Subjective and Objective Sleep Characteristics, Reward-Related Brain Function and Development in Adolescents.

Stephanie Holm
Mentor: Erika E. Forbes, Assistant Professor, Department of Psychiatry, University of Pittsburgh

Background: The onset of adolescence is a time of dramatic changes, including changes in sleep, and a time of new health concerns related to increases in risk-taking, sensation seeking, depression, substance use, and accidents. Therefore, a more complete understanding of the brain regions involved in these risky decisions and how those regions relate to the sleep changes and the hormonal changes that occur in adolescence will help us in the future to better care for adolescents. One piece of this puzzle is reward-related behavior, and at an even more basic level, response to reward in the brain, specifically the striatum, an area which has been frequently implicated in reward processing. My project explores response to reward as measured by fMRI, the relationship between sleep and reward, between puberty and sleep and between puberty and reward.

Methods: I analyzed and interpreted data for four different sets of analyses. The first looked at reward-related brain activation and its relationship to the sleep changes that occur during adolescence. The second was a longitudinal study looking at the changes in sleep architecture during adolescence and how those changes relate to age and puberty. The third set of analyses looked at developmental changes in sleep comparing healthy adolescents to those with psychopathology. The fourth looked at the relationship between reward-related brain function, sensation-seeking and testosterone levels.

Results and Conclusions: Sleep patterns usually associated with puberty (less sleep, later sleep and self-reported worse sleep) were related to less activation in the caudate in response to reward. As our group also showed that less activation in the caudate was associated with pubertal development, this may imply that puberty and the sleep changes that come with it could have a compounding effect, further decreasing caudate activation and leading adolescents to strive for more rewards to reach an appetitive threshold. However, I’ve also provided evidence that not all changes in sleep are puberty-related; the decrease in slow-wave sleep that occurs in adolescence seems to be more related to age. This may be because decreasing slow-wave sleep is related to synaptic pruning and cognitive processes, whereas the changes described above are more related to puberty and emotional processing, such as responses to reward. As further evidence that reward processing is related to puberty, I’ve shown that responses on a validated sensation seeking scale are related to activation in the caudate and that, moreover, that response seems to be a function of testosterone level. Finally, I’ve made clear the importance of a developmental perspective in evaluating sleep in healthy adolescents and those with psychopathology.
Final Report Cover Page

Ethical Status (Check One)
___ My scholarly project did not involve work with human subjects or animals.
___ My scholarly project was reviewed by the University of Pittsburgh IRB and determined to be exempt.
___X_ My scholarly project was approved by the appropriate University of Pittsburgh/UPMC review board (a number must be provided below).

IRB approval #: ___0503010_and_020705___
IACUC approval #: __________
CORID approval #: __________
QA/QI approval #: __________

Student Signature: [Signature] Date: 2/9/11

Mentor Approval: By signing below I attest that I have worked with the above named student longitudinally on his/her scholarly project. I further attest that all scholarly project work was completed in accordance with the appropriate University of Pittsburgh/UPMC review board for human subjects, animals, QA/QI and/or CORID. I have reviewed this final report together with the criteria for evaluation of scholarly project final report and feel the student has satisfactorily completed the scholarly project requirement.

Mentor Signature: [Signature] Date: 2/9/11
(B) Project Bibliography

Published Paper

• Holm SM, Forbes EE, Ryan ND, Phillips ML, Tarr JA and RE Dahl. “Reward-Related Brain Function and Sleep in Pre/Early Pubertal and Mid/Late Pubertal Adolescents,” Journal of Adolescent Health, 45 (2009); 326-334

Papers in Progress


Conference Presentations and Awards

• Sleep Research Society 2010 Honorable Mention Abstract Award for the scientific merit of the abstract “Adolescent Slow-wave Sleep Decreases with Age Rather than Puberty in a Longitudinal Sample.”
(C) Summary of Scholarly Activity

Significance of the Project
The importance of this project was in the ability to bring together a combination of variables: puberty, sleep (both objective and subjective measures, as well as both at-home and in-lab assessment), and real-life mood and brain function. For deeper exploration of the significance of this project, please see the four attached papers and the editorial describing some of my work that is attached as part of the addendum.

Approaches taken to accomplish project goals
Analyses of data from multiple different studies allowed for a thoughtful exploration of these relationships. Study methods used included fMRI, EEG, actigraphy, self-report forms and blood spot and hormone assay. For methodological details, please see the methods sections of each of the four papers attached.

Independence of the Student
Though my mentor informed me of the datasets available in the lab, I formed all the hypotheses and spearheaded the analyses of all the projects within my SP. I also wrote the first draft of everything that I was a first author on, and also took the lead in making revisions when we received them. Furthermore, I helped with other data analysis in the lab, and taught other members of the lab how to use multiple new pieces of software that improved efficiency in our lab. I was also involved in a limited amount of data collection and in some preprocessing of data.

Project Originality
Even though both the sleep changes in adolescence and the increase in risky behavior are well-described, the relationships between them had not previously been described. Furthermore, the relationship between each of these and puberty had still not been fully developed. This project added to scientific knowledge in these areas. For further discussion of the originality of some of this work, please see the editorial attached to the addendum (which references my work extensively).

Project Limitations and Possible Future Directions
As in any studies which utilize fMRI, one major limitation is the fact that brain connectivity is complex and brain activation in discrete areas doesn’t directly map to single functions. Therefore, caution is always warranted in interpreting fMRI studies, including mine—as reward-related brain functioning is undoubtedly more complex than we were able to capture the relationships to sleep and to sensation seeking have to be viewed in that light. Similarly, the technologies that we used to measure sleep (polysomnography, actigraphy and self-report) can only approximate the full experience that combines to be sleep, though we try to get as complete a description as we can by including both objective (PSG or actigraphy) and subjective (self-report) data.

Special Considerations
None.
**Contribution to Analytical Skills**

In working with my mentor and postdocs in the lab, I have greatly improved my statistical skills, which helps me to read the literature much more critically. I now have much experience using general linear models, as well as some experience with repeated measures analysis. I’ve also learned how to do preprocessing and first and second level analyses of fMRI data, and I’ve learned about the techniques used in analyzing longitudinal development data. I have also improved as a scientific writer. Also, the process of overcoming problems we encountered (such as an early error in computing sleep variables that had been made in our lab) taught me the value of thoughtful planning and knowing your data at all possible levels.
(D) Product: Scholarly Project Papers and Presentations

Papers attached are:

• Holm SM, Forbes EE, Ryan ND, Phillips ML, Tarr JA and RE Dahl. “Reward-Related Brain Function and Sleep in Pre/Early Pubertal and Mid/Late Pubertal Adolescents,” *Journal of Adolescent Health*, 45 (2009); 326-334

Abstracts attached are:

(E) Addendum

I have continued to work on sub-projects within my SP throughout the course of my four years of medical school, as evidenced in my bibliography. These have continued to shed light on my broader questions of the relationships between sleep, reward and pubertal development during adolescence.

For further discussion of the significance of my 2008 paper, see the following editorial, published in the same issue of the journal:

Reward-Related Brain Function and Sleep in Pre/Early Pubertal and Mid/Late Pubertal Adolescents

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Manuscript received December 23, 2008; manuscript accepted April 2, 2009

Abstract

Purpose: The onset of adolescence is a time of dramatic changes, including changes in sleep, and a time of new health concerns related to increases in risk-taking, sensation seeking, depression, substance use, and accidents. As part of a larger study examining puberty-specific changes in adolescents’ reward-related brain function, the current article focuses on the relationship between functional neuroimaging measures of reward and measures of sleep.

Methods: A total of 58 healthy participants 11–13 years of age completed a functional magnetic resonance imaging scan using a guessing task with monetary rewards and 4 days of at-home actigraphy and self-reported sleep ratings. Sleep variables included actigraph measures of mean weekend minutes asleep, sleep onset time, and sleep offset time, as well as self-reported sleep quality.

Results: During reward anticipation, less activation in the caudate (part of the ventral striatum) was associated with fewer minutes asleep, later sleep onset time, and lower sleep quality. During reward outcome, less caudate activation was associated with later sleep onset time, earlier sleep offset time, and lower sleep quality.

Conclusions: It has been hypothesized that adolescents’ low reactivity in reward-related brain areas could lead to compensatory increases in reward-driven behavior. This study’s findings suggest that sleep could contribute to such behavior. Because decreased sleep has been associated with risky behavior and negative mood, these findings raise concerns about a negative spiral whereby the effects of puberty and sleep deprivation may have synergistic effects on reward processing, contributing to adolescent behavioral and emotional health problems. © 2009 Society for Adolescent Medicine. All rights reserved.

Keywords: Adolescence; Puberty; Sleep; fMRI; Reward

The onset of adolescence is a time of dramatic physical, cognitive, emotional, behavioral, and social changes. With puberty also comes a new set of health concerns—rapid increases in rates of risk-taking, sensation seeking, depression, substance use, and accidents. Accordingly, there is growing interest in understanding the neurobehavioral underpinnings of these changes, with a particular focus on pubertal increases in risk-taking and sensation seeking [1,2].

The onset of adolescence is also a time of both physiological and social changes that affect sleep [3]. Of particular concern is evidence that sleep deprivation appears to be nearly epidemic among adolescents [4], along with a growing recognition of the importance of sleep for physical health and cognitive and affective function. This myriad of changes in

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early adolescence raises a series of questions about the interactions between domains. One step toward understanding these important questions is to examine the interrelationships among pubertal maturation, neural systems of reward, and sleep patterns in healthy adolescents—the focus of this article.

Health risks associated with reward-related changes at puberty

Puberty is strongly associated with an increase in health consequences related to risky behaviors including substance use, accidents, and sexual behavior [5,6]. Part of this increase in risky behavior appears to reflect pubertal increases in sensation seeking [2,6], which is hypothesized to be related to some maturational changes in aspects of reward seeking. Thus, changes in neural systems of reward processing in adolescence may underpin these behavioral tendencies [7], and there is growing interest in understanding puberty-specific changes in these systems [5,8]. A second area of health problems that may be related to pubertal changes in reward systems is the large increase in the incidence of affective disorders at adolescence [9–11]. There is a growing body of evidence showing that sleep problems predict the onset of depression across the lifespan, and there is evidence of a bidirectional relationship between sleep and mood [12,13]. Moreover, it was recently shown prospectively that chronic insomnia in adolescents increases the risk of affective problems [14], making a study of the relationship between reward and sleep even more relevant.

Adolescent sleep

According to the model developed by Carskadon [3], physiological and psychosocial factors combine in adolescence to make sleep later, shorter, and different between weekends and weekdays. There is also a tendency for a delay in the circadian timing of sleep [3,15,16]. This natural preference for a more delayed sleep timing typically manifests as later sleep onset and offset and decreasing amounts of sleep at night [3,17]. Taken together these contribute to a well-recognized set of problems in adolescents: late night and erratic sleep/wake schedules and insufficient sleep on school nights. Not only do adolescents typically obtain less nighttime sleep than children [4,15], they also tend to be sleepier even if they obtain as much sleep as children [18], suggesting that they need more sleep. This raises further concerns about the short- and long-term health consequences from obtaining insufficient sleep in adolescence—with a particular emphasis on mood, irritability, risk-taking, and accidents [12,13,19–21].

Sleep, reward, and risk-taking

Studies examining the relationship between sleep and decision making [19] have reported that insufficient sleep is associated with changes in reward-related decision making: people take greater risks and are less concerned with negative consequences [20]. However, most of these studies have focused on adults and on measures in laboratory settings. These effects may be amplified in adolescence with the high rates of both risk taking and sleep deprivation. Therefore, it is important to investigate these questions in adolescents in natural sleeping environments.

Neural correlates of reward processing in adolescents

The striatum, part of the brain’s basal ganglia [22], has been shown to be an important region for reward-related brain function, including positive emotions, motivation to pursue reward, and response to reward [23–25]. The caudate, putamen, and nucleus accumbens are parts of the well-connected striatum [22], which undergoes structural and functional change during adolescence; it has many gonadal steroid receptors and develops over the course of puberty [26]. Adolescents exhibit reward-related brain function in many striatal regions, with several studies reporting differing striatal reactivity to reward in adolescents compared with other age groups [7,27]. A limited number of studies have looked at the relationship between puberty and striatal reactivity [8].

