for submission to The Journal of Research on Adolescence.
DRAFT: DO NOT QUOTE

THE ADVANTAGE OF MULTIPLE MEASURES IN STUDIES OF HORMONES AND BEHAVIOR: MELATONIN AND MOODS IN ADOLESCENT BOYS AND GIRLS.

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INTRODUCTION

All species that have been studied have a circadian rhythym of melatonin secretion from the pineal gland. Melatonin increases at night, in association with the onset of darkness. In the morning, inhibition by light causes melatonin in serum to fall. Since the number of daylight hours varies with the seasons at the upper and lower latitudes, there can also be seasonal differences in melatonin secretion. There has been considerable interest recently in a possible role for melatonin in affective disorders (Rosenthal et al., 1984; Rosenthal et al., 1986; Souetre et al., 1989; Thompson et al., 1988; Winton et al., 1989)). This is because some individuals are reported to experience seasonal affective disorders (SAD). These individuals become depressed during the winter with the decrease in the number of daylight hours. Treatment of these individuals with exposure to high intensity light has been reported to reduce depression (Rosenthal et al., 1984; Rosenthal et al., 1986). Small doses of melatonin in normal individuals have been reported to induce fatigue, drowsiness, a decrease in self-reported alertness, and slowed reaction times on at least two different tasks (Arendt et al., 1984; Lieberman et al., 1984; Sherer et al., 1985).

During development in humans, melatonin concentrations in serum continuously decline from infancy into adulthood (Waldhauser et al., 1988). This decrease in serum melatonin is in part due to the increase in body size that occurs during maturation. Melatonin secretion remains constant while the blood volume increases with body size, melatonin is diluted by the greater amount of blood. Independent of the change in average serum concentrations of melatonin, research from non-human animals suggests that melatonin is involved in the timing of puberty (Foster et al., 1986; Yellon and Foster, 1986). It is possible that this is also true in humans, since changes in the neural response to melatonin are associated with puberty (Mauri et al., 1985; Norman and Liwack, 1987).

Due to reports of melatonin influences on affective behavior and the possible changes in melatonin reported to occur during puberty, we decided to examine whether mood and affect are correlated with this hormone in girls 9-10 years old and boys 11-12 years old. When begining a study of hormonal influences on adolescent behavior one of issues that must be addressed is how

and when to obtain biological samples for hormone measurements. Specifically, when should samples be collected sample (time of day, frequency), what type of samples should be collected (urine, serum, saliva) and what hormones should be measured? We chose a repeated sample collection procedure that we feel has many advantages over designs where a small number of samples are collected. There are several advantages that repeated measurements bring to a study of hormones and behavior. First, average concentrations of hormones over a given period of time can be determined, so that there is greater confidence in the values obtained. Second, multiple and frequent measures of both hormones and behavior allow examination of possible short-term causation: do hormones influence behavior, does behavior influence hormones, or do both effects occur? Third, variability of both hormones and behavior can be examined. This study is a preliminary report of some of our initial findings from a study in which 6-hydroxymelatonin (the primary melatonin metabolite in urine) and behavior were repeatedly sampled over a one month period of time.

METHODS

Urine samples were obtained on rising for 3 days a week (Monday, Tuesday, Wednesday) for four consecutive weeks from girls 9-10 years old (N=36) and boys 11-12 years old (N=49). These ages were chosen so that boys and girls would be at the same stage of pubertal development. Daily questionaires requiring individuals to report their moods and behaviors were filled out three evenings each of the four weeks (Sunday, Monday, and Tuesday). At the end of the month, the child was interviewed by one of the experimenters for more general information about mood, affective behaviors and attitudes (End-of-Month questionaire). At this time the child was also weighed.

In our first analyses we chose to assay one sample per week. In order to minimize variation due to the weekend, Tuesday was selected. Only children who had given urine samples on Tuesday morning of all 4 weeks were included in this analysis. Urine was analyzed for 6-hydroxy-melatonin (6-OH-MEL) according to previously published methods (Arendt et al., 1985).

6-OH-Mel concentrations were adjusted for urinary creatinine and body weight. This allows metabolic and body weight influences on excretion of 6-OH-MEL to be minimized.

