Research protocol: part 1

Project (Bridging the Psychological and Neuroendocrine Functions of the Mother and Her Child: Underlying Mechanisms**) summary:**

The neurobiological mechanisms involved in the influence of post-partum maternal mood fluctuations on child development are far from being understood. The present project is based on translational research aimed to understand possible consequences of maternal mental state on neuroendocrine function of the baby. On the day 3-4 after delivery before the discharge from the maternity ward, the mothers were offered questionaires to asses their mood with special attention to potential presence of postpartum blues. If needed, a psychiatric intervention was offered. In both the mother and the baby, neuroendocrine parameters (aldosterone, cortisol, alpha-amylase, selected interleukins) will be analyzed in their saliva. The mothers will be stratified according to vaginal delivery or C-section. The next visit was 7-9 months thereafter. Both the mother and the baby were exposed to "still face" test with 2 cameras recording. The saliva samples from the mother and the baby were collected before and after the test and mother was offered psychometric questionnaires measuring depressive symptomatology. The reults to be obtained will not only bring new original data, but they wil help to recognize the need of specific interventions directed to help mothers in the early postapartum period.

Protocol Title: Bridging the Psychological and Neuroendocrine Functions of the Mother and Her Child: Underlying Mechanisms, 2/25/2026

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Rationale & background information

The existence of a strong relationship between the mother and her child is a generally known phenomenon. However, the physiological mechanisms involved and possible pathophysiological consequences of maternal mood fluctuations are far from being understood. One of the factors which are likely to be of great importance is the presence of maternal mood disorders. This is particularly true for the occurrence of postpartum depression. Postpartum depression is likely to occur in >10 % of mothers (Izakova et al., 2013) and its association with a dysfunction of the hypothalamic-pituitary-adrenocortical (HPA) axis, which results in increased exposure of pregnant women to cortisol has been proven earlier (Caparros-Gonzalez et al., 2017; De Rezende et al., 2016; Iliadis et al., 2015).

On the other hand, little attention has been given to the postpartum blues. It has been described that about 50–80 % of women experience postpartum blues during the first few days after delivery. Such women commonly report tearfulness, mood lability, anxiety, or irritability. The symptoms are mild, typically peak on the 4th day, and disappear spontaneously within two weeks of delivery (Wisner et al., 2010; Earls et al. 2010). If symptoms of depression last >2 weeks, the woman should be evaluated to exclude or confirm a more serious mood disorder. It appears that there is no strong association between the occurrence of postpartum blues and reproductive hormone levels (Chatzicharalampous et al., 2011). Concerning other endocrine alterations, several older studies reported increased morning cortisol concentrations in the blood on the days on which the symptoms appeared compared to the days without symptoms (Ehlert et al., 1990) or in women with postpartum blues compared to a non-blues group (Okano & Nomura, 1992; Taylor et al., 1994). Changes at different levels of the HPA axis in women with postpartum blues were confirmed in more recent investigations (Garcia-Leal et al., 2017; O'Keane et al., 2011).

Despite high prevalence, the biological mechanisms linking transient maternal mood fluctuations to infant stress regulation remain poorly understood, particularly in the context of postpartum blues.

To our knowledge, no study has simultaneously investigated maternal mood symptoms, acute dyadic stress reactivity, and cumulative stress load using a multilevel design at 6–7 months postpartum, thus the main aim of the project is to achieve a deeper understanding of the relationship and bridging mechanisms between the psychological state of the mother and her child, with a particular focus on postpartum blues, as well as on the mechanisms that play a key role in this interaction, approached on a multilevel and multidisciplinary basis. Understanding early dyadic stress regulation may allow identification of early biomarkers of vulnerability and contribute to preventive strategies targeting both maternal and infant mental health.

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Study goals and objectives

The main goal of the project is to achieve a deeper understanding of the relationship and bridging mechanisms between the psychological state of the mother and her child, with a particular focus on postpartum blues, as well as on the mechanisms that play a key role in this interaction, approached on a multilevel and multidisciplinary basis.

