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Variation in the oxytocin receptor gene influences neurocardiac reactivity to social stress and HPA function: A population based study

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ABSTRACT

Oxytocin (OT) is a nonapeptide neurohormone that is involved in a broad array of physiological and behavioral processes related to health including hypothalamic–pituitary–adrenal (HPA) axis functioning, autonomic nervous system (ANS) activity and social behaviors. The present study sought to explore the influence of genetic variation in the oxytocin receptor (SNP; rs53576) on autonomic and neurohormonal functioning across both resting and psychological stress conditions in a population based sample of older adults. Results revealed that A carrier males showed higher levels of resting sympathetic cardiac control as compared to their G/G counter parts. However, G/G participants displayed significantly higher levels of sympathetic reactivity to psychological stress with G/G males showing the highest levels of sympathetic response to stress. Although no significant effects were detected for heart rate or parasympathetic cardiac control across resting and stress conditions, results revealed that G/G participants generally displayed heightened stroke volume and cardiac output reactivity to the psychological stressor. Furthermore, analysis of diurnal fluctuations in salivary cortisol revealed that G/G participants displayed lower awakening cortisol levels and less variation in salivary cortisol across the day as compared to A carrier individuals.

1. Introduction

Oxytocin (OT) is a nonapeptide neurohormone that is involved in a broad array of physiological and behavioral processes including hypothalamic–pituitary–adrenal (HPA) axis functioning, autonomic nervous system (ANS) activity, and social behaviors, including pair bonds and social recognition in both humans and animal models (Carter et al., 2008; Kemp and Guastella, 2011; Bartz et al., 2011). Exposure to a variety of stimuli triggers the hypothalamic paraventricular nucleus to release OT into OT receptor rich cortical, limbic, and brainstem regions associated with emotion and heterarchical control of neuroendocrine and ANS functioning (Gimpl and Fahrenholz, 2001; Carter et al., 2008). Indeed, intranasal administration of OT to humans decreases amygdala activation to threatening stimuli, increases trust, and promotes the encoding of positive social memories (Kosfeld et al., 2005; Guastella et al., 2008). Genetic variation in the OT receptor gene has been shown to be associated with empathy and is associated with attachment style and pro-social temperament (Rodrigues et al., 2009; Bartz et al., 2010; Costa et al., 2009; Tost et al., 2010). Furthermore, polymorphisms in the oxytocin receptor have previously been associated with parenting behaviors (Bakermans-Kranenburg and van Ijzendoorn, 2008), adult attachment styles (Gillath et al., 2008), emotional support seeking (Kim et al., 2010) and self-esteem (Saphire-Bernstein et al., 2011).

In addition to its well-described role in regulating social processes, OT modulates autonomic nervous system activity by exerting direct effects on preganglionic sympathetic (Gilbey et al., 1982; Pardini et al., 1989) and parasympathetic neurons (Higa et al., 2002). Pharmacological administration of OT to rodents has been shown to modulate ANS functioning across various contexts (Grippo et al., 2009; Carter et al., 2008). Similarly, intranasal administration of OT in humans has been shown to increase overall cardiac control (Norman et al., 2010), and modulates phasic activity of parasympathetic nervous system functioning (Gamer and Buchel, 2012). Furthermore, OT dampens HPA axis reactivity to social stress in humans (Heinrichs et al., 2003) and rodents (Windle et al., 1997). OT has been shown to mitigate pathophysiological processes in numerous animal models of human disease (DeVries et al., 2007; Norman et al., 2010) leading some to suggest that OT may be one of the neurobiological mechanisms underlying the potent influence that social factors (e.g. social isolation, social support) have on health (Uvnas-Moberg, 1998).

In addition to pharmacological manipulations, genetic variation in the OT receptor has been shown to be associated with hypothalamic structure and function (Tost et al., 2010) and has been associated

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with loneliness (Lucht et al., 2009; Norman et al., 2010) and attachment style in depressed individuals (Costa et al., 2009). Additionally, OT receptor single-nucleotide polymorphisms (SNPs) have been found to be associated with modified social behavior, hypothalamus structure and function, and altered cardiac startle reflex (Bakermans-Kranenburg and van Ijzendoorn, 2008; Tost et al., 2010; Rodrigues et al., 2009). Therefore, converging evidence from pharmacological and genetic approaches suggests an important role for the OT system in the regulation of a broad array of psychological and physiological processes.

Although the data described above clearly demonstrate the influence of OT on HPA axis and cardiovascular functioning, the vast majority of these studies utilized acute pharmacological manipulations that provide minimal information into the long term effects of OT signaling in humans. In the present study, we evaluate the relationship between a common SNP in the OT receptor gene (rs53576) and its associations with individual differences in cardiovascular and HPA axis function in a population based sample of older adults. Based upon previous research from animal and human studies we hypothesized that variation in the OT receptor would relate to individual differences in autonomic and HPA axis functioning. Furthermore, given the influence of estrogens on OT receptor activity (Young et al., 1998), we hypothesized that sex would moderate the relationship between OT receptor variation and individual differences in autonomic and HPA axis function.

