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Oxytocin Receptor Gene Polymorphisms, Attachment, and PTSD: Results from the National Health and Resilience in Veterans Study

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Abstract

The human oxytocin system is implicated in social behavior and stress recovery. Polymorphisms in the oxytocin receptor gene (*OXTR*) may interact with attachment style to predict stress-related psychopathology like posttraumatic stress disorder (PTSD). The objective of this study was to examine independent and interactive effects of the *OXTR* single nucleotide polymorphism (SNP) rs53576, which has been associated with stress reactivity, support-seeking, and PTSD in prior studies, and attachment style on risk for PTSD in a nationally representative sample of 2163 European-American (EA) U.S. military veterans who participated in two independent waves of the National Health and Resilience in Veterans Study (NHRVS). Results revealed that insecure attachment style [adjusted odds ratio (OR)=4.29; $p<0.001$] and the interaction of rs53576 and attachment style (OR=2.58, $p=0.02$) were associated with probable lifetime PTSD. Among

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individuals with the minor A allele, the prevalence of probable PTSD was significantly higher among those with an insecure attachment style (23.9%) than those with a secure attachment style (2.0%), equivalent to an adjusted OR of 10.7. We attempted to replicate these findings by utilizing dense marker data from a genome-wide association study of 2,215 high-risk civilians; one *OXTR* variant, though not rs53576, was associated with PTSD. Exploratory analyses in the veteran sample revealed that the interaction between this variant and attachment style predicting probable PTSD approached statistical significance. Results indicate that polymorphisms in the *OXTR* gene and attachment style may contribute to vulnerability to PTSD in U.S. military veterans.

Graphical abstract

Keywords

posttraumatic stress disorder; *OXTR*; rs53576; insecure attachment; gene environment interaction

Posttraumatic stress disorder (PTSD), an often chronic and debilitating psychiatric disorder associated with exposure to traumatic life events, is characterized by intrusive thoughts, avoidance of trauma reminders, negative cognitions and mood, and alterations in arousal and reactivity (American Psychiatric Association, 2013). Lifetime and past-year prevalence rates of PTSD are 8.3% and 4.7%, respectively, in community samples (Kilpatrick et al., 2013), with similar rates observed in military populations (e.g., 8.0% and 4.8%; Wisco et al., 2014). Supportive environments can buffer the effects of traumatic stress (Southwick et al., 2016), and as such, low social support is among the most robust risk factors for developing PTSD (Ozer et al., 2003). The oxytocin system plays a key role in social behavior and stress regulation (McCall and Singer, 2012), and has thus been a focus of research attempting to elucidate both social and biological mechanisms of trauma recovery.

Oxytocin is a neuropeptide produced in the hypothalamus that is released from axonal terminals and dendrites, leading to local action and diffusion throughout the brain where the oxytocin receptor is distributed, including the central and basolateral amygdala, medial preoptic area, anterior and ventromedial hypothalamus, ventrolateral septum, anterior cingulate, and hypoglossal and solitary nuclei (Gimpl and Fahrenholtz, 2001; Loup et al., 1991; Boccia et al., 2013). Intranasal oxytocin has been found to increase trust (Kosfeld et al., 2005), positive communication (Ditzen et al., 2009), and empathy (Shamay-Tsoory,

2011); to decrease anxiety and neural correlates of fear expression among individuals with PTSD (Pitman et al., 1993; Koch et al., 2016); and to interact with *in vivo* social support to reduce neuroendocrine and subjective responses to psychosocial stress (Heinrichs et al., 2003). This work suggests that stress-related activation of the oxytocin system may help to engender affiliative behavior, which can attenuate stress responses such as hypothalamic-pituitary-adrenal (HPA) axis reactivity and mitigate the risk of developing psychopathology (Olff, 2012).

The oxytocin receptor gene (*OXTR*) single nucleotide polymorphism (SNP) rs53576 [major allele: G, minor allele: A; minor allele frequency in European-Americans (EAs)=35%; <http://browser.1000genomes.org/index.html>], which maps to the third intron (a noncoding region) of *OXTR*, has emerged as a promising candidate genetic marker of individual differences in social behavior, stress reactivity, and psychopathology. SNPs located in noncoding regions can affect the levels of expression and the spatial expression of a gene and therefore alter gene action (Plomin et al., 2008). Among voles, genetic variation in non-coding regions of *OXTR* contribute to *OXTR* transcription levels, oxytocin receptor density, and individual differences in social cognition and behavior (King et al., 2016).

