RESEARCH ARTICLE

Absence of Oxytocin Alters Neuroendocrine Proteomic and Genomic Expression in Oxytocin Knockout Mice

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Abstract

Gene knockout animal models have proven extremely useful for studying the effects of a single gene product in systems where human studies are not an option. The nine amino acid peptide, oxytocin, has been shown to play dual roles as a peptide hormone in parturition and milk production; and as a neuropeptide in social and maternal behavior. In this study, we investigated the effect of the absence or presence of a functioning oxytocin allele on other endocrine genes. Taking advantage of an oxytocin-null murine model (OTKO-/-), gene expression levels were assayed for the prohormones; pro-oxytocin, pro-vasopressin, and proopiomelanocortin; processing enzymes, prohormone convertase 1, prohormone convertase 2 and carboxypeptidase E; and transcription factors, c-Fos, and c-Jun in OT+/+ and OTKO-/- animals. Significant changes in gene expression levels were found to occur in cortex and hypothalamic tissues of OTKO-/- mice. Specifically, there was an up-regulation of POMC, PC2, c-Fos and c-Jun in hypothalamus. PC2 was also found to be up-regulated in the cortex. Western blot analysis of the affected tissues showed similar results. We conclude that the absence of the peptide hormone oxytocin can affect other peptide hormone systems, including processing enzymes and transcription factors.

Keywords: oxytocin, vasopressin, prohormone convertase, SELDI-TOF, realtime RTPCR, OTKO mice

1.0 INTRODUCTION:

The nine amino acid peptide oxytocin is produced in magnocellular neurons and is involved in behavior,[1] salt homeostasis,[2, 3] cardiovascular relaxation,[4-7] milk injection, [8] myometrial contraction,[9] and social recognition response.[10] Oxytocin is also produced centrally where it acts as a neuropeptide to impact cognitive processing of information as well as maternal,[11] social and sexual behaviors.[12, 13] Oxytocin's role in behavior was determined in studies where differential expression patterns of oxytocin receptors in the amygdala regions of Montane and Prairie voles brains were correlated with differences in social and maternal behavior.[14] Due to the behavioral component associated with oxytocin and its receptors, alterations in oxytocin levels have also been proposed as a potential cause of autism.

Autism is a complex neurodevelopmental disorder of unknown etiology that potentially has multiple causes ranging from genetic to environmental, [15] in which diagnosed individuals exhibit distinctive impairments in cognition, imagination, behavior, communication and social interactions.[16-20] Thus, neuropeptides such as oxytocin provide a logical place to start when investigating neuropsychiatric disorders such as autism. Previous studies have suggested that the lack of oxytocin may be associated with a subset of the autistic spectrum of disorders. [21, 22] Alterations in the oxytocin gene, i.e., single nucleotide polymorphisms or SNPs, resulting in extended forms of the OT peptide were proposed as a possible contributor to the onset of the disorder due to a peptide processing error.[21] In two previous studies on oxytocin levels in autistic children, evidence was presented suggesting that there was a lack of mature oxytocin and an increased amount of "extended" forms of oxytocin that were presumably not processed correctly.[23] That is, in the blood of autistic children, oxytocin was thought to retain its C-terminal glycine, lysine or arginine residues. This hypothesis was examined further in another study by direct sequence analysis of the pro-oxytocin gene in autistic children and by mass spectrometry for the presence or absence of oxytocin and these 'extended forms' in their blood. [24] In the patients examined in that study, and others not reported, there was a substantial lack of sequence variability in the oxytocin gene, i.e., SNPs, nor were extended forms of oxytocin found in the blood. [24] While this does not prove that oxytocin does not play a role in autism, it does suggest that other components of the oxytocin system should be explored, e.g., processing enzymes, transcription factors, and enzymes. Although the results of the previous studies suggest that oxytocin's role in autism may be limited; other studies using intranasal sprays to treat autistic children with oxytocin have shown very promising results toward improved behavioral effects.[25] Thus, more studies on oxytocin and its regulation are warranted.

To better study the effects of the lack of oxytocin on the endocrine system, an animal model was needed. In 1996, Dr. Scott Young developed the oxytocin-knockout (OTKO-/-) mouse model.[26] OTKO-/- mice have an impaired ability to breed, and lack social and maternal behavioral patterns.[26] To further analyze the effects that the absence of a major neuro- and endocrine peptide has on the otherwise intact neuroendocrine system, we have used proteomic and genomic assays for other prohormones, processing enzymes and transcriptional factors involved in neuroendocrine gene expression. We chose to study the expression of the prohormones; pro-

vasopressin and pro-opiomelanocortin; the prohormone processing enzymes; PC1/3, PC2 and carboxypeptidase E (CPE); and the transcriptional factors; c-Jun and c-Fos.

The results presented in this new study provide the first description of using SELDI-TOF MS, semi-quantitative western blotting and semi-quantitative RealTime RTPCR to analyze the effects of knocking out a peptide hormone, i.e., oxytocin, in an animal model. We provide evidence that in the oxytocin-KO mice, other prohormones, processing enzymes and transcriptional factors are affected in different brain regions.

