Title: Understanding Oxytocin's Neural and Behavioral Effects in Adolescents Diagnosed With Autism

Protocol ID: 16082021IG

Upload Date: October 2015

Research plan

The main object of this experiment is to examine on the influence of oxytocin administration on neural activity during a variety of social tasks.

Forty adolescents (ages 12-18) with an existing clinical diagnosis of high-functioning ASD will be offered to take part in an innovative experiment that examines the influence of oxytocin administration on neural mechanisms, social thinking, and social behaviors.

Participants will visit The Gonda Brain Research Center at Bar Ilan University two times (**first and second visit**:1-2 weeks apart) to take part in a double-blind, crossover, and randomized controlled trial. Moreover, we will also recruit 40 typically developing adolescents (ages 11-18) as a control group. The control participants will arrive at The Gonda Brain Research Center at Bar Ilan University only once.

Screening and First Visit. Prior to the first visit, extensive phone screening will be conducted regarding chronic medical problems, cardiovascular risk factors, CNS disease, other mental illnesses, prohibited medications, and menstrual status. Also, participants with retardation, color blindness, impaired vision, impaired hearing, history of significant head injury or neurological illness, current diagnosis of substance dependence, metallic implants will be excluded from the study. The following measures in the domains of language, social communication, repetitive behaviors, sensory hypersensitivity, and psychiatric symptoms will be assessed via parental report to provide more extensive phenotype information: Clinical Evaluation of Language Fundamentals (Semel, Wiig, & Secord, 2003); the Social Responsiveness Scale (SRS: Constantino & Todd, 2003), Test Of Pragmatic Language (TOPL: Phelps-Terasaki & Phelps-Gunn, 2007) and Sensory Profile (Dunn, 1999). We plan to include participants with IQ's$\geq$ 80, with language acquisition sufficient to participate meaningfully in the tasks and to comprehend the nature of their participation.

First study visit procedure. Following characterization, adolescents and their parent/s will visit The Gonda Brain Center twice and will be randomly assigned to receive a single dose of intranasal Oxytocin (detail regarding dosages appear in Gordon et al., 2013) at one of the visits and a placebo nasal spray at the other. This within-subject design will allow each child to have one active treatment with Oxytocin. Prior to the beginning of each study visit, participants' parents will sign an informed consent. Children will also be given an informed assent that explains the study in age-appropriate terms. This study's protocol will be

approved by the University's ethical board as well as by a Helsinki Ethical Committee. Participants will be told that we are studying the effects of Oxytocin on brain function and social behavior.

The participants and researchers will be blind to the treatment condition. Each puff per nostril contains 3 international units [IU], with an identical placebo containing all ingredients except the active Oxytocin. The older age group of participants (aged 16–18) will receive a dose of 24 IU, which has been selected for most adult oxytocin intranasal spray research studies and has been done in children ages 16-19 as well. Those 15 and younger will receive a 75% dose of 18 IU as was demonstrated in children aged 12-15 in the only study conducted in children to date (Guastella et al., 2010).

After administration, participants will fill out the following questionnaires:

- **Autonomic Dysfunction Scale** (ADS: Ming et al., 2010)
- **Early Adolescent Temperament Questionnaire** (EATQ: Capaldi & Rothbart, 1992). The current questionnaire has been designed to specifically tap experiences common to adolescents and is available in self-and parent-report formats. It assesses temperament and self-regulation via adaptation of scales used in studies of children and adults. The revised questionnaire (EATQ-R: Ellis & Rothbart, 1999) also contains two behavioral scales to allow examination of the relationship of temperament to social-emotional functioning. The EATQ-R has been designed to assess temperament in adolescents aged 9-15. The Early Adolescent Temperament Questionnaire-Revised assesses temperament and behavioral scales.

All of the above questionnaires and also the questionnaires from the clinical assessment represent trait measures, which are not expected to be influenced significantly by Oxytocin administration. We will extract trait measures that will be used as mediating variables or controlled for when analyzing Oxytocin's effect on behavior or brain responses.

Since the half-life of Oxytocin is short, 15 minutes after intranasal administration, participants will be taken to the MEG lab for scanning. The following phase of the study will require approximately one hour (including preparation for the scan) during MEG scanning, in which participants will be engaged by paradigms pertaining to social functioning. For example, view faces and objects, view hands enacting social or non-social actions, and/or play socially interactive games.

