ORIGINAL ARTICLE

The Relationship Between Estrogen and the Decline in Delta Power During Adolescence

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Study Objectives: During adolescence, there is a precipitous decrease in slow-wave sleep (SWS) and its spectral correlate, delta power, which may reflect cortical reorganization. The temporal association between the decrease in delta power and puberty suggests that sex steroids may initiate these changes. This association has not been previously investigated.

Methods: To determine whether estrogen triggers the adolescent decline in delta power, we compared delta power in 14 girls with central precocious puberty (CPP) and 6 age-matched, prepubertal controls. Five CPP participants were re-studied 7–14 months after pubertal suppression to determine if the changes in delta power are reversible after restoring a prepubertal hormonal milieu. The change in delta power was also compared between CPP participants and five historic controls from a longitudinal polysomnographic study.

Results: CPP participants (6.7–10.5 years) spent 30% of the night in SWS. Delta power ($3.7 \times 10^6 \pm 2.7 \times 10^5$ μV^2) predominated in the first 2 non-rapid eye movement episodes and decayed exponentially (tau 0.006 minutes). Age-matched controls demonstrated similar sleep staging (24% SWS) and delta dynamics ($3.3 \times 10^6 \pm 5.1 \times 10^5$ μV^2, tau 0.004 minutes). Four out of 5 CPP participants had a significant decrease (26%) in delta power after hormone suppression ($p < .05$), similar to historic controls.

Conclusion: Using an innovative model of girls with CPP studied before and after estrogen suppression, the effects of puberty on the decline in delta power were dissociated from those of chronologic age. The current studies suggest that increased estrogen does not cause the adolescent decline in delta power and indicate that neurodevelopmental changes *per se* or other factors associated with puberty drive these sleep changes.

Keywords: slow-wave sleep, slow-wave activity, central precocious puberty, sex steroids.

Statement of Significance

There is a steep decline in delta power, a marker of sleep depth, during normal adolescence that is thought to result from the homeostatic "pruning" of redundant cortical synapses. The temporal concordance of this decline with normal puberty has led to speculation that sex steroids may mediate this effect. The current studies demonstrate that girls with precocious puberty, despite having premature exposure to estrogen, do not have less deep sleep or delta power than their prepubertal peers. Additional studies are necessary to determine if the change in sleep depth during adolescence represents a pre-programed neurodevelopmental sequence, or is due to other hormonal or non-hormonal changes associated with puberty.

BACKGROUND

Sleep augments hypothalamic gonadotropin-releasing hormone (GnRH) secretion in the early stages of puberty in boys and girls.[1] Gonadal sex steroids (eg, estrogen and testosterone), which increase following stimulation by GnRH-induced gonadotropin secretion, may in turn act on the adolescent brain to shape sleep staging and depth.

Longitudinal polysomnographic (PSG) studies in children age 6–18 years have identified a dramatic decline in slow-wave sleep (SWS) and its associated high levels of delta power (often called slow-wave activity [SWA] in the EEG) that begins at age 11–12 years.[2,3] The observation of a contemporaneous decline in cortical synaptic density and cerebral metabolic rate have led to the hypothesis that the change in delta power is a reflection of the maturational brain reorganization that occurs during adolescence.[4] Recent studies have suggested that these cortical changes are complement-mediated,[5] although the trigger(s) of this profound structural reorganization during adolescence are unknown.

It is noteworthy that the decline in delta power coincides with the average age of normal pubertal onset.[6] The decline in delta power occurs significantly earlier in girls than in boys,[2,3,7] mirroring sex differences in the timing of puberty. In studies that included both physician-determined Tanner staging of puberty and PSG sleep studies at 6-month intervals, Campbell et al. found that the age of most rapid delta decline was in fact related to the age of most rapid pubertal maturation, favoring a causal role for sex steroids.[8] Further, men with congenital GnRH deficiency who fail to undergo puberty have more SWS than age-matched controls and demonstrate a decrease in SWS after treatment with testosterone,[9,10] providing further support for the hypothesis that sex steroids may modulate sleep staging and depth.

The dramatic decline in delta power among young adolescents also coincides with a delay in sleep timing (ie, a tendency to stay up late).[11] While this "evening chronotype" has traditionally been attributed to environmental factors (eg, television, computers), studies demonstrating that this phenomenon is preserved under controlled laboratory conditions,[12] is cross-cultural, and predates modern technological advances[13] suggest that it is also likely to be biologically-mediated. Studies reporting an attenuated chronotype shift in gonadectomized animals[14,15] and a sleep timing delay in girls at a younger age than in boys[16] again suggest that sex steroids may be one such mediator. Other studies, however, have found that delayed sleep onset predates physical signs of puberty,[17] arguing against this hypothesis.