Hypotheses

Because age and puberty are correlated in adolescents and age is measured more precisely and easily, many studies do not disentangle the effects of age from puberty-specific effects [7,27]. Given our interest in puberty-specific changes in neural systems of reward, this study was designed from the outset to allow us to directly examine the effects of pubertal maturation by recruiting subjects in a narrow age range, but varying in pubertal development. In a parallel paper from this study (focusing on puberty-specific changes in reward processing) adolescents who were mid/later pubertal showed less reactivity in reward systems compared with their pre/early pubertal counterparts and compared with adults [8]. In that article, we interpreted our findings as suggesting that less striatal reactivity to reward could lead pubertal adolescents to seek greater levels of excitement. Based on those previous findings, we hypothesized that sleep characteristics associated with adolescent maturation would be associated with alterations in striatal reactivity in response to reward. Because of the possible influence of pubertal development on both sleep and reactivity to reward, we included sexual maturation in our model for sleep and reward processing.

Methods

Participants

All participants provided informed consent according to the guidelines of the University of Pittsburgh Institutional Review Board. Adolescents were recruited from the community through advertisements, flyers, and demographically targeted phone lists. Participants were recruited to be in a narrow age range (11-13 years) but vary in pubertal development. Because on average girls are mid-puberty at
Participants completed daily sleep diaries [34] to report time they went to bed, time they fell asleep, who woke them up, how well they slept, and how difficult it was to wake up. One of the visual analogue scale variables from the sleep diaries, sleep quality (assessed from very bad to very good) was included in our analyses. Reward processing. The fMRI paradigm [10] was an adaptation of a card-guessing paradigm [35] to probe striatal response to reward, with a section to measure anticipation of reward as well as outcome.

During the first 3 seconds of each 27s trial, participants had to guess, via button press, whether the value of a visually presented card with a possible value of 1-9 was higher or lower than five (decision making phase). During the next 12 seconds, the trial type (either reward/neutral or loss/neutral) was presented visually. (During a reward/neutral trial it was impossible to lose money and during a loss/neutral trial it was impossible to win money; anticipation phase.) This was followed by the “actual” numerical value of the card (500ms); outcome feedback (a green upward-facing arrow for win, a red downward-facing arrow for loss, or a yellow circle if they did not win or lose money that trial; 500ms); and a crosshair presented for 11s (outcome includes the actual value, outcome feedback, and first 8 seconds of the crosshair). The baseline condition is the final three seconds of staring at the crosshair before the next trial commences. Trials were presented in four runs, with 12 trials per run and a balanced number of outcome trial types within runs.

As has been previously done with this task [10, 35], participants were told that they would receive $1 for each win, lose 50 cents for each loss, and experience no earnings change for neutral outcomes. Participants were unaware of the fixed outcome probabilities (each participant had $12 of winnings). During practice and between runs, participants’ engagement was maintained by verbal encouragement to stay on task. To maximize sample size, data from only run one were included in analyses. Also, focusing on run one minimizes the influences of fatigue and habituation that can occur with repeated runs [10].

Procedure

Each participant completed a lab session including an fMRI scan and training in using the actigraph and completing sleep diaries. The 4-day sleep study took place on the following weekend (90% after scan; M = 2.86 days, SD = 3.19). We verified that the subjects who completed the sleep study before the scan did not systematically bias our results.
Data Scoring, Processing, and Reduction

Actigraphy-measured sleep. Participants were instructed to wear the actigraph on their non-dominant wrist from Friday afternoon at 4 pm until they awoke Tuesday morning, removing it only for contact sports, swimming, or bathing. Actigraphs recorded continuously. Subjects pressed a button to indicate when they were trying to go to sleep and when they woke up, which inserted a marker into the actigraph record.

Actigraphy data were preprocessed and scored in 60-s epochs using Action W 2.5. Sleep onset and sleep offset times were determined using the actigraph record, supported by the button-press marker and sleep diary. Data were processed using the Cole-Kripke procedure [36]. Coders were trained by scoring records collectively, then by individually scoring the same records, comparing and discussing discrepancies.

Actigraphy variables used were mean minutes asleep, mean sleep onset, and mean sleep offset across the 2 weekend nights. Minutes asleep was operationalized as the total epochs of actigraphy-recorded sleep during time in bed. Sleep onset was the first minute of several contiguous periods of low activity scored as sleep. Sleep offset was the last minute of low-activity scored during the night. As would be expected, some of the sleep variables correlated with each other. Sleep onset time correlated with sleep offset time \( (r = 0.541) \), minutes asleep correlated with sleep offset time \( (r = 0.259) \), and minutes asleep correlated with sleep onset time \( (r = -0.565) \). Although we collected four nights of data, the variability of the weeknight data (some adolescents were on school schedules while some were on holiday schedules) led us to focus exclusively on the weekend data, allowing all subjects to be studied on more natural self-selected schedules. We verified that the subjects who were on holiday schedules did not bias our results. (See Table 2 for sleep variables by development and gender.)

Table 2
Comparison of sleep variable between groups.

<table>
<thead>
<tr>
<th>Developmental group</th>
<th>Gender</th>
<th>Actigraphy-measured sleep</th>
<th>Sleep quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sleep onset</td>
<td>Sleep offset (hh:mm:ss)</td>
</tr>
<tr>
<td>Early</td>
<td>Male</td>
<td>Mean = 23:29:47</td>
<td>Mean = 09:19:04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:52:42</td>
<td>SD = 00:30:27</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Mean = 22:56:06</td>
<td>Mean = 08:41:13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:50:07</td>
<td>SD = 00:45:14</td>
</tr>
<tr>
<td>Both</td>
<td>Mean</td>
<td>Mean = 23:07:53</td>
<td>Mean = 08:54:28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:52:18</td>
<td>SD = 00:43:55</td>
</tr>
<tr>
<td>Late</td>
<td>Male</td>
<td>Mean = 23:36:36</td>
<td>Mean = 08:43:07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:26:44</td>
<td>SD = 00:59:34</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Mean = 23:38:32</td>
<td>Mean = 08:45:00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:56:54</td>
<td>SD = 00:58:16</td>
</tr>
<tr>
<td>Both</td>
<td>Mean</td>
<td>Mean = 23:37:33</td>
<td>Mean = 08:44:04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:12:21</td>
<td>SD = 00:58:08</td>
</tr>
<tr>
<td>Both</td>
<td>Male</td>
<td>Mean = 23:34:46</td>
<td>Mean = 08:52:48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:18:03</td>
<td>SD = 00:55:09</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Mean = 23:21:17</td>
<td>Mean = 08:43:28</td>
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<tr>
<td></td>
<td></td>
<td>SD = 00:57:27</td>
<td>SD = 00:52:36</td>
</tr>
<tr>
<td>Both</td>
<td>Mean</td>
<td>Mean = 23:27:20</td>
<td>Mean = 08:47:39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD = 00:07:10</td>
<td>SD = 00:53:29</td>
</tr>
</tbody>
</table>

Self-reported sleep. The variable used in data analyses, intended to capture the adolescents’ subjective experience of sleep, was mean weekend sleep quality \( (M = 77.3, SD = 15.5) \).

Reward processing. Each participant underwent scanning using a Siemens 3 T Allegra scanner. Blood-Oxygenation-Level-Dependant-Response functional images were acquired with a gradient echo planar imaging (EPI) sequence and covered 34 axial slices (3 mm thick) beginning at the cerebral vertex and encompassing the entire cerebrum and the majority of the cerebellum (Time to Repetition/Time to Echo = 2000/25 ms, Field Of View = 20 cm, matrix = 64 × 64). For each participant, we first acquired a reference EPI scan and visually inspected it for artifacts (e.g., ghosting) and for good signal across the entire volume of acquisition.

Whole-brain image analysis was completed using SPM2 (Statistical Parametric Mapping) [http://www.filion.ucl.ac.uk/spm]. For each scan, images for each participant were realigned to the first volume in the time series to correct for head motion. We confirmed that each participant’s data reflected <4 mm or degrees of motion. Realigned images were spatially normalized into a standard Montreal Neurological Institute template space using a 12-parameter affine model. Normalized images were smoothed to minimize noise and residual difference in gyral anatomy with a 6 mm full-width at half-maximum Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean.

Preprocessed data sets were analyzed using second-level random effect models that account for both scan-to-scan and participant-to-participant variability to determine task-specific regional responses. For each participant and scan, predetermined condition effects at each voxel were calculated using a \( t \)-statistic, producing a statistical image for...
each of the two contrasts of interest: (1) reward anticipation > baseline and (2) reward outcome > baseline. Group-level analyses were thresholded at a voxel level of \( p < .05 \) and an extent of at least 10 contiguous voxels, masked for the effects of the task across the striatal region of interest (ROI), and corrected for multiple comparisons with False Discovery Rate using a functional mask within the activation clusters of interest. Our striatal ROI was constructed using the PickAtlas Tool (v1.04) in SPM2 and defined as a sphere with 20 mm radius, centered on Talairach coordinates (0,10,-10) [37] that encompassed the entire ventral striatum and adjacent regions of the caudate nucleus.

Data Analysis

A general linear model was used in SPSS (v.16.0.1) to analyze the relationship between each sleep variable, gender, and development (GLM Univariate with sleep variable as the dependent variable and gender and development as fixed factors.) Response to reward was examined in SPM using regressions, with the sleep variable as the independent variable, and gender and development as covariates. Because of multicollinearity between age and puberty, age was not included in the final model. The results of the SPM regressions were exported to SPSS for traditional regression analyses yielding \( R^2 \).

Results

Development and sleep

Pubertal maturation was associated with fewer total minutes asleep (\( F = 5.72, \ df = 1, \ p = 0.020, \) mean\(_{early} = 542.2, \ SD_{early} = 38.2, \) mean\(_{late} = 503.5, \ SD_{late} = 61.3 \), with adolescents who were mid/late pubertal obtaining about 39 minutes less sleep time on average than their pre/early pubertal counterparts. There was considerable variation in sleep time in both groups. Development was not associated specifically with sleep onset, sleep offset, or sleep quality. There were no gender differences evident.

Actigraphy-measured sleep and reward-related brain function

In the reward anticipation phase, subjects with fewer minutes asleep and later sleep onset time exhibited less caudate activation (Table 3, Figure 1a). In the reward outcome phase, subjects with later sleep onset time showed less caudate activation, but later sleep offset time was associated with greater caudate activation (Table 3, Figure 1b). There were no significant sleep by development or sleep by gender interactions.

Self-reported sleep and reward-related brain function

In both the reward anticipation and outcome phases, lower caudate activation was associated with lower subjective sleep quality (Table 3, Figure 2). There were no significant sleep by development or sleep by gender interactions.

Discussion

This study provides preliminary evidence of potentially important links between reward-related brain function and sleep during a period of maturational changes to both systems. Getting less sleep or subjectively worse sleep was associated with less striatal reactivity to reward. Also, participants who went to bed later had less striatal reactivity to reward. The overall pattern is that the sleep pattern associated with adolescence—that is, lower quantity and quality of sleep—is associated with less reactivity of reward-related brain systems.

It is currently debated in the developmental neuroscience literature whether adolescents have increased or decreased striatal reactivity in response to reward. Models, such as the Triadic [5], have been proposed to account for findings of increased reactivity, whereas other investigators have found decreased reactivity in adolescence [27] and related to puberty [8]. At the simplest level, the data in this paper are consistent with the hypothesis that less reactivity in reward systems may contribute to real-life patterns of increased risk-taking behavior in adolescence. That is, pubertal adolescents may require more exciting rewards in order to create the same level of neural activation as prepubertal adolescents and thus be more prone to risk taking and sensation seeking. This explanation could also fit with the seemingly paradoxical observations that the onset of adolescence is not only a time of greater sensation seeking but also a time when youth often complain of feeling bored [38]. Thus, the findings in this paper are consistent with the idea that insufficient sleep may exacerbate low-positive-affect in ways that may have important health consequences.