2.0 METHODS AND MATERIALS:

2.1 Oxytocin Knockout (OTKO-/-) Mice.

The murine oxytocin null model (OTKO-/-) [26] was obtained from the Jackson Laboratory (Bar Harbor, Maine). Briefly, knock-out of the oxytocin gene in mice was generated using a neomycin-resistant targeting plasmid inserted into the first exon of the oxytocin gene. The altered oxytocin allele was then detected in the germline of chimeric male mice. Transmission of the mutant allele was confirmed by PCR analysis, immunocytochemistry, and hybridization-histochemistry of the heterozygous and homozygous mutant progeny.[26]

2.2 SELDI-TOF Mass Spectrometry of Pituitaries.

For direct analysis of mouse pituitary peptides, the pituitaries were homogenized in 0.1N HCl containing 1 mM PMSF and the cell debris removed by centrifugation (5 min at 15,000 X g). One microgram of each sample was spotted onto a Weak Cation Exchange (WCX2) ProteinChip® (Ciphergen, Palo Alto, CA) or a Strong Anion Exchange (SAX2) ProteinChip® for 30 min in a humid chamber, washed with 5µl distilled water and allowed to dry. For WCX2 ProteinChips®, matrix, alpha-cyano-4-hydroxycinnamic acid (CHCA) in 50% acetonitrile containing 0.1% TFA (0.5 µl), was added to the spots and allowed to dry. For SAX2 ProteinChips®, 0.5 µl of matrix, i.e., sinapinic acid in 50% acetonitrile containing 0.1% TFA was added to the spots and allowed to dry. The ProteinChips® were analyzed on a Ciphergen SELDI™ Protein Biology System II (Ciphergen; Palo Alto, CA) with a spot protocol that ionized each spot four times over twenty different areas. [27, 28] The spot was initially ionized with 1 laser hit at an intensity of 200, with the sensitivity set at 10, followed by 4 laser hits at 195 intensity. The source voltage was set at 20,000 volts and detector voltage at 1,900 volts. The 80 laser hits were averaged for each spot and the resulting protein profile analyzed to determine the mass of each peak. Ciphergen ProteinChip® 3.2.1 software was used to integrate the area under each peak for use in semi-quantitative analysis.

2.3 Mouse RNA Sample Extraction and Purification

Total RNA was isolated from mouse pituitary, hypothalamus, and cortex using TRIzol® Reagent (Invitrogen; Carlsbad, CA) as previously described. [29] The RNA samples were treated with Turbo™ DNA Free DNase I (Ambion; Austin, TX) to remove DNA contamination. The DNase I enzyme was inactivated by phenol:chloroform extraction, RNA precipitated with ice-cold ethanol, and stored in DEPC-H_2O at -80°C until use.

Quantification of the RNA samples was performed using the RiboGreen® RNA Quantification Reagent Kit (Molecular Probes; Eugene, OR) and detected using the Packard Fusion Fluorescence microplate analyzer

(PerkinElmer®; Shelton, CT) with a 485nM λ excitation filter and a 530nm λ emission filter.

2.4 Real-Time PCR Gene Expression Assay Using Multiplexed TAQMAN® Probes

All gene expression data was collected with an iCycler iQ Real-Time PCR optical detection system (Bio-Rad Laboratories; Hercules, CA). The two-step RT-PCR reactions were carried out with the iScript™ cDNA Synthesis Kit from Bio-Rad Laboratories (Palo Alto, CA) and Bio-Rad IQ™ Supermix was utilized for the amplification step. The protocol for the iScript™ cDNA synthesis reactions was: 4 **µ**ls of the 5x iScript™ reaction mixture, 1 **µ**l iScript™ reverse transcriptase, 5 **µ**ls RNA template (50 ng total RNA/reaction, and 10 **µ**ls of Nuclease-free distilled deionized water. cDNA synthesis conditions were: 25ºC for 5 minutes, 42ºC for 30 minutes, 85ºC for 5 minutes after which the reactions were held at 4ºC. The IQ™ Supermix reaction conditions contained a 1 x concentration of reaction buffer 0.8mM dNTPs, 200nM of each Taqman® probe, 650nM of each primer, and a final $MgCl_2$ concentration of 3.0mM. All Assay-on-Demand™ Taqman® probe sets were purchased from Applied Biosystems (Forester City, CA). Assay-on-Demand™ kits utilized in this investigation are shown in Table 1. Each gene-of-interest was multiplexed with a housekeeping gene, the MAX transcription factor gene (Table 1). [29, 30] To test the fidelity and amplification integrity for all gene-of-interest assays, initial reactions were separated on a 2% agarose gel, stained with ethidium bromide, and visualized on a Fotodyne® UV transilluminator system (Hartland, WI) (Figure 1). Analysis of the Realtime RTPCR data was by $2^{-\Delta\Delta CT}$. [31]

Table 1: PCR Assays on Demand or Primer Sets

Protein	Assay ID
Oxytocin	Mm00726655_s1
Vasopressin	Mm00437761_g1
Pro-opiomelanocortin (POMC)	Mm00435874_m1
Carboxypeptidase E	Mm00516341_m1
Proprotein Convertase subtilisin/kexin type 1	Mm00479023_m1
Proprotein Convertase subtilisin/kexin type 2	Mm00500981_m1
c-Fos	Mm00487425_m1
c-Jun	Mm00495062_s1
MAX Housekeeping Gene	Mm00465485_m1
MAX Housekeeping Gene Forward primer	5'TTACTGAAGAGCGAGC AGATGTC T-3'
Reverse primer	5'TTCCTTATGATTGTGTG GATAGGTTT-3'
Probe sequence	5'CTTGACAACTCTGCTTA CTGCTCAGGCATCT-3'
The MAX probe was coupled at the 3'-end to the Black Hole Quencher and at the 5'end to Texas Red.	