An important limitation of the aforementioned pediatric studies is that due to their observational design, these studies have been unable to clearly discriminate the effects of pubertal maturation from age *per se* on sleep structure or chronotype. Additional limitations include the absence of

sex steroid measurements, and in some studies,[2] the assignment of pubertal stage based entirely on the amount of pubic hair. Development of pubic hair results from the action of both increased gonadal steroids (gonadarche) and increased adrenal steroids (adrenarche), a process that is independent of gonadarche,[18] leading to a potential misclassification of pubertal stage.

To determine whether sex steroids influence sleep staging, depth, and chronotype independently of age, we studied girls with central precocious puberty (CPP) who, by definition, demonstrate a dissociation of age and normal pubertal onset. We hypothesized that if estrogen is, indeed, a biological mediator of the adolescent changes in sleep, then girls with CPP, who have early exposure to estrogen, would have later bedtimes and less delta power than age-matched, prepubertal controls. We also predicted that, because the decrease in delta power across adolescence is thought to reflect irreversible synaptic pruning, girls with CPP who have been treated with a GnRH analogue to suppress sex steroid production would not demonstrate a return of delta power to prepubertal levels.

MATERIALS AND METHODS

Study Participants

Fourteen otherwise healthy girls with CPP, defined as either Tanner II breast development before age 8 years or a history of menarche before age 10 years, and 6 age-matched, prepubertal controls were studied (Figure 1, Supplementary Table S1). Girls with CPP first came to medical attention at 7.5 ± 0.2 years of age (range 6.0–8.5) but reported a history of thelarche (breast development) at 6.8 ± 0.3 years (4.5–8.5) and pubarche (pubic hair development) at 7.1 ± 0.4 years (3.0–8.7) (Figure 1). Nearly 60% were overweight or obese, and 86% were African American or Hispanic. Obesity and minority race/ethnicity are also associated with earlier pubertal development within the normal range. (Supplementary Table S1). The diagnosis of CPP was made by a pediatrician or pediatric endocrinologist based on physical examination findings and a hormone profile indicative of central activation of the reproductive axis defined by increased luteinizing hormone (LH) at baseline or after GnRH stimulation testing. All participants who had cranial imaging (n = 7) had a normal brain MRI with the exception of one participant who had a left temporal arachnoid cyst, a structural abnormality which has been reported in association with CPP,[19] and a neuroepithelial cyst between the splenium of the corpus callosum and vein of Galen; she had no neurological symptoms such as headache, seizures, or developmental delay and no other endocrine disorders. Eight of the girls with CPP were treated with a GnRH analogue (GnRHa; leuprolide acetate [Lupron Depot] or histrelin acetate [Supprelin LA]) to halt pubertal maturation; the remainder declined treatment (Supplementary Table S2). Participants were not on any other medications that affect sleep and were not known to or suspected of having a sleep disorder based on a medical chart review and results of a validated sleep habits questionnaire completed by a parent.[20] The study was approved by the Partners Human Research Committee. Signed informed assent and consent was obtained from each participant and her parent.

