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An epigenetic rheostat of experience: DNA methylation of *OXTR* as a mechanism of early life allostasis

Joshua S. Danoff, Jessica J. Connelly, James P. Morris, Allison M. Perkeybile *

University of Virginia, Department of Psychology, 102 Gilmer Hall, P.O. Box 400400, Charlottesville, VA, 22904, USA

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ABSTRACT

Oxytocin is a neuropeptide hormone which is involved in regulation of social behavior, stress response, muscle contraction, and metabolism. Oxytocin signaling is dependent on its binding to the oxytocin receptor, coded for by the *OXTR* gene. Many studies have examined the role of epigenetic regulation of *OXTR* in neurological and behavioral outcomes in both humans and animal models. Here, we review these studies, critically analyze their findings in the context of oxytocin's role as an allostatic hormone, and provide suggestions for future research. We use *OXTR* as a model for how those in the field of psychoneuroendocrinology should perform epigenetic studies in order to maximize both biological relevance and potential for biomarker development.

1. Oxytocin signaling

Oxytocin is a neuropeptide hormone which regulates many processes in both the central nervous system and the periphery [1]. Oxytocin was first described in 1906 by Sir Henry Dale for its role in uterine contraction [2]. We now know that oxytocin is pleiotropic and is involved in regulating lactation, social behavior, stress response, immune activity, metabolism, food intake, and bone growth [1,3,4]. Oxytocin signaling is dependent on its interaction with the oxytocin receptor, which is the product of the *OXTR* gene, though it also binds with less affinity to vasopressin receptors. Widespread central and peripheral *OXTR* expression supports oxytocin's pleiotropic actions. *OXTR* is expressed throughout the brain, with particularly high levels in olfactory regions, striatum, hippocampus, amygdala, and certain cortical regions [5,6]. In the periphery, oxytocin receptors are present in both male and female reproductive organs, mammary tissue, kidney, and throughout the cardiovascular system [7].

Recently, Quintana and Guastella proposed that oxytocin is not only a social hormone but instead promotes allostasis (see Ref. [8]) in both social and nonsocial contexts [9]. Oxytocin acts to help the organism adapt to changes in the environment and to anticipate changes in the future environment. Quintana and Guastella suggest that by facilitating adaptation to both current and future environments, oxytocin signaling helps to maintain stability throughout the lifespan. For example, oxytocin signaling protects the fetus from the hypoxic and painful conditions of birth, thereby maintaining stability in a changing environment [10,11]. Additionally, the amount of oxytocin in a rat pup's hypothalamus is positively associated with maternal contact and altering early life oxytocin impacts behavior in adulthood, suggesting that oxytocin calibrates an organism to their social environment (reviewed in Ref. [12]).

Importantly, while mammalian species have very similar patterns of oxytocin innervation, species differ greatly in the distribution of oxytocin receptors which allows for differences in species-typical social behaviors (see Ref. [13]). *OXTR* expression is regulated by the epigenetic modification DNA methylation which allows for differential expression within species as well. Here, we review studies in both animal models and humans which study epigenetic regulation of *OXTR* and its relationship to gene expression, behavior, neuroimaging outcomes, and life experiences. In doing so, we propose that epigenetic regulation of *OXTR* expression is a critical element of oxytocin's allostatic activity – perhaps serving as a rheostat to fine tune the organism to its environment. We also address methodological considerations for epigenetic studies in psychoneuroendocrinology and discuss how *OXTR* DNA methylation may serve as a biomarker for various conditions and outcomes in the future.

2. Epigenetic basics and methodological considerations

Gene expression is tightly regulated by epigenetic processes. The two main epigenetic modifications are DNA methylation and post-translational modifications to histone tails. This review will focus on

* Corresponding author.
E-mail address: allison.perkeybile@virginia.edu (A.M. Perkeybile).

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DNA methylation. DNA methylation occurs when a DNA methyltransferase (DNMT) covalently adds a methyl group to cytosine residues [14]. In mammals, somatic cells are generally only methylated when cytosines are immediately followed by a guanine (called a CpG site), though mature neurons do exhibit DNA methylation at cytosines followed by other bases (CpH sites) [15,16]. Many gene promoters have dense regions of CpGs called CpG islands, and DNA methylation of CpG islands is associated with decreased transcription of the gene [17]. CpGs in gene bodies are generally highly methylated, especially in highly expressed genes, and may function to prevent aberrant transcription, facilitate elongation, or coordinate splicing [14,15].

Epigenetic studies are particularly useful in the field of psychoneuroendocrinology for several reasons. First, measures of epigenetic modifications are continuous and provide more variability among subjects than genetic measurements. Second, epigenetic modifications are sensitive to the environment, meaning that epigenetic markers can be a readout of developmental programming by different environments which allows for a biological representation of interactions between the environment and the genome [18]. The first paper to document this phenomenon in the brain was by Weaver et al. who showed that maternal behavior programs DNA methylation of the glucocorticoid receptor gene (*Nr3c1*) in rat hippocampus, which in turn regulates *Nr3c1* expression and stress reactivity [19]. Since this landmark study, many have studied how one's developmental environment affects physiology and behavior via epigenetic modifications [20]. Below we describe methodological considerations for performing epigenetic studies, using *OXTR* as an example.

2.1. Methodological consideration 1: CpG sites to examine

When designing an epigenetic study, it is important to select CpG sites to examine that are informative about the population and relevant to biological function. DNA methylation at chosen CpG sites should be highly variable in the population. If DNA methylation at CpG sites does not vary within the population it presents not only statistical problems but also is likely not related to the phenotype being examined. Furthermore, it is critical that one considers the variability of DNA methylation measurements when interpreting experimental results. For this reason, measures of DNA methylation should be completed in triplicate in order to understand the assay variability for the experiment. For example, when measuring *OXTR* DNA methylation using bisulfite-pyrosequencing, we routinely find that measurements across replicates vary by 1–2%. Thus, we should not attribute group differences in DNA methylation under 2% to actual group differences, since they could result from measurement error. When replicates become cost-prohibitive, creating a standard curve from 0% to 100% methylated controls and/or running a subset of DNA samples in triplicate can be used to determine assay variability.

In order to gain mechanistic understanding of how DNA methylation is related to the phenotype, it is imperative that DNA methylation at the chosen CpG site is biologically relevant, i.e. related to transcription. Work establishing this is well suited for cell culture and animal models, though correlational evidence from human brain tissue is also informative. In *OXTR*, an early study by Kusui et al. used a hepatoblastoma cell culture model to show that a DNA methylation in a specific region of the CpG island around the *OXTR* promoter termed MT2 is critical for

