# Protocol

# Inhaled Oxytocin and HPA Axis Reactivity

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Complete Title:  Inhaled Oxytocin and HPA Axis Reactivity
Short Title: Inhaled Oxytocin and HPA Axis Reactivity
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Mood, Mother and Child: The Psychobiology of Dyadic Resilience
NICHD, R01HD093901

## Table of Contents

## 1. Abbreviations and Definitions of Terms

| Abbreviation | Definition |
|---|---|
| OT | Oxytocin |
| TSST | Trier Social Stress Test |
| CRT | Cortisol |
| ACTH | Adrenocorticotropic hormone |
| MMI | Mood, Mother and Infant Study |
| MMC | Mood, Mother and Child Study |

## 2. Protocol Synopsis

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|---|---|
| Study Title | Inhaled Oxytocin and HPA Axis Reactivity |
| Funder | NICHD |
| Clinical Phase | n/a |
| Study Rationale | This is Randomized Parallel Assignment Quadruple masked protocol to test the effect of inhaled oxytocin vs. placebo on HPA axis reactivity during a standardized social stressor, the Trier social stress test. This work will address a substantial gap regarding the effect of OT on psychobiology in women and test the extent to which differences in OT response contribute to dyadic development. |
| Study Objective(s) | Primary<br>The primary objective of this experiment is to test the extent to which oxytocin compared with placebo alters cortisol trajectory during the Trier Social Stress Test.<br><br>Secondary<br>The secondary objective of this experiment is to test the extent to which oxytocin compared with placebo alters other measures of stress reactivity during the Trier Social Stress Test. We will measure trajectory of ACTH, lagged association between ACTH and CRT, heart rate variability, and pre-ejection period during the Trier Social Stress Test.<br><br>Other<br>We will test the extent to which 1) maternal depression/anxiety and 2) OXTR genotype modifies the effect of oxytocin vs. placebo on measures of stress reactivity. |
| Test Article(s)<br>*(If Applicable)* | Participants will receive six intranasal sprays of oxytocin or placebo manufactured to mimic oxytocin nasal spray containing all equivalent ingredients except oxytocin. Each insufflation delivers 4 IUs of oxytocin for a total oxytocin dosage of 24 IUs. |
| Study Design | Randomized Parallel Assignment Quadruple masked experiment |
| Subject Population key criteria for Inclusion and Exclusion: | This study will follow-up the existing Mood, Mother and Infant (MMI) prospective longitudinal cohort (R01HD073220), comprised of 222 mother-infant dyads who were recruited between May 2013 and April 2017 and completed the 12-month MMI visit. Inclusion Criteria for Inhaled Oxytocin and HPA Axis Reactivity, a sub study of |

| | |
|---|---|
| | the Psychobiology of Resilience in Mother-Child Pairs follow-up study: 1) Participated in the MMI study 2) Both mother and child willing and able to participate in the follow-up visits at 6 to 8 years postpartum. 3) Not pregnant, verified by urine pregnancy test on day of study visit. |
| Number Of Subjects | Our projected enrollment is 150 participants, recruited from the 222 individuals who enrolled in the MMI study. |
| Study Duration | Each subject's participation in the Inhaled Oxytocin and HPA Axis Reactivity study is expected to last 2 hours. Participation in the Mood, Mother and Infant and Mood, Mother and Child study began with recruitment in the third trimester of pregnancy. Child follow-up has currently extended to about 8 years. |
| Study Phases<br>Screening<br>Study Treatment<br>Follow-Up | (1) Screening: Participants in the Mood, Mother, and Infant study are invited to participate in the Mood, Mother, and Child follow-up study. Those who meet inclusion criteria are scheduled for a mother-only visit for the Inhaled Oxytocin and HPA Axis Reactivity sub study.<br>(2) Intervention: Non-pregnant women will be randomized to either 24 IU of nasal OT or placebo prior to the Trier Social Stress Test.<br>(3) Follow-up: Effects of nasal OT will be measured during the study visit, which will last about 2 hours. |
| Efficacy Evaluations | This is not a treatment study; this is a translational study to test the effect of oxytocin on the HPA axis. |
| Pharmacokinetic Evaluations | *NA* |
| Safety Evaluations | Prior to and during a 90-minute period following intranasal OT or placebo, mothers will be monitored for vital signs (heart rate, blood pressure, ear temperature), vascular resistance and mood. |
| Statistical And Analytic Plan | We will use a general linear model to test the hypothesis that participants who are randomized to receive nasal oxytocin experience lower HPA reactivity, operationalized using CRT area under the curve (AUC) during the TSST, to evaluate this proposed causal pathway |
| Data And Safety Monitoring Plan | Study PI, Dr. Alison Stuebe, will provide medical oversight for the randomized trial of oxytocin vs. placebo. Any adverse events, as well as any significant deterioration in mood, health or wellbeing, will be reported directly to Dr. Stuebe (the PI). All moderate and severe adverse events will be reported within one week to the FDA, whether anticipated or unanticipated. |

