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Methylation of the oxytocin receptor gene mediates the effect of adversity on negative schemas and depression

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Abstract

Building upon various lines of research, we posited that methylation of the oxytocin receptor gene (*OXTR*) would mediate the effect of adult adversity on increased commitment to negative schemas and in turn the development of depression. We tested our model using structural equation modeling and longitudinal data from a sample of 100 middle-aged, African American women. The results provided strong support for the model. Analysis of the 12 CpG sites available for the promoter region of the *OXTR* gene identified four factors. One of these factors was related to the study variables, whereas the others were not. This factor mediated the effect of adult adversity on schemas relating to pessimism and distrust, and these schemas, in turn, mediated the impact of *OXTR* methylation on depression. All indirect effects were statistically significant, and they remained significant after controlling for childhood trauma, age, romantic relationship status, individual differences in cell types, and average level of genome-wide methylation. These finding suggest that epigenetic regulation of the oxytocin system may be a mechanism whereby the negative cognitions central to depression become biologically embedded.

Major depression is highly prevalent in Western countries, showing a lifetime prevalence rate of 17% in the United States (Kessler et al., 1994, 2005). Although our knowledge of this disorder has grown dramatically, it has become evident that further advances will require more complex models that integrate multiple levels of human functioning (Clark & Beck, 2010). Decades of research has established that depressed persons tend to have a history of adversity that has given rise to a pessimistic cognitive bias that colors the way they process and orient to everyday events (Beck, 2008; Gonzalol, Kleim, Donaldson, Moorey, & Ehlers, 2012; Liu & Bates, 2014; Romens, Abramson, & Alloy, 2009). A profusion of studies has also shown that psychotherapeutic interventions that target these negative cognitions are an effective treatment for depression (Bulter, Chapman, Forman, & Beck, 2006; Hollon, Steward, & Strunk, 2006).

Researchers have documented the neurobiological concomitants of these pessimistic schemas more recently. These studies show that pessimistic cognitive styles tend to be associated with rather distinct neurological patterns (Clark & Beck, 2010; Disner, Beevers, Haigh, & Beck, 2011; Herwig et al., 2010) and that these patterns become more similar to those manifested by nondepressed individuals following psychotherapeutic treatment of these cognitive biases (Beauregard, 2014; Thomaes et al., 2014). Biologically, changes in neurological patterns may be influenced by shifts in epigenetic regulation (Kandel, 2006; Molfese, 2011).

Epigenetic regulation entails biochemical mechanisms that influence the genome to express (upregulate or downregulate) particular genes (Carey, 2012; Haig, 2012). Epigenetic factors appear to be an important mechanism whereby life experiences become biologically embedded and provide the physiological underpinnings for cognitive, emotional, and behavioral traits tailored for adaptation to environmental demands circumstances (Landecker & Panofsky, 2013; Meaney & Szyf, 2005; Meloni, 2014). Building on this idea, the current study investigates the extent to which the impact of environmental adversity on the development of a pessimistic cognitive style is mediated by epigenetic regulation of the oxytocin system. This idea is developed in the sections to follow. We then test our hypotheses using structural equation modeling and longitudinal data from a sample of middle-aged African American women.

Cognitive Schemas and Depression

The most empirically supported theories of depression emphasize the role of pessimistic cognitive schemas. Aaron Beck (1967, 2008) popularized this perspective in the late 1960s with his contention that depressed persons suffer from a negative cognitive triad consisting of a pessimistic

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view of themselves, their environment, and the future. Later, Abramson, Seligman, and Teasdale (1978) and Abramson, Metalsky, and Alloy (1989) gave impetus to this approach by recasting it using attribution theory. Although these two traditions differ in some respects, they agree that a negative cognitive bias leads individuals to process the vicissitudes of everyday life in a manner that leads to depression. Both camps also agree that these pessimistic schemas usually develop in response to chronic exposure to adverse circumstances, especially those occurring during childhood. Consistent with this general paradigm, a number of studies have confirmed associations between adversity, pessimistic cognitions, and depression (Alloy et al., 2006; Clark & Beck, 2010; Gibb et al., 2001). There is also strong evidence that these cognitive biases can be changed. Hundreds of studies have shown that psychotherapeutic interventions can modify these cognitive styles with the result being a lifting of depression (Butler et al., 2006; Hollon et al., 2006).

Several studies have recently investigated the neurological patterns associated with depression. Consistent with the idea that depression is rooted in a pessimistic orientation, Herwig et al. (2010) found that depressed persons show a neurological response to neutral stimuli that resembles the neurological pattern that both they and nondepressed individuals demonstrate in response to stimuli with a negative valence. In general, neurological studies have found that depressed individuals manifest neurological patterns (especially in limbic and prefrontal cortex structures) that are consistent with a pessimistic bias (Disner et al., 2011). Further, a variety of investigations have reported that psychotherapeutic interventions that target patients' negative cognitions tend to result in both a lifting of depression and a normalization of neurological patterns (Beauregard, 2014; DeRubeis, Webb, Tang, & Beck, 2010).

Overall, this set of findings is consistent with a model of depression where adversity fosters a pessimistic orientation that is sustained by particular underlying neurological mechanisms. This change in biological and cognitive structure, in turn, increases the chances of depression. However, new experiences (including psychotherapy) can change this negative cognitive schema and its supporting neurological pattern, with the result being a reduced risk for depression.

