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# Maternal oxytocin treatment at birth increases epigenetic age in male offspring

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## Abstract

Exogenous oxytocin (OT) is widely used to induce or augment labor with little understanding of the impact on offspring development. In rodent models, including the prairie vole (*Microtus ochrogaster*), it has been shown that oxytocin administered to mothers can affect the nervous system of the offspring with long lasting behavioral effects especially on sociality. Here, we examined the hypothesis that perinatal oxytocin exposure could have epigenetic and transcriptomic consequences. Prairie voles were exposed to exogenous oxytocin, through injections given to the mother just prior to birth, and were studied at the time of weaning. The outcome of this study revealed increased epigenetic age in oxytocin-exposed animals compared to the saline-exposed group. Oxytocin exposure led to 900 differentially methylated CpG sites (annotated to 589 genes), and 2 CpG sites (2 genes) remained significantly different after correction for multiple comparisons. Differentially methylated CpG sites were enriched in genes known to be involved in regulation of gene expression and neurodevelopment. Using RNA-sequencing we also found 217 nominally differentially expressed genes ($p<0.05$) in nucleus accumbens, a brain region involved in reward circuitry and social behavior; after corrections for multiple comparisons 6 genes remained significantly differentially expressed. Finally, we found that maternal oxytocin administration led to widespread alternative splicing in the nucleus accumbens. These results indicate that oxytocin exposure during birth may have long lasting epigenetic consequences. A need for further investigation of how oxytocin administration impacts development and behavior throughout the lifespan is supported by these outcomes.

## Introduction

The neurohormone oxytocin (OT) is involved in both the physiology of birth and the onset of maternal behavior[1,2]. Pulsatile release of oxytocin in the myometrium facilitates

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uterine contractions during birth and prevents postpartum hemorrhage[1]. Evidence indicates that oxytocin can cross the placental barrier[3–6], prompting the hypothesis that oxytocin serves as a maternal-fetal signaling molecule in the peripartum period[7]. In fact, oxytocin signaling in the fetus is critical during birth to protect against hypoxic conditions[8] and acts as an analgesic during birth[9]. While there remains disagreement over the primary source of oxytocin in the fetus (i.e. if it is derived maternally or from the fetal brain), it is clear that maternally derived oxytocin does signal to the fetal brain in some capacity.

In current obstetric practices, synthetic oxytocin (aka Pitocin) is used to induce or augment labor, with potential consequences for both maternal and fetal behavioral outcomes[10]. Rates of labor induction administration in the United States have more than tripled over the last three decades, and nearly a third of births are induced[11]. Accounting for both labor induction and augmentation, it is estimated that half of all births in the United States include some form of synthetic oxytocin administration[12]. Several groups have provided evidence that use of synthetic oxytocin during labor modestly increases risk of neurodevelopmental disorders including autism spectrum disorder[13–15] (ASD) and attention deficit hyperactivity disorder[15,16] (ADHD) in offspring, with some of these studies finding that male children are particularly sensitive to synthetic oxytocin use. Though many factors might explain this association, including known associations of labor induction and augmentation on subsequent maternal behaviors including skin-to-skin contact and breastfeeding[10], it remains possible that maternally administered oxytocin impacts the fetal brain in the peripartum period.

Examination of molecular mechanisms mediating the impact of synthetic oxytocin administration on offspring development and behavior requires the use of an animal model. Many studies in prairie voles and other rodents suggest that administration of oxytocin to a neonate immediately after birth impacts behavioral and neuroanatomical outcomes throughout the lifespan[17]. Male prairie voles exposed to oxytocin as a neonate display increased partner preference behavior as adults[18]. Further, neonatal exposure to oxytocin increases c-Fos staining in the supraoptic nucleus[19] and vasopressin receptors in the cingulate cortex[20]. Though the above studies are informative, they do not simulate labor induction because in these studies exposure to oxytocin occurred after birth. One study simulating labor induction found that maternal oxytocin treatment in mice resulted in widespread differential gene expression in embryonic brains, with enrichment for genes involved in protein localization within the cell[21]. This same group, however, found no differential gene expression in the brains of mice born to saline and oxytocin treated dams at postnatal day 9[21].

We have previously used prairie voles, a monogamous rodent species with human-like oxytocin-dependent social behaviors[22], to examine the impact of maternal oxytocin administration on offspring behavioral and molecular outcomes. We found that offspring of oxytocin-treated dams had increased social behaviors throughout the lifespan, including more frequent and longer ultrasonic vocalizations as pups, increased parental care towards non-kin pups as subadults, and increased time spent with conspecifics during a partner preference test as an adult[6]. Additionally, neuroimaging revealed numerous differences in brain region volumes and functional connectivity[23]. The effects of oxytocin exposure on

brain region volumes were widespread but small while changes in functional connectivity in males exposed to maternally administered oxytocin were more robust[23] . Specifically, oxytocin-exposed males had greater and stronger functional connectivity throughout the brain[23]. Importantly, we have provided evidence that fetuses are acutely sensitive to maternally administered oxytocin, effects of maternally administered oxytocin on offspring are not due to changes in maternal behavior, and maternally administered oxytocin impacts epigenetic regulation of the oxytocin receptor gene (*Oxtr*)[6]. However, how maternally administered oxytocin impacts developmental processes and gene regulation in prairie voles beyond effects on *Oxtr* remains unknown.

