Chapter 3

Poor neonatal adaptation after antidepressant exposure: is the serotonergic system involved?

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Under review
ABSTRACT

Objective
Infants exposed to selective antidepressants (SADs) in utero are at risk to develop poor neonatal adaptation (PNA) postpartum. As symptoms are nonspecific and the etiology of PNA is unknown, the diagnostic process is hampered. We hypothesized that the serotonin metabolism plays a role in the etiology of PNA.

Methods
In this controlled study, infants admitted postpartum from February 2012 to August 2013 were included and followed for three days. Infants exposed to SADs during at least the last two weeks of fetal life were included in the patient group (n=63). Infants not exposed to psychotropic medication and admitted postpartum for another reason were included in the control group (n=126). The neonatal urinary 5-HIAA levels of SAD-exposed infants who developed PNA, SAD-exposed infants who did not develop PNA and control infants were compared.

Results
The course of the 5-HIAA levels over the first three days postpartum differed between infants with and without PNA (p=<0.001) with higher 5-HIAA levels in infants with PNA on day one (2.42 mmol/mol, p=0.001). Presence of maternal psychological distress modified this relationship.

Conclusions
A transient disturbance of the neonatal serotonergic system may play a role in the etiology of PNA. Other factors, including the presence of maternal psychological distress, also seem to play a role.
INTRODUCTION

Poor neonatal adaptation (PNA) is a syndrome caused by exposure to psychotropic medication during pregnancy. It consists of symptoms of restlessness, such as tremors or sleeping difficulties that are mostly mild and self-limiting. However, symptoms of PNA are nonspecific and can also be an expression of other, more severe neonatal pathology, such as perinatal infection. To exclude these other syndromes, invasive tests are frequently performed, which can be harmful and stressful to the infant and parents.

Approximately 2-9% of Western pregnant women use selective antidepressants (SADs) during pregnancy and 20-30% of their infants develop PNA. Symptoms mostly develop between eight and 48 hours postpartum and fade within 72 hours postpartum.

Knowledge of the pathogenesis and etiology of PNA is limited, which makes it difficult to diagnose this syndrome or predict which infant will develop symptoms of PNA. Symptoms of PNA are most likely caused by SAD withdrawal. However, the pathogenesis may also be toxicity or an overlap between withdrawal and toxicity. Furthermore, other factors might also lead to restlessness in infants, such as maternal psychiatric illness and disturbances in hormone or neurotransmitter levels. This multifactorial etiology might explain the variability of PNA.

Laine et al. suggested that the serotonergic activity is involved in the etiology of PNA and that this is reflected by 5-hydroxyindoleacetic acid (5-HIAA), the main serotonin metabolite. It is assumed that the level of 5-HIAA reflects the degree of serotonergic activity. They showed that infants exposed to selective serotonin reuptake inhibitors (SSRIs) had lower 5-HIAA levels in umbilical cord blood compared to non-exposed infants. Infants with a lower 5-HIAA level showed more symptoms of restlessness. However, umbilical cord blood reflects fetal life while PNA develops postpartum. Therefore, the exact role of serotonin in the etiology of PNA is not resolved. Knowledge of the course of neonatal 5-HIAA levels during the first days postpartum can be of additional value.

In the present non-randomized controlled study we examined the course of urinary 5-HIAA levels of SAD-exposed infants during the first three days of neonatal life. Results were compared to a control group of non-exposed infants, to examine the effect of fetal SAD-exposure on the neonatal serotonergic system. Within the group of SAD-exposed infants, urinary 5-HIAA levels were compared between infants who did and did not develop PNA to examine the role of the serotonergic system in the development of PNA.
METHODOLOGIES

Setting and standard procedures
We conducted a non-randomized controlled study in a teaching hospital in Amsterdam. The psychiatric, obstetric, pediatric (POP) clinic of this hospital is a center of expertise for pregnancy and psychiatric disorders and advises women with a psychiatric disorder before, during and after pregnancy. About fifty percent of all pregnant women who visit the POP clinic live in our catchment area and therefore deliver in our hospital. Within eight hours postpartum these women are admitted to the maternity ward together with their infants for an observation period of ≥72 hours. Infants who need more surveillance are admitted to the neonatal care unit (NCU). Trained nurses observe infants for PNA by means of the Finnegan scoring list. This observational tool was originally designed to assess PNA after exposure to opiates but has been widely used for observation of PNA after exposure to SADs. A validated observational tool does not exist. All infants are examined by a pediatrician on a daily basis. At the end of the observation period the pediatrician in charge concludes if PNA has been present or absent. This decision is made upon evaluation of all completed Finnegan scoring lists, the moment of onset and course of symptoms and the physical examination. If necessary other neonatal pathology is excluded.

