INFLUENCE OF GENETIC VARIATION ON SOCIAL BEHAVIORS AND FRONTAL CORTEX DIFFERENCES IN GENERALIZED ANXIETY DISORDER

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Elizabeth Ann Chesna

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DEDICATION

And we know that in all things God works for the good of those who love him, who have been called according to his purpose. – Romans 8:28

First and foremost, I would like to thank the members of my committee for all their help and guidance. I also want to thank my family for their continuous support and love throughout my academic career and for believing in me always. Lastly, I would like to thank my friends and classmates for their input, support, guidance, love, and for making the graduate school experience even more enjoyable.

I would like to dedicate my dissertation to Jeff Sailus, former DNA Section Supervisor at Austin Police Department. Thank you for being my mentor, giving me the opportunity to learn from you, and for trusting me to do a lot of important work and get so much experience in your lab. Thank you also for becoming a close friend. I will always remember your witty comments, the laughs, the memories we made in Austin, and all the things you taught me. Lastly, thank you for how much you believed in me. Your guidance and support helped me realize my true potential and affirmed my love of forensics. I promise to always do my best in this field to make you proud. Rest in peace Jeff, you and the impact you had on my life will never be forgotten.

ABSTRACT

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Certain behaviors have a major impact on the criminal justice system and medical field. The research presented here focuses on antisocial behaviors and generalized anxiety disorder (GAD). Antisocial behaviors such as aggression, criminal behavior, and drug abuse contribute to violent crime. In developed countries, the majority of violent crime is committed by a reduced group of antisocial recidivistic offenders. Currently, the United States has the largest incarceration rate in the world. Identification of genetic variants that influence these behaviors is crucial for the prevention of crime, reduction in recidivism, and the understanding of the etiology of criminal behavior in general. In the first part of this study, a custom primer panel for massively parallel sequencing (MPS) was designed to include 48 single nucleotide polymorphisms (SNPs) potentially associated with social behaviors. Traditional methods, such as single base extension (SBE), are limited in multiplexing capability and time consuming. MPS is more cost effective and allows for a large number of SNPs to be analyzed simultaneously. A preliminary sample set of 100 Caucasian male students were used to assess the validity and concordance of this custom MPS panel. Eight SNPs were genotyped using both SBE and MPS techniques, with all successful profiles being 100% concordant. Participants also completed a survey assessing multiple behaviors and psychological traits. While no significant associations were found in this preliminary sample pool, some trends were observed in behavioral traits. The findings of this study suggest that this panel can be used to simultaneously assess a large number of behavioral and psychological markers. To further explore these results, genetic

variants observed in the preliminary control population were compared to a set of high risk individuals. Therefore, in the second part of this study, 19 markers associated with dopamine (DA) turnover and oxytocin (OXT) were compared between an inmate (N=100) and control (N=100) population. Two SNPs (rs909525 and rs1799836) associated with monoamine oxidase had significantly different major allele frequencies between control and inmate populations (p=0.00002 and p=0.00004 respectively). Moreover, haplotype analysis revealed strong linkage disequilibrium in markers associated with monoamine oxidase A (MAOA), catechol-O-methyl transferase (COMT), and OXT. Two haplotypes associated with MAOA had differences in frequency between controls and inmates. Haplotype GAT was observed more often in inmates than controls (p=0.0012) and GGT was not observed in the inmate population (p=0.000004). Multifactor dimensionality reduction was used to test for gene-gene interaction. Epistasis between markers was not found; however, strong redundancies between rs4680 and rs11476, and rs1799836 and rs740603 were observed. These results provide evidence that marker variation occurs between inmate and control samples and this variation may contribute to behaviors associated with delinquency.

Anxiety disorders also have a major impact on society, as they are the most common type of psychiatric disorder. Among these, generalized anxiety disorder (GAD) is one of the most prevelant. GAD involves persistent anxiety and may worsen over time if left untreated. As a result, an individual's daily life is impaired. Furthermore, there is an economic burden on society and the healthcare system. Imaging techniques, including functional magnetic resonance imaging (fMRI), have allowed for better understanding of structural and functional changes involved in GAD. In the third part of this study, fMRI

was used to assess thickness and surface area differences in GAD patients. Moreover, eleven bilateral frontal regions defined in the Desikan-Kiliany Atlas were compared. A total of 300 participants were included in this study within three groups: GAD patients (N=100), psychiatric controls (N=100), and healthy controls (N=100). Groups were matched for demographic characteristics and other psychiatric conditions. No significant differences were observed for surface area in the left or right hemisphere; however, significant differences were found for thickness in both hemispheres. In the left hemisphere, lower thickness was observed in GAD patients verses healthy controls (p=0.0001) for the pars triangularis and superior frontal region (p=0.0000). Also, significantly lower thickness was observed in psychiatric controls compared to healthy controls (p=0.0000) for the superior frontal region. In the right hemisphere, lower thickness was observed in GAD patients versus healthy controls (p=0.0006) for the caudal middle frontal region and superior frontal region in GAD (p=0.0000). These findings provide evidence that these structures may be involved in GAD. Furthermore, they also suggest GAD may be due to damage from chronic stress as it suppresses neurogenesis, dendritic growth, and synaptic strength.

KEY WORDS: Forensic science, Behavioral genetics, Dopamine, Oxytocin, Serotonin, Inmate, Massively parallel sequencing, Single nucleotide polymorphisms, Anxiety, Generalized Anxiety Disorder, Magnetic resonance imaging, Functional magnetic resonance imaging

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ABBREVIATIONS

5-HT	Serotonin
5-HT1A	Serotonin 1A receptor
5-HT2A	Serotonin 2A receptor
5-HTP	5-hydroxytryptophan
5-HTTLPR	Serotonin-transporter-linked polymorphic region
ACC	Anterior cingulate cortex
ACTH	Adrenocorticotropic hormone
ADH1B	Alcohol dehydrogenase 1B
ADHD	Attention-Deficit/Hyperactivity Disorder
AIM	Ancestry informative marker
ALDH2	Aldehyde dehydrogenase 2
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANKK1	Ankyrin repeat and kinase domain containing 1
ASD	Autism Spectrum Disorder
BDNF	Brain-derived neurotropic factor
BNST	Bed nucleus of the stria terminalis
BOLD	Blood oxygen level dependent
Ca++	Calcium ion
CA1	Cornu Ammonis 1
CA3	Cornu Ammonis 3
CaM-KII	Type II calcium-calmodulin kinase
cAMP	Cylic AMP
CAV3	Caveolin 3
CIAP	Calf Intestinal Alkaline Phosphatase

CMOS	Complementary metal-oxide semiconductors
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CREB	cAMP response element binding protein
CRH	Corticotropin-releasing hormone
CT	Computed Tomography
CU	Callous unemotional
ΔpH	Change in pH
ΔV	Change in surface potential
D’	Normalized coefficient of linkage disequilibrium
D_1	Dopamine receptor type 1
D_2	Dopamine 2 autoreceptor / Dopamine receptor type 2
D_3	Dopamine receptor type 3
D_4	Dopamine receptor type 4
D_5	Dopamine receptor type 5
DA	Dopamine
DβH	Dopamine beta-hydroxylase
ddNTP	Dideoxynucleotide triphosphate
dlPFC	Dorsolateral prefrontal cortex
dmPFC	Dorsomedial prefrontal cortex
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
DOPA	Dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
DRD1	Dopamine receptor 1

DRD2	Dopamine receptor 2
DRD4	Dopamine receptor 4
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion Tensor Imaging
DYNLL2	Dynein light chain 2, cytoplasmic
EPB41L4A	Erythrocyte membrane protein band 4.1 4A
EPSP	Excitatory postsynaptic potential
FAAH	Fatty acid amide hydrolase
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-aminobutyric acid
GABA-A	Gamma-aminobutyric acid-A
GABRA2	Gamma-aminobutyric acid type A receptor alpha
GAD	Generalized Anxiety Disorder
GI	Gastrointestinal
HC	Healthy control
HID	Human identification
HPA	Hypothalamic pituitary adrenal
HPLC	High pressure liquid chromatography
HTR2A	Serotonin receptor 5-hydroxytrytamine receptor 2A
HVA	Homovanillic acid
HWE	Hardy-Weinberg Equilibrium
IFG	Inferior frontal gyrus
ISFET	Ion-sensitive field-effect transistor
IQ	Intelligence Quotient
ISP	Ion sphere particle

LD	Linkage disequilibrium
LOD	Log of the odds of linkage disequilibrium
LTP	Long-term potentiation
MAO	Monoamine oxidase
MAOA	Monoamine oxidase A
MAOB	Monoamine oxidase B
MDR	Multifactor dimensionality reduction
Mg++	Magnesium ion
MPS	Massively Parallel Sequencing
MRI	Magnetic Resonance Imagining
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
N	Sample size
Na+	Sodium ion
NAcc	Nucleus accumbens
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
NSF	N-ethylmaleimide sensitive factor
OFC	Orbitofrontal cortex
OPRM1	Opioid receptor mu 1
OXT	Oxytocin
OXTR	Oxytocin receptor
PC	Psychiatric control
PCR	Polymerase Chain Reaction

PET	Positron Emission Tomography
PFC	Prefrontal cortex
PGB	Pregabalin
PGM	Personal genome machine
PH	Phenylalanine hydroxylase
Pin1	Peptidylprolyl cis/trans isomerase NIMA-interacting 1
PKMζ	Protein kinase M ζ
pM	Pica molar
PTGDS	Prostaglandin D2 synthase
PVN	Paraventricular nucleus of the hypothalamus
SAM	Sympathetic adrenal-medullary
SBE	Single Base Extension
SD	Standard deviation
SLC6A4	Solute carrier family 6 member 4
SNPs	Single nucleotide polymorphisms
SNS	Sympathetic nervous system
SSRI	Selective reuptake inhibitor
STG	Superior temporal gyrus
TH	Tyrosine hydroxylase
TPH1	Tryptophan hydroxylase 1
TPH2	Tryptophan hydroxylase 2
TrKB	Tyrosine kinase B
μL	Microliter
μM	Micro molar
VTA	Ventral tegmental area

CHAPTER I

Development of a Behavioral Genetics Panel Using Massively Parallel Sequencing

Abstract

Traits associated with criminal behavior are influenced by dopamine, serotonin, and oxytocin. In this study, a custom primer panel for massively parallel sequencing (MPS) was designed to include 48 single nucleotide polymorphisms (SNPs) potentially associated with behavior. MPS allows for a large number of SNPs to be analyzed simultaneously, while traditional methods such as single base extension (SBE) are limited in multiplexing capability. Caucasian male students (N=100) were used as a preliminary sample set to determine the validity and concordance of this custom MPS panel. Eight SNPs were genotyped using both MPS and SBE techniques and were fully concordant. Participants also completed a survey assessing multiple behaviors and psychological traits. While no significant associations were found in this preliminary sample pool, some trends were observed in behavioral traits. These results indicate this panel may be used to simultaneously assess a large panel of behavioral and psychological markers.

Keywords

Forensic science, Forensic psychiatry and behavioral science, Behavioral genetics, Massively parallel sequencing, Single nucleotide polymorphisms, Oxytocin, Dopamine turnover, Serotonin

Introduction

In psychiatry and behavioral sciences, the study of traits that are associated with antisocial behaviors is an important area of research (Gold & Appelbaum, 2014). Traits associated with antisocial behaviors include psychopathy, empathy, callous

unemotionality, and moral beliefs; whereas, antisocial behaviors include aggression, criminal behavior, and drug abuse. The ability to identify or predict traits and behaviors is crucial for the prevention of crime, reduction in recidivism, and the understanding of the etiology of criminal behavior in general.

Behavioral genetics, the study of the interaction between the environment and genes and their effect on behavior, is a useful tool to help answer these scientific questions (Bernet, Vnencak-Jones, Farahany, & Montgomery, 2007). Moreover, with the advancement of technology and science, specific genetic information related to behavior can be further investigated independent of environmental factors (Plomin, Owen, & McGuffin, 1994). This approach may be helpful to reduce the potential for data bias. Additionally, more individuals can be studied, allowing for more generalization of data due to an increased sample size. Therefore, there has been increased interest in direct genetic testing to understand correlations between genes and behavior.

Dopamine (DA), serotonin (5-HT), and oxytocin (OXT) are neurotransmitters associated with behavior. DA, or 3,4-dihydroxyphenylethylamine ($C_8H_{11}NO_2$), serves as both a pituitary hormone and catecholamine neurotransmitter (Foley, 2009). The starting point of DA synthesis is typically considered tyrosine. However, phenylalanine can also be converted into tyrosine by the liver or within the neuron by phenylalanine hydroxylase (PH). Tyrosine is then converted to L-DOPA (L-dihydroxyphenylalanine), the precursor to DA by tyrosinase or tyrosine hydroxylase (TH). Next, L-DOPA is converted to DA by DOPA decarboxylase (Elsworth & Roth, 1997) (Figure 1). DA is a strong reinforcing agent, making it critical in the reward pathway. Furthermore, it is important in many physiological processes including motor functions (Wooten *et al*., 1989).

DA receptors are large G-protein coupled receptors that include five subtypes (D_1, D_2, D_3, D_4, and D_5). Although there are five subtypes, they are often classified into two groups: D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4). D_1 receptors are present in multiple areas of the brain with high concentrations in the hippocampus, nucleus accumbens (NAcc), frontal and temporal cortex, substantia nigra, and hypothalamus. They are responsible for many DA cognitive functions including attention and memory. Furthermore, D_1 receptors also help mediate rewarding effects after drug abuse (Seeman, 2009; Sadock, Sadock, & Ruiz 2009; Grandy, Miller & Li, 2016). D_2 receptors are found mostly in the ventral tegmental area (VTA), NAcc, basal ganglia and septum. They are considered important in the mediation of behavioral and motor activity. Moreover, they help regulate mood and emotional stability (Rhang, 2003; Stahl, 2008).

Four major DA pathways exist within the brain: nigro-stiatal, tuberinfundibular, mesocortical, and mesolimbic. In the nigro-stiatal pathway, DA is involved in movement, motor control and function, and learning of motor skills (Schacter & Weger, 2009; Malenka, Nestler, & Hyman, 2009). For the tuberinfundibular pathway, DA inhibits the release of prolactin (Ben-Jonathan & Hnasko, 2001). The mesocortical and mesolimbic pathways are associated with behavior. More specifically, they make up the reward pathway (Figure 2). In the mesocortical pathway, dopaminergic neurons originate in the VTA and terminate in the prefrontal cortex (PFC). Mesocortical DA helps regulate emotional and cognitive behavior. The mesolimbic pathway is often referred to as the pleasure pathway. Dopaminergic neurons originate in the VTA and terminate in the NAcc, amygdala, and hippocampus. Mesolimbic DA is responsible for pleasure, reward, and

addiction. Furthermore, this pathway is responsible for reinforcing behaviors (Bjorklund & Dunnet, 2007; Ayano, 2016).

Although DA is the main neurotransmitter involved in the reward pathway, gamma-aminobutryic acid (GABA) and glutamate are also important. GABA neurons within the VTA will inhibit DA release. In contrast, drugs that inhibit or indirectly inhibit GABA will result in increased output of DA. For example, opiates (such as heroin) bind to opiate receptors in the VTA and cause hyperpolarization of GABA. Moreover, the neuron is less likely to fire an action potential, also referred to as decreased neuronal excitability. Hyperpolarization of GABA (inhibition of the inhibitory neurotransmitter and therefore excitation) results in increased output of DA. The excitatory neurotransmitter glutamate is also important in the reward pathway. Descending glutamate pathways extend from the PFC to the VTA, NAcc, and amygdala. The amygdala is important in emotional response (Carlson, 2012).

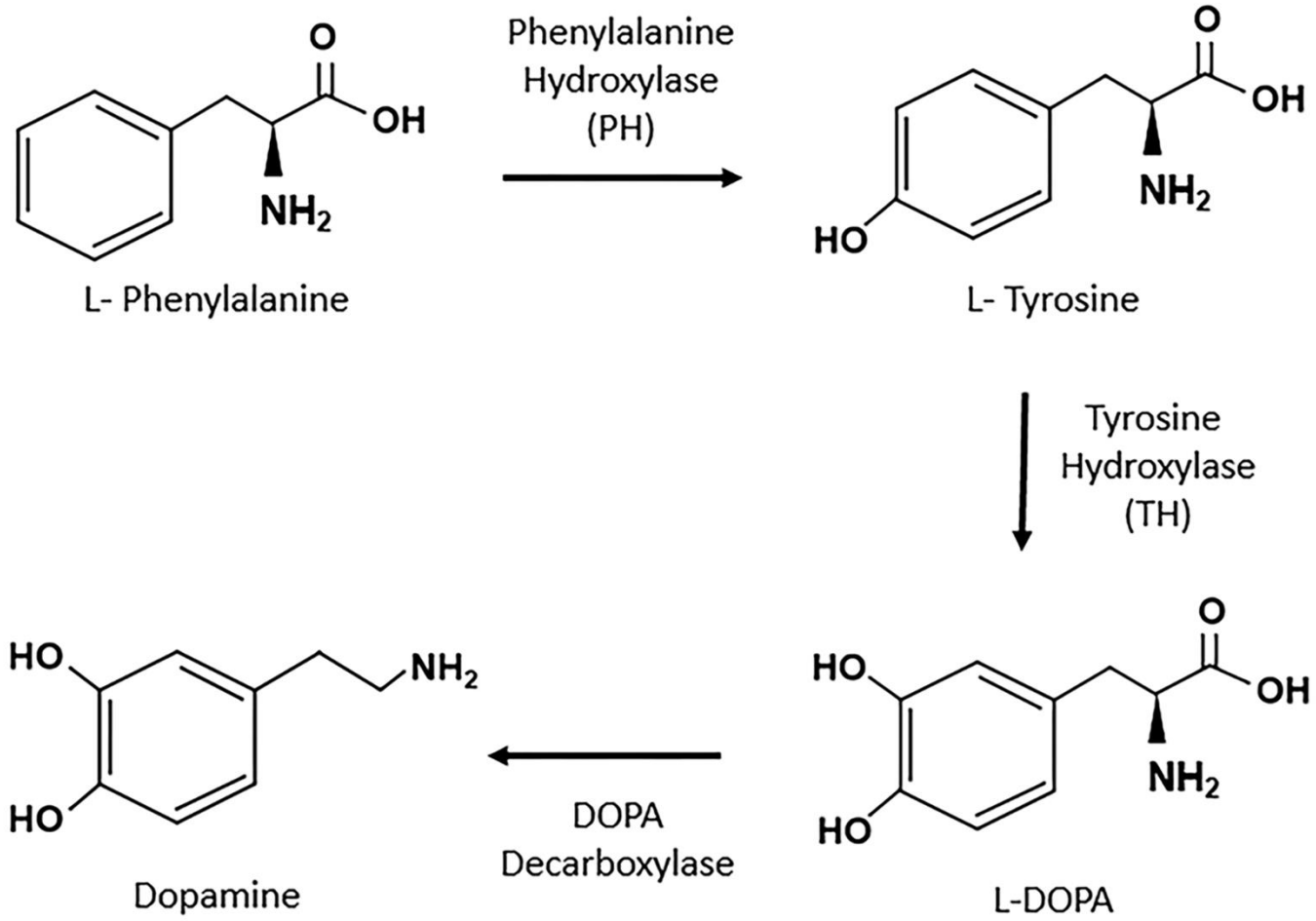

Figure 1. Primary metabolic pathway for synthesis of dopamine.

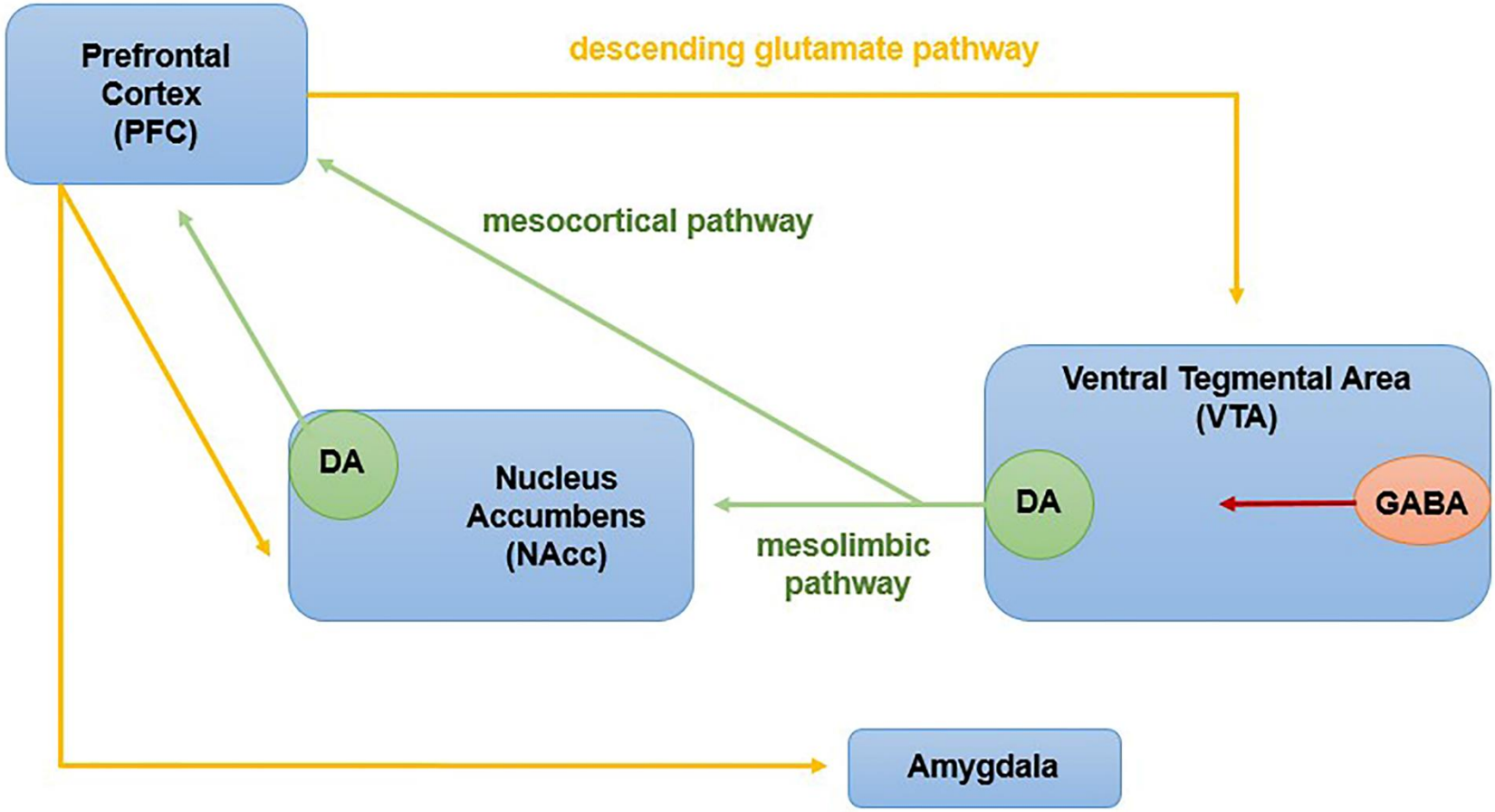

Figure 2. Anatomy of the reward pathway.

