Short Sleep as an Environmental Exposure—Carskadon et al

INTRODUCTION

Depression has been linked to a polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR in SLC6A4). Nevertheless, the association is indirect and findings have been mixed. Caspi and colleagues indicate that environmental moderators play a significant role in the link between genetic background and symptom development; that is, exposure to stressful life events constitute a moderating influence on the association between 5-HTTLPR genotype and depression. The mechanism(s) by which stressful life events may influence the development of mood disorders in genetically susceptible individuals have not been elucidated.

A link between insomnia and depression is well known and considered a bidirectional process. Thus, for example, sleep difficulty is a prominent feature of depressive illness, including trouble falling asleep, staying asleep, waking too early in the morning, or, in some, the atypical manifestation of hypersomnolence. On the other hand, epidemiologic studies show that past insomnia predicts future development of depression in older humans as well as young adults. Short sleep per se has been associated with mood problems, and a recent study found that teens reporting short sleep or extended sleep were at highest risk of suicidal tendencies.

The serotonin neurotransmitter system is a central actor in depression, most notably in depression pharmacotherapy, which features drugs that affect serotonin reuptake. Serotonin also plays a prominent role in sleep neuroregulation: activity in serotonin-rich neurons of the raphé nucleus inhibits the expression of rapid eye movement (REM) sleep. The interaction of REM sleep systems and depressed mood has been identified for decades. For example, short REM sleep latency is a feature common to a subgroup of patients with major depressive disorder, and suppression of REM sleep either operationally or pharmacologically improves mood of depressed patients. Furthermore, these authors demonstrated that reversal of this blunting required a comparable amount of daily recovery sleep. These data led us to speculate...
METHODS

First-year students who accepted admission to Brown University in April 2009 and April 2010 were invited by e-mail to join the project. The 2009 invited cohort included all incoming students (n = 1,498); the 2010 cohort included only students age 18 yr and older (n = 1,340). Parental permission was sought for participants younger than 18 yr, a procedure exclusive to the 2009 cohort. All procedures were approved by the Rhode Island Hospital/Lifespan Institutional Review Board for the Protection of Human Subjects. Participants were compensated for completing 3 and 7 consecutive days. The outcome survey was available to complete each day from early evening until early the next morning, and access was restricted by requiring students to log in with their identification number and a password. Participants were incentivized to complete diaries each day with a $1.00 payment and small bonuses for completing 3 and 7 consecutive days. The outcome survey included questions about sleep and behaviors, as well as our primary mood outcome measure, the Centers for Epidemiology Studies-Depression (CES-D) scale. Analyses were performed using SPSS version 19 statistical package (IBM, Armonk, NY).

RESULTS

As shown in Table 1, phenotype groups did not differ with respect to sex distribution (chi-square = 1.39; df = 3; P = 0.71), age (F_{3,131} = 0.67, P = 0.57), racial distribution (chi-square = 14.1; df = 12; P = 0.295), or number of diary nights (F_{3,131} = 0.04, P = 0.99). As designed, TST (F_{3,131} = 125.87, P < 0.001) and subsequently through the first 8-9 wk after the start of their first semester.

SLEEP, Vol. 35, No. 6, 2012
CES-D (F$_{3,31} = 69.48$, $P < 0.001$) showed significant group differences driven by the phenotype grouping parameters, although Fisher LSD demonstrated that the average TSTs did not differ between the 2 shorter TST groups or between the 2 longer TST phenotype groups. Furthermore, the CES-D scores did not differ between the 2 high CES-D groups and between the 2 low CES-D groups. Table 2 shows significant differences in racial distribution and TST among the 3 genotype groups: the S’S’ group had a significantly higher proportion of Asian students, i.e., 74% of Asian students were S’S’. In addition, Fisher LSD indicates that students in the S’S’ group had shorter TSTs than those in the S’L’ and L’L’ groups. Sex distribution, age, number of diary nights, and CES-D scores did not differ by genotype group.

Table 3 provides the distribution of genotypes for each of the phenotypic groups. Results of an exact test for Hardy-Weinberg proportions using Markov chain–Monte Carlo implementation$^{33}$ indicate that our observed genotype frequencies do not differ from Hardy-Weinberg equilibrium ($P = 0.598$). A significant

**Table 1**—Phenotype group demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Shorter sleep and high CES-D</th>
<th>Shorter sleep and low CES-D</th>
<th>Longer sleep and high CES-D</th>
<th>Longer sleep and low CES-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>40</td>
<td>34</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Age in years (SD)</td>
<td>18.1 (0.4)</td>
<td>18.2 (0.7)</td>
<td>18.0 (0.3)</td>
<td>18.1 (0.5)</td>
</tr>
<tr>
<td>M/F</td>
<td>13/27</td>
<td>15/19</td>
<td>12/17</td>
<td>14/18</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>16</td>
<td>19</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Asian</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>African</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Other*</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>No. of diaries (SD)</td>
<td>51 (11)</td>
<td>50 (11)</td>
<td>50 (12)</td>
<td>51 (11)</td>
</tr>
<tr>
<td>TST in hr (SD)</td>
<td>6.5 (0.4)</td>
<td>6.5 (0.5)</td>
<td>7.9 (0.3)</td>
<td>7.9 (0.3)</td>
</tr>
<tr>
<td>CES-D score (SD)</td>
<td>21.9 (7.4)</td>
<td>6.7 (3.5)</td>
<td>22.7 (8.6)</td>
<td>7.1 (3.6)</td>
</tr>
</tbody>
</table>

*Race was answered as multiracial, missing, or preferred not to answer. CES-D, Center for Epidemiologic Studies-Depression; M/F, male/female; TST, total sleep time.

