for PB sleep duration or brain, liver and serum concentration of PB and its metabolites.

Diurnal fluctuations occur in the activities of hepatic steroid hydroxylases (Colas et al., 1969) and tryptophan pyrrolase (Rapport et al., 1966). Phillips and Berry (1970) noted a circadian pattern in mouse liver phosphoenolpyruvate from pyruvate). Gielen et al. (1970) found a circadian pattern for cholesterol-7- α hydroxylase activity (involved in the biosynthesis of bile acids), which is controlled by the adrenocortical system.

Mouse kidney transamidase activity is circadian (Van Pilsum and Halberg, 1964).

8. CNS amines

a. Serotonin (5HT)

Scheving et al. (1968a), who measured 5HT levels every hour, found a circadian rhythm for whole rat brain, which peaked during the light phase. Dixit and Buckley (1967) reported a similar pattern for rat brain 5HT, during normal and reversed photoperiods. Peak 5HT occurs at the time of lowest corticosterone (2300 hours, mid-light phase) and 6 hours before the corticosterone peak. Scapagnini et al. (1971) noted that 5HT levels in the rat hippocampus and amygdala followed a circadian pattern peaking at 2000 hours and troughing at 0400 hours. The plasma corticosterone pattern is similar, with a peak at 2000 hours and a trough at 0800 hours. Friedman and Walker (1968, 1969) found that rat caudate nucleus (CN) and midbrain (MB) 5HT followed a circadian pattern, with a peak at 1800 hours and a minimum between 2400-0600 hours. They related the 5HT levels to the sleep-wakefulness cycle. Reiss et al., (1968) indicated that 5HT in the inferior colliculus and the red nucleus-medial tegmentum of the cat was greater at 1900 hours than 0700 hours. Hery et al. (1972) obtained similar results for rat cortex, hypothalamus and brain stem 5HT. 5HT synthesis decreased significantly in the brain stem in the dark (2100 hours) as compared with the light phase (1500 hours) and was dependant on the initial tryptophan (TP) levels, not the rate of conversion of TP to 5HT. They further indicated that although 5HT synthesis was maximal during the light period (formation of 5HT from TP), its release was maximal during the dark (formation of 5-hydroxyindole acetic acid from 5HT). Okada (1971) studied the maturation of the circadian rhythm of rat whole brain 5HT.

The pattern appeared at days 35-37, preceding the onset of the adult S-W pattern, which matures on days 49-52. Asano (1971) obtained similar results. R  ther et al. (1967) reported that whole brain 5HT levels of rats maintained under constant light or constant dark were elevated over that of rats under a normal light-dark photoperiod.

b. Norepinephrine (NE)

Scheving et al. (1968a) found an ultradian pattern for NE levels in the whole brain of the rat while Friedman and Walker (1968, 1969) reported a circadian pattern for rat CN and MB NE, which was maximal at the end of the dark phase. Reis et al. (1968) demonstrated a diurnal cycle in NE levels of the cat cervical cord, pons, substantia nigralateral tegmentum, anterior hypothalamus, tuber cinereum and the pineal. Generally, NE content was maximal at the middle or end of the light period. The pattern for the anterior hypothalamus and pineal peaked in the dark phase. Constant light abolished the pattern in the pineal but not in the cervical cord or anterior hypothalamus. Reis and Wurtman (1968) demonstrated a circadian alteration in the NE concentrations in parts of the cat hypothalamus and cervical spinal cord. They did not find diurnal fluctuations in other brain areas, but they measured levels only at the onset of the light and dark periods. Reis and Gutnick (1970) demonstrated a circadian pattern for cat cervical and sacral-coccygeal spinal cord NE concentrations, peaking at 1900 hours, while that of the thoracic cord exhibited a biphasic pattern. Manshardt and Wurtman (1968) reported a circadian rhythm (peaking during the dark period) for hypothalamic NE, but not for striatum or midbrain. Walker et al. (1971) found that NE levels in the hypothalamus and caudate nucleus were greater at 0300 hours than

at 1500 hours. Asano (1971) indicated that the adult circadian pattern for whole brain NE does not appear until days 35-37, after birth. The NE levels peaked in the dark, similar to the pattern for brain parts (vide supra). Ancill et al. (1970) examined whole rat brain NE, DA and 5HT concentrations but found a circadian pattern only for 5HT levels, which was maximal at 1200 hours (mid-light period) and minimal at 2400 hours.

c. Dopamine (DA)

Scheving et al. (1968a) reported an ultradian pattern for whole brain DA of rats. Piepho and Friedman (1968) found a circadian pattern for rat CN DA, and inverse pattern for caudate homovanillic acid, a degradative product of DA.

d. Achetylcholine (ACh)

Friedman and Walker (1972) reported circadian patterns for rat CN and MB, and mouse whole brain ACh. Peak ACh levels occurred at 2400 hours in the rat, 0600 hours in the mouse while the trough was at 1200 hours in the rat MB and 1800 hours in the rat CN and mouse brain. Hanin et al. (1970) also found a circadian pattern for whole rat brain ACh, which peaked at 1400 hours. The pattern was found in grouped rats only and emerged after 18 days in controlled environmental conditions.

e. Miscellany

Friedman and Walker (1968, 1969) demonstrated a circadian rhythm for CN and MB histamine, which peaked at 0600 hours in the normal and in the caudate nucleus of the adrenalectomized rat. Piepho and Friedman (1971) found circadian patterns for hindbrain and spinal cord glycine, which were significantly higher during the dark phase.

C. Circadian rhythms in drug response

1. CNS depressants

Davis (1962) demonstrated in mice that sleep duration (loss of the righting reflex) produced by a constant dose of PB (60 mg/kg) varied over the day, peaking during the light period and troughing during the dark period. Grouped mice showed a longer sleeping time during the day than did isolated mice. Pauly and Scheving (1964) found that the maximum toxicity of PB (75-90 mg/kg, IP) in rats occurred during the dark phase, while the doses were least toxic toward the end of the light period. Emlen and Kem (1963) reported that the rate of recovery from PB anesthesia was more rapid during the active (dark) than the inactive (light) phase. Lindsay and Kullman (1966) demonstrated in mice that PB toxicity was least during the dark period. Scheving et al. (1968d) found a circadian pattern in rats for PB sleep duration, which was longest at 2000 hours and shortest at 0900 hours. Friedman and Walker (1969) also reported that sleep duration in rats due to PB (50 mg/kg, IP) exhibited a circadian pattern. The duration was longest during the light phase (1800 hours), and shortest during the dark period (0600-2400 hours).

Nair and Casper (1969) found in rats that hexobarbital (HB; 150 mg/kg, IP) sleeping time was significantly greater at 1400 hours than at 0200 hours. Haus and Halberg (1959) noted that the percentage of deaths due to ethanol was greater at 1600-2000 hours, than for the rest of the day. Marte and Halberg (1961) found that the maximum toxicity of chlordiazepoxide in mice occurred at 2400 hours.

2. CNS stimulants

Scheving et al. (1968e) showed that amphetamine toxicity in rats was greater in the dark than in the light phase. The pattern was relatively unaltered by blinding but abolished by constant light. Walker (1972) found similar results in toxicity for isolated or aggregated mice, although the latter were more sensitive to the lethal effects. Ancill et al. (1970) found that amphetamine (10 mg/kg, IP) produced no alteration in the circadian locomotor cycle of the rat, while lower doses (1.25 and 2.5 mg/kg, IP) significantly altered the pattern, in a dose-dependant fashion. The increases in activity were much greater between 0800-2000 hours. While control locomotor activity does not increase until the dark period, the low doses of amphetamine increased motor activity prior to the onset of the dark period.

Pauly and Scheving (1964) reported that the onset time for rats to undergo continuous tremors from tremorine (100 and 64 mg/kg, SC) depended on the time of administration. The maximal latency to tremor occurred during the light phase (0200 hours) and the minimum occurred during the dark (2100 hours), for the two doses tested. Webb and Russell (1966) found that seizure component times due to the convulsant, hexafluorodiethyl ether, followed a circadian pattern. Seizure times and the percentage of animals affected were greater at 1000 hours (light) than at 2100 hours (dark). Lutsch and Morris (1967) demonstrated that in mice, lidocaine produced convulsions in a significantly higher percentage of animals at 2100 hours (dark) than at 1500 hours (light).

Halberg et al. (1955) reported a circadian periodicity in the incidence of mortality and audiogenic seizures in mice, which were maximum at 2000 hours (early dark period). Schreiber and Schlesinger (1971) showed that

audiogenic seizure susceptibility in mice was greater during the night than the day. Day-night differences in susceptibility appear at 22 days of age and persist until 30 days of age, reaching a maximum difference at 26 days of age. Several species of mice tested on day 27, exhibited a circadian pattern in audiogenic seizure severity scores. Schreiber and Schlesinger (1972) found that reversing the photoperiod reversed the seizure susceptibility pattern. Increased susceptibility to audiogenic seizures was directly related to lower levels of NE and 5HT in the brain. Piepho (1972) demonstrated in vehicle-treated mice that the extensor latency time of minimal electroshock seizure (MES) is shorter during the dark phase, while there were no circadian differences in the MES threshold. Peak toxicity of the convulsants, allylglycine and strychnine in mice occurred during the light phase, corresponding to minimal brain GABA and glycine concentrations. The anti-convulsant activities of acetazolamide, diphenylhydantoin and phenobarbital do not differ in effectiveness from day to night. However, meprobamate was more effective in protecting mice from seizures during the light phase.

3. Allergic response

Reinberg and Sidi (1966) demonstrated a circadian pattern in the allergic response of humans on a standardized routine, in terms of the area of erythema and wheal after intradermal histamine. The maximum response occurred at the onset of the sleeping (dark) period. The protective response of the antihistamine, periacetine, was more rapid and of shorter duration when administered at 1900 hours than at 0700 hours. Reinberg (1967) described several related studies testing the intradermal allergic response to histamine and compound 48/80 and urinary 17 OHCS and 17-ketosteroids. The peak response

occurred at about 2300 hours, at the time of minimum urinary corticosteroid excretion.

DeVries et al. (1962) reported a circadian pattern in the hyperactivity of the bronchial tree to histamine in patients with asthma and bronchitis, which was most sensitive during the night. Reinberg (1967) indicated that asthma attacks correlated with times when adrenal steroid release (urinary 17OHCS and 17-ketosteroids) and potassium excretion were low.

4. Drugs altering monoamine levels

Saelens et al. (1968) reported that in mice pargyline increased motor activity in the dark phase, while alpha-methyl-m-tryosine had an opposite effect. Black et al. (1969) found that reserpine produced a dose-dependant depletion of whole rat brain NE, which was maximal during the dark period. Walker et al. (1971) demonstrated that reserpine depleted hypothalamic and caudate NE to the same extent in the day or night, although NE concentrations in controls was greater during the dark. DL-DOPA with pargyline increased NE concentrations in these areas, which were greater during the day than the night. Scapagnini et al. (1971) found that although the 5HT levels in the hippocampus, amygdala and hypothalamus were greater in the dark than the light period, p-chlorophenylalanine depleted their 5HT content to about the same extent in the light and dark periods. Zigmond and Wurtman (1970) showed that the accumulation of rat brain ^3H -catecholamines after ^3H -tyrosine administration was significantly higher in the light period than the dark period. Reis et al. (1969) studied the NE-depleting action of morphine in several brain regions of the cat. They found a significant correlation between NE depletion of an area and the presence of a daily NE rhythm in the area.

Friedman and Walker (1972) described the circadian fluctuations in the toxicity of several cholinomimetics. ACh (IV and IP), neostigmine, pilocarpine and oxotremorine are most toxic during the dark period. The atropine (IP) pattern is inverted. IV scopolamine and atropine methyl nitrate also show maximum toxicity during the light period.

Wurtman et al. (1970) determined that rat brain S-adenosylmethionine concentrations were maximal in the dark and minimal during the light period.

5. Analgetics

Reinberg et al. (1967) found that human urinary salicylate excretion was circadian. The duration of salicylate excretion was longest when the drug was given at 1900 hours. Lutsch and Morris (1971) reported a circadian pattern for analgesia in mice exposed to a constant dose of morphine. It was maximum at 0300 hours. Reversal of the photoperiod reversed the analgesia response pattern.

6. Miscellany

Scheving et al. (1972) measured 5HT in rat spleen over a 24-hour period. A circadian pattern for 5HT concentration was maximum at 2400-0700 hours, in several experiments. Carlstrom and Laurell (1968) studied the effect of nicotinic acid on the diurnal variation of plasma FFA. Mean plasma FFA concentration was elevated, in an ultradian pattern; plasma triglycerides and cholesterol were reduced.

D. CNS Amines

1. Serotonin (5HT)

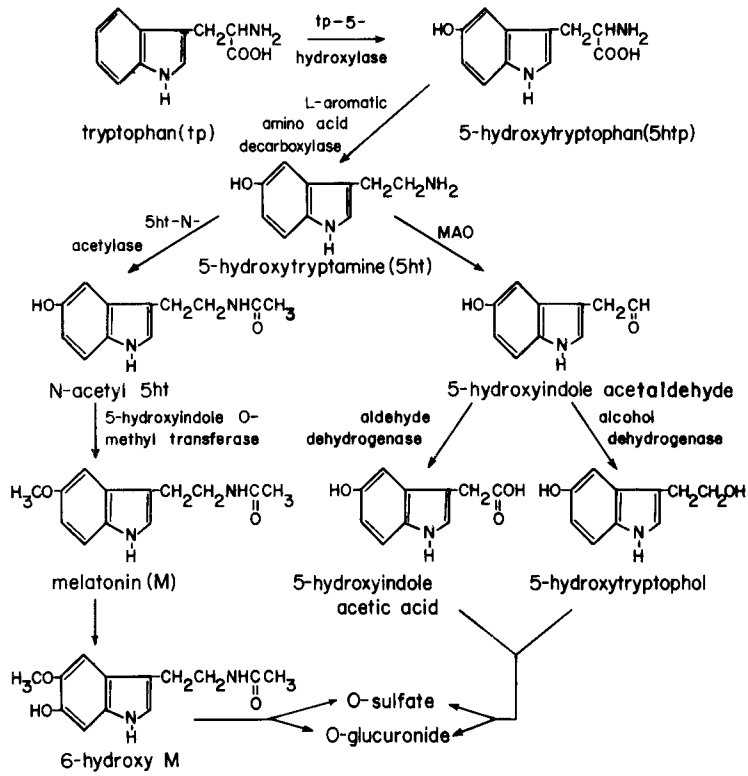
a. Metabolism

Serotonin or 5-hydroxytryptamine (5HT), an indolealkylamine, was first isolated by Page (1954) from blood platelets and from intestinal mucosa by Erspamer (1954). Rapport et al. (1948) isolated a vasoconstrictor from serum which they named serotonin and which had identical properties to synthetic 5HT. 5HT is found in animals (eg. mammals to coelentrates) and plants (eg. bananas, nuts, etc.) (cf. Garattini and Valzelli, 1965; Erspamer, 1966). Large amounts are found in the gastrointestinal tract associated with the enterochromaffin cell system and blood platelets.

The presence of 5HT in the CNS was demonstrated by Twarog and Page (1953) and Page (1954). Distribution in the mammalian brain is uneven: in the hypothalamus, septal area and midbrain concentrations are relatively high, while in the cortex, cerebellum and white matter they are low (Bogdanski et al. 1957). Giarman and Day (1959) found the bovine pineal is rich in 5HT. Subsequent studies in such species as the rat, monkey, etc. demonstrated that 5HT is higher in the pineal gland than in any other mammalian organ examined (eg. in rat 57-73 $\mu\text{gm/gm}$; Owman, 1963).

5HT is synthesized from an essential amino acid, tryptophan (TP) in two steps (cf. Figure 1). The first step is the hydroxylation of TP to 5-hydroxytryptophan (5HTP) and the second is the decarboxylation of 5HTP to 5HT. The first reaction, catalyzed by the enzyme, tryptophan-5-hydroxylase (TPH; EC 1.99.1.4), is the rate-limiting step in 5HT synthesis. Following its synthesis, 5HTP is almost immediately decarboxylated to 5HT by the enzyme

INDOLEAMINE METABOLISM



5HTP decarboxylase (5HTPD; EC 4.1.1.18). This intermediate is not normally detectable in the brain or plasma. The enzyme is not specific, since it is capable of decarboxylating other L-aromatic amino acids, including the NE precursor DOPA. Therefore, the designation L-aromatic amino acid decarboxylase (AAADC) is a better term for the enzyme. Both enzymes are found in the brain and periphery. Brain 5HT can be synthesized from plasma TP taken into the brain by an active process.

The cellular distribution of 5HT in the CNS was determined by histochemical fluorescence techniques, which delineate intraneuronal 5HT (Falck *et al.*, 1962). With this technique serotonergic pathways in the CNS, which are especially prominent in the raphé nuclei of the brain stem were traced (Hillarp *et al.*, 1966). Ascending 5HT-containing fibers go to the diencephalon and telencephalon from the rostral raphé nuclei, while pathways from the caudal portion descend to the lumbar and sacral cord. Pujol *et al.* (1971) demonstrated in cats, that lesions of the raphé system decrease diencephalon and telencephalon 5HT and produce insomnia proportional to the extent of destruction of 5HT-containing cell bodies in the raphé system.

5HT is stored in the CNS in free (active, mobile) and bound (inactive, reserve) forms. The bound form is stored as a complex with ATP in synaptic vesicles in nerve endings. Such nerve endings and vesicles can be isolated by ultra-centrifugation techniques and visualized by transmission electron microscopy and electron microscopic autoradiographic methods.

Degradation of 5HT is primarily via oxidative deamination by monoamine oxidase (MAO; EC 1.4.3.4), an enzyme found in the brain and most tissues. MAO is widely distributed and localized intracellularly within the mito-

chondrial membrane. It is present in nerve cells, but not glia, and located in the mitochondria around the storage granules found in nerve endings. It deaminates a number of amines to unstable aldehydes which are subsequently transformed to corresponding alcohols or acids by reduction or oxidation, respectively. MAO deaminates compounds in which the amine group is attached to the terminal carbon. MAO forms the unstable 5-hydroxyindoleacetaldehyde (5HIAc) from 5HT, which is oxidized primarily to 5-hydroxyindoleacetic acid (5HIAA), although a small amount is reduced to the alcohol, 5-Hydroxytryptophol (5HTPL). MAO inhibitors increase 5HT tissue levels by blocking its destruction. Several metabolites of 5HT, such as melatonin, 5HTPL, and 5-methoxytryptamine (5MT) are physiologically active (vide infra for further discussion).

b. Physiological Effects

1) Serotonin

There is little doubt that 5HT is a neurotransmitter in the CNS, if the traditional criteria used to establish putative transmitters are applied to 5HT. These include CNS localization of 5HT itself, its synthetic and degradative enzymes, release of 5HT after electrical stimulation and effects of microiontophoretic 5HT similar to normal physiological responses (Werman, 1966).

The neurophysiological effects of 5HT on the CNS have been extensively studied. Marrazzi and Hart (1956) found that 5HT depressed transcallosal responses. Krnjevic and Phillis (1963) found that 5HT depressed cortical activity induced by both glutamate and peripheral stimulation. Intracarotid administration of 5HT in cats produced an initial arousal pattern, followed

by a prolonged hypersynchrony (sleep-like) (Koella and Czicman, 1966). After midpontine transection of the brain stem, cauterization of the area postrema or application of 5HT-blocking agents to the fourth ventricle, 5HT produced only arousal. They concluded that 5HT acts upon receptor sites in the area postrema and this stimulating effect is transmitted to cell bodies in the solitary tract nucleus (Moruzzi, 1960). Bronzino et al. (1972) demonstrated a similar EEG synchronization effect of 5HT applied to the area postrema. Bradley and Wolstencraft (1965) found that application of 5HT to single neurons of the pons and medulla had variable effects: 40% of the neurons examined were excited by 5HT, while 49% were inhibited. When stimulation is induced via the optic tract, 5HT depresses the lateral geniculate body (LGB), but is without effect with antidromic stimulation or after application of excitant amino acids (Curtis and Davis, 1962; 1963). 5HT also has depressant actions on the hypothalamus (Bloom et al., 1963), olfactory bulb (Bloom et al., 1964), hippocampus (Biscoe and Straughan, 1965) and spinal cord (Phillis et al., 1968).

2) Metabolites

The actions of metabolites of 5HT and other indoleamines (IA) must be considered. Part of the interest generated in these substances is derived from a number of IA compounds which have psycho-pharmacological activity, such as LSD, DMT (N,N-dimethyltryptamine), DET (N,N-diethyltryptamine), bufotenine (5-hydroxy-N,N-dimethyltryptamine) and psilocybine (4-phosphoryloxy-N,N-dimethyltryptamine). Attempts have been made to relate schizophrenia and other psychiatric disorders to aberrant metabolism of IA which might cause the synthesis of these compounds in humans. Woolley and

Shaw (1954) hypothesized that the hallucinogenic effect of LSD was based on 5HT antagonism but this theory has since been refuted by demonstrating that bromo-LSD, which is a potent 5HT antagonist, has no hallucinogenic activity. A number of active hallucinogens (vide supra) are formed by the methylation of 5HT and tryptamine on the terminal amino position (N-methylation).

Axelrod (1962) isolated a N-methylating enzyme system in rabbit and human lungs and found it absent in mice, rats and cats. Morgan and Mandell (1969) reported the presence of this enzyme in brains of chicks and rats.

Melatonin (M, 5-methoxy-N-acetyltryptamine), is formed from 5HT (for a detailed discussion, vide infra). McIsaac (1961) postulated (under unspecified conditions) a disorder of M metabolism, whereby the M side chain closes, forming 10-methoxyharmalan, which has hallucinogenic properties. Szara (1961) reported that 6-hydroxy derivatives of DMT and DET are the active forms of these compounds and hallucinogenic activity is correlated with urinary 6-hydroxy derivatives (Szara and Hearst, 1962). An enzyme is found in the liver (Jepson et al., 1962) which degrades M by 6-hydroxylation. Hydroxy indole O-methyl transferase (HIOMT), the enzyme which methylates the 5-hydroxy group of N-acetyl 5HT to form M, is also able to methylate other IA such as 5HT, 5HIAA and 5HTPL, to form 5-methoxytryptamine (5MT), 5-methoxy-indoleacetic acid and 5-methoxytryptophol (5MTPL), respectively. Arutyunyan et al. (1964) reported sleep induction and other central actions of 5MT (mexamine). Taborsky (1971) found 5HT, 5HTPL and 5MTPL produced sleep in young chicks. The action of 5HTPL was greatly potentiated by pyrazole but not by iproniazid or disulfiram. Iproniazid decreased 5HT sleep. Other investigators have demonstrated sleep-inducing properties for 5HTPL

(Feldstein, 1971), and 5-hydroxyindolacetaldehyde (5HIAc; Sabelli and Giardina, 1970), the immediate oxidative product of 5HT by MAO (see Fig. 1). Ethanol alters the metabolism of 5HIAc by shifting product formation away from 5HIAA towards 5HTPL (Feldstein et al., 1967).

Hoffer and Osmond (1967) have advanced a theory for schizophrenia which involves the transformation of Epi (adrenaline) to adrenochrome by an oxidative ring closure, to form an indole-like compound.

3) Effect of Precursors

The role of 5HT in the CNS has been studied by using depletors, inhibitors of synthesis and precursors. The immediate precursor of 5HT, 5HTP, has been used to increase central 5HT, since 5HT does not cross the blood brain barrier (BBB). 5HTP injections in cats, dogs and rabbits (Bogdanski et al., 1958; Monnier and Tissot, 1958; Costa et al., 1960; Tabushi and Himwich, 1970) produce a high voltage, slow wave EEG and sedation. After a latency of 15-20 minutes, doses of 30-50 mg/kg (IP or IV) of DL-5HTP (or 15-25 mg/kg of L-5HTP) induce a state resembling SWS, which lasts for 5-6 hours, without PS. In rats, small doses of 5HTP reduce spontaneous activity (Joyce and Mrosovsky, 1964) and can cause tremor and agitation (Udenfriend et al., 1957) although 5HT itself has a sedative effect (Pierre and Cahn, 1955). Similar effects are seen in mice. In rabbits, small doses of 5HT and 5HTP induce sedation, while large doses result in excitation. Ledebur and Tissot (1965) injected 5HTP directly into the mediolateral part of the caudal brain stem, inducing SWS, while injections in the dorsolateral pontine tegmentum induced cortical desynchronization. Tabushi and Himwich (1970) found that a low dose of 5HTP (50 mg/kg, IV) delayed the onset of PS, while higher doses

(100-200 mg/kg) markedly increased SWS. The anti-5HT agent, methysergide (1 mg/kg), increased the alert stage and decreased both SWS and PS. Wyatt et al. (1971a) have shown different effects of 5HTP in humans. PS increased while SWS decreased slightly, apparently compensating for the increased PS. These results differ from those obtained in cats (Jouvet, 1967b) in which 5HT was implicated in SWS.

Increasing the dietary intake of TP increases the level of brain 5HT (Green et al., 1962), while animals on a TP-deficient diet have reduced 5HT levels (Gal and Drewes, 1962). Brain 5HT is derived by hydroxylation of TP, occurring mainly, if not wholly in the brain. Weber and Horita (1965) demonstrated that the increase in cerebral 5HT in rats after IP TP was unaffected by evisceration and that perfusion of cerebral hemispheres with a TP solution resulted in elevated 5HT levels on the perfused side only. Moir and Eccleston (1968) studied changes in concentrations of 5-hydroxyindoles (5HI) in whole rat brain and various regions of dog brain after TP and 5HTP injections and concluded that intracerebral TP hydroxylation is the primary factor controlling cerebral 5HT formation. These and other investigators (Garattini and Valzelli, 1965) questioned the use of 5HTP to increase central 5HT because it is also decarboxylated in parts of the brain where 5HT is not normally present.

TP levels in human plasma (Wurtman et al., 1968b) and certain rat tissues (Wurtman et al., 1968c) undergo diurnal variation. Although 5HT concentrations in the rat brain and pineal gland also vary with time of day (vide supra), a relationship between these and the rhythms of plasma or tissue TP content is still conjectural.

In normal humans TP reduces sleep latency and slightly increases sleep length and increases total sleep time. In insomniacs it reduces sleep latency and the number of awakenings (Hartman, 1967; Hartman et al., 1971b). Oswald et al. (1966) found an increased onset of PS sleep with TP in humans.

4) Blockade of Synthesis or Storage of 5HT.

a) p-Chlorophenylalanine (pCPA)

Koe and Weissman (1966) reported that pCPA depletes 5HT in brain, peripheral tissues and blood, in rats and dogs. Brain 5HT and 5HIAA content are reduced to very low levels, while brain NE and DA decreased only slightly. pCPA reduces the normal increase in 5HI compounds after TP loading, inhibits liver TP hydroxylase and suppresses the TP- and phenylalanine-hydroxylating activity of livers from animals treated with it. They concluded and others have since substantiated (Jequier et al., 1967) that pCPA acts by inhibiting TPH, the rate limiting step in 5HT synthesis. Bloom and Giarman (1970) observed that pCPA (316 mg/kg, IP) decreases rat pineal 5HT content more than 90% within a day without affecting NE content. Pineal 5HT concentrations return to normal levels 4-6 days after treatment. Granular vesicle opacity of pineal nerve endings observed by the electron microscope is also lost one day after pCPA treatment and recovers with a time-course similar to tissue 5HT recovery.

Tenen (1967) found that depleting doses of pCPA in rats had no effect on circular activity cage locomotion but depressed jiggle cage activity. Volicer (1969) examined the effects of pCPA in mice and rats and found spontaneous activity decreased only in rats.

The involvement of 5HT in SWS prompted Delorme et al. (1966a) to study the effects of pCPA on sleep in cats. They observed it produced a state of insomnia or permanent wakefulness, with inhibition of PS and SWS. This pattern of disruption of sleep by pCPA in cats resembles the time-course of 5HT depletion seen in rats (latency of 16 hours and duration of about 3 days). Mouret et al. (1967, 1968) obtained similar results in chronically-implanted rats and cats with doses of pCPA which almost completely deplete central 5HT. The loss and recovery of sleep correlated with the time-course of central 5HT change in rats similarly treated. The loss of sleep in pCPA-treated cats (440 mg/kg) was immediately suppressed by 5HTP (40 mg/kg) and restored to normal for 10-12 hours. Koella et al. (1968) obtained equivalent results with pCPA and 5HTP treatment in chronically-implanted cats. Torda (1967) reported substantially the same results with chronically-recorded, pCPA-treated rats, and in sleep-deprived rats in which she measured 5HT content (75 hours after injection). Whole brain 5HT from animals deprived of sleep (for 15 hours) was slightly elevated from controls, indicating that pCPA reduction of brain 5HT is a direct and not a secondary effect produced by sleep-deprivation.

Pujol et al. (1971) extensively examined the metabolism of 5HT with respect to the insomnia produced by pCPA and lesions of the raphé system, in chronically-recorded cats. After a 12-hour latency, a single dose of pCPA (500 mg/kg) eliminated PS and SWS, which was maximal 36-40 hours after injection and which recovered after 4 or 5 days without any sleep rebound. Lesions of the raphé system produced permanent arousal, with no PS and very little SWS, which correlated with the completeness of the lesion. Cortical 5HT and

5HIAA were greatly decreased; formation of ^3H -5HT from ^3H -TP decreased, but ^3H -TP uptake and synthesis of ^3H -5HTP were unaffected. 5HTP injections in cats with raphe lesions did not abolish the arousal pattern.

Cremeta and Koe (1966) found that no complaints of insomnia were reported by normal humans given up to 3 gm/day (about 40 mg/kg) of pCPA.

b) Reserpine (R)

R depletes tissue 5HT (and other amines) from their storage sites, probably by interfering with the intracellular binding (Pletscher et al., 1956). R causes almost complete depletion of amine stores, which, depending on the dose used, recover to normal levels in about a week. There are some differences in the mechanism of depletion of the amines since maintaining R-treated animals at 4°C. depletes CAs but not 5HT. The animals are not sedated but become so after returning to room temperature, when central 5HT decreases (Garattini and Valzelli, 1958; Sulser and Brodie, 1960). In addition to the actions of R on binding, it also blocks uptake of endogenous and exogenous amines into nerve endings (Brodie et al., 1960) in vivo and in vitro. The sedative effects of R last only several days compared to the longer time-course of brain amine depletion (Häggendahl and Lindquist, 1964). Reserpinized animals have been given the amine precursors 5HTP and DOPA, in an attempt to ascertain whether 5HT or NE depletion accounts for the sedation. 5HT increases the sedation, although large doses cause excitation. On the other hand, when DOPA was injected the R signs were reversed, suggesting that the depletion of CAs was responsible for the depression (Monnier and Tissot, 1958; Jouvet, 1968).

In the cat, a single dose of R (0.5 mg/kg) suppressed SWS for 12-14 hours and PS for 22-24 hours, the latter returning to control only 5-6 days later. A selective and prolonged period of PGO (ponto-geniculate-occipital) spiking (30-50/minute) appears within several hours of injection and lasts for 50-60 hours (Matsumoto and Jouvét, 1964; Jouvét, 1967a). This PGO activity is identical to PGO activity during PS. Recordings were made at the same level of the pons, mesencephalon, lateral geniculate and occipital cortex and accompanied by lateral eye movements and twitches of the eyes and ears. However, the appearance of the cat is quite different from sleep and there is no decrease of the neck muscle tone. Hoffman and Domino (1969) compared the effects of R on the sleep cycle in cats and humans. Low doses (0.01-0.16 mg/kg, IM) decreases SWS in cat and man. In the cat PS decreases for 48 hours without any rebound, while in man a fall in PS occurred on the first night with a rebound increase on the subsequent 2-3 nights. Coulter *et al.* (1971) examined the EEG sleep patterns of 20 subjects following single and repeated oral doses (1.0 mg) of R. Both treatments produced an increase in PS and a decrease in SWS.

5HTP (30-50 mg/kg, IV) injected 2-3 hours after R immediately suppresses the PGO activity and induces EEG and behavioral sleep for 4-6 hours, after which the characteristic fast cortical activity and PGO spiking of R reappear. Similar dosages of DOPA increase the PGO activity 50-80/minute for 4-6 hours. After a 20-30 minute latency, brief periods of SWS occur which are always followed by behavioral and EEG PS, demonstrating an almost normal pattern, which returns to the R pattern after 5-6 hours (Jouvét, 1968).

c) MAO Inhibition

MAO inhibitors (MAOIs) which act upon both IAs and CAs by inhibiting their catabolism and thereby increasing their central concentration have actions on the sleep-wakefulness cycle. Inhibitors such as iproniazid, pargyline and nialamide have a specific and selective suppressive effect on PS, while increasing SWS, probably as a secondary response. After a single injection of nialamide (10 mg/kg), PS is suppressed for 90-100 hours; it recovers after one week. PS suppression occurs even in animals deprived of PS (Jouvet, 1968). Recovery of PS after MAOIs occurs without the rebound of PS seen after PS deprivation. Chronic administration of phenylisopropylhydrazine (1-4 mg/kg), which inhibits brain MAO without impairing liver MAO, can produce PS suppression for several weeks (Delorme, 1966). These studies also demonstrated a correlation between the loss of PS by nialamide and the disappearance of the histochemical MAO stain in the pontine tegmentum at the level of the locus coeruleus, where lesions selectively suppress PS.

Wyatt et al. (1969) administered MAOIs to humans and found marked PS suppression. Akindele et al. (1970) studied the actions of the MAOI, phenelzine and nialamide on human sleep. After 5-22 days of phenelzine (60-90 mg daily) PS was abolished, coinciding with the time of mood improvement. No effect of nialamide (75 mg/day for 17 days or 500 mg once, IM) on PS were noted.

c. The Pineal Gland

• As a result of recent, extensive study, the mammalian pineal can no longer be considered a vestigial organ (Wurtman et al., 1968a). The gland

(weighing 1.0 mg or less in rodents) is unusually rich in such biogenic amines as NE, H, 5HT and other IAs, as well as the enzymes which metabolize these compounds. Although the pineal gland develops embryologically from the roof of the diencephalon (Kelly, 1962), it maintains no direct neural connections with the brain. It is on the "peripheral" side of the BBB and possesses peripheral innervation (vide infra). The pineal is of interest for several reasons. The 5HT synthesis and content is higher than any other organ. Two enzymes, which acetylate, then methylate 5HT are unique to the pineal and form melatonin, which has marked physiological and pharmacological effects. The concentrations of these compounds and enzymes are subject to alterations by environmental stimuli such as light, and physiological factors. Some pineal biochemical reactions can be regulated by the autonomic nervous system.

1) Melatonin (M)

a) Localization

Probably the most important biochemical discovery made in recent decades concerning the pineal gland was the isolation and identification of its characteristic indole, M. McCord and Allen (1917) demonstrated that bovine pineal extracts contained a substance which lightens amphibian skin. Beginning in 1947, Lerner and coworkers set out to isolate and characterize the active principle of the extracts, using over 250,000 bovine pineal glands. Through a series of elegant experiments (Lerner et al., 1959, 1960) the active substance was found to be 5-methoxy-N-acetyltryptamine or M (see Figure 1).

M is synthesized only in the pineal, although small amounts have been found in peripheral nerves. It is formed in a two-step process in which 5HT is N-acetylated (Weissbach et al., 1960) and then is methoxylated on C5-hydroxy group (Axelrod and Weissbach, 1961). Acetyl 5HT methyl transferase (EC 2.1.1.4), the enzyme catalyzing the first reaction is widely distributed in mammalian tissues, however, the second enzyme, which transfers a methyl group from S-adenosylmethionine to N-acetyl-5HT (HIOMT) is found in the pineal and thus determines the selective localization of M synthesis (Axelrod et al., 1961). Spectral characteristics for pineal gland, retinal and Harderian gland HIOMT have been described recently (Cardinali et al., 1972). Although N-acetyl-5HT is the best substrate for HIOMT, it is not the only one. The enzyme can also methylate other indolamines such as 5HT, 5HIAA and 5HTPL, forming small amounts of 5MT, 5MIAA and 5MTPL, respectively (Axelrod and Weissbach, 1961; McIsaac et al., 1965).

M is degraded mainly in the liver, by hydroxylation at the sixth carbon (about 80% of M) and excreted in the urine in the conjugated forms as the O-sulfate and O-glucuronide. This enzyme is specific in forming a 6-hydroxy indole. Thus it plays no role in 5HT synthesis (Jepson et al., 1962). In addition, traces of injected M appear in the urine unchanged and as 5MIAA (Kopin et al., 1960; Wurtman et al., 1964c).

The disappearance of injected M (acetyl-³H) from blood and its tissue distribution have been studied in mice and rats (Kopin et al., 1960) and cats (Wurtman et al., 1964c). ³H-M appears to be taken up by all tissues, including the brain, indicating that unlike 5HT, its access to the CNS is not hindered by the BBB. In the cat, one hour after injection it is

concentrated in the pineal, parts of the eye and ovaries (Wurtman et al. , 1968). Other endocrine tissues and peripheral nerves selectively take up $^3\text{H-M}$ to a small degree, while adipose tissue has the smallest uptake, indicating that the uptake in such organs as ovary, pineal and adrenal gland is unrelated to their relatively high lipid contents. Anton-Tay and Wurtman (1969) have shown that $^3\text{H-M}$ injected into the blood or the lateral cerebral ventricles is selectively concentrated in the hypothalamus and midbrain.

M has been shown by several investigators to have pronounced effects on the reproductive system of rodents and birds (Wurtman et al., 1968a; Tait et al., 1969; Reiter and Franchini, 1969). Relatively low doses decrease rat ovarian weight, retard vaginal opening, increase pituitary LH content and depress the proportion of daily vaginal smears showing estrus phases (Chu et al., 1964; McIsaac et al., 1964). Male gonadal growth is similarly reduced. Baschieri et al. (1963) found that daily injections of M (1.0 mg/kg) decreased thyroid cell height and ^{131}I uptake

b) Pharmacological Effects

Marczynski et al. (1964) examined the effects of M implants on the EEG of unrestrained cats. Small amounts of crystalline M (15-30 μgm) were administered by cannulae directly into the preoptic region of the hypothalamus, nucleus centralis or brain stem reticular formation (RF) and the animals observed for behavioral and EEG effects. Implants in the preoptic region had the most pronounced actions, which appeared after 15-30 minutes and lasted 2-3 hours. The EEG exhibited cortical synchronization with an increase in amplitude and slowing of subcortical activity, while at the same time the animals went to sleep. Implants in the nucleus

centralis medialis led to qualitatively similar EEG activity and behavior, however brain stem implants produced no consistent changes. Administration of pineal extracts or M decreases the spontaneous motor activity in the rat (Reiss et al., 1963b; Wong and Whiteside, 1968).

Arutyunyan et al. (1964) examined the pharmacological properties of M. Subcutaneous injection of M (25 mg/kg) produced a slight behavioral depression in cats lasting 1-1.5 hours. Similar doses (25-50 mg/kg, IV) in mice were without effect. Doses of M (10-20 mg/kg, IV) in cats and rabbits resulted in slow, high-amplitude waves in the EEG of cortical and subcortical structures 5-10 minutes after injection, which lasted 1-2 hours. M (20 mg/kg, SC) significantly prolonged the hypnotic effects of HB (60 mg/kg, IP) and chloral hydrate (200 mg/kg, IP) in mice. This effect might be due, in part, to the ability of M (50-100 mg/kg, SC) to reduce body temperature 2-3°C. In the rabbit, M (25 mg/kg) had no hypothermic action (Barchas et al., 1967). In similar experiments, Barchas et al. (1967) found M (25 mg/kg, IP) increased HB (100 mg/kg, IP) sleeping time in mice 50 per cent. Fraschini and Martini (1970) examined the effect of M on PB sleep. They injected M into the lateral cerebral ventricles of rats immediately after PB (30 mg/kg, IP). M (30-120 µgm) potentiated PB sleep in a dose-dependent fashion (about a 14-45% increase). These results seem specific for M, since comparable intraventricular doses of 5HT with PB produced small decreases in sleeping time. M (2.5 mg/kg, IV or IP) in 4-day old chicks (which have an incomplete BBB) immediately caused the animals to assume a roosting posture for about 45 minutes (Barchas et al., 1967). A dose of M (10 mg/kg) did not alter blood pressure in cats or the contractile force of the heart or the EEG in dogs.

Hishikawa et al. (1969) found in young chickens that M (10-60 mg/kg, IP) had a powerful sedative and hypnotic effect which appeared within 1-2 minutes and lasted for 30-60 minutes. M-induced sleep was characterized by a slow, high voltage EEG, similar to normal SWS. Pinealectomized rats show increased motor activity and an activated EEG (Reiss et al., 1963a; Nir et al., 1969a). Recently Anton-Tay et al. (1971) gave human volunteers IV doses of M (0.25-1.25 mg/kg in ethanol). Initially the EEG was "deactivated", followed in 15-20 minutes by sleep lasting about 45 minutes. Similar results occurred in several epileptics but not in Parkinson patients who experienced minimal sleep effects but did improve in their condition.

Anton-Tay et al. (1968) administered M (about 1.0 mg/kg, IP) to female rats and measured 5HT and NE in the MB, hypothalamus and cerebral cortex over a 3 hour period. Cortical 5HT decreased 14% 20 minutes after M and was still depressed after 60 minutes, while its concentration in the MB and hypothalamus increased significantly. After 3 hours the cortical and hypothalamic 5HT concentration had returned to normal, but that in the MB continued to increase. A weakness in experimental design is the use of "zero-time" controls instead of vehicle-treated animals (diluted ethanol) sacrificed at specific times after injection, thereby obscuring possible (and probably) amine changes due to ethanol (Feldstein, 1971) and effects due to circadian rhythms. In preliminary experiments with rats sacrificed one hour after 0.5 mg of M (total dose), whole brain 5HT was elevated significantly while NE content was unchanged. In mice, M (30 mg/kg, IP) given every 3 hours for 18 hours caused no change in gross behavior or NE content of the brain or heart.

Klein et al. (1970) and Berg and Klein (1971) have studied several aspects of M synthesis using cultured rat pineals. Treatment of cultures with NE or dibutyryl adenosine 3', 5'-monophosphate (DAMP) caused a 6-10-fold increase of N-acetyltransferase activity and subsequent M formation. They concluded that N-acetyltransferase, which forms N-acetyl 5HT, is elevated by NE through a cyclic AMP mechanism dependent on protein synthesis, since cycloheximide and actinomycin D block the augmented formation of labeled M from labeled TP. Wurtman et al. (1971) examined these effects of NE and found they could be blocked by the β -adrenergic blocking drug propranolol but not the α -adrenergic blocker, phenoxybenzamine. Neither altered the response to DAMP.

2) Anatomy

The work of Rowan (1925) which demonstrated that gonadal function in the junco finch involved regulation by environmental lighting gave the first hint of the importance of light on physiological systems. His observations indicated that the endocrine system was not closed, but could be influenced greatly by environmental stimuli. Fiske et al. (1960, 1962) showed that pineal weight and synthetic activity in rats kept in constant light decreased 25%, while constant darkness had no effect (compared with animals maintained under diurnal lighting conditions). Axelrod et al. (1965) has shown that pineal weight varies over a 24-hour period, and is lowest at the end of the light period. Pineal extracts depress gonadal weight and function (Wurtman et al., 1959), while pinealectomy or constant light increase ovarian weight (Wurtman et al., 1961). Injections of M reduced gonadal weight and function in animals maintained in normal lighting. These

results suggested that the pineal contained a factor which inhibited gonadal function in the rat, whose synthesis or release was probably related to environmental lighting.

The rat pineal gland is rich in such biogenic amines as 5HT (Quay, 1963), histamine (Machado et al., 1965), DA and NE (Pellergrino de Iraldi and Zieher, 1966). Quay (1963) has shown that the concentration of pineal 5HT is not constant but exhibits a characteristic diurnal variation when animals are maintained under diurnal lighting conditions (usually 12 or 14 hours light, 12 or 10 hours dark). It is highest during the day and falls during the night, in these nocturnal animals. Quay (1965) demonstrated the same pattern for pineal 5HIAA. Rats placed in continuous darkness for one week retained their pineal 5HT rhythm (Snyder et al., 1964, 1965), although this might not have been sufficient time to abolish the 5HT rhythm. Blinded animals retain a similar pattern of pineal 5HT under normal illumination, constant light or constant dark. This rhythm is extinguished in 5 days when normal animals are placed in constant light.

M, synthesized from pineal 5HT and in vitro M-forming activity have similar diurnal patterns although peaks and troughs do not correspond to that of pineal 5HT (Quay, 1965). Wurtman et al. (1963b) reported that continuous light decreased HIOMT activity relative to that of animals kept in darkness. If the nerves entering or leaving the superior cervical ganglion (SCG) were cut, pineal HIOMT activity was no longer alterable by continuous light or darkness (Wurtman et al., 1964a,b). Axelrod et al. (1965) observed in rats kept under diurnal lighting, a diurnal pattern of pineal HIOMT activity, which was greatest at midnight. Animals kept in darkness showed a marked

rise in HIOMT activity, which could be prevented by puromycin and partially blocked by actinomycin D, agents which interfere with DNA-directed protein synthesis.

Kappers (1960) demonstrated that the pineal is richly innervated by post-ganglionic sympathetic axons, which have their cell bodies in the SCG. In the rat, these provide the only innervation to the pineal. Axons originating in the retinal ganglion cells travel via the optic nerve to the optic chiasm, where they separate into several components (Hayhow et al., 1960). The largest and most important nerve bundle contributes crossed and uncrossed fibers to the primary optic tract (POT) which transmit to the occipital cortex where images are perceived. The superior and inferior fasciculi of the accessory optic system leave the optic chiasm. The superior fasciculus terminates in three nuclei within the MB tegmentum, while the inferior fasciculus enters the lateral hypothalamus, running with fibers of the median forebrain bundle (MFB) before terminating in the nucleus of Bochenek in the rostral midbrain tegmentum. Retinohypothalamic fibers have been described which leave the chiasm dorsally and terminate in the adjacent medial hypothalamus in some species (Krieg, 1932), although other studies have questioned their existence (Hayhow et al., 1960).

Studies by Moore and Axelrod and coworkers have elegantly determined the pathways by which light influences pineal function, with regard to M and HIOMT activity. Rats with bilateral lesions of the MFB (where it passes through the lateral hypothalamus) or sham-operated animals were placed in continual light or darkness for 30 days (Axelrod et al., 1966). HIOMT activity and pineal weight in sham-operated animals kept in darkness was five

times greater than in animals kept in light, while animals with lesions showed no differences between the light and dark-treated animals. Four procedures were performed in rats: 1) both eyes were enucleated; 2) one eye was enucleated and the MFB on the ipsilateral side transected (interrupting the photic input to both inferior accessory optic tracts, IAOT); 3) bilateral lesions were placed in the optic tract at the LGB (producing a functionally blind rat with an input to IAOT intact); 4) both IAOT and primary optic tracts were severed (only retinohypothalamic fibers maintained intact in order to determine if they mediate pineal function), (Moore et al., 1967). Animals prepared as outlined above were kept under continual light or dark for 30 days, then their pineals were assayed for HIOMT activity. In enucleated rats or animals in which the IAOT had been interrupted, light and dark had no effect on M synthesis but the characteristic changes in HIOMT activity did occur in animals with only POT lesions, implicating the IAOT in these effects. Wartman et al. (1969) demonstrated that although cholinergic innervation is not important in the pineal response to light, the facilitation of HIOMT activity in darkness is mediated by the parasympathetic system. The results of these experiments indicate that impulses produced in the eye by light travel in the optic nerve, are transmitted through the IAOT, reaching the nucleus of Bochenek in the MB. From there they descend through the spinal cord, where, at the upper thoracic cord, preganglionic sympathetic fibers enter the SCG. Postganglionic sympathetic fibers (nervi conarii) transmit the impulse to their termination in the pineal gland.

A possible mechanism by which pineal IAS might exert their actions centrally has been suggested by several workers (Koella, 1969a; Fraschini and

Martini, 1970). Since M is not found in the general circulation, it and other IAs may exert their effects by being transported through the cerebral spinal fluid (CSF), in a direct, internal circulation of the brain (Sheridan et al., 1969). They have demonstrated such transport in the hamster. In the experiments of Fraschini and Martini (1970) intraventricular injections of M potentiated PB sleep. Koella (1969b) hypothesized that because of the high concentration in the pineal of 5HT, M and some other active IAs, these substances could "leak" via the recessus pinealis, into the posterior part of the third ventricle, then flowing with the CSF into the fourth ventricle where they can exert a hypersynchronizing action on the area postrema, nucleus tractus solitarii and upper brain stem, areas which have been shown to be sensitive to such hypersynchronizing actions of IAs.

3) Other Pineal Amines

Pineal NE is present in relatively high concentrations in the rat (Pellegrino de Iraldi and Zieher, 1966; Wurtman and Axelrod, 1966). At the end of the light (sleep) period NE was three times that at the end of the dark (awake) period. The increase in pineal NE at the onset of the dark period is in phase with the rapid increase in M formation (Quay, 1965) and occurs when pineal 5HT is falling sharply (Quay, 1963). Thus the work of Klein and coworkers suggest that the stimulation of N-acetyltransferase and subsequent M production, by NE might occur physiologically. The NE rhythm is abolished by blinding or maintaining rats in constant light or constant darkness after as little as one day (Wurtman et al., 1967a). This further suggests that the NE rhythm, like the HIOMT but not the 5HT rhythm is exogenous and dependent on environmental light input to the pineal. Sectioning

the preganglionic fibers to the SCG (Pellegrino de Iraldi and Zieher, 1966) or unilateral MFB transection and contralateral enucleation (Wurtman et al., 1967a) also abolish the pineal NE rhythm, to a constant, intermediate level.

Other rhythms in the pineal include greater incorporation of circulating ^{32}P during the dark than light (Roth, 1965) and increase content of ethanol soluble lipids during the day than the night (Quay, 1961).

2. Catecholamines

a. Metabolism

The CAs NE, Epi and DA are 3,4-dihydroxy derivatives of phenylethylamine (see Figure 2). Elliot (1905) first postulated that an Epi-like substance might be released from sympathetic nerves and thus be responsible for chemical transmission at synapses. The presence of an adrenergic "sympathin" in extracts of mammalian brain was first described by von Euler (1946). Von Euler (1948) finally established that NE is the sympathetic transmitter by chemically isolating NE in splenic nerves. Holtz (1950) confirmed this finding in brain and spinal cord and demonstrated that NE content was much greater than that of Epi. Until 1954 it was generally suspected that brain NE was located mainly in blood vessels. Vogt (1954) measured CAs by bioassay and first demonstrated their relatively high concentration in specific anatomical areas, in cat and dog brain. The distribution pattern is generally similar in other species. In man, rat, cat and monkey, the highest concentrations are in the hypothalamus and range from 1-3 $\mu\text{gm/gm}$. The concentrations in other areas can be ranked as follows: MB and pons, medulla oblongata and striatum followed by a group of structures with very low concentrations, such as the hippocampus, cerebral cortex, cerebellum and spinal cord. In the corpus striatum, particularly the CN, where the NE concentration is relatively low, DA is found in very high concentrations (3-8 $\mu\text{gm/gm}$).

Since 1962, the highly specific and sensitive histochemical fluorescence microscopy technique has permitted the direct visualization of the biogenic amines, NE, DA, Epi and 5HT, at the cellular level. The use of these methods confirmed that NE present in peripheral mammalian tissues and

specific CNS areas is localized almost exclusively in sympathetic or adrenergic nerves (Carlsson et al., 1962). In the presence of formaldehyde gas the amines are converted to fluorescent isoquinoline derivatives which are green in the case of the NE and DA products and yellow for the 5HT derivative. In many areas of the brain fine nerve fibers with extensive terminal arborizations or varicosities containing CA or 5HT have been demonstrated. Using these techniques and selective stereotaxic placement of lesions in the CNS, Heller and Moore (1965) and Ungerstedt (1971) described NE, DA or 5HT pathways in the brain. More recently, the use of density-gradient centrifugation and autoradiographic localization of ^3H -NE and electron microscopy have combined to reveal that NE is localized largely within specific storage particles or synaptic vesicles contained in adrenergic nerve endings.

Gross mesencephalic and specific hypothalamic lesions produce a fall in CA and 5HT concentrations and fluorescence in the brain. After it was shown that thoracic cord section decreases spinal cord NE (Magnusson and Rosengren, 1963), several NE-containing bulbospinal systems were described (Dahlstrom and Fuxe, 1965). They are localized in the medulla and send axon processes into the spinal cord. Ascending systems containing NE neurons originate in the lower brain stem (medulla, pons and MB) and extend to the hypothalamic, preoptic area, limbic system and cortex. Lesions in the MFB of the lateral hypothalamus and in the dorsomedial brain stem tegmentum significantly decreased whole brain NE and 5HT, while lesions in the ventrolateral tegmentum decreased NE only (Heller and Moore, 1965).

Lesions have been used to demonstrate a nigro-striatal system of DA-containing neurons which originate within the substantia nigra and project

to the striatum. Other DA neurons are located in the MB and project rostrally, primarily to the olfactory tubercle. The only monoaminergic cell bodies located above the MB, with the exception of a DA cell group in the retina, is a small set of neurons localized near the median eminence, which is thought to be of importance in neuroendocrine regulation. Areas where there is the greatest number of nerve endings (not cell bodies) contain the highest NE concentration.

b. Biosynthesis

As first suggested by Blaschko in 1939, the synthesis of CAS begins with tyrosine. Tyrosine is transported into the brain and central adrenergic neurons by specific active transport mechanisms. Once within the neuron it is hydroxylated by the cytoplasmic enzyme, tyrosine hydroxylase (TH; E C 1.14.3.a) to dihydroxyphenylalanine (DOPA; see Figure 2). Direct demonstration of enzymatic hydroxylation of tyrosine was only achieved recently (Nagatsu et al., 1964). They found that TH is easily saturated by substrate and is therefore the rate limiting step in the biosynthesis of CAS. Several CAS also inhibit TH, suggesting that this inhibition is involved in the regulation of CAS synthesis. (TH does not hydroxylate tyramine, D-p-tyrosine or DL-m-tyrosine to DA.) Alpha methyl-p-tyrosine (AMPT), a competitive inhibitor of TH is converted to α -methyl DOPA, depleting tissue CAS but not 5HT (Spector et al., 1965; Anden et al., 1966).

DOPA is decarboxylated within the cytoplasm to form DA which is taken up into storage granules by an active process. The decarboxylating enzyme is sometimes known as DOPA decarboxylase although it is the same non-specific L-aromatic amino acid decarboxylase (AAADC) that forms 5HT from 5HTP. The

wide distribution and high activity of this enzyme in many tissues has made it difficult to achieve sufficient enzyme inhibition to decrease CA levels. In DA-neurons there is no further synthesis, while in NE-neurons the DA is hydroxylated, within storage granules by dopamine- β -hydroxylase (DH; E C 1.14.2.1). The enzyme also catalyzes the conversion of several other phenylethylamines other than DA, to their β -hydroxylated products (Kaufman and Friedman, 1965), as long as they contain an aromatic ring with a 2- or 3-carbon side chain terminating in an amino group. The potent inhibitor of DH, disulfiram, decreases brain and heart NE content, probably by complexing with copper ions of the enzyme.

In chromaffin tissue, such as the adrenal medulla and certain areas of the brain, the cytoplasmic enzyme, phenylethanolamine-N-methyltransferase (PNMT; E C 2.1.1) methylates NE to form Epi (Wurtman and Axelrod, 1966). Presumably the NE passes from the storage granules for this step and newly formed Epi is taken back into storage granules. PNMT is strongly inhibited by its substrate NE and product Epi at concentrations (of Epi) normally present in the adrenal medulla.

c. Degradation

Armstrong et al. (1957) first reported that a 3-O-methylated 4-hydroxylated catechol acid, 3-methoxy-4-hydroxymandelic acid ("vanillilmandelic acid, VMA") is a urinary metabolite of both NE and Epi (see Figure 2). Subsequently the O-methylated amines normetanephrine (NM) and metanephrine (MET) were also found in the urine. Metabolic degradation of CAs primarily involved two enzymes: monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). Since both act on a wide variety of amine substrates and each

is active on the products of the other, a spectrum of CA metabolites can be identified in urine.

N-methylation and β -hydroxylation decrease the susceptibility of phenylethylamines to MAO. The relative proportion of oxidized to reduced products in the deaminating steps varies among species and under different conditions. DA is a better substrate for MAO than Epi or NE, so large amounts of the deaminated metabolites of DA (dihydroxyphenylacetic acid, DOPAC and homovanillic acid, HVA) are excreted in the urine.

d. MAO Inhibition

Inhibition of MAO elevates tissue levels of biogenic amines such as NE, DA and 5HT (Spector, 1963) and decreases the excretion of deaminated metabolites of amines, such as VMA, 3-methoxy-4-hydroxyphenylglycol and HVA, while the excretion of NM, octopamine and tyramine increases (Sjoerdsma, 1966). Although MAO inhibition does not prolong the physiological effects of NE, MAO is important in regulating the intracellular level of amines by metabolizing any amines which leak into the axoplasm from storage granules.

The second pathway for CA metabolism is the O-methylation of the catechol ring by COMT (E C 2.1.16), which requires S-adenosylmethionine as a methyl donor. NE and Epi are metabolized to the corresponding 3-O methylamines NM and MET (see Figure 2). It is localized extraneuronally, primarily in the liver and kidney, as well as in brain tissue (highest in the area postrema and lowest in the cerebellar cortex) and within the red blood cell, and is responsible for inactivating CAs released into the circulation or in tissues lacking abundant adrenergic innervation. The O-methylated metabolites can be conjugated, often with a sulfate or deaminated by MAO

to form O-methylated glycols or acids. COMT is inhibited by dichloromercuribenzoate, suggesting it has a sulfhydryl group at the active site. Polyphenols such as pyrogallol inhibit COMT in vitro but are less effective in vivo.

MAO and COMT act upon CAs to produce physiologically inactive metabolic products, but neither enzyme plays a primary role in terminating the physiological actions of CAs. The rapid inactivation of CAs is due to uptake back into the presynaptic nerve endings. This is an active and specific uptake mechanism, which can be blocked by cocaine and antidepressant drugs, such as imipramine and some sympathomimetics.

e. Pharmacological Effects of CAs in the CNS

Bass (1914) produced sleep when he applied Epi under the dura or into the brains of dogs. Leimdorfer (1948) demonstrated that intracisternal Epi injections produced drowsiness in cats. Epi and NE injected into the lateral ventricle of chronically-implanted cats produced a state resembling light anesthesia (Feldberg and Sherwood, 1954).

Other pharmacological investigations of the role of CAs in central phenomena utilized systemic injections of amines (see Rothballer, 1959; Jouvet, 1972a, b). In moderate doses, NE and Epi produce cortical activation paralleling the increase in peripheral sympathetic tone. The principle site of action appeared to be in the upper pons and lower mesencephalon, since lesions in these areas abolish the activating effect of NE. Weilmalherbe et al (1959) demonstrated that NE and Epi did not pass the BBB. Key and Marley (1962) compared the effects of injected (IV or IP) sympathomimetics in young chicks, which have an incomplete BBB and older birds,

with chronically implanted electrodes. Amines such as NE, Epi and DA produced behavioral sleep with cortical slowing, while in adults these same drugs induced arousal. Mandell and Spooner (1968) summarize over twenty studies on the behavioral effects of NE and Epi administered so as to cross the BBB (intracisternally, intraventricularly or IV in animals with immature BBB). In a variety of species (mice, rat, chick, cat, dog and man) the predominant effect was sedation and/or somnolence. Torda (1968) found that microinjections of NE into the preoptic area of the reticular activating system (RAS) during deep sleep significantly shortened the latency and duration of PS and the latency of arousal. NE given during PS resulted in arousal almost immediately. Cordeau et al. (1971) found Epi and NE (50 µg-3 mg) introduced in the third ventricle or cisterna magna of cats produced wakefulness and/or excitation.

Both NE and DA appear to play roles in sleep. Since they do not penetrate the BBB, precursors (such as DOPA) are used to elevate their central concentration. Precursors or inhibitors of synthesis alter the CNS content of both amines produce differing effects due to their dissimilar localization and central effects (vide infra).

Peripheral injection of DOPA greatly increases CNS DA concentration and to a lesser extent central NE (Chambers et al., 1971). Accompanying the increase in brain CA content is a marked increase in EEG and behavioral alerting in chicks and young chickens (Key and Marley, 1962; Spooner and Winters, 1965) and in rabbits and cats (Monnier and Tissot, 1958; Jouvét, 1967a). Ledebur and Tissot (1965) injected DOPA directly into the caudal brain stem or in the dorsolateral pontine tegmentum, inducing a fast

cortical activity in the rabbit. DOPA, DA (both IP) and amphetamine (SC) produced alert EEG patterns in implanted chicks even though the blood pressure responses are similar to the rise produced by NE, which is behaviorally depressant (Spooner and Winters, 1967). Jouvett (1967b) reported that in cats DOPA (30-50 mg/kg) caused an increase in waking, with almost total disappearance of SWS and PS for 6 hours. On the other hand, dihydroxyphenylserine, the direct precursor of NE, (Blaschko et al., 1950) increases both SWS and PS in the rat (Havlicek, 1967). Wyatt et al. (1970a) showed that in humans, L-DOPA led to a significant decrease in the duration of PS sleep. Gunne et al. (1971) tested L-DOPA in six patients with narcolepsy but did not find it superior to amphetamine in its waking action.

f. Inhibition of Synthesis

Disulfiram blocks CA synthesis by inhibiting DH (Moore, 1969). Wise and Stein (1969) administered disulfiram to rats in a self-stimulation bar-pressing paradigm and found that animals stopped pressing the bar and appeared sedated.

AMPT, a relatively specific inhibitor of CA synthesis, inhibits TH (Weissman and Koe, 1965), producing a fall in NE and DA content in rat brain (Dominic and Moore, 1969). Torda (1968) administered AMPT to rats (80 mg/kg, 3 times, 6 hours apart, IP or by surgical implant) and found no EEG changes for the first 3 hours, but after 18 hours NE was reduced to almost zero, SWS increased and PS and wakefulness shortened. In AMPT-pretreated animals, microinjections of NE into the preoptic area or RAS "temporarily reversed the EEG changes", shortening SWS and lengthening PS. Hartmann et al. (1971a)

reported that in the rat oral AMPT (50-100 mg/kg) increased PS while AMPT (75 mg/kg, IP) decreased PS, corresponding to the time of maximum decrease in brain NE. In the cat, King and Jewett (1971) found AMPT (3-400 mg/kg, IP) increased PS and brain NE was depressed. Jouvet (1972_a) administered AMPT (150-200 mg/kg, IP) to cats with lesions in the raphe system, when behavioral and EEG waking was almost permanent (2-6 days after lesioning). Behavioral sedation and cortical synchronization began 4-6 hours after AMPT lasted 24 hours and then returned to behavioral and EEG insomnia. Weitzman et al. (1969) found that in the monkey, AMPT significantly decreased PS and behavioral waking with a proportionate increase in SWS. Wyatt et al. (1971_b) administered AMPT to five patients, resulting in an elevation in total PS (a lengthening of REM episodes instead of a greater frequency of episodes). Various effects of AMPT on sleep include increased duration of PS both lengthened SWS and PS in the cat (King and Jewett, 1969), increased duration of SWS in the rat (Branchey and Kissin, 1970) or no apparent effects in rats (Marantz and Rechtschaffen, 1967).

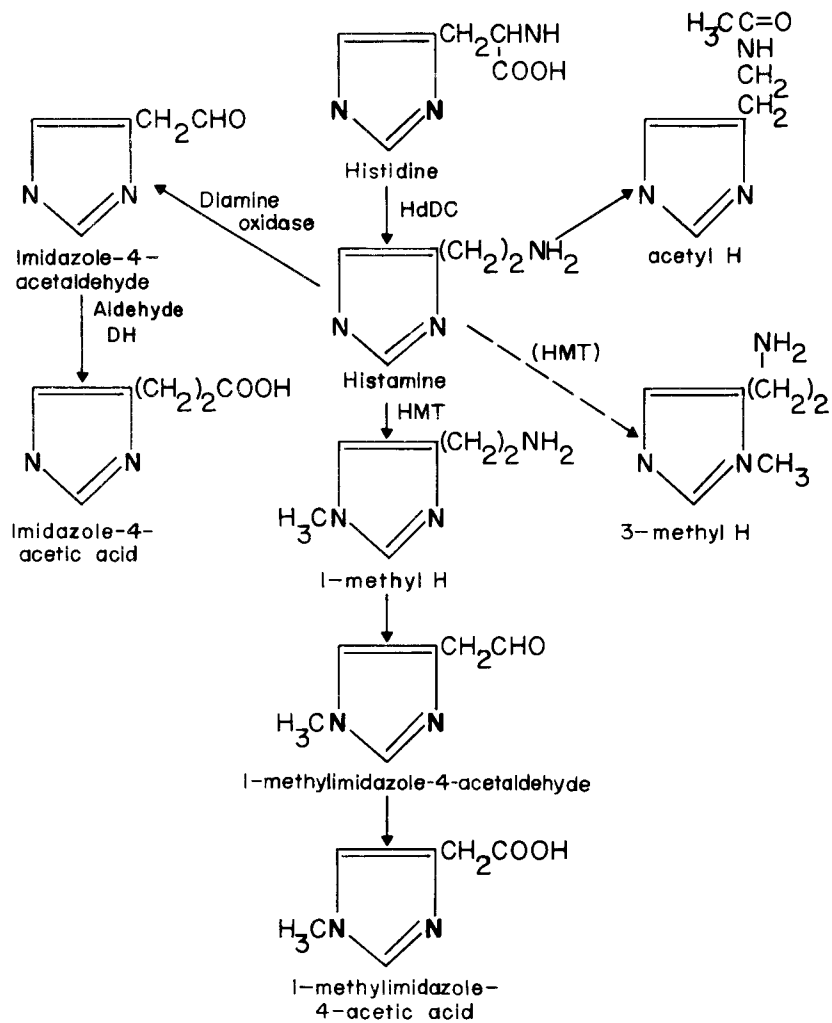
Several investigators have studied the effects on sleep of alpha-methyl DOPA, which blocks the decarboxylation of both 5HTP and DOPA, thus decreasing 5HT and DA transiently and NE for several days. Dusan-Peyrethon et al. (1968) found methylDOPA (100-200 mg/kg) in cats, caused a profound decrease in PS (for 10-22 hours) and a relative increase in SWS. In humans, Baekeland and Lundwall (1971) administered alpha-methylDOPA (250 mg, 3 times daily and 500 mg at bedtime) and found an increase in PS and a decrease in stage 4 sleep.