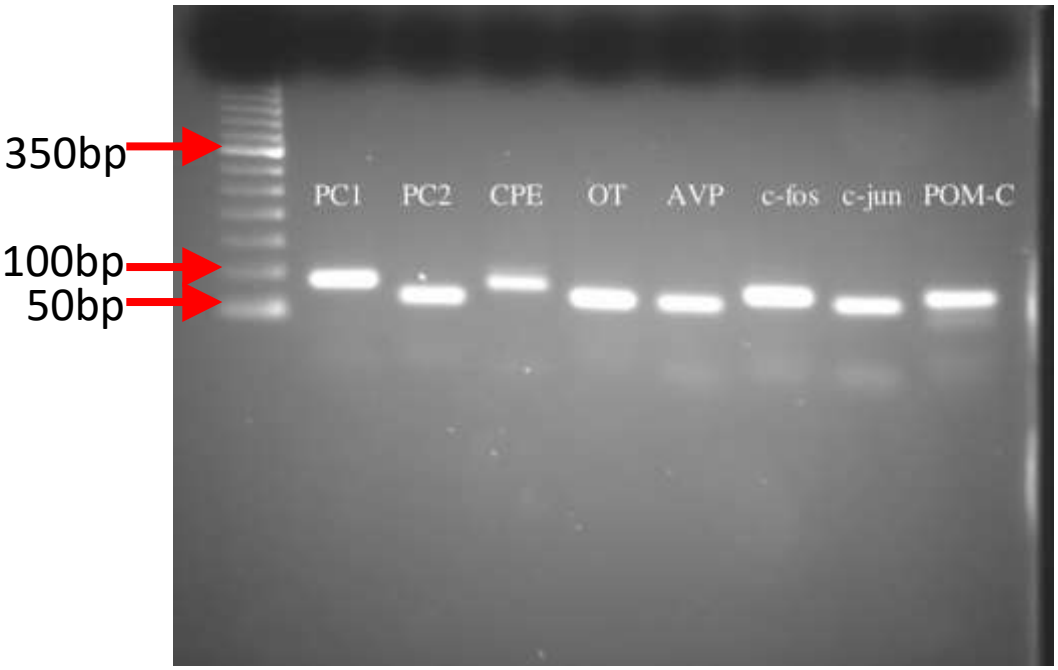

Figure 1: **Verification of PCR Product for Each Primer Set.** A standard PCR was run using hypothalamic tissue. A 50 BP standard marker (Invitrogen) was run in Lane 1. Each primer set was tested prior to this to verify conditions to yield one band at the correct size.

2.5 Western Blotting

Tissue was prepared for western blotting by homogenization in Tris/EDTA buffer, pH 7.8. The tissue homogenate was centrifuged for 5 minutes at 16,000 x g, µg/sample the supernatant protein normalized to 30 µg/sample using the Bio-Rad protein assay. Proteins were separated on a 10% SDS gel and transferred to PVDF membranes. The membranes were probed with appropriate primary antibodies for 1 hour, followed by visualization using ECL on a Fuji-LAS3000 high sensitivity camera. Antibodies used were: c-Fos and c-Jun (Stressgen), PC1/3 and PC2 (Dr. Donald Steiner, Chicago, IL; and Dr. Iris Lindberg, University of Maryland, College Park, respectively), and CPE. [32] All bands were normalized to actin by either probing at the same time or in the case of proteins that were too close in size to actin, after stripping the blot. Band densities were determined using the FUJI ImageGuage software V.3.1.

3.0 RESULTS:

3.1 PCR Determination of the mRNA for Prohormones, Processing Enzymes, and Transcription Factors.

A test PCR was conducted to determine that each primer was correct and working before continuing the study (Figure 1). As expected, each primer set provided a single band on an agarose gel that correlated with the expected size PCR product.

3.2 SELDI-TOF MS Analysis of Peptide Hormone Expression.

Oxytocin and AVP peptide hormones are localized to the posterior pituitary. To analyze the pituitary peptidome, we used the technique of surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS) that has been used for other studies involving pro-oxytocin and pro-vasopressin. [24, 27, 28] In SELDI-TOF MS, tissue is extracted in a buffer and applied to a chemically-modified ProteinChip® that is

designed to retain proteins/peptides based upon their chemical properties, e.g., anion, cation or hydrophobic. After the proteins are applied, the ProteinChip® is washed to remove proteins/peptides not compatible with the ProteinChip® chemical-properties, and an energy absorbing matrix is applied to enhance the ionization of the proteins/peptides retained on the ProteinChip®. For the present investigation, we analyzed the peptides, oxytocin, arg-vasopressin (AVP), and the associated neurophysin regions for both peptide hormones. Other peptide hormones, i.e., ACTH, α-MSH, β-endorphin were analyzed in the anterior pituitary and hypothalamus.