The present paper elaborates on this general model by adding epigenetics. Although most gene regulation is a response to the immediate demands of the environment, epigenetics focuses on changes in gene expression that last for months, years, or even a lifetime (Carey, 2012; Francis, 2011). Epigenetic regulation entails biochemical mechanisms that influence the genome to express (upregulate or downregulate) particular genes. This occurs when a methyl group attaches to a CpG segment of DNA (Carey, 2012; Francis, 2011). The effect of methylation, especially when it occurs in the promoter region of a gene, is to inhibit gene expression. For our purposes, there is rapidly accumulating evidence regarding the role of epigenetic factors in neural development. Studies have shown, for example, that epigenetic modification (e.g., methylation) of particular genes influences perception, emotion, memory, cognition, learning, and neural plasticity (Kandel, 2006; Molfese, 2011). In other words, there is reason to believe that epigenetic changes are part of the process whereby adverse experiences and a pessimistic cognitive structure become biologically embedded. In the following section, we argue that these epigenetic changes likely include DNA methylation of the oxytocin receptor gene (*OXTR*).

The *OXTR* Gene

The neuropeptide oxytocin is a peripheral hormone and central neuromodulator that has been shown to influence a number of social and affective responses. For our purposes, studies indicate that intranasally administered oxytocin tends to increase optimism, trust, generosity, and empathy (Baumgartner, Heinrichs, Vonlanthen, Fishbacker, & Fehr, 2008; Kosfeld, Heinrichs, Zak, Fischbacker, & Fehr, 2005; MacDonald & MacDonald, 2010; Zak, 2012). Further, it reduces anxiety while enhancing emotional control and the probability that threatening situations will be perceived as challenges rather than as stressors (Kubzansky, Mendes, Appleton, Block, & Adler, 2012). Research suggests that these cognitive and emotional responses are supported biologically by reducing cortisol, decreasing activation of the amygdala, and augmenting connectivity between the amygdala and brain structures supporting affective control (Cardoso, Ellenbogen, Orlando, Bacon, & Joober, 2012; Gamer, Zurowski, & Buchel, 2010, Kirsch et al., 2005; Puglia, Lillard, Morris, & Connelly, 2015).

These findings suggest that oxytocin fosters a cognitive and emotional orientation that is the obverse of the pessimistic, distrusting cognitive schemas displayed by depressed individuals. In addition, the biological consequences of administering oxytocin largely appear to be counter to the biological concomitants of depression. These observations support the hypothesis that depressed persons suffer from low levels of oxytocin and that oxytocin might be administered to treat their disorder. Contrary to this idea, however, research investigating the link between plasma oxytocin levels and various social and emotional responses has produced mixed results (McCullough, Churchland, & Mendez, 2013). Further, when oxytocin is administered to individuals who experienced love withdrawal or rejection as children, they do not demonstrate the prosocial changes (e.g., trust, empathy, and generosity) described above (Huffmeijer et al., 2013; Olff et al., 2013). Similarly, intranasal administration of oxytocin often produces little effect when given to psychiatric patients (Bertsch, Schmidinger, Neumann, & Herpertz, 2012; Olff et al., 2013).

However, it is quite possible that these puzzling findings are explained by epigenetic markers within the oxytocin system that regulate the impact of exogenously administered oxytocin (Kumsta, Hummel, Chen, & Heinrichs, 2013; Puglia et al., 2015). To achieve its effect, the oxytocin molecule must be able to bind to the *OXTR* gene. To the extent that this gene is highly methylated (turned off), it becomes increasingly difficult for circulating oxytocin to bind, with the result that increased levels of oxytocin will have little downstream

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effect. Building upon research showing that environmental context can influence epigenetic effects (Beach, Lei, Brody, Dogan, & Philibert, 2016, in press; Simons et al., 2016), we expect that chronic exposure to adversity fosters methylation of *OXTR*. This would reduce responsiveness to oxytocin, thereby fostering a cold, guarded orientation when interacting with others (supported biologically by increased cortisol production, greater activation of the amygdala, etc.). Such calibration of *OXTR* would be functional from an evolutionary perspective because it prepares the individual for a hostile, unpredictable environment where others are not trustworthy (Simons & Klopack, 2015). Cognitively, this orientation would be similar to the negative cognitive schemas that place a person at risk for depression.

Methylation of *OXTR*

The extent to which a gene is expressed is controlled in large measure by its promoter region. The promoter region is usually located on the gene's first exon and is a segment of DNA that initiates transcription of the gene. DNA methylation of the promoter region of *OXTR* varies within the population (Jack, Connelly, & Morris, 2012), and this variation has been linked to reduced transcription of the gene (Gregory et al., 2009; Kusui et al., 2001). High methylation of the *OXTR* promoter has been linked to particular neurological patterns (Jack et al., 2012). A functional magnetic resonance imaging study by Puglia, Lillard, Morris, and Connelly, (2015), for example, found that methylation in the promoter region of *OXTR* is associated with greater responsiveness to negative stimuli in several brain regions crucial for social and emotional processing (e.g., amygdala, fusiform, lateral occipital cortex, and insular cortex). These individuals also showed less amygdala coupling, a pattern associated with reduced habituation and emotion regulation.

Further, methylation of various sites in the *OXTR* promoter have been associated with behavioral phenotypes such as callous–unemotional traits (Cecil et al., 2014; Dadds et al., 2014), autism (Gregory et al., 2009), and anorexia nervosa (Kim, Kim, Kim, & Treasure, 2014). Although these phenotypes have in common characteristics suggestive of a compromised oxytocin system (e.g., low empathy, distrust, and estrangement from others), they also entail quite different forms of psychopathology and problem behavior. However, while most of these studies have focused on methylation within the *OXTR* promoter, the specific sites found to be significant vary across phenotypes. The sites found to be significant for callous–unemotional traits (Cecil et al., 2014), for example, differ from those that have been linked to autism (Gregory et al., 2009). It is possible that these sites of *OXTR* that vary independently perform somewhat different functions and so may be implicated in different disorders. At a minimum, it appears that methylation patterns in this region reflect several different orthogonal processes. In the present study, we investigate the extent to which there are methylated sites within the *OXTR* promoter that are related to the pessimistic and distrustful schemas that place a person at risk for depression. We expect that these methylated sites likely differ somewhat from those that prior research has linked to phenotypes such as autism or calloused–unemotional traits.