5-HT ($C_{10}H_{12}N_2O$), also referred to as 5-hydroxytryptamine, is found in both the central nervous system (CNS) and the periphery. More specifically it is found in the midline of the brain stem, blood platelets, enteric neurons, and enterochromaffin cells in the gastrointestinal (GI) tract (Lucki, 1998; Yadav, 2012; Hensler *et al.,* 2013). As a result, it allows for bidirectional communication between the GI tract and the CNS (Jia & Rajani, 2017). Biosynthesis of 5-HT involves the conversion of L-tryptophan to 5-hydroxy-L-tryptophan with tryptophan hydroxylase 1 and tryptophan hydroxylase 2 (TPH1/2). From there, 5-hydroxytryptophan (5-HTP) decarboxylase converts 5-hydroxy-L-tryptophan to 5-hydroxytryptamine, or 5-HT (Figure 3). 5-HT's main functions include regulation of mood, appetite, digestion, social behaviors, and sexual behaviors (Beecher *et al.,* 2019).

OXT ($C_{43}H_{66}N_{12}O_{12}S_2$) is a neuropeptide synthesized in the hypothalamus with functions in the peripheral reproductive tissue and central nervous system (Ross *et al.*, 2009; Figure 4). After secretion, it is stored or circulated through the bloodstream (Brownstein *et al.*, 1980). Although OXT is a key component in the birthing process, equal concentrations are found in the posterior pituitary and plasma in both men and women. OXT plays a major role in social behaviors including bonding, trust, and empathy (Gimpl *et al.*, 2001).

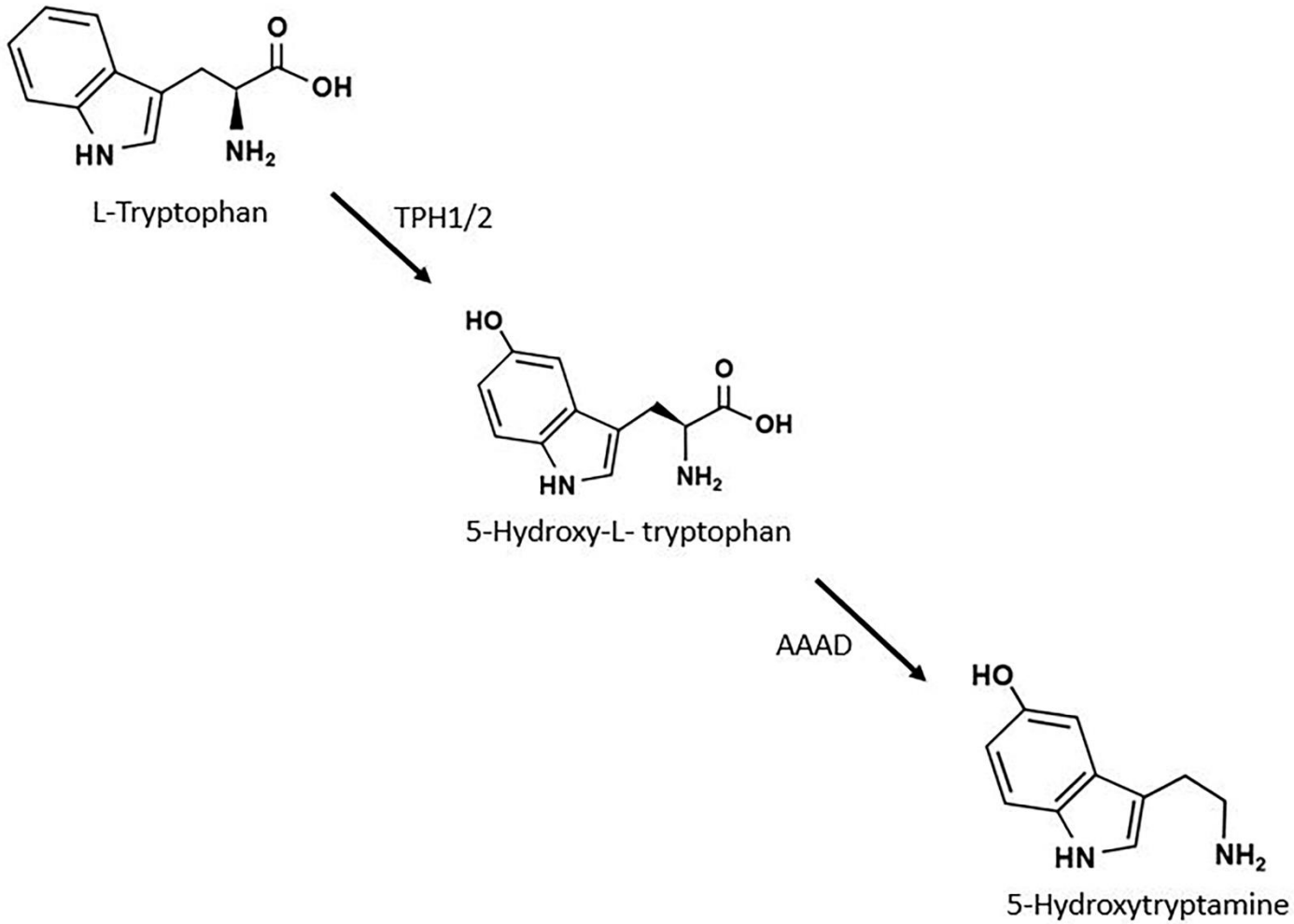

Figure 3. Synthesis of serotonin.

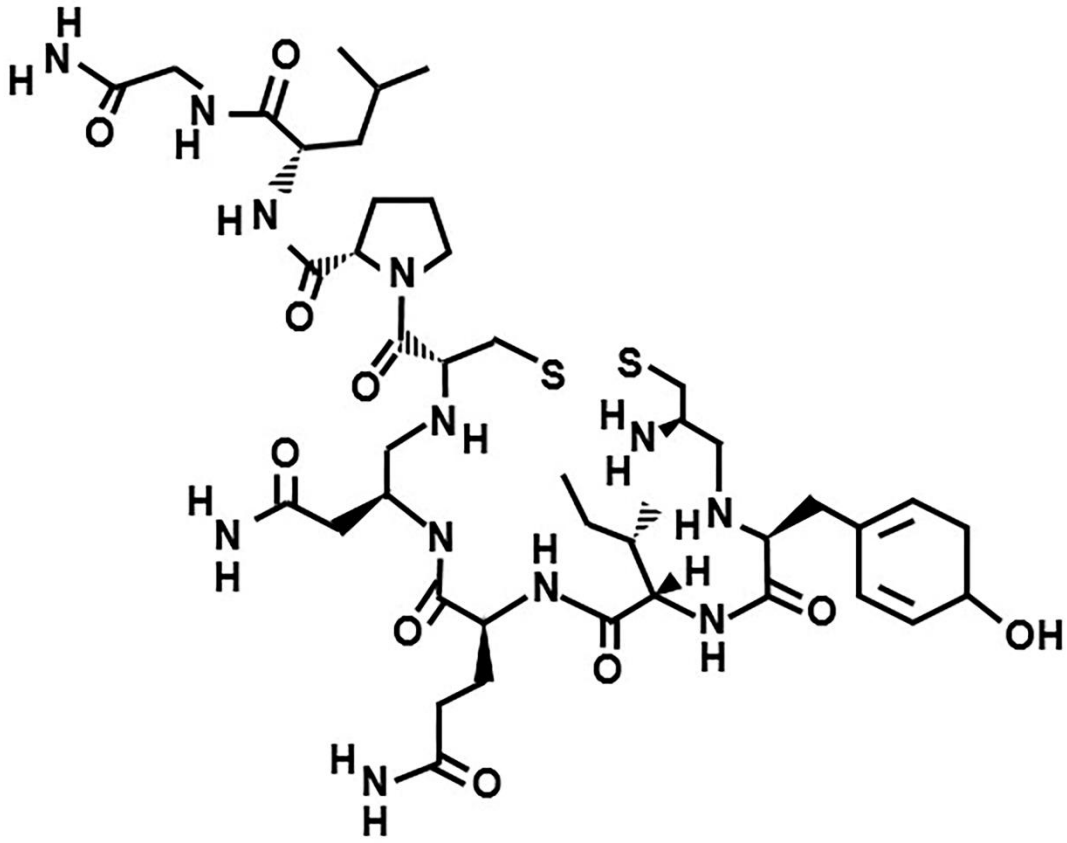

Figure 4. Oxytocin chemical structure.

Many studies have proposed links between the imbalance of these neurotransmitters and behaviors that could lead to criminal activity such as aggressive and addictive behaviors (Nelson & Trainor, 2007; Koob, 2006; Johansson *et al.*, 2013; Pagani *et al.*, 2015; Gimpl & Fahrenholz, 2015). Neurotransmitters are responsible for the communication among neurons in the brain. If a central neurochemical imbalance exists, the signaling along this complicated neural network may be impaired. This cascading effect can lead to physical and behavioral problems depending on the neurotransmitter(s) involved. For example, abnormal levels of DA have been shown to cause Parkinson's disease, aggressive behavior, and impulsive behavior (Gallagher *et al*., 2015; Zai *et al*., 2012; Chester *et al.,* 2015). Levels of neurotransmitters within the brain may be regulated through various mechanisms such as synthesis, transportation, and metabolism. Therefore, genes associated with the neurotransmitters, receptors, transporters, and enzymes involved in the metabolic pathway may all affect the level of neurotransmitters within the synapse (Dennis & Cheng, 2011; Grigorenko *et al.*, 2010). These genes contain polymorphic sites that can be studied to relate or link to certain behavioral traits. Single base variations, or single nucleotide polymorphisms (SNPs), have previously been shown to correlate with certain behavioral traits. For example, SNPs located on genes within the oxytocin receptor (OXTR) have been associated with callous unemotional traits, neuroticism, human pair-bonding, aggression, and empathy (Gimpl & Fahrenholz, 2001; Beitchman *et al*., 2012; Walum *et al*., 2012; Wu, Li, & Su, 2012). SNPs located on genes associated with 5-HT have been linked to aggressive behavior, schizophrenia, and psychopathy traits (Pagani et al., 2015; Gimpl & Fahrenholz; Lucki, 1998). Similar to OXT, the reduced expression of

5-HT has been found to cause aggressive behavior (Johansson *et al.*, 2012; Pagani *et al.*, 2015).

One of the most common methodologies for SNP analysis is single base extension (SBE). With SBE, primers are designed to attach immediately adjacent to the SNP. Fluorescently labeled dideoxynucleotides (ddNTPs) are incorporated preventing further extension. Finally, the products are separated and detected using capillary electrophoresis (Borsting & Moreling, 2015). Although this technique can easily be transferred to laboratories currently performing fragment analysis, the major limitation is multiplexing capability. Only a limited number of SNPs can be analyzed simultaneously.

More advanced DNA sequencing methods have been used in the medical field to reveal correlations with genetic diseases and cancer. As a result, the behavioral genetics field is focusing on using DNA sequencing to determine if an individual is predisposed to exhibit certain behavior. While some associations between SNPs and behavior have been made, many studies have been limited on the number of SNPs due to conventional methods like SBE.

Massively parallel sequencing (MPS) is a newer technology designed to overcome many of the limitations of prior sequencing methods. Previous techniques were limited by specialized nucleotides, electromagnetic intermediates such as X-ray, and imaging technology (Sanger, Nicklen, & Coulson, 1977; Smith et al., 1986; Metzker, 2010). Therefore, non-optical sequencing methods were developed. More specifically, the process of using integrated circuits, complementary metal-oxide semiconductors (CMOS) was explored. CMOS has allowed for fast and large scales of photonic imaging (Theuwissen, 2008).

Rather than detecting photons, an electrochemical detection method was developed to the measure the hydrogen ions released by DNA polymerase (Sakurai & Husimi, 1992). Specifically, the ion-sensitive field-effect transistor (ISFET) was found to work with the CMOS process and detect hydrogen ions (Yeow et al., 1997; Bausells, Carrabina, Errachid, & Merlos, 1999; Milgrew, Hammond, & Cumming, 2004; Hizawa, Sawada, Takao, & Ishida, 2006). The Ion Torrent™ Personal Genome Machine (PGM) uses MPS technology and sequences DNA based on a change in pH (ΔpH). Each ion chip consists of many layers including sensor elements that have a single floating gate over an ISFET. Within each well on the chip, there is a bead containing the DNA template. Underneath, is a metal-oxide sensing layer (Figure 5). During sequencing, protons are releases after nucleotides become incorporated into the DNA strand. This proton release causes a change in pH within the well. As a result, the metal-oxide-sensing layer surface potential changes (ΔV), causing a ΔV in the underlying field-effect transistor (Rothberg *et al.*, 2011).

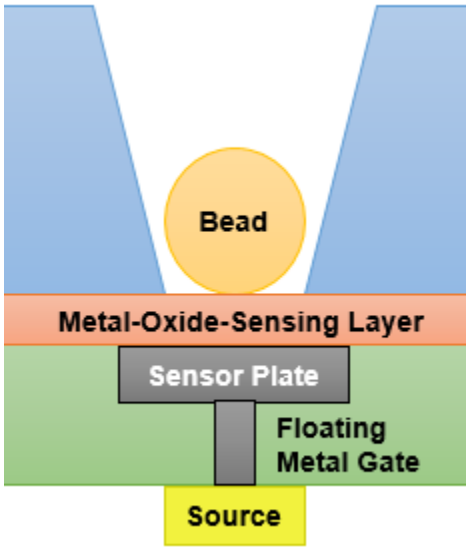

Figure 5. Simple schematic of well and chip structure.

Before sequencing, DNA is fragmented and ligated to adapters. The adaptor-ligand libraries are then clonally amplified onto the beads. A magnetic bead-based process is used to enrich the template beads. DNA polymerase and primers are bound to the templates on the chip through loading port. Each bead settles into an individual sensor well via centrifugation. During sequencing, one nucleotide flows at a time. When the specific nucleotide is complementary to the template base adjacent to the sequencing primer, the nucleotide will incorporate into the forming strand. As a result, the length of the sequencing primer increases by one base (or more, if homopolymer) and a proton is released with the hydrolysis of the incoming nucleotide triphosphate. The proton release decreases the pH at proportion to the number of nucleotides incorporated (Rothberg *et al.,* 2011).

MPS is advantageous because it allows for the simultaneous analysis of a high number of SNPs in a large number of individuals in a single chip. Introducing barcodes allows for samples to be pooled and sequenced simultaneously. This high throughput sequencing is more cost and time effective than previous methods. MPS is currently being used in the medical field to predict diseases and personalize treatment/therapy (Berglund, Kiialainen, & Syvanen, 2011; Cai *et al.,* 2014; Nakabayashi *et al.,* 2018; Wakai *et al.,* 2019). For example, several mutations correlated with the autosomal-recessive disease cystic fibrosis were identified using this technology (Elliot, Radecki, Bellal-Moghis, & Kammesheidt, 2012). Furthermore, frequent mutations found in cancerous tumors have also been observed using the Ion Torrent™ (Cai *et al.,* 2014). These findings indicate that the same concept can be applied to behavioral analysis. A custom panel of SNP markers

can be comprehensively designed in order to include markers (in genes of receptors, transporters, or metabolic enzymes) to study their association with behaviors.

In this study, a 48 SNP custom primer panel was designed based on previous literature (Table 1). SNPs associated with oxytocin, either the gene itself or the receptor gene were included. Moreover, one SNP located downstream of the OXTR, Caveolin 3 (CAV3), was also incorporated into the panel since linkage disequilibrium (LD) overlap between OXTR and CAV3 may contribute to Autism Spectrum Disorder (Campbell *et al.*, 2011). SNPs associated with 5-HT were also selected: some associated with the 5-HT receptor 5-hydroxytrytamine receptor 2A (HTR2A) and one on the tryptophan hydroxylase 1 gene (TPH1), a gene involved in the production of TPH, a rate-limiting enzyme involved in the synthesis of 5-HT (Saetre *et al.*, 2010).

SNPs associated with the metabolism of DA (DA turnover) were also included. DA is metabolized by several enzymes including monoamine oxidase A (MAOA), monoamine oxidase B (MAOB), catechol-O-methyltransferase (COMT), and dopamine beta-hydroxylase (DBH). Therefore, SNPs on these genes were selected. SNPs associated with DA receptors were also incorporated into this panel, including dopamine receptor 2 (DRD2), dopamine receptor 4 (DRD4), and ankyrin repeat and kinase domain containing 1 (ANKK1), which is closely related to DRD1 (Neville, Johnstone, & Walton, 2004).

Other SNPs associated with drug response, metabolism, and addiction were also selected for this custom panel. These include enzymes involved in alcohol and drug metabolism: aldehyde dehydrogenase 2 (ALDH2), alcohol dehydrogenase 1B (ADH1B), and fatty acid amide hydrolase (FAAH). SNPs on drug receptor genes were also included (opioid receptor mu 1 (OPRM1) and gamma-aminobutyric acid type A receptor alpha 2

(GABRA2)). Furthermore, one SNP associated with the brain-derived neurotropic factor (BDNF) gene was included. BDNF is important in cell communication, learning, and memory. Moreover, it is a key component in synaptic plasticity and in long-term potentiation (Greenberg, Xu, Lu, & Hempsted, 2009).

The purpose of this study was to explore the use of MPS in the field of behavioral genetics by developing a panel including multiple SNPs possibly associated with behavior. Forty-eight neural SNPs were analyzed using MPS. Eight SNPs were also analyzed using SBE to compare the results of two techniques, and determine the reliability of the MPS panel. Genotypes of SNPs associated with OXT, 5-HT, and DA turnover were compared to a self-reported survey to determine if there was an association with behavior. It is hypothesized that polymorphic variants at these SNP markers will be associated with antisocial behavioral traits. Furthermore, it is expected that these SNP markers will serve as a large panel of SNPs used to analyze multiple behaviors at once.

Table 1

Forty-eight SNPs included in the custom MPS panel, their associated gene, and potential associations based on previous literature.

SNP	Gene	Potential Associations	Previous Studies
rs877172	OXT	Social behavior, borderline personality disorder and inappropriate intense anger	Walum (2012); Gadow (2013); Moul (2015)
rs4813625	OXT	Stress-induced dopamine release, anxiety, emotional well-being	Love (2012)
rs1042778	OXTR	Aggressive behaviors, prosocial behavior, perspective	Malik (2012); Isreal (2009); Christ (2016)
rs11476	CAV3	Autism Spectrum Disorder	Campbell (2011)
rs237902	OXTR	Negative symptoms, psychopathy	Montag (2013)
rs53576	OXTR	Empathy and stress reactivity, affect, prosocial behavior	Rodrigues (2009); Lucht (2009); Kogan (2011)
rs6770632	OXTR	Aggressive behaviors	Malik (2012)
rs6311	HTR2A	Paranoid schizophrenia, suicidal behavior, childhood adversity), suicide attempts, anger and aggression	Galaktionova (2012); Pearson (2014); Pawlak (2016); Giegling (2006)
rs6314	HTR2A	Paranoid schizophrenia, withdrawn behavior	Galaktionova (2012); Rubin (2013)
rs1800532	TPH1	Suicide attempts, schizophrenia	Pawlak (2016); Saetre (2010)
rs3788862	MAOA	Pain sensitivity, tension in females, aggression and impulsivity	Kim (2006); Gonzalez (2019); Grigorenko (2010)
rs909525	MAOA	Aggression and impulsivity, complex suicide	Grigorenko (2010); Cugura (2018)
rs979605	MAOA	Violence, aggression and impulsivity	Quellet-Morin (2016); Grigorenko (2010)
rs1799836	MAOB	Antisocial behavior, anger and impulsivity	Caspi (2002); Grigorenko (2010)
rs2283729	MAOB	Agreeableness and pain sensitivity	Kim (2006); Horjales-Araujo (2013)
rs165599	COMT	Violent behavior in schizophrenia, perceived stress during pregnancy and childhood IQ, smoking behavior	Gu (2009); Lamb (2014); Lerman (2007)
rs4680	COMT	Violent behavior in schizophrenia, working memory, distress tolerance, schizophrenia, nicotine dependence	Gu (2009); Wang (2013); Amstadter (2012); Pelka-Wysiecka (2013); Beuten (2006)
rs737865	COMT	Violent behavior in schizophrenia, smoking behavior, anger	Gu (2009); Lerman (2007); Calati (2011)
rs740603	COMT	Pain sensitivity, schizophrenia, nicotine dependence	Kim (2006); Li (2012); Beuten (2006)
rs129882	DBH	Attention deficit hyperactivity disorder	Tong (2015)
rs1611115	DBH	Heroin abuse, alcohol dependence	Pavlov (2012); Preuss (2013)
rs739398	DBH	Aggressive behavior	Grigorenko (2010)
rs1076560	DRD2	Alcohol dependence, opioid dependence, schizophrenia	Sasabe (2007); Clarke (2014); Luykx (2017)
rs1799732	DRD2	Protein expression of receptor, suicide ideation in alcoholism	Arinami (1997); Johann (2005)
rs1800497	ANKK1	ADHD, alcohol dependence, cocaine dependence, food reinforcement, DRD2 density	Pan (2015); Blum (1991); Noble (1993); Avena (2009); Jonsson (1999)

rs1800955	DRD4	Transcriptional activity of DRD4, reduced sensitivity of reward	Okuyama (1999); Hattori (2009)
rs6265	BDNF	Depression, smoking cessation	Ribeiro (2007); The Tobacco and Genetics Consortium (2010)
rs1535255	CNR1	Alcohol dependence, impulsivity, and agreeableness	Herman (2006); Ehlers (2007); Juhasz (2009)
rs806368	CNR1	Cannabis dependence, impulsivity, depressive symptoms, cocaine dependence	Agrawal (2009); Zuo (2009); Ehlers (2007); Mitjans (2013); Clark (2013)
rs806379	CNR1	Transcriptional efficacy of the CNR1 gene	Zhang (2004)
rs2023239	CNR1	Transcriptional efficacy of the CNR1 gene , neural functioning associated with substance use, reactivity to alcohol cues and more positive treatment outcomes, impulsivity, and agreeableness	Zhang (2004); Filbey (2010); Hutchison (2008); Ehlers (2007); Juhasz (2009)
rs1049353	CNR1	Post-traumatic stress disorder, alcohol dependence	Lu (2008); Marcos (2012)
rs6454674	CNR1	Schizophrenia disease severity, alcohol dependence, cocaine dependence, bipolar disorder	Copoglu (2015); Marcos (2012); Clarke (2013); Alpak (2014)
rs806380	CNR1	Alcohol dependence, cannabis dependence	Marcos (2012); Agrawal (2009)
rs324420	FAAH	Brain activation when shown marijuana cues, drug use and addiction, marijuana withdrawal and experience of happiness following use, bipolar disorder and major depression	Filbey (2010); Flanagan (2006); Sipe (2002); Haughey (2008); Schacht (2009); Montelone (2010)
rs1799971	OPRM1	Opioid dependence	Haerian (2013)
rs671	ALDH2	Enzymatic activity of ALDH2, alcohol consumption and dependence	Edenberg (2004); Sherva (2009)
rs1229984	ADH1B	Enzymatic activity of ADH1B, alcohol consumption and dependence	Edenberg (2004); Sherva (2009)
rs279826	GABRA2	Alcohol dependence	Edenberg (2004)
rs279836	GABRA2	Drinking patterns (drunkenness), alcohol dependence	Dick (2014); (Edenberg (2004)
rs279844	GABRA2	Alcohol sensitivity	Lind (2008)
rs279845	GABRA2	Drinking patterns (drunkenness), alcohol dependence	Dick (2014); Edenberg (2004)
rs279858	GABRA2	Drinking patterns (drunkenness), alcohol dependence	Dick (2014); Edenberg (2004)
rs279867	GABRA2	Drinking patterns (drunkenness)	Dick (2014)
rs279871	GABRA2	Drinking patterns (drunkenness), alcohol dependence	Dick (2014); Edenberg (2004)
rs497068	GABRA2	Drinking patterns (drunkenness), alcohol dependence	Dick (2014); Edenberg (2004)
rs567926	GABRA2	Early onset of alcohol abuse, aggressive behavior	Strac (2012)
rs9291283	GABRA2	Alcohol abuse and dependence, conduct disorder in adolescence, early onset of alcohol abuse, aggressive behavior	Melroy (2014); Strac (2012)

Methods and Materials

Samples

Buccal swabs from Caucasian male students at Sam Houston State University (N=100) were previously collected. Each individual completed a survey designed to assess several behavioral categories including empathy, aggression, and psychopathy (see Appendix). All personally identifiable information was previously removed in accordance with Sam Houston State University policy. All protocols used in this study were approved by the Sam Houston University State Institutional Review Committee.

