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Authors: E. Solomonova, Y.E.A. Lee, S. Robins, L. King, N. Feeley, I. Gold, B. Hayton, E. Libman, C. Nagy, G. Turecki, P. Zelkowitz

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Sleep quality is associated with vasopressin methylation in pregnant and postpartum women with a history of psychosocial stress

Solomonova, E[1,2,3]., Lee, Y.E.A[2]., Robins, S[2,3]., King, L[1,3]., Feeley, N[2,4,5]., Gold, I[1,6]., Hayton, B[1,3]., Libman, E[2,3]., Nagy, C[7,8,9] .,Turecki, G[1,8,9]. & Zelkowitz, P*[1,2,3].

[1] Department of Psychiatry, McGill University
[2] Lady Davis Institute for Medical Research, Jewish General Hospital
[3] Department of Psychiatry, Jewish General Hospital
[4] Center for Nursing Research, Jewish General Hospital
[5] Ingram School of Nursing, McGill University
[6] Department of Philosophy, McGill University
[7] Department of Neurology and Neurosurgery, McGill University
[8] McGill Group for Suicide Studies
[9] Douglas Mental Health University Institute

*Corresponding author:
Dr. Phyllis Zelkowitz
Director of Research, Department of Psychiatry, Jewish General Hospital
Senior Investigator, Lady Davis Institute for Medical Research, Jewish General Hospital
Department of Psychiatry, McGill University
phyllis.zelkowitz@mcgill.ca
514-340-8222 ext. 25258

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Highlights:

- During pregnancy and postpartum, sleep is associated with vasopressin methylation
- Psychosocial stress may mediate the relationship between stress and vasopressin
- Sleep during perinatal period is worse in women with history of psychosocial stress

Abstract

Background: The relationship between disturbed sleep and stress is well-documented. Sleep disorders and stress are highly prevalent during the perinatal period, and both are known to contribute to a number of adverse maternal and foetal outcomes. Arginine vasopressin (AVP) is a hormone and a neuropeptide that is involved in stress response, social bonding and circadian regulation of the sleep-wake cycle. Whether the AVP system is involved in regulation of stress response and sleep quality in the context of the perinatal mental health is currently unknown. The objective of the present study was to assess the relationship between levels of cumulative and ongoing psychosocial risk, levels of disordered sleep and AVP methylation in a community sample of pregnant and postpartum women.

Methods: A sample of 316 participants completed a battery of questionnaires during the second trimester of pregnancy (PN2, 12-14 weeks gestation), third trimester (PN3, 32-34 weeks gestation), and at 7-9 weeks postpartum (PP). Disordered sleep was measured using the Sleep Symptom Checklist at PN2, PN3 and PP; cumulative psychosocial risk was assessed with the Antenatal Risk Questionnaire (ANRQ) at PN2; salivary DNA was collected at the follow-up (FU, 2.9 years postpartum); and % methylation were calculated for AVP and for two of the three AVP receptor genes (AVPR1a and AVPR1b). Women were separated into high (HighPR) and low (LowPR) psychosocial risk groups, based on their scores on the ANRQ.

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Results: Women in the HighPR group had significantly worse sleep disturbances during PN2 ($p<.001$) and PN3 ($p<.001$), but not at PP ($p=.146$) than women in the LowPR group. In HighPR participants only, methylation of AVP at intron 1 negatively correlated with sleep disturbances at PN2 (r_s=-.390 , $p=.001$), PN3 (r_s=-.384, $p=.002$) and at PP (r_s= -.269, $p=.032$). There was no association between sleep disturbances and AVPR1a or AVPR1b methylation, or between sleep disturbances and any of the AVP methylation for the LowPR group. Lastly, cumulative psychosocial stress was a moderator for the relationship between AVP intron 1 methylation and disordered sleep at PN2 ($p<.001$, adjusted R^2=.105), PN2 ($p<.001$, adjusted R^2=.088) and PP ($p=.003$, adjusted R^2=.064).

Conclusions: Our results suggest that cumulative psychosocial stress exacerbates sleep disorders in pregnant women, and that salivary DNA methylation patterns of the AVP gene may be seen as a marker of biological predisposition to stress and sleep reactivity during the perinatal period. Further research is needed to establish causal links between AVP methylation, sleep and stress.

Keywords: sleep; pregnancy; postpartum; psychosocial stress; vasopressin; DNA methylation

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1. Introduction

Sleep disorders are prevalent during pregnancy and postpartum, and contribute to a number of adverse psychological and physiological outcomes for both mother and child. Psychosocial stress is known to affect sleep quality and to contribute to maternal and infant distress. Sleep regulation and reactivity to psychosocial stress have both been associated with the activity of arginine vasopressin (AVP). In this paper, we investigated the relationship between disordered sleep, cumulative psychosocial stress and DNA methylation of AVP- and its receptors AVPR1a and AVPR1b.

1.1. Sleep and stress during pregnancy

Many women experience disturbed sleep during pregnancy, and specific physiological and hormonal changes are associated with each trimester (Balserak & Lee, 2017). Sleep disorders during pregnancy have been associated with a number of adverse outcomes including preterm birth (Okun et al., 2011), postpartum depression (Okun, 2015), intrauterine growth restriction and admission to neonatal intensive care (Carnelio et al., 2017).

During the first trimester, longer sleep time, daytime sleepiness and frequent napping are common, likely due to the cascade of hormonal changes associated with this period (Hedman et al., 2002). During the second trimester sleep quality improves and daytime sleepiness diminishes, however, nocturnal awakenings increase, and sleep disorders, including snoring and restless legs syndrome become more common (Balserak & Lee, 2017). By the third trimester, many women experience restless legs syndrome (Chen et al., 2017) and sleep-disordered breathing including snoring and obstructive sleep apnea (Truong & Guilleminault, 2018). After birth, sleep quality typically improves. Disordered sleep in the postpartum period is often characterized by changes in sleeping arrangements and by frequent awakenings to attend to the

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infant, which effectively produce an altered circadian rhythm and sleep fragmentation in new mothers (Stremler et al., 2017).