Taken together, the initial findings suggest that getting less sleep in adolescence could represent a key element in a negative spiral of health-relevant effects. Obtaining less sleep may impact neural systems of reward in ways that exacerbate mood and behavioral problems. It is noteworthy that the pattern of reduced striatal reactivity to reward is consistent with findings on reward-related brain function in adolescents with major depressive disorder [9,10]. More generally, these results raise several provocative questions, particularly about the direction of the relationship between sleep and reward processing. For example, rather than sleep impacting reward systems, changes in reward systems may be influencing sleep (e.g., increased pursuit of late-night rewarding social activities could reduce total quantity of sleep). Another possibility is that a third factor changing with development—for example, alterations in dopamine system function with puberty—might impact both sleep and reward processing.

We also found that, contrary to our hypotheses, earlier wake time was associated with less striatal reactivity to reward. Because actigraphy-measured total sleep was correlated with actigraphy-measured wake-up time, it is possible...
that total sleep time, which is also reflected in earlier wake time, may be driving the pattern of association between sleep characteristics with brain function. In addition, our findings differ from those of the only extant study of sleep and reward-related brain function. In that study [39], which used an experimental sleep deprivation procedure in adults, participants showed no statistically significant difference in response to reward receipt after sleep deprivation.

It also is important to acknowledge several limitations to this study. These data are cross-sectional and correlational, and thus they do not allow any conclusions about directionality. Longitudinal data are needed to investigate the direction of influence between sleep and reward processing.

This study is also limited by the use of only two nights of weekend sleep data, the lack of subjective data regarding in-scanner experience, and the absence of behavioral data on reward processing or risk taking.

Although these results are preliminary, they raise concerns about a negative synergy of health risks emerging in early adolescence that center on sleep and reward. These include evidence that sleep deprived people take more risks [20], that puberty is associated with decreased reward activation and increased risk taking [2], that puberty is associated with sleep changes leading to very high rates of sleep deprivation [3], and that puberty is associated with sharply increasing rates of depression, suicide, risk taking, and substance use [1].
This study further highlights the need for conceptual advances and more comprehensive models for understanding the interrelationships between these developing regulatory systems in adolescents. Although some conceptual models have been described [3,12], we are at an early point in understanding how these complex, overlapping systems of sleep/arousal and affect regulation mature across adolescence and how puberty-specific changes in reward systems may play a key role in some aspects of these changes in ways that have clinical relevance.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Association of sleep measures with reward-related striatal function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain region</strong></td>
<td><strong>Talairach coordinates of maximum voxel in cluster</strong></td>
</tr>
<tr>
<td></td>
<td><strong>+ or – Correlation</strong></td>
</tr>
<tr>
<td><strong>Reward anticipation</strong></td>
<td></td>
</tr>
<tr>
<td>Minutes asleep</td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>Positive</td>
</tr>
<tr>
<td>Sleep onset time</td>
<td>Negative</td>
</tr>
<tr>
<td>Sleep offset time (no suprathreshold clusters)</td>
<td>Negative</td>
</tr>
<tr>
<td>Subjective sleep quality</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Reward outcome</strong></td>
<td></td>
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<tr>
<td>Minutes asleep (no suprathreshold clusters)</td>
<td>Negative</td>
</tr>
<tr>
<td>Caudate</td>
<td>Positive</td>
</tr>
<tr>
<td>Caudate</td>
<td>Negative</td>
</tr>
<tr>
<td>Caudate</td>
<td>Negative</td>
</tr>
<tr>
<td>Sleep offset time</td>
<td>Positive</td>
</tr>
<tr>
<td>Subjective sleep quality</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Note: Results are from region of interest analyses focusing on the effects of task in the striatum. Development (i.e., early or late pubertal stage) and gender were included in the model as covariates. Reward anticipation and reward outcome were fMRI task conditions. Cluster threshold was 10 voxels, p < .05 with False Discovery Rate correction. df = (1, 54) for objective sleep variables and (1, 49) for the subject sleep variable. The square of the correlation coefficient (R^2) was calculated for the full model (i.e., using gender, development, and sleep to predict brain function).
By examining variables that have not previously been studied together—puberty, sleep in natural environments (using both objective and subjective measures), and reward-related brain function—this study begins to illuminate interrelationships between three physiological processes that have major health implications in adolescence. By focusing on a narrow age range with considerable variability in pubertal development, this study addresses questions about sleep and reward-related brain function that are likely to be directly linked to puberty rather than age or social-setting differences (e.g., high school vs. middle school). Because of mounting concerns about adolescent sleep [4] and because sleep may be related to both depression and risk taking [19,21,40], it will be important to promote a better understanding of the relationship between sleep and reward in young adolescents.

Acknowledgments

We thank Jennifer Jakubcak, Donna Moyle, Kelsey Ronan, and Alexander Johnston for all their skillful help in collecting, processing, and understanding our data. We also express our appreciation to the adolescents and their families for their generous participation in this study. This work was supported by National Institutes of Health grant K01 74769 (E.E.F.), NARSAD Young Investigator Award (E.E.F.), National Institute on Drug Abuse grant DA018910 (R.E.D.), National Institutes of Health grant T32 HD049354-04 (R.E.D.), NARSAD Independent Investigator Award (M.L.P.), National Institutes of Health grant R01 MH 076971 (M.L.P.), and National Institute of Mental Health P50 MH080215 (N.D.R.).

References

[34] Bertocci MA, Dahl RE, Williamson DE, et al. Subjective sleep complaints in pediatric depression: A controlled study and comparison...


Title: Adolescent Slow-Wave Sleep Decreases with Age rather than Puberty in a Longitudinal Sample

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Running Head: Adolescent Slow-Wave Sleep Decreases with Age

Word Count: 1935
References: 15
Summary

There is growing interest in understanding the role of sleep in learning, plasticity, and brain development including changes that occur during adolescence. Time spent in slow wave sleep declines over the course of puberty. However, since advancing pubertal status is correlated with age, it can be difficult to separate these effects. Using a longitudinal sample that covers pubertal development, these analyses aim to uniquely separate the effects of puberty from those of age. 28 participants (Age range: 6.6-15.9 years) completed multiple polysomnographic studies over the course of 7 years (Mean=4.5 studies, SD =1.2), and also completed sleep diaries. Research nurses performed tanner staging for pubertal development, and participants were classified as pre/early (Tanner breast/genital score =1-2) or mid/late (Tanner score =3-5). Mixed models were conducted using pubertal status and age as predictor variables for each sleep variable. Variables included were sleep latency, time spent in each Stage of non-REM sleep (1-4), total REM sleep, REM activity, REM sleep latency, sleep efficiency, self-reported sleep quality and ease of waking. In this longitudinal sample, slow-wave sleep decreased with age. Decreases were unrelated to pubertal status. Results suggest that after accounting for age-related sleep changes, pubertal status was unrelated to changes in slow-wave sleep. The results from this study support growing evidence that changes in slow wave sleep across development may be linked to neuro-maturational changes that are linked to age and experience, in contrast to other sleep and circadian changes in adolescence which may be tied more directly to pubertal maturation.

Key Terms: puberty, slow-wave sleep, brain development, adolescence
INTRODUCTION

There are numerous changes in sleep during adolescence, including delayed sleep phase, decreased amount of sleep (Carskadon, 2002) and decreases in the amount and intensity of slow-wave sleep (Feinberg et al., 2006). As with any maturational change occurring during adolescence, the role of puberty must be considered in these sleep changes. Studies of adolescents’ natural sleep show that increasing pubertal status is associated with later bedtimes, less sleep (Sadeh et al., 2009) and less deep sleep (Jenni and Carskadon, 2004). Some aspects of sleep—such as the circadian shift—may be closely related to pubertal maturation while others may not. The process of disentangling puberty-specific from puberty-independent neuro-maturational processes has both scientific and practical significance—particularly in light of the evidence that puberty has been occurring at earlier ages in recent times (Dahl, 2008).

Research in the last few years has produced conflicting results regarding the relationship between aspects of sleep architecture and puberty. Decreased slow-wave sleep has been consistently related to puberty in cross-sectional studies and other studies where the effects of puberty and age could not easily be disentangled (Jenni and Carskadon, 2004). For example, delta-wave power density (the power of the waves in the delta frequency range divided by the number of minutes of non-REM sleep) has been reported to decrease with increasing pubertal maturation (Jenni and Carskadon, 2004), but was recently shown by Feinberg et al (Feinberg et al., 2006) in a longitudinal sample to be related to age but not puberty. It is noteworthy that in that sample the decrease in delta power density occurred earlier in girls (Campbell et al., 2005)—a pattern often seen in puberty-related processes, since adolescent girls tend to begin puberty younger than boys. Together, these findings suggest that the relationship between changes in slow-wave sleep and age may be more complex than it appears.
Though slow wave sleep is related to the homeostatic process of sleep (process S), it is not believed that this change in slow wave sleep in adolescence is related to changing nighttime homeostatic regulation (Jenni et al., 2005). Although adolescents seem to accumulate sleep pressure more slowly throughout the day (and therefore have less total slow wave sleep), the slow wave sleep contained in their NREM sleep periods dissipates along the same curve that is seen among children (Jenni et al., 2005). It has been suggested that this decline in total amount of slow-wave sleep may instead correspond to the synaptic pruning during adolescence (Feinberg and Campbell, 2010), whereby the decreased metabolic activity as a result of pruned, efficient processes does not accumulate sleep pressure as rapidly as the less efficient processes of children.

Many aspects of cognitive development in adolescence are a function of age and not puberty (Dahl, 2008). Adolescents appear to slowly develop adult-like higher order cognitive processes in a way that is unrelated to pubertal development. The nearly linear increase in white matter during adolescence (Blakemore and Choudhury, 2006), which continues well into adulthood, may represent myelination of the frontal cortex, important in the development of executive functioning. Also occurring in adolescence is the slow process of synaptic pruning which continues into the early 20s, and has been hypothesized to be experience-driven (Blakemore and Choudhury, 2006), meaning that the pathways that are most used get reinforced, allowing for more efficient cognitive functioning. Thus, it follows that slow wave sleep, which is thought to be related to changes in the developing brain (Jenni et al., 2005, Feinberg and Campbell, 2010), may be more related to age than puberty, because many of the cognitive changes are a function of age. Age and puberty may interact to influence disparate aspects of
sleep in different ways; therefore it is important to put adolescent sleep changes into the context of the developing brain.

This paper will describe an analysis of sleep architecture changes in relation to age and puberty in a longitudinal sample of healthy children and adolescents spanning the entire range of pubertal development. As this is a unique sample of well-characterized normal subjects who returned to our lab for multiple sleep studies over a span of years, we hope to add further evidence to our knowledge of the interrelations among sleep, age and puberty during adolescent development.

METHODS

Participants. 28 (71% male; 89% white) participants completed two or more lab-based PSG sleep studies (Mean =4.5; SD= 1.2) over seven years. Participants were recruited through newspaper advertisements as part of the control group for a program project studying neurobehavioral changes in pediatric affective disorders. These participants had no history of psychiatric disorders and were not endorsing psychiatric symptoms at the times they were studied. They were evaluated using the Kiddie Schedule for Schizophrenia and Affective Disorders Present and Lifetime Version (Kaufman et al., 1997) with both parent and child as the reporter. These participants were at low familial risk for depression with no first degree relatives with any lifetime affective disorder episodes, no first or second-degree relatives with a history of manic episodes, schizophrenia or schizoaffective disorder, and fewer than 20% of second degree relatives with a diagnosis of MDD (using the KSADS-E or SADS-L where appropriate, history on unavailable relatives was obtained from the parent using the Family History-RDC technique). All parts of this study had IRB approval and all participants and parents were informed of the
risks and benefits of participating. All parents provided informed consent; participants over 14 gave written consent and under 14 provided verbal assent.

Puberty. Participants underwent physical examination by a research clinician to determine stage of pubertal development (Marshall and Tanner, 1968). Consistent with our approach to examining affective aspects of pubertal development (Holm et al., 2009), we classified participants as **pre/early pubertal** if they were Tanner stage 1 or 2 (n= 79 lab visits, representing 26 participants) and as **mid/late pubertal** if they were Tanner stage 3, 4, or 5 (n= 44 lab visits, representing 21 participants) on the scale that assesses breast/genital development, an index reflecting changes in gonadal steroids, which influence neural development and affect-related brain function. Although some might argue for the advantages of examining each stage of puberty, there is no evidence that these 5 relatively arbitrary stages are physiologically separate stages; to be consistent with previous analyses from our research group we focused on comparisons between pre/early pubertal and mid/late pubertal status (for more in depth discussion of measures and analyses of puberty see (Shirtcliff et al., 2009)).