DNA Extraction and Quantitation

DNA was extracted on the QIAcube® (QIAGEN, Hilden, Germany) using the QIAamp® DNA Investigator Kit (QIAGEN). DNA quantitation was performed on a StepOne™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) using SYBR® Green Master Mix (Thermo Fisher Scientific). Each DNA sample (2µL) was added to a master mix consisting of 0.5µL 20uM D21S11 primers (GenBank Accession number AP000433) (Integrated DNA Technologies, Coralville, IA), 0.8µL bovine serum albumin (BSA, 8mg/mL, Sigma-Adrich), 9.2µL deionized water (diH_2O), and 12.5µL SYBR® Green Master Mix (Thermo Fisher Scientific). PCR cycling consisted of the following parameters: 10min at 95°C, followed by 40 cycles of 15s at 95°C and 1min at 60°C. Data was considered acceptable with standard curve R^2 values of 0.99 or greater.

Single Base Extension Method

PCR Amplification

Samples were prepared using the Type-it® Microsatellite PCR kit (QIAGEN) with a DNA target of 0.2ng. Two previously optimized multiplexes (see Appendix) were used

to analyze a total of 8 SNPs. Each DNA sample (2.5µL) was added to 10µL PCR master mix, 6.5µL 5X Q Solution (QIAGEN), 1.25µL 2µM primer mix (Table 2) (Integrated DNA Technologies), 0.4µL BSA (8mg/mL) (Agilent Technologies) and 0.85uL diH_2O). A positive sample (2.5µL control DNA) and negative control were prepared and included in each run. The total volume per reaction was 12.5µL and DNA amplification was performed on the Mastercycler Gradient (Eppendorf). In order to remove primers and dNTPs, post PCR clean-up was performed. Additionally, samples and controls were analyzed on a 2% agarose gel stained with 2 µL of SYBR® Safe DNA Gel Stain (Thermo Fisher Scientific) to verify successful amplification. Calf alkaline phosphatase (5µL 1U/µL, Promega, Madison, WI, USA), diH2O (2.5µL), and Exonuclease I (2uL of 1U/uL, Affymetrix, Santa Clara, CA, USA) were added to each sample. This solution was incubated at 37°C for 1 hour followed by 75°C for 15 minutes.

Table 2

Amplification primers for each marker and the associated gene.

Gene	Marker	Fwd/Rev	Primer Sequence
SLC6A4	rs25531	Fwd	CCTAGGATCGCTCCTGCATC
		Rev	GGAGATCCTGGGAGAGGTG
OXT	rs877172	Fwd	CAGACTCTCCTGCCCTCTTG
		Rev	CTCATGCCAGTGACTCATGC
OXT	rs4813625	Fwd	GAGGGGTTGTTGAACAGGTG
		Rev	CTGCCCTCTTGTTGAGGAAG
MAOA	rs979605	Fwd	ATGTCAAGTTGAGCTCACG
		Rev	AAGAACTGGTGTGAGGAGC
MAOA	rs909525	Fwd	TAGGCTGCAATGTCAGATGG
		Rev	CTACAGGCAATCCCTGAGC
MAOA	rs3788862	Fwd	AGCATCAGAGGAAAGCAGC
		Rev	CAGATGGTATGGAGATGGGAG
MAOB	rs2283729	Fwd	AAGCGCAAGCTATGAAACAGGC
		Rev	AGCTATGAAGCCAGCCATATGC
MAOB	rs1799836	Fwd	TGGAGTGTTCTGGCCTTTAC
		Rev	ACATAGCCTACCACAGACTCTG

The primer concentration was 2µM for all amplification primers.

Single Base Extension/Minisequencing

Single base extension was performed using the SNaPshot® Multiplex Kit (Thermo Fisher Scientific) according to the manufacturer's protocol (Applied Biosystems, 2010). The concentration of each primer was optimized prior to starting this research (Table 3). A reaction clean-up was performed for each sample. One microliter of calf intestinal alkaline phosphatase (CIAP, 1U/µL, Promega) was added to each minisequencing product to remove any unincorporated ddNTPs. All samples were placed on the GeneAmp® PCR System 9700 (Thermo Fisher Scientific) with the following parameters: 37°C for 60 min, 75°C for 15 min, and a final incubation at 4°C. An additional post-extension treatment was followed using CIAP (1µL, Promega).

Table 3

SBE primers, associated gene, primer direction, and concentration from stock.

Gene	Marker	Primer Sequence	F/R	Concentration (µM)
SLC6A4	rs25531	GCATCCCCCCTGCACCCCC	F	0.25
OXT	rs877172	GATGAGCTCTGTGACCTGCT	R	0.25
OXT	rs4813625	TCTCTGGGCCACTGCTG	F	1
MAOA	rs979605	GACAACTATTTCTAGAATTTGCA	F	0.2
MAOA	rs909525	GTGAAGGCCAGGTACAGAGGAAAT	F	0.05
MAOA	rs3788862	GTCCCACTAGGCAAGCCTCCTAAAAGCA	F	0.05
MAOB	rs2283729	GCCTGGAACTATGTCTTATTTAATTTCCG	R	0.1
MAOB	rs1799836	GGAGCAGATTAGAAGAAAGATGGTGTC	F	0.05

Genotyping

Minisequencing products (0.5µL) were added to 9.5µL of master mix (9uL Hi-Di™ Formamide and 0.5µL LIZ 120 Size Standard (Thermo Fisher Scientific)). Samples were

denatured by incubation at 95° for 3 min and run using a 3500 Genetic Analyzer (Thermo Fisher Scientific)) as per manufacturer's instructions using POP7 polymer and 50cm capillary array (injection voltage: 1.2kV, injection time: 30s, run voltage (13kV), run time: 1300s). Data were analyzed using GeneMapper® ID Software v4.1 (Thermo Fisher Scientific, 2009).

Massively Parallel Sequencing

Panel Design

A custom panel comprised of 46 amplicons was designed using the Life Technologies panel design tool (www.ampliseq.com) (Thermo Fisher Scientific Ion Ampliseq Designer). This 2x primer pool covered 48 SNPs (Table 1) including 7 of the SNPs analyzed using SBE in order to determine concordance between the techniques.

PCR Amplification

Samples (N=92) were prepared using a DNA target of 10ng. Each DNA sample (6μL) was added to a 14uL PCR master mix (4μL 5X Ion AmpliSeq™ HiFi Mix (Thermo Fisher Scientific) and 10μL 2X Ion AmpliSeq™ custom primers (Thermo Fisher Scientific). The total volume per reaction was 20μL and DNA amplification was performed on the GeneAmp® PCR System 9700 (Thermo Fisher Scientific). Amplification was programmed with the following parameters: 99°C for 2 min, 20 cycles of 99°C for 15s and 60°C for 4min, and a final hold at 10°C. After amplification, primers were partially digested by adding 2μL FuPa Reagent (Thermo Fisher Scientific). Partial digestion was performed on the GeneAmp® PCR System 9700 (Thermo Fisher Scientific) with the following parameters: 50°C for 10 min, 55°C for 10 min, 60°C for 20 min, and a final hold at 10°C.

Adapter Ligation and Purification

A barcode adapter mix was made consisting of 2µL Ion P1 Adapter (Thermo Fisher Scientific) and 4µL nuclease-free water. The mix (6uL) was aliquoted into tubes and an Individual Ion Xpress™ Barcode X1 (2µL, Thermo Fisher Scientific) was added to each sample. Switch solution (4µL, Thermo Fisher Scientific) was added to each sample along with the appropriate diluted barcode (2µL). DNA ligase (2µL, Thermo Fisher Scientific) was added to each sample and ligation was performed on the GeneAmp® PCR System 9700 (Thermo Fisher Scientific) with the following parameters: 22°C for 20 min, 72°C for 10 min, and a final hold at 10°C. Samples were purified using Agencourt® AmpPure® X Reagent (45µL, Beckman Coulter Life Sciences, Brea, CA) and DynaMag™ -96 Side Magnet (Thermo Fisher Scientific) according to the manufacturer's protocols. Two 70% ethanol washes (150µL) were performed and DNA was eluted from beads using 50µL of low TE (Life Technologies, 2019).

Library Quantitation

Samples were quantified using the Ion Library Quantitation Kit (Thermo Fisher Scientific). A 100-fold dilution for each sample was prepared with 5uL sample and 495µL di$H_2$0. All diluted samples (9µL) and standards were quantified in duplicate with a master mix consisting of 10µL 2X TaqMan® Master Mix and 1µL 20X Ion TaqMan® Assay. Samples were quantified according to the manufacturer's protocol on the ABI 7500 Real-Time PCR System (Thermo Fisher Scientific) using the following parameters: 50°C for 2 min, 95°C for 2 min and 40 cycles of 95°C for 15s and 60°C for 1 min (Life Technologies, 2019).

Preparation for Ion Chef (Templating)

Each library was diluted to 50pM and all libraries were pooled together. Twenty-five microliters of each library mix was added to the reagent tubes in the Ion Chef. Ion Chef reagents and each Ion 316™ Chip Kit v2 (Thermo Fisher Scientific) were loaded according to the manufacturer's protocol (Life Technologies).

MPS Sequencing

The Ion PGM was initialized according to the manufacturer's protocol (Thermo Fisher Scientific). For each run, the second chip was loaded onto the Ion PGM within four hours of the first chip. The second chip was stored in the refrigerator and was brought to room temperature for 20 min prior to sequencing (Life Technologies).

MPS Data Analysis

Samples were analyzed using the Ion Torrent Suite v4.6 (Thermo Fisher Scientific). A custom BED file was designed to determine the alleles called. The samples were viewed and analyzed using the HID SNP Genotyper plugin (Life Technologies). Only SNPs associated with DA, OXT, and 5-HT were used for analysis in this study.

Statistics

Allele and genotypic frequencies were compared to those published in PubMed (www.ncbi.nlm.nih.gov/pubmed). Hardy-Weinberg equilibrium and linkage disequilibrium were analyzed using Genetic Data Analysis Software (Weir, 1996). Logistic and linear regression analysis were performed using SPSS® Software (IBM, 1968). Bonferroni correction for multiple comparisons was applied where necessary.

Results/Discussion

Single Base Extension

One hundred DNA samples were genotyped using two SBE multiplexes. Eight SNPs were analyzed: rs25531(SLC6A4), rs877172 (OXT), rs4813625 (OXT), rs2283729 (MAOB), rs1799836 (MAOB), rs3788862 (MAOA), rs909525 (MAOA), and rs979605 (MAOA). Profiles were obtained for each sample and genotypes were recorded. Major allele frequencies and genotype frequencies were consistent with those published in PubMed for populations with similar characteristics. Hardy-Weinberg equilibrium and linkage disequilibrium were estimated for each SNP. No departures were detected for Hardy-Weinberg equilibrium ($p>0.05$). Linkage disequilibrium (LD) estimations revealed departures for rs877172/rs4813625 ($p<0.001$), rs737865/rs740603 ($p<0.0003$), and rs737865/rs4680 ($p<0.0003$), indicating non-random association.

Massively Parallel Sequencing

Before data analysis, loading density was inspected for each chip. All chips had loading densities over 75%. The ion sphere particle (ISP) loading, final library, and number of reads were also observed to ensure quality data (Figure 6). Enrichment, clonal vs. polyclonal, and final library results were also provided by the Ion Torrent software (Figure 7). High total reads (>2.5 million) and percent library (>75%) were deemed indicative of successful library preparation and sequencing.

No major differences were noted between chip 1 (6ng target) and chip 2 (10ng target). Therefore, it was concluded that a DNA target of 6ng is sufficient to produce usable data. The distribution of coverage and mean coverage was also calculated for each marker

(Figure 8). A large distribution of coverage was observed at each marker; however, the mean average was relatively consistent (number of reads ranging from 558 to 1136).

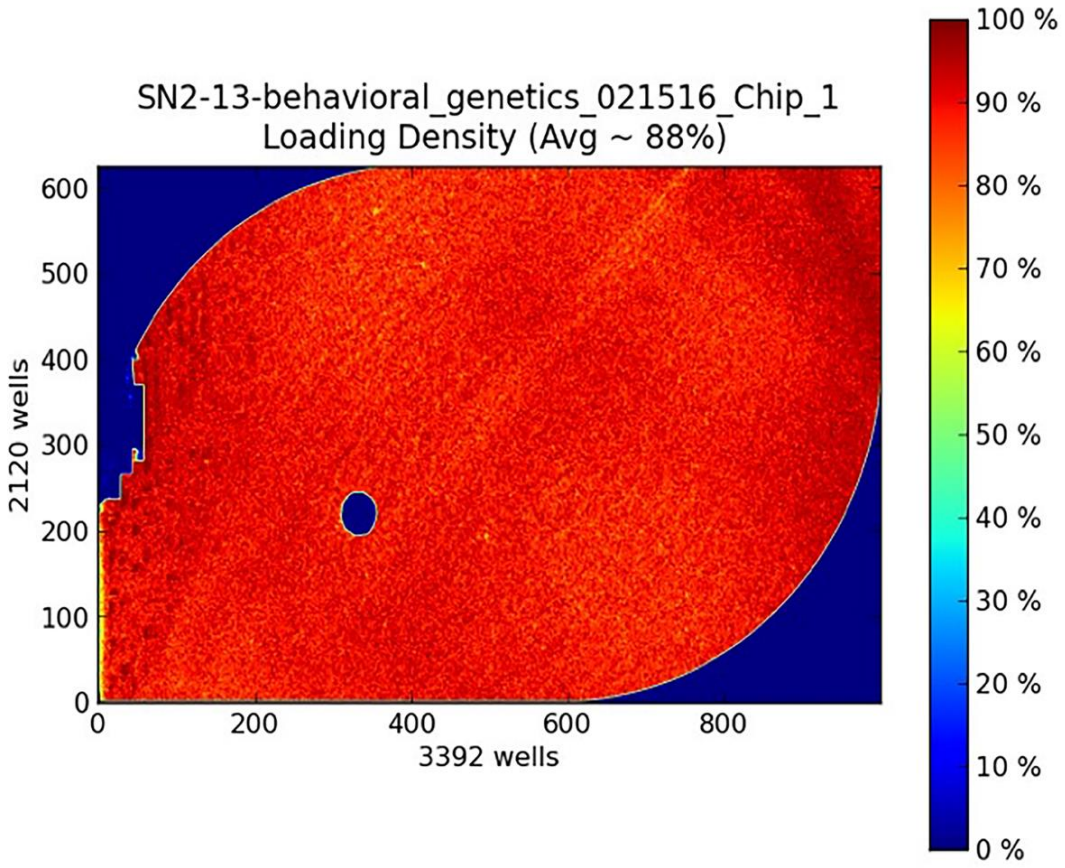

Figure 6. Example of ion sphere particle (ISP) loading density. The chip depicted here had the highest loading density (88%). All chips used in this experiment yielded loading densities above 75%.

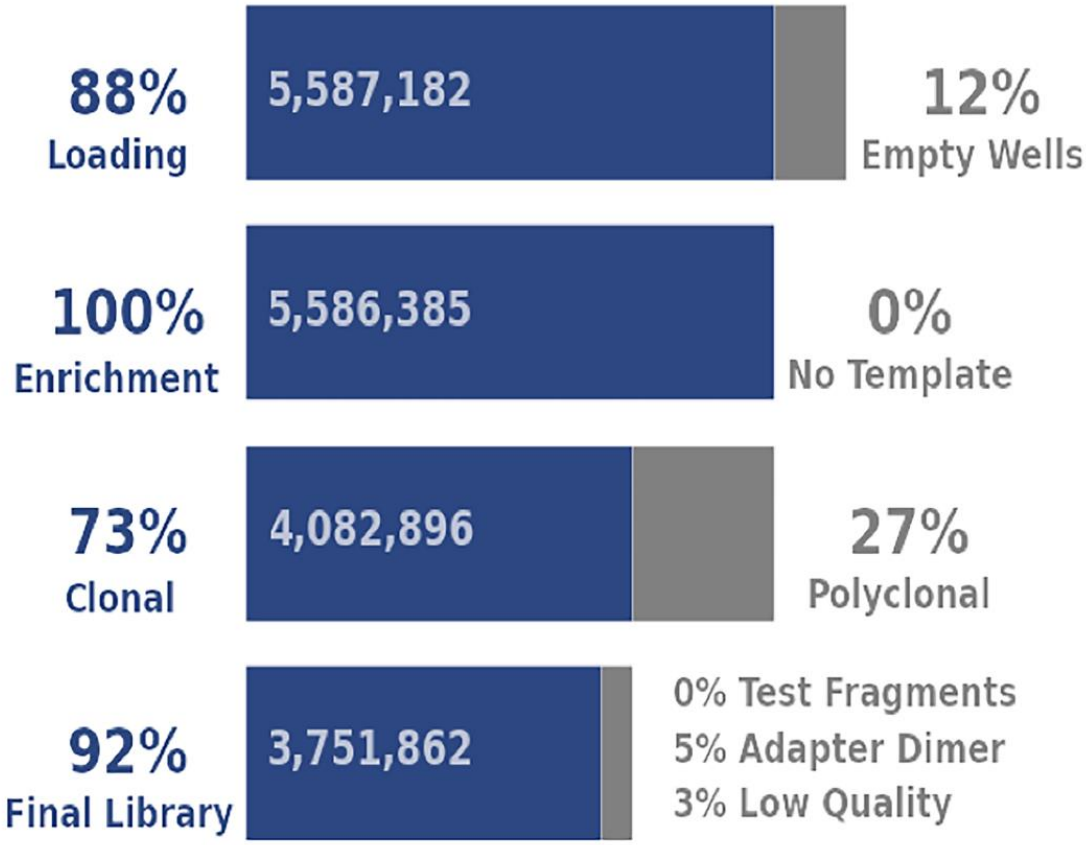

Figure 7. Example of Ion Torrent software ion sphere particle (ISP) summary. Loading density, enrichment, clonal reads, and final library were included. The graph depicted here is for the same chip in Figure 6.

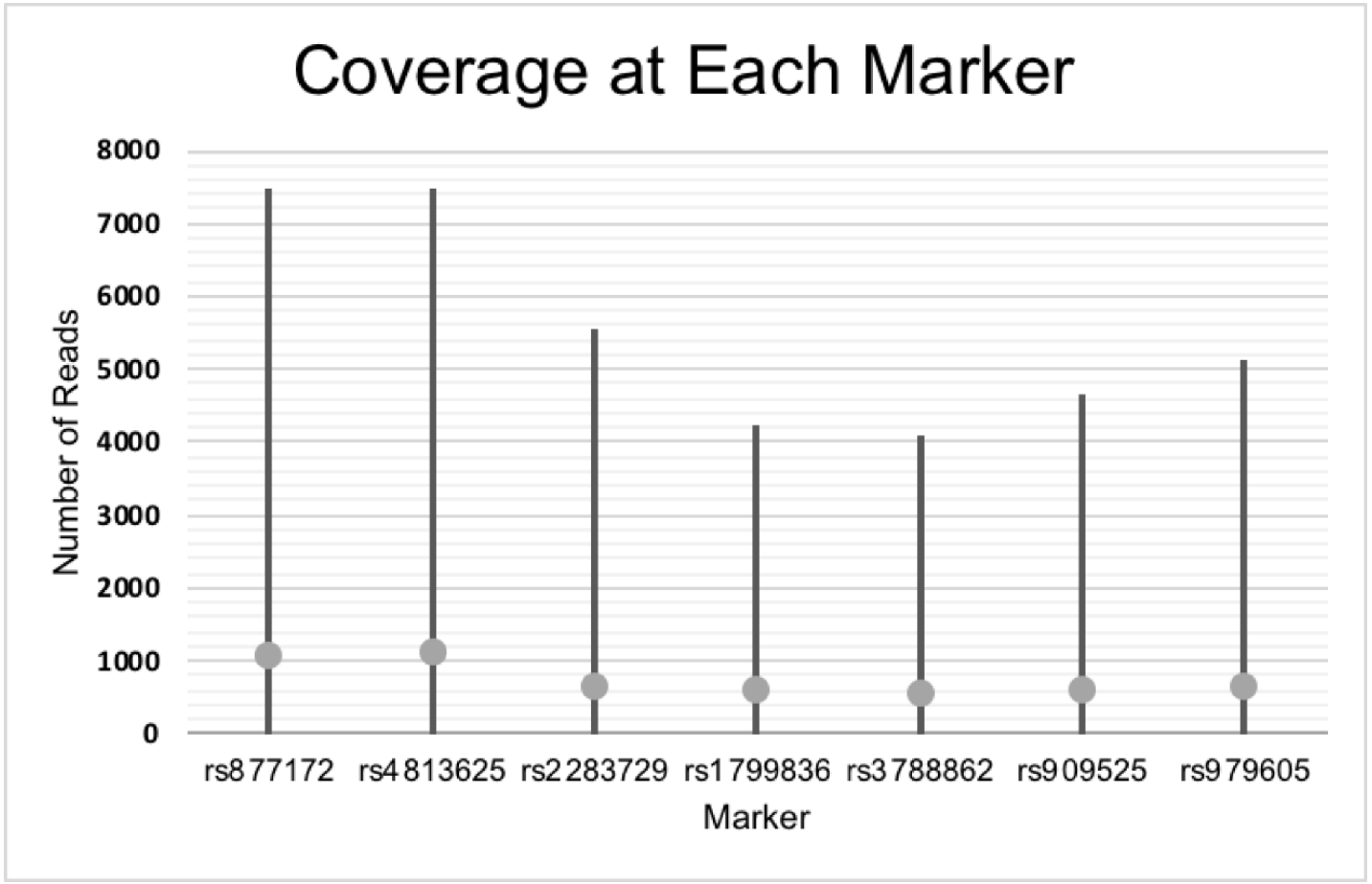

Figure 8. Distribution of coverage and mean coverage for each SNP marker with massively parallel sequencing. Raw data was obtained from the Ion Torrent Software and a custom BED file was created to read data for this custom primer panel.

