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Sleep and Serotonin in Two Strains of *Mus musculus*[1,2]

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MITLER, M. M., H. B. COHEN, J. GRATTAN, J. DOMINIC, T. DEGUCHI, J. D. BARCHAS, W. C. DEMENT AND S. KESSLER. *Sleep and serotonin in two strains of Mus musculus.* PHARMAC. BIOCHEM. BEHAV. 1(5) 501–507, 1973.–Two strains of *Mus musculus*, C57BL/10J and BALB/CJ, were studied in an attempt to check for any naturally occurring correlation between sleep and brain serotonin. The C57BL/10J compared with the BALB/CJ had more slow wave sleep throughout the day and particularly during hours of darkness. During peak sleep periods, C57BL/10J also had more REM sleep. At three different times of the day (1200, 1600, and 0400 hr) neurochemical assays were done on brain stem and cerebral cortex for tryptophan and serotonin levels and for trytophan hydroxylase activity. An inspection of the ordering of means for strains suggested that the greater amount of slow wave sleep for C57BL/10J was paralleled by higher brain stem and cortex tryptophan levels, higher cortex tryptophan hydroxylase activity, and higher cortex serotonin levels. An inspection of temporal trends across strain and time of day suggested that slow wave sleep may vary negatively with brain stem tryptophan hydroxylase activity, brain stem serotonin level, and cortex tryptophan level. While no simple sleep-serotonin relationship obtained, because of such trends the data were interpreted as being generally consistent with the hypothesis of an active serotonergic sleep inducing mechanism in brain.

Sleep Serotonin *Mus musculus* Brain biogenic amines

RECENTLY, there has been much interest in the role that 5-hydroxytryptamine (serotonin) might play in the organization of sleep and wakefulness. Jouvet [9,10] has proposed, on the basis of brain lesion and pharmacological studies, that several groups of neurons in the brain stem raphe system, known to contain exclusively serotonin [2], constitute an active sleep-inducing system. These studies suggested that sleep reduction varies directly with the experimental reduction of brain serotonin. However, subsequent work in our laboratory has cast some doubt on the generality of the direct relation between sleep and serotonin. Dement *et al.* [5] and Dement, Mitler and Henriksen [4] have shown that sleep in the cat is profoundly reduced as an initial response to serotonin depletion by chronic parachlorophenylalanine treatment, but that later, while serotonin levels are still at their lowest values, sleep returns to as much as 70 percent of control values.

We felt, therefore, that it would be useful to begin assessment of serotonin's role in sleep without the use of lesions or drugs by comparing sleep in two strains of *Mus musculus* having differences in brain serotonin. Such a genetic approach attempts to relate behavioral differences to endogenous differences in serotonin and avoids neurophysiological operations or drug administration. Furthermore, since comparisons were intraspecific, our data were not subject to the many difficulties involved in interpreting cross-species comparisons. (See Hodos and Campbell [7] for a critical discussion of cross-species approaches.)

C57BL/10 and BALB/C strains were selected for study since earlier work has shown that C57BL/10 mice have lower levels of serotonin than do BALB/C in brain stem sections [11,15]. We now report results of sleep comparisons along with extensive data on serotonin chemistry in these two strains.

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Method

Sleep Analyses

Animals. Four C57BL/10J (C strain) and four BALB/CJ (B strain) male mice, supplied by Jackson Laboratories of Bar Harbor, Maine, were used. The mice were 70–120 days of age at the time of surgery.

Surgery. Surgical implantation of recording leads was performed under pentobarbitol sodium anesthetic following the methods outlined by Mitler and Levine [13]. While still anesthetized, each mouse was placed in an individualized cage fashioned of two translucent plastic dishpans fitted top to top. The free end of a cable, permanently fixed to the skull, was plugged into a freely turning commutator which input to an eight-channel Model III electroencephalograph in another room.

Recording procedure. Animals were housed individually and studied four at a time. Data for the entire C group were gathered first since no B mice were then available. However, all sleep data were gathered within an 11-week interval (3/29/71 to 6/10/71). Housing and recording conditions were identical for both strains. Individualized, translucent recording cages were placed in an isolated recording room having an 18–22°C temperature range and a 12L–12D lighting schedule (light onset at 0700 hr). Ample food, water, and bedding material were provided so that the mice could be left unhandled and undisturbed for the entire 13-day run.