3. Histamine

Histamine (H, β -imidazolyethylamine; Fig. 3) was first synthesized in 1907 and isolated by Barger and Dale (1910), long before its biological significance was known. Dale and Laidlaw (1910) investigated the pharmacology of H and recognized its effects to be similar to those of many tissue extracts. They further noticed that the immediate response of sensitized animals to an otherwise innocuous protein was the same as H poisoning. It was not until 1927 that Best, Dale, Dudley and Thorpe isolated H from liver and lung samples. Lewis (1927) and coworkers established that H was liberated from cells of the skin in allergic responses. For further discussion of some of the peripheral actions of H, see Kahlson and Rosengren (1968) and Goodman and Gilman (1970).

a. Localization.

H has probably attracted less attention than most other biogenic amines in the nervous system because investigations on H have been hampered by problems with interfering substances in tissue determination methods and the lack of specific inhibitors of synthesis or depletors. These technical problems have resulted in a great deal of variation in reported tissue H values from laboratory to laboratory. The postganglionic sympathetic nerves are high in H content (60 $\mu\text{g}/\text{gm}$, which is about four times their NE content), while the spinal cord is lowest (0.1-1 $\mu\text{g}/\text{gm}$). Other nerves have intermediate H levels (von Euler, 1956). The regional distribution of H is similar to that for NE and 5HT. H is highest in the hypothalamus (twice the concentration of 5HT), intermediate in the MB and lowest in the cortex and white matter. Taylor et al. (1972), using an extremely sensitive and specific H assay,



confirmed these results and showed wide differences in H content of various brain nuclei. The posterior lobe and stalk of the hypophysis, area postrema, choroid plexus and pineal body contain H, although the H in the posterior hypophysis may be in mast cells. Mast cells, which contain large amounts of H in the periphery, are virtually absent in the CNS (Olsson, 1968). Several studies have examined the subcellular localization of brain H. Michaelson and Coffman (1967) reported that H is located primarily in the crude mitochondrial pellet, while others demonstrate a greater proportion in the microsomal pellet (Carlini and Green, 1963; Kataoka and DeRobertis, 1967).

Early fluorometric studies of tissue histamine content were hampered by the presence of an interfering substance, which has since been identified as the polyamine, spermidine. Michaelson (1967) pointed out the overlap of fluorescence emission curves of H and spermidine. In the early methods, spermidine was extracted and yielded a fluorophor with the same fluorescence characteristics as H, thus giving false high H values.

Tabor and Tabor (1964) and Kremzner et al. (1970) reported that the spermidine concentration is uniformly high in the CNS, although the concentration in white matter is three to four times that in gray matter.

Few studies have examined the pharmacology of spermidine or its function and effects in the brain. Friedman and Rodichok (1970) evaluated the toxicity of IP spermidine on a 24-hour basis. The LD₅₀ pattern is bimodal, with peaks just prior to the onset of the dark and light periods. Spermidine levels in mouse whole brain, myelencephalon, and liver peak midway through the dark period (Friedman and Rodichok, 1972). Histamine levels

in the brain exhibited a similar circadian pattern, corresponding to the motor activity rhythms of these animals.

b. Synthesis

H is formed from histidine (Hd); see Fig. 3) by the action of Hd decarboxylase (HdDC; EC 4.1.1.22). There is a good, though not exact, correlation between the distribution of H and HdDC activity in the brain (White, 1959). In areas where this correlation fails, such as the area postrema, which is rich in H but poor in HdDC activity, H may be concentrated from the blood by mast cells. There has been some controversy as to whether HdDC was the same non-specific decarboxylase involved in the formation of 5HT and DA, but recent evidence indicates that there is a distinct H-forming enzyme (Schwartz et al., 1970). There are two main pathways of H metabolism; ring N-methylation by imidazole-N-methyl transferase (HMT, EC 2.1.1.8) to form methyl H, while oxidation of the side chain by histaminase forms imidazoleacetaldehyde and then imidazoleacetic acid by aldehyde dehydrogenase (see Fig. 3). After H is formed in the soluble portion by HdDC, H is taken up into a particulate form (probably similar to the vesicles storing other biogenic amines) protecting it from destruction by such enzymes as HMT which is in the soluble portion. The activity of HMT is very high in guinea pig brain but relatively low in rat brain (Brown et al., 1959). White (1959) demonstrated in cat brain that methyl H is the major metabolite, indicating that histaminase activity is absent. Snyder et al. (1966) found in the rat that most of intraventricularly-injected H was recovered as imidazoleacetic acid.

c. Physiological effects

1) Histamine and related substances

Feldberg and Sherwood (1954), by intraventricular H injection in cats produced increased respiration, vomiting, muscular weakness as well as some sedation lasting about one hour. Sawyer (1955) obtained similar results in anesthetized rabbits, and concluded that H activates a rhinencephalic mechanism (olfactory bulb, MFB and basal ganglia), which stimulates the adenohypophysis. In unanesthetized or etherized rabbits, H evoked an arousal reaction. Monnier et al. (1970) reported that a H infusion of the rabbit third ventricle produced marked electrographic arousal with a decrease in delta waves. This effect was not blocked by acetylsalicylic acid, indicating that the effect was not merely pain-mediated, but probably due to a direct central awakening action, such as on the ascending MB RF and the hypothalamic and hippocampal systems. Friedman and Walker (1969) demonstrated a diurnal fluctuation in CN H in rats, which peaked during the dark phase, when animals are awake. Monnier et al. (1970) found a five-fold increase in release of "H-like" substances in the hemodialysate associated with arousal compared with relaxed animals.

Krnjevic and Phillis (1964) found that the activity of single pyramidal Betz cells of cats was "slightly moderated" by Hd and more rapidly by H iontophoretically-applied. However, the same depressing effect was also achieved with CAs, ephedrine, hydroxytryptamine or GABA. Small amounts of H depressed cerebral cortical neuron excitability, as did Hd and imidazoleacetic acid (Phillis et al., 1968). Larger amounts caused both depression and excitation, the latter possibly due to the metabolite, methyl H. These

effects, as well as the effects of NE, ACh and 5HT, were antagonized by anti-histamines (mepyramine maleate). H applied iontophoretically to thalamic neurons had no effect (Curtis and Davis, 1962), perhaps due to the barbiturate anesthesia used. However, Bradley (1968) observed depressant effects on 50% of the brain stem reticular neurons examined. Curtis et al. (1961) found no effect of H on spinal neurons, possibly because of barbiturate anesthesia. In cats anesthetized with nitrous oxide and methoxyflurane or anemically-decerebrated, NE, 5HT and H applied extracellularly to spinal neurons increased membrane polarization, decreased the amplitude of the EPSP and IPSP and blocked synaptic or antidromic invasion of some motoneurons (Phillis et al., 1968), effects resembling an inhibitory transmitter. However, iontophoretic strychnine and IV picrotoxin failed to antagonize the actions of these amines.

Sadre and Tiwari (1966) demonstrated that H, like chlorpromazine, potentiated the hypnotic actions of PB and chloral hydrate in mice. The hypnotic effects of PB in rats (Bovet et al., 1958) and rabbits (Goldstein et al., 1963; Monnier et al., 1967) were reversed by IV H.

2) Modification of CNS H content by drugs

Boissier et al. (1970) administered low doses of Hd (100 mg/kg, IP) and found a maximum increase in cerebral H content one hour later. After 90 minutes no significant changes in the H content of several brain parts could be demonstrated. Taylor and Snyder (1972) utilized high doses of Hd (500 and 1000 mg/kg, IP) and achieved maximum mouse whole brain H content elevation of 110 and 169%, respectively, one hour after injection. Multiple Hd injections elevated brain H further. One hour after Hd (1000 mg/kg, IP), H content in all mouse brain parts examined (except medulla

oblongata) was significantly increased.

Taylor and Snyder (1971) studied the effects of various HdDC inhibitors on the formation of ^3H -histamine from intraventricularly injected ^3H -histidine. Histidine can be decarboxylated to H in mammalian tissues by the "specific" HdDC, which is inhibited by α -hydrazinohistidine or NSD1055 (cf. Snyder, 1972), or by the aromatic amino acid DC, which also decarboxylates DOPA and 5HTP and is inhibited by α -methyl DOPA (Lovenberg et al., 1962). α -hydrazinohistidine and NSD-1055 administered IP significantly lowered ^3H -histamine content in the hypothalamus and thalamus-MB. α -methyl DOPA failed to affect ^3H -histamine concentration in any brain region. None of the drugs altered brain levels of ^3H -histidine or endogenous Hd. Doses of 200-500 mg/kg of α -hydrazinohistidine and NSD-1055 lowered hypothalamic H by 40% while α -methyl DOPA had no effect. Taylor and Snyder (1972) studied the effect of these inhibitors and 4-thiazolylmethoxyamine on in vitro HdDC activity. All four compounds inhibited HdDC activity significantly, although the effect of α -methyl DOPA was much less than the others. Menon et al. (1971) studied five HdDC inhibitors for their effects on rat brain H, NE, DA and 5HT content and behavioral activity. 4-Thiazolylmethoxyamine (100 and 300 mg/kg, IP) depletes whole brain H to 10% of control 3 days and 1 day, respectively, after administration. One day after a dose of 100 mg/kg, 5HT content was increased while NE and DA content decreased slightly. After three days DA was further decreased, NE slightly elevated and 5HT, approximately normal. Taylor and Snyder (1972) examined whole mouse brain amine content one hour after Hd (1000 mg/kg, IP). 5HT was significantly decreased but NE, DA and acid and neutral indoleamines were unchanged.

Taylor and Snyder (1971; 1972) tested the effect of a large variety of drugs on H content of the rat hypothalamus and mouse brain, respectively. In the former study, reserpine, pCPA, chlorpromazine, pargyline, tranylcypromine, atropine, physostigmine, L-DOPA and 6-hydroxydopamine had no effect on H content. In the latter study, H content was significantly lowered by L-3-methylhistidine and reserpine, while dexbrompheniramine and PB elevated it. Adam and Hye (1966) examined in the cat the effects of a number of compounds on H content of several areas of the hypophysis, hypothalamus and the massa intermedia. Compound 48/80 lowered H in the posterior hypophysis and stalk but had no effect in other areas. Reserpine depleted, while iproniazid and phenothiazines increased hypothalamic H content. Green and Erickson (1964; 1967) demonstrated that chlorpromazine (20 and 40 mg/kg) elevated, while diphenhydramine (50 mg/kg) significantly lowered rat and guinea pig brain H respectively. Boissier et al. (1970) examined H content in several rat brain areas one hour after diphenhydramine (50 mg/kg), but found no significant alterations from control.

Merritt et al. (1964) reported sedation in rats and a 63% decrease in brain NE, 24 hours after 15 mg/kg, IP of decaborane (DB). Merritt and Schultz (1966) showed in whole rat brain that this dose of DB depleted DA levels 60% after 4 hours, with a return to normal 24 hours later. They suggested that the decarboxylase system might be inhibited by DB. Merritt and Sulkowski (1967) demonstrated in vitro that AAADC was rapidly and completely inhibited by DB (15 mg/kg, IP) within 2 hours and did not begin to recover until after 72 hours. Whole rat brain 5HT was depleted significantly after 4 hours, reaching a maximum at 24 hours, followed by a slow

recovery. Medina et al. (1969) reported that DB (15 mg/kg, IP) depleted rat brain H to a maximum at 24 hours. H levels recovered slowly, requiring more than one week to return to control. They showed that DB inhibited HdDC almost 90% within 2 hours after administration. Unfortunately, these authors did not measure other biogenic amines, such as NE, DA or 5HT, so that their inter-relationships could be compared. Schayer and Reilly (1971) found that DB inhibited brain and stomach HdDC and in vivo H formation in the brain and skin.

4. Acetylcholine (ACh) in the CNS

The cholinergic system in the CNS has been the subject of several recent and thorough reviews (Karczmar, 1969, 1970, 1973; Potter, 1970).

a. Formation and Localization

ACh is formed from acetyl CoA and choline, by the enzyme choline acetyltransferase (choline acetylase, ChAc, EC 2.3.1.6). In multicellular animals, ChAc is found normally only in nerves. The placenta of higher primates, although not innervated, contains considerably more ACh and ChAc than most nervous tissues (Hebb and Ratkovic, 1962). The highest ChAc activity is found in the CN, retina, corneal epithelium and spinal roots (3-4 mg ACh/gm/hour synthesized). Dorsal spinal roots and the cerebellum contain only trace amounts. Intracellular ChAc is found predominantly in the crude mitochondrial fraction (from sucrose density, differential centrifugation data) of homogenized mammalian brain. This fraction contains mitochondria, membrane fragments and synaptosomes with enclosed synaptic vesicles. Upon further resolution, the enzyme is found to be in the

cytoplasm and not in the vesicle, therefore it must be concluded that ACh is made in the cytoplasm and concentrated in the vesicles. ACh released from nerve endings acts postsynaptically and its actions are terminated by acetylcholinesterase (AChE or acetylcholine acetyl-hydrolase, ED 3.1.1.7), which hydrolyzes it to form acetate and choline. The choline is taken back into the presynaptic ending by an active process, for the resynthesis of more ACh.

ACh has been established as a transmitter (using the criteria outlined by Werman, 1966; vide supra) only in the motor neuron collaterals of the Renshaw cells (Eccles, 1969). It is likely that other synapses in the CNS are also cholinergic although it is technically very difficult to establish this conclusively.

b. Pharmacological Effects

ACh injected directly into the carotid artery induces a state of cortical activation (Bremer and Chatonnet, 1949; Monnier and Romanowski, 1962; Marczynski, 1967). Yamamoto and Domino (1967) reported the same effect after IV injection. In the intact animal, physostigmine, prostigmine and nicotine increase cortical and behavioral arousal in the intact animal (Jouvet, 1972b). Microelectrophoretic studies have shown that some central neurons are excited and others are depressed by ACh (Curtis and Crawford, 1969). Areas affected by ACh include CN, ventral basal thalamus, cochlear nucleus, brain stem and pyramidal cells of the cortex.

Microinjection of ACh with a chemitrode into the bulbar, mesencephalic, pontine or rostral RF produces EEG synchronization and a hypnogenic action in the cat, that is identical to the EEG and behavioral appearance of SWS (Courville et al., 1962). Similar injections of ACh in encephale

isolé cats produced EEG synchronization comparable to that in unanesthetized intact cats (without peripheral blood pressure changes), indicating that the effect is central. Cordeau et al. (1961) found that the administration of ACh with physostigmine produced EEG synchronization and behavioral arousal. ACh alone, applied to all areas of the RF produced EEG synchronization and sleep. Yamaguchi et al. (1964) placed crystalline ACh into areas of the RF rostral to those studied by Cordeau and coworkers, resulting in sleep. The effect was more pronounced when ACh was administered in the mesencephalic RF. Application of carbachol did not produce sleep. Although the animals were catatonic, the EEG was desynchronized. Hernandez-Peon et al. (1963) also obtained arousal when carbachol was applied to the lateral mesencephalic RF. They suggested that the mesencephalic arousing system is activated by cholinergic influences, as well as adrenergic. Cho et al. (1962) found that the potent muscarinic compound, oxotremorine produced flaccid paralysis and areflexia, associated with deep sleep, when injected into the brain stem RF of conscious cats and dogs. These effects could be antagonized by atropine (IV, IM or by central microinjection). A descending sleep system has been postulated which follows the MB limbic circuit described by Nauta (1958) (ie. the MFB from the preoptic region through the lateral hypothalamus into the MB limbic area). ACh injections into this circuit is followed by sleep, while lesions or atropine injections made caudally to the ACh injection suppress its sleep-inducing effect (Velutti and Hernandez-Peon, 1963). Since cholinceptive neurons are present in the cortex, diencephalon, brain stem and other areas, and some sites respond preferentially to muscarinic or nicotinic compounds, the exact mechanisms of the EEG activation induced by

cholinergic agents is uncertain.

Jasper and Tessier (1971) measured the rate of liberation of free ACh from the surface of prostigmine treated cerebral cortex of freely moving cats during sleep and arousal. The rate of release was lowest during SWS and increased during PS and waking, and seemed to be related to the EEG pattern, rather than the behavior per se. Administration of hemicholinium (which blocks ACh synthesis) into the lateral ventricle of cats, increased SWS, decreased waking and had no effect on PS (Domino and Stawiski, 1971). A number of studies have attempted to correlate brain ACh metabolism and the state of arousal (cf. Jouvet, 1972b). EEG arousal elicited by midpontine transection, mesencephalic RF and septal stimulation via peripheral or central sensory stimulation or by amphetamine is accompanied by an increase in ACh release at the cortical level and a decrease in bound brain ACh. Cortical synchronization from precollicular transection or physiological sleep is accompanied by a decrease in the cortical release of free ACh and an increase in bound ACh. Barbiturate anesthesia further reduces ACh release (Kurokawa et al., 1963).

Hanin et al. (1970) reported a diurnal variation of whole rat brain ACh. Peak concentrations occur at 1400 hours (2 hours into the light phase) and trough between 2200-0600 hours, primarily in the dark, when rats are awake. These results are in line with the data cited above, showing that arousal is accompanied by a decrease of bound ACh (and a release of cortical ACh), while the reverse occurs during sleep. Friedman and Walker (1972) found that peak ACh levels of the rat MB and CN occurred at 2400 hours, while whole mouse brain ACh levels peaked at 0600 hours. Trough values occurred

at 1200 hours in rat MB and 1800 hours for rat CN and mouse brain. These results differ from the Hanin study and Saito (1971), who found that whole brain ACh content peaks at 6 hours in the light and troughs throughout the dark period. These differences point to the complexity of the system and the difficulty of demonstrating a clear-cut correlation between ACh levels and the arousal state. Additional aspects of circadian variation of cholinergic function is discussed under the biological rhythms section.

Monnier and Herkert (1972) recently reported that the ACh concentration in hemodialysates of aroused rabbits (via RF stimulation) was significantly greater than that from sleeping or sleep-resistant donors.

E. Sleep-wakefulness Phenomena

1. Sleep Phenomenology

Berger (1930) discovered the electroencephalogram (EEG) and first investigated its nature. Numerous investigators have since studied the changes occurring in the EEG, when subjects (human and animal) changed from the alert, wakeful state to drowsiness, light sleep and deep sleep (as measured by the arousal threshold). Mauthner (1890) and von Economo (1926, 1929) were probably the first to express the idea that sleep was not passive but an actively-induced, controlled phenomenon. Hess (1924) argued that sleep is a vegetative function to be classified with such activities as respiration, circulation, temperature regulation, etc. He further stated that sleep was not simply a state of quiescence but was accompanied by active physiological processes, under control of the central and autonomic nervous systems.

The studies of Loomis and coworkers (1937, 1938) and Davis and coworkers (1938, 1939) revealed that in the transition from the resting awake state (with its typical, more or less continuous alpha waves 8-12 Hz) to deep sleep, the human subject goes through typical stages, which they labelled A-E. Stage A is characterized by an intermittent loss of alpha waves. In stage B the alpha pattern disappears completely and is replaced by a fast low voltage, irregular pattern. In stage C spindle-shaped bursts of 14 Hz are observed. Slow (1.5-3 Hz) and finally very slow (0.6-1.5 Hz) delta waves characterize stages D and E, respectively. Brazier (1949) observed that during the transition from the waking state to sleep, the locus of maximum EEG activity shifts from the occipitoparietal towards the frontal region. Some workers also point out a characteristic pattern, the K-complex and sharp vertex spike,

transient phenomena appearing in the sleep EEG either spontaneously or in response to rhythmic sensory stimulation.

Derbyshire et al. (1936) and Klaue (1937) observed that states which clinically appeared to be deep sleep contained occasional cortical EEG desynchronizations. Dement and Kleitman (1957) first studied this new kind of sleep, referred to by Jouviet as paradoxical sleep (PS). It is characterized by a low voltage, fast cortical EEG pattern, hardly distinguishable from an activated pattern. It is accompanied by the appearance of theta waves (4-6 Hz) in the hippocampus and by spindles of 6-8 Hz in the pontine reticular formation. This stage is also known as rapid eye movement (REM) sleep, desynchronized sleep, dream sleep, activated sleep and rhombencephalic sleep (by Jouviet, who considered that it is probably organized by structures in the rhombencephalon). Subjects awakened during this period often recall dreams, while they rarely recall dreams when awakened from the other sleep stages. Certain characteristic phasic events of this sleep stage include, in addition to the eye movements, muscular twitching, cardiorespiratory irregularities and bursts of monophasic sharp waves from the pons, lateral geniculate nuclei, oculomotor nuclei and visual cortices. These PGO spikes seem to represent some sort of primary triggering process for the phasic events, especially the eye movements (Dement, 1970).

Dement and Kleitman (1957) suggested a relatively simplified classification of the various sleep stages, which has been adapted by many workers in the field. Stage 1 is characterized by "modified" alpha waves and corresponds to late stage A and stage B; stage 2, showing spindling with low background is about equivalent to C; stage 3 is identified by delta waves

and spindling; stage 4, containing slow waves only, is comparable to D and E. They added a stage 1 REM or emerging stage 1 (from stage 2), which contains rapid eye movements and a low voltage, fast EEG pattern. Sometimes stages 1-4 are referred to collectively as non-REM (NREM) or slow wave sleep (SWS), as opposed to REM sleep. Rechtschaffen and Kales (1968) present precise definitions of these states in the human, making it possible to classify any 20-30 second epoch into one of the NREM states, REM sleep or wakefulness. In the older EEG terminology, the delta pattern (0.5-3.5 waves per second) corresponds to stage 3 and 4; theta (4-7 waves per second) to stage 1 and 2; alpha (8-13 waves per second) to awake but not visually stimulated and beta (14-25 waves per second) to arousal.

A characteristic sign of sleep is the low level of muscular activity probably related to the reduced reactivity of CNS structures involved in the organization of motor output. The electrical threshold of the motor cortex is elevated during sleep in the monkey (Lilly et al., 1956) and the cat (Hughes and Mazurowski, 1958). Marchiafava and Pompeiano (1964) found the flexor motor output produced by pyramidal tract stimulation to be reduced with the onset of SWS and further decreased during low voltage fast sleep. The complete inactivity of the neck muscles during PS is so characteristic it is used to verify fast low voltage sleep. As first observed by Tarchanoff (1894), spinal reflexes such as the "knee jerk" are reduced and often missing during sleep. Giaquinto et al. (1963) and Gassel et al. (1964) found that cats with a motor nerve stimulator and monitored for EMP and EEG, spinal reflexes were only slightly affected during SWS, but during PS monosynaptic reflexes were abolished.

2. Sleep Deprivation

Another approach to study sleep-wakefulness phenomena is sleep deprivation and subsequent biochemical and neurophysiological examination for induced changes. There is a physiological need for sleep, since deprivation can lead to malfunction, permanent damage and death. Temporary sleep loss is made up for by increased total sleep, by an increase in the length of the various stages. Extensive reviews of this subject have appeared (Wilkinson, 1961; Oswald, 1962; Kleitman, 1963). Tyler (1955) found sleeplessness in man (30-75 hours) produced symptoms resembling schizophrenia; i.e., instability, inattention, memory loss and hallucinations. Rodin et al. (1962) studied the EEG of human subjects deprived of sleep for as long as 120 hours and found "marked evidence of drowsiness" within 24 hours after the start of the experiment. Pieron (1913) found dogs deprived of sleep for 500 hours were hyperirritable but in good condition. Upon microscopic examination of the prefrontal cortex of these animals, he found cell shrinkage, vacuolization of cytoplasm and disappearance of Nissl substance. The amount of damage was proportional to the degree of sleep loss and was apparently reversible. Stern (1937) found that the blood-CSF barrier of dogs became more permeable after prolonged sleep deprivation.

a. PS Deprivation

Jouvet et al. (1964) selectively deprived chronically-implanted cats of PS by placing them on small platforms surrounded by water. Animals that lapsed into PS, with muscle relaxation would fall into the water, and soon learned to develop SWS without PS. Although having the normal amount

of slow sleep at the termination of the PS deprivation, animals were sleepy, weak and photophobic. During recovery, PS periods were prolonged and occurred at shorter intervals. The recovery of cats deprived of PS by electrical stimulation of the reticular formation when they lapsed into PS was studied by Siegel and Gordon (1965). They observed PS rebound during the first recovery day, in which PS occupied 53% of total sleep, versus only 33% on baseline days.

b. Neurochemical Alterations After Sleep Deprivation

Pujol et al. (1968) reported that in rats, NE turnover is increased during PS and decreased during PS deprivation. In examining the effect of pCPA on sleep, Torda (1967) found that 15 hours of PS deprivation increased brain 5HT slightly but not significantly. Tsuchiya et al. (1969) found no change in telencephalon (TE), diencephalon (DE) and mesencephalon-pons-medulla (MPM) ACh, NE and 5HT content after 10 hours of total sleep deprivation. After 24 hours of deprivation TE ACh increased and DE NE and 5HT decreased. After 96 hours of PS deprivation TE ACh decreased, DE and MPM NE content decreased but 5HT did not change. Stern et al. (1971) measured NE and 5HT in the cerebral hemispheres, DE and brain stem of 1) PS-deprived rats (3, 5, 6 and 8 days, without allowing recovery), 2) after 5 days of control stress treatment and 3) in normal rats. Amine levels in the three regions were unchanged after 3-8 days of PS deprivation. Pargyline (20 mg/kg, IP, 2.5-3.5 hours before sacrifice) after 5 days of PS deprivation or stress increased NE and 5HT over that of normals, suggesting their increased synthesis. They concluded that the enhanced amine synthesis after PS deprivation might

be due to non-specific stress, rather than loss of PS per se. Steinberg et al. (1969) found that human urinary NE and Epi were elevated significantly following 24 hours of sleep deprivation. NM, MET and VMA were not changed significantly.

Karadzic et al. (1971) examined free amino acids in frontal and occipital cortex, CN, thalamus, hippocampus and mesencephalic RF in PS-deprived, normals and stressed cats. Seven of eleven amino acids measured in the brain changed significantly after deprivation. Aspartic acid, cysteine, lysine, arginine and glycine increased significantly in several areas and gaba and threonine were altered significantly in 4 out of 6 and 5 out of 6 areas, respectively. Glutamic acid, serine, tyrosine and histidine were not altered significantly.

3. Drugs Acting on Sleep Stages

a. Reserpine

In cats reserpine suppresses SWS 12-14 hours and PS for 22-24 hours which recovers after 5-6 days. It selectively triggers PGO activity (30-50 per minute) for 50-60 hours (Jouvet, 1967b). The PGO activity is similar to PGO activity of PS, but without the decrease in neck muscle tone. 5HTP (30-50 mg/kg), 2-3 hours after reserpine totally suppresses the PGO activity and induces EEG and behavioral SWS for 4-6 hours. The same dosage of DOPA after reserpine increases PGO activity and an almost normal level of PS and SWS is evident for 4-6 hours (Matsumoto and Jouvet, 1964). This is part of the evidence for Jouvet's theory (1967 a,b) that serotonergic mechanisms are responsible for SWS and CA mechanisms are involved in PS. The reserpine-induced PGO activity is enhanced by eserine, decreased by atropine

(over 1.0 mg/kg) but totally suppressed by PB, imipramine, alpha-methyl DOPA and MAOI (Delorme, 1966).

b. MAO Inhibitors (MAOIS)

MAOIs are potent PS suppressors (Jouvet et al., 1965), although there is some species variation in the response to them. Pargyline, nialamide, iproniazid and phenylisopropylhydrazine are selective PS suppressors. PS is suppressed for 90-100 hours after nialamide (10 mg/kg) and a stepwise recovery occurs without any rebound in about a week. Nialamide can even suppress PS after 72 hours of operational PS-deprivation. In man after one week of MAOI treatment, a progressive and significant reduction of PS occurred (Wyatt et al., 1969). Karczmar et al. (1970) have demonstrated a pharmacological model of PS by giving eserine to reserpinized animals, which could be abolished by atropine.

c. α -Methyl-p-tyrosine (AMPT)

AMPT significantly increases PS in rats (Hartman et al., 1971a) Torda, 1968), cats (King and Jewett, 1971) and man (Wyatt et al., 1971a; Baeklund and Lundwall, 1971). On the other hand Weitzman et al. (1969) showed it decreased PS in monkeys. α -methyl m-tyrosine (AMMT) or α -methyl DOPA reduce PS and increase SWS (Jouvet, 1967b). α -methyl DOPA still reduces PS after selective PS deprivation (Dusan -Peyrethon et al., 1968)

d. p-Chlorophenylalanine (pCPA)

A 5HT depletor, pCPA, produces waking behavior (Mouret et al., 1967; Torda, 1967) and decreased both PS and SWS in cats (Delorme et al.,

1966b; Koella et al., 1968 Karadzic, 1968; Pujol et al., 1971). 5HTP (10-50 mg/kg) restores sleeping patterns to normal (Jouvet, 1968; Pujol et al., 1971). p-Chloromethamphetamine (15-30 mg/kg), which also selectively depletes 5HT (Pletscher et al., 1964) induces a constant state of arousal for 16-18 hours, with recovery after 60 hours (Delorme et al., 1966a).

e. Gamma-butyrolactone

Jouvet et al. (1961) demonstrated the gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) at relatively low doses (50 mg/kg) induced both types of sleep states (vide infra). The induction of PS was not observed in the rat and was inconsistent in the normal cat (Marcus et al., 1967; Winters and Spooner, 1966). GHB triggers PGO spikes and PS in chronic pontile cats (Jouvet, 1972b). Matsuzake et al. (1964) demonstrated that the PS-triggering action of short chain fatty acids was not limited to the C4 butyrate compounds but was more pronounced with C5 and C6 compounds. The C4 to C6 agents produce PS in the decorticated, mesencephalic and pontile cats, but not in cats with lesions of the pontine tegmentum (Jouvet et al., 1961) or after midpontine transections (Tokizane, 1966). After atropine (which suppresses the final tonic phase of PS), GHB or GBL are still able to induce PS in pontile cats, while after MAOI (nialamide) which blocks the first phase of PS (PGO activity) short chain fatty acids are unable to induce PS (Delorme et al., 1965).

4. Localization of "Sleep Centers"

Attempts to delineate a "sleep center" in the CNS have utilized lesioning and electrical and chemical stimulation, coupled with neurophysiological recording to reveal neuronal pathways and/or nuclei which might be

involved in the normal sleep-wakefulness cycle. The effects of various CNS lesions will first be examined, followed by an analysis of stimulation experiments and finally a discussion of attempts to find humoral sleep-inducing factors.

a. Lesion Experiments

Bremer (1936) demonstrated that transection of the spinal cord at the first cervical vertebra (encephale isolé preparation) resulted in an alert animal with a desynchronized EEG. Animals with a mesencephalic transection at the level of the superior colliculus (cerveau isolé) were stuporous and exhibited a synchronized EEG analagous to SWS. He concluded this state was due to exclusion of the bulbomesencephalic RF, assuming that in natural sleep the RF is depressed and normal ascending, activating impulses are reduced or eliminated. He further stated that the functional depression of the RF plays a major or even a primary role in cumulative "defacilitation" because of its central position in the arousal sequence. Ranson (1939), using lateral hypothalamic lesions, produced sleep in monkeys which lasted 4-8 days. Sleep of short duration was produced by lesions in the rostral hypothalamus or at the junction of the hypothalamus and thalamus. He concluded that sleep is a deviation from the waking state (deactivation) and that wakefulness is largely dependent upon hypothalamic drives. If sympathetic discharge from the descending pathways of the posterior hypothalamus is abolished, these waking drives are eliminated, producing sleep. Large bilateral lesions of the posterior hypothalamus in cats produced sleep lasting several days (Rangström, 1947). Sleeping animals could be aroused by various sensory stimuli. His conclusion was that the lesions destroyed a large

number of cells containing specific hyponogenic substances, which are transported to other brain areas, to produce sleep. Villablanca (1962) observed that in cerveau insolé cats kept alive for 20-60 days the sleep-wakefulness cycle (as measured by EEG and behavioral indices) was reestablished. The SWS during the first 10 postoperative days was attributed to surgical trauma. This indicated that the waking state could be organized by structures rostral to the lower MB. Nauta (1946) found that bilateral transection of the hypothalamus in or near the mammillary bodies or suprachiasmatic region produced sleep, while unilateral or bilateral medial or rostral hypothalamic lesions did not alter the S-W cycle in the rat. He concluded that lesions which interrupt fiber bundles such as the Vicq d'Azyr and MFB interfere with normal waking capacity. These fibers are known to connect the mammillary region with the anterior thalamic nuclei, the lateral hypothalamic area and septal region. He postulated an "intensive relay" of impulses from the posterior hypothalamus ("waking center") through the lateral hypothalamus and an active sleep center in the anterior hypothalamus. "Since animals whose mammillary and suprachiasmatic areas had been transected behaved like animals with transections at the mammillary level only, he deduced that the sleep center exerts an inhibitory influence on the waking center and not directly on the cortex and other major parts of the nervous system. Nauta suggested that the impulses from the anterior sleep center are conveyed to the waking center via the medial forebrain bundle. The waking center, in turn, exerts an activating influence upon the lateral hypothalamus and thence upon the cortex, and inhibitory influence upon a hypothetical center 'L' for 'body sleep'" (Koella, 1967, p. 102).

Lesions of the basal forebrain (preoptic region, diagonal band of Broca) in cats, reduced the time spent in the drowsy state, although the time asleep was not altered (Serman et al., 1964). Batini et al. (1958) transected the rostral brain stem at the pons (rostromedial-pretrigeminal preparation), inducing permanent EEG and behavioral sleep. From these experiments Moruzzi (1960) concluded that the "entire reticular formation of the midbrain with the support of the tonic sensory inflow which is likely to occur through cranial nerves still connected to the cerebrum (I, II, III) is by itself not adequate for maintaining a state of vigilance. The phasic aspect of EEG activation is still present, however, in the cat whose midbrain is connected to the cerebrum; clear-cut EEG arousal is elicited in this preparation, by olfactory stimuli." Lesions slightly caudal (midpontine pretigeminal) produce behavioral and EEG arousal. However low doses of barbiturate readily evoke slow waves and spindling. In light of this Moruzzi (1960) states that "there is a region in the rostral part of the pons which seems to be of critical importance for the maintenance of the EEG patterns of wakefulness. Since the classical sensory pathways are cut both in the prepontine and in the midpontine preparations, it would follow that the transection does not act by severing fibers from lower levels."

Hemisection of the MB at the superior colliculus or midcollicular level produced slow waves and spindles in the ipsilateral cortex, which gradually disappeared within 4-5 days (Cordeau and Mancia, 1959). As such patterns also occurred with the lateral sensory paths intact, they concluded that ascending activating influences from the lower brain stem travel in two distinct paths to supply the two cortices. Hodes (1963) had similar

findings with hemisection or unilateral procaine injections of the cord.

Jouvet et al. (1966) demonstrated in cats that an 80-90% destruction of the raphé system (the medial 5HT-containing neurons of the brain stem, from the nucleus raphé obscurus to the caudal medulla) resulted in permanent wakefulness. After 3-4 days very short periods of behavioral and EEG SWS reappear. There was a correlation between the extent of destruction of the raphe system, the decrease in cerebral 5HT and the loss of sleep. Kostowski et al. (1968) and Pujol et al. (1971) obtained similar results. Lesions of the pontine tegmentum selectively abolish PS without significantly impairing either waking or SWS (Jouvet, 1962). Histochemical methods show a dense group of neurons high in NE content located in the area of the locus coeruleus. Total destruction of the locus coeruleus causes complete PS-suppression (Jouvet and Delorme, 1965) and produce a significant decrease of NE in the brain rostral to the lesion (Roussel, 1967). Control lesions nearby do not affect sleep.

b. Stimulation Experiments

Hess (1944, 1949) performed the pioneer studies on electrically-evoked sleep using point by point stimulation of the entire DE and surrounding areas of the rat brain. Following repetitive stimulation via low voltage, rounded DC pulses, cats exhibited reduced activity and other presomnic signs (such as eyelids drooping, smaller pupils, lying down and curling up). Soon after they appeared to be asleep. He could reverse the sleep effect with higher voltages and/or higher frequencies of stimulation. In these animals the electrode tips were in the thalamus, in the lower two-thirds of

the massa intermedia, near the midline (medial thalamus). Electrodes placed in the surrounding area produced only "partial sleep", with depressed motor activity. These findings point to the specificity of the medial thalamic area as a hypogenic site and the production of sleep at will, rather than coincidentally. Hess' findings have since been confirmed by Akert and co-workers (1950, 1952, 1953) who further showed a striking similarity between the behavioral and EEG patterns after the electrical stimulation. Akimoto et al. (1956) produced behavioral and EEG sleep in the dog with low voltage, low frequency stimulation of the thalamus. Monnier and coworkers (1950, 1963) induced sleep by stimulation of the intralaminar thalamic nuclei of cats and rabbits. Low voltage and frequency were necessary, as higher frequencies and voltages produced arousal. Jouvett (1962) also reported sleep effects of low frequency stimulation of the medial thalamic nuclei in cats. Following low voltage and low frequency stimulation of the hippocampus of the cat, Parmeggiani (1960, 1962) observed a number of presomnic signs. Penaloza-Rojas et al. (1964) induced a sleep-like state by low frequency stimulation of various areas of the cerebral cortex. Sterman and Clemente (1962 a,b) stimulated the preoptic area (basal forebrain) of freely-moving cats and observed sleep, which included EEG spindling.

Moruzzi and Magoun (1949) demonstrated that the brain stem RF is responsible for cortical and behavioral arousal through the ascending reticular activating system (ARAS). The ARAS is composed of the brain stem core from the medulla to the rostral part of the mesencephalon, with cortical projections. They could reproduce the electrocortical features of natural wakefulness by stimulation of the ARAS as well as the classical specific

afferent pathways, in the periphery of the brain stem. The specific system (medial and lateral fillets) ascends the stem outside the RF, and in addition sends collaterals to the RF, which merge diffusely and tend to maintain the activating tone of the ARAS. The specific system is relatively-unaffected by anesthetics, while the nonspecific RF is very sensitive, due to its polysynaptic organization (Larrabee and Posternak, 1952). Favale et al. (1961) similarly produced sleep in cats by stimulation of various areas of the brain stem RF, such as the MB tegmentum and some nuclei of the medulla. Jouvet (1962) also reported sleep effects after stimulation of the MB RF of cats. When he stimulated the pontine RF with low voltage stimuli at low frequency he produced PS (lasting as long as 15 minutes). Strong stimuli usually produced arousal. Caspers and Winkel (1954) produced behavioral and EEG sleep effects after stimulation of the MB RF in the rat. Magnes et al. (1961) in cats produced widespread cortical synchronization by low frequency stimulation near the nucleus of the solitary tract.

Koella (1967) attempted to synthesize the results of lesion and stimulating experiments, to establish the structure of the sleep-controlling apparatus. He concluded that all structures which upon stimulation yield sleep or sleep-like signs constitute the "sleep center". This heterogeneous structure is made up of components which qualitatively and quantitatively subserve different functions. Low rate stimulation of the nucleus of the solitary tract produces cortical synchronization (Magnes et al., 1961), while lesions of this area increase the effectiveness of arousal stimuli. The authors interpret these findings to mean that there is a two-way network connecting the solitary tract nucleus with the ascending mesencephalic RF, forming a negative feedback

system which limits phasic arousal. Koella considers the bulbar synchronizing structures a sleep-facilitating apparatus, acting via inhibitory influences on the activating or arousal system, in a subordinate capacity. It is responsible for short, phasic sleep, rather than long, tonic periods of sleep. Similarly, the MB RF is important for short, phasic periods of awakening. Reduction of the ascending, activating influence therefore, results in sleep. Lesions in this area produce semi-permanent sleep in the TE and behavioral sleep signs. Stimulation in this area might activate inhibitory pathways, which have been shown to have inputs into the RF from the lower brain stem, thalamus and cerebral cortex. Koella considers the basal forebrain, including the anterior hypothalamus, as another subordinate controlling structure. Waldvogel (1945) elicited yawning by stimulation of the area between the anterior commissure and infundibulum. Parmeggiani (1962) evoked many presomnic signs in the cat by hippocampal stimulation and occasionally produced light sleep. The pons seems to control a phase of sleep, but acts only when slow sleep is already present. Stimulation of the tegmentum also produces sleep signs for a short duration.

Koella concludes from various lines of experimental evidence that the thalamic hypnogenic area qualifies as the "head ganglion" of sleep. Thalamic stimulation produces sleep of long duration, preceded by presomnic signs. This type of sleep includes PS episodes seen in normal sleep. After repeated stimulation it becomes progressively easier to reinduce sleep after awakening. Lower voltages are needed to produce sleep in the thalamus than in other areas such as the lower brain stem and hypothalamus. The recruiting response, best elicited by low-rate stimulation of the midline thalamus, closely resembles

to sleep spindles. The thalamic-induced recruiting response can be recorded in the MB RF (as well as the cortex), suggesting a thalamo-reticular pathway, which might also convey inhibitory impulses to the ascending activating system, as previously mentioned.

c. Humoral Sleep Factors

1) Cerebrospinal Fluid (CSF)

Early studies related to an active induction of sleep envisioned a humoral factor primarily responsible for the induction of sleep. Legendre and Pieron (1910, 1911) sleep-deprived dogs for as long as 18 days, withdrew some of their CSF and injected it into the fourth ventricle of non-fatigued dogs, which promptly showed signs of drowsiness and sleep for 2-6 hours. Dogs treated with CSF from non-fatigued animals remained alert. Schnedorf and Ivy (1939) confirmed these basic findings but emphasized that the central "depressing" effects of the injection was in part due to concomitant elevation of intracranial pressure and to hyperthermia, which usually followed the intracisternal injection. They attempted without success to detect ACh in the CSF.

Pappenheimer et al. (1967) studied the perfusate from sleep-deprived goats whose ventricular systems were cannulated. When injected into the ventricular system of cats and rats, the perfusate induced signs of sleep (inactivity) lasting up to 18 hours, while there was no change in the activity of animals receiving CSF from non-sleep-deprived animals. No EEG studies of the recipient animals have been reported. The factor responsible for the inactivity has not yet been identified, but it has been reported to be dialy-

zable (molecular weight under 1000-2000). Ringle and Herndon (1969) made some unsuccessful attempts to induce sleep in rats with CSF from sleep-deprived rats.

Drucker-Colin et al. (1970) perfused sleep-deprived cats via push-pull cannulae inserted in the MB RF. The outgoing perfusate from the cats during the PS-rebound induced sleep in recipient cats when injected through the push-pull cannulae inserted in their midbrains.

2) Plasma

Demole (1927) observed that blood calcium was decreased during sleep and predicted that brain calcium would increase during sleep. He injected several microliters containing 0.25-2 mg of CaCl_2 into the infundibular and tuber cinereum region of the hypothalamus, producing sleep after several hours latency. Other areas of the hypothalamus and other brain structures were unresponsive. Koella (1967) cites several other studies which confirmed these results. Cloetta et al. (1934) found calcium to be increased during sleep, particularly in the infundibular region. These findings suggest that a shift of calcium from the blood to certain parts of the brain stem might be an important hypnogenic factor. The mechanism of this shift is unknown but it might be due to an increased permeability of limiting membranes, as indicated by Stern (1937).

Purpura (1956) utilized a cross circulation arrangement in cats and showed that 30-80 seconds after arousal was produced by reticular stimulation in the "donor" animal, it occurred in the non-stimulated recipient cat. In order to test for sleep-inducing factors in the blood, Kornnüller et al.

(1961) and Monnier, Koller and Graber (1963) used the crossed blood circulation technique in cats and rabbits, respectively. Stimulation of the medio-central intralaminary thalamus of the donor induces a significant increase in cortical waves. Both groups observed that after a delay of less than a minute the recipient shows a statistically significant increase in cortical slow activity (usually after the fourth stimulation of the thalamus). On the other hand, stimulation of the MB RF, which induces arousal in the donor, significantly reduces cortical slow waves in the recipient. These results suggest that hypnogenic and alerting substances may be transported in the plasma and can change the state of consciousness of recipient animals. Monnier and Hösli (1964, 1965) demonstrated that a dialysate of cerebral blood from rabbits during sleep induced by electrical stimulation of the thalamus, when injected into recipient rabbits produced EEG evidence of sleep. Recipients receiving a dialysate from non-stimulated control rabbits had waking patterns. They excluded changes in visceral activities (blood pressure, heart rate respiration and body temperature), electrolyte concentration and pH. The dialysis techniques indicated that the active substances were of low molecular weight. Comparisons between the "sleep dialysate" and other pharmacological substances indicated that its effects generally resembled those of chlopromazine and substance P. Apparently the sleep dialysate factor obtained from the rabbit is active only after electrically-induced sleep, since Ringle and Herndon (1968) failed to show any sleep-promoting effects from the reconstituted plasma dialysates from 72 hours sleep-deprived rabbits, in recipient rabbits, rats or mice. The fact that a blood perfusate can induce sleep only after stimulation of the thalamus, but not when taken

from sleep-deprived rabbits shows that it is necessary to differentiate between electrically-induced sleep, normal sleep and the condition produced by prolonged sleep deprivation. Hypnogenic factors released under these conditions are not necessarily related to substances which are produced during and induce or facilitate the onset of normal sleep. On the other hand, the thalamus per se is not involved in the sleep mechanism, since its total destruction does not significantly alter the sleep-waking cycle (Angeleri et al., 1969). Finally, the fact that Siamese twins, sharing the same blood circulation, may sleep independently (Alckseyeve, 1958) makes it unlikely that sleep depends exclusively upon blood-borne factors.

3) Neurohumors

The studies of Koella and Czicman (1966) reported that intracarotid 5HT produced an initial brief arousal followed by a prolonged phase of hypersynchrony, presumably acting on the area postrema. This stimulating effect is transmitted to the neurons in the solitary tract nucleus, which has been shown by Moruzzi (1960) to have hypersynchronizing properties. pCPA depletes brain 5HT, by inhibiting its synthesis and produces insomnia in the cat, which develops and recovers following the time course of brain 5HT content (Jouvet, 1969). 5HTP, the 5HT precursor, restores sleep to the normal pattern for 10-12 hours when given at the peak of the pCPA effect. In addition, Pujol et al. (1971) and others have shown that lesions of the raphe system deplete brain 5HT and induce permanent arousal. Matsumoto and Jouvet (1964) found that reserpine produced insomnia in the cat; eliminating SWS for days and PS for 2-4 days. 5HTP restored SWS immediately without PS while DOPA restored PS, indicating 5HT was involved in SWS, while CAs were necessary for

PS. Feldberg and Sherwood (1954) injected NE and Epi into the lateral ventricle of chronically-prepared cats, producing a sleep-like state after 20 minutes that lasted about 3 hours. Others have demonstrated alerting actions of NE (Torda, 1968; Cordeau et al., 1971). The case for NE in sleep phenomena therefore, is less clear than for 5HT.

Sharpless and Rothballer (1961) and Söderberg (1962) have shown that stimulation of the RF may release a blood factor with an activity similar to vasopressin. Other studies have shown that stimulation of monoamine bundles or terminals might release monoamines (McLennan, 1964; Stein and Wise, 1969; Arbuthnott et al., 1970) or monoamine metabolites which might be eliminated either in the blood or CSF, where they could act on other (unknown) brain areas. Jouvet (1972b) points out that central "hypnogenic" stimulation or sleep deprivation might release a greater amount of transmitters or metabolites than physiological sleep.

The daily fluctuations of brain biogenic amines are discussed in detail under the Biological rhythm section but it is appropriate that these changes are briefly mentioned here. In newborn rats, which have not yet acquired a circadian rhythm of sleep there is no alteration of brain 5HT (Okada, 1971), while in the adult rat, with a well-established sleep cycle, there is a significant increase in endogenous brain 5HT during the day (when these nocturnal animals sleep) and a decrease during the night (Dixit and Buckley, 1967; Friedman and Walker, 1968). The latter investigators found a reversed pattern for MB and CN NE content, suggesting that this amine might have a waking role. This is supported by the work of Torda (1968) and Cordeau et al. (1971).

Jouany et al. (1960) have shown that GHB induces sleep which is not distinguishable from natural sleep. At higher doses the effect resembles anesthesia. Bessman and Fishbein (1963) have shown that GHB occurs in the rat and human brain, where it is derived from GABA.

Kroll (1933) prepared water extracts from brains of sleeping cats and rabbits and injected them intraventricularly or intracisternally into waking cats and rabbits. The donor animals had been put to "sleep" by intraventricular injection of CaCl_2 , by Pernocton (butallylonal) or by electrosleep (Passing a current longitudinally through the head). Recipient animals promptly slept when treated with the brain extracts from "sleeping" animals, while recipients of extracts from waking donors did not sleep. Kroll also demonstrated that brain extracts from hibernating hamsters and porcupines induced sleep in cats for several days (but not in monkeys). There were several uncertainties in these experiments, such as the possibility that the extracts might contain sufficient Pernocton or CaCl_2 to induce sleep or that the electrical current might produce electronarcosis rather than sleep.

Hunter and Rigal (1966) reported that plasma human growth hormone (HGH) was elevated during the night. Quabbe et al. (1966) suggested that nocturnal peaks of HGH might occur preferentially during periods of deeper sleep. Takahashi et al. (1968) found that a plasma HGH peak appeared at the onset of deep EEG sleep and smaller peaks appeared during subsequent deep sleep phases. If sleep was delayed or interrupted, the HGH peak also shifted. Peak HGH secretion was not correlated with changes in plasma glucose, insulin or cortisol. Honda et al. (1969) obtained similar results and suggested that secretion is inhibited during PS while SWS increases HGH secretion by

stimulating the secretion of hypothalamic HGH releasing factor. Sassin et al. (1969) supported this conclusion and demonstrated that reversal of the sleep-wake cycle was accompanied by a concomitant plasma HGH pattern reversal, perhaps both due to the same neural mechanism.

F. Barbiturates

Barbiturates belong to the general class of hypnotic-sedatives. These agents are general depressants, depressing a wide range of cellular functions in many organ systems. The type compound, barbituric acid, first synthesized from urea and malonic acid by von Baeyer, is not a central depressant. The derivatives of barbituric acid obtained by replacing the two hydrogens on the C5 position by alkyl or aryl groups are called barbiturates and frequently have hypnotic potency. In 1903, Fischer and von Mering introduced diethylbarbituric acid (barbital), to medicinal use.

1. Distribution

In general, structural changes resulting in increased lipid solubility are associated with a decreased duration of action, a decrease in latency to onset of activity, more rapid metabolic degradation and often increased hypnotic potency. Barbituric acid derivatives with only one substituent group are without central depressant activity and the dimethyl or ethylmethyl derivatives are very weak. Barbiturates with an ethyl group and a longer chain are more potent than barbital, but when the chain length exceeds 4 or 5 carbons, activity decreases. The present use of barbiturates is well stated by Goodman and Gilman (1970): "... more than 2500 barbiturates were synthesized, many were carefully studied pharmacologically, and approximately 50 were marketed for clinical use. Today, a dozen or so barbiturates are widely used; of these, five or six would probably be sufficient to meet most therapeutic needs."

A number of factors, both physiochemical and physiological influence the hypnotic or anesthetic potency of barbiturates. Some of the former include water solubility, ionization, distribution between water and lipids, rate of entry into the brain compared with other tissues, protein binding, volume of distribution in the body and rates of metabolism and excretion. There is no impenetrable barrier to barbiturate diffusion, so if the drug is in the plasma long enough, it is distributed to all tissues and fluids, including the fetal circulation, since it crosses the placental barrier.

a. Physiochemical Factors

In their undissociated form, the barbiturates have a rather high affinity for non-polar solvents, which correlates with their pharmacological properties. The more highly lipid soluble compounds are short-acting agents, with a rapid onset of action. A fraction of the barbiturate is reversibly bound to plasma proteins, chiefly albumin (Goldbaum and Smith, 1954). Protein binding appears to depend on the same structural features that determines affinity for non-polar solvents. With the exception of body fat, the capacity of tissues to concentrate barbiturates depends largely on protein binding. Fat depots can contain very high concentrations of the ultrashort acting barbiturates which possess high lipid solubility. Highly lipid-soluble drugs, such as thiopental, reach maximum concentrations in the brain within two or three passages of the blood. During the first few minutes after injection, thiopental is found in highest concentration in the brain areas that have the greatest blood flow (cortex, geniculate bodies and colliculi). On the other hand, barbital and phenobarbital, with low partition coefficients, penetrate

the BBB slowly, and 15 minutes or more might be needed for sleep induction after IV injection, but they have a longer duration of action. Regional differences in brain blood flow have little effect on the distribution of slowly-penetrating agents. If the alkalinity of the plasma is increased, as after hyperventilation or by infusion of sodium bicarbonate solution, the plasma concentration of the undissociated, lipid-soluble form of the drug falls. This results in the outward flux of the drug from tissue to plasma, lightening anesthesia because brain concentrations of the drug are decreased.

b. Physiological Factors

Differences in barbiturate metabolism have been shown among species (Quinn et al., 1958) and strains (Vessell, 1968a, b). Metabolism varies with age, especially between the adult and newborn. In the latter, liver metabolizing enzymes are lacking (Jondorf et al., 1958). Dietary influences, such as deficiencies (Conney et al., 1961) and fasting, both solids (Dixon et al., 1960) and liquids (Borcelleca and Manthei, 1957) alter drug metabolism. The differences in drug metabolism between sexes has been shown in the rat to be due to the influence of sex hormones (Quinn et al., 1958; Remmer 1958a). The pharmacological action of HB is prolonged after adrenalectomy (Remmer, 1958a, b) but decreased after thyroxin pretreatment (Conney and Garren, 1961). Barbiturate metabolism is altered by such pathological states as the presence of tumors (Rosso et al., 1968), liver disease (see below, under Metabolism), CNS lesions (Nair et al., 1970; Kostowski and Dolfini, 1969) or X-irradiation (Nair and Zeitlin, 1967). Other drugs can

alter barbiturate response through inhibition or stimulation of metabolism (see below) or pharmacological antagonism or potentiation. Changes in ambient temperature can alter the rate of barbiturate metabolism and its pharmacological effects (see below).

2. Termination of Activity and Metabolism

Three processes are responsible for termination of the central depressant actions of barbiturates: physical redistribution, metabolic degradation and renal excretion. These tend to decrease plasma concentration, resulting in a withdrawal of the drug from its CNS site(s) of action. Physical redistribution plays an especially-important role for the highly lipid soluble, ultrashort acting compounds like thiopental. The rapid emergence from hypnosis occurs as the barbiturate shifts from the brain to other tissue compartments (Areas of high blood flow such as the brain, exhibit maximum drug concentrations within minutes, while areas with lessor blood flow, such as muscle and fat, require much longer periods to become saturated.) Thus as these tissues slowly take up the drug, plasma concentration drops, causing the outward diffusion from the brain and subsequent loss of anesthesia, even though very little drug is metabolized,

Many barbiturates are transformed to inactive metabolites in the liver. Their metabolites are usually less lipid soluble, more polar and more water soluble, factors which tend to increase their renal excretion. Metabolism is usually via oxidation of the side chains in position 5, although ethyl groups are resistant, so barbital is excreted almost unchanged. The reaction occurs in vitro in the microsomal fraction of liver homogenates, requiring

molecular oxygen and NADPH, to form an epoxide, which is hydroxylated to the glycol. Secondary reactions such as oxidation of primary or secondary alcohols to carboxylic acids or ketones are carried out by enzyme in the nonparticulate fraction of liver homogenates. These products may appear in the urine as free compounds or the glucuronic acid conjugates. N-alkyl barbiturates may be converted to active metabolites by oxidation, such as metharbital and mephobarbital forming barbital and phenobarbital, respectively. In addition, desulfuration of thiobarbiturates and ring cleavage occur to a very small extent. Only barbital is primarily dependant upon renal excretion, although as much as 30% of phenobarbital, diallylbarbituric acid and aprobarbituric acid may be excreted unchanged by the kidney.

Since barbiturates are metabolized primarily in the liver, it is important to know whether they and other drugs have hepatotoxic effects. In normal subjects, hypnotic doses of barbiturates do not alter the results of any of the usual clinical tests of hepatic function. The large doses ingested daily by barbiturate addicts do not injure the liver in any detectable way. Even in patients with severe liver disease, the tests reveal no deleterious changes (Sessions et al., 1954). The barbiturates do not disappear less rapidly from the blood than in normal individuals. Experimental animals with a large portion of the liver removed surgically or severely damaged by carbon tetrachloride do sleep longer than normal controls after the administration of most barbiturates. Other drugs, such as SKF 525A and JB 516, specifically inhibit the liver microsomal metabolizing system and greatly enhance most barbiturate hypnosis. In experimental animals, nutritional deficiencies, diabetes and obstructive jaundice can prolong the action

of barbiturates. Stress can prolong barbiturate action, possibly due to adrenocortical action on hepatic drug metabolizing enzymes.

Of great importance is the fact that barbiturates can induce the enzymes responsible for their own metabolism. All barbiturates are not equally effective in this respect; phenobarbital has a very prominent action, while HB is quite weak. This accounts, in part, for the development of tolerance (and cross tolerance) to barbiturates. This phenomena can even be seen on the ultrastructural level in the liver, in terms of greatly increased amounts of endoplasmic reticulum (which contains the degradative enzymes) after only several doses of the barbiturate (Chedid and Nair, 1972). In addition, the barbiturates stimulate the production of enzymes responsible for metabolizing many other drugs, such as that of the coumarin anticoagulants. Barbital, which is not metabolized by the liver (and whose effects therefore are not prolonged by severe liver injury as mentioned above or environmental temperature changes as mentioned below), markedly enhances the enzyme activity responsible for metabolizing other barbiturates.

In vitro study of drug metabolism by microsomal enzyme has shown that a narrow temperature range is imperative to retain enzyme activity (+37°C. for rat liver; Leadbeater and Davies, 1964). Rink et al. (1956) found that in the isolated perfused rat liver a temperature fall from +37° to +24°C. extended the half-life of thiopental from 46 minutes to 537 minutes. This is equally important for in vivo experiments. The response produced by the drug should be correlated with body temperature, and one should consider the influence that alters physiological temperature regulation. Barbiturates render most laboratory animals almost poikilothermic, since these drugs

paralyze temperature regulation (Fuhrman, 1947). Sotaniemi (1967) summarizes several studies related to temperature and barbiturate metabolism and concludes "that the duration of sleep produced by short-acting barbiturates was extended in cold environment, whereas no similar prolongation of sleep was noted with long-acting barbiturates, such as barbital and phenobarbital".

3. Central Effects

Barbiturates are similar to the gaseous anesthetics in producing all degrees of CNS depression, ranging from mild sedation to coma and death. The degree of depression depends on the particular compound, dose, route of administration, certain physiological factors (see above), the degree of CNS excitability and previous experience with drugs which might alter metabolism.

The principal use of barbiturates is the production of sleep. In controlled studies, they shorten the average time of onset to sleep and lengthen the duration of sleep (Lasagna, 1956). Superficially barbiturate-induced sleep resembles physiological sleep, but, as should be apparent by now, normal sleep is extremely complex, and drug-induced sleep adds further to this complexity. In some individuals, small doses of barbiturates may produce excitement instead of sedation, probably because of an action similar to what is seen with ethanol intake; ie. by selective depression of inhibition. Subtle distortions of mood and impairment of judgement, vigilance, fine and gross motor movements and auditory discrimination can occur after barbiturate administration and can persist for many hours beyond the hypnotic effects. Some of these effects might be due to brain or plasma levels of metabolites, which are usually without effect but do remain in the body long after the

parent drug concentration has fallen to low levels.

a. EEG Effects

Small doses of barbiturates increase the high frequency EEG (15-35 Hz), producing "barbiturate activation", which appears first in the frontal cortex and then spreads to the parietal and occipital cortex. It is accompanied by clouding of consciousness and occasionally euphoria. As the dose is increased, large amplitude, random slow waves (2-8 Hz) similar to those occurring during normal sleep appear and consciousness is lost, although response to strong, painful stimuli is retained. Higher doses further decrease the amplitude with occasional brief periods of electrical silence---the "burst suppression" pattern. The periods of electrical activity disappears, a pattern similar to the depression produced by volatile anesthetics. The EEG during barbiturate-induced sleep resembles normal sleep except for the occasional bursts of spindles (14 Hz) and REM episodes, often preceded by several seconds of "saw tooth" waves (2-3 Hz). During the regular administration of barbiturates, the amount of REM sleep per night is reduced at first, but gradually returns to normal, as tolerance to the REM-suppression develops. When the drug is discontinued, there is a rebound increase in REM sleep and irregularities in REM sleep may continue for many nights.

b. CNS Excitability

In anesthetic doses, all barbiturates are effective antidotes to convulsant drugs and are capable of suppressing convulsions of strychnine poisoning, tetanus and status epilepticus. Phenobarbital has a selective anticonvulsant action, making it useful for symptomatic prevention of

epileptic seizures, especially of the grand mal type. Unlike other barbiturates, in anesthetic doses it completely abolishes the electrical excitability of the monkey motor cortex. In smaller doses, in most structures it elevates the threshold for minimal motor response and initiation of an afterdischarge shortens the duration of the afterdischarge and prolongs the recovery time for convulsive capacity.

c. Analgesia

Barbiturates, unlike the volatile anesthetics, lack significant ability to obtund pain in doses not impairing consciousness. Any analgetic effects are probably due to reduction of the psychic phase of the pain, rather than to a changed perception of pain. Ultrashort barbiturates such as thiopental, HB and methoxital are commonly injected IV to induce or sustain surgical anesthesia; to supplement inhalation anesthetics. They provide a rapid and pleasant induction, absence of salivation and reduce post-anesthetic excitement and vomiting.

d. Respiration

Barbiturates are respiratory depressants, affecting the drive to respiration and the rhythmic respiratory movements. These drives include that of the respiratory centers in the medullary and pontine RAS (neurogenic), a chemical drive, dependent on PCO_2 and arterial and CSF pH and a hypoxic drive. In the waking state approximately two-thirds of normal ventilation is due to the neurogenic drive. Sleep induced by hypnotic doses of barbiturates involves no more respiratory depression than occurs in normal sleep. With higher doses the neurogenic drive is first affected, leaving the direct

action of CO_2 and hydrogen ions on the respiratory centers, which also is lost as the dose is increased further. Death from barbiturate poisoning is usually attributed to respiratory failure by direct paralysis of the medullary respiratory center. The neural elements controlling muscles for respiration are resistant to barbiturates and can continue to discharge after other rhythmic activity has ceased. Analeptics, such as picrotoxin and pentylenetetrazol stimulate respiration either by resensitizing the medullary center to CO_2 or acting directly on the center.

4. Peripheral Effects on Barbiturates

a. Cardiovascular (CV)

In sedative or hypnotic doses barbiturates do not produce significant CV effects, except for a slight decrease in blood pressure and heart rate and a slight increase in cerebral blood flow, such as occur in normal sleep or in a recumbent position. Larger or IV doses produce a marked hypotension for several minutes in normals and hypertensives. Doses large enough to cause anesthesia generally produce a sustained decrease in the mean arterial and pulse pressure. The action on heart rate is variable, but the tendency is for an increase. In high doses barbiturates can exert direct effects on cardiac and vascular musculature, such as reducing contractile force. Cardiac output may be reduced, probably due to blood redistribution into the splanchnic bed and extremities. The important circulatory effect of barbiturates comes from their central depressing influence on vasomotor tone and control. Barbiturates do not sensitize the heart to Epi and are beneficial in reducing irregularities produced by cyclopropane.

b. Gastrointestinal (GI)

The oxybarbiturates tend to decrease GI tone and the amplitude of the rhythmic contractions. Hypnotic doses decrease colon and small intestine motility and increase the motility but decrease emptying time of the stomach, similar to that during normal sleep. Gastric and pancreatic juice secretion is reduced. In vitro barbiturates depress intestinal loop activity and reduce histamine-stimulated secretion in gastric pouches.

c. Urogenital

Hypnotic doses of barbiturates have only slight effects on urinary excretion, possibly due to sleep alone. In adults anesthetic doses decrease glomerular filtration rate, urine flow and electrolyte excretion. Large doses depress the tone and contractility of the ureters and urinary bladder. The uterus is resistant to barbiturates and hypnotic doses do not significantly impair uterine activity during labor, however anesthetic doses do decrease the force and frequency of uterine contractions and since they cross the placental barrier, may impair the infants' respiration.

5. Mode and Sites of Action

As general depressants, barbiturates depress the activity of nerves skeletal, smooth and cardiac muscle and reduce tissue oxygen consumption, as well as respiration of cell-free preparations of liver and brain mitochondria. These effects require a wide range of concentrations, generally higher than those necessary to produce CNS depression and it is unlikely that a common mechanism is involved.

a. Biochemical Effects

During barbiturate anesthesia there is a decrease in energy-yielding reactions in the brain; oxygen uptake and lactate and heat production are decreased. Certain barbiturates have been shown to depress uptake of inorganic phosphate in mitochondrial preparations with only a slight decrease in oxygen uptake, therefore it has been suggested that this "uncoupling" of phosphorylation from oxidation might be responsible for their depressant effects. Unfortunately not all barbiturates produce this uncoupling and some compounds which do are not hypnotics (eg. salicylates). In vivo effects include a decrease in the level of brain inorganic phosphate, an increase incorporation of phosphate into ATP and an increase in the level of phosphocreatine. These results suggest that the depression of oxygen consumption is due to reduced neuronal activity (Jenkins, 1969, p. 239).