Participants
From February 2012 to August 2013 infants were included. The patient group consisted of infants who were admitted for observation of possible PNA and whose mothers used one or more SAD during at least the last two weeks of pregnancy. Two weeks is the maximum time for SADs to reach their effective dosage. SADs were defined as SSRIs, serotonin norepinephrine reuptake inhibitors (SNRIs) or noradrenergic and specific serotonergic antidepressants (NaSSAs). If the mother also used another type of psychotropic drug, the infant was excluded because exposure to other psychotropic medication might also cause PNA. Infants were included in the control group if the mother did not use psychotropic medication during pregnancy and if the mother-infant dyad was admitted for another neonatal or maternal reason with an expected hospital stay of ≥72 hours. Most infants who fulfil these criteria are born by caesarean section while the type of birth of infants in the patient group reflects the normal population. Therefore, the control group was constructed of three equal sized groups of infants born by planned caesarean section, emergency caesarean section and vaginal delivery.

Exclusion criteria for both groups were insufficient knowledge of the Dutch or English language, mental retardation of at least one of the parents, multiple
pregnancies, use of illicit drugs or regular alcohol use (>2 units per week) during the last trimester of pregnancy, use of systemic corticosteroids during pregnancy or when counselling or participation in this study would interfere with the clinical course. When possible, parents were informed on the study prior to delivery. Otherwise, parents were informed within 24 hours postpartum. All subsequent eligible patients were included. The study was approved by the medical ethics committees of the OLVG west Hospital and VU Medical Center in Amsterdam, the Netherlands. The authoritative parent(s) of all infants signed an informed consent form.

**Determinants and outcome measure**

First, we compared urinary 5-HIAA levels between infants exposed and not exposed to SADs. Thereafter, we solely examined the SAD-exposed infants and compared 5-HIAA levels in infants with and without PNA.

Exposure to SADs in utero was determined by medical interview during pregnancy at the POP clinic and verified postpartum. On the first day postpartum the mother completed a questionnaire with respect to demographic characteristics, medication and intoxications during pregnancy. Five pediatricians established PNA and were blinded for the 5-HIAA levels of infants. The outcome measure was the urinary 5-HIAA level during the first three days postpartum.

**Urine collection**

Urine was collected by means of the ‘Peespot’, a filter containing 2 mM ascorbic acid and 2 mM ethylenediamine tetraacetate to improve preservation of 5-HIAA. The correlation between urine collected with the Peespot and mid-stream urine is 1.0 (data not shown). Two filters were placed in each diaper. Saturated Peespots were sent to the clinical laboratory of our hospital within 30 minutes after diaper change. Peespots were centrifuged and urine was stored at -20°C. Time points at which filters were removed were categorized as 0-24, >24-48 and >48 hours postpartum.

**5-HIAA analysis**

To determine 5-HIAA, high performance liquid chromatography (Shimadzu) with fluorescence detection was used with an excitation of 275 nm and emission of 345 nm. Fifty µl of the sample was mixed with 1.0 ml elution buffer (NH4)2HPO4 (pH 4.5) after which 20 µl was injected into the HPLC system (Lichrocart 25 cm RP C18). A gradient of (NH4)2HPO4 and methanol buffer was used to elute the 5-HIAA component. The amount of 5-HIAA in the sample was quantified by calculation of the area relative to those of the internal standard (Sigma). Peak areas were
compared to the calibrator (Chromsystems). Creatinine was determined in each sample by dry chemistry (Vitros 5.1FS, Johnson&Johnson). 5-HIAA was adjusted for the creatinine level as the 5-HIAA level in a single urine sample depends on the concentration of urine.