The first nine days were allowed for recovery from surgery, acclimation to the environment, and acceptable test recordings to be obtained. At 0800 hr on the tenth day after surgery, four days of continuous electroencephalographic and electromyographic recording began.

Scoring. Each day was divided into three eight-hour segments: 0800–1600 hr, 1600–2400 hr, and 2400–0800 hr. Records were scored for the behavioral states of wakefulness, slow-wave sleep (SWS), and REM sleep. Resolution was carried to the nearest three-second division with the restriction that only state changes lasting more than six sec would be scored.

Neurochemical Analyses

Animals. Seventy-two C and 72 B male mice (70–120 days of age) supplied by Jackson Laboratories of Bar Harbor, Maine, were used. All animals were housed individually for at least three weeks and kept on the same 12L–12D light schedule as that used in our sleep comparisons. All studies were performed during a three-week period (9/21/72 to 10/7/72).

Tryptophan levels. The brain stems (from anterior hypothalamus to medulla excluding cerebellum) and cortexes of eight C and eight B mice were removed and frozen on dry ice during each of the hours: 11:30–12:30, 19:30–20:30, and 3:30–4:30 of a single day. Thus, for each strain × eight-hour period × brain segment cell, we had eight samples. Tryptophan levels were later assayed flurometrically using the method of Denckla and Dewey [6].

Tryptophan hydroxylase activity. Using the same dissection procedures and sacrifice schedules, the *in vitro* activity of tryptophan hydroxylase was measured in the brain stems and cortexes of 24 C and 24 B mice using a modification of the method of Ichiyma and associates [3,8].

Serotonin levels. The brain stems and cortexes of 24 C and 24 B mice were removed as above. Serotonin levels were flurometrically determined using the method of Barchas, Erdelyi and Angwin [1].

RESULTS AND DISCUSSION

Sleep Data

Records showed no qualitative strain difference in electroencephalograms.

The percents of each eight-hour period spent in quiet sleep and active sleep for each animal were analyzed with strain × recording day × eight-hour period analyses of variance with days and periods being treated as repeated measures. The effect of recording day was not significant for either dependent variable ($F = 0.53$ and 2.50, both $df = 3/18$, both $p > 0.08$ for quiet and active sleep respectively). Therefore data are collapsed across recording days. Entries have been transformed from the raw data to reflect percent of day instead of percent of period.

Slow-wave sleep. The C mice spent more time in SWS than did B mice ($F = 13.25$, $df = 1/6$, $p < 0.001$). Both groups showed pronounced nocturnality as indicated by SWS difference among eight-hour periods ($F = 49.62$, $df = 2/12$, $p < 0.001$). Cell contrasts, however, indicated that SWS differences were pronounced only for 1600–2400 hr and 2400–0800 hr. (For such contrasts the two-tailed critical values for $t_{\alpha=0.025}$ and $t_{\alpha=0.01}$ were 2.23 and 2.76 respectively computed from the formula of Winer [19, p. 324]. The t for 0800–1600 hr, 1600–2400 hr and 2400–0800 hr were 0.05, $p > 0.05$; 2.41, $p < 0.025$; and 2.93, $p < 0.01$, respectively.)

REM sleep. With respect to percent of time spent in REM sleep (Table 2), the strains did not differ significantly ($F = 2.02$, $df = 1/6$, $p > 0.21$). Most REM sleep occurred during the period of greatest SWS, 0800–1600 hr, as reflected by the eight-hour period effect ($F = 50.18$, $df = 2./12$, $p < 0.001$). The strain × eight-hour period interaction only approached significance ($F = 2.54$, $df = 2/12$, $p < 0.25$). However, in spite of the nonsignificant strain and strain × eight-hour period effects for percent REM sleep, we decided to contrast strains for 0800–1600 hr separately, since so little REM sleep occurred elsewhere. Such a contrast suggested that during these hours C mice had more REM sleep than did B mice ($t = 2.56$, $p < 0.025$).