2. Methods

2.1. Participants

Data for this study were collected annually between 2002 and 2006, as part of Chicago, Health Aging and Social Relations Study (CHASRS). CHASRS is a longitudinal population based study of non-Hispanic White, Black, and non-Black Hispanic persons born between 1935 and 1952 and living in Cook County, IL. The sample was selected using a multistage probability design in which the first stage involved identifying a subset of households estimated to have high probability of containing at least one adult age 50–65 years. The final sample consisted of 93 individuals who ranged from 50 to 68 years of age on the first testing occasion. The study was approved by the Institutional Review Board of the University of Chicago, and all participants gave informed consent.

2.2. Procedure

For each of five annual visits to our laboratory, participants arrived between 8:00 and 9:00 a.m., whereupon informed consent was obtained before beginning a day of assessments that included standard psychological surveys, health and medication interviews, and anthropometric measurements. Participants collected saliva samples as they engaged in their normal daily activities for three consecutive days in years 1, 3, 4 and 5. On each of the 3 days, participants provided three saliva samples at specific times of the day: upon awakening, 30 min after awakening, and before bedtime. Furthermore, in years 2 and 3 of the study participants completed a well validated psychological stress test that consisted of mental arithmetic performed in front of an audience.

Cardiovascular measures were obtained prior to lunch for all participants. Sensors for electrocardiograph, impedance cardiograph, and blood pressure recording were attached to participants. Participants were then seated in a comfortable padded chair. During a 15-minutes adaptation period, participants completed questionnaires while experimenters established good signal quality. Participants then sat quietly for an additional 5 min prior to recording of resting cardiovascular activity. In years 2 and 3, participants completed a mental arithmetic task immediately following the 5 minute baseline session and these data were collapsed into a single data point. Finally, in year 5 blood samples were taken for measurement of the estrogens estrone, estradiol and estriol.

2.3. Cardiovascular measures

Cardiovascular measures of sympathetic and parasympathetic cardiac control, respectively, were derived from pre-ejection period (PEP) and high (respiratory) frequency (0.12–0.40 Hz) heart rate variability (HF HRV). HF HRV is a rhythmic fluctuation of heart rate in the respiratory frequency band (respiratory sinus arrhythmia), and has been shown to be a relatively pure index of parasympathetic control (Berntson et al., 1997). HF HRV was derived by spectral analysis of the interbeat interval series derived from the ECG, following previously specified procedures (Berntson et al., 1997). The interbeat interval series was time sampled at 4 Hz (with interpolation) to yield an equal interval time series. This time series was detrended (second-order polynomial), end tapered, and submitted to a fast Fourier transformation. HF HRV spectral power was then integrated over the respiratory frequency band (0.12–0.40 Hz). HF HRV is represented as the natural log of the heart period variance in the respiratory band (in ms^2). All autonomic variables were averaged across the 5 years of the CHASRS study to better capture stable individual differences in autonomic functioning. All autonomic variables displayed relatively high Cronbach alphas across this 5 year span: HR (0.89), PEP (0.88), RSA (0.92), SV (0.82) and CO (0.84).

PEP is derived from the electrocardiogram and the impedance cardiogram, and is the period between the electrical invasion of the ventricular myocardium (Q wave of the ECG) and the opening of the aortic valve. The impedance cardiogram was obtained using the standard tetrapolar electrode system and procedures described elsewhere (Sherwood et al., 1990). The ECG and basal thoracic impedance (Z_0) were measured using a Bionex system (Mindware, Gahanna, OH). Software (Mindware, Gahanna, OH) was used to analyze the dZ/dt waveforms to obtain impedance-derived measures (i.e., PEP). For each subject, ECG and impedance data were ensemble averaged for each minute to produce estimates of the PEP. PEP depends on the time development of intraventricular pressure, and it is widely used as an index of myocardial contractility. Because variations in contractility are largely under sympathetic control, PEP is commonly used as a noninvasive measure of sympathetic cardiac control (Berntson et al., 1997), with lower PEP values representing higher levels of sympathetic cardiac control. PEP values were scored minute-by-minute and are represented in milliseconds. Impedance and ECG data were also used to derive stroke volume (SV) which details the volume of blood discharged with each cardiac contraction as well as cardiac output (CO), a measure of the volume of blood pumped by the heart over 1 min (i.e., SV*HR).

2.4. Diurnal cortisol rhythm

The diurnal cortisol data had a hierarchical structure with three levels: occasion at which the saliva sample was collected (at awakening, 30 min after awakening, evening; Level 1), day (Sunday, Monday, Tuesday; Level 2), and individual (Level 3). These data were therefore analyzed with multilevel models for longitudinal data (e.g., Singer and Willett, 2003). We analyzed a series of hierarchical models. Model 1 was an empty model without any predictors. The estimates of this model were used to calculate the intraclass correlation coefficient, that is, the proportion of the total variance that is due to each level of analysis (Hox, 2010). In Model 2, we added hours since awakening as a Level-1 variable. Since time at awakening was coded with zero, the intercept of this model reflects the average cortisol level at awakening. The slope reflects the rate of change in cortisol over time. Note that diurnal cortisol trajectories are typically not strictly linear but follow a more complex non-linear pattern (McEwen, 2007). However, non-linear change models cannot be

appropriately modeled with three measurement occasions, as is the case in our data (Singer and Willett, 2003). In Model 3, we examined the influence of oxytocin receptor variation on the overall level of cortisol (main effect). In Model 4, we additionally examined the influence of oxytocin receptor variation on the rate of change (interaction between slope and gene). Finally, we added demographic covariates (sex, age, ethnicity) in Model 5. Sex and age were centered on the grand mean. Ethnicity was reflected in two dummy variables (reference category: [Caucasian], Dummy 1: [African American], Dummy 2: [Hispanic]). Hence, the overall intercept of this model now reflects the predicted level of cortisol at awakening for Caucasians with average sex and average age. Diurnal cortisol rhythm data were collected in years 1, 3, 4 and 5 and of the CHASRS study and since there were no significant differences across time we chose to collapse across years in order to reduce error variance.