The nucleus accumbens is a candidate brain region for molecular mechanisms underlying prairie vole social behaviors including those affected by early life oxytocin administration[24,25]. Further, we have recently provided evidence that males are specifically sensitive to a different early life experience (parental care, particularly by fathers) which impacts gene expression, synapse density and morphology in nucleus accumbens, and the social behavior of alloparenting, displaying parental care to offspring other than one's own[26]. Here, we provide evidence that maternally administered oxytocin increases the pace of development, indexed by epigenetic age, in nucleus accumbens of weaning-age male offspring. We also provide evidence of widespread DNA methylation changes localized to exons and introns and depleted in promoters and 5' untranslated regions. Finally, we show that while maternal oxytocin treatment leads to limited changes in gene expression, there is widespread alternative splicing of mRNA transcripts.

## Methods

### Animal Model and Experimental Design

Subjects were laboratory-bred prairie voles (*Microtus ochrogaster*), descendants of wild-caught stock captured near Champaign, Illinois. Breeding pairs were housed in large polycarbonate cages (44 cm × 22 cm × 16 cm). Animals were given high-fiber Purina rabbit chow and water *ad libitum*, cotton nestlets for nesting material, and were maintained on a 14:10 light:dark cycle. All procedures were conducted in accordance with NIH standards for animal welfare and approved by the Institutional Animal Care and Use Committee at Indiana University, Bloomington.

A timed mating paradigm was used to predict the estimated date of delivery. Female prairie voles are induced into estrus and ovulation by the presence of a mate. Females were introduced to stud males and allowed to interact for 24 hours. After these 24 hours, a perforate cage divider was introduced to prevent early mating while allowing females to remain exposed to male odors and dirty bedding to induce estrus. After 72 hours, the cage divider was removed and mating was observed. The expected day of birth is 21.5 days after mating. On the expected day of birth, animals were checked for physical characteristics indicating successful term pregnancy, including weight gain, shape of abdomen, and prominence of nipples. Animals deemed term pregnant were weighed and injected i.p. with 0.5 mg/kg freshly reconstituted oxytocin (Bachem, Torrance, CA) or saline in an equivalent volume. Litters that were born within 24 hours of injection were included in the study and reared to postnatal day 20 (day of birth: postnatal day 0). Only one animal was used from

each litter to avoid genetic and litter effects. Weights were measured at postnatal day 7 and postnatal day 20. We chose to investigate weaning-age animals because at weaning age, species-typical social behaviors which are disrupted by early life exposure to exogenous oxytocin emerge. On postnatal day 20, offspring were deeply anaesthetized with isoflurane and euthanized via cervical dislocation and rapid decapitation. Brains were extracted, flash frozen on dry ice, and stored at −80°C until dissection. A total of 8 animals (4 saline, 4 oxytocin) were used for epigenetic age and genome-wide methylation measurements. A subset of 6 animals (3 saline, 3 oxytocin) were used for RNA-sequencing analysis.

The dose of oxytocin used in this study is a subthreshold dose which does not induce labor in prairie voles (Supplementary Figure 1A). We note that in the past, we have used this dose and others and found varying effects on offspring based on sex and oxytocin dose[6,23]. While this dose is higher than what most pregnant women (though not all) would receive to induce labor[14,27–29] , it is possible prairie voles require more oxytocin because of their heightened oxytocin system. A study in rats found that labor induction requires similar amounts of oxytocin in humans and rats[30]. Notably, non-pregnant adult female prairie voles have over twice as much circulating oxytocin than non-pregnant adult female rats[31], which might explain why the dose used in our study did not induce labor.

An additional set of 7 animals from a previous study of early life experience were used to compare epigenetic age acceleration of offspring in the saline treatment group and offspring born to mothers unmanipulated prior to birth. Methods describing the generation of these animals and measurement of epigenetic age are described in these studies[26,32]. Based on previous findings that the male brain is more sensitive to early oxytocin exposure with regard to oxytocin receptor density and functional connectivity, only males were used for the present analysis.

### Tissue Dissection and Nucleic Acid Isolation

Brains were equilibrated to −20°C prior to sectioning. Nucleus accumbens was dissected by first making a coronal cut to remove the olfactory bulbs, then making a second coronal cut 2 mm caudal to the frontal pole, and extracting nucleus accumbens by bilateral punches (1 mm in diameter, 2 mm in depth). DNA and RNA were extracted using the AllPrep DNA/RNA mini kit (Qiagen, Valencia, CA) following manufacturer instructions. DNA was stored at −20°C until further use. RNA was stored at −80°C until further use.

