Effects of Genotype and Sleep on Temperament

Andrée-Anne Bouvette-Turcot, MSc[a,b,c], Michael Pluess, PhD[d], Annie Bernier, PhD[c], Marie-Hélène Pennestri, PhD[a,b], Robert Levitan, MD[e], Marla B. Sokolowski, PhD[f], James L. Kennedy, MD[e], Klaus Minde, MD[b], Meir Steiner, MD[g,h], Irina Pokhvisneva, MSc[a,b], Michael J. Meaney, PhD[a,b,i], Hélène Gaudreau, PhD[a,b], on behalf of the MAVAN research team

abstract

BACKGROUND AND OBJECTIVES: Sleep problems are frequent in young children; however, children vary in the degree to which they are affected by poor sleep quality. We investigated whether a polymorphism in the serotonin transporter gene, which is linked to emotional function, is a potential moderator of the influences of sleep duration on infant temperament using longitudinal data.

METHODS: We examined the interactive effects of average sleep duration between 6 and 36 months of age and the 5-HTTLPR genotype on negative emotionality/behavioral dysregulation at 36 months in 209 children recruited into a longitudinal birth cohort study. Triallelic genotyping of 5-HTTLPR was performed by looking at *SLC6A4* genotype, focusing on the serotonin transporter-linked polymorphic region (5-HTTLPR) including the SNP polymorphism (rs23351). Child sleep habits were assessed with a maternal self-report questionnaire.

RESULTS: After controlling for demographics and both previous and concurrent maternal depression, multiple linear regression analyses revealed a significant interaction effect of average sleep duration for the first 3 years of life and 5-HTTLPR genotype on child negative emotionality/behavioral dysregulation such that the effects were exclusive to those with low-expressing 5-HTTLPR genotypes.

CONCLUSIONS: The results suggest differential susceptibility to the effect of sleep duration early in life, which reiterates that the short allele of the 5-HTTLPR represents a marker of increased environmental sensitivity regarding emotional development. Differential susceptibility theory posits that certain factors may increase an individual's susceptibility to the environment, in either a positive or negative fashion.

WHAT'S KNOWN ON THIS SUBJECT: Sleep disturbances in infants associate with individual differences in temperament. However, little is known about interindividual differences and potential moderating factors, such as genotype.

WHAT THIS STUDY ADDS: The results suggest that the cumulative effect of total sleep duration during the first 3 years of life on temperament is moderated by child 5-HTTLPR genotype following a differential susceptibility model.

[a]*Ludmer Center for Neuroinformatics and Mental Health, and* [b]*Douglas Mental Health University Institute, McGill University, Montreal Canada;* [c]*Department of Psychology, University of Montreal, Montreal, Canada;* [d]*Queen Mary University of London, London, United Kingdom;* [e]*Center for Addiction and Mental Health, and* [f]*Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada;* [g]*Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, Canada;* [h]*Women's Health Concerns Clinic, St. Joseph's Healthcare, Hamilton, Canada; and* [i]*Singapore Institute for Clinical Sciences, Singapore*

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Sleep disturbances in infants are often associated with individual differences in temperament, including irritability, fussiness, and poor rhythmicity.[1–4] For example, measures of sleep continuity, as assessed by video recording at 6 months of age, predict both maternal and paternal perceptions of temperament.[1] In young children, lack of sleep results in irritable mood, lower tolerance to frustration, emotion lability, and inattention.[5–7] Nap deprivation in 36-month-old children results in increased expressed negativity to the presentation of neutral and negative pictures and decreased expressed positivity to positive pictures.[8] Furthermore, when facing an emotional challenge (solvable and unsolvable puzzles), nap-deprived children displayed dampened positive emotions to solvable puzzles as well as increased negative emotions to unsolvable puzzles. Vriend and colleagues[9] reported that 1 hour of sleep restriction for 4 consecutive nights resulted in increased sleepiness, less positive affective response to stimuli, and increased difficulty in emotion regulation, in children aged 8 to 12 years. These findings establish a causal influence of sleep on emotional outcomes across childhood. Furthermore, the relation between sleep difficulties and negative affectivity in children can persist into adolescence, which underlines the importance of studying the relation between sleep quality and temperament early in development.[10,11] However, research in this area lacks longitudinal designs that include early developmental periods as well as studies that consider individual differences.