2.5. Cortisol assay procedures

Following collection, samples were stored at −80°(C) until shipped at room temperature to the Labor fur Stress-Monitoring at the University of Gottingen, Germany for assay by using an RIA protocol. Lower and upper limits of detection were 0.15 and 25 ng/ml. Five identical control samples were included in each assay to test inter- and intraassay consistency. Intraassay coefficients of variation (CV) ranged from 2.8% to 8.4% (mean 4.6%); the average interassay CV was 3.4%. To correct a strong positive skew in the data, cortisol values were natural-log transformed before use in analysis.

2.6. Estrogens assay procedure

Frozen serum samples were shipped on dry ice to the Laboratory of Proteomics and Analytical Technologies at SAIC (Frederic, MD, USA) and were assayed for endogenous estrone, estradiol and estriol according to an established protocol (Xu et al., 2007). Briefly, stock and working standard solutions of estradiol and stable isotope labeled estradiol were prepared. Calibration standards and quality control samples were then prepared using charcoal-stripped human serum with no detectable levels of estrogens. Next, the unknown serum samples and the calibration standards and quality control samples were hydrolyzed, extracted, and derivatized. All samples were then analyzed using capillary liquid chromatographic/electrospray ionization/tandem mass spectrometric analysis. The precision or percentage of recovery of a low concentration (8 pg/mL) of assay ranged from 91% to 113%. Serum estrogens from study participants were quantified using Xcalibur Quan Browser (Thermo Electron, Waltham, MA, USA). All concentrations were subjected to a natural log transformation in order to correct for positive skew.

2.7. DNA collection and analysis

Genomic DNA was extracted from leukocytes (Qiagen, Valencia CA), tested for purity and mass by spectrophotometry (Nanodrop ND1000), and genotyped for the SNP in the OT receptor (rs53576), using commercial TaqMan Genotyping Assays (Applied Biosystems Inc., Foster City CA) performed on a iCycler real-time PCR instrument (BioRad Inc., Hercules CA) as previously described (Cole et al., 2010). The SNP examined in this study consisted of an adenine (A) or guanine (G) within intron 3 of the OT receptor gene. In total, 11 individuals were A/A, 32 individuals were A/G and 49 individuals were G/G. The sampling did not deviate from Hardy–Weinberg equilibrium ($X^2 = 2.39$, $p > 0.05$). We chose to compare G/G individuals with individuals carrying at least one copy of the A allele (A carrier) as previously reported (Rodrigues et al., 2009).

3. Results

As displayed in Table 1, the OT receptor (rs53576) the A allele was not evenly distributed across gender and ethnicity. Therefore, demographic variables were included as covariates for all results reported below participants were of similar age, sex and ethnic makeup. Furthermore, G/G and A carrier participants had similar levels of education and income ($p > 0.05$). Additionally, all significant results described below were independent of individual differences in circulating estrone, estradiol and estriol.

3.1. Cardiovascular function

A 2 (genotype) × 2 (sex) ANOVA on resting cardiovascular measures revealed no main effects for genotype or sex ($p > 0.05$). Similarly, no gene × sex interactions were found for HR, RSA, SV, or CO ($p > 0.05$). However a significant gene × sex interaction ($F_{1,61} = 5.26$, $p = 0.02$) was detected for PEP (Fig. 1C). This effect reflected the fact that A allele carrier males displayed significantly higher levels of sympathetic cardiac control as revealed by lower resting PEP levels (Fig. 1C).

A mixed model 3 (baseline, stress, recovery) × 2 (genotype) × 2 (sex) ANOVA revealed the expected effects of the mental arithmetic task; increased HR, decreased PEP, decreased RSA, increased SV and CO (F's > 5.76, p's < 0.05; Table 2). Furthermore, a significant effect of sex was detected for PEP across the stress manipulation reflecting the fact that women generally displayed lower PEP (higher sympathetic tone) levels across all conditions (Table 2). No time × sex effects were observed ($p > 0.05$). A significant time × genotype interaction was detected for PEP ($F_{1,176} = 3.26$, $p = 0.04$; Fig. 2A) reflecting significantly elevated sympathetic nervous system function (lower PEP) across both stress and recovery conditions in G/G participants (Table 2). Additionally, a significant time × genotype × sex interaction was detected for PEP ($F_{1,168} = 3.81$, $p = 0.02$; Fig. 2A) reflecting the fact that male G/G participants displayed significantly elevated sympathetic reactivity to stress as compared to A carrier males while G/G women showed comparable responses to A carrier females (Fig. 2A, Table 2). No genotype or sex effects were found for HR or RSA (Table 2). The mixed models ANOVA revealed a significant time × genotype effect for both SV ($F_{1,168} = 3.29$, $p = 0.04$, Fig. 2B) and CO ($F_{1,168} = 4.35$, $p = 0.01$, Fig. 2C) reflecting the fact that G/G participants generally displayed heightened SV and CO responses to the mental arithmetic task. All of the above mentioned relationships were maintained after controlling for age, ethnicity, income, education, self reported cardiovascular disease, medication use, and circulating estrogens.