It should be noted here that our results for C57BL/10J are similar to those reported by Valatx, Bugat and Jouvet [18] for the related strain C57BR/cd/Orl. Daily SWS was 43.9% (compared to our 44.63%) and daily REM sleep was 5.7% (compared to our 4.82%).

Another facet of the sleep differences is shown in Table 3 which presents REM sleep as a percentage of total sleep (100 × percent REM sleep/percent REM sleep plus percent SWS). On this measure there was no overall strain effect ($F < 1.0$). The effect of eight-hour period was pronounced ($F = 14.46$, $df = 2/12$, $p < 0.001$). Most important, however, was the strong strain × eight-hour period interaction effect ($F = 10.86$, $df = 2/12$, $p < 0.001$). This interaction is a statistical manifestation of two clear strain differences. First, from Table 3 it is clear that the percent of total sleep spent in REM sleep was greatest for C animals during 0800–1600 hr while for B animals the greatest value for this measure occurred during 1600–2400 hr. Second, it appears that the composition of C sleep has pronounced circadian variation while the composition of B sleep is relatively uniform throughout the day.

TABLE 1

MEAN (± S.D.) DAILY PERCENT OF SLOW WAVE SLEEP AS FUNCTIONS OF STRAIN AND 8-HR PERIOD

	0800–1600 hr	1600–2400 hr	2400–0800 hr	Overall
C57BL/10J	19.77 ± 0.72	10.53 ± 2.33	14.32 ± 1.57	44.63 ± 3.35
BALB/CJ	19.70 ± 1.56	6.89 ± 0.17	9.90 ± 4.12	36.49 ± 3.05

$F_{strain} = 13.25, df = 1/6, p<0.001$
$F_{8\text{-hr period}} = 49.62, df = 2/12, p<0.001$
$F_{strain \times 8\text{-hr period}} = 2.18, df = 2/12, p$ NS
$t_{0800-1600\ hr} = <1, p$ NS
$t_{1600-2400\ hr} = 2.41, p<0.025$ (computed from the formula of Winer [19, p. 324])
$t_{2400-0800\ hr} = 2.93, p<0.01$ (computed from the formula of Winer [19, p. 324])

TABLE 2

MEAN (± S.D.) DAILY PERCENT OF REM SLEEP AS FUNCTIONS OF STRAIN AND 8-HR PERIOD

	0800–1600 hr	1600–2400 hr	2400–0800 hr	Overall
C57BL/10J	2.96 ± 0.63	0.89 ± 0.21	0.98 ± 0.29	4.82 ± 0.85
BALB/CJ	2.15 ± 0.68	0.91 ± 0.36	0.81 ± 0.21	3.87 ± 1.04

$F_{strain} = 1.55, df = 1/6, p<$NS
$F_{8\text{-hr period}} = 56.49, df = 2/12, p<0.001$
$F_{strain \times 8\text{-hr period}} = 1.95, df = 2/12, p<0.25$
$t_{0800-1600\ hr} = 2.56, p<0.025$ (computed from the formula of Winer [19, p. 324])
$t_{1600-2400\ hr} = <1, p$ NS
$t_{2400-0800\ hr} = <1, p$ NS

TABLE 3

MEAN (±S.D.) PERCENT OF TOTAL SLEEP SPENT IN REM SLEEP AS FUNCTIONS OF STRAIN AND 8-HR PERIOD

	0800–1600 hr	1600–2400 hr	2400–0800 hr	Overall
C57BL/10J	13.14 ± 2.54	8.09 ± 1.74	6.27 ± 1.27	9.17 ± 1.55
BALB/CJ	9.71 ± 2.75	11.58 ± 4.43	8.27 ± 2.00	9.86 ± 2.91

$F_{strain} = 0.175, df = 1/6, p$ NS
$F_{8\text{-hr period}} = 14.46, df = 2/12, p<0.001$
$F_{strain \times 8\text{-hr period}} = 10.86, df = 2/12, p<0.001$

Sleep cycle length. We also examined sleep cycle length during 0800–1600 hr. The cycle was defined as the mean duration from one REM sleep period offset to the next REM sleep period offset, regardless of what or how many other states intervened. The strains did not differ significantly on this measure (16.7 ± 5.29 min vs. 12.79 ± 4.00 min for C and B mice respectively, F = 1.35, df = 1/6, $p>0.25$). Therefore, the mean of 14.78 ± 5.56 min is our best estimate of sleep cycle length in *Mus musculus*.