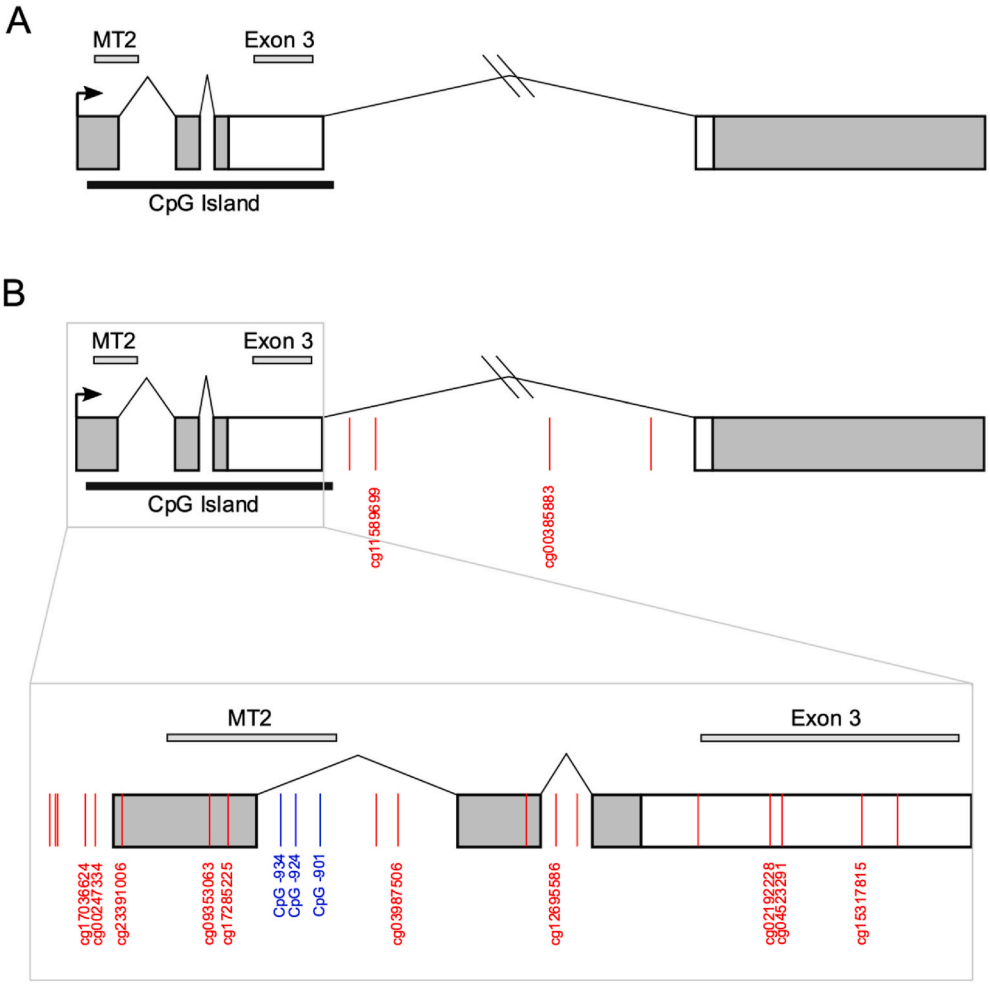

Fig. 1. Human *OXTR* gene schematic and commonly studied CpG sites. A) Gene schematic of human *OXTR* (though the structure is similar in many rodent model species, including prairie voles. Boxes represent exons and the lines represent introns. Coding regions are in white and untranslated regions are in gray. The black arrow indicates the transcription start site. The black bar below the gene denotes the CpG island. Above the gene in light gray boxes are two regions of interest, MT2 and exon 3, where DNA methylation is commonly examined. B) The gene schematic of *OXTR* is reproduced to indicate CpG sites (vertical red lines) measured by the Illumina MethylationEPIC 850k array. CpG sites which have been associated with behavioral or neurological outcomes are labeled with the cg number provided by Illumina. Blue lines indicate CpG sites −934, −924, and −901 (named according to position relative to the translation start site) which are associated with gene expression in the human brain [22]. This schematic shows CpG sites in the following region which includes *OXTR* and 2000bp upstream and downstream of *OXTR*: hg19 chr3:8,790,137–8,813,294. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

regulation of *OXTR* gene expression, though they did not examine the role of DNA methylation in exon 3 (see Fig. 1A for a gene schematic) [21]. We have recently shown that DNA methylation at specific CpG sites in this region, −901, −924, and −934 (named according to position relative to the translation start site) are negatively correlated with gene expression in human temporal cortex while other CpG sites are not [22]. Additionally, using a prairie vole animal model, we showed that DNA methylation at CpG sites homologous to −901, −924, and −934 are sensitive to early life experience and associated with gene expression in the nucleus accumbens [22,23]. We also provide evidence that DNA methylation in MT2 is more informative of *Oxtr* gene expression than DNA methylation in exon 3, though further work in human brain tissue or cell cultures would help to corroborate our findings. This type of work in an animal model and in human tissue demonstrates the biological relevance of DNA methylation at these particular CpG sites in MT2. This information then allows us to confidently focus our studies on these sites while working to understand their role in shaping outcomes of interest.

Many studies use Illumina arrays to measure DNA methylation across the genome. The available Illumina arrays measure DNA methylation at 22 CpG sites around *OXTR* (see Fig. 1B for CpG sites on the Illumina MethylationEPIC 850k array). These CpG sites mostly cover the CpG island, but many of these CpG sites have not been functionally associated with gene regulation. DNA methylation at these CpG sites may be correlated with DNA methylation at other sites which have been functionally characterized (as suggested by research in prairie voles), but further studies using human tissue are necessary [22]. As such, the interpretation of studies using this method must be cautious.

2.2. Methodological consideration 2: tissue choice

DNA methylation is tissue and cell type specific [15,16,24]. This presents a methodological problem for studies in psychology and neuroscience: the relevant tissue is not accessible in live subjects. Many studies measure DNA methylation in peripheral tissues as a proxy for the brain, most notably blood and saliva. Using Illumina arrays, several studies have compared DNA methylation in the brain to blood and not surprisingly find that most CpG sites do not have similar DNA methylation levels in the blood and brain [25–28]. For CpG sites not on the Illumina array, it is beneficial to look at the correlation of DNA methylation in brain and blood tissue, possibly in animal models if available. For example, we have recently shown that DNA methylation at CpG sites homologous to sites −901, −924, and −934 are highly correlated in prairie vole nucleus accumbens and blood ($r^2 > 0.42$ for these CpG sites), and DNA methylation of these sites in the blood are negatively correlated with gene expression in nucleus accumbens [23]. We have also shown that DNA methylation of these sites is highly correlated in blood tissue and saliva in humans, indicating saliva tissue may also be informative for the state of the brain for these specific CpG sites [29,30]. Collectively, these studies indicate that peripheral measures of methylation at specific CpG sites in *OXTR* are informative for human studies of the brain, though the mechanism that leads to correlated methylation levels in saliva, blood, and brain remains to be elucidated. It should be noted that not all CpG sites in *OXTR* have high cross-tissue correlation of DNA methylation, particularly when looking at CpG sites on the Illumina arrays. One reason for this may be that the CpG sites in MT2 where DNA methylation has high cross-tissue correlation are studied because they are functionally related to gene expression and may serve to regulate *OXTR* expression in both neural and immune tissues.

Some studies have made use of CpG sites which do not have correlated DNA methylation in brain and peripheral tissues to answer interesting questions. The "signature model" proposed by Aberg et al., suggests that peripheral DNA methylation may be a marker of developmental events which are also related to behavioral outcomes [31]. For example, the Dutch Famine study showed that periconceptional exposure to famine is associated with differences in DNA methylation in blood at the *IGF2* locus and increased risk for schizophrenia [32,33]. Though DNA methylation of *IGF2* in blood is not mechanistically related to schizophrenia, it is an epigenetic signature of the famine risk factor.

The last consideration of tissue choice is cell composition. Commonly studied tissues like blood and brain are composed of many different cell types, each with their own epigenetic state. Group level changes in DNA methylation may simply reflect changes in the relative abundance of neurons and glia (in the brain) or immune cells (in blood) instead of bona fide changes in gene regulation (Fig. 2). For example, a recent study found an epigenomically distinct subset of Autism Spectrum Disorder (ASD) patients which differed from both controls and other ASD patients [34]. The authors then investigated the estimated cell type composition of the blood samples used in the study and found that this distinct group had a higher proportion of granulocytes and lower proportion of lymphocytes compared to both the remaining ASD patients and the controls. The authors conclude that the epigenomic differences in this subset are due to changes in immune cell types. Our group has investigated how *OXTR* DNA methylation differs across cell types in

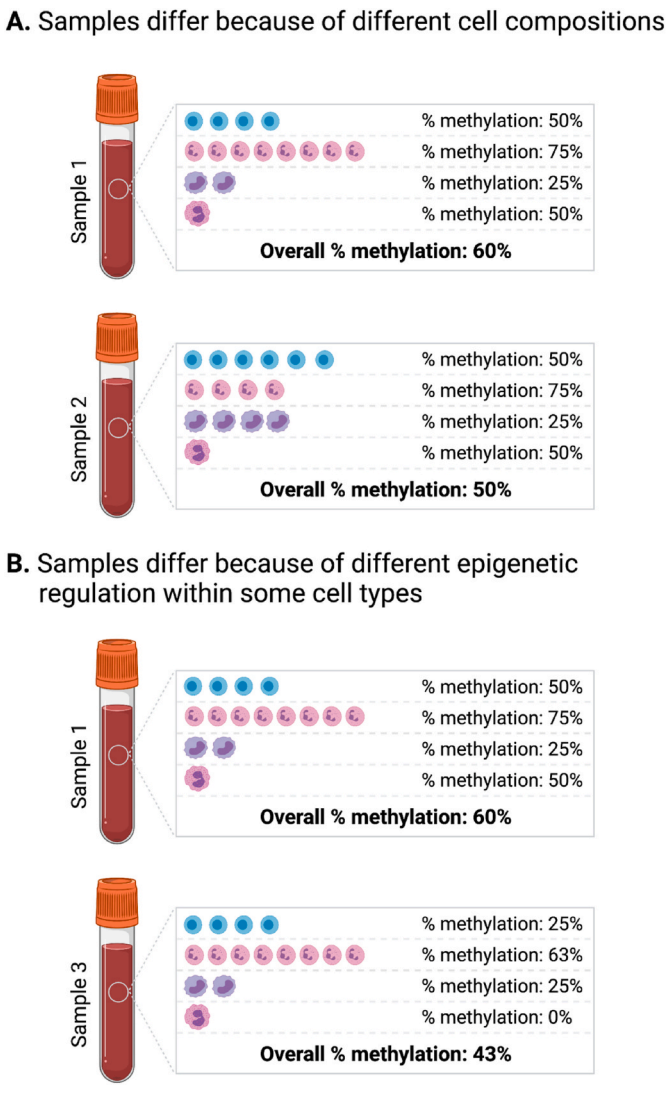

Fig. 2. Sources of variation in DNA methylation in heterogenous tissues. A) Samples 1 and 2 differ in overall DNA methylation because of changes in proportions of cell types. Individual cell types do not differ in DNA methylation levels among the two samples. B) Samples 1 and 3 differ in overall DNA methylation because of changes in DNA methylation levels of individual cell types. The proportion of each cell type are not different in the two samples. Image created with BioRender (biorender.com).

whole blood and found no significant difference in DNA methylation at CpG site −934 among different cell types [35]. These results suggest that even if differences in cell type proportions are present in whole blood, it would not affect *OXTR* methylation measurements. Further work characterizing *OXTR* DNA methylation across tissues and cell types would clarify how to account for tissue type in future studies. In studies where cell type compositions are not measured, the interpretation of the results must recognize both changes in cell type composition and changes in epigenetic regulation as possible reasons for observed differences in DNA methylation.