Doses of barbiturates which cause sedation without loss of consciousness are without effect on cerebral circulation or metabolism (Kety et al., 1948). However larger doses always decrease cerebral oxygen consumption, with variable effects on blood flow. It seems that barbiturates exhibit no direct action on cerebral vasculature and changes are secondary to other effects.

b. Neurophysiological Effects

It is generally believed that the synapse is the site of action of hypnotic compounds, since chemical transmission is more susceptible to interference by barbiturates than is conduction along nerve or muscle fibers. At skeletal neuromuscular junctions barbiturates render the postsynaptic membrane insensitive to the depolarizing effects of ACh (Thesleff, 1956).

Barbiturates enhance the neuromuscular blocking effects of d-tubocurarine and decamethonium. The response of intestinal smooth muscle and certain neuroeffector organs, such as the submaxillary gland, to ACh and other spasmogenic agents is reduced by barbiturates. Little work has been done on the effect of barbiturates on adrenergic junctions, but some barbiturates may cause presynaptic liberation of transmitter. Barbiturates selectively depress sympathetic ganglia transmission in concentration without effect on nerve conduction, junctions or smooth muscle (Exley, 1954). The response of ganglion cells to preganglionic stimulation and choline esters is diminished by barbiturates, but they are not depolarized and respond to potassium chloride. Transmission is severely impaired by doses that do not prevent the release of ACh from preganglionic endings, although high doses of barbiturates may also reduce the efflux of ACh from stimulated fibers. This effect might account for the fall in blood pressure produced by some barbiturates.

Using intracellular recording techniques, the monosynaptic EPSP has been shown to be depressed by barbiturates in concentrations that have no effect on resting transmembrane potentials or the electrical properties of the membrane, probably by decreasing the release of transmitter (Løynning *et al.*, 1964). Polysynaptic pathways are somewhat more susceptible to barbiturates than monosynaptic reflexes (Wikler, 1945) but this cannot account for their hypnotic effects, since more selective polysynaptic depressants (eg. mephanesin) have little hypnotic effect.

Barbiturates have some local anesthetic action, increasing the threshold to electrical stimulation, reducing the amplitude of the spike potential and negative afterpotential, prolonging the relative and absolute

refractory periods and slowing conduction velocity (Heinbecker and Bartley, 1940). Large doses elevate the threshold for sensory stimulation. Schoepfle (1957) demonstrated that a solution of thiopental was five times more potent than equivalent amounts of procaine in rendering single fibers of frog sciatic nerves completely inexcitable with little or no effect on resting membrane potential or transmembrane resistance. In lobster axons, barbiturates reduce the maximum sodium and potassium transmembrane conductance and slow the rate of onset of sodium conductance after small depolarizing potentials.

c. Reticular Activating System (RAS)

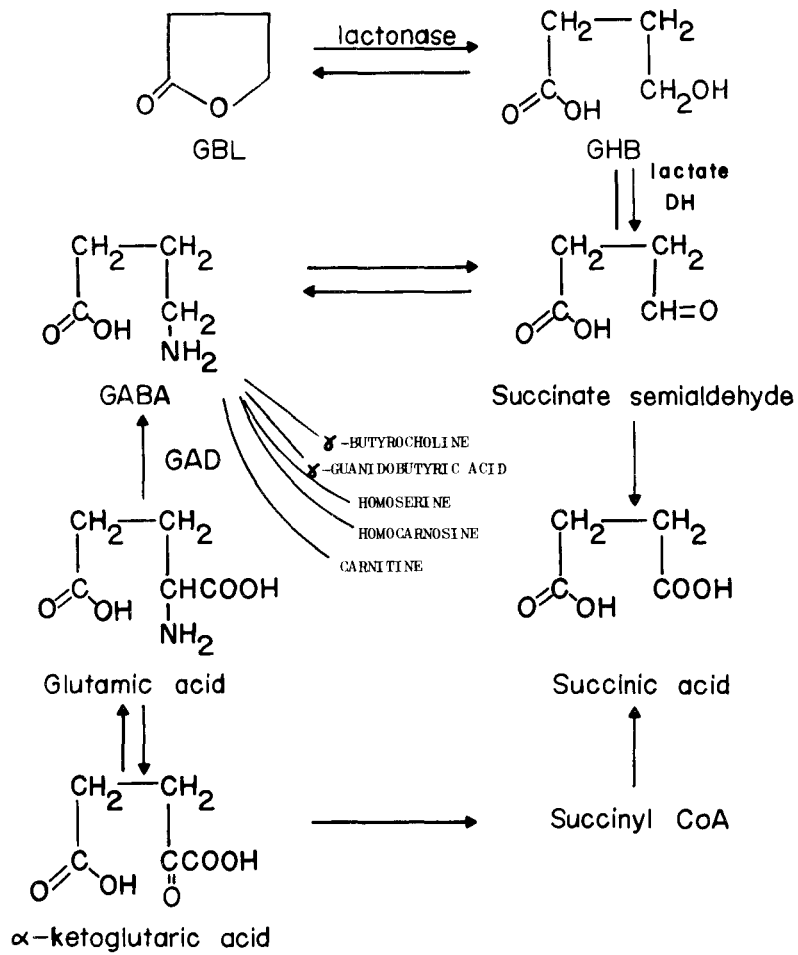
Although attempts have been made to localize the action of barbiturates to certain gross regions of the brain stem, such as the MB tegmentum or hypothalamus, drugs of this class apparently act at all levels of the nervous system. Nevertheless, the complex RF is exquisitely sensitive to the depressant effects of the barbiturates and other anesthetic agents. Whatever their effects elsewhere in the nervous system, it is the effect on the reticular system that seems to be responsible for the inability to maintain wakefulness under the influence of barbiturates. The present view of the importance of the RF in drug-induced sleep is based on a large body of evidence accumulated since the pioneering work of Moruzzi and Magoun (1949) (see review by Killam, 1962). In doses that only slightly affect transmission in the primary sensory pathways, barbiturates increase the threshold of the RF to direct electrical stimulation and depress the potentials evoked in the system by a variety of sensory stimuli. Lesions in the MB RF can reproduce

the characteristic EEG of barbiturate-induced sleep, while lesions of the primary sensory pathways do not.

G. Gamma-butyrolactone

1. Sedative Actions.

The sedative or hypnotic activity of short chain fatty acids (from C2 to C10) was studied by several investigators (Samson and Dahl, 1955; White and Samson, 1956; Holmqvist and Ingvar, 1957) before the introduction of modern techniques of continuous EEG recordings. Primary interest has since focused on two C4 compounds, gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL), a cyclic form that is converted to GHB by the action of a liver and plasma lactonase (Roth and Giarman, 1966; see Fig. 4). Jouany et al., (1960) who observed that low doses of butyric acid produced sleep and higher doses, anesthesia, were subsequently prompted to examine the action of the derivatives, GHB and GBL. Benda and Perles (1960) and Laborit et al. (1960) examined the EEG and physiological responses after GBL and GHB. They observed sleep-like states and anticonvulsant activity, as well as general anesthesia at higher doses. After a short latency, 50 mg/kg of GBL or GHB induce both states of sleep and a secondary increase in PS (Jouvet et al., 1961). The induction of PS was inconsistently found in the normal cat and was not observed in the rat (Marcus et al., 1967; Winters and Spooner, 1965). C5 and C6 compounds are even more effective than the C4 agents in triggering PS (Matsuzaki et al., 1964; Delorme et al., 1966a). In decorticate, mesencephalic or pontile cats, PS can always be induced almost immediately after IV injection, provided the compound is injected after the absolute refractory period (of 10-15 minutes) following the last PS episode. After a short latency, C4 and C6 compounds consistently produce a typical PS phase. The C4-C6 compounds appear to act at the level of the pons, since they still



induce PS in pontile cats but are ineffective after mediopontine transection (Tokizane, 1966; Matsuzaki, 1969) or after lesions of the dorsolateral pontine tegmentum (Jouvet et al., 1961). These compounds antagonize the inhibitory effects of atropine upon PS in pontile cats but are ineffective in inducing PS during the PS-suppressor effect of MAOIs (Delorme et al., 1965).

2. Active Form

Jenny et al. (1962) and others reported a similarity of the EEG patterns between anesthetic doses of GHB and PB. Winters and Spooner (1965; 1966) showed that at high doses (200-600 mg/kg), GHB produces a polygraphic pattern similar to PB and alpha-chloralose. They reported that after GHB a progression of various types of epileptiform EEG patterns were obtained. They suggested that GHB induced a state similar to generalized non-convulsant epilepsy, which could act to disconnect the cortex from subcortical regions. An alternate conclusion is suggested by the finding of Wikler (1952), who demonstrated a "divorce" or dissociation phenomena between the EEG and behavioral effects of several drugs.

The aforementioned studies stimulated neurochemical investigations of the mechanism of action of these compounds. There has been a great deal of controversy as to whether GHB or GBL is the active product and which of these is a normal constituent in the brain that might play a role in normal sleep, as a "natural hypnogen". Giarman and Roth (1964) initially were unable to demonstrate GHB in the brain, although Bessman and Fishbein (1963) did. Subsequently Roth and Giarman (1970) corroborated this finding when they utilized a more sensitive technique than in their earlier studies. Bessman

and Skolnik (1964) reported that the onset and duration of anesthesia correlated with the presence of the lactone in the brain, after either GHB or GBL was administered to rats. On the other hand, Giarman and Roth (1964) found that when rats were given GBL (500 mg/kg, IV) high brain concentrations ($10^{-2}M$) were achieved within one minute, followed by a decline due to redistribution from the brain and rapid conversion to GHB. When brain GHB content reached about 70 $\mu\text{g/gm}$, the righting reflex was lost. Recovery occurred when brain GHB fell below this critical level, suggesting that anesthesia is related to brain GHB content and not GBL. Guidotti and Ballotti (1970) obtained similar results. Roth et al. (1966) added further evidence that GHB was the active form in experiments that examined the effects of GHB and GBL in blocking transmission through the SCG and after direct injection into the CNS. Only GHB produced depression in the rat after intracisternal injection, while a greater delay in the onset of transmission blockade in the SCG was seen with GBL than GHB, suggesting that the delay might be due to conversion to the active form. When administered into the thalamus and hippocampus of unanesthetized monkeys by micro-injection, GHB immediately produced a slow-wave, high-amplitude EEG. GBL was without effect. Besson et al. (1971) found that anesthetic doses of GHB greatly reduced the dorsal root potential produced by segmentary stimulation, while the dorsal root potential produced by cortical stimulation was unaffected. They previously described (Besson et al., 1968) a similar action common to a variety of anesthetics, which act presumably by a presynaptic mechanism.

3. GABA in the CNS and Relationship to GBL

The close structural similarity between GBL and GABA (cf. Fig. 4) suggested that GBL might act on the GABA system. The physiological actions of GABA are interrelated with L-glutamic acid (GA), the main precursor of GABA (cf. Fig. 4). This conversion is catalyzed by the enzyme glutamic acid decarboxylase (GAD; EC 4.1.1.15) and is essentially irreversible (cf. Roberts, 1960). The major pathway of GABA degradation is via transamination (GABA: 2-oxoglutarate aminotransferase; EC 2.6.1.19) with α -ketoglutarate to form GA and succinic semialdehyde, requiring pyridoxal phosphate as a cofactor (Baxter and Roberts, 1958).

High levels of GABA are found in the peripheral nerves of crustaceans and in the mammalian CNS (Awapara et al., 1950; cf. Baxter, 1970). Kuffler and Eyzaguirie (1955) proposed that GABA might play an inhibitory role in the CNS. An inhibitory factor (Factor I) isolated from the brain was found to consist primarily of GABA (Bazemore et al., 1957). In the monkey brain, GABA levels are highest in the substantia nigra, globus pallidus and hypothalamus (Fahn and Cote, 1968). The thalamus and cerebral cortex contain lesser amounts, while white matter contains the least. Cerebellar GABA content is highest in axons of cells shown electrophysiologically to be inhibitory (Purkinje, basket, stellate and Golgi type II), supporting the theory that GABA has an inhibitory role in the CNS. GA is the most abundant amino acid in brain tissue (Tallan, 1962). Highest levels are found in the cerebrum and lowest levels in the spinal cord (Shaw and Heine, 1965). Extracellular administration of GABA produced hyperpolarization and increased membrane conductance in cortical neurons (Krnjevic and Schwartz, 1967), Deiter's cells (Obata et al., 1967)

and spinal motoneurons (Curtis et al., 1968) and is capable of blocking most types of cortical activity (Bradley, 1969). Peripheral injection of GABA produces no significant effects on evoked cortical potentials and intra-carotid injections have only brief, transient effects (cf. Baxter, 1970), probably due to the lack of penetration of the BBB. Young animals have incomplete BBB. In chicks, GABA (1.5 mg/kg) produces ataxia, incoordination, depression and a lack of responsiveness to visual stimuli (Scholes, 1965).

A number of recent studies have attempted to correlate the central levels of GABA and related compounds with the physiological state and pharmacological treatment of the animal. A homeostatic function for GABA during hypoxia is possible since after exposure of rats to 8% oxygen for 10 minutes brain levels are significantly elevated (Wood, 1967). Exposure of animals to hyperbaric oxygen lowers brain GABA content and induces seizures. Seizure susceptibility is inversely proportional to GABA levels (Wood et al., 1969). GABA injections (IP) protect animals against hyperbaric oxygen-mediated convulsions, but the effect is not specific and can be achieved with other amino acids and salts (Wood and Watson, 1964). Some convulsant drugs (eg. semicarbazide and thiosemicarbazide) lower brain GABA content, while others (such as hydrazine) elevate GABA levels (cf. Baxter, 1970). Anticonvulsant agents (such as hydroxylamine and glutamic acid hydrazide, GAH), which elevate GABA, do not protect against the convulsant effect of thiosemicarbazide at times when CNS GABA levels are distinctly elevated. Although GAH, hydroxylamine and AOAA all elevate GABA levels, only the latter two protect against pentylene-tetrazol convulsions, illustrating that there is no direct correlation with convulsions and CNS GABA content.

Apart from the possible involvement of GHB and GBL with GABA, only limited evidence links GABA with sleep phenomena. Deprivation of sleep (Mandel and Godin, 1964; Micic et al., 1967) and induction of sleep with strong light (Godin and Mandel, 1965) elevate rat brain GABA. In cats with brain stem transections at the upper MB (cerveau isolé), the cortical EEG shows continuous spindling characteristic of sleep. This "activated sleep" is accompanied by a release of GABA from the perforated cortical pial surface of $2 \mu\text{g/hr./cm}^2$. This rate is three times that in normal, intact, aroused animals or animals with brain stem transections of the upper cervical cord (encephale isolé) with aroused EEG patterns (Jasper et al., 1965). The pattern is reversed for the rate of release for GA. In rats treated with ethanol and GBL or GHB, the combination produced sleep (loss of the righting reflex) of much greater duration than that obtained by adding the durations of individual components (McCabe et al., 1971).

Several alternate routes of metabolism for GABA have been proposed, based on the product recovery and isolation of enzymes capable of catalyzing these transformations. Their physiological significance is unclear but the fact that several of them involve other brain amines, suggests an inter-relationship of these amine systems. GABA can combine with arginine to form gamma-guanidobutyric acid, which is found in the CNS and has an inhibitory action when applied to the mammalian cerebral cortex (Takahashi et al., 1961). Large amounts of gamma-guanidobutyric acid have been found at the epileptic focus of human cerebral cortex (Jinnai et al., 1966). Two histamine related products are formed from GABA, homocarnosine (GABA-histidine) and homoan-serine (GABA-L-methyl histidine). Both compounds are found in the brain.

Gamma-amino- β -hydroxybutyric acid (GABOB) was detected in rat brain and has inhibitor actions (Hayashi, 1959) although there is some controversy about its presence in the CNS. GABOB has been used clinically to treat epilepsy (Nishimoto et al., 1964). GABA is trimethylated in brain tissue in vitro to form gamma-butyrobetaine (Hosein et al., 1962) and this product can be hydroxylated in vivo and in vitro to carnitine, although no significant amount of carnitine is formed from ^{14}C -GABA administered peripherally to rats and mice (Lindstedt and Lindstedt, 1965). Gamma-butyrobetaine, carnitine and the CoA esters, acetyl-L-carnitine and acetyl-L-carnityl CoA have "ACh-like" actions (Hosein and Orzeck, 1966). Gamma-aminobutyrylcholine is found in the brain and although it does not cross the BBB, it has anticonvulsant effects. Homopantothenic acid and alpha-gamma-diaminobutyric acid which are structurally related to GABA have also been found in the brain.

4. Involvement of Other Amines with GBL

Giarman and Schmidt (1963) demonstrated that GHB increased ACh content in the brain stem (the dorsal pontine tegmentum). Parenteral administration of GHB, GBL or 1,4-butanediol (which is also converted to GHB, in vivo) results in a marked increase in brain DA with little or no effect on such amines as NE and 5HT (Gessa et al., 1966; Roth and Suhr, 1970) or GABA (Giarman and Schmidt, 1963). Roth and Suhr (1970) showed that the elevated brain DA has the same regional distribution as the normal endogenous amine. Aghajanian and Roth (1970), utilizing fluorescence microscopy, demonstrated that the selective increase in DA occurs exclusively within the terminals of DA-containing neurons. This is in contrast to the generalized increase in brain fluorescence

after MAOIs. The GHB-induced increase in DA is due to newly-formed DA, since it does not occur after inhibition of DA synthesis with AMPT, but is still seen after amine depletion with reserpine (Gessa et al., 1968). These investigators showed that GHB and related compounds do not inhibit MAO or COMT. In addition to the elevation in DA, Roth and Suhr (1970) and Roth (1971) demonstrated a corresponding reduction in subcortical HVA. The former study also reported that GBL produced a selective increase in the specific activity of brain DA, but not NE, in rats injected with ^{14}C -tyrosine (IV). They concluded that GBL (and GHB) increase brain DA primarily by selectively blocking its release from DA-containing neurons.

5. Mechanism of Action

A number of mechanisms of action for GBL and GHB have been proposed, ranging from physical to neurochemical, and are summarized here. These compounds might inhibit the metabolic activity of cerebral tissue, as they do in muscle and yeast (Samson et al., 1956). They might act upon cerebral metabolism by affecting the pentose shunt (cf. Laborit, 1964) or the metabolism of GABA (Fishbein and Bessman, 1964; Roth and Giarman, 1969). Mitoma and Neubauer (1968) demonstrated that GHB is converted to GABA, although there is no elevation of brain GABA after GHB administration. Godin et al. (1968) demonstrated that GHB increases cerebral glucose, decreases lactate and the incorporation of glucose carbon into GA, glutamine, aspartic acid and GABA in rat brain. A similar elevation of glucose in mouse brain, without a correlation with sedation or anesthesia was reported by Leonard and Watkinson (1971). Godin et al. (1968) showed that GHB can form succinate or GABA after

oxidation to succinic semialdehyde by lactate DH (cf. Fig. 4). DeFeudis and Collier (1970) reported a similar conversion of GHB to GABA and indicated that it proceeds via Krebs cycle intermediates.

The selective increase in brain DA and ACh (and decrease in HVA) suggests that the action of GBL might involve these amines. Since the locus coeruleus contains both ACh and DA (Shute and Lewis, 1966; Gerardy et al., 1969) and pontine neurons seem to participate in triggering PS, the effects of the C4-C6 compounds might be related to their selective effect on the pons. In the intact animal, the effects upon striatal DA might conceal the pontine effect.

Several investigators have suggested that the C4-C6 fatty acids might act at the membrane level (Dahl, 1968; Rizzoli and Galzigna, 1970) by reacting with membrane lipids and perhaps interfering with the movement of critical ions. Butyrate can bind with lecithin (in synaptic structures), 5HT and DA by forming a molecular complex when it passes through synaptic membranes. The formation of this complex might alter the equilibrium in the synaptic cleft and induce the release of additional transmitter or it might interfere with ACh release (Rizzoli and Galzigna, 1970). This hypothesis might also explain the absolute refractory period, when it is impossible to trigger PS pharmacologically or electrically because insufficient transmitter is available in the nerve terminals. When a sufficient amount of transmitter is subsequently synthesized, the fatty acids are then able to trigger PS.

H. Alteration of Drug-induced Sleep

Various physiological and pharmacological factors have been shown to alter the effects of hypnotic drugs. Environmental light is one which can bring about circadian changes in the effects of a variety of drugs, including hypnotics. This aspect of drug effectiveness, only recently considered, probably deserves consideration in almost all pharmacological studies and will be discussed in the section on Biological rhythms. Other factors also discussed below, are under the headings of physiological and pharmacological changes.

1. Physiological Effects

Vesell (1968a, b) thoroughly examined the duration of action of hexobarbital (HB) anesthesia (125 mg/kg, IP) in a number of species of mice. Some species sleep longer and have correspondingly lower liver hexobarbital oxidase (HBO) activity than animals that have short sleep durations. Genetic crosses between long-sleepers retained the long sleep duration and HBO activities, while crosses between long and short sleeping species had sleeping times and HBO activities intermediate to the two. The trait of male mice sleeping longer than females was maximal in inbred strains and diminished by outbreeding. The duration of HB-sleep in a NIH strain decreases progressively and significantly from one week of age and plateaus at three weeks, corresponding to the time-course of development of liver HBO activity. The age, sex, strain and species of experimental animals therefore are important in drug response. HB sleeping time decreased, while HBO activity increased in animals removed from their usual hardwood bedding and placed on red cedar (softwood) bedding for six days. Softwoods contain enzyme-inducing substances,

such as terpenes. The changes in sleeping time and enzyme activity were reversed by putting the mice back on hardwood bedding. A painful stimulus applied at regular intervals significantly shortens HB sleep without altering HBO activity. Crowding of animals also tends to shorten HB sleep. Mice maintained at 20°C. slept longer and had correspondingly lower HBO activity than those kept at 25°C. or 30°C. However, mice given HB are unable to survive environments of 15°C, or 37°C.

a. Temperature

Fuhrman (1946) summarized some of the earlier work done in mammals and lower animals on body temperature and drug action, including toxic and hypnotic effects. A number of investigators report that rats and mice at lower environmental temperatures sleep longer with a given dose of a barbiturate. Fuhrman (1946) reported that mice maintained at 27°C. slept almost four times as long after the onset of PB-induced sleep as those maintained at 37°C. There was no difference in sleeping time for barbital-treated animals at the two temperatures, indicating that the prolonged PB sleep was due to its decreased metabolism. Buchel and Tanguy (1967) reported similar findings in mice for doses of PB and HB producing sleep of a duration greater than 50 minutes. Mice maintained at a simulated altitude of 18,000 feet (hypobaric) for five days had a shorter loss of the righting reflex with HB, zoxazolamine and mephenesin but not with PB (Hawkins et al., 1971).

b. Diet

Kalyanpur et al. (1968) examined the influence of dietary factors on PB and barbital-induced sleep duration. These include high protein and high carbohydrate diets as well as vitamins (thiamine, nicotinic acid, tryptophane, and the B complex) and steroids (desoxycorticosterone, 4-chlorotestosterone, aldosterone, progesterone, stilbestrol, with the latter three administered on both an acute and chronic basis). Thiamine, nicotinic acid, biotin, progesterone, high carbohydrate and high protein diets significantly prolong PB sleep. Tryptophane, nicotinamide and pyridoxine and the steroids (except for progesterone administered acutely) decreased PB sleep. Lamson et al. (1951) tested glucose and related substances for their ability to restore hypnosis to guinea pigs awakening from barbiturate hypnosis. IP doses of glucose, galactose, levulose, several Krebs' cycle intermediaries, ascorbate, yeast, glutamic acid, glycerol and epinephrine were effective in "re-introducing" sleep in awakening animals, while sucrose, nicotinamide, pantothenic acid, glycine, alanine, ATP and DPN were not. There were marked species differences in the response; guinea pigs reacted to all active substances, while rabbits, hamsters, pigeons and chickens respond to a lesser extent. Rats do not respond to glucose but do to lactate and pyruvate. Mice respond very slightly, while goldfish and tadpoles do not respond at all. Lactate, pyruvate and glutamate decreased the onset of barbital anesthesia, presumably by increasing its rate of entry into the brain. The effect was antagonized by ACh. The authors hypothesized that the various active metabolites act by decreasing ACh synthesis, since they inhibit choline acetylase, which in turn would allow for a higher level of brain barbital.

c. Sex

The duration and intensity of drug response is often greater in the female adult than in the male adult rat. Brodie (1956) reported that female rats given HB slept four times as long as males, and had correspondingly lower metabolizing enzyme activity. Female rats administered testosterone slept less and had higher enzyme activity than controls, while males given estradiol slept longer and had lower enzyme activity than controls. These results indicate that the female is not more sensitive to the drug, but that the sex hormones influence drug metabolism. However, Brodie (1956) further stated that in mice, guinea pigs, rabbits and dogs such sex differences were not demonstrable.

Selye (1947, p. 59) demonstrated that steroids themselves are able to induce sleep. In order of decreasing potency, the steroids listed are: pregnanediol, desoxycorticosterone acetate, progesterone, testosterone and estradiol. Winter and Flataker (1952) studied the effects of steroids on the response of mice to HB anesthesia. Sleep duration was shortened by cortisone (SC) and ACTH (IP) treatment but not by desoxycorticosterone acetate (SC). Rupe et al. (1963) demonstrated that in rats stressed by hind leg ligation for 2.5 hours prior to testing, the duration of sleep from PB, HB and meprobamate, decreased in comparison to unstressed controls. The effect was absent in adrenalectomized rats and could be restored by treatment with ACTH or corticosterone. Similarly, Bousquet et al. (1965) showed that the duration of response to HB, PB, meprobamate and zoxazolamine was significantly reduced in stressed animals, while that to barbital and phenobarbital was unaffected.

The duration of response to HB in adrenalectomized or hypophysectomized rats was unalterable, suggesting that the effects of stress on drug response are mediated over the pituitary-adrenal axis. Rümke and Noordhoek (1969) reported that lynestrenol (a progesterone-like compound) decreased HB sleeping time, while enhancing its in vivo liver metabolism.

d. Hormones

Feeding mice 2% desiccated thyroid in their food prolonged PB and thiopental-induced sleep (Prange et al., 1966). Propylthiouracil (0.2% in the drinking water) decreased PB sleep but not thiopental sleep. In rats, thyroxin (100-1000 μgm , IM) only slightly prolonged PB in sleep, while thyroidectomy greatly prolonged PB sleep. The authors concluded that propylthiouracil and thyroid feeding of mice prolonged sleep by decreasing the activity of the metabolizing system, even though in rats thyroxin produced only slight increases in PB-sleep. Prolongation of PB in thyroidectomized rats is probably due to the general decrease in metabolic rate and the accompanying decreased body temperature. Conney and Garren (1961) reported that pretreatment of male rats with thyroxin prolonged the duration of action of HB by decreasing the activity of the liver enzyme metabolizing system.

e. Pathology

Kostowski and Dolfini (1969) reported that lesions of the midbrain raphe increase PB sleep in rats, even though such lesions have been shown to decrease SWS (Mouret et al., 1967) and cause persistent behavioral and EEG arousal (Kostowski et al., 1968). Bilateral lesions in the posterior hypothalamus suppress HBO activity, while lesions in the caudate nucleus,

hippocampus, preoptic hypothalamus or cerebral cortex produced no change (Nair et al., 1970). However sleeping time was significantly prolonged after hippocampal, preoptic and posterior hypothalamic lesions. Caudate but not cortical lesions decreased HB sleep. They suggested that HBO activity is regulated by the posterior hypothalamus, possibly through the release of hypophyseal tropic hormones. Exposure of young rats to sublethal X-irradiation inhibited the normal rapid increase in HBO activity occurring at three weeks of age, but was reversed three weeks later (Yam and DuBois, 1967). The X-irradiation appears only to delay the increase of enzyme activity. Exposure of the head only or hypophysectomy without irradiation also inhibit development of the HBO system, indicating a possible involvement of central hormonal regulation of HBO activity. Starvation prolonged HB sleep (Dixon et al., 1960; Fujii et al., 1968) while cachexia produced by feeding rats a rancid, standard biotin deficient diet decreased PB sleep (Peters and Boyd, 1966).

2. Pharmacological Effects

Kato and Chiesara (1962) reported that a number of centrally-acting drugs decreased the duration of PB sleep (25 mg/kg, IP) in rats. They include phenobarbital, phenaglycodol, glutethimide, nikethamide, meprobamate, chlorbutol and chlorpromazine. The effect developed 24 hours after treatment and was maximal after another 24 hours. PB metabolism in liver slices of similarly-treated animals was increased. Boissier et al., (1967a,b) examined the central effects of sympatholytics on a subhypnotic dose of PB (25-30 mg/kg) in mice and rats. At the higher doses employed, phenoxybenzamine,

dibenzamine, phentolamine, yohimbine, dihydroergotamine, propranolol, guanethidine, α -methyl-DOPA and chlorpromazine caused a high percentage of mice to sleep, when administered 35-65 minutes prior to PB and they reduced spontaneous activity and rectal temperature. Bretylium (8-32 mg/kg) was without effect. Consolo et al. (1965) and Sadre and Tiwari (1966) also reported the prolongation of PB sleep by chlorpromazine. Peters (1972) studied the prolongation of PB hypnosis in mice by the β -blocker, alprenolol. The increased duration of sleep was not related to an altered tissue distribution or hypothermia. Alprenolol decreased NADH^+ phosphate oxidase, cytochrome c reductase and cytochrome P450 reductase activities and cytochrome P450 content. Naik et al. (1969) demonstrated potentiation of PB sleep by the analeptics nikethamide and pentylenetetrazol and antagonism by picrotoxin. Paradoxially, all three compounds produced a similar elevation in brain PB levels.

The effect of pretreatment with α -methy-L-tyrosine (same as AMPT) on the duration of PB sleep, alone and in combination with chlorpromazine, reserpine or meprobamate was tested by Menon et al. (1967). The only significant increases in PB sleep occurred in AMPT-treated animals concomitantly given reserpine or chlorpromazine. Richards et al. (1965) examined, with respect to sleep duration and arousal time (from an electrical stimulus), the effects of pretreatment of mice with chlorpromazine, reserpine, meprobamate and hydroxyzine, given immediately before HB (100 mg/kg, IP). All prolonged sleep significantly at the higher doses and at their lowest doses with meprobamate and hydroxyzine. Smith (1961) reported that reserpine (100 $\mu\text{gm}/\text{kg}$, SC) given 20 hours prior to barbital (200 mg/kg, IP) significantly

potentiated barbital sleep in rats. LSD (1 mg/kg) given 30 minutes prior to barbital antagonized this effect of reserpine. However, Salmoiraghi and Page (1957) demonstrated that low doses of LSD or bromo LSD (2 μ gm/kg, one hour prior to HB) enhanced the prolongation of HB sleep (70 mg/kg) produced by 5HT (20 mg/kg, 10 minutes prior to HB) and reserpine (5 mg/kg, one hour prior to HB). Child et al. (1961) demonstrated that reserpine (2 mg/kg, IP) pretreatment, 1-6 hours before its administration, shortened barbitone-induced sleep. Sleep returned to normal by the 2nd day, increased on days 3-4 then became normal again. The rate of penetration of barbitone into the brain as measured by brain levels was reduced by reserpine pretreatment.

To produce stress in rats, Bousquet et al. (1965) administered histamine (5 mg/kg, IV) 2.5 hours prior to HB and obtained decreased HB sleeping time. Winter and Flataker (1952) demonstrated in mice that the antihistamine, diphenhydramine (10 mg/kg, 30 minutes prior to the anesthetic) prolonged sleep induced by HB or ether. Winter (1948) reported that the antihistamines, diphenhydramine, pyranisamine, tripeleminamine, and promethazine, prolonged HB (100 mg/kg, IP) sleep in mice and that diphenhydramine prolonged PB (50 mg/kg, IP) sleep. He also found that diphenhydramine prolonged PB-sleep (35 mg/kg, IP) in guinea pigs. Pierre and Cahn (1956) found that 5HT (5 mg/kg and 20 mg/kg) administered 5 minutes and 10 minutes respectively after thiopental (40 mg/kg, IP) significantly prolonged sleep, but not after PB, thialbarbital, urethane or ether. Buchel et al. (1960) also reported prolonged sleeping times in mice after 5HT (50 mg/kg, IP and SC) which decreased after iproniazid (150 mg/kg, IP) or JB 516 (10 mg/kg, IP) given 24 hours prior to the HB. Iproniazid was effective in blocking the prolongation of HB sleep produced

by 5 HTP (25 mg/kg, IV) given 5 minutes prior to HB. Baldieri (1965) showed that pargyline (2.5-10 mg/kg, IP), given 15 minutes prior to PB (30 mg/kg, IP) prolonged PB sleep in rats.

Proctor et al. (1964) demonstrated that several anticholinesterases enhanced the hypnotic activity of HB in mice. TEPP prolonged HB sleep and its effect could be antagonized by atropine but not by atropine methyl bromide. Perphenazine combined with TEPP greatly enhances HB sleep and was similarly blocked by atropine but not atropine methyl bromide. Barnes and Meyers (1964) reported that eserine, given concomitently with PB, decreased sleep, as did amphetamine, alone or with eserine. Neostigmine had no effect on PB sleeping time, suggesting that the cholinergic mechanisms activated were central.

Significant prolongation of PB sleep (65 mg/kg) in mice was produced after a one hour pretreatment with aminopyrine and phenylbutazone (both at 25 mg/kg; Eckhardt et al., 1958). Prednisone (25 mg/kg), morphine (1 or 25 mg/kg) or acetylsalicylic acid (25 or 250 mg/kg, orally) were without effect. The effective drugs significantly elevated brain PB content. However, Coldwell and Peters (1968) reported that a one hour pretreatment with acetylsalicylic acid (0.2-1.9 mg/kg, orally) significantly potentiated hypnosis in rats, at several doses of PB or thiopental. Quinine and quinidine (both 50 mg/kg, IP) prolonged PB-induced sleep in mice and rats (Boulos et al., 1970). Both drugs decreased the rate of loss of PB content in goat plasma, without a change in the volume of distribution and were potent competitive inhibitors of PB metabolism in rat liver homogenates. Quinine did not alter PB brain levels significantly in mice, 5 minutes after PB or upon awakening.

Seto and Keup (1969) tested a number of alkylmethoxybenzene and alkylmethylene-dioxybenzene essential oils on PB (50 mg/kg) and ethanol (3.5 gm/kg)-induced sleeping time in female mice. Myristicin, isomyristicin, safrole and elemicin at doses of 20 mg/kg double PB sleeping time. At a dose level of 50 mg/kg, asarone, isoelemicin, ortho-anethole, eugenol methyl ester and isoeugenol methyl ester double sleeping time over controls. Asarone (at 75 mg/kg) was the only essential oil capable of doubling the sleeping time over control, ethanol-treated mice. Several other essential oils tested were much less effective in prolonging PB or ethanol-induced sleep. Jori et al. (1969) reported that in rats eucalyptol (500 mg/kg, SC, 18 or 36 hours pretreatment) significantly decreased PB sleeping times and brain PB content. Other constituents of essential oils, such as guaiacol, menthol, oil of Pinus pumilio, α - and β -pinene, are not effective in altering PB sleep duration.

Conney (1967) reviewed the pharmacological implications of liver microsomal enzyme induction by a wide spectrum of drugs, including hypnotics, anesthetic gases, CNS stimulants, anticonvulsants, tranquilizers, muscle relaxants, antihistamines, alkaloids, insecticides and steroids. Some of these act as competitive inhibitors of drug metabolism, in oxidative and demethylation reactions. Remmer (1962), Conney and Burns (1959) and other investigators demonstrated the stimulating effect of barbiturates on microsomal metabolizing enzymes. Most inducers are like phenobarbital. They stimulate various pathways of metabolism by the liver microsome system, including oxidation, reduction, glucuronide formation and deesterification. Polycyclic aromatic hydrocarbons stimulate a more limited group of reactions,

such as some hydroxylating pathways. The course and intensity of induction also differs. Phenobarbital, given daily, produces a maximum in induced enzyme activity (3-10 fold increase over control) after at least three days, while a single IP injection of dieldrin (40 mg/kg) or DDT (200 mg/kg) in rats elevates HB and acetophenetidin metabolism to maximum (2-3 times control) in 5-10 days (Ghazal et al., 1964). Enzyme induction results in a more rapid metabolism and usually a decreased drug effect, i.e., diminished toxicity or duration of hypnotic sleep. In some cases, however, the metabolite might be the active form, leading to exaggerated effects.

Brodie (1956) described the potent enzyme inhibitory action of SKF 525A. Animals treated with this compound and then administered a hypnotic, such as HB, sleep much longer than controls. SKF 525A, which has no sedative action of its own, also prolongs the action of amphetamine. It acts by inhibiting hydroxylation, N-dealkylation, some O-dealkylation, deamination, sulfoxide formation, reduction of azo and aromatic nitro compounds, some hydrolysis reactions and the formation of glucuronides. However, there are species and drug exceptions to the above.

The alteration of the effects of the non-barbiturate hypnotics, chloral hydrate and ethanol should be briefly considered. Fastier et al. (1957) reported that chloral hydrate (250 mg/kg, IP) sleeping time was prolonged by adrenaline, noradrenaline, phenylephrine, methoxamine, 5HT, histamine, ergotamine, yohimbine and atropine. They stated this effect was not due to circulatory actions but possibly due to lowering body temperature. Buchel et al. (1960) demonstrated in mice that 5HT (10 mg/kg, SC) produced sleep when given 3 hrs prior to a subhypnotic dose of chloral hydrate

(26 mg/kg, IP). (The peak effect occurred 30 minutes after 5HT). Chloral hydrate sleep (325 mg/kg, IP) was prolonged by 5HT (50 mg/kg, SC and IP, up to 60 minutes prior) or 5HTP (25 mg/kg, IV, 5 minutes prior). Buchel and Levy (1960) found that in mice, reserpine (5 mg/kg) produced sleep after a subhypnotic dose of chloral hydrate (200 mg/kg), which was antagonized by JB 516 (5-10 mg/kg) and ipronized (50-100 mg/kg). Reserpine (2 and 5 mg/kg) prolonged a hypnotic dose of chloral hydrate (300 mg/kg), which was antagonized by JB 516 (2.5 and 5 mg/kg, 20 minutes prior) and iproniazid (50 mg/kg, 22 and 46 minutes prior) but not by iproniazid (25 mg/kg). 5HTP (5-25 mg/kg, IV) and DOPA (10-25 mg/kg, IV) reinduced sleep in animals awakening from chloral hydrate (200-225 mg/kg). The effects of both compounds were antagonized by iproniazid (150 mg/kg, IP) given 24 hours prior to the hypnotic. Sadre and Tiwari (1966) showed that histamine (10 mg/kg) and chlorpromazine (10 mg/kg), alone or together, prolonged sleep induced by chloral hydrate (400 mg/kg) in mice. Lechat and Levy (1969) reported that in mice, DL-DOPA (150 mg/kg, IP) 10 minutes prior to chloral hydrate (250 and 325 mg/kg, IP), doubled sleep duration, and was antagonized by the MAOI furazolidone (500 mg/kg, orally, 24 hours prior).

Blum et al. (1971) reported that in mice, pyrazole, an inhibitor of liver alcohol dehydrogenase, produced a dose-dependant prolongation of ethanol-induced sleep, when given 30 minutes prior to ethanol. Blum et al. (1972b) showed that glycine (0.9 mmoles/100 gm, IP) given 5 minutes after ethanol significantly prolonged sleep over controls. This effect appears to be due to central actions and not to altered ethanol metabolism.

Carlsson et al. (1972) demonstrated in rats and mice that AMPT (40 or 200 mg/kg), which in itself causes no significant depression of motor activity, reduced the normal increase in motor activity produced by small doses of ethanol (1 or 2 gm/kg). Blum et al. (1972a) found that AMPT (3 x 80 mg/kg or 400 mg/kg) enhanced ethanol-induced sleep time in mice. AMPT-L-DOPA treated animals had a reduction of the AMPT-alone increase in ethanol sleep, with a 60% increase in whole brain DA. L-DOPA alone (3 x 400 mg/kg/day) appears to prolong ethanol sleep, although they did not mention if this increase was significant.

III. MATERIALS AND METHODS

A. Experimental Animals

Male albino rats of the Sprague-Dawley strain (Holtzman Breeders, Madison, Wisconsin) weighing 300-400 grams each, were used in these experiments. Males were chosen in order to reduce variability introduced by hormonal cycles, which is greater in the female. They were housed in groups of six in stainless steel wire mesh cages, 29 x 23 x 23 cm. A uniform mixture of Pel-e-cel (absorbant compressed cellulose; Paxton Products, Paxton, N. J.) and Litter-green (McFadden Industries, Inc., Kansas City, Missouri) was used in trays beneath cages, such that animals had no direct contact.

Male albino mice of the Swiss-Webster strain (Simonson), weighing 20-35 gm each, were maintained in clear plastic cages, 28.5 x 18 x 13 cm, 6-8 per cage.

Purina Rat Chow pellets and tap water were provided ad libitum. The composition of the Rat Chow consisted of approximately 23% protein, 5% fat, 4% crude fiber and 6% ash, plus vitamins and minerals (figures provided by Ralston Purina Co., St. Louis, Missouri). Feeding, watering and cage cleaning were performed at random times of day to prevent these procedures from causing an exogenous rhythm.

B. Animal Housing Conditions

The rack of animal cages was kept in a quiet, well ventilated, windowless room, measuring 2.7 x 3.7 x 4.1 meters. Stable ambient temperature of $23 \pm 1^{\circ}\text{C}$., relative humidity of $25 \pm 5\%$ and barometric pressure of

730-760 nm of mercury were monitored by a Lambrecht KG recorder.

Animals were adapted to a lighting schedule of 0800-2000 hours C.S.T. light and 2000-0800 hours dark, using an automatic 24-hour timer. Fluorescent Vita-lites supplied by Duro-Test Corp. (North Bergen, N. J.) provided a lighting spectrum approximating daylight. Cages were 5-6 feet from the lights, resulting in a radiation intensity of 20-50 μ watt/cm², as measured on a radiant flux meter (12-46 footcandles, using a Gossen Luna-Pro light meter). All animals were adapted to these conditions for a minimum of three weeks prior to use in experiments.

During the dark phase, various procedures, such as feeding, drug injections, etc., were performed under red light (25 watts, about 750 μ watts/cm² radiant energy but about 8 footcandles of light).

C. Experimental Procedures

1. Measurement of drug-induced sleep.

The duration of drug-induced sleep was measured as the time from the loss (onset) to the recovery of the righting reflex. Recovery is defined by Vesell (1968b) as the ability of the animal to place both forefeet simultaneously on the ground twice within 15 seconds, when placed on its back. According to Vesell (1968a, b) unstimulated animals will sleep longer than stimulated ones. In order to standardize stimulation the tails of the animals were pinched approximately every three minutes, once they began to emerge from deep sleep (as evidenced by slight movements of their limbs).

The durations of PB-sleep were first determined for the various

pretreatments at each of the four times (0600, 1200, 1800, 2400 hours) during the 24-hour period. A second set of animals were given the same pretreatment dosages and PB at these times, but sacrificed half-way through the previously-determined sleeping times ($t 1/2$). Vehicle-treated controls were sacrificed at the same time as treated animals. From these results a control curve could be established, showing a time course for monoamine changes at the four times. The amine content of brain parts from treated animals could then be compared with the control curve at the appropriate time. During the dark phase, injected animals were maintained under red light or in darkness, and removed quickly to an adjacent, dimly-lit room for decapitation.

2. Drug dosages

Pentobarbital sodium (PB) was dissolved in 0.9% saline (25 mg/ml) and administered (50 mg/kg, IP) to control groups which received only the vehicle used in pretreatment, and treated groups, which received one of the pretreatments described below. Gamma-butyrolactone (GBL), a liquid, was diluted with saline, to yield a final GBL concentration of 350 mg/ml in 0.9% saline. GBL (350 mg/kg, IP) was administered to controls and pretreated animals. In order to examine their effects with respect to the time of day, these drugs were administered at six-hour intervals.

To investigate the role of biogenic amines in drug-induced sleep, several pretreatments which alter CNS amine levels, were utilized prior to testing for the duration of sleep produced by PB or GBL. Pretreatments include amine depletors and precursors. Pretreatment drug doses and times of administration were chosen to optimize the change in CNS levels of one or

more monoamine being studied. These were selected, whenever possible, from previously-published reports.

The DA (and NE) precursor, DL-DOPA, was administered at a dose of 500 mg/kg, IP (equivalent to 250 mg/kg of L-DOPA, the active isomer), one hour prior to the sleep-inducing drug. Because of the low water solubility of this high dose, and the desire to use low injection volumes (about 1.0 ml or less), the DOPA was suspended in 1% methyl cellulose (250 mg/ml). Pargyline, an MAO inhibitor (6 mg/kg, IP, in 0.9% saline) was given 30 minutes after DOPA. This treatment primarily elevates DA and NE to a lesser extent (Chamber et al., 1971). The DOPA-pargyline treatment is used to raise and maintain the elevated brain amine levels. Alpha methyl p-tyrosine (AMPT, 200 mg/kg, IP, dissolved in 1N NaOH and neutralized with an equal volume of 1N HCl to produce a fine suspension, 66.67 mg/ml) was administered 4 hours prior to PB or GBL. This amine acid analogue of tyrosine depletes NE stores by competing for tyrosine hydroxylase. p-Chlorophenylalanine (pCPA) has been shown by several investigators to deplete brain 5HT while only decreasing CA concentrations slightly (Koe and Weissman, 1965; Mouret et al., 1967). Three doses of 100 mg/kg, IP (in 1% methyl cellulose) were administered 72, 48 and 24 hours prior to the administration of PB.

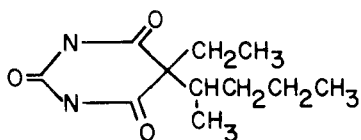
A set of experiments were conducted in mice, testing the effect of cholinergic drugs on PB-sleep. Neostigmine or physostigmine (0.3 mg/kg, IP) were administered 30 minutes prior to PB (50 mg/kg, IP).

The following table lists the drugs used, summarizes their doses and the intervals prior to the administration of PB or GBL. In every

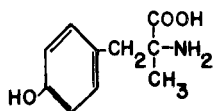
instance, the hypnotics were given at six-hour intervals (i.e., 0600, 1200, 1800 and 2400 hours). The pretreatments were always timed with respect to these times. Injections, during the dark phase, were conducted under red light. (Some of the drugs used are shown in Fig. 5).

<u>DRUG</u>	<u>DOSE (mg/kg)</u>	<u>TIME PRIOR TO HYPNOTIC INJECTION</u>	<u>REFERENCE</u>
pCPA	3 x 100	72, 48, 24 hours	Koe and Weissman, 1966
L-AMPT	200	4 hours	Hollinger, 1969
DL-DOPA- pargyline	500 6	1 hour 30 minutes	Walker <u>et al.</u> , 1971
L-Hd	100	1 hour	Boissier <u>et al.</u> , 1970
	500	1 hour	Taylor and Snyder, 1972
	1000	1 hour	Taylor and Snyder, 1972 Campos and Jurupe, 1970
L-TP	500	1 hour	Moir and Eccleston, 1969 Campos and Jurupe, 1970
DPH	50	1 hour	Boissier <u>et al.</u> , 1970
DB	15	24 hours	Medina <u>et al.</u> , 1969
M	25 50	(same time) "	Barchas <u>et al.</u> , 1967
DL-5HTP	100	1 hour	
Neostigmine	0.3	30 minutes	Proctor <u>et al.</u> , 1964
Physostigmine	0.3	30 minutes	Proctor <u>et al.</u> , 1964
5MT	20	1 hour	
N-acetyl 5HT	20	1 hour	
5HIAA	20	1 hour	
5MIAA	20	1 hour	

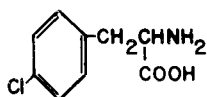
Pentobarbital



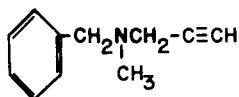
α -methyl-p-tyrosine



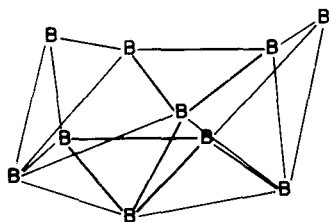
p-chlorophenylalanine



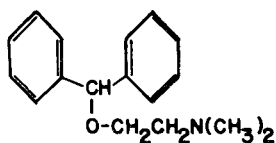
Pargyline



Decaborane (B₁₀H₁₄)

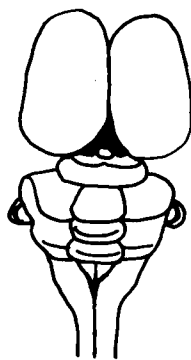


Diphenhydramine

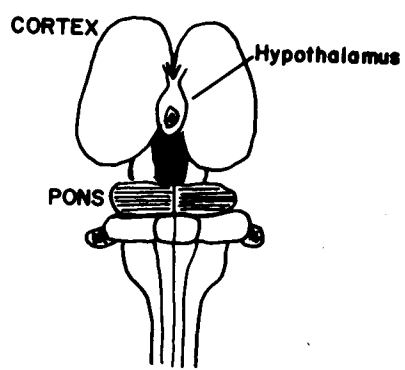


3. Brain Dissection

Rats were decapitated by means of a Harvard decapitator. The skin covering the skull was incised with a pointed surgical scissors and the brain exposed by an incision beginning at the foramen magnum and following the midline suture. The dura was incised ventral surface upward. The brain was freed from the skull by severing the cranial nerves and placed on a saline-moistened gauze pad which rested on an ice-cold aluminum block. Using fine curved tweezers, the hypothalamus (Fig. 6) was removed by undercutting it from the rest of the brain and placed in liquid nitrogen within 2 minutes after sacrifice. The brain was reoriented to its normal position, the cerebral hemispheres separated to expose the caudate nuclei (CN, Fig. 6), which were removed by undercutting and then placed in the liquid nitrogen (-195 °C). The cerebrum was removed by cutting the cerebral peduncles above the stria terminalis. This constitutes the anterior border of the tissue designated "midbrain" (MB, Fig. 6). The posterior limit was cut from the top of the pons on the ventral side to the bottom of the inferior colliculi on the dorsal side. The tissue was then placed in liquid nitrogen. Midbrain includes the thalamus and subthalamus, as well as the subcollicular tissue. The cerebellum was removed from the remaining brain. The region designated as "stem" (lower brain stem) (Fig. 6) was obtained by cutting posteriorly at the decussation of the pyramidal tracts (sulcus medianus dorsalis). This tissue was placed in liquid nitrogen in less than 5 minutes after sacrifice. The tissues were maintained in liquid nitrogen for several minutes to insure complete freezing. The brain parts were then sealed in

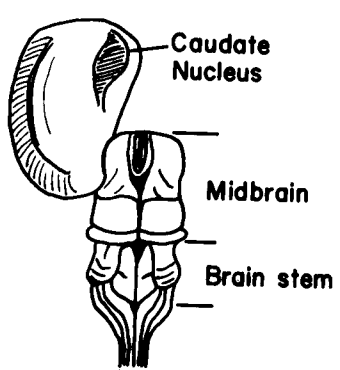


DORSAL

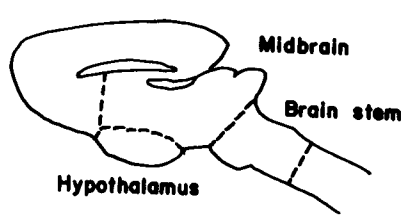


CORTEX
Hypothalamus
PONS

VENTRAL



Caudate Nucleus
Midbrain
Brain stem



Midbrain
Brain stem
Hypothalamus

Figure 6: Brain Dissection

Parafilm, labelled and stored in the deep freezer (-25°C.) until they were analysed for amines (vide infra).

The reasons for selecting brain areas instead of utilizing the whole brain for analyses are as follows: It has become increasingly clear that analysis of the amines of the whole brain results in a "homogenizing" of important differences among various brain areas, which have unique anatomical, physiological and neurochemical characteristics. The hypothalamus (Hpth), a center for autonomic function, has high concentrations of NE and lesser amounts of 5HT. It contains a number of nuclei which send fibers out to other areas of the brain and peripheral nervous system. The MB lies above the Hpth and is often combined with it for amine analysis. The MB contains moderate amounts of NE and lesser amounts of 5HT. It includes NE-containing areas and the head of the 5HT-containing raphé system, which are involved in the control of sleep. The MB includes the superior and inferior colliculi which are involved in visual and auditory reflexes, respectively, processes that are modulated during sleep. The brain stem, caudal to the MB, contains the NE-containing locus coeruleus, as well as the main portion of the raphé system. The CN is important because it is part of the basal ganglia, which is involved with motor control. The CN has very high concentrations of DA and high ACh concentrations and high AChE activity.

4. Brain Amine Analysis

Monoamine levels in the CNS were determined fluorometrically using slight modifications of the method of Shellenberger and Gordon (1971). The basis of this and similar methods (Chang, 1964; Ansell and Beeson, 1968)

is the adsorption of catecholamines on alumina (batch technique) and the removal of serotonin from the remaining supernatant by organic extraction. An acid eluate of the alumina is buffered to neutral pH and oxidized for form a fluorescent trihydroxyindole derivative. Serotonin is determined by reaction with ninhydrin in a pH 7.0 buffer to form the fluorescent compound.

a. Reagents:

1. 0.4 N perchloric acid: to 56.8 ml of 70.8% solution per liter of distilled water, add 1.0 gm sodium metabisulfite ($\text{Na}_2\text{S}_3\text{O}_4$) and 0.5 gm disodium EDTA per liter.
2. Tricine solution: 17.9 gm Tricine (N-tris(hydroxymethyl) methylglycine) and 25 gm disodium EDTA in each liter of 0.525N NaOH (42 ml per liter of distilled water of a 50% NaOH solution, 12.5 N).
3. 0.5 M borate buffer, pH 10.0: 30.92 gm per liter of distilled water of boric acid (results in pH of about 3.4) then adjusted to pH 10 with NaOH pellets and saturated with NaCl and n-heptanol (about 1 ml).
4. 0.05 M phosphate buffer, pH 7.0: 3.11 gm $\text{H}_2\text{H}_2\text{PO}_4$ · H_2O and .34 gm Na_2HPO_4 per liter distilled water.
5. Phosphate buffer - EDTA solution: Add 9.0 gm disodium EDTA to 1.0 liter of 0.1 M phosphate buffer (4.27 gm Na_2HPO_4 and 9.52 gm KH_2PO_4 per liter distilled water) and adjust to pH 7.0 with 5.0 N NaOH (made from 50% NaOH).
6. 0.1 N iodine reagent: 4.0 gm KI and 1.0 gm iodine are dissolved in distilled water and q.s. to a final volume of 80 ml.
7. Alkaline sodium sulfite: Dilute 1.0 ml (250 mg/ml in distilled water), sodium sulfite (kept in freezer until used), to 10 ml

with 5.0 N NaOH

8. 0.1 M ninhydrin: 445.4 mg ninhydrine dissolved in 25 ml distilled water (the ninhydrin solution is stored in the freezer).

b. Amine Separation

At the time of analysis individual brain parts are weighed on a Roller-Smith torsion balance (Model LG) and placed in 3 mls of cold 0.4 N perchloric acid contained in Kontes Duall tissue grinder tubes (size 22) and maintained in crushed ice (see Fig. 7). The tissues are then homogenized using a motor-driven Teflon pestle (Kontes). After homogenization the tubes are kept in ice for about 10 minutes and then centrifuged for 10 min. at maximum speed (2200 rpm or 1000 g) on an IEC Model K centrifuge (radius head, 7.5 inches). The supernatant was removed and placed in 35 ml Wilkens-Anderson reaction vessels. The pellets were rehomogenized in 2.5 ml of 0.4 N perchloric acid, centrifuged and the second supernatants added to the first. Standard tubes were prepared at this point utilizing high and low concentrations of each standard in 5.5 ml of 0.4 N perchloric acid. Perchloric acid alone served as a reagent blank.

The pH of each supernatant is then adjusted to 7.8 ± 0.2 (usually 7.8 - 7.9) with Tricine buffer, using a Beckman fine tip combination electrode calibrated to pH 8.0 with standard buffer on a Beckman Model 72 pH meter. To this supernatant about 300 mg of activated alumina was added. The alumina (aluminum oxide, Woelm) was prepared by washing several hundred grams in tap water overnight in a Hurricane Photo Washer. In this device water enters from the bottom and agitates the alumina constantly so that the fine particles are washed away. The remaining alumina was washed in boiling HCl

Figure 7: Amine analysis flowsheet

TISSUE
homogenized in
3.0 and 2.5ml of
0.4N perchloric acid

adjust to pH 7.8 with TRICINE

SHAKE WITH 300mg ALUMINA, centrifuge

ALUMINA(CAs)

Supⁿ(5HT)

- 1) wash with distilled H₂O
- 2) elute with 0.05N perchloric acid, take 1.0ml aliquots
- 3) ADD 1.5ml phosphate buffer, pH 7.0
+
0.2ml iodine , wait 2 min.
+
0.5ml alkaline sulfite
+
0.4ml glacial acetic acid
- 4) HEAT 2 min. @ 95°C., then cool in ice bath
- 5) READ NE, 380nm A, 495nm E
- 6) reheat for 10 min.
- 7) cool, wait 30 min.

READ DA, 325nm A, 380nm E

- 1) NaCl saturate, add 15.0ml heptanol
- 2) adjust to pH 9.8 with K₂CO₃
- 3) vortex mix 1-1.5 min., centrifuge
- 4) aspirate aqueous phase
- 5) wash heptanol with pH 10 borate buffer, centrifuge
- 6) transfer 13.5ml heptanol
ADD 2.5ml phosphate buffer, pH 7.0
- 7) SHAKE, centrifuge
- 8) aspirate heptanol
- 9) to 1.0ml aliquots,
ADD 0.1ml ninhydrin
HEAT 25 min. @ 100°C., cool 1 hour
- 10) READ 5HT, 385nm A, 490nm E

(Anton and Sayre, 1962), then in distilled water until the pH of the water reached 3.4. It was dried in batches in a large watch glass under a General Electric infrared heat lamp and stored in sealed polyethylene (Nalgene) bottles in a dessicator.

The reaction vessels were then shaken by hand and a horizontal shaker for a total of 20 min. to suspend all the alumina, and centrifuged for 5 min. at 1000 r.p.m. (200 g). The supernatants were transferred to another set of reaction vessels for the 5HT analysis. The alumina was washed twice with 20 mls deionized, distilled water, pH 7.0 (distilled water from the tap passed through Barnstead mixed resin cartridge, D5041). Most of the first wash was aspirated. After the second wash the alumina was centrifuged at 1000 r.p.m. for 5 min., and the wash aspirated carefully and completely. The washed alumina was stored during the isolation of 5HT in the refrigerator (4°C), until the catecholamines were eluted for analysis. To the supernatant from the alumina, containing the 5HT about 3 gms of NaCl was added and shaken to assure salt and saturation. 15 ml of n-heptanol (redistilled from Eastman 381) to remove impurities which cause high blanks and lower recoveries) was added to the salt-saturated solutions. (Vacuum distillation of the heptanol is necessary if 5HT recoveries from heptanol are low, because of interfering impurities.) To extract the 5HT from the aqueous to the organic phase, the solutions were adjusted to pH 9.8 ± 0.4 using about 300 mg K_2CO_3 (0.3 ml of a 1 gm per ml distilled water solution). Immediately after addition of the K_2CO_3 each vessel was shaken to avoid 5HT loss resulting from localized regions in the solution of high pH. All reaction vessels are mixed on a vortex mixer for 3-5 min., then centrifuged at 1000 r.p.m. Using a

water-vacuum aspirator with a Pasteur pipette on the end and a small hole in the tubing just above the top of the pipette, the tip of the pipette is plunged quickly through the upper heptanol layer into the aqueous phase, the crystals of NaCl and K_2CO_3 gently dislodged and the bottom layer aspirated by covering the hole in the tubing with the index finger. With care and practice this can be done with very little loss of heptanol. The heptanol layer was then washed twice with 50 ml of pH 10 borate buffer, vortex mixed for 60-90 seconds, and centrifuged at 1000 r.p.m. for 5 min. The borate buffer layer could be removed by aspiration as described above or directly by careful pipetting (in either case without any borate contamination) 13.5 ml of the upper heptanol layer and placing it in a clean reaction vessel. 2.5 ml of 0.05 M phosphate buffer, pH 7.0, was added to the 13.5 ml of heptanol and the vessels were vortex mixed for 40-60 seconds, centrifuged at 1000 r.p.m. for 5 min and the heptanol aspirated (using a vacuum flask to save it for distillation and reuse). 1.0 ml samples of the phosphate buffer are removed for 5HT analysis, taking care to exclude heptanol, which interferes with the fluorescence after the ninhydrin reaction.

c. Estimation of the amines:

To 1.0 ml of the phosphate buffer placed in Corning disposable culture tubes, 0.1 ml of 0.1 M ninhydrin reagent was added and heated in an oven at 100°C. for 25 min. after removal before reading the fluorescence in an Aminco-Bowman SPF. The samples are left at room temperature for 1 hour. An activation peak of 385 nm and emission peak of 490 nm (uncorrected) are used. For tissues blanks duplicate 1.0 ml samples of the phosphate buffer

aliquots, heated and cooled without ninhydrin, are read immediately after adding 0.1 ml ninhydrin reagent.

3.0 ml of 0.05 N perchloric acid was added to the washed alumina, vortex and hand shaken for about 5 min to elute the catecholamines, then centrifuged for 5 min at 1000 r.p.m. 1.0 ml aliquots of the perchloric acid eluate are placed in disposable test tubes and brought to pH 6.5 ± 0.2 with 1.5 ml of 0.1 M phosphate-buffer-EDTA. 0.2 ml of 0.1 N iodine reagent are added to each tube, shaken immediately and exactly 2 min later 0.5 ml of the alkaline sulfite are added and shaken. After 2 min the samples are acidified to pH 4.4 - 4.8 with 0.4 ml of glacial acetic acid. Duplicate 1.0 ml aliquots for tissue blanks are developed by adding the alkaline sulfite first, then the iodine and acetic acid. The tubes were heated in a water bath (95 - 100°C.) for 2 min, cooled in ice water for 3-5 min, then NE fluorescence read at room temperature (activation peak at 380 nm, emission peak at 495 nm, uncorrected). After reading for NE, the tubes were returned to the water bath and heated for 10 min to develop the DA fluorophore, then removed and transferred to an ice bath for cooling. The fluorescence produced in this manner is stable indefinitely as long as the samples are kept at 4°C but will decline 15-20% after 30 min at room temperature before becoming stable indefinitely. The DA fluorescence is read after 30 min or the next day at an activation peak of 325 nm and emission peak of 380 nm (uncorrected).

Tissue content of amines is calculated to give $\mu\text{g}/\text{mg}$ tissue weight, after correcting for tissue blank readings, by comparison with standards extracted through the procedure. After correcting standards and samples for the blanks, the fluorescence units of the standards are plotted on the Y-axis versus the concentration on the x-axis.

The corrected fluorescence units for the samples are used with the standard plots to determine the tissue concentration of each amine. Results are expressed in terms of $\mu\text{gm/gm}$ wet tissue weight by multiplying the tissue content times the tissue factor (1.0 divided by the tissue weight in grams).

All drugs utilized in these studies were extracted through the amine procedure, to check for possible interference with the amines themselves. The only drug found to produce interference was DOPA, which interfered with the DA analysis. To remove the DOPA, the CAS were eluted with two 2 ml aliquots of 0.05 N perchloric acid, adjusted to pH 6.5 with tricine buffer and applied to pH 6.5 buffered AG 50 W-X4 resin columns (Glisson et al., 1972). NE and DA are adsorbed, while DOPA is not. NE and DA were eluted with 12 ml of 1 N HCl, the pH readjusted and processed in the usual manner.

5. Fluorometry Utilizing the Aminco-Bowman Spectrophotofluorometer

Determinations of tissue amine levels by fluorescent methods were performed on an Aminco-Bowman Spectrophotofluorometer (SPF) with the off-axis attachment and transistorized photomultiplier microphotometer with a RCA 1P21 photomultiplier tube. After the xenon lamp has warmed up for about thirty minutes, the photomultiplier is switched on, and its voltage set at 800 volts. The microphotometer is then adjusted to zero. Before each reading session the lamp is adjusted to peak response (greatest sensitivity) against a quinine sulfate standard ($2.5 \mu\text{g/ml}$ in 0.1 N sulfuric acid) using an activation wavelength of 250 nm and emission at 350 nm (uncorrected). In this case, as with other standards, the wavelengths cited are uncorrected and for each reading session activation and emission wavelengths must be deter-

mined precisely by "peaking" them with the respective standards (usually within 5-10 nm of the "uncorrected" wavelengths cited). Reading the quinine standard first provides a day-to-day check on the sensitivity of the total SPF system. If the reading has dropped off, the lamp position is adjusted to restore readings to previous levels. If the reading cannot be restored by these procedures, it indicates that the mirrors might require cleaning or that the lamp is failing.

The low concentration internal standard was usually set at 40 on the microphotometer scale, the other standards read and tissue samples compared with the standard curve established, plotting concentration (x-axis) versus corrected fluorescence units (y-axis).

6. Rectal Temperature

Rectal temperature was measured in rats using a Yellow Springs Telethermometer, with a rectal temperature probe (No. 401). The probe was lubricated with the light mineral oil and inserted approximately 7 cm into the colon, for 1.5 - 2.0 minutes before the temperature was recorded.

In control and treated animals, rectal temperatures were obtained every 15-20 minutes. Temperature was plotted on the y-axis, versus time on the x-axis. In order to compare the temperature pattern of controls and treated animals, the intercepts of the plots for individual animals were noted at ten-minute intervals (i.e., 5, 15, 25, 35 minutes, etc.) and the means at each time determined and replotted to represent an average pattern for control and treated animals. This permits comparison of differences between control and treated groups, as well as between different times of day.