Sleep Study. All participants came for multiple sleep studies lasting 4 nights. They received an intravenous catheter (IV) on their first day, and had blood draws from the IV throughout the study. Night one was excluded from the analysis as an adaptation night. Nights three and four were excluded because the participants received a pharmacologic agent prior to these nights, possibly altering their sleep. Therefore, only data from night two was used for these analyses. For two weeks prior to the study, participants went to bed between 9 and 10 pm and rose roughly 10 hours later (by 8 am); they kept sleep diaries and maintained that schedule in the lab. As a result of this fixed schedule, the pubertal circadian shift could not be assessed in this sample.

PSG Sleep. Sleep was scored in 30-s epochs, using standard criteria (Rechtschaffen, 1968),
resulting in the following variables: minutes of stage 1 sleep (ST1), minutes of stage 2 sleep (ST2), minutes of slow-wave sleep (SWS; the sum of stage 3 and stage 4), sleep efficiency (SE), minutes of time in REM sleep (RT), REM activity-the number of REM units (RA), REM latency (RL), activity (A) and sleep latency (SL).

Self-reported sleep. Participants completed sleep diaries each morning about the previous night (as in (Holm et al., 2009)). The two visual analogue scale variables, sleep quality and ease of waking, were included in our analyses. For consistency, only data from night two in the lab (the same night as the EEG data) were included. Of 126 sleep studies (from the 28 participants), self-report data were available for 120 studies, with six studies missing the data either because of the participants’ non-compliance, or due to errors in data management.

Statistical Analyses. Statistical analyses were conducted using mixed models, with person and family as random effects to account for nesting of time points within participants and to account for five sibling pairs. Pubertal status and age were fixed predictor variables, and a model was computed for each sleep variable. For all sleep variables, socioeconomic status at the time of the participant’s first sleep study was correlated with each sleep variable. SES was not significantly related to the sleep variables, and was excluded from the analyses. Gender and race were also excluded, as this sample had inadequate variation in gender (71% male) and in race (89% white) for meaningful analyses.

RESULTS

In this longitudinal sample, time spent in slow-wave sleep decreased with age (See Figure 1; F=16.448, p<.001). Decreases were unrelated to pubertal status (See Figure 2; F=0.537, p=0.465). The other PSG variables (Amount of stage 1 sleep, stage 2 sleep, and REM sleep, as
well as sleep efficiency, REM activity, REM latency, activity and sleep latency) did not show significant relationships with either age or pubertal status.

The self-reported sleep variables (Sleep Quality and Ease of Waking) did not show significant relationships with either age or pubertal status.

DISCUSSION

Using a longitudinal sample that provides the opportunity to disentangle the effects of age and puberty, this study adds to recently accumulating evidence that some changes in sleep architecture may be more related to age than puberty. The body of literature currently implies that adolescents have a circadian shift that is related to puberty (Sadeh et al., 2009); this shift most often manifests itself as later bedtimes and sleeping less, with a greater difference between weekend and weekday sleep patterns (likely secondary to societal pressures) (Carskadon, 2002). However, this study confirms that a change in sleep architecture—amount of deep sleep— is not related to puberty, at least as measured by physical development, after controlling for the effects of age.

These changes in sleep occur in the setting of many other changes in behavior and brain development during adolescence. Much cognitive development occurring through adolescence appears to be strongly related to age (Dahl, 2008, Blakemore and Choudhury, 2006). But many aspects of emotional processing that change in adolescence are related to puberty, including increased sensation seeking, or the tendency to pursue high-arousal activities (Martin et al., 2002).

The association of sleep with puberty, age, emotion and cognition in adolescence is complex and can have critical influence on adolescent risky behavior. It has been previously suggested that older adolescents are prone to risky behaviors and to their negative consequences
due to a combination of the puberty-related emotional instability and the age-related protracted
development of executive functioning (Dahl, 2008). Changes in adolescent sleep likely
complement both aspects of this model. Sleep deprivation—which occurs with puberty—
potentially exacerbates emotional changes (Dahl, 2008). Additionally, we propose that reduced
deep sleep—which we have shown is associated with age—may be related to the development of
improved executive functioning. Because deep sleep is postulated to reflect synaptic pruning
during development (Feinberg and Campbell, 2010), it may play a role in the balance of affective
systems and self-control systems.

Given the limitations of our sample size, racial and gender homogeneity of our sample,
and use of a single measure of puberty, it will be important to flesh out, preferably in a larger
longitudinal sample, what the changes in different aspects of sleep mean for real-life adolescent
sleep and how it changes over adolescence with puberty, age and life-experience.
REFERENCES


Figure 1. Using a Mixed models analysis, age was significantly related to slow wave sleep in this longitudinal sample. For illustrative purposes only, data was separated by age of the participant at the time of the study (rounded to the nearest half age) and the mean at each age was graphed, with error bars representing standard deviation.

Figure 2. Using a Mixed models analysis, puberty was not significantly related to slow wave sleep in this longitudinal sample. For illustrative purposes only, data was separated by pubertal status at the time of each study, as well as by age of the participant at the time of the study (rounded to the nearest half age) and the mean at each age was graphed. Slow wave sleep decreases with age along the same trajectory regardless of pubertal status.
Rebel with a Cause: Brain Activation Associated with Testosterone Levels May Explain Increased Sensation Seeking in Adolescents

Stephanie M. Holm, Ronald E. Dahl, Carol Worthman, Mary L. Phillips, Neal D. Ryan and Erika E. Forbes

Introduction

In the 1955 film, James Deans’ iconic character is shown engaging in many different risky behaviors, including heavy drinking and drag racing to the edge of a cliff. Though the reviews of the day were horrified at the depictions of “unhappy youth on another delinquency kick” (Land, 1983) but the film is in many ways timeless. The main theme stems from the underlying increase in risky behavior that occurs in adolescence, which contributes greatly to morbidity and mortality in this age group (Steinberg, 2004). It is thought that this increase may be due to developmental changes in neural function relevant to sensation seeking (SS), or the tendency to pursue highly stimulating activities (Martin et al., 2002, Forbes and Dahl, 2010).

Sensation-Seeking

The sensation seeking scale was originally developed in the Zuckerman lab (Zuckerman et al., 1964) as a scale for measuring the extent to which individuals pursue stimulating things or activities. In the 1990’s, this scale was adapted and validated for use in children (Russo et al., 1991, Russo et al., 1993). Sensation seeking (SS) has been shown to be related to risky real world behavior in adolescence. For example, elevated sensation seeking scores in young adolescents are related to increased drug and alcohol use (Martin et al., 2002). Recently, a dual-systems model has been described (Steinberg et al., 2008, Dahl, 2008), which explains the increase in risky behavior during adolescence as a mismatch between two systems: increasing SS (which occurs early in adolescence because it is related to puberty) and increasing self-regulation (which occurs slowly over the course of adolescence). In this model, the reason that SS is so
important in early adolescence is because adolescents have not yet developed as much self-regulatory capacity; these two systems are out of balance.

Reward circuitry

Also relevant to a discussion of risky behavior are imaging studies that have described the areas of the brain that are important in how people respond to rewards. The reward circuit that exists within the basal ganglia circuitry and has been well described in both animal and human studies (Delgado, 2007). In particular, the caudate nucleus (located in the dorsal portion of the ventral striatum) is thought to be particularly important in synthesizing information to create goal-directed behavior. To put it differently, the caudate is important in actually pursuing rewards (Haber and Knutson, 2010).

Relationship between Sensation-Seeking and Reward Circuitry

Using functional magnetic resonance imaging, SS has been related to activity under emotional stimuli in brain areas implicated in emotional reactivity (Joseph et al., 2009). SS scores were also shown to relate to brain activity in the thalamus and insula in people watching scary films (Straube et al., 2010), and though these regions are not generally considered to be part of the reward circuitry, they are heavily connected to that circuit. Additionally, evidence from other modalities suggests that sensation seeking scores are related to dopamine receptor density in the ventral striatum (Gjedde et al.).

Relationship between Reward Circuitry and Hormones

Hormonal influence on neurons can be substantial and is posited to be an important part of adolescent brain development (Ernst et al., 2008, Sisk and Foster, 2004). Testosterone produced during adolescence is known to be related to structural and functional changes in brain regions important for emotional processing (Nelson et al., 2005). Menstrual phase variation in
responses to reward has also been demonstrated (Andreano and Cahill, 2010), providing further evidence for a hormonal role in reward-related neural circuits.

*Relationship between Sensation-Seeking and Hormones*

Hormonal effects on SS are obvious from the gender difference in SS scores, with males scoring higher than females (Daitzman et al., 1978). Moreover, it has been suggested that even changes in utero hormone exposure (i.e. in opposite sex twins) can affect SS later in life (Resnick et al., 1993). SS has been found to be greater in adolescents with higher circulating levels of testosterone (Daitzman and Zuckerman, 1980).

*Connecting SS, Hormones and Reward Circuitry*

Because both increases in SS during adolescence and changes to reward responsiveness may be related to the hormonal changes associated with puberty, the relationships between hormonal levels, SS scores, and reward-related brain functioning are worthy of further inquiry. Elucidating these relationships may help to further explain the role of each of these factors in the pursuit of risky behaviors during adolescence. While the relationships between each of these pairs has been described, we hoped to bring a more compete understanding of how all three factors are related. The current study examined the hypothesis that the relationship between testosterone and SS in adolescents may occur through reward related brain function.

*Method*

*Participants*

All participants provided informed consent according to the guidelines of the University of Pittsburgh Institutional Review Board. Adolescents were recruited from the community through advertisements, flyers, and demographically targeted phone lists. Participants were returning for their second visit as part of this study, two years after their initial visit. They were
originally recruited to be in a narrow age range but vary in pubertal development (see (Holm et al., 2009)). At this two-year follow up, they are not only older ($M=14.5$ years, $SD=0.8$), and all are mid/late pubertal (Tanner 3/4/5). Adolescents were free of current and lifetime psychiatric disorders, did not have braces, and had no history of head injury, serious medical illness, psychotropic medication use, alcohol use, or illicit drug use.

129 adolescents were initially enrolled in the larger study, however subjects were excluded for being lost to time 2 follow-up ($n=20$), excessive head movement during the scan ($n=11$), not participating in a scan (most often due to braces) ($n=11$), being withdrawn from the study ($n=3$), missing SS data ($n=1$) or missing hormone data ($n=21$), resulting in a final sample of 62 adolescents for the analyses reported in this paper.

**Materials**

*Pubertal development.* Adolescents underwent physical examination by a trained research nurse to determine stage of pubertal development (Marshall and Tanner, 1968). Consistent with our approach to examining affective aspects of pubertal development (Holm et al., 2009), we classified participants as *pre/early pubertal* if they were Tanner stage 1 or 2 and as *mid/late pubertal* if they were Tanner stage 3, 4, or 5 on the tanner scale that assesses breast/genital development, an index reflecting changes in levels of gonadal steroids, which influence neural development and affect-related brain function (Sisk and Foster, 2004). Consistent with our previous studies focusing on sleep and neuroendocrine function, our *a priori* approach was to examine categorical comparisons, rather than using continuous Tanner score, with 100% of this sample classified as mid/late pubertal adolescents.

*Reward processing.* The fMRI paradigm (Forbes et al., 2009) was an adaptation of a card-guessing paradigm (Delgado et al., 2004), with a section to measure anticipation of reward as well as outcome.
During the first three seconds of each 27s trial, participants had to guess, via button press, whether the value of a visually presented card with a possible value of 1-9 was higher or lower than five (decision making phase). During the next 12 seconds, the trial type (either reward/neutral or loss/neutral) was presented visually. (During a reward/neutral trial it was impossible to lose money and during a loss/neutral trial it was impossible to win money; anticipation phase.) This was followed by the “actual” numerical value of the card (500ms); outcome feedback (a green upward-facing arrow for win, a red downward-facing arrow for loss, or a yellow circle if they did not win or lose money that trial; 500ms); and a crosshair presented for 11s (outcome includes the actual value, outcome feedback and first 8 seconds of the crosshair). The baseline condition is the final three seconds of staring at the crosshair before the next trial commences. Trials were presented in four runs, with 12 trials per run and a balanced number of outcome trial types within runs.