While the functionality of *OXTR* rs53576 in humans is not fully explicated, the A allele has been associated with morphometric alterations of the hypothalamus and amygdala, which were in turn associated with increased functional connectivity between these structures during the processing of emotionally salient social cues (Tost et al., 2010). The minor A allele has also been associated with several maladaptive outcomes, including reduced empathy (Rodrigues et al., 2009), prosocial behavior (Kogan et al., 2011), psychological resources, optimism, self-esteem (Saphire-Bernstein et al., 2011), and reward dependence (Tost et al., 2010); greater depressive symptomatology (Saphire-Bernstein et al., 2011); and higher physiological and subjective stress reactivity (Rodrigues et al., 2009). In contrast, the G allele has been associated with positive social behaviors, including sociality (Li et al., 2015), seeking emotional support when distressed (Kim et al., 2010), and being more likely to benefit from social support (Chen et al., 2011).

PTSD is thought to develop, in large part, as a function of gene by environment (i.e., GxE) interactions (Ressler, 2016; Sheerin et al., 2017), and the behavioral expression of certain genotypes is sensitive to influence from the social environment (Kim et al., 2011). Individual differences in perceptions of the social environment, such as adult attachment style, serve as critical mechanisms of stress recovery and the development of social bonds (Charuvastra & Cloitre, 2008), such that attachment may serve as an environmental moderator of *OXTR* effects. A secure attachment style is characterized by a sense that important relationships are dependable and trustworthy. In contrast, an insecure attachment style is characterized by emotional lability, and either jealousy and fear of intimacy (avoidant attachment style) or the tendency to seek constant reciprocation and validation from relationships (anxious/ambivalent attachment style; Hazan and Shaver, 1987). Given that insecure attachment in adulthood is associated with reduced responsiveness to social support (Ditzen et al., 2008), insecurely attached individuals may be at greater risk of developing PTSD when exposed to trauma.

The *OXTR* SNP rs53576 has previously been found to interact with attachment style to predict social anxiety; individuals with an A allele and less secure attachment reported more social anxiety than G homozygotes (Notzon et al., 2016). In the only published study of *OXTR* rs53576 genotype and PTSD symptoms, among individuals with an A allele, a perceived negative social environment was associated with significantly increased PTSD symptoms (regardless of economic stress), while among G homozygotes, a negative social environment predicted elevated PTSD symptoms only for individuals reporting high economic stress (Lucas-Thompson and Holman, 2013). Thus, the G/G genotype may be protective against poor mental health outcomes in the context of negative social environments (e.g., insecure attachment).

The current study aimed to examine the extent to which *OXTR* SNP rs53576 genotype and attachment style independently and interactively related to probable lifetime PTSD in a contemporary, nationally representative sample of EA U.S. military veterans. We hypothesized that (1) a greater minor allele dosage would be associated with higher odds of PTSD; (2) insecure attachment would be associated with higher odds of PTSD; and (3) among veterans with insecure attachment, individuals with a greater minor rs53576 allele dosage would be at greatest risk for PTSD. Given the dearth of research on *OXTR* and PTSD, we also utilized dense marker data from a genome-wide association study (GWAS) to identify additional *OXTR* SNPs that could be associated with PTSD. Previous GWAS of PTSD have identified risk variants implicated in a variety of processes, including neuroprotection, neuronal function, and immune function (Guffanti et al., 2013; Kilaru et al., 2016; Liberzon et al., 2014; Logue et al., 2013; Nievergelt et al., 2015; Stein et al., 2016; Xie et al., 2013), but, to date, *OXTR* rs53576 not been associated with PTSD in these studies.

Methods

Participants

Veteran sample—Data were drawn from the National Health and Resilience in Veterans Study (NHRVS), a contemporary, nationally representative cohort study of U.S. veterans. Data from two independent samples (1,657 EA veterans in 2011, 506 EA veterans in 2013) were combined in this study to increase statistical power (total N=2163). A detailed description of the NHRVS sample is provided elsewhere (Wisco et al., 2014). The NHRVS sample was drawn from a research panel of more than 50,000 U.S. households maintained by GfK Knowledge Networks, Inc. (Menlo Park, CA). Knowledge Networks maintains KnowledgePanel, a probability-based, online, non-volunteer access survey panel of a nationally representative sample of more than 50,000 U.S. adults that covers approximately 98% of U.S. households, including mobile phone-only households. The GfK Knowledge Networks recruitment protocol relies on probability-based sampling of addresses from the U.S. Postal Service's Delivery Sequence File (DSF); the key advantage of the address-based sampling methodology is that it allows sampling of almost all U.S. households. To permit generalizability of study results to the entire population of U.S. veterans, post-stratification weights based on demographic distributions (i.e., age, sex, race/ethnicity, level of education, Census region, metropolitan area) drawn from the most recent Current Population Survey