7. Statistical analyses

a. Calculation of mean, standard deviation and standard error of the mean

The calculation of the arithmetic mean (\bar{X}), the standard deviation (S.D.) and the standard error of the mean (S.E.) were computed on a Smith-Corona Cogito 1016PR calculator utilizing the tape input (Iota 1) to enter prerecorded programs. The program provided by Smith-Corona (Statistics 1001) uses the following formulas:

$$\bar{X} = \frac{\sum X}{N} \quad \text{where } X = \text{individual values}$$

$$N = \text{total number of } X \text{ values}$$

$$S.D. = \sqrt{\frac{(X - \bar{X})^2}{N - 1}} = \sqrt{\frac{\sum (X - \bar{X})^2}{N - 1}}$$

$$S.E. = S.D. / \sqrt{N}$$

As the program is written, after all entries have been made, the calculator prints out values corresponding to the following:

$$N, \quad X, \quad X^2, \quad \bar{X}, \quad S.D. \text{ and } S.E.$$

b. Calculation of t and determination of P, the degree of significance

The unpaired t-test utilized, computed t as follows:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\left(\frac{(N_1-1)(S.D._1)^2 + (N_2-1)(S.D._2)^2}{(N_1-1) + (N_2-1)} \right) \left(\frac{1}{N_1} + \frac{1}{N_2} \right)}}$$

The output from the program gives the value of t for a comparison between the two groups, when the group size (N), mean (\bar{X}) and the standard deviation (S.D.) for each group are entered. The P value corresponding to the t calculated is found in a table of the significance limits of the Student t -test distribution, using the appropriate degrees of freedom. The P values obtained are expressed as being less than a degree of confidence (per cent probability), read on the $2P$ scale. A value of P less than 0.05 (95% confidence limit) was used as the criterion for significant differences between groups.

IV. RESULTS

A. Duration of Hypnotic Sleep on a 24-Hour Basis

1. Pentobarbital

The duration of sleep produced by PB (50 mg/kg, IP) was examined by two procedures. Initially it was administered to untreated groups of rats at 0600, 1200, 1800 and 2400 hours and the duration of PB-induced sleep determined (Table 1, Fig. 8). The duration of sleep with PB alone is maximal at 1200 hours and minimal at 2400 hours. The difference between the peak and trough are significant at the $P < .01$ level. The peak value also differs significantly from that at 1800 hours ($P < .01$). The results at 1800 and 2400 hours do not differ significantly from each other. There is no significant difference when results at 0600 hours are compared with the other times. However, the difference between 0600 and 2400 hours has borderline significance ($P < 0.1, > 0.05$).

A second pattern for PB-sleep was obtained by the administration of PB together with the pretreatment vehicle, at the same time that the drug treated group received their drug doses. This vehicle-treated group constituted the "CONTROL" group, for comparison with the treated groups. Several controls were run with each treated group. They were pooled with other similar controls to obtain the control values at the four times in the 24-hour period. As seen in Fig. 8, this pattern does not exhibit the sharp peak and trough seen with the PB-alone. However, because of the large number of animals used for this pooled control, significant differences were

Table 1: The duration of pentobarbital sleep

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
Pentobarbital (alone)	104.8+18.6 (5)	115.6+17.8 (5)	87.3+ 8.1 (6)	86.7+ 8.9 (6)	D E ⁺
Pentobarbital (pooled)	115.5+11.7 (18)	119.5+ 9.7 (17)	109.3+14.1 (17)	101.3+ 8.9 (16)	C d E ⁺

Table indicates the duration of drug-induced sleep, in minutes, for animals receiving PB alone and the pooled controls receiving the vehicles used in the drug pretreatment studies. Sleeping times are means \pm standard deviation, with the number of animals used in parentheses.

+ Letter designations indicate point to point comparisons within each 24-hour patterns as follows: aA (0600 vs 1200 hours), bB (0600 vs 1800 hours), cC (0600 vs 2400 hours), dD (1200 vs 1800 hours), eE (1200 vs 2400 hours) and fF (1800 vs 2400 hours). Lower case indicates $P < 0.05$ and capitals indicate $P < 0.01$ for significant differences.

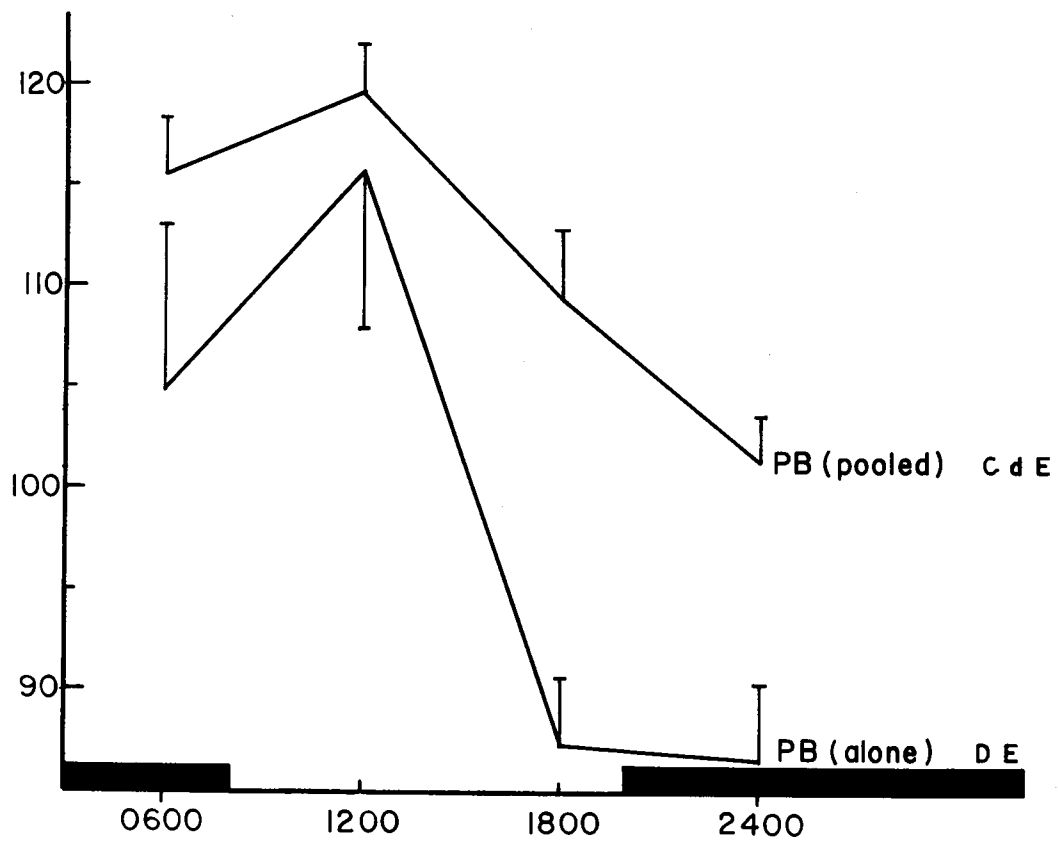


Figure 8: Pentobarbital-Induced Sleep

obtained at 0600 and 2400 hours ($P < .001$), 1200 vs 1800 hours ($P < .02$ and 1200 vs 2400 hours ($P < .001$).

The results obtained by both procedures corroborate earlier studies demonstrating a peak duration of drug-induced sleep during the light period and a trough during the dark period.

2. Gamma-butyrolactone

The effect of administration of GBL (350 mg/kg, IP) at six-hour intervals can be seen in Fig. 9, (Table 9, GBL (alone) and GBL (pooled)). The duration of sleep with GBL (alone) was longest during the light phase, at 1800 hours and differs significantly from the trough at 0600 hours at the 0.05 level. The peak also differs significantly from the adjacent measurements (1200 and 2400 hours) with $P < 0.05$. The sleep pattern of saline injected (GBL (pooled) animals (vide infra)) differed quantitatively but not qualitatively from GBL (alone).

B. Effects of Drug Pretreatment on Pentobarbital Sleep Duration

1. Drugs Affecting the NE and DA Systems

a. DOPA-pargyline

DOPA-pargyline pretreatment (500 and 6 mg/kg, IP, respectively) very significantly prolongs the duration of PB-sleep over controls at all times tested, with $P < 0.001$. (Figs. 10 - 12, Table 3). Although these increases over respective controls ranged from 103% at 0600 to 146.3% at 2400 hours, the pattern must be considered flat from a statistical examination since there are no significant increases in sleep duration between any of the DOPA-pargyline-treated groups, during the 24-hour period.

Table 2: The duration of gamma-butyrolactone sleep

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
GBL (alone)	68.2+9.3 (5)	79.3+6.7 (3)	110.3+16.3 (4)	77.0+11.2 (5)	B d F ⁺
GBL (pooled)	98.0+3.6 (6)	110.1+4.5 (8)	96.4+16.2 (11)	88.3+10.0 (7)	A c d E ⁺

Table indicates the duration of drug-induced sleep, in minutes, for animals receiving GBL (350mg/kg) alone and the pooled controls also receiving the vehicle used in the drug pretreatment study. Sleeping times are means \pm standard deviation, with the number of animals used in parentheses.

+For explanation of letter designations see Table 1.

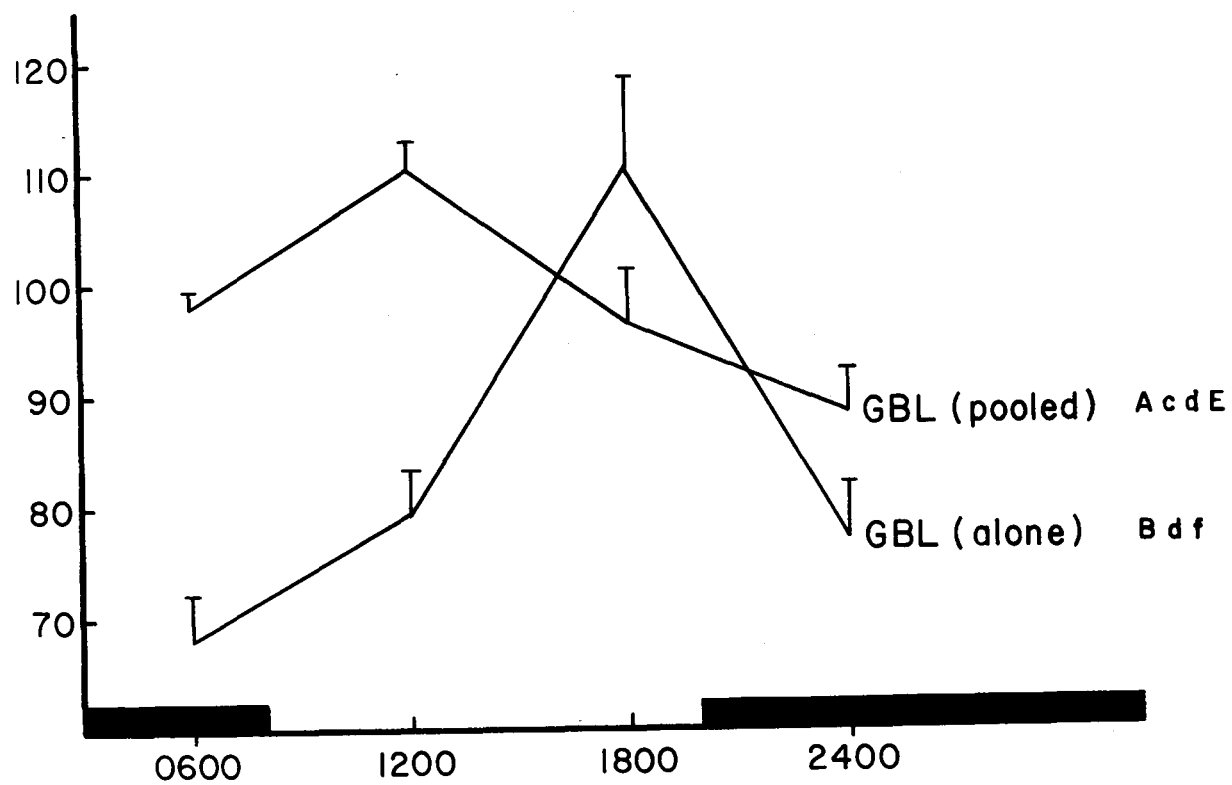


Figure 9: GBL-Induced Sleep

Table 3: The duration of pentobarbital-sleep after pretreatment with drugs affecting the NE and DA systems

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
Treatment					
CONTROL	115.5+11.7 (18)	119.5+ 9.7 (17)	109.3+14.1 (17)	101.3+ 8.9 (16)	C d E ⁺
DOPA + pargyline	234.5+37.9** (5)	245.5+22.8** (4)	237.3+14.1** (5)	249.6+22.8** (5)	
AMPT	112.8+13.5 (6)	143.1+23.7* (4)	106.8+ 7.0 (3)	177.3+14.5** (7)	C e F ⁺

Table indicates the duration of drug-induced sleep, in minutes, for animals receiving the pre-treatments plus PB (50mg/kg, IP). Sleeping times are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from controls are indicated by * ($P < 0.05$) and ** ($P < 0.01$).

⁺For explanation of letter designations see Table 1.

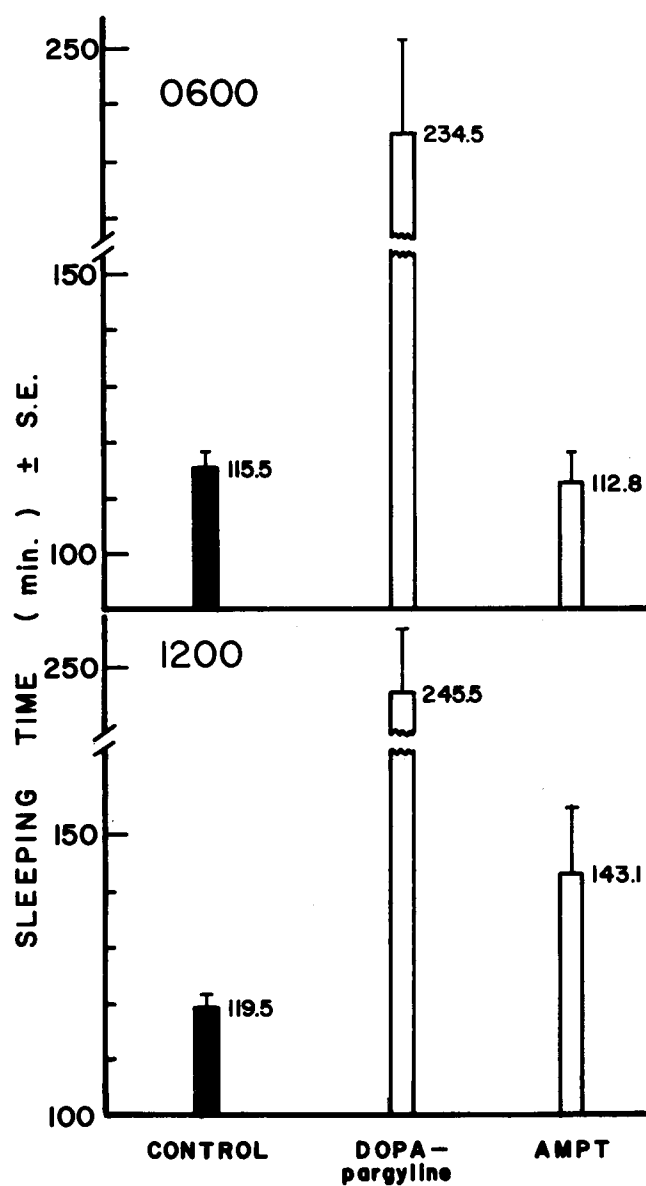


Figure 10: Pentobarbital-induced sleep after pretreatment with drugs affecting the NE and DA systems, 0600--1200 hours

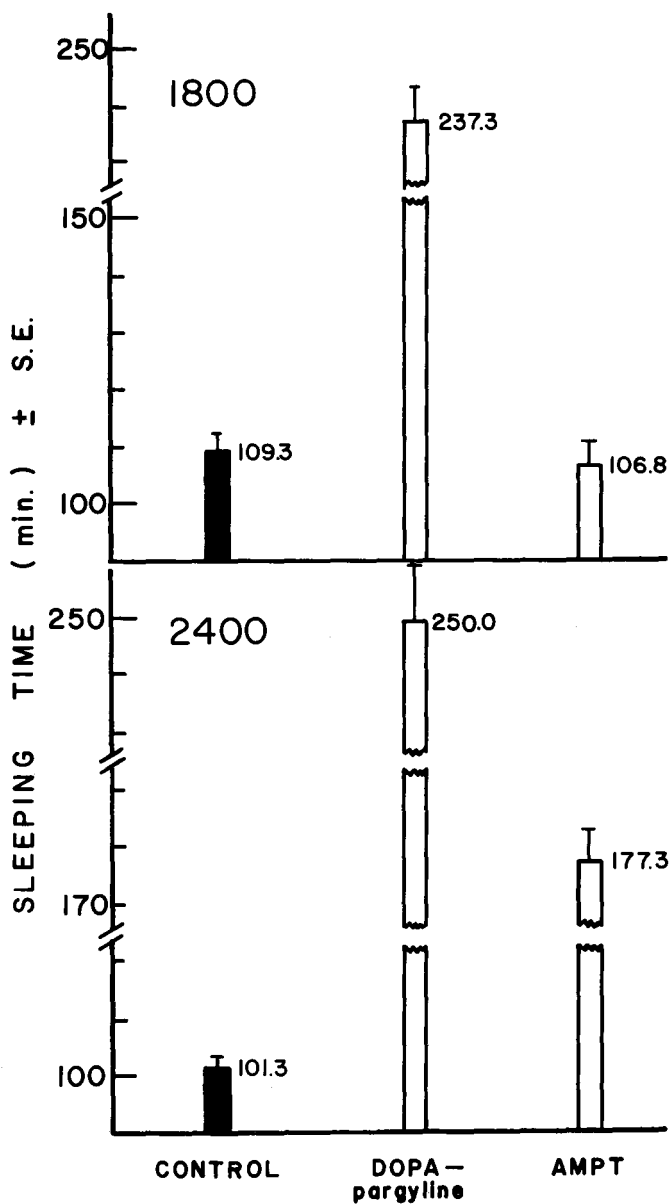


Figure 11: Pentobarbital-induced sleep after pretreatment with drugs affecting the NE and DA systems, 1800-2400 hours

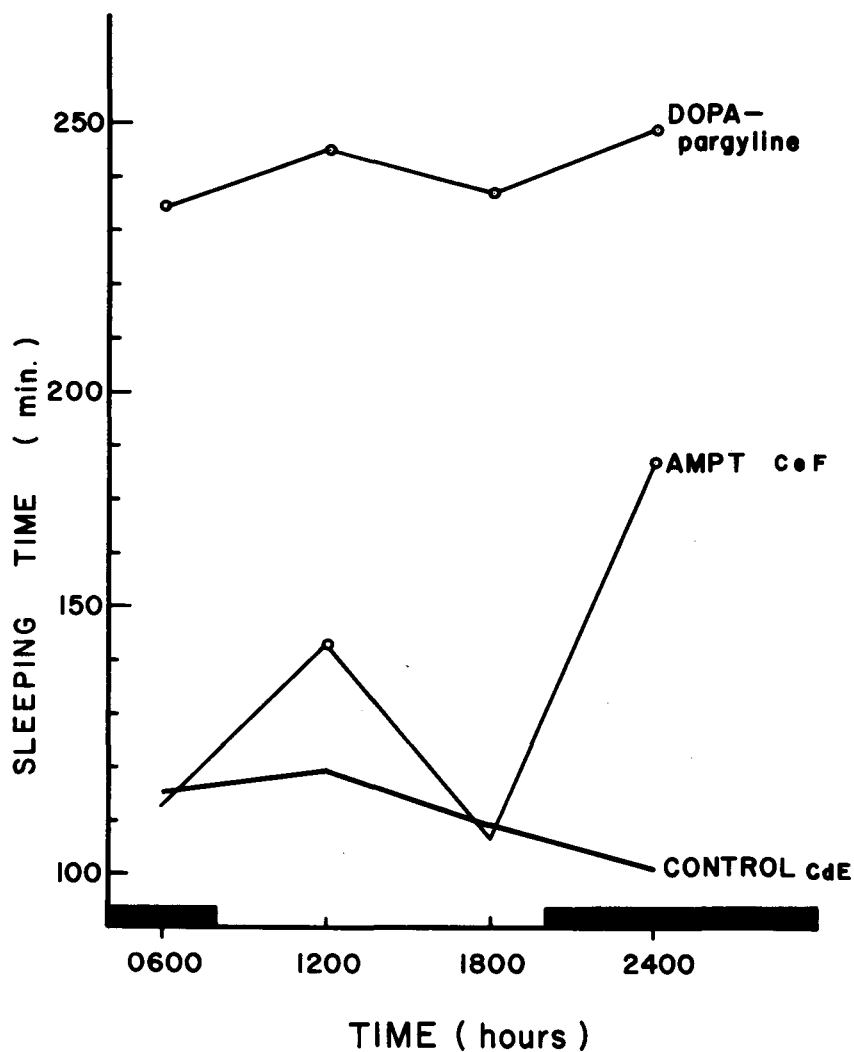


Figure 12: Circadian pattern of pentobarbital-induced sleep after pretreatment with drugs affecting the NE and DA systems. (Open circles indicate significant differences from CONTROL, $P < 0.05$ or greater; for explanation of letter designations see Table 1).

b. Alpha-methyl p-tyrosine

The effects of pretreatment with AMPT (200 mg/kg, IP) are more complex (Figs. 10-12, Table 3). A biphasic pattern is obtained, with peaks at 1200 and 2400 hours. There is no significant difference in the duration of sleep between control and AMPT-treated animals at 0600 and 1800 hours. However, there are significant increases in sleep duration over controls at the two peaks, viz. at 1200 (19.8% increase) and 2400 hours (75.0% increase), at the 0.05 and 0.001 level, respectively. There are also significant differences in sleep duration of AMPT-treated animals, when the following comparisons are made: 0600 vs 2400 hours ($P < 0.001$); 1200 vs 2400 hours ($P < 0.05$) and 1800 vs 2400 hours ($P < 0.005$).

2. Drugs Affecting the 5HT System

a. p-Chlorophenylalanine

pCPA, a 5HT depletor, very significantly prolongs PB-sleep at each point in the 24-hour pattern ($P < 0.001$ level; Figs. 13-17 , Table 4). The increases over control are as follows: 0600 (19.5%), 1200 (34.4%), 1800 (49.8%) and 2400 hours (45.9%). The pattern is diurnal, peaking at 1200 to 1800 hours (Fig. 17). The duration of sleep differed significantly between 0600 and 1200 hours ($P < 0.05$) and between 0600 and 1800 hours ($P < 0.005$) but not between other time points.

b. Tryptophan

TP, of secondary precursor of 5HT, significantly increased PB-sleep over control at each point in the curve ($P < 0.001$ (Figs. 13-17, Table 4), as follows: 43.4% (0600), 32.3% (1200), 59.1% (1800) and

Table 4: The duration of pentobarbital-sleep after pretreatment with drugs affecting the 5HT system

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
Treatment					
CONTROL	115.5±11.7 (18)	119.5± 9.7 (17)	109.3±14.1 (17)	101.3± 8.9 (16)	c d E ⁺
pCPA	137.9±12.7** (6)	161.1±17.3** (4)	163.7± 8.6** (5)	147.9±24.5** (5)	a B ⁺
TP	165.6±15.5** (9)	158.0±22.1** (6)	173.8± 7.3** (6)	154.0±16.6** (5)	f ⁺
5HTP	131.3±16.3 (3)	144.0±15.4** (6)	104.3± 5.5 (3)	146.3±21.1** (3)	D ⁺
M25	114.6±22.2 (4)	155.8± 6.6** (4)	131.2±10.3* (4)	125.2± 9.3** (5)	A D E ⁺
M50	192.3±32.0** (3)	157.3±13.0** (4)	141.5±15.4** (5)	159.8±24.1** (4)	b ⁺

Table indicates the duration of drug-induced sleep, in minutes, for animals receiving the pre-treatments plus PB (50mg/kg, IP). Sleeping times are means ± standard deviation, with the number of animals used in parentheses. Significant differences from controls are indicated by * (P < 0.05) and ** (P < 0.01).

⁺For explanation of letter designations see Table 1.

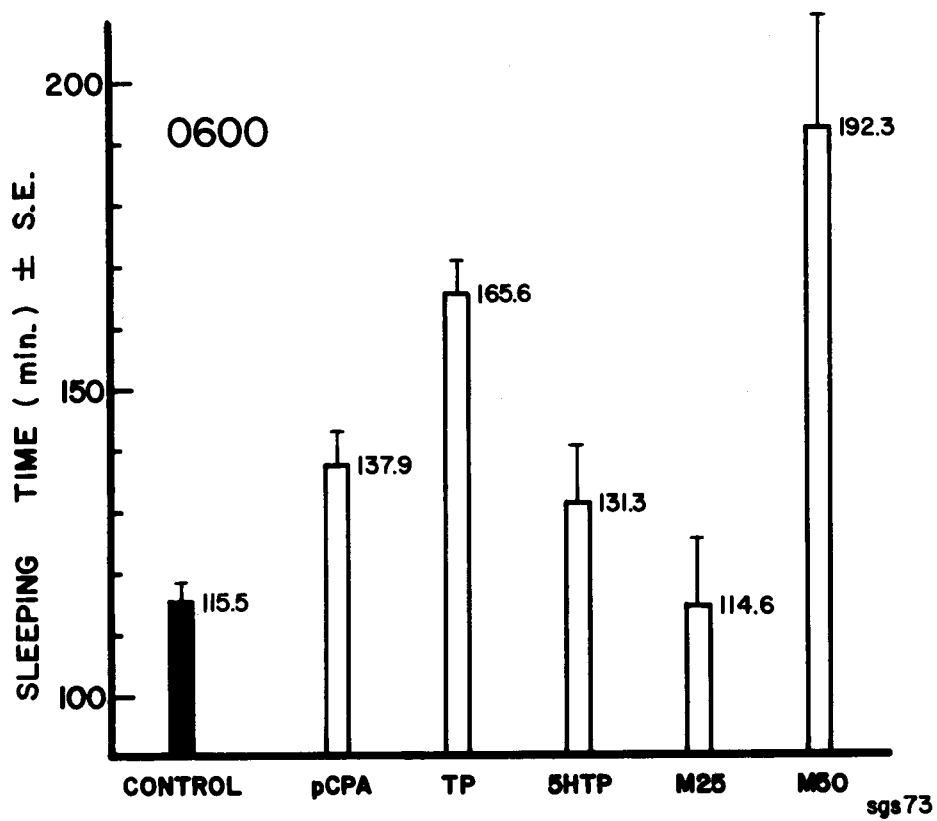


Figure 13: Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 0600 hours

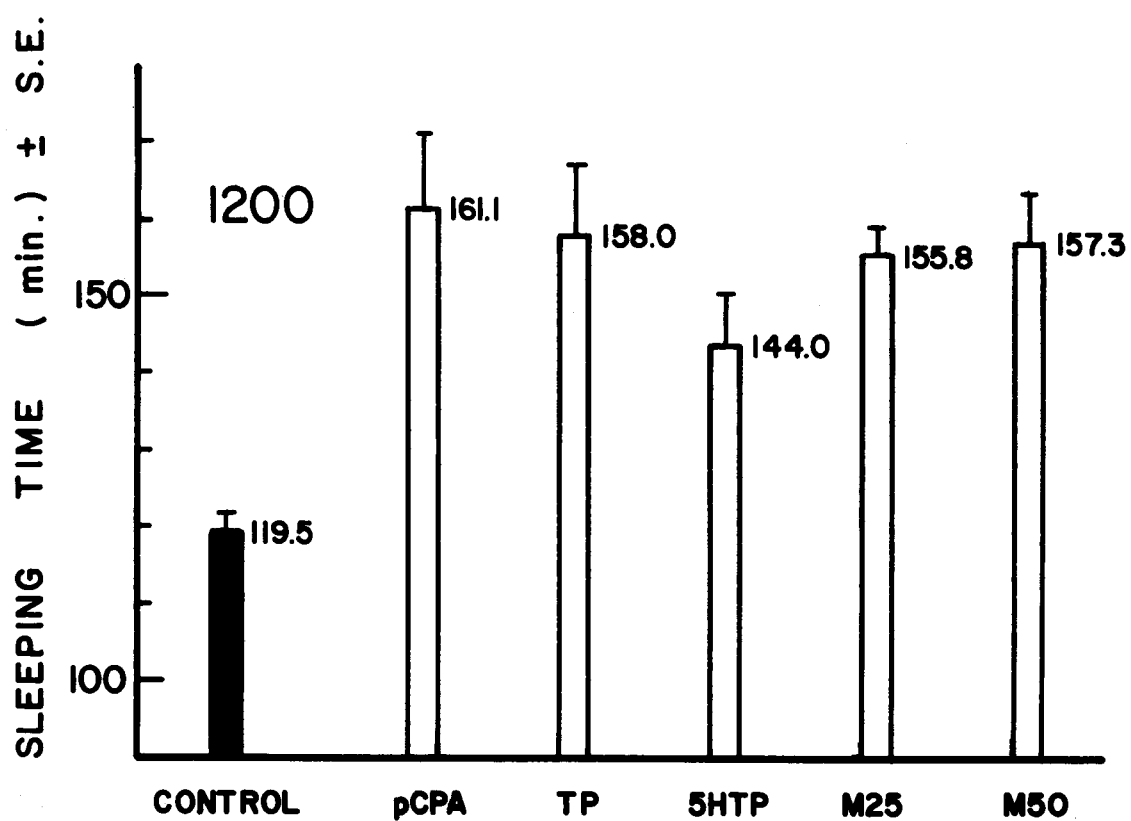


Figure 14: Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 1200 hours

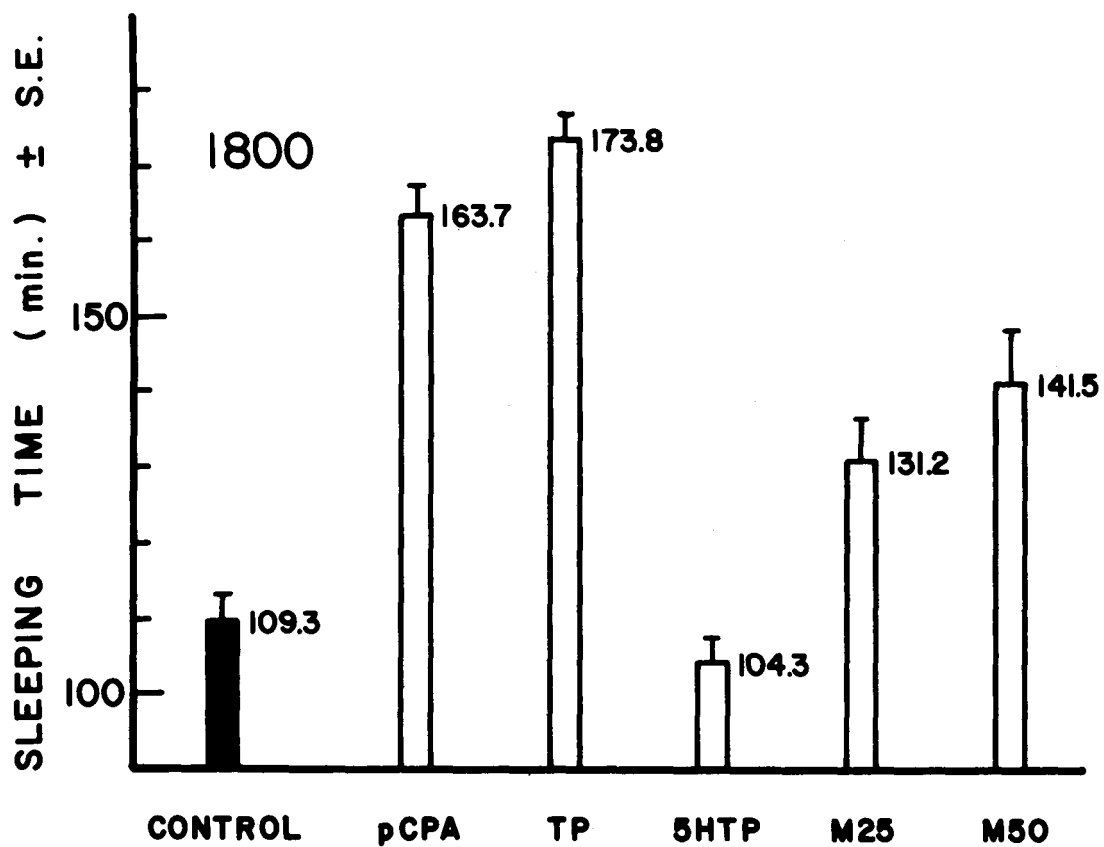


Figure 15: Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 1800 hours

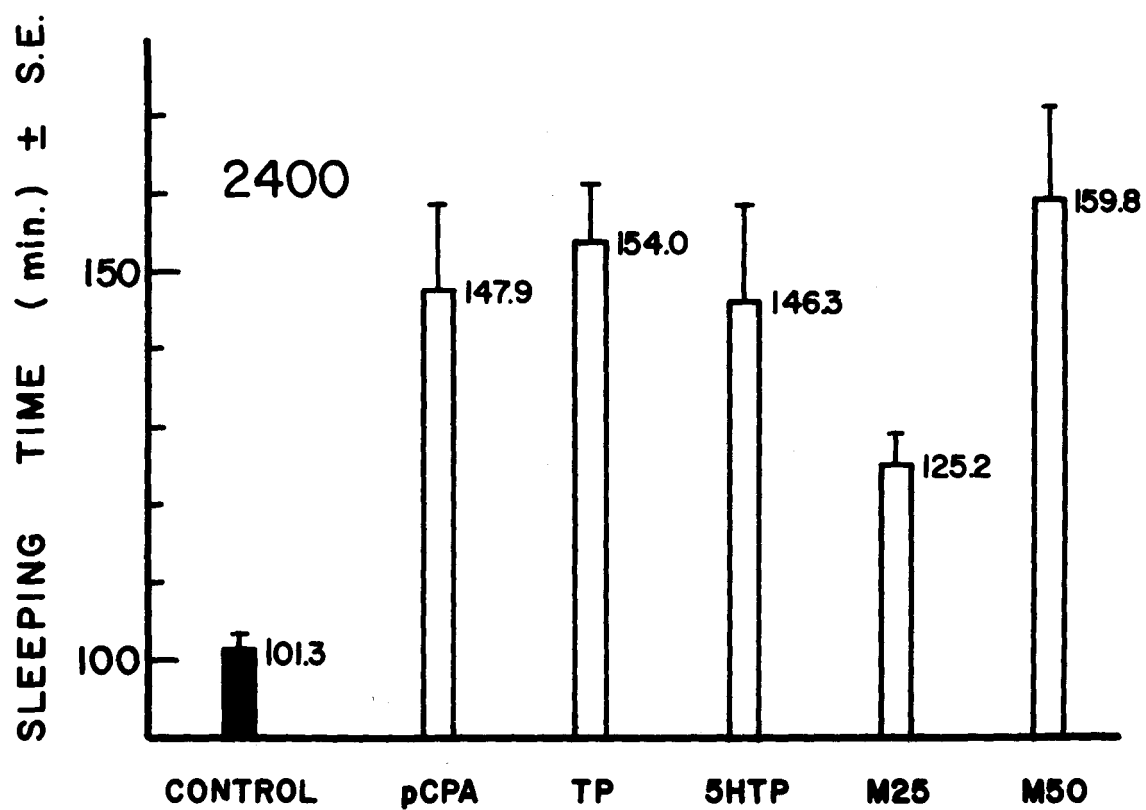


Figure 16: Pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system, 2400 hours

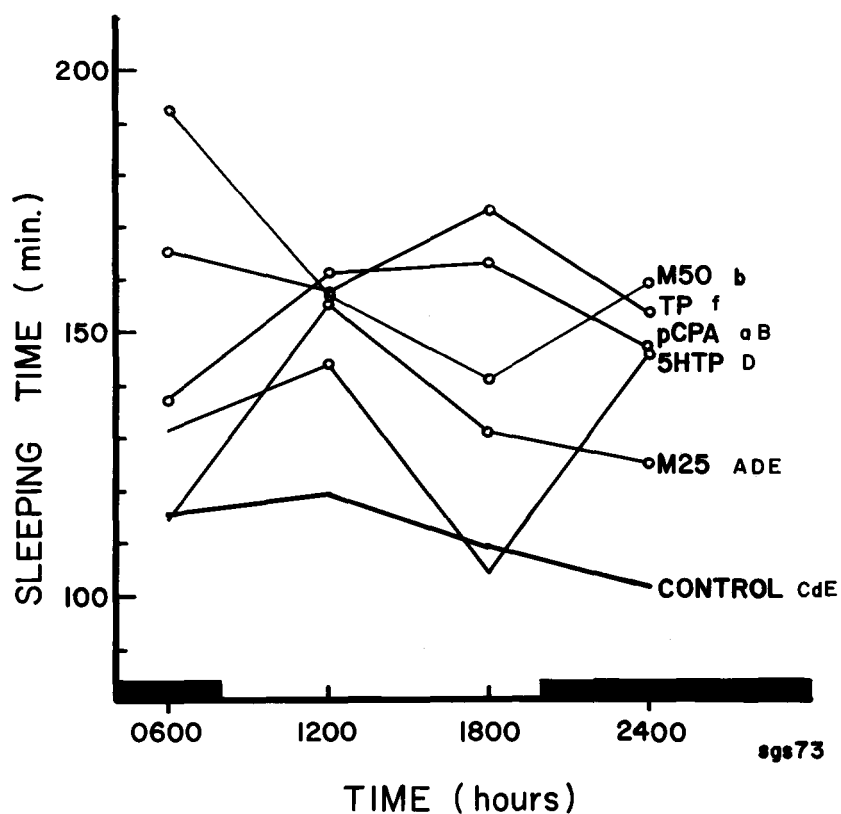


Figure 17: Circadian pattern of pentobarbital-induced sleep after pretreatment with drugs affecting the 5HT system. (Open circles indicate significant differences from CONTROL, $P < 0.05$ or greater; for explanation of letter designations see Table 1).

52.0% (2400). The pattern was relatively flat. The only significant variation in the duration of sleep was the decrease from the 1800 hour value at 2400 hours ($P < 0.05$).

c. 5-Hydroxytryptophan

5HTP, the primary precursor of 5HT, produced a relatively flat pattern in PB-sleep duration (Figs. 13-17, Table 4). Significant differences from control occurred at 1200 and 2400 hours (in each case, $P < 0.001$), while the difference between the sleep duration at 0600 hours and controls was approximately $P < 0.05$. The increases over control were 20.5% at 1200 hours, 34.7% at 2400 hours and 13.7% at 0600 hours. The only significant differences among treated groups was between the 1200 and 1800 hour groups ($P < 0.01$).

d. Melatonin

Melatonin, (M), which was administered at two dose levels (25 and 50 mg/kg, IP), yielded qualitatively and quantitatively different results for each dose (Figs. 13-17, Table 4). Both increase PB-sleep at every time point (except for the 25mg/kg (M25) dose at 0600 hours). Melatonin-modified sleep at a dose of 25mg/kg peaks at 1200 hours during the light period, while at the higher dose (50mg/kg, M50) the curve is shifted to peak at 0600 hours, during the dark period. The M25 dose significantly increases PB-sleep duration over controls at 1200 hours ($P < 0.001$; 30.4%), 1800 hours ($P < 0.05$; 20.0%) and 2400 hours ($P < 0.001$; 23.6%). At this dose significant differences occur between treated groups for 0600 vs. 1200 hours ($P < 0.05$), 1200 vs. 1800 hours ($P < 0.05$) and 1200 vs. 2400 hours ($P < 0.001$). At the M50 dose, the PB-sleep duration for all treated groups is significantly

greater than their respective controls ($P < 0.001$). It ranges from 29.5% at 1800 hours to 66.6% at 0600 hours. In a comparison of M25 with M50 treatment the only significant difference occurs between the 0600 and 1800 hours groups ($P < 0.05$).

e. Compounds Structurally-related to 5HT

The effects of several compounds structurally related to 5HT were tested in mice for their ability to enhance PB-sleep at a single time point (1500 hours) during their normally-quiet period. The compounds were administered one hour prior to PB, at a dose of 20mg/kg (in 1% methyl cellulose). Table 5 shows significant increases in PB-sleep is produced by 5-methoxytryptamine ($P < 0.02$; 74.6%) and N-acetyl 5HT ($P < 0.025$; 105.4%), a precursor of M. 5HIAA and 5-methoxy indoleacetic acid are without significant effects.

3. Drugs Affecting the Histamine System

The possible role of CNS histamine (H) in PB-induced sleep was examined utilizing its precursor, histidine (Hd), a depletor, decaborane (DB) and an antihistamine, diphenhydramine (DPH).

a. L-Histidine

Three doses of L-Hd, were administered to rats. The lowest dose (100 mg/kg, Hd100) significantly increases PB-sleep over controls at 0600 hours ($P < 0.05$; 15.6%), 1200 hours ($P < 0.001$; 22.0%) and 2400 hours ($P < 0.05$; 21.2%) (Figs.18-22, Table 6). The only significant difference between treated groups is between the 1200 and 1800 hours groups ($P < 0.05$). The intermediate Hd dose (500mg/kg, Hd 500) increases PB-sleep significantly over controls at 1800 hours ($P < 0.001$; 47.6%), but decreases PB-sleep

Table 5: The effects of compounds structurally-related to 5HT on pentobarbital-sleep, in mice.

<u>Compound</u>	<u>PB-sleep duration</u>
CONTROL	31.9 \pm 5.1 (7)
5MT	55.6 \pm 12.4 (8)*
N-acetyl 5HT	65.4 \pm 11.7 (7)*
5HIAA	40.5 \pm 13.3 (8)
5MIAA	40.5 \pm 10.4 (8)

Table indicates the duration of drug-induced sleep, in minutes, for animals receiving the pretreatment plus PB (50mg/kg, IP) at 1500 hours only. Sleeping times are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from controls are indicated by * ($P < 0.05$).

Table 6: The duration of pentobarbital-sleep after pretreatment with drugs affecting the histamine system

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
Treatment					
CONTROL	115.5+11.7 (18)	119.5+ 9.7 (17)	109.3+14.1 (17)	101.3+ 8.9 (16)	c d E ⁺
Hd100	133.5+12.2* (4)	145.8+ 9.7** (4)	124.0+11.0 (3)	122.8+18.7* (5)	d ⁺
Hd500	122.3+14.0 (3)	131.8+25.9 (4)	161.3+27.6** (3)	87.8+ 9.3* (4)	c e F ⁺
Hd1000	152.3+ 3.5** (3)	142.3+34.7* (4)	168.4+19.2** (5)	114.6+20.6* (5)	c F ⁺
DB	196.0+35.6** (6)	202.0+59.5** (6)	213.5+27.9** (4)	217.7+52.4** (6)	
DPH	289.8+38.7** (4)	230.3+12.4** (4)	177.8+54.1** (4)	281.8+60.4** (4)	a b f ⁺

Table indicates the duration of drug-induced sleep, in minutes, for animals receiving the pre-treatments plus PB (50mg/kg, IP). Sleeping times are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from controls are indicated by * (P < 0.05) and

** (P < 0.01).

⁺For explanation of letter designations see Table 1.

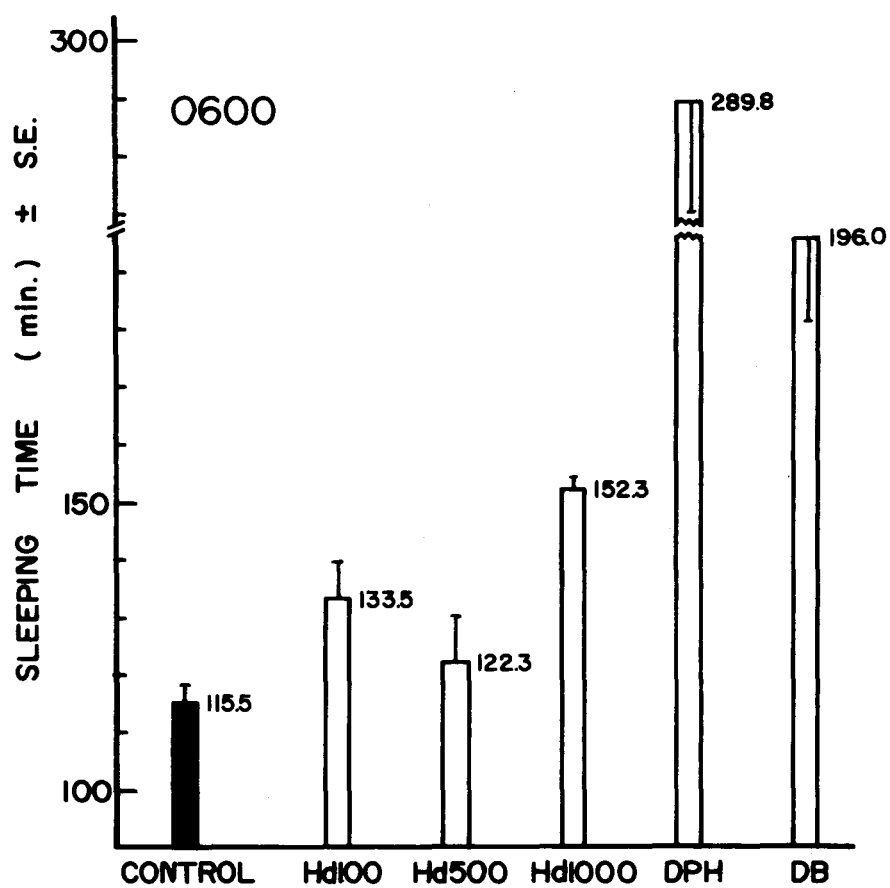


Figure 18: Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 0600 hours

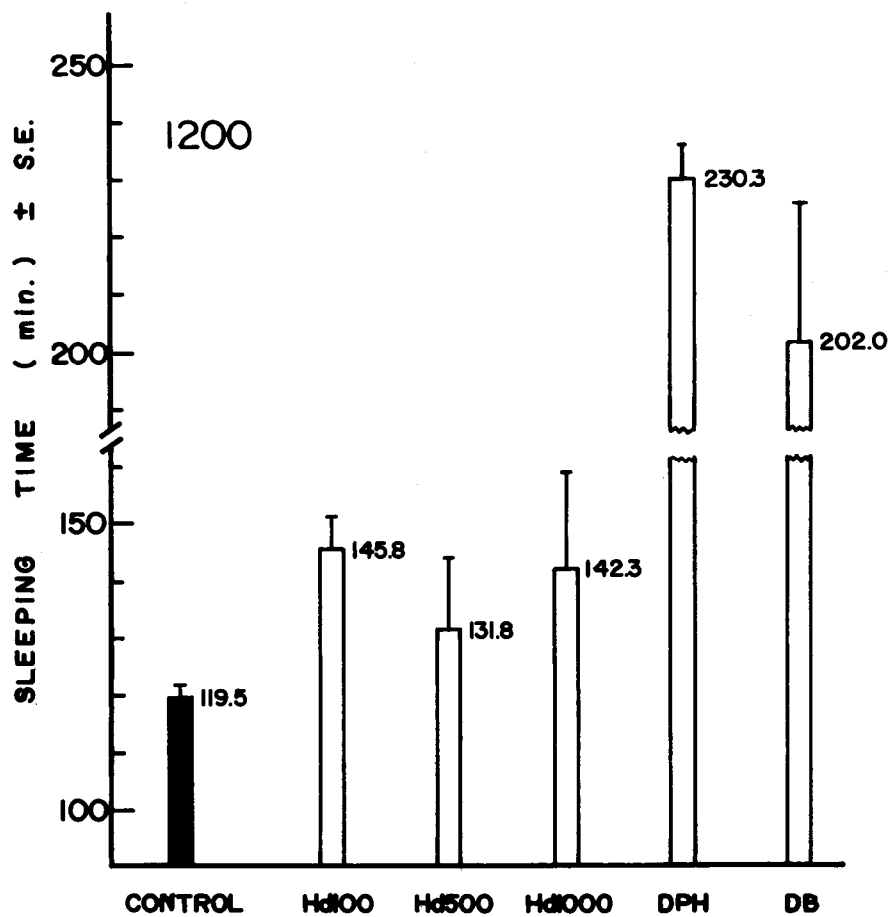


Figure 19: Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 1200 hours

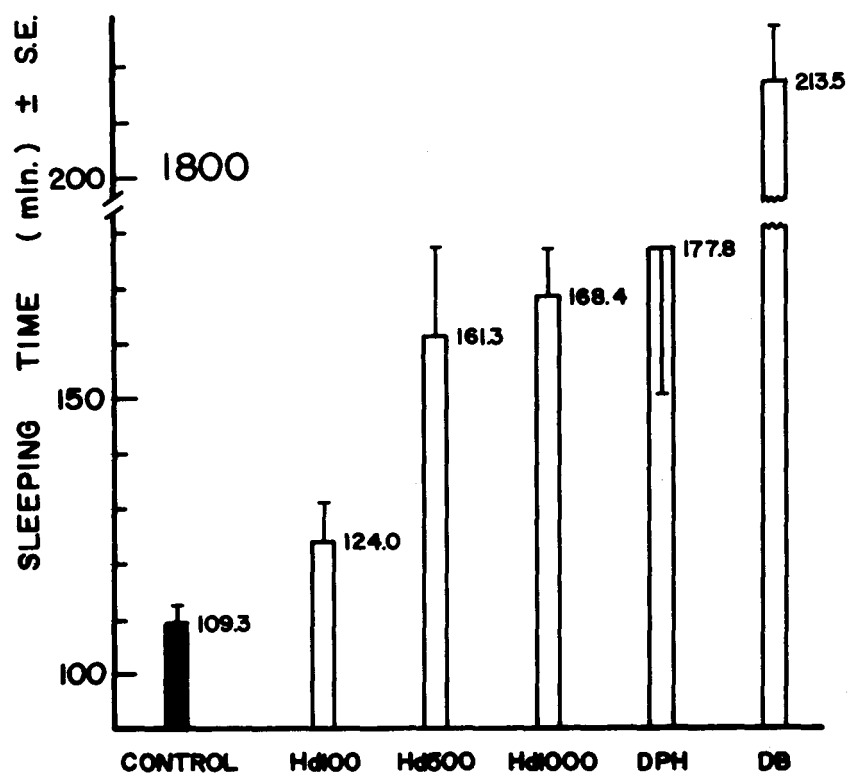


Figure 20: Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 1800 hours

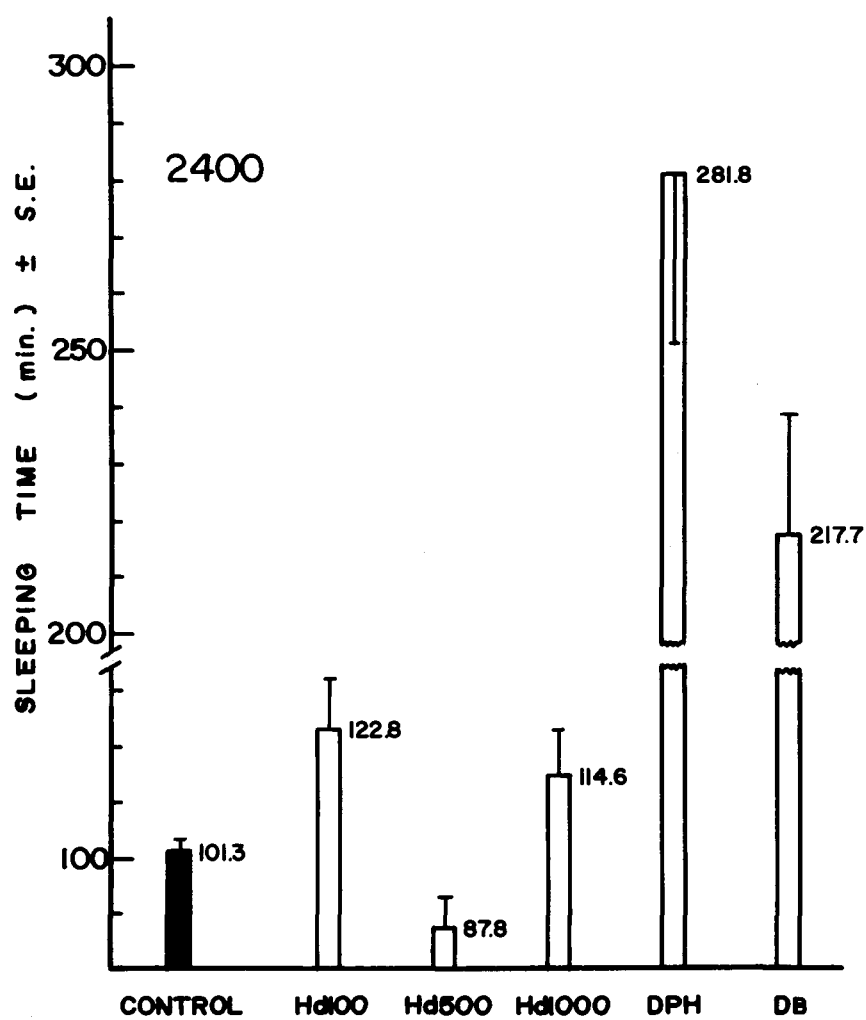


Figure 21: Pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system, 2400 hours

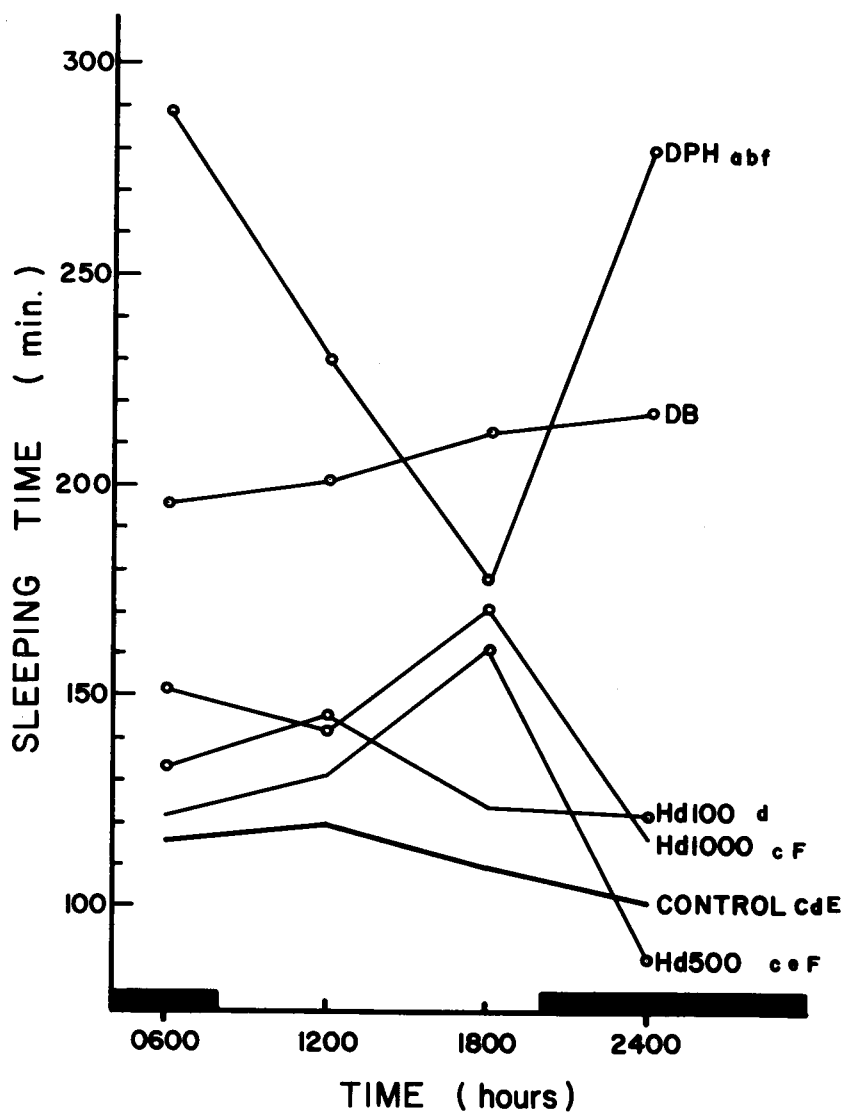


Figure 22: Circadian pattern of pentobarbital-induced sleep after pretreatment with drugs affecting the histamine system. (Open circles indicate significant differences from CONTROL, $P < 0.05$ or greater; for explanation of letter designations see Table 1).

significantly at 2400 hours ($P < 0.05$; 13.4%). The trough value for this treatment occurs at 2400 hours and differs significantly ($P < 0.05$) from the values at 0600, 1200 and 1800 hours. The highest dose of Hd (1000 mg/kg, Hd 1000) produces a similar pattern, exhibiting a peak duration of PB-sleep at 1800 hours and a trough at 2400 hours. PB-sleep duration increases significantly over controls at 0600 hours ($P < 0.001$; 31.9%), 1200 hours ($P < 0.05$; 19.1%), 1800 hours ($P < 0.001$; 54.1%) and 2400 hours ($P < 0.05$; 13.1%). The trough value at 2400 hours differs significantly from the peak value at 1800 hours ($P < 0.05$) and from the value at 0600 hours ($P < 0.05$). Differences observed between the dosage groups at a given time are as follows: at 0600, the difference in the duration of PB-sleep between the Hd100 and Hd500 groups is not significant, but the difference between the Hd 1000 value and that for Hd 500 is significant ($P < 0.025$) and borderline for the Hd 100 dose. There are no significant differences between the doses at 1200 hours. At 1800 hours there is a significant difference in sleep duration between the Hd 100 and Hd 1000 groups ($P < 0.05$). The Hd 500 value at 2400 hours differs significantly ($P < 0.05$) from the other two groups.

b. Decaborane

Pretreatment with DB very significantly prolongs PB-sleep over controls at each point tested ($P < 0.001$; Figs. 18-22, Table 6). The increases over controls were as follows: 0600 hours (69.8%), 1200 hours (69.1%), 1800 hours (95.4%) and 2400 hours (114.8%). The pattern does not exhibit a significant diurnal variation. No significant differences in sleep duration occur between the treated groups at the times tested.

c. Diphenhydramine

DPH significantly increases PB-sleep at each experimental time point ($P < 0.001$; Figs. 18-22 , Table 6). The increases over control are as follows: 0600 hours (151.0%), 1200 hours (92.7%), 1800 hours (62.7%) and 2400 hours (178.1%). The pattern is circadian, with a peak at 2400-0600 hours and a trough at 1800 hours. The differences in PB-sleep duration at the peak (2400-0600 hours) and the trough are significant ($P < 0.05$).

4. Drugs Affecting the Cholinergic System

The effect in mice of physostigmine (0.3 mg/kg, IP) and neostigmine (0.3 mg/kg, IP) tertiary and quaternary anticholinesterases, respectively, on hypnosis induced by PB (50 mg/kg, IP) was examined. The pattern for saline pretreated is relatively flat and the controls do not exhibit significant diurnal fluctuations, (Fig. 23) although there appears to be a peak at 1800 hours and a trough at 2400 hours.

With physostigmine the only significant increase over controls occurs at 2400 hours ($P < 0.05$; 71.8%; Fig. 23 Table 7). There are no significant differences between each of the physostigmine-treated groups. Neostigmine pretreatment produces significant increases in sleep duration at each experimental time point. The following increases were noted: 0600 hours (105.2%; $P < 0.001$), 1200 hours (67.6%; $P < 0.01$), 1800 hours (57.8%; $P < 0.06$) and 2400 hours (101.8%; $P < 0.02$).

Table 7: The duration of pentobarbital-sleep after pretreatment with two cholinesterase inhibitors, in mice

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
Treatment					
CONTROL	36.3+22.2 (12)	36.5+22.5 (11)	41.7+26.0 (9)	26.3+18.3 (11)	
Neostigmine	74.4+17.1** (9)	61.1+ 8.2** (9)	65.8+19.8 (8)	53.0+23.4* (8)	c ⁺
Physostigmine	44.7+30.5 (10)	41.0+28.1 (11)	55.2+19.7 (10)	45.1+18.2* (9)	

Table indicates the duration of drug-induced sleep, in minutes, for mice receiving the pretreatments plus PB (50mg/kg, IP). Sleeping times are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from controls are indicated by *(P < 0.05) and ** (P < 0.01).

⁺For explanation of letter designations see Table 1.

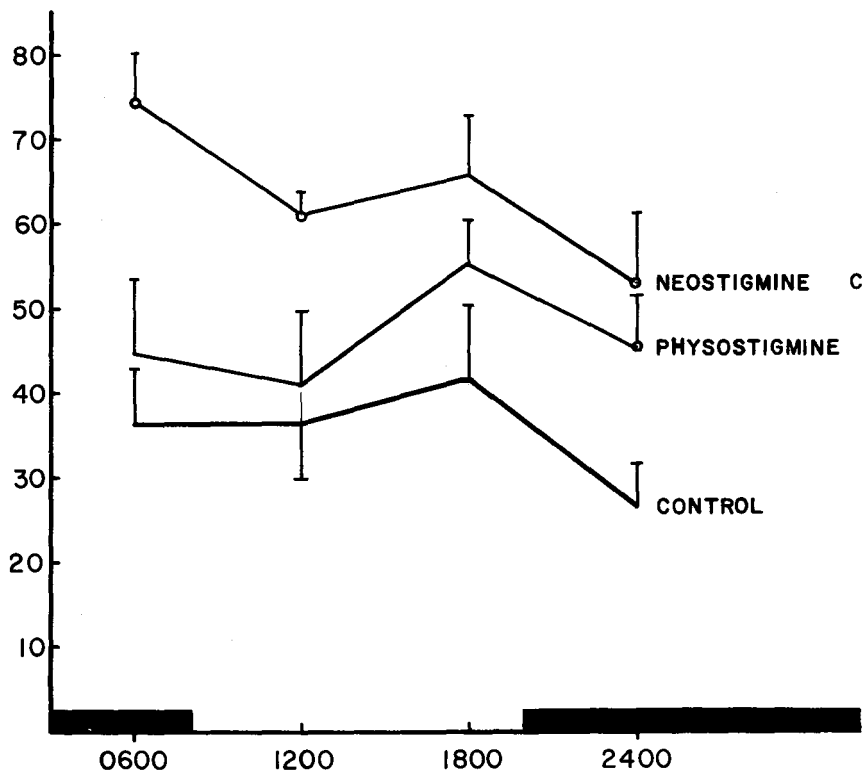


Figure 23: Pentobarbital-induced sleep after pretreatment with two anti-cholinesterases. (Open circles indicate significant differences from CONTROL, $P < 0.05$ or greater; for explanation of letter designations see Table 1).

C. Effects of Histidine Pretreatment on GBL Sleep Duration

Pretreatment of rats with Hd (100 and 500mg/kg, IP) one hour prior to GBL (350mg/kg, IP) resulted in only one significant difference in GBL-sleep duration over saline-treated controls. It occurred at 1800 hours for the Hd500 (29.7%, P 0.05; Table 8).

D. CNS Amines at $t \frac{1}{2}$ Following Pretreatment and PB Regimens

1. Time-course for Amine levels in Controls

Vehicle-treated controls were sacrificed at approximately the same time as the treated animals (i.e., $t \frac{1}{2}$ or half-way through the previously-determined sleep duration for each pretreatment drug) and the brain amine concentrations determined at the four experimental times during the 24-hour period. The time-course of amine concentrations are plotted for each of the four experimental times (Figs 24-27). The X-axis represents the time after the induction of PB-sleep. The times plotted on the X-axis depend on the $t \frac{1}{2}$ time at which each pretreatment group (control and treated) was sacrificed. The Y-axis represents the amine concentrations ($\mu\text{gm}/\text{gm}$ wet tissue weight).