Although developmental research often assumes that most children are equally affected by the same environmental factors, a growing number of studies provides evidence that individual characteristics appear to modulate the influence of early life experiences.[12] For instance, differential susceptibility theory posits that certain factors (eg, personality,[13] cortisol reactivity,[14] genes,[12] childhood socialization factors[15]) may increase an individual's general susceptibility to the environment, enhancing the detrimental impacts of adverse environments, as well as the positive effects of supportive settings.[16] Furthermore, genetic differential susceptibility theory, which has been put forward by Bakermans-Kranenburg and van IJzendoorn,[17] specifically emphasizes the moderating role of genetic variance that act as plasticity markers in gene × environment paradigms.[18]

A significant proportion of the differential susceptibility literature has focused on a common variation in the serotonin transporter gene (5-HTTLPR).[19,20] Two functional alleles, long (L) and short (S), result from a 43-bp insertion/deletion in the promoter region of 5-HTT. The S, as opposed to the L, allele has been associated with a significantly reduced in vitro basal transcription of 5-HTT mRNA.[21]

The presence of the S allele is related to numerous outcomes. For instance, individuals carrying an S allele are at greater risk for adulthood depression and other emotional impairments if they experienced early adversity,[19,22–28] whereas in the same context, the L allele appears to be protective.[19] Such findings, and other gene × environment studies investigating 5-HTTLPR moderating role of early care quality,[12,29,30] suggest that the 5-HTTLPR polymorphism influences sensitivity to the environmental context.

Furthermore, serotonin is known to play a role in the regulation of behavioral states[31,32] by suppressing rapid eye movement sleep[31,33] and maintaining the circadian rhythm of the sleep-wake cycle.[34] Importantly, sleep and wakefulness are complex processes, and observed individual differences are most likely influenced by both genetic and environmental factors. Evidence from studies examining genetic data in sleep research have found that persistent short sleep schedule in university students was associated with increased depressed mood in carriers of the 5-HTTLPR SS genotype, compared with SL or LL carriers.[35] The authors proposed that short sleep duration, as an adverse "environmental exposure," interacts with the genetic vulnerability in the prediction of mood disorders as well as other problematic phenotypes (eg, anxiety, fear, hostility, suicidality, and attention bias).

Reported associations between sleep and temperament tend to be moderate in magnitude.[4,10,36] One reason for these moderate effect sizes may be that effects vary between individuals as a function of moderating factors, including genetic ones. Building on existing but scarce literature on sleep and emotional development,[37] this study aimed to investigate genetic moderation of sleep duration in early childhood on child temperament.

Considering the influence of the 5-HT systems on both emotional function and sleep, we hypothesized that S allele carriers would be more affected by sleep duration (from 6 to 36 months), such that they would display higher negative emotionality/behavioral dysregulation (NE/BR; 36 months) in the context of shorter sleep duration over time. However, on the basis of the differential susceptibility theory, we expected S-allele carriers not only to be more negatively affected by shorter average sleep duration but also more positively affected by longer average sleep duration, compared with other 5-HTTLPR genotypes. This study is, to our knowledge, the first to examine the relation between total sleep duration in the first years of life and

NE/BR using longitudinal data from a birth cohort study.

METHODS

Participants

Our community sample consisted of 209 mothers recruited in Montréal (Québec) and Hamilton (Ontario), Canada, at 13 to 20 weeks' gestation from antenatal care clinics at the time of routine ultrasound or through advertisements at hospitals (see Table 1 for demographic information). Participants were part of the Maternal Adversity, Vulnerability, and Neurodevelopment (MAVAN) study, which examines the development of individual differences in phenotypes associated with multiple forms of psychopathology. Eligibility criteria for mothers included age 18 or older, singleton gestation, and fluency in French or English. Women with severe chronic illness (other than hypertension, asthma, or diabetes) and other serious medical conditions (eg, placenta previa) were excluded. Only babies born at 37 weeks or later and above 2000 g were included in the MAVAN cohort. Mothers were first assessed during their pregnancy (~26 weeks) and then followed at multiple time points that included home visits and laboratory sessions. Written, informed consent was obtained from all participants. Ethics approval was obtained from the Douglas Mental Health University Institute (McGill University, Montreal) and St-Joseph Healthcare/McMaster University, Hamilton.