(U.S. Census Bureau, October, 2011) were computed by GfK Knowledge Networks statisticians and applied in inferential analyses. All participants gave informed consent and the Human Subjects Subcommittee of the Veterans Affairs (VA) Connecticut Healthcare System and VA Office of Research & Development approved the study.

Civilian sample—The Yale-Penn sample included small nuclear families and unrelated individuals collected to study the genetics of substance dependence. Families were ascertained from treatment centers and advertisements that recruited affected sibling pairs (ASPs) meeting DSM-IV criteria for cocaine or opioid dependence. Probands were excluded from further study if they had ever received a diagnosis of a major psychotic illness. Other family members of the ASPs were recruited when available, regardless of affection status. Case-control subjects were recruited from substance abuse treatment centers and through advertisements at the University of Connecticut Health Center, Yale University School of Medicine, the Medical University of South Carolina, the University of Pennsylvania, and McLean Hospital.

Assessments

NHRVS veteran sample genotyping—Participants provided saliva samples collected using Oragene DNA (OG-250) kits for DNA analysis. DNA was extracted using prepIT-L2P reagent (DNA Genotek, Ontario, Canada) according to the manufacturer's directions. Samples were genotyped with the PsychChip genomewide association analysis (GWAS) array (Illumina, San Diego, CA, USA). Genotypes were called using GenomeStudio software (version 2011.1) and genotyping module V1.8.4 (Illumina, San Diego, CA, USA). Ninety samples with missing genotyping rate >5% were excluded from analysis. Criteria for SNP inclusion included: minor allele frequency (MAF) >0.01%, missing genotyping rate per SNP <5%, and Hardy-Weinberg equilibrium (HWE) p -value >10⁻⁵, resulting in 423,415 autosomal SNPs and 2,737 samples. Genome-wide identity-by-descent (IBD) sharing was estimated for all pairwise samples in PLINK (Purcell et al., 2007) to detect duplicates and potentially related samples using 93,814 independent SNPs with MAF >0.01. A total of 9 duplicate pairs and 12 additional pairs with a high level of IBD sharing (>0.1) were detected. One subject of each duplicate or related pair was randomly chosen for removal, retaining 2,718 independent samples (2,270 EAs).

To examine population stratification of the remaining unrelated EA samples, principal components (PCs) for the GWAS data were computed using EIGENSOFT (Price et al., 2006) based on a set of SNPs (64,219) in common with HapMap3, which were in low linkage disequilibrium (LD) and had MAF >0.01. Outlier EA samples, defined as samples whose ancestry was at least three standard deviations from the mean on one of the largest two PCs, were detected and removed from the PC analysis ($n=95$). Following best practice guidelines of IMPUTE2 (Howie et al., 2009), we imputed 1000 genome variants into the EA samples. Pre-phasing was performed with SHAPEIT (Delaneau et al., 2012) to infer haplotypes for the EA samples based on 295,837 autosomal SNPs with MAF >0.01. We then conducted imputation on pre-phased haplotypes using IMPUTE2 against reference data from the 1000 Genomes Phase III integrated variant set. After post-imputation quality control [SNP missing rate<0.05, MAF>0.005, imputation quality score (info) >0.5, and

HWE $>10^{-6}$], 10,377,932 SNPs remained for analysis. For the current study, the *OXTR* rs53576 SNP was imputed with quality score (info=0.84), indicating that it was well-imputed (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). We extracted the *OXTR* rs53576 SNP from the imputed data of EA samples for candidate gene analysis. In the veteran sample, MAF (A) was 35.5%; there was no evidence of deviation from Hardy-Weinberg expectations for the *OXTR* rs53576 genotype ($\chi^2=0.10$, $p=0.75$).