The neuropeptides oxytocin and vasopressin were detected by SELDI-TOF MS in the OT+/+ animals (Figure 2). In contrast, in the OTKO-/- mice, vasopressin but not oxytocin was observed indicating that the mice were true OTKO-/- mice (Figure 2). Furthermore, oxytocin was detected in a heterozygote individual (Figure 1A). Further verification that oxytocin was absent was provided by SELDI-TOF MS analysis for the neurophysin-I carrier portion of the pro-oxytocin prohormone as previously described (28). Neurophysin I was identified in all OT+/+ animals (Figure 2) but was absent in the OTKO-/- animals (Figure 2).

3.3 Semi-Quantitative RealTime PCR Analysis of Prohormones, Prohormone Processing Enzymes and Transcription Factor Expression Levels.

Semi-quantitative RealTime RTPCR was used for the genomic analysis of each brain region. In this type of PCR, genes of interest are analyzed by a fluorescently labeled probe that binds specifically to PCR products during the PCR protocol to produce a "realtime" analysis of the increase in cDNA formed by the PCR. We used pre-designed primer-probe sets from ABI for each of the prohormones, processing enzymes and transcription factors. The MAX housekeeping gene was probed with a primer probe set of our own design that allowed for multiplexing each gene of interest in the same well as the Max Housekeeper gene. [29, 30]

The process of transforming a prohormone to a peptide hormone requires a highly regimented synthetic pathway.[33] For a prohormone such as pro-oxytocin, the pathway involves expression in the oxytocinergic neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN), followed by processing in the regulated secretory granules and release from the neurons in response to stimuli. [34, 35] The processing of prohormones requires the presence of specific enzymes that recognize the paired-basic residue processing sites. The prohormone processing enzymes, PC1/3 and PC2[36-41] were chosen because they are known to play a role in processing pro-oxytocin to oxytocin in mice.[28] Carboxypeptidase E, an enzyme that cleaves the basic amino acid residues, i.e., lysine and arginine, from the processed peptide hormones, was also chosen for analysis. [42] In addition, other prohormones in the pituitary may be influenced by the lack of oxytocin and thus, are of interest. We chose two other prohormones, pro-vasopressin and pro-opiomelanocortin, as they directly influence the endocrine system and may in turn be modulated by the presence or absence of oxytocin. Expression of pro-oxytocin is known to be regulated by two transcription factors, i.e., c-Fos and c-Jun. [43] For the semi-quantitative RealTime PCR analysis of these enzymes, transcription factors and prohormones, we chose three brain regions, i.e., hypothalamus, pituitary, and prefrontal cortex (cortex).