Table 1
Studies of DNA methylation of *OXTR* in model systems.

Study	Species	Tissue	*OXTR* Region	Method	Phenotype/ Manipulation	Results
Studies in Human Cells						
Kusui et al., 2001 [21]	Human	HepG2 cells, liver, peripheral blood, myometrium	MT1, MT2, MT3, MT4	Methylation-sensitive restriction enzyme digest and PCR amplification	Genomic deletion of MT2; tissue type	Deletion of MT2 resulted in increased expression of the gene in luciferase reporter assay. MT2 methylation was highest in liver tissue (where *OXTR* is not expressed) and lowest in term myometrium (where *OXTR* is highly expressed).
Cho et al., 2019 [76]	Human	Dermal fibroblasts	MT2	Bisulfite-sequencing	Age and senescence	Fibroblasts from older donors had higher DNA methylation than fibroblasts from young donors. Pharmacological demethylation of *OXTR* in fibroblasts from older adults inhibited senescence.
Studies in Rodents						
Mamrut et al., 2013 [36]	Mouse	Uterus, mammary gland	Promoter	Bisulfite-sequencing	Pregnancy	Following parturition, *Oxtr* methylation in uterus decreased and was negatively related to *Oxtr* expression. Following parturition, *Oxtr* methylation in mammary glands increased and was positively associated with *Oxtr* expression.
Harony-Nicolas et al., 2014 [37]	Mouse	Brain	Promoter	Bisulfite-sequencing	Gene expression in various brain regions	DNA methylation of *Oxtr* varied across brain region. Brain regions with higher DNA methylation at CpG site 7 had higher levels of *Oxtr* expression.
Towers et al., 2018 [77]	Mouse	Hippocampus	MT2, Exon 3	Bisulfite-sequencing	*Tet1* knockout mouse	*Tet1* knockout mice had increased DNA methylation of both MT2 and Exon 3 in hippocampus, which was associated with decreased gene expression.
Beery et al., 2016 [38]	Rat	Hippocampus, striatum, hypothalamus, PBMC	MT2 (5′ portion)	EpiTYPER and bisulfite-pyrosequencing	Maternal care received	High levels of maternal care were associated with increased *Oxtr* methylation in blood (PBMCs). Maternal care was not associated with change in DNA methylation in hippocampus. Average levels of DNA methylation of each CpG site were similar across brain regions examined. Within individuals, DNA methylation was not correlated across brain regions.
Perkeybile et al., 2919 [23]	Prairie vole	Nucleus accumbens, whole blood	MT2	Bisulfite-pyrosequencing	Parental care received (induced by handling or natural)	Handling resulted in higher parental care and lower DNA methylation at CpG sites −901, −924, −934_1, and −934_2 of MT2 in offspring. DNA methylation at these sites was negatively correlated with *Oxtr* expression. DNA methylation at these sites in blood was positively correlated with DNA methylation in nucleus accumbens and negatively correlated with *Oxtr* expression in nucleus accumbens. Among offspring raised by natural (unmanipulated) parenting, those raised by low care parents had higher DNA methylation at these CpG sites.
Kenkel et al., 2019 [41]	Prairie vole	Brain	MT2	Bisulfite-pyrosequencing	Maternal oxytocin administration before labor	Maternal oxytocin administration increased DNA methylation in fetal brain. DNA methylation was negatively associated with *Oxtr* expression.
Danoff, Wroblewski et al., 2021 [22]	Prairie vole	Nucleus accumbens	MT2, Exon 3	Bisulfite-pyrosequencing	Parental care received (induced by handling)	Handling increased parental care and resulted in decreased DNA methylation in both MT2 and exon 3. DNA methylation in MT2 was negatively associated with *Oxtr* expression. There was no relationship between DNA methylation in exon 3 and *Oxtr* expression.
Studies in other model animals						
De Leon et al., 2020 [78]	Macaque	Whole Blood	MT2	EpiTYPER	Social behavior and oxytocin levels in CSF	There was no association of *OXTR* methylation with social behavior or oxytocin levels in CSF.
Cimarelli et al., 2017 [79]	Dog	Saliva	Promoter	Bisulfite-pyrosequencing	Response to approach by human stranger	Male dogs with lower *OXTR* methylation were more likely to approach the stranger and less likely to remain passive or hide behind their owner.
Banlaki et al., 2017 [80]	Canines	Buccal cells	Promoter	EpiTYPER	Species and breed	*OXTR* methylation differed among wolves, border collies, golden retrievers, and Siberian huskies.

3. Epigenetic regulation of *oxtr* in model systems

As previously mentioned, Kusui et al. used cell culture and luciferase reporter assays to show that *OXTR* expression is regulated by DNA methylation in a region called MT2, which overlaps exon 1 and intron 1 (see Fig. 1A) [21]. Since this study, there has been much work done in animal models to determine how DNA methylation in this region and other regions of the gene relates to gene expression, early life experience, and overt behaviors. Table 1 provides a list of these studies, some of which we will further discuss in this section.

Several groups have studied epigenetic regulation of *Oxtr* in common laboratory rodents including mice and rats. In mice, *Oxtr* expression in uterus and mammary glands is dynamic across pregnancy and associated with changes in *Oxtr* methylation in the promoter region [36]. Using a similar strategy as Kusui et al., Mamrut et al. also show that DNA methylation in a region of the promoter (bp −956 through −541, numbered according to transcription start site) is critical for repressing gene expression [36]. The same group then investigated the role of this region in regulating *Oxtr* in the brain. They found that DNA methylation of this region varies across brain regions, though only one CpG site was significantly correlated with gene expression across brain regions [37]. These findings demonstrate biological relevance of *Oxtr* DNA methylation in peripheral reproductive tissue at a time in which this tissue is undergoing rapid changes in its reliance on and involvement with the oxytocin system, but this relevance does not extend universally to central tissues.

A study using rats examined the effects of maternal care on DNA methylation of a region homologous to MT2 in brain and blood tissue. Beery et al. report that offspring of higher care mothers have increased DNA methylation of *Oxtr* at specific CpG sites in peripheral blood mononuclear cells (PBMCs), but no change in DNA methylation in hippocampus [38]. They also report that CpG sites have similar methylation values across brain regions on average, though the correlation within individual animals is weak. In striatum and hippocampus, there is evidence of a positive association between DNA methylation at specific CpG sites and *Oxtr* expression, though these relationships were not significant after correcting for multiple comparisons. The lack of relationship between DNA methylation and gene expression may indicate that the DNA methylation at these sites is not regulating *Oxtr* expression in these regions of rat brains. Other CpG sites yet to be studied in rats may be more sensitive to early life experience and relevant to gene regulation. Notably, the CpG sites that regulate *OXTR* expression in humans (ie. −934, −924) are not conserved in rats, indicating that there may be different mechanisms regulating *Oxtr* expression in rats and that other animals may be a better model for human *OXTR* regulation [23].

Our group has studied the effects of early life experience on DNA methylation of *Oxtr* in prairie voles. We chose the prairie vole as a model for this work (opposed to other rodents) because prairie voles display human-like oxytocin-dependent social behaviors including pair bonding and biparental care [13]. Prairie voles are highly sensitive to their early life social environment: those raised by high contact parents reach some developmental markers earlier and display higher levels of social behaviors later in life [39,40]. Moreover, prairie voles have high conservation of the MT2 and exon 3 regions of *Oxtr* and CpG sites directly implicated in regulation of *OXTR* expression in the human brain are more conserved in prairie voles than rats or mice [22,23]. Our results indicate that DNA methylation of *Oxtr* is reduced by high care parenting in prairie vole nucleus accumbens in both the MT2 and exon 3 regions, though the effect is stronger in MT2 than in exon 3 [22,23]. We also show that DNA methylation in MT2 is negatively associated with *Oxtr* expression but DNA methylation in exon 3 is not. Within MT2, a subset of CpG sites at the 3′ end of the region which contains sites homologous to human CpGs −901, −924, and −934 is most sensitive to early life experience and most related to *Oxtr* expression [22]. Importantly, DNA methylation at these sites is positively correlated in nucleus accumbens and blood, and DNA methylation of these sites in blood is negatively associated with *Oxtr* expression in nucleus accumbens [23]. These results indicate that DNA methylation of these CpG sites in peripheral tissues are informative about the levels of *Oxtr* expression in the brain, which is important for studies in human subjects. We have also shown that *Oxtr* methylation is sensitive to other early life experiences. In a study which simulated the common practice of labor induction via oxytocin administration, we exposed pregnant prairie voles to oxytocin via intraperitoneal injection and examined the effect on the brain of the offspring. We found that as parturition approaches, fetal brains show lower DNA methylation in *Oxtr* MT2 which is associated with higher *Oxtr* expression [41]. However, high doses of maternal oxytocin administration disrupted this response, resulting in increased DNA methylation of *Oxtr* MT2 in fetal forebrains and long lasting effects on social behavior, indicating this epigenetic process is likely regulated by maternal oxytocin [41]. Overall, these studies support the use of prairie voles as a model of *Oxtr* epigenetics, particularly when investigating social behaviors. Furthermore, these studies show that *Oxtr* DNA methylation is responsive to the environment and environmental tuning of *Oxtr* expression via epigenetic mechanisms may increase the organism's fitness for that environment. Oxytocin signaling is neuroprotective to the hypoxic and inflammatory conditions surrounding birth [42,43]. Thus, maternal oxytocin may signal to the fetus to prepare for birth by increasing oxytocin receptor availability (via DNA hypomethylation), consistent with its role as an allostatic hormone (Fig. 3). By disrupting timing and/or level of maternal oxytocin signaling, this response is disrupted, with long lasting effects on offspring behavior.