Specific Objectives at the Hospital Level

To identify possible mood changes in mothers using self-report scales aimed at confirming or excluding the presence of postpartum blues at hospital discharge (3rd–4th day postpartum).
To assess neuroendocrine parameters in saliva samples from mothers and their newborns collected at hospital discharge (3rd–4th day postpartum).
To determine the extent of the newborn's acute stress response by measuring stress hormone concentrations in saliva collected after heel prick capillary blood sampling for mandatory newborn metabolic screening.
To compare psychometric and neuroendocrine parameters of mothers and newborns according to mode of delivery (vaginal birth vs. cesarean section).

Specific Objectives at the Research Clinic Level

To assess possible mood changes in mothers using self-report scales focused on depressive symptomatology when the child is 6–7 months old.
To evaluate neuroendocrine parameters in saliva samples from mothers and their children at the child's age of 6–7 months.
To assess behavioral and neuroendocrine responses of mothers and their children during the "Still Face Test" using analysis of recordings from two cameras.
To determine the level of cumulative everyday stress in mothers over the previous three months by measuring hair cortisol concentrations.

Study design

This study is designed as an observational analytical cohort study with retrospective assessment of cumulative stress exposure and concurrent evaluation of behavioral and neuroendocrine stress responses.

84-healthy mother-neonate dyads were recruited. The inclusion criteria were a physiological course of gravidity, term birth (37 weeks 0 days to 41 weeks 6 days of gestation), eutrophic neonate with good post-delivery adaptation (Apgar score 7 or more in the first minute, pH in umbilical cord blood above 7.2). The exclusion criteria include the chronic somatic diseases of the mother before or during gravidity, a psychiatric disorder of the mother, occurrence of gestosis, gestational diabetes or liver disease in pregnancy, BMI above 30 at the beginning of pregnancy, surgical conductance of the labor (forceps, vacuum extractor), drug abuse, asphyxia of the neonate (Apgar score <6 in the 5th minute and/or pH in umbilical cord blood in the first minute below 7.2).

The mothers and simultaneously their neonates were being investigated twice, namely 3–4 days (72–96 h) postpartum and 7–9 months postpartum. The mothers were recruited at the Department of Neonatology of the Faculty of Medicine of the Slovak Medical University, and the 1st Department of Obstetrics and Gynecology, Faculty of Medicine of the Comenius University, University Hospital, Bratislava, Slovakia. Demographic and clinical data on both the mother and the newborn are recorded. The first examinations were done at the maternity hospital on the day of the discharge in the afternoon (13.00–16.00 h) to avoid the influence of daily rhythms on endocrine parameters. The mothers were administered psychometric questionnaires detailed below. Saliva samples were collected from both the mother and her neonate under resting, non-stress conditions as well as in response to compulsory heel blood sampling in the form of the heel-prick test described below. At the 7–9 month postpartum, a follow-up visit was held at the Research Clinic, Biomedical Research Center, Slovak Academy of Sciences again in the afternoon hours. The mothers were being administered psychometric questionnaires detailed below. Thereafter the mother and her baby were exposed to a still-face stress test described below. The samples of saliva from both the mother and the baby were collected before and after the test. A 3 cm long strip of hair (starting from the scalp) was obtained from the mother at the time of both visits. The analyses of neuroendocrine stress markers and evaluation of psychometric scales in the whole sample, a multiple regression analysis will be performed in this study. The focus will be given to correlations with maternal postpartum blues scores.

Methodology

Assessment of Depressive Symptoms in the Postpartum Period

Psychological state with regard to depressive and anxiety symptoms will be assessed using self-report scales.

At the first assessment (hospital discharge, 3rd–4th day postpartum), mothers will complete the Kennerley-Gath questionnaire for detection and evaluation of postpartum blues (Kennerley & Gath, 1989) and a slightly modified version of the Edinburgh Postnatal Depression Scale validated in Czech (Břicháček, 2000; Mohr, 2015).

At the follow-up assessment (6–7 months postpartum), the Beck Depression Inventory, the Zung Self-Rating Depression Scale, and the Edinburgh Postnatal Depression Scale will be used.