**Potential effect modifiers and confounders**

Maternal and neonatal stress levels may be associated with exposure to SADs, PNA as well as with the 5-HIAA level.\(^8,11-15,22,23\) We examined whether maternal, pregnancy and neonatal stressors were effect modifiers or confounders in both comparisons. The presence of maternal psychological distress was measured by means of the hospital anxiety and depression scale (HADS) on the first day post-partum. This validated instrument consists of 14 questions. A total score on the anxiety and/or depression subscale of eight or higher indicates depression and anxiety and is indicative for elevated psychological distress.\(^24,25\) Pregnancy stress was defined as complications during pregnancy, such as hypertension. Neonatal stress was defined as small for gestational age (birth weight of <10\(^{th}\) percentile according to the Dutch perinatal registration based on ethnicity\(^26\)), prematurity (gestational age <37 weeks) or complications during or after birth, such as infection.

In the relationship between PNA and 5-HIAA, in addition delivery stress, type and dosage of antidepressant and duration of antidepressant usage were examined as potential effect modifiers or confounders. Delivery stress, indicated as type of delivery, was categorized as vaginal delivery, planned- or emergency caesarean section. The duration of antidepressant usage was dichotomised in usage during part of pregnancy or the entire pregnancy. Type of antidepressant was categorized in SSRI, SNRI, NaSSA or a combination of SADs. Dosage was defined as normal in case of the minimal effective dosage and respectively low and high when the dosage was lower or higher than the minimal effective dosage.\(^18\) In case two types of SADs were used, the highest dose was taken into account.

**Sample size calculation**

The required sample size was calculated to detect a difference in the course of 5-HIAA over time between infants with and without PNA with a power of 80%, significance level of 5% and standard deviation (SD) of 2 mmol/mol. We assumed a prevalence of PNA of 40\(^{th}\)\(^27\) and a correlation of 0.6 between urine samples of the same infant. Furthermore, we assumed that in 30% of infants it would not be possible to collect any urine. To detect a clinically relevant difference of 1.8 mmol/mol (<1 SD), 63 patients were needed. With a patient:control ratio of 1:2 (126
controls), it was possible to detect a clinical relevant difference of 0.9 mmol/mol (<0.5 SD) between SAD-exposed and non-exposed infants.

**Statistical analysis**

Statistical analyses were performed with SPSS version 21 (IBM, New York, USA). The baseline characteristics of infants exposed and not exposed to SADs were summarized by means of descriptive statistics. Because the group of non-exposed infants was selected to contain an equal number of infants for each of the three types of delivery, whereas the exposed group was a representative sample, no formal statistical comparison of baseline characteristics between groups was performed.

Baseline characteristics of infants with and without PNA were compared. The only continuous (normally distributed) variable was age, which was compared by the independent sample t-test. Categorical variables were analyzed by means of the chi square test. If more than 20% of the expected cell counts were less than five, the Fisher exact test was performed.

Generalized estimated equations (GEE) were used to investigate the between-group difference in the course of the 5-HIAA level over the first three days postpartum. This type of longitudinal data analysis takes into account that repeated measurements taken from the same person are correlated and uses all available data (including those of infants with only one or two 5-HIAA measurements). The model included main effects of a categorical factor for time and group and the interaction between time and group. We first performed an overall test for presence of an interaction between time and group. If the interaction was significant, post hoc tests were performed to investigate the difference in the 5-HIAA level between groups on the three separate days. When comparing the exposed group to the control group analyses were adjusted for type of delivery by including this as a factor in the model. For all GEE analyses an exchangeable correlation structure was used. The estimated means obtained from the GEE analyses were plotted.

We assessed several factors as potential effect modifiers or confounders. If the interaction term between a factor, time and group was significant, we considered this factor as an effect modifier. We stratified our results according to the strongest effect modifier based on the p-value of the interaction term. Factors were considered as confounders if they did not fulfill the criteria for effect modification and if one or both regression coefficients of the interaction between time and group changed with 10% or more. The number of patients restricted the number of confounders. The strongest confounders were added. A p-value of <0.05 was considered significant.
RESULTS

Inclusion of patients
In Figure 1 in- and exclusion of patients is described. Of infants exposed to SADs, 63 infants were included (71%). In 44 infants (70%) at least one urine sample was collected and analyzed. Of infants not exposed to SADs, 126 infants were included (43%). In 80 infants (63%) at least one urine sample was collected and analyzed.

Figure 1. In- and exclusion of infants. In the upper Figure, inclusion of infants of the patient group is presented. In the bottom Figure, inclusion of infants of the control group is presented.