**Table 2**—Genotype group demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>S’S’</th>
<th>S’L’</th>
<th>L’L’</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>50</td>
<td>61</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Age in years (SD)</td>
<td>18.1 (0.5)</td>
<td>18.1 (0.4)</td>
<td>18.0 (0.2)</td>
<td>$F_{2,132} = 1.16$, $P = 0.32$</td>
</tr>
<tr>
<td>M/F</td>
<td>19/31</td>
<td>24/37</td>
<td>11/13</td>
<td>$\chi^2 = 0.44$, $df = 2$, $P = 0.81$</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td>$\chi^2 = 23.624$, $df = 8$, $P = 0.003$</td>
</tr>
<tr>
<td>Caucasian</td>
<td>19</td>
<td>35</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>20</td>
<td>7</td>
<td>0</td>
<td>$F_{2,132} = 1.00$, $P = 0.370$</td>
</tr>
<tr>
<td>African</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>$F_{2,132} = 3.124$, $P = 0.047$</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Other*</td>
<td>7</td>
<td>12</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>No. of diaries (SD)</td>
<td>50 (12)</td>
<td>50 (10)</td>
<td>53 (11)</td>
<td></td>
</tr>
<tr>
<td>TST in hr (SD)</td>
<td>6.9 (0.7)</td>
<td>7.2 (0.9)</td>
<td>7.3 (0.7)</td>
<td></td>
</tr>
<tr>
<td>CES-D score (SD)</td>
<td>16.0 (10.1)</td>
<td>14.5 (9.6)</td>
<td>12.7 (9.9)</td>
<td></td>
</tr>
</tbody>
</table>

*Race was answered as multiracial, missing, or preferred not to answer. CES-D, Center for Epidemiologic Studies-Depression; M/F, male/female; TST, total sleep time.

**Table 3**—Distribution (n) of genotypes within phenotype groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Shorter sleep and high CES-D</th>
<th>Shorter sleep and low CES-D</th>
<th>Longer sleep and high CES-D</th>
<th>Longer sleep and low CES-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>S’S’</td>
<td>23</td>
<td>12</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>S’L’</td>
<td>14</td>
<td>14</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>L’L’</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

CES-D, Center for Epidemiologic Studies-Depression.
overrepresentation of the S’S’ genotype for the 5-HTTLPR was seen for participants who reported shorter sleep and high CES-D scores relative to other groups (chi-square = 15.04; df = 6; P = 0.02). Figure 1 illustrates the proportion of participants carrying the genotypes who were shorter sleepers with high CES-D scores: almost one-half of the S’S’ group showed this phenotype, whereas approximately one-fourth with the S’L’ genotype and only one-eighth of the L’L’ genotype had this phenotype.

The bidirectional nature of the sleep and depressed mood association, i.e., that depressed mood can predict poor sleep and vice versa, raises concern that preexisting depression might be the determining factor in the observed genetic association. Thus, to exclude the possibility that the shorter sleep times observed in the daily diaries were biased by ongoing depressive symptoms that preceded the transition to university, we repeated this analysis after removing participants whose CES-D scores from a precolligate prospective survey were above a clinically meaningful cutoff score of 16. Although 32 participants were excluded on this basis, the shorter sleep/high CES-D phenotype remained distinguished from the others by overrepresentation of S’S’ genotype (chi-square = 12.90; df = 6; P = 0.045).

**DISCUSSION**

Our findings indicate that 1st-year university students who carry 2 alleles of the low-expressing polymorphism of the serotonin transporter gene reported more depressed mood in the presence of a persistent pattern of short nocturnal sleep. Sleep difficulties and depression have been linked in the clinical pantheon since the earliest descriptions of mood disorders (e.g., Hippocrates, 4th century BC). Yet, not all who suffer from sleep disturbance or short sleep are depressed. We propose that a short sleep pattern—whatever the source (insomnia, stress, lifestyle choices)—constitutes an “environmental exposure” that interacts with genetic vulnerability (5-HTTLPR S’S’ genotype), leading to increased likelihood of depressed mood in susceptible individuals. This model nests well within the current theories on 5-HTTLPR and its role in biased attention for emotionally salient cues and plasticity to environmental influences. For example, a short sleep pattern might reduce the cognitive resources required to shift attention away from negative stimuli. This reduced ability to disengage from negative stimuli would suggest that negative environmental influences might have a more profound effect on mood in relatively sleep-deprived individuals who tend to have biased attention for such exposures. Although our outcome in this analysis was mood, we expect to see a similar association of 5-HTTLPR and chronic short sleep exposure with other domains such as hostility, attention bias, anxiety, fear, suicidality, and so forth.

