Relationships between neuronal V1aR expression and single-nucleotide polymorphisms in *Microtus ochrogaster*

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1 Abstract

Studying neurobiological models of social cognition has emerged as an important way to understand the complex processes that account for social behaviors. The neuropeptides vasopressin and oxytocin are known to act on their central nervous system receptors to shape social cognition and behavior. In the prairie vole, a monogamous rodent often used for studying social cognition because of its ability to form pair-bonds, diversity in expression of vasopressin 1a receptors (V1aR) has been linked with non-coding variation at the *avpr1a* locus. In particular, V1aR expression in the retrosplenial cortex, a region of the brain that is critical in social cognition, is correlated with a single-nucleotide polymorphism (SNP) in the intron of the gene, implying that this region of the gene is significant in the regulation of *avpr1a* expression.

The aim of this study was to develop an *in vivo* method to link genetic variation to variation in neuronal and behavioral phenotypes, and to apply the assay to the prairie vole model system. This was done by adapting the haploChIP method, which compares the expression of two alleles in heterozygous cells by using binding affinity to RNA Polymerase II as a marker for gene transcription. Chromatin immunoprecipitation targeting RNA Polymerase II did not demonstrate significant differences in binding between the two alleles for the *avpr1a* SNP. Preliminary results for a more specific marker of active transcription, Serine-2 Phosphorylated RNA Polymerase II, shows more promise in establishing a relationship between *avpr1a* intron variation and V1aR expression in the retrosplenial cortex. Further study, therefore, may reveal an important regulatory role for the *avpr1a* intron and thus provide insight into the complex processes that underlie social interactions.

2 Background

2.1 Introduction

The physiology underlying social interactions showcases the incredible complexity of the brain's regulation of behavior. The intricacy of the neural networks and molecular pathways that govern social cognition act as obstacles to understanding it on a mechanistic level, but taking a neurobiological approach has shown promise in resolving the bases of social behaviors. Deficiencies in social cognition can severely damage the ability of individuals to function in the sophisticated social networks characteristic of many mammalian species, such that the study of attachment disorders in social psychology has become an area of active clinical research. A product of the multidisciplinary approach to studying social cognition has been the development of neurobiological models of attachment and other social behaviors that provide insight into sociality. The purpose of this study is to develop an *in vivo* assay for linking genetic variation to variation in neuronal and behavioral phenotypes, and to then apply the assay to a model system: the social behavior of the monogamous prairie vole *Microtus ochrogaster*.

2.2 Vasopressin and Oxytocin

Reward pathways provide behavioral reinforcement necessary for the formation of social memory, which underlies attachment and other social behaviors. Dopamine and opioids are both significant in attachment due to their broadly critical role in reward pathways.[1] However, the closely related neuropeptides oxytocin (OT) and arginine vasopressin (AVP) have been specifically implicated in the formation of social attachment in a wide range of mammalian species, and are more generally important in regulating a diverse array of species-specific social behaviors. Though these peptides are found only in mammals, related forms are present in all vertebrates, and an ancestral version exists in many invertebrates. These homologs have been

implicated in reproductive behaviors in these other species, suggesting that their function in conspecific interaction has been conserved.[2] The evolutionary conservation of these peptides has made them useful in understanding the basis of social behavior, as generalizations can be made about their function across species.

The hormonal functions of OT and AVP in the periphery are well characterized. They are synthesized in the hypothalamus and secreted into circulation by the posterior pituitary gland. OT is released during labor to stimulate uterine contractions, and causes milk let down in response to nipple stimulation during nursing.[3] AVP functions peripherally as an antidiuretic, concentrating urine to preserve water. In addition, both neuropeptides have receptors, OTR for OT and (primarily) V1aR for AVP, that are important in social behavior.[2] These receptors' presence in the brain provides the molecular basis for OT and AVP mediated regulation of social behavior.

OT's significance in maternal care of offspring, a type of social bonding present in almost all mammalian species, provided the first basis for studying these related neuropeptides roles in the brain in the context of attachment.[2,4] Since then, studies have found associations between OT and AVP and several social behaviors such as social recognition, conspecific aggression, mate bonding, and trust.[2,5-7] These molecules have even demonstrated measurable effects on human social behavior.[7-8] The bulk of recent research on the role of the neuropeptides OT and AVP in social attachment has focused on the prairie vole.

