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Seasonal Variation in CSF 5-HIAA Concentrations in Male Rhesus Macaques

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*Seasonal changes in cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) concentrations were assessed on multiple occasions in 103 free-ranging male rhesus macaques (*Macaca mulatta*). At the time of sampling subjects ranged between the ages of 2 and 6 years. CSF samples were collected between the hours of 0900 and 1600 throughout the Fall, Winter, and Spring from 1990 through 1994. Data were analyzed in a general linear mixed model with random intercepts. Results indicated that CSF 5-HIAA concentrations decreased with age. CSF 5-HIAA concentrations were significantly increased in the Fall (October and November), which is the height of the breeding season, with no evidence of differences between Winter and Spring. There was also some evidence that the seasonal variation in CSF 5-HIAA concentrations was blunted in younger, more immature subjects.*
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The study of serotonin (5-HT) and its major metabolites is of continuing interest because impaired 5-HT mediated neural functioning has been associated with a variety of psychopathological problems such as inappropriate aggression (Brown et al. 1979; Higley et al. 1992a, 1996b; Linnoila et al. 1983; Mehlman et al. 1995; Virkkunen et al. 1994a), excessive alcohol use (Ballenger et al. 1979; Coccaro 1989; Higley et al. 1996a,b; Virkkunen et al. 1994), impaired social interactions (Higley et al. 1994, 1997; Raleigh et al. 1980; Raleigh and McGuire 1991), as well as sleep difficulties (Zajicek et al. 1997), and reduced social competence (Higley et al. 1996c; Kruesi et al. 1990) in both humans and nonhuman primates.

Some of these serotonin-mediated psychopathological problems have been shown to vary with season (Maes et al. 1993a,b). Paralleling this observation, a growing body of research indicates that the 5-HT system shows circannual seasonal variation in humans. For example, several studies have shown seasonal variation in platelet 5-HT uptake, although the time of the year for Vmax varies from study to study (Arora et al. 1984). Similarly, Corona and colleagues (1982) found monthly differences in platelet 5-HT levels, with the highest levels in November, and Maes and colleagues (1995) found that plasma tryptophan concentrations were lower in the Spring months, with a nonsignificant overall peak in the November-February period. After

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examining the human hypothalamus *post mortem*, Carlsson and coworkers (1980) discovered a seasonal variation in hypothalamic 5-HT concentrations, with a maximum peak during the autumn period of October-November, and a seasonal low in late Winter/early Spring. In addition, Losonczy et al. (1984) found that cerebrospinal fluid (CSF) concentrations of the major 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) were higher in the Fall and Winter months.

Seasonal variation in the prolactin response to mCPP and tryptophan, with the highest levels of prolactin present in the Fall and Winter months, were also observed by Brewerton and colleagues (1992). Another study performed by Åsberg et al. (1980) found that CSF 5-HIAA concentrations in normal human controls were higher during Summer months (July-August), although this failed to reach statistical significance. Likewise, Brewerton et al. (1988) found that CSF 5-HIAA concentrations in humans were higher in the Summer months and lower in the Spring, although levels were elevated in the Fall as well. In a separate study, he and his colleagues also found CSF 5-HIAA concentrations to be higher in the Summer months than all other months (Brewerton et al. 1995). Unlike most other studies, Csernansky et al. (1988) found that in patients with psychotic disorders, CSF 5-HIAA concentrations were lower in the Fall, specifically in September, whereas O'Rourke and colleagues (1987) found no seasonal variation in CSF 5-HIAA concentrations. Finally, the highest CSF 5-HIAA concentrations in the late autumn months and the lowest in the Spring were observed by Wirz-Justice and Richter (1979). The above studies indicate an overall trend for lower 5-HT functioning in the Spring, with higher 5-HT functioning in the Summer and, at times, in the Fall. Nevertheless, differences between studies exist and remain largely unexplained.

Climatic or meteorological influences that might be related to seasonal differences in CSF 5-HIAA concentrations (e.g., percent sunlight, percent humidity, percent rainfall and temperature) have been examined as well. Brewerton and colleagues (1995) found that, independent of seasonal variations, CSF 5-HIAA concentrations were correlated with percent sunshine, temperature, and relative humidity. When analyzed separately by gender, there was an inverse relationship between CSF 5-HIAA concentrations and temperature, although only in men. Such findings suggest that climatic variables have the potential to affect the functioning of the 5-HT system and possibly to increase the risk for 5-HT-related psychopathology.