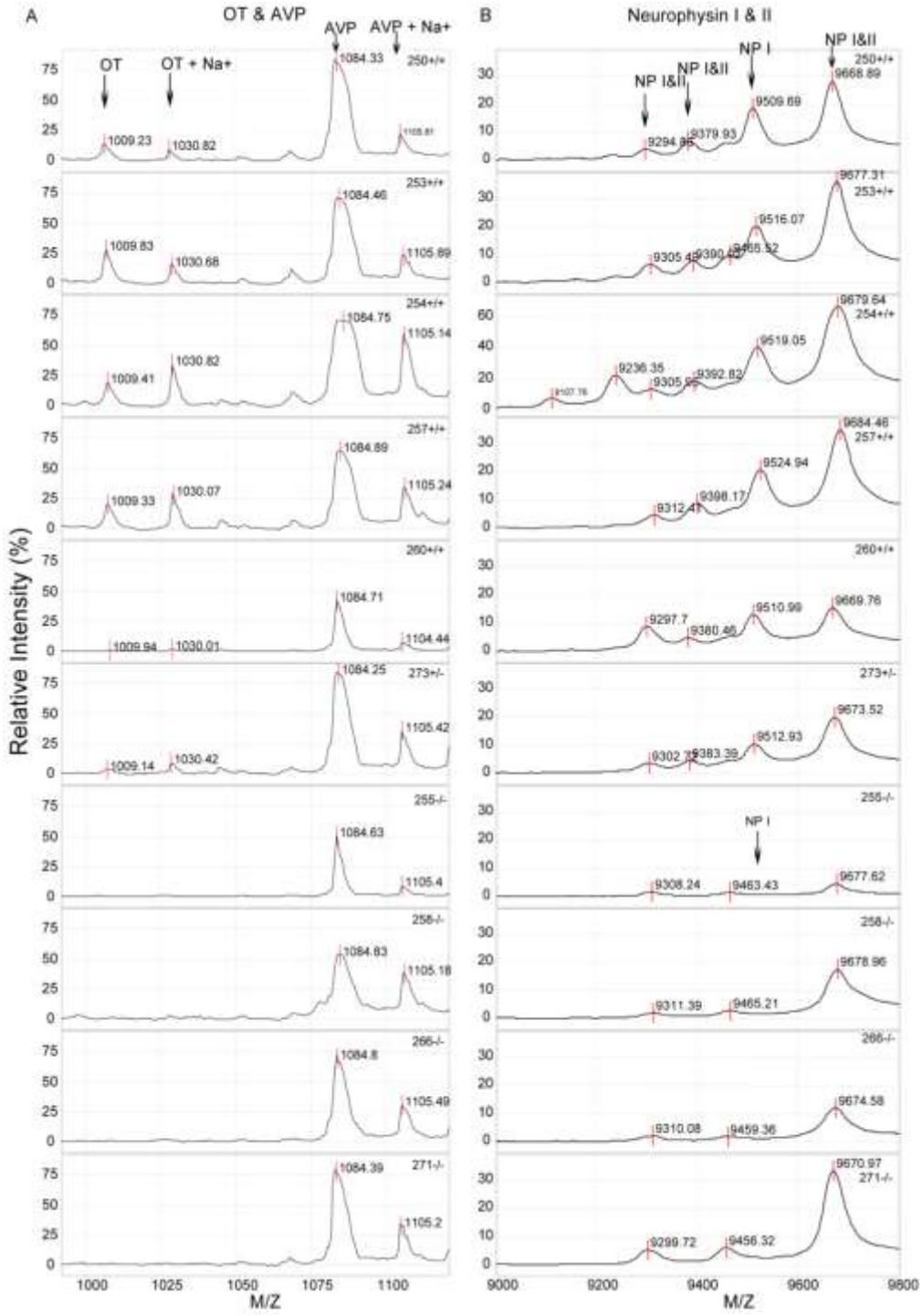

Figure 2: **Phenotype verification of OTWT and OTKO animals by SELDI-TOF Mass Spectrometry.** A) Oxytocin and vasopressin analysis in OTKO-/- and OT+/+ animals by SELDI-TOF Mass Spectrometry. B) Neurophysin-I region analysis in OTKO-/- and OT+/+ animals by SELDI-TOF Mass Spectrometry. The numbers in the top right corner of each spectra indicate the mouse ID and whether it was OT+/+, OT+/-, or OTKO-/-. OT is indicated by arrows at ~1009 daltons and its sodium adduct at ~1030 daltons. AVP is indicated by arrows at 1084 daltons and its sodium adduct at ~1105 daltons. Neurophysin I in (B) indicates the neurophysin region of the pro-oxytocin prohormone and Neurophysin II indicates the corresponding region in pro-vasopressin. There were 5 OT+/+ mice and 6 OTKO-/- mice.

3.4 Expression of POMC, PC2, c-Fos, and c-Jun mRNA was Increased in the Hypothalamus

As expected, RealTime PCR analysis determined that oxytocin was present in OT+/+ but not OTKO-/- hypothalamic tissue (Table 2). Pro-vasopressin was expressed in both OT+/+ and OTKO-/- mice, although there was no significant difference in expression levels (Table 2). In contrast, POMC mRNA expression levels were increased 3-fold ($p<0.005$) in hypothalamic tissue (Table 2).

There was no significant change in PC1/3 mRNA expression (Table 2). However, a significant 2-fold increase ($p<0.01$) in hypothalamic PC2 mRNA was observed in OTKO-/-, compared to OT+/+ hypothalami. Likewise, an apparent 2-fold increase that was not significant ($p<0.056$) was observed in CPE mRNA levels (Table 2).

Both transcription factors exhibited increased mRNA expression levels in the OTKO animals. Gene expression levels of c-Fos doubled in the OTKO animals ($p<0.007$) and c-Jun increased 3-fold ($p<0.003$) in the OTKO group (Table 2).

3.5 Expression of c-Fos was decreased in Pituitaries

In the pituitary samples, c-Fos expression was found to be decreased by 85%. As was expected, expression of vasopressin was not observed in either OTKO-/- or OT+/+ groups (Table 2). In contrast, oxytocin gene expression was detected in the OT+/+ group but not OTKO-/- pituitaries (Table 2). There was no difference in POMC mRNA expression levels.

No significant changes were observed for PC1/3, PC2, CPE, or c-Jun enzyme mRNA levels in the pituitary (Table 2).