One potential factor contributing to sleep disorders is psychosocial stress. Elevated levels of chronic psychosocial stress have been linked to a variety of disorders in both humans and in non-human animals, and to adverse pregnancy outcomes, such as preterm birth (Facchinetti et al., 2007) and child psychopathology (Glover et al., 2018). There likely exists a bi-directional relationship in which stress may cause disordered sleep (Kalmbach et al., 2018), and disordered sleep, including chronic sleep deprivation and circadian misalignment, increases the stress response (McEwen & Karatsoreos, 2015). In accordance with current allostatic load theories of pregnancy and stress, sleep loss during pregnancy has been proposed to be both a result of stress during the perinatal period and a stressor in its own right, contributing to physical and mental distress in pregnant women (Palagini et al., 2014).

1.2. Arginine Vasopressin, sleep and psychosocial stress

Both stress response and sleep regulation depend in part on the activity of arginine vasopressin (AVP). AVP is a hormone and a neuropeptide that, together with oxytocin (OT), plays an important role in affiliative and social behaviors (Insel, 2010), including a special role in pregnancy and in maternity (Carter, 2017); and in circadian sleep-wake regulation (Kalsbeek et al., 2010). AVP is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and in the suprachiasmatic nucleus, and two of AVP's receptors, AVPR1a and AVPR1b, are found in the brain, where AVP acts centrally as a neurotransmitter (Insel, 2010). AVP is released in the hypothalamic-pituitary-adrenocortical (HPA) axis as part of the stress response. Animal research shows that AVP expression is sensitive to early social stress (Murgatroyd et al., 2015) and to sustained adult stress (Goncharova, 2013). In rodent models of

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early life stress, decreased AVP activity in the paraventricular nucleus and in the hypothalamus has been associated with such outcomes as altered maternal behaviours, including anxiety (Murgatroyd et al., 2015).

The field of epigenetics aims to describe heritable changes to gene expression not brought about from a cell's DNA sequence (Holliday & Pugh, 1975). DNA methylation, an epigenetic process involved in cellular differentiation and the regulation of gene expression (Schubeler, 2015), describes the covalent binding of a methyl group at the 5 position of a cytosine-phosphate-guanine (CpG) dinucleotide. DNA methylation is studied to measure effects on gene regulation, and investigations aim to capture promoter regions that include transcription start sites. In somatic cells, CpG rich areas, (CpG islands), are composed of 300-3000 base pairs and are often found in promoter regions of genomic DNA. These islands are often maintained in a hypomethylated state to allow for gene expression; hypermethylation is tied to transcriptional silencing (Lester et al., 2016). The effect of DNA methylation found elsewhere in the gene body is dependent on a myriad of factors including CpG composition, the functional chromatin state of the DNA in these locations, enhancer and repressor regions, and transcription factor binding (Jones, 2012). DNA methylation is an epigenetic indicator that can potentially be used as a biomarker for health and disease (Schubeler, 2015), which is relatively stable (Woodfine et al., 2011), but also sensitive to environmental pressures (Meaney & Szyf, 2005).

Activity of AVP in the context of perinatality is closely associated to that of OT: both hormones are involved in maternal care, mother-infant bonding, attachment and reaction to stress (Carter, 2017). Our group has previously reported that psychosocial stress may act as a moderator for the relationship between circulating levels of oxytocin and depression (Zelkowitz et al., 2014). This suggests that the regulation of the OT-AVP system may depend, to a degree,

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on the epigenetic changes associated with lived adversity and/or with inherited patterns of reactivity to adversity. AVP is part of the stress response of the HPA axis, and disturbed sleep is associated with HPA axis activation by increasing the allostatic load on the system. The relationship between disturbed sleep during pregnancy, history of cumulative psychosocial stress and methylation levels in AVP and its receptor genes AVPR1a and AVPR1b has not been examined.

The present study investigated 1) whether subjective sleep quality during pregnancy and postpartum was differentially associated with peripheral measures of methylation patterns of AVP/R1a/R1b in women who have, and have not, experienced high levels of psychosocial stress; and 2) if psychosocial stress moderated the relationship between AVP/R1a/R1b methylation and sleep disturbances during pregnancy and postpartum.

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2. Material and Methods

2.1. Participants and study protocol

A community sample of 316 women was recruited between 2009 and 2012 at a general hospital and a birthing center in Montreal, Quebec, Canada. Inclusion criteria were: being 18 years or older, being pregnant with a single child, and being able to complete questionnaires in either French or English. The study methodology is described elsewhere (Zelkowitz et al., 2014). Participants completed a battery of questionnaires during the second trimester of pregnancy (PN2, 12-14 weeks gestation), during the third trimester (PN3, 32-34 weeks gestation), at 7-9 weeks postpartum (PP) and at a follow-up home visit at 2.9 years postpartum (FU), when saliva samples for DNA analyses were obtained. The study had a high retention rate: out of 316 eligible women who participated at PN2, 300 (94.9%) completed questionnaires at PN3, and 287 (90.8%) at PP. 189 mothers (62.6%) participated at FU, and we obtained a valid DNA sample from 171 (90.5%) participants. The study was approved by the research ethics committees both at the hospital and at the birthing center used for participant recruitment. Written informed consent was obtained from participants at all time-points.

2.2. Sleep measures

Subjective sleep quality was measured using the Sleep Symptom Checklist (SSC), which screens for sleep disturbances such as insomnia, daytime consequences of insufficient sleep such as fatigue, sleep-disordered breathing and restless legs syndrome. This instrument has been validated against standard polysomnography in a sample of older primary care patients, and was shown to be sensitive to detecting major sleep disorders (Bailes et al., 2008). The checklist consists of 19 items targeting the severity of symptoms experienced during the last month, rated

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on a Likert-type scale ranging from “not at all”=0, to “very”=3. Three subscales were calculated, reflecting different qualities of disordered sleep: insomnia, daytime symptoms and sleep disorders (restless legs and sleep-disordered breathing). The sum of the scores indicates the overall levels of sleep disturbance, with higher scores signaling higher levels of disturbed sleep. Participants filled out the SSC at PN2, PN3 and PP.