Concordance of MPS Results to SBE

To confirm accurate and reproducible results, haplotypes and genotypes derived from SBE (N=100) were compared with those from MPS (N=92). Not all samples yielded results using MPS. Samples with a small number of reads or very different positive and negative coverage would not call alleles. Two reads per nucleotide per SNP were required to determine a successful haplotype/genotype using MPS. Ninety-one percent of samples were successful. Of the successful profiles, there was 100% concordance between the two techniques. These results indicate this MPS panel is robust enough to analyze at least 48 SNPs in one hundred individuals simultaneously. This is a major improvement compared to the limited multiplexing capability of SBE.

Associations with Behavior

Five main traits and behaviors as well as subcategories were investigated for possible associations with the resulting haplotypes and genotypes. In total, twenty categories were studied: aggression with proactive and reactive subcategories; depression; psychopathy with the dimensions' egocentricity, callous unemotional (CU) traits, and antisocial lifestyle traits; empathy including perspective taking, fantasy scale, and empathetic concern; perceptions of wrongdoing; and antisocial behavior with subcategories involving drug use and distribution, and varying criminal activities (severe crime, property crime, and violent crime).

First, mean scores for each behavior were graphically depicted across genotypes for each SNP. The data was then analyzed using linear and logistic regression. The skewness and kurtosis of the data was calculated with SPSS® Software. Acceptable dispersion of the data was demonstrated with a value of ±3 for each category. Thus, the

majority of the data was analyzed using linear regression. Due to the irregular dispersion of the data, logistic regression was required to explore the statistical associations for antisocial behavior and most of its subcategories. No SNPs showed significant associations after Bonferroni correction was applied (p=0.05/160=0.0003). It is expected that few significant associations would be observed in a preliminary sample set with similar characteristics.

Although no significant associations were found, some trends were observed. For rs25531, in the SLC6A4 gene, an association was observed for drug and antisocial behavior (Table 4). It was found that individuals with the GA genotype were more likely to show a combination of drug use (drug 2 category) and antisocial behavior (b=1.658, N=100, p=0.006) than individuals with the genotype AA (Table 4). Individuals with the GG genotype were also more likely to exhibit a combination of antisocial behavior and drug use/distribution or providing alcohol to a minor (drug 1 category) (b=1.418, N=100, p=0.015) (Table 4). These results indicate that 5-HT may play a role in antisocial behavior and drug use. This finding is consistent with previous studies showing that serotonin influences social adversity and social anxiety (Caspi *et al.*, 2003). This SNP has also been linked to cigarette smoking and alcohol consumption (Rasmussen *et al.*, 2009). Furthermore, studies have also found polymorphisms at this site to be associated with ADHD, autism, hyperactivity, and maternal sensitivity (Gadow *et al.*, 2013; Mileva-Seitz *et al.*, 2011). An association with one OXT SNP (rs877172) and behavior was also observed. With a decrease in the number of A alleles, individuals were more likely to exhibit antisocial behavioral and commit property crimes (b=-1.109, N=100, p=0.017) (Table 4). Thus, individuals with the CC genotype were most likely to exhibit this type of

behavior, followed by those with the CA genotype, and those with the AA genotype. This indicates there may be an association between OXT and antisocial behavior and property crime. These results are consistent with previous studies that demonstrate OXT influences social behavior (Beitchman *et al.,* 2012; Moul *et al.,* 2015). Variants at this marker have been associated with borderline personality disorder and inappropriate intense anger (Stanley & Siever, 2010). OXT polymorphisms have also been associated with childhood-onset aggression (Malik *et al.,* 2012).

Table 4

Behaviors and associated SNPs.

Behavior	**SNP (p-value)**
Drug-associated antisocial behavior 1	rs25531 (p=0.015); rs979605 (p=0.045)
Drug-associated antisocial behavior 2	rs25531 (p=0.006); rs979605 (p=0.037)
Depression	rs739398 (p=0.019)
Callous unemotional traits	rs1611115 (p=0.003); rs2283729 (p=0.030); rs4680 (p=0.033)
Perceptions of wrong	rs740603 (p=0.014); rs1799836 (p=0.049)
Property crime + antisocial behavior	rs877172 (p=0.017)

SNPs involved with dopamine turnover were also associated with specific traits and antisocial behaviors. One MAOA SNP, rs979605, exhibited an association with drug-associated antisocial behavior including recent drug use (drug-associated ASB 2) (b=-1.629, N=100, p=0.037) and recent drug use/distribution (drug-associated ASB 1) (b=-1.194, N=100, p=0.045) with C being the risk allele (Table 4). Variants within this marker have previously been linked to violence (Quellet-Morin *et al.,* 2016). Two SNPs were also associated with traits indicating increase risk for antisocial behavior; for example, rs2283729 (MAOB) and CU traits (b=-0.276, N=100, p=0.030) and rs4680 (COMT) (b=0.175, N=100, p=0.033) (Table 4). This could demonstrate the overall dismissal of the

general public and possible punishment. Individuals with the G allele at rs2283729 were more likely to recognize wrong or criminal actions but not be apathetic to the consequences. Other studies have found polymorphisms at this marker to be associated with agreeableness and pain sensitivity (Kim *et al.*, 2006; Horjales-Araujo *et al.*, 2013). In addition, another MAOB SNP, rs1799836, demonstrated an association with perceptions of wrong (b=0.214, N=100, p=0.049) with A being the risk allele (Table 4). This SNP has been linked to smoking (additive behavior) and aggression (Perkovic *et al.*, 2016). One variable number tandem repeat (VNTR) in the promotor region of the MAOA gene has been linked to MAOA functional activity. Low MAOA activity has been associated with childhood maltreatment and resulting antisocial behavior. In contrast, high MAOA activity was shown to mediate the effects early childhood maltreatment has on development of antisocial behavior (Caspi *et al.*, 2002).

Additional SNPs associated with dopamine turnover analyzed using MPS were also compared to behavior. An association was found between the COMT SNP rs4680 and depression (b=-0.121, N=100, p=0.031) suggesting that by increasing the number of G alleles, the risk for depression increases (Table 4). Thus, those with the GG or AG genotype are more likely to experience depression than those with the AA genotype. In contrast, individuals with AA or AG genotypes at rs4680 are more likely to exhibit CU traits such as a lack of guilt and empathy (b=0.167, N=100, p=0.033) than those with the GG genotype. Another SNP within the COMT gene, rs740603, showed an association with impulsive and risk-taking lifestyles dimension of psychopathy (b=-0.195, N=100, p=0.014) (Table 4). This dimension of psychopathy was positively associated with the number of G alleles in the rs740603 SNP. Two SNPs within the dopamine beta-hydroxylase (DβH) gene

demonstrated associations with a behavior or trait. Specifically, rs739398 showed a relationship with depression (b=0.155, N=100, p=0.019), and rs1611115 showed an association with CU (b=0.256, N=100, p=0.003 (Table 4). Those with the genotype AA and AG at rs739398 were more likely to exhibit depression while those with the genotype TT or CT at rs1611115 were more likely to demonstrate CU behavioral traits. Previous COMT research has focused on the valine to methionine substitution in codon 158 and found it to be linked to increased aggression and antisocial behavior (Perkovic *et al.*, 2016). Most studies with DBH have focused on ADHD; however, one study found that the TT genotype at rs1611115 may increase the risk of heroin abuse (Xie *et al.*, 2013).

The results presented here indicate that this custom primer panel can be used to simultaneously analyze 48 markers potentially associated with behavior using MPS. High loading density, reads, and percent library were observed for each MPS chip, with 6ng of target DNA yielding usable data. Of the successful profiles, there was 100% concordance between the two techniques confirming the accuracy of this new custom panel. While no significant associations were found in this preliminary sample set, some trends were still observed for antisocial behaviors and traits. It is expected that significant differences and more variation will be found when comparing this data set to more high risk individuals.

Conclusion

The results of this study demonstrated that MPS has the potential to be used in the behavioral sciences and forensic psychiatry field to analyze several SNPs related to multiple behaviors simultaneously. Moreover, this large panel of behavioral SNPs may be used in early prevention or treatment of psychiatric disorders which have a large impact the medical field and criminal justice system. Although no significant associations were

found in this preliminary data set, some trends were observed. Specifically, these behaviors included drug-associated antisocial behavior, depression, perceptions of wrongdoing, drug use/distribution, property crimes, and the psychopathic dimensions callous unemotional and antisocial lifestyle behavioral traits. These results affirm that OXT, 5-HT, and DA can influence behavior. A major limitation of this study is that it consists of a small sample size (N=100). In order to confirm these associations, replicate studies should be performed. Furthermore, samples from high risk individuals (e.g. inmates) and samples from multiple ethnicities should be included in the future. These types of prediction (association) studies require a large sample size in order to improve the accuracy and the reproducibility of the results. Future studies should also focus on setting a proper threshold for MPS results and investigating the mechanism behind the associations between neurotransmitters and behavior. The use of animal models or pharmacological studies may also be useful in describing the exact mechanism of these pathways. Moreover, the role the environment plays on these individuals, specifically factors like childhood adversity and criminality of peers, requires investigation.

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CHAPTER II

Sequence variation in genes affecting dopamine turnover and oxytocin in a sample of male inmates

Abstract

Behavior is a complex process influenced by both genetics and the environment. Some neurotransmitters including oxytocin and dopamine have been associated with social behavioral traits. Certain genes (such as genes of receptors, transporters, and enzymes involved in metabolic pathways of these neurotransmitters) are associated with these neurotransmitters. These genes contain polymorphic sites, single nucleotide polymorphisms (SNPs), which can be studied to relate or link them to certain behavioral traits. While some associations between SNPs and behavior have been made, this study analyzes multiple SNPs in both male inmate (N=100) and control (N=100) populations. This study included a total of 19 SNPs associated with oxytocin (OXT) and dopamine (DA) turnover. Two SNPs (rs909525 and rs1799836) associated with monoamine oxidase had significantly different major allele frequencies between control and inmate populations ($p=0.00002$ and $p=0.00004$ respectively). Moreover, haplotype analysis revealed strong linkage disequilibrium in markers associated with monoamine oxidase A (MAOA), catechol-O-methyl transferase, and oxytocin. Two haplotypes associated with MAOA had differences in frequency between controls and inmates. Haplotype GAT was observed more often in inmates than controls ($p=0.0012$) and GGT was not observed in the inmate population ($p=0.000004$). Multifactor dimensionality reduction was used to test for gene-gene interaction. Epistasis between markers was not found; however, strong redundancies between rs4680 and rs11476, and rs1799836 and rs740603 were observed. These results

provide evidence that marker variation occurs between inmate and control samples and this variation may contribute to behaviors associated with delinquency.

Keywords

Forensic science, Behavioral genetics, Single nucleotide polymorphism, Oxytocin, Dopamine turnover, Inmate, Haplotype, Epistasis

Introduction

Behavior depends on multiple neural pathways that are the focus of current research. Behavior is affected by both genetics and the environment. Although genetics is only one component in the development of behavior, knowledge of genetic influences can provide insight on the etiology of certain types of behavior. Several neurotransmitters have been correlated with social behavior. More specifically, oxytocin (OXT) and dopamine (DA) are two neurotransmitters that play a main role in social behavior. Beginning to understand the influence of OXT and DA on behavior may help explain underlying causes for aggressive and antisocial behavior.

DA is a neurotransmitter that acts as a strong reinforcing agent. It is involved in the reward system, making it critical in addictive behavior, depression, and schizophrenia (Grigorenko *et al.*, 2010). Levels of DA within the brain may be regulated through various mechanisms such as synthesis, transportation, and metabolism. Moreover, DA levels in the brain can be affected by changes in enzymatic activity, which may in turn influence behavior. DA is synthesized from the amino acid L-tyrosine and broken down into norepinephrine by dopamine beta-hydroxylase (DBH; Figure 9).

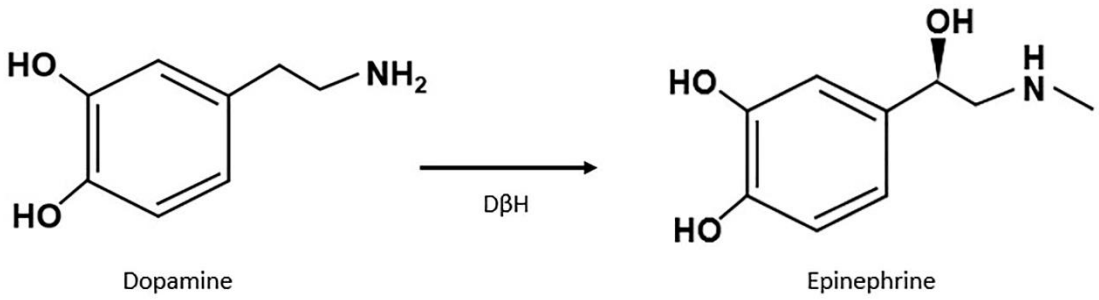

Figure 9. Breakdown of dopamine into epinephrine.

Dopamine is also primarily metabolized into dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase (MAO) (Elsworth *et al.*, 1997). Monoamine oxidase has two forms that are 73% homologous: monoamine oxidase A (MAOA) and monoamine oxidase B (MAOB) (Bortolato *et al.*, 2009). Both MAO enzymes are critical in the breakdown of DA (Craig *et al*, 2009). DOPAC is further catabolized by catechol-O-methyl transferase (COMT) into homovanillic acid (HVA) which is excreted in urine (Witte *et al.*, 2012) (Figure 10).

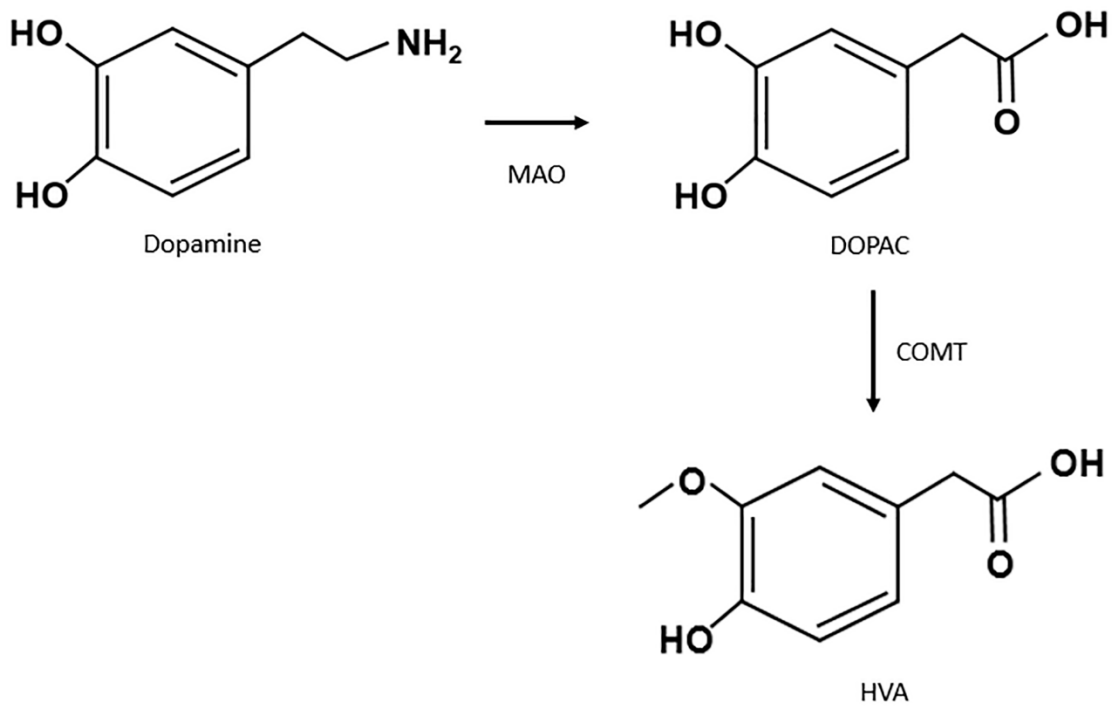

Figure 10. Breakdown of dopamine into metabolites DOPAC and HVA.

As previously mentioned, OXT functions in the peripheral reproductive tissue and central nervous system (Ross *et al.*, 2009). OXT is associated with bonding, trust, and callous unemotional traits. Callous unemotional traits include lack of empathy, remorse and guilt and are known to correlate with antisocial behavior in children (Gimpl *et al.*, 2001). Furthermore, these traits increase the likelihood of psychopathy in adults (Moul *et al.*, 2015).

Previous studies have shown that various SNPs located within genes associated with OXT, DA, and their receptors, transporters, and related metabolic enzymes correlate with certain behavioral traits (Manuck *et al.*, 1999). Imbalanced levels of OXT and DA are known to correlate with social behavior; therefore, expression of specific alleles may

be related to the regulation of these neurotransmitters. The study of these variants can help determine their genetic influence on behavior.

There is limited data analyzing genetic links to criminal behavior with inmate samples. The focus of previous work has been on a MAOA upstream variable number tandem repeat (MAOA-uVNTR) and a serotonin transporter linked polymorphic region (5-HTTLPR) (Armstrong *et al.,* 2014; Tiihonen *et al.,* 2014; Wells *et al.,* 2017; Boisvert *et al.,* 2017; Armstrong *et al.,* 2017).

In this study, SNPs associated with DA turnover, OXT, and the oxytocin receptor (OXTR) were analyzed. Since DA is metabolized by several enzymes including MAOA, MAOB, COMT, and DBH, several SNPs located on these genes were incorporated. More specifically, three SNPs located on the MAOA gene (rs3788862, rs909525, and rs979605) were selected. Previously, rs3788862 has been associated with pain sensitivity (Kim *et al.,* 2006), tension in females (Gonzalez *et al.,* 2019), aggression and impulsivity (Grigorenko *et al.,* 2010). rs909525 has been associated with aggression, impulsivity (Grigorenko *et al.,* 2010), and complex suicide (Cugura *et al.,* 2019). rs979605 has also been associated with aggression and impulsivity (Grigorenko *et al.,* 2010); however, it also is thought to play a role in violence (Quellet-Morin *et al.,* 2016). SNPs located on the MAOB gene (rs2283729 and rs1799836) were also included. rs2283729 has been associated with agreeableness and pain sensitivity (Kim *et al.,* 2006; Horjales-Araujo *et al.,* 2013) and rs1799836 with antisocial behavior (Caspi *et al.,* 2002), anger, and impulsivity (Grigorenko *et al.,* 2010). DBH SNPs included rs161115, rs129882, and rs739398. rs161115 is associated with heroin abuse (Pavlov *et al.,* 2012) and alcohol dependence (Preuss *et al.,* 2013); rs129882 with attention deficit hyperactivity disorder (ADHD) (Tong *et al.,* 2015); and rs739398

with aggressive behavior (Grigorenko *et al.*, 2010). Four SNPs located on the COMT gene were also selected: rs737865 is associated with violent behavior in schizophrenia (Gu *et al.,* 2009), smoking behavior (Lerman *et al.,* 2007), and anger (Calati *et al.,* 2011); rs740603 with pain sensitivity (Kim *et al.,* 2006), schizophrenia (Li *et al.,* 2012), and nicotine dependence (Beuten *et al.*, 2006); rs165599 with violent behavior in schizophrenia (Gu *et al.,* 2009), perceived stress during pregnancy and childhood IQ (Lamb *et al*., 2014), and smoking behavior (Lerman *et al.,* 2007); and rs4680 with violent behavior in schizophrenia (Gu *et al.,* 2009), working memory (Wang *et al.,* 2013), distress tolerance (Amstadter *et al.,* 2012), schizophrenia (Pełka-Wysiecka *et al.,* 2013), and nicotine dependence (Beuten *et al.,* 2006).

SNPs associated with oxytocin, either the gene itself or the receptor gene, were included. More specifically, two SNPs located within the OXT gene (rs877172 and rs4813625) were incorporated. rs877172 has been associated with social behavior (Walum *et al.,* 2012; Gadow *et al.,* 2013), borderline personality disorder, and inappropriate intense anger (Moul *et al.,* 2015); and rs4813625 with stress-induced dopamine release, anxiety, and emotional well-being (Love *et al.,* 2012). Three OXTR SNPs were also selected: rs53576, rs1042778, and rs6770632. rs53576 has been associated with empathy and stress reactivity (Rodrigues *et al.,* 2009), affect (Lucht *et al.,* 2009), and prosocial behavior (Kogan *et al.,* 2011); rs1042778 with aggressive behaviors (Malik *et al.,* 2012), prosocial behavior (Israel *et al.,* 2009), and perspective (Christ *et al.,* 2016); and rs6770632 with aggressive behaviors (Malik *et al.,* 2012).

One SNP located downstream of the OXTR, rs11476 (CAV3 gene), was also incorporated into this study since linkage disequilibrium overlap between OXTR and

CAV3 may contribute to autism spectrum disorder (ASD) (Campbell *et al.,* 2011). Another SNP (rs25531) located on the SLC6A4 (solute carrier family 6 member 4) gene was included. This gene is a protein coding gene for the serotonin transporter and rs25531 has previously been associated with ADHD, ASD (Gadow *et al.,* 2013), prosocial behavior, and social anxiety (Stoltenberg *et al.,* 2013).