Yale-Penn genotyping and imputation—The Yale-Penn sample was genotyped using one of two genotyping arrays: (1) the Illumina HumanOmni1-Quad v1.0 microarray containing 988,306 autosomal SNPs, (2) the Illumina Infinium Human Core Exome microarray, which contains 265,919 exome-focused SNPs and approximately 240,000 tagging SNPs which allow genome-wide imputation. SNP genotype imputation was performed with IMPUTE2 (Howie et al., 2009) using genotyped SNPs and the March 2012 1000 Genomes reference panel (1000 Genomes Project, 2012; <http://www.1000genomes.org/>), which contains phased haplotypes for 1,094 individuals of various ancestries: 379 of European descent (CEU, FIN, GBR, IBS, and TCI), 286 of Asian descent (CHB, JPT, and CHS), 181 admixed American samples (PUR, CLM, and MXL), and 246 samples of African descent (ASW, LWK, YR). All SNPs had imputation quality scores >0.80 .

Attachment Style—The Attachment Style Questionnaire (ASQ; Hazan and Shaver, 1987) was used to assess adult attachment style. Participants were asked to choose which of three statements corresponding to attachment style best described their feelings and attitudes in relationships: secure (“I find it relatively easy to get close to others and am comfortable depending on them and having them depend on me...”), insecure-avoidant (“I am somewhat uncomfortable being close to others. I find it difficult to trust them completely and to allow myself to depend on them...”), and insecure-anxious/ambivalent (“I find that others are reluctant to get as close as I would like. I often worry that my partner does not really love me or will not want to stay with me...”). The ASQ has good convergent validity with longer measures of attachment (Sperling et al., 1996). The two insecure attachment styles were collapsed given the low frequency of endorsement of anxious/ambivalent attachment, lack of difference in PTSD rates for the two attachment styles ($\chi^2=0.14$, $p=0.71$), and evidence for associations between both types of insecure attachment and PTSD (Elwood et al., 2007; Muller et al., 2000).

PTSD—Probable lifetime PTSD was assessed with the PTSD Checklist (PCL) in the veteran sample. The Trauma History Screen (THS) was used to assess lifetime exposure to 14 potentially traumatic events (Carlson et al., 2011). Participants are asked to identify their ‘worst’ traumatic events endorsed on the THS and then rate how bothered they have been by each PTSD symptom ever in their lifetime on a 5-point scale ranging from “Not at all” to “Extremely.” The DSM-IV (Weathers et al., 1993) and DSM-5 (Weathers et al., 2013) versions were used in the 2011 and 2013 samples, respectively, with excellent internal consistency in both samples ($\alpha=0.94-0.95$). Respondents who did not endorse any potentially traumatic events on the THS ($n=206$) were assigned scores corresponding to no

symptoms. Cut scores for a probable PTSD diagnosis (PCL-S 50 [Weathers et al., 1993]; PCL-5 33 [Bovin et al., 2016; Friedman et al., 2016] were chosen based on previous research that calibrated cut scores against scores on the gold-standard instrument for assessing PTSD (i.e., the Clinician Administered PTSD Scale).

The Semi-structured Assessment for Drug Dependence and Alcoholism (SSADDA; Pierucci-Lagha et al., 2005, 2007) was used to assess DSM-IV PTSD among individuals who endorsed trauma exposure in the Yale-Penn civilian sample. Interrater and test-retest reliability for the SSADDA PTSD module are 0.59 and 0.76, respectively.

Data Analysis

Descriptive statistics were computed to summarize sociodemographic, military, and trauma-related variables in the combined veteran sample, and separately for veterans based on whether they screened positive for PTSD. Multivariable binary logistic regression analyses were conducted using SPSS 22.0 to evaluate the independent and interactive effects of *OXTR* rs53576 minor allele dosage and attachment style on risk for lifetime PTSD. All analyses were adjusted for age, sex, marital status, combat veteran status, number of lifetime traumas (from THS), nature of worst traumatic event (i.e., assaultive [i.e., attacked with a weapon, physical or sexual assault as an adult or child] vs. non-assaultive [e.g., natural disaster, serious transportation accident] trauma), and the top 10 PCs from the population stratification analysis. Alpha was set to 0.05. All reported raw frequencies are unweighted. Post-stratification weights were applied to means, percentages, and inferential statistics to ensure generalizability to the general population of U.S. veterans. Interpretation of the magnitude of odds ratios was informed by Chen and colleagues' guidelines (Chen et al., 2010).

Within the Yale-Penn civilian sample, case-control status was regressed on imputed SNP allele dosage adjusted for age, sex, and the first five ancestry PCs using logistic regression models solved with generalized estimating equations to account for the correlation between related individuals.