These and other work in model systems (see Table 1) provide an excellent starting point for understanding how epigenetic regulation of *Oxtr* can modulate oxytocin's allostatic activity. They also highlight the complexity of the control of these markers and the potentially different mechanisms guiding central and peripheral *Oxtr* regulation. Overall, further work in animal models and cell culture should focus both on understanding the molecular cascades linking early life experiences and other environmental events to changes in *Oxtr* methylation and gene expression as well as how *Oxtr* methylation contributes to behaviors.

4. Epigenetic regulation of *OXTR* in humans

4.1. *OXTR DNA methylation in human development*

Table 2 describes studies of *OXTR* methylation and developmental outcomes in humans. In this section, we will focus on studies relating *OXTR* methylation to major developmental events. First, we will discuss the role of *OXTR* methylation in parturition. The first paper to look at the role of DNA methylation in regulating oxytocin receptor expression found that DNA methylation of MT2 is lower in term pregnant myometrium than nonpregnant myometrium, as would be expected given the upregulation of oxytocin receptors prior to parturition [21]. Two studies have examined how delivery mode affects *OXTR* MT2 methylation in placenta. Both studies found that there is increased DNA methylation in spontaneous preterm vaginal deliveries compared to term vaginal deliveries, and one study also found increased DNA methylation at one CpG site in cesarean delivery (no labor) compared to term and preterm labor vaginal deliveries [44,45]. These studies suggest that DNA hypomethylation is a mechanism for preparing the placenta for vaginal birth. Dysregulation of this mechanism may lead to complications: increased DNA methylation and reduced *OXTR* expression in the umbilical vein has been documented in pre-eclampsia complications [46]. Further studies are warranted to understand how *OXTR* methylation regulates pregnancy outcomes and if this is related to psychological outcomes in infants and children as they develop. Studies in model systems which mechanistically examine how *OXTR* methylation changes in reproductive tissues through pregnancy and with different birth modes will also help to define the role of *OXTR* epigenetic regulation in pregnancy outcomes.

Prenatal factors, including maternal stress, influence the physiology

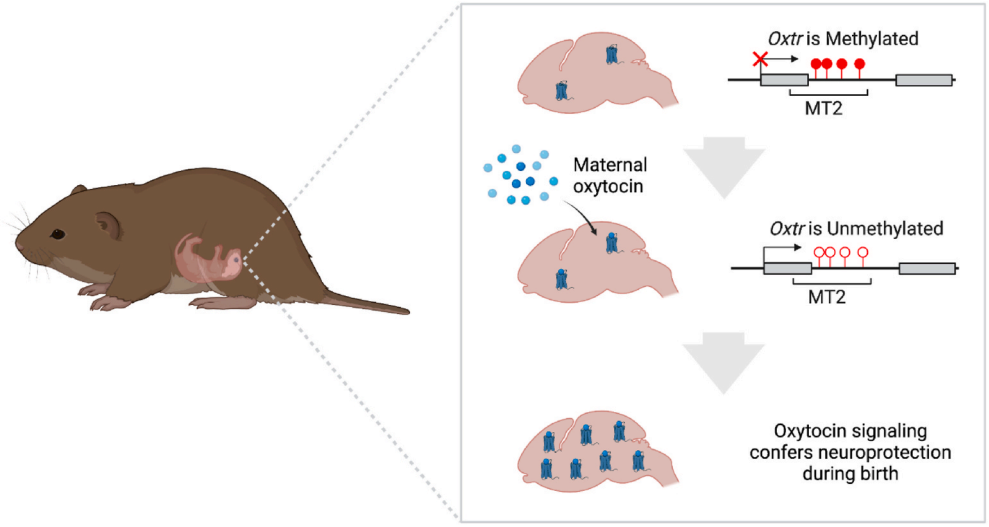

Fig. 3. DNA methylation is a mechanism of allostasis: preparing the fetus for birth. As parturition approaches, maternal oxytocin signals to the fetal brain to prepare for birth. DNA hypomethylation of *Oxtr* results in increased gene expression and oxytocin receptor availability in the brain, which confers neuroprotection to hypoxic and inflammatory conditions of birth. Image created with BioRender (biorender.com).

and psychology of offspring throughout the lifespan [47]. One study found that maternal depression is not related to DNA methylation in MT2 or exon 3 in placenta, though DNA methylation at CpG site −860 is mildly associated with anti-depressant exposure [48]. A few studies have looked at the effects of prenatal risk factors on *OXTR* methylation in cord blood. Unternaehrer et al. found that DNA methylation in exon 3 is negatively associated with maternal stress and depressivity [49]. Milaniak et al. found that DNA methylation in introns 1 and 2 is associated with resilience to prenatal stress, meaning that children with higher DNA methylation in cord blood had fewer conduct problems at age 7 than their prenatal risk would predict [50]. Together these studies suggest methylation may be a marker for prenatal stress in the maternal-fetal environment, but that the region of the promoter examined is an important factor in interpreting findings. However, a third study found no effect of prenatal stress on DNA methylation in cord blood [51], highlighting the need for additional work here. Additionally, another study found that perinatal maternal depression was not associated with DNA methylation in exon 3 of children assessed as toddlers [52]. More studies are necessary to determine the role of prenatal risk in setting up the epigenetic regulation of *OXTR* with particular focus on how this changes over the child's lifespan.

Our group has performed studies connecting *OXTR* methylation to neurobiological and behavioral outcomes in infants. DNA methylation at CpG −924 in MT2 was associated with hemodynamic response (assessed by fNIRS) in inferior frontal cortex upon viewing emotional faces [29]. This association had valence specificity: DNA methylation is negatively related to inferior frontal cortex activity when viewing happy faces but positively associated with activity when viewing angry or fearful faces. Another study using EEG found that DNA methylation at CpG −934 is negatively associated with brain signal entropy during social perception, and that brain signal entropy is positively associated with parent reported social behavior [30]. We have also shown that *OXTR* methylation is dynamic during infancy. Maternal engagement at 5 months is predictive of changes in *OXTR* methylation in infant saliva between 5 months and 18 months such that higher maternal engagement predicts a decrease in DNA methylation [53]. In this study, DNA methylation at 18 months was positively associated with infant temperamental discomfort, suggesting that maternal engagement with infants can change infant temperament by modulating the oxytocin system. These results, namely that higher maternal care modulates *OXTR* methylation and modulates offspring behavior, are consistent with research in rodent models [22,23]. Furthermore, these results suggest that increased maternal care may prepare the infant for future interactions with its mother by decreasing *OXTR* methylation and increasing brain response to happy faces, which may increase the salience of maternal engagement.

Studies in children and adolescents have found that *OXTR* methylation is associated with behavioral outcomes. For example, two studies have found that *OXTR* methylation is associated with callous-unemotional traits. Cecil et al. showed that DNA methylation across the CpG island in cord blood is prospectively associated with higher callous-unemotional traits at age 13, but only in subjects with low internalizing problems [54]. However, in this study, DNA methylation assayed from whole blood at ages 7 and 9 were not associated with callous-unemotional traits. Another study found that DNA methylation in whole blood in the MT2 region is higher in children with high levels of callous-unemotional traits at ages 9–16 but not ages 4–8, indicating there is a developmental component to this association [55]. Other studies in children have associated DNA methylation of *OXTR* with social communication, theory of mind abilities, and attachment behaviors [51,56,57]. Only one study thus far has looked at the relationship between DNA methylation in children and neuroimaging outcomes. Fujisawa et al. found that childhood maltreatment was associated with increased DNA methylation at MT2 CpGs −1119/-1121 (assayed as one unit) in saliva, and that DNA methylation at these sites was negatively correlated with gray matter volume in the left orbitofrontal cortex [58].