3.2. Diurnal cortisol rhythm

In Model 1, we estimated the proportion of total variance accounted for by each level: 58.7% of the total variance in cortisol was due to within-day variation, and 41.3% of the total variance was due to between-person variation. The proportion of variance due to intra-individual day-to-day fluctuations in cortisol was less than 0.001%, indicating that individual cortisol levels are highly stable

Table 1
Demographics of participant population.

	G/G	A Carrier
n	49	43
Age	57.43 ± (0.6)	57.70 ± (0.6)
% Female	59.2	44.2
% African American	36.7	23.3
% Hispanic	26.5	32.6
Education (in years)	14.17 ± (0.5)	13.33 ± (0.4)
Income (in thousands $)	68.99 ± (71.3)	71.61 ± (84.6)

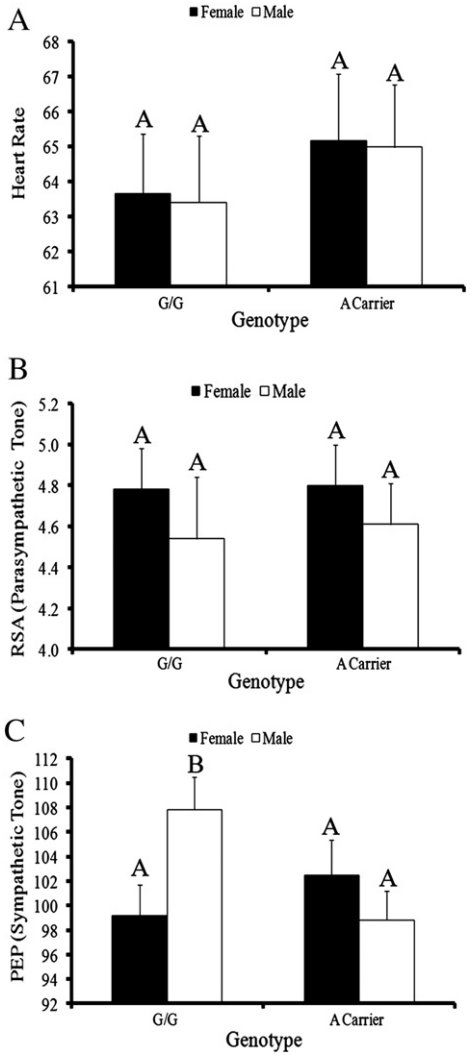

Fig. 1. Resting cardiovascular measures across genotype and sex. (A) No significant sex or genotype differences were found for HR or (B) RSA. (C) A significant gene × sex effect was detected for PEP reflecting the fact that G/G males displayed higher PEP (lower sympathetic tone) values than all other groups while G/G females had comparable PEP values to that of A carrier females. Data are presented as mean ± S.E.M. Different letters represent statically significant differences ($p<0.05$). HR = heart rate in beats per minute, RSA = respiratory sinus arrhythmia in milliseconds^2, PEP = pre-ejection period in milliseconds.

across different days. In Model 2, we modeled linear changes in cortisol over the course of one day. As expected, the slope was negative and significantly different from zero ($b=-.22$, $t_{349}=-11.18$, $p<.01$; Table 3), indicating that cortisol levels tend to decrease over the day. No effects for age, sex, or ethnicity were detected ($p>0.05$).

In Models 3 to 5, we examined the influence of the OT receptor genes on the diurnal trajectories. The results for these models are reported in Table 3. The OT receptor gene had no effect on the overall level of cortisol (Model 3, Table 3), but it significantly moderated the diurnal trajectory of cortisol (Model 4, Table 3). Specifically, A carriers displayed a relatively larger diurnal variation over the course of the day as compared to G/G participants (Fig. 3, Table 3). Additionally, A carriers display significantly higher levels of cortisol upon awakening (Fig. 3, Table 3). Controlling for age, sex, and ethnicity reduced the differences in awakening cortisol levels but did not change the association between receptor variation and the slopes (Model 5; Table 3).

4. Discussion

The results of this study demonstrate that genetic variation in the OT receptor is associated with alterations in sympathetic cardiac control as well as alterations in HPA axis functioning in a population based sample of older adults. These data build upon and extend previous animal and human research utilizing pharmacological manipulations to demonstrate the association between the OT system and metabolic processes. Furthermore, these data provide the first investigation into the relationship between the OT receptor system and the specific contributions of the parasympathetic and sympathetic branches of the ANS to cardiovascular processes across basal and psychological stress conditions. The results of the present data are consistent with previous reports demonstrating elevations in autonomic cardiac control following intranasal oxytocin administration (Norman et al., 2010). These data also suggest that variations in the OT receptor impact HPA axis activity. Given the key role of OT signaling in the processing of social information, such results imply that social psychological processes may play a significant role in structuring the overall activity of peripheral neural and endocrine systems.