Our sleep data thus show that C mice have more daily SWS than B mice (44.63% vs. 36.49%) and that this difference is probably not attributable to differences in sleep cycle length. Our findings also suggest that while daily REM sleep did not differ significantly between strains (4.82% vs. 3.87% for C and B, respectively), C mice did show significantly more REM sleep during the eight-hour period of greatest total sleep (2.96% vs. 2.15%). Furthermore, the data indicate that the percent of total sleep spent in REM sleep is greatest during the period of greatest total sleep and varies in a clear diurnal fashion only for C mice. For B mice this variable peaks during the eight hours with least sleep and shows less pronounced diurnal periodicity.

Neurochemical Data

In a preliminary study [12] we found that C mice did not differ from B mice in brain stem levels of serotonin (0.930 ± 0.32 μg/g vs. 0.965 ± 0.12 μg/g, $t<1.0$). Furthermore, we found that C mice had higher serotonin levels in cortex than did B mice (0.504 ± 0.06 μg/g vs. 0.408 ± 0.03 μg/g, $t = 7.12$, $df = 55$, $p<0.005$). Activity of tryptophan hydroxylase, a crucial enzymatic step in the biosynthesis of serotonin from tryptophan, was lower in C mice than in B mice for brain stem samples (3.36 ± 0.52 nmoles/g/hr vs. 4.10 ± 0.77 nmoles/g/hr, $t = 2.70$, $df = 22$, $p<0.01$) and higher than B mice for cortex samples (1.04 ± 0.21 nmoles/g/hr vs. 0.64 ± 0.13 nmoles/g/hr, $t = 2.93$, $df = 16$, $p<0.01$).

Those preliminary neurochemical data were gathered during 0800–1600 hr in animals on a light schedule identical to that used in the sleep study. With such data, we could see no simple relationship between sleep and serotonin. However, the facts that we neurochemically sampled during only a few hours of the day and that there were no strain differences in SWS time during that sampled segment could explain why no clear sleep-serotonin relationship was then noted. The more detailed data reported here deal with three different points in the serotonin biosynthetic pathway beginning with tryptophan and ending with serotonin and tap three important times since the data were gathered from animals killed during the 60 min surrounding each midpoint of the three eight-hour periods used in our sleep analyses.

The data for tryptophan levels, tryptophan hydroxylase activity, and serotonin levels were analyzed by strain × eight-hour period × brain segment analyses of variance.

Table 4 summarizes our findings for tryptophan levels by strain, eight-hour period and brain segment. These data show no strain or eight-hour period effects. Brain stem level of tryptophan was clearly greater than cortex level (F = 503.43, df = 1/4, $p<0.001$). There was a suggestion of a strain × brain segment interaction (F = 3.18, df = 1/14, $p<0.1$), and further contrasts disclosed that C mice had slightly higher tryptophan levels in brain stem than did B mice (t = 1.85, df = 46, $p<0.10$). From Table 4 it is clear that this strain difference was due to values in the 1600–2400 and 2400–0800 periods.

Table 5 presents data for tryptophan hydroxylase activity. There was only a slight strain effect (F = 3.36, df = 1/14, $p<0.1$) indicating that C mice tended to have lower enzyme activities. There was no eight-hour period effect. Across groups, tryptophan hydroxylase activity was sharply higher for brain stem than for cortex (F = 618.80, df = 1/14, $p<0.001$). Interestingly there was a significant strain × brain segment interaction (F = 25.83, df = 1/14, $p<0.001$) which derives from the C group's having lower activity measures in brain stem ($t = 5.52$, $df = 46$, $p<0.001$) and higher activity measures in cortex ($t = 9.01$, $df = 46$, $p<0.001$).