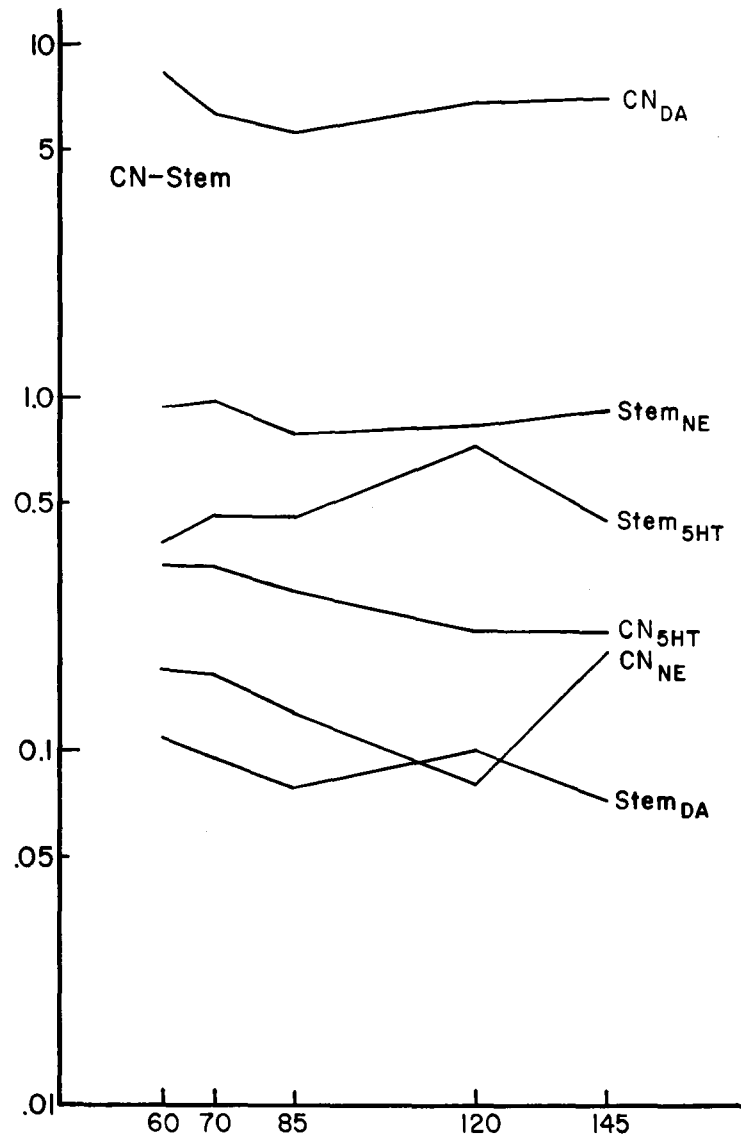
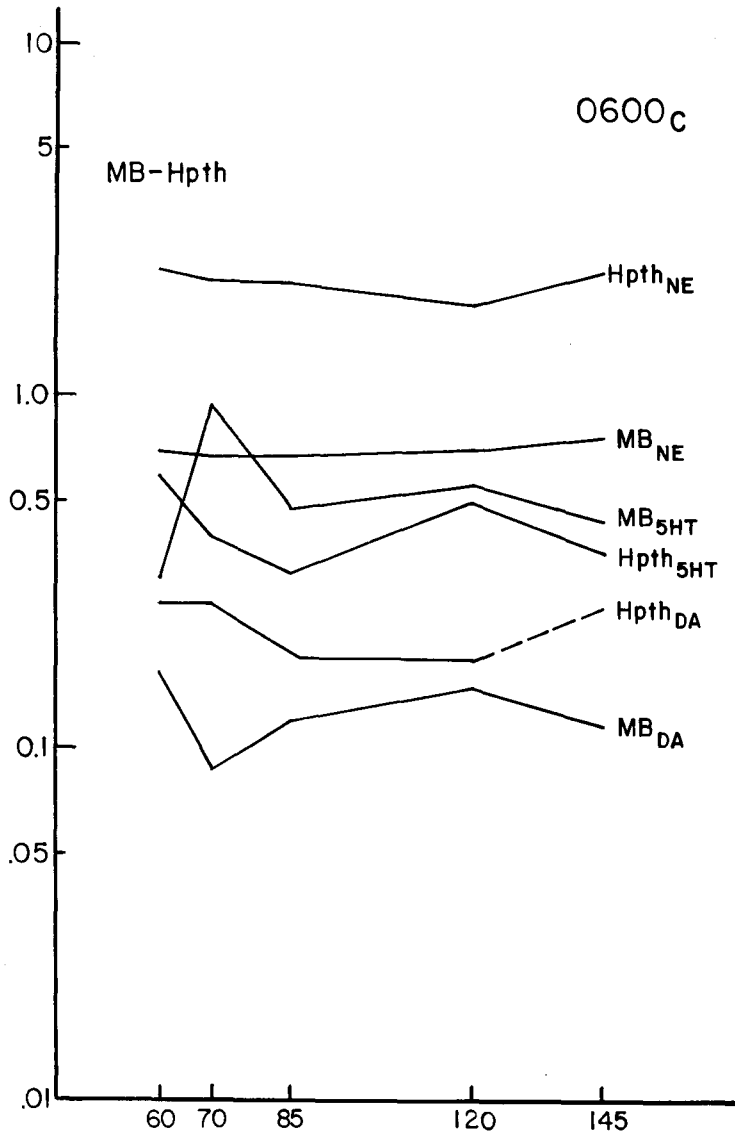
Table 8: The duration of gamma-butyrolactone-sleep after pretreatment with histidine

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
Treatment					
CONTROL	98.0+ 3.6 (6)	110.1+ 4.5 (8)	96.4+16.2 (11)	88.3+10.0 (7)	A c d E [†]
Hd100	91.9+1 .9 (4)	108.7+ 1.5 (3)	100.1+15.1 (4)	91.5+15.6 (4)	
Hd500	98.3+ 9.5 (4)	103.1+13.1 (8)	125.0+ 5.7* (2)	93.8+ 4.0 (6)	b F [†]

Table indicates the duration of drug-induced sleep, in minutes, for animals receiving the histidine pretreatment plus GBL (350mg/kg, IP). Sleeping times are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from controls are indicated by * (P < 0.05).

[†]For explanation of letter designations see Table 1.

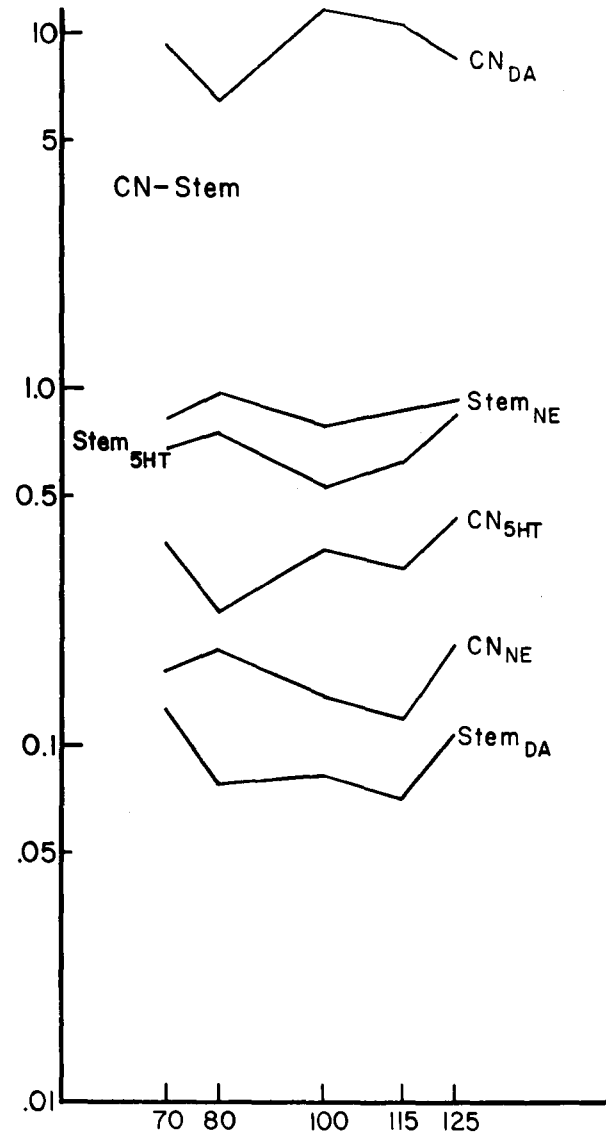
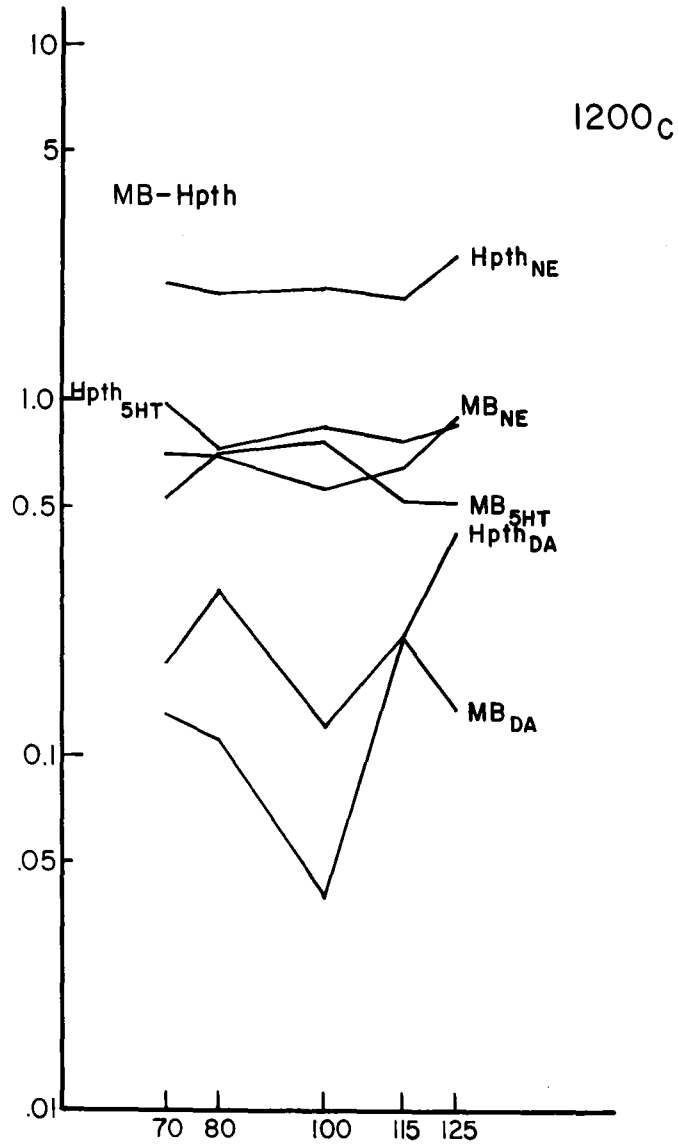
Figure 24



Legend for Figure 24

The time-course of CNS amine concentrations of CONTROLS -- 0600 hours. Mean concentrations for CONTROLS, which are compared to the TREATED groups, are plotted at their corresponding $t \frac{1}{2}$ time.

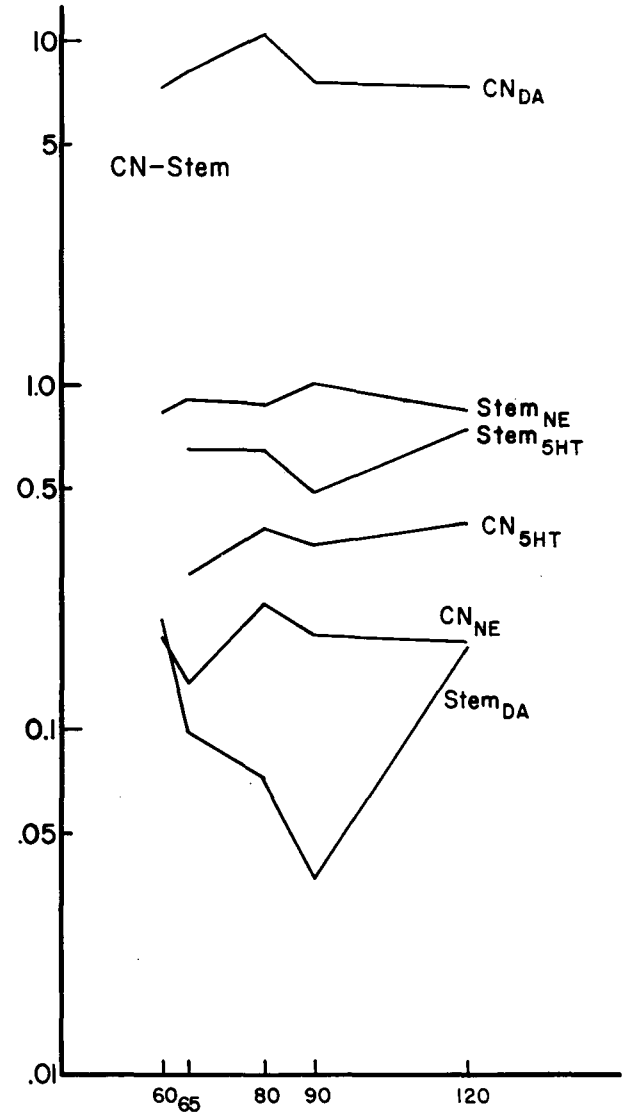
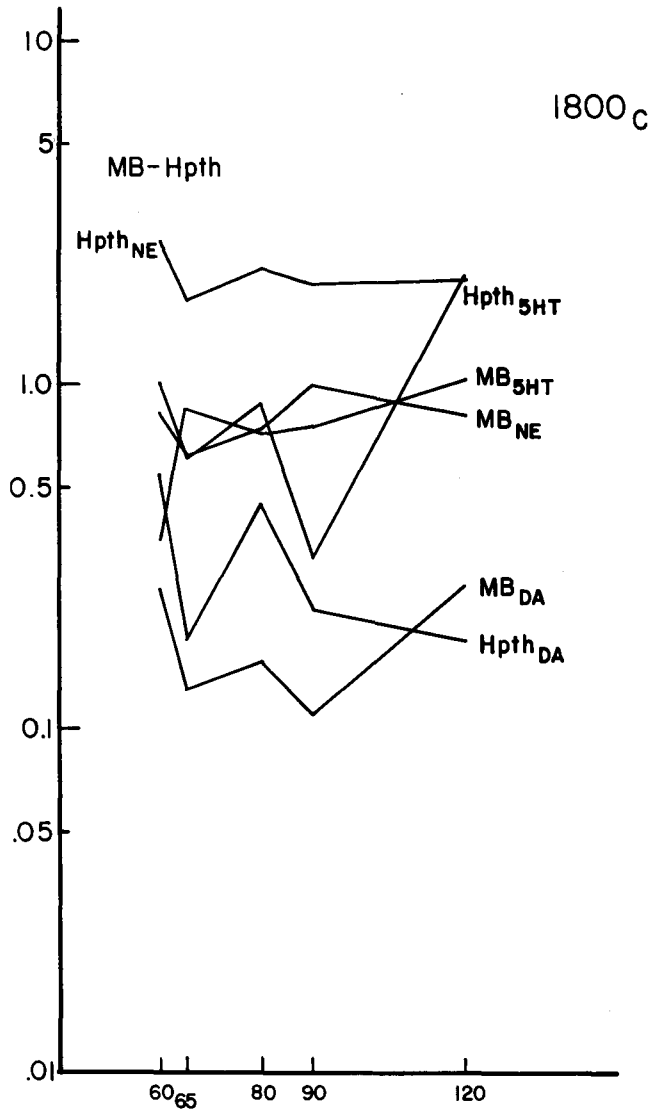
Figure 25



Legend for Figure 25

The time-course of CNS amine concentrations of CONTROLS -- 1200 hours. Mean concentrations for CONTROLS, which are compared to TREATED groups, are plotted at their corresponding $t \frac{1}{2}$ time.

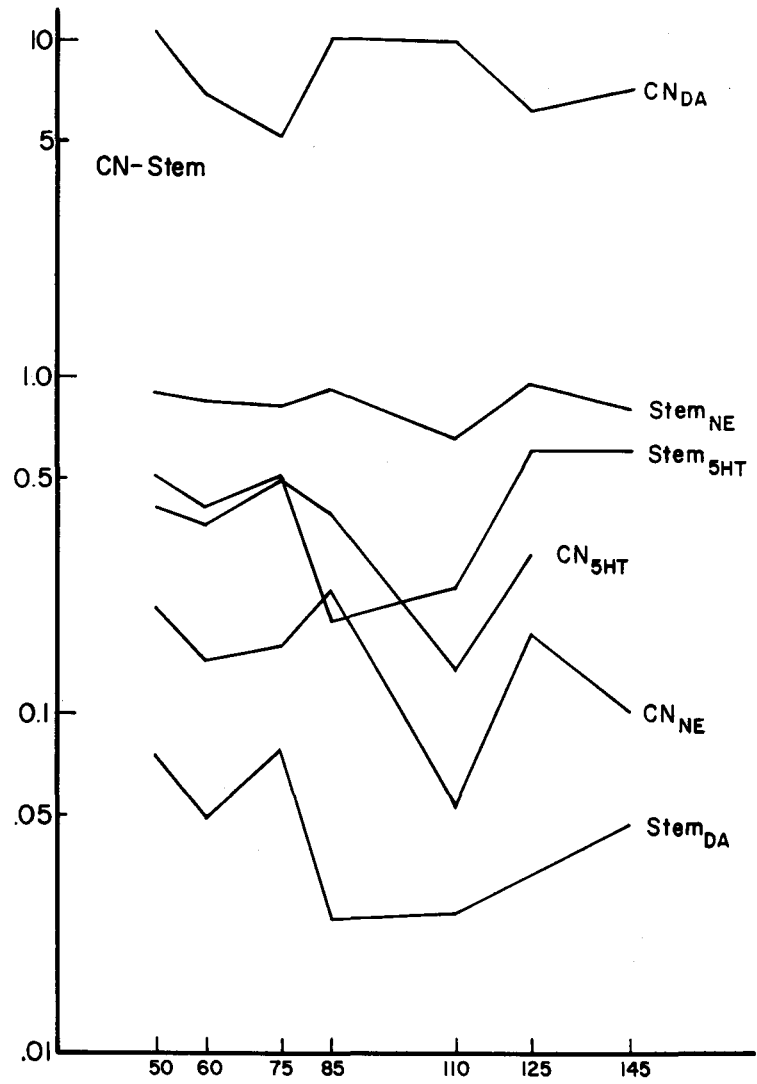
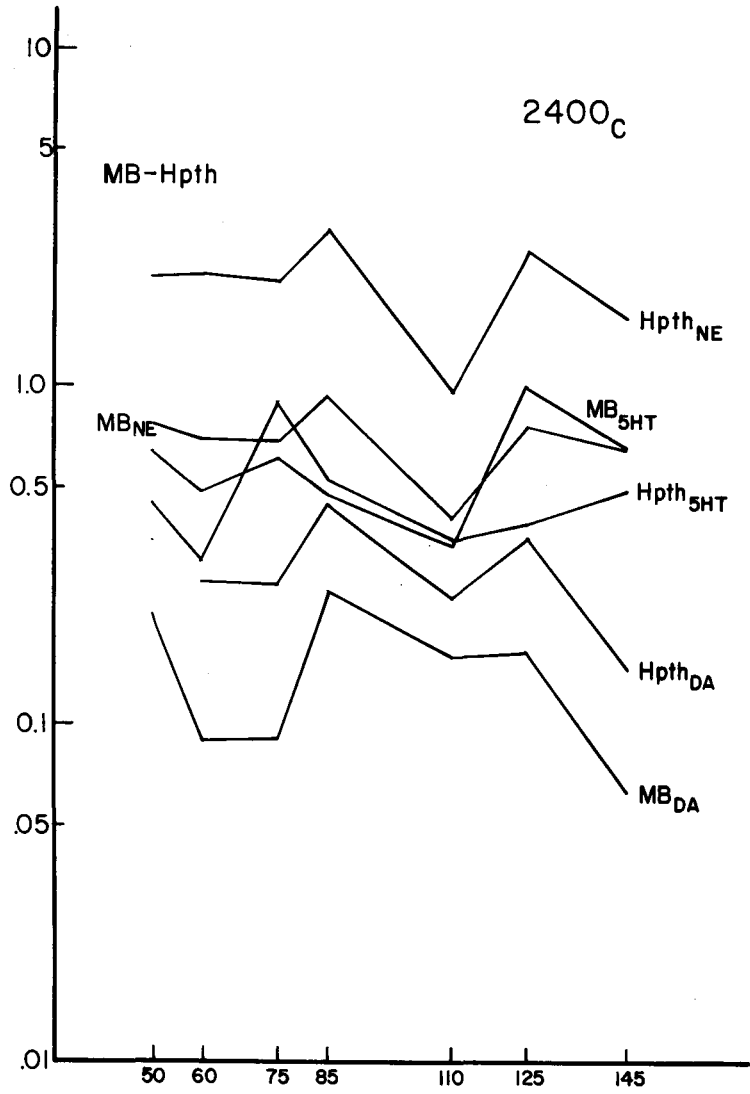
Figure 26



Legend for Figure 26

The time-course of CNS amine concentrations of CONTROLS -- 1800 hours. Mean concentrations for CONTROLS, which are compared to TREATED groups, are plotted at their corresponding $t \frac{1}{2}$ time.

Figure 27



Legend for Figure 27

The time-course of CNS amine concentrations of CONTROLS -- 2400 hours. Mean concentrations for CONTROLS, which are compared to TREATED groups, are plotted at their corresponding $t_{\frac{1}{2}}$ time.

At 0600 hours, the only significant alteration in the MB amine time-course pattern (Fig. 24) occurs between the 5HT values at 60 and 70 minutes ($P < 0.02$). There is a significant reduction of CN NE levels at 120 minutes as compared with the value at 145 minutes ($P < 0.02$). Brain stem 5HT is elevated significantly at 120 minutes, over the values at 85 or 145 minutes (both $P < 0.001$). Hypothalamic DA concentrations exhibit a significant difference between the values at 70 and 85 minutes ($P < 0.05$).

At 1200 hours MB NE levels differ significantly between 80 and 100 minutes ($P < 0.05$). MB DA levels exhibit significant differences between 80 and 100 minutes ($P < 0.01$) and 100 and 115 minutes ($P < 0.025$). There are significant variations in CN DA concentrations between the following times: 70 vs. 80 minutes ($P < 0.05$), 80 vs. 100 minutes ($P < 0.001$) and 115 vs. 125 minutes ($P < 0.01$). Hypothalamic DA content at 145 minutes is increased significantly from that at 115 minutes ($P < 0.05$) (Fig. 25).

At 1800 hours, significant differences between MB DA levels occur between 60 and 65 minutes ($P < 0.01$) and 80 and 90 minutes ($P < 0.05$). MB 5HT content between 60 and 65 minutes differs significantly. Brain stem DA reaches a minimum at 90 minutes, which differ significantly from the value at 120 minutes ($P < 0.05$). Hypothalamic NE levels at 60 minutes are elevated from that at 65 minutes ($P < 0.025$). Hypothalamic 5HT content is reduced significantly at 90 minutes, and differs from 80 minutes ($P < 0.05$) and 120 minutes ($P < 0.001$) (Fig. 26).

At 2400 hours there are significant differences in MB NE levels between the following times: 75 vs. 85 minutes ($P < 0.01$), 85 vs. 110 minutes ($P < 0.001$) and 110 vs. 125 minutes ($P < 0.005$). Between 75 and 85

minutes and 125 and 145 minutes MB DA levels differ significantly (both $P < 0.005$). MB 5HT is elevated at 125 minutes, differing significantly from that at 100 ($P < 0.002$) and 145 minutes ($P < 0.05$). CN NE concentration is decreased significantly at 110 minutes from the values at 85 minutes ($P < 0.02$) and 125 minutes ($P < 0.05$). There are several significant differences between CN DA concentrations: 50 vs. 60 minutes ($P < 0.005$), 75 vs. 85 minutes ($P < 0.02$) and 110 vs. 125 minutes ($P < 0.025$). Brain stem NE levels are significantly lower at 110 minutes than at 125 minutes ($P < 0.02$). Brain stem DA levels vary significantly: 75 vs. 85 minutes ($P < 0.05$), 85 vs. 110 minutes ($P < 0.02$), 110 vs. 125 minutes ($P < 0.02$) and 125 vs. 145 minutes ($P < 0.05$). Hypothalamic DA concentrations reach a minimum at 145 minutes differing significantly from the value at 125 minutes ($P < 0.01$). Hypothalamic 5HT concentrations differ significantly between the 85 and 110 minutes determinations ($P < 0.05$) (Fig. 27).

2. Drugs affecting the NE and DA systems

a. DOPA-pargyline

DL-DOPA (500 mg/kg, IP) and pargyline (6 mg/kg, IP) administered to rats one hour and 30 minutes, respectively, prior to PB (50 mg/kg, IP). DOPA-pargyline treatment significantly increased DA ($P < 0.05$ or less) above control in almost all parts of the brain examined (15/16). (Table 9, Figs. 28 - 29). At 0600 hours the following increases were obtained: MB (724.7%, $P < 0.02$), CN (44.0%, $P < 0.05$), brain stem (1045.7%, $P < 0.005$) and hypothalamus (1457.5%

Table 9: CNS concentrations of NE, DA and 5HT after DOPA-pargyline pretreatment with PB, on a 24-hour basis.

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.69+.14 (3)	.88+.03 (2)	.81+.17 (3)	.75+.04 (3)
T	.74+.05 (3)	.93+.08 (4)	.79+.17 (4)	.79+.02 (4)
Hpth NE				
C	1.79+.25 (3)	2.52+.54 (2)	2.02+.25 (3)	2.55+.44 (3)
T	1.97+.17 (3)	2.41+.18 (4)	2.37+.62 (4)	2.71+.21 (4)
CN NE				
C	.08+.03 (3)	.19+.06 (2)	.18+.02 (3)	.17+.05 (3)
T	.19+.04* (3)	.32+.07 (4)	.42+.14* (4)	.44+.07** (4)
Stem NE				
C	.83+.04 (3)	.93+.16 (2)	.85+.13 (3)	.96+.08 (3)
T	.92+.02* (3)	.99+.13 (4)	1.00+.26 (4)	1.08+.19 (4)
MB DA				
C	.15+.005 (2)	.13+.008 (2)	.26+.13 (3)	.16+.03 (3)
T	1.21+.29* (3)	1.13+.40* (4)	3.09+.22** (3)	2.64+.46** (3)
Hpth DA				
C	.18+.06 (3)	.42+.04 (2)	.18+.05 (3)	.35+.04 (2)
T	2.74+.67** (3)	4.17+.96** (4)	9.81+.75** (4)	6.55+1.41** (3)
CN DA				
C	6.81+1.58 (3)	8.47+.61 (2)	7.37+2.04 (3)	6.12+.93 (3)
T	9.80+.33* (3)	10.41+.88 (3)	12.49+2.66* (4)	11.54+2.18* (3)
Stem DA				
C	.10+.04 (3)	.11+.07 (2)	.17+.01 (3)	.23+.004 (2)
T	1.15+.28** (3)	1.30+.44* (3)	3.66+.89** (4)	2.26+.52* (3)

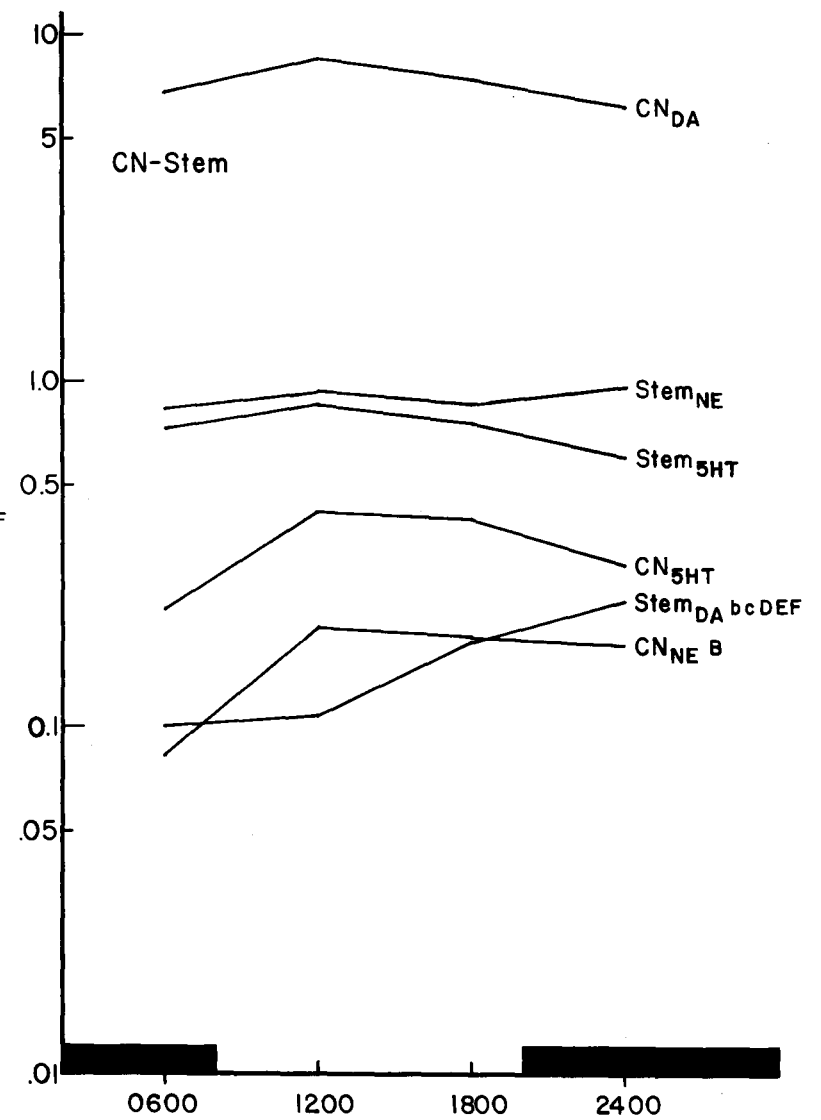
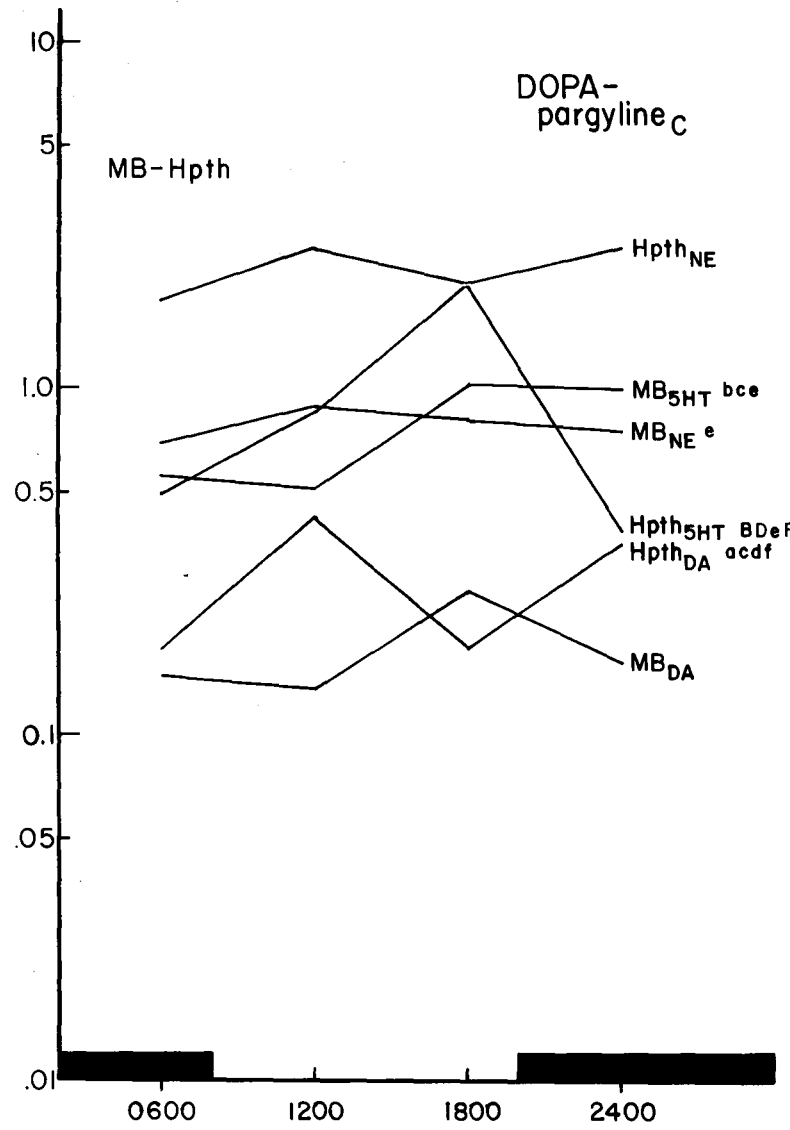
Table 9 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.56+.11 (3)	.57+.03 (2)	1.02+.23 (3)	1.00+.15 (3)
T	.37+.06 (3)	.44+.05 (3)	.36+.06* (2)	.40+.08** (3)
Hpth 5HT				
C	.49+.15 (3)	.85+.16 (2)	2.03+.06 (2)	.39+.15 (3)
T	.48+.10 (3)	.87+.09 (3)	2.26+.92 (3)	1.16+.22** (3)
CN 5HT				
C	.22+.05 (3)	.42+.12 (2)	.40+.12 (3)	.30+.13 (3)
T	.25+.05 (3)	.60+.15 (3)	.47+.10 (3)	.29+.07 (3)
Stem 5HT				
C	.73+.02 (3)	.85+.07 (2)	.74+.17 (3)	.60+.12 (3)
T	.57+.10 (3)	.65+.07* (3)	.44+.08 (3)	.48+.09 (3)

Table indicates amine concentrations (ugm/gm) of NE, DA and 5HT in midbrain (MB), hypothalamus (Hpth), caudate nucleus (CN) and brain stem (Stem) of rats receiving DOPA-pargyline pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means + standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * (P<0.05) or ** (P<0.01).

C = Control; T= Treated

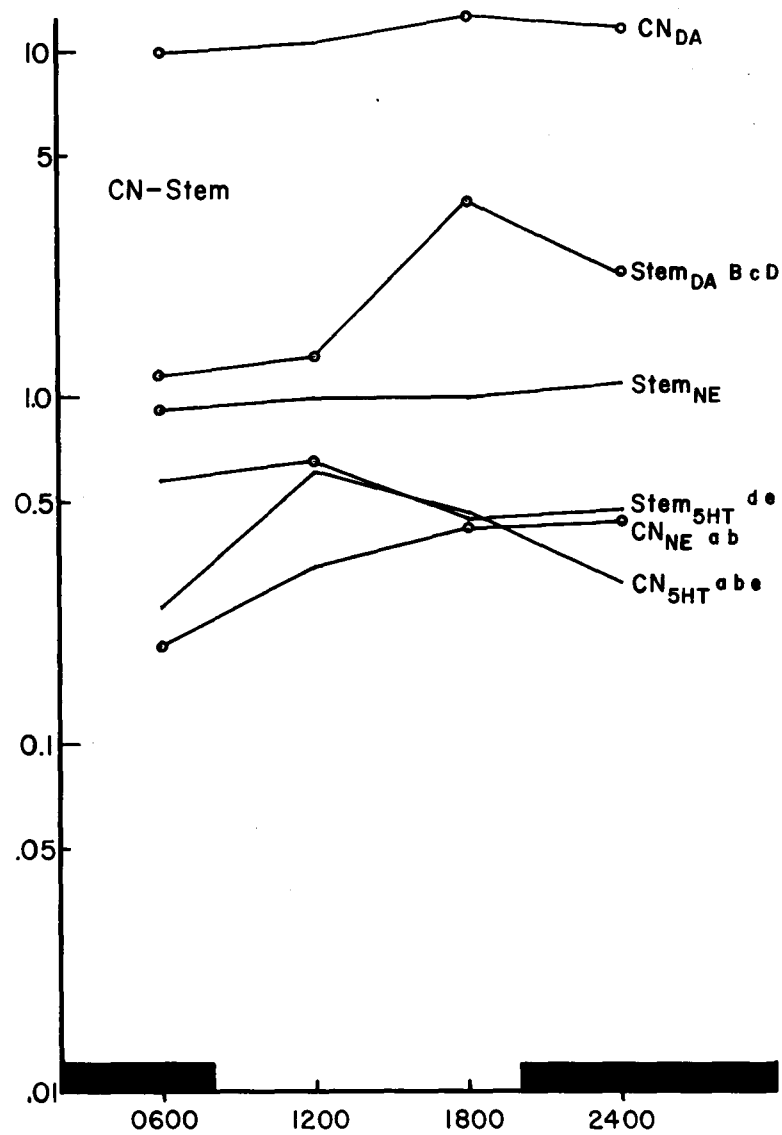
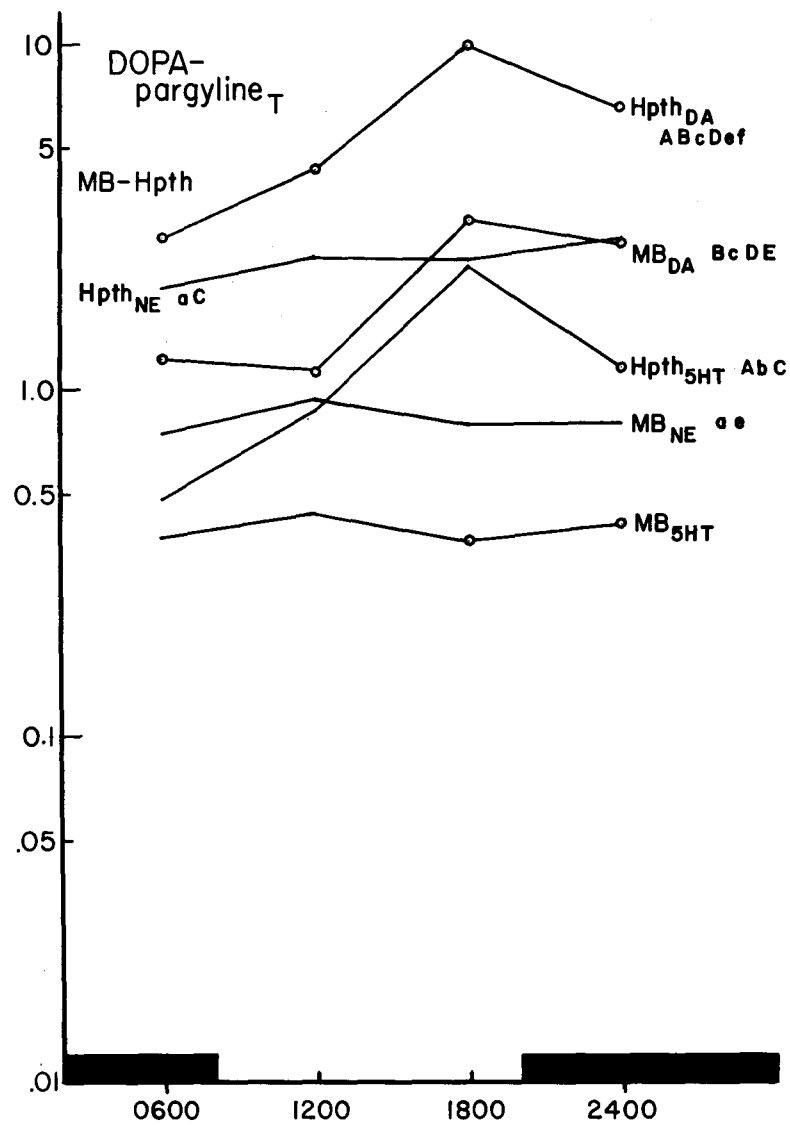
Figure 28



Legend for Figure 28

The effects of DOPA-pargyline pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- CONTROLS. Mean amine concentrations are plotted on a three-cycle scale (Y-axis), with the X-axis the daily time (hours). Letter designations adjacent to the amine pattern indicate point to point comparisons within each 24-hour pattern: a A (0600 vs 1200 hours), b B (0600 vs 1800 hours), c C (0600 vs 2400 hours), d D (1200 vs 1800 hours), e E (1200 vs 2400 hours) and f F (1800 vs 2400 hours). Lower case indicates $P < 0.05$ and capitals indicate $P < 0.01$ for significant differences.

Figure 29



Legend for Figure 29

The effects of DOPA-pargyline pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notation of figure, see legend to Figure 28.

P 0.005). NE levels also increase in the CN (137.5%, P 0.02) and brain stem (10.4%, P < 0.025). At 1200 hours, DA levels increase as follows: MB (745.5%, P < 0.05), brain stem (1112.3%, P < 0.05) and hypothalamus (891.6%, P < 0.01). On the other hand, 5HT levels decrease significantly in the brain stem (23.0%, P < 0.05). At 1800 hours DA concentrations are significantly elevated in the MB (1094.4%, P < 0.001), CN (69.6%, P < 0.05), brain stem (1992.4%, P < 0.005) and hypothalamus (5394.6%, P < 0.001). MB 5HT decreases significantly (64.4%, P < 0.05), while CN NE increases significantly (132.1%, P < 0.05). At 2400 hours the significant increases in DA levels were: MB (1536.6%, P < 0.001), CN (89.0%, P < 0.02), brain stem (879.5%, P < 0.02) and hypothalamus (1753.4%, P < 0.01). Significant increases occur for CN NE (154.1%, P < 0.005) and hypothalamus 5HT (200.4%, P < 0.01). Of the 24-hour patterns obtained for the three amines, 9 of 12 are circadian, while the remainder are unvarying. Fluctuation of patterns was found for NE in the MB, CN and hypothalamus; for DA in MB, brain stem and hypothalamus and for 5HT in CN, brain stem and hypothalamus. For details of this aspect of the study refer to Figs. 28-29, Table 9 and to Appendix B.

b. Alpha-methyl-p-tyrosine

AMPT (200mg/kg, IP) was administered four hours prior to PB.

In almost all cases, NE and DA levels are significantly decreased from controls (Table 10, Figs, 30-31). At 0600 hours, NE concentrations are significantly lower in the MB (52.6%, P < 0.005), CN (61.2%, P < 0.001), brain stem (45.8%, P < 0.005) and the hypothalamus (53.9%, P < 0.005). DA levels are reduced significantly in the CN (77.8%, P < 0.001) and hypothalamus (86.3%,

Table 10: CNS concentrations of NE, DA and 5HT after AMPT pretreatment with PB, on a 24-hour basis

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.69+.04 (4)	.70+.12 (4)	.82+.08 (3)	.92+.04 (4)
T	.33+.14** (4)	.39+.03** (4)	.37+.04** (5)	.38+.05** (4)
Hpth NE				
C	2.27+.44 (4)	2.12+.34 (6)	2.62+.13 (3)	2.94+.59 (4)
T	1.05+.15** (4)	1.01+.23** (4)	1.03+.19** (5)	1.11+.18** (4)
CN NE				
C	.17+.10 (2)	.16+.04 (5)	.19+.03 (3)	.23+.06 (4)
T	.07+.04** (4)	.13+.03 (4)	.10+.03* (5)	.17+.05 (4)
Stem NE				
C	.94+.13 (4)	.83+.09 (6)	.84+.08 (3)	.92+.15 (4)
T	.51+.02** (3)	.43+.05** (6)	.40+.07** (5)	.45+.03** (4)
MB DA				
C	.12+.02 (3)	.13+.05 (3)	.25+.01 (2)	.25+.01 (2)
T	.11+.02 (3)	.09+.01 (4)	.10+.02** (4)	.14+.03** (4)
Hpth DA				
C	.36+.10 (3)	.18+.07 (4)	.54+.17 (3)	.44+.15 (4)
T	.05+.02* (3)	.15+.07 (4)	.30+.09 (2)	.12+.07* (3)
CN DA				
C	8.24+1.00 (4)	9.26+1.52 (5)	7.33+1.49 (3)	10.02+1.88 (4)
T	1.83+.39** (4)	1.99+.53** (4)	2.20+.81** (5)	2.87+.33** (4)
Stem DA				
C	.11+.05 (3)	.13+.06 (4)	.26+.08 (2)	.02+.003 (2)
T	.08+.006 (3)	.29+.10* (6)	.17+.06 (5)	.03+.03 (3)

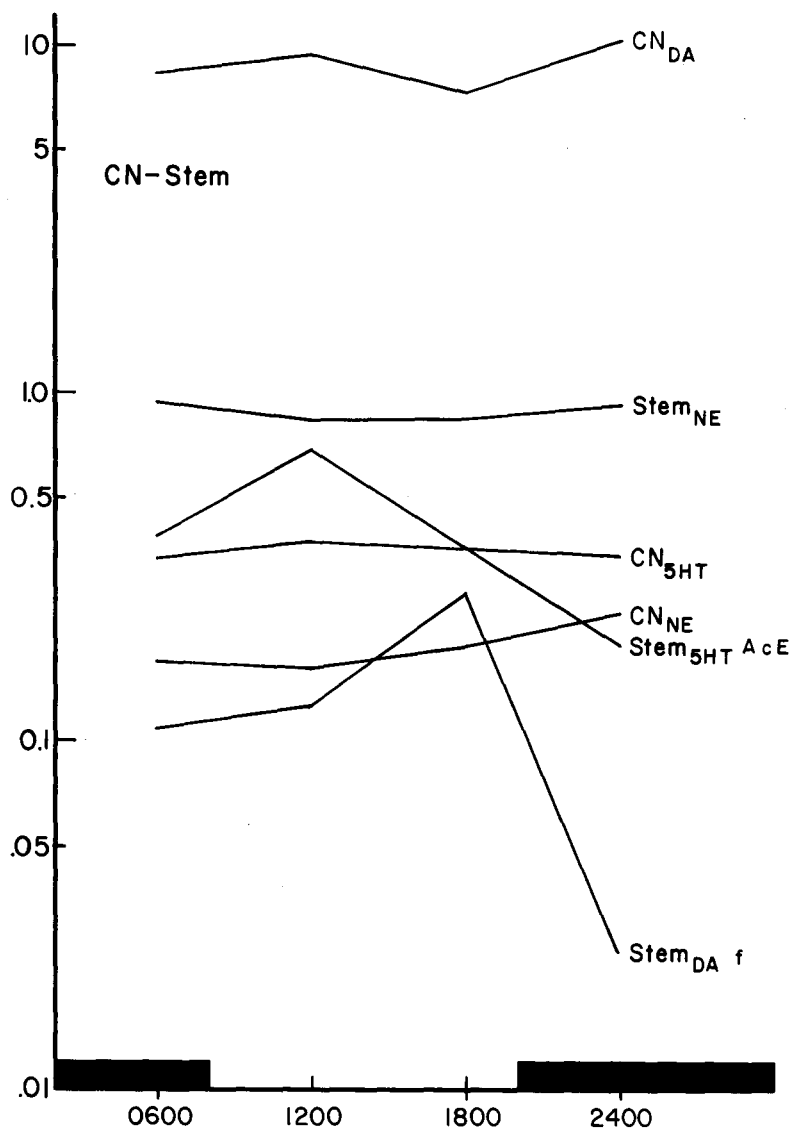
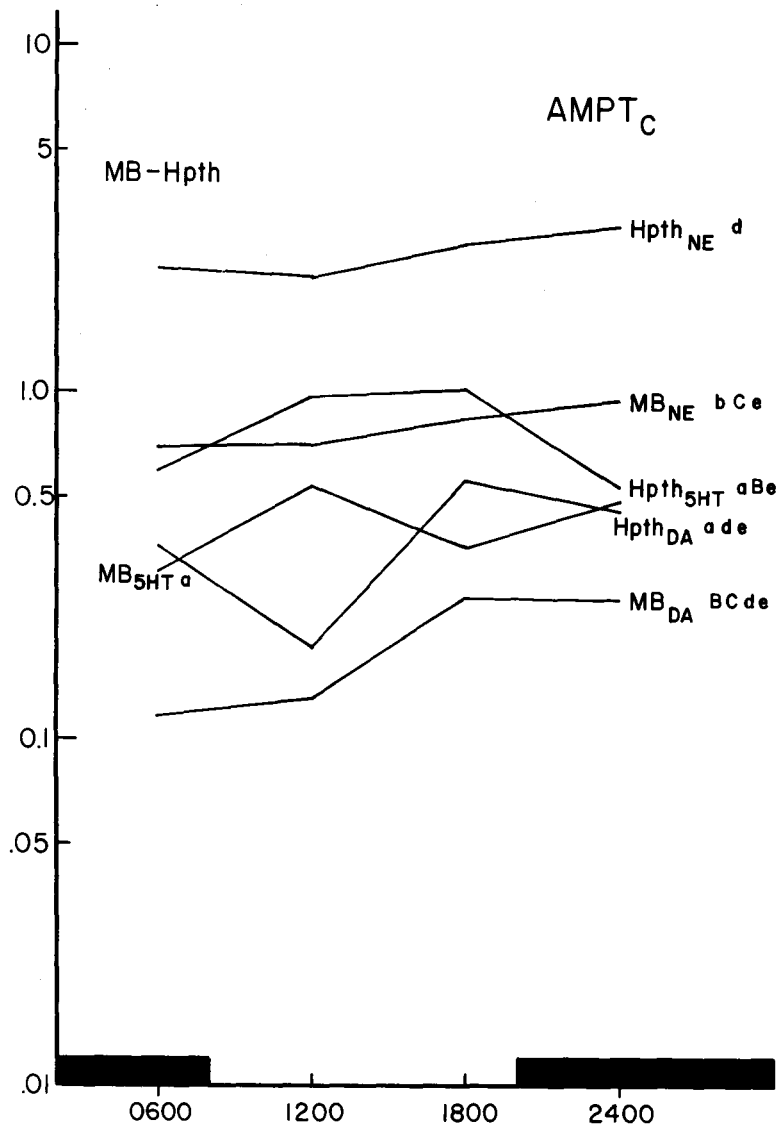
Table 10 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.30 \pm .08 (4)	.53 \pm .08 (3)	.35 \pm .06 (2)	.48 \pm .20 (4)
T	.60 \pm .02** (3)	.85 \pm .14** (3)	.39 \pm .07 (3)	.48 \pm .08 (3)
Hpth 5HT				
C	.59 \pm .09 (4)	.96 \pm .18 (3)	1.00 \pm .13 (3)	.52 \pm .05 (3)
T	.68 \pm .18 (4)	.53 \pm .07* (3)	(.97) (1)	.48 \pm .23 (4)
CN 5HT				
C	.33 \pm .21 (4)	.37 \pm .01 (3)	---	.34 \pm .17 (4)
T	.31 \pm .06 (3)	.37 \pm .05 (3)	---	.16 \pm .05 (4)
Stem 5HT				
C	.39 \pm .04 (3)	.68 \pm .05 (4)	---	.19 \pm .04 (3)
T	.58 \pm .05** (3)	.92 \pm .28 (3)	---	.16 \pm .05 (3)

Table indicates amine concentrations ($\mu\text{gm}/\text{gm}$) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving AMPT pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * ($P < 0.05$) or ** ($P < 0.01$).

C = Control; T = Treated

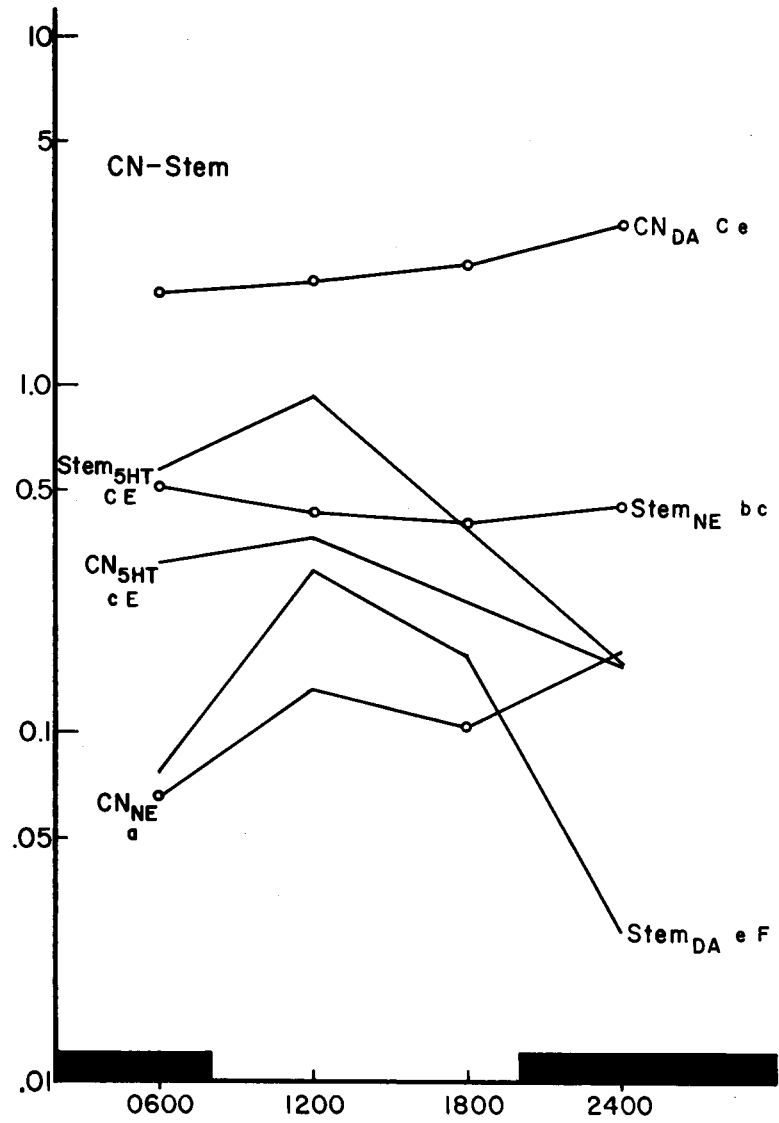
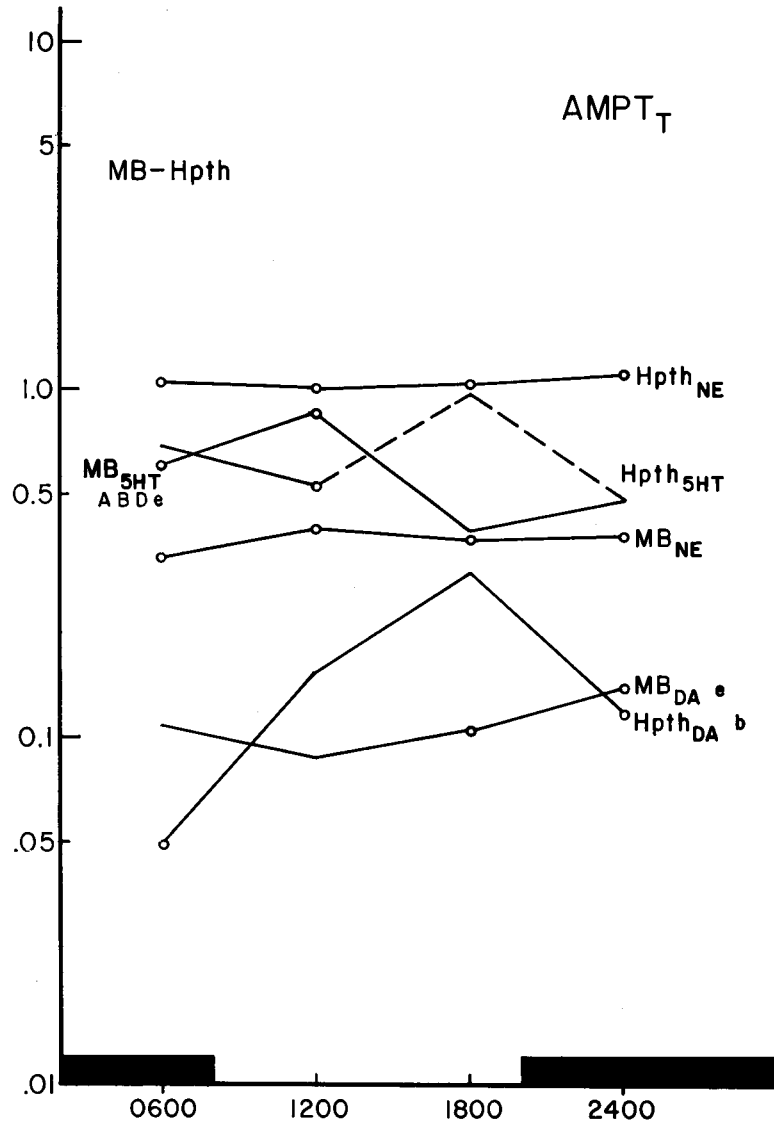
Figure 30



Legend for Figure 30

The effects of AMPT pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- CONTROL. For complete explanation of notations of figure, see legend to Figure 28.

Figure 31



Legend for Figure 31

The effects of AMPT pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notations of figure, see legend to Figure 28.

$P < 0.01$). On the other hand, 5HT concentrations are elevated significantly in the MB (99.9%, $P < 0.005$) and brain stem (48.3%, $P < 0.01$).

At 1200 hours, NE levels decrease significantly in the MB (43.6%, $P < 0.01$), brain stem (48.2%, $P < 0.001$) and hypothalamus (52.1%, $P < 0.005$). DA concentrations are lowered significantly in the CN (78.5%, $P < 0.001$), but increase in the brain stem (131.8%, $P < 0.05$). 5HT levels increase significantly in the MB (60.3%, $P < 0.025$), but decrease in the hypothalamus (44.5%, $P < 0.02$).

At 1800 hours NE levels decrease significantly in the MB (55.4%, $P < 0.001$), CN (43.9%, $P < 0.02$), brain stem (51.6%, $P < 0.001$) and hypothalamus (60.5%, $P < 0.001$). DA concentrations are reduced significantly in the MB (58.6%, $P < 0.001$) and CN (70.0%, $P < 0.001$).

NE levels at 2400 hours are decreased significantly in the MB (58.9%, $P < 0.001$), brain stem (50.8%, $P < 0.001$) and hypothalamus (62.2%, $P < 0.001$) and there is a significant reduction of DA levels in the MB (44.0%, $P < 0.01$), CN (71.4%, $P < 0.001$) and hypothalamus (74.7%, $P < 0.02$). Of the 12 amine patterns, after AMPT treatment 7 display diurnal rhythms. These can be examined in detail in Figs.30-31, Table 10 and in Appendix B .

3. Drugs Affecting the Serotonin System

a. p-Chlorophenylalanine

pCPA (3 x 100mg/kg, IP) depletes brain 5HT significantly in all brain tissues examined, at each time point studied, with relatively minor effects on CAs (Table 11, Figs.32-33). At 0600 hours, 5HT concentrations are reduced significantly in the MB (97.0%, $P < 0.01$), CN (87.8%, $P < 0.01$), brain stem (94.8%, $P < 0.01$) and hypothalamus ($P < 0.01$). At 1200 hours, 5HT

Table 11: CNS concentrations of NE, DA and 5HT after pCPA pretreatment with PB, on a 24-hour basis

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.67+.08 (3)	.69+.06 (3)	.74+.11 (4)	.68+.10 (3)
T	.65+.03 (3)	.64+.11 (5)	.59+.14 (5)	.67+.10 (5)
Hpth NE				
C	2.11+.21 (3)	1.99+.31 (3)	2.18+.23 (4)	2.00+.18 (3)
T	1.94+.53 (3)	1.76+.27 (4)	1.28+.20** (5)	2.03+.34 (5)
CN NE				
C	.16+.03 (3)	.19+.07 (3)	.23+.06 (3)	.16+.04 (3)
T	.19+.02 (3)	.19+.04 (5)	.14+.03* (5)	.18+.02 (5)
Stem NE				
C	.97+.12 (3)	.97+.18 (3)	.87+.09 (4)	.82+.10 (3)
T	.86+.13 (3)	.70+.05* (5)	.70+.07** (5)	.67+.10 (5)
MB DA				
C	.09+.03 (3)	.11+.02 (3)	.16+.01 (3)	.09+.03 (3)
T	.09+.02 (3)	.08+.02* (5)	.11+.03 (4)	.10+.03 (5)
Hpth DA				
C	.26+.02 (3)	.29+.06 (2)	.44+.12 (3)	.26+.06 (3)
T	.28+.05 (3)	.25+.06 (5)	.12+.03** (4)	.18+.08 (4)
CN DA				
C	6.35+1.05 (3)	6.42+.87 (3)	10.06+2.78 (3)	5.17+1.03 (3)
T	4.71+1.16 (3)	4.87+.80* (5)	7.33+.49 (5)	5.41+.80 (5)
Stem DA				
C	.09+.02 (2)	.08+.02 (3)	.07+.02 (4)	.08+.01 (3)
T	.03+.04 (3)	.08+.01 (5)	.08+.03 (4)	.07+.04 (5)

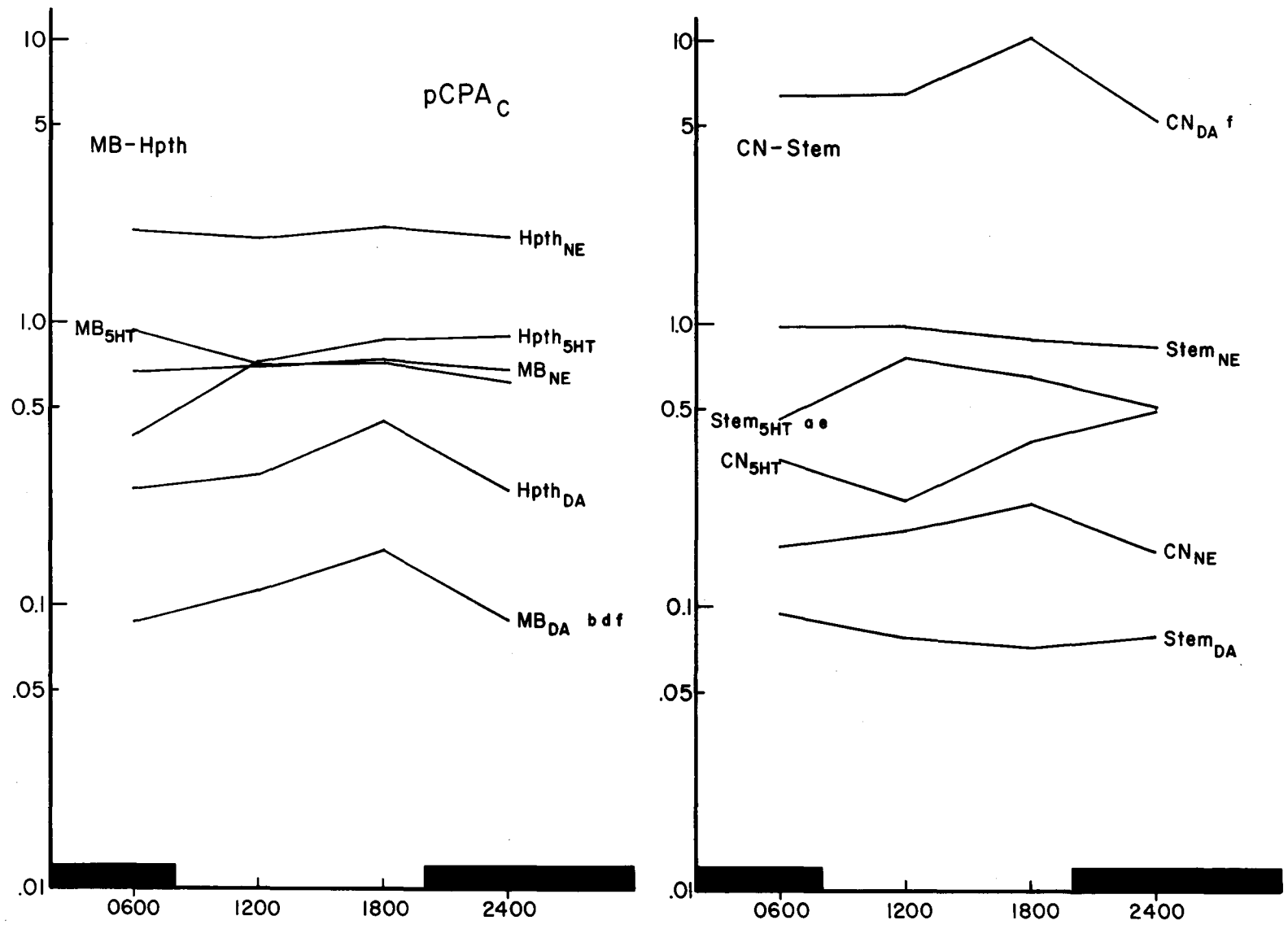
Table 11 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.93+.31 (3)	.70+.20 (2)	.71+.20 (4)	.61+.09 (2)
T	.03+.01** (3)	.08+.04** (5)	.08+.02** (4)	.36+.09* (4)
Hpth 5HT				
C	.40+.11 (2)	.72+.20 (3)	.87+.15 (2)	.89+.41 (3)
T	(0)**	.24+.12** (5)	.08+.07** (3)	.05+.003* (3)
CN 5HT				
C	.33+.07 (3)	.24+.02 (2)	.38+.13 (4)	.49+.14 (3)
T	.04+.07** (3)	.02+.02** (4)	.10+.03** (4)	.13+.01* (3)
Stem 5HT				
C	.46+.14 (3)	.75+.09 (3)	.64+.12 (2)	.50+.08 (3)
T	.02+.04** (3)	.10+.03** (5)	.03+.02** (4)	.07+.03** (5)

Table indicates amine concentrations ($\mu\text{gm}/\text{gm}$) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving pCPA pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means \pm standard deviation, with the number of animals used in parentheses.

Significant differences from control are noted by * ($P < 0.05$) or ** ($P < 0.01$).
C = Control; T = Treated

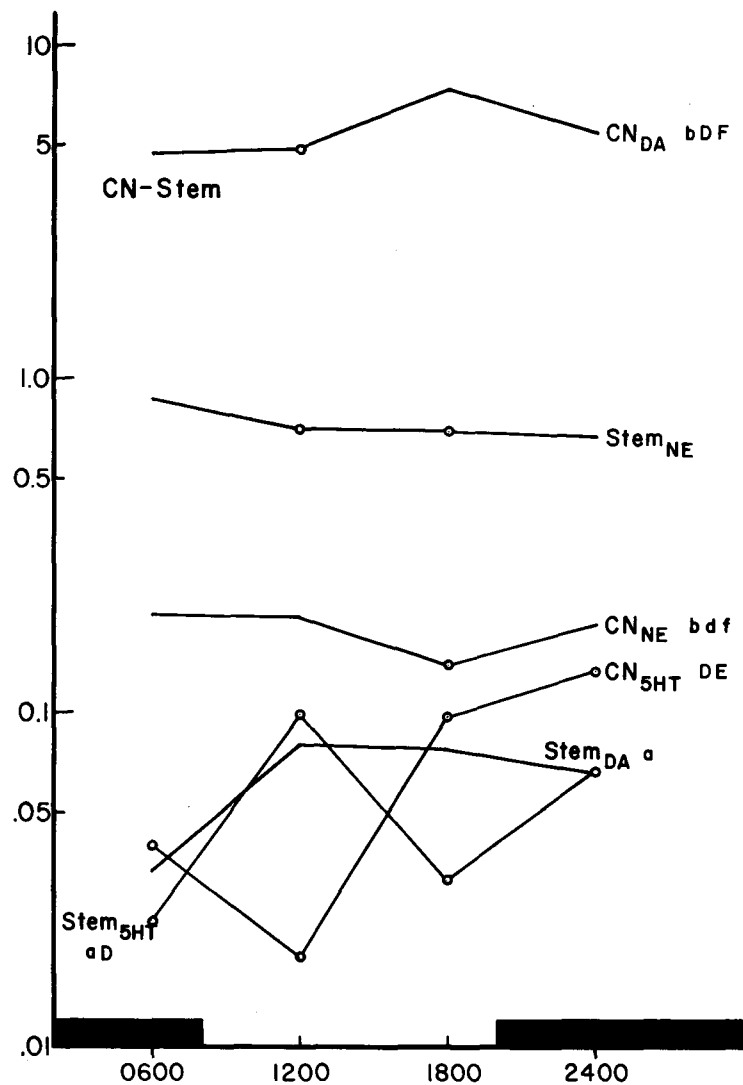
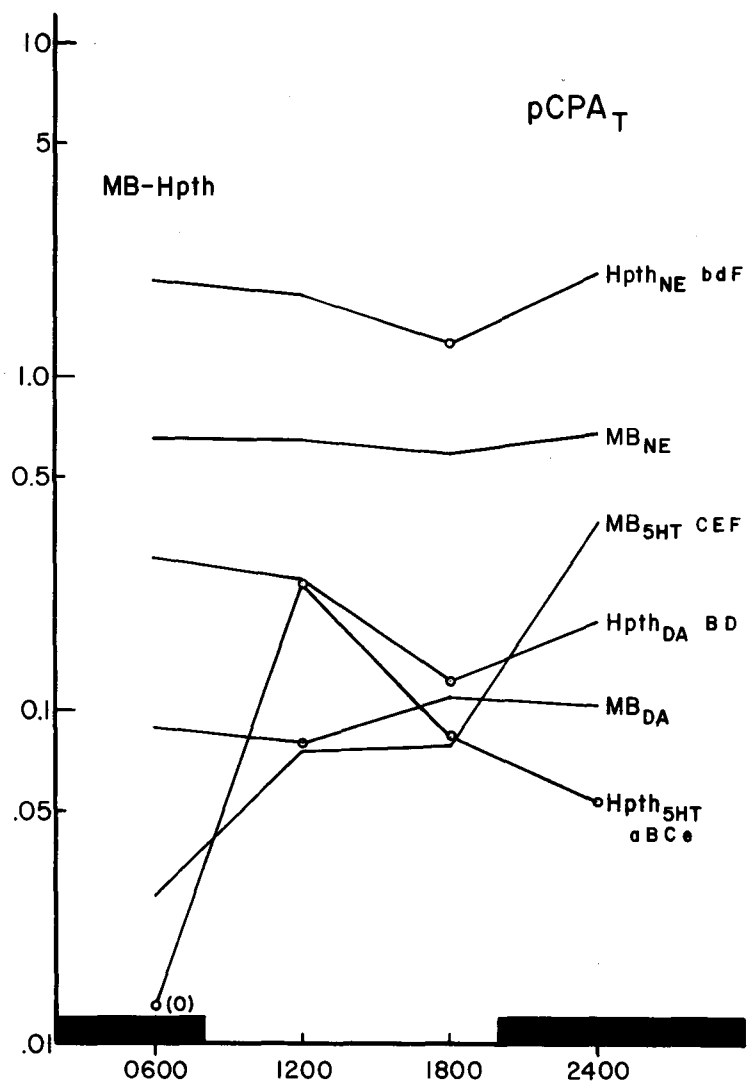
Figure 32



Legend for Figure 32

The effects of pCPA pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- CONTROL. For complete explanation of notations of figure, see legend to Figure 28.