2.3. Psychosocial stress

Cumulative psychosocial stress was measured at PN2 using the Antenatal Risk Questionnaire (ANRQ). This instrument measures early and ongoing psychosocial stress, specifically targeting factors associated with perinatal depression (Austin et al., 2005). It consists of 12 items pertaining to adverse life events, such as stressful life events, mental health problems, history of physical and sexual abuse, history of maternal support during childhood, and current social support. The items are scored either as “yes/no” or on a Likert-type scale from 1 to 5, with 5 indicating higher incidence of adversity. “Yes/no” questions are scores as “no”=0, and “yes”=5. Scores range from 5 to 67; a cutoff score of 23 is used to identify high stress women who are at risk for prenatal depression, with the receiver operating characteristic (ROC) area under the curve of 0.69 (acceptable) at this cutoff point. For the present analyses, women were divided into high psychosocial risk (HighPR) and low psychosocial risk (LowPR) groups, using this standard cut off score (Priest et al., 2008).

2.4. Vasopressin methylation measures

Salivary DNA was collected at the FU time point. Since DNA methylation patterns are documented to be relatively stable during lifetime (Heijmans et al., 2008) it is possible to make some inferences based on a DNA sample obtained 3.5 years after the beginning of the study.

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Participants were instructed not to eat or drink for 30 minutes prior to saliva collection. Using the Oragene™ OG-500 collection kit participants provided 2 mL of saliva. This kit contains an enzymatic solution that prevents DNA degradation, allowing the stable storage of samples at room temperature indefinitely. The methodology for DNA collection, quality checks, extraction and methylation is described elsewhere (King et al., 2017). Methylation data from the following 4 key genomic regions was captured: AVP intron 1 and downstream area, and two AVP receptor genes, AVPR1a exon 1 area and AVPR1b upstream area. The areas were mapped to the publicly available coordinates provided by the Human GRCh37/hg19 assembly of the USCS Genome Browser (Kent et al., 2002). The following individual CpG sites were captured: **AVP** intron 1 on chromosome 20 (20:3064043, 20:3064059, 20:3064081, 20:3064090, 20:3064102, 20:3064119, 20:3064126, 20:3064135, 20:3064145), and downstream area (20:3063006, 20:3063018, 20:3063026, 20:3063037, 20:3063049, 20:3063060); **AVPR1a** exon 1 on chromosome 12 (12:63543831, 12:63543856, 12:63543858, 12:63543861, 12:63543864, 12:63543872, 12:63543874, 12:63543878, 12:63543908, 12:63543932, 12:63543936, 12:63543950, 12:63543954, 12:63543968, 12:63543976, 12:63544013, 12:63544015, 12:63544040, 12:63544055, 12:63544065, 12:63544068, 12:63544079, 12:63544097, 12:63544101, 12:63544103, 12:63544114, 12:63544116, 12:63544120, 12:63544123, 12:63544125, 12:63544127, 12:63544149, 12:63544161, 12:63544163, 12:63544171, 12:63544175, 12:63544203, 12:63544206, 12:63544217, 12:63544241, 12:63544243, 12:63544256, 12:63544262, 12:63544264, 12:63544270); and **AVPR1b** upstream on chromosome 1 (1:206223538, 1:206223546, 1:206223560, 1:206223562, 1:206223572, 1:206223579, 1:206223590, 1:206223592, 1:206223628, 1:206223650, 1:206223655, 1:206223675). Methylation for each of these individual sites was read (minimum of 20 reads per site) and then

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the% methylation scores were averaged to create one average methylation score for each gene region. See Figure 1 for the location of the captured sites on AVP, AVPR1a and AVPR1b genes.