Seasonal variation in CNS 5-HT functioning has been and continues to be of interest for researchers investigating changes in human psychopathological disorders that vary by season, such as suicide, violence, depression, or seasonal affective disorder (Maes et al. 1993a,b; Rosenthal et al. 1984). Because the amount of time humans spend indoors varies from individual to individual in an uncontrolled fashion, it is difficult to assess the impact of seasonal and climatic variables on CNS 5-HT functioning and 5-HT-related psychopathology. Indeed, this uncontrolled variable may explain the mixed findings that have emerged in this area. One possible method to better assess the impact of climatic variables on CNS 5-HT functioning would be to sample from nonhuman primates that live outdoors year-round. Yet, while nonhuman primates have been widely used to study 5-HT-mediated psychopathology, to date we know of no studies that have systematically investigated seasonal variation of CSF 5-HIAA concentrations in nonhuman primates. Findings from subjects that live outdoors could provide important information about the overall impact of seasonal and climatic changes on CNS 5-HT functioning. Given that rhesus macaques are frequently used as models for the study of CNS 5-HT and its influence on behavioral development, we felt it was important to examine how CNS 5-HT functioning varies circannually in this particular species.

The focus of the present study was to determine whether seasonal fluctuations in CSF 5-HIAA concentrations exist in free-ranging rhesus macaques and, if so, to assess the effect of age and development on seasonal differences. Since rhesus monkey infants spend a relatively large percentage of their day in contact with their mothers, infants and juveniles are often buffered from external stressors and climatic variables that may influence CNS 5-HT activity. Furthermore, seasonal changes in sex hormones do not appear until the onset of puberty (Rose et al. 1978). To the degree that the change in hormonal status modulates CNS 5-HT, it could also be postulated that prepubertal subjects would not show seasonal variation in CSF 5-HIAA concentrations equivalent to that of adults. We felt that studying a wide range of macaques in a free-ranging population, where the influence of artificial lighting and imposed feeding schedules were not present, would help to draw relevant conclusions about seasonal effects on the 5-HT system. Furthermore, the research setting we chose, while having a characteristic seasonal pattern of temperature, was not so extreme in annual temperatures that variation in CNS 5-HT would simply reflect possible effects of heat and cold stressors on the 5-HT system. Therefore, we hypothesized that in a free-ranging population of male rhesus monkeys there would be seasonal variation in concentrations of the 5-HT metabolite, 5-HIAA, and that it would be most pronounced in postpubescent subjects.

METHODS

Subjects and Conditions of Capture

One hundred and three free-ranging male rhesus monkeys (*Macaca mulatta*) , ranging from 2 to 6 years of age,

living on a coastal island of South Carolina (Morgan Island: 475 total acres, 32° north latitude), were sampled at various months reflecting typical seasonal variation over a 5-year period. Subjects were captured and their CSF sampled after they jumped into inescapable food-baited corrals. Because this capture system is passive, not all subjects were captured at the same time (average number of subjects captured during each sampling was 30).

As in other natural rhesus macaque populations (Lindburg 1971), these free-ranging male subjects resided in mixed-sex groups. From 1990 through 1994, monkeys were captured monthly except in April, and during the peak Summer months of July, August, and September, during which time the temperatures in this area were too hot to work humanely with anesthetized subjects.

Data Collection

All CSF samples were obtained between 0800 and 1600 hours. After being caught inside the corral, subjects were anesthetized with a standardized dose (15 mg/kg) of ketamine hydrochloride, and 3 ml of CSF were obtained from the cisterna magna of each subject using a 5 ml syringe and a 22 gauge needle. CSF was aliquoted, placed in cryotubes, and immediately frozen on dry ice. Samples were stored in a cryogenic chest filled with dry ice for no more than 12 hours. They were then placed in a freezer and maintained at −70°C until they were assayed. Samples obtained from each year were assayed separately and within 12 months of collection. CSF was assayed for 5-HIAA using gas chromatography and mass spectroscopy using the method described by Polinsky and colleagues (1988). Intra-assay variation was found to be 5% for MHPG, 2% for HVA, and 6% for 5-HIAA. Inter-assay variation was 8% for MHPG, 5% for HVA, and 10% for 5-HIAA. Pooled controls were assayed in either duplicate or triplicate on any given day that data were generated.

The reproducibility of the derivitization procedure degraded noticeably when ambient temperature was greater than 70°F and humidity was greater than 30%. Consequently, samples were not assayed during this time (most Summer months). Records were kept of: 1) the elapsed time from when subjects were injected with ketamine HCL; 2) the time of day when the CSF was obtained; and 3) the day of the week when each sample was obtained.

To assess the relationship of climatic variables on seasonal variation, climatic data were obtained from the National Climatic Data Center (NCDC) in Beaufort, SC, located about 30 miles from the research population. Sky condition, temperature, and precipitation are recorded hourly at the NCDC every day.

Description of Sample Analysis

The data consisted of 415 CSF 5-HIAA concentration measurements from the 103 male rhesus monkeys. Individual monkeys were sampled as few as one, and as many as twelve times over the five-year period. Half of the data came from juvenile and adolescent monkeys aged 2 to 3 years, and half from adult monkeys aged 4 to 6 years. More samples were obtained from 2- and 3-year-olds early in the project, whereas the majority of samples from 4- to 6-year-olds were obtained late in the project.