Results

Preliminary Analysis

A t-test examining whether attachment style was associated with *OXTR* rs53576 minor allele dosage in the veteran sample was conducted to rule out a spurious gene-environment interaction (Moffitt et al., 2005). This association was found to be non-significant ($t=0.38$, $p=0.70$).

Descriptive Statistics

Veteran sample—Only EA veterans with *OXTR* rs53576 dosage data were included in the current study; 138 veterans were excluded. Sociodemographic, military, trauma, and clinical characteristics of the veteran sample are presented in Table 1.

Civilian sample—The Yale-Penn civilian sample included 1,195 PTSD cases (686 AA and 509 EA) and 4,096 trauma exposed controls (2,390 AA and 1,706 EA). The AA cases were excluded to match the EA veteran sample, leaving 2,215 participants. The sample was 41.7% female and had a mean age of 39.2 years. A total of 91.5% of participants endorsed some type of substance dependence (alcohol, nicotine, cocaine, opioids, or cannabis), with 78% of participants endorsing some type of illicit substance dependence specifically (i.e., cocaine, opioids, or cannabis).

Prediction of Probable PTSD Diagnosis

Candidate gene approach in the veteran sample—Table 2 shows the results of multivariable binary logistic regression analyses. We observed a medium-to-large main effect of attachment style in predicting probable PTSD. The main effect of *OXTR* rs53576 minor allele dosage was non-significant. When the *OXTR* rs53586 genotype $\times$ attachment interaction was removed from the model to test for unconditional main effects, the insecure attachment effect was retained (OR=8.66, 95%CI=5.22–14.37, $p<0.001$); the effect of minor allele dosage remained non-significant ($p=0.076$). There was a significant small-to-medium magnitude interaction of rs53576 minor allele dosage and attachment style in predicting PTSD. As displayed in Figure 1, the highest odds of probable PTSD were observed among individuals with a G/A or A/A genotype who reported insecure attachment. That is, among individuals with an A allele, the prevalence of probable PTSD was significantly higher among those with an insecure attachment style (23.9%) versus those with secure attachment style and no minor alleles (2.0%), equivalent to an adjusted odds ratio of 10.7 [95% confidence interval (CI)=4.01–25.36; $p<0.001$] and a large effect size.

Denser SNP map extracted from GWAS in civilian sample—GWAS results for the *OXTR* region in the civilian sample are presented in Figure 2. *OXTR* rs53576 was not significant ($p=0.18$). Using the Li and Ji (2005) method to correct for the number of independent LD blocks within the gene, only *OXTR* rs2300549, located in intron 2, surpassed the experiment-wide significance threshold for association ($p=0.00147$). In order to rule out potential confounding by substance dependence diagnosis, we added illicit substance dependence diagnosis to the model as a covariate; results for rs2300549 were essentially unchanged ($p<0.003$). *OXTR* SNP $\times$ sex interactions were non-significant. Follow-up analyses in the veteran sample revealed that this SNP, which was also imputed with high quality (0.93), did not have an unconditional main effect on probable PTSD ($p=0.83$) but exhibited a nearly significant interaction with attachment style to predict PTSD ($p=0.059$).

Discussion

To our knowledge, this is the first study in U.S. military veterans to assess the association between *OXTR* rs53576 genotype and PTSD. Results show that the interaction between *OXTR* SNP rs53576 genotype and attachment style was associated with probable lifetime PTSD in a large, contemporary, nationally representative sample of U.S. military veterans. Veterans with insecure attachment were at significantly higher risk of meeting screening criteria for PTSD if they had at least one rs53576*A compared to G homozygotes. This

finding parallels results showing that insecurely attached individuals with an A allele were more socially anxious than G homozygotes (Notzon et al., 2016), and that *OXTR* rs53575 genotype interacted with negative social environments to predict PTSD symptoms (Lucas-Thompson and Holman, 2013). Further, the association between insecure attachment style and greater likelihood of probable PTSD is consistent with longitudinal observations of the effect of insecure attachment on PTSD symptoms (Franz et al., 2014) and extends previous findings in military samples (Currier et al., 2012; Escolas et al., 2012) to a nationally representative sample, and is clinically relevant given that an insecure attachment style has been linked to reduced treatment response among veterans with PTSD (Forbes et al., 2010).