The purpose of this study was to analyze nineteen SNPs potentially associated with behavior using single base extension (SBE). Genetic variant observation was compared between a male inmate (N=100) and a control population (N=100). Furthermore, inmate genotypes were compared to survey data associated with aggression, sociability, and arrest rates.

Methods

Samples

Buccal swabs from male students at Sam Houston State University (N=100) and male inmates from a southern, metropolitan county jail (N=100) were previously collected. The average age of student sample members was 21.16 (SD = 2.02) and the inmate sample averaged 31.63 years of age (SD = 11.20). Inmates completed a survey designed to assess several behavioral categories including empathy, aggression, and psychopathy (see Appendix). All personally identifiable information was previously removed in accordance to Sam Houston State University policy. All protocols used in this study were approved by the Institutional Review Board. Samples in both populations consisted of all males. Control samples were matched to inmate samples based on ethnicity and gender (58% African American, 25% Hispanic, 12% Caucasian, and 5% other). Inmate offenses included violent offenses (44%), drug offenses (22%), property offenses (16%), and other (18%). Ten

ancestry informative markers (AIMs) were used to confirm reported ethnicity: rs722869, rs1858465, rs1876482, rs1344870, rs1363448, rs952718, rs2352476, rs714857, rs1823718, and rs735612 (Kosten *et al.*, 2013; Table 5). AIM testing was only performed for 179 samples, as DNA was not available for the remaining 21 samples (Figures 11 and 12).

Table 5

Ancestry informative markers used to confirm self-reported ancestry.

Marker	**Locus**	**Chr.**	**ALFRED UID**	**# pop.**
rs722869	VRK1	14	SI003730N	121
rs1858465	Intergenic between LOC100506650 and LOC645163	17	LO008926Z	112
rs1876482	Intergenic between FAM49A and ZFYVE9P2	2	LO009036S	155
rs1344870	Intergenic between SGOL1 and VENTXP7	3	SI007821S	119
rs1363448	PCDHGB1	5	LO149652B	134
rs952718	ABCA12	2	SI004800M	113
rs2352476	Intergenic between MIR4468 and RPS17P12	7	LO010459T	65
rs714857	Intergenic between INSC and SOX6	11	SI001818S	113
rs1823718	Intergenic between C15orf59 and TBC1D21	15	LO007105N	134
rs735612	RYR3	15	LO000926R	72

DNA Extraction and Quantitation

DNA was previously extracted on the QIAcube (QIAGEN, Hilden, Germany) using the QIAamp DNA Investigator Kit (QIAGEN). DNA quantitation was performed on a StepOne™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA) using SYBR® Green Master Mix (Thermo Fisher Scientific). Each DNA sample (2µL) was added to the master mix consisting of 0.5µL 20uM D21S11 primers (GenBank Accession

number AP000433) (Integrated DNA Technologies, Coralville, IA), 0.8µL bovine serum albumin (BSA, 8mg/mL; Sigma-Aldrich, St. Louis, MO), 9.2µL deionized water (diH_2O), and 12.5µL SYBR® Green Master Mix (Thermo Fisher Scientific) using the following parameters: 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C.

PCR Amplification

Samples were prepared using the Type-it® Microsatellite PCR kit (QIAGEN) with a DNA target of 0.2ng. Four multiplex assays were used to genotype a total of nineteen SNPs (Table 6). Each DNA sample (2.5µL) was added to 10µL PCR master mix, 6.5µL 5X Q Solution (QIAGEN), 1.25µL Primer Mix (Table 6) (Integrated DNA Technologies), 0.4µL BSA (8mg/mL, Sigma-Aldrich) and 0.85µL diH_2O). A positive sample (2.5µL control DNA) and negative control were prepared and included each the run. The total volume per reaction was 12.5µL and DNA amplification was performed on the GeneAmp® PCR System 9700 (Thermo Fisher Scientific). In order to remove unincorporated primers and dNTPs, post PCR clean-up was performed. Calf alkaline phosphatase (CIAP, 5µL 1U/µL, Thermo Fisher Scientific), diH2O (2.5µL) and Exonuclease I (2µL of 1U/µL, Thermo Fisher Scientific) were added to each sample.

Table 6

List of SNPs with their associated gene, chromosome, and observed alleles.

SNP	Gene	Chr.	Allele
rs3788862	MAOA	X	A/G
rs909525	MAOA	X	A/G
rs979605	MAOA	X	C/T
rs2283729	MAOB	X	A/G
rs1799836	MAOB	X	A/G
rs161115	DBH	9	C/T
rs129882	DBH	9	C/T
rs739398	DBH	9	C/A
rs737865	COMT	22	C/T

rs740603	COMT	22	A/G
rs165599	COMT	22	G/A
rs4680	COMT	22	G/A
rs877172	OXT	20	G/T
rs4813625	OXT	20	G/C
rs11476	CAV3	3	A/T
rs53576	OXTR	3	G/A
rs1042778	OXTR	3	G/T
rs6770632	OXTR	3	G/T
rs25531	SLC6A4	17	G/A

Single Base Extension

Single base extension (SBE) was performed SNaPshot Multiplex Kit (Thermo Fisher Scientific) according to manufacturer's protocol (Thermo Fisher Scientific). The concentration of each SBE primer (Table 7) (Integrated DNA Technologies) was optimized prior to starting this research. A reaction clean-up was performed for each sample. One microliter of CIAP (1U/μL; Thermo Fisher Scientific) was added to each minisequencing product to remove any ddNTPs. All samples were placed on the GeneAmp® PCR System 9700 with the following parameters: 37°C for 60 min, 75°C for 15 min, and a final soak at 4°C.

Table 7

List of primer concentrations.

Multiplex	SNP	Gene	PCR Primer Conc. (uM)	SBE Primer Conc. (uM)
1	rs25531	SLC6A4	2	0.25
1	rs877172	OXT	2	0.25
1	rs4813625	OXT	2	1.0
2	rs2283729	MAOB	2	0.1
2	rs1799836	MAOB	2	0.05
2	rs3788862	MAOA	2	0.05
2	rs909505	MAOA	2	0.05
2	rs979605	MAOA	2	0.2
3	rs740603	COMT	2	2.0
3	rs737865	COMT	2	2.0
3	rs739398	DBH	2	2.0
3	rs1611115	DBH	2	2.0
3	rs165599	COMT	2	2.0
3	rs4680	COMT	2	2.0
3	rs129882	DBH	2	2.0
4	rs11476	CAV3	0.2	0.1
4	rs53576	OXTR	0.2	0.1
4	rs6770632	OXTR	0.1	0.1
4	rs1042778	OXTR	0.1	0.1

Genotyping

Minisequencing products (0.5µL) were added to 9.5µL of master mix (9µL Hi-Di™ Formamide and 0.5µL LIZ 120 Size Standard (Thermo Fisher Scientific)). Samples were separated and detected on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) as per manufacturer's instructions using POP7 polymer and 50cm capillary array (injection voltage: 1.2kV, injection time: 30s, run voltage (13kV), run time: 1300s). Data was analyzed using GeneMapper® ID Software v4.1 (Thermo Fisher Scientific).

Statistical Analysis

Allele and genotypic frequencies were compared to those published in PubMed. Hardy-Weinberg equilibrium and Haplotype analysis were performed using Haploview software (Barrett *et al*, 2005; www.broadinstitute.org/haploview/haploview). D' (normalized coefficient of linkage disequilibrium) and LOD (log of the odds of linkage disequilibrium between two loci) were estimated. Multifactor Dimensionality Reduction (MDR) was used to determine gene-gene interaction (Hahn *et al*, 2003). Logistic and linear regression analysis for survey data was performed using IBM® SPSS® Statistics. Bonferroni correction for multiple comparisons was applied where necessary.

Results

Inmate – Control Analysis

Allelic Analysis

Control and inmate DNA samples were genotyped using single base extension. Nineteen SNPs were analyzed within four multiplexes. Profiles were obtained for each sample and genotypes were recorded. Only one departure from Hardy-Weinberg equilibrium was detected (rs739398; $p<0.001$). In this case, a heterozygote deficit was observed and as a result, this marker was not used in further analyses. A Bonferroni correction was applied for multiple comparisons, with an adjusted p-value of 0.0028 (0.05/18). Major allele frequencies were compared for each marker in inmates and controls (Figure 13). Major differences were observed for two SNPs: rs1799836 (MAOB) and rs909525 (MAOA). For rs1799836, the allele A was observed more often in inmates than controls (N=100, p=0.0000426) (Table 8). In contrast, the allele G was observed more often in inmates for marker rs909525 (N=100, p=0.0000199) (Table 8).

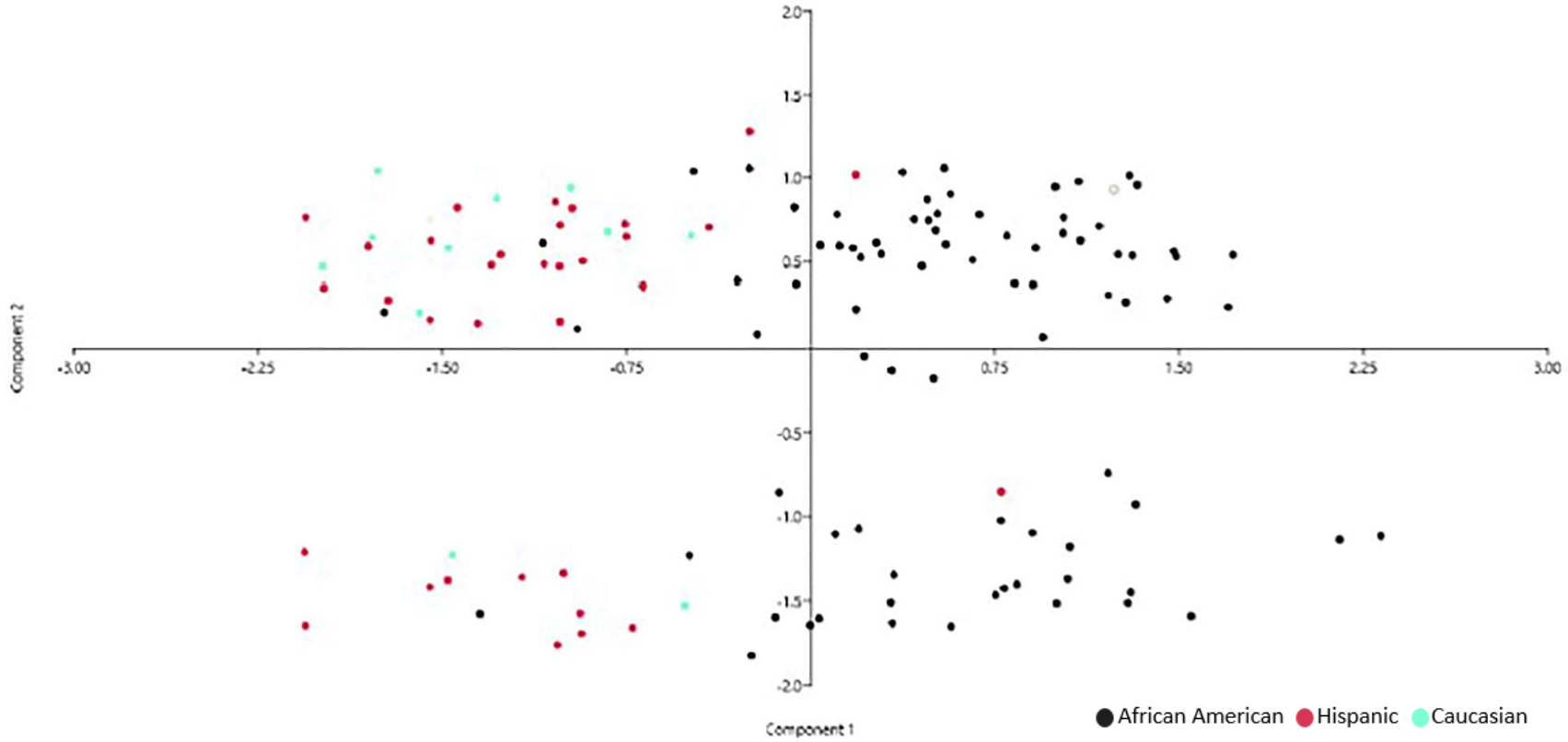

Figure 11. PCA analysis for AIMs to confirm ethnicity.

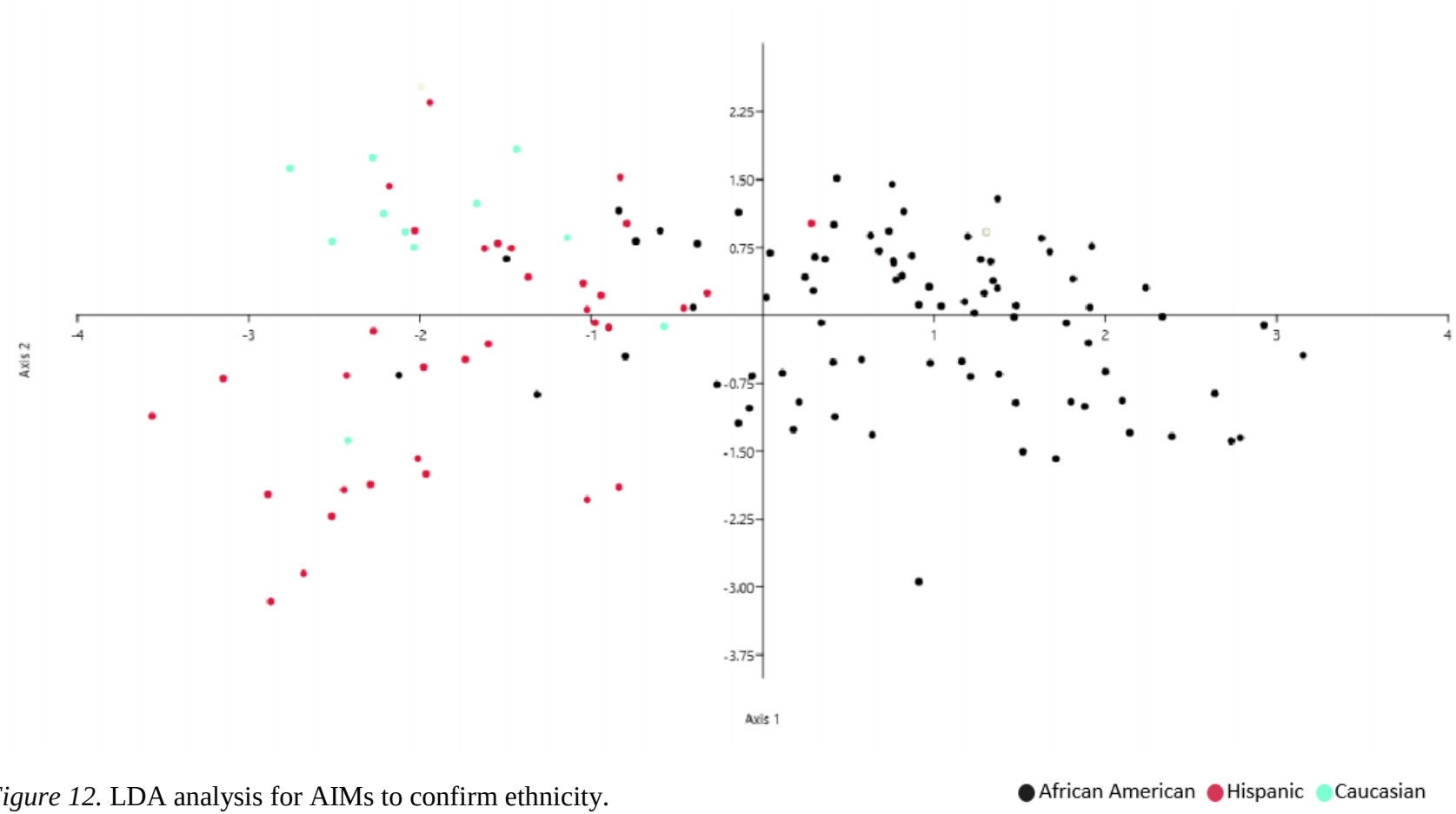

Figure 12. LDA analysis for AIMs to confirm ethnicity.

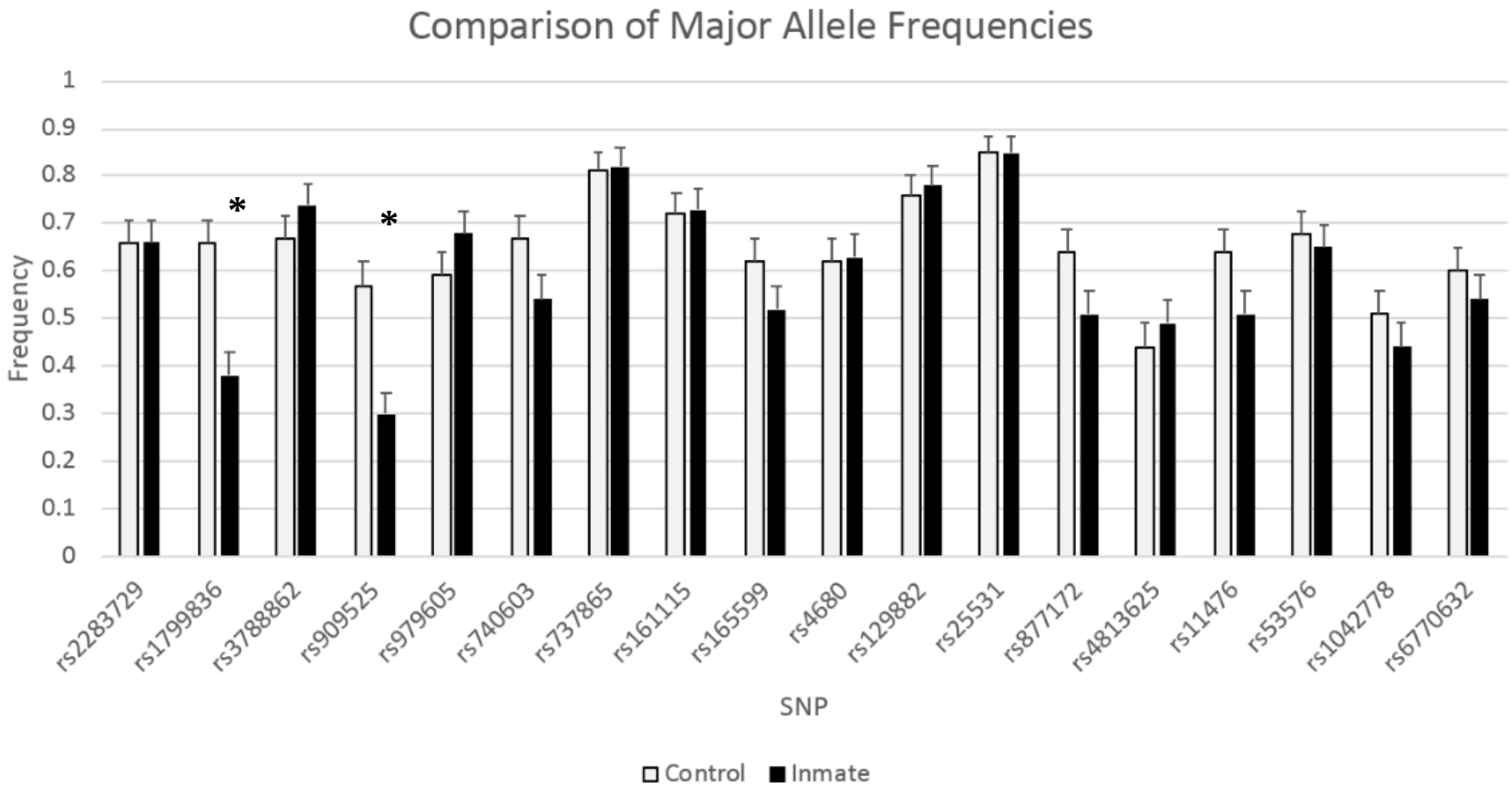

Figure 13. Comparison of major allele frequencies in inmate and control populations. Bonferroni correction for multiple comparisons was used (0.05/18 markers = 0.0028). *indicates a $p < 0.00002$.

Table 8

Frequencies of associated allele in control and inmate populations.

SNP	Gene	Associated Allele	Inmate: Control Ratio	P-value
rs909525	MAOA	A	0.722, 0.434	0.0000199*
rs1799836	MAOB	G	0.622, 0.343	0.0000426*

*indicates significance after Bonferroni correction applied (0.05/18 markers = 0.0028).

Haplotype Analysis

Haplotype analysis revealed high linkage disequilibrium (LD) (LOD≥ 2 and D´>0.8 depicted as bright red (Haploview)) between MAOA markers rs3788862 and rs909525, COMT markers rs737865 and rs740603, and OXT markers rs4813625 and rs877172 (Fig. 2). Weak or no LD (LOD≥2 and D´<0.8 depicted by shades of pink red; LOD<2 and D´<0.8 depicted by white (Haploview)) was observed for the remaining markers. For MAOA, two haplotypes were found to have statistically significant differences in frequency between controls and inmates (Table 9). Haplotype GAT was observed more often in inmates than controls (0.165 vs. 0.030; Fisher's exact test, N=100, p=0.0012). Furthermore, the haplotype GGT was not observed in the inmate population (0.000 vs. 0.172; Fisher's exact test, N=100, p=0.0000036).