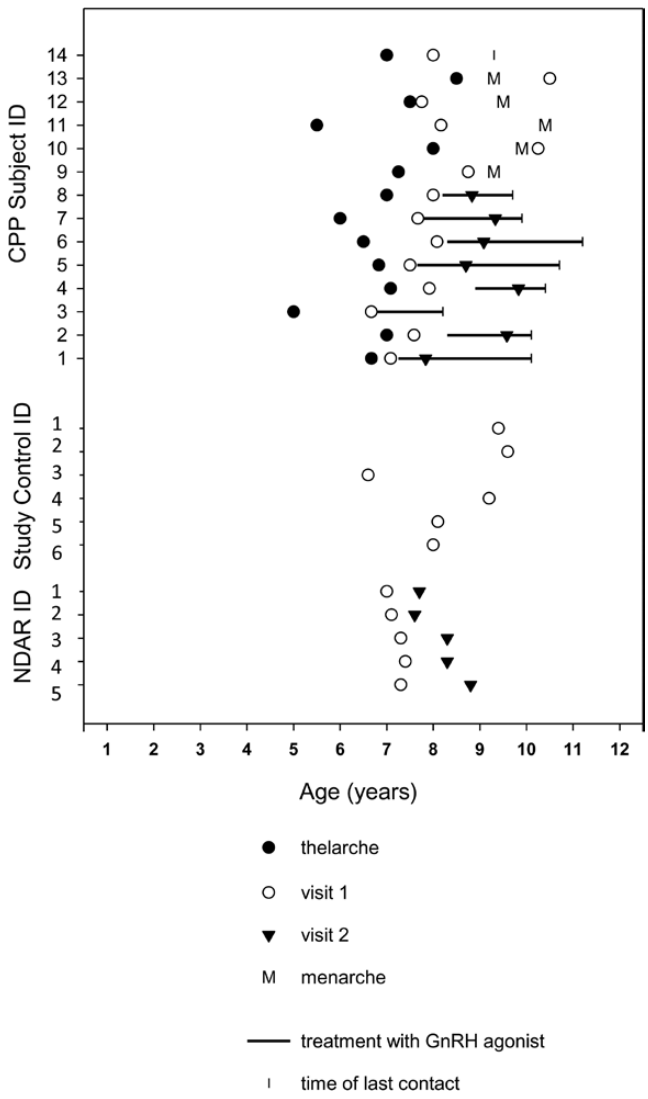

Figure 1—Pubertal milestones and GnRH agonist treatment in relation to sleep study visits in 14 girls with central precocious puberty (CPP). Age at all sleep study visits is given for study controls (n = 6) and historic NDAR controls (n = 5). All participants underwent at least one sleep study visit. Seven CPP participants were studied before (o) and after (▾) treatment with a GnRH agonist (—) to suppress sex steroid production. Time of last contact is denoted by (I), thelarche by (●) and menarche by (M).

Experimental Protocol

Overnight PSG sleep studies were conducted in participants and controls at the Clinical Research Center of the Massachusetts General Hospital in individual, dedicated sleep rooms according to standard methodology[21] using EEG (total of 6 frontal, central and occipital leads), electro-oculogram, electrocardiogram, and pulse oximetry (ALICE LE PSG system, Sleepware software, Phillips Respironics). Seven of the eight participants with CPP who underwent pubertal suppression partook in two study visits, one before and one after initiating treatment with a GnRHa. Data from one of the post-treatment study visits was lost due to equipment malfunction. The eighth participant with CPP completed the first study visit but was then lost to

follow-up. The six participants with CPP who declined treatment and the six controls underwent one PSG study.

Participants ate dinner before each sleep study. Caffeine was prohibited. PSG recording began 10 minutes before lights out and continued until natural awakening the following morning. Lights were turned off between 20:00 and 22:30, based on each participant's habitual bedtime. A single blood sample was drawn the morning after the sleep study (06:00–08:00) to measure LH, follicle-stimulating hormone (FSH), estradiol (E2) [three biochemical markers of gonadarche], and dehydroepiandrosterone sulfate (DHEAS, a biochemical marker of adrenarche). A morningness/eveningness (M/E) score, a surrogate for chronotype, was determined using the Children's ChronoType Questionnaire (CCTQ), a questionnaire completed by parents that has been validated for use in school age children.[22] Participants with M/E scores ≤ 23 were classified as morning types, 24–32 as intermediate types, and ≥33 as evening types, as previously described.[22]

Data Analysis

Sleep Stage Scoring

Each 30-second epoch was visually scored as wake, non-rapid eye movement (NREM, stages N1, N2, or N3) sleep, or REM sleep by a single registered PSG technician according to American Academy of Sleep Medicine criteria.[21] Sleep efficiency was defined as the percent of time spent in bed asleep, and wake after sleep onset (WASO) was defined as the time spent awake after the first epoch of scored sleep until final awakening. Sleep latency was defined as the duration of time from lights out to the first epoch of sleep.

Spectral Analysis

Spectral analysis was performed on girls with CPP before (n = 14) and after (n = 6) treatment and on contemporaneous controls (n = 6). Slow-wave activity (SWA; 0.5–4 Hz range) during N2 and N3 was determined using the spectral analysis program SpectralTrainFig (https://github.com/DennisDean/SpectralTrainFig) with 10 × 4-second sub-epochs, a 50% Tukey (tapered cosine) window, and a 30-second sleep staging scoring window within Matlab (MathWorks, Inc., version R2013b, Natick, MA). Artifacts were identified using SpectralTrainFig or manually as any delta power ≥ 5× the average delta power in N2 and N3 during the night and were removed. Similar analyses were performed for EEG data in the other frequency bands (theta 4.5–8 Hz, alpha 8–12 Hz, sigma 12–15 Hz, and beta 15–23 Hz). NREM–REM sleep cycles (composed of NREM episodes and REM episodes) were determined as previously described,[23] using a modified Feinberg and Floyd method that involves insertion of an epoch of REM sleep (iREM) when the first REM episode is "skipped," a common occurrence in children. To account for inter-subject differences in sleep duration, only the first 8.2 hours of sleep, the length of the shortest sleep episode observed in any subject, was included in spectral analyses.

Reproductive Hormone Assessment

Serum samples were analyzed for LH, FSH, and E2 using a chemiluminescent microparticle immunoassay (CMIA; Architect, Abbott Diagnostics). The Architect CMIA has a minimum detectable concentration (MDC) of 0.07 IU/L for LH and coefficients of variation (CVs) of < 5% for quality control sera (QCS) containing 4–50 IU/L. The MDC for FSH is 0.05 IU/L and CVs are < 5% for QCS containing 5–75 IU/L. LH and FSH levels are expressed in international units per liter as equivalents of the Second International Pituitary Reference Preparation (80/552 for LH and 78/549 for FSH). The MDC for the E2 assay is 10 pg/ml, and the interassay CVs are 9.6% and 3.9% for QC samples containing 36 and 184 pg/ml, respectively. The E2 assay has been standardized and calibrated against liquid chromatography/tandem mass spectrometry.[24] DHEAS was measured using an immunoassay (Quest Diagnostics).

