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Effects of Genotype and Sleep on Temperament.

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Longitudinal effects of sleep and 5-HTTLPR on temperament: Support for differential susceptibility

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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.

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Contributors' statement

Andrée-Anne Bouvette-Turcot: Ms Bouvette-Turcot carried out the statistical analyses, she was involved in the interpretation of the data and she wrote most of the manuscript.

Michael Pluess: Dr. Pluess was involved in the interpretation of the data. He critically reviewed the manuscript and approved the final manuscript as submitted.

Annie Bernier: Dr. Bernier was involved in the interpretation of the data. She critically reviewed the manuscript and approved the final manuscript as submitted.

Marie-Hélène Pennestri: Dr. Pennestri was involved in the interpretation of the data. She critically reviewed the manuscript and approved the final manuscript as submitted.

Robert Levitan: Dr. Levitan was involved in the conception and design of the study. He reviewed and approved the final manuscript as submitted.

Marla Sokolowski: Dr. Sokolowski was involved in the conception and design of the study. She co-led the genetic component of the study. She reviewed and approved the final manuscript as submitted.

James Kennedy: Dr. Kennedy was involved in the conception and design of the study. He co- led the genetic component of the study. He reviewed and approved the final manuscript as submitted.

Klaus Minde: Dr. Minde was involved in the interpretation of the data. He critically reviewed the manuscript and approved the final manuscript as submitted.

Meir Steiner: Dr. Steiner was involved in the conception and design of the study. He reviewed, revised the manuscript and approved the final manuscript as submitted.

Michael Meaney: Dr. Meaney is the principal investigator of this study. He was involved in the conception and design of the study. He reviewed and approved the final manuscript as submitted.

Hélène Gaudreau: Dr. Gaudreau coordinated and supervised data collection, She was involved in the conception and design of the study. She was involved in the interpretation of the data and she wrote most of the manuscript.

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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.

Method: 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 three 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.

Conclusion: The results suggest differential susceptibility to the effect of sleep duration early in life, which reiterates that the short allele of the 5-HTTLPR represent 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, either positive or negative.

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Introduction

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 one hour of sleep restriction for four consecutive nights resulted in increased sleepiness, less positive affective response to stimuli, and increased difficulty in emotion regulation, in children aged 8-12 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 on 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 provide evidence that individual characteristics appear to modulate the influence of early life experiences (e.g., [12]). Genetic differences, for instance, may enhance or dampen the impacts of environmental conditions on child development as suggested by the *Differential Susceptibility Theory* which posits that certain

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factors (e.g., 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 [12].

A significant proportion of the differential susceptibility literature has focused on a common variation in the serotonin transporter gene (5-HTTLPR*)* [16] (see [17] for a review). Two functional alleles, long (L) and short (S), result from a 43bp 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 [18].

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 [16, 19-25] whereas in the same context, the L allele appears to be protective [16]. Such findings, and other gene x environment studies investigating 5-HTTLPR moderating role of early care quality [12,26,27], 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 [28,29], by suppressing REM sleep [28,30] and maintaining the circadian rhythm of the sleep-wake cycle [31]. 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 found that persistent short sleep schedule in university students was associated with increased depressed mood in carriers of the 5-HTTLPR SS genotype, compared to SL or LL carriers [32]. The authors proposed that short sleep duration, as an adverse 'environmental exposure', interacts with the genetic vulnerability in the prediction of

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mood disorders as well as other problematic phenotypes (e.g., anxiety, fear, hostility, suicidality, and attention bias).

Hypotheses

Reported associations between sleep and temperament tend to be moderate in magnitude [4,10,33]. One reason for these moderate effect sizes may be that effects vary between individuals as a function of moderating factors, including genetic ones. Building upon already existing but scarce literature on sleep and emotional development [34], this study aims 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, based on the differential susceptibility theory, we expected S-allele carriers to not only be more negatively affected by shorter average sleep duration but also more positively affected by longer average sleep duration, compared to 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.