# 3. Background and Rationale

## 3.1. Introduction

Mothers who were enrolled in the Mood, Mother and Infant study[1] will be eligible to participate in the 6-year follow-up maternal visit. At the time of this visit, mothers will be randomized to a single 24 IU dose of nasal oxytocin or placebo. Following administration of the study drug, women will participate in the Trier Social Stress Test (TSST), and blood samples will be collected to quantify HPA axis reactivity.

## 3.2. Name and Description of Investigational Product or Intervention

Active drug: Six intranasal sprays of oxytocin. Each insufflation delivers 4 IUs of oxytocin for a total oxytocin dosage of 24 IUs.

Placebo: Six intranasal sprays of placebo manufactured to mimic oxytocin nasal spray containing all equivalent ingredients except oxytocin.

## 3.3. Non-Clinical and Clinical Study Findings

Limited evidence suggests that exogenous OT has mixed effects on women with perinatal depression. Mah et al administered nasal OT and placebo in a within-subject cross-over study among women with PND. Nasal OT increased protective behavior towards the infant, but did not affect sensitive caregiving; moreover, with OT, women were more likely to report a harsh response to infant crying[2-4]. No studies to our knowledge have examined exogenous OT among mothers of older children. In the present proposal, we plan a randomized controlled trial of nasal OT vs. placebo among MMC mothers. This work is innovative, because few studies have tested the effect of nasal oxytocin on HPA axis reactivity, and the overwhelming majority of subjects studied have been male[5,6]: of 147 individuals in the extant literature who received OT or placebo prior to the TSST, only 24 were women. These studies found varying associations between OT and HPA axis activity, with moderation by factors such as concurrent social support[7] emotional regulation abilities[8], substances use[9] and childhood trauma history[22,10]. These differential effects of OT on HPA axis may contribute to perinatal depression or anxiety. We will test the effect of nasal OT vs. placebo among 150 women with detailed, longitudinal data, including patterns of endogenous OT secretion during breastfeeding and maternal experience of trauma in her own childhood.

## 3.4. Relevant Literature and Data

Perinatal depressive symptoms are associated with dysregulation of the HPA axis. Jolley et al[11] measured ACTH and cortisol during an exercise stressor at 6 and 12 weeks postpartum and found expected correlations between ACTH and cortisol among euthymic women (N=13), but not among women with depressive symptoms (N=9). Heinrichs compared lactating mothers who breastfed vs. held their infants 30 minutes before the TSST and found that recent breastfeeding blunted cortisol, but not ACTH[12]. This blunting is thought to be mediated by oxytocin, and a growing literature implicates dysregulation of OT in PND. Findings in our laboratory using extracted samples demonstrate an inverse association between OT during breastfeeding and maternal

symptoms of depression and anxiety at 2 months postpartum[13]. We further found that OT AUC differentially impacted HPA axis activity during the TSST in women with or without mood symptoms[14]. These results implicate OT and HPA axis dysregulation in the psychobiology of perinatal depression; in the proposed study, we will determine whether these differences persist at 6 to 8 years postpartum.

### 3.5. Study Objectives

#### 3.5.1. Primary Objective

The primary objective of this experiment is to test the extent to which oxytocin compared with placebo alters cortisol trajectory during the Trier Social Stress Test.

#### 3.5.2. Secondary Objective

The secondary objective of this experiment is to test the extent to which oxytocin compared with placebo alters other measures of stress reactivity during the Trier Social Stress Test. We will measure trajectory of ACTH, lagged association between ACTH and CRT, heart rate variability, and pre-ejection period during the Trier Social Stress Test.