2.3 Introduction to the Prairie Vole

Prairie voles have a monogamous mating system, with males and females forming life-long pair bonds, sharing and defending a nest, and raising young.[9-10] Monogamy is relatively rare among mammals. In fact, other closely related vole species in the *Microtus* genus do not

form pair bonds.[11] The strength of pair bonding relationships has made the prairie vole one of the most important model systems for studying attachment and its neurobiological basis.

Though most prairie vole individuals form monogamous pair bonds, there is still variation in mating strategies and sexual fidelity. Though most male voles are monogamous, many will take on a wanderer strategy, mating with females without forming a pair-bond.[10] The behavioral variation that prairie voles exhibit enables the use of comparative studies that facilitate obtaining an understanding of the biological processes governing social cognition. The comparative approach takes advantage of differences in behavioral phenotypes by finding correlations, and thus identifying candidates for causal relationships, between behavioral variation and variation in brain structure, expression phenotypes, or genotype.

2.4 Attachment and Neurophysiology

V1aR expression patterns, knockout experiments, and understanding of dopaminergic reward pathways provide the basis for deducing the neural circuits that underlie attachments.[11] The medial amygdala projects AVP fibers onto the ventral pallidum and lateral septum, whereas OT fibers project from the hypothalamus onto the nucleus accumbens, prefrontal cortex, and medial amygdala.[12-13]

The retrosplenial cortex, sometimes called the posterior cingulate, is known to be important for spatial memory, which has been related to sexual fidelity and mating strategy in prairie voles.[14-15] V1aR expression in the retrosplenial cortex is highly variable in the prairie vole, making it an area that potentially accounts for variation in social cognition, which in turn affects patterns of social affiliation and sexual fidelity.[16] Recent studies in humans have also implicated the posterior cingulate in romantic love and obsession.[17] These studies suggest that

the retrosplenial cortex plays a critical role in the cognitive processes that underlie prairie vole pair bonding.

2.5 The Prairie Vole and the V1aR Receptor

Previous studies have demonstrated that receptors for the neuropeptides oxytocin and vasopressin play vital roles in social behavior in prairie voles. Regions of high OTR and AVP expression differ greatly between the prairie vole and the non-monogamous but phylogentically closely related montane or meadow voles, with regions implicated in pair-bond formation tending to show higher expression in the monogamous prairie vole.[18-19] In addition, injecting antagonists to these neuropeptide receptors in the parts of the brain where expression differs causes a considerable decline in pair-bonding.[20-22] Similarly, administration of OT or AVP into certain regions of the prairie vole brain facilitates pair-bond formation, as can induced overexpression of OTR and V1aR.[20-21] V1aR overexpression through viral vectors in the promiscuous meadow voles has even been shown to increase partner preferences.[23]

As mentioned before, prairie voles exhibit within-species variation in mating strategy. In addition to the across species differences in V1aR distribution, considerable variation in V1aR distribution exists within the prairie vole species, and individual expression between brain regions covaries.[16] In the same way that sexual behavior is correlated to V1aR expression in comparisons between monogamous and promiscuous *Microtus* species, evidence shows that the variation in V1aR distribution predicts sexual fidelity within the prairie vole species, further validating the importance of the receptor in attachment.[14] Furthermore, studies suggest that polymorphism in the *avpr1a* gene, particularly in regulatory regions, predicts brain expression of V1aR, and may therefore account for the observed phenotypic variation.[24-27]

Studies have explored the idea that variation in microsatellite length in *avpr1a* accounts for differences in expression. Microsatellite length at the *avpr1a* locus is polymorphic in the prairie vole, and laboratory studies have shown that this variation correlates with V1aR binding in some regions of the brain.[24-25,28] However, current evidence seems to discredit the theory that microsatellite length actually drives behavioral variation. While correlations between expression and *avpr1a* length were found in field populations, behavior does not appear to be influenced by microsatellite length.[26] It is possible that non-repetitive sequence polymorphism actually drives variation in expression and behavioral phenotypes, and that genetic linkage between sequence polymorphism and length polymorphism has led to the observed correlations.[26-27]