Following this session, participants will be debriefed, asked to report any side effects and how they feel, asked if they think they received Oxytocin or not. Finally, participants may need to finish answering some questionnaires or behavioral tasks. If the patient/parent wishes for the patient to participate in the optional eye scanning while out of the MEG, then this will also be performed.

Second study visit procedure. During the second visit, the procedures will be repeated except that if the patient received Oxytocin, they would receive a placebo or vice versa. The interventions will be counterbalanced. Same as in the first visit, after the administration, participants will be taken to the MEG lab for scanning. The screening will be repeated in case there have been any significant changes to the patient's medical status. The same questionnaires, behavioral tasks, and neuropsychiatric tests will be repeated.

Control participants

Only a handful of studies examined the neural basis of adolescents' social understanding in the MEG. Due to this gap in the literature, we will recruit control aged match subjects. Control participants will arrive only for one session in The Gonda Brain Center in Bar Ilan University. Similar to the patient group, during this session, participants will undergo a social interaction task with their parent and afterward will be taken to the MEG lab for scanning. Differently from the patient group, control subjects **won't receive Oxytocin or placebo.** The following phase of the study will require approximately one hour (including preparation for the scan) during MEG scanning, in which participants will be engaged by paradigms pertaining to social functioning as mentioned above.

At the end of each visit, in both the experiments, participants will be offered food (granola bar/fruit) and water, thanked and paid. Overall, the study will take approximately 15 hours [First experiment (clinical assessment and session T1-T3): 7.5 hours, without the intervention's meeting; Second experiment (visits V1-V3): 6.5 hours]. Participants will be paid according to their participation in the different parts of the study:

- V1- 150 NIS (2.5 hr)
- V2- 350 NIS (2.5 hr)

Overall, participants who take part in both parts of the experiments will be paid a total of 500 NIS for their participation, and a meeting with a member of the clinical team that performed the assessment will be offered to them in order to discuss the assessment process free of charge. Control participants will be paid 150 NIS (for approximately 2 hours). In addition, participants who, for any reason will ask to terminate the experiment, participants who will manifest distress or participants who will not cooperate with the study's procedure will be excused from participation.

Possible inconvenience:

The experimental tasks require the participants to concentrate for a long period of time, and difficulty may arise. Therefore, participants will be able to take as many breaks as long as they need. In addition, brain imaging requires a preparation phase in which participants should lay still without moving their heads while the researcher digitizes their head shape. Also, when performing the various experimental tasks, participants will be asked to lay still, not to move, and to reduce to minimum eye blinks. During the brain scans, a professional researcher will be in touch with the participants through a set of cameras and microphones. If necessary, there is an option for the researcher to stay in the room during the brain scans. As mentioned before, in response to every sign of distress, the experiment will be stopped.

Oxytocin's identification

Name: Oxytocin (OT)

Trade Name: Pitocin

Description: Peripherally, Oxytocin causes the contraction of uterine smooth muscle and of the myoepithelial cells within the mammary gland. In the central nervous system, Oxytocin is a modulator of social behaviors, facial recognition, fear, anxiety, and trust. oxytocin is metabolized by hepatic oxytocinases and disposed by biliary and renal excretions.

Molecular Formula: C43H66N12O12S2

Chemical Structure: Cysteine - tyrosine - isoleucine - glutamine - asparagine - cysteine - proline - leucine - glycine (CYIQNCPLG)

Pharmacologic Class: Neuropeptide

Formulation: Each 1.5 mL vial contains 60 units/mL (.1056 mg/mL).

Route of Administration: Intranasal

Storage and Stability: Refrigerator (2°-8°C)

prescriptions information: Oxytocin will be delivered from "Maayan Haim" pharmacy. If needed- prescriptions will be signed by Dr. Irit Mor (MD).

The proposed study will use targeted manipulations with intranasal oxytocin administrations in conjunction with neurocognitive and neuroimaging paradigms to assess how Oxytocin may impact social brain functioning in adolescents with ASD.

Dr. Mor will also serve as a Medical Monitor and Safety Officer to assist the PI and research team with the monitoring of any side effects of Oxytocin administration. Please explain who this Medical Monitor will be with his/her credentials for functioning in this capacity.