TABLE 4

MEAN (± S.D.) TRYPTOPHAN LEVEL (μG/G) AS FUNCTIONS OF STRAIN, BRAIN SEGMENT, AND MIDPOINT OF 8-HR PERIOD

	0800–1600 hr		1600–2400 hr		2400-0800 hr		Overall	
C57BL/10J	Brain Stem	1.63 ± 0.106	Brain Stem	1.73 ± 0.193	Brain Stem	1.68 ± 0.135	Brain Stem	1.680 ± 0.149
	Cortex	0.762 ± 0.145	Cortex	0.916 ± 0.211	Cortex	0.922 ± 0.163	Cortex	0.867 ± 0.184
BALB/CJ	Brain Stem	1.60 ± 0.228	Brain Stem	1.59 ± 0.168	Brain Stem	1.55 ± 0.283	Brain Stem	1.581 ± 0.221
	Cortex	0.765 ± 0.123	Cortex	0.873 ± 0.224	Cortex	0.785 ± 0.166	Cortex	0.808 ± 0.175

F_{strain} = 3.97, df = 1/14, $p<0.1$
$F_{8\text{-hr period}}$ = 2.00, df = 2/21, p NS
$F_{brain\ segment}$ = 503.43, df = 1/14, $p<0.001$
$F_{strain \times 8\text{-hr period}}$ = <1 df = 2/21, p NS
$F_{strain \times brain\ segment}$ = 3.18, df = 1/14, $p<0.1$
$t_{overall\ brain\ stem}$ = 1.82, df = 46, $p<0.10$
$t_{overall\ cortex}$ = 1.14, df = 46, p NS

TABLE 5

MEAN (± S.D.) TRYPTOPHAN HYDROXYLASE ACTIVITY (NMOLES/G/HR) AS FUNCTIONS OF STRAIN, BRAIN SEGMENT AND MIDPOINT OF 8-HR PERIOD

	0800–1600 hr		1600–2400 hr		2400–0800 hr		Overall	
C57BL/10J	Brain Stem	3.48 ± 0.403	Brain Stem	3.50 ± 0.290	Brain Stem	3.16 ± 0.769	Brain Stem	3.38 ± 0.529
	Cortex	1.26 ± 0.172	Cortex	1.16 ± 0.171	Cortex	1.08 ± 0.187	Cortex	1.17 ± 0.186
BALB/CJ	Brain Stem	4.26 ± 0.617	Brain Stem	4.43 ± 0.642	Brain Stem	4.26 ± 0.759	Brain Stem	4.32 ± 0.650
	Cortex	0.673 ± 0.146	Cortex	0.730 ± 0.249	Cortex	0.694 ± 0.131	Cortex	0.699 ± 0.176

$F_{strain} = 3.36$, $df = 1/14$, $p<0.1$
$F_{8\text{-hr period}} = <1$ $df = 2/21$, p NS
$F_{brain\ segment} = 618.80$, $df = 1/14$, $p<0.001$
$F_{strain \times 8\text{-hr period}} = <1$ $df = 2/21$, p NS
$F_{strain \times brain\ segment} = 25.83$, $df = 1/14$, $p<0.001$
$t_{overall\ brain\ stem} = 5.49$, $df = 46$, $p<0.001$
$t_{overall\ cortex} = 9.01$, $df = 46$, $p<0.001$

TABLE 6

MEAN (± S.D.) SEROTONIN LEVEL (μG/G) AS FUNCTIONS OF STRAIN, BRAIN SEGMENT AND MIDPOINT OF 8-HR PERIOD

	0800–1600 hr		1600–2400 hr		2400–0800 hr		Overall	
C57BL/10J	Brain Stem	1.01 ± 0.138	Brain Stem	1.00 ± 0.230	Brain Stem	0.991 ± 0.137	Brain Stem	1.00 ± 0.166
	Cortex	0.509 ± 0.056	Cortex	0.499 ± 0.052	Cortex	0.515 ± 0.074	Cortex	0.508 ± 0.059
BALB/CJ	Brain Stem	0.983 ± 0.111	Brain Stem	1.03 ± 0.194	Brain Stem	1.02 ± 0.136	Brain Stem	1.00 ± 0.145
	Cortex	0.421 ± 0.074	Cortex	0.425 ± 0.058	Cortex	0.427 ± 0.051	Cortex	0.424 ± 0.055