### Pan-mammalian Methylation Array and Epigenetic Clock Scores

DNA methylation was measured from 250 ng bisulfite-converted genomic DNA using the custom Illumina chip “HorvathMammalMethylChip40”. Beta values were normalized using SeSaMe and used as input to calculate epigenetic age on the universal clocks[33,34]. Epigenetic age acceleration was calculated by subtracting age (20 days) from the UniversalClock2 estimate, an epigenetic age measurement which was developed from many mammalian species including prairie voles.

### Statistical Analysis of Developmental Outcomes

We used the 2-sample test for equality of proportions to compare the proportion of dams giving birth within 24 hours following injection of either saline or oxytocin. Offspring weights were analyzed using a mixed effects linear model with a fixed effect of the interaction of age, sex, and oxytocin treatment and a random effect of litter ID to account for genetic and litter effects. Post-hoc comparison of weights between treatment groups was completed using the emmeans package[35]. Epigenetic age acceleration was compared between saline and oxytocin treatment groups using the Wilcoxon rank-sum test. Epigenetic age acceleration was compared between saline-exposed offspring and offspring from uninjected mothers using the Wilcoxon rank-sum test.

### Differential DNA Methylation Analysis

DNA methylation was compared between oxytocin and saline treatment groups using *limma* in R[36,37]. Reported *p* values were adjusted using the Benjamini-Hochberg method implemented in *limma*. Probes were filtered to remove any probe which failed in any sample, leaving 15550 of 37554 probes. Nominally differentially methylated probes (unadjusted $p < 0.05$) were tested for enrichment of specific genomic features (compared to CpGs with no difference in methylation) using the two-proportions Z-test. Gene ontology testing was performed using the enrichGO function in the clusterProfileR package by comparing genes with at least one differentially methylated CpG site (unadjusted $p<0.05$) to the background list of all genes with a CpG site annotated to the 15550 probes with good signal. For gene ontology testing, input genes were converted to mouse entrez IDs using biomaRt[38].

### RNA-sequencing

RNA quality was assessed using Agilent Tape Station. All samples had RNA Integrity Number (RIN) scores greater than 7. Libraries were generated from 500 ng RNA using the NEBNext Ultra Directional RNA Library Prep Kit (New England Biolabs, Ipswich, MA) with mRNA magnetic isolation. Multiplexed libraries were sequenced at 2 × 75 base pair, paired-end reads on the Illumina NextSeq 500 platform at Genome Analysis and Technology Core, University of Virginia (RRID: SCR_018883), with 25 million reads per sample. The raw sequencing data was subjected to pre-processing steps of adapter removal and quality-based trimming using TrimGalore with removal of auto-detected Illumina adapters and trimming of low-quality ends up to a threshold of Q20[39]. Reads that became shorter than 35 bp due to either adapter removal or quality trimming were discarded. Quality control was completed with MultiQC[40]. Transcript abundances in the dataset were quantified using pseudoalignment approach of Kallisto[41], against the annotated transcriptome of prairie vole (*Microtus ochrogaster, MicOch v1.0,* Ensembl release version 96), which includes 19,648 annotated genes. Novel transcript identification for examining alternative transcript usage was performed using STAR2 alignments[42] and Stringtie[43].

### Statistical analysis of RNA-sequencing data

Analysis of RNA-sequencing data was completed in R version 3.5.2[37]. Differential expression analysis was completed using the DESeq2 package, which identifies significance

at the genome-wide level[44]. Only genes with greater than 1 transcript per million on average across samples (13,785 genes) were included in the DESeq2 analysis. Isoform switches were analyzed using the IsoformSwitchAnalyzeR package with the DEXseq workflow using default parameters[45–47]. Domains and other functional features of putative proteins from each transcript were identified using the following programs: protein coding potential of transcripts was determined using CPC2[48]; protein domains were identified using Pfam[49]; intrinsically disordered regions were identified using NetSurfP-2[50]; signal peptides were identified using SignalP[51].

## Results

### Maternal oxytocin administration increases epigenetic age in juvenile offspring

We first examined if early exposure to endogenous oxytocin impacts neurodevelopmental trajectories by examining epigenetic age. Male offspring were chosen for this study because maternal oxytocin administration leads to a small but significant increase in weight, indicating accelerated development (Supplementary Figure 1B). Epigenetic age is widely used in humans to estimate biological age and determine the impact of environmental factors on the aging process[52]. Recently, a pan-mammalian epigenetic array was developed along with an epigenetic age estimator that applies to all mammals[34,53]. Notably, this epigenetic age estimator was developed using data from many mammalian species, including prairie voles. To test if perinatal exposure to exogenous oxytocin impacts epigenetic age, we modeled labor induction by administering oxytocin or saline (as a control) to pregnant prairie voles on the expected day of birth, then examined epigenetic consequences in the nucleus accumbens of male juvenile (postnatal day 20) offspring (Figure 1A). We found that maternal oxytocin administration, compared to the control, led to an acceleration in epigenetic age (Figure 1B, $p$=0.029). In fact, maternal oxytocin administration leads to increased epigenetic age of over 12 days (mean of saline group = −4.99 days, mean of oxytocin group = +7.65), though it is difficult to translate the magnitude of the difference given that much more rodent neurodevelopment occurs postnatally compared to humans[54,55]. In a separate comparison, epigenetic age acceleration in the saline group did not differ significantly from offspring born to mothers not treated prior to birth ($p$=0.649, Supplementary Figure 1C). These results indicate that in male prairie voles, early exposure to exogenous oxytocin around the time of birth can accelerate epigenetic aging and suggests that neurodevelopment might also be accelerated.