Statistical Methods

To determine the dissipation of SWA across the night for each participant, the SWA for each NREM episode was first expressed as the average SWA during the episode divided by the average SWA across the night × 100 and plotted at the midpoint of the episode. A decaying exponential function was then applied, as previously described,[25] to determine the decay rate (tau) of delta power across the night, SWA at sleep onset (SWA_0) (eg, y-intercept or amplitude), and SWA at the horizontal asymptote of the decline (SWA_∞). Total SWA per night and the percent of SWA that occurred during the first two NREM episodes were also calculated and compared across study groups.

Differences in sleep staging, SWA, and hormonal levels between controls and CPP participants or between CPP participants during visit 1 and visit 2 were analyzed using ANCOVA, controlling for age. Comparisons between group decay rates were performed using an exponential mixed model fitted to the delta values with group, time, and group × time interactions. Correlations between total delta power and hormone levels were determined using Pearson correlations. Undetectable hormone levels were assigned the lower limit of detection of the assay (eg, E2 < 10 treated as 10).

To determine whether the change in delta power observed in CPP participants between visits 1 and 2 is different from what is observed during the course of normal adolescence, the percent change in delta power was calculated and compared between CPP participants and historic controls. Delta power measurements from five historic age-matched healthy female controls who underwent in-home PSG studies every 6 months from age 6 to 10 years as part of the University of California (UC) Davis Sleep Laboratory's longitudinal study of sleep and EEG changes across adolescence were obtained from the NIH-supported National Institute of Mental Health (NIMH) Data Repositories (NDAR) (10.1073/pnas.0812947106). Note that because a different spectral analysis software program was used in the NDAR study, only the percent change in delta power, but not raw delta power measurements, were compared across contemporaneous and historic participants.

All data are expressed as mean ± SE (range) unless otherwise indicated, and $p < .05$ is considered significant.

RESULTS

Reproductive Phenotyping

CPP Participants Sleep Study 1 (Pre-treatment)

Participants were admitted for the first sleep study at age 8.2 ± 1.1 years (6.7–10.5), 7.1 ± 2.3 months (2.0–27.0) after the diagnosis of CPP (Figure 1, Supplementary Table S1),

before receiving any suppressive treatment. They had Tanner II-V breasts and Tanner I-V pubic hair, and two (#10, #13) were post-menarchal (Table 1). Basal (un-stimulated) reproductive hormones measured the morning after the first sleep study were consistent with CPP (LH > 0.3 IU/L[26,27] in 11 of 14 participants). Of note, a low basal LH does not exclude CPP; the sensitivity of this test has been reported to be as low as 60%.[28] Only seven of the CPP girls with pubic hair (Tanner stage > II) demonstrated concentrations of DHEAS consistent with adrenarche (> 40 mcg/dL) and thus pubic hair development may have been due to a combination of adrenal and ovarian steroids. Low DHEAS levels in the remaining girls suggest that sexual hair development was due to ovarian androgen production alone.

CPP Participants Sleep Study 2 (Post-treatment)

Six participants declined suppressive treatment and did not complete a second study visit. Seven of the remaining eight participants who underwent pubertal suppression with a GnRH analogue completed a second sleep study 10.0 ± 1.0 (7–14) months after treatment initiation, on average 12.7 ± 2.0 (8–24) months after the first study visit (Figure 1). One participant was lost to follow-up. All of these participants had been pre-menarchal at the first visit and remained so. Breast tissue regressed, remained stable, or progressed by 1 Tanner stage. Progression was associated with a delay in initiating suppressive treatment after the first sleep study. Reproductive hormone testing revealed a low morning LH and/or a high FSH to LH ratio and undetectable E2 levels (Table 1, Supplementary Table S2), consistent with pubertal suppression. DHEAS levels increased between the two study visits in all participants, consistent with previous studies demonstrating normal progression of adrenarche in children with CPP during GnRHa treatment.[29]

Age-Matched Contemporaneous Study Controls

The six controls were 8.6 ± 0.5 years (6.6–9.6) at the time of the sleep study and were predominantly of normal weight (67%) and Caucasian (83%). They were pre-menarchal and had no clinical or biochemical signs of central puberty (eg, vaginal discharge, accelerated linear growth, breast development). One girl (#6) had previously been diagnosed with premature

Table 1—Pubertal Development and Sex Steroid Levels in Girls With Central Precocious Puberty (CPP) and Control Participants.