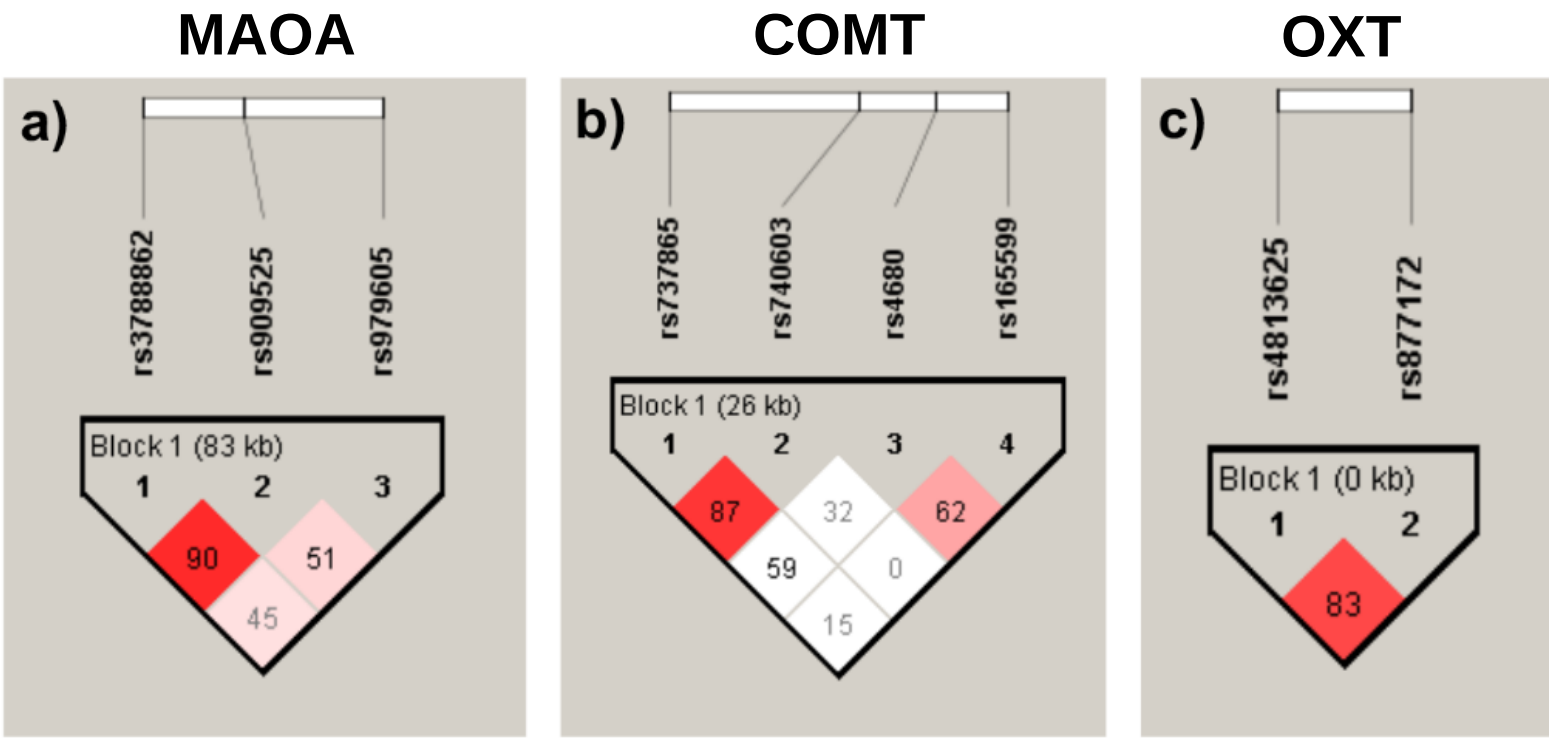

Figure 14. Haplotype analysis for markers that exhibited linkage disequilibrium. High linkage disequilibrium (LD) is depicted as bright red (LOD≥ 2 and D′>0.8), weak LD depicted as shades of pink red (LOD≥2 and D′<0.8), and no LD depicted by white (LOD<2 and D′<0.8) (Haploview).

Table 9

Haplotype associations for MAOA markers. Fisher's exact test was used to calculate p-value.

Haplotype Associations	Frequency	Inmate: Control Ratio	P value
GAC	0.464	0.546, 0.384	0.0075
AGT	0.179	0.155, 0.202	0.0965
GAT	0.097	0.165, 0.030	0.0012*
AGC	0.087	0.093, 0.081	0.1933
GGT	0.087	0, 0.172	0.0000036*
GGC	0.071	0.031, 0.111	0.0194
AAC	0.010	0.010, 0.010	0.5025

*indicates significance after Bonferroni correction applied (0.05/18 markers = 0.0028).

Multifactor Dimensionality Reduction

Multifactor dimensionality reduction was used to test for gene-gene interactions. No epistasis was found; however, a strong redundancy between rs4680 (COMT) and rs11476 (CAV) was observed. Also, a strong redundancy was found between rs1799836 (MAOB) and rs740603 (COMT) (Fig. 3).

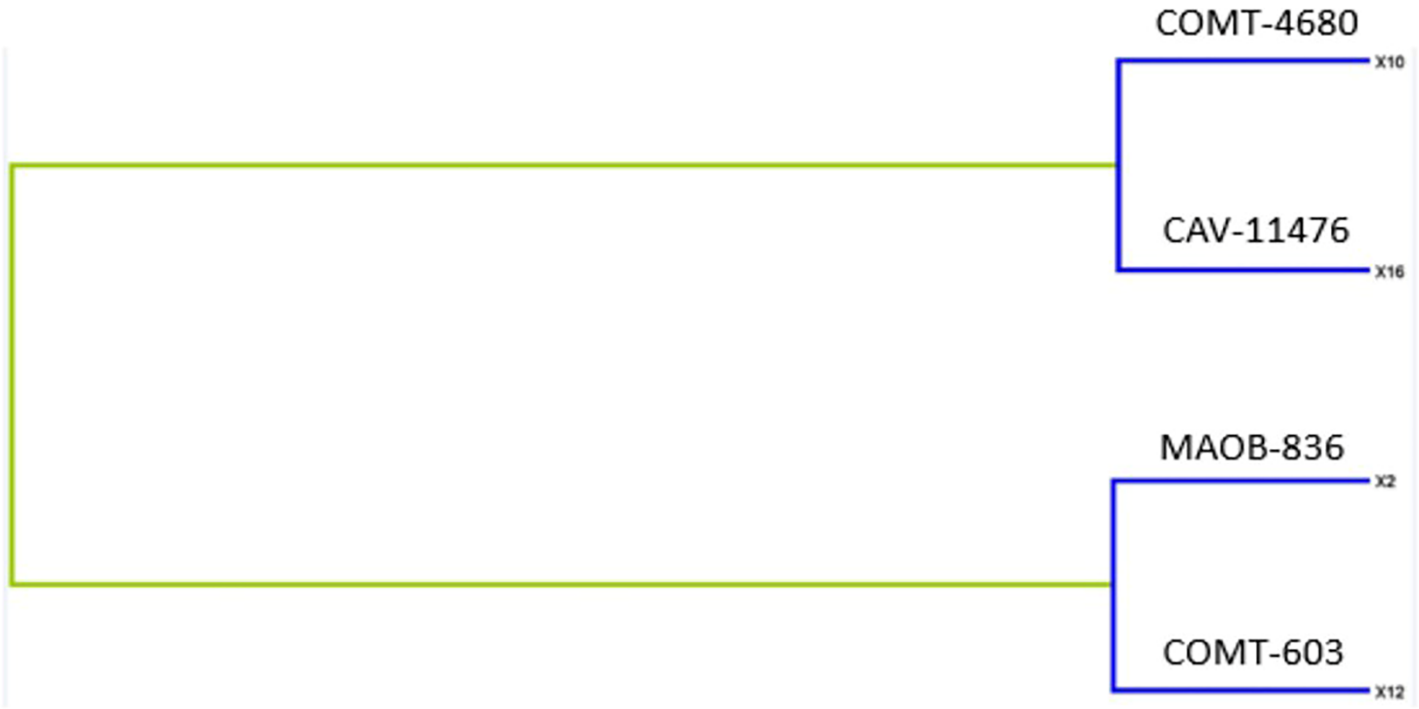

Figure 15. Multifactor dimensionality reduction analysis for gene-gene interactions. Red depicts a synergistic relationship (epistasis). Blue depicts a redundancy, or correlation.

Within-Inmate Analysis

Survey data collect from each individual was used to determine if there were associations between genotype and behavior. The following behaviors were used in the analysis: aggressive violence, serious antisocial behavior, low self-control, prior violent crime rate, prior property crime rate, and prior drug crime rate. Regression analysis revealed no significant associations after Bonferroni correction ($p=0.05/18=0.0028$) was applied.

Discussion

This study evaluated genetic variants in a control versus inmate population. Two markers associated with MAO (rs1799836 and rs909525) showed significant differences in major allele frequency. The A allele was observed more often in inmates than controls for rs1799836. This rs1799836 polymorphism has previously been associated with the efficiency of the MAOB intron 13 removal. A change from the G to A allele at this marker may cause higher protein expression and increased MAOB enzyme activity (Jakubauskiene *et al.*, 2012). Furthermore, the A allele was also found to predict putaminal dopamine turnover, causing increased dopamine turnover in early Parkinson's disease (Lohle *et al.*, 2017). The G allele was observed more often in inmates for marker rs909525 in this study. This marker has been more extensively studied in suicidal behavior. The A allele was associated with suicidality and higher reward dependence in suicide attempters (Antypa *et al.*, 2013b; Balestri *et al.*, 2017). Other studies have associated this polymorphism with anger and aggression. Antypa (2014a) found that males homozygous for the G allele scored higher in measures of expressing anger outward. Another study found carriers of this allele to be more aggressive (Chen *et al.*, 2015). The results of the present study differ

from a previous study performed by Grigorenko (2010) on Russian incarcerated adolescents. They analyzed twelve SNPs associated with DA turnover, and no significant differences in single genetic variants were observed between the investigated groups (Grigorenko *et al.,* 2010). No significant variation was observed in this study between controls and inmates for COMT, DBH, OXT, and OXTR. The focus of COMT research has been on the valine to methionine substitution in codon 158. The Met158 allele has been linked to increased aggression and antisocial behavior in schizophrenics (Pavlov *et al.*, 2012) as well as a predisposition for future aggression in children whose mother smoked while pregnant (Brennan *et al.,* 2001). Multiple SNPs involving DBH have been studied with limited sample sizes, and varying levels of association with ADHD have been discussed. However, in one large study, a C allele at rs129882 has been linked to an increased risk for ADHD (Tong *et al.*, 2015). Additionally, the TT genotype at rs1611115 (1021TT) has been shown to increase the risk of heroin abuse (Xie *et al.,* 2013). SNPs involved with oxytocin have been associated with callous unemotional traits, neuroticism, human pair-bonding, and empathy (Gimpl *et al.*, 2001; Beitchman *et al.*, 2012; Walum *et al.*, 2012; Wu *et al.*, 2012). Furthermore, Johansson (2012) found that some OXTR SNPs showed significant associations for the interactive effects between SNPs and alcohol on aggressive behavior.

Haplotype analysis revealed high LD between MAOA markers rs3788862 and rs909525. Furthermore, the frequencies of two haplotypes (GAT and GGT) were significantly different between inmate and control samples ($p=0.0012$ and 0.000036 respectively). More specifically, the haplotype GGT was not observed in the inmate population. Therefore, the absence of this haplotype may be related to delinquency. These

findings are different than previously reported by Grigorenko (2010). Multifactor dimensionality reduction was used to test for gene-gene interactions. Although no epistasis was observed with MDR, two strong redundancies were found: (1) rs4680 (COMT) and rs11476 (CAV) and (2) rs1799836 (MAOB) and rs740603 (COMT). This indicates that COMT and CAV, and COMT and MAOB may together influence the levels of neurotransmitters in the brain.

Behavioral studies in mice and humans have affirmed that oxytocin and dopamine are key components in behavior. For example, normal female mice can determine which males are infected using social cues. When male mice are infected, the females are aversive to those odors. Kavaliers (2003) found that when the oxytocin gene was deleted, female mice were unable to discriminate between the infected and uninfected males. These findings indicate the oxytocin plays a factor in social odor discrimination. The influence of oxytocin on emotion in humans has also been explored. Subjects that underwent the administration of intranasal oxytocin showed an increase in the ability to recognize fear, indicating oxytocin influences fear recognition (Fisher-Softy *et al*, 2010). Furthermore, in transgenic mice, the MAOA deficiency resulted in lower thresholds for aggression regardless of the aggressiveness of the intruder as well as an increase in overall aggression (Vishnivetskaya *et al.*, 2007).

High pressure liquid chromatography (HPLC) methods have also been used to measure neurotransmitters. Qi (2009) used HPLC to measure dopamine and two of its metabolites after oxytocin and methamphetamine had been administered intracerebroventricularly. By increasing oxytocin, there was an increase in the levels of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic

acid (HVA) in the prefrontal cortex. In contrast, increasing levels of oxytocin decreased serotonin's metabolite 5-hydroxyindolacetic acid but showed little effect on serotonin itself (Qi *et al.,* 2009).

One SNP associated with serotonin was used in this study. The OXTR is expressed in the serotonergic raphe nuclei in the brain, suggesting there is a mechanism in which they both influence behavior together. OXTR knockout male mice showed less aggression suggesting oxytocin plays a role in aggression. In female mice, there was no change in aggression (Pagani *et al.,* 2015). This suggests that in females there may be a compensatory mechanism with serotonin when the oxytocin receptor is absent in that region of the brain. Furthermore, it indicates that serotonin and oxytocin together influence behavior.

Although no significance was found between genotype and behaviors tested in the survey, it does not indicate these polymorphisms have no influence on behavior. Previous studies suggest that gene-environment interaction is responsible for increased risk for criminal behavior. Wells (2017) found that proximal life stress in MAOA-L allele carriers (who have experienced distal stress) has been associated with an increase in delinquency and crime. This allele, when coupled with parental criminality, also showed an increase in self-reports of criminal behavior and rates of violent and property arrests. However, it was found that the interaction of MAOA-L with abuse, predicted less serious delinquent and criminal behavior (Armstrong *et al.*, 2014). Furthermore, MAOA-L carriers exposed to early stress were more sensitive to the effects of later stress on self-control (Boisvert *et al.,* 2017). Armstrong (2017) also found that 5-HTTLPR genotype was not directly associated with violent crime and property offense arrests; however, heart rate and genotype together influenced violent arrest rates.

There were some limitations in this study. Limited access to inmate DNA resulted in a small sample consisting of males only. Each sample set was also a mixture of African Americans, Hispanics, Caucasians, and other. Although inmate and control samples were proportionally matched, further studies may focus on each ethnic group individually. Inmate samples consisted of violent offenders, drug offenders, property offenders, and other. Future studies may be performed with a wider or more specific range of offenses. Studies involving gene-environment interactions may also provide more information on the influence of these polymorphisms and behavior.

Antisocial and aggressive behavior have become a major problem as the United States currently has the largest incarceration rate in the world (2,162,400 people were incarcerated in 2016; Bureau of Justice Statistics). The strong heritability of criminal activity in addition to environmental influences and gene-gene interaction indicates that a genetic underlying can help explain aggressive and antisocial behavior. This current study builds on limited data from inmate samples. The results of this study provide evidence that genetic variation occurs between inmate and control DNA.

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CHAPTER III

Frontal Cortex Thickness and Surface Area Differences in Psychiatric Patients with Generalized Anxiety Disorder

Abstract

Anxiety disorders, specifically Generalized Anxiety Disorder (GAD) are highly prevalent in the United States. As a result, there has been a major interest in understanding the underlying mechanisms involved. Imaging techniques, such as functional Magnetic Resonance Imaging (fMRI) have allowed for major progress to be made in determining the pathology and etiology of GAD. Previous structural studies have focused on volume changes in the brain. In this study, thickness and surface area differences were assessed for eleven bilateral frontal regions defined in the Desikan-Kiliany Atlas. A total of 300 participants were included in this study within three groups: GAD patients (N=100), psychiatric controls (PC; N=100), and healthy controls (HC; N=100). Groups were matched for demographic characteristics and other psychiatric conditions. No significant differences were observed for surface area in the left or right hemisphere; however, significant differences were found for thickness in both hemispheres. In the left hemisphere, lower thickness was observed in GAD patients verses healthy controls ($p=0.0001$) for the pars triangularis and superior frontal region ($p=0.0000$). Also, significantly lower thickness was observed in psychiatric controls compared to healthy controls ($p=0.0000$) for the superior frontal region. In the right hemisphere, lower thickness was observed in GAD patients versus healthy controls ($p=0.0006$) for the caudal middle frontal region and superior frontal region in GAD ($p=0.0000$). These findings provide evidence that these structures may be involved in GAD. Furthermore, they also suggest

GAD may be due to damage from chronic stress as it suppresses neurogenesis, dendritic growth, and synaptic strength.

Keywords

Generalized Anxiety Disorder, Anxiety, Psychiatric patients, Surface area, Thickness, Pars triangularis, Superior frontal region, Caudal middle frontal region

Introduction

Anxiety disorders have a major impact on the population. Among these disorders, Generalized Anxiety Disorder (GAD) is one of the most prevalent. As a result, there has been increased interest in understanding the structures and mechanisms involved in the development of GAD. In recent years, there has been significant progress in determining the genetic and environmental influences. Furthermore, imaging techniques have also allowed for differences in brain structure and function to be observed in patients with GAD.

Anxiety disorders are the most common type of psychiatric disorder (Kjernisted & Bleau, 2004). Anxiety refers to excessive fear or worry in response to a stimulus (Tian, et al., 2016). Occasional anxiety is a normal experience; however, anxiety disorders involve persistent anxiety and may worsen over time. Often the individual's everyday life is hindered as a result. Anxiety can generally be described in two major categories: acute (state anxiety) and chronic (trait anxiety) (Gross & Hen, 2004). Although the two are related, they differ in psychological measures and how they influence the cognitive process (Stein, 2009). Anxiety is broken down into six categories: panic disorder, post-traumatic stress disorder, social phobia, specific phobia, obsessive-compulsive disorder, and generalized anxiety (DSM) (Stein, 2009). Over thirty percent of adults and adolescents in

the United States experience an anxiety disorder during their lifetime (National Institute of Mental Health, 2017).

GAD is a relatively newer diagnosis, first appearing as a distinct category in the DSM-III. It is now one of the most common psychiatric disorders encountered by primary care specialists. For example, in the United States, approximately 9 million people are diagnosed during their lifetime (Jetty, Charney, & Goddard, 2001). GAD is more common in females, with prevalence highest in midlife (Bandelow & Michaelis, 2015). The main feature of GAD is excessive or unreasonable worry (Stein, 2009). There are six main criteria required for an individual to be diagnosed with GAD. In summary, the heightened anxiety must occur most days for over six months and it must be difficult to control the worry. These feelings also are associated with at least three of the following symptoms: restlessness, quick to fatigue, difficulty concentration, irritability, tension of muscles, and sleep problems. In order to be diagnosed with GAD, the symptoms must cause significant distress in daily life. Lastly, the feelings must not be explained by other mental disorders or attributed by substance use or medical condition (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition, 2013).

Currently, there is some controversy over the diagnostic criteria of GAD. In the DSM-III, symptoms were only required for one month; whereas, they are required for six months in the current diagnostic criteria. Kessler (2005) found that subthreshold cases (meeting all the criteria except for duration of symptoms) are still very similar in threshold criteria. These threshold criteria include duration, impairment, age of onset, comorbidity, parental GAD, and sociodemographic traits (Kessler, et al., 2005).

Comorbidity

Anxiety disorders have high comorbidity rates with other psychiatric disorders. For example, anxiety and major depression are highly comorbid. More than fifty percent of patients that visit a physician during a depressive or anxiety episode have a second comorbid anxiety or depressive disorder (Hirshfeld, 2001). Furthermore, physical symptoms, depressive symptoms, and functional impairments were also found to be additive in individuals with anxiety and depression (De Waal, Arnold, Eekhof, & van Hemert, 2004). Substance abuse disorder is also frequently found in patients with GAD. Grant (2004) found a significant and positive association between substance use disorders and mood or anxiety disorders. Another study found a strong genetic correlation between GAD and neuroticism (Hettema, Prescott, & Kendler, 2004). GAD alone significantly impacts the life of an individual, comorbidity with other psychiatric disorders can further hinder recovery and promote reoccurrence.

GAD Treatments

Several pharmacological treatment options are available to patients with GAD. Generally, initial recommended treatment is a selective serotonin reuptake inhibitor (SSRI) (Baldwin, Waldman, & Allgulander, 2011; Bandelow, Zohar, Hollander, Kasper, & Möller, 2008). All SSRIs have a similar mechanism of action: increase 5-HT via inhibition of its uptake pump (Vaswani, Linda, & Ramesh, 2003). Although all SSRIs share a therapeutic mechanism, the side effects and efficacy of drug differs. Biological substrates and pathways involved with serotonin may contribute to drug efficacy and negative effects. Furthermore, this may be the result of metabolism differences (poor metabolizers versus rapid metabolizers) within cytochrome P450 enzymes. Overall, meta-analyses revealed

fluoxetine (Prozac) had the greatest response rate and remission; whereas, sertraline (Zoloft) was best for tolerability (Baldwin, Woods, Lawson, & Taylor, 2011).

Benzodiazepines are sometimes prescribed for GAD and have a much more rapid onset of action compared to SSRIs. Most benzodiazepines, including diazepam (Valium), clonazepam (Klonopin), alprazolam (Xanax), and lorazepam (Ativan) have been successful in treating GAD (Shader & Greenblatt, 1993). Benzodiazepines are gamma-aminobutyric acid-A (GABA-A) agonists. Moreover, they induce conformational changes to enhance the affinity for GABA binding (Longo & Johnson, 2000). Benzodiazepines are recommended for immediate and short-term use. Although they are effective anxiolytics, they also cause sedation, dizziness, and other CNS depressant effects. Long-term treatment can have negative implications on the health of an individual. For example, long-term benzodiazepine treatment is known to cause cognitive impairment and can also decrease the efficacy of GABA-A receptors, similar to that in alcoholism (Stewart, 2005; Longo & Johnson, 2000). Benzodiazepines also have a high risk for abuse potential and severe withdrawal effects (Vgontzas, Kales, & Bixler, 1995).

Pregabalin (PGB) is also used for treatment of GAD. It is considered an anticonvulsant and is a derivative of the neurotransmitter GABA (Sills, 2006). PGB is both effective and rapid for GAD. Furthermore, it does not have major withdrawal symptoms similar to that of benzodiazepines (Pande et al., 2003). Buspirone is another common drug prescribed for GAD. It is a partial agonist for the serotonin 1A receptor (5-HT1A). Furthermore, it is thought to be an antagonist for dopamine 2 (D2) autoreceptors with weak affinity to serotonin 2A receptors (5-HT2A). However, the exact mechanism of action for

buspirone is still unknown. Buspirone has been found to combat GAD with similar effectiveness to benzodiazepines, with less withdrawal symptoms (Loane & Politis, 2012).

Genetic and Environmental Influences

Several studies have been performed to better understand the etiology and pathology associated with GAD. Some research suggests that genetic and environmental factors play a role in the development. Compared to other anxiety disorders, GAD is less influenced by genetics (Martin, Ressler, Binder, & Nemeroff, 2009). However, one study suggested that GAD may be associated with polymorphisms located on three genes: prostaglandin D2 synthase (PTGDS), dynein light chain 2, cytoplasmic (DYNLL2), and erythrocyte membrane protein band 4.1 4A (EPB41L4A) (Donner, et al., 2008). Other studies suggest genes involved with DA and 5-HT may influence the development of GAD. For, example a variant within the MAOA gene has been associated with GAD compared to panic disorder and depression (Tadic, et al., 2003). Although these limited number of studies suggest a few candidate genes specific to GAD, most studies have been unsuccessfully replicated (Martin, Ressler, Binder, & Nemeroff, 2009). Instead, it is more likely that many genes together influence GAD (Moffitt, et al., 2007).