The Model to Be Tested

Based on the arguments presented in the previous sections, we test the general model shown in Figure 1. Our measure of adult adversity is a composite that focuses on two chronically stressful conditions (financial pressure and living in a high-crime area) assessed over a 4-year period. Past research (see Thoits, 2010) has provided strong evidence that such conditions predict depression. Further, given the profusion of studies linking negative cognitions to depression (Alloy et al., 2006; Beck, 2008; Clark & Beck, 2010; Gibb et al., 2001), we expect that much of the effect of adult adversity on depression is explained by development of a more negative cognitive style. Given the hundreds of studies reporting that relatively brief psychotherapeutic interventions can temper a negative cognitive bias, we expect that persistent exposure to adversity likely augments commitment to such an orientation. We include two types of negative cognitions: pessimism (a negative schema regarding the future) and distrust (a negative schema regarding others). We expect that the each of these schemas will mediate a portion of the effect of adversity on depression. Because of its strong overlap with depression, and its more tenuous connection with oxytocin, we did not include a negative view of self among the hypothesized mediators. However, the really unique aspect of our model is the prediction that much of the effect of adversity on cognitive change is indirect and can be explained by methylation of *OXTR*. Note that we do not expect *OXTR* to directly impact depression. Rather, in our view, methylation of the oxytocin promoter provides biological support for distrusting and pessimistic schemas. It is these schemas that in turn elevate the probability of negative mood and depression.

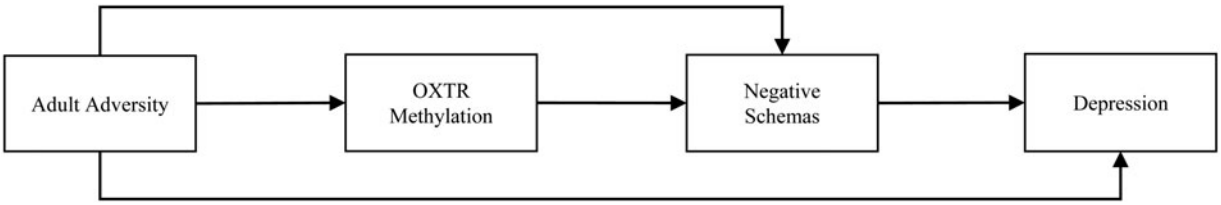

Figure 1. The general model.

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Overall, our model indicates several mediating effects. Specifically, it predicts (a) a significant indirect effect of adversity on pessimism and distrust through methylation of *OXTR*; (b) a significant indirect effect of *OXTR* on depression through pessimism and distrust; and (c) a significant indirect effect of adversity on depression through methylation of *OXTR* and the two negative schemas: pessimism and distrust. To strengthen the test of the causal arguments assumed by our general model, we use longitudinal data that allows us to assess change in our two hypothesized cognitive mediators as well as change in depression. Unfortunately, *OXTR* methylation was assessed at only one time point.

Method

Participants

We tested our hypotheses using data from Waves 2, 3, 4, and 5 for 100 of the primary caregivers in the Family and Community Health Study (FACHS). FACHS is a longitudinal study of several hundred African American families that was initiated in 1997. Using a stratified random sampling procedure, the sampling strategy was intentionally designed to generate families representing a range of socioeconomic statuses and neighborhood settings. The details regarding recruitment are described by Gibbons, Gerrard, Cleveland, Wills, and Brody (2004) and Simons and colleagues (2011). The first wave of FACHS data was collected in 1997–1998 from 889 African American children and their primary caregivers (PCs; 829 women, 60 men). At study inception, about half of the sample resided in Georgia ($n = 467$) and the other half in Iowa ($n = 422$). All of the children were in the fifth grade at the beginning of the study. At Wave 1, 36% of the families were below the poverty line, and 51% of the PCs identified as single parents. The third, fourth, and fifth waves of data were collected from 2001 to 2002, 2004 to 2005, and 2007 to 2008, respectively. Of the 889 PCs interviewed at Wave 1, 693 were interviewed again at Wave 5 (77.26% of the original sample).

At Wave 5, 100 African American women were randomly selected from the roster of PCs to participate in an epigenetic assessment. Costs associated with the blood draws and epigenetic assays necessitated the drawing of a subsample. Mean chronological age for the group was 48.52 years (SD = 9.30), 18% had less than a 12th-grade education, and 25% were married. The majority (68.5%) lived in the inner city, 12.2% lived in the suburbs, and 19.3 lived in rural areas. All 100 respondents are of African ancestry. Comparisons with participants who did not provide methylation status or complete all study measures did not reveal any significant differences on demographic variables and outcome.

Procedures

At each wave, computer-assisted interviews were administered in the respondent's home and took on average about 2 hr to complete. The instruments were presented on laptop computers. Questions appeared in sequence on the screen, which both the researcher and the participant could see. The researcher read each question aloud, and the participant entered an anonymous response using a separate keypad. In addition, participants were also asked to provide a blood sample at Wave 5. A certified phlebotomist drew four tubes of blood (30 ml) from each participant and shipped the sample the same day to a laboratory at the University of Iowa for preparation. Typical DNA yield for each pellet was between 10 and 15 mg of DNA.