The data presented in this study determined that G/G individuals have significantly higher sympathetic cardiac reactivity in response to a psychological stressor, which is consistent with a recent study reporting elevated heart rate reactivity in non-depressed G/G participants (Riem et al., 2011). However, these data are somewhat in contrast with a previous report suggesting A allele carriers display heightened startle reflex (Rodrigues et al., 2009). However, we

Table 2
Mean ± SEM across stress conditions across sex and genotype. HR = heart rate measured in beats per minute, PEP = pre-ejection period measured in milliseconds, RSA = respiratory sinus arrhythmia measured in milliseconds^2, SV = stroke volume measured in milliliters, CO = cardiac output measured in liters per minute.

	G/G			A Carrier					
	Female	Male	Overall	Female	Male	Overall	Time	Time × Gene	Time × Gene × Gender
HR Baseline	64.43 ± (2.1)	67.36 ± (2.4)	65.89 ± (1.6)	65.59 ± (2.6)	65.38 ± (2.2)	65.49 ± (1.7)	$F_{2,168}=154.22$, $p<0.01$	$F_{2,168}=1.40$, $p=0.25$	$F_{2,168}=1.18$, $p=0.31$
HR Stress	72.28 ± (1.9)	76.84 ± (2.3)	74.56 ± (1.5)	73.21 ± (2.5)	72.09 ± (2.1)	72.65 ± (1.6)			
HR Recovery	67.19 ± (1.9)	71.58 ± (2.3)	69.38 ± (1.5)	68.94 ± (2.5)	67.93 ± (2.1)	68.44 ± (1.6)			
PEP Baseline	99.46 ± (1.9)	109.57 ± (2.3)	104.52 ± (1.5)	101.96 ± (2.4)	102.69 ± (2.1)	102.32 ± (1.6)	$F_{2,176}=8.45$, $p<0.01$	$F_{2,176}=3.26$, $p=0.04$	$F_{2,168}=3.81$, $p=0.02$
PEP Stress	97.85 ± (1.8)	105.15 ± (2.2)	102.00 ± (1.4)	101.41 ± (2.2)	102.43 ± (2.0)	101.92 ± (1.5)			
PEP Recovery	98.25 ± (1.8)	106.08 ± (2.2)	102.65 ± (1.4)	100.33 ± (2.2)	101.77 ± (2.0)	101.05 ± (1.5)			
RSA Baseline	4.74 ± (0.2)	4.63 ± (0.3)	4.69 ± (0.2)	4.95 ± (0.3)	4.34 ± (0.2)	4.64 ± (0.2)	$F_{2,164}=22.91$, $p<0.01$	$F_{2,164}=0.18$, $p=0.83$	$F_{2,164}=1.89$, $p=0.15$
RSA Stress	4.19 ± (0.2)	4.08 ± (0.3)	4.14 ± (0.2)	3.99 ± (0.3)	4.01 ± (0.3)	4.00 ± (0.2)			
RSA Recovery	4.52 ± (0.2)	4.24 ± (0.3)	4.38 ± (0.2)	4.34 ± (0.3)	4.11 ± (0.2)	4.24 ± (0.2)			
SV Baseline	69.72 ± (4.8)	75.08 ± (5.5)	72.40 ± (3.6)	75.24 ± (5.8)	79.38 ± (5.0)	77.31 ± (3.8)	$F_{2,168}=5.76$, $p<0.01$	$F_{2,168}=3.29$, $p=0.04$	$F_{2,168}=0.45$, $p=0.64$
SV Stress	75.04 ± (4.7)	81.70 ± (5.5)	78.37 ± (3.6)	74.27 ± (5.8)	82.67 ± (5.0)	78.47 ± (3.8)			
SV Recovery	73.35 ± (5.0)	77.83 ± (5.8)	75.59 ± (3.8)	74.57 ± (5.8)	81.05 ± (5.3)	77.81 ± (4.0)			
CO Baseline	4.44 ± (0.3)	5.01 ± (0.3)	4.72 ± (0.2)	4.75 ± (0.4)	5.04 ± (0.3)	4.90 ± (0.3)	$F_{2,168}=63.20$, $p<0.01$	$F_{2,168}=4.35$, $p=0.01$	$F_{2,168}=0.13$, $p=0.87$
CO Stress	5.42 ± (0.3)	6.18 ± (0.4)	5.80 ± (0.2)	5.23 ± (0.4)	5.85 ± (0.3)	5.54 ± (0.3)			
CO Recovery	4.84 ± (0.3)	5.50 ± (0.4)	5.17 ± (0.3)	4.84 ± (0.4)	5.40 ± (0.3)	5.17 ± (0.3)			