Table 2: Fold Expression of Prohormones, Processing Enzymes, and Transcription Factors in the Normal and OTKO Mice

	OT	Cortex	Hypo	Pit
OT	+/+	1.23 ± 0.32	0.62 ± 0.46	0.84 ± 0.39
	-/-	0	0	0
VP	+/+	0	1.15 ± 0.32	0
	-/-	0	1.45 ± 0.40	0
POMC	+/+	1.07 ± 0.43	1.12 ± 0.25	1.09 ± 0.31
	-/-	1.28 ± 0.50	3.51 ± 0.56*	1.19 ± 0.57
PC1/3	+/+	1.04 ± 0.07	1.01 ± 0.12	1.01 ± 0.12
	-/-	1.08 ± 0.09	1.05 ± 0.19	1.06 ± 0.19
PC2	+/+	1.06 ± 0.24	1.04 ± 0.12	1.06 ± 0.15
	-/-	3.34 ± 0.49*	2.19 ± 0.36*	1.21 ± 0.36
CPE	+/+	1.39 ± 0.31	1.20 ± 0.25	1.10 ± 0.25
	-/-	1.79 ± 0.68	2.33 ± 0.54	0.73 ± 0.11
cFos	+/+	1.10 ± 0.21	1.06 ± 0.15	1.94 ± 0.66
	-/-	0.89 ± 0.19	2.42 ± 0.36*	0.29 ± 0.10*
cJun	+/+	1.04 ± 0.13	1.13 ± 0.25	1.24 ± 0.38
	-/-	1.02 ± 0.09	2.93 ± 0.32*	0.79 ± 0.26

Expression for each was determined by 2^{-DDCT}. (31)
*Gray highlighting indicates significant differences ($p<0.05$).

3.6 PC2 Expression was Increased in the Cortex

The mRNA expression levels for PC2 increased 3-fold ($p<0.0029$) in the OTKO-/- group when compared against the OT+/+ animals (Table 2). In the cortex samples, vasopressin mRNA was not detected in either OT+/+ or OTKO-/- groups (Table 2), while oxytocin gene expression was only observed in the OT+/+ animals. None of the other mRNA levels were significantly affected.

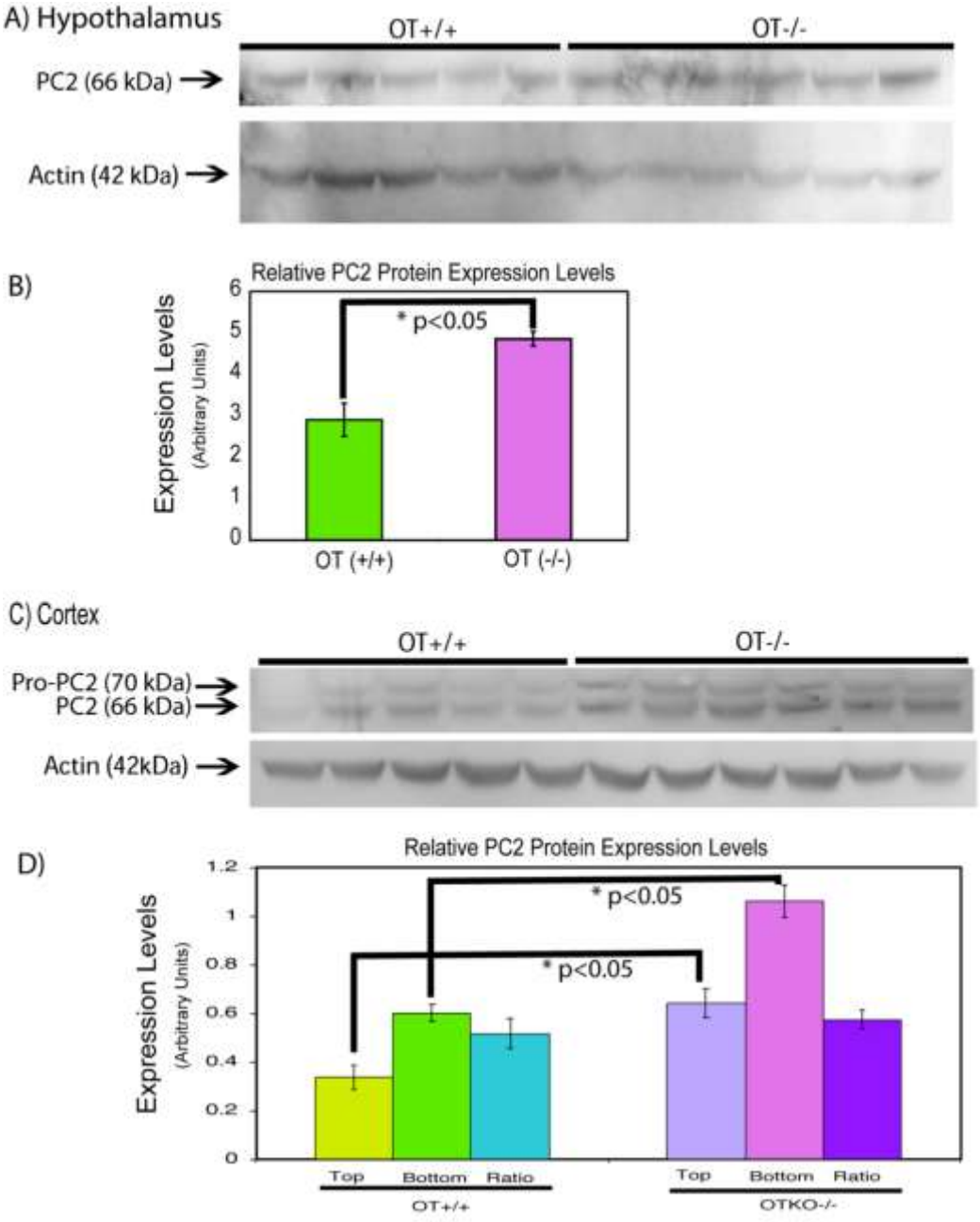

Figure 3: **PC2 Protein is Increased in Hypothalamus and Cortex.** Western blot analysis of Prohormorne Convertase 2 (PC2) in both hypothalamus (A&B), and cortex (C&D). Antibody dilution for PC2 was 1:1000. The results represent the mean ± the SEM, with an n of 5 for OT+/+ and 6 for OTKO-/-.