	VISIT 1				VISIT 2[a]			
	Tanner stage breast/pubic hair	LH (IU/L)	E2 (pg/ml)	DHEAS (mcg/dL)	Tanner stage breast/pubic hair	LH (IU/L)	E2 (pg/ml)	DHEAS (mcg/dL)
CPP								
1	III/I	0.2	22	43	III/III	0.7	<10	61
2	II/III	0.4	<10	39	III/IV	0.2	<10	61
3	II/II	0.8	20	31	—	—	—	—
4	II/II	0.1	<10	59	III/IV	0.4	<10	103
5	III/III	0.3	<10	40	I/III	0.3	<10	68
6	III/III	1.8	45	12	III/III	0.2	<10	23
7	V/III	3.0	66	28	IV/III	0.8	<10	41
8	III/III	4.5	65	20	III/IV	0.3	<10	22
9	III/III	4.0	41	96	—	—	—	
10	V/V	4.6	34	—	—	—	—	
11	III/III	<0.1	<10	42	—	—	—	
12	II/I	2.5	33	60	—	—	—	
13	V/IV	2.3	70	83	—	—	—	
14	III/III	2.3	39	28	—	—	—	
Controls								
1	I/I	0.1	12	37	—	—	—	
2	I/I	0.3	<10	42	—	—	—	
3	I/I	<0.1	<10	35	—	—	—	
4	I/I	<0.1	12	47	—	—	—	
5	I/I	0.2	<10	64	—	—	—	
6	I/III	0.1	<10	122	—	—	—	

LH = luteinizing hormone; E2 = estradiol; DHEAS = dehydroepiandrosterone sulfate, "—" = test not performed.
[a]Visit 2 was conducted after pubertal suppression with a gonadotropin releasing hormone agonist in CPP participants 1, 2, and 4–8.

adrenarche based on the presence of pubic hair at age 7.5 years, and three other girls (ages 8.1–9.6 years) appeared to be in the early stages of normal adrenarche based on a DHEAS level > 40 mcg/dL in the absence of sexual hair (Table 1).

Sleep Staging in CPP Participants and Study Controls

During visit one (Pre-treatment), CPP participants slept an average of 8.9 ± 0.2 hours (7.8–10.5) and demonstrated sleep staging typical for age[30] (Figure 2, Supplementary Table S3) with 399.0 ± 12.7 minutes (321.5–504.0) spent in NREM sleep, 100.6 ± 5.1 minutes (73.5–132.5) in REM sleep, and 32.1 ± 6.6 minutes (11.5–91.5) awake after sleep onset. Sleep was well-consolidated with a sleep efficiency of 94.0% ± 1.2% (83.3%–97.8%). Participants fell asleep between 20:51 and 23:30 with a sleep latency of 37.6 ± 23.6 minutes (7.0–80.0). When re-studied 8–24 months later while receiving pubertal suppressive therapy (visit 2), CPP participants did not demonstrate any significant differences in sleep duration (9.0 ± 0.4 hours [8.1–10.0]), number of minutes or percent time spent in each sleep stage (Figure 2), sleep efficiency (95.8% ± 1.8% [89.3–98.7]), or sleep latency (11.8 ± 6.6 minutes [0.0–37.0]) relative to their pre-treatment study visit.

Study controls slept an average of 9.1 ± 0.2 hours (8.3–9.8), similar to the CPP participants and typical for age[30] (Figure 2, Supplementary Table S3). Time spent in NREM sleep was 403.8 ± 18.4 minutes (384.0–423.5), in REM sleep was 109.5 ± 5.7 minutes (94.5–130.0), and WASO was 34.1 ± 12.6 minutes (5.0–89.0), a sleep pattern that also did not differ from participants with CPP (Figure 2, Supplementary Table S3). Sleep efficiency was 94.0% ± 2.1% (84.9–99.0), and participants fell asleep between 20:37 and 22:28 with a sleep latency of 20.5 ± 7.1 minutes (11.0–28.0).

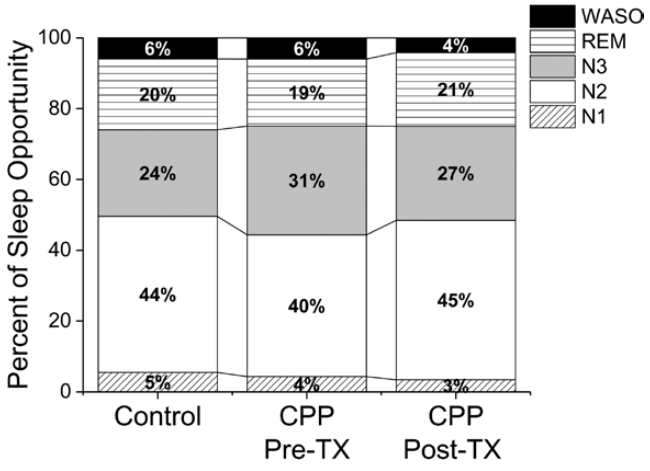

Figure 2—Average percent distribution of sleep staging during the sleep opportunity for the control, central precocious puberty before treatment (CPP Pre-TX) and central precocious puberty after treatment (CPP Post-TX) groups. WASO = Wake after sleep onset; REM = Rapid Eye Movement; N3 = Non-REM stage 3; N2 = Non-REM stage 2; and N1 = Non-REM stage 1. There were no significant differences between percent of time spent in each sleep stage between conditions (control vs. CPP Pre-TX or CPP Post-TX) or visits (CPP Pre-TX vs. CPP Post-TX, all $p > .05$).