Method

Participants

Our community sample consisted of 209 mothers recruited in Montréal (Québec) and Hamilton (Ontario) at 13-20 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

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associated with multiple forms of psychopathology. Eligibility criteria for mothers included age 18 or over, singleton gestation, and fluency in French or English. Women with severe chronic illness (other than hypertension, asthma, or diabetes) and other serious medical conditions (e.g., 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.

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Measures

Sleep Duration. Mothers were asked to fill in questionnaires regarding their child's sleep habits over the last 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 [35,36]. Provided that consolidation of nocturnal sleep has been shown to occur around 12 months of age (e.g., [37]), 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 (i.e., a combination of both nighttime and daytime sleep). Sample questions include: a) At what time do you put your child to bed for the night? (bedtime); b) What is the total length of your child's sleep during the night? (night sleep duration); c) At what time does s/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, in order to derive an average sleep duration measure. The mixed model for sleep duration was fitted to the 209 subjects with non-missing value for the outcome

(NE/BR at 36 months), mother and child genotype. The resulting random intercepts were used as a measure of average sleep duration across the first three 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 ul total genomic DNA was combined with 1X MBI Fermentas PCR buffer containing (NH4)2SO4, 1.5 mM MgCl2 (MBI Fermentas), 0.0325 µg each primer ([38]); 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, ON) thermal cycler: denaturing for 30 sec at 95°C, annealing for 30 sec at 61°C and extension for 1 min at 71°C, and a final extension at 72°C for 10 min. Five microlitres of the PCR product was combined with 1X New England Biolabs Buffer 2, 10 U MspI restriction enzyme (New England Biolabs) 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 to GeneScan 500 ROX size standard using GeneMapper (version 4.0). 10% of samples were genotyped in duplicate. Error rate was below 1%. When children were aged 36 months, buccal swabs were also collected for mothers. Maternal genotype was used in our analyses as a covariate.

There is evidence that two 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 [25,39] . The L_AL_A genotype is associated with a greater 5-HTT binding potential in human putamen [40] and midbrain [41] as well as with higher mRNA expression *in vitro* [39]. We grouped the L_G and S alleles since

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these variants are functionally similar with respect to 5-HTT expression [39]. We compared L_AL_A homozygote infants to S/L_G allele carriers.

Negative Emotionality/Behavioral Dysregulation. Infant NE/BR was measured using a composite score derived from the Early Childhood Behaviour Questionnaire (ECBQ [42]) at 36 months. The ECBQ is a maternal-report questionnaire comprised of 201 items grouped in 18 subscales and is based on a 7-point Likert scale ranging from "never" to "always". The questionnaire yields 18 sub-scores: 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 one factor we termed "negative emotionality/behavioral dysregulation" that was comprised of 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 [43]; see Table 1 for mean and standard deviation).

Potential confounds. Demographic information (i.e., gender and SES) 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 (CES-D) [44], a self-report, 20-item measure was used to assess mood state. The CES-D is one of 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%; Table 2) is consistent with the literature on

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Caucasian populations [39]. Tests of Hardy-Weinberg Equilibrium (HWE) did not deviate from expected values for both groups of children (χ^2 =1.6; n = 209; *ns*) and mothers (χ^2 =1.1; n = 209; *ns*). 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 > .05).

Multiple imputations. Multiple imputations [45] 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 non-missing 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 non-missing 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 (SES), 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.

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These analyses yielded a significant interaction between average sleep duration over time and child 5-HTTLPR genotype (β = -.67, t = -2.74, p = .01). 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). Simple slopes results revealed significant average sleep duration effect on child NE/BR only for children who carry either one or two copies of the S allele (β = -.55, t = -4.44, p < .001). In contrast, for those homozygous for the long allele (L_AL_A), no such association was detected (β = .13, t = .62, *ns*). 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 = .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 = .58 and PA = .56) [46].

Discussion

We examined whether the relation between average sleep duration during the first three 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 one or two copies of the S allele, shorter sleep duration was associated with higher ratings

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of NE/BR. In contrast, amongst 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 above [47], and as confirmed by RoS analyses [48]. Carriers of at least one 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 [49]. As such, short sleep duration may represent an indicator of non-optimal environmental opportunity as opposed to a biological/constitutional marker. Furthermore, such inter-individual 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 (e.g.,[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 [50]. The short allele of the serotonin transporter has long been considered "vulnerability" or "risk" allele because of enhanced detrimental consequences associated with early adversity [16]. 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 [51]. This hypothesis is also

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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 [52].