As has been previously done with this task (Forbes et al., 2009, Delgado et al., 2004, Forbes et al., 2006), participants were told that they would receive $1 for each win, lose 50 cents for each loss, and experience no earnings change for neutral outcomes. Participants were unaware of the fixed outcome probabilities (each participant had $12 of winnings). During practice and between runs, participants’ engagement was maintained by verbal encouragement to stay on task. To maximize sample size, data from only run one were included in analyses. Also, focusing on run one minimizes the influences of fatigue and habituation that can occur with repeated runs (Forbes et al., 2009).

_Sensation Seeking Scale for Children._ At time two, the sensation seeking scale for children was used (Russo et al., 1991), which is based off the original adult sensation seeking scale by Zuckerman (Zuckerman et al., 1964). Responses were scored 0,1 with the appropriate items
reverse scored. Due to a typographical error on our questionnaire, one item had to be omitted from the final analyses.

**Procedure**

Each participant completed a lab session including an fMRI scan, had blood spot sampling via a minimally invasive finger stick (Worthman and Stallings, 1997) assayed for testosterone ($M=213.7$ ng/dL, $SD=194.7$) and completed the Sensation Seeking Scale for Children (SSSC) ($M=15.1$, $SD=3.2$) (Russo et al., 1991).

**Data Scoring, Processing and Analyses**

*Reward processing.* Each participant was scanned using a Siemens 3T Allegra scanner. BOLD functional images were acquired with a gradient echo planar imaging sequence and covered 34 axial slices (3mm thick) beginning at the cerebral vertex and encompassing the entire cerebrum and the majority of the cerebellum ($TR/TE = 2000/25$ ms, $FOV= 20$ cm, matrix = 64x64). For each participant, we first acquired a reference EPI scan and visually inspected it for artifacts (e.g., ghosting) and for good signal across the entire volume of acquisition. All participants included in this report were free of such problems.

Whole-brain image analysis was completed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm). For each scan, images for each participant were realigned to the first volume in the time series to correct for head motion. We confirmed that each participant’s data reflected <4 mm or degrees of motion. Realigned images were spatially normalized into a standard Montreal Neurological Institute template space using a 12-parameter affine model. Normalized images were smoothed to minimize noise and residual difference in gyral anatomy with a 6mm full-width at half-maximum Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean.
Preprocessed data sets were analyzed using second-level whole brain random effect models that account for both scan-to-scan and participant-to-participant variability to determine task-specific regional responses. For each participant and scan, predetermined condition effects at each voxel were calculated using a $t$-statistic, producing a statistical image for each of the 2 contrasts of interest: (1) reward anticipation > baseline and (2) reward outcome > baseline. Group-level analyses were thresholded at a voxel level of $p<.01$ and an extent of at least 20 contiguous voxels; they were masked for the effects of the task.

Data Analysis. Response to reward was examined in SPM using regressions, with the sensation seeking (SS) score as the independent variable. Because of our a priori hypotheses about striatal function and its relevance to SS, the mean brain activation over the clusters in the striatal regions were then extracted for further analyses. A Pearson correlation was used in PASW (v.18.0) to analyze the relationships between gender, testosterone level and sensation seeking score; Pearson correlations were also used to analyze the relationship between testosterone level and the brain activation that had been shown in SPM to be related to SS.

Results

We found no significant relationship between gender and sensation seeking (SS), but, as expected, boys had higher testosterone than girls ($F=116.252, p<.001$). Also, testosterone level was significantly related to SS ($F=4.429, p<.05$).

Regressions indicated that SS was positively correlated with brain activation in a number of brain regions (see Table 1). Focusing on the results in the striatum, because these regions are highly relevant for sensation seeking, behavior and reward function (Haber and Knutson, 2010), SS was positively correlated with caudate response to both reward anticipation (35 voxels,
$T=2.94, p<0.01$, Talairach coordinates [-9,-1,20]) and reward outcome (180 voxels, $T=3.72$, $p<0.001$, Talairach coordinates [1,1,15]; Figure 1). In both boys and girls, testosterone was positively correlated with SS-relevant caudate activation during reward anticipation ($F=6.363$, $p=.018$ boys; $F=5.924, p=.021$ girls; Figure 2), but not during reward outcome.
<table>
<thead>
<tr>
<th>Brain Region</th>
<th>+ or - Correlations</th>
<th>Hemisphere</th>
<th>Talairach coordinates of maximum voxel</th>
<th>Cluster size</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
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<tr>
<td><strong>Reward Anticipation</strong></td>
<td></td>
<td></td>
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<td><em>Fusiform Gyrus, BA 37</em></td>
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<td>-8</td>
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<td>23</td>
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<td>-5</td>
<td>102</td>
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<td>-1</td>
<td>20</td>
<td>35</td>
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<tr>
<td><em>Precuneus, BA 19</em></td>
<td>positive</td>
<td>Right</td>
<td>20</td>
<td>-81</td>
<td>40</td>
<td>23</td>
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<tr>
<td><strong>Reward Outcome</strong></td>
<td></td>
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<td>1</td>
<td>15</td>
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<tr>
<td><em>Culmen</em></td>
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<td>24</td>
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<tr>
<td><em>Precuneus, BA 7</em></td>
<td>positive</td>
<td>Left</td>
<td>-</td>
<td></td>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

Table 1. Results of the regression of sensation seeking score with brain activity during the guessing task. Thresholds were p<0.01 and cluster size of 20.
Figure 1. Regression between Sensation Seeking and Caudate activation during Reward Outcome.

Sensation Seeking

Caudate Activation During Reward Outcome Over the Entire Cluster, located at (1, 1, 15)
Figure 2. Regression between Testosterone and Caudate activation during Reward Anticipation, in the cluster most strongly related to Sensation Seeking, split by gender.
References


Developmental Changes in Sleep May Differ in Adolescents with Affective Psychopathology

Stephanie M. Holm, Erika E. Forbes, Neal D. Ryan, Laura Trubnick, Jennifer Jakubcak, Ronald E. Dahl (University of Pittsburgh)

Introduction: Because affective disorders involve sleep disruptions and increase in incidence sharply at adolescence, when sleep patterns are also shifting, examining contributions of both affective psychopathology and puberty to adolescents’ is highly relevant. This study characterized sleep changes in relation to puberty in adolescents with major depressive disorder (MDD), anxiety disorders, and co-morbid MDD and anxiety.

Methods: 334 participants (age range=7.4-17.0 years) completed a polysomnography (PSG) study and self-report measures of sleep. Participants had current MDD (n=118) or anxiety disorder, some with co-morbid MDD (n=104); or no history of psychiatric disorder (healthy group; n=112). Participants were classified as pre/early pubertal or mid/late pubertal status based on Tanner scores from physical exam. Sleep variables included were sleep latency, time spent in each stage of non-REM sleep (1-4), total REM sleep, REM activity (RA), REM latency, sleep efficiency, and self-reported sleep quality and ease of waking.

Results: Stage 3 and stage 4 sleep decreased with age. The healthy group reported higher sleep quality than the MDD or anxiety groups. The interaction of puberty and diagnostic group predicted RA ($F=3.18; \text{df}=3; p<.05$), and Stage 4 sleep ($F=3.20; \text{df}=3; p<.05$). RA was lower in the mid/late than the pre/early pubertal group, but only for healthy adolescents. Among pre/early adolescents, the anxiety group had less Stage 4 sleep than the healthy or MDD groups.

Discussion: This study allows us to evaluate how pubertal development and its relationship to sleep may differ among adolescent clinical populations, elucidating sleep-related developmental psychopathology issues.
Introduction

The development of both sleep difficulties and psychiatric difficulties in youth can lead to problems with psychiatric functioning and sleep later on in life. Sleep problems early in life have recently been shown to be an indicator of continued sleep difficulties later in life [1]. Sleep and psychopathology have also been shown to be inextricably linked over time; with sleep predicting future difficulties with mood [2-4] and vice versa [5].

Sleep trouble and psychiatric disorders are frequently comorbid. Sleep disturbance is one of the diagnostic criteria of a depressive episode [6]; and moreover there are generally accepted sleep architecture changes in adults with MDD [7]. A number of these differences have been evident in REM sleep in particular. For example, depressed adults show shortened REM latency and higher REM density [7]. In depressed men, REM activity is positively correlated with depressive symptomatology [8]. Other findings in depression include decreased slow wave sleep (at least in men), and more time awake [7]. Adults with anxiety disorders spend less time than their healthy counterparts in NREM sleep, especially slow-wave sleep [9].

Recent work on the relationship between psychiatric disorders and sleep disturbances in young people [10-12] indicate the importance of considering development when examining the association between these types of problems. There have been similarities between findings with adults and findings with youth, and there have been important differences. Although children and adolescents with depression have complaints of poor sleep, as do their adult counterparts, the depressed youth appear not to have changes in sleep architecture relative to healthy peers [10]. Anxious youth do show
decreased NREM sleep (particularly slow wave sleep), similar to adults with anxiety [12], although the anxious youth seem much less bothered by sleep difficulties than anxious adults are [9, 12]. Furthermore, the association between affective disorders and sleep may be moderated by pubertal development. For example, puberty has been shown to influence the changes in cortisol secretion around the time of sleep onset that occur in youth with affective disorders [11].

Adolescence is a particularly crucial period for our understanding of sleep, depression and anxiety and how they are interrelated because sleep findings in people with psychopathology are different in children and adults, and there is a large increase in the rate of affective disorders during adolescence[13]. Evidence that aspects of affective processing in adolescence are related both to sleep [14] and to puberty [15] further underscores the importance of considering the effect of puberty on these processes and their relationship.

This study uses data from a series of sleep studies as well as data on pubertal development in young people with depression and anxiety to test relationships between sleep, psychopathology and pubertal development. Parts of the sample have been reported on previously [10, 12], however hypotheses about sleep and diagnosis have not been tested in the entire sample. Based on prior work [10, 12, 16], we hypothesize that depressed youth will report more sleep difficulties on the subjective measures (i.e., self-report), but that anxious youth will show more disturbed sleep on objective measures (i.e., sleep EEG). We also predict possible effects of puberty on REM sleep, as differences in REM sleep are apparent in adults with depression[7], but not children[10].

Methods
Participants

Participants included 334 youth (48.2% female; 83% European American) who were recruited as part of a long running program project study on neurobehavioral changes in pediatric affective disorders. Participants were recruited through Western Psychiatric Institute and Clinic’s inpatient and outpatient programs, as well as through local newspaper advertisements. The participants in these analyses were diagnosed with either no psychopathology \( n=112 \), current major depressive disorder (MDD) \( n=118 \), or an anxiety disorder with or without concurrent depression (ANX) \( n=104 \) (these included Anxiety disorder NOS \( n=43 \), Generalized anxiety disorder \( n=42 \), overanxious disorder \( n=4 \), panic disorder \( n=2 \), social phobia \( n=1 \), separation anxiety disorder \( n=11 \) and co morbid generalized anxiety and separation anxiety \( n=1 \)) (Table 1). They were evaluated using the Kiddie Schedule for Affective Disorders and Schizophrenia--Present and Lifetime Version [17] with both parent and child used as reporters. Any participants with diagnoses other than those listed above were excluded from this analysis. All parts of this study had IRB approval and all participants and parents were informed of the risks and benefits of participating in the study. All parents provided informed consent; participants over age 14 gave written consent and those under 14 provided verbal assent.

189 healthy subjects were originally recruited and studied, but this group was age and gender-matched to the clinical groups (with all non-European American subjects left in the sample to more closely approximate the clinical groups) to reach a final control group size of 112 subjects.
Puberty

Participants underwent physical examination by a trained research clinician to determine stage of pubertal development[18]. Consistent with our approach to examining affective aspects of pubertal development[19], we classified participants as pre/early pubertal if they were Tanner stage 1 or 2 for breast/genital development (n = 182) and as mid/late pubertal if they were Tanner stage 3, 4, or 5 (n = 136) [20]. Sixteen participants were missing tanner data, either because a physical exam was refused, or a trained staff member was not available at the time of their study.