Spectral Power in CPP Participants, Study Controls, and Historic NDAR Controls

The CPP participants had an average total delta power of $3.7 \times 10^6 \pm 2.7 \times 10^5$ μV² (range $1.9–5.4 \times 10^6$) before treatment, with the majority of delta power (66.8% ± 0.03%) falling within the first two NREM episodes. Age-matched, prepubertal study controls demonstrated a similar magnitude and distribution of delta power ($p = .39$ and $p = .21$, Figure 3, A and B).

After 7–14 months of minimal estrogen exposure due to GnRHa treatment (visit 2), delta power was significantly lower ($p < .05$) than at visit 1, decreasing by 32.6 ± 8.1% in four CPP participants and remaining stable in the fifth CPP participant (Figure 3, A, B, and C).

There were no significant differences in the other frequency bands (theta, alpha, sigma or beta) between study controls and CPP participants before or after GnRHa-treatment (Supplementary Table S4).

To determine whether the decrease in delta power observed in CPP participants in association with estrogen suppression equals or exceeds the natural decline in delta power of early adolescence, we compared the percent change in delta power in CPP participants between visits 1 and 2 with the percent change in delta power over a 6–12 month period in age-matched female controls from the NDAR database. These historical controls demonstrated variable delta power trajectories, from a 37% decrease to a 66% increase in delta power, indicating that the change in delta power observed among CPP participants falls within the expected range for girls in this age group (Figure 3C).

We next compared delta activity dynamics across the night in CPP participants at visit 1, CPP participants at visit 2, and study controls (Figure 4). All three groups showed an exponential decline in delta power, a reflection of dissipating sleep pressure, with no differences in decay constants (0.004, 0.006, and 0.003 minutes), SWA_0 (204.3%, 257.4%, and 258.1%), or SWA_∞ (12.6%, 37.2%, and 48.2%) between study controls, CPP participants at visit 1, and CPP participants at visit 2, respectively.

In a combined analysis of CPP participants and study controls, we found no correlation between DHEAS levels and delta power ($r = -0.38$, $p = .1$) or between E2 levels and delta power ($r = -0.01$, $p = .9$).

Chronotype in CPP Participants and Study Controls

CPP participants and study controls demonstrated a similar distribution of chronotype scores, with the average score in both groups falling within the "intermediate" category, defined as scores of 24–32 (CPP: 27.5 ± 1.7 [20–43]; controls: 25.2 ± 3.1 [17–33]). Suppressive therapy with a GnRHa and/or the passage of time had no effect on CPP participants' morningness/eveningness preference, and chronotype did not correlate with chronologic age or stage of breast development in combined analyses of CPP participants and study controls ($r = -0.06$, $p > .05$ for both tests).

DISCUSSION

The temporal association between adolescence and activation of the reproductive axis suggests that sex steroids may be one trigger of the structural reorganization of the cortex and subsequent decline in delta power. However, there has been no