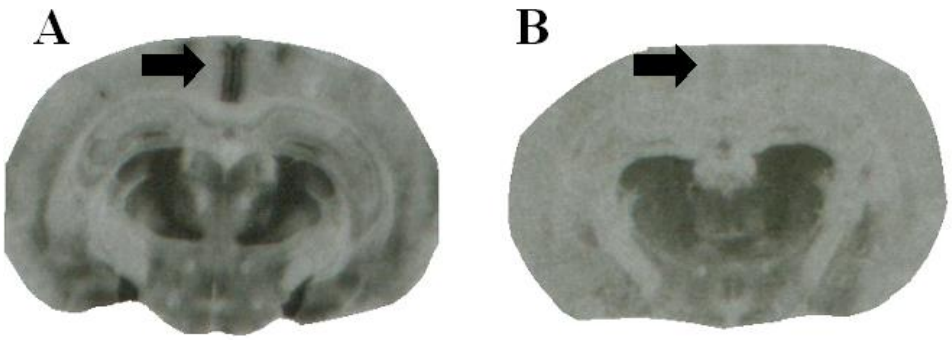

Figure 2.1: Representative autoradiograms of ^{125}I-AVP binding to brain slices demonstrating high and low V1aR expression in the retrosplenial cortex (marked by arrow) and its correlation with genotype at SNP 2403 in the *avpr1a* intron. A) Comes from an individual that is homozygous for the high-expressing allele at SNP 2403, while B) comes from and individual that is homozygous for the low-expressing allele.

Another possible source of phenotypic variation has been discovered in a single nucleotide polymorphism (SNP) within the intron of the *avpr1a* gene at position, which predicts expression better than microsatellite length does. A guanine (G) base at this location within the gene is correlated with low V1aR expression in the retrosplenial cortex and therefore weaker pair-bonds while a thymine (T) is correlated with high V1aR expression in the retrosplenial

cortex and stronger pair-bonds. The location of this SNP along with the observed correlation implies that the *avpr1a* intron serves as an important regulatory region for the gene.[29]

2.6 *In vivo* Assay for Allelic Variation

During transcription, phosphorylation of the C-Terminal Domain (CTD) of RNA polymerase II (RNA Pol II) drives initiation and the transition from initiation to elongation, with different phosphorylation states of the CTD marking different stages of transcription. Serine 5 phosphorylation of the CTD is characteristic of early stages of elongation, and tends to be associated with promoter regions, while serine 2 phosphorylation of the CTD is characteristic of later stages, and tends to be associated with transcribed regions.[30] The amount of phosphorylated Pol II associated with chromatin is also correlated with the presence of transcripts.[31]

Haplotype-specific chromatin immunoprecipitation (ChIP), or haploChIP takes advantage of these properties of transcription to detect allele-specific differences in gene expression *in vivo*.[32] This is done by comparing *in vivo* quantities of bound phosphorylated RNA Pol II to two different alleles from heterozygotes for a gene of interest. As a first step for this study, we tried adapting this method by first testing a less specific antibody, unphosphorylated RNA polymerase II. We also simplified quantification of data from ChIP products by using pyrosequencing rather than mass spectrometry. In general, this method has not been used to link variation in phenotypes related to social cognition and genetic polymorphism.