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 two time points, including a time that corresponds to the completion of the outcome measure (i.e., 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 (e.g., actigraphy) to complement the assessment of sleep 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 one of 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 co-existing components of common features.

Our results also convey promising evidence for stepping away from commonly used one-way/dual-risk paradigms. Instead, prevention and intervention programs should evolve toward specifically tailored approaches that take into account genetic susceptibility to non-optimal environments along with promotion and enhancement of enriched home/environmental conditions. A first and important step would be to increase general awareness regarding increased

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individual sensitivity to shorter sleep durations. Future research should also investigate persistent short sleep schedules and trajectories and their further longitudinal impacts.

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Table 1

Means (standard deviation) of demographic variables, predictor variables, and outcome variables in offspring by 5-HTTLPR genotype postpartum on original (not imputed) sample

	5-HTTLPR	
	L_AL_A	One or two copies of S or L_G
Sample size	63	146
Gender[a]	30	66
Maternal depression 6M	12.07 (9.99)	10.19 (9.56)
Maternal depression 36M	13.35 (10.91)	11.51 (8.93)
6m total sleep duration (hours)	13.29(1.45)	12.92 (2.29)
12m total sleep duration (hours)	13.17 (1.36)	13.03 (1.54)
18m total sleep duration (hours)	12.72 (1.23)	12.84 (1.33)
24m total sleep duration (hours)	12.53 (1.05)	12.33 (1.24)
36m total sleep duration (hours)	11.71 (1.23)	11.97 (1.01)
NE/BR	.18 (.80)	-.00 (1.11)

[a] Number of females

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Table 2a

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	.48	40.19	6.22	19.14	100.00

Table 2b

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

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Table 3

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	t
1. Child gender	.21	1.61
SES	-.06	-.28
Maternal depression 6M	.02	1.72^{t}
Maternal depression 36M	.03	2.89^{**}
Maternal 5-HTTLPR genotype	-.07	-.49
2. Total sleep duration (E)	-.40	3.77^{**}
Child 5-HTTLPR genotype (G)	-.12	-.79
3. Interactive Term (GxE)	-.67	-2.74^{**}
R^2 (adj.)		.27
df		(84, 193)