Our candidate SNP (rs53576) selected *a priori* had a non-significant unconditional main effect on PTSD when tested independently in our veteran sample, and was not associated with PTSD when tested using a GWAS approach in our civilian sample. Extracting data acquired using a GWAS approach allows for detection of new associated variants in or near a potential candidate gene, and we did find nominal significance for another *OXTR* SNP (rs2300549). We therefore tested this variant identified via GWAS in our veteran sample, and while the main effect was null, there was preliminary evidence that it interacts with attachment style to predict PTSD. To our knowledge, this SNP has not previously been linked to behavioral phenotypes or specific biological function. It is possible that *OXTR* rs53576 may be a marker for variants elsewhere in the oxytocin receptor gene that also have effects on PTSD risk when interacting with environmental variables. This newly-implicated SNP may be considered in future work examining *OXTR* polymorphisms, attachment style, and PTSD.

While *OXTR* polymorphisms have been implicated in stress recovery, social functioning, and the development of psychopathology, the specific functional roles of these variants remain to be determined (Aspé-Sánchez et al., 2016). Oxytocin released in the context of supportive social environments is thought to influence stress response systems via oxytocin receptors in the hypothalamic-limbic circuits, particular in the amygdala, which has been implicated in emotional processing and social behavior (Meyer-Lindenberg et al., 2011). Given oxytocin's role in social reward processing (Donaldson and Young, 2008), it is plausible that *OXTR* polymorphisms may be associated with variable perceptions of social reward value. However, very few studies have related rs53576 or other *OXTR* variants to human plasma oxytocin levels, and results have thus far been inconsistent, showing both lower levels of oxytocin plasma in rs53576 G homozygotes (Moons et al., 2014) and no difference between individuals with G/A and A/A genotypes (Parker et al., 2014). The genetic risk for social dysfunction conferred by the *OXTR* rs53576 minor allele may translate to a neural mechanism that predisposes individuals to negative appraisals of social stimuli, which may compromise stress-reducing effects of social support and exacerbate the effect of cognitive representations of relationships as threatening, untrustworthy, or unstable.

Additional studies of biological function of *OXTR* genotypes are most promising for determining proximal outcomes of direct genetic effects, which are notoriously small and difficult to replicate (Bakermans-Kranenburg and van IJzendoorn, 2014). Further, brain and peripheral oxytocin levels and the expression of *OXTR* are likely influenced by multiple underlying mechanisms. As such, studies of epigenetic regulation of *OXTR* are warranted

and could help to elucidate how psychosocial stress exposure may regulate *OXTR* expression (Kumsta et al., 2013). Finally, replications of GxE effects such as that observed in our veteran sample are needed to evaluate more fully the functional relevance of the *OXTR* rs53576 genotype in shaping PTSD risk.

The limitations of the study include a focus on EA, predominantly male veterans,; research on non-EA and female veterans is needed. Given higher rates of PTSD in women (Olff et al., 2007), preliminary evidence of rs53576 genotype $\times$ sex interactions predicting activity in limbic areas (Tost et al., 2010), and sex differences in fear and stress responses (Taylor et al., 2000), future studies could explore the potential role of sex in *OXTR* rs53576 $\times$ environment interactions on stress- and trauma-related phenotypes like PTSD. Lucas-Thompson and Holman (2013) found that men's functioning in response to economic stress was moderated by both *OXTR* genotype and negative social environments, whereas among women, functioning was associated with negative social environments regardless of genotype and economic stress. These findings suggest the possibility that environmental variables exert more direct influences on women, and are more likely to interact with genetic vulnerabilities in men. While biomedical studies with intention and adequate power to examine sex differences in variables like *OXTR* remain sparse (Mazure & Jones, 2015), such work could elucidate whether the pattern of findings in the current study hold for or differ in men and women. Further, while the current study had more statistical power to detect small genetic effects than many previous studies, the number of veterans reporting lifetime PTSD remained small, limiting our ability to draw conclusions pertaining to clinical populations. The civilian sample had a higher prevalence of PTSD (22%) but was not nationally representative in nature. As such, the findings in the Yale/Penn sample are not a true replication, and the evidence for association of *OXTR* with PTSD in this sample was weak. Our measure of attachment was necessarily brief and cannot speak to attachment security in young childhood; however, the emphasis on current attachment style is less influenced by retrospective memory biases and more relevant to veterans' present functioning. Further, the low prevalence of anxious/ambivalent attachment and the lack of an item corresponding to disorganized attachment precluded us from examining potentially interesting associations between these attachment styles, *OXTR* minor allele dose, and PTSD. Critically, the cross-sectional design of the current study precluded causal inferences, particularly about the association between attachment and PTSD diagnosis. Rather than the proposed social causation model, which posits that insecure attachment conferred risk for PTSD, findings could be explained by social selection, such that PTSD led to less secure attachment (Kaniasty & Norris, 2008).