Sleep Study

All participants came at least once to the lab for a polysomnography (PSG) study lasting 3-4 nights. For those participants that completed sleep studies on multiple visits, only the first visit was used for these analyses. Analyses focused on sleep variables from night 1, as this was the only night with consistent procedures across all subjects (subsequent nights included pharmacologic challenges). Moreover, this allowed us to examine the group differences in sleep adaptation to a new environment.

Objective Sleep

Sleep was scored in 30-s epochs, using standard criteria[22]. The following variables were computed: amount of stage 1 sleep (ST1), amount of stage 2 sleep (ST2), amount of stage 3 sleep (ST3), amount of stage 4 sleep (ST4), sleep efficiency (SE), amount of time in REM sleep (RT), REM activity, or the total number of rapid eye movements over the course of the night (RA), REM onset latency (RL), and sleep
latency (SL).

*Self-reported subjective sleep*

Participants completed sleep diaries every morning about the previous night [10], reporting time they went to bed, time they went to sleep, when they woke up, how well they slept, and how easy it was to wake up. The two visual analog scale variables, sleep quality and ease of waking, were included in our analyses. Data from sleep diaries was available for only half of the participants (155 participants) because sleep diaries were not used during the early years of the study, some participants did not complete these items, or data was lost due to errors in data management.

*Statistical Analyses*

Statistical analyses used general linear models with race, pubertal status, age and diagnosis (MDD, ANX, and healthy) as predictor variables for each sleep variable and included only the interaction between puberty and diagnosis in the model (as it was the only one for which we had a hypothesis). For diagnostic group main effect findings, post-hoc analyses were performed using pair wise t-tests to compare the sleep variable between groups. For each of these post-hoc analyses, we examined equality of variance. If the variance was significantly unequal between groups, we report the results of testing for groups with unequal variances. For each continuous independent variable for which a significant relationship was found between the predictor variable and the sleep variable, we computed $R^2$.

Socioeconomic data were available for 89% of the participants ($M= 41.4$, $SD=13.3$). Preliminary analyses indicated that SES was not significantly related to
any of the sleep variables, and was therefore omitted from the analyses. Tanner stages were unevenly balanced across gender ($\chi^2(df)=22.355(1)$, $p<.001$) with boys disproportionately pre/early in pubertal development (Table 1). This precluded analyses relevant to development within gender groups. Six sleep variables were significantly different between genders (Stage 2 sleep, Stage 4 sleep, sleep efficiency, RA, Sleep Quality and Ease of Waking), with girls showing more stage 2 sleep ($F(df)=5.570(1)$, $p<.05$; girls 283.7 (39.0); boys 271.0 (49.2)), boys showing more stage 4 sleep ($F(df)=4.478(1)$, $p<.05$; girls 41.07 (32.8); boys 45.5 (31.9)), girls showing more efficient sleep ($F(df)=5.640(1)$, $p<.05$; girls 88.4 (5.4); boys 85.2 (9.3)), girls showing less REM activity ($F(df)=4.337(1)$, $p<.05$; girls 101.5 (91.4); boys 107.2(92.65)), girls showing lower self-rated sleep quality ($F(df)=5.422(1)$, $p<.05$; girls 64.1(28.3); boys 73.9 (24.0) and girls showing lower self-rated ease of waking ($F(df)=5.353 (1)$, $p<.05$; girls 57.7 (31.3); boys 69.25 (29.2)). For those six variables, analyses were re-run including gender in the model to determine whether gender seemed to influence associations between diagnostic group and sleep. Results were largely the same.

As would be expected, age was significantly different in pubertal development groups ($F(df)=417.886(1)$, $p<.001$) with pre/early pubertal participants younger (Pre/Early Pubertals 10.4 years old(1.4); Mid/Late Pubertals 14.0 years old (1.7)). Age is not significantly different between diagnostic groups (Table 1).
Race was also unbalanced across diagnostic groups ($\chi^2(df)=7.457(2), p<.05$), with a high percentage of European Americans (EA) in the healthy group compared to the MDD and ANX groups (Table 1).

**Results**

**Subjective Sleep**

Main analyses revealed a main effect for diagnostic group for *sleep quality* ($F=12.88; df=3; p<.05$). Post-hoc analyses revealed that MDD participants ($t=3.23; p<.005$) and ANX participants ($t=3.10; p<.005$) reported lower sleep quality than healthy participants, but did not differ from each other (Figure 1, Table 1).

**Objective Sleep**

Main analyses revealed a main effect of diagnostic group on *stage 2* ($F=8.91, df=3; p<.05$) and on *stage 3* ($F=15.04, df=3; p<.05$). Post-Hoc analyses revealed that ANX participants spent more time in *stage 2* than MDD participants ($t=3.34, p<.005$) and more time than healthy participants ($t=3.37, p<.005$), the healthy and MDD participants did not differ from each other. Post-hoc analyses revealed that healthy participants spent less time in *stage 3* than the MDD participants ($t=1.98, p<.05$), but that the healthy and ANX groups were not significantly different and neither were the MDD and ANX groups (Table 1).

Analyses also revealed a main effect of pubertal development on *stage 1* ($F=4.925, df=1, p<.05$) with mid/late pubertals spending more time in stage 1 than pre/early pubertals (Mid/late $M(sd)= 27.0 (13.9)$ minutes; Pre/Early $M(sd)= 22.7 (12.3)$ minutes) and a main effect of race on sleep latency ($F=5.621, df=1, p<.05$) with non-
European American subjects experiencing a shorter sleep latency (Non-European American $M(sd)$= 22.8 (13.4) minutes; European American $M(sd)$= 31.3 (26.7) minutes).

As in other studies of the development of sleep [23-26], age was associated with decreasing amounts of stage 3 ($F=9.80; df=1; p<.01; R^2=0.04$) and stage 4 sleep ($F=38.07; df=1; p<.001, R^2=0.28$) (Figure 2). Age was also associated with decreasing REM latency ($F= 6.046, df=1, p<.05, R^2=0.03$).

The interaction of diagnostic group X development predicted Stage 4 sleep ($F=3.20; df=3; p<.05$; Figure 3b). Within all groups, mid/late pubertal adolescents exhibited less stage 4 sleep than pre/early adolescents (Healthy $t=7.00, p<.001$; MDD $t=6.56, p<.001$; ANX $t=3.82, p<.001$). Post hoc analyses were also performed within each developmental group. Within the pre/early pubertal group: the ANX group spent less time in stage 4 than the healthy group ($t=5.47, p<0.001$) or the MDD group ($t=5.69, p<0.001$), which did not differ from each other. Within the Mid/Late pubertal group, all groups spent an equal amount of time in stage 4.

The interaction of diagnostic group X development also predicted RA ($F=3.18; df=3; p<.05; Figure 3a$). Post-hoc analyses within each group revealed that RA appeared to decrease with typical pubertal development: healthy mid/late pubertal participants showed less RA than healthy early/pre pubertal participants ($t=4.13; p<.001$). This decrease was not evident in the MDD or ANX groups. Post hoc analyses within each developmental group revealed that within the pre/early pubertal group, the ANX group had less RA than the MDD group ($t=2.16, p<0.05$), but was statistically equivalent to the RA in the healthy group. (The MDD and healthy groups also had the same amount of RA). Within the mid/late pubertal group, the MDD group had more REM activity than
the healthy group or ANX group ($t=3.84, p<.001$ for MDD versus healthy; $t=2.57, p<.05$ for MDD versus ANX) (the healthy and ANX groups did not differ).

**Discussion**

In this study of affective disorders, pubertal development, and sleep in a large sample of adolescents, we found that diagnostic status and development interacted to influence two objective sleep variables: RA and stage 4 sleep. In contrast, diagnostic status alone was associated with subjective sleep: lower sleep quality. Age was associated with decreases in stage 3 and stage 4 sleep. These findings represent a replication of two sets of previous findings: (1) youth with MDD and anxiety disorders report sleep difficulties but exhibit few differences from healthy youth in sleep EEG [10, 12, 16]; and (2) slow wave sleep decreases with age [27].

Our results for the interaction of puberty and affective disorder to influence RA and stage 4 sleep suggest that the reason that sleep patterns in adolescents with affective disorders are unclear may be because of effects of development. This may be an explanation for why the sleep changes in adolescent affective disorders have thus far eluded us, while sleep in adult affective disorders is so well described. Maintaining a developmental perspective may be an important consideration in studies of sleep and affective disorders in adolescence. The fact that puberty may play a role in the sleep changes in adolescent affective disorders is particularly important because sleep disturbances are central to affective disorders and puberty is a major risk factor for affective disorders [15].

This lack of decrease in REM activity with pubertal development among the depressed adolescents may represent the development of the REM disinhibition that is
well-described in adults [7]. It has been shown that depressed children do not have differences in their REM sleep[10], while adults do. Though these data are not longitudinal, it may be that this disinhibition of REM occurs as depressed adolescents go through puberty. Because the onset of puberty is also the time when the risk for depression goes up significantly, future researchers could explore how central REM disinhibition might be to the onset and course of depression.

Our findings for puberty effects on sleep within the anxiety disorder group suggest that anxious children and teens may be showing more “mature” sleep patterns early in adolescence when compared with healthy or depressed counterparts. Though the anxious youth also seemed to decrease in the amount of stage 4 sleep that they get across puberty, the pre/early pubertal anxious teens had less stage 4 than the pre/early healthy subjects, almost as if the anxious subjects had begun the developmental decrease in stage four earlier. Also, the RA of the anxious subjects did not decrease across pubertal development (as in the healthy group), and the anxious teens at all levels of development have the same RA as the mid/late pubertal healthy teens. More work is necessary, but it may be that children and adolescents with anxiety may have sleep patterns similar to more developed healthy adolescents.

This study has multiple limitations. The study design was cross sectional, which limits our ability to draw conclusions about within-person change with development. Also, using only the first night of sleep data could introduce adaptation confounds into our data.

In summary, the findings of the current study suggest that development plays an important role in the sleep disturbances of young people with affective disorders.
Considering puberty in particular shows promise for helping to elucidate the timing and nature of associations between affective disorders and sleep problems, as puberty could influence the onset and course of sleep problems in those with affective disorders.
References

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Figure 1. Plot of mean self-reported sleep quality within the diagnostic groups. Error bars represent 1 standard error of the mean. *p<0.05, **p<0.01, *** p<0.001.

Figure 2. Minutes of stage 4 sleep versus age, by diagnostic group. Regression line is for the entire sample. $R^2=0.28$
Figure 3. Development by diagnosis interactions. (a) Average REM activity (RA) for developmental groups, by diagnostic group. (b) Average Stage 4 sleep for developmental groups, by diagnostic group. Error bars represent 1 standard error of the mean. *p<0.05, ** p<0.01, ***p<0.001.
Rebel with a Cause: Brain Activation Associated with Testosterone Levels May Explain Increased Sensation Seeking in Adolescents

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Adolescence is a time of enormous increase in risky behavior, which contributes greatly to morbidity and mortality in this age group (Steinberg, 2004). It is thought that this increase may be due to developmental changes in neural function relevant to sensation seeking (SS), or the tendency to pursue highly stimulating activities (Martin et al, 2002; Forbes and Dahl, 2010).

SS has been found to be greater in adolescents with higher circulating levels of testosterone (Daitzman and Zuckerman, 1980). SS also has been related to activity under emotional stimuli in brain areas implicated in emotional reactivity (Joseph et al 2009). Finally, testosterone produced during adolescence is known to be related to structural and functional changes in brain regions important for emotional processing (Nelson et al, 2005). The current study examined the hypothesis that the relationship between testosterone and SS in adolescents may occur through reward-related brain function.