$F_{strain} = 2.36$, $df = 1/14$, p NS
$F_{8\text{-hr period}} = <1$ $df = 2/21$, p NS
$F_{brain\ segment} = 578.10$, $df = 1/14$, $p<0.001$
$F_{strain \times 8\text{-hr period}} = <1$ $df = 2/21$, p NS
$F_{strain \times brain\ segment} = 3.67$, $df = 1/14$, $p<0.1$
$t_{overall\ cortex} = 5.11$, $df = 46$, $p<0.001$

Table 6 presents serotonin levels. Analysis disclosed no strain or eight-hour period effects. As with values for tryptophan level and tryptophan hydroxylase activity, brain stem serotonin levels were higher than cortex levels ($F = 578.10$, $df = 1/14$, $p<0.001$). There was no strain × eight-hour period interaction. However, the strain × brain segment interaction approached significance ($F = 3.67$, $df = 1/14$, $p<0.1$) due to C mice's clearly higher serotonin levels in cortex ($t = 5.11$, $df = 46$, $p<0.001$).

The present studies confirm and extend our preliminary findings. As before, we found no strain differences in brain stem serotonin levels, but clearly higher cortical serotonin levels in C than in B mice. The data also confirm our previous findings that tryptophan hydroxylase activity was lower for C than for B mice in brain stem, but higher for C than for B mice in cortex.

The present attempts to examine serotonin biochemistry at three different points in the day have not disclosed significant diurnal variation in tryptophan level, tryptophan hydroxylase activity or serotonin level on an across-strain basis. One explanation for such a lack of diurnal variability may be that too few time points were sampled. However, our data on serotonin's amino acid substrate, tryptophan, do not parallel findings for other compounds in the biosynthetic pathway. Data suggest that C have clearly higher tryptophan levels than do B especially in brain stem, but only during the 1600–2400 and 2400–0800 hr periods.

COMBINED RESULTS AND DISCUSSION

The hypothesis that sleep is controlled by some central serotonergic mechanism was this research's conceptual starting point. Therefore, only neurochemical associations with SWS data will be considered here. It is obvious that no simple sleep-serotonin relationship was found, however certain noteworthy trends may be seen.

There are at least two systematic approaches for examining combined sleep and neurochemical data, a static strain comparison approach and a dynamic correlation approach. Both approaches involve regarding strain and strain × eight-hour period means for SWS and for the neurochemical measures as single observations, rank ordering them, and then asking questions about joint trends. Such procedures, while *post hoc* in nature, are useful in assessing relationships which may guide future work.

First strains were compared by collapsing across time of day. Using this static approach we simply considered ordering of means for quiet sleep and the neurochemical variables and made strain comparisons. Over all time periods, the C group had higher SWS percentages and higher tryptophan levels in both brain stem and cortex than did the B group, thus suggesting a direct relationship between SWS and tryptophan level. Furthermore, inspection of data for the three time periods disclosed that C compared with B mice had more SWS and higher tryptophan levels both in brain stem and in cortex only during the 1600–2400 and 2400–0800 hour periods; during the 0800–1600 hour period, strain differences on these measures were minimal. There was also a direct association between SWS and cortex tryptophan hydroxylase activity; brain stem activity values, however, appeared to be ordered inversely with respect to SWS percentages. For serotonin level, the direct relationship also holds in cortex data; strain means for brain stem serotonin levels are identical.

Thus, in four of six cases (the two exceptions being brain stem measures of tryptophan hydroxylase activity and serotonin level) rank order strain comparisons indicated that sleep time was directly associated with serotonin-related neurochemical variables. Restating, on a static strain comparison basis, SWS times are paralleled by higher brain stem and cortex tryptophan levels, higher cortex tryptophan hydroxylase activity, and higher cortex serotonin levels. These four trends are consistent with Jouvet's sleep-serotonin hypothesis. The positive sleep-tryptophan relationship is of particular interest in light of the recent work by Tagliamonte *et al.* [16,17] which raise the possibility that tryptophan level may vary directly with serotonin metabolism. It is of interest that three of these four direct sleep-neurochemical relations derive from cortex, while both inconsistencies derive from brain stem data. Such inconsistencies may mean that serotonergic terminals important for electrophysiological SWS lie in cortex. Therefore, statically speaking, while serotonin related measures have higher absolute values in brain stem, the smaller values of cortex samples may be more relevant to SWS.