Despite these limitations, results of the current study illustrate the direct association between attachment style and PTSD, and provide preliminary evidence for the interactive role of *OXTR* polymorphisms and attachment style in increasing risk for PTSD. These findings may inform biosocial models of PTSD and suggest that individuals with one or more rs53576*A alleles may benefit from post-trauma interventions that target the oxytocin system (Olff, 2012) and efforts to improve attitudes and feelings toward relationships (Markowitz et al., 2015), which may facilitate social coping that plays a critical role in trauma recovery (Charuvastra and Cloitre, 2008). Further research is needed to determine the proximal mechanisms of *OXTR* polymorphisms in trauma recovery, and to evaluate

whether pharmacotherapies targeting the oxytocin system may be useful for preventing and/or treating PTSD (Frijling et al., 2014; Koch et al., 2014). Continued examination of potential genetic and interpersonal risk and protective correlates of PTSD can ultimately help to inform our understanding of the development and maintenance of PTSD among trauma-exposed individuals.

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Disclosures

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RE: John H. Krystal, MD

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Consultant

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Patents and Inventions

1. Seibyl JP, Krystal JH, Charney DS. Dopamine and noradrenergic reuptake inhibitors in treatment of schizophrenia. US Patent #:5,447,948. September 5, 1995
2. Vladimir, Coric, Krystal, John H, Sanacora, Gerard – Glutamate Modulating Agents in the Treatment of Mental Disorders US Patent No. 8,778,979 B2 Patent Issue Date: July 15, 2014.
3. Charney D, Krystal JH, Manji H, Matthew S, Zarate C., - Intranasal Administration of Ketamine to Treat Depression United States Application No. 14/197,767 filed on March 5, 2014; United States application or PCT International application No. 14/306,382 filed on June 17, 2014
4. Arias A, Petrakis I, Krystal JH. – Composition and methods to treat addiction. Provisional Use Patent Application no.61/973/961. April 2, 2014. Filed by Yale University Office of Cooperative Research.
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6. Zarate, C, Charney, DS, Manji, HK, Mathew, Sanjay J, Krystal, JH, Department of Veterans Affairs “Methods for Treating Suicidal Ideation”, Patent Application No. 14/197.767 filed on March 5, 2014 by Yale University Office of Cooperative Research

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Highlights

- Nationally representative gene $\times$ environment interaction studies of PTSD lacking
- Oxytocin receptor gene polymorphism rs53576 implicated in stress responses
- rs53576*A allele $\times$ insecure attachment associated with increased risk of PTSD
- A denser SNP set in civilians revealed another *OXTR* variant associated with PTSD
- Findings highlight role of the oxytocinergic system in trauma recovery

Figure 1.

Probable lifetime PTSD diagnosis by *OXTR* rs53576 SNP and attachment style in veteran sample participants in which minor allele imputation hard call threshold=0.90 (n=1570). Interaction of *OXTR* rs53576 minor allele carriage $\times$ attachment style was statistically significant (see Table 2). ** $p < 0.01$, *** $p < 0.001$. Secure attachment with 0 minor alleles is the reference. CIs: secure/1–2 minor alleles (0.36–2.57), insecure/0 minor alleles (2.24–14.73), insecure/1–2 minor alleles (4.43–26.02). Analyses are adjusted for age, sex, marital status, combat status, number of traumatic life events, nature of worst event (assaultive vs. non-assaultive trauma), and ancestral proportion scores.

Figure 2.

Regional Manhattan plot of the chromosome 3 denser SNP set surrounding *OXTR*. Single nucleotide polymorphisms are color-coded based on the linkage disequilibrium with the most significant single nucleotide polymorphism in the *OXTR* region. *OXTR* rs2300549 ($p=0.0011$), located at 8800747, surpassed the experiment-wide significance threshold for association ($p=0.00147$) after correcting for the number of independent LD blocks within the gene.