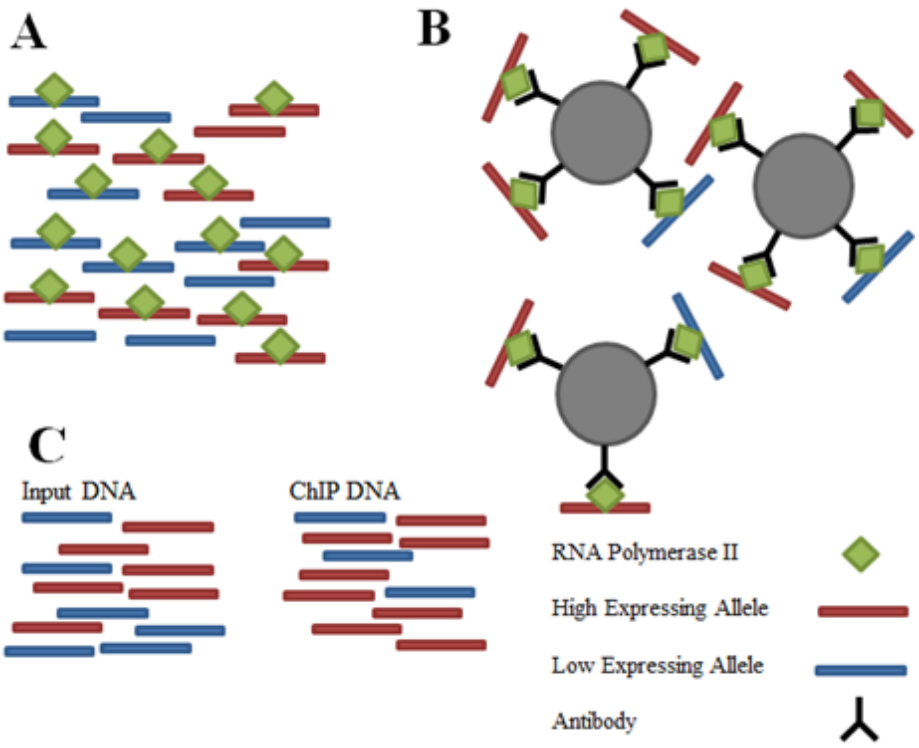

Figure 2.2: Diagram outlining the haploChIP process for RNA Pol II ChIP. A) Chromatin cross-linked to RNA Pol II from heterozygotic cells is isolated. B) Beads coated in antibodies against RNA Pol II bind to Pol II, which isolates the two different *avpr1a* alleles at a ratio that reflects the relative expression levels of the alleles. C) Cross-links are reversed, DNA is isolated from the antibodies and beads, and can be compared to Input DNA, DNA that has not been treated by immunoprecipitation. Differences in allele ratios in the product can be quantified and compared by methods such as pyrosequencing.

2.7 Serine-2 Phosphorylated RNA Polymerase II Chromatin Immunoprecipitation

Another objective of this study is to test the hypothesis that the aforementioned *avpr1a* intronal SNP accounts for variation in sexual fidelity as well as V1aR expression in the retrosplenial cortex. By employing ChIP with antibodies targeting Serine 2 phosphorylated RNA Polymerase II, we will determine whether the SNP genotypes are correlated with transcription levels. This will provided us insight into the genotypic source of variation in V1aR expression and correlated social behavior, and could potentially implicate the intron as a significant regulatory site for *avpr1a*.

3 Methods

3.1 Antibody Optimization

We purchased ChIP grade antibody against RNA polymerase II phosphorylated at Serine 2 from Abcam. To optimize the amount of antibody used in immunoprecipitation, we performed three separate ChIP reactions on chromatin from a single individual. We used: 1.4µg, 3.5µg, and 5.6µg of antibody per 100µL of chromatin, with quantities selected based on the previously optimized RNA polymerase II ChIP. We amplified the final ChIP products and compared the yield of each concentration of antibody by Gel Electrophoresis. We followed optimizations made in previous experiments for quantities of other antibodies.

3.2 Primer Selection

We optimized sequencing primers for the ability to selectively amplify *avpr1a* rather than a paralogous *avpr1a* pseudogene found in the prairie vole.[28] For the optimization reaction, we used 12.5µL of Flexi GoTaq® Colorless Master Mix, 0.25µL of 1µL of forward primer, 0.25µL of 1µL of reverse primer, 0.5µL of genomic DNA at 10-50ng/µL, and 11.5µL of water. The thermal cycler protocol consisted of 3 minutes at 94˚C, 55 cycles of 95˚C for 30 seconds, variable annealing temperature for 30 seconds, and 72˚C for 20 seconds, and 1 minute at 72˚C. We used annealing temperatures of 57.5, 60, and 62˚C, selected based on primer melting temperatures. We then performed gel electrophoresis on the products to compare yields at different temperatures and purified optimal yield reactions using the QIAquick PCR Purification Kit from Qiagen. We then submitted samples for Sanger Sequencing to determine whether the pseudogene was present in the final product and chose a primer set that contained minimal quantities of pseudogene.

3.3 Raising Voles

We captured wild voles in Ava, Illinois. We then genotyped the voles at SNP 2403 using Sanger Sequencing and crossed the voles. We then outcrossed heterozygotes from the F1 generation with field caught animals. Since the age of wild-caught voles was unknown, the age of pairing for the parental and F1 generations varies. We genotyped the offspring and paired heterozygotes at around post-natal day 45. We used these paired heterozygotes for chromatin immunoprecipitation.