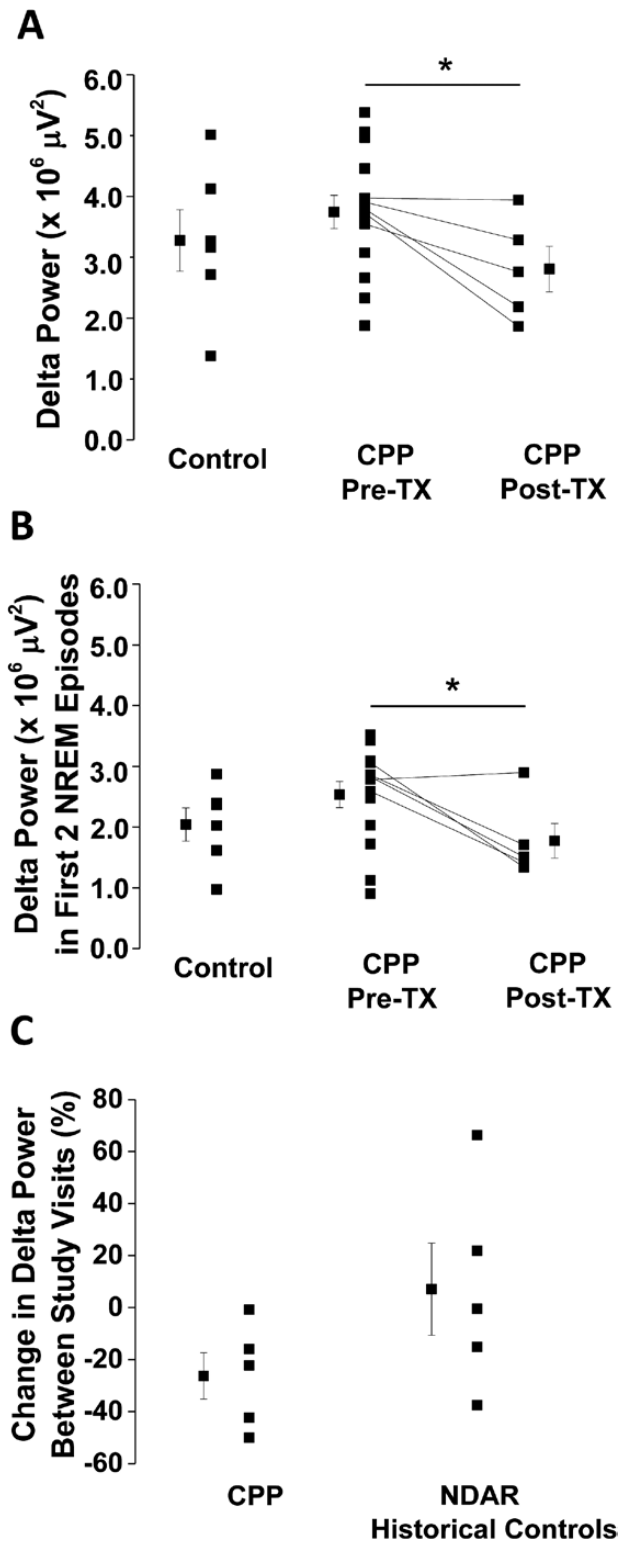

Figure 3—Delta power across the entire sleep opportunity (A) and in the first 2 NREM episodes (B) for the study controls, central precocious puberty subjects before treatment (CPP Pre-TX) and central precocious puberty subjects after treatment (CPP Post-TX). The percent change in delta power between study visits for CPP subjects and NDAR historic controls is shown in (C). Subject-level data is connected by a line and the off-set black squares indicate mean ± SEM. Asterisks denote significant differences between groups ($p < .05$).

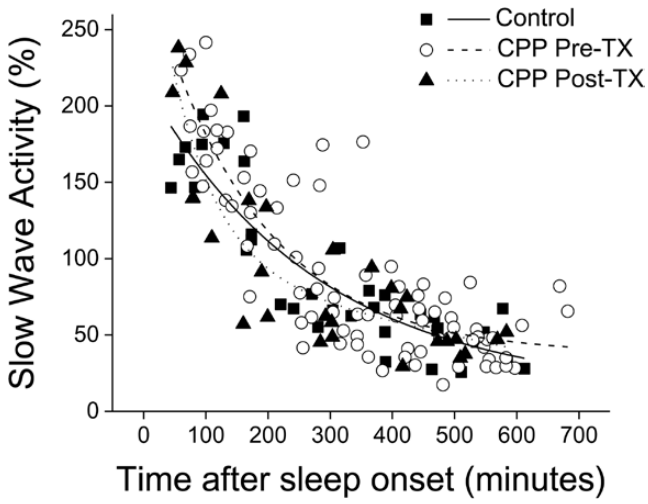

Figure 4—Decay of slow-wave activity across the sleep opportunity for the control (▪), central precocious puberty before treatment (CPP Pre-TX, o) and central precocious puberty after treatment (CPP Post-TX, ▲) groups. Data are plotted on the x-axis as the midpoint of each non-REM (NREM) episode and on the y-axis as percent of the average SWA in stages NREM 2 and 3 across the entire sleep episode. Lines represent the exponential decay function for control (−), CPP Pre-TX (- - -), and CPP Post-TX (. . . . .) groups. There were no significant differences in decay rates between groups ($p > .05$).

previous attempt to disentangle the effects of chronologic age and puberty on delta power by studying children with disorders of puberty. Using a model of girls with CPP, the current studies demonstrate that premature estrogen exposure is not associated with a premature decrease in delta power and that estrogen withdrawal, through hormone suppression with a GnRHa, does not alter the physiologic decline in delta power in girls. Taken together, these data suggest that estrogen does not mediate the dramatic decline in delta power during adolescence.

The current studies utilized a unique study design comparing participants with CPP and age-matched, prepubertal controls. Girls with CPP represent a natural model of premature estrogen exposure. Importantly, girls with CPP recapitulate the normal physiology and sequence of events in female pubertal development (breast buds, pubic hair, peak height velocity, followed by menarche) with the only difference being a shift in the timing of pubertal onset. Thus, with this model, estrogen exposure is both endogenous and physiological and can be quantitated with serum measurements and correlated with physical exam findings.