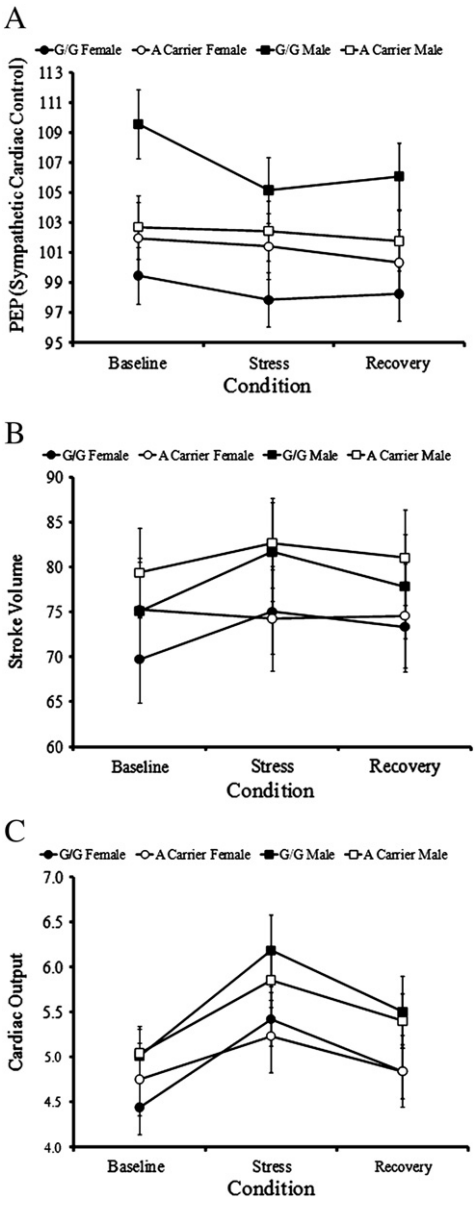

Fig. 2. Cardiovascular responses mental arithmetic stress (A) A significant time × genotype interaction was detected for PEP reflecting significantly elevated sympathetic nervous system function (lower PEP) across both stress and recovery conditions in G/G participants. Additionally, a significant time × genotype × sex interaction was detected for PEP reflecting the fact that male G/G participants displayed significantly elevated sympathetic reactivity to stress while G/G women showed comparable responses to A carrier females. (B) A significant time × genotype effect for SV reflecting the fact that G/G participants generally displayed heighted SV to the mental arithmetic task. (C) Similar to SV, A significant time × genotype effect for CO reflecting the fact that G/G participants generally displayed heighted CO to the mental arithmetic task. Data are presented as mean ± S.E.M. See Table 2 for statistics. PEP = pre-ejection period measured in milliseconds, SV = stroke volume measured in milliliters, CO = cardiac output measured in liters per minute.

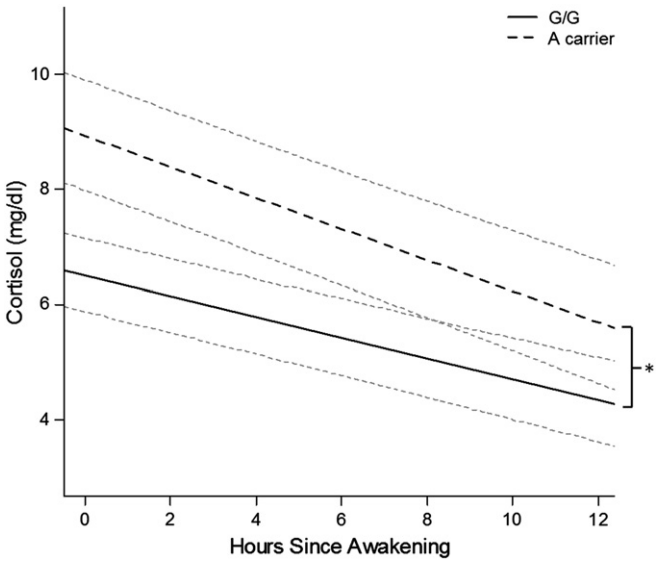

Fig. 3. Diurnal variation in cortisol as a function of OT receptor SNP. G/G participants displayed lower levels of cortisol upon awakening and displayed less diurnal variation throughout the day. Values are based on the Model 4 analysis discussed in the methods section. The small dashed lines represent standard error. * denotes statistically significant difference ($p<0.05$).

believe there are numerous factors which contribute to the apparent discrepancy. Firstly, whereas the Rodrigues et al. study utilized a startle reaction paradigm to test reactivity, our study utilized a mental arithmetic psychological stress paradigm. Psychological stressors commonly elicit distinct health-relevant cardiodynamic and HPA axis activity profiles in contrast to more traditional stress induction methods such as the cold pressor task or the startle reflex (Berntson et al., 1994). Secondly, the Rodrigues et al. study used heart rate as an indicator of stress reactivity which provides an important endpoint measure of cardiac function, but does not allow for conclusions regarding the contribution of sympathetic and parasympathetic cardiac control. Given the fact that heart rate can change through any number of combinations of sympathetic and parasympathetic patterns of activity, it only provide limited information onto the potential mechanisms and implications underlying OT receptor variation and hemodynamic processes. Finally, the sample used in Rodrigues et al., was comprised of university aged participants while this study utilized a population based sample of older individuals, and previous data have clearly demonstrated that ageing results in dramatic changes in numerous cardiodynamic processes (Kaye and Esler, 2008).

The finding that naturally occurring variation in the OT receptor influences HPA axis and autonomic activity in older individuals provides a further mechanism through which the OT system may influence health outcomes. Specifically, the findings of heightened sympathetic cardiac control and blunted HPA axis diurnal variation in G/G individuals are of particular interest. Indeed, sympathetic nervous system activity has been shown to be positively associated with morbidity and mortality,

Table 3
Slope and intercept values for diurnal variation in cortisol across genotype.