Family studies have also been used to better understand the underlying genetics. Ninan (2001) found no familial association specific to GAD, but instead found greater instances of mood and anxiety disorders in first-degree relatives of individuals with GAD (Ninan, 2001). Another biometrical twin modeling study found minimal familial aggregation for GAD. Furthermore, it was concluded that there was no sex specific genetic influence (Hettema, Prescott, & Kendler, 2001). Twin studies have shown that approximately thirty percent of GAD is influenced by additive genetics (multiple genes)

and the rest is explained by environment of the individual, not the shared environment (Hettema, Neale, & Kendler, 2001; Tambs, Czajkowsky, & Roysamb, 2009).

The role of environment in the development of GAD has also been explored. For example, friendship difficulty as a child has been associated with GAD (Degan, Almas, & Fox, 2010). GAD has also been linked to several childhood risk factors including maltreatment, inhibited temperament, and low socioeconomic status. Furthermore, internalizing problems, conduct problems, and high negative emotionality are possible contributors to the development of GAD (Moffitt, et al., 2007). An individual's interpretation of their experiences may also contribute to GAD. Ruscio (2004) performed a study with high worry individuals with and without GAD. It was found that GAD worriers had less control over negative thoughts, greater hyperarousal, and favored negative beliefs about worry. As a result, they proposed GAD is different compared to other forms of worry and associated with unique experiences and appraisals (Ruscio & Borkovec, 2004).

Imaging Techniques

Imaging techniques have also allowed for great progress to be made in understanding what happens in the brain for individuals with GAD. These techniques include computed tomography (CT), positron emission tomography (PET), diffusion tensor imaging (DTI), and magnetic resonance imagining (MRI).

CT makes use of x-ray technology. Rather than using a fixed x-ray beam, the beam rotates around the patient. As the x-ray beam leaves the patient, the signal is picked up by a detector directly across. This technique produces cross-sectional images (slices) that provide more information than a fixed x-ray. Each image slice can be viewed individually

or can be stacked on the computer to create a three-dimensional image. Dense structures can easily be viewed with CT; however, contrast agents may be needed to observe abnormalities in soft tissues (National Institute of Biomedical Imaging and Bioengineering). PET scans measure function rather than structure. With this technique, a radioactive tracer is injected into the patient. Typically, PET scans are used to measure glucose consumption. However, they also can be used to measure oxygen consumption and blood flow (Berger, 2003). DTI is used to indirectly measure neural circuits via movement of water. Tissue microstructure can impede the diffusion of water. Since DTI is sensitive to water diffusion within tissue, it can measure tissue microstructure changes (Basser, Mattiello, & LeBihan, 1994). Moreover, DTI can be used to assess white matter integrity (Banz, Yip, Yau, & Potenza, 2016).

MRI can also be used to measure structural differences within the body. This technique is preferred because it does not use damaging radiation. Instead, MRI machines use strong magnets. With each radiofrequency pulse, the nuclei of spinning hydrogen atoms in the body align to the magnetic field. When the radiofrequency pulse ends, the nuclei flip back to the original position. The sensor measures the energy released and the time it takes for realignment. Since different tissues within the body have different amounts of water (and hydrogen atoms), different amounts of energy are emitted. The computer analyzes the signal and produces pictures (Berger, 2002).

Functional magnetic resonance imaging (fMRI) has played a major role in understanding neural mechanisms because it makes use of functional imaging rather than static neuroanatomy. fMRI measures activity via blood flow. More specifically, it works by measuring blood oxygen level dependent (BOLD) change. When hemoglobin is

oxygenated it is diamagnetic; whereas, it is paramagnetic when deoxygenated. This difference in magnetic property will produce small differences in the magnetic resonance signal of the blood. Since blood oxygenation changes with activity level, brain activity can be measured (Chen & Glover, 2015). This type of imaging allows for observation of structural differences, altered functional connectivity, and fiber tract identification within the brain.

Anatomy and Physiology Important in Stress Response

In order to understand regions of interest in most research involving GAD, it is important to know some of the main anatomical structures involved in the processing of a stressful event. In response to a stimulus, the central nucleus of the amygdala is activated. The amygdala triggers the cascade of responses that prepare the body to react to a situation (Wilson, 2016). Acute stress causes a release of hormones / neurotransmitters that in turn affect the memory process (Roozendaal, 2009). More specifically, this activation stimulates the hypothalamic pituitary adrenal (HPA) axis. Many of the acute and chronic responses of stress are the result of the central nucleus outputs to several brain regions. For example, output to the parabrachial nucleus increases respiration and output to the dorsal motor nucleus of vagus can cause ulcers (Davis, 1992).

After a threat is identified, the hippocampus helps compare the situation to what the brain already knows about safety and danger. The PFC is accessed after the amygdala and hippocampus have performed their duties. The PFC is important in logic, planning, and attention. When the threat has passed, it puts together data points into a coherent narrative (Wilson, 2016). Each of these areas in the brain are crucial in the reaction to a stressor, formation of memory, and learned behavior.

When an individual encounters a threatening stimulus, several physiological responses occur. These situations typically require a lot of energy; therefore, the autonomic and endocrine responses are catabolic (break down molecules to release energy). The main systems involved are the sympathetic adrenal-medullary (SAM) system and the HPA axis. Catecholamine stress hormones are released under the SAM system. During a stressful situation, the hypothalamus and sympathetic nervous system (SNS) stimulate the adrenal medulla, releasing epinephrine and norepinephrine. These in turn activate the SNS. Epinephrine affects the metabolism of glucose and causes stored nutrients to become available. Norepinephrine increases blood flow to the muscles as the result of increased cardiac output (Carlson, 2012; McCarty, Horwatt, & Konarska, 1998; McCarty, 1985; Berne, 1958).

Another stress hormone is cortisol which is excreted by the adrenal cortex. It is a considered a glucocorticoid because of its effect on glucose metabolism. Furthermore, it helps convert proteins and fats into usable energy and helps stimulate blood flow. Release of glucocorticoids is controlled by the activity of the HPA axis. This secretion is controlled by the neurons of the paraventricular nucleus of the hypothalamus (PVN). PVN neurons secrete the peptide corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH). The entrance of ACTH into general circulation stimulates the adrenal cortex to secrete glucocorticoids (Carlson, 2012).

Changes in Behavior / Long-Term Potentiation

Simply stated, learning is a relatively permanent change in behavior or mental process due to experience (from a cellular perspective as well). Advances in research

techniques have shown that synaptic changes accompany forms of learning. This long-term potentiation (LTP) includes N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (glutamatergic), and pre- and postsynaptic changes, especially in hippocampal cells (Shi et al., 1999; Makino & Malinow, 2009).

The concept of LTP was first discovered by Bliss & Lomo (1973) after performing high frequency electrical stimulations in brains of rabbits. Specifically, their focus was on stimulation of the perforant path. Most input to the hippocampus comes from the entorhinal cortex. Axons of neurons in the entorhinal cortex pass through the perforant path and form synapses with the granule cells of the dentate gyrus. The cells extend axons along the mossy fiber tract. Within the hippocampus there are pyramidal cells of regions CA3 and CA1. Schaffer collaterals project from the CA3 to CA1 area (Bliss & Lomo, 1973; Berger, 1984). Bliss & Lomo (1973) found that high frequency stimulation of the perforant path led to increased excitability of neurons downstream (CA3, CA1 field). They also observed the effects of repeated stimulation. Re-stimulation (with the same amount of frequency) showed even higher excitability. Furthermore, even with decreased re-stimulation, higher excitability was observed downstream. They also found that the increase in excitatory postsynaptic potential (EPSP) was relatively long-term (up to months at a time) (Bliss & Lomo, 1973). Therefore, their results provided evidence for what has been deemed LTP.

Early LTP is local, consisting of functional/structural changes at the synapse. It is also short-term; rapidly decaying and independent of protein synthesis. Upon the onset of an action potential, glutamate is released. Glutamate binds to postsynaptic receptors AMPA and NMDA. When glutamate binds to the AMPA receptor, the ion channel opens

and there is an influx of sodium ions (Na+). This Na+ influx results in local depolarization, or EPSP. This depolarization causes magnesium ions (Mg++) to be kicked out of the NMDA receptor. Then Na+ and calcium ions (Ca++) (glutamate binding still required) can flow through the ion channel of NMDA. This influx of positive ions creates strong depolarization; therefore, an action potential is created. Ca++ influx activates the enzyme type II calcium-calmodulin kinase (CaM-KII) which flips the other AMPA receptors currently not facing the synapse (aka externalization of the AMPA receptors). However, more glutamate is still required. CaM-KII also activates the enzyme nitric oxide synthase (NOS) which synthesizes nitric oxide (NO). NO is considered a retrograde messenger. The gas travels back to the pre-synapse triggering more glutamate release. The glutamate can now also bind to the new receptors (flipped receptors) available (Lynch et al., 1984; Shi et al., 1999; Silva et al., 1992; Shen & Meyer, 1999; Lledo et al., 1995; Endoh et al., 1994; Carlson, 2012).

Late LTP is global and requires cell nucleus activity as well as genetic transcription and translation. It is also longer term; more durable and requires protein synthesis. During late LTP, CaM-KII activates cAMP which activates CREB (transcription factor). This transcription factor binds to the promotor region of a DNA strand, resulting in genetic transcription and translation of the protein brain derived neurotropic factor (BDNF). When BDNF is released, the tyrosine kinase B (TrKB) receptors are activated. This receptor activation then activates mammalian target of rapamycin (mTOR). mTOR stimulates many transcription factors responsible for cytoskeletal reorganization and the growth of new dendrites and terminal buttons (synaptogenesis) (Frey et al., 1988;

Soderling, 2000; Barco, Alarcon, & Kandel, 2002; Carlson, 2012; Bekinschtein et al., 2007).

Some important proteins, enzymes, and other roles of CaM-KII are also significant in LTP. Typically, Pin1 inhibits the translation of mRNA to the protein PKM-zeta. CaM-KII will block Pin1 and allow for translation to take place (mRNA to PKM-zeta protein). PKM-zeta is responsible for the activation of the NSF enzyme. In early LTP, NSF plays a role in movement and expression of AMPA receptors. PKM-zeta also inhibits Pin1. As a result, even more PKM-zeta is produced. This causes the AMPA receptors to remain expressed; therefore, it is important in late LTP (Migues et al., 2010; Xia & Storm, 2005; Sacktor, 2011).

Brain Structural Differences in GAD

Previous research has focused on volume differences in GAD. These include volume of brain structures, regions within structures, and gray and white matter. Several studies found volume differences for the amygdala and regions of the prefrontal cortex. Schienle (2011) found that individuals with GAD had larger amygdala and dorsomedial prefrontal cortex (dmPFC) volumes. Furthermore, gray matter volumes were different in regions associated with regulation of emotion and anticipation of anxiety (Schienle, Ebner, & Schafer, 2011). Milham (2005) found that gray matter volume was reduced in the left amygdala in patients with anxiety disorders. Hilbert (2015) observed lower volumes of white matter in the dorsolateral prefrontal cortex (dlPFC) in GAD patients and Andreescu (2017) found gray matter differences in the OFC associated with higher worry severity. These findings suggest differences in structures involved in the processing of a stressful stimuli may attribute to the development of GAD. Changes associated with the amygdala

are expected, as it is responsible for the cascading effects in response to stress. The dlPFC seems to be a distinct region of the prefrontal cortex important in GAD. The dlPFC is known for its role in executive functions including selective attention and working memory. This region supports the response to sensory information. Moreover, it may indirectly influence emotional reactivity with alterations to perceptual attention systems (Corbetta & Shulman, 2002; Ochsner, Silvers, & Buhle, 2012).

Other studies observed some structural differences outside the amygdala and frontal regions. Gray matter differences were observed in the ACC and putamen in GAD patients associated with worry severity (Andreescu et al., 2017). The ACC is involved in cognitive processes including decision making and cost-benefit calculation (Apps, Rushworth, & Chang, 2016). The putamen is involved in higher-level learning, but is also important in stimulus-response and habit (Grahn & Parkinson, 2008). Therefore, alterations in the ACC and putamen may affect correct perception and processing of a threat or stressor. Gray matter volume differences were also found in basal ganglia structures in GAD subjects (Hilbert *et al.*, 2015). Basal ganglia structures are involved in motor control, attention, and cognitive and emotional functions. The basal ganglia receive information from the neocortex and project to the thalamus which project back to the frontal cortex (Graybiel, 2000). Differences in the basal ganglia volume suggest there may be impairment of information circulating back to the prefrontal cortex.

Structural differences in pediatric GAD have also been investigated. De Bellis (2000) found that pediatric patients with GAD had larger total amygdala and right amygdala volumes. Both white and gray matter superior temporal gyrus (STG) volumes were also significantly larger in pediatric GAD patients (De Bellis, 2002). Furthermore,

Milham (2005) observed reductions in left amygdala gray matter volume. These results are similar to those in adults with GAD; however, the STG is also implicated in pediatric findings. The STG is important in the processing of auditory information. It is also associated with the visual analysis of social information via gaze and body movement (Boddaert *et al.*, 2004). These findings suggest pediatrics with GAD may have a hard time reading and understanding social ques, contributing to their anxiety. Furthermore, these results indicate structural differences can already be observed in pediatric GAD patients.

Altered Functional Connectivity in GAD

Differences in functional connectivity have also been of interest to the medical community. Functional connectivity refers to the temporal correlation in the high amplitude (low-frequency) spontaneously generated BOLD signal for different brain regions. More simply put, it refers to the spontaneous BOLD fluctuations (Fox & Raichle, 2007). Functional connectivity tests can be performed while an individual is performing a task or at a resting-state. Resting-state functional connectivity measures BOLD values between different structures of the brain while a patient is at rest (van den Heuvel & Hulshoff Pol, 2010).

Most functional connectivity research confirms that the amygdala and prefrontal cortex are important in GAD. Hilbert (2014) measured functional connectivity in the amygdala and prefrontal cortex. Decreased functional connectivity and abnormal activation was observed for both the amygdala and prefrontal cortex (Hilbert, Lueken, & Beesdo-Baum, 2014). Other studies have more closely identified specific regions of the amygdala and prefrontal cortex that contribute to the development of GAD. Moreover, they suggest structures in the limbic system may also be involved. Makovac (2015) found

lower connectivity between the right amygdala and three right hemisphere regions: superior frontal gyrus, paracingulate/anterior cingulate cortex, and supramarginal gyrus. Fonzo (2016) also found decreased connectivity between right amygdala and right dorsal cingulate and prefrontal cortex. Another study specifically observed the basolateral and centromedial amygdalar subregions. In individuals with GAD, both subregions showed significantly less distinct connectivity (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009). The findings of these functional studies include that same brain structures/regions having structural differences. Furthermore, they provide insight to the increased or decreased connectivity / activation between these structures.

GAD Studies Using Other Techniques

Diffusion tensor imaging (DTI) can also be coupled with fMRI. A few studies have used this technique to determine if white matter abnormalities are present in GAD. Phan (2009) observed lower fractional anisotropy in the right uncinated fasciculus white matter. White matter abnormalities were also observed in adolescents with GAD. Individuals had reduced fractional anisotropy in four areas: inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, bilateral uncinated fasciculus, and corona radiate (Liao, Yang, Zhang, He, & Li, 2014).

Some emotion regulation studies have been performed with GAD patients. Fitzgerald (2017) had GAD patients view negative images and observed over-engagement of the amygdala and frontal regions. In another study, when subjects were processing fearful faces, decreased connectivity in the right anterior insula and dorsal ACC (Klumpp, Angstadt, & Phan, 2012). Other advanced techniques have been used to better understand the neural circuitry involved in GAD. Optogenetics is a relatively newer technique that

overcomes many of the limitations encountered in previous techniques. As the name suggests, it combines optics and genetic manipulation. Tye (2011) found that stimulation of the basolateral terminals within the central nucleus of the amygdala in mice created an acute, reversible anxiolytic effect. In contrast, inhibition of that pathway produced anxiety-related behaviors (Tye, et al., 2011). Another study performed by Ohmura (2014) explored activation of serotonergic neurons in the median raphe nucleus and dorsal raphe nucleus. They discovered activation of the neurons in the median raphe nucleus produced anxiety; whereas, activation in the dorsal raphe nucleus produced no effect on anxiety-like behavior (Ohmura, Tanaka, Tsuematsu, Yamanaka, & Yoshioka, 2014). Another study found evidence that the bed nucleus of the stria terminalis (BNST) helped modulate anxiety. More specifically, certain projections within the oval and anterodorsoal BNST had contrasting effects on anxiety (Kim, et al., 2013). Optogenetic testing in mice has helped scientists better understand the underlying circuitry associated with GAD. It supplements functional connectivity studies by allowing for manipulation of certain pathways (inhibition or activation) to determine specific pathways and regions involved.

Purpose of Study

Although significant progress has been made in the understanding of GAD, the underlying neural circuitry is still not well known. Furthermore, structural studies involving GAD have been limited to volume. The purpose of this study was to use fMRI to observe surface area and thickness differences in patients with GAD. Moreover, to determine if GAD and other psychiatric disorders can be distinguished by these differences. The study focused on the frontal cortical regions of the brain.

Methods

Participants

Psychiatric patients were recruited from the Menninger Clinic in Houston, TX and healthy controls were recruited from the community. Personally identifiable information was removed and all procedures were approved by the Internal Review Board at Baylor College of Medicine.

Study Groups

A total of 300 participants were included in this study within three groups: GAD patients (N=100), psychiatric controls (PC; N=100), and healthy controls (HC; N=100). Groups were matched for demographic characteristics including age, gender, and race (Table 10). Furthermore, patients were also matched for other psychiatric conditions.

Table 10

Demographic characteristics for each group of patients.

	GAD Group	**PC Group**	**HC Group**
N	100	100	100
Age (years), mean (SD)	29.3 (9.9)	30.3 (8.4)	31.4 (11.6)
Gender, n male (%)	88	88	86
Race, n Caucasian (%)	58	57	52

Imaging

Images were collected at the Core for Advanced MR Imaging at Baylor College of Medicine. A 3 T Siemens Trio MR scanner was used to capture the high-resolution structural T1 MRI data with the following parameters: 4.5 min structural MPRAGE sequence (TE = 2.66 ms, TR = 1200 ms, flip angle = 12°, 256 x 256 matrix, 160 one mm axial slices at 1 x 1 x 1 mm voxels).

Volumetric Parcellation

Freesurfer v. 5.3 was used to perform automated volumetric parcellation using T1-weighted structural images (surfer.nmr.mgh.harvard.edu). Eleven bilateral regions defined in the Desikan-Kiliany Atlas were chosen as regions of interest: pre-central, superior frontal, caudal middle frontal, rostral middle frontal, pars orbitalis, pars triangularis, pars opercularis, paracentral, frontal pole, lateral orbitofrontal, and medial orbitofrontal (Desikan et al., 2006).

Statistics

Groups were matched for demographic and clinical characteristics. Differences were assessed using a t-test. T-tests were also used to determine significant structural differences between groups (GAD vs. HC, GAD vs. PC, and PC vs. HC). A Holm-Bonferroni method was used to correct for multiple comparisons assuming 22 regions (0.05/23 = 0.002).

Results

Surface area and thickness differences were recorded in all patients. No significant differences were observed for surface area in the left or right hemisphere (Figures 14 and 15). In contrast, significant differences were observed for thickness in both hemispheres. In the left hemisphere, lower thickness was observed in GAD patients verses healthy controls ($p=0.0001$) for the pars triangularis. Also, significantly lower thickness was observed in the superior frontal region in GAD patients compared to healthy controls ($p=0.0000$) and psychiatric controls compared to healthy controls ($p=0.0000$) (Figure 16).

In the right hemisphere, lower thickness was observed in GAD patients versus healthy controls ($p=0.0006$) for the caudal middle frontal region. Significantly lower

thickness was also observed in the superior frontal region in GAD patients compared to healthy controls (p=0.0000) (Figure 17). However, these differences were not observed compared to psychiatric controls.

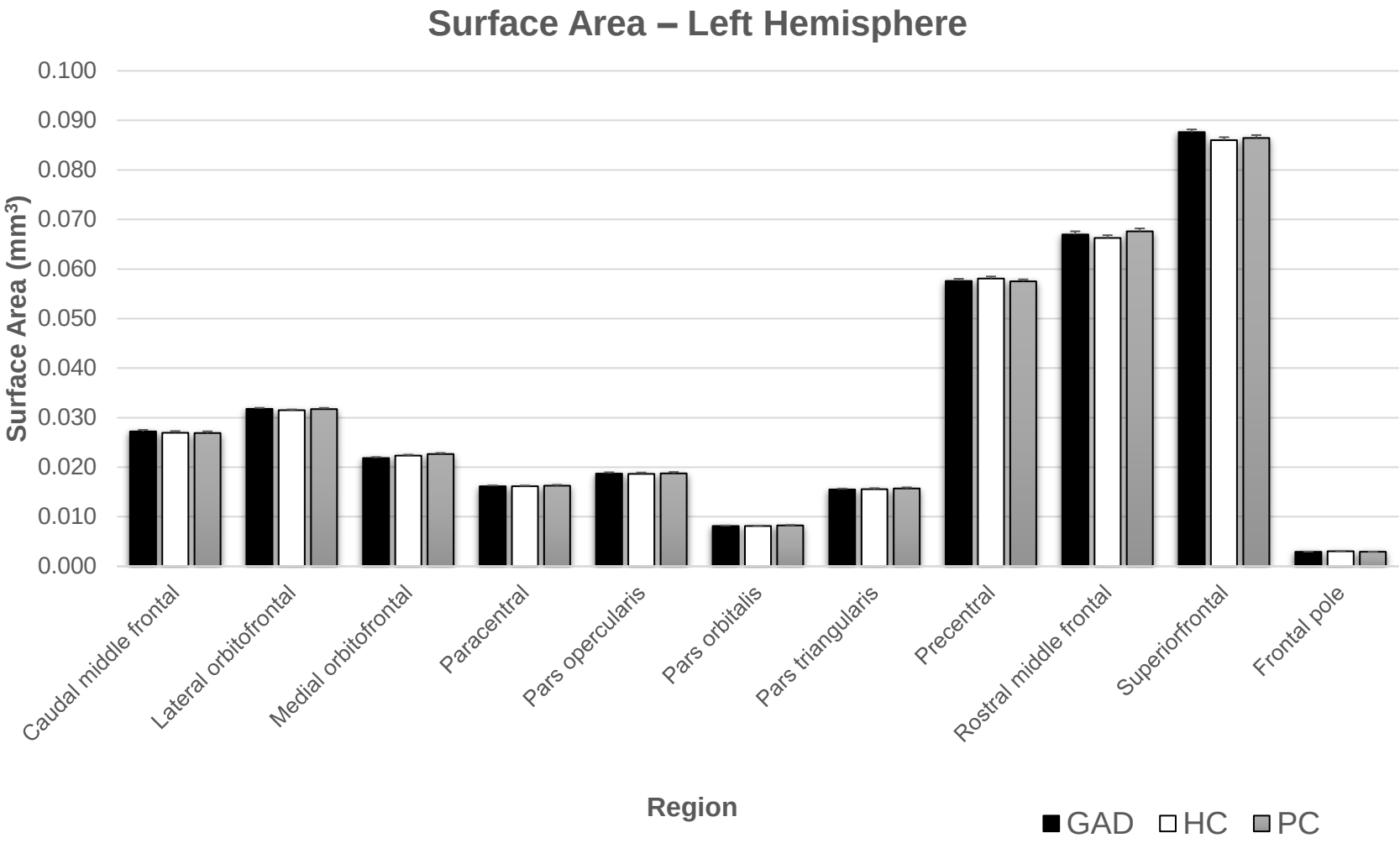

Figure 14. Comparison of surface area differences observed in the left hemisphere.