Measures

Sleep Duration

Mothers were asked to fill in questionnaires regarding their child's sleep habits over the previous few weeks at 6, 12, 18, 24, and 36 months, providing information on bedtime, wakeup time, and sleep duration. Twenty-five questions pertaining to sleep were adapted from the Self-Administered Questionnaire for the Mother.[38,39] Provided that consolidation of nocturnal sleep has been shown to occur around 12 months of age,[40] along with the establishment of circadian rhythms, and that our sample encompass infants aged 6 to 36 months, we targeted questions related to total sleep duration (ie, a combination of both nighttime and daytime sleep). Sample questions include the following: (1) At what time do you put your child to bed for the night? (bedtime); (2) What is the total length of your child's sleep during the night? (night sleep duration); and (3) At what time does she or he wake up in the morning? (wake time).

A mixed model (growth curve) with random intercept was fitted with sleep duration as the repeated outcome across time, to derive an average sleep duration measure. The mixed model for sleep duration was fitted to the 209 subjects with nonmissing value for the outcome (NE/BR at 36 months) and mother and child genotype. The resulting random intercepts were used as a measure of average sleep duration across the first 3 years.

Infant Genotype

Buccal swabs were collected at 36 months. DNA extraction and 5-HTTLPR genotyping was performed at the Center for Addiction and Mental Health, in Toronto (Canada). For the *SLC6A4* LPR variant, 4 μL total genomic DNA was combined with 1×MBI Fermentas polymerase chain reaction (PCR) buffer containing (NH4)2SO4, 1.5 mM MgCl2 (MBI Fermentas, Burlington, Canada), 0.0325 μg each primer[41]; forward primer labeled with 5′ HEX fluorescent tag), 0.16 mM each dNTP (MBI Fermentas) and 1 U Taq polymerase (MBI Fermentas) to a total volume of 25 μL. The PCR reactions were subjected to an initial denaturation for 3 min at 95°C, followed by 40 cycles of amplification in an AB 2720 (Thermofisher Scientific, Burlington, Canada) thermal cycler: denaturing for 30 seconds at 95°C, annealing for 30 seconds at 61°C and extension for 1 minute at 71°C, and a final extension at 72°C for 10 minutes. Five microliters of the PCR product was combined with 1×New England Biolabs Buffer 2, 10 U MspI restriction enzyme (New England Biolabs, Whitby, Canada) in a total volume of 30 μL was digested overnight at 37°C. Digested products were electrophoresed on an AB 3130-Avant Genetic Analyzer as per manufacturer's directions, and product sizes determined by comparison with GeneScan 500 ROX size standard using GeneMapper (version 4.0). Ten percent of samples were genotyped in duplicate. Error rate was <1%. When children were aged 36 months, buccal swabs were also collected for mothers. Maternal genotype was used in our analyses as a covariate (Genotype polymorphism

TABLE 1 Means (SD) of Demographic Variables, Predictor Variables, and Outcome Variables in Offspring by 5-HTTLPR Genotype Postpartum in Original (Not Imputed) Sample

	5-HTTLPR	
	L_AL_A	1 or 2 copies of S or L_G
Sample size	63	146
Gender, female	30	66
Maternal depression 6 mo	12.1 (10.0)	10.2 (9.6)
Maternal depression 36 mo	13.4 (10.9)	11.5 (8.9)
6 mo total sleep duration (h)	13.3 (1.5)	12.9 (2.3)
12 mo total sleep duration (h)	13.2 (1.4)	13.0 (1.5)
18 mo total sleep duration (h)	12.7 (1.2)	12.8 (1.3)
24 mo total sleep duration (h)	12.5 (1.1)	12.3 (1.2)
36 mo total sleep duration (h)	11.7 (1.2)	11.97 (1.0)
NE/BR	0.2 (0.8)	0.0 (1.1)

L_AL_A, homozygous for the long allele.

frequencies are provided for children in Table 2 and for mothers in Table 3).