Table 1

Sociodemographic, military, and trauma and clinical characteristics of the veteran sample

	Full Sample (n=2163)	No PTSD (n=2010; 92.3%)	PTSD (n=153, 7.7%)
	Weighted mean (SD) or n (weighted %)	Weighted mean (SD) or n (weighted %)	Weighted mean (SD) or n (weighted %)
Sociodemographic and Military Characteristics			
Age	62.93 (14.55)	63.80 (14.06)	51.50 (15.51) *
Male sex	2058 (92.1%)	1929 (93.2%)	129 (79.1%) ***
Some college or higher	1938 (66.8%)	1791 (66.6%)	147 (69.2%)
Married/living with partner	1765 (75.3%)	1656 (76.5%)	109 (61.9%) ***
Currently employed	829 (34.7%)	764 (34.6%)	65 (36.3%)
Household income \$60,000/year	1186 (43.5%)	1119 (44.0%)	67 (37.4%)
Combat veteran	808 (33.9%)	717 (31.9%)	91 (57.2%) ***
Number of years served in military	6.85 (7.41)	6.81 (7.46)	7.17 (6.82)
Trauma and Clinical Characteristics			
Number of lifetime traumatic events	3.35 (2.60)	3.09 (2.36)	6.47 (3.30) ***
Index traumatic event			
Sudden death of loved one	617 (33.8%)	589 (34.9%)	28 (21.4%)
Life-threatening illness/injury	325 (16.2%)	310 (17.1%)	15 (6.4%)
Military-related trauma	164 (8.5%)	133 (7.3%)	31 (21.5%)
Assaultive trauma	175 (8.2%)	147 (7.6%)	28 (14.3%) **
OXTR rs53576 genotype			
Minor allele (A)			
A/A	171 (11.3%)	155 (11.1%)	16 (14.8%)
A/G	704 (45.1%)	645 (45.1%)	59 (48.5%)
Two major alleles (G/G)	702 (43.7%)	654 (43.7%)	48 (36.7%) *
Attachment Style			
Secure	1654 (71.7%)	1618 (76.1%)	36 (17.2%) ***
Insecure	606 (28.3%)	473 (23.9%)	133 (82.3%)
Avoidant	529 (24.2%)	413 (20.5%)	116 (69.4%)
Anxious/ambivalent	77 (4.2%)	60 (3.5%)	17 (12.9%)

Note: Significant "No PTSD" vs. "PTSD" t-test and chi-square group differences indicated as

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$.

The DSM-IV version of the PCL (Weathers et al., 1993) was used in the 2011 sample (score range=17–85), and the DSM-5 version (PCL-5; Weathers et al., 2013) was used in the 2013 sample (score range=0–80). Allele counts based on hard call of genotypes with maximum probability=0.90.

Table 2

Results of multivariable binary logistic regression analyses evaluating relations between OXTR rs53576 minor allele dosage, attachment style, and lifetime probable PTSD diagnosis in veteran sample

	OR	95% CI	<i>p</i>
<i>OXTR</i> rs53576 minor allele dosage	0.68	0.34–1.36	0.28
Insecure attachment style	4.29	2.06–8.94	<0.001 **
rs53576 minor allele dosage*Attachment style	2.58	1.15–5.76	0.02 *
Age	0.97	0.95–0.98	<0.001 **
Sex	3.17	1.69–5.95	<0.001 ***
Marital status	0.84	0.53–1.34	0.46
Combat veteran status	2.08	1.32–3.26	0.001 **
Number of lifetime traumatic events	1.41	1.30–1.52	<0.001 ***
Nature of worst trauma	0.84	0.43–1.64	0.60

Notes. OR=Adjusted Odds Ratio; CI=confidence interval.

*
 $p < .05$,

**
 $p < .01$,

***
 $p < .001$.

Analyses are adjusted for age, sex, marital status, combat veteran status, number of traumatic life events, nature of worst trauma (assaultive vs. non-assaultive), and ancestral proportion scores. When the minor allele dosage*attachment interaction was removed from the model, the main effect of attachment was retained ($p < 0.001$) and the effect of minor allele dosage remained nonsignificant ($p = 0.076$). To control for the effects of potential confounds, we supplemented the primary analyses examining a full model that included covariate-by-environment and covariate-by-gene interaction terms (Keller, 2014). While the model may have overfit the data given the number of covariates, the GxE interaction was retained (OR=2.70, 95% CI=1.08–6.77, $p < 0.05$). None of the covariate-by-environment nor covariate-by-gene interactions were statistically significant, with the exception of the sum of lifetime traumatic events and attachment style ($p < 0.01$).