Measures

Distrust. We assessed distrust at Waves 4 and 5 using seven items adapted from the scale Experiences in Close Relationships—Revised (Fraley, Waller, & Brennan, 2000). The instrument asks respondents to report the extent to which they agree (1 = *strongly disagree*, 7 = *strongly agree*) with items such as "I do not like people getting too close to me" and "I find it difficult to trust others." Scores were summed across items to form a composite measure of distrust. The coefficient α was 0.70 at both waves. The correlation between waves was .41.

Pessimism. Pessimism was assessed with the five-item short form of the Life Orientation Test (Scheier & Carver, 1985). The instrument consists of items such as "If something can go wrong for me, it will," "I am always pessimistic about the future," and "I rarely count on good things happening to me." Respondents indicated their degree of agreement using a 4-point scale (1 = *strongly disagree*; 4 = *strongly agree*). The coefficient α was about 0.65 at both waves. The correlation between waves was .34.

Depression. At Waves 4 and 5, respondents completed five items from the Mini-Mood and Anxiety Symptom Questionnaire (Clark & Watson, 1997). This scale asks respondents to indicate how much of the time during the past week they had felt depressed, discouraged, hopeless, like a failure, and worthless. Potential responses range from 1 (*not at all*) to 3 (*extremely*). The cronbach α was 0.82 at both waves. The correlation between waves was .50.

Adult adversity. This construct was assessed at Waves 2, 3, and 4 using a composite measure consisting of two components: unmet material needs and neighborhood disorder. The four-item Unmet Material Needs Scale was developed by Conger and Elder (1994) and focuses on the extent to which respondents were unable during the preceding year to afford the basic necessities of life such as food, clothing, housing, and medical care. Response format for these items ranged from 1 (*strongly disagree*) to 5 (*strongly agree*). The coefficient α for this instrument was roughly 0.86 at each wave. Neighborhood crime was assessed with a revised version of the community deviance scale developed for the Project on Human Development in Chicago Neighborhoods (Sampson, Raudenbush, & Earls, 1997). The four-item mea-

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sure is concerned with how often various criminal acts occur within the respondent's community. It includes behaviors such as drinking in public, selling or using drugs, groups of teenagers hanging out on the street, and gang violence. The coefficient α ranged from 0.86 to 0.88. Scores were standardized and then summed across the two subscales to form a composite measure of adult adversity for each wave. We averaged the scores across waves.

OXTR methylation. A certified phlebotomist drew four tubes of blood (30 ml) from each participant and shipped it the same day to a laboratory at the University of Iowa for preparation. Typical DNA yield for each pellet was between 10 and 15 mg of DNA. The Illumina (San Diego, CA) 450K Human Methylation Beadchip was used to assess genome-wide DNA methylation. Samples were randomly assigned to 12 slides with groups of 8 slides being bisulfite converted in a single batch. There was no technical variability (chip/batch effects) in the current study. Four replicates of the same DNA sample were also included to monitor for slide to slide and batch bisulfite conversion variability with the average correlation coefficient between the replicate samples being .99. The resulting data were inspected for complete bisulfite conversion and average beta values for each targeted CpG residue determined using the Illumina Genome Studio Methylation Module, Version 3.2. The manufacturer recommended approach for determination of betas at each CpG site is beta value = $M/(M + U + 100)$, where M and U are raw methylated and unmethylated signal intensities, respectively. Details regarding processing and preparation of the methylation data are described by Dogan and colleagues (2014). Most research on methylation of *OXTR* has focused on a CpG island on chromosome 3 located between 8808961 and 8811280. The Illumina 450K Human Methylation Beadchip includes 12 sites from this area. The means and ranges obtained for these 12 probes are presented as online-only Supplementary materials (Table S.1).

Control variables. To avoid overestimated results, we controlled for age (mean age = 44 years), relationship status (*single* = 1, *married or cohabitating* = 0), and childhood trauma. The latter variable was assessed at Wave 1 using five retrospective items from Kessler, Davis, and Kendler (1997). Respondents reported whether they had experienced various stressful events (e.g., parental divorce or family violence) when they were growing up. Sixty-nine percent of respondents reported they had experienced at least one of these stressful events when they were growing up. The Spearman–Brown coefficient for this scale was 0.70.

Further, in addition to childhood adversity, age, and relationship status, our models controlled for average level of methylation. Adversity has been shown to increase the methylation of various genes (Beach et al., 2016, in press). By controlling for average level of methylation, we addressed the possibility that adversity had fostered a general increase in methylation among our respondents and that any association between adversity and methylation of *OXTR* in our data was merely a reflection of this general increase.

Finally, our models controlled for individual differences in cell type. Ficoll purified peripheral mononuclear cell DNA pellets of the sort used in the current investigation comprised several different cell types (CD4, CD8, CD14, CD19, and CD56; Reinius et al., 2012), leading to potentially misleading observations if individual differences in predictors are also associated with individual differences in mixture of cell types. Therefore, to account for individual differences in cell types, we utilized the regression calibration approach developed by Houseman et al. (2012). This involved identifying highly informative CpG sites by contrasting the methylation profiles associated with our five purified cell types (CD4+ T cells, CD8+ T cells, CD14+ monocytes, CD19+ B cells, and CD56+ natural killer cells). Data characterizing the full 450K methylation profile for each of the purified cell types was contributed by Reinius et al. (2012; GEO database under accession number GSE35069), and loci differentiating cell types were identified using regression in MethLAB, Version 1.5 (Kilaru, Barfild, Schroeder, Smith, & Conneely, 2012). The top 100 sites identified in this manner were selected for further analysis. These sites were the ones that were most highly differentially methylated with respect to cell type, and observations for these 100 loci from our current data set of African American women were imported into SPSS version 22 (IBM, 2012) for factor analysis. Because one of the loci was on the x chromosome, it was dropped from further consideration, and we performed a principal components analysis on the remaining 99 loci to identify factors that would characterize individual differences in cell type in the most parsimonious manner. Based on the Scree plot of this principal components analysis, the first four factors were retained and included as controls in our structural equation modeling (SEM) analyses.