There is evidence that 2 functional variants of the L allele (L_A and L_G) result from a single nucleotide polymorphism (A→ G, rs25531) in the 5-HTTLPR region[28,42]. The L_AL_A genotype is associated with a greater 5-HTT binding potential in human putamen[43] and midbrain[44] as well as with higher mRNA expression in vitro.[42] We grouped the L_G and S alleles because these variants are functionally similar with respect to 5-HTT expression.[42] We compared L_AL_A homozygote infants to S/L_G allele carriers.

NE/BR

Infant NE/BR was measured using a composite score derived from the Early Childhood Behavior Questionnaire (ECBQ[45]) at 36 months. The ECBQ is a maternal-report questionnaire comprising 201 items grouped in 18 subscales and is based on a 7-point Likert scale ranging from "never" to "always." The questionnaire yields 18 subscores: activity level/energy, attentional focusing, attentional shifting, cuddliness, fear, frustration, discomfort, high-intensity pleasure, impulsivity, inhibitory control, low-intensity pleasure, motor activation, perceptual sensitivity, positive anticipation, sadness, shyness, sociability, and soothability. The ECBQ items were entered into a principal component analysis to obtain 1 factor we termed "NE/BR" that comprised positive ratings of discomfort, fear, frustration, activity level, motor activation, and sadness, and negative ratings of attentional focusing, cuddliness, inhibitory control, and soothability (as previously validated by Bouvette-Turcot et al[46]; see Table 1 for mean and SD).

Potential confounds

Demographic information (ie, gender and socioeconomic status) was obtained during the first home visit. Maternal depression level was assessed at 6 and 36 months' postpartum to account for the fact that maternal reports of their children might be influenced by mood. The Center for Epidemiologic Studies Depression Scale,[47] a self-report, 20-item measure, was used to assess mood state. This measure is among the most common screening tests for depression.

RESULTS

Preliminary Analyses

5-HTTLPR Genotype Frequency and Demographics

The frequency of mothers and children with the L_AL_A genotype (25%–30%; Tables 2 and 3) is consistent with the literature on Caucasian populations.[42] Tests of Hardy-Weinberg equilibrium did not deviate from expected values for both groups of children and mothers (all *P*s $>$ .05). Comparisons using *t* tests, assuming equal variances, showed that the 5-HTTLPR genotypes of both mothers and children were unrelated to either demographic information or main study variables (all *p*'s $>$ 0.05).

Multiple Imputations

Multiple imputations[48] were used to impute missing data for all variables except 5-HTTLPR genotype and NE/BR. The number of imputed data sets was 10, and results were averaged across the 10 imputed data sets. All nonmissing information among the variables was used in the algorithm to impute missing data, using the R package "mice" (http://cran.r-project.org/web/packages/mice/index.html). The outcome measure, child NE/BR, was not imputed, and it was not used as a predictor for the imputation of missing data in the covariates. After multiple imputations, the complete sample size for the analysis was 209 (subjects who have nonmissing data for both NE/BR and the 5-HTTLPR genotype). Number of imputed cases varied from 22 to 71 across measures.

Main Analyses

For each imputed data set, an average sleep duration over time was first obtained from the mixed model analysis, and then hierarchical regressions were performed. Variables were entered as follows: Block 1, child gender, socioeconomic status, maternal depression at 6 months' postpartum, maternal depression at 36 months' postpartum, and mother 5-HTTLPR genotype; Block 2, average sleep duration over time and child 5-HTTLPR genotype; Block 3, interactive term of average sleep duration over time and child 5-HTTLPR genotype. The results of the multiple regression analyses were pooled across imputed data sets together to obtain estimates of the covariate effects and their variance-covariance matrix.