Although focused on estrogen exposure, the current studies do not support a role for other reproductive hormones in the decline in delta power during adolescence. In CPP, premature activation of the hypothalamic GnRH neuronal network is the inciting event, stimulating pituitary secretion of FSH and LH, which then trigger ovarian estrogen production. While the GnRH neuropeptide has traditionally been thought to be confined to the hypothalamic median eminence, the identification of the GnRH peptide[31] and the GnRH receptor[32] in human cerebral cortex indicates the potential for extra-hypothalamic functions. In the current studies, however, premature exposure to GnRH

was not associated with an earlier decline in delta power, arguing against a role for GnRH in adolescent brain maturation. As female puberty is accompanied by a modest rise in ovarian-derived testosterone in girls with normally-timed[33] or precocious puberty,[34] the current model can be extended to determine the effect of premature testosterone exposure on delta power. While we did not measure testosterone levels in the current studies, our data suggest that, at least in girls, testosterone is unlikely to be responsible for the decline in delta power during adolescence.

We also found no association between peripheral blood DHEAS levels, the primary biochemical marker of adrenarche, and delta power in combined analyses of girls with CPP and study controls. A potential role for an adrenal-derived hormone in the decline in delta power during adolescence was suggested by Campbell et al. based on the strong association between the age of most rapid delta decline and the age of most rapid increase in pubic hair in girls.[8] Note that while the same association was observed in the boys in that study, pubic hair develops in boys in response to both testicular and adrenal-derived androgens, whereas in girls with normally-timed puberty, pubic hair typically reflects adrenarche. Furthermore, whereas DHEAS has been shown to act centrally in animal models,[35] in humans, DHEAS in peripheral blood does not easily penetrate the blood brain barrier.[36] Thus, the neuroactivity ascribed to DHEAS likely comes from hormone synthesized de novo within the brain[37] rather than from the adrenal. Thus, it is quite possible that central and peripheral DHEAS profiles during development are discordant due to different control mechanisms. Taken together, it is unlikely that DHEAS plays a major role in the decline in delta power during adolescence.

Our finding of similar chronotype scores in girls with CPP and prepubertal controls also fails to support the hypothesis that sex steroids induce a change in chronotype during adolescence. This hypothesis stems from cross-sectional studies in adolescents demonstrating an association between more advanced pubertal stages and a delay in circadian phase preference[11] and is supported by rodent studies linking sex steroids to circadian physiology.[15] Importantly, previous studies of chronotype changes during adolescence suffered not only from the limitations inherent to a cross-sectional study design, but performed pubertal staging according to pubic hair rather breast development, which as previously discussed, does not reflect central puberty or correlate with estrogen exposure.

The current studies had several limitations that may have affected our conclusion. In order to create a relatively homogenous CPP cohort, we utilized very strict inclusion criteria which limited our sample size and hence the power to detect differences in sleep between CPP participants and study controls. Campbell et al. demonstrated a 20% decline in delta power (*SD* 14.7%) in boys and girls between the ages of 11 and 13 years (from[3] and personal communication with authors). Using this *SD*, we determined that with six CPP participants, we would have sufficient power (0.8) to detect a 17% within-participant increase in delta power between study visits. As we studied 14 girls with CPP and six controls, the results of our study are unlikely to be due to a type II error. Our study controls were rigorously determined to be prepubertal both clinically and biochemically and were age-matched but were not matched according to BMI or race. As we did not have longitudinal data on controls to compare with data from CPP participants before and after GnRHa treatment, we relied on normative EEG data from the NDAR dataset that was collected using in-home PSG. These study limitations therefore mandate replication in a larger group of CPP and control subjects who have been carefully phenotyped and followed longitudinally. While CPP participants were studied on average 1.3 years after breast development, it is also possible that not all girls were exposed to estrogen levels of sufficient magnitude or duration to induce changes in delta power; several (n = 5) girls had either undetectable estrogen levels or were still in early puberty (Tanner II breast development) at the time of the first sleep study. However, delta power was not inversely correlated with pubertal stage and therefore it is unlikely that inadequate estrogen exposure would account for our findings. Lastly, we did not control for potential differences in sleep duration or bedtime before the sleep study visit and did not include an in-hospital adaptation night which may have introduced additional variability into the single study night measurements. However, the recent demonstration that sleep restriction, achieved by delaying bedtime, in young adolescents does not alter delta power on the night of restriction or two nights later[38] provides some reassurance that any differences in sleep duration or bedtime before the study visit are unlikely to have altered our results.

In conclusion, using a unique model of girls with CPP to dissociate the effects of estrogen exposure from chronologic age, we find that estrogen does not appear to play a significant role in the dramatic decline in delta power that characterizes normal adolescence, nor do we find evidence for a connection between estrogen exposure and circadian phase delay. As with other neurodevelopmental milestones, the structural brain reorganization of adolescence that results in changes in delta power and circadian phase is most likely programmed very early in life but may be influenced by prenatal exposures and/or genetic variation. Recent genetic and molecular biology studies implicating microglia and the complement cascade in cortical remodeling[5] point to variation at the level of the brain's resident immune system as a natural starting point for further investigation.

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SUPPLEMENTARY MATERIAL

Supplementary data are available at *SLEEPJ* online.

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