The dynamic approach involves correlation of sleep and neurochemical means over strain and time of day. Table 7 presents the rank order correlation matrix for mean percent SWS and means for tryptophan level, tryptophan hydroxylase activity, and serotonin level. We wish to emphasize that these correlations are not meant to be statistical analyses. They serve only as tools for organizing our discussion of the complicated sleep-serotonin trends among strain and time of day. Each correlation derives from six pairs of numbers taken from strain × eight-hour period cells. Thus, the correlation between SWS and brain stem tryptophan level (Row 1, Column 2) was computed by pairing ranks of cell means in Table 1 and brain stem cell means in Table 4.

With an N of six, none of the correlations with SWS (Row 1) are statistically significant ($r_{\alpha 0.05} = 0.829$) However, the most noteworthy correlations with SWS are the

TABLE 7

RANK ORDER CORRELATION MATRIX FOR PERCENT SWS AND THE SIX NEUROCHEMICAL MEASURES

	PSW	BTL	BTA	BSL	CTL	CTA	CSL
PSW	1.000	0.429	−0.543	−0.657	−0.486	0.314	0.657
BTL		1.000	−0.657	−0.543	0.429	0.657	0.829
BTA			1.000	0.371	−0.257	−0.657	−0.829
BSL				1.000	0.086	0.143	−0.543
CTL					1.000	0.343	0.257
CTA						1.000	0.657
CSL							1.000

Coefficients were computed from all pairs of the six strain × eight-hour periods means taken from Tables 1, 4, 5, and 6. The variable codes are as follows: PSW = percent slow-wave sleep, BTL = brain stem tryptophan level, BTA = brain stem tryptophan hydroxylase activity, BSL = brain stem serotonin level, CTL = cortex tryptophan level, CTA = cortex tryptophan hydroxylase activity, and CSL = cortex serotonin level.

negative ones for brain stem tryptophan hydroxylase activity (−0.543), brain stem serotonin levels (−0.657) which approached significance, and cortex tryptophan level (−0.485). Note that such inverse relationships are not inconsistent with the direct relationships observed with the static approach. The static approach indicated that, in four of six cases, strain differences in sleep were directly paralleled by strain differences in serotonin-related neurochemical variables. The inverse relationships suggested by the dynamic approach indicate that, for example, whether there is much or little overall brain stem tryptophan hydroxylase activity, when sleep time is higher, tryptophan hydroxylase activity is lower. Further, while differences in cell means for the neurochemical variables may be small, on a rank order basis the dynamic approach could be taking account of small but relevant changes over time. In light of what is known about the relative magnitudes of storage and functional pools of putative neurotransmitters, small changes in neurochemical measures may be very important indeed.

In summary, then, if we accept these correlations as indicative of dynamic sleep-serotonin trends, they suggest that during peak sleep times serotonin production is at a low (tryptophan hydroxylase activity down) and serotonin utilization is at a high (serotonin level down). Such conditions would be expected from a sleep inducing neuronal complex whose impulse traffic increases during sleep. Thus, the dynamic approach also yields results which are consistent with the notion of an active serotonergic sleep-inducing mechanism. Further, such relationships are consistent with the observations of Sinha *et al.* [14]. These workers killed cats during either wakefulness, SWS, or REM sleep. Assays on several brain stem areas disclosed reduced serotonin levels in animals killed during SWS compared with those killed during wakefulness.

Finally, our data suggest that the relationship between sleep and serotonin formulated from lesion and drug experimentation, may also be seen in cross-strain observational studies. In future cross-strain studies, larger and more frequent samplings can yield an additional advantage over manipulative studies. More data points will allow the use of multivariate statistical techniques in examining the interrelationships among sleep and neurochemical variables. Such statistical techniques may ultimately allow sleep parameters to be predicted.

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