CSF 5-HIAA measurements were collected in Winter, Spring, and Fall seasons. Winter data were defined as measurements collected during the months of January, February, and March. Spring measurements were collected in May and June, and Fall measurements were collected during the months of October and November. Roughly one third of the measurements were taken in each season. There were no Spring data in 1990. In 1991 and 1992, roughly one third of the measurements were taken in each season. In 1993 and 1994 there were proportionately more Spring and fewer Winter observations, compared with earlier years.

The mean and standard deviation for the CSF 5-HIAA concentrations were 228.7 and 59.8 pmol/ml, respectively. The time of day that CSF 5-HIAA measures were obtained from monkeys was missing for about 40% of the samples. The remaining samples were equally distributed across the morning and afternoon hours. The frequency distribution for four categories of cloud cover at time of data collection is shown in Table 1. Temperature ranged from 1.66° C to 28.88° C, with a mean of 16.66° C. It was raining during data collection 43% of the time. The recorded humidity ranged from 68% to 95%, with a mean of 88%. Cloud cover and day of week were not recorded at the time of CSF collection in 24 and 168 cases, respectively; thus, temperature, precipitation data, and humidity data were absent for 192 out of the 415 records.

Statistical Analyses

Relationships between CSF 5-HIAA concentrations and age, season, climate, time-of-day, and year covariates were assessed using a regression modeling framework.

Table 1. Frequency Distribution of Cloud Cover

Cloud Cover	Frequency of Occurrences	% of Total Days
Clear, <10% sky cover	37	9.5
Scattered, 10–50% sky cover	153	39.1
Broken, 60–90% sky cover	136	34.8
Overcast, >90% sky cover	65	16.6

Frequency missing = 24.

In order to account for correlations among repeated observations on monkeys, random effects regression models (Diggle et al. 1994), also known as two-level hierarchical linear models (Byrk and Raudenbush 1992), were fit to the data. Models with random intercepts and models with both random intercepts and random slopes were tested. The random intercept models assume that the repeated observations on a monkey deviate around the monkey's individual mean CSF 5-HIAA concentration and, conditional on the covariates in the model, the collection of all of the monkeys' individual means are normally distributed around a grand population mean.

All monkeys were assumed to share a common age slope. Models with random slopes relax the assumption of a common age slope. However, for the CSF 5-HIAA data, there was insufficient evidence of heterogeneity of slopes among monkeys to warrant retention the random slope terms in the model. Therefore, the more parsimonious random intercept modeling framework was adopted. Preliminary exploration and model fitting indicated significant variation in CSF 5-HIAA associated with year of data collection. Consequently, indicator variables representing year of data collection were included in all subsequent models. Exploratory analysis of the consistency of the year effect across age groups and seasons is presented below.

RESULTS

Several possible relationships between CSF 5-HIAA concentrations and age were explored by comparing results among models with either: 1) a linear function of age; 2) a quadratic function of age; 3) a cubic function of age; or 4) dummy coded age (no functional form assumed). Results indicated that the simple linear function of age provided a reasonable representation of the data. Therefore, all results presented assume the simple linear form.

Model 1 Covariates: Age, Season, and Year

$$Y_{ij} = \alpha_{0i} + \beta_1(Winter) + \beta_2(Spring) + \beta_3(Fall) + \beta_4(Age_{ij}) + \beta_5(Yr90) + \beta_6(Yr91) + \beta_7(Yr92) + \beta_8(Yr93) + \varepsilon_{ij},$$

where Y_{ij} = CSF 5-HIAA concentration for monkey i at age j, α_{0i} = random intercept parameter, assumed distribution Normal $(0,\tau^2)$; *Winter* = 1 if data collected during winter months, 0 else, *Spring* = 1 if data collected during spring months, 0 else, *Fall* = 1 if data collected during fall months, 0 else, Age_{ij} = centered age in years, (centered by subtracting the mean age (4 years) from the monkey's age in years at time of data collection), *Yr90* = 1 if data collected in 1990, 0 else, *Yr91* = 1 if data collected in 1991, 0 else, *Yr92* = 1 if data collected in 1992, 0 else, *Yr93* = 1 if data collected in 1993, 0 else, and ε_{ij} = residual error, assumed distribution Normal $(0,\sigma^2)$.