### Maternal oxytocin administration leads to differential DNA methylation of genes related to neurodevelopment

To further understand epigenetic consequences of maternal oxytocin administration, we examined differential DNA methylation in the nucleus accumbens. We found 900 nominally differentially methylated CpG sites (annotated to 589 genes; 370 CpG sites were hypermethylated in the oxytocin-exposed animals and 530 sites were hypomethylated in the oxytocin-exposed animals, Figure 2A, Supplementary Table 1). After correcting for multiple comparisons, two CpG sites remain significant: one in an intron of splicing factor *Srsf5* and another in an intron of calcium channel subunit *Cacna1e*.

We next tested whether differentially methylated CpG sites are enriched for specific genomic regions (Figure 2B). We found that CpG sites with increased methylation following oxytocin treatment are highly enriched for exonic regions ($p$=5.6e-5). Notably, CpG sites with lower methylation following oxytocin treatment are less likely to be in exons ($p$=0.012), evidence that maternal oxytocin treatment preferentially leads to increased methylation of exonic CpG sites. DNA methylation is typically high in exons, where DNA methylation regulates transcription initiation at alternative transcription start sites and regulates splicing of pre-mRNA[56–59]. CpG sites with lower methylation are also less likely to be found in unannotated (intergenic) regions ($p$=0.003), promoters ($p$=0.009), and 5' untranslated regions ($p$=0.0004). CpG sites with lower methylation in the oxytocin group are enriched for intronic ($p$=1.9e-14) and intergenic upstream ($p$=0.005) regions. This indicates that maternally administered oxytocin might impact expression or splicing of alternative transcripts, given the enrichment of differentially methylated CpG sites in introns and exons and the depletion of differentially methylated CpG sites in genic regions associated with transcription initiation, such as the promoter and 5' untranslated region. Finally, these enrichments are consistent with oxytocin treatment accelerating development, as it has previously been shown that CpG sites that show higher DNA methylation with aging are enriched for exons while those that with lower DNA methylation with aging are enriched for introns[60].

We then performed gene ontology analysis to determine biological processes of genes with differential methylation (Figure 2C). We found 10 biological processes with significant enrichment; most of these processes are related to neurodevelopment and cell proliferation. Specific biological processes of interest include central nervous system development ($p$=0.035), cell proliferation in hindbrain ($p$=0.035) and cell surface receptor signaling pathway involved in cell-cell signaling ($p$=0.045). Altogether, differential methylation of CpG sites following maternal oxytocin administration supports the conclusion that oxytocin administration prior to birth accelerates neurodevelopment.

## Maternal oxytocin administration leads to widespread alternative transcript usage

We next examined how maternal oxytocin treatment impacts gene expression in the nucleus accumbens. Using RNA-sequencing, we found that there are 229 genes differentially expressed between the saline and oxytocin treatment groups, 126 of which have higher expression in the oxytocin-exposed group (Figure 3A, Supplementary Table 2). However, only six genes remain differentially expressed after correcting for multiple comparisons. Given the enrichment of differential methylation resulting from maternal oxytocin treatment at CpG sites in exons and introns, we hypothesized that there might be alternative splicing as a result of perinatal oxytocin exposure because of the known role of DNA methylation in these gene regions in regulating alternative splicing[58,59]. Using novel isoform detection in the RNA-sequencing data (see GEO Series GSE240856 for .gtf file containing coordinates of novel transcripts and genes), we analyzed the impact of perinatal oxytocin exposure on alternative splicing among genes in the nucleus accumbens. We find that perinatal oxytocin exposure leads to differential transcript usage (i.e. alternative splicing) in 233 genes at genome-wide significance (Figure 3B, Supplementary Table 3). The prevalence of differential transcript usage is striking compared to differential gene expression, where only

six genes were differentially expressed at genome-wide significance. We did not perform gene ontology analysis on genes with differential transcript usage because of the number of novel transcripts belonging to genes which are not annotated in the prairie vole genome.