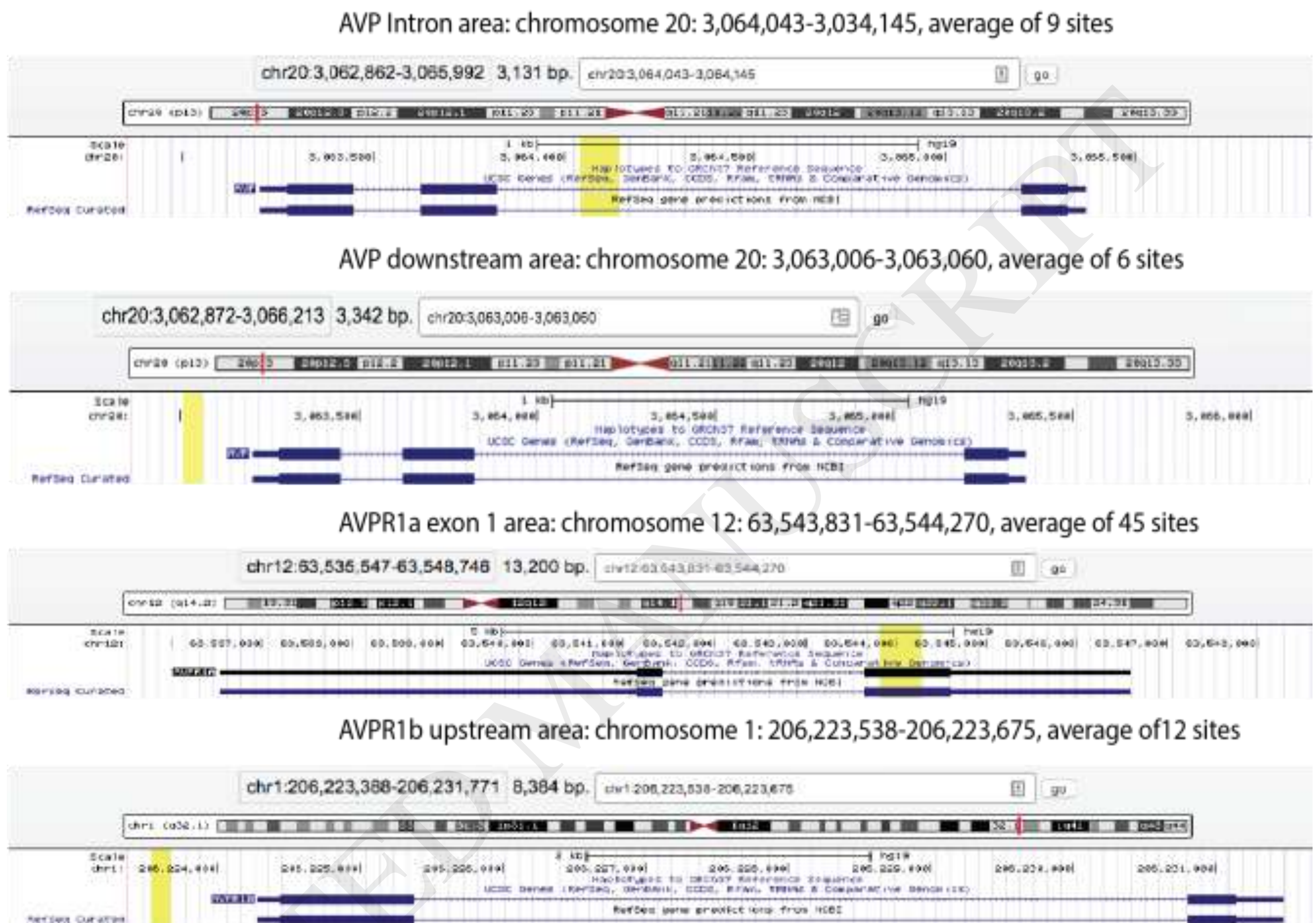

Figure 1. UCSC Genome Browser (http://genome.uscs.edu) locations of captured sites for AVP (intron1, and downstream areas), AVPR1a (exon 1) and AVPR1b (upstream) are highlighted in yellow. Captured sites were averaged and a single score with average % methylation was used.

2.5. Data analyses

Due to non-normal distribution of AVPR1a and AVPR1b scores, and of scores on the sleep disorders subscale of the SSC at PN2 and PP, we have used non-parametric tests for these variables. Group (HighPR and LowPR) and time-point (PN2, PN3 and PP) differences in sleep quality were assessed using a mixed-design repeated measures ANOVA and follow-up paired-

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samples and independent samples t-tests. An independent samples Mann-Whitney test with HighPR/LowPR status as a between subject effect and with AVPR1a, AVPR1b, AVP intron 1, and AVP downstream methylation scores as dependent variables was used to test group differences on methylation scores. Spearman rank-order correlations were used to investigate patterns of associations between sleep quality and methylation scores. The purpose of the correlational analyses was exploratory and descriptive, therefore correction for multiple testing was not applied.

Three separate linear regression analyses were performed to test for a possible moderating role of the AVP methylation in the relationship between reported experience of psychosocial stress (ANRQ scores) and sleep quality during pregnancy and postpartum. Standardized z scores for ANRQ and AVP intron 1 % methylation, as well as the interaction term (ANRQ x AVP i) were used as predictors, and standardized z scores for SSC at PN2 and at PN3 were used as dependent variables (Dawson, 2014). All analyses were performed using IBM SPSS 24.0 software for Macintosh.

3. Results

3.1. Descriptive statistics

Descriptive statistics are reported in Table 1. At PN2, there were 104 participants (32.9%) in the HighPR group, and 209 participants (66.1%) in the LowPR group. Three participants (.9%) did not provide answers on the ANRQ questionnaire.

At PN3, 98 participants (32.7%) were in the HighPR group, and 199 (66.3%) were in the LowPR group. At PP, 94 participants (32.8%) were in the HighPR group, and 190 (66.2%) were

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in the LowPR group. Finally, at FU, 72 participants (38.1%) were in the HighPR group, and 115 (60.8% were in the LowPR group).

Participants who dropped out from the study at the PP did not differ from those who completed the study at PN2 and PN3 on SSC and ANRQ scores, and on the demographic variables. Participants who dropped out from the study at the FU time-point, however, tended to have less total years of schooling (M(dropped out)=15.69±3.21, M(retained)=16.99±2.86; $p<.001$); had lower ANRQ total score (M(dropped out)=16.98±10.18; M(retained)=20.11±10.37, $p=.009$) so tended to be in the Low PR group (74.6%, $\chi^2(1)=5.83$, $p=.016$) ; and tended to be born in Canada (67,7% Canadian born; $\chi^2(1)=11.17$, $p=.001$).

Table 1. Descriptive Statistics for the Main Variables

	n	*M*	*SD*	*Range*
SSC scores				
PN2	316	14.89	7.52	0-46
PN3	298	16.71	7.84	1-41
PP	287	9.51	6.44	0-40
ANRQ	313	18.85	10.39	5-50
AVP %Methylation				
AVP downstream	186	25.40	6.35	0-39.80
AVP intron 1	171	10.10	5.94	0-28.78
AVPR1a	186	16.98	2.77	0-23.76
AVPR1b	187	1.86	1.24	0-6.43

Notes: M=mean; SD=standard deviation; SSC=Sleep Symptom Checklist; PN2 = second trimester of pregnancy; PN3 = third trimester of pregnancy; PP = 2 months postpartum; ANRQ=Antenatal Risk Questionnaire; AVP=Arginine Vasopressin

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3.2. Sleep quality and psychosocial stress

We found a significant main effect of time point in levels of disordered sleep (F(2)=152.24, p<.001, η_p^2=.36) as well as a significant group x disordered sleep quality (F(2)=3.07, p=.047, η_p^2=.01) effect (see Table 2).