#### 3.5.3. Other objectives:

We will test the extent to which maternal depression/anxiety modifies the effect of OT on CRT, ACTH, lagged association between ACTH and CRT, heart rate variability, and pre-ejection period during the Trier Social Stress Test.

We will test the extent to which OXRT genotype modifies the effect of OT on CRT, ACTH, lagged association between ACTH and CRT, heart rate variability, and pre-ejection period during the Trier Social Stress Test.

## 4. Investigational Plan

### 4.1. Study Design

This is Randomized Parallel Assignment Quadruple masked experiment to test the effect of inhaled oxytocin vs. placebo on HPA axis reactivity during a standardized social stressor, the Trier social stress test. Mothers who were enrolled in the Mood, Mother and Infant study will be eligible to participate in the 6-year follow-up maternal visit. At the time of this visit, mothers will be randomized to a single 24 IU dose of nasal oxytocin or placebo. Following administration of the study drug, women will participate in the Trier Social Stress Test (TSST), and blood samples will be collected to quantify HPA axis reactivity during a 90-minute period following study drug administration.

### 4.2. Allocation to Treatment Groups and Blinding (if applicable)

Non-pregnant women will be randomized to either 24 IU of nasal OT or placebo. The Investigational Drug Service will use a random number generator to prepare a randomization table. Participants will receive a nasal insufflation bottle of oxytocin intranasal spray or placebo intranasal spray manufactured to mimic oxytocin nasal spray.

## 4.3. Study Duration, Enrollment and Number of Subjects

Each subject's participation in the Inhaled Oxytocin and HPA Axis Reactivity study is expected to last 2 hours. Participation in the Mood, Mother and Infant[1] and Mood, Mother and Child[15] study began with recruitment in the third trimester of pregnancy, with ongoing follow-up through the child's 6 to 8th birthday.

## 4.4. Study Population

This study will follow-up the existing Mood, Mother and Infant (MMI) prospective longitudinal cohort (R01HD073220)[1], comprised of 222 mother-infant dyads who were recruited between May 2013 and April 2017 and completed the 12-month MMI visit. In the MMI study, 222 mothers ages 18-45 and their infants were enrolled. Participants were recruited from community clinics in the third trimester of pregnancy and continued to participate in the study through 12 months postpartum. At the 12-month visit, mothers were invited to continue to be followed via online surveys at 6-month intervals; more than 80% of women who have completed the MMI study to date have continued to participate. Enrolled participants in the MMI study met the following inclusion and exclusion criteria:

### 4.4.1. MMI Inclusion Criteria:

- Singleton pregnancy;
- Intention to breastfeed (due to the centrality of breastfeeding to the oxytocin assessment);
- Intention to remain within 40 miles of the University of North Carolina - Chapel Hill through infant's first birthday;
- Ability to communicate in English.

### 4.4.2. MMI Exclusion Criteria:

- Maternal diagnosis of Axis I disorders other than unipolar depression or anxiety disorders. Women with a history of bipolar disorder were excluded, given their increased risk of postpartum psychosis.
- Active substance abuse at enrollment in the 3rd trimester of pregnancy (Tobacco, alcohol, illicits);
- Major congenital anomaly;
- Chronic medication/medical condition contraindicated for breastfeeding;
- Current use of tricyclic antidepressants, which alter cortisol and heart rate variability.

At enrollment, all participants underwent a Structured Clinical Interview Non-Patient version (SCID-NP).

### 4.5. Inclusion Criteria for Inhaled Oxytocin and HPA Axis Reactivity

This is a trial embedded within the Psychobiology of Resilience in Mother-Child Pairs follow-up study. Subjects were included who:

- Participated in the MMI study
- Both mother and child willing and able to participate in the follow-up visits at 6 to 8 years postpartum.
- Were not pregnant, verified by urine pregnancy test on day of study visit.