Baseline characteristics
In Table 1, the baseline characteristics of SAD-exposed and non-exposed infants are presented. Of the 44 SAD-exposed infants, 24 (55%) were diagnosed with PNA. None of these infants needed pharmacological treatment. In Table 2, the baseline characteristics of infants exposed to SADs are stratified into infants with and without PNA.
Table 1. Baseline characteristics of infants who were exposed and not exposed to selective antidepressants in utero and their mothers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Infants exposed to SADs (n=44)</th>
<th>Infants not exposed to SADs (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age in years, median (range)</td>
<td>34 (27-41)</td>
<td>33.5 (21-42)</td>
</tr>
<tr>
<td>Married/cohabiting, n(%)</td>
<td>43 (98)</td>
<td>75 (94)</td>
</tr>
<tr>
<td>Complications during pregnancy&lt;sup&gt;a&lt;/sup&gt; (pregnancy stress), n(%)</td>
<td>13 (23)</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Smoking during last trimester of pregnancy, n(%)</td>
<td>1 (2)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>HADS&lt;sup&gt;b&lt;/sup&gt; on first day postpartum (maternal stress), n(%)</td>
<td>Anxiey and/or depression score elevated (≥8)</td>
<td>13 (30)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>0</td>
</tr>
<tr>
<td>Type of delivery (delivery stress), n(%)</td>
<td>Vaginal</td>
<td>36 (82)</td>
</tr>
<tr>
<td></td>
<td>Planned caesarean section</td>
<td>3 (7)</td>
</tr>
<tr>
<td></td>
<td>Emergency caesarean section</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Gender male, n(%)</td>
<td>23 (52)</td>
<td>42 (53)</td>
</tr>
<tr>
<td>Neonatal stress, n(%)</td>
<td>15 (34)</td>
<td>32 (40)</td>
</tr>
<tr>
<td>Prematurity</td>
<td>4 (9)</td>
<td>10 (13)</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>3 (7)</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Birth complications&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3 (7)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Neonatal complications during hospital stay&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8 (18)</td>
<td>25 (31)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Complications during pregnancy: hypertension, preeclampsia, cholestasis, hyper- and hypothyroidism, diabetes
<sup>b</sup> HADS: hospital anxiety and depression scale
<sup>c</sup> More than one cause of neonatal stress was possible.
<sup>d</sup> birth complications: 5 minute Apgar score <7, shoulder dystocia
<sup>e</sup> neonatal complications during hospital stay: infection requiring antibiotics, hyperbilirubinemia, respiratory distress, hypoglycaemia.

Table 2. Baseline characteristics of infants exposed to selective antidepressants with and without poor neonatal adaptation (PNA) and their mothers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Infants with PNA&lt;sup&gt;a&lt;/sup&gt; (n=20)</th>
<th>Infants without PNA&lt;sup&gt;a&lt;/sup&gt; (n=24)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age in years, mean (SD)</td>
<td>35.3 (3.7)</td>
<td>33.0 (4.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Marital status married/cohabiting, n(%)</td>
<td>20 (100)</td>
<td>23 (96)</td>
<td>1.00</td>
</tr>
<tr>
<td>Complications during pregnancy&lt;sup&gt;b&lt;/sup&gt; (pregnancy stress), n(%)</td>
<td>10 (50)</td>
<td>3 (13)</td>
<td>0.01</td>
</tr>
<tr>
<td>Smoking during last trimester of pregnancy, n(%)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Type of antidepressant, n(%)</td>
<td>SSRIF</td>
<td>16 (80)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>SNRI&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1 (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaSSA&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2 (10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combination of SSRIF and NaSSA&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1 (5)</td>
<td>2 (8)</td>
</tr>
</tbody>
</table>
Comparison between SAD-exposed and non-exposed infants

There was no significant difference in the course of the 5-HIAA level over the first three days postpartum between SAD-exposed and non-exposed infants ($p=0.23$, adjusted for type of delivery, neonatal- and pregnancy stress). Presence of maternal psychological distress appeared to be an effect modifier. After stratification, the course of 5-HIAA over time in infants exposed to maternal psychological distress did not differ between groups ($p=0.39$ adjusted for type of delivery and neonatal stress), similar to the course of 5-HIAA over time in infants not exposed to maternal psychological distress ($p=0.48$, adjusted for type of delivery, neonatal- and pregnancy stress).