Table 2. Mixed-Design Repeated Measures Analysis of Variance of Scores on Sleep Symptom Checklist by Psychosocial Risk Group

	df	*SS*	*MS*	*F*	*p*	η_p^2
Within subjects (sleep over time)	2	7481.35	3740.68	152.24	<.001	.36
Interaction	2	150.86	75.430	3.07	.047	.01
Between subjects (psychosocial risk)	1	1148.01	1148.01	10.72	.001	.04

Follow-up independent samples t-tests revealed that women in the HighPR group had significantly higher rates of disordered sleep than women in the LowPR group at PN2 (M(HighPR)=16.95±7.55, M(LowPR)=13.85±7.33, t(311)=-3.490, p<.001) and PN3 (M(HighPR)=19.14±8.35, M(LowPR)=15.56±7.35, t(293)=-3.759) but not at PP (M(HighPR)=10.33±7.31, M(LowPR)=9.15±5.97, t(282)=-1.456, p=.146). Follow-up paired-sample t-tests run separately for each group revealed significant differences between all three time points for both groups. For the HighPR group, sleep quality was significantly worse at PN3 (M=19.14±8.45) than at PN2 (M=17.22±7.64, t(96)=-2.792, p=.006); and significantly better at PP (M=10.27±7.40) than at PN3 (M=18.75±8.21, t(90)=10.146, p<.001). Further, incidence of sleep disorders was significantly lower at PP (M=10.33±7.31) than at PN2 (M=16.74±7.52, t(93)=9.232, p<.001). A similar pattern was observed for the Low PR group: sleep quality significantly decreased from PN2 (M=13.82±7.45) to PN3 (M=15.56±7.35, t(197)=-3.902,

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$p<.001$), and significantly improved from PN3 (M=15.53±7.39) to PP (M=9.05±5.95, t(186)= 12.592, p<.001) finally, sleep quality at PP (M=9.15±5.97) was significantly better than at PN2 (M=13.85±7.40, t(189)=8.787, p<.001). All these differences were significant after Bonferroni correction for multiple comparisons (Bonferroni correction: $\alpha_{altered}$=.05/6 comparisons =.008). Further, follow up independent samples t-tests revealed that in the HighPR group sleep quality was significantly worse at PN2 and at PN3 than in the LowPR group. The groups did not differ in SSC scores at PP. These results are plotted in Figure 2.

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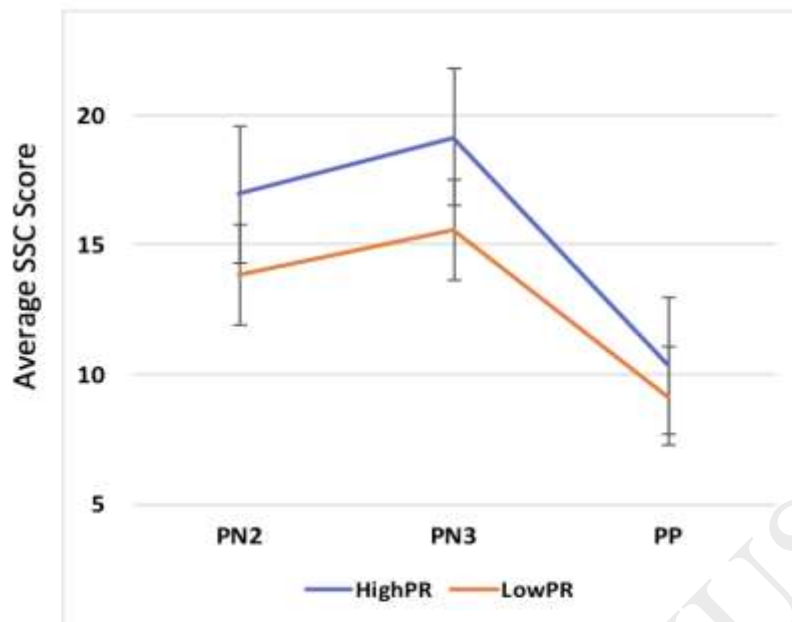

Figure 2. Sleep Symptom Checklist (SSC) mean scores±SD in women with high (HighPR) and low (LowPR) levels of cumulative psychosocial risk during the second (PN2) and third (PN3) trimesters of pregnancy and at 2 months postpartum (PP). High SSC scores indicate higher incidence of sleep disorders. All paired samples t-tests and independent samples t-tests, except for group differences at PP, p<.008 (Bonferroni correction for multiple comparisons: $\alpha_{altered}$=.05/6 comparisons =.008).

3.3. Psychosocial risk and AVP

No group differences (HighPR vs. LowPR) were found on any of the AVP methylation scores.

3.4. Sleep and AVP methylation in relation to psychosocial risk

Different patterns of relationship between sleep and AVP methylation patterns were observed in the HighPR and LowPR groups: levels of sleep disturbances significantly negatively correlated with methylation of AVP intron 1 at PN2 (r_s=-.390 , p=.001), PN3 (r_s=-.384, p=.002) and at PP (r_s= -.269, p=.032), and with AVP downstream area at PN2 (r_s=-.274, p=.021), but not at PN3 or PP, and only in the HighPR group. Methylation in the regions captured for AVPR1a

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nor AVPR1b was not significantly associated with sleep quality. No significant correlations were found between SSC score and AVP downstream, AVPR1a or AVPR1b for the LowPR group.

3.5. Correlation analyses: sleep characteristics and AVP intron 1 methylation

Since total scores on the SSC were associated with AVP intron 1 methylation at all the three time-points, we conducted an exploratory correlation analyses to investigate which subscales of SSC (insomnia, daytime symptoms, and sleep disorders) specifically were associated with AVP intron 1 methylation in the HighPR group. At PN2, all three subscales were negatively correlated with AVP intron 1 methylation (insomnia: r_s =-.262, p=.036; daytime symptoms: r_s =-274, p=.029; sleep disorders: r_s =-.384, p=.002), at PN3 insomnia (r_s =-.324, p=.011) and sleep disorders (r_s =-.384, p=.002), but daytime symptoms were not associated with AVP intron 1 methylation; and at PP only insomnia (r_s=-.314, p= .011) was associated with AVP intron 1 methylation. Scatterplots of correlational analyses are presented in Supplement 1.