## 5. Study Procedures

The Mood, Mother and Infant Study is a prospective observational cohort study that began enrollment in May of 2013. Women were recruited in the 3rd trimester of pregnancy and followed prospectively through 12 months postpartum. Mother-infant pairs who completed the 12-month visit will be invited to participate in the current Psychobiology of Resilience in Maternal-Child Pairs follow-up study. The Inhaled Oxytocin and HPA Axis Reactivity trial described here is a randomized controlled trial embedded within the Psychobiology of Resilience study.

### 5.1. Screening/Baseline Visit procedures

When the study staff member speaks with the participant to ascertain interest in participating in the MMC visits, the staff member will screen the participant for any contraindications to inhaled oxytocin and review the risks and benefits of OT administration. Before the visit, the study staff member will email the participant the consent document. Prior to the visit, a study staff member who has completed DocuSign Part 11 training will speak with the participant by phone and review the full consent document. Following this discussion, the participant will sign an electronic consent. Once the participant has signed the electronic consent, Dr. Stuebe will review and sign the order for IDS.

### 5.2. Intervention/Treatment procedure for study visit

Non-pregnant women will be randomized to either 24 IU of nasal oxytocin (OT) or placebo. The Investigational Drug Service (IDS) will use a random number generator to prepare a randomization table. Participants will be block randomized by risk status at enrollment in the Mood, Mother and Infant (MMI) study, as verified by structured clinical diagnostic interview (No history of depression or anxiety, Past depression or anxiety, current depression or anxiety). Both participants and study personnel will be blinded to allocation group. At the end of the protocol, participants will be asked which condition they believed they were in ('oxytocin,' 'control,' 'not sure') to ascertain success of blinding. Forty minutes after treatment, women will undergo the Trier Social Stress Test (TSST), comprised of a speech task and a math task; the TSST reliably induces large and consistent HPA and cardiovascular responses[16,17]. The TSST is administered as follows: Pre-Task Instructions: (5 minutes) Subjects will be introduced to 3 people (the 'selection committee') and asked to assume the role of a job applicant. Anticipation Period: The subject prepares her speech for 3 minutes in the presence of the committee. Speech: The committee asks the subject to deliver her talk for 5 minutes while being video and audio-recorded. If the subject finishes early, the committee

responds with prepared questions to ensure that she speaks for the entire 5 minutes. These questions are designed to be non-harassing but to create a feeling of lack of predictability/controllability (e.g., "Do you have any enemies?") Serial Subtraction (PASST): The committee will ask the subject to subtract the number 7 from 2000 as quickly and accurately as possible for 5 minutes. For each mistake, the committee says "Stop -- mistake -- start over at 2000." Stress Recovery: The subject sits quietly alone for 20 minutes.

**5.3. Follow- up procedures : NA**

**5.4. Unscheduled visits: NA**

**5.5. Concomitant Medication documentation: NA**

**5.6. Rescue medication administration ( if applicable): NA**

**5.7. Subject Completion/ Withdrawal procedures**

All participants will also be reminded before starting the procedures that they would be free to withdraw at any time without penalty, and may contact our IRB review board or the Principal Investigator (numbers are provided) if she feels that rights regarding privacy or risk to health have been infringed. The right of the participants to withdraw from the study or to refuse any procedure will be made absolutely clear. The original consent form will be kept in a secured location in a locked office, and a copy will be provided to the participant.

**5.8. Screen failure procedures**

Some mothers who are eligible for Inhaled Oxytocin and HPA Axis Reactivity are expected to be pregnant at the time of the follow-up study. Because oxytocin can cause contractions, the Inhaled Oxytocin and HPA Axis Reactivity visit will not be scheduled while the mother is known to be pregnant, and will be deferred until 6 months postpartum. All mothers without a known pregnancy will be screened with a urine pregnancy test on arrival to the Mother Visit; if the pregnancy test is positive, the nasal OT and TSST protocol will not be performed.

**5.9. STUDY EVALUATIONS AND MEASUREMENTS**

Mobile Impedance Cardiographs (MindWare Tech Ltd, Gahanna, OH) will be used to measure cardiac rate, interbeat interval and pre-ejection period (PEP) in mothers and children. MindWare HRV software will be used to derive respiration and to calculate high frequency HRV and respiratory sinus arrhythmia from the IBI series as indices of parasympathetic activity. Respiratory rate will be used in all HRV analyses. MindWare Impedance Cardiography Analysis software will calculate cardiac time intervals (PEP, LVET), stroke volume and cardiac output. PEP will index sympathetic activation.

