Oxytocin Gene Polymorphisms Influence Human Dopaminergic Function in a Sex Dependent Manner

Supplemental Information

Supplemental Methods

Participants

We genotyped and analyzed data from 55 healthy volunteers who participated in a pain-stress study conducted at the University of Michigan. Recruitment criteria have been previously detailed (1) but briefly, participants were recruited via advertisement then screened using the Structured Clinical Interview for DSM-IV (non-patient version, SCID-NP) to ensure they had no current or past history of medical, neurological, or psychiatric illness, including substance abuse or dependence. Subjects were not taking any psychotropic medications or hormone treatments, including birth control for at least six months and were non-smokers.

Genotyping

Assessment of Population Stratification Using Ancestry Informative Markers

The samples were genotyped for 186 ancestry markers (AIMs) (2). The same AIMs were genotyped in 1051 individuals from the 51 worldwide populations represented in the HGDP-CEPH Human Genome Diversity Cell Line Panel (http://www.cephb.fr/HGDP-CEPH-Panel). Structure 2.2 (http://pritch.bsd.uchicago.edu/software.html) was run simultaneously using the AIMs genotypes from our sample and the 51 CEPH populations to identify population substructure and compute individual ethnic factor scores. This ancestry assessment identifies seven ethnic factors (2). In our study sample the predominant mean (SD) [median] ethnic factor scores were: European: 0.66 (0.40) [0.92]; African: 0.14 (0.30) [0.00]; Asian: 0.10 (0.23) [0.02];

Mid Eastern: 0.06 (0.15) [0.02]; and Far Eastern: 0.02 (0.08) [0.00]. Since the sample was predominantly Caucasian, the European ethnic factor score was included as a covariate in all analyses to account for the variability in allele frequencies across ethnicities. This factor score was highly skewed; therefore a binary median-split score was derived.

Behavioral Questionnaires

Measures of well-being were obtained from participants at the time of enrollment. These assessments covered three main domains: emotional well-being (EWB), psychological well-being (PWB) and social well-being (SWB). The EWB is a brief measure that reflects an individual's general feelings of positive affect which is a reflection of both contextual and personality factors while the PWB and SWB scales relate to the degree to which individuals feel they thrive in their personal and social lives (3-5).

Positron Emission Tomography (PET) Studies

PET Acquisition and Preprocessing

PET scanning protocols were identical to those used in previous publications (1). Briefly, images were reconstructed using iterative algorithms into a 128 x 128 pixel matrix in a 28.8 cm diameter field of view. Attenuation correction was performed through a 6 min transmission scan (^{68}Ge source) obtained before the PET study. Small head motions during emission scans were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered to each other with the same software (6). Time points were then decay-corrected during reconstruction of the PET data.

The total activity of [^{11}C] raclopride administered to each subject was in tracer quantities (i.e. subpharmacological doses). Fifty percent of the [^{11}C] raclopride dose was administered as a bolus with the remainder delivered as a continuous infusion by a computer-controlled automated pump to more rapidly achieve steady-state tracer levels. Under these conditions, equilibrium conditions across kinetic compartments are achieved approximately 35 min after tracer administration (7).

Twenty-eight image frames were acquired over 90 minutes with an increasing duration (30 seconds up to 10 minutes) and were coregistered to each other (6). Dynamic image data for each of the receptor scans were transformed, on a voxel-by-voxel basis, into two sets of images: (a) a tracer transport measure (K_1 ratio), and (b) a receptor-related measure, binding potential at equilibrium (BP_{ND}), the latter using data obtained from 35-45 min (baseline) or 60–80 min (pain stress) after tracer administration. This measure was obtained using the ratio of brain activity to activity in the cerebellum *minus* 1 (7, 8). Activation of dopamine (DA) D2/D3 neurotransmission is detected as a reduction in BP_{ND} (i.e. lower levels of DA D2/D3 receptor availability during the challenge). This was calculated as the difference between the baseline state and the activated, pain stress state (i.e. baseline-stress).

PET-MRI Coregistration

The T1-weighted magnetic resonance (MR) and PET images of each subject were coregistered to each other using a mutual information algorithm as previously described (9). For this purpose, K_1 images were first aligned to the MR, and the transformation matrix applied to the coregistered BP_{ND} scans of the same series. The MR scans were then anatomically standardized to stereotactic coordinates (Montreal Neurologic Institute, MNI) by linear and non-linear warping, and the resulting transformation matrix applied to K_1 and BP_{ND} images (9, 10).

The accuracy of coregistration and warping algorithms were confirmed for each subject individually by comparing the transformed MR and PET images to each other and the MNI atlas template.

Supplemental Results

Baseline DA D2/D3 Receptor Availability

As we observed a significant gene by sex interaction for rs4813625 in stress-induced DA D2/D3 activation, we sought to determine whether genetic variation at that single nucleotide polymorphism was also associated with differences in DA D2/D3 receptor availability at baseline. For this purpose, we performed an additional analysis on the baseline data. In SPM, an analysis of covariance model was used to examine baseline DA D2/D3 BP_{ND} data using sex and genotype as between-subject factors with AIMs score included as a covariate. SPM analyses revealed significant regional effects for rs4813625 in a large area encompassing the left putamen and left ventral striatum (*x,y,z* coordinates, -13, 8, -3; $F = 15.93$, cluster size = 10,434 mm^3; $p_{FWE} = 0.005$). Post-hoc analyses in SPSS, however, revealed no significant effects of gene [$F(1,45) = 1.37$, $p = 0.247$] or gene by sex interaction [$F(1,45) = 3.46$, $p = 0.069$] though a significant effect of sex [$F(1,45) = 13.74$, $p = 0.001$] was noted where females exhibited greater D2/3 receptor availability (mean BP_{ND} ± SD, females, 2.22 ± 0.25; males, 1.94 ± 0.22). The lack of a gene effect at baseline suggests the association between rs4813625 genotype and stress-induced DA D2/D3 activation is related to variations in presynaptic function rather than differences in overall receptor availability.

Supplemental References

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