Analytic strategy

SEM using Mplus 7.11 (Muthén and Muthén, 1998–2012) was employed to test our mediation hypotheses. In order to provide a stronger test of causal priorities, our SEM models include controls for earlier assessments of distrust, pessimism, and depression. This allows us to examine the impact of adversity and *OXTR* methylation on change in these variables. To assess the goodness of fit of our models, we used the chi-square test, the comparative fit index, and the Steiger root mean square error of approximation (RMSEA; Browne & Cudeck, 1992). The comparative fit index was truncated to the range of 0 to 1, and values close to 1 indicate a very good fit (Bentler, 1990). An RMSEA smaller than 0.05 indicates a close fit, whereas RMSEAs between 0.05 and 0.08 suggest a reasonable fit (Browne & Cudeck, 1992). The significance of mediation or indirect effects was tested using the bootstrapping option in Mplus. This approach uses 1,000 resamples of the data with bias corrected 95% confidence intervals (see Mallinckrodt, Abraham, Wei, & Russell, 2006).

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Results

Preliminary analysis

Following the example of others (Cecil et al., 2014; Dadds et al., 2014), factor analysis was used to reduce the 12 probes (available in the Illumina 450K Human Methylation Beadchip) in our region of interest into a smaller set of factors. As shown in Table 1, this analysis identified four factors. Follow-up analysis (see online-only supplementary Table S.2) showed that Factor 2 was significantly related to adult adversity (0.262, $p > .01$), distrust (0.213, $p > .05$), and pessimism (0.225, $p > .05$), a pattern consonant with our study hypothesis, whereas Factors 1 and 3 were not related to any of the study variables and Factor 4 was only associated with distrust. Thus, our analysis proceeded using Factor 2 as our measure of *OXTR* methylation. This factor contained four CpG sites (cg08535600, cg09353063, cg17285225, and cg23391006).

Figure 2 shows the location on *OXTR* of this factor, as well as the other three factors identified in our analysis. The figure also depicts the areas investigated in various prior studies of *OXTR* methylation. Using the approach of Cecil et al. (2014), black rectangles indicate the area of the CpG island investigated by each study, and significant CpG sites identified by these studies are shown as black circles. Studies focusing on callous–unemotional traits have found effects for sites located in our Factor 4 (Cecil et al., 2014) and in locations between our Factor 4 and our Factor 2 (Dadds et al., 2014); sites associated with autism have been located still further upstream from those included in our Factor 2 (Gregory et al., 2009); and sites correlated with anorexia nervosa (Kim et al., 2014) have been identified in the same general location as the four sites in our Factor 2.

Table 1. *Summary of exploratory factor analysis results for the OXTR CpG sites (N = 100)*

	Factors			
	I	II	III	IV
CpG sites				
cg02192228	0.957			
cg04523291	0.947			
cg15317815	0.918			
cg27501759	0.820			
cg17285225		0.873		
cg08535600		0.775		
cg23391006		0.684		
cg09353063		0.629		
cg12695586			0.911	
cg19619174			0.852	
cg03987506				0.805
cg00078085				0.780
Eigenvalues	3.860	2.847	1.272	1.072
Variance (%)	32.163	23.725	10.597	8.932

Note: Principal component analysis with varimax rotation was used. Kaiser–Meyer–Olkin (KMO) = 0.742; Bartlett's test of sphericity: $\chi^2 = 705.743$, $df = 66$, $p < .0001$.

Model testing

The correlation matrix for the study variables is presented in Table 2. The pattern of associations is in keeping with the mediation model to be tested. Adult adversity is significantly correlated with depression (.319) and both distrust (.278) and pessimism (.272). Distrust and pessimism in turn show significant associations with depression (0.454 and 0.311, respectively). Further, adult adversity is correlated .262 with *OXTR* methylation, and *OXTR* methylation is associated 0.213 and 0.225 with distrust and pessimism, respectively. With the exception of childhood adversity, all of the control variables show significant associations with at least some of the study variables, underscoring the importance of including them in our SEM.

Before testing our full SEM model including *OXTR* methylation, we tested a baseline model that assessed the indirect effects of adult adversity on change in depression as mediated through changes in distrust and pessimism. Controlling for childhood trauma, age, and relationship status, adult adversity predicted both increased distrust ($\beta = 0.277$, $p = .001$) and increased pessimism ($\beta = 0.272$, $p = .003$) that in turn predicted increased depression (distrust → depression: $\beta = 0.365$, $p < .001$; pessimism → depression: $\beta = 0.178$, $p = .026$). These indirect effects were significant, supporting the hypothesis that the adverse circumstances disproportionally suffered by African Americans promote cognitive schemas that increase the probability of depression.

Figure 3 shows the results of our full SEM including oxytocin Factor 2. Note that the model includes prior assessments of distrust, pessimism, and depression. Thus, the model focuses upon the extent to which adult adversity and methylation of *OXTR* predict changes in the cognitive schemas and depression, thereby providing a more stringent test of the assumed causal priorities. The model controls for childhood trauma, age, and relationship status. The four cell-type factors are included as covariates. The model fit indices indicate that the model provides a good fit to the data. Further, these findings remain unchanged when the average genome-wide methylation score for each respondent is included as a covariate (see online-only supplementary Figure S.2).