Saliva Sampling

Maternal saliva samples will be collected using Salivette® devices (Sarstedt, UK). Participants will be asked to place cotton swabs in their mouths and allow them to saturate with saliva for 60–120 seconds.

Saliva collection from newborns and 7-month-old infants will be performed either by the mother or a research staff member using centrifuge tubes with a special cotton swab (SalivaBio Infant Swab, Salimetrics, USA). Based on preliminary results, the swab will be kept in the mouth for 90–120 seconds.

Saliva samples will be stored at −20°C until analysis.

Heel Prick as a Stressor

Newborns will undergo mandatory heel prick capillary blood sampling for neonatal screening tests prior to hospital discharge. The blood collection will be scheduled in the afternoon on the day of discharge.

The heel skin will be thoroughly disinfected, punctured with a small lancet, and a small amount of blood will be collected onto filter paper.

Saliva samples from both mother and newborn will be collected 10 minutes before the heel prick and 25 minutes afterward, corresponding to the expected increase in cortisol concentration.

“Still Face Test”

Infants aged 6–7 months and their mothers will participate in the “Still Face Test,” introduced by Tronick et al. (1978), a standardized procedure used to study emotional

and stress regulation. The test is based on a stress-inducing situation created by the absence of parental responsiveness.

The procedure consists of three phases:

Play phase (3 minutes) – mother interacts normally and plays with the infant (control condition).

Still face phase (2 minutes) – mother maintains a neutral, expressionless face without any verbal or non-verbal response.

Reunion phase (3 minutes) – mother resumes normal interaction and play.

Mother and child will remain alone in the examination room during the test and will be recorded by two cameras and facial action units will be analysed by open-source software tools (OpenDBM).

Saliva samples for hormone measurement will be collected from both mother and child before the test and 20 minutes after the test.

Measurement of Neuroendocrine Parameters in Saliva

Salivary cortisol concentrations will be measured using a commercially available ELISA kit (IBL International, Hamburg, Germany).

Salivary α-amylase activity will be measured using ELISA kits (Salimetrics, UK).

Salivary aldosterone concentrations will be determined using a modified method (Hlavacova & Jezova, 2008; Hlavacova et al., 2013) with a commercial kit (Immunotech, Prague, Czech Republic).

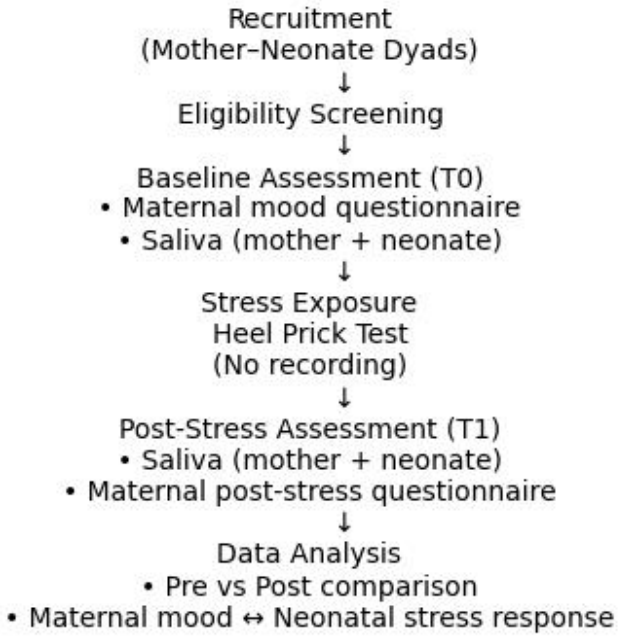
Salivary interleukin-6 concentrations will be measured using ELISA kits (Salimetrics, USA).