Oxytocin is a nonapeptide hormone produced in the supraoptic nucleus and paraventricular nucleus of the hypothalamus by magnocellular neurosecretory cells and released into the blood from the neurohypophysis. Oxytocin is a key regulating hormone of innate human emotions and behaviors including aggression, attachment, fear, and social cognition. In recent studies, Oxytocin has been shown to act on the limbic system, most notably the amygdala.

Clinical studies of the effects of intranasal oxytocin administration have been conducted successfully worldwide in the last six years. In the US, intranasal administration of Oxytocin is used in clinical studies, to our knowledge, Oxytocin was never found to have been withdrawn from investigation or marketing in any country due to any safety or efficacy related reasons.
The application of the substance is considered safe for administration to human subjects under the conditions of the study since intranasal Oxytocin at this dosage is known from decades of use to be safe and well tolerated, as evinced by their routine usage in protected patient populations, such as nursing mothers (The nasal form of Oxytocin – Syntocinon, manufactured by Novartis Pharma – is listed in the FDA website as being used routinely from the 1962 until 1995). The only contraindications are known hypersensitivity reactions to Oxytocin. The dosage proposed in this study conforms to clinical doses and have been used previously in normal volunteers for cognitive and imaging studies by others and ourselves (Born et al., 2002; Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005; Leake, Weitzman, & Fisher, 1980). Any side effects for this application form are rare, and no serious side effects have been recorded for intranasal application of up to 60 IU oxytocin (Epperson, McDougle, & Price, 1996). Nausea and

vomiting and transient cardiac arrhythmias have been described rarely. In addition, anaphylactic reactions are described in the datasheet, although the medical literature only contains case reports related to the intravenous application of Oxytocin. Furthermore, a previous IND exemption (#75429) states, "Intranasal oxytocin has previously been approved for marketing in the United States from March 20, 1962, until its withdrawal from the market on August 7, 1997." The Federal Register notice issued on that date [Federal Register, Vol. 62, No. 152, Docket No. 97N-0326, pp. 42575 – 42577] clearly states that this withdrawal was at the request of the manufacturer because the drug was no longer being marketed. No safety reasons were cited in connection with the withdrawal. The intranasal form of Oxytocin remains on the market outside of the United States.

As there may be many confounders for the intranasal administration of medications, i.e. swallowing the medication or depositing it in the nasopharynx will decrease absorption, Dr. Ilanit Gordon will be in charge of the nasal administration procedure. Dr. Gordon has been extensively involved in Oxytocin intranasal administration in the past. Prior to participants' self-administration, Dr. Gordon (or a research team member she has trained) will explain and demonstrate to them exactly how they should use the nasal spray in order to make sure administration is optimal. Additionally, nasal spray bottles will be fitted with the intranasal delivery system in order to optimize the delivery of Oxytocin.

Use of Placebo: In a double-blind, crossover, and randomized controlled trial, participants will be assigned to receive a single dose of Oxytocin and a placebo nasal spray 1-2 weeks apart. Nasal sprays will be prepared by the "Shor Tabachnik" (each puff per nostril will contain three international units [IU]), with an identical placebo containing all ingredients except the active Oxytocin. As all participants will receive both Oxytocin and Placebo, we will not be withholding drugs from any participants. Placebo and Oxytocin spray containers will look identical. A designated research assistant in the lab will be the only person who will know which sprays contains active Oxytocin and which sprays do not. They will receive this information from the pharmacy preparing the sprays. This individual will be assigned to give the sprays to the experimenter, who will be blind to the contents of the spray. We will ask participants at the end of each trial if they believe they received Oxytocin or placebo in order to control for their assumptions regarding treatment.

Although there is a placebo administration in this protocol, no standard of care medications for ASD will be withheld from participants. The use of Oxytocin and the placebo in this study are to evaluate the administration of Oxytocin on brain function. Oxytocin is not being given for a therapeutic purpose.