3.6. Moderation analysis: interaction between psychosocial risk and AVP intron 1 methylation in relation to sleep quality

At **PN2**, ANRQ and AVP intron 1 methylation scores significantly predicted SSC scores ($F(2, 166)=5.814$, p=.004, adjusted R^2=.054). ANRQ score was a significant predictor (p=.002), but AVP % methylation was not (p=.153). Adding the interaction term (ANRQ x AVP intron 1) improved the model fit ($F(3, 165)=7.589$, p<.001, adjusted R^2=.105, ΔR^2= .056). ANRQ was again a significant predictor (p=.005), AVP % methylation was not a significant predictor (p=.428), and the ANRQ x AVP interaction term was a significant predictor (p=.001).

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Similarly, at **PN3**, ANRQ and AVP % methylation significantly predicted SSC scores (F(2, 164)=6.453, *p*=.002, adjusted R^2=.062). Both ANRQ (*p*=.007) and AVP intron 1 methylation score (*p*=.016) were significant predictors of SSC score. With the addition of the interaction term (ANRQ x AVP intron 1), the model fit was improved (F(3,163)=6.340, *p*<.001, adjusted R^2=.088, ΔR^2 = .032). ANRQ (*p*=.013) and ANRQ x AVP % methylation interaction term (*p*=.018) were significant predictors, while AVP % methylation score was a non-significant trend predictor (*p*=.054).

Likewise, at **PP**, AVP intron 1 methylation scores significantly predicted SSC scores (F(2, 166=3.220, *p*=.042, adjusted R^2=.026). ANRQ score was a significant predictor (*p*=.025), but AVP intron 1 % methylation score was not (*p*=.222). With the addition of the interaction term (ANRQ x AVP intron 1), the model fit was improved (F(3,165)=4.848, *p*=.003, adjusted R^2=.064, ΔR^2=.044). ANRQ (*p*=.047) and ANRQ x AVP intron 1 interaction term (*p*=.006) were both significant predictors, while AVP intron 1 methylation score was not a significant predictor.

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4. Discussion

This study aimed to investigate the relationship between disordered sleep during pregnancy and postpartum, DNA methylation of the AVP system genes (AVP, AVPR1a and AVPR1b) and psychosocial stress. First, we found that women with more cumulative psychosocial stress had a higher incidence of sleep disorders during second and third trimesters of pregnancy, but not during the postpartum period, than women with low cumulative history of psychosocial stress. This suggests that there may be a snowball effect for stressors: psychosocial stress adding to the stressor of pregnancy may increase the incidence of sleep disorders.

Second, our results demonstrate a relationship between psychosocial stress, peripheral (saliva) AVP intron 1 methylation, and disordered sleep. Specifically, in women with high levels of cumulative psychosocial stress, disordered sleep was associated with hypomethylation of the AVP intron 1 area. Further, psychosocial stress was found to moderate the relationship between AVP methylation and sleep disorders during pregnancy and at postpartum, suggesting that AVP may be indirectly associated with sleep regulation, depending on the underlying levels of exposure to psychosocial stress. This indicates that neurochemical regulation of both sleep and sociality may have some of the same underlying mechanisms, which may be, at least in part, instantiated through epigenetic regulation of the AVP system genes.

4.1. Sleep quality during pregnancy and in postpartum is associated with cumulative psychosocial stress

We found that sleep was significantly more disordered in women with history of high levels of cumulative psychosocial stress than in women with low levels of cumulative psychosocial stress during the second and third trimesters of pregnancy, but not at postpartum.

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This is consistent with the view that, in addition to lived experience of stressful events, pregnancy itself can be considered a stressor, which may, in turn, increase the incidence of sleep disorders (Palagini et al., 2014), and that physiological changes specific to pregnancy trigger sleep disorders. Although the postpartum period is characterized by its own challenges (insufficient sleep, demands of infant care, changing/novel sleeping arrangements), it is possible that these stressors are qualitatively different from and more widespread than those associated with pregnancy. The fact that the group differences disappeared at postpartum indicates that there may be a pregnancy-specific interplay between being at risk for psychosocial stress, sleep reactivity and pregnancy-associated stressors.

Since both stress and insufficient/disordered sleep have previously been linked to adverse outcomes for both mother and infant, identifying women at high risk for sleep disorders during pregnancy is relevant for clinical practice. Even though in the present study we did not use any of the objective measures of sleep (polysomnography, or actigraphy), the Sleep Symptom Checklist, has previously been validated as a measure that correlates well with the polysomnographic markers of sleep disorders (Bailes et al., 2008). Interventions such as cognitive-behavioral therapy for insomnia (CBT-I) and a sleep enhancement program (Lee et al., 2016) were shown to be effective in improving sleep quality. Further, CBT-I during pregnancy may be effective in reducing symptoms of depression and anxiety (Tomfohr-Madsen et al., 2017). Thus, focusing on sleep interventions during pregnancy may decrease levels of physical and mental distress during pregnancy and in the postpartum, and may potentially decrease rates of negative outcomes for both mother and infant.