** $p < .01$; ^{t} $p < .10$

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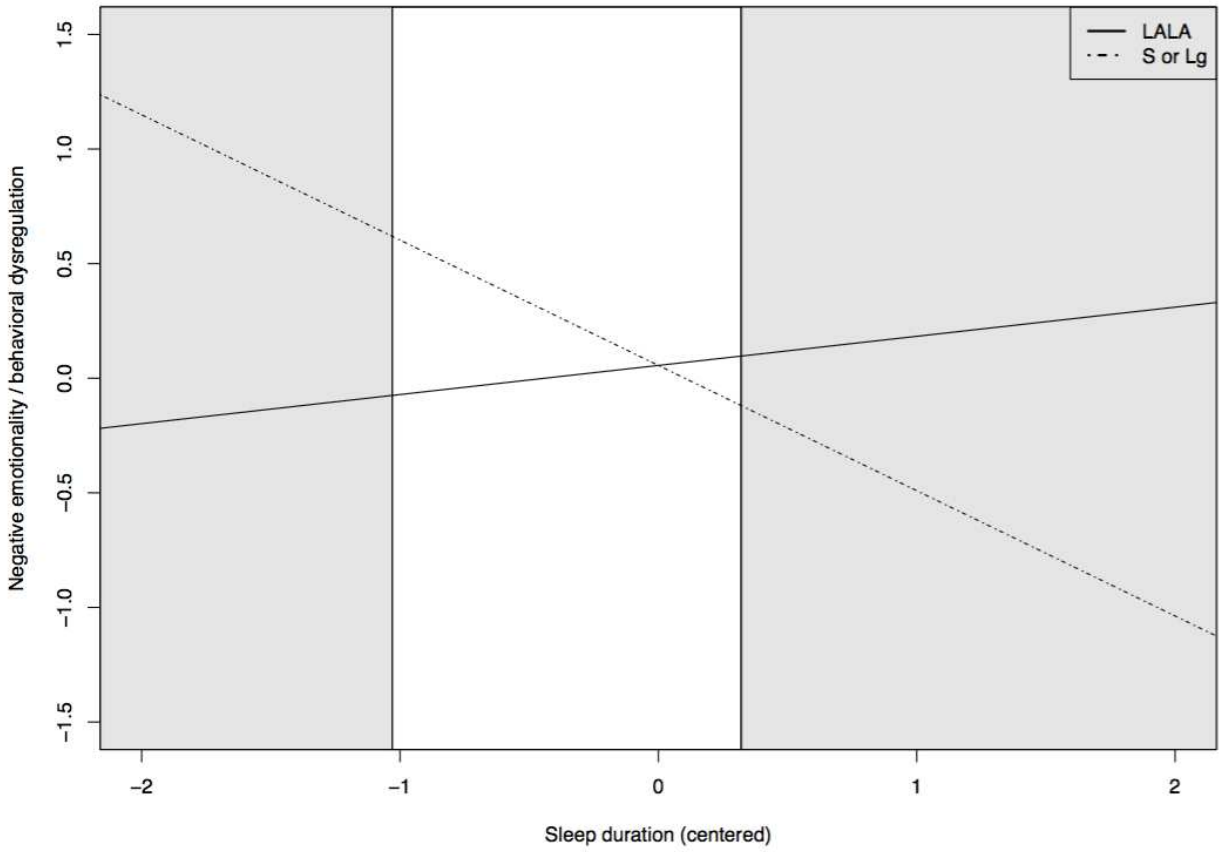
Figure 1

Interaction effect and RoS of total sleep duration and 5-HTTLPR genotype on negative emotionality/behavioral dysregulation at 36 months



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2015-0080.RX – *Longitudinal effects of sleep and 5-HTTLPR on temperament: Support for differential susceptibility.* Andrée-Anne Bouvette-Turcot, Michael Pluess, Annie Bernier, Marie-Hélène Pennestri, Robert Levitan, Marla B. Sokolowski, James L. Kennedy, Klaus Minde, Meir Steiner, Michael J. Meaney, and Hélène Gaudreau, *on behalf of the MAVAN research team*

EDITOR/REVIEWER COMMENTS *Paste each of the editor and reviewer queries here.*	AUTHOR'S RESPONSE *Paste your answer to the editor and reviewer queries here.* *If you alter your manuscript to address this query, you MUST paste the relevant altered text here.*	REFERENCE PAGE *Provide the location where the change can be found.*	CHANGE APPROVED? FOR EDITORIAL USE ONLY
Editor's comment 1. The introduction can be shortened.	The introduction has been shortened. We removed the following paragraph: "Endogenous susceptibility factors may be behavioral/psychological (e.g., personality; [13]), physiological (e.g., cortisol reactivity; [14]), or genetic (e.g., [12]). In the context of early emotional development, studies have shown that increased negative emotionality, a well-documented temperament profile, may result from gene x environment interactions [15,16]. Negative emotionality is an early emerging trait that encompasses high levels of sub-components such as fear, anger, and sadness [17]. Although infant temperament has a strong hereditary component [18], it is also affected by early life experiences [19]."	We removed lines 1-7, Page 5.	
Editor's comment 1. However, the discussion can be lengthened.	The discussion was lengthened and adapted to clinician readers. "As such, short sleep duration may represent an indicator of non-optimal environmental opportunity as opposed to a biological/constitutional marker." "Our results also convey promising evidence for stepping away from commonly used one-way/ dual-risk paradigms. Instead, prevention and intervention programs should evolve toward specifically tailored approaches that take into account genetic susceptibility to non-optimal 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."	Page 12, lines 12-14 Page 13, lines 16-21	