Effect	Model 3					Model 4					Model 5				
	Coef.	*SE*	*t*	*df*	*p*	Coef.	*SE*	*t*	*df*	*p*	Coef.	*SE*	*t*	*df*	*p*
Intercept	6.95	0.54	12.95	72	<0.001	6.51	0.64	10.17	72	<0.001	7.84	0.80	9.78	68	<0.001
Time	−0.22	0.03	−8.45	349	<0.001	−0.18	0.03	−6.93	348	<0.001	−0.18	0.03	−6.86	348	<0.001
Genotye	1.41	0.86	1.64	72	0.105	2.43	0.96	2.54	72	0.013	1.83	0.95	1.93	68	0.058
Genotye × Time						−0.09	0.04	−2.30	348	0.022	−0.09	0.04	−2.34	348	0.020

especially in relation to cardiovascular disease (Airaksinen, 1999; Billman, 2006; Hohnloser, 2005). Similarly, alterations in the diurnal rhythm of HPA axis function are related to clinical depression and all cause mortality (Keller et al., 2006; Marklund et al., 2004). Future studies with larger sample sizes will be necessary in order to better address the causal influences of OT receptor variation on health outcomes.

There are a number of limitations of the present study. Firstly, the relatively low sample size necessitates that the gender differences presented in this study be interpreted with caution. Furthermore, this study only evaluated one of the many possible oxytocin receptor SNP's and future studies will be necessary in order to determine if the findings presented here extend to other SNP's. The fact that the distribution of the A alleles was not uniform across gender and ethnicity deserves further investigation and while we statistically controlled for these variables in all analysis, future studies will need to evaluate the potential moderational effects that gender and ethnicity may play in oxytocin receptor SNP's and their influence on physiology.

In conclusion, these data indicate that genetic variation in the OT receptor system may play a significant role in structuring basal and stress-responsive activity of the sympathetic and parasympathetic nervous system and diurnal fluctuation in HPA axis function in older adults. When combined with the existing literature on the effects of OT on cardiovascular and HPA axis function, these findings provide further evidence of the important role OT plays in physiological and psychological processes.

References

Airaksinen, K.E., 1999. Autonomic mechanisms and sudden death after abrupt coronary occlusion. Ann. Med. 31, 240–245.

Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2008. Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. Soc. Cogn. Affect. Neurosci. 3, 128–134.

Bartz, J.A., Zaki, J., Ochsner, K.N., Bolger, N., Kolevzon, A., Ludwig, N., et al., 2010. Oxytocin selectively improves empathic accuracy. Psychol. Sci. 21, 1426–1428.

Bartz, J.A., Zaki, J., Bolger, N., Ochsner, K.N., 2011. Social effects of oxytocin in humans: Context and person matter. Trends in Cognitive Sciences 15, 301–309.

Berntson, G.G., Cacioppo, J.T., Binkley, P.F., Uchino, B.N., Quigley, K.S., Fieldstone, A., 1994. Autonomic cardiac control: III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. Psychophysiology 31, 599–608.

Berntson, G.G., Bigger Jr., J.T., Eckberg, D.L., Grossman, P., Kaufmann, P.G., Malik, M., et al., 1997. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 34, 623–648.

Billman, G.E., 2006. A comprehensive review and analysis of 25 years of data from an in vivo canine model of sudden cardiac death: implications for future anti-arrhythmic drug development. Pharmacol. Ther. 111, 808–835.

Carter, C.S., Grippo, A.J., Pournajafi-Nazarloo, H., Ruscio, M.G., Porges, S.W., 2008. Oxytocin, vasopressin and sociality. Prog. Brain Res. 170, 331–336.

Cole, S.W., Arevalo, J.M.G., Takahashi, R., Sloan, E.K., Lutgendorf, S.K., Sood, A.K., et al., 2010. Computational identification of gene-social environment interaction at the human IL6 locus. Proc. Natl. Acad. Sci. U. S. A. 107, 5681–5686.

Costa, B., Pini, S., Gabelloni, P., Abelli, M., Lari, L., et al., 2009. Oxytocin receptor polymorphism and adult attachment style in patients with depression. Psychoneuroendocrino 34, 1506–1514.

DeVries, A.C., Craft, T.K., Glasper, E.R., Neigh, G.N., Alexander, J.K., 2007. 2006 Curt P. Richter Award winner: social influences on stress responses and health. Psychoneuroendocrinology 326, 587–603.

Gamer, M., Buchel, C., 2012. Oxytocin specifically enhances valence-dependent parasympathetic responses. Psychoneuroendocrinology 37 (1), 87–93.

Gilbey, M.P., Coote, J.H., Fleetwood-Walker, S., Peterson, D.F., 1982. The influence of the paraventriculo-spinal pathway and oxytocin and vasopressin on sympathetic preganglionic neurons. Brain Res. 251, 283–290.

Gillath, O., Shaver, P.R., Baek, J.M., Chun, D.S., 2008 Octt. Genetic correlates of adult attachmentstyle. Pers. Soc. Psychol. Bull. 34 (10), 1396–1405.

Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: structure, function, and regulation. Physiol. Rev. 81, 629–683.

Grippo, A.J., Trahanas, D.M., Zimmerman II, R.R., Porges, S.W., Carter, C.S., 2009. Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. Psychoneuroendocrinology 34, 1542–1553.

Guastella, A., Mitchell, P., Mathews, F., 2008. Oxytocin enhances the encoding of positive social memories in humans. Biol. Psychiatry 64, 256–258.

Heinrichs, M., Baumgartner, T., Kirschbaum, C., Ehlert, U., 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol. Psychiatry 54, 1389–1398.

Higa, K.T., Mori, E., Viana, F.F., Morris, M., Michelini, L.C., 2002. Baroreflex control of heart rate by oxytocin in the solitary–vagal complex. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282, R537–R545.

Hohnloser, S.H., 2005. Ventricular arrhythmias: Antiadrenergic therapy for the patient with coronary artery disease. Journal of Cardiovascular Pharmacology and Therapeutics, 10 (Suppl 1), S23–31.

Hox, J.J., 2010. Multilevel Analysis: Techniques and Applications, 2nd ed. Lawrence Erlbaum, Mahwah.

Kaye, D., Esler, M.D., 2008. Autonomic control of the aging heart. Neuromolecular Med. 10, 179–186.

Keller, J., Flores, B., Gomez, R.G., et al., 2006. Cortisol circadian rhythm alterations in psychotic major depression. Biol. Psychiatry 60, 275–281.

Kemp, A.H., Guastella, A.J., 2011. The role of oxytocin in human affect : a novel hypothesis. Curr. Dir. Psychol. Sci. 204, 222–231.

Kim, H.S., et al., 2010. Culture, distress, and oxytocin receptor polymorphism (OXTR)interact to influence emotional support seeking. Proc. Natl. Acad. Sci. U. S. A. 107, 15717–15721.

Kosfeld, M., Heinrichs, M., Zak, P.J., Fischbacher, U., Fehr, E., 2005. Oxytocin increases trust in humans. Nature 435, 673–676.

Lucht, M., Barnow, S., Sonnenfeld, C., et al., 2009. Associations between the oxytocin receptor gene OXTR and affect, loneliness and intelligence in normal subjects. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 860–866.

Marklund, N., Peltonen, M., Nilsson, T.K., Olsson, T., 2004. Low and high circulating cortisol levels predict mortality and cognitive dysfunction early after stroke. J. Intern. Med. 256, 15–21.

McEwen, B.S., 2007. The physiology and neurobiology of stress and adaptation, Central role of the brain. Physiol. Rev. 87, 873–904.

Norman, G.J., Cacioppo, J.T., Morris, J.S., Malarkey, W.B., Berntson, G.G., Devries, A.C., 2010. Oxytocin increases autonomic cardiac control: moderation by loneliness. Biol. Psychol. 863, 174–180.

Pardini, B.J., Lund, D.D., Schmid, P.G., 1989. Organization of the sympathetic postganglionic innervation of the rat heart. J. Auton. Nerv. Syst. 28 (3), 193–201.

Riem, M.M.E., Pieper, S., Out, D., Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2011. Oxytocin receptor gene and depressive symptoms associated with physiological reactivity to infant crying. Soc. Cogn. Affect. Neurosci. 6, 294–300.

Rodrigues, S.M., Saslow, L.R., Garcia, N., John, O.P., Keltner, D., 2009. Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. Proc. Natl. Acad. Sci. U. S. A. 106, 21437–21441.

Saphire-Bernstein, S., Way, B.M., Kim, H.S., Sherman, D.K., Taylor, S.E., 2011. Oxytocin receptor gene (OXTR) is related to psychological resources. Proc. Natl. Acad. Sci. U. S. A. 108 (37), 15118–15122 Sep 13.

Sherwood, A., Allen, M.T., Fahrenberg, J., Kelsey, R.M., Lovallo, W.R., van Doornen, L.J., 1990. Methodological guidelines for impedance cardiography. Psychophysiology 27 (1), 1–23.

Singer, J.D., Willett, J.B., 2003. Applied longitudinal data analysis. Modeling change and event occurence. Oxford, New York.

Tost, H., Kolachana, B., Hakimi, S., Lemaitre, H., Verchinski, B.A., Mattay, V.S., et al., 2010. A common allele in the oxytocin receptor gene OXTR impacts prosocial temperament and human hypothalamic-limbic structure and function. Proc. Natl. Acad. Sci. U. S. A. 10731, 13936–13941.

Uvnas-Moberg, K., 1998. Oxytocin may mediate the benefits of positive social interaction and emotions. Psychoneuroendocrinology 238, 819–835.

Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology 138, 2829–2834.

Young, L.J., Wang, Z., Donaldson, R., Rissman, E.F., 1998. Estrogen receptor alpha is essential for induction of oxytocin receptor by estrogen. Neuroreport 9, 933–936.

Xu, X., Roman, J.M., Issaq, H.J., Keefer, L.K., Veenstra, T.D., Ziegler, R.G., 2007. Quantitative measurement of endogenous estrogens and estrogen metabolites in human serum by liquid chromatography-tandem mass spectrometry. Anal. Chem. 79, 7813–7821.