Blood will be collected at baseline, during the speech and math tasks, and at 10 and 20 minutes of recovery, as HPA-axis responses are reliably found 10-30 minutes following the TSST. Evidence regarding optimal timing of stress testing is conflicting. Visits will be scheduled for 1 pm to increase likelihood of detecting a stress response unopposed by the circadian influence, based on the experience of investigators in our laboratory and published studies of postpartum women. These investigators have found menstrual

cycle phase does not affect TSST results; therefore visits will not be scheduled based on cycle phase.

At baseline, 40 minutes after nasal OT, at the end of the TSST, and at the end of recovery, participants will complete a brief task assessment questionnaire, rating on a scale of 0-10 to what extent then felt 1) Stressed, Worried, Nervous, or Tense; 2) Happy, Relaxed, Comfortable, or Satisfied; 3) Irritated, Annoyed, 'Pissed off,' or Furious; 4) Depressed, Sad, Down, or Unhappy; and 5) Overwhelmed, Unable to Control Things, or Discouraged.

At the end of the protocol, participants will be asked which condition they believed they were in ('oxytocin,' 'control,' 'not sure') to ascertain success of blinding.

## 5.10. Efficacy Evaluation: NA

## 5.11. Pharmacokinetic Evaluation: NA

## 5.12. Safety Evaluations

Prior to and during a 90-minute period following intranasal OT or placebo, mothers will be monitored for vital signs (heart rate, blood pressure, ear temperature), vascular resistance and mood.

## 5.13. STATISTICAL CONSIDERATIONS

### 5.13.1. Primary Endpoint

The primary endpoint of this analysis is CRT trajectory during the TSST.

### 5.13.2. Secondary Endpoint

Our secondary end points comprise other measures of stress reactivity during the TSST, including trajectory of ACTH, lagged association between ACTH and CRT, heart rate variability, and pre-ejection period.

### 5.13.3. Other endpoint

In exploratory analyses, we will test the extent to which 1) maternal depression/anxiety and 2) OXTR genotype.

## 5.14. Statistical Methods

Our primary objective is to test the extent to which nasal oxytocin compared with placebo alters the trajectory of CRT during the TSST. We will use repeated measures analysis to quantify the effects of group (OT vs. placebo) on CRT trajectory during the TSST. We will first establish the shape of unconditional trajectory models for CRT using generalized linear mixed models with a random intercept and slopes to account for individual differences in CRT trajectories. We will test the fit of quadratic, cubic, and exponential effects of time as well as linear spline models. The most parsimonious unconditional trajectory model that fits the data well will be used for hypothesis testing. The intercept of the CRT trajectories will be placed at peak CRT. We will regress growth parameters (intercept and slopes) on treatment arm. A significant effect of treatment arm on the intercept will indicate a difference in peak CRT across groups, and a

significant effect of treatment arm on slopes will indicate differences in the rate of recovery during the TSST.

For secondary endpoints, we will similarly use repeated measures analysis to quantify the effects of group (OT vs. placebo), time, and group x time on each measure's trajectory during the TSST. We will use repeated measures analysis with an interaction term to test the extent to which maternal depression/anxiety and OXTR genotype modifies the effect of oxytocin vs. placebo on measures of stress reactivity.

### 5.15. Sample Size and Power

Our projected sample size is 150 participants whose cortisol is assessed 8 times during the visit. Heinrichs et al (2001) assessed the effect of breastfeeding vs. holding a baby on cortisol levels among postpartum women undergoing the Trier Social Stress Test. Cortisol levels were similar prior to the social stressor, and then diverged, with values that were 1.03, 1.22, and 0.73 standard deviations higher among those who had held their baby compared with those who had breastfed. Because CRT growth parameters are individual-level parameters, power for detecting the effect of individual randomization to treatment arm on CRT trajectories can be simplified into an independent samples t-test. We used SAS 9.4 to estimate the minimum detectable difference for a two-sample t-test based on sample size, with 80% power and an alpha of 0.05. With only 30 participants per group, we are powered to detect a standardized effect of 0.74 standard deviations (Table). This effect is in line with the smallest plausible effect size in Heinrichs et al.