Editor's comment 2. Please provide more detail regarding the self-administered questionnaire for the mother in the Methods section.	We provided more details regarding the self-administered questionnaire for mother. "Provided that consolidation of nocturnal sleep has been shown to occur around 12 months of age (e.g.,[37]), 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 (i.e., a combination of both nighttime and daytime sleep). Sample questions include: a) At what time do you put your child to bed for the night? (bedtime); b) What is the total length of your child's sleep during the night? (night sleep duration); c) At what time does s/he wake up in the morning? (wake time). »	Page 7, lines 13-19	
Editor's comment 3. Why was total sleep duration (i.e., both nighttime and daytime sleep) used for instead of nighttime alone?	Provided that literature has shown that consolidation of nocturnal sleep occurs around 12 months along with the establishment of the circadian rhythms and since our population includes infants from 6 to 36 month of age, we targeted total sleep duration instead of solely night sleep duration. This allows us to capture established interindividual variability in sleep duration (e.g., Iglowstein et al., 2003).	No change	
Reviewer 1 comment 1. First, it might be important to elucidate the role of sleep duration as an index of the environment that has a differential impact on child development.	We addressed the concern of sleep duration as an index of the environment in the manuscript. "As such, short sleep duration may represent an indicator of non-optimal environmental opportunity as opposed to a biological/constitutional marker. Furthermore, such inter-individual 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]."	Page 12, lines 11-16	
Reviewer 1 comment 2. Second, GxE studies have been harshly criticized for their lack of replicability as most of them seem to be underpowered. It would be good to add a power analysis. Better still, it would be great to have a replication in a similar study (e.g.., ALSPAC).	Imputation analyses yielded multiple data sets, hence it was not possible to conveniently run a power analysis. However, a replication study is planned with an Asian sample. Data are currently collected and we expect to have the full data in the coming year with the goal to present them as a follow up study.	No change	
Reviewer 1 comment 3. Third, the evidence seems to point at differential susceptibility; interpretations of the results in terms of vantage susceptibility are not supported by the data and the statistical analyses.	In our manuscript, we mention that the results are consistent with differential susceptibility. After adding mother's genotype in the analyses, the differential susceptibility finding is even stronger. In lights on our new results, we also removed the discussion regarding vantage sensitivity.	Page 12, lines 1-5	
Reviewer 1 comment 4. Fourth, in the Methods at some points maternal genotype is referred to: if data on 5HTTLPR for mothers is available in the current sample, it would of course be wonderful to include in the analyses.	We agree with the reviewer that it is important to include maternal genotype in the analyses. We ran new analyses on subjects for which maternal genotype was available (n=209) and this analysis yielded significant results (β = -.67, t = -2.74, p = .01). Related results, tables and figures were updated accordingly (frequency table for mother genotype).	Page 14-17	
Reviewer 1 Minor point: crucial contributions to theory and research on genetic differential susceptibility have not	We added references in the introduction related to theory and research on differential susceptibility.	Page 5, lines 1 and 5	