3.4 Dissection and Chromatin Isolation

We sacrificed pair-bonded, heterozygous voles for the G/T allele and dissected out the retrosplenial cortex, which we placed in cold PBS. We then cross-linked the tissue by placing it in 37% formaldehyde for 15 minutes, and then added glycine to quench the cross links. We washed the tissue twice with cold PBS, and homogenized it with a pestle and homogenizing tube. After homogenization, we incubated the tissue in cell lysis buffer for 15 minutes on ice, followed by incubation in nuclear lysis buffer for 15 minutes on ice. We then sonicated the samples on ice with 5 10-second bursts with 10 seconds between bursts. We then centrifuged samples at 4˚C, aliquoted the supernatant into new microcentrifuge tubes, and snap-froze it in liquid nitrogen. We stored samples at -80˚C after snap-freezing. For Serine-2 Phosphorylated RNA Pol II ChIP, all solutions mentioned above contained 10mM sodium pyrophosphate to minimize phosphatase activity on RNA Polymerase.

3.5 Chromatin Immunoprecipitation

We incubated rabbit antibody specific to the C-Terminal Domain of Serine 2 phosphorylated RNA Polymerase II, or antibody specific to RNA Polymerase II for the preliminary experiment, with Dynabeads from Invitrogen at 4˚C overnight in fresh PBS with

BSA. We washed the beads with PBS with BSA, added 100µL chromatin, and incubated the chromatin and beads overnight again in PBS with BSA and freshly made RIPA buffer. After incubation, we washed the beads with wash buffer 8 times, and again with a final wash buffer. All solutions up to this point contained 10mM sodium pyrophosphate for Serine-2 Phosphorylated RNA Pol II ChIP. We then isolated DNA from the beads by incubating the beads in elution buffer at 65˚C for 10 minutes, vortexing every 2 minutes. We centrifuged the samples and transferred the supernatant to a new tube. We reversed crosslinks by incubating the samples overnight at 65˚C, with Input DNA samples starting at this step. We then incubated samples for 2 hours at 37˚C with Proteinase K and glycogen. We extracted DNA using phenol and chloroform, followed by ethanol precipitation. We then resupsended the pellet in 30µL of TE and 10µg of RNase A and incubated for 2 hours at 37˚C. We then purified the samples using the QIAquick PCR Purification Kit from Qiagen. We combined ChIP products from the same specimens and subjected these products to ethanol precipitation again to concentrate the DNA. We resuspended in 20µL TE and stored the products at -80˚C.

3.6 PCR Amplification and Sequencing

We designed primers specific for the intron of *avpr1a* as described earlier. We ordered a reverse primer labeled with biotin from IDT for pyrosequencing. Each reaction contained 5µL of DNA (for ChIP) or 4µL of DNA (for Input), 0.5µL of 1µM forward primer, 0.5µL of 1µM reverse primer, 12.5µL of Flexi GoTaq® Colorless Master Mix, and water for a total of 25µL. The thermal cycler protocol consisted of 3 minutes at 94˚C, followed by 55 cycles of 30s at 95˚C, 30s at 60˚C, and 30s at 72˚C, followed by 2 minutes at 72˚C. We submitted samples to EpigenDx for pyrosequencing, along with data for polymorphic sites within the sequenced region.

4 Results

4.1 RNA Pol II ChIP

Prior to performing ChIP targeting Serine-2 Phosphorylated RNA Pol II, we performed ChIP using a less specific RNA Pol II antibody. We submitted 10 samples of ChIP and Input DNA, DNA not treated by immunoprecipitation, to EpigenDx for pyrosequencing. To check for the presence of the pseudogene, we treated a site that differs between the pseudogene and functional gene as a polymorphic site. In addition to SNP 2403, two other polymorphic sites were identified for sequencing. Two neighboring regions along the intron were sequenced, with the first region containing the pseudogene marker, SNP 2403, and one other polymorphic site, and the second region containing the third polymorphic site.