62 participants (M=14.5 years, SD=0.8; all mid/late pubertal (Tanner 3/4/5) by physical exam) with no history of medical or psychiatric problems participated in a functional magnetic resonance imaging (fMRI) scan, had blood spot sampling via a minimally invasive finger stick (Worthman and Stallings, 1997) assayed for testosterone (M=213.7 ng/dL, SD=194.7) and completed the Sensation Seeking Scale for Children (SSSC) (M=15.1, SD=3.2) (Russo, et al 1991).
The fMRI session was on a Siemens Allegra 3T scanner using echoplanar imaging. Blood oxygenation level-dependent (BOLD) response was measured during a monetary reward task that separately examines reward anticipation and reward outcome and has been shown to reliably engage the striatum, an important brain region for reward processing (Balleine et al, 2007; Haber & Knutson, 2010). Processing was conducted using SPM2 and second-level analyses were performed in SPM8. Regression models were conducted with brain function as the dependent variable and SS as an independent variable, using a threshold of $p<0.01$ and a cluster size of 20 voxels. Results were masked for the effects of the task. To constrain analyses by relevance to SS, data from significant striatal clusters in these regressions were then extracted and testosterone was regressed with the SS/reward relationship, because of our a priori hypothesis about caudate function and SS, as well as evidence that this task strongly activates the caudate.

We found no significant relationship between gender and SS, but testosterone was related to gender ($F=116.252, p<.001$). Also, testosterone level was significantly related to SS ($F=4.429, p<.05$). Regressions indicated that SS was positively correlated with caudate response to both reward anticipation (35 voxels, $T=2.94, p<0.01$, Talairach coordinates -9,-1,20) and reward outcome (180 voxels, $T=3.72, p<0.001$, Talairach coordinates 1,1,15; Figure 1). In both boys and girls, testosterone was positively correlated with SS-relevant caudate activation during reward anticipation ($F=6.363, p=.018$ boys; $F=5.924, p=.021$ girls; Figure 2).

Although SS was not directly related to circulating testosterone level, we found that testosterone was associated with the neural response to reward that had been
correlated to SS. This hormone-brain-experience finding suggests that functional neuroimaging may help explain how hormones influence individual differences in behavior during adolescence.
Figure 1. Regression between Sensation Seeking and Caudate activation during Reward Outcome.

\[ R^2 = 0.19 \]
Figure 2. Regression between Testosterone and Caudate activation during Reward Anticipation, in the cluster most strongly related to Sensation Seeking, split by gender.
Conclusion: WD sleep was reduced for children as they transitioned to kindergarten. This is likely due to reduced nap opportunities in the kindergarten setting. Caretakers advanced the WD nocturnal sleep period presumably to prevent sleep loss as children transition to kindergarten. Earlier WE bedtimes and rise times suggest an advance in the circadian period. Further research is warranted to better understand the effect of sleep loss and changes in circadian processes on behavioral and physiological functioning.

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0935

adolescent slow-wave sleep decreases with age rather than puberty in a longitudinal sample

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Introduction: Time spent in slow wave sleep declines over the course of puberty. However, as advancing pubertal status is correlated with age, it can be difficult to separate these effects. Using a longitudinal sample that covers the age range of pubertal development, these analyses aim to uniquely separate the effects of puberty from those of age.

Methods: 28 participants (Age range: 6.6-15.9 years) completed multiple polysomnography (PSG) studies over the course of 7 years (Mean = 4.5 years). Participants spent at least two nights in the lab at every study (max = 5) and also completed sleep diaries. Tanner staging for pubertal development was performed by trained research nurses, and participants were classified as pre/early (Tanner breast/genital score = 1 or 2) or mid/late (Tanner score = 3-5) pubertal status. PSG data were scored by coders trained to reliability. Results from night one were used in the analyses because of procedural differences in subsequent nights. Mixed models were conducted using pubertal status and age as predictor variables for each sleep variable. Variables included were sleep latency, time spent in each Stage of non-REM sleep (1-4), total REM sleep, REM activity, REM sleep latency, sleep efficiency, self-reported sleep quality and ease of waking.

Results: In this longitudinal sample, stage 3 sleep, stage 4 sleep, and REM latency decreased with age. Decreases were unrelated to pubertal status.

Conclusion: Results suggest that age, independent of pubertal development, accounts for some of the normal changes in sleep at this point in the lifespan, consistent with recent findings from other studies. Adolescence is a time of simultaneous biological, social, and emotional changes, and all of these could contribute to changes in sleep. Since some of the changes in sleep are related to pubertal development, it is interesting that changes in sleep architecture seem to be more closely related to age.

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Measuring Sleep Latency in Pediatric Insomnia Trials: Role of Actigraphy in Relation to Polysomnography

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Introduction: Polysomnography (PSG) is a standard outcome measure for insomnia trials. However, actigraphy may have advantages over PSG in tolerability, cost, and ease of repeated measurements. We studied the tolerability and ability of actigraphy to detect change in sleep latency (SL) in children with autism participating in a pilot trial of supplemental melatonin.

Methods: Children had a clinical diagnosis of autism confirmed by the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview-Revised. All children had sleep-onset insomnia, with SL > 30 minutes at least 3 nights a week. After 3 weeks of baseline data were obtained, melatonin (Natrolo®) was begun at 1 mg, with dose increased every three weeks (to 3 mg, then 6 mg) until the child achieved an actigraphically-measured SL < 30 minutes on 5 or more nights/week. Actigraphy (Phillips Respironics) was obtained each night for 17 weeks, with PSG obtained at baseline and each dose of melatonin in a subset of children.

Results: Fifteen children participated, age 6.5 years (2.2) [mean (standard deviation (SD))]. All children tolerated actigraphy, with average percentage of 0.72 scorable nights for each child. Four of seven children tolerated baseline PSG studies. Sleep latency measured simultaneously by actigraphy and PSG was highly correlated (r = 0.77; P < 0.001; Spearman correlation) and did not differ for PSG [50.5 (46.5)] vs. actigraphy [47.6 (52.8)]; P = 0.50; Mann-Whitney test. Actigraphy-based SL was 44.5 minutes (23.8) at baseline and 23.7 minutes (8) at end of study (P = 0.05; Wilcoxon-signed rank test). Actigraphy-based SL was stable in the 2nd and 3rd weeks, as compared to the first week, of each dosing period.

Conclusion: In this 17-week trial, actigraphy was accurate, better tolerated than PSG, and able to detect change in SL after intervention, supporting its use as an outcome measure in pediatric insomnia trials.

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Resident Bone Marrow Stem Cells Are Recruited to Peripheral Circulation in Children With OSA: Relevance to Endothelial Function

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Introduction: Endothelial dysfunction is a potential complication of obstructive sleep apnea syndrome (OSAS) in children, and has been ascribed to systemic inflammatory changes. However, not all children with OSAS will manifest endothelial dysfunction. We hypothesized that the variability in endothelial function in OSAS may be related to the ability to recruit repair mechanisms such as bone marrow derived stem cells (BMSC).

Methods: Pre-pubertal non-hypertensive children with or without polysomnographically-confirmed OSAS were recruited. Endothelial function was assessed in a morning fasted state, using a modified hyperemic test involving cuff-induced occlusion of the radial and ulnar arteries. Blood was drawn and 3 types of BMSC were assessed by flow cytometry, namely endothelial progenitor cells (EPC), hematopoietic stem cells (HSC), and very small embryonic-like stem cells (VESL).

Results: 25 children with OSAS (mean age 7.6 ± 1.5 years, mean BMI z-score: 1.23 ± 0.6) and 10 age-, gender-, ethnicity-, and BMI-matched controls (CO) were studied. Compared to CO, significant delays to peak capillary reperfusion after occlusion release (Tmax) occurred in the OSAS children as a group, but substantial individual variability was present. Similarly, EPC, HSC, and VSEL counts were significantly higher in OSAS children compared to CO (P < 0.01). However, Tmax was significantly and inversely correlated with EPC (P < 0.01), but not with HSC or VSEL.

Conclusion: OSAS in children is associated with increases in BMSC in peripheral blood. Endothelial dysfunction is a frequent, yet not universal consequence of OSAS in children. The variance in endothelial functional phenotype in pediatric OSAS appears to be associated with the ability to recruit BMSC, and more specifically EPC.

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B. Clinical Sleep Science - V. Psychiatric and Behavioral Disorders and Sleep

0683
DEVELOPMENTAL CHANGES IN SLEEP MAY DIFFER IN ADOLESCENTS WITH AFFECTIVE PSYCHOPATHOLOGY
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Introduction: Because affective disorders involve sleep disruptions and increase in incidence sharply around the same time sleep patterns shift in early adolescence, characterizing sleep in adolescents with psychopathology is highly relevant. This study characterized sleep changes in relation to age and puberty in adolescents with major depressive disorder (MDD), anxiety disorders, and co-morbid MDD and anxiety.

Methods: 411 participants (Age mean = 11.3; range = 6.0-17.0 years) completed a polysomnography (PSG) study, and were evaluated using the K-SADS for diagnosis: healthy (n = 189), MDD (n = 122), anxiety (n = 54) and co-morbid MDD/Anxiety (n = 46). Participants spent at least two nights in the lab and completed sleep diaries. Tanner staging was performed by trained research nurses, and participants were classified as pre/early (Tanner breast/genital score = 1-2) or mid/late (3-5) pubertal status. PSG data were scored by coders trained to reliability. Only results from night one were included because of procedural differences in the subsequent nights. Statistical analyses used linear models with pubertal status, age and diagnosis (MDD, ANX, MDD/ANX and healthy) as predictor variables for each sleep variable. Sleep variables included were sleep latency, time spent in each stage of non-REM sleep (1-4), total REM sleep, REM activity (RA), REM latency, sleep efficiency, and self-reported sleep quality and ease of waking.

Results: As in other studies of adolescent development, stage 3 and stage 4 sleep decreased with age. Clinical status predicted stage 2 sleep, sleep quality, and ease of waking. The puberty X diagnostic interaction predicted RA (F = 3.655, df = 3, P = .013), with healthy pre/early-pubertal subjects exhibiting greater RA (Mean = 148.1, SD = 87.5) than healthy mid/late-pubertal (Mean = 68.9, SD = 83.4).

Conclusion: This study allows us to evaluate how pubertal development and its relationship to sleep may differ among adolescent clinical populations, elucidating sleep-related developmental psychopathology issues.

0684
RELATIONSHIPS BETWEEN SLEEP AND MOOD IN ADOLESCENTS WITH BIPOLAR DISORDER
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Introduction: A growing body of research suggests strong relationships between sleep and mood in adult bipolar disorder (BD), with multiple studies documenting that changes in sleep duration predict subsequent changes in the polarity of mood. However, very little is known about the relationships between sleep and mood functioning in individuals with early-onset BD. We sought to prospectively examine the associations between sleep and morning mood in a sample of adolescents with BD and matched comparison groups of youth with attention-deficit hyperactivity disorder-combined type (ADHD-C) and those without psychopathology.

Methods: Participants included 13 adolescents (ages 11-17) diagnosed with BD who were between mood episodes, 14 diagnosed with ADHD-C, and 21 healthy controls. Sleep diaries were collected over four consecutive nights. A modified version of the Positive and Negative Affect Scale (PANAS) was used to collect parent-report ratings of Negative Affect (NA), Positive Affect (PA) and Irritable Affect (IA) each morning. Random coefficient regression was used to examine associations between nightly total sleep time (TST) and subsequent morning mood.

Results: Total sleep time was a significant predictor of morning NA, F(1, 162.29) = 5.99, P = .015, with reductions in TST associated with increases in NA. This effect was not statistically different between groups. TST was also a predictor of morning IA, F(1, 160.33) = 5.47, P = .021. The interaction term was significant for those with BD, b = -.009, t(1, 161.00) = -2.20, P = .030, indicating that the negative association between TST and IA was particularly strong in this group. TST was not a significant predictor of morning PA.

Conclusion: These results support the hypothesis that adolescents with BD may be particularly sensitive to slight sleep alterations and are more likely than their peers to experience negative morning mood as a result of decreases in sleep time.

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0685
SLEEP-WAKE BEHAVIOUR IN YOUNG PATIENTS WITH BIPOLAR DISORDER AND ADHD
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Introduction: The early stages of bipolar disorder (BDP) and attention deficit hyperactivity disorder (ADHD) share a number of common traits, making an accurate diagnosis problematic. Diagnostic recognition at the earlier stages of symptom progression, provide for specific sets of targeted interventions. In order to identify potential distinct biomarkers between these two disorders, we have first examined sleep-wake patterns using actigraphy.

Methods: To date n = 10 patients with BDP (6m, mean age 23.13 ± 4.08) and n = 7 patients with ADHD (7m, mean age 18.43 ± 5.59) were recruited from 2 community based mental health clinics. All participants completed at least 2 weeks of actigraphy and sleep diaries. Differences in sleep variables between the two groups were analysed using t-tests.