3.7 PC2 Protein is Increased in the Hypothalamus and Cortex while c-Fos is Decreased in the Pituitary

In the hypothalamus, significant increases in mRNA were observed for four proteins, PC2, c-Fos, c-Jun, and POMC. When these proteins were examined by western blotting in the hypothalamus (Figure 3A&B), only PC2 was observed to be increased by 1.75-fold ($p<0.05$) similar to the increase in mRNA. c-Fos and c-Jun levels were not significantly changed (data not shown). POMC is cleaved to peptide hormones and the prohormone is not easily observable by western blots. Mass spectrometry failed to identify changes in ACTH, α-MSH, or β-endorphin in the OTKO-/- mice (data not shown).

In the cortex, only PC2 mRNA was significantly increased. Western blot analysis of cortex proteins showed two bands that were tentatively identified as representing Pro-PC2 (~70 kDa) and the cleaved active enzyme, PC2 (~66 kDa) (Figure 3C). Densitometric analysis of these bands normalized to actin indicated that there was a significant 1.8-fold increase in the top, Pro-PC2 band ($p<0.001$), and a 1.8-fold increase in the bottom PC2 band ($p<0.001$) (Figure 3D).

Pituitary mRNA levels for c-FOS were shown to be decreased. When pituitary protein levels were examined by western blotting there was a significant 15% decrease in c-FOS protein ($p<0.001$).

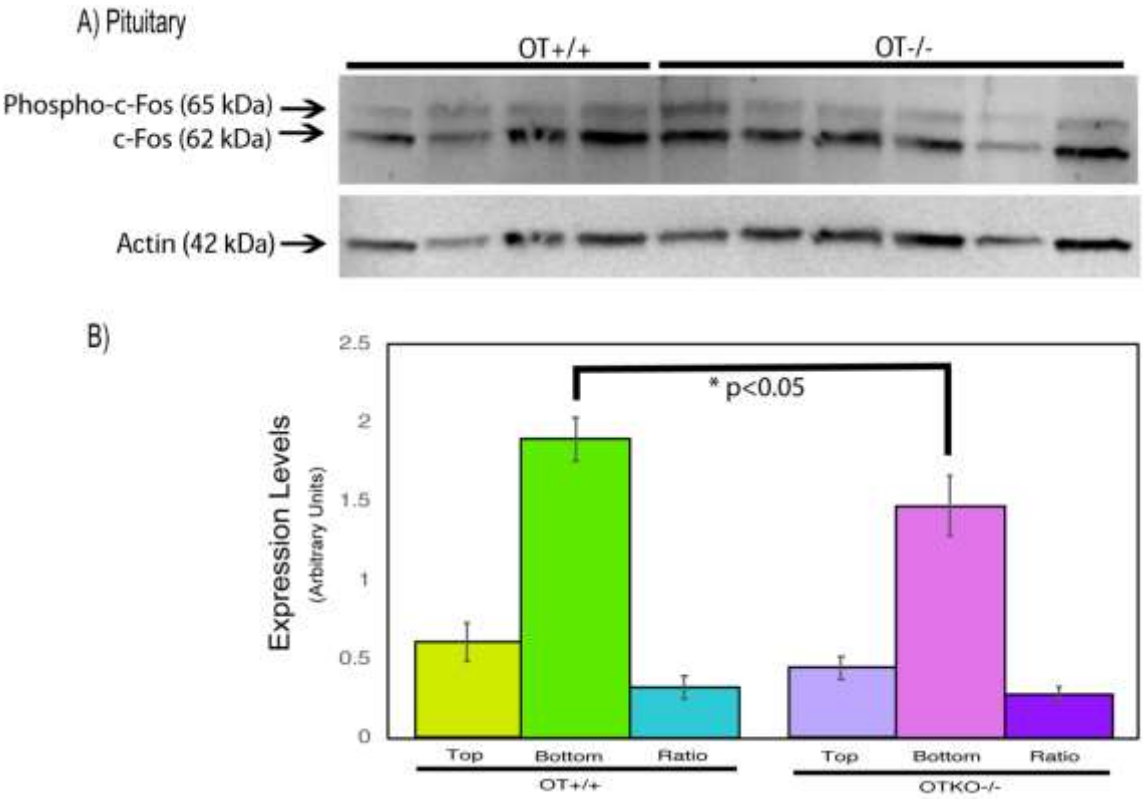

Figure 4: **c-Fos Protein is Decreased in Pituitary Tissue**. Pituitary tissue homogenate was probed for c-Fos by western blots. Antibody dilution for c-Fos was 1:500. The results represent the mean ± the SEM, with an n=4 for OT+/+ and n=6 for OTKO-/-.