Table 2. Baseline characteristics of infants exposed to selective antidepressants with and without poor neonatal adaptation (PNA) and their mothers. (continued)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Infants with PNA (^a) (n=20)</th>
<th>Infants without PNA (^a) (n=24)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of antidepressant usage, n(%)</td>
<td></td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td>Entire pregnancy</td>
<td>15 (75)</td>
<td>20 (83)</td>
<td></td>
</tr>
<tr>
<td>Part of pregnancy</td>
<td>5 (25)</td>
<td>4 (17)</td>
<td></td>
</tr>
<tr>
<td>Dosage of antidepressant, n(%)</td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Low</td>
<td>0 (0)</td>
<td>4 (17)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6 (30)</td>
<td>7 (29)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>14 (70)</td>
<td>13 (54)</td>
<td></td>
</tr>
<tr>
<td>Type of delivery (delivery stress), n(%)</td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>Vaginal</td>
<td>16 (80)</td>
<td>20 (83)</td>
<td></td>
</tr>
<tr>
<td>Planned caesarean section</td>
<td>2 (10)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>Emergency caesarean section</td>
<td>2 (10)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>HADS(^f) first day postpartum (maternal stress), n(%)</td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Anxiety and/or depression score elevated (≥8)</td>
<td>4 (20)</td>
<td>9 (38)</td>
<td></td>
</tr>
<tr>
<td>Gender male, n(%)</td>
<td>9 (45)</td>
<td>14 (58)</td>
<td>0.38</td>
</tr>
<tr>
<td>Neonatal stress, n(%)(^b)</td>
<td>5 (25)</td>
<td>10 (42)</td>
<td>0.25</td>
</tr>
<tr>
<td>Prematurity</td>
<td>0 (0)</td>
<td>4 (17)</td>
<td>0.11</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>2 (10)</td>
<td>1 (4)</td>
<td>0.58</td>
</tr>
<tr>
<td>Birth complications(^e)</td>
<td>0 (0)</td>
<td>3 (13)</td>
<td>0.24</td>
</tr>
<tr>
<td>Neonatal complications during hospital stay(^f)</td>
<td>3 (15)</td>
<td>5 (21)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

\(^a\) PNA: poor neonatal adaptation \(^b\) complications during pregnancy: hypertension, preeclampsia, cholestasis, hyper- and hypothyroidism, diabetes \(^c\) SSRI: selective serotonin reuptake inhibitor \(^d\) SNRI: serotonin and norepinephrin reuptake inhibitor \(^e\) NaSSA: noradrenergic and specific serotonergic antidepressant \(^f\) HADS: hospital anxiety and depression scale \(^g\) More than one cause of neonatal stress was possible. \(^e\) birth complications: 5 minute Apgar score <7, shoulder dystocia \(^f\) neonatal complications during hospital stay: infection requiring antibiotics, hyperbilirubinemia, hypoglycaemia.

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**Comparison between SAD-exposed and non-exposed infants**

There was no significant difference in the course of the 5-HIAA level over the first three days postpartum between SAD-exposed and non-exposed infants ($p=0.23$, adjusted for type of delivery, neonatal- and pregnancy stress). Presence of maternal psychological distress appeared to be an effect modifier. After stratification, the course of 5-HIAA over time in infants exposed to maternal psychological distress did not differ between groups ($p=0.39$ adjusted for type of delivery and neonatal stress), similar to the course of 5-HIAA over time in infants not exposed to maternal psychological distress ($p=0.48$, adjusted for type of delivery, neonatal- and pregnancy stress).
Comparison between exposed infants who did and did not develop PNA

There was a significant difference in the course of the 5-HIAA level over the first three days postpartum between infants with and without PNA (p=<0.001, adjusted for type of delivery, neonatal stress, dosage of antidepressant and duration of antidepressant usage). Presence of maternal psychological distress and type of antidepressant modified this relationship. We stratified our data based on the presence of maternal psychological distress, which showed that the course of 5-HIAA over time in infants exposed to maternal psychological distress differed between groups (p=<0.001, adjusted for dosage of antidepressant). The course of 5-HIAA over time in infants not exposed to maternal psychological distress also differed between groups (p=<0.001, adjusted for neonatal stress, type of delivery and dosage of antidepressant) (Figure 2).