Figure 33



Legend for Figure 33

The effects of pCPA pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notations of figure, see legend to Figure 28.

levels decrease significantly in the MB (89.3%, $P < 0.001$), CN (92.1%, $P < 0.001$), brain stem (86.8%, $P < 0.001$) and hypothalamus (66.9%, $P < 0.01$). DA levels decrease significantly in the MB (29.1%, $P < 0.05$) and CN (24.2%, $P < 0.05$), while NE is reduced in the brain stem (27.7%, $P < 0.02$).

At 1800 hours 5HT content is reduced significantly in the MB (89.0%, $P < 0.001$), CN (74.5%, $P < 0.005$), brain stem (95.0%, $P < 0.001$) and hypothalamus (90.5%, $P < 0.001$). NE levels are reduced in the CN (39.7%, $P < 0.02$), brain stem (19.9%, $P < 0.01$) and hypothalamus (41.6%, $P < 0.001$). Hypothalamic DA is reduced significantly (72.7%, $P < 0.005$). At 2400 hours there is a significant decrease of 5HT in the MB (40.4%, $P < 0.05$), CN (72.9%, $P < 0.02$), brain stem (86.6%, $P < 0.001$) and hypothalamus (94.1%, $P < 0.025$). Fluctuating patterns were seen in 8 of 12 amine patterns studied. For additional details, cf. Figs.32-33, Table 11 and Appendix B.

b. Tryptophan(Figures 34-35, Table 12)

TP (500mg/kg,IP) was administered one hour prior to PB. At 0600 hours 5HT concentrations in treated animals are increased significantly in the MB (40.5%, $P < 0.005$) and the hypothalamus (130.7%, $P < 0.005$), NE in the hypothalamus is reduced significantly (15.7%, $P < 0.05$). At 1200 hours TP decreased DA significantly in the MB (30.4%, $P < 0.05$), brain stem (81.0%, $P < 0.005$) and hypothalamus (29.3%, $P < 0.05$). CN NE levels decrease significantly (48.5%, $P < 0.05$). At 1800 hours, hypothalamic 5HT is increased significantly (692.0%, $P < 0.01$), while MB NE decreases significantly (26.2%, $P < 0.05$). At 2400 hours, brain stem 5HT levels increase significantly (42.9%, $P < 0.05$), while hypothalamic DA decreases significantly (42.0%, $P < 0.025$).

Table 12: CNS concentrations of NE, DA and 5HT after TP pretreatment with PB, on a 24-hour basis

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.67+.04 (3)	.69+.06 (3)	.98+.08 (2)	.68+.10 (3)
T	.62+.03 (4)	.65+.05 (5)	.73+.11* (5)	.70+.09 (5)
Hpth NE				
C	2.07+.11 (3)	1.99+.31 (3)	1.96+.02 (2)	2.01+.18 (3)
T	1.75+.17* (4)	2.10+.50 (5)	2.21+.35 (5)	2.04+.27 (5)
CN NE				
C	.13+.02 (3)	.19+.07 (3)	.19+.03 (2)	.16+.04 (3)
T	.12+.02 (4)	.10+.02* (5)	.15+.04 (5)	.14+.04 (5)
Stem NE				
C	.79+.04 (3)	.97+.18 (3)	1.10+.14 (3)	.82+.10 (3)
T	.76+.08 (4)	.80+.03 (5)	.92+.08 (5)	.79+.08 (5)
MB DA				
C	.12+.05 (3)	.12+.02 (3)	.11+.002 (2)	.09+.03 (3)
T	.12+.006 (4)	.08+.02* (5)	.13+.05 (4)	.14+.03 (5)
Hpth DA				
C	.18+.03 (3)	.29+.06 (2)	.22+.01 (2)	.26+.06 (3)
T	.28+.14 (4)	.20+.02* (5)	.22+.12 (4)	.15+.02* (4)
CN DA				
C	5.63+1.17 (3)	6.42+.87 (3)	7.58+.10 (2)	5.17+1.03 (3)
T	4.06+.73 (4)	5.31+.82 (5)	6.99+.81 (5)	6.58+1.74 (5)
Stem DA				
C	.08+.005 (2)	.08+.02 (3)	.04+.03 (2)	.08+.006 (3)
T	.08+.02 (4)	.01+.01** (3)	.11+.04 (5)	.08+.02 (5)

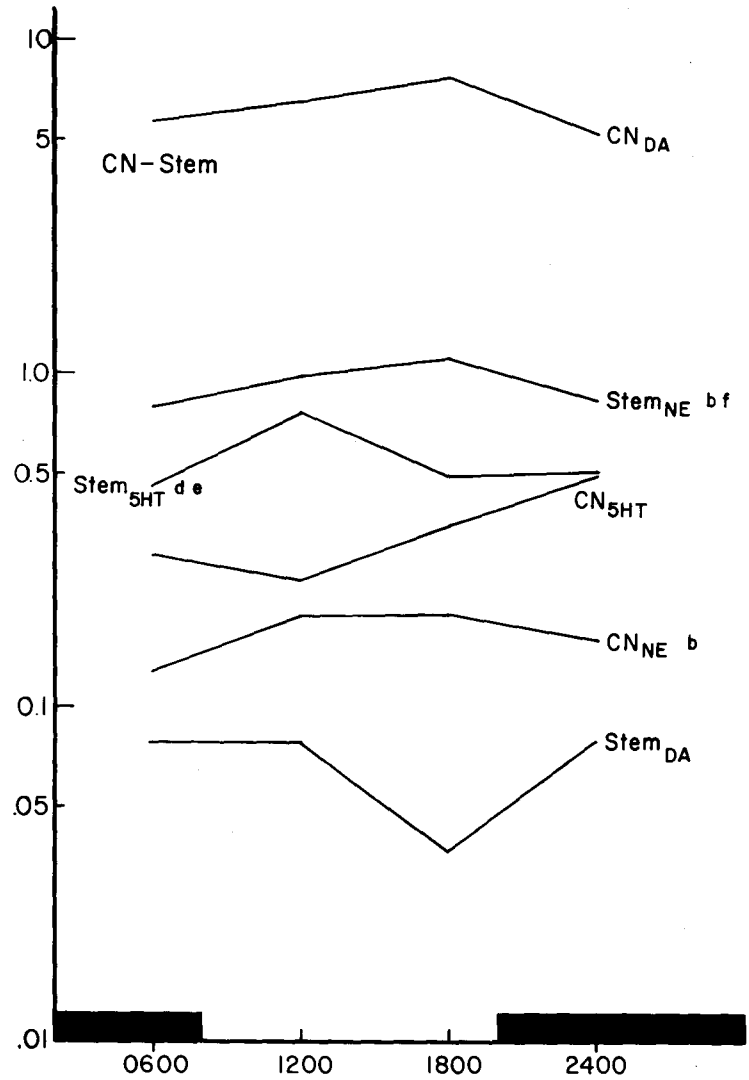
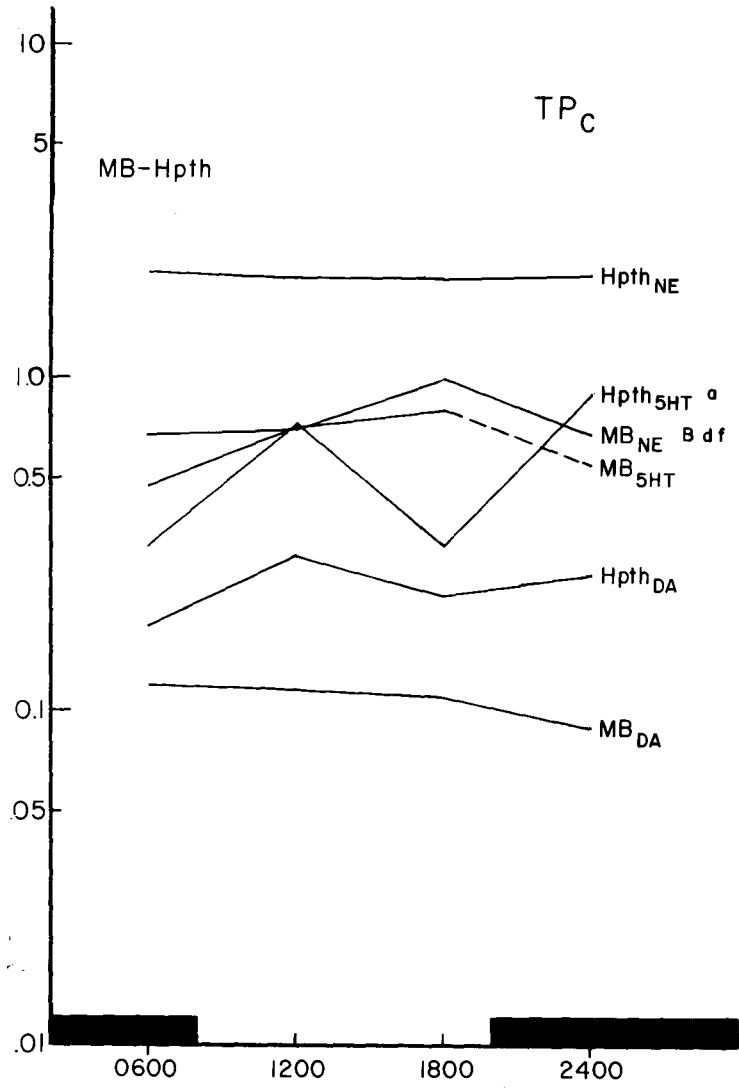
Table 12 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.47+.04 (3)	.70+.20 (2)	.80+.07 (2)	(.55) (1)
T	.60+.12** (4)	.79+.16 (5)	1.49+.43 (4)	.63+.20 (5)
Hpth 5HT				
C	.31+.04 (3)	.72+.20 (3)	.31+.01 (2)	.89+.41 (3)
T	.72+.11** (3)	.82+.05 (4)	2.47+.44** (3)	.80+.11 (5)
CN 5HT				
C	.28+.06 (3)	.24+.02 (2)	.34+.08 (2)	.49+.14 (3)
T	.29+.06 (4)	.37+.08 (5)	.51+.19 (5)	.45+.12 (5)
Stem 5HT				
C	.46+.02 (2)	.76+.09 (3)	.49+.05 (2)	.50+.08 (3)
T	.53+.13 (4)	.78+.08 (5)	.67+.16 (5)	.72+.13* (5)

Table indicates amine concentrations ($\mu\text{gm}/\text{gm}$) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving TP pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * (P 0.05) or ** (P 0.01).

C = Control; T = Treated

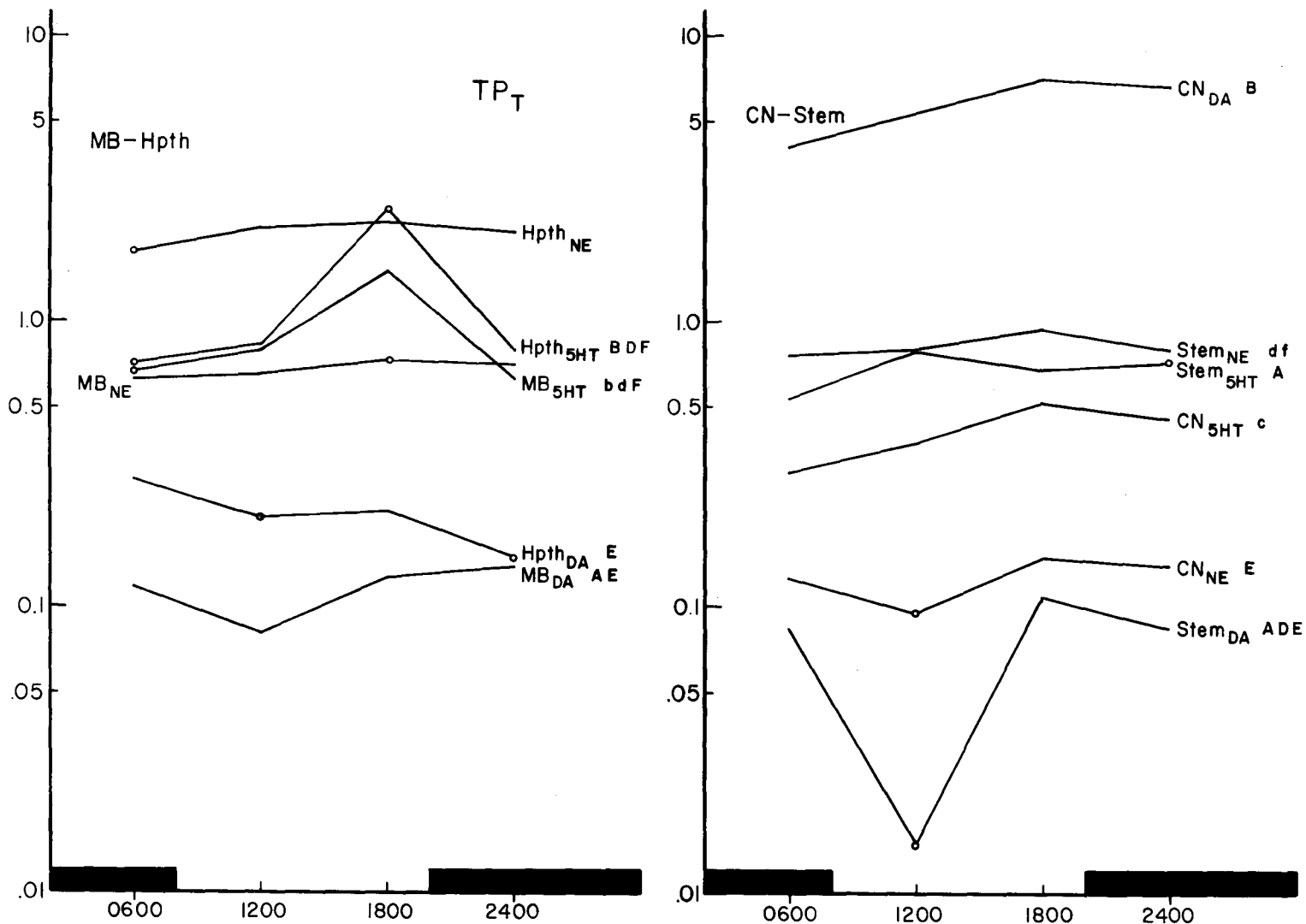
Figure 34



Legend for Figure 34

The effects of TP pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- CONTROL. For complete explanation of notations of figure, see legend to Figure 28.

Figure 35



Legend for Figure 35

The effects of TP pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notations of figure, see legend to Figure 28.

A majority (10/12) amine patterns showed significant variation over the 24-hour experimental period. Details of these findings can be seen by referring to Figs.34-35, Table 12 and Appendix B.

c. Melatonin(Figures36-37, Table 13)

M (25mg/kg, IP) was administered simultaneously with PB. At 0600 hours M increases MB 5HT levels significantly (76.3%, $P < 0.01$) from controls, while MB DA decreases significantly (43.9%, $P < 0.02$) (Table 13, Figs.36-37). At 1200 hours brain stem 5HT levels are reduced (46.3%, $P < 0.01$) and hypothalamic DA decreases (76.1%, $P < 0.02$). At 1800 hours, M lowers CN DA significantly (11.8%, $P < 0.05$). Diurnal variations were obtained in 8/12 amine patterns. These are described in detail in Fig.37, Table 13 and in Appendix B.

4. Drugs affecting the Histamine System

a. L-Histidine

L-Hd (500mg/kg, IP) was administered one hour prior to PB. At 0600 hours, MB 5HT is significantly increased over controls (107.8%, $P < 0.02$). DA concentrations are reduced in the brain stem (67.1%, $P < 0.05$) but elevated in the hypothalamus (332.8%, $P < 0.005$). At 1200 hours, brain stem 5HT decreases significantly (28.3%, $P < 0.02$). At 2400 hours, significant elevations occur in MB DA (135.0%, $P < 0.005$) and hypothalamic 5HT (73.6%, $P < 0.01$) concentrations. Details of the diurnal fluctuations of amine patterns (7 of 12) after Hd500 are indicated in Fig.39, Table 14 and Appendix B.

b. Decaborane(Figures40-41, Table 15)

DB (15mg/kg, IP) was administered 24 hours prior to PB. The

Table 13: CNS concentrations of NE, DA and 5HT after M25 pre-treatment with PB, on a 24-hour basis

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.69 ₍₄₎ ±.04	.69 ₍₃₎ ±.06	.61 ₍₃₎ ±.12	.70 ₍₃₎ ±.10
T	.73 ₍₄₎ ±.12	.63 ₍₄₎ ±.08	.64 ₍₅₎ ±.11	.68 ₍₅₎ ±.04
Hpth NE				
C	2.27 ₍₄₎ ±.44	1.99 ₍₃₎ ±.31	1.76 ₍₃₎ ±.40	2.13 ₍₃₎ ±.21
T	1.98 ₍₅₎ ±.49	1.69 ₍₄₎ ±.49	1.75 ₍₅₎ ±.19	1.99 ₍₅₎ ±.41
CN NE				
C	.17 ₍₂₎ ±.10	.19 ₍₃₎ ±.07	.14 ₍₃₎ ±.09	.14 ₍₃₎ ±.04
T	.14 ₍₄₎ ±.04	.10 ₍₄₎ ±.02	.15 ₍₅₎ ±.01	.14 ₍₅₎ ±.02
Stem NE				
C	.94 ₍₄₎ ±.13	.97 ₍₃₎ ±.18	.91 ₍₃₎ ±.07	.85 ₍₃₎ ±.05
T	.89 ₍₅₎ ±.14	.79 ₍₄₎ ±.14	.91 ₍₅₎ ±.14	.87 ₍₅₎ ±.04
<hr/>				
MB DA				
C	.12 ₍₃₎ ±.02	.11 ₍₃₎ ±.02	.13 ₍₂₎ ±.01	.09 ₍₃₎ ±.04
T	.07 ₍₄₎ ±.01*	.09 ₍₄₎ ±.05	.12 ₍₄₎ ±.01	.09 ₍₄₎ ±.03
Hpth DA				
C	.26 ₍₃₎ ±.09	.29 ₍₂₎ ±.06	.18 ₍₂₎ ±.03	.26 ₍₃₎ ±.09
T	.17 ₍₄₎ ±.01	.07 ₍₄₎ ±.03*	.16 ₍₄₎ ±.03	.22 ₍₄₎ ±.01
CN DA				
C	8.24 ₍₄₎ ±1.00	6.42 ₍₃₎ ±.87	8.12 ₍₃₎ ±.19	6.83 ₍₃₎ ±.11
T	6.90 ₍₅₎ ±.87	7.06 ₍₃₎ ±1.37	7.17 ₍₅₎ ±.58*	7.15 ₍₅₎ ±1.09
Stem DA				
C	.11 ₍₃₎ ±.05	.08 ₍₃₎ ±.02	.10 ₍₂₎ ±.03	.05 ₍₃₎ ±.05
T	.06 ₍₅₎ ±.02	.05 ₍₃₎ ±.03	.05 ₍₅₎ ±.03	.03 ₍₄₎ ±.001

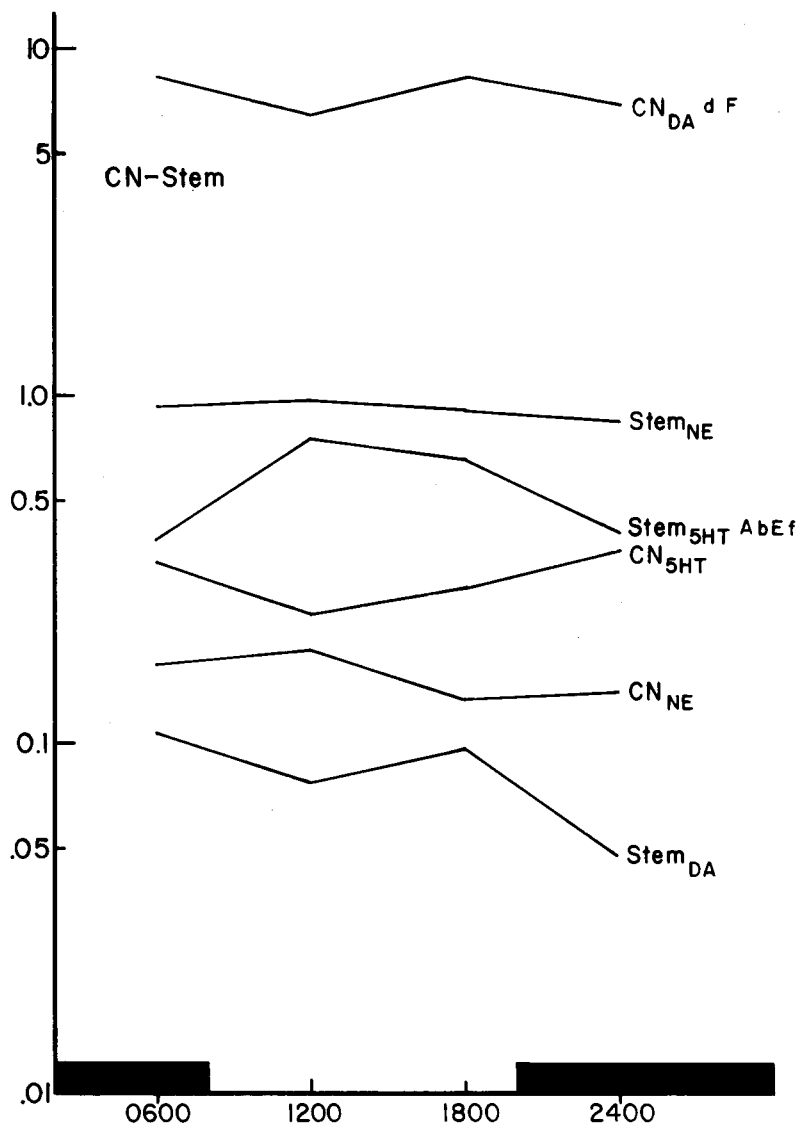
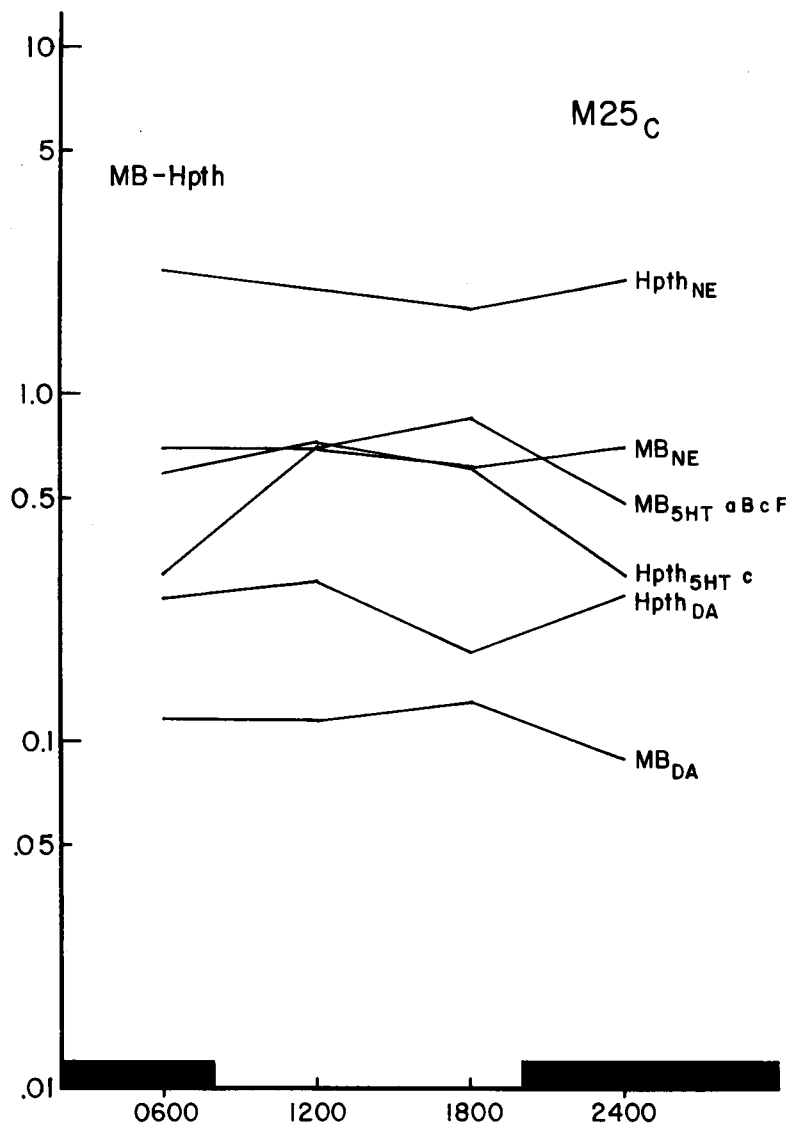
Table 13 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.30 ₍₄₎ ±.08	.70 ₍₂₎ ±.20	.85 ₍₃₎ ±.09	.48 ₍₃₎ ±.08
T	.53 ₍₄₎ ±.09**	1.05 ₍₄₎ ±.21	.97 ₍₅₎ ±.17	.61 ₍₅₎ ±.15
Hpth 5HT				
C	.59 ₍₄₎ ±.09	.72 ₍₃₎ ±.20	.61 ₍₃₎ ±.25	.30 ₍₂₎ ±.04
T	.64 ₍₅₎ ±.15	.84 ₍₄₎ ±.05	.38 ₍₄₎ ±.21	.28 ₍₄₎ ±.10
CN 5HT				
C	.33 ₍₄₎ ±.21	.24 ₍₂₎ ±.02	.28 ₍₃₎ ±.04	.36 ₍₂₎ ±.04
T	.43 ₍₅₎ ±.09	.26 ₍₄₎ ±.02	.47 ₍₅₎ ±.13	.46 ₍₄₎ ±.13
Stem 5HT				
C	.39 ₍₃₎ ±.04	.76 ₍₃₎ ±.09	.66 ₍₃₎ ±.11	.41 ₍₃₎ ±.07
T	.60 ₍₅₎ ±.31	.41 ₍₄₎ ±.12**	.82 ₍₅₎ ±.14	.38 ₍₄₎ ±.18

Table indicates amine concentrations (ugm/gm) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving M25 pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means ± standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * (P < 0.05) or ** (P < 0.01).

C = Control; T = Treated

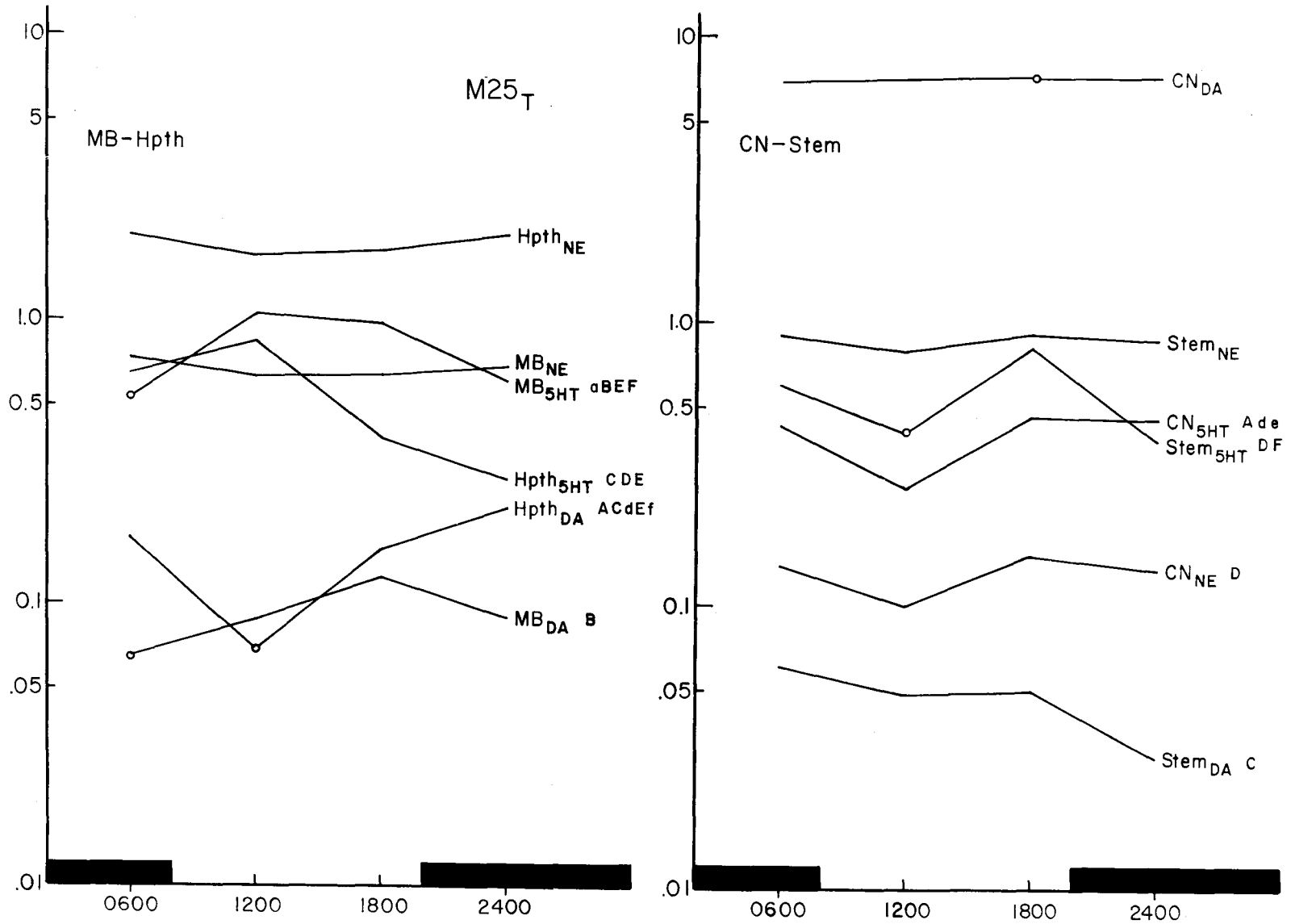
Figure 36



Legend for Figure 36

The effects of M25 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- CONTROL. For complete explanation of notations of figure, see legend to Figure 28.

Figure 37



Legend for Figure 37

The effects of M25 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notations of figure, see legend to Figure 28.

Table 14: CNS concentrations of NE, DA and 5HT after Hd500 pre-treatment with PB, on a 24-hour basis

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.69+.04 (4)	.70+.12 (4)	.74+.11 (4)	.77+.10 (4)
T	.70+.08 (4)	.75+.08 (5)	.76+.13 (7)	.85+.08 (4)
Hpth NE				
C	2.27+.44 (4)	2.12+.34 (6)	2.18+.23 (4)	2.11+.35 (4)
T	2.57+.46	2.31+.53	2.13+.37	1.77+.37
CN NE				
C	.17+.10 (2)	.16+.05 (5)	.23+.06 (3)	.21+.08 (4)
T	.52+.14 (3)	.17+.04 (5)	.22+.03 (7)	.18+.04 (4)
Stem NE				
C	.94+.13 (4)	.83+.09 (6)	.87+.09 (4)	.90+.11 (4)
T	.89+.14 (4)	.78+.10 (5)	.93+.10 (7)	.90+.11 (4)
<hr/>				
MB DA				
C	.12+.02 (3)	.13+.05 (3)	.16+.01 (2)	.21+.07 (3)
T	.26+.17	.26+.07	.11+.04	.50+.05**
Hpth DA				
C	.26+.09 (3)	.18+.07 (4)	.44+.12 (3)	(.35) (1)
T	1.11+.28** (4)	.22+.07 (3)	.37+.11 (6)	(.29) (1)
CN DA				
C	8.24+1.00 (4)	9.26+1.52 (5)	10.06+2.78 (3)	10.48+.87 (3)
T	7.91+1.28 (3)	11.96+3.02 (2)	9.06+1.46 (7)	9.82+1.84 (4)
Stem DA				
C	.11+.05 (3)	.13+.06 (4)	.07+.02 (4)	.08+.02 (3)
T	.04+.02* (4)	.09+.03 (2)	.07+.01 (2)	.13+.07 (3)

Table 14 (continued)

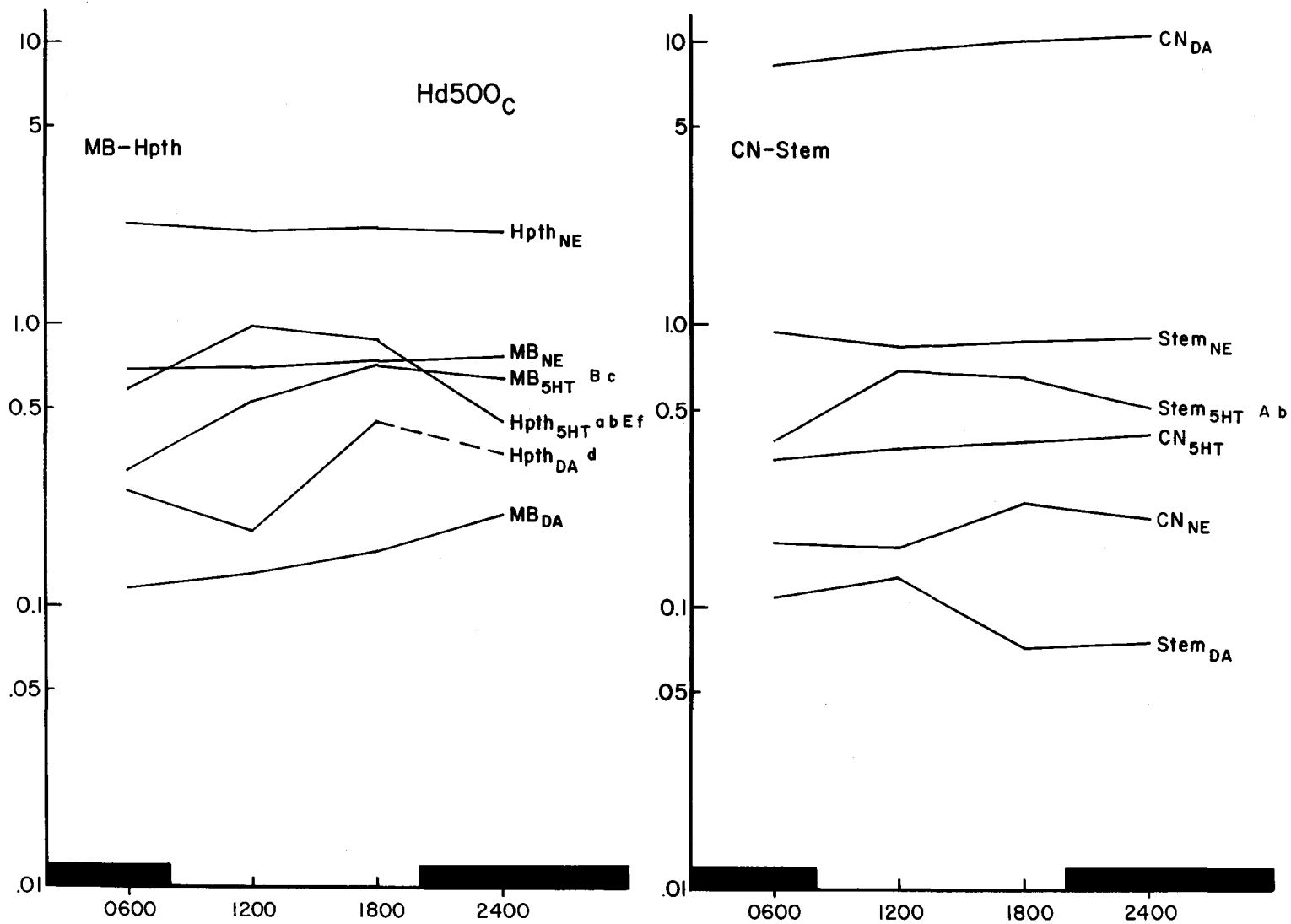
218

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.30 \pm .08 (4)	.53 \pm .07 (3)	.71 \pm .20 (4)	.64 \pm .19 (4)
T	.63 \pm .18*	.60 \pm .14	.66 \pm .15	.80 \pm .13
Hpth 5HT				
C	.59 \pm .09 (4)	.98 \pm .18 (3)	.87 \pm .15 (2)	.45 \pm .10 (4)
T	.73 \pm .24 (4)	.79 \pm .15 (4)	1.02 \pm .04 (2)	.78 \pm .10** (3)
CN 5HT				
C	.33 \pm .21 (4)	.36 \pm .007 (2)	.38 \pm .13 (4)	.41 \pm .08 (4)
T	.30 \pm .15 (4)	.29 \pm .12 (2)	.39 \pm .09 (5)	.44 \pm .10 (3)
Stem 5HT				
C	.39 \pm .04 (3)	.68 \pm .05 (4)	.64 \pm .12 (2)	.51 \pm .19 (4)
T	.35 \pm .11 (4)	.49 \pm .10* (5)	.65 \pm .14 (7)	.62 \pm .17 (3)

Table indicates amine concentrations ($\mu\text{gm}/\text{gm}$) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving Hd500 pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * ($P < 0.05$) or ** ($P < 0.01$).

C = Control; T = Treated

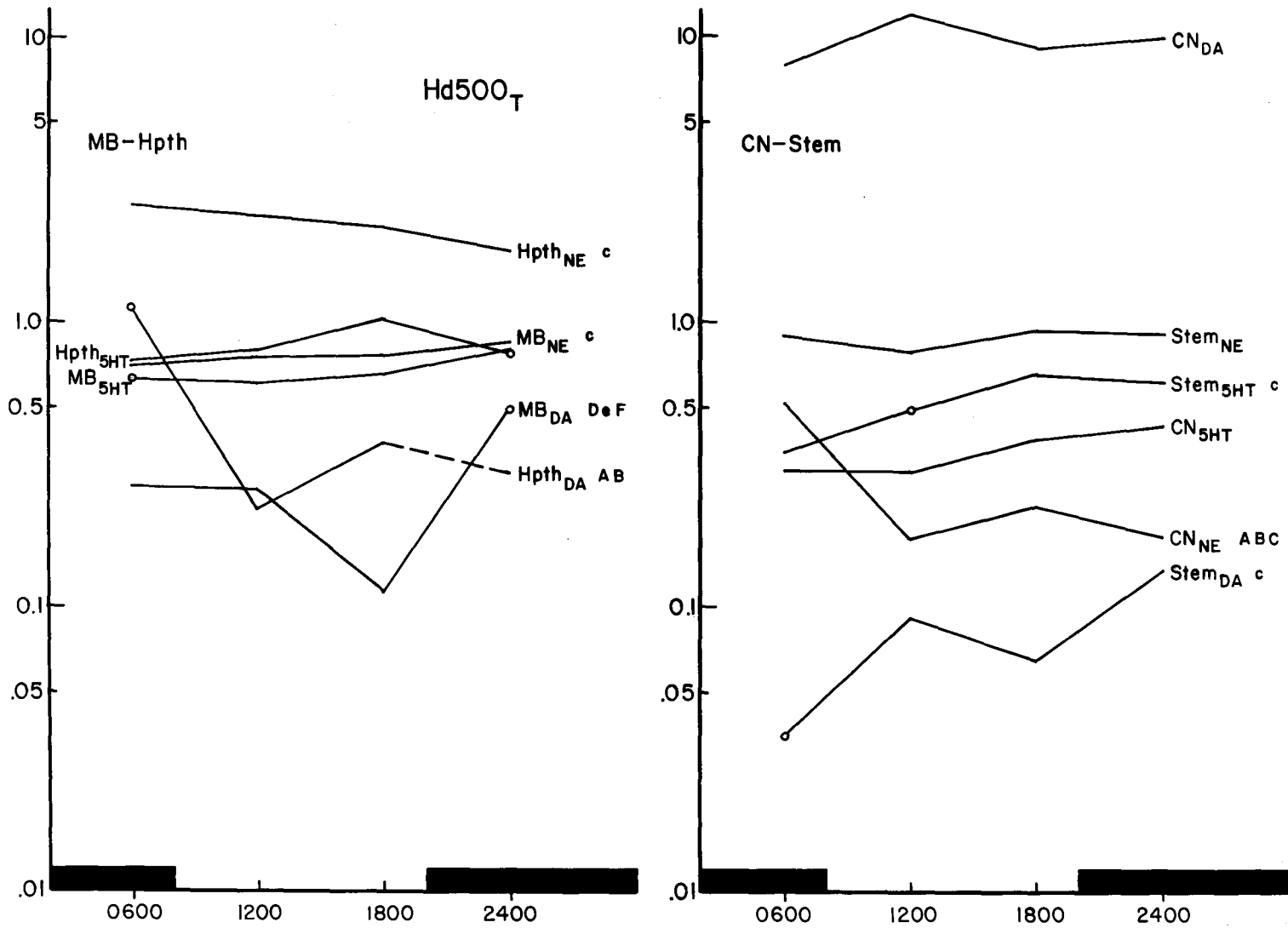
Figure 38



Legend for Figure 38

The effects of Hd500 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- CONTROL. For complete explanation of notations of figure, see legend to Figure 28.

Figure 39



Legend for Figure 39

The effects of Hd500 pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notations of figure, see legend to Figure 28.

Table 15: CNS concentrations of NE, DA and 5HT after DB pretreatment with PB, on a 24-hour basis 221

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.68+.09 (3)	.56+.02 (2)	.90+.12 (3)	.40+.02 (2)
T	.28+.05** (5)	.22+.03** (5)	.24+.03 (4)	.17+.01** (3)
Hpth NE				
C	1.93+.18 (3)	2.04+.22 (3)	1.99+.14 (3)	.96+.09 (2)
T	.42+.19** (4)	.43+.09** (4)	.47+.07** (4)	.23+.09** (4)
CN NE				
C	.10+.02 (3)	.14+.02 (3)	.18+.02 (3)	.05+.03 (2)
T	.10+.06 (5)	.07+.03 (5)	.09+.02 (3)	.05+.01 (4)
Stem NE				
C	.81+.04 (3)	.79+.14 (3)	.97+.13 (3)	.66+.006 (2)
T	.37+.05** (5)	.35+.04** (5)	.33+.04** (4)	.31+.06** (4)
<hr/>				
MB DA				
C	.13+.03 (3)	.04+.003 (2)	.18+.06 (3)	.16+.07 (2)
T	.39+.12* (5)	.13+.05 (5)	.17+.07 (3)	.10+.02 (3)
Hpth DA				
C	.18+.04 (3)	.12+.007 (2)	.20+.03 (3)	.23+.02 (2)
T	.76+.05** (4)	.14+.05 (3)	.21+.03 (4)	.12+.06 (2)
CN DA				
C	6.22+1.37 (3)	11.63+ .11 (3)	7.47+1.07 (3)	9.81+ .86 (2)
T	8.21+ .57* (5)	11.71+ .70 (5)	11.64+ .28 (3)	10.47 + .55 (4)
Stem DA				
C	.09+.02 (3)	.08+.03 (3)	.11+.03 (3)	.03+.02 (2)
T	.31+.14** (4)	.10+.04 (4)	.39+.13* (3)	.43+.07** (3)

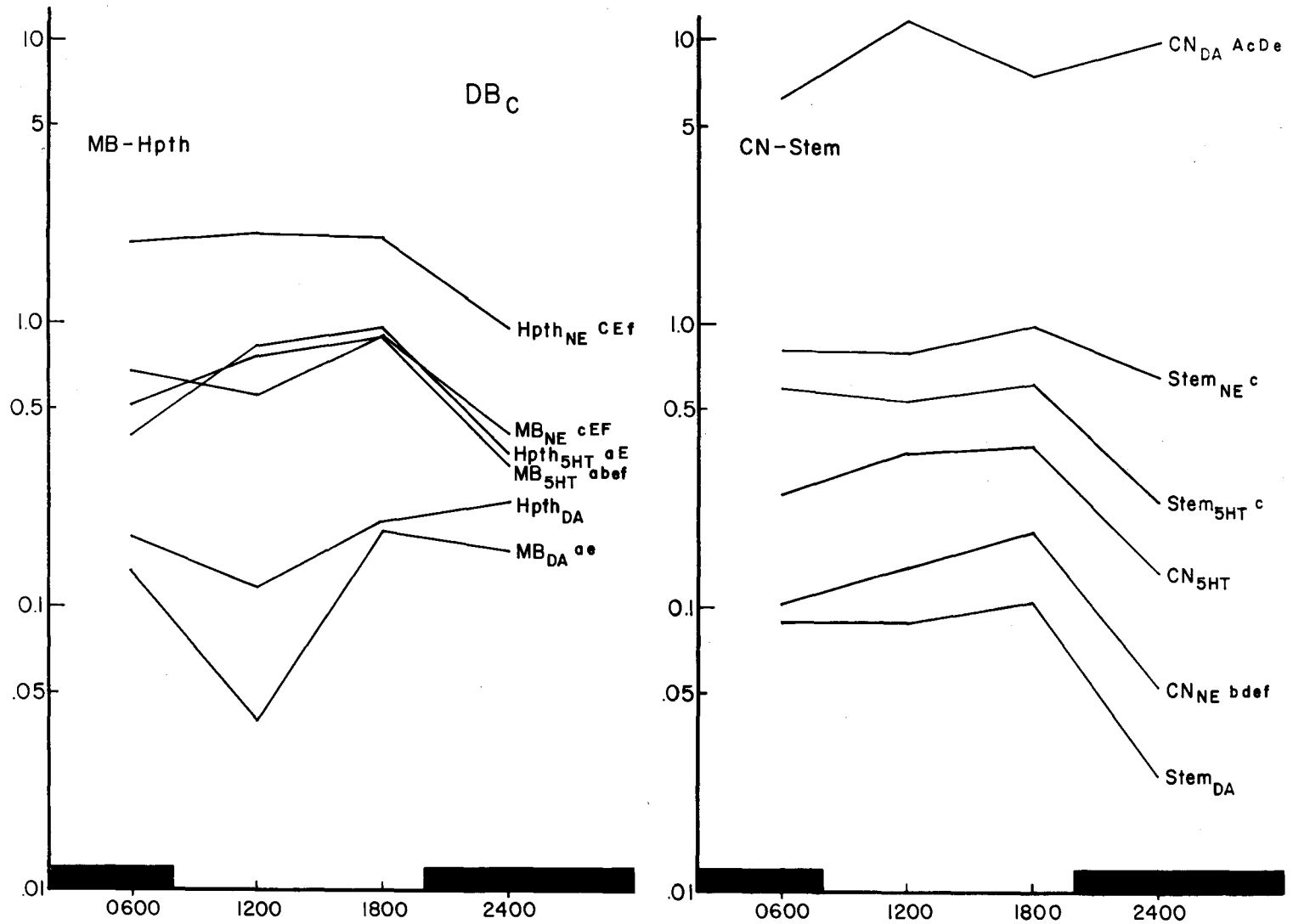
Table 15 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.52+.07 (3)	.76+.04 (2)	.89+.15 (3)	.33+.09 (2)
T	(.28) (1)	.54+.08* (3)	.19+.02** (3)	.14+.02* (3)
Hpth 5HT				
C	.40+.14 (2)	.84+.09 (3)	.95+.53 (3)	.35+.05 (2)
T	1.77+.52* (3)	.18+.05** (4)	.14+.06 (4)	.07+.04** (4)
CN 5HT				
C	.25+.05 (3)	.35+.14 (3)	.37+.10 (3)	.13+.05 (2)
T	.67+.52 (4)	.20+.03* (5)	.10+.001* (2)	.06+.03 (3)
Stem 5HT				
C	.59+.02 (3)	.53+.15 (2)	.62+.11 (3)	.03+.02 (2)
T	.21+.04** (2)	.82+.18 (3)	.16+.07* (2)	.10+.02 (2)

Table indicates amine concentrations (ugm/gm) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving DB pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * (P < 0.05) or ** (P < 0.01).

C = Control; T = Treated

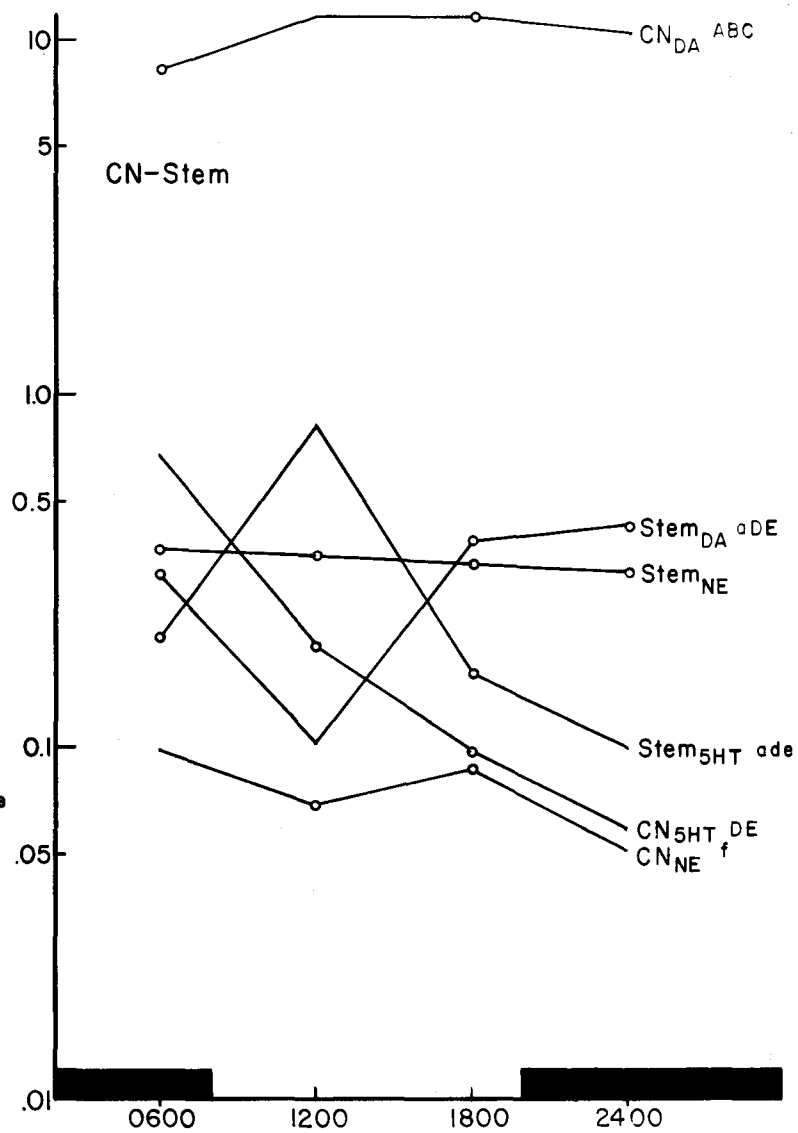
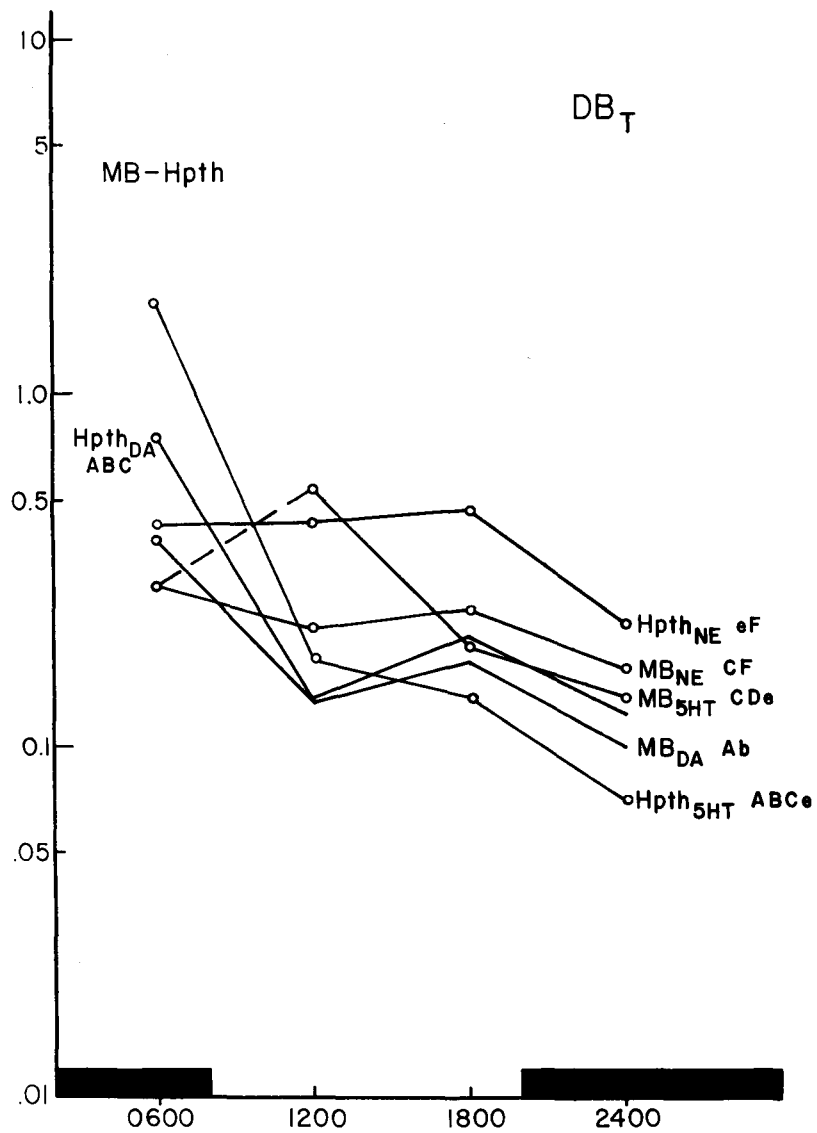
Figure 40



Legend for Figure 40

The effects of DB pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT --- CONTROL. For complete explanation of notations of figure, see legend to Figure 28.

Figure 41



Legend for Figure 41

The effects of DB pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notations of figure, see legend to Figure 28.

control values for the 0600 group at 100 minutes were obtained by interpolating between the amine values determined for 85 and 120 minutes, in order to compare differences between the treated and control groups at this time.

At 0600 hours, there is a significant decrease of MB NE (58.1%, $P < 0.001$) but MB and CN DA increase (190.4% and 32% respectively, each $P < 0.05$). Brain stem NE levels decrease significantly (54.8%, $P < 0.001$). Brain stem DA increases and 5HT is reduced from control (65.2% and 65.4% respectively, each $P < 0.005$). Hypothalamic NE decreases (78.1%, $P < 0.001$), while significant increases occur in the levels of hypothalamic DA (325.5%, $P < 0.001$) and 5HT (340.6%, $P < 0.02$) values.

At 1200 hours NE levels are reduced significantly in the MB (61.1%, $P < 0.001$), CN (49.3%, $P < 0.02$), brain stem (55.6%, $P < 0.001$) and hypothalamus (78.7%, $P < 0.001$). 5HT concentrations are decreased significantly in the MB (29.0%, $P < 0.05$), CN (44.5%, $P < 0.05$) and hypothalamus (78.9%, $P < 0.001$). At 1800 hours, only a single control animal was available, limiting the opportunity for statistical analyses. Relative to this control, NE levels appear reduced in the MB, CN, brain stem and hypothalamus, while DA levels are elevated in the MB, CN and brain stem. 5HT levels appear to be decreased in the MB, CN, brain stem and hypothalamus.

At 2400 hours, NE levels are significantly decreased in the MB (58.3%, $P < 0.001$), brain stem (65.5%, $P < 0.005$) and hypothalamus (76.3%, $P < 0.001$). DA concentrations are elevated significantly in the brain stem (1554.7%, $P < 0.005$). 5HT levels decrease significantly in the MB (58.3%, $P < 0.05$) and hypothalamus (79.5%, $P < 0.005$). Significant circadian variations in 11 of 12 amine patterns examined were obtained after DB pretreatment.

c. Diphenhydramine(Figures 42-43, Table 16)

DPH (50mg/kg, IP) was administered one hour prior to PB. At 0600 hours, DPH increases MB 5HT concentrations over controls (39.2%, $P < 0.025$) but hypothalamic NE is reduced significantly (25.6%, $P < 0.001$). At 1200 hours, there is a significant elevation of CN 5HT (97.0%, $P < 0.02$). At 1800 hours MB NE levels are reduced significantly (19.4%, $P < 0.01$).

Eight of twelve amine patterns vary significantly during the 24-hour period of measurement. The details of these variations can be seen in Fig.43 Table 16 and Appendix B.

5. Gamma-butyrolactone(Figures 44-45, Table 17)

Amine concentrations in MB, CN, brain stem and hypothalamus were evaluated half-way through the previously-determined sleep period at 0600, 1200, 1800 and 2400 hours (Table 17). The results over the 24-hour period for controls are plotted separately (Fig. 44) from GBL-treated (Fig. 45). The controls, receiving saline, of course did not sleep.

There are significant changes in amine concentrations of GBL-treated animals, compared with controls, in the following cases: MB 5HT, 1200 hours (77.8% increase, $P < 0.001$); CN DA, 1200 hours (41.3% increase, $P < 0.05$); brain stem 5HT, 1200 hours (50% increase, $P < 0.05$); MB 5HT, 1800 hours (64.7% increase, $P < 0.05$), hypothalamic DA, 1800 hours (128.1% increase, $P < 0.02$) and hypothalamic NE, 2400 hours (20.4% decrease, $P < 0.05$).

Circadian fluctuations in the amine patterns were seen in 6 of 12 cases. The details of these variations can be seen in Fig. 45, Table 17 or Appendix B.

Table 16: CNS concentrations of NE, DA and 5HT after DPH pretreatment with PB, on a 24-hour basis

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.76+.06 (4)	.64+.17 (5)	.98+.08 (2)	.65+.11 (4)
T	.70+.05 (6)	.76+.20 (3)	.79+.05** (6)	.52+.04 (3)
Hpth NE				
C	2.22+.19 (4)	1.91+.38 (5)	1.96+.03 (2)	1.59+.46 (4)
T	1.65+.08** (5)	1.63+.50 (4)	2.05+.16 (5)	.99+.21 (3)
CN NE				
C	.19+.03 (3)	.12+.05 (5)	.19+.03 (2)	.10+.05 (4)
T	.18+.02 (4)	.18+.05 (3)	.20+.04 (6)	.16+.07 (3)
Stem NE				
C	.93+.06 (4)	.88+.16 (5)	1.10+.14 (2)	.81+.13 (4)
T	.89+.10 (6)	.73+.16 (4)	.99+.09 (6)	.74+.06 (3)
<hr/>				
MB DA				
C	.11+.02 (3)	.21+.06 (4)	.11+.002 (2)	.06+.001 (3)
T	.13+.007 (6)	.20+.06 (3)	.15+.04 (6)	.06+.003 (3)
Hpth DA				
C	(.25) (1)	.22+.07 (3)	.22+.01 (2)	.14+.05 (4)
T	.18+.03 (3)	.26+.02 (2)	.26+.10 (5)	(.03) (1)
CN DA				
C	7.01+.21 (4)	10.48+.58 (5)	7.58+.10 (2)	7.06+1.57 (4)
T	7.49+.53 (5)	13.33+2.03 (3)	6.63+1.59 (6)	7.99+ .26 (3)
Stem DA				
C	.07+.003 (2)	.07+.003 (3)	.05+.01 (2)	.05+.02 (3)
T	.08+.02 (4)	.07+.01 (3)	.06+.02 (6)	.03+.003 (3)

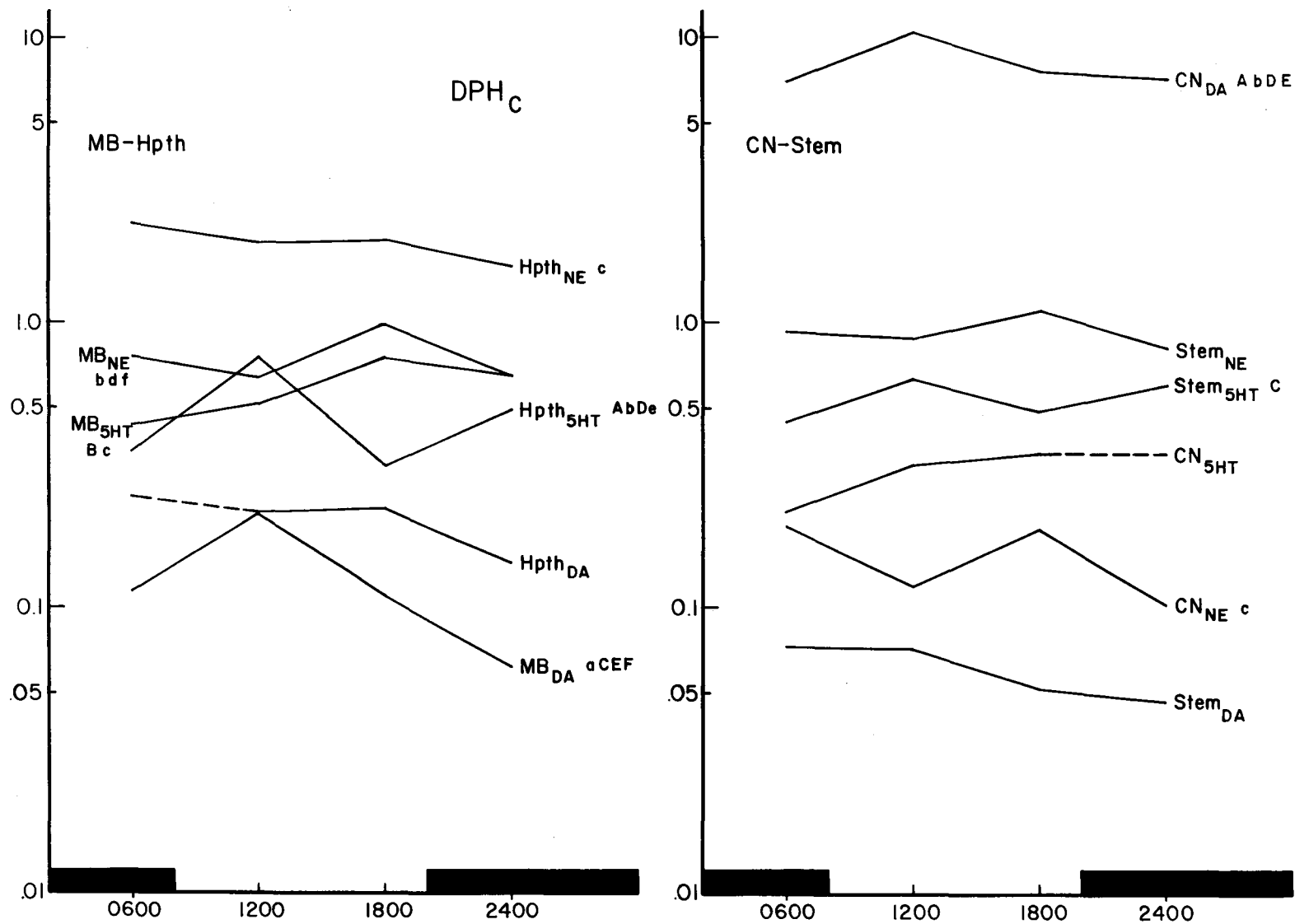
Table 16 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.44+.05 (4)	.52+.19 (5)	.75+.07 (2)	.66+.10 (3)
T	.61+.09* (3)	.57+.12 (3)	.75+.12 (6)	.63+.14 (3)
Hpth 5HT				
C	.35+.01 (3)	.76+.05 (3)	.31+.007 (2)	.49+.12 (3)
T	.42+.06 (3)	.91+.15 (3)	.55+.14 (6)	.45+.03 (3)
CN 5HT				
C	.22+.06 (4)	.32+.06 (2)	.34+.08 (2)	(.34) (1)
T	.15+.02 (4)	.62+.01* (2)	.44+.06 (6)	(.18) (1)
Stem 5HT				
C	.45+.03 (3)	.63+.15 (4)	.49+.05 (2)	.60+.06 (4)
T	.44+.23 (3)	.64+.15 (4)	.45+.09 (6)	.51+.17 (3)

Table indicates amine concentrations ($\mu\text{g}/\text{g}$) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving DPH pretreatment and PB (50mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * ($P < 0.05$) or ** ($P < 0.01$).