To illustrate the impact of an isoform switch, we provide one example gene with significant differential transcript usage. *Camk2b*, which encodes a subunit of the calcium-calmodulin dependent protein kinase II, is a critical kinase which enables synaptic plasticity at excitatory synapses and is implicated in reward processes in the nucleus accumbens[61,62]. CaMKII is also notably part of the oxytocin receptor signal pathway[63]. Structurally, the CaMKIIβ protein consists of the protein kinase (catalytic) domain (Figure 3C, exons 1–10, shown in purple), a regulatory region associated with autophosphorylation (Figure 3C, exons 11 and 12, shown in gray as they were not identified by Pfam), a variable linker region (Figure 3C, exons 13–20, shown in orange and gray), and an association domain necessary for oligomerization (Figure 3C, exons 21–23, shown in green)[64]. *Camk2b* alternative splicing in the brain is developmentally regulated, with embryos favoring expression transcripts without exon 13 (ENSMOCT00000004130, also termed $\beta_e$) and adults expressing transcripts that include this exon (ENSMOCT00000004126 and MSTRG14953.1, also termed β' and β respectively)[65]. Additionally, there is a transcript which includes proline-rich exons 18–20 and is expressed in skeletal muscle (ENSMOCT00000004120, also termed $\beta_M$)[66]. We find that while total expression of *Camk2b* only differs minimally and not at genome-wide significance (Figure 3D, Supplementary Table 2), animals perinatally exposed to oxytocin have increased usage of the canonical brain-expressed transcripts (in particular $\beta_e$ and β') while the saline control group primarily expresses the $\beta_M$ transcript originally found in skeletal muscles (Figure 3E,F). Though the role of the $\beta_M$ transcript is not well defined in neurons, this transcript has reduced affinity for binding F-actin[67], an interaction which both localizes the CaMKII complex to dendritic spines and regulates the stability of cytoskeletal structures in spines[68].

## Discussion

Our results from this animal model suggest that the common obstetric practice of using oxytocin to induce labor may hold the potential to impact neurodevelopmental processes and gene regulation of offspring, with effects that can be detected far past the drug exposure. Our data in prairie voles indicate that male offspring exposed to maternally administered oxytocin have accelerated neurodevelopment, measured here by an index of epigenetic age. We also provide evidence that maternally administered oxytocin leads to widespread changes in DNA methylation, and differentially methylated CpG sites are enriched in exons and introns and are depleted from promoters and 5' UTRs. Finally, we show that while maternal oxytocin treatment leads to limited differential gene expression, it does result in widespread alternative splicing, including of oxytocin-responsive gene *Camk2b*. Exposure to exogenous oxytocin in the perinatal period led to accelerated aging and altered transcriptional and post-transcriptional processes.

One intriguing hypothesis resulting from these studies might be that maternally derived oxytocin signaling, which peaks during birth[69], organizes neurodevelopmental processes. Oxytocin administration to mothers changes the timing of oxytocin signaling to the fetus,

with the potential to change the neurodevelopmental trajectories of offspring, resulting in increased epigenetic age. An alternative (though not mutually exclusive) hypothesis might be that a bolus of oxytocin signaling, experienced prior to the time when oxytocin signaling typically occurs, might desensitize or downregulate the oxytocin receptors and result in reduced oxytocin signaling at birth and reduced protection against hypoxia[8].

Some clinical research indicates that oxytocin administration might exacerbate birth hypoxia[70,71]. Additionally, a study in rats reported that maternal oxytocin administration led to worse metabolic outcomes in neonates following an anoxic challenge[72]. However, a second study which used an oxytocin dosing regimen more similar to that used for human labor induction found that oxytocin administration did not impact oxidative stress in the fetal brain[30]. Previous work in rats suggests that birth hypoxia accelerates development of dendritic arbors in nucleus accumbens[73], though the impact of maternal oxytocin administration on this outcome is not known. Further work examining how oxytocin administration relates to hypoxia at birth might clarify the mechanisms underlying the results presented in this study. This hypothesis is not tested in the present study since we did not use an additional hypoxic challenge, only allowing us to suggest that maternal oxytocin administration might make fetuses more sensitive to the hypoxic conditions of physiological birth.

Our results on manipulation of oxytocin in neonatal male prairie voles do suggest that maternal oxytocin administration and/or its consequences for the neonate might lead to changes in the epigenome and transcriptome of the nucleus accumbens later in life. Specifically, administration of oxytocin to the mother led to widespread changes in DNA methylation of genes related to neurodevelopment, with differentially methylated CpG sites being enriched in exons and introns (Figure 2). Accordingly, we find that while maternal oxytocin administration led to some changes in gene expression, the effects were particularly pronounced in alternative splicing and transcript usage (Figure 3). DNA methylation of introns and exons is known to regulate alternative splicing[58,59], suggesting that maternal oxytocin administration might result in differential transcript usage via differential DNA methylation. More work including a longer time-course study of critical developmental timepoints will be necessary to determine if these results are indicative of an accelerated developmental trajectory, and whether differential DNA methylation and alternative splicing persists until adulthood.

Our results in male prairie voles indicate that maternal oxytocin administration alters the epigenome and transcriptional regulation in the nucleus accumbens in weaning-age animals. Notably, maternal oxytocin administration leads to increased epigenetic age, suggesting that animals exposed to exogenous oxytocin, or its consequences might experience a faster pace of development. We speculate whether possible mechanisms leading to these outcomes might alter sensitivity to the hypoxic conditions of birth or have other developmental consequences. Oxytocin administration prior to and during labor is widely used in obstetrics as a tool for reducing poor birth outcomes but cannot be assumed to be without consequences.

