Intranasal Oxytocin:
A Neuropharmacological Intervention for Maternal Neglect?

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Study Protocol

The purpose of this study is to explore how oxytocin may modify early maternal brain and behavioral responses to infant cues. This study will examine a potential pharmacological intervention for maternal neglect which targets core neurobiological deficits. This may eventually be used to supplement and augment other psychosocial and behavioral interventions.

Initial enrollment: Recruitment occurred during the postpartum period using EPIC medical records and advertising via newspaper, internet, public postings, etc. Once eligibility was confirmed, subjects were scheduled for a study visit where they provided informed consent before participating in any study activities.

Visit 1: 4 Months Postpartum – Adult Attachment Interview (AAI). During this visit, each enrolled woman participated in a modified version of the Adult Attachment Interview (AAI), a semi-structured 1½-2 hour-long interview involving specified questions and follow-up inquiries relating to childhood relationships with attachment figures. The modified version was chosen because of its theoretical links with patterns of information processing in the brain. Each digitally recorded interview was transcribed (with personally identifying details altered to preserve anonymity), and coded blindly to classify each woman's adult attachment pattern. We also collected sociodemographic and breastfeeding data, and screened for postpartum depression using the Edinburgh Postnatal Depression Scale (EPDS).

Visits 2A&B and 3A&B: Intranasal Oxytocin vs. Placebo Administration. In this double-blinded cross-over placebo-controlled trial of intranasal oxytocin, maternal brain and behavioral responses to infant cues were assessed within and between subjects. Each participant was administered both the active (oxytocin) and inactive (placebo) nasal spray just prior to separate behavioral or fMRI scanning sessions, with the order of administration balanced across subjects. All involved were blinded to the identity of the oxytocin or placebo sprays. Before each visit, the mother was asked to abstain from tobacco, food and drink (except water) for at least 2 hours prior to the visit. We attempted to schedule visits during the latter half of the mother's menstrual cycle, to minimize potential confounding from fluctuations in estrogen levels. Mothers without regular menstrual cycles were attempted to be scheduled one month apart. Mothers who were still breastfeeding were asked to feed their infant at least one hour prior to the visit.

For the four visits involving intranasal drug administration (two behavioral testing visits and two fMRI scanning visits), the following protocol applied: participants were given a urinary pregnancy test prior to the start of each visit. If a mother tested positive, she was excluded from the study due to theoretical concern about possible effects of oxytocin on uterine contraction. Just prior to drug administration, the mother completed the Positive and Negative Affect Scale (PANAS) to rate her current emotions, for comparison to post-spray. The mother then self-administered a dose of either oxytocin (3 puffs per nostril [4 IU per puff] = 24 IU total) or a placebo spray containing only the inactive ingredients of the oxytocin solution. Both experimenters and subjects were blind to the treatment they are receiving. A stopwatch was started at the moment the subjects began the intranasal administration, so that the behavior assessment or fMRI scanning could begin approximately 50 minutes later. Forty minutes after drug administration, the PANAS was repeated.

Visits 2A&B: 5-6 Months Postpartum – Behavioral Response. On arrival, the mother and infant were escorted into a behavioral observation room at the Clinical Nutrition Research Center, where the infant was introduced to some developmentally appropriate toys. Questionnaires completed at home were reviewed (Infant Behavior Questionnaire [IBQ], Parenting Stress Index [PSI] and demographics and breastfeeding questionnaire), and if incomplete, were completed at this time. Each mother then self-administered the nasal spray, which had been previously randomized by the investigational pharmacy to be either oxytocin or placebo. During the waiting period, the Bayley Scales of Infant Development screener was completed with the infant, to exclude developmental delays in areas of fine motor, gross motor, language or social development. This was performed by a research associate who has been trained in the use of this standardized tool. Fifty minutes after the nasal spray was administered, each mother rejoined her infant to participate in a videotaped "free play" interaction on the floor for 3 minutes. The recording was later coded for dyadic interactions using a