Results: Average sleep duration was 530.7 ± 55.2mins in patients with ADHD and 568.3 ± 59.3mins in patients with BDP. Compared to patients with BDP, patients with ADHD had significantly greater average WASO (t = 2.63, P = 0.019) and a trend for less variability in sleep duration across the data collection period (t = -2.21, P = 0.050). The patients with BDP experienced a mixture of short and long sleep durations throughout the assessment period. In addition, there was a trend for patients with ADHD to have greater activity levels during waking periods: 506.0 ± 129.6mins versus 399.7 ± 94.9mins (t = 1.96, P = 0.069).

Conclusion: Even in this small sample, differences in sleep-wake behaviour between patients with BDP and ADHD is evident. The decreased stability in sleep duration in patients with BDP may reflect underlying neurobiological mechanisms, including reports of circadian disturbance that are typically observed in BDP. Increased activity levels, both during wake and sleep periods in patients with ADHD, would appear characteristic of symptoms of this disorder.

0686
COMORBIDITY AND PSYCHOSTIMULANT USE AS POTENTIAL MODERATORS OF THE RELATIONSHIP BETWEEN ADHD AND SLEEP IN CHILDREN
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Introduction: It has been suggested that the presence of psychiatric comorbidity and the use of psychostimulant might moderate the relationship between sleep disturbances and Attention-Deficit/Hyperactivity Disorder (ADHD) in children. However, support for this hypothesis

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Results: We found that LL-rearing was associated with pronounced alterations in the ventral hypothalamic region. In LL-reared rats, there was an increase in retinal input to and a significant reduction in light-induced Fos-expression in the ventral hypothalamus. These differences were reduced, but not eliminated, by a switch to LD environment at 3 months. Conclusion: These data provide novel information about the influence of light on the postnatal development of subcortical visual nuclei, and support a mechanism whereby the effects of light-rearing on the timing of sleep/wakefulness might be explained.

0057
PUBERTAL MATURATION, REWARD-RELATED BRAIN FUNCTION, AND SLEEP IN ADOLESCENTS
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Introduction: Puberty is a time of dramatic changes, including changes in sleep. The onset of adolescence is also a period of new health concerns related to increases in risk-taking, sensation-seeking, depression, substance use, and accidents. The larger study examined pubertally-specific changes in adolescents' reward-related behavior and included functional neuroimaging of reward as well as objective and subjective measures of sleep. This presentation will focus on the relationship between sleep and reward.

Methods: 58 participants age 11-13 completed four-days of home actigraphy and self-reported sleep ratings and a functional magnetic resonance imaging scan using a guessing task with monetary rewards that separated reward anticipation from reward outcome. Sleep variables included in the analyses were mean weekend minutes asleep and self-reported sleep quality.

Results: During reward anticipation, both fewer minutes asleep (t=2.67, p<.01, 23 voxels at 0, 1, 18) and lower sleep quality (t=2.44, p<.01, 13 voxels at -4, 2, 11) were associated with less activation in the caudate. During reward outcome lower sleep quality was associated with less activation in the caudate (t=2.86, p<.005, 214 voxels at -6, 6, 11).

Conclusion: Our results support the hypothesis that sleep patterns in adolescence are associated with altered patterns of activation in reward circuitry in ways that could have important health implications. One set of hypotheses about response to reward in adolescents is that adolescents' low reactivity in reward-related brain areas could lead to compensatory increases in reward-driven behavior. These findings suggest that sleep characteristics could also contribute to such behavior. Because decreased sleep has been associated with risky behavior and negative mood, these findings raise concerns about a negative spiral of effects whereby maturational effects of puberty and sleep deprivation may have synergistic effects on reward processing, contributing to adolescent behavioral and emotional health problems.

0058
GABAA RECEPTORS IMPLICATED IN REM SLEEP CONTROL
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Introduction: The sublaterodorsal nucleus (SLD) is one brain area identified in the rat as a REM sleep induction zone. Local application of GABAA receptor antagonists to the SLD can induce REM sleep. This finding is consistent with local GABAA receptors exerting negative control over REM sleep. Here we sought to determine the GABAergic innervation and subunit composition of the GABAA receptors implicated in REM sleep control. Two GABAergic afferents were studied, that from the caudal, ventrolateral periaquiductal grey (vPAG) and nucleus pontis oralis (PnO).

Methods: Long-Evans Hooded rats were unilaterally injected in the vPAG or PnO with a solution of biotinylated dextran amine (BDA, 10k MW) and sacrificed 10 days later. Coronal sections were studied through the SLD after multiple labeling of orthogradely transported BDA, the vesicular GABA transporter (VGAT) to visualize GABAergic terminals, and an antibody to one of the GABAA receptor subunit proteins. Colocalization of immunoreactivity was visualized with fluorescence, laser scanning, confocal microscopy. The experimental strategy sought to find triple-labeled varicosities in SLD that would identify the source of GABA terminals and post-synaptically apposed receptor subunits.

Results: We found GABAergic terminals (BDA/VGAT labeled varicosities) in SLD that have their source in neurons located in the PnO and vPAG. Many of these terminals have a somatic location where they can provide a potent inhibitory effect. Conclusive evidence for the colocalization of a receptor subunit is still being sought with a variety of antibodies.

Conclusion: Based on studies of c-Fos expression, PnO and SLD contain GABAergic neurons with a REM-off pattern of activity. The withdrawal of GABA release by these neurons in the SLD may be an important mechanism in control of REM sleep. Identification of the GABAergic subtype(s) mediating these effects in SLD could provide a novel target for therapeutic pharmacological agents.

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0059
EFFECTS OF CIRCADIAN PERIOD AND SLEEP PRESSURE ON HYPOCRETIN-MEDIATED SLEEP-TO-WAKE TRANSITIONS
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Introduction: The Hypocretins (Hcrts, also called Orexins) are two neuropeptides expressed in the lateral hypothalamus that play a crucial role in boundary state control. Previously, our laboratory demonstrated that in vivo photostimulation of Hcrt neurons genetically targeted with ChR2, a light-activated cation channel, is sufficient to increase the probability of an awakening event during both slow-wave sleep (SWS) and rapid eye movement (REM) sleep. In the current study, we ask whether Hcrt-mediated sleep-to-wake transitions are affected by circadian period and sleep pressure.

Methods: We stimulated Hcrt neurons in mice during either SWS or REM sleep and then measured the latency from sleep to wakefulness. We also stimulated mice immediately following 2 or 4 hours of sleep deprivation by gentle handling.

Results: We found that stimulation of Hcrt neurons increased the probability of an awakening event throughout the entire circadian period but that this effect was lost with sleep pressure induced by 2 or 4 hours of sleep deprivation. Interestingly, photostimulation of Hcrt neurons was still sufficient to increase activity in Hcrt neurons after sleep deprivation, even though this stimulation did not cause an increase in transitions to wakefulness. We also demonstrate that photostimulation of Hcrt neurons increases neural activity in the downstream arousal-promoting locus coeruleus and tuberomammillary nucleus, but not after 2 hours of sleep deprivation. Finally, stimulation of Hcrt neurons was still sufficient to increase the probability of an awakening event in histamine-deficient HDC KO animals.

Conclusion: These results suggest that the Hcrt system promotes wakefulness throughout the circadian period by exciting downstream targets, which themselves are inhibited with increased sleep pressure.
Despite the ubiquity of sleep across species, surprisingly little is known about its functions. It is known that during human adolescence, sleep patterns typically undergo marked changes. Electroencephalogram (EEG) studies indicate a 50% reduction of deep (stage 4) sleep and a 75% reduction in the peak amplitude of delta waves during nonrapid-eye movement sleep in adolescence [1]. Changes in circadian rhythms impart a tendency for later sleep onset. School schedules are often incompatible with a corresponding delay in sleep offset, leading to a less than optimal amount of sleep for the majority of adolescents.

Adolescence is also a time of dramatic changes in body, brain, and behavior. Converging evidence from structural and functional magnetic resonance imaging, electrophysiological, and postmortem studies indicate dynamic changes in the adolescent brain. Three robustly replicated themes emerging from these studies are: (a) greater “connectivity” exemplified by increases in white matter volumes, functional magnetic resonance imaging (fMRI) correlations among disparate regions during tasks, and EEG coherence; (b) a changing balance between earlier maturing/puberty-related limbic systems and later-maturing frontal executive function systems; and (c) a pattern of childhood peaks followed by adolescent decline for gray matter volumes [2]. If these gray matter volume reductions are partially accounted for by decreases in synaptic density, it may be related to the EEG changes noted above, as EEG signals emanate from spatially coherent activity of synapses [3]. A recent study examined the possible relationship between anatomical brain changes and EEG changes by acquiring both MRI and quantified EEG in 138 healthy subjects from ages 10 to 30 years [4]. Curvilinear reductions in gray matter volume of the frontal and parietal cortex were matched by similar curvilinear reductions in EEG power of the corresponding regions, supporting the connection between gray matter volume reductions, EEG changes, and synaptic pruning.

The behavioral manifestations associated with these changes vary considerably, depending on social context, but adolescents in all social mammals tend to demonstrate greater propensities for affiliation with same age peers and increases in sensation seeking and risk taking.

The link between these adolescent changes in sleep, brain, and behavior remain poorly understood. In “Reward-Related Brain Function and Sleep in Pre/Early Pubertal and Mid/Late Pubertal Adolescents,” published in the current issue of the Journal, the authors [5] provide an early step to address this void of knowledge. Specifically, they examine the relationship between various measures of sleep and fMRI measures during a task designed to assess reward anticipation and reward outcome.

The study of reward systems during adolescence is particularly relevant because of their centrality to decision making. Many of the sources of adolescent morbidity and mortality are directly related to decision making. Whether to use drugs, have unprotected sex, drive recklessly, or take other risks are all influenced by how the brain’s reward systems assess cost and benefit. One of the challenges addressed by the study is to untangle the pubertal/hormonal effects of adolescence from other maturational effects. To partially isolate puberty-specific effects the authors examined subjects in a relatively narrow age range who differed on Tanner stage. Perhaps because of the relative ease of manipulating hormone levels in animal models a preponderance of the literature focuses on pre/post puberty comparisons, although future studies should also address the many brain and sleep changes occurring well after the completion of puberty. The results of the study indicate that poorer sleep is related to less activation of the caudate nucleus during reward anticipation and reward outcome. It is hypothesized that this lower activation may result in compensatory increases in the reward-driven behavior characteristic of teens. As with all studies relating imaging findings to behavior, caution is merits in interpretation, particularly with regard to causality. There is rarely a one-to-one correspondence between a particular brain region and a discrete function. Most functions involve many regions, and most regions are involved in many
functions. Longitudinal studies following changes in an individual’s sleep, fMRI activations, and behavior will help to elucidate the brain/sleep/behavior causal relationships. Also of interest would be to assess whether genetic differences relate to individual variance in sleep parameters.

In addition to the possible association of sleep changes with depression, substance abuse, and accidents noted in the article, there may also be connections with other disorders typically emerging during adolescence, such as schizophrenia. Based on his interpretation of the dramatic decreases in delta sleep of healthy adolescents as reflecting robust synaptic pruning, Irvin Feinberg, in 1982, postulated that schizophrenia may be a consequence of an exaggeration of typical synaptic elimination [6]. Subsequently, studies of membrane phospholipids, prefrontal metabolism, and frontal cortical gray matter changes have lent support to this “exaggeration of typical adolescent changes” hypothesis for schizophrenia [7–10].

An important point made by Holm and his colleagues [5] is that biological tendencies for sleep changes during adolescence do not mean that interventions are futile. The tendencies can be modulated by efforts to promote more sleep, and the effects of such interventions can be assessed to further elucidate the relationship between sleep quality and behavioral outcomes.

All in all, the current article represents an important but early step in our exploration of the relationship between sleep, brain development, and behavior in adolescence. Given that one-third of our lives are spent asleep and that the amount and quality of sleep has profound impact on our health, cognition, and behavior, it seems prudent to devote more resources to research this understudied domain. The dramatic changes in sleep, brain, and behavior during the second decade of life make this an even more compelling mandate for adolescent health research.

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References