Table 2a. Model 1 Parameter Estimates for Seasonal Variation in CSF 5-HIAA Concentrations with Associated Tests of Fixed Effects

Variable	Mean	SE	df	t-value	Probability
Winter	253.6	8.2	305	31.03	.0001
Spring	249.9	7.7	305	32.36	.0001
Fall	282.3	9.3	305	30.36	.0001

Table 2b. Model 1 Parameter Estimates for the Mean Difference in CSF 5-HIAA Concentrations with Increasing Age and Across Calendar Years, with Associated Model 1 Test Parameters

Variable	Mean Difference	SE	df	t-value	Probability
Age	−17.4	3.7	305	−4.74	.0001
YR90	−73.6	15.5	305	−4.74	.0001
YR91	−68.5	11.2	305	−6.12	.0001
YR92	−34.7	10.1	305	−3.43	.0007
YR93	−10.6	8.2	305	−1.29	.1976

The results of Model 1, shown in Tables 2a and 3, indicate a statistically significant variation in CSF 5-HIAA measurements associated with age, season, and year of data collection. The model shown in Table 2b predicts an average decrease of 17.4 pmol/ml per year as the subjects get older. The means for Winter, Spring, and Fall were 253.6 pmol/ml, 249.9 pmol/ml, and 282.3 pmol/ml, respectively. These parameter estimates shown in Table 2a represent the mean CSF 5-HIAA concentrations for each season, in monkeys of average age (4 years), for data collected during year 1994. The predicted mean for monkeys older than 4 years can be obtained by subtracting 17.4 pmol/ml for each additional increase in one year of age (see Table 2b). The predicted mean for younger monkeys can be obtained by adding 17.4 pmol/ml for each year less than four. The predicted means corresponding earlier calendar years (1990–93) are lower, as described below.

As shown in Figure 1, mean CSF 5-HIAA averaged 29 to 33 pmol/ml higher in the Fall season than Winter or Spring ($p < .05$). There is, however, substantial overlap of the Winter and Spring confidence intervals. We conclude that CSF 5-HIAA concentrations were significantly higher in the Fall than in the Winter or Spring, but find no evidence of differences between Winter and Spring. Table 3 indicates significant variation in CSF 5-HIAA concentrations associated with the year of data collection. The regression coefficient for the 1990

Table 3. Test of Fixed Effects

Variable	df	F-ratio	Probability
Season	2/305	14.34	.0001
Age	1/305	22.46	.0001
Year	4/305	11.94	.0001

dummy variable was −73.6. This means that after accounting for differences due to age and season, the expected mean CSF 5-HIAA concentration in 1990 was 74 pmol/ml lower than that of 1994 (see Table 2b).

In order to determine whether the year-to-year differences in concentration levels were due to an anomaly in the data or were consistent across age groups and seasons, mean CSF 5-HIAA with 95% confidence intervals for each age group, year, and season were plotted, as shown in Figure 2. The plot reveals, for example, that a comparison of the Winter data of three-year-olds across calendar years indicates higher means in 1992 and 1994 relative to 1990 and 1991 (note that the plot of a mean without a confidence interval, e.g., three-years-old, 1993, indicates a data point for a single monkey). The estimated means of Winter data for four-year-olds also appeared to be higher in later years. The same trend of elevated mean CSF 5-HIAA concentrations in later years is evident for the Spring data for each age group. With a few exceptions, the same trend also holds for the Fall data. Thus, across age groups and seasons, there appear to be consistent differences in mean CSF 5-HIAA concentrations over the years.

The model indicates that increasing calendar year is associated with increased CSF 5-HIAA concentrations. Compared to 1994 average concentrations, the mean concentrations were 68 pmol/ml lower in 1991, 35 pmol/ml lower in 1992, and 11 pmol/ml lower in 1993. However, as shown in Table 4, the number of younger subjects obtained across calendar years decreased.

Additional models were fit to the data that included calendar year by age and calendar year by season interactions. None of the interactions were statistically significant. Thus, there were no indications that the age or the season effect varied by year. Model 1 included data from all five years. If similar age and season effects were found in each year separately, it would have increased our confidence that we found a reasonable model to describe age and seasonal changes in mean CSF 5-HIAA concentrations. Therefore we fit five separate models, one for each of the five years, of the form that follows.

Model 2 Covariates: Age and Season (a Separate Model for Each Year)

$$Y_{ij} = \alpha_{0i} + \beta_1(Winter) + \beta_2(Spring) + \beta_3(Fall) + \beta_4(Age_{ij}) + \varepsilon_{ij}$$

The results of this model testing age and seasonal changes in CSF 5-HIAA concentrations showed elevated mean concentrations in the Fall, and negative age slopes indicating decreased concentrations for older monkeys (see Figure 3).

The results for 1991 data, however, were statistically different than those for other years. Close inspection of the data showed a greater seasonal effect for older animals. This observation motivated fitting of models with an age by season interaction. Models of the following form were fit to the data from each year.