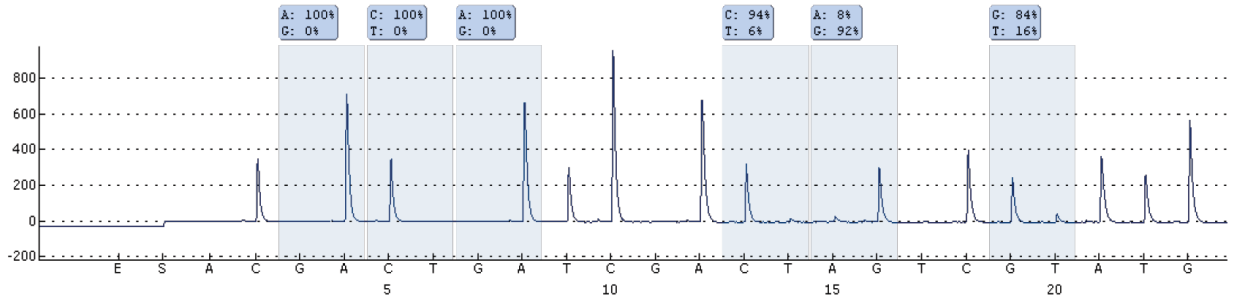

Figure 4.1: Representative image of pyrosequencing data returned from EpigenDx. Boxed areas indicate sites that are identified on submission as polymorphic. The comparative heights of peaks within the boxes indicate the relative quantities of different alleles, with the percentages above the boxes showing the percentage of each allele present in the sequenced sample. This image was taken from sequencing data for a single individual in the first sequenced region. The last polymorphic region in the above figure is SNP 2403.

Results returned from EpigenDx indicated the percent of each allele found at indicated polymorphic sites. The average pseudogene content was 8.10±0.38% (mean±standard error) in input samples and 8.12±0.86% in ChIP samples. According to a paired two-tailed t-test, there was not a significant difference ($p>0.05$) in pseudogene content between Input and ChIP samples. A paired two-tailed t-test revealed no significant difference ($p>0.05$) in relative high-

expressing (T) allele content between pre and post immunoprecipitation treatments for SNP 2403. There also was not a significant difference ($p>0.05$) between pre and post immunoprecipitation treatment allele ratios in the samples at SNP 2391. However, at SNP 2449 there was a weakly significant enrichment of the A allele from pre to post immunoprecipitation ($p = 0.054$) (Figures 4.2 and 4.3).

Site	Allele	Mean Input	Mean ChIP	p-value
Pseudogene	**A**	**8.10**	**8.12**	**0.9826**
SNP 2403	**T**	**41.1**	**38.0**	**0.6314**
SNP 2391	**C**	**94.4**	**94.5**	**0.6712**
SNP 2449	**A**	**74.4**	**86.0**	**0.0544**

Figure 4.2: Summary of results for pyrosequencing of the prairie vole *avpr1a* intron from input and ChIP treated chromatin from RNA Pol II ChIP. Each row shows the mean percent of a particular allele in Input and ChIP samples, averaged across the ten individuals. The specific nucleotide represented in the data is indicated in the allele column. P-values were obtained from a paired two-tailed t-test comparing the percent of a specific allele in Input samples to those in ChIP samples.

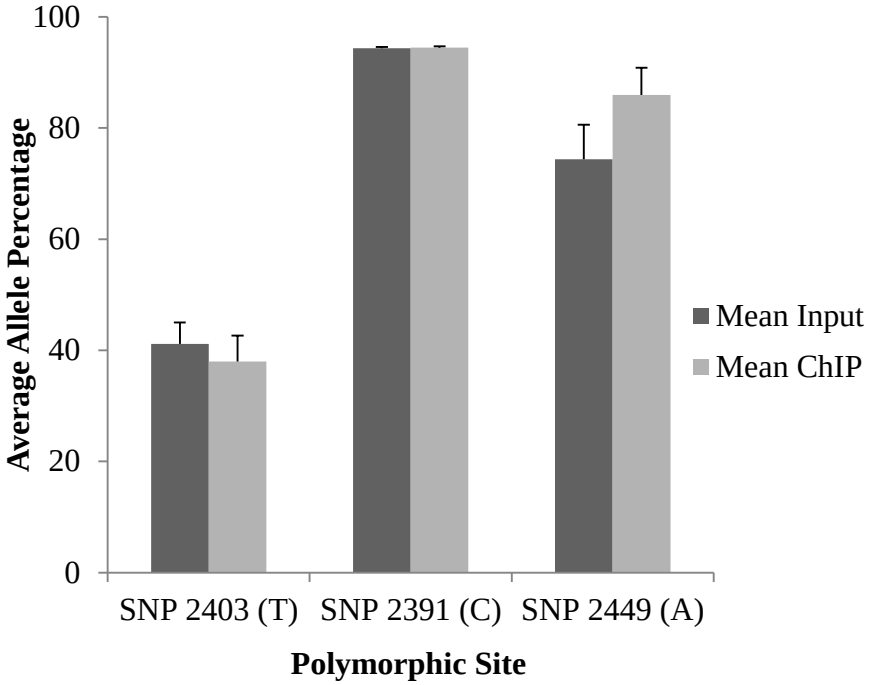

Figure 4.3: Graphical representation of average allele content of Input and ChIP samples at three SNP sites for RNA Pol II ChIP. Each bar represents the mean percent of an allele present across

all ten individuals for Input (dark) and ChIP (light) samples. Error bars indicate standard error. The allele represented by the graph is indicated in parentheses next to the name of the SNP site.