Consistent with the study hypotheses, the effect of adult adversity on distrust and pessimism is largely mediated by methylation of *OXTR*. In addition, the effect of *OXTR* methylation on depression is fully mediated by its association with distrust and pessimism. Table 3 summarizes the results using the bootstrapping method in MPlus with 1,000 replications to test the significance of these various indirect effects. The table shows that all of these indirect pathways are significant. This includes the path adult adversity → mOXTR → distrust → depression, as well as the path adult adversity → mOXTR2 → pessimism → depression. Overall, the results provide support for the hypothesized model.

Discussion

Epigenetic factors such as methylation appear to be one important mechanism whereby life experiences become biologically embedded and can serve as the physiological

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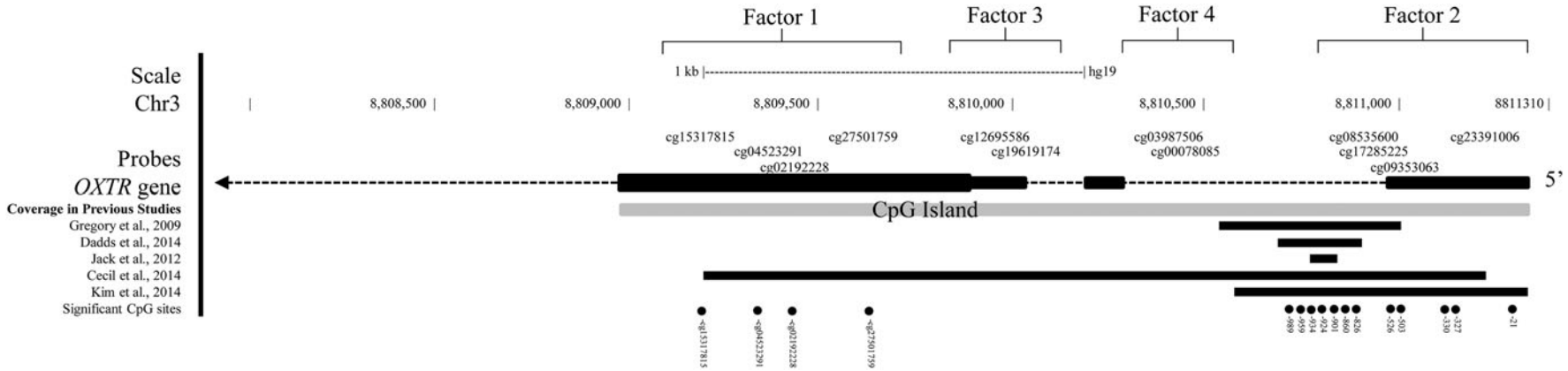
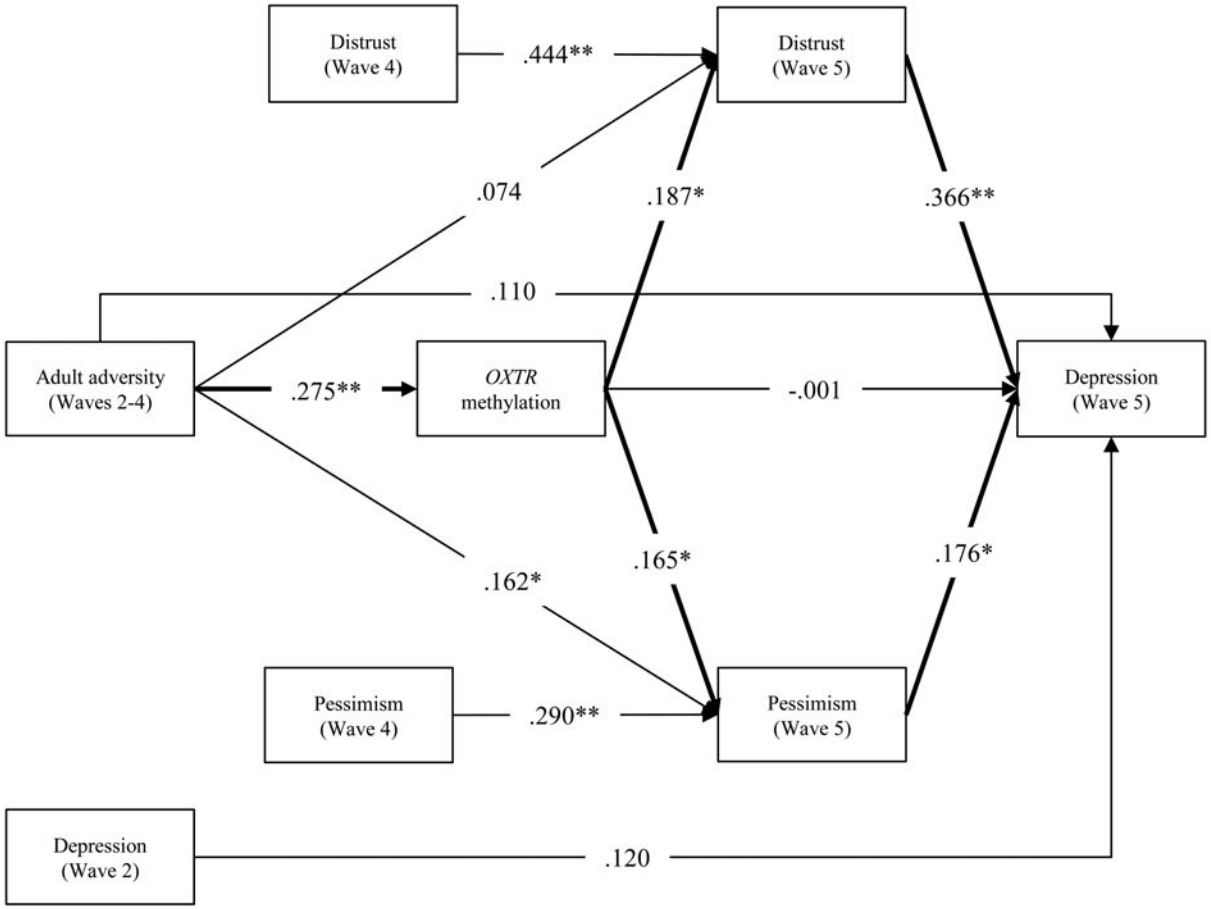