On day one, the 5-HIAA levels were higher in infants with PNA compared to infants without PNA (2.42 mmol/mol, p=0.001, adjusted for type of delivery, neonatal stress, dosage of antidepressant and duration of antidepressant usage). On day two and three, there was no significant difference between groups (day two 0.32 mmol/mol, adjusted p-value=0.71, day three -0.73 mmol/mol, adjusted p-value=0.36).

Figure 2. Course of the urinary 5-HIAA level over time with 95% Confidence Interval (CI) of infants with and without poor neonatal adaptation (PNA), controlled for confounders. A. Entire group of infants, corrected for neonatal stress, type of delivery, dosage of antidepressant and duration of antidepressant use. B. Infants of mothers with psychological distress, corrected for dosage of SAD. C. Infants of mothers without psychological distress corrected for neonatal stress, type of delivery and dosage of SAD.
DISCUSSION

To the best of our knowledge, this is the first study on neonatal urinary 5-HIAA levels during the first three days postpartum in relation to exposure to SADs and the development of PNA. Comparison of SAD-exposed and non-exposed infants showed no difference in the course of 5-HIAA levels. The presence of maternal
psychological distress modified this relationship. This supports earlier studies which demonstrated that maternal stress influences the neonatal serotonin metabolism and modifies the effects of SSRIs on the fetus.\textsuperscript{29,30}

Infants with PNA showed a significant different course of 5-HIAA levels compared to infants without PNA whereby the 5-HIAA levels were higher on day one which levelled off on day two and remained stable at day three. There are a few possible explanations for this finding. It is likely that symptoms of PNA are caused by a disorganized central serotonergic system, which has difficulties in adapting to the abrupt decrease of serotonin in the synaptic cleft. The postsynaptic serotonergic receptor down-regulation, caused by high serotonin supply during SAD-exposure in utero, results in decreased serotonin binding leading to a higher level of unbound serotonin. This may lead to increased serotonin breakdown and increased 5-HIAA levels. Another possibility is the terminal auto-receptor feedback mechanism, whereby the sudden decrease of serotonin results in increased serotonin turnover that leads to an increased 5-HIAA levels. Our results are conflicting with the results of Laine et al. which showed increased serotonergic symptoms in combination with decreased 5-HIAA levels.\textsuperscript{9} A possible explanation for this difference might be the manner of symptom evaluation: Laine et al. used a serotonergic symptom score which mainly addresses symptoms to toxicity,\textsuperscript{9} while we regarded PNA as symptoms of withdrawal as scored with the Finnegan list.

Presence of maternal psychological distress modified the relationship between PNA and 5-HIAA, possibly due to the influence of maternal stress on both the neonatal serotonin metabolism and symptoms of restlessness in infants.\textsuperscript{13} In either the entire group of SAD-exposed infants and after stratification, the course of 5-HIAA over time differed between infants with and without PNA. In all groups, the 5-HIAA levels were higher in infants with PNA on day one, which equalized on day three. This indicates a transient relationship between PNA and 5-HIAA.

Main strengths of this study are the non-invasive design, analysis of the course of 5-HIAA over three days postpartum, inclusion of a control group and measurement and adjustment for several stressors. However, this study has several limitations. Our aim was to examine the central cerebral serotonin metabolism of infants in a non-invasive manner. Measurement of 5-HIAA in urine is preferred as it is non-invasive and stable compared to measurement of 5-HIAA in liquor and plasma.\textsuperscript{31} However, there is debate whether urinary 5-HIAA levels are a reliable representation of the central serotonin metabolism as less than 5-10\% of the body’s serotonin is present in the brain.\textsuperscript{32} Few studies reported significant correlations between plasma and liquor- and between plasma and urine 5-HIAA levels.\textsuperscript{33,34} The correlation between liquor and urine 5-HIAA levels is unknown. Possibly urinary 5-HIAA levels mainly represent the peripheral serotonergic sys-
tem. As PNA is most likely of central origin our finding of higher 5-HIAA levels in infants with PNA contradicts this. Another limitation is the establishment of PNA. Although PNA was assessed in a systematic way, inter-observer differences might have influenced our results.

In conclusion, the 5-HIAA levels were higher in infants with PNA compared to infants without PNA on the first day postpartum, possibly due to a transient disturbance of the serotonergic system. Other factors, such as maternal psychological distress, also seem to play a role. Additional studies are needed to further unravel the etiology of PNA whereby the role of other monoamines, hormones and genetics can be explored.

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REFERENCES