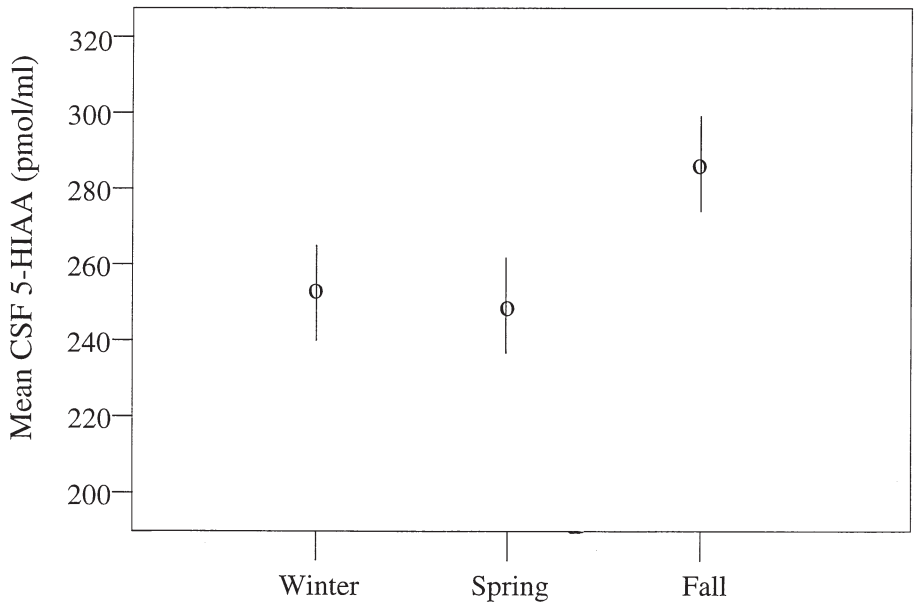

Figure 1. The fitted seasonal mean CSF 5-HIAA concentrations with 95% confidence intervals across all subjects and years. The Y-axis units are in pmol/ml, with the axis going from 200 to 320 pmol/ml. The X-axis shows each of the three seasons during which CSF was sampled.