Figure 2. Location of oxytocin receptor gene (*OXTR*) methylation probes within the CpG island (hg19; chr3:8808962–8811280) included in the study, and the grouping of these CpGs into factors shown in the top of the figure. Black rectangles indicate the area of the *OXTR* CpG island investigated by previous research on DNA methylation. Significant CpG sites identified in those studies are shown as black circles.

Table 2. *Correlations, means, and standard deviations among study variables (N = 100)*

	1	2	3	4	5	6	7	8	9	10	11	12
1. Adult adversity (W2–W4)	—											
2. *OXTR* methylation	.262**	—										
3. Distrust (W5)	.278**	.213*	—									
4. Pessimism (W5)	.272**	.225*	.262**	—								
5. Depression (W5)	.319**	.113	.454**	.311**	—							
6. Childhood adversity	.037	.082	.061	.097	−.008	—						
7. Relationship status (single)	.223*	.020	.223*	.059	.123	.038	—					
8. Chronological age	−.018	.206*	.134	.051	−.124	−.144	.243*	—				
9. Factor 1 cell type	−.267**	.080	−.082	−.007	−.089	.002	−.114	−.138	—			
10. Factor 2 cell type	.033	.051	−.074	−.068	.048	−.010	.004	−.083	.009	—		
11. Factor 3 cell type	−.103	−.186†	.034	−.116	−.039	−.040	−.145	.028	.006	−.002	—	
12. Factor 4 cell type	.057	.417**	.241*	.157	.103	−.067	−.073	.202*	−.014	−.008	.014	—
Mean	0.008	0.000	23.978	9.865	6.240	1.830	0.450	48.520	−0.003	−0.001	−0.001	0.001
SD	0.616	1.000	7.060	2.066	1.837	1.400	0.500	9.296	34.245	11.878	5.925	5.014

Note: Factors 1 to 4 are the four principle components reflecting cell-type variation in the current data. W2–5, Waves 2–5.



Cell-types as covariates

Adult adversity → Factor 1, β = -.267, p = .001
Adult adversity → Factor 2, β = .033, p = .714
Adult adversity → Factor 3, β = -.103, p = .305
Adult adversity → Factor 4, β = .057, p = .574
OXTR methylation ↔ Factor 1, β = .185, p = .020
OXTR methylation ↔ Factor 2, β = .069, p = .404
OXTR methylation ↔ Factor 3, β = -.182, p = .065
OXTR methylation ↔ Factor 4, β = .391, p = .000
Factor 1 → Depression, β = -.051, p = .688
Factor 2 → Depression, β = .080, p = .309
Factor 3 → Depression, β = .015, p = .837
Factor 4 → Depression, β = -.001, p = .992

Figure 3. Oxytocin receptor gene (*OXTR*) methylation as a mediator of the effect of stressful environment on depression. $N = 100$. $\chi^2 = 64.500$, $df = 54$, $p = .103$. Values presented are standardized parameter estimates. Childhood trauma, chronological age, and relationship status are controlled. The bold words indicate that the test of indirect effect is significant. †$p < .10$, *$p \leq .05$, **$p \leq .01$ (two-tailed tests).

Table 3. *Summary of indirect effects*

Paths	Total Effect	Indirect Effect	Portion of Variance for Mediator
Adult adversity → *OXTR* methylation → Distrust → depression	0.615* 0.132; 1.168	0.056* 0.009; 0.193	9.106%
Adult adversity → *OXTR* methylation → Pessimism → depression	0.615* 0.132; 1.168	0.024* 0.001; 0.116	3.902%

Note: The values presented are unstandardized parameters. Bootstrapping with 1,000 replications.
*$p \leq .05$ (two-tailed test).

underpinnings for cognitive, emotional, and behavioral traits tailored for adaptation to environmental demands (Landecker & Panofsky, 2013; Meloni, 2014; Simons & Klopack, 2015). Building on this idea, the current study investigated the extent to which individuals might adapt to environmental adversity by downregulating the *OXTR* gene. Several studies have shown that oxytocin is involved in social skills and cognition relating to empathy, trust, and optimism (Baumgartner et al., 2008; Kosfeld et al., 2005; MacDonald & MacDonald, 2010; Zak, 2012). Hence increased methylation (i.e., downregulation) of the promoter region of the receptor gene might be seen as providing the biological underpinnings for a vigilant, distrusting orientation. This negative cognitive set might be viewed as a functional adaptation given the person's past experience. However, it would also place the individual at risk for depression. A profusion of studies have linked a negative cognitive style to development of depressive disorder (Alloy et al., 2006; Beck, 2008; Clark & Beck, 2010; Gibb et al., 2001). Based upon this idea, we posited that methylation of the *OXTR* gene would mediate the effect of adult adversity (financial pressure or high-crime neighborhood) on increased commitment to negative schemas (pessimism and distrust) and, in turn, the development of depression. Our results provided support for this model.