These analyses yielded a significant interaction between average sleep duration over time and child 5-HTTLPR genotype (β = –0.67, *P* = .01) (Table 4). This interaction was explored both graphically and statistically with regions of significance analyses (RoS) that were conducted with a Web-based program developed by Fraley (http://www.yourpersonality.net/interaction) (Fig 1). Simple slopes results revealed significant average sleep duration effect on child NE/BR only for children who carry

TABLE 2 Triallelic 5-HTTLPR Child Genotype Polymorphism Frequencies

	Genotype						
	L_AL_A	L_AL_G	L_GL_G	SL_A	SL_G	SS	Total
Count	63	8	1	84	13	40	209
Percentage	30.14	3.83	0.48	40.19	6.22	19.14	100.00

TABLE 3 Triallelic 5-HTTLPR Maternal Genotype Polymorphism Frequencies

	Genotype						
	L_AL_A	L_AL_G	L_GL_G	SL_A	SL_G	SS	Total
Count	71	19	1	75	8	48	209
Percentage	27.80	9.10	0.50	35.90	3.80	23.00	100.00

either 1 or 2 copies of the S allele (β = –0.55, $P < .001$). In contrast, for those homozygous for the long allele (L_AL_A), no such association was detected (β = 0.13, P = .54). Furthermore, the RoS on X test (an approach that allows to examine the values of X for which the moderator and outcome variables are significantly related) revealed lower and upper bounds of significance within the observed predictor variable, confirming that the interaction pattern was consistent with differential susceptibility (for lower bound, X = –1.03, and for higher bound, X = 0.32; simple slopes were significant outside this region). Finally, the proportion of interaction (PoI) and the proportion affected/percentage above (PA) indexes were computed. The PoI represents the proportion of the total area between the lines of an interaction plot, bounded by ± 2 SD on the predictor, which is above the crossover point. The PA quantifies the proportion of subjects who fall above the crossover point for the interaction. PoI and PA indexes supported differential susceptibility (PoI = 0.58 and PA = 0.56).[49]

TABLE 4 Interactive Effects of Average Short Sleep Duration Between 6 and 36 Months and Child 5-HTTLPR in Relation to Child NE/BR at 36 Months, Accounting for Pooled Estimated Confounding Effects

Model and Steps	β	P
1. Child gender	0.22	.75
Socioeconomic status	–0.01	.95
Maternal depression		
6 mo	0.01	.09
36 mo	0.03	.01
Maternal 5-HTTLPR genotype	–0.05	.75
2. Total sleep duration (environment)	0.13	.54
Child 5-HTTLPR genotype (gene)	–0.10	.47
3. Interactive term (gene × environment)	–0.67	.01

R^2 (adjusted) = 0.27; *df*, 84, 193.

DISCUSSION

We examined whether the relation between average sleep duration during the first 3 years of life and NE/BR in 36-month-old children was moderated by the 5-HTTLPR genotype. Our results revealed a significant relation between average sleep duration from ages 6 to 36 months and NE/BR but only for children with the 5-HTTLPR S allele. For children carrying either 1 or 2 copies of the S allele, shorter sleep duration was associated with higher ratings of NE/BR. In contrast, among those homozygous for the L_A variant, child NE/BR was not related to average sleep duration. Hence, shorter sleepers who were also S-carriers displayed increased scores of temperament characteristics including frustration, fear, discomfort, sadness, and inattention. Importantly, 5-HTTLPR genotype alone was unrelated to average sleep duration over time or child NE/BR scores, ruling out a main effect hypothesis.

Our results are consistent with differential susceptibility theory as described earlier[50] and as confirmed by RoS analyses.[51] Carriers of at least 1 copy of the S allele had not only the highest NE/BR scores at shorter average sleep durations but also had the lowest NE/BR scores at longer average sleep duration, which was not the case for their L_AL_A counterparts.

Our results underline individual differences in emotional and regulatory responses to longitudinal sleep durations. Indeed, not every individual will react in the same way to variations in sleep duration, as documented in adult literature.[52] As such, short sleep duration may represent an indicator of nonoptimal environmental opportunity as opposed to a biological/constitutional marker. Furthermore, such interindividual differences in susceptibility to sleep duration and their effects on NE/BR are worth identifying early in life given that early temperament has been found to be a good proxy measure for later psychopathology.[43]