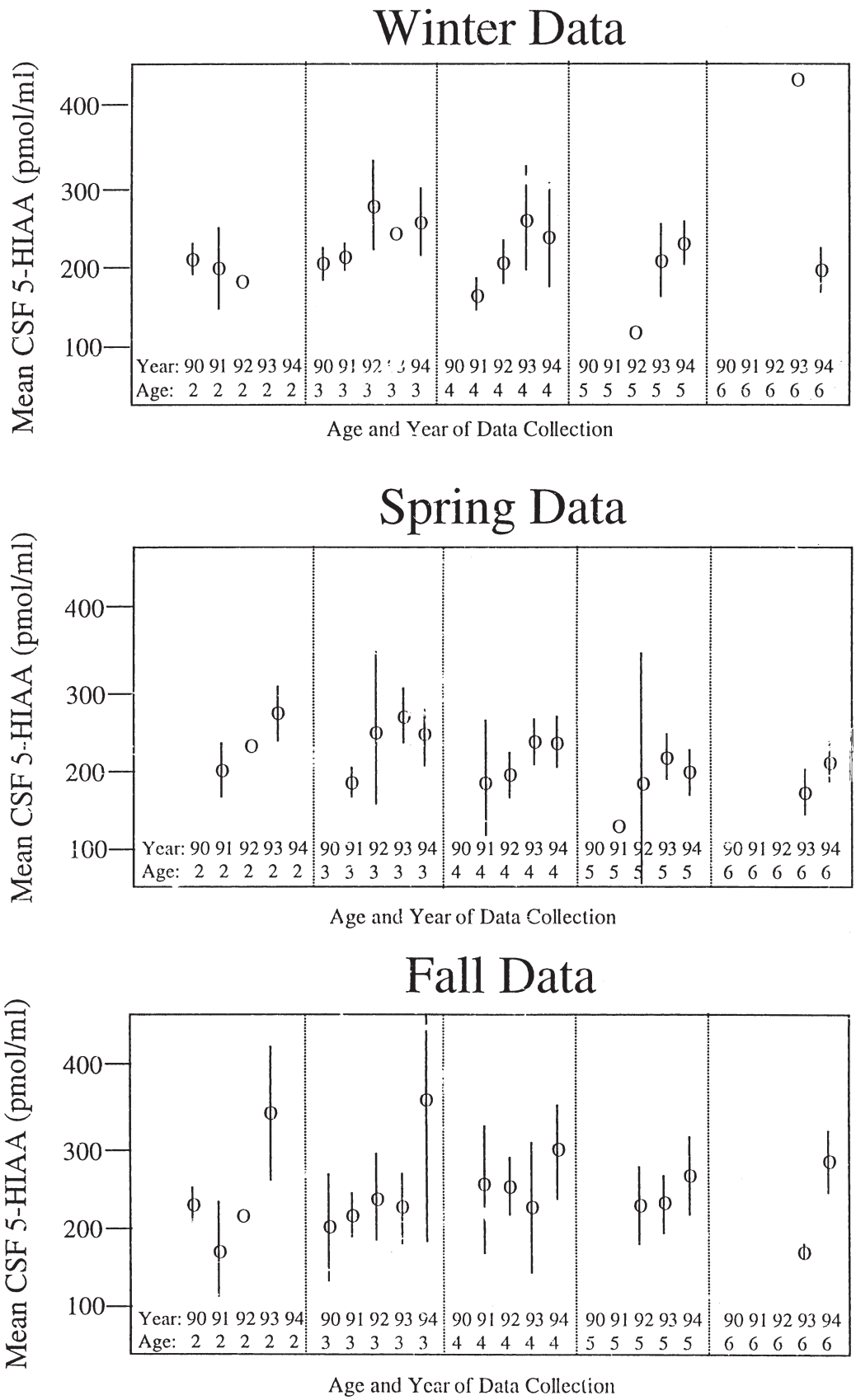

Figure 2. Mean CSF 5-HIAA concentrations graphed by each of the three seasons that were studied, plotted as a function of age and year with 95% confidence intervals across all subjects and years. The Y-axis units are in pmol/ml, with the axis going from 100 to 400 pmol/ml. The X-axis shows each age and year during which CSF was sampled.

Table 4. Number of Subjects by Age and Year of Data Collection

Year/Age	<2	3	4	5	6+	Total
1990	31	8	0	0	0	39
1991	10	40	9	1	0	60
1992	1	7	22	6	0	36
1993	10	11	10	25	5	61
1994	0	8	11	13	40	72
Total	59	76	52	45	45	277

Model 3 Covariates: Age, Season, and Age by Season Interaction

$$Y_{ij} = \alpha_{0i} + \beta_1(Winter) + \beta_2(Spring) + \beta_3(Fall) + \beta_4(Age_{ij}) + \beta_5(Winter*Age_{ij}) + \beta_6(Spring*Age_{ij}) + \beta_7(Fall*Age_{ij}) + \varepsilon_{ij}$$

Results of fitting the models to each year indicated a significant age by season interaction for the 1991 data only, the first year when both younger and some older aged animals were captured. For 1991, the year with the most three-year-old subjects ($n = 40$), there was evidence of increased seasonal effect for older animals, when compared with the younger animals. However, this result was not found in the data for any of the other later years when the sample sizes for younger animals were smaller, nor was it found in an all-years combined model. A similar, albeit nonsignificant pattern was seen in the 1992 data.

The possibility of an association between CSF 5-HIAA concentration and temperature was explored by adding linear, quadratic, and cubic functions of temperature to Model 1. No significant temperature effects were found in any of the three models. Similar functions of temperature were fit to the data after dropping the season variable from the model (i.e., models of the form).

Model 4 Covariates: Temperature, Age, and Year

$$Y_{ij} = \beta_0 + a_{0i} + \beta_1(Ctemp) + \beta_2(Ctemp^2) + \beta_3(CTemp^3) + \beta_4(Age_{ij}) + \beta_5(Yr90) + \beta_6(Yr91) + \beta_7(Yr92) + \beta_8(Yr93) + \varepsilon_{ij},$$

where *Ctemp* is temperature in degrees Celsius (C) centered at the mean. Cubic and quadratic terms for temperature were not found to be significant and were dropped from the model. There was, however a statistically significant linear effect for temperature. The model predicted a decrease of 8 pmol/ml on CSF 5-HIAA for each increase of 10°C. This, however, may have been a result of seasonal-controlled differences in temperature. Because temperature and season are correlated, several alternative models were fit to control for seasonal effects and test for temperature contributions to CSF 5-HIAA concentrations. The results of these tests indicated no significant temperature effects after controlling for seasonal effects.

Humidity, precipitation, cloud cover, and time-of-day variables were added to models in a similar manner as described for the temperature measure. Other exploratory techniques included plotting the climatic and time-of-day variables against CSF 5-HIAA concentrations, and plotting the residuals from Model 1 against the climatic and time-of-day variables. None of these variables were significant predictors in the models, nor were any relationships between any of them and CSF 5-HIAA concentrations found in the plots.

DISCUSSION

Consistent with some previous findings (Åsberg et al. 1980; Brewerton et al. 1988; Carlsson et al. 1980; Wirz-Justice and Richter 1979), but not all (Blennow et al. 1993; O'Rourke et al. 1987), the data presented in this study reinforce the existence of seasonal variation in 5-HT functioning. With an increase in concentrations during the Fall months, the data suggest that CSF 5-HIAA follows a natural circannual rhythm. A comprehensive analysis showed significant associations between CSF 5-HIAA concentrations and age, season, and year of data collection. Mean CSF 5-HIAA concentrations decreased by about 17 pmol/ml for each year increase in age across the age range of two to six years. Mean concentrations in the Fall were elevated 29 to 33 pmol/ml relative to Winter and Spring. There was a general decrease in the mean CSF 5-HIAA concentrations of the subjects as they increased in age (from two to six years of age), consistent with findings from normal human subjects (Bowers and Gerbode 1968; Langlais et al. 1985; Rogers and Dubowitz 1970). We expected to find this trend in rhesus monkeys since previous studies have shown that CSF 5-HIAA concentrations are stable across time between individuals, but decrease with age as a group (Higley et al. 1992b, 1996a,c,d; Kraemer et al. 1989).