4.2 Serine-2 Phosphorylated RNA Pol II ChIP

After performing the preliminary experiments using an unphosphorylated RNA Pol II antibody, we performed ChIP using an antibody against Serine-2 Phosphorylated RNA Pol II, which is a more specific indicator of gene expression. Samples from three individuals were submitted for pyrosequencing. The average pseudogene content for the samples was 3.91±6.22% for Input samples and 6.22±0.47% for ChIP samples. According to a paired two-tailed t-test, there was not a significant difference ($p>0.05$) in pseudogene content between Input and ChIP samples. Paired two-tailed t-tests also revealed no significant difference ($p>0.05$) between pre and post immunoprecipitation allele ratios in samples at any of the three polymorphic sites (Figures 4.4 and 4.5).

Site	Allele	Mean Input	Mean ChIP	p-value
Pseudogene	**A**	**3.91**	**6.22**	**0.4110**
SNP 2403	**T**	**36.6**	**50.1**	**0.3899**
SNP 2391	**C**	**96.3**	**95.6**	**0.7610**
SNP 2449	**A**	**83.3**	**83.5**	**0.9778**

Figure 4.4: Summary of results for pyrosequencing of the prairie vole *avpr1a* intron from input and ChIP treated chromatin from Serine-2 phosphorylated RNA Pol II ChIP. Each row shows the mean percent of a particular allele in Input and ChIP samples, averaged across the three individuals. The specific nucleotide represented in the data is indicated in the allele column. P-values were obtained from a paired two-tailed t-test comparing the percent of a specific allele in Input samples to those in ChIP samples.

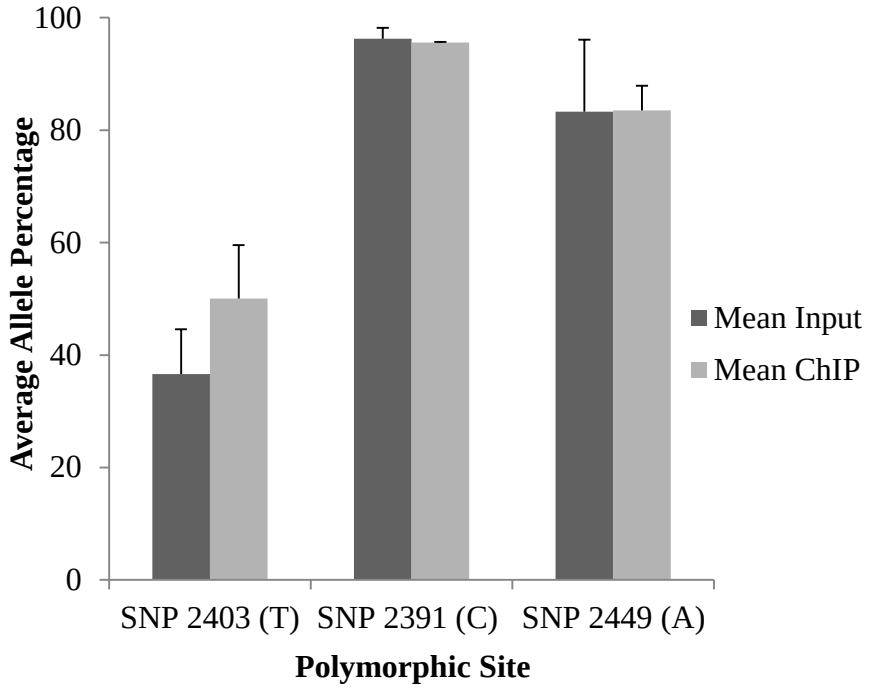

Figure 4.5: Graphical representation of average allele content of Input and ChIP samples at three SNP sites for Serine-2 phosphorylated RNA Pol II ChIP. Each bar represents the mean percent of an allele present across all three individuals for Input (dark) and ChIP (light) samples. Error bars indicate standard error. The allele represented by the graph is indicated in parentheses next to the name of the SNP site.