There has been high interest in work studying how childhood experiences relate to DNA methylation observed in adults. Consistent with work in animals, several studies have found that increased maternal care is associated with reduced DNA methylation of *OXTR* and adverse early experience is associated with increased DNA methylation [59–62], though not all studies find this association [63]. A recent meta-analysis indicates a small but significant effect of childhood adversity on *OXTR* methylation, though the meta-analysis did not differentiate between DNA methylation in MT2 and Exon 3 [64]. *OXTR* methylation has been repeatedly shown to mediate or moderate the effects of childhood experience on adult behaviors and outcomes. For example, Smearman et al. show that *OXTR* methylation interacts with child abuse to predict depression and anxiety symptoms [60]. Another study showed that childhood trauma and social instability is associated with increased DNA methylation in MT2 as adults, which in turn is negatively associated with fatherhood involvement displayed [62]. We show that high DNA methylation in MT2 blunts the relationship between childhood neighborhood harshness and BOLD response associated with reward anticipation in the caudate and orbitofrontal cortex [65].

Table 2
Studies of DNA methylation of *OXTR* in Humans: Developmental Populations and Outcomes.

Study	Tissue	*OXTR* Region	Method	Phenotype	Results
Pregnancy Outcomes, Prenatal Environment, and Infancy					
Kim et al., 2013 [44]	Placenta	MT2	Bisulfite-sequencing	Delivery mode	Preterm labor (vaginal delivery) resulted in increased DNA methylation at site −934 in decidua compared to term labor (vaginal delivery) and term cesarean delivery groups. Cesarean delivery resulted in increased methylation at site −959 in amnion in term cesarean delivery compared to term labor and preterm vaginal deliveries.
Behnia et al., 2015 [45]	Amnion	Exon 1	Methylation-sensitive restriction digest	Delivery mode	Preterm birth was associated with in increased DNA methylation of *OXTR* compared to term labor (vaginal delivery) and term not in labor (cesarean delivery). Among birth modes, there was no significant differences in *OXTR* gene expression, but there was increased protein expression of the 70 kDa isoform in preterm labor compared to the other two groups. The term labor group had decreased protein expression of the 45 kDa isoform compared to the other two groups.
Gao et al., 2019 [46]	Umbilical vein	Exon 1	Bisulfite-sequencing (NGS)	Pre-eclampsia	Increased *OXTR* methylation was associated with pre-eclampsia complications and decreased gene expression.
Galbally et al., 2018 [48]	Placenta	MT2, Exon 3	EpiTYPER	Maternal depression and anti-depressant use	Maternal depression diagnosis was not associated with DNA methylation. Maternal antidepressant use was associated with DNA methylation at one CpG site (MT2 -860).
Unternaehrer et al., 2016 [49]	Cord blood	Exon 3	EpiTYPER	Maternal stress during pregnancy	DNA methylation was negatively associated with number of stressful life events, maternal depressivity, and salivary cortisol.
Milaniak et al., 2017 [50]	Cord blood	Introns 1 and 2	HM450 Array	Prenatal risk and conduct problems at age 7	Higher DNA methylation was associated with resilience to prenatal risk (meaning less conduct problems than would be predicted by prenatal risk).
Krol, Puglia et al., 2019 [29]	Saliva	MT2 −924	Bisulfite-pyrosequencing	fNIRS response to emotional faces	DNA methylation was positively correlated with right inferior frontal cortex response to angry and fearful faces. DNA methylation was negatively associated with right inferior frontal cortex response to happy faces.
Krol et al., 2019 [53]	Saliva	MT2 −924	Bisulfite-pyrosequencing	Maternal engagement	Maternal engagement at 5 months was negatively correlated with change in DNA methylation between 5 months and 18 months (meaning high maternal engagement was associated with decrease in DNA methylation). DNA methylation at 18 months was positively associated with infant temperamental discomfort.
Puglia et al., 2020 [30]	Saliva	MT2 −934	Bisulfite-pyrosequencing	Brain signal entropy	Infants with lower DNA methylation had higher brain signal entropy, which was positively associated with parent reported social behavior.
Studies in Children and Adolescents					
Cecil et al., 2014 [54]	Cord blood and whole blood	CpG Island spanning exons 1-3	HM450 Array	Callous-unemotional traits	In children with low internalizing problems, higher *OXTR* methylation at birth was associated with callous-unemotional traits at age 13. Higher DNA methylation was also associated with reduced risk exposure in these children. In both children with low and high internalizing problems, parental risks were associated with higher DNA methylation. Neither DNA methylation at age 7 nor age 9 was associated with callous-unemotional traits.
Dadds et al., 2013 [55]	Whole blood	MT2	EpiTYPER	Callous-unemotional traits	Callous unemotional traits were associated with higher DNA methylation in older children (ages 9–16) but not in younger children (4–8 years old).
Moore et al., 2017 [81]	Buccal cells	MT2 and exon 3	HM450 Array	Neonatal contact	There was no association between neonatal contact and DNA methylation of *OXTR* assessed in early childhood.
Rijlaarsdam et al., 2017 [51]	Cord blood	Exon 3	HM450 Array	Prenatal environment, autistic traits	DNA methylation was not associated with prenatal stress. DNA methylation in cord blood was positively associated with communication problems at age 6 in participants with G/G genotype at SNP rs53576.
Fujisawa et al., 2019 [58]	Saliva	MT2	EpiTYPER	Childhood maltreatment, gray matter volume	Maltreated children had higher DNA methylation at CpG sites −1121/-1119 (assayed as one unit). Children with higher DNA methylation at these CpG sites had lower gray matter volume in left orbitofrontal cortex.
MacKinnon et al., 2019 [56]	Saliva	Exon 3	Bisulfite-sequencing (NGS)	Theory of mind	*OXTR* methylation was negatively associated with theory of mind abilities in children with mothers who display low levels of maternal structuring behavior.
Lecompte et al., 2020 [57]	Buccal cells	Exon 3	Bisulfite-sequencing (NGS)	Attachment behaviors	DNA methylation was negatively associated with maternal structuring behavior and child's controlling-caregiving behavior.
Studies of Childhood Experience completed in Adults					

(*continued on next page*)

Table 2 (*continued*)

Study	Tissue	*OXTR* Region	Method	Phenotype	Results
Unternaehrer et al., 2015 [59]	Whole blood	Exon 3	EpiTYPER	Maternal care received	Higher maternal care received was associated with lower DNA methylation of adults in the 3′ portion of exon 3. Men had lower DNA methylation in this region than women.
Smearman et al., 2016 [60]	Whole blood	MT2, exon 3	HM450 Array	Child abuse, depression and anxiety as adults	Child abuse was associated with higher methylation of 2 CpG sites in exon 3, though these associations did not survive correction for multiple comparisons. There were significant interactions of DNA methylation and child abuse history in predicting depression and anxiety symptoms, though the direction of the associations depended on the location of the CpG site.
Gouin et al., 2017 [61]	Whole blood	Promoter, MT2, and enhancer in intron 3	Bisulfite-pyrosequencing	Early life adversity	Early life adversity was associated with increased DNA methylation in the promoter and MT2 region and decreased DNA methylation in the enhancer at age 27 in females only. DNA methylation was associated with childhood anxiousness in females only.
Beach et al., 2018 [82]	Whole blood	Exon 1	HM450 Array	Substance abuse initiation	DNA methylation at age 20.5 was associated with higher probability of substance abuse initiation at age 13 in participants with 5-HTTLPR genotype s/s or s/l, but there was no effect in those with 5-HTTLPR l/l genotype.
Womersley et al., 2019 [63]	Whole blood	Exon 3	Bisulfite-sequencing (NGS)	Limbic brain volumes	*OXTR* methylation was not related to childhood emotional neglect or hippocampal or amygdalar volumes.
Brown et al., 2020 [62]	Saliva	MT2	Not reported	Childhood trauma, social instability and fatherhood behaviors (displayed)	Childhood trauma and social instability were associated with increased DNA methylation of *OXTR* which in turn was negatively associated with fatherhood involvement.
Gonzalez et al., 2020 [65]	Whole blood	MT2 -924 and −934	Bisulfite-pyrosequencing	Early life stress; BOLD response during reward anticipation task	In participants with low *OXTR* methylation (at age 28–29), childhood neighborhood harshness was positively associated with caudate and orbitofrontal BOLD response during the reward anticipation task (at age 28–29). There was no relationship in those with high *OXTR* methylation.
Parianen Lesemann et al., 2020 [83]	Saliva	MT2	Methylation sensitive high resolution melt curve analysis	N170 response to faces, perceived trustworthiness	DNA methylation interacted with childhood trauma to predict N170 response to emotional faces, though the interaction was not significant after correcting for multiple comparisons. There was no effect of *OXTR* DNA methylation on the participants' ratings of trustworthiness.
Studies of Older Adults					
Ebner et al., 2019 [84]	PBMCs	MT2 −934	Bisulfite-pyrosequencing	Attachment	DNA methylation was negatively associated with attachment avoidance in both young adults and older adults. DNA methylation was positively associated with attachment anxiety only in young adults.
Needham et al., 2015 [85]	PBMCs	Entire gene	HM450 Array	Life course socioeconomic status	Study completed in samples from MESA study. Participants were aged 55–94 years. Low childhood SES was associated with increased DNA methylation in non-promoter CpGs but there was no association with CpGs at promoter. Persistent low SES and upward mobility were associated with increased DNA methylation at non-promoter sites. DNA methylation was not related to gene expression.