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been referred to (e.g. work by Ellis, Bakersman, Boyce, Dodge).			
Reviewer 2. Main concern: I would recommend the authors work at simplifying the explanations of the analysis and presentation of results.	We simplified the description of our mixed model analyses. We also gave more precisions on the region of significance analyses in the Result section.		
Reviewer 2 comment 1. Abstract a. Please include how sleep was assessed in the methods.	We added a description on how sleep was assessed. "Child sleep habits were assessed with a maternal self-report questionnaire. »	Page 3, line 11.	
Reviewer 2 comment 2 Method a) Does the computation of average sleep duration account for changes over time? That is, some children will start as long sleepers and others as short sleepers but over time, will change their sleep habits so that the long sleepers may become short sleepers etc.	We acknowledge this important comment with regards to shorts vs long sleepers. However, this study was designed to assess sleep duration as a continuous variable and thus did not allowed to discriminate long sleepers vs short sleepers. Nonetheless, we agree that future research should investigate continuity or discontinuity in sleep schedule as mention in our limitation sections (see P14). Furthermore, we are currently developing a trajectory analyses to follow up on this matter.	No change	
Reviewer 2 comment 3 Discussion a) The limitation regarding parent report of sleep duration is not solely accounted for by presence of maternal depression. In addition to the usual tendency to over-estimate sleep, these reports were retrospective which are subject to recall bias. The longitudinal nature helps with the bias (each subject is their own control), but even then, the level of attention paid to sleep over the previous two-week period will change as the infant ages and sleep behaviors changes. Please add this into the limitations.	We now discuss the limitation of parent-report of sleep duration in relation to changes in infant age and sleep behavior. "However, one may question the lack of objective measures (e.g., actigraphy) to complement the assessment of sleep 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."	Page 13, lines7-8	
Reviewer 2 comment 3 Discussion b) There were a high proportion of missing data which were replaced using imputations. Please provide an explanation of how this will impact the generalizability of the results.	We acknowledge that the way results were reported could have lead to a misinterpretation as per the number of missing data. We clarified this information in the text. Furthermore, we would like to draw the reviewer's attention to the fact that multiple imputations work well even on smaller samples (e.g., n=50) and with far more missing data (e.g., 50%) (Graham, 2009). "After multiple imputations, the complete sample size for the analysis was 209 (subjects who have non-missing data for both NE/BR and the 5-HTTLPR genotype). Number of imputed cases varied from 22 to 71 across measures."	Page 10 lines 11-13. Page 11, lines 17-19 were removed	
Reviewer 2 comment 3 Discussion c) I found it odd that the conclusions did not address the impact of potential gene susceptibility as suggested by the results.	We added clinical implication related to the differential susceptibility. "Our results also convey promising evidence for stepping away from commonly used one-way/ dual-risk paradigms. Instead, prevention and intervention programs should evolve toward specifically tailored approaches that take into account genetic susceptibility to non-	Page 13, line 16-21	

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The authors state "parents with children presenting with temperaments and/or behavior difficulties should *be* encouraged to promote longer sleep schedules." This type of clinical recommendation is not new. I would suggest that those versed in the importance of sleep for behavior would suggest increasing sleep duration as a matter of course if the child was reported to not be getting enough. Also, the results do not show a main effect of sleep duration so this conclusion is not really warranted. The novel aspect of this study is the interaction between sleep and 5-HTTLPR. I would recommend including the clinical implications of this finding.	optimal 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."		
Reviewer 3 comment 1. Grammar and style. I would recommend a native English speaker review for grammatical errors but below are a few examples that should be addressed before publication: from expected values for both groups of children . . ."	We reviewed the grammatical errors in the manuscript.		
Reviewer 3 comment 1 p. 4 (line 40) temperament early on in development.	The sentence has been modified.		
Reviewer 3 comment 1 p. 5 (line 8) (e.g., personality; p. 5 (line 37) The presence of the S allele . . . numerous negative outcomes (delete health)	The sentence has been modified.		
Reviewer 3 comment 1 p. 6 (line 16) Change sentence to "Evidence from studies examining genetic data in sleep research, . . ."	The sentence has been modified.		
Reviewer 3 comment 1 p. 7 (line 3) insert affected so "but also more positively affected by longer sleep duration"	The sentence has been modified.		

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Reviewer 3 comment 1 p. 9 (line 32) composite score derived from based on the Early Childhood Behaviour Questionnaire	The sentence has been modified.		
Reviewer 3 comment 1 p. 10 (line 27) "did not deviate	The sentence has been modified.		
Sample and Methodology. The sample selection and measures used are appropriate. Some obvious limitations are the maternal-report for both sleep and temperament but it is an important first study of these interactions in young children and the authors are cognizant of these limitations. The authors should explain the following sentence: "to use all the available longitudinal sleep data in the best possible way, ..."	We removed this sentence since it was confusing.	Page 7, line 20	
Reviewer 3 comment 4 I would recommend adding as room allows to the conclusion paragraph about how the study findings might be used or inform clinical practice.	We added clinical implication related to the differential susceptibility. We added clinical implication related to the differential susceptibility. "Our results also convey promising evidence for stepping away from commonly used one-way/ dual-risk paradigms. Instead, prevention and intervention programs should evolve toward specifically tailored approaches that take into account genetic susceptibility to non-optimal 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."	Page 13, lines 13-21	

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