Measurement of Hair Cortisol

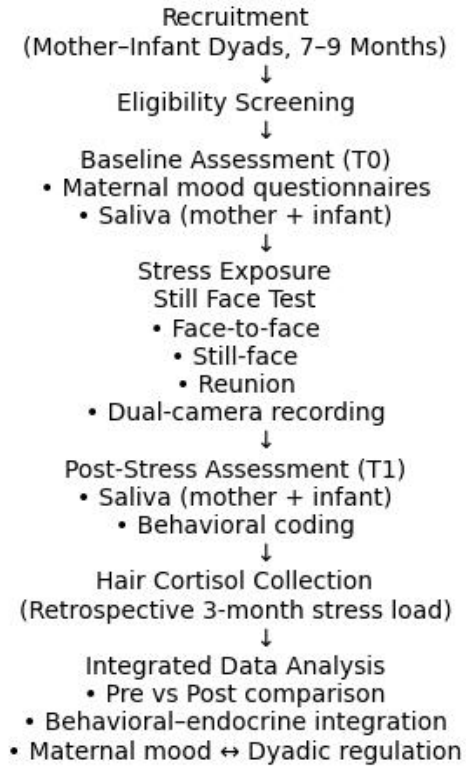
Hair cortisol extraction will be performed using a modified protocol (Balagova & Jezova, 2018) and analyzed by ELISA (IBL International, Hamburg, Germany).

Hair grows approximately 1 cm per month (Wennig, 2000). Therefore, cortisol concentrations measured in 3 cm hair segments (starting from the scalp) reflect cumulative cortisol secretion over the previous three months.

Flow diagram 1. At the hospital



Flow diagram 2. at Research Clinic 7-9 months after delivery



Safety considerations

This study involves retrospective analysis of previously collected biological samples and recorded data. No new biological sampling, stress exposure, or psychological testing will be conducted for the purposes of the current analysis. Therefore, no additional physical or psychological risk is posed to participants as part of this study. All biological samples (saliva and hair) and behavioral recordings were obtained according to established clinical and research procedures.

Follow-up

All data will be pseudonymized prior to analysis. Personal identifiers will be removed, and each participant will be assigned a study code. Data will be stored on secure, password-protected systems in accordance with applicable data protection regulations (e.g., GDPR). Only authorized research personnel will have access to the data.

Because the study involves only retrospective laboratory and data analysis, no adverse events related to the current research activities are anticipated.

Should any previously unrecognized clinically relevant findings emerge during analysis, appropriate procedures for notification will be followed in accordance with institutional and ethical guidelines.

Data management and statistical analysis

All data will be pseudonymized and stored on secure, password-protected servers. Biological, questionnaire, and behavioral data will be linked using coded study identifiers. Data cleaning will include range checks, verification of extreme values, and assessment of normality.

Descriptive statistics will summarize participant characteristics and baseline measures. Pre- and post-stress differences in endocrine parameters will be analyzed using paired t-tests or non-parametric equivalents, as appropriate. Associations between maternal mood symptoms and maternal/infant stress responses will be examined using correlation and multivariable linear regression models adjusting for relevant covariates (e.g., maternal age, infant sex, sampling time).

Where repeated measures are available, linear mixed-effects models will be used to account for within-subject and dyadic clustering. Interaction analyses will explore whether chronic stress exposure (hair cortisol) moderates acute stress reactivity.

Statistical significance will be set at $\alpha = 0.05$ (two-tailed). Missing data will be assessed and handled using appropriate methods (e.g., mixed models or multiple imputation where necessary). Sample size is based on detecting moderate effect sizes with 80% power at the 5% significance level.

Expected outcomes of the study

Findings obtained from the evaluation of mechanisms bridging the mother's psychological state and her child will be applied to the optimization of preventive measures necessary to reduce the negative consequences of severe stress situations (pandemics, climate change, social isolation) on the healthy development of the young generation. Results obtained in the area related to the COVID-19 pandemic and its consequences can be considered promising for further development and research of preventive and therapeutic approaches for viral infections and other negative phenomena of contemporary life.

Duration of the project

Approximately 4 years.

Ethics

App. Ethical Approval p_1/ p_2/ p_3

Informed consent forms

App. Informed_consent_EN, Informovany_suhlas

Research protocol: part 2

Budget and support of the project:

Approved budget from APVV-18-0283: euros

Collaboration with other scientists or research institutions

Department of Psychiatry and Psychotherapy, Philipps-University Marburg, Marburg, Germany

Curriculum Vitae of investigators

Separated email attachement.