These studies indicate that *OXTR* methylation is sensitive to early experiences and may mediate neural and behavioral outcomes related to early life experiences. Specifically, these studies provide evidence that maternal engagement may prepare the child for future social interactions by modulating *OXTR* methylation. Further work in longitudinal samples will help to clarify the relationship between pre- and postnatal environment, epigenetic regulation of *OXTR*, and adult outcomes. Additionally, further developmental work should also focus on outcomes in older adults, where the literature is largely empty (see Table 2).

4.2. Associations of OXTR DNA methylation and neuroimaging outcomes in adults

In order to understand how DNA methylation of *OXTR* is related to neural outcomes in living subjects, we and others have used both structural and functional neuroimaging approaches (see Table 3 for a complete list of neuroimaging studies and Table 4 for studies of behavior and psychopathologies in adults). We have shown that peripheral DNA methylation in MT2 is positively associated with amygdala volume, and another group has shown that peripheral DNA methylation in exon 3 is not related to amygdala volumes [63,66]. We and others have also shown that DNA methylation in peripheral blood mononuclear cells is related to BOLD response in many brain regions associated with social or affective processes. For example, Jack et al. show that DNA methylation of CpG −934 in MT2 is associated with BOLD response in left superior temporal gyrus and cingulate gyrus while observing ambiguous social stimuli. We have also shown that DNA methylation in MT2 is associated with BOLD response in brain regions typically associated with the salience network, including amygdala, insular cortex, anterior cingulate and inferior frontal cortex [29,67]. These seminal studies featured passive viewing tasks or perceptual discriminations that were easy for adult participants. Therefore, it is difficult to make specific inferences about the functional relevance of the positive relationship between DNA methylation and increased BOLD response in these regions. In a more recent study, we engaged participants in a difficult, divided social

Table 3
Studies of DNA methylation of *OXTR* in Humans: Neuroimaging Studies in Neurotypical Adults.

Study	Tissue	*OXTR* Region	Method	Phenotype	Results
Jack et al., 2012 [86]	PBMCs	MT2 -934	Bisulfite-pyrosequencing	BOLD response to ambiguous social stimuli	DNA methylation was positively associated with BOLD response to ambiguous social stimuli in left superior temporal gyrus and cingulate gyrus.
Puglia et al., 2015 [67]	PBMCs	MT2 -934	Bisulfite-pyrosequencing	BOLD response to emotional faces	DNA methylation was positively associated with BOLD response to angry and fearful faces in the left amygdala, insular cortex, posterior superior temporal sulcus, right fusiform gyrus, anterior cingulate cortex, and lateral occipital cortex. DNA methylation was negatively associated with functional connectivity between the right amygdala and several areas, including: anterior cingulate cortex, fusiform gyrus, inferior frontal gyrus, and insular cortex.
Puglia et al., 2018 [35]	PBMCs	MT2 -934	Bisulfite-pyrosequencing	BOLD response during selective social attention	DNA methylation was positively associated with BOLD response during selective social attention in: dorsolateral prefrontal cortex, anterior cingulate cortex, and parietal lobule. Higher DNA methylation was associated with lower functional connectivity between the dorsolateral prefrontal cortex and the salience network during the task. There were significant behavioral interactions, such that those with high autistic traits had a positive relationship between DNA methylation and visual cortex activation during the task. Those with high anxiety levels had a negative relationship between DNA methylation and visual cortex during the task.
Lancaster et al., 2018 [66]	PBMCs	MT2 -934	Bisulfite-pyrosequencing	Heart rate variability, amygdala gray matter volume	DNA methylation negatively correlated with high-frequency heart rate variability. DNA methylation was positively correlated with amygdala gray matter volume.
Chen et al., 2019 [87]	Saliva	MT2 −924/-934, −901	EpiTYPER	Intranasal oxytocin, BOLD response during prisoner's dilemma	In women, intranasal oxytocin administration blunted the positive relationship between DNA methylation at CpG −901 and left occipital pole BOLD response in cooperative outcomes. In men, intranasal oxytocin and DNA methylation at CpG −924/-934 (assayed as one unit) interact to predict BOLD response in right precuneus, left postcentral gyrus, and right occipital pole in the unreciprocated cooperation condition.
Krol, Puglia et al., 2019 [29]	PBMCs	MT2 -924	Bisulfite-pyrosequencing	BOLD response to emotional faces	DNA methylation was positively correlated with BOLD response in the right inferior frontal cortex when viewing angry faces.

attention task [35]. We were particularly interested in whether the interplay between attentional control and salience networks was predictive of performance on the task. Similar to prior research, we found activation in the attentional control network was associated with behavioral performance [68,69]. Moreover, individuals with higher DNA methylation had greater activation in this network when selectively attending to social information. Higher DNA methylation was also associated with decreased functional coupling of the salience and attentional control networks. Finally, we found that the interaction of the two networks was such that task performance for individuals low in connectivity between networks improved with greater recruitment of the attentional control network, whereas individuals with high connectivity between networks showed poorer performance with increased activation of the attentional control network. This finding illustrates the difficulty of relating individual variability in DNA methylation with behavioral performance and regional and functional connectivity findings in neuroimaging studies. Future studies should capitalize on groups with known social deficits (for example, patients with autism spectrum disorder), developmental trajectories of social and cognitive function in children, and an increased focus on intrinsic and task-based functional connectivity.

4.3. Connecting early experience, OXTR DNA methylation, and adult behavior

If we have a goal of eventually using *OXTR* DNA methylation as a biomarker for various outcomes in humans, it is imperative that we work to understand how *OXTR* methylation relates to experiences across the lifespan. *OXTR* methylation may serve as a link between early life experiences and future behaviors, such that early life experiences prepare organisms for their future environments. In a series of studies of a longitudinal sample of rural African American men, Kogan and colleagues have performed pathway analyses to understand how DNA methylation in MT2 (in saliva) relates to the effects of early life experience on adult behaviors [62,70–72]. Results from these studies indicate that childhood trauma and social instability are positively correlated with *OXTR* methylation. *OXTR* methylation mediates the relationship between these factors and diverse behaviors in adulthood, including substance abuse, romantic relationship support displayed to one's partner, and fatherhood involvement displayed towards children [62,70,71]. Additionally, participants with higher methylation of *OXTR* are more likely to develop defensive relational schemas, which ultimately increases substance abuse and depression [72]. These studies provide an example of how a richly phenotyped longitudinal dataset can provide insight into how *OXTR* methylation affects the development of adult behaviors.

This type of integrative work, while challenging, can greatly inform our understanding of life experiences and how *OXTR* regulation can create individual variability in behavioral and physiological phenotypes. Studies in animal models are an excellent fit for furthering this type of work. The quicker time scale available in a model system as well as the ability to manipulate the early environment in ways that are not possible in human populations will allow researchers to develop large, complex datasets that can serve as a guide for asking similar questions in humans.

5. Epigenetic regulation of *OXTR* expression as a mechanism of allostasis

We have reviewed a number of studies investigating DNA methylation of *OXTR* across the lifespan in animal models and in humans. While discrepancies in methodologies and sites of study remain, what is clear is that epigenetic regulation of *OXTR* via DNA methylation occurs in response to life events, likely contributing to individual variation in behavior and physiology. If, as proposed by Quintana and Guastella [9], oxytocin promotes allostasis, evidence presented here shows that this likely happens in part through regulation of *OXTR* expression (Fig. 4). Both early life events and adult experiences can alter, perhaps permanently, the epigenetic state of *OXTR*, which in turn can change expression of *OXTR*. These changes in the receptor system in turn can change activity of the peptide. For example, studies in rodents show that increased parental care early in life decreases *Oxtr* methylation which leads to increased *Oxtr* expression. This is adaptive to the social

Table 4
Studies of DNA methylation of *OXTR* in Humans: Behaviors and Psychopathologies.