C = Control; T = Treated

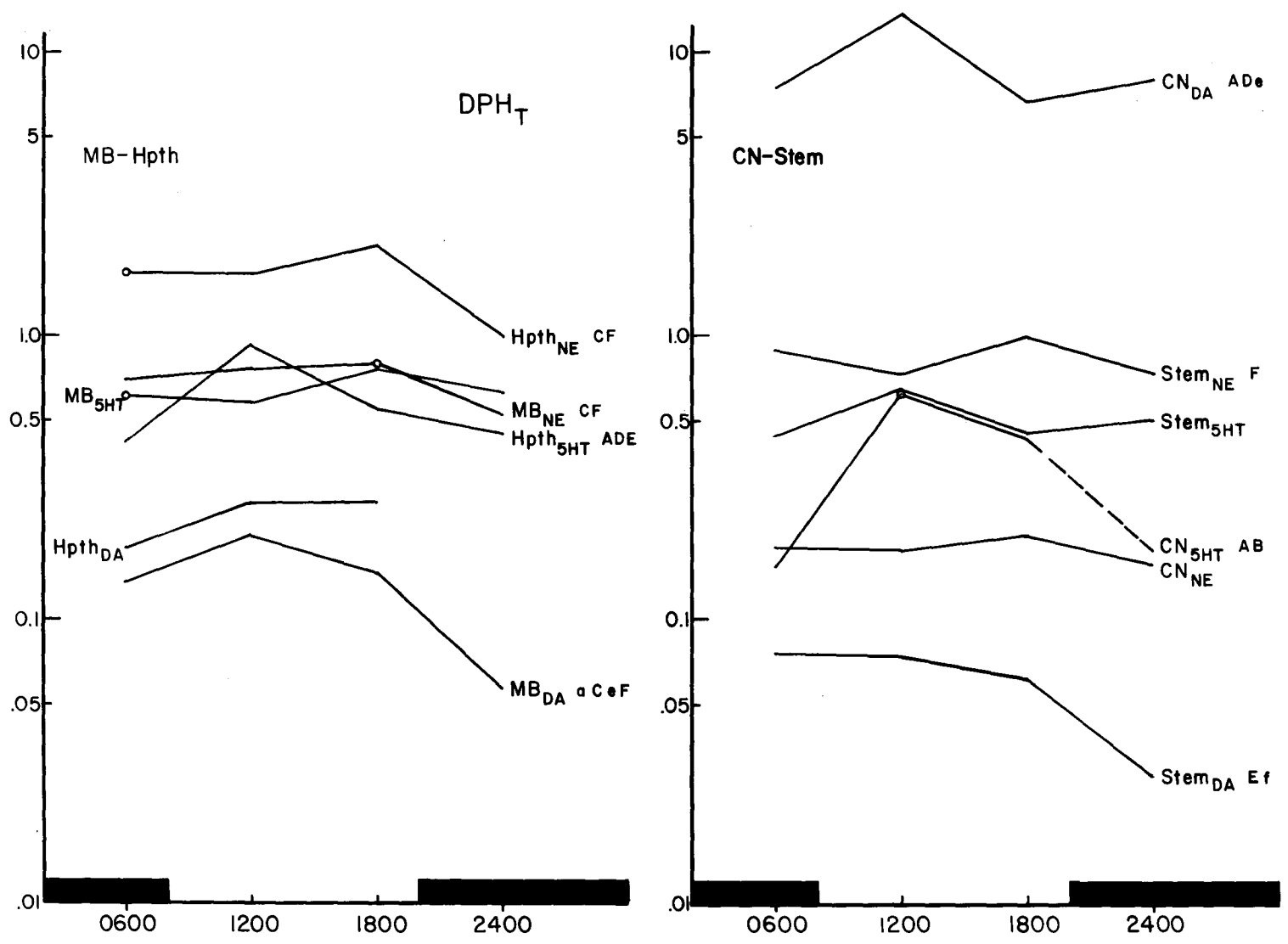
Figure 42



Legend for Figure 42

The effects of DPH pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- CONTROL. For complete explanation of notations of figure, see legend to Figure 28.

Figure 43



Legend for Figure 43

The effects of DPH pretreatment with pentobarbital, on the CNS concentrations of NE, DA and 5HT -- TREATED. For complete explanation of notations of figure, see legend to Figure 28.

Table 17: CNS concentrations of NE, DA and 5HT after GBL, on a 24-hour basis

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE				
C	.80+.07 (4)	.90+.08 (4)	.83+.04 (4)	.87+.09 (4)
T	.88+.05 (4)	.91+.08 (5)	.85+.09 (5)	.88+.07 (5)
Hpth NE				
C	2.39+.37 (4)	2.79+.49 (4)	2.74+.40 (4)	3.23+.16 (4)
T	2.33+.05 (3)	2.72+.51 (5)	2.43+.25 (5)	2.57+.47* (5)
CN NE				
C	.18+.02 (4)	.20+.02 (4)	.21+.02 (4)	.17+.05 (4)
T	.17+.007 (4)	.22+.04 (4)	.19+.05 (5)	.23+.08 (4)
Stem NE				
C	1.03+.13 (4)	1.05+.06 (4)	.95+.08 (4)	1.21+.009 (3)
T	1.08+.13 (4)	1.21+.15 (5)	1.02+.09 (4)	1.14+.14 (5)
MB DA				
C	.18+.03 (3)	.18+.05 (4)	.20+.05 (4)	.21+.04 (4)
T	.25+.05 (4)	.21+.05 (5)	.26+.06 (5)	.26+.06 (4)
Hpth DA				
C	.34+.05 (4)	.61+.13 (3)	.44+.12 (4)	.44+.03 (3)
T	.33+.13 (4)	.46+.06 (4)	1.00+.32* (4)	.44+.15 (4)
CN DA				
C	6.46+.90 (4)	6.17+.66 (4)	7.75+1.36 (4)	5.72+1.61 (4)
T	7.72+1.00 (4)	8.72+1.66* (4)	9.40+1.04 (5)	8.33+2.22 (4)
Stem DA				
C	.20+.04 (4)	.05+.01 (4)	.11+.01 (4)	.06+.03 (3)
T	.16+.06 (4)	.05+.007 (4)	.14+.03 (4)	.10+.03 (4)

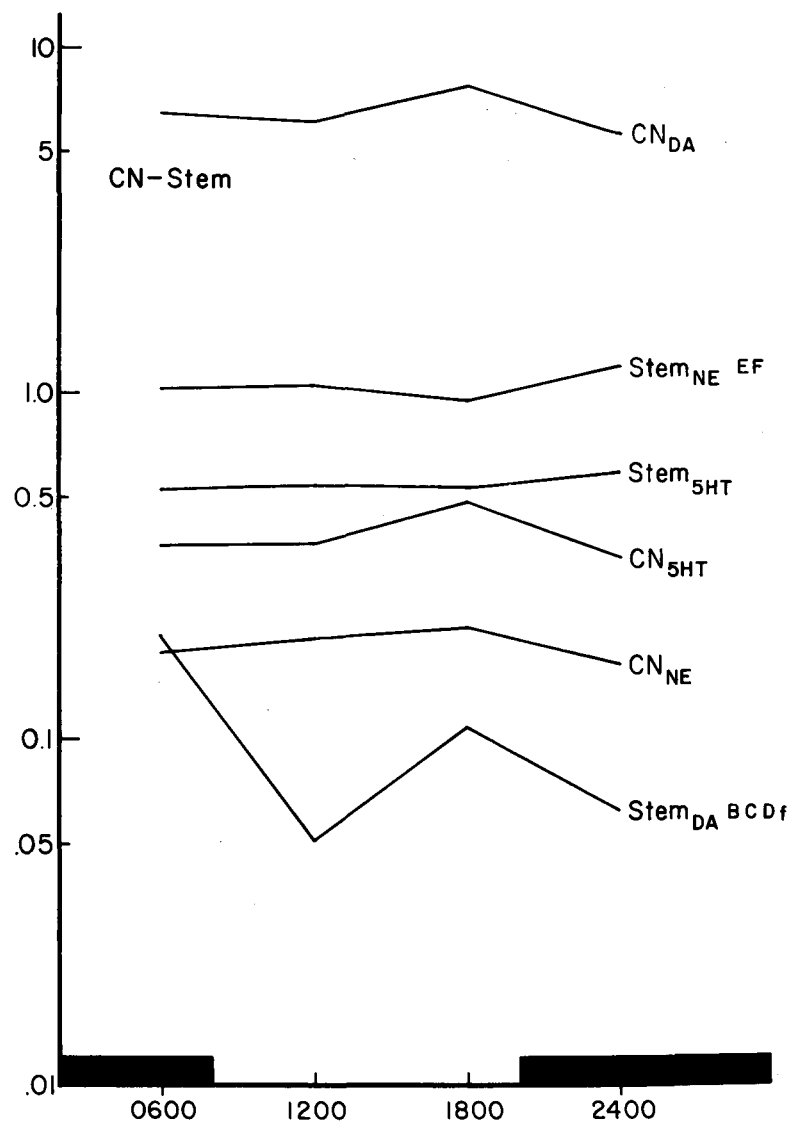
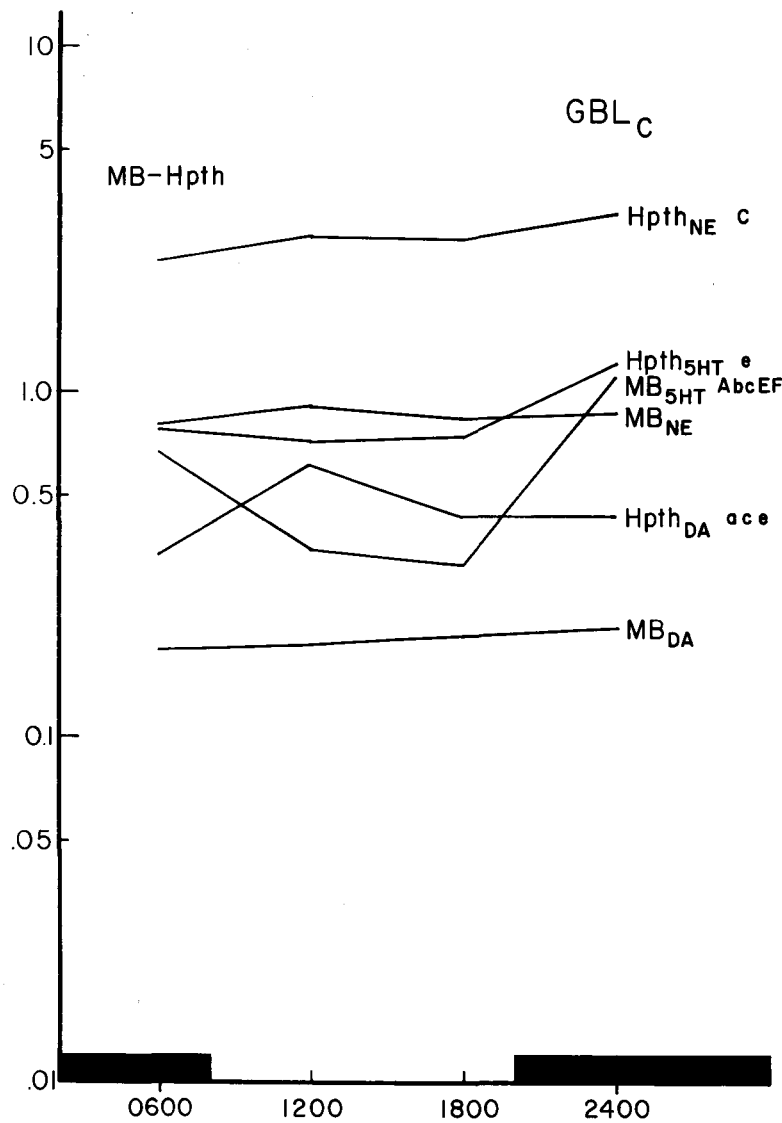
Table 17 (continued)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB 5HT				
C	.67+.10 (3)	.35+.05 (3)	.31+.14 (4)	1.09+.23 (4)
T	.54+.30 (4)	.62+.07** (5)	.52+.07** (5)	1.42+.44 (4)
Hpth 5HT				
C	.78+.28 (3)	.71+.14 (4)	.74+.36 (3)	1.20+.22 (3)
T	.86+.07 (4)	.82+.14 (5)	.81+.18 (5)	1.32+.45 (5)
CN 5HT				
C	.36+.06 (2)	.37+.06 (3)	.48+.13 (4)	.34+.16 (3)
T	.28+.08 (4)	.40+.03 (3)	.67+.31 (5)	.38+.07 (3)
Stem 5HT				
C	.53+.13 (4)	.54+.06 (3)	.53+.13 (4)	.60+.08 (4)
T	.47+.04 (3)	.81+.15* (4)	.62+.03 (4)	.73+.10 (4)

Table indicates amine concentrations ($\mu\text{g}/\text{gm}$) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving GBL (350mg/kg, IP). Animals were sacrificed midway through the previously-determined sleeping time. Values are means \pm standard deviation, with the number of animals used in parentheses. Significant differences from control are noted by * ($P < 0.05$) or ** ($P < 0.01$).

C = Control; T = Treated

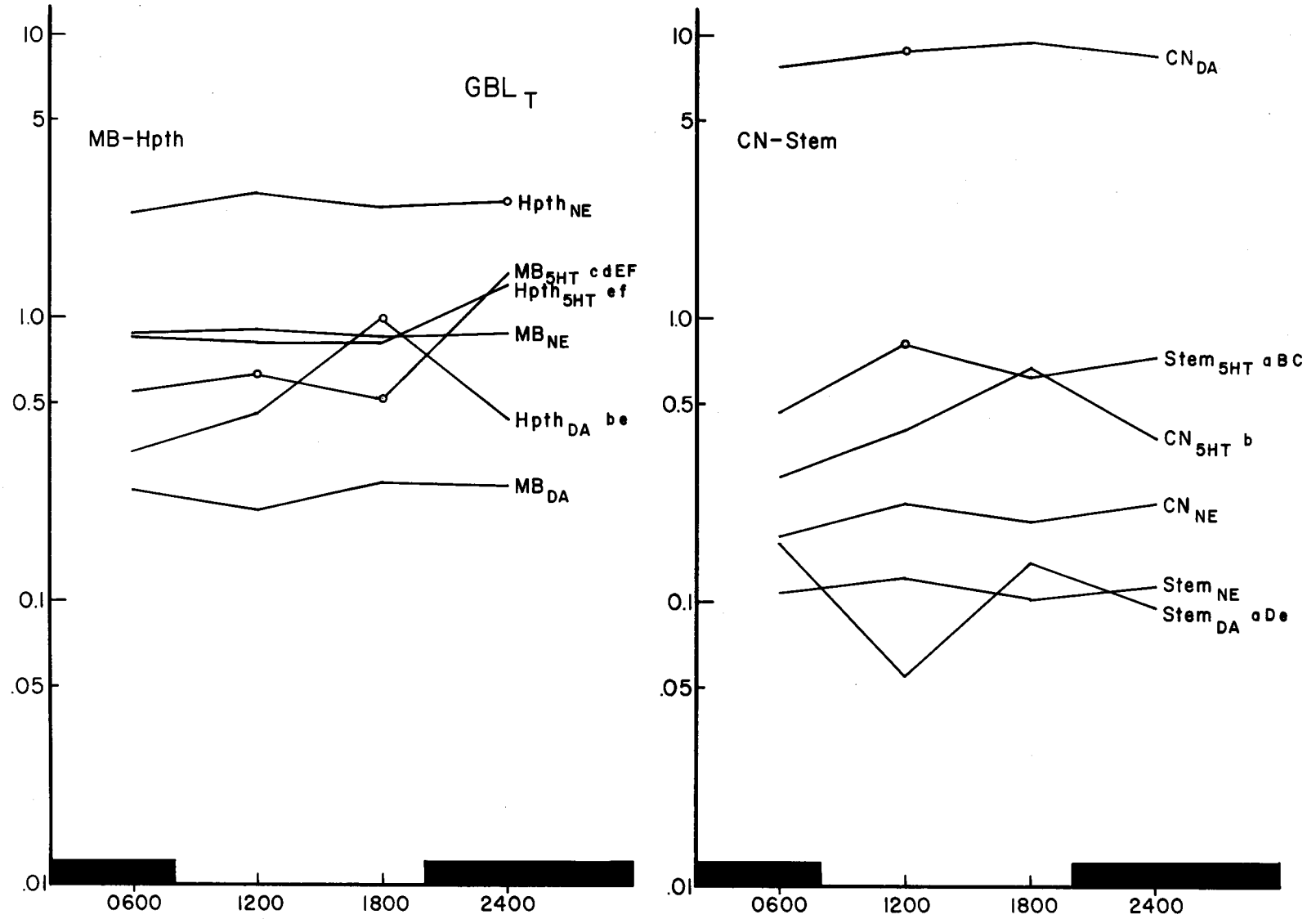
Figure 44



Legend for Figure 44

NE, DA and 5HT concentrations in the CNS for GBL--CONTROL animals. For complete explanation of figure, see legend to Figure 28.

Figure 45



Legend for Figure 45

NE, DA and 5HT concentrations in the CNS for GBL--TREATED animals. For complete explanation of notations of figure, see legend to Figure 28.

E. The Effect on Rectal Temperature of Pentobarbital Alone and in Combination with Drug Pretreatments

1. Untreated animals

Rectal temperatures were measured in groups of eight rats at 0600, 1200, 1800 and 2400 hours (Table 18). The 24-hour pattern for rectal temperature peaks at 2400 hours ($38.44 \pm 0.38^{\circ}\text{C}.$) and is minimal between 1200-1800 hours ($36.90 \pm 0.54 - 36.88 \pm 0.68^{\circ}\text{C}.$) (Fig. 46). The trough values differ significantly from the peak value (each at $P < 0.001$). The peak also differs significantly from the value at 0600 hours ($P < 0.05$). The 0600 hours value differs significantly from those at 1200 hours ($P < 0.005$) and 1800 hours ($P < 0.01$).

2. Treated animals

Rectal temperatures were measured in vehicle-treated control and drug-treated animals during the determinations of sleep duration after PB. The patterns for the mean rectal temperatures of controls and treated animals are plotted at ten-minute intervals for each of the four experimental times during the 24-hour period (Figs. 47-50). These plots of the temperature means demonstrate the hypothermia produced by PB (controls) and the influence of drug pretreatment on this pattern. Values are tabulated in Table 19-22.

Of all the pretreatment groups examined, the major temperature effects are produced only by TP (hypothermia), 5HTP (hypothermia) and DOPA-pargyline (resistance to hypothermia).

At 0600 hours, rectal temperatures in TP-treated animals are 1.86 to $3.81^{\circ}\text{C}.$ lower than controls, and remain lower after the temperature begins to return to normal in controls. The pattern for the TP-treated animals differs

Table 18: The 24-hour pattern of rectal temperature in untreated rats

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>	
Rectal temperature (°C.)	37.9±0.6 (8)	36.9±0.5 (8)	36.9±0.7 (8)	38.4±0.4 (8)	A B c E F ⁺

Table indicates rectal temperature (°C.) measured at six-hour intervals. Values are means ± standard deviation, with the number of animals used in parentheses.

⁺For explanation of letter designations see Table 1.

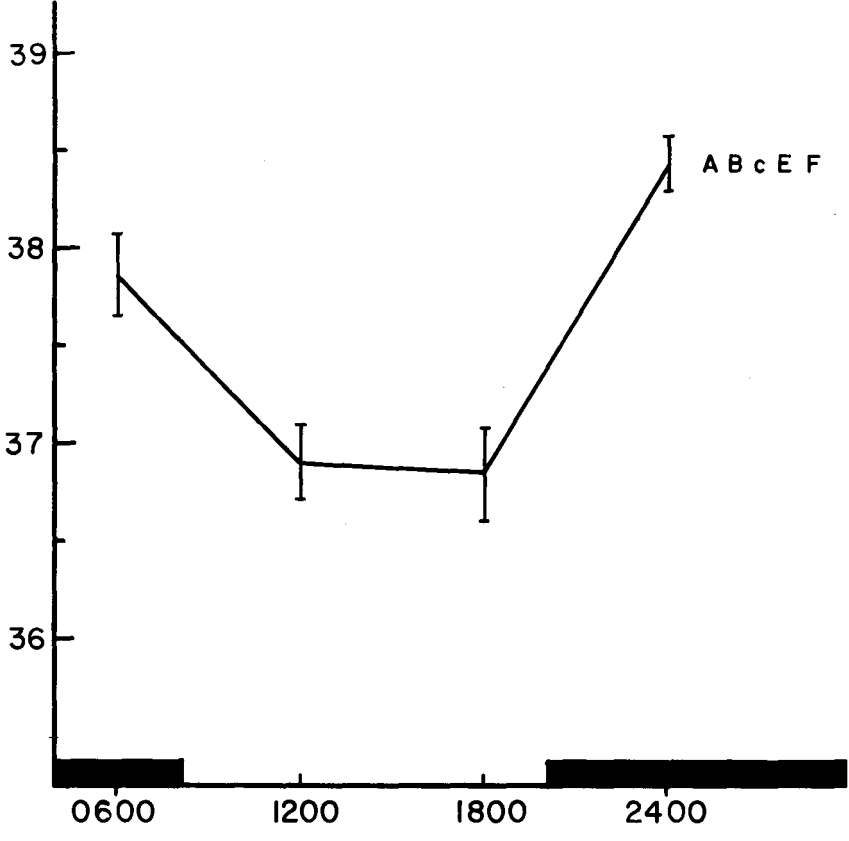


Figure 46: Rectal temperature (^oC.) in untreated rats

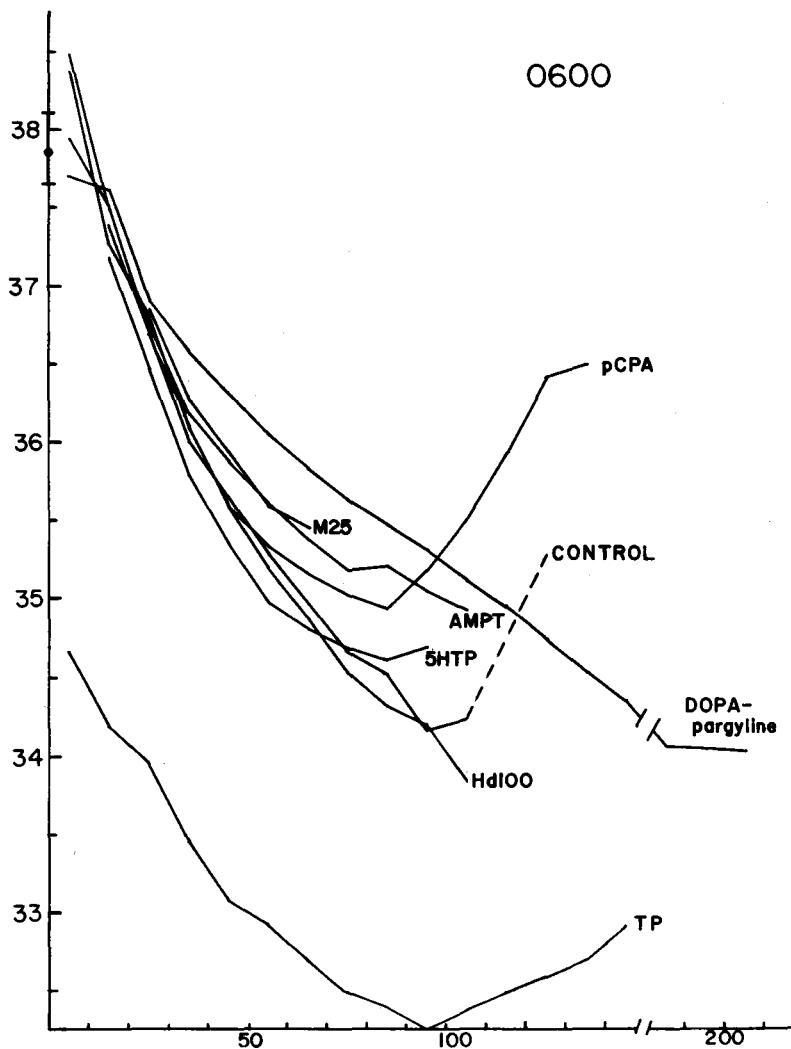


Figure 47: Rectal temperature in CONTROLS and drug pretreated groups, with pentobarbital -- 0600 hours. The Y-axis is temperature, in $^{\circ}\text{C}$. and the X-axis is minutes after the onset of pentobarbital sleep.

Table 19: The time-course of PB hypothermia in controls and treated rats, 0600

TIME (min.)	<u>CONTROL</u>	<u>DOPA-</u> <u>parg.</u>	<u>AMPT</u>	<u>pCPA</u>	<u>TP</u>	<u>5HTP</u>	<u>M</u>	<u>Hd100</u>
5	38.5 [±] .5 (7)	37.7 [±] 1.4 (5)	37.9 [±] .2 (5)	38.4 [±] .4 (3)	34.7 [±] .3* (3)			
15	37.5 [±] .7 (11)	37.3 [±] 1.3 (5)	37.5 [±] .5 (5)	37.6 [±] .2 (3)	34.2 [±] .4* (3)	37.2 [±] .9 (4)		37.4 [±] .3 (3)
25	36.7 [±] .6 (16)	36.9 [±] 1.2 (5)	36.7 [±] .6 (6)	36.8 [±] .3 (4)	34.0 [±] .4* (3)	36.5 [±] .9 (4)	36.9 [±] .2 (3)	36.7 [±] .2 (6)
35	36.1 [±] .7 (16)	36.6 [±] 1.2 (5)	36.2 [±] .8 (6)	36.1 [±] .2 (5)	33.5 [±] .6* (3)	35.8 [±] 1.0 (4)	36.3 [±] .3 (4)	36.1 [±] .2 (6)
45	35.7 [±] .7 (16)	36.3 [±] 1.3 (5)	35.9 [±] .9 (6)	35.6 [±] .2 (6)	33.1 [±] .6* (3)	35.4 [±] 1.0 (4)	35.9 [±] .3 (4)	35.6 [±] .3 (6)
55	35.3 [±] .7 (16)	36.1 [±] 1.3 (5)	35.6 [±] .9 (6)	35.3 [±] .3 (6)	32.9 [±] .6* (3)	35.0 [±] 1.0 (4)	35.6 [±] .4 (3)	35.2 [±] .3 (6)
65	35.0 [±] .7 (15)	35.8 [±] 1.5 (5)	35.4 [±] 1.0 (6)	35.2 [±] .5 (6)	32.7 [±] .6* (3)	34.8 [±] 1.1 (4)	35.5 [±] .2 (2)	34.9 [±] .3 (6)
75	34.7 [±] .8 (13)	35.6 [±] 1.6 (5)	35.2 [±] 1.1 (6)	35.0 [±] .6 (6)	32.5 [±] .5* (3)	34.7 [±] 1.2 (4)		34.5 [±] .4 (6)
85	34.5 [±] .9 (13)	35.5 [±] 1.6 (5)	35.2 [±] 1.3 (5)	34.9 [±] .8 (6)	32.4 [±] .5* (3)	34.6 [±] 1.2 (4)		34.3 [±] .4 (6)
95	34.2 [±] .8 (10)	35.3 [±] 1.7 (5)	35.1 [±] 1.5 (5)	35.2 [±] .7* (6)	32.3 [±] .5* (3)	34.7 [±] 1.2 (4)		34.2 [±] .5 (6)
105	34.2 [±] 1.0 (9)	35.1 [±] 1.7 (5)	34.9 [±] 1.3 (5)	35.5 [±] .9* (6)	32.4 [±] .6* (3)	34.3 [±] .8 (3)		33.8 [±] .7 (3)
115		35.0 [±] 1.8 (5)		35.9 [±] .8 (6)	32.5 [±] .8 (3)			
125		34.7 [±] 1.7 (5)		36.4 [±] 1.1 (5)	32.6 [±] .9 (3)			
135		34.5 [±] 1.7 (5)		36.5 [±] 1.4 (3)	32.7 [±] 1.0 (3)			
145		34.4 [±] 1.6 (5)			32.9 [±] 1.1 (3)			
==== 185		34.1 [±] 1.2 (5)						

significantly from the control pattern at all time points (5-105 minutes, $P < 0.001 - 0.005$). The curves for the other treatments do not differ significantly from control, except for the pCPA values at 95-105 minutes, which are significantly elevated above controls ($P < 0.025$).

At 1200 hours, the fall in rectal temperature of DOPA-pargyline-treated animals proceeds at a slower rate than for controls. The DOPA-pargyline pattern is elevated significantly from the control pattern during 25 to 95 minutes ($P < 0.01 - 0.001$), ranging from 1.53 to 2.85°C. The rectal temperature in 5HTP-treated animals is significantly lower than the controls during 5-95 minutes ($P < 0.001$), ranging from 1.62 to 3.22°C. The temperature effects of the other treatments at this time are not significantly different from control, except for the following: M25 lower than control during 75-85 minutes (0.92 and 0.37°C., respectively, each $P < 0.025$) and pCPA higher than control at 65 minutes (0.88°C., $P < 0.05$).

At 1800 hours, the rectal temperature in DOPA-pargyline-treated animals is also above control values. They are significantly greater than the controls during 15-85 minutes ($P < 0.05 - < 0.001$), ranging between 0.88 to 2.01°C. TP-treated animals have a significant lowering of temperature compared with controls during 5 to 95 minutes ($P < 0.001$), ranging between 2.17 to 3.75°C. In 5HTP-treated animals the rectal temperature is significantly lower than the control temperature for 15 to 25 minutes and at 65 minutes ($P < 0.05$), with differences ranging from 1.04 to 1.12°C.

At 2400 hours, the rectal temperatures of DOPA-pargyline-treated animals are significantly elevated above controls during 15 to 95 minutes (1.60-3.00, $P < 0.001$). The 5HTP pattern is significantly lower than the control

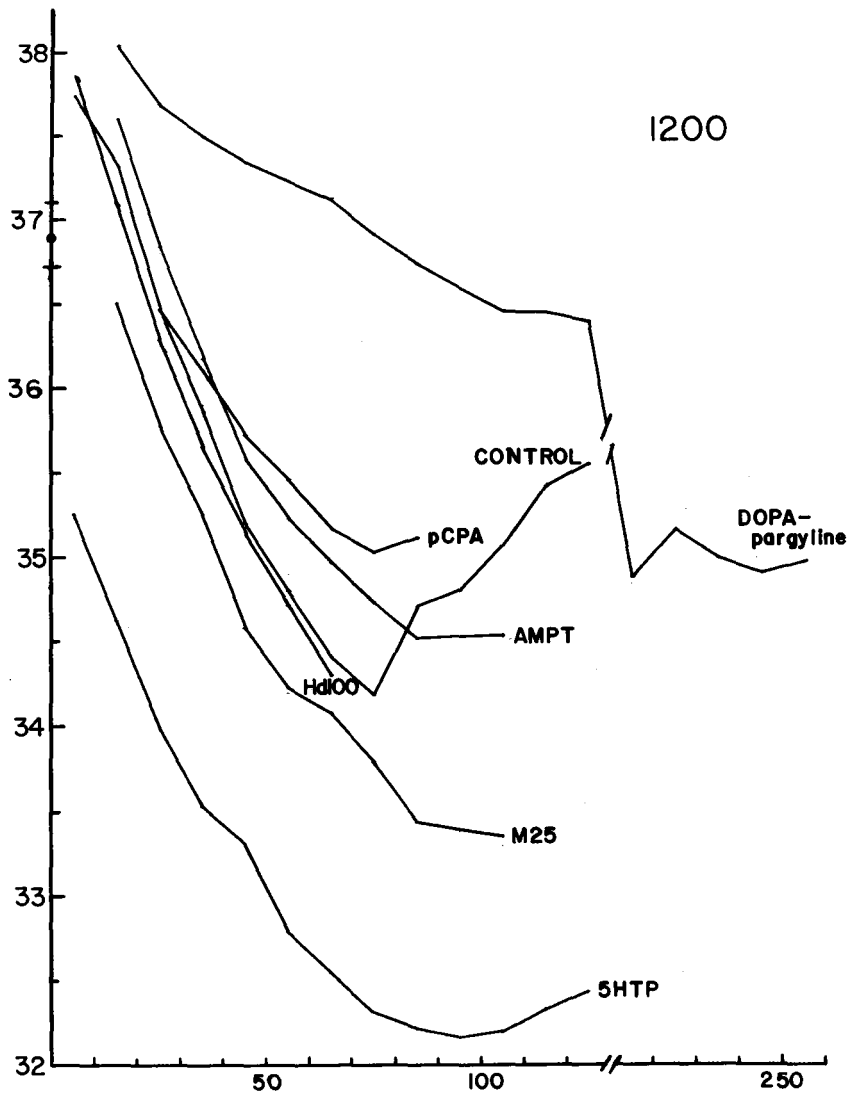


Figure 48: Rectal temperatures in CONTROLS and drug pretreated groups, with pentobarbital -- 1200 hours. Figure legends are the same as Figure 46.

Table 20: The time-course of PB hypothermia in control and treated rats, 1200

TIME (min.)	CONTROL	DOPA- parg.	AMPT	pCPA	5HTP	M	Hd100
5	37.7±.3 (3)				35.3±.3** (4)		37.9±.1 (4)
15	37.3±.5 (8)	38.0±.9 (4)	37.6±.2 (3)		34.6±.2** (4)	36.5±.5 (2)	37.1±.3 (5)
25	36.5±.6 (11)	37.7±.8** (4)	36.8±.2 (5)	36.5±.3 (3)	34.0±.2** (4)	35.8±.4 (4)	36.3±.3 (5)
35	35.2±.6 (13)	37.5±.6** (4)	36.2±.2 (5)	36.1±.3 (4)	33.5±.2** (4)	35.3 ±.2 (4)	35.7±.3 (5)
45	34.8±.6 (13)	37.3±.5** (4)	35.6±.2 (5)	35.7±.4 (4)	33.1±.2** (4)	34.6±.4 (6)	35.1±.4 (5)
55	34.4±.6 (13)	37.2±.5** (4)	35.2±.2 (5)	35.5±.4 (4)	32.8±.2** (4)	34.2±.4 (6)	34.7±.4 (5)
65	34.3±.7 (9)	37.1±.6** (4)	35.0±.2 (5)	35.2±.4 (4)	32.6±.2** (4)	34.1±.3 (5)	34.3±.5 (5)
75	34.7±.5 (6)	36.9±.6** (4)	34.7±.3 (5)	35.0±.3 (4)	32.3±.2** (4)	33.8±.3* (3)	
85	34.8±.6 (6)	36.7±.5** (4)	34.5±.4 (5)	35.1±.3 (4)	32.2±.2** (4)	33.4±.1* (2)	
95	35.1±.6 (6)	36.6±.5** (4)	34.5±.4 (5)		32.2±.2** (4)	33.4±.1* (2)	
105	35.4±.9 (4)	36.5±.5 (4)	34.5±.7 (5)		32.2±.3 (4)	33.4±.2 (2)	
115	35.6±.9 (2)	36.5±.5 (4)			32.3±.2 (3)		
125		36.4±.4 (4)			32.4±.2 (3)		
====							
215		34.9±.8 (4)					
225		35.1±.6 (3)					
235		35.1±.6 (3)					

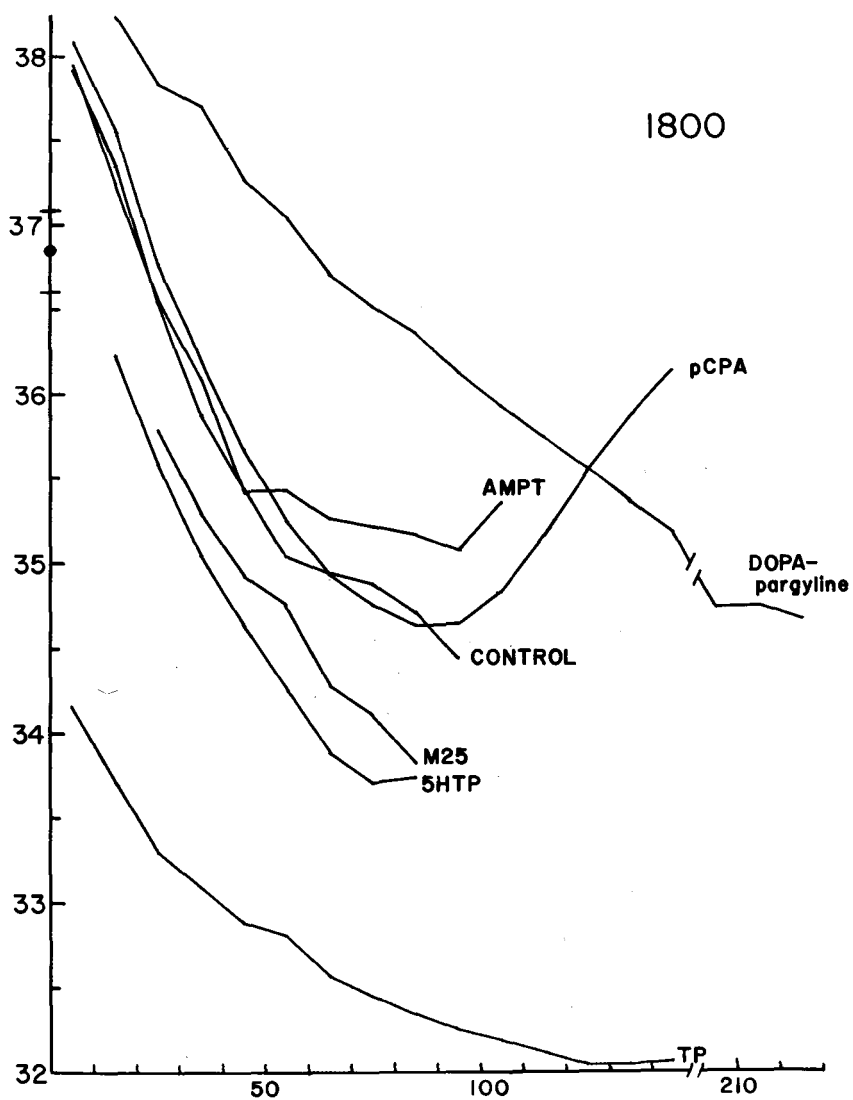


Figure 49: Rectal temperature in CONTROLS and drug pretreated groups, with pentobarbital -- 1800 hours. Figure legends are the same as Figure 46.

Table 21: The time-course of PB hypothermia in control and treated rats, 1800

TIME (min.)	CONTROL	DOPA- parg.	AMPT	pCPA	TP	5HTP	M
5	37.9 \pm .5 (8)	38.8 \pm .3 (5)	38.0 \pm .6 (3)	38.1 \pm .3 (5)	34.2 \pm .2 (3)		
15	37.4 \pm .8 (10)	38.2 \pm .2 [*] (5)	37.2 \pm .8 (3)	37.6 \pm .3 (6)	33.7 \pm .2 (3)	36.2 \pm .1 (3)	
25	36.5 \pm .8 (12)	37.8 \pm .3 ^{**} (5)	36.6 \pm 1.0 (3)	36.8 \pm .3 (5)	33.3 \pm .3 (3)	35.6 \pm .1 [*] (4)	35.8 \pm .2 (3)
35	35.9 \pm .8 (13)	37.7 \pm .5 ^{**} (5)	36.1 \pm 1.0 (3)	36.2 \pm .3 (5)	33.1 \pm .2 (3)	35.0 \pm .3 (4)	35.3 \pm .2 (5)
45	35.4 \pm .8 (14)	37.3 \pm .4 ^{**} (5)	35.4 \pm 1.1 (3)	35.7 \pm .4 (5)	32.9 \pm .2 (3)	34.6 \pm .3 (4)	34.9 \pm .2 (5)
55	35.0 \pm .9 (14)	37.0 \pm .4 ^{**} (5)	35.4 \pm 1.0 (3)	35.2 \pm .4 (5)	32.8 \pm .2 (3)	34.3 \pm .3 (4)	34.6 \pm .3 (5)
65	34.9 \pm .9 (13)	36.7 \pm .4 ^{**} (4)	35.3 \pm .9 (3)	34.9 \pm .5 (5)	32.6 \pm .3 (3)	33.9 \pm .2 (4)	34.3 \pm .4 (5)
75	34.9 \pm 1.1 (12)	36.5 \pm .4 ^{**} (4)	35.2 \pm .6 (3)	34.8 \pm .6 (5)	32.5 \pm .3 (3)	33.7 \pm .2 (4)	34.1 \pm .3 (4)
85	34.7 \pm 1.4 (9)	36.4 \pm .3 [*] (4)	35.2 \pm .3 (3)	34.6 \pm .6 (5)	32.4 \pm .3 (3)	33.7 \pm .2 (4)	33.8 \pm .3 (2)
95	34.4 \pm 1.6 (4)	36.1 \pm .3 (4)	35.1 \pm .2 (3)	34.6 \pm .8 (5)	32.3 \pm .3 (3)		
105		35.9 \pm .3 (4)	35.4 \pm .4 (3)	34.8 \pm 1.0 (5)	32.2 \pm .3 (3)		
115		35.7 \pm .4 (4)		35.2 \pm 1.2 (5)	32.1 \pm .3 (3)		
125		35.6 \pm .4 (4)		35.5 \pm 1.5 (5)	32.1 \pm .3 (3)		
135		35.4 \pm .4 (4)		35.9 \pm 1.6 (5)	32.1 \pm .3 (3)		
145		35.2 \pm .4 (4)		36.1 \pm 1.5 (5)	32.1 \pm .3 (3)		
===== 205		34.7 \pm .4 (4)					

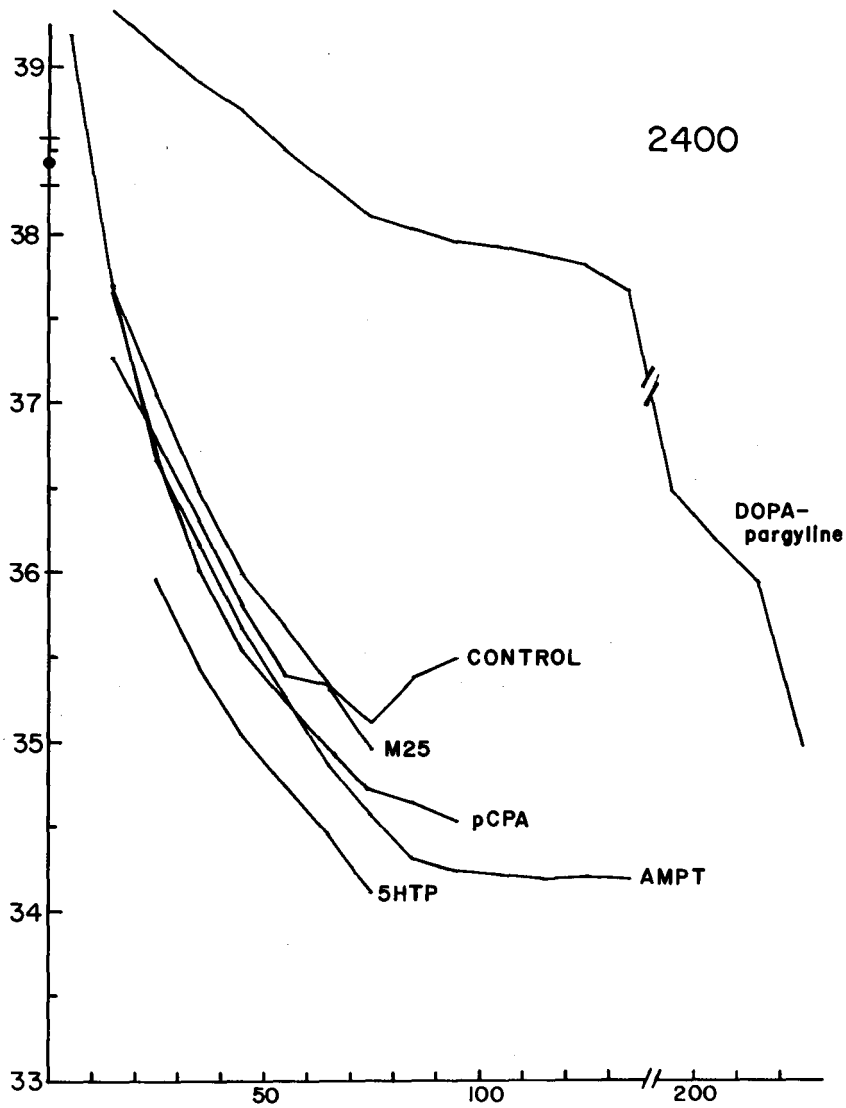


Figure 50: Rectal temperature in CONTROLS and drug pretreated groups, with pentobarbital -- 2400 hours. Figure legends are the same as Figure 46.

Table 22: The time-course of PB hypothermia in control and treated rats, 2400

TIME (min.)	CONTROL	DOPA- parg.	AMPT	pCPA	5HTP	M
5			39.2±.01 (2)			
15	37.7±.3 (4)	39.3±.8** (4)	37.7±.7 (5)	37.7±.1 (2)		37.3±.4 (2)
25	37.1±.1 (10)	39.1±.7** (5)	36.7±.5 (7)	36.7±.9 (5)	36.0±.5** (4)	36.8±.4 (3)
35	36.5±.2 (11)	38.9±.7** (5)	36.2±.6 (8)	36.0±1.0 (5)	35.4±.3** (4)	36.3±.4 (4)
45	36.0±.3 (11)	38.7±.8** (5)	35.7±.4 (8)	35.5±1.2 (5)	35.0±.3** (4)	35.8±.5 (6)
55	35.7±.4 (11)	38.5±.8** (5)	35.3±.3* (8)	35.2±1.3 (5)	34.7±.4** (4)	35.4±.5 (6)
65	35.3±.5 (8)	38.3±.7** (5)	34.9±.3* (8)	35.0±1.3 (5)	34.5±.4 (4)	35.3±.5 (4)
75	35.1±.6 (8)	38.1±.7** (5)	34.6±.3 (8)	34.7±.3 (5)	34.1±.3 (4)	35.0±.5 (2)
85	35.4±.6 (6)	38.0±.7 (5)	34.3±.4 (8)	34.6±1.3 (5)		
95	35.5±1.1 (4)	38.0±.6 (5)	34.2±.4 (8)	34.5±1.4 (4)		
105		37.9±.6 (5)	34.2±.6 (8)			
115		37.9±.6 (5)	34.2±.7 (8)			
125		37.8±.5 (5)	34.2±.8 (8)			
135		37.7±.6 (5)	34.2±.8 (7)			
==== 195		36.5±1.1 (5)				
205		36.2±1.0 (5)				

pattern during 25 to 75 minutes ($P < 0.02 - 0.001$) with differences ranging from 0.88 to 1.08°C . The AMPT pattern is reduced significantly below that of control for 55 to 65 minutes after PB, with differences of 0.43 and 0.47°C ., respectively ($P < 0.05$).

DB ($15\text{mg}/\text{kg}$, IP) in rats produces a significant fall in rectal temperature, 24 hours after its administration. The subsequent administration of PB causes a more profound and significant decrease in rectal temperature. In male mice, the rectal temperature 24 hours after DB is less depressed than in the rats. After PB, the fall in rectal temperature is double that of saline or methyl cellulose controls.

DPH ($50\text{mg}/\text{kg}$, IP) in mice, produces a marked fall in rectal temperature, from 38.1 ± 0.9 to $34.3 \pm 0.7^{\circ}\text{C}$. After PB temperature decreases further to 30.5 ± 0.9 , as compared to $35.7 \pm 2.4^{\circ}\text{C}$. for saline or methyl cellulose controls.

V. GENERAL DISCUSSION

A. Introduction

Normal sleep is a complex process, and drug-induced sleep further complicates the process. It is evident that a variety of drug treatments and/or conditions (environmental, physiological, pathological, etc.) can influence the state of sleep considerably as has been illustrated by experiments described in the thesis and elsewhere. Sleep induced by drugs has not been studied to any great extent from the standpoint of circadian alteration, i.e., time-linked drug effects, or with a consideration of the internal milieu of the organism as it influences drug affect. This is an important consideration because of the periodicity basic to sleep-wakefulness phenomena. Since biological systems and organisms are in a dynamic state, studies of their functions ought to include these time-linked aspects.

For these reasons an examination of alterations in the duration of drug-induced sleep has been made on a 24-hour basis. Concomitantly brain amine levels in specific CNS areas, body temperature changes, as well as their interrelationships have been studied.

A basic question of this study revolves around the possible modes by which sleep induced by hypnotics might be affected. Several possibilities will be enumerated and subsequently examined as they might apply to data derived from specific drug treatments.

The following are to be considered:

- 1) amine background upon which the hypnotic acts
- 2) the influence of temperature on metabolism and hypnosis

- 3) actions on metabolizing enzymes (of amines and the hypnotic)
- 4) the binding of barbiturate and amines and their bioavailability

B. Drugs affecting the NE and DA system

1. DOPA-pargyline

DOPA-pargyline pretreatment significantly prolongs PB-sleep at each time tested (Table 3). The curve over the 24 hour period is essentially flat indicating that at this dosage, sleep duration has reached a ceiling response. Amine measurements made half-way through the sleep period in such animals indicate significant increases in DA concentrations in all brain parts, at almost every experimental time point (15/16 values). NE levels increased significantly in only 4 of 16 values, viz, the CN at 0600, 1800 and 2400 hours; brain stem at 0600 hours. 5HT levels are decreased in the MB at 1800 and 2400 hours. Of these amine changes, the increased DA concentration seems to correlate best with the prolongation of sleep (15/16 instances). In the CN at 1200 hours there is no significant change in DA concentration at a time when sleep duration is increased over controls.

These results are in opposition to the reports of excitatory effects for DOPA or DA, administered intraventricularly, intracisternally and iontophoretically (Spooner and Winters, 1965). The changes in brain NE and 5HT concentrations coincide only infrequently with the prolongation of PB-sleep in this treatment group. In the face of a PB challenge the usual excitatory effect of DOPA or DA is inoperative and other mechanisms must be

considered. Lesions of the DA-containing areas in the mesencephalic ventral tegmentum (in the substantia nigra) produce behaviorally-comatose animals, but with a relatively normal EEG sleep-wake cycle (Jones et al., 1969). On the other hand, Connor (1970) demonstrated that direct iontophoretic application of DA to CN neurons produced spike rate depression. Lammers and van Rossum (1968) reported bizarre stereotypic behavior in rats after the administration of DOPA in combination with a peripheral decarboxylase inhibitor. When animals were isolated, they remained in a catatonic state. The behavior, which was maximal after one hour, lasted over an eight-hour period. The prolongation of PB sleep in DOPA-pargyline rats might be a reflection of catatonia superimposed on depression. Ansel and Markham (1970) noted drowsiness in two of four normal patients given L-DOPA chronically, although they did not appear sedated. Messiha et al. (1970) found that DA urinary excretion was elevated in both depressed and manic patients, and returned to normal only in the latter case after lithium therapy. In the treatment of Parkinson patients, depression has been reported (Cherington, 1970; Damasio et al., 1970). These studies suggest that under some conditions DOPA and/or DA can exert depressant effects.

Blum et al. (1972a) demonstrated that DOPA enhanced ethanol-induced sleep, which seems to corroborate the present study. These investigators suggested that the enhanced sleep might be due to the naturally-occurring, alcohol-like metabolite of DA, 3,4-dihydroxyphenylethanol (DOPET), which augments sleep. A similar argument could be invoked for the results obtained in the present study. To establish this it would be necessary to demonstrate this compound's formation in vivo, and correlate its presence with sleep.

Although there are no significant differences between sleep periods at the experimental times, the amine levels of DOPA-pargyline treated animals do exhibit significant diurnal fluctuations (Fig . 29). These amine changes include MB, brain stem, and hypothalamic DA and hypothalamic 5HT, all of which peak significantly at 1800 hours. CN 5HT, brain stem 5HT and MR NE levels exhibit significant peaks at 1200 hours. CN and hypothalamic NE values are minimal at 0600 hours and maximal at 1800-2400 hours. These results indicate that the influence of pretreatment on amine levels in CNS varies with the tissue and time of the day. This could be of clinical importance in the selection of the proper time of day, for example, for Parkinsonian patients to ingest L-DOPA, for maximum therapeutic efficacy.

Rectal temperature in DOPA-treated animals decreases at a significantly slower rate than in the controls, indicating that this treatment produces a resistance to the normal hypothermia induced by PB. Myers and Yaksh (1968) reported that in the rat, intraventricular NE and DA (DOPA after a latency) cause hyperthermia. At every experimental point, except 0600 hours, the pattern is elevated above controls, and continues to decrease, even when animals recover from sleep. A fall in body temperature might significantly decrease hepatic PB metabolism and thereby prolong its action, but the temperature effect found favors a less depressed metabolic rate than in controls. It seems unlikely therefore that the body temperature changes play an important role in these effects.

Another possible basis for DOPA-pargyline effects on sleep might be attributed to the binding of PB (Lasser et al., 1963; Pagnini et al., 1971) and CAs to plasma proteins. Danon and Sapira (1972) reported that labelled

CAs (including NE, DOPA and DA) are bound to human serum albumin, in vitro. These gel filtration studies demonstrated that the fraction of NE bound ranges from 20% at high protein concentrations to 90% at concentrations of $3 \times 10^{-8}M$. Goldbaum and Smith (1954) reported that 37% of PB is bound in vitro to bovine serum albumin. Pagnini et al. (1971) found that a number of drugs which are bound to serum albumin in a manner similar to PB, enhance the duration of PB narcosis in mice. Increasing the concentrations of these drugs in vitro results in a further reduction of PB bound to serum albumin. The drugs were without effect on liver metabolism of PB. In mice treated with one of these drugs (sulfaethylthiazole), brain levels of PB were significantly higher than in animals receiving PB alone. These findings suggest that the large dose of DOPA or the DA formed from it (and/or possibly their metabolites), might be bound to plasma proteins, thereby displacing PB from sites it would otherwise bind to. With more free PB available to act upon the CNS structures, hypnosis would be prolonged.

It is not likely that the prolonged hypnotic sleep is due to inhibition of the metabolizing enzyme. Nair (personal communication) indicated that the half-life of the enzyme oxidizing PB is about 15 hours. Since DOPA-pargyline are administered 60 and 30 minutes, respectively, prior to PB, there is relatively little time for them to alter enzyme activity.

2. Alpha-methyl-p-tyrosine

AMPT pretreatment significantly prolongs PB-sleep at 1200 and 2400 hours (Table 3, Figs. 11-12), and significantly decreases NE and DA levels from controls (Table 10, Fig. 31). However 5HT levels are

unchanged in 10/14 values, increase in 3/14 and decrease in 1/14 values. Brain stem 5HT and DA, CN 5HT and MB 5HT levels exhibit diurnal patterns with peaks at 1200 hours and troughs at 2400 hours, in the dark. Hypothalamic DA also peaks significantly in the light (1800 hours) and troughs in the dark (0600 hours). CN NE and DA, and MB DA have maxima in the dark phase. These results demonstrate that even when CNS CAs are depleted, the reduced amine concentrations vary with the time of day.

Amine reductions from control were as follows: MB NE, 43.6% - 58.9%; brain stem NE, 45.8 - 51.6% and hypothalamic NE, 52.1 - 62.6%. CN NE decreases significantly only at 0600 hours (61.3%) and 1800 hours (43.9%). The percentage depletion from controls for NE in all tissues examined ranges between 43.6 - 62.2% in 14/16 instances (Table 10). The percentage depletion from control for CN DA (70.0 - 78.5%, 4/4 values) and hypothalamic DA (74.7 - 86.3%, 2/4 values) is somewhat higher than for NE. The extent of depletion of NE in the tissues over the 24 hour period studied is relatively constant. AMPT is a tyrosine hydroxylase inhibitor, exerting its amine-depleting effects by blocking CA synthesis. The degree of depletion of NE and DA is indicative of different rates of turnover (Iversen and Glowinski, 1966). Udenfriend and Zaltzman-Nirenberg (1963) reported that in the guinea pig brain the half-time of DA was 2 hours while that of NE was 4 hours. The greater depletion of DA could also be due to its greater release.

Rech et al. (1968) reported that AMPT induces behavioral depression in rats, related to the CA depletion. Weitzman et al. (1969) found that AMPT decreases REM sleep, with a compensatory increase in NREM sleep. Hartman

et al. (1971) reported that in the rat oral AMPT increased PS, while IP AMPT (75 mg/kg) disturbed sleep and decreased PS. Torda (1968) found no obvious EEG changes 3 hours after AMPT (80 mg/kg, IP), but did after 18 hours (3 doses 6 hours apart). SWS increased and PS and wakefulness decreased. King and Jewett (1971) described that AMPT in cats enhanced REM sleep, at a time when NE levels are significantly reduced. Sjoerdsma et al. (1965) found that AMPT produced sedation in humans. Jouvet (1972b) indicated that the effects of AMPT on PS were variable, but in all species behavioral and EEG waking were decreased, which could be counteracted by DOPA administration. These studies indicate that after AMPT administration waking is reduced. Such depression might be expected to enhance PB-sleep. This is demonstrated in the present study at 2400 as well as 1200 hours. The latter result occurs during the normal sleep period of rodents, when hypnotically-induced sleep reaches a maximum. The second, more prominent peak at 2400 hours, comes at a time when the rats are normally awake and active. There are several amine differences at 2400 hours compared to 1200 hours that might contribute to the augmented sleep. These differences include: significantly-reduced MB and hypothalamic DA at 2400 hours. MB 5HT and brain stem DA are elevated and hypothalamic 5HT is reduced compared to controls at 1200 hours, but not at 2400 hours. Since the MB includes the anterior portion of the 5HT-containing raphe system, which numerous lesion and pharmacological experiments (cf. Jouvet, 1972b) indicate is vital for sleep, facilitation of PB-sleep might be attributed to the augmented MB 5HT levels. This effect would have to be balanced somewhat by the decreased hypothalamic 5HT which would tend to counter sleep (vide

infra regarding reduced 5HT and depression). There are no significant differences for 5HT from control at 1200, 1800 or 2400 hours for brain stem, which contains the posterior portion of the raphé system.

An examination of the time-course of rectal temperatures in treated animals reveals a pattern that is in essence similar to that of the control. This would tend to eliminate temperature changes as a factor for the significant increase in PB-sleep at 1200 hours. At 2400 hours, rectal temperature is significantly lower in AMPT-treated animals than controls (at 55 and 65 minutes, 0.43 and 0.49°C. lower, respectively) but such small differences in body temperature are not likely to be effective in prolonging PB-sleep at 2400 hours.

The effects of AMPT on PB-binding to serum proteins or the PB-metabolizing enzyme are probably minor. AMPT was administered 4 hours prior to PB, which probably is insufficient time to alter the PB-metabolizing enzyme system (Nair, personal communication). Danon and Sapira (1972) reported that L-tyrosine is not bound to albumin, but AMPT was not studied. Notwithstanding its inhibitory effect, there is some indication that AMPT itself can be hydroxylated to alpha-methyl DOPA and subsequently form alpha-methyl DA and alpha-methyl NE (Maitre, 1965). Danon and Sapira (1972) did find that alpha-methyl DOPA was bound to the same extent as DOPA. Moore et al. (1967) reported a number of toxic effects after AMPT, but which do not appear until 20-48 hours after administration. These effects include hypothermia (6.0°C. lower than controls), lethargy, and weight loss. AMPT-treated animals have higher blood glucose levels within 2 hours after injection, which return to

normal 24 hours later. An interesting aspect of the AMPT-sleep pattern is its similarity to the bimodal circadian plasma glucose pattern obtained by Frideman and Walker (1969). They explained the nature of their pattern on the basis of a diurnal rhythm for the initial glucose peak and a feeding rhythm for the second peak. Lawson et al. (1951) demonstrated that glucose was effective in restoring sleep to animals awakening from drug-induced sleep. Finally, one cannot help but think that the possibility exists for the displacement of PB from plasma by several substances entering the plasma concomitantly with AMPT itself or its metabolites and dietary constituents.

C. Drugs Affecting the 5HT System

1. p-Chlorophenylalanine

pCPA treatment significantly prolongs PB-sleep above controls at all experimental time points. Furthermore, the 24-hour pattern of sleep duration is circadian, with a peak at 1200-1800 hours (Figs.13-17). This compound produces a significant reduction of 5HT levels at each point of measurement during the 24-hour period (16/16). Several concomitant reductions in DA levels occur during the light phase, viz., 29.1% decrease in MB DA ($P < 0.05$) at 1200 hours, 24.2% decrease in CN DA ($P < 0.05$) at 1200 hours and a 72.7% decrease in hypothalamic DA ($P < 0.005$) at 1800 hours.

MB 5HT exhibits a maximum at 2400 hours in the dark phase, with significant differences between this peak and 0600, 1200 and 1800 hours. Hypothalamic NE is minimal at 1800 hours, differing significantly from 0600, 1200 and 2400 hours. CN NE and hypothalamic DA are also minimal at 1800

hours, differing significantly from the values at 0600 and 1200 hours. CN DA is maximal at 1800 hours, differing significantly from 0600 and 1200 hours levels. Hypothalamic 5HT exhibits a trough at 0600 hours (5HT concentrations reduced to zero) and a peak at 1200 hours, with significant differences between the trough and the other three time points and the peak and the 2400 value. CN 5HT levels are minimal at 1200 hours and maximal at 2400 hours, during the dark phase. Brain stem DA is minimal at 0600 hours, while brain stem 5HT is maximal at 1200 hours.

The amine patterns after pCPA exhibit diurnal variations, which, in general are at their normal levels for CAs and reduced for 5HT. The extent of depletion is great, i.e., in 12/16 measurements it is greater than 85%. MB 5HT is depleted to the smallest extent (40.4%) at 2400 hours, when the prolongation of sleep by PB is least. The depletion of hypothalamic 5HT at 1200 hours (66.9%) or CN 5HT at 1800 hours (74.5%) occurs when the augmentation of PB-sleep is maximal. In the pCPA-depleted state, brain stem and hypothalamic 5HT are at their peak levels, at 1200 hours, although these tissues are at 13.2% and 33.1% of their respective control levels. The fact that the sleep pattern, although shifted to an augmented level, still maintains a 24-hour variation, reflecting the normal pattern would suggest that the relative, rather than absolute 5HT values play a role in the modulation of sleep. Superimposed upon this effect is the general depressant activity of PB itself. The brain stem contains most of the 5HT-containing raphe system, which is important in normal sleep (cf. Jouvet, 1972b). The concomitant reduction in CN NE and hypothalamic NE and DA are factors that might

facilitate PB sleep. Many studies suggest that NE and DA are important for waking (cf. Marczyński, 1967; Marley and Stephenson, 1972; Jouvet, 1972b).

In view of several reports of the insomnic effect of pCPA (Delorme et al., 1966a; Koella et al., 1968; Mouret et al., 1968; Pujol et al., 1971), one would not have expected the prolongation of sleep obtained in the present studies. In pCPA-induced sleep changes, both REM and SWS are decreased. Dement et al. (1972) reported in the cat, that the early effect of pCPA is an increase in REM sleep for the first 24-48 hours (no dosage is given). This is followed by a reduction in REM and SWS after the third day. The prolonged deprivation of REM and SWS over the three-day period in which pCPA was administered could explain the enhanced effect of PB seen in these studies, as a rebound phenomenon. The circadian variation in the prolongation might then be a reflection of the variation of the CA present, as well as a change in relative, rather than absolute levels of 5HT.

Central depression can occur with reduced 5HT levels. Shaw et al. (1967) and Pare et al. (1969) demonstrated that 5HT levels were significantly lower in the brain stem of depressed subjects. Bourne et al. (1968) found that 5HIAA was also reduced in such cases. These findings, together with others (vide supra) seem to indicate that depression can occur whenever 5HT levels deviate from a specific optimal range of values.

Whether binding of pCPA to plasma proteins is involved in this augmentation of PB-sleep is not certain, since it is not known if pCPA binds significantly to plasma proteins, although phenylalanine does not (Danon and Sapira, 1972). However, pCPA is fairly slowly cleared from the plasma. Koe

and Weissman (1966) reported that 3 days were required for a single dose of pCPA (316 mg/kg, IP), to fall to one-half its maximum plasma level. With such a slow clearance, the consecutive administration of pCPA would result in its accumulation in the plasma.

pCPA affects the rectal temperature only slightly under the conditions of these experiments, compared to controls. Occasional changes, in the direction of hyperthermia compared to controls, occurred (at 0600 hours, at 95-105 minutes, 1.11 and 1.27°C.; and 1200 hours, at 65 minutes, 0.88°C). These changes would very likely mitigate against prolonged sleep.

2. Tryptophan

TP prolongs PB-sleep significantly over controls at each of the experimental times (Table 4), but the only significant difference between the TP groups is between the 1800 and 2400 hours values. TP significantly elevates 5HT at 0600 hours in the MB and hypothalamus and at 1800 hours in the hypothalamus. Amine concentrations decrease in the following areas: hypothalamic NE levels at 0600 hours; in the MB, brain stem and hypothalamic DA and CN NE at 1200 hours; in MB NE at 1800 hours; and hypothalamic DA levels at 2400 hours. TP, a secondary precursor of 5HT, was given to elevate brain 5HT, which it did in only 4/16 instances, perhaps because the time interval and dose schedule used was insufficient. The use of 5HTP to increase central 5HT has been criticized because of the widespread distribution of the decarboxylating enzyme leads to formation of 5HT in CNS areas in which it is not normally present (Moir and Eccleston, 1968). Exogenous TP follows the physiological uptake pathways, which involve hydroxylation of TP at intra-

neuronal sites and uptake into the CNS 5HT stores. Exogenous 5HTP is taken up by the brain and decarboxylated in the cytoplasm, and subsequently and quite readily attacked by MAO.