Although polysomnographic aspects of sleep have been associated with psychopathology, the role of sleep in our model is based on chronic exposure to longer or shorter sleep rather than sleep electroencephalographic features. Thus, the quantity of sleep over an extended time frame is a central factor that can bias outcome depending on genetic background. A key strength of our approach, therefore, was to capture sleep exposure on a night-to-night basis for an average of 50 nights, rather than to ask for a retrospective estimate or a few nights of polysomnography. This approach enhances the conclusion that shorter sleep exposure over time is associated with symptoms of depressed mood in susceptible individuals. The conclusion is further strengthened by the prospective nature of the study, which allowed us to test the hypothesis and confirm the findings after excluding participants with prior evidence of depressed mood.

Another advantage of our approach is a clear mechanistic pathway from the environmental exposure, i.e., chronic short sleep, to the outcome, i.e., depressed mood. Thus, the physiology of 5-HT1A receptors and 5-HTTLPR—and the interplay between these 2 components of the serotonin system—suggest a possible mechanism by which exposure to short sleep can manifest as increased depressive symptoms in genetically vulnerable individuals. Central 5-HT1A receptors are present in the dorsal raphé nuclei as presynaptic autoreceptors where they inhibit cell firing and decrease serotonin release. In addition, postsynaptic 5-HT1A receptors occur in numerous brain regions that are implicated in mood regulation, e.g., the hippocampus, lateral septum, and cingulate and entorhinal cortex, as well as the raphé nuclei. Decreased serotonergic neurotransmission through 5-HT1A has been implicated in depression and suicide, and Roman and colleagues observed decreased 5-HT1A neurotransmission after 8 days of sleep restriction in the rat. In addition, evidence that low expression of 5-HTTLPR can further reduce 5-HT1A neurotransmission has been shown in 5-HT transporter knockout mice in whom density and expression of 5-HT1A receptors is low. Similarly, a positron emission tomography study in human adults showed that individuals with at least 1 S’ allele for the serotonin transporter have decreased 5-HT1A receptor binding. Thus, exposure to short sleep duration in the presence of S’ alleles of 5-HTTLPR may represent an additional insult to the serotonin system, leaving those with the 5-HTTLPR short alleles most vulnerable to depressed mood from shortened sleep. Figure 2 provides a schematic model of these effects.
We recognize that our findings are limited by a relatively small sample size; however, the project is ongoing and future analyses will examine this finding with more participants. In addition, we plan to use a larger sample to examine whether cumulative or aggregate genetic scores across several genes may help to identify other pathways that affect the association of sleep length to mood disorders, and whether genes that are known to affect sleep “need” and sleep length act as moderator factors in response to the shorter or longer sleep exposures. As with all genetic association studies, we acknowledge a risk of unmeasured third variables accounting for the results, including the possibility of population stratification and linkage disequilibrium between measured and causal variants. A larger sample will allow us to examine these issues.

We note that other outcomes such as weight gain, \(^{41}\) cardiovascular consequences, \(^ {42}\) risk-taking behavior, \(^ {43}\) substance abuse, \(^ {44}\) and impulsivity \(^ {45}\) have been linked to short sleep, though with inconsistent expression. As with depressed mood, individual differences may be understood better by using a gene by “environmental” exposure (G × E) approach. In other words, exposure to chronic levels of insufficient or disrupted sleep may manifest a preexisting vulnerability in genetically susceptible individuals, whereas exposure to longer, less disrupted sleep may lead to improved outcomes.

ACKNOWLEDGMENTS

The authors thank Tifenn Raffray, MD, and Tamara Bond, PhD, for assisting with the design of the surveys, Brandy Roane, PhD, for assistance with data collection and extraction, Caroline Gredvig-Ardetto for data management, Michelle Loxley for Illume programming, Kayla Beaucage for DNA assays, and the staff of the Sleep for Science Research Laboratory for assisting with sample collection. This research was supported by the Sleep Research Society Foundation Elliot D. Weitzman, MD, Research Grant and National Institute of Mental Health grant, MH079179, awarded to MAC, 1S10RR023457-01A1 and Shared equipment grants (ShEEP) from the Medical Research Service of the Department of Veteran Affairs, awarded to JEM, and K23MH086689 awarded to KMS.

DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

REFERENCES

25. Dyson R, Renk K. Freshmen adaptation to university life: depressive
24. Hicks T, Heastie S. High school to college transition: a profile of the
23. Paykel ES. Life events and affective disorders. Arch Gen Psychiatry 1992;49:651-68; discussion
69-70.
4. Carskadon MA, Thompson GA. Short sleep as an environmental exposure—Carskadon et al

3. Carskadon MA, Thompson GA. Short sleep as an environmental exposure—Carskadon et al
1. Carskadon MA, Thompson GA. Short sleep as an environmental exposure—Carskadon et al