Study	Tissue	*OXTR* Region	Method	Phenotype	Results
Gregory, Connelly et al., 2009 [88]	Temporal cortex, whole blood	MT2	Bilsufite-sequencing	Autism Spectrum Disorder	ASD patients had increased DNA methylation at CpGs −860, −934, and −959 in peripheral blood. In post-mortem samples of temporal cortex, ASD patients had increased DNA methylation at CpGs −924 and −934. ASD patients had decreased *OXTR* expression in cortex, and DNA methylation was negatively associated with gene expression (see Ref. [22]).
Unternaehrer et al., 2012 [89]	Whole blood	Exon 3	EpiTYPER	Acute stress	DNA methylation increased following acute stressor.
Kim et al., 2014 [90]	Buccal cells	MT2	Bisulfite-sequencing	Anorexia nervosa	Patients with anorexia nervosa had higher DNA methylation at 5 CpGs in MT2 (including −924 and −901) compared to healthy controls. DNA methylation at certain CpGs were associated with eating disorder symptomology, autism quotient, and depression and anxiety.
Ziegler et al., 2015 [91]	Whole blood	Exon 3	Bisulfite-sequencing	Social Anxiety Disorder	Patients with social anxiety disorder had lower *OXTR* DNA methylation. DNA methylation was negatively associated with the Social Phobia Scale and Social Interaction Anxiety Scale. There was a negative association of DNA methylation and amygdala response to social-phobia related words.
Reiner et al., 2015 [92]	Whole blood	Exons 1 and 2	Bisulfite-sequencing	Major Depressive Disorder	*OXTR* DNA methylation in exon 1 was lower in patients with MDD, and this effect was stronger in patients with G/G genotype at rs53576. DNA methylation in exon 2 was not associated with MDD.
Bell et al., 2015 [93]	Whole blood	MT2 -934	Bisulfite-pyrosequencing	Postpartum depression	Women who do not display depression during pregnancy, have the G/G allele of rs53576, and have high DNA methylation are more likely to develop postpartum depression than women with low DNA methylation or at least one A allele of rs53576.
Chagnon et al., 2015 [94]	Saliva	Exon 3	Bisulfite-pyrosequencing	Anxiety and depression in older women	DNA methylation was higher in women with anxiety and depression who have the A/A genotype of rs53576.
Rubin et al., 2015 [95]	Whole blood	MT2 -934	Bisulfite-pyrosequencing	Schizoaffective disorders, emotion processing, gray matter volume	Schizophrenia patients had higher DNA methylation than controls. DNA methylation did not differ from controls in patients with bipolar disorder or schizoaffective disorder. DNA methylation was negatively correlated with emotion processing in women only. Several diagnosis by DNA methylation interactions were predictive of gray matter volume of brain regions related to social cognition.
Cappi et al., 2016 [96]	PBMC	Exon 3	Bisulfite-pyrosequencing	Obsessive-compulsive disorder	DNA methylation was higher in OCD patients compared to controls. DNA methylation was negatively associated with depressive symptoms, but not anxiety symptoms.
Yuksel et al., 2016 [97]	Whole blood	MT1-MT4	Methylation-specific enzymatic digestion	Autism spectrum disorder	Patients with autism spectrum disorder had lower DNA methylation in MT1 and MT3 compared to controls. No differences between ASD patients and controls in MT2 or MT4.
Grove et al., 2016 [98]	Whole blood	Exon 3	Bisulfite-pyrosequencing	Cognition in patients with psychotic disorders	DNA methylation at one CpG site was negatively correlated with cognitive ability.
Kimmel et al., 2016 [99]	Whole blood	Intron 2, near an estrogen responsive element	HM450 array	Postpartum depression	*OXTR* methylation was lower in women with postpartum depression. Within PPD cases, women who were antenatally depressed as well had lower DNA methylation. DNA methylation was negatively associated with serum estradiol levels in PPD cases but not controls.
Simons et al., 2017 [100]	Whole blood	Exon 1	HM450 array	Major depressive disorder	Adult adversity was positively associated with *OXTR* methylation, which in turn was positively associated with distrust and pessimism. Distrust and pessimism were positively associated with depression.
King et al., 2017 [52]	Saliva	Exon 3	Bisulfite-sequencing (NGS)	Perinatal depression	Mothers with persistent (prenatal and postpartum) depression had increased DNA methylation compared to those with no depression, prenatal depression, or postpartum depression. There was no effect of maternal depression status on *OXTR* DNA methylation in children.
Haas et al., 2018 [101]	Saliva	MT2 −924/-934	EpiTYPER	Openness to experience	DNA methylation was negatively associated with the openness dimension of the Big 5 personality traits.
Ein-Dor et al., 2018 [102]	Saliva	MT2	qMethyl	Attachment avoidance	DNA methylation was positively associated with attachment avoidance in individuals with low anxiety.
Aghajani et al., 2018 [103]	Saliva	Exon 3	Bisulfite-sequencing	BOLD response in individuals with conduct disorder	There was an interaction between conduct disorder diagnosis, callous-unemotional traits, and *OXTR* DNA

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Table 4 (*continued*)

Study	Tissue	*OXTR* Region	Method	Phenotype	Results
					methylation in predicting the following outcomes: BOLD response to emotional faces in midcingulate, insular, tempoparietal, and precuneal cortices, and functional connectivity between amygdala and ventromedial prefrontal cortex.
Nawjin et al., 2019 [104]	Whole blood	Exon 3	Bisulfite-sequencing (NGS)	Post-traumatic stress disorder	DNA methylation was increased in female PTSD patients compared to controls, but not in male PTSD patients compared to controls. DNA methylation was positively associated with anhedonia subscale. In female PTSD patients, there were negative associations between DNA methylation at 2 CpG sites and amygdala response to negative faces. In control females, these associations were positive.
Kogan et al., 2019 [70]	Saliva	MT2	EpiTYPER	Substance abuse	Prosocial ties were negatively correlated with *OXTR* DNA methylation. DNA methylation was positively correlated with substance abuse problems. There was a significant indirect effect of childhood trauma on *OXTR* DNA methylation via prosocial ties.
Bang et al., 2019 [105]	Whole blood	MT2 (CpGs −959, −934, and −924), exon 3	Bisulfite-pyrosequencing	Schizophrenia	DNA methylation in MT2 was reduced in patients with recent onset schizophrenia and ultra-high risk participants compared to healthy controls. DNA methylation at CpG −959 was negatively associated with anhedonia-asociality in women with recent onset schizophrenia and ultra-high risk participants. There was a negative association of DNA methylation at CpG −959 and striatal-amygdala functional connectivity in women but not in men. DNA methylation in exon 3 was not significantly associated with any outcome.
Thaler et al., 2019 [106]	PBMCs	MT2, exon 3	HM450 array	Anorexia nervosa	DNA methylation differed among active anorexia, anorexia in remission, and no eating disorder patients, though the direction of associations differed among the CpG sites. DNA methylation at some CpG sites was significantly associated with social behavior.
Kogan et al., 2019 [71]	Saliva	MT2	EpiTYPER	Childhood trauma, socioeconomic instability, and romantic relationship support	Childhood trauma is positively associated with socioeconomic instability, which in turn is positively associated with *OXTR* DNA methylation. DNA methylation is negatively associated with romantic relationship support provided to main partner.
Andari et al., 2020 [107]	Saliva	MT2	EpiTYPER	Autism spectrum disorder, resting state functional connectivity	CpGs −901, −924/-934 (assayed as one unit), and −989 were hypermethylated in ASD patients compared to controls. DNA methylation of CpGs −1119/-1121 (assayed as one unit) was positively associated with social responsiveness score. DNA methylation at CpG −989 was negatively associated with autism symptom severity. DNA methylation at CpG −989 was negatively associated with resting state functional connectivity of superior temporal sulcus and posterior cingulate cortex and positively associated with resting state functional connectivity of ventral striatum and ventromedial prefrontal cortex.
Park et al., 2020 [108]	Whole blood	MT2, exon 3	Bisulfite-pyrosequencing	Obsessive-compulsive disorder	DNA methylation at CpGs −959 and −934 were reduced in OCD patients (including when restricted to only drug-naïve patients). DNA methylation at CpG −959 was negatively associated with symptom severity.
Moser et al., 2020 [109]	Saliva	Enhancer in intron 3	Bisulfite-sequencing (NGS)	Borderline personality disorder	There was no association of *OXTR* methylation with BPD.
Kimura et al., 2020 [110]	Whole blood	MT2	HM450 Array	William's syndrome	Patients with William's syndrome had increased *OXTR* methylation and decreased *OXTR* expression.
Kogan et al., 2020 [72]	Saliva	MT2	EpiTYPER	Substance abuse, depressive symptoms	In men with high *OXTR* methylation, there is a positive association of contextual stressors and defensive/hostile schemas, which are related to substance abuse via social bonding risk factors.
Schiele et al., 2021 [111]	Whole blood	Exon 3	Bisulfite-sequencing	Response to treatment for obsessive-compulsive disorder	DNA methylation was higher at baseline in OCD patients than controls. DNA methylation was negatively correlated with responsiveness to OCD therapy.
Siu et al., 2021 [112]	Whole blood (ASD), Saliva, (OCD and ADHD)	MT2	Bisulfite-pyrosequencing	Neurodevelopmental disorders	In ASD males, there was hypomethylation of CpGs −982 and −860 compared to controls. Females with ADHD had lower levels of DNA methylation at CpGs −989, −924, and −934 compared to controls. Females with OCD had higher levels of DNA methylation at CpGs −924 and −934 compared to controls. Males with OCD had higher levels of DNA

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Table 4 (*continued*)

Study	Tissue	*OXTR* Region	Method	Phenotype	Results
Warrener et al., 2021 [113]	Saliva	MT2 -901, −924, and −934	Bisulfite-pyrosequencing	Depression and suicidality in war veterans	methylation at CpGs −826 and −835 compared to controls. DNA methylation at CpG −924 was negatively correlated with depression symptoms. DNA methylation at CpG −901 was positively correlated with PTSD symptoms. DNA methylation was not associated with suicidality.