This study is not without limitations. It was conducted in an animal model and data are at present available only for males. Whether similar findings might occur in females and whether these findings have implications for human development remains to be tested. Further, we acknowledge the small sample used in this study. However, the strength of these findings supports the need to expand investigation into the role of oxytocin in epigenetic regulation and its possible consequences across the lifespan in both male and female offspring using larger samples in both animal models and human studies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

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## Data availability

RNA-sequencing and epigenetic array data are available at NCBI Gene Expression Omnibus with accession GSE240832.

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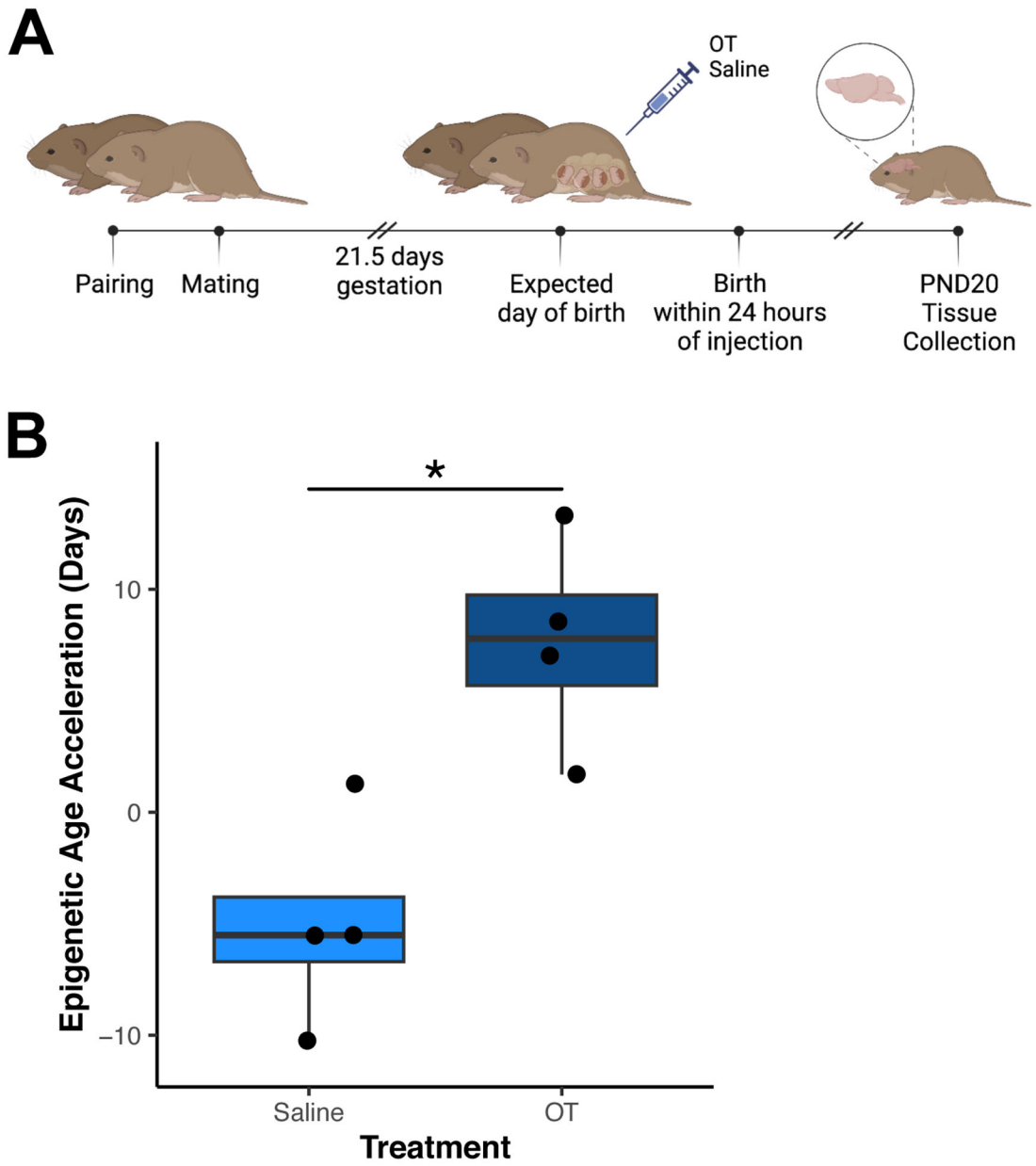


**Figure 1. Maternal oxytocin administration leads to epigenetic age acceleration in nucleus accumbens of male juveniles.**

A) Experimental timeline, B) Maternal oxytocin administration leads to epigenetic age acceleration (Wilcoxon rank-sum test, W=16, $p$=0.029). 0 days on the y axis indicates that an animal has the same epigenetic age as chronological age (postnatal day 20). * $p$<0.05