According to differential susceptibility theory, individuals are differentially affected by both negative and positive environmental influences rather than just being more or less vulnerable to the negative effects of adverse influences.[53] The short allele of the serotonin transporter has long been considered "vulnerability" or "risk" allele because of enhanced detrimental consequences associated with early adversity.[19] However, this allele might be viewed as a "plasticity" allele rather than a "vulnerability" allele. Indeed, the S allele might confer adaptive advantages in high-quality environments, in line with the assumption that the S allele associates with increased sensitivity to environmental cues.[54] This hypothesis is also supported in the animal literature such that there is evidence that an orthologous 5-HTTPLR promoter polymorphism in the rhesus macaque determines sensitivity to both negative and positive maternal influences.[55]

One limitation to this study is the use of maternal reports of both sleep duration and NE/BR. However, the inclusion of maternal depression at 2 time points, including a time that corresponds to the completion of the outcome measure (ie, 36 months), prevents potential confound with affect at the time of the child report. Controlling for maternal depression ensures that ratings of NE/BR and sleep are not merely a reflection of mothers' mood. However, one may question the lack of objective measures (eg, actigraphy) to complement the assessment of sleep

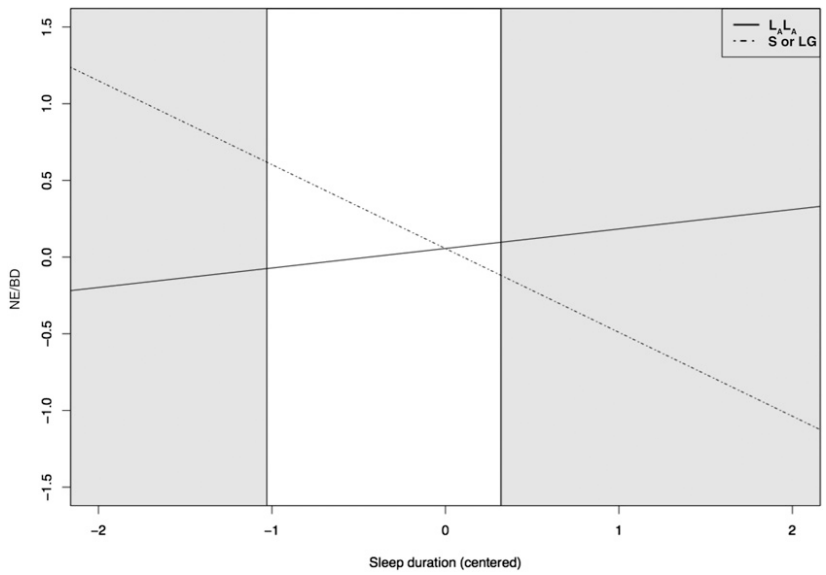

FIGURE 1
Interaction effect and RoS of total sleep duration and 5-HTTLPR genotype on NE/BD at 36 months.

with questionnaires and to account for the fact that, as children get older, capturing the changes in their sleep duration might get more challenging for parents to report. Notwithstanding these limitations, this study is among the first to apply the differential susceptibility theory to sleep duration and 5-HTTLPR especially in a longitudinal design, providing support for genetic moderation of sleep duration effects on early temperament. Our results also emphasize the importance of integrating longitudinal designs to children sleep studies. Indeed, our results show that early sleep duration is associated with early emotional development. However, our correlational design does not allow for conclusions beyond the fact that sleep duration and NE/BR are coexisting components of common features.

Our results also convey promising evidence for stepping away from commonly used 1-way/dual-risk paradigms. Instead, prevention and intervention programs should evolve toward specifically tailored approaches that take into account genetic susceptibility to nonoptimal environments along with promotion and enhancement of enriched home/environmental conditions. A first and important step would be to increase general awareness regarding increased individual sensitivity to shorter sleep durations. Future research should also investigate persistent short sleep schedules and trajectories and their further longitudinal impacts.

ABBREVIATIONS

5-HTTLPR: serotonin transporter gene
ECBQ: Early Childhood Behavior Questionnaire
MAVAN: Maternal Adversity, Vulnerability, and Neurodevelopment
NE/BR: negative emotionality/behavioral dysregulation
PCR: polymerase chain reaction
RoS: regions of significance

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Address correspondence to Andrée-Anne Bouvette-Turcot, MSc, University of Montreal Psychology, C.P. 6128, Succ. Centre-ville, Montreal, Quebec, Canada H3C3J7. E-mail: andree-anne.bouvette-turcot@umontreal.ca

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