MB and hypothalamic 5HT concentrations peak at 1800 hours and are greater than levels at 0600, 1200 and 2400 hours. Brain stem NE is maximal at 1800 hours and CN DA is maximal at 1800-2400 hours. The elevated 5HT in the hypothalamus (1800 hours) and brain stem (2400 hours) and decreased brain stem and CN DA at 1200 hours might be expected to favor sedation. MB and brain stem DA and CN NE levels are minimal at 1200 hours. Reduced levels of NE and DA at 1200 hours might be expected to reduce their alerting effects and facilitate depression by PB. It should be pointed out that the results favoring sleep that were obtained at 1200-1800 hours occur during the period of normal sleep in these animals.

Several clinical studies have reported that TP ingestion by humans has a facilitatory effect on sleep (Wyatt et al., 1970b; Hartman et al., 1971b). Wyatt et al. (1970b) found increased total and NREM sleep and decreased REM sleep. Pretreatment with a decarboxylase inhibitor enhanced the sedative effect, while reducing the unpleasant side effects. However, these investigators concluded that TP does not act through a 5HT mechanism, since 5HTP increases REM sleep, but TP did not. They further report that TP still has the same effects in pCPA-treated patients, in which the conversion of TP to 5HT is inhibited. Hartman et al. (1971b) found that TP reduced sleep latency and slightly increased total sleep time, which was dose-dependent. They also reported that similar doses of L-histidine or L-tyrosine did not have these

effects.

As seen in Figs. 47, 49 and Tables 19, 21, TP markedly lowers body temperature from control levels, from the onset of sleep, throughout the period of measurement. For example 5 minutes after the onset of sleep temperature fell 3.81°C. (from 38.48 in controls to 34.67°C.) at 0600 hours and 3.75°C. (from 37.92 in controls to 34.70°C.) at 1800 hours. As pointed out earlier, the reduction of body temperature results in prolongation of PB-sleep, by decreasing its rate of metabolism. These changes in body temperature are of such magnitude that they might readily contribute to the enhancement of sleep.

The binding of TP to serum albumin might also contribute to prolongation of PB-sleep. McManemy and Oncley (1958) and McManemy *et al.* (1961) reported in fasting humans and *in vitro*, respectively, that 75% of plasma L-TP is bound to albumin. This binding is stereospecific and pH dependent, involving the indole ring. However, tryptamine and 5HT are not bound significantly. The large dose of TP utilized (500 mg/kg, IP) could raise plasma TP levels significantly to displace PB and enhance its actions.

3. Melatonin

Two doses of melatonin (M25 and M50) were used in the hypnotic study but only the lower dose was used for the brain amine determinations. The M25 dose prolongs PB-sleep significantly at 1200, 1800 and 2400 hours, with a peak at 1200 hours and a trough at 2400 hours. The M50 dose prolongs PB-sleep significantly at each experimental time, but shifted the peak to 0600 hours and the trough to 1800 hours. M has behavioral and EEG hyper-

synchronizing effects (Marczynski et al., 1964; Arutyunyan et al., 1964; Hishikawa et al., 1969). Barchas et al. (1967) demonstrated in mice that M (25 mg/kg, IP) prolonged HB sleeping time by 50%. Arutyunyan et al. (1964) found that M prolonged chloral hydrate- and hexenal-induced sleep. The present study is the first report of a diurnal pattern for this enhanced hypnotic effect of M. The peak effect of the M25 dose occurs in the light phase, when rats normally sleep.

The M25 dose had relatively little effect on brain amines. At 0600 hours the effects of increasing 5HT and decreasing DA in the MB, favor sleep, which is prolonged at this time. At 1200 hours, the decrease of brain stem 5HT favors waking, while reduced hypothalamic DA favors sleep. Prolonged PB-sleep is the effect at this time, indicating that the overall effect of these amine changes is a facilitation of sleep. The primary CN amine, DA, is reduced significantly at 1800 hours, when PB-sleep is prolonged. Since the CN is involved with motor activity, the reduced DA level might reflect the inactivity of animals at this time. At 2400 hours the amines changes are not significant and PB-sleep is less prolonged. These amine results are in the same direction as those obtained by Anton-Tay et al. (1968), who, in the female rat, found elevations of MB 5HT, 1 and 2 hours after a considerably lower dose of M, as well as increased hypothalamic 5HT at 1 hour and reduced cortical 5HT at 20 and 60 minutes. There were no significant changes in NE in these areas. The sites of the prominent amines changes are the same as those which have been shown to selectively concentrate ³H-M (Noble et al., 1967; Anton-Tay and Wurtman, 1969).

The M25 dose had no significant effects on body temperature effects of PB, except for a reduction at 75-85 minutes in the 1200 hours group (Table 19-22, Figs. 47-50). Barchas et al. (1967) found that M (25 mg/kg) had no effect on body temperature in rabbits, while Arytyunyan et al. (1967) reported that in mice, dose of M (50-100 mg/kg, SC) lower body temperature 2-3°C. Hypothermia obtained with M in the present study is probably a factor in the prolongation of PB-sleep.

The effect of the M50 dose paradoxically is reversed, with a maximum at 0600 hours in the dark and a minimum at 1800 hours, but PB-sleep is prolonged to the same extent as the M25 dose at 1200 and 1800 hours. The M50 results are subject to several possible interpretations. Doubling the M dose does not result in a uniform enhancement of PB-sleep over the M25-treated groups. The increased prolongation at 0600 and 2400 hours with M50 (motor and metabolic) occurs in the dark phase, suggests that there might be a relationship to the greater activity of the animals at these times. Doubling the M dose might produce brain monoamine changes favoring longer sleep. Because M is structurally similar to 5HT it might displace 5HT from its active site, and to a greater extent with the M50 than the M25 dose. $^3\text{H-M}$ selectively concentrated in the MB and hypothalamus (Anton-Tay and Wurtman, 1969). Greater effects on these areas, which normally contain significant amounts of 5HT, would favor the depressant actions of M and 5HT. The extent of M-hypothermia might be greater for the M50, when the animals normal temperature is high, resulting in a greater fall when combined with PB, to yield a slower rate of PB metabolism. Finally, with the M50 dose toxic effects

might be more prominent during the active period.

4. 5HTP and 5HT-related Compounds

5HTP significantly prolonged PB-sleeping time over controls at 1200 and 2400 hours (Figs. 13-17, Table 5), although there is an increase at 0600 hours that is of borderline significance. This bimodal pattern is similar to that obtained with AMPT, in which there is prolongation during the normal sleep period (1200 hours) and at 2400 hours, when rats are active. In a limited study of the brain amines after 5HTP, at 1200 hours, 5HT was elevated in MB, hypothalamus and to a lesser extent in the CN. There was a concomitant increase in MB, CN and brain stem DA, as well as CN NE. A metabolite of DA might be involved in the enhanced PB-sleep after 5HTP (vide supra). Because 5HT plays an important role in the production of sleep (Jouvet, 1972b) the increase in 5HT produced in many brain areas by 5HTP might be expected to prolong PB sleep throughout the 24 hour period. The question to be asked then is why was there no prolongation at 1800 hours?

The 5HTP temperature pattern at 1200 hours is significantly lower than control (between 5-95 minutes, $P < 0.001$), with absolute differences ranging between 1.62 - 3.22°C. (Table 20, Fig. 48). Significant decreases occur at 1800 hours at the 15, 25 and 65 minutes measurements (each, $P < 0.05$). There are also significant differences between 5HTP-treated and control groups at 2400 hours, at 25-55 minutes (each, $P < 0.001$) and at 65-75 minutes (each, $P < 0.05$). The absolute reductions in body temperature are less than at 1200 hours, ranging from 0.88 - 1.08°C. There are no significant differences between temperatures for 5HTP-treated and control groups at 0600 hours and

most of the measurements at 1800 hours. The prolongation of PB-sleep at 1200 and 2400 hours could be explained in part, on the basis of reduced body temperatures at these times. Barofsky and Feldstein (1970) found that 5HTP (50-200 mg/kg, IP) produced hypothermia. They attributed this effect to the formation of "tryptophols" (tryptophol, 5-methoxytryptophol and 5-hydroxytryptophol), which can lower body temperature in a dose-dependent manner. Pretreatment with a MAO inhibitor prevented the 5HTP-induced hypothermia, suggesting that the "tryptophol" metabolite, rather than 5HT, is the active hypothermic agent. Feldstein et al. (1970) and Taborsky (1971) reported that 5-hydroxytryptophol and 5-methoxytryptophol induce sleep as well.

The effects of several 5HT-related compounds were tested for their ability to prolong PB-sleep in mice. 5HIAA and 5-methoxy indoleacetic acid were without significant effect, while 5-methoxytryptamine and N-acetyl-5HT were active. Rectal temperatures were not determined, but it is probable that these compounds share some of the hypothermic properties of the tryptophols. These studies indicate that the activity of metabolites should be considered in any analysis of the effects of biogenic amines.

D. Drugs Affecting the Histamine System

1. Histidine

Hd significantly prolonged PB-sleep over controls in the following instances: Hd100 dose at 0600, 1200 and 2400 hours; Hd500 dose at 1800 hours; and at the Hd1000 dose at the four experimental time points (Table 6 , Figs. 18-22). PB-sleep is reduced significantly with the Hd500 dose at 2400 hours.

PB-sleep in the Hd-pretreated groups follows a circadian pattern, with peaks at 1200 hours (Hd100) and at 1800 hours (Hd500 and Hd1000), during the normal sleep period for rats. Monnier and Hatt (1969) reported that H activates the CNS reflexly when administered systemically and by a direct action on the reticulo-thalamic activating systems after intraventricular injection. Inferentially, Friedman and Walker (1969) suggested a waking effect of H as the result of finding that H concentrations in the CN are maximal during the normal waking period of rats. The results of the present study do not support such a role for H in PB-sleep, although H might be active in the sleep-wakefulness cycle of physiological sleep.

Although brain H levels were not measured in the present study there is abundant evidence to show that Hd elevates H in the brain (Taylor and Snyder, 1972; Schwartz et al., 1972). Taylor and Snyder (1972) found in the mouse, that H was significantly elevated in the hypothalamus, MB-thalamus-hippocampus, cortex, striatum, medulla-pons and cerebellum, one hour after Hd (1000 mg/kg, IP). PB (60 mg/kg, IP) alone, one hour prior to determination, produced a significant elevation (26%) of whole mouse brain H.

After Hd500, DA in the brain stem decreases and hypothalamic DA increases at 0600 hours, while MB 5HT is elevated. At 1200 hours, brain stem 5HT is reduced. At 2400 hours there are elevations in MB DA and hypothalamic 5HT. At 1800 hours, when the peak prolongation of PB-sleep occurs, with the Hd500 and Hd1000 doses, there are no significant changes in the levels of any of the amines studied, in any of the brain parts examined, suggesting that these amine changes are of minor importance in the prolongation of PB-sleep.

The changes that do occur at other times tend to balance out. Taylor and Snyder (1972) found no significant alterations in whole mouse brain NE, DA or acidic and neutral indoles, one hour after Hd (1000 mg/kg, IP) but did find a significant decrease (35%) in 5HT.

There is no simple explanation forthcoming for the significant decrease in sleep at 2400 hours compared to control, produced with the Hd 500 dose.

There are no significant alterations in body temperature at 0600 and 1200 hours of rats pretreated with Hd100 (Tables 19, 20, Figs. 47, 48). Unfortunately this aspect of the study is incomplete, but the formation of H from Hd, might be expected to produce hypothermia according to the findings of Packman et al. (1953). Shaw (1971) reported that in mice, H (intraventricular, 1-10 ug) or 125 mg/kg H (SC) produced hypothermia. Packman et al. (1953) obtained hypothermia in rats, with several doses of H (SC).

The prolongation of PB-sleep by Hd appears to be unrelated to changes in levels of CNS monoamines studied. Displacement of PB binding to serum proteins is probably not involved because Hd does not bind to proteins to a significant extent (McManemy et al., 1961). The effects might be due to a direct depressant effect of H, which prolongs PB-sleep in a pattern following the normal sleep cycle of the rat. Phillis et al. (1968) described depressant effects of iontophoretic H on cortical neurones, which could be antagonized by antihistamines. The greater depression at 0600 hours by the Hd1000 as compared to Hd500 dose could be explained by the higher levels of H produced. Although they found that iontophoretic methyl H had similar effects on cortical neurons as H, Taylor and Snyder (1972) showed no significant trans-

formation of ^3H -methyl H from ^3H -histamine formed from ^3H -histidine in rat brain. This argues against H metabolites being responsible for some of these effects. The overall result of increasing H in many brain areas by Hd pre-treatment would probably favor depression, facilitating the action of PB.

Several investigators have reported that the H levels in neonatal rat brain are five times higher than in adults (Pearce and Schamberg, 1969; Ronnberg and Schwartz, 1969). Snyder (1972) found that telencephalon and diencephalon H exhibit a peak 5-10 days after birth, with a decline to adult levels by day 17. The increased H might be related to the maturing CNS, since high H levels occur at times of rapid neuronal growth. However, this elevated CNS H might also be related to the increased amount of time new-born animals sleep, if H has the depressant effects mentioned above.

2. Decaborane

DB significantly prolonged PB sleep. The pattern is relatively flat, indicating that a ceiling effect has been reached. DB was used to deplete brain H, but also affected levels of NE, DA and 5HT. NE concentrations are significantly depleted in almost all tissues and at all experimental times (14/16). The extent of depletion in the hypothalamus (76.3 - 78.7%) is greater than that in the MB (58.1 - 61.1%) or brain stem (54.8 - 65.6%).

Merritt et al. (1964) reported that rat brain NE was depleted 63%, 24 hours after DB (15 mg/kg, IP). In this study 5HT is also depleted in 9/16 cases. Both of these amines are involved in central temperature regulation (Bligh and Cottle, 1966; Myers and Yaksh, 1969). Their depletion might be expected to disrupt mechanisms involved in heat production and loss.

DB inhibits decarboxylase activity, in vivo and in vitro, but also increases the rate of release of amines (Merritt and Sulkowski, 1967). The decarboxylase inhibition accounts for the depletion of NE and 5HT. Medina et al. (1969) demonstrated that DB (15 mg/kg, IP) depleted whole rat brain H maximally 24 hours after injection, by inhibiting Hd decarboxylase. H was depleted about 60%, suggesting other, resistant pools of H exist. The depletion of H, by DB, might be expected to increase circulating H significantly, with a resultant H-induced vasodilatation which would further the heat loss (vide supra discussion of H-induced hypothermia; Packman et al., 1953).

The elevated DA in the MB at 0600 and 1800 hours and at 0600 hours in the hypothalamus might be expected to favor a waking effect. At 0600 and 2400 hours DA is reduced in the brain stem. Brain stem 5HT concentrations are also reduced. The H-depleting effects of DB fit with the arousal actions of H described earlier, in that the decrease in the levels of an alerting substance would tend to facilitate sleep. Sleep after Hd is augmented, partly because high concentrations of H exert a depressant effect. Hd pretreatment has fewer effects on other brain monoamines, while DB produced many, which contribute to the overall prolongation of PB-sleep obtained. A more specific H-depletor might better reveal the exact involvement of H in sleep. NE-depleting actions of DB favor sleep, as do the effects interfering with temperature regulation.

Since DB inhibits decarboxylating enzymes, it might also inhibit the liver oxidizing enzyme system, responsible for metabolizing PB. Administration of DB 24 hours prior to PB would allow sufficient time for enzyme inhi-

bition to occur, which would result in a reduced PB metabolism and therefore a longer duration of sleep.

3. Diphenhydramine

DPH significantly prolonged PB-sleep at all times examined and did so in a circadian manner (Table 6, Figs. 18-21). The effect is minimal at 1800 hours, and maximal at 2400-0600 hours. DPH was used because in its clinical use it frequently produces sedation. The maximum effect on PB-sleep occurs during the dark phase, unlike in the PB pattern, in which the peak occurs at 1200 hours, during the sleep period of rats. Clearly, the pattern does not follow that of sedatives, which produces longer sleep duration during the normal sleep period of animals.

DPH effects on amine levels are not striking (Table 16, Fig. 41). The increase of MB 5HT at 0600 hours favors enhanced sleep because this amine has a significant function in the raphe system. The reduction in hypothalamic NE also contributed to this effect. This period corresponds to a time of maximal enhancement of PB-sleep by DPH. The elevation of CN 5HT at 1200 hours is the only significant amine change at a time which also favors sleep. However, 5HT is of secondary importance in the CN. At 1200 hours, DPH-sleep duration is prolonged to an intermediate extent. The lowering of MB NE at 1800 hours would favor enhanced sleep, which does occur at 1800 hours, although at this time DPH prolongs PB-sleep to the least extent. There are no significant amine changes at 2400 hours, although the prolongation of PB-sleep returns to its maximal level.

The effect of antihistamines (beyond antagonizing H) are numerous and depend on structural features of molecular types. Most antihistamines have a dual central action: the initial depression seen at therapeutic doses shifts to EEG activation and seizures, if the dose is increased sufficiently. Douglas (1970) states that there is not yet a clear-cut explanation of the diverse actions of antihistamines, but a partial explanation might involve their anticholinergic effects or direct antagonism of CNS H.

Green and Erickson (1964) reported that DPH (50 mg/kg, oral, one hour prior to sacrifice) reduces whole rat brain H. DPH (15 mg/kg, SC, 2 hours prior to sacrifice) increases guinea pig brain stem and cerebellum H (Green and Erickson, 1967). In whole mouse brain, Taylor and Snyder (1972) found no change in H, 4 hours after DPH (20 mg/kg, IP). Boissier et al. (1970) reported that DPH (50 mg/kg, IP, one hour prior to sacrifice) did not alter H levels in whole rat brain or in several rat brain areas, including hypothalamus, CN and thalamus.

Winter (1948) demonstrated in mice, the prolongation of HB (100 mg/kg, IP) or PB (50 mg/kg, IP) sleep, by DPH and other antihistamines (10 mg/kg, IP). No explanation of mechanism involved was given, except for citing its clinical sedative side effects. The results of the present study clearly corroborate this observation, but extend it by demonstrating that this prolongation varies significantly with the time of day.

There are several other factors which could influence the duration of PB-sleep after DPH. The antihistamine might prolong sleep by blocking the alerting activity of H in the CNS, reported by Monnier and Hatt (1969) or

Friedman and Walker (1969). DPH has anticholinergic effects (Douglas, 1970), which could antagonize central ACh, which, according to Friedman and Walker (1972) is maximal in the rat when these animals are in an alert state. Antagonizing ACh at times when it is normally maximal (i.e., 2400-0600 hours) might account for the longer duration of PB-sleep, because of the association of ACh with states of central excitation. Packman et al. (1953) reported that in mice, DPH produced significant and maximal depression of body temperature one hour after its administration. Such a hypothermia could facilitate the prolongation of PB-sleep.

Antihistamines also affect neural transmission and neuromuscular function. Abdel-Aziz and Bakry (1973) reported that DPH blocked transmission in a rat phrenic nerve-diaphragm preparation and reduced the action of ACh on the frog rectus muscle. This blocking action might delay the recovery of the righting reflex in PB-treated animals. A local anesthetic effect for antihistamines was also reported by Douglas (1970).

The basis of the circadian differences in PB-sleep with DPH might be due to its monoamine changes, as well as the multiple effects just described, interpreted according to the continuum in the development of catalepsy by sedative drugs elaborated by Winters et al. (1967; 1972). Faingold and Berry (1972) demonstrated that IV infusion of DPH in cats (3.0 mg/kg/minute) initially produced synchronized cortical activity, followed by an abrupt change to a desynchronized pattern approximately 5 minutes prior to the cortical seizure. The dose required to produce a seizure was 37 mg/kg. The dose utilized in the present study (50 mg/kg) is greater than that required for

seizure in the cat, although in the cat administration was IV and differences in species sensitivity must be considered. The use of PB in this study could obscure the later desynchronizing phase.

E. Gamma-butyrolactone

GBL (350 mg/kg, IP) produced a maximum duration of sleep at 1800 hours and a minimum at 0600 hours (Table 17, Fig. 44, 45), a pattern similar to that for PB-treated animals, in which the peak duration of sleep occurs during the normal sleep phase for rats. Significant increases over control for 5HT levels occur in the MB at 1200 and 1800 hours and in the brain stem at 1200 hours. These areas contain the raphe system. Significant elevations in CN DA at 1200 hours and hypothalamic DA at 1800 hours, and CN DA at 1800 and 2400 hours (with borderline significance: P about 0.05) favor waking although the possibility of structurally-related depressant amines cannot be discounted. A balance between these effects must exist, since the GBL pattern follows both normal and PB-sleep in a facilitory manner.

Winters et al. (1967; 1972) have criticized designating the effects of GBL (or GHB) as "sleep". They found that GHB produced the following progression of effects: hypersynchrony, spiking with polyphasic bursts (7-15 Hz) and periods of electrical silence and hypersynchronous high frequency generalized seizures. They claim that this is a "catatonic" state, or a "generalized non-convulsive epilepsy or seizure". They cite several other drugs used for anesthesia, which follow this GHB continuum, including ether, alpha-chlorolose and phencyclidine. The present study demonstrates a diurnal

pattern for GBL-induced sleep which is similar to that of PB. At this dose the hypersynchronizing effects described by Winters and coworkers might predominate and induce sleep, while facilitating normal sleep. If this is not the case, and the results of GBL administration is to produce catatonia, the present data would indicate that such a catatonia follows a circadian pattern.

Roth and Suhr (1970) found that GBL (3 x 500 mg/kg, IP for three hours) significantly elevated whole rat brain DA and 5HT, without any altering brain NE. (The results of the present study are similar although a considerably lower dose was used). They found that GBL (or GHB) specifically blocks DA release without affecting synthesis, but did not elucidate the mechanism of its CNS depressant action. They did find a good correlation between the sedative action and the increase in brain DA. Bustos et al. (1972) indicated that the blockade of DA release is probably due to the reduced impulse flow in the nigro-neostriatal pathway, but did not elaborate on how this might affect sleep. In the present study brain DA levels increased only infrequently, therefore it cannot be directly related to the circadian pattern for the duration of GBL-induced sleep. Giarman and Schmidt (1963) reported that GBL increased ACh in whole mouse brain and rat MB and brain stem. Since microinjection of ACh into several areas of the RF results in behavioral and EEG sleep (Courville et al., 1962; vide supra), this effect of GBL on brain ACh might be related to its depressant actions.

F. Anticholinesterases

Physostigmine in mice increases PB-sleep significantly only at 2400 hours (Table 7 , Fig. 23). The pattern is flat. Neostigmine significantly prolongs PB-sleep at 0600, 1200 and 2400 hours, while significance is borderline at 1800 hours. The pattern has a peak at 0600 hours and a trough at 2400 hours. Since neostigmine is a quaternary compound and does not cross the BBB, it must exert its effects peripherally by some indirect mechanism.

Neostigmine inhibits cholinesterase, resulting in increased ACh at cholinergic sites. Its effects at the neuromuscular junction, spinal cord and ganglia are based on a combination of its cholinesterase inhibition and direct effects, which can result in muscle paralysis. Friedman and Walker (1972) demonstrated that rat MB and CN ACh levels were maximal at 2400 hours (and minimal for MB at 1200 hours and at 1800 hours for CN). They found that in mice, the neostigmine toxicity pattern, which was maximal at 0600 hours, and minimal at 1800 hours resembles the pattern of the duration of PB-sleep in mice after neostigmine. The LD50 of neostigmine ranged from 0.23 - 0.36 mg/kg (IP) during the 24-hour period. The dose utilized in the present study (0.30 mg/kg, IP) is in the middle of this circadian LD50 range. The peak prolongation of PB-sleep at 0600 hours might be due to an interaction of PB with the toxic effects of neostigmine at various pharmacological sites.

VI. SUMMARY

This study was undertaken in order to clarify the influence of some CNS amines on the narcosis produced by a classic hypnotic agent, pentobarbital and to a lesser extent, by another type of soporific agent, gamma-butyrolactone (GBL). It was prompted by a number of earlier investigations, including those from our laboratory, which reported significant variations over a 24-hour period in the duration of drug-induced sleep, in the concentrations of biogenic amines in whole brain and portions of the brain. This study required a consideration of the normal circadian fluctuation of biogenic amines in various brain regions and the effects of pretreatment with various precursors, depletors, inhibitors of synthesis or metabolism, and other drugs related to biogenic amines, with respect to the duration of drug-induced sleep. An attempt to correlate alterations in brain amine levels in several brain areas with sleep duration seemed warranted. Since any drug or compound capable of changing body temperature presumably could alter the time-course of PB-sleep by influencing such activities as metabolism, an examination of temperature effects was also undertaken.

The experimental animals (adult male rats and mice) were adapted under stable laboratory conditions to an automatically-timed light-dark cycle (0800-2000 hours light) for at least three weeks prior to use. The onset and duration of sleep, measured by the loss and recovery of the righting reflex, were determined every six hours (at 0600, 1200, 1800 and 2400 hours), after IP doses of PB (50mg/kg) or GBL (350mg/kg). Pretreatment regimens were instituted at a sufficient time prior to the administration of the hypnotic so that the levels of the amine(s) under examination might be changed. Pre-

treated and vehicle-injected control animals were used to determine alterations in sleeping time. Similarly treated animals, sacrificed midway through the previously-determined sleep period were used for the spectrophotofluorometric determinations of biogenic amine levels in the midbrain, hypothalamus, caudate nucleus and brain stem. The duration of sleep in control rats after PB, 50mg/kg, IP, ranged from a low of 101.3 ± 8.9 minutes at 2400 hours during the dark phase of the illumination cycle, to 119.5 ± 9.7 minutes, at 1200 hours, during the light phase. Peak and trough values are significantly different ($P < 0.01$) and correlate inversely with rectal temperature, which peaks at 2400 hours, when these nocturnal animals are most active and troughs at 1200 hours, when they are quiescent. As a generalization, a majority of the treatment regimens resulted in some prolongation of PB-sleep. The following types of modified PB sleeping patterns were obtained with the pretreatment regimens used:

Type I: a normal diurnal or circadian pattern, in which the peak occurs during the usual sleeping period. In rats and mice this is during the light phase of the illumination cycle.

Type II: a relatively flat pattern indicating that the system was responding maximally to a particular treatment.

Type III: a relatively flat pattern in which one point departs significantly from the overall trend.

Type IV: an inverted diurnal or circadian pattern, in which peak effects are observed during the dark phase of the illumination cycle.

Type V: a bimodal or ultradian pattern in which a peak and trough occur in the light as well as in the dark phase of the illumination cycle.

Treatments significantly prolonging PB-sleep in a Type I circadian fashion include pCPA (a 5HT depletor), histidine (a precursor of histamine) and melatonin (a naturally-occurring derivative of 5HT).

pCPA administered in 100mg/kg doses 72, 48 and 24 hours prior to PB significantly prolonged PB-sleep at every experimental time point ($P < 0.001$). The increases ranged from 19.5% at 0600 hours to 49.8% at 1800 hours. The peak duration at 1800 hours differs significantly from the trough at 0600 hours ($P < 0.01$).

Histidine was administered IP one hour prior to PB at three different dose levels. At 100mg/kg, histidine significantly prolonged sleep from 15.6 to 22.1% at 0600, 1200 and 2400 hours, but not at 1800 hours. At 500mg/kg, PB-sleep increased significantly at 1800 hours by 47.6% ($P < 0.001$), but decreased significantly at 2400 hours by 13.4% ($P < 0.05$). At 1000mg/kg, PB-sleep is significantly prolonged over the entire 24-hour period, from a low of 13.1% at 2400 hours to a high of 54.1% at 1800 hours.

Melatonin, given concomitantly with PB potentiates sleep in a dose-dependent manner. Sleep is significantly prolonged (from $P < 0.05$ to < 0.001) by 50mg/kg at every time point and at every time point except 0600 hours with 25mg/kg. Sleep is prolonged by 20.0 to 30.4% at the lower dose and by 29.5 to 66.6% at the higher dose. There is a shift of the peak effect with 25mg/kg from 1200 hours to 0600 hours with 50mg/kg. The pattern with the M50 dose therefore must be categorized as a Type IV PB-sleep pattern.

Highly significant potentiation of PB-sleep ($P < 0.001$) was achieved after pretreatment with DOPA-pargyline (a precursor amine plus a MAOI) and decaborane (an inhibitor of histamine synthesis). These patterns were Type

II. DL-DOPA (500mg/kg, IP) and pargyline (6.0mg/kg, IP) administered one hour and 30 minutes, respectively, prior to PB, increased the duration of sleep from 103% at 0600 hours to 146.3% at 2400 hours. Decaborane (15.0mg/kg, IP) administered 24 hours before PB prolonged sleep at the subsequent 24-hour period from 69.1% at 1200 hours to 114.8% at 2400 hours.

Type III PB-sleep patterns were obtained with tryptophan (a secondary precursor of 5HT) and 5HTP (the immediate precursor of 5HT). Tryptophan (500 mg/kg, IP) administered one hour prior to PB, potentiated sleep significantly ($P < 0.001$) from 32.0% at 1200 hours to 59.1% at 2400 hours. Sleeping time was significantly lower at 2400 hours than at 1800 hours ($P < 0.05$). After 5HTP (100mg/kg, IP) administered one hour before PB, sleep was significantly prolonged (except for the measurement at 1800 hours) over control at 0600 ($P < 0.05$), 1200 ($P < 0.001$), and 2400 ($P < 0.001$) hours from 13.7 to 34.7%. The duration of sleep at 1800 hours was significantly lower than at 1200 hours ($P < 0.01$).

A Type V bimodal sleep pattern was obtained after pretreatment with AMPT (200mg/kg, administered IP 4 hours before PB). The two significant peaks of 19.8% and 75% above control occurred at 1200 ($P < 0.05$) and 2400 ($P < 0.001$) hours, respectively. The two troughs at 0600 and 1800 hours were not significantly different from controls.

Diphenhydramine (50mg/kg, administered one hour prior to PB) exhibited a Type IV pattern with the peak occurring in the dark phase of the illumination cycle. The significant increases in sleep ($P < 0.001$ at each time point) ranged from 62.7% at 1800 hours to 178.1% at 2400 hours. Peak and trough differed significantly from each other.

A tertiary anticholinesterase, physostigmine and a quaternary anticholinesterase, neostigmine were examined in mice for their effects on PB-sleep. Both were administered at a dose of 0.3mg/kg, IP, one hour prior to PB (50mg/kg, IP). The duration of sleep in control mice ranged from 26.3 ± 18.3 minutes at 2400 hours to 41.7 ± 26.0 minutes at 1800 hours. A diurnal rhythm was not obtained in these controls because of the variability of the data, although there is the suggestion of a peak at 1800 hours. Physostigmine prolonged sleep at each time point but only at 2400 hours is an increase of 71.8% significant ($P < 0.05$). On the other hand, neostigmine prolongs sleep significantly at each time point (except at 1800 hours, where significance is borderline) from 67.6% ($P < 0.01$) at 1200 hours to 105.2% ($P < 0.001$) at 0600 hours.

The effect on sleep of compounds structurally-related to 5HT were administered to mice at a dose of 20mg/kg, IP, one hour prior to PB at a single time point (1500 hours), favorable to eliciting a hypnotic effect. Significant prolongation of sleep occurred with 5-methoxytryptamine (74.6%; $P < 0.02$) and N-acetyl-5HT (105.4%; $P < 0.025$). 5HIAA and 5-methoxyindole acetic acid were without significant effect.

The duration of sleep produced in rats by 350mg/kg, IP, of gamma-butyrolactone varies with the time of day it is administered. It ranges from 68.2 ± 9.3 minutes at 0600 hours to 110.3 ± 16.3 minutes at 1800 hours. The difference between peak and trough is statistically significant ($P < 0.05$). Pretreatment with histidine 100 and 500mg/kg produced augmented sleep only at 1800 hours, with the higher dose. The difference was significant at $P < 0.05$.

Pretreatment with DOPA-pargyline significantly elevated DA levels in various brain parts above control throughout the 24-hour period, in 15/16 instances. On the other hand, NE levels were elevated in only 4/16 instances (in CN at 0600, 1800 and 2400 hours; in the brain stem at 0600 hours), while 5HT decreased in 2/16 instances (in the brain stem at 1800 and 2400 hours). In 9 of 12 amine patterns a fluctuating pattern was obtained over the 24-hour period of measurement. The remaining 3 amine patterns, viz., MB-5HT, CN DA and brain stem NE, were unvarying. The fall in rectal temperature after PB is significantly reduced by DOPA-pargyline pretreatment. Prolongation of sleep with DOPA-pargyline correlates best with the increase in DA levels. The possibility that DA might displace PB bound to plasma proteins or that DA is converted to a metabolite exerting a hypnotic effect is considered in attempting to explain the augmentation of PB-sleep by this regimen.

AMPT significantly reduced brain NE (13.6 to 62.2%) and DA (43.9 to 86.3%) levels in 14/16 and 8/16 instances, respectively, during the 24-hour period of examination. 5HT rose significantly in 3/14, fell in 1/14 and was unchanged in 10/14 instances. Sleep correlates best with the decreased levels of NE and DA. Of 12 amine patterns, 5 are unvarying and 7 display diurnal rhythms. Temperature reductions after AMPT exhibit minimal differences from PB alone and do not appear to account for the increased duration of sleep. Similarly, the effects of AMPT on the protein binding of PB are probably minor.

pCPA significantly decreased 5HT levels in all tissues throughout the 24-hour period (16/16 instances), from 40.4 to 97.0%, with only minor changes in CA levels. Body temperature reduction is probably of minor significance in the prolongation of PB-sleep. The effect of pCPA on the protein binding of

PB is unknown, but the cumulative effect could be significant. The prolongation of sleep correlates in this case with the widespread depletion of 5HT. 8 of 12 amine patterns fluctuate during the 24-hour period.

TP increased 5HT in 4/16 instances (40.5 to 69.2%), decreased NE in 3/16 (15.7 to 48.5%) and decreased DA in 4/16 instances (29.3 to 81.0%), suggesting that the amine changes were not of major importance in lengthening sleep. However, the significant increase in hypothalamic 5HT with a concomitant fall in MB NE at 1800 hours might explain the peak effect in sleep seen at that time. Of 12 amine patterns, 10 show significant variation throughout the 24-hour period. Two other factors undoubtedly contribute to TP-augmented PB-sleep, *viz.*, the probable displacement of bound PB because TP is significantly bound to plasma protein and the marked hypothermic effect TP produces. Within five minutes of the onset of PB sleep, rectal temperature was significantly lower than control by almost 4°C. This difference was maintained for more than two hours and was greater at 1800 than 0600 hours.

The amine effects with M were studied only at the M25 dose. Only 5/48 amine levels changed from control significantly (four decreases, MB DA (43.9%), CN DA (11.8%), brain stem 5HT (46.3%) and hypothalamic DA (76.1%); 1 increase, MB 5HT (76.3%)). Temperature effects of this dose are minor and displacement of protein bound PB is improbable. This suggests that the effect might be due to a direct depressant action.

Although Hd500 significantly prolonged PB-sleep at 1800 hours, no significant changes in the levels of the amines studied occurred in any of the brain parts examined, suggesting that neither NE, DA nor 5HT play a significant role in Hd-prolongation of PB-sleep. Only 6 of 48 amine levels changed significantly from control. Four increased and two decreased at times when

sleep was not significantly different from controls. Binding and PB displacement does not seem to be involved in facilitated PB-sleep, since Hd does not bind to plasma to any extent. Two possibilities to explain the effect observed include a direct depressant effect of the elevated histamine generated by Hd and/or histamine hypothermia. Temperature studies in the rat were limited to the Hd100 dose and were not striking. 7 of 12 amine patterns after Hd500 exhibited diurnal variation during the 24-hour period.

DB, a histamine depletor, markedly reduces NE levels in 14/16 instances from 54.8 to 78.7%, an action favoring sleep. (Depletion of NE was consistently greater in the hypothalamus than in MB and brain stem.) 5HT was also depleted in 9/16 instances by 29.0 to 78.7%. On the other hand, DA increased in 7/16 cases. The latter two amine changes, it can be argued, favor waking. However, 5HT levels normally are inversely related to NE levels. When this relationship is altered sleeping time is extended. The rise in DA levels is probably not sufficient to overcome the loss of functional NE. Furthermore, the conversion of DA to a soporific metabolite cannot be discounted. Both NE and 5HT are involved in heat production and loss. The profound hypothermia produced by DB might reflect the reduced levels of these amines and their involvement in temperature regulation. Additionally, vasodilatation resulting from the mobilization of histamine by DB could contribute to heat loss and reduced metabolic activity. The degree of amine mobilization by DB suggests that it could raise the level of circulating amines and thus could interfere with PB binding. 11 of 12 amines exhibit significant diurnal variations after DB.

The very significant prolonged Type IV PB-sleep pattern after DPH does

not appear to reflect brain amine changes to any extent. Only 2/16 5HT values, 2/16 NE and no DA values were significantly different from controls. However, 8 of 12 amine patterns display diurnal changes (These are detailed in Figs. 42-43, Table 16 and Appendix B.) The DPH-augmented PB-sleep and its inverted diurnal pattern can be explained on the basis of some of the following diverse pharmacological and toxic actions of antihistamines: 1) antagonism of the CNS alerting effects of histamine, 2) hypothermia, especially during maximal metabolic activity, 3) local anesthetic, anticholinergic and neuromuscular-blocking actions, 4) time-related change in sensitivity to the toxic actions, which include, sedation and cortical seizure activity leading to catalepsy. There is no evidence available to suggest that DPH is bound to any extent by plasma proteins.

The few significant amine changes after GBL (6/48) occur during the light phase, the normal sleeping time MB 5HT increases 77.8 and 64.0% respectively, at 1200 and 1800 hours, while brain stem 5HT increased 50.0% at 1200 hours. CN DA at 1200 hours and hypothalamic DA at 1800 hours increased 41.3 and 128% respectively. The only decrease occurring is in hypothalamic NE (20.4% at 2400 hours). These data in essence corroborate the findings of Roth and Suhr (1970) obtained in whole rat brain, with a considerably higher dose. They correlated sedative effects of GBL with the increased DA levels. The effect of 5HT might supersede any other amine, since the 5HT increases occur in the raphe system, where according to Jouvet (1972b) sleep centers are located. The possibility that what is termed "GBL-sleep" is really a state of catatonia has been suggested by Winters et al. (1972). If this is true, then this catatonia follows a circadian pattern.

The question concerning the relationship of biogenic amine background to the hypnotic effect of PB can be answered in the following manner: PB sleep is augmented by raising brain levels of DA or by reducing 5HT levels or by decreasing NE levels or by combination of these effects. The amine changes and sleep effects in general display a variety of diurnal or circadian patterns, when measured over periods of 24 hours. The probability that other amines must participate in these effects is inferred from data obtained with compounds that mobilize acetylcholine and histamine. Other factors influencing the duration of PB-sleep include: hypothermic effects of compounds, (a possible alteration of the protein binding of the hypnotic) and a direct depressant effect of the compounds themselves. GBL "sleep" follows the normal circadian sleep pattern of rats. Amine changes are not extensive, but when normal sleep is expected GBL increases DA, and 5HT levels and decreases NE levels.

Appendix A: Drugs, Chemicals and Equipment

1. Drugs

N-acetyl-5-hydroxytryptamine (Sigma)
L-alpha-methyl-p-tyrosine (Merck)
DL-p-chlorophenylalanine (Sigma)
Decaborane (Alpha Inorganics)
Diphenhydramine HCl (Sigma)
DL-DOPA (DL-3,4-dihydroxyphenylalanine; K & K)
Gamma-butyrolactone (Eastman)
L-histidine (Sigma)
5-hydroxyindoleacetic acid (Sigma)
Melatonin (Sigma)
5-methoxyindoleacetic acid (Sigma)
5-methoxytryptamine
Neostigmine methylsulfate (Roche)
Pargyline (Abbott)
Pentobarbital sodium (Abbott)
L-tryptophan (Sigma)
Physostigmine salicylate (Merck)

2. Chemicals

Acetic acid (Baker)

Alumina (Grade I, Woelm)

Boric acid (Baker)

Disodium EDTA (Fisher)

n-heptanol (Eastman, Sigma)

Iodine (Baker)

Perchloric acid (Baker)

Potassium carbonate (Fisher)

Potassium iodide (Fisher)

Potassium phosphate, monobasic (Mallinkrodt)

Sodium chloride (Fisher)

Sodium phosphate, monobasic (Merck)

Sodium phosphate, dibasic (Merck)

Sodium sulfite (Baker)

Ninhydrin (Pfanstiehl)

Tricine (Calbiochem)

3. Equipment

Aminco-Bowman Spectrophotofluorometer

Beckman, Model 72 pH Meter, with electrode #39030

Christian Becker, Model EA-1 Torsion Balance

Eberbach, 2-speed shaker

IEC centrifuge (Model K)

Hythermco oven

Precision Scientific heating bath (Model 83)

Scientific Products Deluxe Mixer (vortex)

Yellow Springs Telethermometer (with probes 401 and 403)

Harvard decapitator

Paragon 24-hour timer (Model 4000-0)

Appendix B: Analyses of Amine patterns

1. Drugs affecting the NE and DA systemsa. DOPA-pargyline (Figures 28-29, Table 9)

Analyses of the 12 24-hour amine patterns in DOPA-pargyline treated animals reveals that 9 are circadian. At 1200 hours, the MB NE peak is significantly different from the values obtained at 0600 ($P < 0.02$) and 2400 hours ($P < 0.025$). MB DA maxima occur at 1800-2400 hours, while minima are at 0600-1200 hours. The concentration of MB DA at 1800 hours differs significantly from that at 0600 and 1200 hours (each at a $P < 0.001$). The DA level at 2400 hours also differs significantly from that at 0600 ($P < 0.02$) and 1200 hours ($P < 0.01$). CN NE concentrations exhibit a trough at 0600 hours, which differs significantly from 1200 and 1800 hours levels (each at $P < 0.05$). CN 5HT exhibits peak concentrations at 1200 hours, which differs significantly from 0600 hours ($P < 0.02$) and 2400 hours ($P < 0.05$) values. 5HT concentrations at 0600 and 1800 hours also differ significantly ($P < 0.05$). Brain stem DA levels peak at 1800 hours, differing significantly from 0600 and 1200 hours values, at $P < 0.01$. DA levels at 0600 and 2400 hours also differ significantly ($P < 0.05$). Brain stem 5HT levels reach a maximum at 1200 hours, which differs significantly from the values at 1800 hours ($P < 0.025$) and 2400 hours ($P < 0.005$). Hypothalamic DA levels peak at 1800 hours, which is significantly different from the levels at 0600 ($P < 0.001$), 1200 ($P < 0.001$) and 2400 hours ($P < 0.02$). There are also significant differences between the following points: 0600 vs. 1200 hours ($P < 0.005$), 0600 vs. 2400 hours ($P < 0.05$). Hypothalamic 5HT levels exhibit a peak at 1800 hours and a trough at

0600 hours, which differ significantly ($P < 0.05$). The trough at 0600 hours also differs significantly from 1200 and 2400 (both $P < 0.01$).

b. Alpha-methyl-p-tyrosine (Table 10, Figs. 30, 31)

In AMPT-treated animals, 9 of 12 amine patterns exhibit diurnal fluctuations. MB DA exhibits a significant difference between levels at 1200 and 2400 hours ($P < 0.02$; Fig. 31). The MB 5HT pattern has a peak at 1200 hours, which differs significantly ($P < 0.01$), from the trough at 1800 hours. The peak also differs significantly from the values at 0600 hours ($P < 0.001$) and 2400 hours ($P < 0.02$). The trough at 1800 hours also differs significantly from values at 0600 hours ($P < 0.01$). CN NE levels at 0600 and 1200 hours differ significantly ($P < 0.05$). The values of CN 5HT exhibit a minimum at 2400 hours, which differs significantly from those at 0600 ($P < 0.02$) and 1200 hours ($P < 0.005$). The maximum brain stem NE concentration at 0600 hours differs significantly from the values at 1800 and 2400 hours (both $P < 0.05$). Significance is borderline at 1200 hours. Brain stem DA levels exhibit a minimum at 2400 hours, that differs significantly from the values at 1200 hours ($P < 0.02$) and 1800 hours ($P < 0.01$). The minimum brain stem 5HT concentration at 2400 hours differs significantly from that at 0600 hours ($P < 0.001$) and 1200 hours ($P < 0.01$). Hypothalamic DA levels at 0600 and 1800 hours differ significantly ($P < 0.02$).

2. Drugs Affecting the Serotonin System

a. p-Chlorophenylalanine (Table 11, Figs. 32,33)

The amine patterns of pCPA-treated animals exhibit circadian fluctuation in 9 of 12 instances. MB 5HT levels in pCPA-treated animals

exhibit a maximum at 2400 hours, which differs significantly from the levels at 0600 hours ($P < 0.005$), 1200 hours ($P < 0.005$) and 1800 hours ($P < 0.001$). CN NE decreases to a minimum at 1800 hours, which differs significantly from the values obtained at 0600 hours ($P < 0.05$), 1200 hours ($P < 0.005$) and 2400 hours ($P < 0.025$). CN DA exhibits a peak at 1800 hours, which differs significantly from the concentrations at 0600 hours ($P < 0.025$), 1200 hours ($P < 0.001$) and 2400 hours ($P < 0.005$). CN 5HT is maximal at 2400 hours, differing significantly from the value at 1200 hours ($P < 0.001$). The value at 1200 hours differs significantly from that at 1800 hours ($P < 0.01$).

Brain stem DA is minimal at 0600 hours, and differs significantly from the value at 1200 hours ($P < 0.05$). Brain stem NE is maximal at 0600 hours, differing significantly from the values at 1200 hours (P about 0.05) and 1800 hours ($P < 0.01$). Hypothalamic NE has a trough at 1800 hours, which differs significantly from the values at 0600 hours ($P < 0.05$), 1200 hours ($P < 0.02$) and 2400 hours ($P < 0.005$). The hypothalamic DA levels trough at 1800 hours and differ significantly from those at 0600 hours ($P < 0.005$) and 1200 hours ($P < 0.01$). The peak value of hypothalamic 5HT at 1200 hours, differs significantly from that at 0600 hours ($P < 0.02$) and 2400 hours ($P < 0.05$). The 5HT value at 0600 hours also differs significantly from that at 1800 hours ($P < 0.01$) and 2400 hours ($P < 0.001$).

b. Tryptophan (Figures 34-35, Table 12)

TP (500mg/kg, IP) treated animals exhibited circadian fluctuations of 24-hour amine levels in 10 of 12 cases. The pattern of MB DA in treated animals exhibits a minimum concentration at 1200 hours, which differs significantly from the values at 0600 and 2400 hours (both $P < 0.01$). MB 5HT has a

peak at 1800 hours and trough at 2400 hours. These differ significantly ($P < 0.025$). The peak at 1800 hours also differs significantly from the values at 1200 ($P < 0.02$) and 2400 hours ($P < 0.01$). CN NE exhibits a minimal concentration at 1200 hours, which differs significantly from the value at 2400 hours ($P < 0.01$). CN DA concentration is minimal at 0600 hours, and differs significantly from the peak level at 1800 hours ($P < 0.001$). CN 5HT levels describe a similar pattern with a trough at 0600 hours, that differs significantly from the 2400 hours values ($P < 0.05$). The brain stem NE pattern is maximal at 1800 hours, and differs significantly from the values at 1200 hours ($P < 0.02$) and 2400 hours ($P < 0.05$). The brain stem DA pattern has a trough at 1200 hours, which differs significantly from the concentrations at 0600, 1800 and 2400 hours (all $P < 0.001$). The minimum brain stem 5HT levels at 0600 hours differs significantly from that at 1200 hours ($P < 0.01$). Hypothalamic DA levels are minimal at 2400 hours, and differ significantly from the value at 1200 hours ($P < 0.005$). Hypothalamic 5HT exhibits peak levels at 1800 hours and trough values at 0600 hours, which differ significantly from each other ($P < 0.005$). The peak value also differs significantly from that at 1200 and 2400 hours (both $P < 0.001$).

c. Melatonin(Figures 36-37, Table 13)

In M-treated animals, amine patterns fluctuated in a diurnal manner in 8 out of 12 instances. The pattern of MB DA has a minimum at 0600 hours, that differs significantly from the peak concentration at 1800 hours ($P < 0.001$). MB 5HT levels are maximal at 1200-1800 hours. The value of 5HT at 0600 hours differs significantly from that at 1200 ($P < 0.05$) and 1800 hours ($P < 0.005$) and the value at 2400 hours also differs significantly from 1200

and 1800 hours (each at $P < 0.01$). CN NE is minimal at 1200 hours and is significantly lower than the value at 1800 hours ($P < 0.005$). CN 5HT concentration also exhibits a minimum at 1200 hours, which differs significantly from the levels at 0600 hours ($P < 0.01$), 1800 hours ($P < 0.02$) and 2400 hours ($P < 0.05$).

Brain stem DA levels are maximal at 0600 hours, and significantly higher than the minimum level at 2400 hours ($P < 0.01$). The peak concentration of brain stem 5HT at 1800 hours is significantly different from levels at 1200 and 2400 hours (both $P < 0.005$). Hypothalamic DA levels exhibit a maximum at 2400 hours and a minimum at 1200 hours, which differ significantly from each other ($P < 0.005$). The trough value at 1200 hours also differs significantly from the levels at 0600 hours ($P < 0.001$) and 1800 hours ($P < 0.05$). Hypothalamic 5HT concentrations are maximal at 0600-1200 hours and minimal at 2400 hours. The trough differs significantly from 0600 ($P < 0.01$) and 1200 hours ($P < 0.001$). There is also a significant difference between the levels at 1200 and 1800 hours ($P < 0.005$).

3. Drugs Affecting the Histamine System

a. L-Histidine (Figures 38-39, Table 14)

Amine levels after Hd500 were altered in a circadian manner in 7 of 12 instances. The 24-hour pattern of MB NE levels reaches a maximum at 2400 hours and differs significantly from the levels at 0600 hours ($P < 0.05$). MB DA levels are minimal at 1800 hours and maximal at 2400 hours, and differ significantly from each other ($P < 0.001$). The trough value at 1800 hours is significantly lower than that at 1200 hours ($P < 0.01$), while the peak level at 2400 hours differs significantly from that at 1200 hours ($P < 0.025$). CN NE

concentration exhibits a peak at 0600 hours, which is significantly greater than the values obtained at 1200 hours ($P < 0.005$), 1800 ($P < 0.001$) and 2400 hours ($P < 0.01$). Brain stem DA and 5HT levels differ significantly between 0600 and 2400 hours ($P < 0.05$). The difference in brain stem DA levels between 0600 and 1200 hours is significant ($P < 0.05$). The difference in hypothalamic NE levels between 0600 and 2400 hours is also significant ($P < 0.05$). Hypothalamic DA levels are maximal at 0600 hours and differ significantly from 1200 hours ($P < 0.005$) and 1800 hours ($P < 0.001$).

b. Decaborane (Figures 40-41, Table 15)

DB-treated animals had brain amine patterns that showed diurnal fluctuations for 11 of 12 instances. MB NE levels exhibit a minimum at 2400 hours, which differs significantly from levels at 0600 and 1800 hours (both $P < 0.01$). MB DA is maximal at 0600 hours, differing significantly from the values at 1200 hours ($P < 0.005$), 1800 hours ($P < 0.05$) and 2400 hours ($P < 0.01$). MB 5HT concentration is maximal at 1200 hours, and differs significantly from that at 1800 and 2400 hours (both $P < 0.005$). The 5HT value at 2400 hours also differs significantly from that at 1800 hours ($P < 0.05$). CN NE concentrations are minimal at 2400 hours and differs significantly from the value obtained at 1800 hours ($P < 0.05$). The pattern of CN DA exhibits a minimum at 0600 hours, which differs significantly from the values obtained at 1200, 1800 and 2400 hours (each at $P < 0.001$). CN 5HT levels are minimal at 2400 hours, and differ significantly from the level at 1200 hours. The 5HT values at 1200 and 1800 hours differ significantly ($P < 0.001$).

The brain stem DA pattern has a minimum at 1200 hours, which differs significantly from the values at 0600 hours ($P < 0.05$), 1800 hours ($P < 0.01$)

and 2400 hours ($P < 0.001$). Brain stem levels of 5HT are maximal at 1200 hours and differ significantly from the values obtained at 0600 hours ($P < 0.025$), 1800 hours ($P < 0.05$) and 2400 hours ($P < 0.02$). Hypothalamic NE levels reach a minimum at 2400 hours, differing significantly from that at 1200 hours ($P < 0.025$) and 1800 hours ($P < 0.01$). Hypothalamic DA concentrations are maximal at 0600 hours, differing significantly from the values at 1200, 1800 and 2400 hours (each at $P < 0.001$). Hypothalamic 5HT is minimal at 2400 hours, differing significantly from the values at 0600 hours ($P < 0.005$) and 1200 hours ($P < 0.02$). The maximum at 0600 hours differs significantly from that at 1200 and 1800 hours (each at $P < 0.005$).

c. Diphenhydramine (Table 16, Figs. 42, 43)

DPH-treated animals had brain amines patterns that showed diurnal variations in 8 of 12 instances. The 24-hour pattern of MB NE has a minimum at 2400 hours, which differs significantly from the values at 0600 hours ($P < 0.005$) and 1800 hours ($P < 0.001$) (cf. Fig. 43). MB DA concentrations are also minimal at 2400 hours, and differs significantly from the levels at 0600 ($P < 0.001$), 1200 ($P < 0.02$) and 1800 hours ($P < 0.005$). There is also a significant difference between MB DA levels at 0600 and 1200 hours ($P < 0.05$). CN DA concentrations are maximal at 1200 hours, and differ significantly from 0600 ($P < 0.001$), 1800 ($P < 0.001$) and 2400 hours ($P < 0.02$). CN 5HT levels are minimal at 0600 hours, and differ significantly from 1200 and 1800 hours values (each at $P < 0.001$). There is a significant difference between brain stem NE levels at 1800 and 2400 hours ($P < 0.005$). Brain stem DA content is minimal at 2400 hours, and differs significantly from that at 1200 ($P < 0.01$) and 1800 hours ($P < 0.05$). Hypothalamic NE reaches a minimum at 2400 hours,

which is significantly lower than the values at 0600 and 1800 hours (both $P < 0.001$). Hypothalamic 5HT levels peak at 1200 hours and differ significantly from values at 0600, 1800 and 2400 hours (each at $P < 0.01$).

4. Gamma-butyrolactone (Figures 44-45, Table 17)

a. Saline controls

The 24-hour patterns for the monoamine levels in the brains of controls exhibited a number of significant diurnal fluctuations. The pattern of MB 5HT has a peak at 2400 hours and a trough at 1800 hours (Fig. 44). The trough at 1800 hours does not differ significantly from the value at 1200 hours. The peak value at 2400 hours differs significantly from the values at 0600 ($P < 0.05$), 1200 ($P < 0.005$) and 1800 hours ($P < 0.005$). There are also significant differences between the concentrations at 0600 hours and that at 1200 hours ($P < 0.01$) and 1800 hours ($P < 0.02$). Brain stem NE has a peak value at 2400 hours, which differs significantly from the values at 1200 hours ($P < 0.01$) and 1800 hours ($P < 0.005$). The pattern for brain stem DA is bimodal, with peaks at 0600 and 1800 hours, which differ significantly from each other ($P < 0.01$). The primary peak at 0600 hours is significantly greater than the value at 2400 hours ($P < 0.01$). The secondary peak at 1800 hours differs significantly from the DA concentrations at 1200 hours ($P < 0.005$) and 2400 hours ($P < 0.005$). The hypothalamic NE pattern has a peak value at 2400 hours, which differs significantly from that at 0600 hours ($P < 0.01$), but not from the values at the other times. Hypothalamic DA exhibits a diurnal pattern, with a peak at 1200 hours and trough at 0600 hours, which differ significantly ($P < 0.02$). The DA concentration at 2400 hours differs significantly from the values at 0600 hours ($P < 0.05$) and 1200 hours ($P < 0.05$). There is a significant

difference between the hypothalamic 5HT at 1200 and 2400 hours ($P < 0.02$), but not the other values.

b. GBL-treated(Figure 45, Table 17)

The MB 5HT pattern for the GBL-treated animals exhibits a peak at 2400 hours, which differs significantly from the values obtained at 0600 hours ($P < 0.02$), 1200 hours ($P < 0.005$) and 1800 hours ($P < 0.005$)(Fig. 45). The value at 1200 hours differs significantly from that at 1800 hours ($P < 0.005$). The difference between the concentrations of CN NE at 0600 and 1200 hours is of borderline significance ($0.1 > P > 0.05$). The CN 5HT pattern exhibits circadian fluctuations, with a maximum at 1800 hours and a minimum at 0600 hours, which differ significantly ($P < 0.05$). There is borderline significance for the difference between the values at 0600 and 1200 hours ($0.1 > P > 0.05$). The brain stem DA pattern for GBL-treated animals is bimodal, with peaks at 0600 and 1800 hours. The minimum DA value at 1200 hours differs significantly from concentrations at 0600 hours ($P = 0.02$), 1800 hours ($P < 0.005$) and 2400 hours ($P < 0.05$). The brain stem 5HT pattern is diurnal, with a peak at 1200 hours that differs significantly ($P < 0.02$) from the trough at 0600 hours. The trough also differs significantly from the values at 1800 hours ($P < 0.005$) and 2400 hours ($P < 0.01$). Hypothalamic DA concentrations exhibit a peak at 1800 hours that differs significantly from 0600 and 2400 hours values (each $P < 0.02$). The peak hypothalamic 5HT concentration at 2400 hours differs significantly from the values at 1200 and 1800 hours (both $P < 0.05$).

Appendix C: Additional amine studies

1. Pentobarbital at the point of awakening

The concentrations of NE, DA and 5HT in the MB, CN, brain stem and hypothalamus were determined at 0600, 1200, 1800 and 2400 hours at the time of awakening from PB-sleep (50mg/kg, IP; Table 23, Fig. 51). MB DA varies significantly during the 24-hour period. The minimal concentration at 2400 hours is significantly less than that at 0600 hours ($P < 0.05$), 1200 hours ($P < 0.01$) and 1800 hours ($P < 0.025$). CN NE concentrations, which are maximal at 2400 hours and minimal at 1800 hours, differs significantly in the following comparisons: 0600 vs 1800 hours ($P < 0.05$) and 1800 vs 2400 hours ($P < 0.05$). Brain stem NE concentrations are maximal at 2400 hours, and differ significantly from the values at 0600 hours ($P < 0.005$), 1200 hours ($P < 0.001$) and 1800 hours ($P < 0.001$). The pattern of brain stem DA exhibits a clearcut diurnal pattern with a minimum at 1800 hours and a maximum at 0600 hours. The peak differs significantly from values obtained at 1200 hours ($P < 0.05$) and 1800 hours ($P < 0.005$), but not from those at 2400 hours. The trough differs significantly from the concentration at 2400 hours ($P < 0.01$).

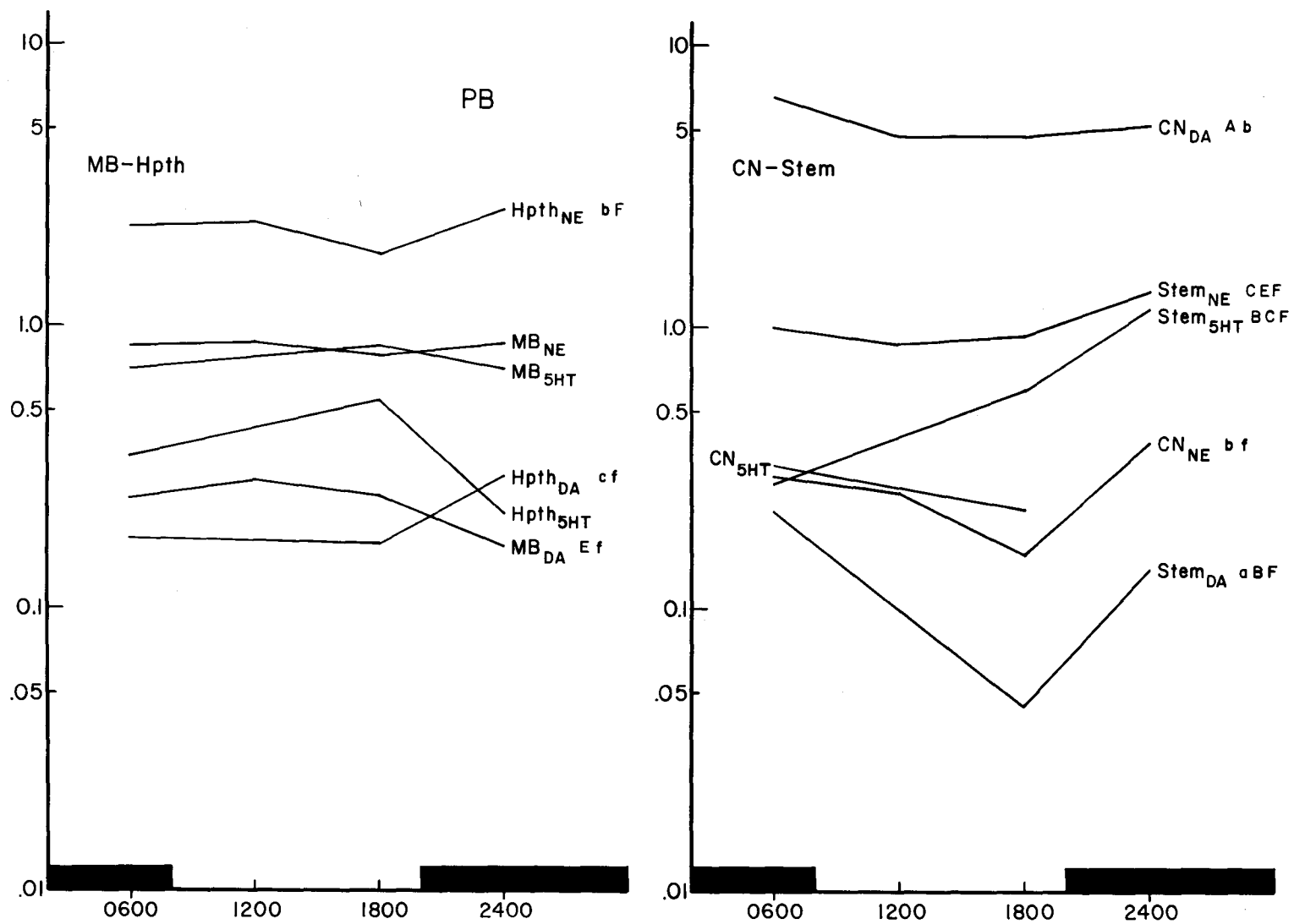
Brain stem 5HT concentrations exhibit a maximum at 2400 hours, which differs significantly from those at 0600 hours ($P < 0.001$) and 1800 hours ($P < 0.005$). There is a significant difference between the 0600 and the 1800 hours values ($P < 0.01$). Hypothalamic NE has a minimal value at 1800 hours, which differs significantly from that at 0600 hours ($P < 0.025$) and 2400 hours ($P < 0.005$). Hypothalamic DA is maximal at 2400 hours and differs significantly from the values at 0600 and 1800 hours (each $P < 0.05$). Hypothalamic 5HT concentrations during the 24-hour period are not significantly different from each other.

Table 23: CNS concentrations of NE, DA and 5HT at the time of awakening from PB (50mg/kg, IP)

	<u>0600</u>	<u>1200</u>	<u>1800</u>	<u>2400</u>
MB NE	.85+.10 (5)	.87+.16 (5)	.78+.13 (5)	.87+.10 (6)
Hpth NE	2.27+.22 (5)	2.32+.73 (5)	1.80+.28 (6)	2.61+.34 (6)
CN NE	.29+.07 (5)	.26+.06 (5)	.16+.09 (5)	.39+.11 (4)
Stem NE	.99+.10 (5)	.87+.10 (5)	.93+.06 (6)	1.33+.16 (5)
MB DA	.24+.07 (5)	.28+.07 (4)	.25+.05 (4)	.17+.04 (6)
Hpth DA	.18+.07 (5)	---	.17+.08 (6)	.29+.07 (4)
CN DA	6.48+.80 (5)	4.77+.74 (5)	4.72+1.42 (6)	5.14+1.67 (6)
Stem DA	.22+.07 (5)	.07+.02 (2)	.05+.02 (5)	.14+.05 (4)
MB 5HT	.71+.12 (5)	---	.85+.15 (5)	.71+.30 (6)
Hpth 5HT	.34+.13 (5)	---	.54+.25 (5)	.22+.04 (3)
CN 5HT	.32+.14 (5)	---	.23+.08 (5)	---
Stem 5HT	.28+.08 (4)	---	.59+.17 (6)	1.15+.24 (4)

Table indicates amine concentrations ($\mu\text{gm}/\text{gm}$) of NE, DA and 5HT in the four areas indicated in Table 9, for animals receiving PB alone. Animals were sacrificed at the time of awakening from PB. Values are means \pm standard deviation, with the number of animals used in parentheses.

Figure 51



Legend for Figure 51

The effects of pentobarbital on the CNS concentrations of NE, DA and 5HT in rats, at the time of awakening. For complete explanation of notations of figure, see legend to Figure 28.

2. Amine concentrations after 5HTP

CNS concentrations of NE, DA and 5HT were determined in animals administered 5HTP (100mg/kg, IP) one hour before PB (50mg/kg, IP), at the time of awakening from PB-sleep. The determination was made at 1200 hours, a time in which this pretreatment increased PB-sleep significantly (to 144.0 ± 15.4 minutes, $P < 0.001$). These results are summarized in the following Table.

	<u>MB</u>	<u>CN</u>	<u>Brain stem</u>	<u>Hypothalamus</u>
NE	$.80 \pm .07$ (4)	$.41 \pm .12$ (4)	$.87 \pm .02$ (4)	$1.91 \pm .07$ (4)
DA	$.33 \pm .20$ (3)	$14.70 \pm .88$ (4)	$.22 \pm .09$ (3)	$.66 \pm .25$ (4)
5HT	$1.40 \pm .20$ (4)	$.61 \pm .16$ (4)	$.85 \pm .20$ (4)	$1.46 \pm .14$ (4)

Values represent means \pm standard deviation, with the number of animals used in parentheses.

In comparison to similarly treated controls, 5HT is significantly elevated in all brain areas, except brain stem. Brain DA was elevated in all four areas, but there were no significant changes in NE levels.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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