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4.2. Relationship between AVP intron 1 methylation, psychosocial stress and sleep during pregnancy and in postpartum

This is the first study to show an association between AVP methylation, psychosocial stress and sleep quality in pregnant and postpartum women. Our exploratory study showed that hypomethylation of sites within AVP intron 1 was associated with higher levels of disordered sleep, but only in women with high self-reported cumulative psychosocial stress. No association was found between psychosocial stress, sleep and AVP, AVPR1a or AVPR1b methylation, and no association was found between any of the AVP methylation measures and sleep for the low psychosocial stress group. Further, while methylation of AVP was not in itself a significant predictor of disordered sleep, we found a significant interaction effect between cumulative psychosocial stress and AVP intron 1 methylation, predicting higher rates of disordered sleep in pregnancy and postpartum. This suggests that the AVP system plays an indirect role in influencing sleep quality during the perinatal period, and that this role depends on other underlying contextual factors, such as psychosocial stress. Further, this moderating role of psychosocial stress in the relationship between AVP and sleep points to the possibility that AVP system may be differentially attuned, via epigenetic influences, to stress and sleep reactivity in individuals who have experienced higher levels of psychosocial adversity.

In order to better elucidate the relationship between specific sleep disturbances and AVP intron 1 methylation, we also conducted an exploratory correlational analysis, which included the specific subscales of the SSC: insomnia, daytime symptoms and sleep disorders (sleep-disordered breathing and restless legs). We found that all three subscales were related to AVP intron 1 methylation during the second trimester of pregnancy; that during the third trimester insomnia and daytime sleepiness were correlated with AVP intron 1 methylation; and, finally,

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that at postpartum the relationship between sleep disturbances and AVP was primarily driven by insomnia. These results are exploratory, do not survive a conservative error correction, and are derived from a self-report questionnaire and are not confirmed by polysomnography, thus should be interpreted with caution. Despite these limitations, our work provides a starting point for future focused research on AVP's role in the interaction between sleep and psychosocial stress during the perinatal period. Further, this paper provides validation for the use of the Sleep Symptom Checklist to screen sleep disorders during pregnancy and postpartum.

In humans, DNA methylation of AVP has been investigated in response to early life stress and victimization (Marzi et al., 2018), however, no association was observed between experience of victimization and changes in AVP methylation profiles. In rats, selective REM sleep deprivation is associated with downregulation of AVP mRNA compared to controls (Narwade et al., 2017) which revert to normal levels after three consecutive days of rest, suggesting that an epigenetic mechanism may be involved in regulation of sleep-wake schedule and in response to acute sleep deprivation. Thus, AVP methylation may be a relatively stable mark that persists following early life changes, but is also relatively dynamic and responds to acute challenges. The limited body of literature on the relationship between AVP methylation, stress and sleep provides a starting point for our research; however, more work is needed to clarify the likely multifactorial nature of AVP's involvement in these complex processes.

To better understand the chromatin state of the gene region captured on AVP, along with any regulatory mechanisms that may be attributed to our CpG sites, we made reference to both the human hg 19 assembly of the WashU EpiGenome Browser (http://epigenomegateway.wustl.edu) and the USCS Genome Browser (http://genome.ucsc.edu) using human reference sequence GRCh37produced by the Genome Reference Consortium. These databases provide information

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on transcription factors that bind in this region of DNA as well as histone modifications which function by signaling chromatin remodeling complexes that ultimately bring about transcriptional activation (signaled by H3K4me3), enhancement (H3K4me2, H3K4me1, H3K4me2, H3K27ac) or repression (H3K9me2, H3K9me3, H3K27me3) (Zhou et al., 2011). We then investigated a cell line that could act as a proxy for our buccal cells and chose two lymphoblastoid cell lines (see Supplement 2). Examining the activity of these cell lines using the WashU EpiGenome Browser revealed that a bivalent enhancer is located at the site of altered methylation. A bivalent chromatin state is simultaneously comprised of both active and repressive histone modifications, which keep gene expression low but poised for rapid activation (Bernhart et al., 2016)

The intronic area of AVP was associated with sleep quality. Intron 1 is placed between Exon A and B, which encode the essential and highly conserved neurophysin (carrier protein), for circulating AVP. This intronic region has previously been found to correlate to other parts of the AVP gene in its effect (Smith et al., 2017) where increased DNA methylation in both the AVP promoter and CpG shore regions (< 2kb flanking CpG Islands) was associated with higher socioeconomic disadvantage and an inferior social environment. Although introns are non-coding areas of the gene, previously considered as “junk DNA”, their function is becoming better understood. Recent evidence points to many possible functions of introns, including gene regulation processes such as alternative splicing, enhancement of gene expression, transcription initiation and termination, and genomic organization (Chorev & Carmel, 2012; Jo & Choi, 2015). In a recent study, Holocaust survivors and their children showed a distinct pattern of methylation of the intronic region of the FKBP5 gene (Yehuda et al., 2016), an area previously associated with history of childhood abuse (Klengel et al., 2013). Thus, introns are potential

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areas of interest for epigenetic changes associated with environmental pressures and transgenerational transmission of environmental sensitivities via foetal programming.

DNA methylation appears to be both relatively stable and relatively plastic (Meaney & Szyf, 2005; Woodfine et al., 2011). Genes associated with the HPA axis are likely to be particularly sensitive to stress-induced changes in DNA methylation (Hing et al., 2014). For example, AVP's paralogue oxytocin has been investigated in relation to stress and DNA methylation of oxytocin's only receptor, the OXTR. In both central and peripheral (blood) samples prairie vole pups receiving low parental attention in early life had higher levels of OXTR methylation than pups who received more nurturing (Perkeybile et al., 2019). Importantly, methylation in blood and brain were highly correlated to each other, and negatively correlated to gene expression in the nucleus accumbens, supporting the use of peripheral samples as a proxy for central activity. Thus, cumulative and ongoing psychosocial stress (as measured by the ANRQ) may also have an effect on AVP methylation patterns. Still, while DNA methylation is dynamic, reversible and responsive to experience, there is evidence that global DNA methylation patterns are relatively unchanging and inherited, and can be seen as stable biomarkers of individual patterns of gene expression (How Kit et al., 2012). It is not currently known how stable/dynamic AVP methylation patterns are, although recent evidence suggests levels of methylation in both the glucocorticoid receptor gene (NR3C1) and the FK506 Binding Protein 5 (FKBP5) gene, both of which are involved in HPA axis regulation, are stable over a two-year period (Di Sante et al., 2018). Our results must be considered preliminary. In our previous work, using the same community sample as the present study (Zelkowitz et al., 2014), we reported that circulating oxytocin may play a buffering role, protecting mothers in the high psychosocial stress group against stress-related disorders, such as depression. In the present study, we found that intronic