4.0 DISCUSSION:

In the present study, we investigated the effects that result when a single gene, pro-oxytocin, has been "knocked-out" to create a peptide hormone "void" in the endocrine system. The broader effect that this single gene knockout had on other prohormones, prohormone processing enzymes, and transcription factors was analyzed using proteomic, peptidomic, and genomic methods.

The initial results of the SELDI-TOF MS peptidomic analysis were as expected, i.e., oxytocin is not present in OTKO-/- mice and is present in the OT+/+ mice. Furthermore, the neurophysin I region that results from cleavage of pro-oxytocin to oxytocin is also not present in the OTKO-/- mice. This is consistent with a previous study analyzing the impact of the absence of the PC2 prohormone processing enzyme on prohormone processing.[28] In that study, oxytocin and its neurophysin were similarly absent from the pituitary peptidome of OTKO-/- mice. Analysis of the peptidomic profiles generated from the pituitary tissues showed no change in AVP production in the OTKO-/- mice.

In the current study, the SELDI-TOF ion peak intensity for oxytocin is observed to be much smaller than that for AVP. Mass spectrometry used to identify oxytocin and AVP in the pituitary samples relies on the ionization of a molecule that makes it 'fly' to the detector. Although oxytocin and AVP have nearly identical amino acid sequences, i.e., CYIQNCP**L**G-NH_2 for OT versus CYFQNC**R**G-NH_2 for AVP, the lack of a charged residue such as the arginine **(R)** in AVP, makes OT less ionized, thus having a lower intensity. The addition of a sodium ion to OT provides a greater charge, causing it to ionize better and thus have a larger intensity at 21 daltons larger than the normal size, i.e., 1030 d versus 1009 d for the sodium adduct.[28]

Analysis of the different genes of interest by Semi-Quantitative RealTime PCR revealed changes in mRNA expression in different tissues. First, the mRNA for the processing enzyme PC1/3 showed no change in any of the tissues from the OTKO-/- mice. In contrast, both the mRNA and protein levels of the processing enzyme, PC2 were found to be increased in the hypothalamus and cortex, but not in pituitary. In the cortex, the pro-form of PC2 was also identified in the western blot and was similarly increased. However, the ratio of pro-PC2 (~70 kDa) to mature PC2 (~66 kDa) was not significantly different suggesting that the cleavage of pro-PC2 to mature-PC2 was not positively or negatively modified. This suggests that these tissues were sensitive to the lack of pro-oxytocin or the peptide oxytocin and thus responded by upregulating the PC2 enzyme. A previous study suggested that in mice, both PC1/3 and PC2 can process pro-oxytocin.[28] However, in humans with Prader-Willi Syndrome, the processing of pro-oxytocin is impaired by the absence of mature PC2 enzyme. [44, 45] This suggests that in humans, PC2 is the predominant enzyme for processing oxytocin. Differences in the amino acids at or near the cleavage site for pro-oxytocin between humans and mice have been proposed to be the cause for such differential processing. [28] The data from the present study are consistent with this explanation, i.e., that PC2 is the primary enzyme responsible for processing pro-oxytocin and that lack of the substrate causes an increase in the compensatory production of PC2. That PC1/3 is not up-regulated further suggests that it is not directly linked to the processing of pro-oxytocin or that it is a redundant enzyme in mice and is not as important for this purpose.

In this study we also investigated the expression of c-Fos and c-Jun, two transcription factors involved in regulating prohormone synthetic pathways. The decrease in c-Fos in the pituitary suggests that it may be required for oxytocin expression and that c-Jun is not. Previous studies have shown an increase in c-Fos with osmostimulation of the SON region of the brain. [46] The disconnect between the amount of decrease in c-Fos in mRNA and protein levels (85% versus 15%) may be due to the relative time for protein degradation allowing more enzyme to be present even with less mRNA.

4.1 Relevance of Knockout Mice

In previous work, the complete deletion of the OT gene from mice resulted in a 'defective social memory' that was represented by increased anxiety/lessened fear response, more aggressive behavior, and a limited maternal behavior. [47] Other work has shown that OT may be involved in regulation of salt intake, heart rhythm, and stress management. [48-50] Observations in other animal models have shown oxytocin and vasopressin receptor localization to have great impacts on animal behaviors such as monogamy, polygamy and sexual behavior. [51] By investigating the various mechanisms within the hypothalamic-pituitary-adrenal (HPA) axis, researchers hope to obtain a better understanding for diagnosing and treating varied complex neuropsychiatric disorders in which abnormal behaviors are observed, e.g., autism.

The results presented here do not provide a clear answer to the understanding of such a complex disorder as autism that exhibits a wide spectrum of phenotypes. [52] However, the results do suggest that future studies investigating other possible components contributing to the biological basis of autism should not only investigate the changes occurring in the regulation of certain behavior-impacting neuropeptides, but also receptor expression and localization within specific regions of the brain, e.g., the amygdala.

5.0 Acknowledgements:

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