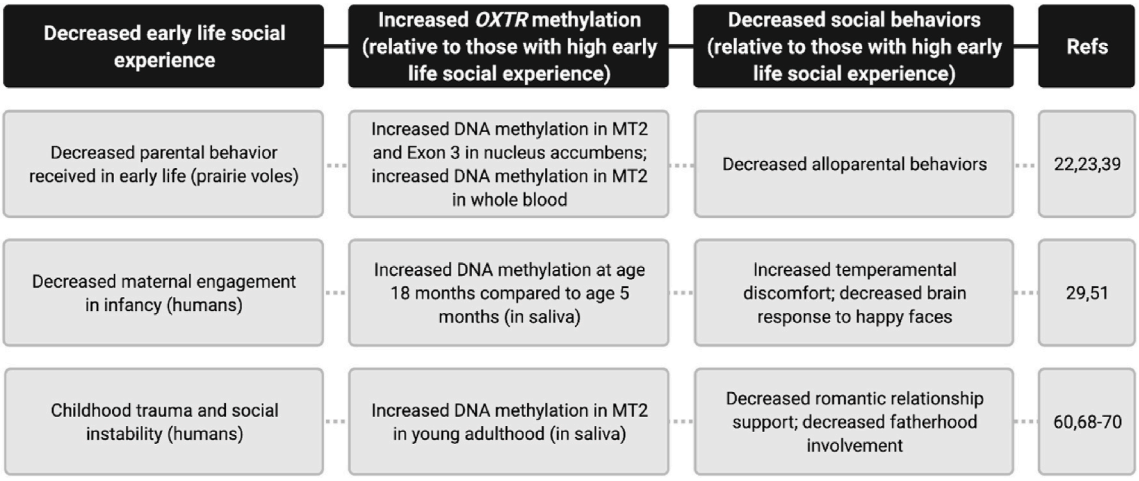

Fig. 4. DNA methylation of the oxytocin receptor as a mechanism of early life allostasis. A model for DNA methylation of the oxytocin receptor is presented (darker boxes). Converging lines of evidence in both human and animal models (lighter boxes) support epigenetic programming of the oxytocin receptor by early life social experience, which in turn prepares the organism for future experiences in that social environment. Image created with BioRender (biorender.com).

environment and prepares the animal for a successful life in that environment. Similarly in humans, decreased *OXTR* methylation associated with greater amounts of parental care may prepare the individual for a social life that is more congruous with their early life experiences. Another example is the physiology of parturition: studies have documented increased *OXTR* methylation in the amnion and decidua in preterm birth compared to term labor, suggesting that as pregnancy reaches term, DNA methylation in the uterus decreases (leading to higher *OXTR* expression) to prepare for parturition. Dysregulation of DNA methylation may lead to obstetric complications such as pre-eclampsia. Epigenetic regulation of *OXTR* thus allows the oxytocin system to more accurately and efficiently respond to environmental cues in the future. Importantly, this means that high DNA methylation of *OXTR* may be optimal in some environments while lower DNA methylation of *OXTR* is advantageous in other environments. This also suggests that epigenetic *dysregulation* of this system (where the level of DNA methylation does not match the environment and context) is a key avenue of study to begin to understand aberrant oxytocin activity in a wide range of behavioral and physiological outcomes and in situations where allostasis is not achieved or maintained.

6. Future directions: furthering our understanding of *OXTR* Methylation's role in allostasis

Although the above evidence supports *OXTR* methylation as a mechanism underlying allostatic effects of oxytocin, many questions remain. While *OXTR* methylation is often associated with early life experiences, some studies differ on the direction of this association. We must remember that these associations may be tissue and CpG site specific. Additionally, while there is evidence for DNA methylation dependent expression of *OXTR*, DNA methylation is not the only factor regulating *OXTR* expression. Nor is *OXTR* the only gene involved in oxytocin signaling. The evidence reviewed indicates that *OXTR* methylation in the central nervous system in part contributes to oxytocin's allostatic actions. It also suggests that *OXTR* methylation in peripheral tissues (blood or saliva) may be a signature of life experiences which report on how these life experiences affect *OXTR* methylation in the brain. However, it remains a challenge for the field, particularly in human studies, to connect peripheral measurements of *OXTR* methylation with oxytocin signaling in the central nervous system mechanistically. While future studies in animal models may be able to address this, studies in rodents thus far have been largely correlative.

In particular, many new tools are now available to modify DNA methylation *in vitro* and *in vivo* which can be used to gain insight into how DNA methylation at particular CpG sites are affected by life experience and how they modify *OXTR* expression and behavior. Previous methods to alter DNA methylation such as pharmacological manipulations (for example, 5-azacytidine, zebularine, or indirectly through the histone deacetylase inhibitor trichostatin A) had global effects, meaning that the changes to DNA methylation could not be targeted to a specific gene but instead affected the entire genome. However, using CRISPR-Cas9 systems which allow for target specificity, it is now possible to change DNA methylation at a specific gene locus by coupling a deactivated Cas9 (which prevents cutting of the DNA) conjugated to epigenetic enzymes like DNMT3a (which methylates cytosines) or Tet1 (which initiates demethylation) [73,74]. This method has been used successfully *in vitro* and *in vivo*. Such tools should be used to study how DNA methylation at specific regions of *OXTR* regulates gene expression and contributes to allostasis.

7. Is *OXTR* DNA methylation a biomarker?

The previous research described in this article provides an excellent basis for biomarker development. Biomarkers in psychiatry should be specific and sensitive, capture individual variability, and be standardized and reproducible [75]. In order for *OXTR* methylation in peripheral

tissue to serve as a biomarker, we must first understand the typical development of this marker in longitudinal samples across the lifespan. *OXTR* methylation across development will likely depend on the CpG site and tissue being measured. Along these lines, we must perform studies aimed at understanding the dynamics of peripheral *OXTR* methylation in adulthood in response to both endogenous (for example, hormonal fluctuations associated with menstrual cycle) and exogenous (for example, medication or profound stress) factors. Future studies should also characterize how *OXTR* relates to specific behavioral domains, instead of diagnoses, since many psychiatric conditions have overlapping symptoms. This may help to understand the specificity and sensitivity of *OXTR* methylation in predicting psychiatric outcomes. It is likely that *OXTR* on its own will not be sensitive and specific, but in combination with other biomarkers such as genetic variants or neuroimaging outcomes *OXTR* methylation may be informative.

In parallel to this, further studies are necessary in model systems to provide a mechanistic understanding of how early life experiences affect *OXTR* methylation and how *OXTR* methylation controls gene expression in the brain. This work will require multidisciplinary approaches from biochemistry, molecular genetics, cell biology, neuroscience, and animal behavior. Using animal models will further allow for studying *OXTR* methylation on a much quicker timescale, albeit in cross-sectional samples. Importantly, work in appropriate animal models will help us understand cross-tissue correlations of *OXTR* methylation from relevant tissues such as blood, brain, uterus, and mammary gland. These studies will complement studies in humans and aid in biomarker development. A final future direction involves developing targeted treatments and therapies to push the oxytocin system towards typical levels in individuals who are at risk for or already have aberrant oxytocin systems. Given the oxytocin system's role in mediating or moderating the effect of early life experience on adult behavior, for example, an approach such as this may help to reduce negative long-term outcomes in at-risk populations. This work could use *OXTR* methylation as a biomarker of treatment efficacy. Again, this work will require multidisciplinary efforts in both animals and humans, but remains a future goal for clinical psychiatrists and psychologists.

Lastly, in order to fully understand the utility of *OXTR* methylation as a potential biomarker, we must not only know what *OXTR* methylation reports on but also what it *does not* report on. While we review the literature, we acknowledge that studies which do not find associations between *OXTR* methylation and biobehavioral outcomes often do not get published. Additionally, even in studies where there is a significant association between *OXTR* methylation and such outcomes, we cannot know if associations with other outcomes were tested and insignificant unless they are reported. As such, we recommend that authors explicitly state if their studies are exploratory (testing the association between *OXTR* methylation and several outcomes) or hypothesis-driven (testing only a select few outcomes). Authors should also state if *OXTR* is the only gene where methylation was tested as a marker of these outcomes, particularly when Illumina arrays are used to measure epigenome-wide methylation. We also encourage the reporting and publishing of negative results to fully understand the role of *OXTR* methylation in allostasis and potential uses as a biomarker.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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