14-point sensitivity scale in the CARE-Index. A modified still-face procedure was then conducted. The mother was then taken to another area of the building, while the infant was videotaped to obtain face images used during the subsequent scanning visits. During the brief separation period, the infant was secured in a mounted car seat, and videotaped while using age appropriate toys to elicit smiling (and neutral) facial expressions. This did not usually last no more than 10 minutes. The mother did not observe the videotaping to ensure that each infant face image was novel when presented during the subsequent visits. Crying faces were also elicited by briefly leaving the infant alone in the room, if necessary, secured in the car seat, while the video recorder was running. The infant was observed at all times from behind a one-way mirror, and only left to cry for around 35-40 seconds. Infant cry was also recorded at that time. Infant face images with various degrees of affect–happy, neutral and sad–were then extracted from the digital video recording, for use in the subsequent fMRI procedure (Visit 3). The images were standardized for size, orientation and background using Adobe Photoshop. All of the face images were rated on degree of affect using a 5-point adaptation of the Self-Assessment Manikin (SAM), categorizing each image into one of five affect groups: very happy, happy, neutral, sad or very sad. These faces were matched on age, sex, ethnicity and degree of affect with a large database of "unknown" infant faces, collected during a previous study. Approximately one month later the basic procedure was repeated for each subject, except that those who received intranasal oxytocin were given placebo, and vice versa.

Visit 3A&B: 8-9 Months Postpartum – Brain Response. Around three months after the first behavioral response session, each mother attended two scanning sessions at the Human Neuroimaging Laboratory, Baylor College of Medicine. Intranasal oxytocin or placebo was again administered as described above. Infant face images from Visit 2A were used in the Visit 3A scanning session, and Visit 2B with 3B, to ensure that the repeat scanning session used comparable but unique face images. The images from both sessions were matched on degree of facial affect.

Prior to intranasal drug administration on the first scanning visit, the mother completed the Positive and Negative Affect States (PANAS) questionnaire. During the waiting period, the mother completed questionnaires. Fifty minutes after the administration of the nasal spray, the mother participated in the functional MRI scanning session, passively viewing a series of unique infant face images and hearing cries of her own infant and of an unknown infant. Each parent was informed that their "brain activity will be monitored using functional MRI while you are shown pictures of your own baby". Using an event-related fMRI design, randomly presented images were viewed for 2 seconds, with a random inter-stimulus interval of 2, 4 or 6 seconds. The 60 images were equally divided into 3 affect groups – happy, neutral or sad, with the intensity of happy and sad affect balanced between the "own" and "unknown" faces. The order of the images from each of the 6 groups (own-happy [OH], own-sad [OS], unknown-happy [UH], unknown-sad [US]) were pseudo-randomized within the run, but not between subjects. All imaging was performed using a 3 Tesla Siemens MRI scanner. Visual images were generated using a computer controlled LCD projector, and presented to the mother via an overhead mirror display. Regional brain activation was assessed by measuring changes in blood-oxygen-level-dependent functional MRI signal (BOLD-fMRI). Subjects participated in a single whole-brain functional run of around 185 scans each. High-resolution T1-weighted structural images were acquired. After the scanning session, each mother was asked to rate each of the infant face images on how they thought the infant was feeling, as well as their own feelings of pleasure or arousal, using an adaptation of the Self-Assessment Manikin.

Statistical Analysis Plan

fMRI Data Processing and Analysis:
Imaging data for each subject was preprocessed and analyzed using the following steps:

Head motion correction was performed by spatial alignment of all brain volumes to the first volume by rigid body transformations. Slice scan time correction was performed using sinc interpolation, based on the repetition time (TR) and order of slice scanning (ascending interleaved). Following linear trend removal, low frequency nonlinear drifts of 3 or less cycles was removed using a temporal high pass filter. Spatial smoothing will not performed. The anatomical dataset underwent iso-voxel scaling to 1 x 1 x 1 mm resolution and was transformed into sagittal orientation. It was then transformed into AC/PC and MNI standard space. Functional runs for each subject were then coregistered with the anatomical 3-dimensional dataset, iso-voxel transformed, and then transformed into standard ACPC and MNI coordinate space, resulting in normalized 4-dimensional volume-time course data.

For each functional run of the event-related data, a protocol file was created, representing the timing of each stimulus event. The four infant face stimulus types in the design matrix included Own-Happy (OH), Own-Sad (OS), Unknown-Happy (UH), and Unknown-Sad (US). Each predictor was convolved with a double-gamma hemodynamic response function (Friston, Fletcher, Josephs et al. 1998). Using the General Linear Model (GLM), group effects was evaluated using a random effects analysis, with a percent time course transformation applied to each subject separately. In the random effects analysis, statistical maps was created for each individual subject before being subjected to a second level of statistical analysis, allowing generalization to the sample population of first-time mothers. Main effects and possible interaction effects of infant “identity” and “affect” were explored using 2-factor repeated measure ANOVAs. Group t-maps (2-tailed) were also generated after specifying a particular contrast in stimulus types (e.g. OH>UH), and were visualized on an averaged 3D anatomical image, which was created from all of the individual subject images. Corrections were made for multiple comparisons.