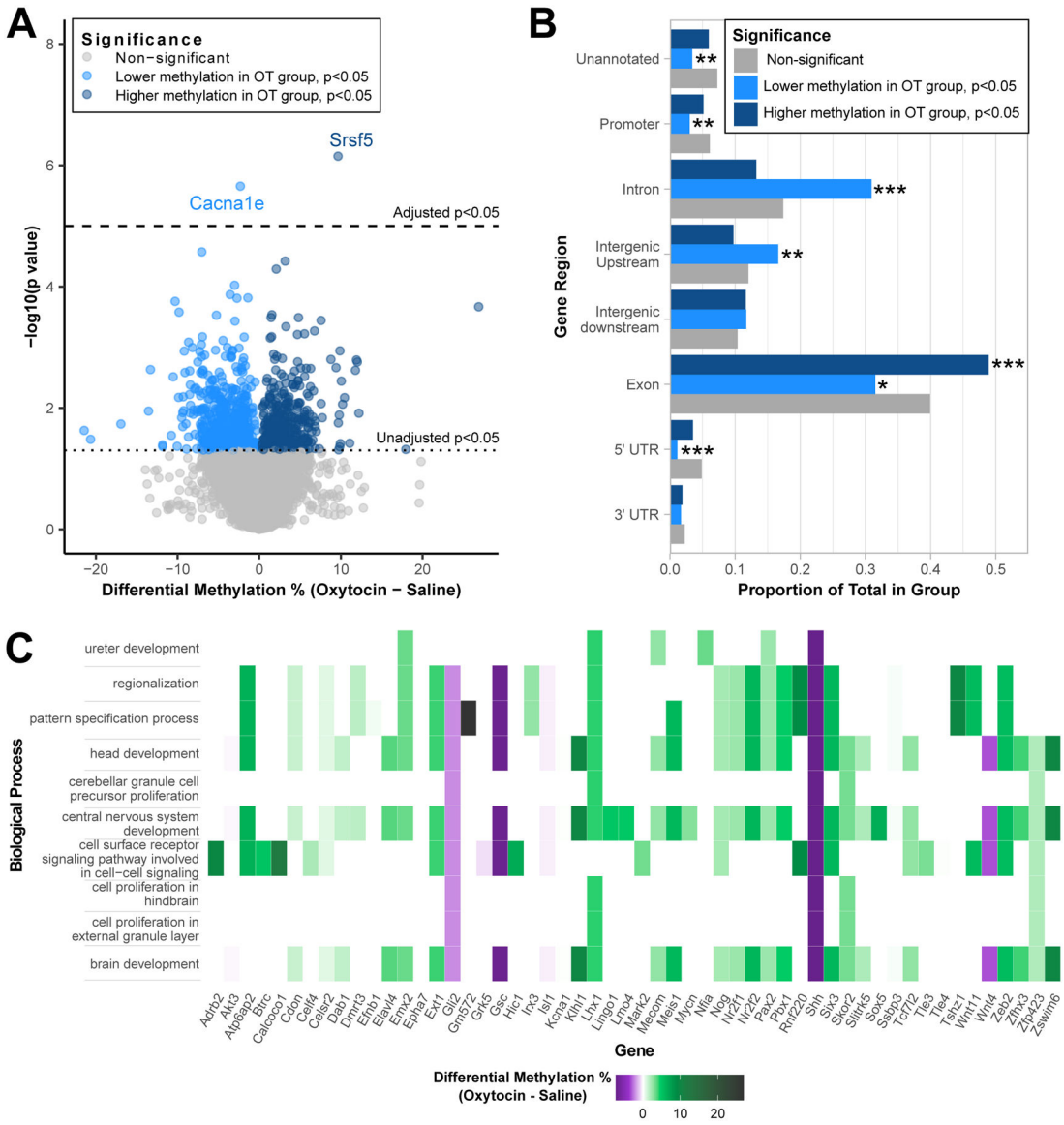


**Figure 2. Maternal oxytocin administration leads to differential methylation of genes related to neurodevelopment.**

A) Volcano plot showing differential methylation in nucleus accumbens associated with maternal oxytocin administration. CpG sites above the dotted line have unadjusted (nominal) p<0.05 and CpG sites above the dashed line have adjusted p<0.05. CpG sites with lower methylation in the OT group are shown in light blue and CpG sites with higher methylation in the OT group are shown in dark blue. Two CpG sites remain significant after correction for multiple comparisons and are labeled with their annotated genes, *Cacna1e* and *Srsf5*. B) Gene regions enriched for differential methylation. Proportions of CpGs in each gene region were compared separately for hypermethylated CpGs and hypomethylated CpGs; both groups were compared to non-significantly differentially methylated CpGs using the two-proportions z-test. Resulting *p*-values were corrected for multiple tests using the Benjamini-Hochberg method and are denoted using stars. C) Heatmap showing significantly enriched gene ontology biological processes and the differential methylation of genes involved in

the process. Differential methylation is shown using color with green indicating higher methylation in the oxytocin group and purple indicating lower methylation in the oxytocin group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