Our finding that CSF 5-HIAA concentrations are higher in the Fall season are in agreement with some humans studies of variation in 5-HT functioning. Not all human studies of seasonal changes in function support our findings, however, and there is some disagreement across different studies when these changes occur. While most studies of normal humans showed lowest concentrations of either 5-HT or 5-HIAA in the Spring and highest concentrations during the Summer (Åsberg et al. 1980; Brewerton et al. 1988; Brewerton 1989; Brewerton et al. 1993; Wirz-Justice and Wehr 1984), animal and human studies also report high peripheral 5-HT measures and CSF 5-HIAA concentrations in autumn (Arora et al. 1984; Brewerton et al. 1988; Carlsson et al.

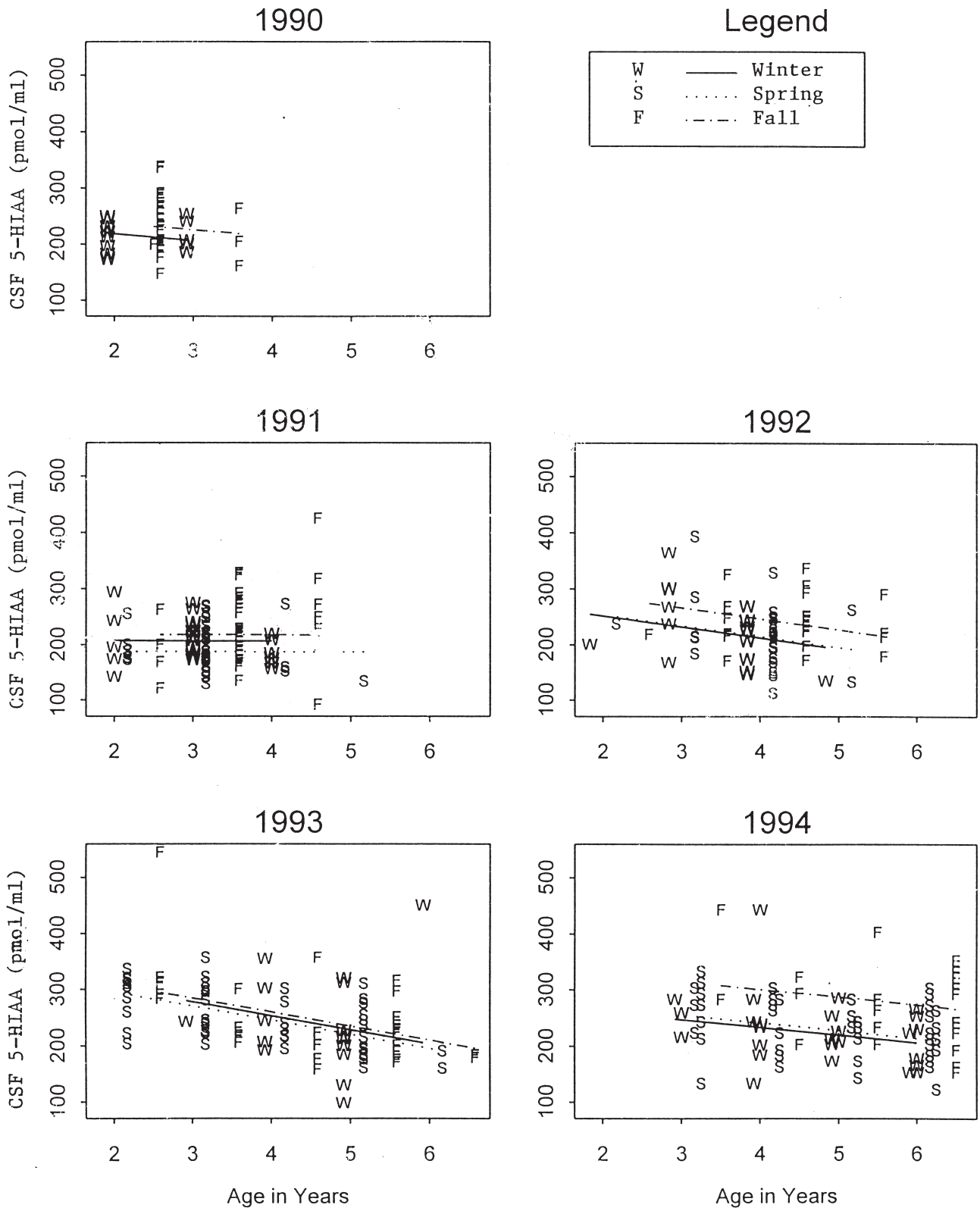

Figure 3. CSF 5-HIAA concentrations versus age overlaid with fitted regression lines for age and season. For each year, separate plotting symbols represent data collected in Winter, Spring and Fall seasons. The results from all five years show elevated mean concentrations in the Fall, and negative age slopes indicating decreased concentrations for older monkeys.