Daily and End of Month questionaire responses were analyzed by factor analysis and mood composites were generated. These measures have been published in ???. For each composite, the mean value was used for the subsequent analyses. Comparisons between mood composites and 6-OH-MEL were analyzed using Spearman's Ranked Correlation Coefficient analysis for non-parametric analyses.

RESULTS

End of Month responses. The monthly average (mean) of urinary 6-OH-MEL for each child was compared with the child's responses on the End of Month questionaire. Results from the boys and girls were analyzed separately. In order to test whether the mean value for 6-OH-MEL had the same predictive value as individual values of 6-OH-MEL, correlations between responses on the End of Month questionaire and individual 6-OH-MEL values were also conducted.

Girls. Melatonin excretion was negatively correlated with four related mood composites. Girls with a low mean 6-OH-MEL rated themselves higher on traits of: "outgoing and friendly", "communal", "involved", and "not depressed". "Body image" also tended to be positively correlated with 6-OH-MEL as well, but this trait did not reach statistical significance (Table 1). When these same analyses were repeated using either the first or last value obtained for 6-OH-MEL fewer strong correlations were found (Table 1). While the trend for an association between these moods and 6-OH-MEL was always present, statistical significance was frequently not obtained for correlation coefficients betwen these mood composites and individual measures of 6-OH-MEL.

Boys Two items were correlated with urinary 6-OH-MEL in boys. Boys with higher 6-OH-MEL tended to be less "self-conscious" (Rosenberg, 1979) about what other people thought of them than those with lower 6-OH-MEL (Table 2). Boys with high 6-OH-MEL also tended to report that their parents were more strict with them than did boys with lower 6-OH-MEL. 6-OH-MEL in boys was not related to "involved" or "communal" or other items on which girls showed a strong negative correlation. In fact, as is shown in Table 2, there was a tendancy for a positive

correlation between these items and 6-OH-MEL. As was true for girls, individual 6-OH-MEL values in boys were not as consistent at predicting hormone-behavior relations on the End of Month responses as were the average values (Table 2).

Daily Measures.

Analyses were carried out both with means obtained for each child during the month of moods and 6-OH-MEL as well as with the day to day concurrence between 6-OH-MEL and behavior.

Monthly means of moods and 6-OH-MEL.

The mean 6-OH-MEL in girls (N=36) was negatively correlated with average composites of "self esteem" (rho=-0.282, $p<0.05$) and "aggression" (rho=-0.327, $p<0.02$). As is shown in Table 3, other measures of moods also tended to be correlated with the mean 6-OH-Mel in girls, but did not reach statistical significance. In boys (N=49), "affection" was positively correlated with 6-OH-MEL (rho=0.390, $p<0.01$). No other measure was significantly correlated with boys' daily responses (Table 3).

Daily concurrance of moods and 6-OH-MEL. There are a number of ways that hormones and behavior could be related. The most simple relations would be direct associations with either hormone secretion predicting mood or vice versa. Since we had collected behavioral data both the evening before and the evening after morning sample collection, we were able to test whether there were direct relations between hormones and behavior in either direction.

Girls. When the daily excretion of 6-OH-MEL was compared with daily reports of moods, consistent correlations were found only for self-esteem in females. High concentrations of 6-OH-Mel in the Tuesday morning specimen were correlated with low self-esteem on the questionaire Tuesday night (rho=-0.255, $p<0.01$). Daily self-reports of aggression and happy were not consistently correlated with the daily variation in 6-OH-Mel (Table 4). No questionaire responses obtained the preceeding evening (i.e. Monday evening) were correlated with urinary 6-OH-MEL the following morning (Table 5).

Boys. In males, affection was correlated with daily changes in 6-OH-Mel (rho=0.315, $p<0.01$). However, in this case increased self-report of affectionate behavior on Monday night's

questionaire preceeded an increase in 6-OH-MEL Tuesday morning (Table 6). There were a number of mood composites reported for Monday night that appeared to be correlated with 6-OH-MEL during individual weeks but none other than affection that were significant for all four weeks. There were no correlations between 6-OH-MEL and Tuesday evening self report of moods (data not shown).