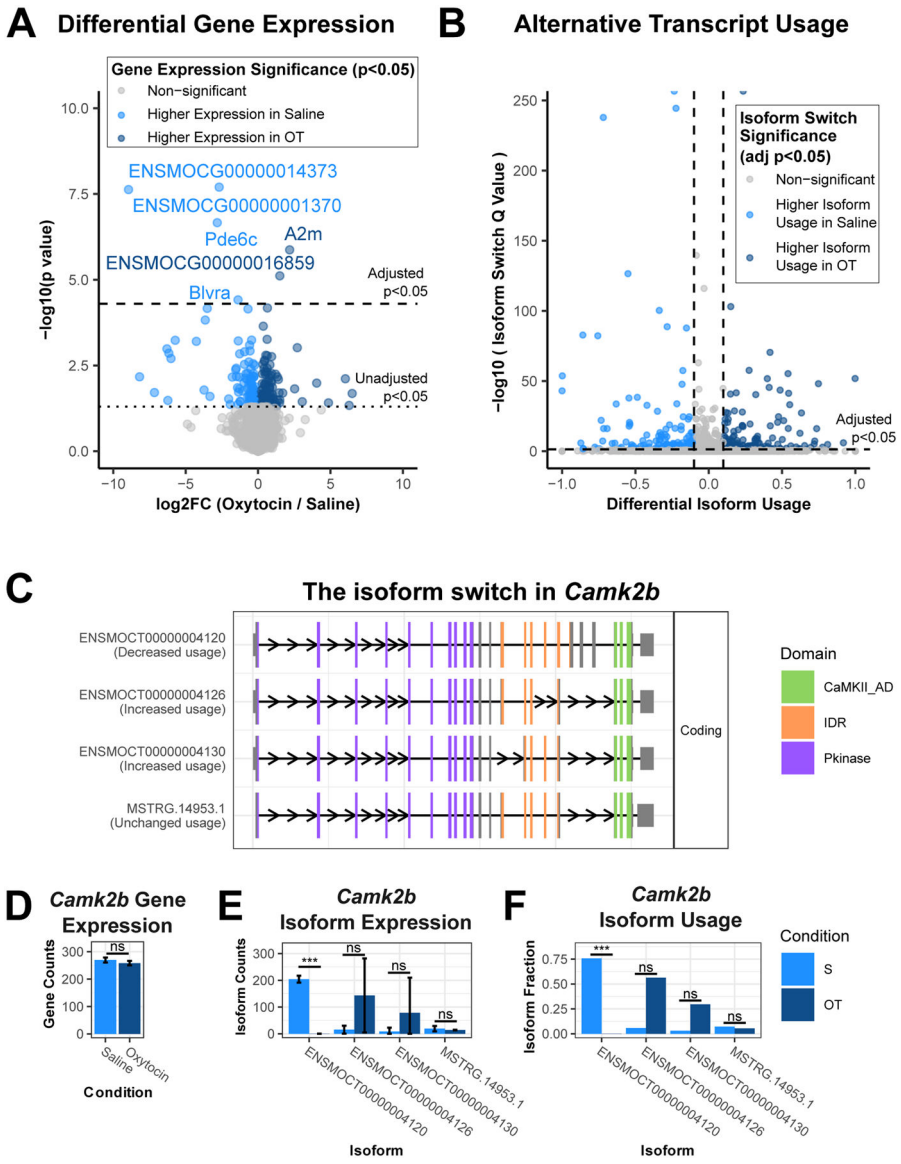


**Figure 3. Maternal oxytocin administration leads to differential transcript usage.**

A) Volcano plot showing differential gene expression in nucleus accumbens associated with maternal oxytocin administration. Genes above the dotted line have unadjusted (nominal) p<0.05 and genes above the dashed line have adjusted p<0.05. Genes with higher expression in the saline group shown in light blue and genes with higher expression in the OT group are shown in dark blue. Six genes remain significant after correction for multiple comparisons and are labeled with their annotated genes. B) Volcano plot showing differential transcript usage in nucleus accumbens associated with maternal oxytocin administration. Transcripts with higher expression in the saline group are shown in light blue and transcripts with higher expression in the OT group are shown in dark blue. Because of the abundance of significant isoform switches, only genes with transcripts with differential expression after correcting for multiple comparisons (above the dashed line) are indicated by color. C) Gene schematic of *Camk2b* showing four different isoforms expressed in nucleus accumbens. D)

*Camk2b* expression does not differ among offspring from saline-treated dams and oxytocin-treated dams at genome-wide significance. E) Quantification of *Camk2b* isoform expression. Expression of isoform ENSMOCT00000004120 is significantly reduced in oxytocin-exposed offspring ($p<0.001$). F) *Camk2b* isoform usage among offspring from saline-treated dams and oxytocin-treated dams. Saline-exposed offspring preferentially use transcript ENSMOCT00000004120 ($p<0.001$) while oxytocin-exposed offspring preferentially, though non-significantly, use transcripts ENSMOCT00000004126 and ENSMOCT00000004130.