Data Analysis

MEG analysis

MEG time series data will be preprocessed and analyzed using Matlab® R2011b and FieldTrip toolbox for MEG analysis (Open Source Software for Advanced Analysis of MEG, Oostenveld et al., 2011). Data are cleaned for line frequency and 24 Hz building vibration artifacts (Tal and Abeles, 2013). The data from task conditions are then segmented into non-overlapping 2-s epochs. Each epoch is visually examined for muscle and jump artifacts. Contaminated epochs will be discarded. To ensure the removal of all heartbeat, eye, and muscle artifacts, an independent component analysis (ICA) will be performed on the data. Segmented data will be down-sampled to 339 Hz to speed up data decomposition. The data will then be decomposed into a set of independent components ordered by the degree of their explained variance. Components indicating heartbeats or eye movements are determined and discarded. The remaining components are then used to reconstruct the pre-down-sampled data.

Sensor-level analyses will be conducted in both time-locked and phase-locked manners using a derived data approach.

1. Event-related magnetic fields (ERFs) will be collected by averaging trials from different conditions. In order to identify the timing of ERFs, we will compute a global measure of activity, root means square (RMS) of all 248 sensors. This stimulus-locked analysis will be used to explore the temporal dynamics of a stimulus-related component in 'reading the mind in the eyes' task such as M-170. In this task, two scores will be produced: peak latency and amplitude of the RMS component for both conditions (eyes VS. vehicles), in both OT and placebo.

2. Analysis of low and high frequencies oscillations - time-frequency representations (TFRs) of the spectral power will be executed in order to evaluate the differences in frequencies' power between OT and placebo in both tasks. Segmented 1-s epochs will be multiplied by a Hanning taper and subjected to a Fast Fourier Transformation (FFT) for the frequencies ranging from 0.5 to 100 Hz. This will result in a power spectrum with a frequency resolution of 0.5 Hz for each epoch. The power spectra will then be averaged across the epochs of each condition, thus obtaining the mean power for each condition and participant.

The next step involves calculating, for each frequency of each sensor of each participant, a power percent-in-signal-change (PSC) metric, for estimating power differences in OT versus placebo for each task condition. Each participant's PSC values for each contrast the is then collapsed across all sensors and averaged across the delta (0.5–3.5 Hz), theta (4–7.5 Hz), alpha (8–12.5 Hz), beta (13–25 Hz), gamma (25.5–59.5 Hz), high-gamma (60–80 Hz), and very-high-gamma (80.5–100 Hz) frequency bands. To reduce dimensionality prior to localization, 1-sample t-tests will be performed for each frequency band and for each comparison against the null hypothesis that the PSC measures came from a continuous, normal distribution with a zero mean. Results will then be Bonferroni-corrected. Finally, 2D scalp topographies of the mean PSC in the significant frequency bands and comparisons will be created and compared between conditions.

Source-space projection analyses will involve localization for the frequency bands, which evidenced significant PSC in the sensor-level data. Sources will be estimated using Synthetic Aperture Magnetometry (SAM: Robinson & Vrba, 1999). SAM is an adaptive nonlinear minimum-variance beamformer algorithm. It calculates the signal covariance from the MEG sensor data and uses it in conjunction with a forward solution for the dipoles at each 3D brain voxel to construct optimum spatial filters. The spatial filtering suppresses the interference of unwanted signals from other locations. For source estimation, data will be band filtered (using

the SAM default IIR filter) for each participant and condition in the frequency bands specified through the sensor-level analysis. Covariance matrices and SAM weights will be computed for each five cubic-mm voxels using the data from the task conditions in each signal change calculation for each frequency-band-filtered time-series data. For each voxel, the data will be multiplied by the weights, creating a "virtual sensor" time-series, which is transformed via FFT to the frequency domain to derive power values. Finally, PSC values will be computed for each comparison, participant, and voxel.ROI analyses will focus on specific brain areas that were associated with ASD in our prior studies of the biological motion and Reading the Mind in the Eyes paradigms and specific brain regions that are known to be related to OT functioning (insula, amygdala, cingulate, NAcc). In these regions, experimental condition differences and treatment effects (OT vs. placebo), as well as condition treatment interactions, will be examined. In general, GLM analyses and random-effects contrasts will be employed to test our focused hypotheses.

Functional connectivity analysis - we will examine the functional connectivity between the FFA and the specific brain regions mentioned above, using phase-amplitude coupling (PAC). This method examines if

the phase of the lower frequency band (alpha) modulates the amplitude of the higher frequency band (beta or gamma) across different brain regions. Next, we will compare the strength of the connectivity to behavioral measures and the severity of the symptoms.