1980; Corona et al. 1982; Maes et al. 1995; Wirz-Justice and Richter 1979). Had CSF samples been obtained during July and August, we may have seen the same Summer peak as in other studies.

It is not clear why our findings may disagree with some of the available human literature in terms of low CSF 5-HIAA concentrations in the Spring. As a species, rhesus macaques share similar behavioral and physiological characteristics with humans. However, unlike humans previously studied, they have distinct changes in behavior and physiology associated with a breeding season. For these reasons, and because our subjects live at a more southern latitude than has been studied in humans, there may be a shift in the seasonal variability of 5-HT functioning. In fact, circannual fluctuations may vary in animals depending on the latitude of habitat (Åsberg et al. 1980). Examination of a population of nonhuman primates living at a different latitude would address this question.

With the exception of the 1991 (and possibly the 1992) data, results of fitting models to individual years indicated consistent age and seasonal effects identical to those obtained by combining the data, although the 1990 data can probably be discounted since there were no results for animals age four and older. Similarly, as the subjects aged, there were only a few prepubescent monkeys sampled by 1994. The significant age by season interaction for the 1991 data and the similar, albeit nonsignificant, results for the 1992 data suggest a plausible finding that the increase in CSF 5-HIAA concentrations associated with the Fall season was greater in older animals. Assuming that the behavioral and hormonal changes associated with the Fall breeding season affect older males differently than younger males and that the hormonal changes modify CNS 5-HT functioning, such a result is possible. The evidence for a differential seasonal effect depending on age was weak or nonexistent in the remaining years, or when examining the data of all years combined. Additional data would be necessary to answer this question fully.

Evidence of seasonal variations in the rhesus monkey serotonergic system, as measured by CSF 5-HIAA concentrations, suggests an evolutionary old variation that may have been preserved from a common ancestor. While cause and effect relationships cannot be established from a correlational study, one possible explanation is that the seasonal increase in 5-HT functioning seen in this study is related to the increased sexual behavior seen during the breeding season. October and November months represent the apex of the breeding season for rhesus monkeys on Morgan Island. Aggression (Eaton et al. 1981), as well as testosterone levels, are typically higher in primate species such as macaques during the months in which they are breeding (Bernstein 1993; Rose et al. 1978). Because androgen receptors are present on the raphe, the testosterone surge may concurrently lead to increased CNS 5-HT turnover. On the other hand, it may also be that the increased CSF 5-HIAA concentrations observed during the breeding season are a result of the stress of sexual competition, where wounding and trauma are commonly occurring stressors.

Finally, the mean of CSF 5-HIAA concentrations for all subjects was higher in each successive calendar year. While it is true that the number of total subjects increased in each successive calendar year with fewer young subjects (i.e., age 2–3 years; see Table 4), the increase across each year was not a consequence of obtaining more young subjects. Because our subjects' CSF 5-HIAA concentrations decreased with age, sampling more old animals on each calendar year would have resulted in a lower overall mean. One explanation could be that many of our male subjects with lower than average CSF 5-HIAA concentrations fail to survive to adulthood. This hypothesis is supported by our published data (Higley et al. 1996b), where 20% of the male population died over the four-year period of the study, and 90% of those who died were from the lowest quartile of CSF 5-HIAA concentrations. Thus, the overall population mean of CSF 5-HIAA may have increased as a result of differential survival of subjects with high CSF 5-HIAA concentrations. Alternatively, the differences may reflect assay variations. However, this seems unlikely given that the same methodology was used for all of the assays and the interassay reliability was very low. Moreover, the average values for other studies from our laboratory did not vary with the years. Currently, we are in the process of sampling female subjects, who remain in their natal group and have a lower mortality rate, in order to assess these possibilities. It is important to note, however, that the seasonal variation in CSF 5-HIAA concentrations was present in different years of the study, independent of any possible assay differences between years.

It has been shown that changes in the amount of exposure to sunlight affects the human serotonergic system which, in turn, can cause changes in behavior (Brewerton 1991). In the present study, however, there was no evidence for an association between CSF 5-HIAA concentrations and temperature, humidity, rainfall, cloud cover, or time of day.

The seasonal rise in CSF 5-HIAA concentrations in this population of rhesus monkeys is of significant interest since these animals are studied year round in both behavioral and physiological research. These findings may help to further the understanding of seasonal variation in 5-HT functioning as it relates to a variety of species-specific behaviors such as aggression or violence and possibly reproduction. More importantly, the data suggest that future studies of seasonally-mediated serotonergic functioning in nonhuman primates can be conducted in a controlled fashion to better understand

behavioral and neurochemical trends in human subjects. Seasonality of CSF metabolites should be considered when using nonhuman primates as models for human behavioral and physiological comparisons.

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