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AVP methylation is negatively associated with sleep disorders during pregnancy and in the postpartum, and that this relationship is moderated by the cumulative psychosocial stress. It is possible, then, that the OT-AVP system functions differentially, depending on the individual's exposure to psychosocial stress. Thus, in women who have AVP intron 1 hypomethylation, there is a higher incidence of perinatal sleep disorders. While it is as yet unknown whether hypomethylation of this AVP region translates into higher or lower levels of circulating AVP in the brain, we hypothesize that this methylation is involved in regulating transcription and thus affects quantity of circulating hormone. The stability of AVP and its receptor's gene expression has been studied in relation to circulating levels of AVP during pregnancy (Marshall & John, 1995) and central expression during aging (Van Der Woude et al., 1995). For example, women experience increments in intravascular volume during pregnancy, osmolality is decreased and at the same time, volume-sensing AVP release mechanisms adjust to sense this new level as normal. While this osmoregulation mechanism is well accepted, AVP stability in relation to aging and age-related disease expression is not fully established.

The AVP system is also linked with regulation of both the HPA axis and the oxytocin system, both of which are associated with stress response, including early life stress (Murgatroyd et al., 2015) and adult stress (Goncharova, 2013), and with pregnancy and maternal behaviors. It is possible, then, that the behaviour of the AVP system changes depending on history of psychosocial stress. Thus, the relationship between AVP methylation and sleep disorders observed in the current study, that was specific to the high psychosocial stress group, may represent a biomarker to react to stressors (such as pregnancy) in a way that involves higher levels of sleep dysregulation. This is consistent with the environmental susceptibility model of stress response (Belsky & Pluess, 2009), which proposes an interaction between an individual

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tendency to develop certain patterns of reactivity to life events and environmental pressures. Moreover, sleep reactivity has also been recently proposed as a trait, that, together with other predisposing factors, such as genes, is sensitive to stressors (Drake et al., 2014; Kalmbach et al., 2018). Thus, the AVP profile observed in the current study may be seen as a biological embedding of the interactive relationship between stress response and sleep reactivity.

The AVP system, then may contribute to dysregulated sleep during pregnancy and in the postpartum. Disordered sleep and psychosocial stress likely function as a bi-directional relationship, and both contribute to possible adverse physical and psychological outcomes for mothers and their infants (for a summary of the proposed model, see Figure 3).

No relationship was observed between sleep and AVPR1a or AVPR1b methylation patterns on the areas we interrogated. While AVPR1a's association with social phenomena, such as autism spectrum disorder and pair bonding, is well documented (Roach et al., 2018), no study, to our knowledge, has linked AVPR1a to sleep or circadian regulation. With regards to AVPR1b, which is expressed both in the pituitary and in the suprachiasmatic nucleus, the circadian "master clock", it has been reported that AVPR1b rs28373064 gene, in interaction with the *clock* rs6832769 gene, affects levels of prosociality in a sample of Chinese men (Ci et al., 2014). This suggests that social and circadian regulation are dependent on some of the same underlying molecular processes. Further, we did not capture the AVPR2 receptor gene, hypermethylation of which was associated, in mice, with circadian entrainment (Azzi et al., 2014). Finally, it is possible that the genes of the AVP system, while involved in stress response, social behaviors and circadian regulation, are only partially sensitive to sleep disturbances of non-circadian nature. Disordered sleep during pregnancy is mostly characterized by such phenomena as

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insomnia, sleep-disordered breathing, daytime fatigue and restless legs syndrome (Balserak & Lee, 2017), which may be relatively independent of circadian rhythm.

One important limitation of the current study concerns the use of salivary DNA which contained exfoliated epithelial cells and leucocytes. Our analysis did not adjust for cell composition. While the genotyping and the epigenome data between salivary and blood-derived DNA are highly correlated, epigenetic markers of DNA expression may have tissue-specific qualities (Langie et al., 2017). To date, salivary DNA is increasingly used to identify epigenetic biomarkers of health and disease due to the fact that the procedure is non-invasive, the sample is stable and yields high quality DNA. Another limitation is the fact that we collected salivary DNA at the follow-up time point, 2.9 years into the postpartum period. While methylation patterns are relatively stable (Woodfine et al., 2011), it is possible that they may have been different during pregnancy. More research is needed to understand the dynamics of AVP expression in salivary DNA and its relationship to circulating and central hormone levels.

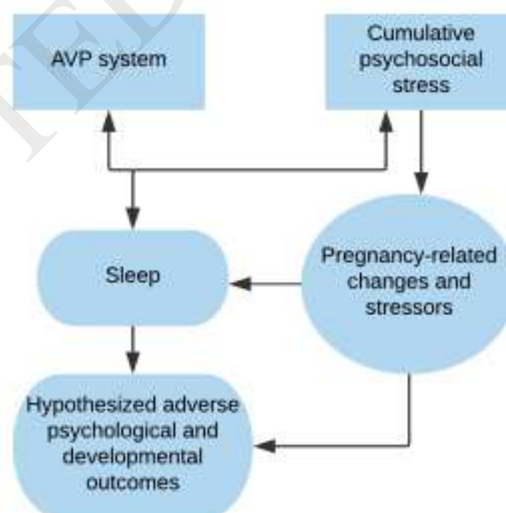

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Figure 3. Hypothesized model of the relationship between lived experience of psychosocial stress, proposed epigenetic mechanisms by which patterns of stress response become biologically embedded, sleep quality and potential outcomes.

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