**Table: Minimum detectable difference by N per group**

| N per Group | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean Diff | 0.74 | 0.68 | 0.63 | 0.60 | 0.57 | 0.54 | 0.52 | 0.50 | 0.48 | 0.46 |

### 5.16. Interim Analysis: NA

## 6. Study Intervention (Device, Drug, or Other Intervention)

The study drug and placebo are obtained from Apotheke Roter Ochsen (Schaffhausen, Switzerland). The study drug was shipped to the UNC Investigational Drug Service, which took responsibility for storage, distribution, drug return and destruction.

## 7. Study Intervention Administration (If Applicable)

Randomization: Non-pregnant women will be randomized to either 24 IU of nasal oxytocin (OT) or placebo. The Investigational Drug Service (IDS) will use a random number generator to prepare a randomization table. Participants will be block randomized by risk status at enrollment in the Mood, Mother and Infant (MMI) study, as verified by structured clinical diagnostic interview (No history of depression or anxiety, Past depression or anxiety, current depression or anxiety).
Masking: Participants will receive a nasal insufflation bottle of oxytocin intranasal spray or placebo intranasal spray manufactured to mimic oxytocin nasal spray. A licensed provider will supervise administration of the study drug. Both participants and study personnel will be blinded to allocation group. At the end of the protocol, participants will

be asked which condition they believed they were in ('oxytocin,' 'control,' 'not sure') to ascertain success of blinding.

## 8. Safety Management

Study PI, Dr. Alison Stuebe, will provide medical oversight for the randomized trial of oxytocin vs. placebo. In addition, a licensed provider will administer the study drug and be present for the Mother Visit. If a participant reports an unanticipated problem or adverse event, Dr. Stuebe will be notified immediately, and she will follow-up directly with the participant. If medical intervention is required, treatment allocation will be disclosed to the participant. The study coordinator will monitor AEs at study visits, tabulating events weekly. Any adverse events, as well as any significant deterioration in mood, health or wellbeing, will be reported directly to Dr. Stuebe (the PI). All moderate and severe adverse events will be reported within one week to the FDA, whether anticipated or unanticipated.

## 9. Data Collection and Management

Participant contact information required for scheduling study visits and phone visits will be maintained and shared by team members only as required. This information will be stored in locked filing cabinets in locked rooms and/or transmitted on secure computers only using masked study id's. All other data communicated to research team (biostatisticians, lab personnel) will be masked.

## 10. Recruitment Strategy

This study will follow-up the existing Mood, Mother and Infant (MMI) prospective longitudinal cohort (R01HD073220), comprised of 222 mother-infant dyads who were recruited between May 2013 and April 2017 and completed the 12-month MMI visit. In the MMI study, 222 mothers ages 18-45 and their infants were enrolled. Participants were recruited from community clinics in the third trimester of pregnancy and continued to participate in the study through 12 months postpartum. At the 12-month visit, mothers were invited to continue to be followed via online surveys at 6-month intervals; more than 80% of women who have completed the MMI study continued to participate in follow-up surveys. We will contact MMI participants and invite them to participate in the Mood, Mother, and Child study, of which Inhaled Oxytocin and HPA Axis Reactivity is a component.

## 11. Consent Process

Each participant has previously enrolled in the Mood, Mother, and Infant Study. As with the MMI study, each participant in the Mood, Mother, and Child Study will receive a verbal and written explanation of the purpose, procedure and potential hazards of this protocol. If a participant agrees to participate in the MMC study, they will receive a verbal and written explanation of the purpose, procedures and potential hazards of the protocol for both the mother and child. When the study staff member speaks with the participant to ascertain interest in participating in the MMC visits, the staff member will screen the participant for any contraindications to inhaled oxytocin and review the risks and benefits of OT administration. Before the visit, the study staff member will email the participant the consent document. Prior to the study visit, a study staff member who has completed DocuSign Part 11 training will speak with the participant by phone and

review the full consent document. Following this discussion, the participant will sign an electronic consent.

## 12. Plans for Publication

We plan to submit our findings to a peer-reviewed publication, such as *Biological Psychiatry*

## 13. References


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## 14. Appendix

Study visit schematic: Inhaled Oxytocin and HPA Axis Reactivity