Discussion

From these results the power of the repeated sampling procedure is apparent. On behavioral measures that were selected to provide a more general picture of affect in our subjects (End of Month measures), moods were more consistently associated with average values of hormone concentrations over the month than with individual hormone samples. In contrast, on behaviors and moods intended to reflect daily variation in affect, the overall associations between hormones and behavior as well as the direction of hormone-behavior relations could be assessed. Correlations between average values or measures suggested a number of possible hormone-behavior relations. When day to day relations were analyzed, only "self esteem" in girls and "affection" in boys showed specific correlations that indicated consistent hormone -> behavior or behavior -> hormone relations (respectively).

A number of points about the value of multiple hormone samples can be addressed with these data. For example, consider the relations between the End of Month measures and average vs. individual values of 6-OH-MEL. Significant correlations are found between means of 4 samples collected over a month. Yet, not all individual samples contributing to the mean show the same strong correlation. Individual hormone samples were less reliable than were average values. Since the End of Month measures were selected to provide a more general profile of affect in these children, these data suggest that a comparable hormonal profile (i.e. a profile of hormone samples collected over time) yeilds the more valuable information about hormone-behavior relations.

The value of multiple samples of both hormones and behavior should also be apparent from the analyses of the daily measures. When both hormones and behavior are collected at multiple time points, it is possible to consider whether there are specific temporal constraints associated with

hormone-behavior relations. Analyses of these temporal relations in our data indicate that there are very different associations for boys and girls. We saw that low nocturnal 6-OH-MEL predicted greater "self esteem" that evening in girls (i.e. hormone -> behavior). In contrast, in boys self report of "affection" preceeded an increase in urinary 6-OH-MEL the following morning (i.e. behavior -> hormone). Due to the small number of hormone samples, the influence of variability in hormones within individuals was not assessed in this study, but with larger numbers of samples this additional analysis has been fruitful (Eccles et al., 1988).

We began these experiments expecting to find an association between melatonin production and affective behavior. This hypothesis was not born out specifically by our data. However, the idea that high melatonin secretion may be associated with reduced energy level and fatigue (Arendt et al., 1984; Lieberman et al., 1984; Sherer et al., 1985) is supported by the hormone behavior relations found in girls. High concentrations of 6-OH-MEL in girls were correlated with low self-reported indices of active childhood activities (i.e. "involved", "not depressed", outgoing and friendly" and "communal"). On the other hand, it could be that pubertal influences on energy level in girls modulate nocturnal melatonin secretion since self-reort of "energy" on Monday night was associated with Tuesday morning 6-OH-MEL according to regression analysis. Since there are reports that girls experiencing precocious puberty and normal boys and girls in later stages of puberty are more likely to report tiredness (Sonis et al., 1985; Susman et al., 1983), there may be interactions between melatonin secretion and energy levels during puberty.

Given the different ages of our boys and girls direct comparisons between the two sexes were not conducted. However, the finding that hormone-behavior relations in boys are very different than those in girls is quite interesting. Whether this reflects a developmental difference or a gender difference remains to be determined. Nevertheless, the data from the daily measures from the boys and girls illustrates very well that correlations between hormones and behavior may be in either direction. Behavior may modulate hormonal secretions as well as hormones modulating behavior. Only temporal analyses of the relations can determine the direction of such an association.

ACKNOWLEDGEMENT. A preliminary report of these findings has been published as a brief communication in Neuroscience Letters. This research was supported by a grant from the National Science Foundation to Drs. J. Eccles, J. Becker and W. Holmes. Dr. Becker is supported by a Research Career Development Award from the NIH. Are there other grants to be cited?

Table 1. Correlation Coefficients for Relations in Girls Between Mean 6-OH-MEL and Individual Values of 6-OH-MEL vs. Mood Composites from the End of Month Questionaire.

MOOD COMPOSITE	CORRELATION COEFFICIENT[1] FIRST 6-OH-MEL	LAST 6-OH-MEL	MEAN 6-OH-MEL
"involved"	-0.365*	-0.262	-0.346*
"not depressed"	-0.246	-0.349*	-0.365*
"outgoing and friendly"	-0.453**	-0.306	-0.387*
"communal"	-0.312	-0.401**	-0.502**
"body image"	0.197	0.168	0.239

1. Spearman Correlation Coefficients were obtained by comparisons between individual or mean values for 6-OH-MEL and the mean values for the mood composites described in Table 1.