Next, activation patterns were compared between oxytocin treatment groups and attachment groups using a mixed-model repeated measures analysis of variance (ANOVA), with fixed effects analyses for 1) “infant face category” as a repeated measure within-subjects variable, 2) “oxytocin treatment group” as a within- and between-subjects variable, and 3) “attachment group” as a between-subjects variable. Subjects were treated as a random effect within each group and were used to test for face affect x treatment group x attachment group interaction effects on brain function. Mean beta weights was calculated and compared between the two treatment and attachment groups, in *a priori* regions of interest (VTA, ventral striatum, medial prefrontal cortex), using the t-test and the Mann-Whitney U-test for non-parametric data.

Brain/Behavior Analyses:
The relationship between brain response to the visual infant stimuli described above and interview derived measures was examined within the general linear model framework. Measures examined in this manner included ratings of the stimuli obtained immediately after scanning, adult attachment classifications, and ratings of the parent-infant interaction session (“maternal sensitivity” score). Initially, percent signal change data was extracted from regions of interest, including mesocorticolimbic pathway (VTA, ventral striatum, medial prefrontal cortex), nigrostriatal pathways (substantia nigra, dorsal striatum, dorsolateral PFC), as well as the amygdala, hippocampus, anterior insult and hypothalamus, and was entered into regression models to determine whether there was a linear relationship between brain activation in response to the stimuli and behavioral scores. We focused on these regions given data from animal and human studies reviewed that implicate them in attachment and/or fear neurocircuitry (Leibenluft, Goblin, Harrison et al. 2004a; Lorberbaum, Newman, Horwitz et al. 2002a; Nitschke, Nelson, Rusch et al. 2004; Seifritz, Esposito, Neuhoff et al. 2003; Strathearn et al., 2008; Swanson, 2000). Regression models also tested for group differences in the relationship between brain activation and behavior. Regions of interest were defined using high resolution 3D

images normalized to standardized stereotactic space (Collins, Zijdenbos, Kollokian et al. 1998). Measures of the striatum included caudate and putamen, outlined on each slice in which these structures were visible. Regression analyses were further employed to identify areas that show a continuous linear dose-response function.

Analyses by Hypotheses:
Since the study is a crossover design, the model initially contains terms testing the order of treatment (oxytocin/placebo) and the interaction between order and treatment to ensure detection of any unexpected carry-over effect from treatment. Since a sufficient washout period was provided and different pictures were shown on the two occasions, we expected these terms to be non-significant, in which case they were removed from the model.

Hypotheses 1-3 was tested using a generalized estimating equation model (SPSS version 17.0), which accounts for repeated measures in the crossover design as well as different types of dependent variables. The basic model contains terms for testing each of these hypotheses including treatment, attachment and treatment x attachment. Interpretation began with the treatment x attachment interaction. If this interaction was not significant, it was omitted from the model. Attachment was initially be treated as a 4-category variable (Types A, B, C and A/C), with the main comparisons being between Types A and B, and between Types C and B. However, we also combined categories to examine secure (Type B) vs. insecure (Types A, C, A/C) attachment. In addition, for the brain-related outcomes, we looked at infant affect as an additional within-subject factor, and examined possible 3-way interactions – treatment x attachment x affect. If this interaction was significant the data was split on attachment group and reanalyzed to assess the treatment x affect interaction within attachment groups. If this interaction was not significant, it was removed. A positive finding for this interaction would indicate splitting on attachment group and reanalysis is in order to assess treatment effect within these groups. Main effects of treatment, attachment, and affect remained in the model. Hypothesis 2(ii) involving counts showed a Poisson distribution outcome while the others showed a normal distribution.

We will also included in the model stratification variables such as maternal age, ethnicity, breastfeeding status and income. Finally, we explored differences between attachment groups on measures of psychopathology, such as depression (from BDI), anxiety (from SSTAI) and parenting experience (from the PSI). If differences emerged between attachment groups in these measures, we considered including them as covariates in analyses.

Hypothesis 4 assessed the correlation between maternal brain reward activation (ventral striatum and medial prefrontal cortex) and three indirect measures of maternal neglect using a linear model.