5 Discussion

5.1 Interpretations

The relatively low pseudogene content in all samples gave us reasonable confidence in the specificity of the amplification primers for the functional gene. The lack of a significant difference between Input and ChIP samples is not surprising, since there is no prior evidence that the functional gene is bound to RNA Pol II at a higher rate than the pseudogene, and the pseudogene already accounts for a low proportion of the amplified products.

The lack of a significant difference between Input and ChIP percentages of the high-expressing T allele at SNP 2403 does not provide strong evidence that this polymorphic site accounts for observed variation for V1aR expression. Despite this, the ability to follow the

experiment to its conclusion and obtain meaningful pyrosequencing data demonstrates that in principle the haploChIP method may be applied for linking genotypic variation at candidate regulatory regions to phenotypic variation.

There is a weakly significant difference between the Input and ChIP percentages of the enhanced allele at SNP 2449. Since SNP 2449 is not far downstream of SNP 2403, there is high degree of genetic linkage, so there is still a possibility that the intronal region around SNP 2403 functions as a regulatory site for *avpr1a* transcription. Immunoprecipitation targeting a more specific indicator of gene expression may therefore lead to significant enrichment of the high expressing allele. From these results, it is also possible that SNP 2449, or even SNPs further downstream, actually are the determining factor for variation in V1aR expression, and that linkage to SNP 2403 has caused the observed correlation between SNP 2403 genotype and V1aR expression in the retrosplenial cortex.

To further test if SNP 2403 accounts for phenotypic variation, we performed the experiment again using an antibody against Serine-2 Phosphorylated RNA Pol II, a more specific indicator of gene expression. Only three samples were submitted initially to determine if further assays would have the potential to reveal a role for SNP 2403 in determining V1aR expression phenotype. Though the pyrosequencing results from these samples do not demonstrate any statistically significant enrichment in alleles associated with high expression according to paired two-tailed t-tests, the differences in mean Input and ChIP quantities of the high expressing allele imply that increasing the sample size may produce more promising results.

5.2 Future Experiments

The immediate follow up to this study would be to perform additional replicates of ChIP against Serine-2 Phosphorylated RNA Pol II to see if a larger sample size yielded statistically

significant enrichment of the high-expressing allele. If a significant enrichment is discovered, it would support the hypothesis that the intron of *avpr1a* serves as a regulatory site and that SNP 2403 has a substantial impact on the region's regulatory effects. Such a finding would indicate that we have successfully connected polymorphism in a high level cognitive process, spatial memory, which has implications on multiple facets of social behavior, with its fundamental genetic basis.

Should Serine-2 Phosphorylated RNA Pol II ChIP provide evidence that the *avpr1a* intron is a regulatory site, it would be interesting to see how regulatory factor binding varies based on SNP 2403 genotype, and thus determine what molecular machinery is responsible for *avpr1a* regulation. We know that SNP 2403 is an important location in the regulatory region and that regulatory effects vary based on its genotype. Therefore, any transcription factors known to bind to sequences equivalent to those around SNP 2403 and that vary in their binding affinity based on the presence of a G or T at the corresponding location are potentially important regulators of *avpr1a* expression. Such transcription factors can be identified using transcription factor affinity prediction (TRAP), and the same haploChIP method can be applied targeting matched transcription factors.[33] If a transcription factor accounts for differences in V1aR expression in the retrosplenial cortex due to SNP 2403, then we would expect it to be bound to SNP 2403 and for it to bind differently to the two different alleles. Such an experiment may identify factors that are important in regulating memory and social cognition on a broader scale.

5.3 Conclusion

In the case of the prairie vole, the significance of the V1aR, and therefore AVP – a neuropeptide whose function has been conserved through evolutionary time – in the retrosplenial cortex for social memory has been linked to attachment and mating strategies. This study

demonstrated the potential for the neurobiological expression patterns that underlie behavioral phenotypic differences to be explained by allelic variation. We have thus seen how genetic differences on the level of a single base, such as the SNP within the *avpr1a* intron, can influence a wide variety of behaviors, such as space use and sexual fidelity for the prairie vole. Though these exact patterns of differential regulation due to SNPs may not be well conserved for *avpr1a*, the same method can be applied to connect heritable differences in social cognition to genetic variation in other genes, parts of the brain, behaviors, and species.

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