* $p<0.05$
** $p<0.02$

Table 2. Correlation Coefficients for Relations in Boys Between Mean 6-OH-MEL and Individual Values of 6-OH-MEL vs. Mood Composites from the End of Month Questionaire.

MOOD COMPOSITE	CORRELATION COEFFICIENT[1] FIRST 6-OH-MEL	LAST 6-OH-MEL	MEAN 6-OH-MEL
"involved"	-0.298	-0.211	-0.219
"self-consciousness"	-0.245	-0.313*	-0.284*
"strict parents"	-0.311*	-0.184	-0.344**
"communal"	0.208	0.039	0.129
"body image"	-0.144	-0.242	-0.221

1. Spearman Correlation Coefficients were obtained by comparisons between individual or mean values for 6-OH-MEL and the mean values for the mood composites described in Table 1.

* $p<0.05$
** $p<0.02$

Table 3. Correlation Coefficients for Relations Between 6-OH-MEL and Mood Composites from the Daily Questionaire.

MOOD COMPOSITE[1]	CORRELATION COEFFICIENT[2]	
	GIRLS	BOYS
"affection"	r_s = -0.061	r_s = -0.390**
"aggression"	r_s = -0.327*	r_s = 0.04
"energy"	r_s = -0.08	r_s = 0.049
"good mood"	r_s = -0.166	r_s = 0.186
"happy"	r_s = -0.246	r_s = 0.225
"nervous"	r_s = 0.120	r_s = 0.01
"self esteem"	r_s = -0.285*	r_s = 0.077

1. Mood composites were obtained for each day. The mean of these measures was obtained for the month and used in the above analysis.
2. Spearman Correlation Coefficients were obtained by comparisons between the mean of 4 values for 6-OH-MEL and the mean values for the mood composites.

* $p<0.05$
** $p<0.01$

Table 4. Correlation Coefficients for Relations in Girls Between Tuesday morning 6-OH-MEL and Mood Composites from the Tuesday Evening Daily Questionaire (6-OH-MEL -> MOOD).

		CORRELATION COEFFICIENT[1]			
MOOD COMPOSITE	ALL TUESDAYS	WEEK 1	WEEK 2	WEEK 3	WEEK 4
"self esteem"	-0.255*	-0.299*	-0.231	-0.341*	-0.212
"happy"	-0.176	0.072	-0.245	-0.378**	-0.153
"aggression"	-0.143	-0.138	-0.286	-0.039	-0.107

1 Spearman Correlation Coefficients were obtained by comparisons between the individual values for 6-OH-MEL and the values for mood composites on each corresponding Tuesday.

* $p<0.05$
** $p<0.01$

Table 5. Correlation Coefficients for Relations in Girls Between Tuesday morning 6-OH-MEL and Mood Composites from the Monday Evening Daily Questionaire (MOOD -> 6-OH-MEL).

MOOD COMPOSITE	CORRELATION COEFFICIENT[1] ALL MONDAYS	WEEK 1	WEEK 2	WEEK 3	WEEK 4
"self esteem"	-0.135	-0.215	-0.180	-0.079	-0.10
"happy"	-0.093	0.153	-0.063	-0.026	-0.251
"aggression"	-0.038	-0.086	-0.076	-0.054	-0.033

1 Spearman Correlation Coefficients were obtained by comparisons between the individual values for 6-OH-MEL and the values for mood composites on each corresponding Monday.

Table 6. Correlation Coefficients for Relations in Boys Between Tuesday morning 6-OH-MEL and Mood Composites from the Monday Evening Daily Questionaire (MOOD -> 6-OH-MEL).

MOOD COMPOSITE	CORRELATION COEFFICIENT[1] ALL MONDAYS	WEEK 1	WEEK 2	WEEK 3	WEEK 4
"affection"	0.315*	0.306*	0.415**	0.363*	0.259*
"happy"	0.130	0.012	0.099	0.382*	0.069
"nervous"	0.207	0.145	0.172	0.359*	0.248
"aggression"	0.094	0.064	0.284*	-0.051	0.064

1. Spearman Correlation Coefficients were obtained by comparisons between the individual values for 6-OH-MEL and the values for mood composites on each corresponding Monday.

*$p<0.05$
**$p<0.01$

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