The Illumina 450K Human Methylation Beadchip provides probes for 12 sites located within or near the promoter region of *OXTR*. Factor analysis condensed these 12 sites into four factors. One of these factors (which consists of four sites) showed significant correlations with all of our study variables, whereas the other three factors were unrelated to the study variables. Using this factor as an indicator of *OXTR* methylation, our SEM analyses corroborated the hypothesized model: *OXTR* methylation mediated the effect of adult adversity on increases in pessimism and distrust, and the effect of *OXTR* methylation on increased depression was mediated by the effects of pessimism and distrust. These findings have several implications.

First, our findings suggest a link between adult adversity and methylation patterns. Past research has emphasized the way that childhood environments may calibrate various biological systems (Miller, Chen, & Parker, 2011). The present study shows that adult stress has an association with methylation of the oxytocin receptor that is independent of childhood effects. These results suggest that calibration of various biological systems may continue throughout the life course. Future research needs to go beyond examination of childhood effects to investigate the extent to which biological systems remain plastic during the adult years. Establishing that this is the case would have important public health consequences.

Second, past research has focused on epigenetic changes related to biological processes such as inflammation and the immune system (e.g., Beach et al., 2016, in press; Romens, McDonald, Svaren, & Pollak, 2014; Simons et al., 2016). As far as we are aware, ours is the first study to show that epigenetic processes are associated with various self-reported, negative cognitive styles of the sort commonly linked to depression. Our results suggest that everyday attitudes and schemas may become biologically embedded through epigenetic processes such as DNA methylation. In large part, this may be the reason they are often so difficult to change. Changing biologically embedded cognitive patterns would require persistent, compelling evidence that the world is different from what one had thought to be the case.

Third, recently there has been much enthusiasm regarding the therapeutic use of oxytocin with psychiatric patients (Bartz & Hollander, 2006; Cochran, Fallon, Hill, & Frazier, 2013; McDonald & McDonald, 2010). This enthusiasm is based in large measure on the many studies showing that intranasal administration of oxytocin is associated with increased social skills, trust, empathy, and optimism, attitudes and emotions that are contrary to those associated with most psychiatric disorders. Unfortunately, research on the use of oxytocin with psychiatric patients has produced few positive effects (Oliff et al., 2013). This lack of therapeutic effects may well be a consequence of methylation of the *OXTR* gene. Downregulation of this gene limits the ability of an organism to respond to oxytocin. In addition, our results suggest that persistent exposure to adversity, a condition experienced by many if not most psychiatric patients, tends to methylate the *OXTR* gene, potentially decreasing the impact of exogenously administered oxytocin.

The present study included several strengths. The data set consisted of longitudinal assessments of several of the variables in our model and contained the data necessary to incorporate important controls such as age, childhood trauma, and cell type. However, the study suffered from certain limitations. For example, like virtually every other methylation study, our assessment of *OXTR* methylation was limited to a single point in time. Although adult adversity was related to methylation of

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OXTR after controlling for variables such as childhood trauma, the conclusion that adult adversity affects *OXTR* methylation requires demonstration that adult stress predicts *change* in *OXTR* methylation. Further, the percent of total variance in depression accounted for by the full biologically embedded pathway from chronic stress to depression was modest, suggesting potential for other avenues of influence from stress to depression.

There are also weaknesses relating to the sample. Given the cost of blood draws and methylation assays, our sample size is small. Further, our sample is limited to middle-aged African American woman. One might argue that this group is particularly appropriate given the model being tested because women are at greater risk of depression than men and African Americans experience substantially more exposure than other groups to adverse conditions such as financial pressure and neighborhood crime. Still, our results clearly need to be replicated with samples more heterogeneous with regard to sex and ethnicity. Finally, although we posited that methylation within the promoter region of *OXTR* would mediate the effect of adversity on negative cognitions, we made no predictions regarding the specific sites that were likely to be important. Future studies need to replicate our finding that these particular sites serve as mediators of the impact of adverse circumstances on distrust and pessimism.

In summary, our results suggest that the negative cognitions associated with depression may be rooted in, or be an adaption to, persistent adversity. Further, contrary to what is often assumed, our findings suggest that this adversity is not limited to childhood experiences. In the present study, adult adversity predicted increased commitment to negative cognitive schemas, whereas childhood trauma had little effect. The major contribution of our study, however, was the finding that methylation of the *OXTR* gene mediated the effect of adult adversity on negative cognitions. This suggests that the oxytocin system may play an important role in the development of depression by serving as one of the mechanisms whereby negative cognitions become biologically embedded.

However, we do not want to oversimplify or overstate the case. It is biologically implausible that *OXTR* is regulated in isolation of other related genes. Given the vast array of social behaviors with deep biological significance that are linked to oxytocin, *OXTR* gene expression is undoubtedly tied to and interacts with the regulation of a wide variety of genetic systems and processes. Although methylation of the *OXTR* promoter appears to be involved in a broad spectrum of psychopathological phenotypes involving low empathy and lack of connection to others, the specific combination of methylated sites and the biological processes that they set in motion likely vary between these different forms of psychopathology (e.g., autism, callus–unemotional trait, attachment disorders, and depression). We hope that future studies that employ much larger samples will be able to identify the particular constellation of *OXTR* sites implicated in each of these disorders and the broader set of epigenetic systems that they signal and with which they participate.

Supplementary Material

To view the supplementary material for this article, please visit http://dx.doi.org/10.1017/S0954579416000420.

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