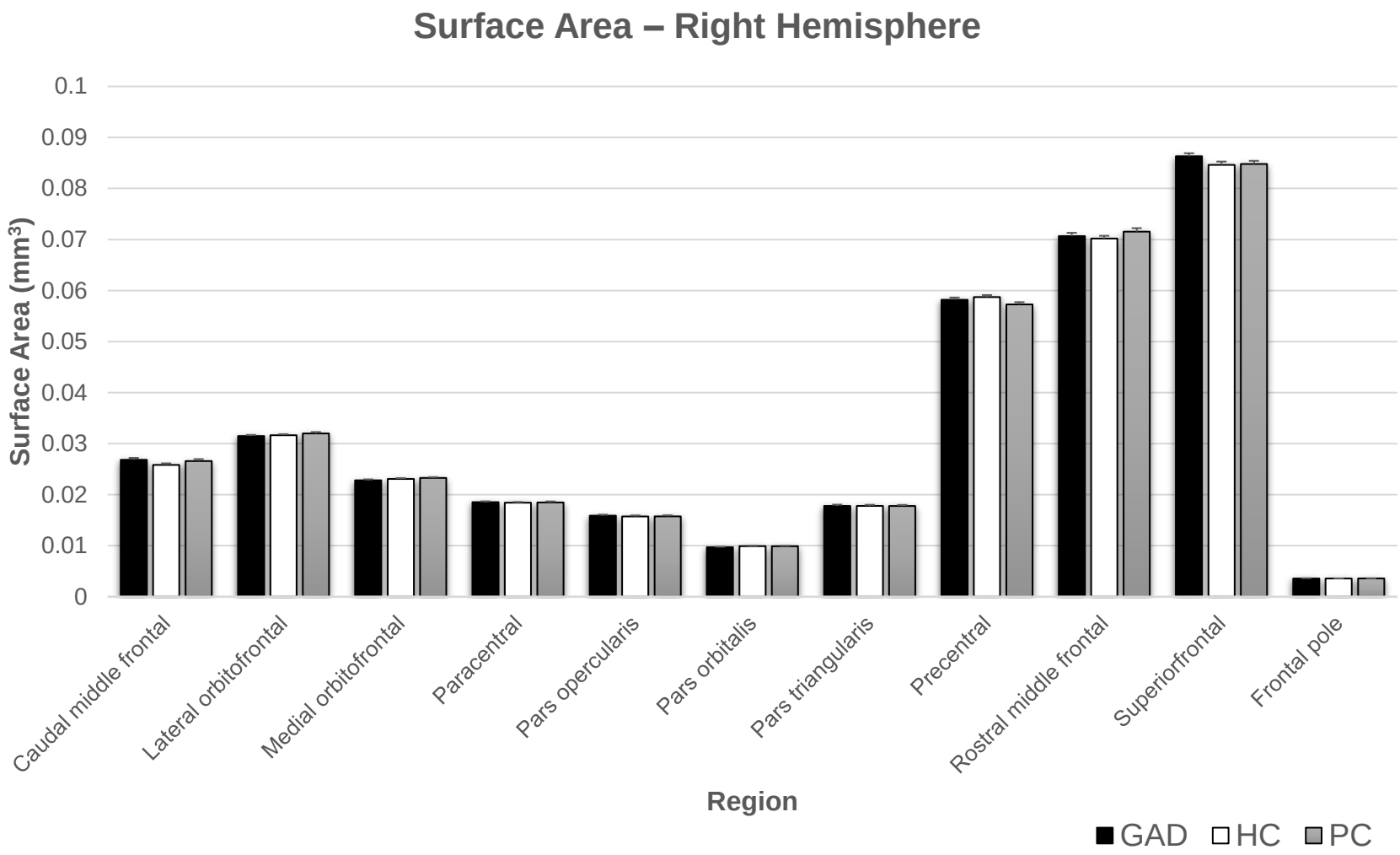

Figure 15. Comparison of surface area differences observed in the right hemisphere

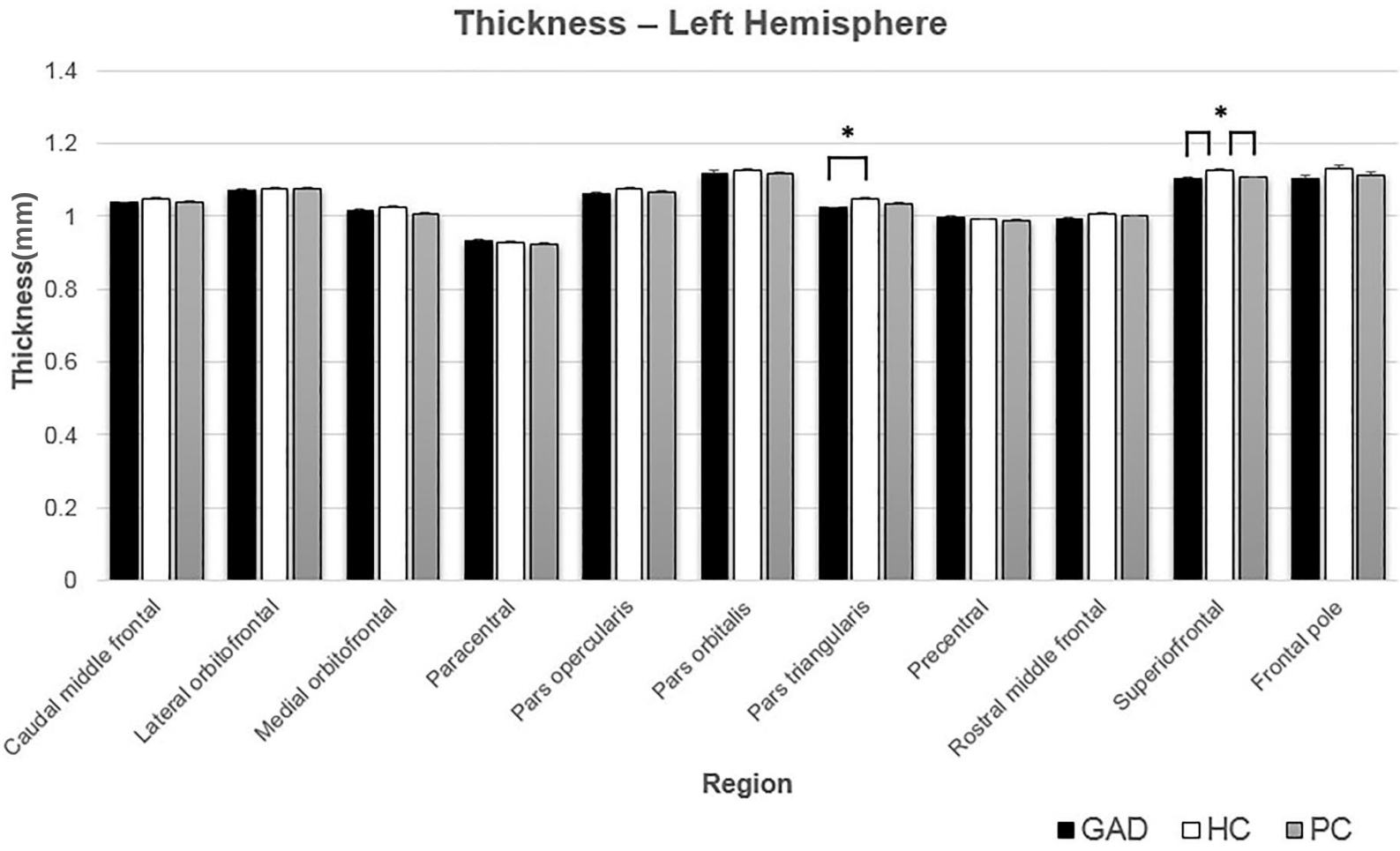

Figure 16. Comparison of thickness differences observed in the left hemisphere. *indicates significance Holm-Bonferroni correction for multiple comparisons (0.05/23 = 0.002)

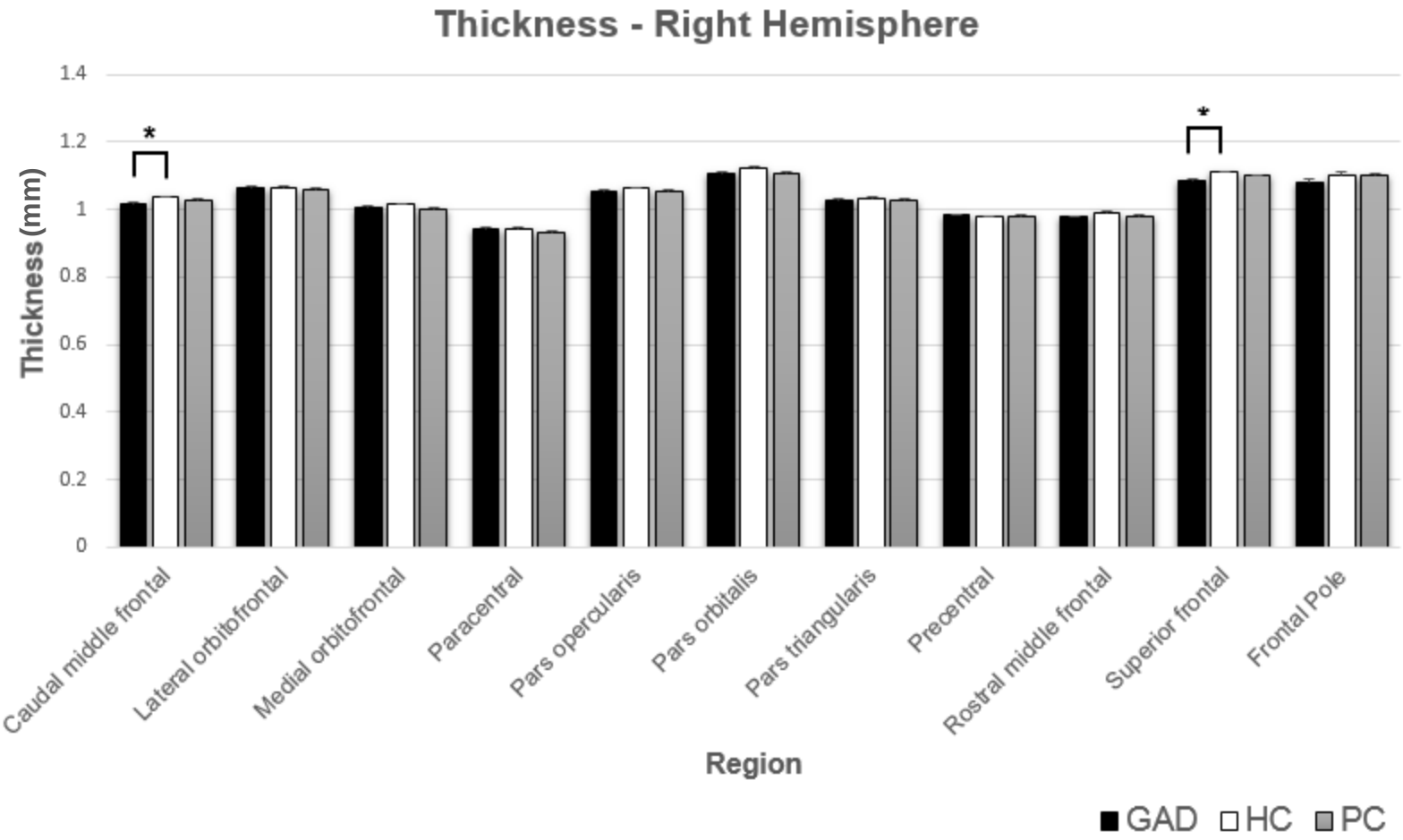

Figure 17. Comparison of thickness differences observed in the right hemisphere. *indicates significance Holm-Bonferroni correction for multiple comparisons (0.05/23 = 0.002).

Discussion

The results of this study indicate thickness differences can be observed in patients with GAD. Most previous research surrounding anxiety suggests there is a disconnect in emotion regulation, or response to stressful stimuli, in limbic structures and PFC. The findings in this study provide insight to the specific PFC regions implicated in GAD that are involved in the regulation of worry. Three regions displayed significant differences within the brain: pars triangularis, caudal middle frontal, and superior frontal.

Low cortical thickness in the pars triangularis (left hemisphere) was observed in GAD patients. The pars triangularis is located in Broca's area and part of the inferior frontal gyrus (IFG). It is especially important in semantic processing, comprehension of speech, and working memory. Moreover, it plays a role in judgement and decision-making (Newman *et al.,* 2003; Rodd, Davis, & Johnsrude, 2005; Rogalsky, Matchin, & Hickok, 2008; Gonzalez, Dana, Koshino, & Just, 2005). Reduced thickness in this area has previously been associated with panic disorder, behavioral problems in children, and anxious symptoms in depression (Kang, Lee & Lee, 2017; Dabbs *et al.,* 2013; Zhao *et al.,* 2017).

Reduced thickness in the right hemisphere of the caudal middle frontal gyrus was also observed in GAD patients. The caudal middle frontal gyrus plays a role in attention, problem solving, working memory, and response inhibition (Andersson *et al*., 2009; Sanchez-Benavides *et al.,* 2010; Swick, Ashley, & Turken, 2008). It has also been implicated in schizophrenia, panic disorder, aversion, and social anxiety (Kikinis et al., 2010; Sakai *et al.,* 2006; Syal *et al.,* 2012). Decreased cortical thickness in this area has

been observed in adolescent marijuana users (Lopez-Larson *et al.,* 2011). Furthermore, reduced volume in this area was associated with depression (Han *et al.,* 2014).

Significantly lower cortical thickness in the left hemisphere was observed in the superior frontal region in GAD patients compared to healthy controls. Furthermore, this was also observed in psychiatric controls compared to healthy controls. This suggests the superior frontal region may be involved in GAD as well as other psychiatric disorders, potentially contributing to comorbidity. Significantly reduced thickness was also observed in the superior frontal region in GAD patients compared to healthy controls in the right hemisphere. The superior frontal gyrus is important in higher cognitive functions, working memory, attention, movement, cognitive control and response selection (Boisgueheneuc *et al.*, 2006; Nagahama *et al.,* 1999; Tamm, Menon, & Reiss, 2002). Reduced thickness in this area has been associated with impulsiveness, reasoning, and cognitive control (Schilling *et al.*, 2013; Tully, Lincoln, Liyanage-Don, & Hooker, 2014).

Overall, these regions play in role in several functions. Specifically, their function in judgement, decision making, response, attention, and problem-solving all may influence altered responses to stress and cause excessive worry. Each of these tasks are critical in the perception and response to stress. Furthermore, these three regions are important in working memory, indicating the same mechanisms involved in formation of memory (LTP) may be altered in GAD.

Most previous structural difference studies involving GAD have been volume studies. However, one study found cortical thickness differences in the OFC and IFG in GAD patients (Andreescu *et al.,* 2017). No thickness differences were observed in the

OFC in this study; however, lower cortical thickness was found in the pars triangularis, which is part of the IFG.

Many studies have found that chronic stress produces detrimental effects on the brain. Stress triggers the release of hormones that can cause damage to and kill cells within the limbic system and frontal cortex. Elevated levels of glucocorticoids over time has been found to atrophy hippocampal cells and cells in the PFC. Long-term stress also produces morphological and chemical changes (Lupien *et al.*, 1998; McEwen, 2009). Chronic stress has been found to suppress neurogenesis and affect plasticity. Moreover, corticosterone treatment has been found to retract dendrites in the CA3 hippocampus (Gould et al., 1997; Sousa et al., 2000; Sapolsky, 2003).

Life stress has also been associated with decreased levels of BDNF. BDNF plays a major role in LTP, and is critical for synaptic plasticity and dendritic growth (Post, 2007). BDNF is also thought to mediate the effects of stress on the hippocampus (Manji *et al.*, 2003). Abnormalities in signaling of this growth factor is also thought to decrease cognition (Grande, 2010). The suppression of neurogenesis and reduction of dendritic branching and length found with chronic stress may contribute to the reduced thickness found in GAD patients.

Pharmaceutical treatment for GAD usually involves an SSRI. Until recently, the general consensus for treating most psychiatric disorders relied on the monoamine theory. This theory refers to psychiatric disorders, specifically depression, being the result of depleting levels of monoamines including 5-HT, norepinephrine, and dopamine within the synapse (Delgado, 2002). Many typical antidepressants (ADs) increase levels of monoamines in the brain. For example, SSRIs prevent the reuptake of 5-HT and result in

more 5-HT in the synaptic cleft (Vaswani, Linda, & Ramesh, 2003). Although typical ADs have shown to increase monoamine levels within twenty-four to forty-eight hours, it usually takes about four to six weeks for these medications to be effective. This indicates there may be another underlying mechanism contributing to psychiatric disorders including GAD.

A novel antidepressant, ketamine, has recently been used for rapid treatment of depression, typically in the case a patient is having suicidal thoughts. Ketamine is a NMDA antagonist, blocking the NMDA receptor. While ketamine has shown success in clinical trials, other NMDA antagonists such as MK-801, AP5, and memantine have failed (Lenze et al., 2012; Smith et al., 2013; Zarate et al., 2006; Ibrahim et al., 2012). As a result, it is suspected that a mechanism unique to ketamine is responsible for its rapid and long-lasting effects.

When ketamine is broken down in the body, one of the minor metabolites produced is hydroxynorketamine (HNK). HNK is considered an active metabolite because it still produces drug effects after being processed by the body. The metabolite HNK increases AMPA activity (in addition to ketamine blocking the NMDA receptor). AMPA activation (ion influx and depolarization) activates the ERK signal transduction pathway. ERK then activates CREB. This transcription factor binds to the promotor region of a DNA strand, resulting in genetic transcription and translation of BDNF. When BDNF is released, TrKB receptors are activated. This receptor activation then activates mTOR. (Li et al., 2010; Autry et al., 2011). Again, mTOR stimulates many transcription factors responsible for neurogenesis, including cytoskeletal reorganization and the growth of new dendrites and

terminal buttons (synaptogenesis) (Laplante *et al.*, 2012; Sarbassov *et al.*, 2004; Duman et al., 2018).

This mechanism is similar to that of LTP. The rapid neurogenesis and alleviation of symptoms observed with ketamine is the result of directly targeting the glutamate system. Since the discovery of ketamine's mechanism of action, it is suspected that the downstream effects of typical ADs trigger neurogenesis, rather than the previous monoamine theory. More specifically, it is now thought that typical ADs over time increase glutamate AMPA receptors and increase BDNF brain production (via AMPA-BDNF upregulation). (Coyle & Duman, 2003; Castren & Rantamaki, 2010). For, example SSRIs have been found to increase AMPA receptors in the hippocampus and PFC (Martinez-Turrillas et al., 2002). Successful treatment of GAD with antidepressants may be attributed to the neurogenesis of the brain regions with reduced thickness.

There were some limitations in this study. Only adult patients were used and samples consisted mostly of males. Future studies should include pediatric and adolescent GAD patients and a more diverse population. Also, this research only included structural differences. Functional and optogenetic studies may provide more information to the underlying neural circuits involved in GAD. Future studies should also focus on determining differences specific to GAD compared to other anxiety and psychiatric disorders.

In conclusion, lower cortical thickness was observed in patients with GAD in three frontal regions. Specifically, significant differences were found for the pars triangularis, caudal middle frontal, and superior frontal. These regions are important in formation of memory and critical in perception and response to a threat. It is suspected the reduced

thickness observed in GAD patients may be due to the effects of chronic stress and learned behavior on the brain. Furthermore, it may be the result of suppressed neurogenesis and dendritic reduction.

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CHAPTER IV

Conclusions

Specific behaviors have a major impact on the criminal justice system and medical field. Two types of maladaptive behaviors are of particular interest due to their influence on crime: aggression and antisocial behaviors. These behaviors have become a major problem as the United States currently has the highest incarceration rate (Sherman, 2000; Bureau of Justice Statistics). Additionally, these behaviors are also two of the leading causes of mental health referrals (Beitchman *et al.*, 2012). In developed countries, the majority of violent crime is committed by a reduced group of antisocial recidivistic offenders and more than 50% of severe antisocial behavior is attributable to genetic factors (Tiihonen *et al.*, 2015; Tracy, Wolfgang, & Figilo, 1990; Ferguson, 2010). Furthermore, criminal records of biological parents have predicted violent and non-violent criminality among their children (Hjalmarsson & Lindquist, 2013). The strong heritability and environmental issues surrounding criminal activity indicates that a genetic underlying can help explain at least some features related to these behaviors. Therefore, this research focused on genetic variation associated with antisocial behavior traits.

MPS technology has been used extensively in the medical field to predict diseases and personalize treatment options. Moreover, it is widely used in ancestry panels purchased by the public. This study aimed to explore the use of MPS in the behavioral genetics and forensic psychiatry fields. Previous studies involving SNPs and behavior have been performed with traditional techniques such as SBE. Although robust, they are limited in multiplexing capibilities, with a maximum of 10 SNPs per panel. In this study, a novel custom designed MPS panel was used to analyze 48 SNPs simultaneously. Data analysis

revealed high loading densities (>75%), total reads (>2.5 million), and percent library (>75%) for each chip. Moreover, no major differences in quality metrics were observed between using a 6ng or 10ng target, suggesting 6ng is sufficient to produce usable data. Over 90% of samples had successful profiles. Haplotype and genotype results from MPS were compared to those of SBE to confirm the accuracy. Of the successful profiles, there was 100% concordance between the two techniques. This large MPS panel of behavioral markers overcomes the limited multiplexing capability of analyzing SNPs using traditional methods. The newly developed panel may be used to determine if an individual is predisposed to exhibit certain behavior. Furthermore, it may be helpful in predicting biological vulnerabilities and providing early intervention and treatment.

This study also includes genetic variation data in high-risk individuals. Currently, only a few groups have access to inmate samples including one Finnish and one Russian group. Specifically, this research builds on the work of Grigorenko (2010) that investigated 12 SNPs in 4 genes associated with DA turnover in a group of male Russian incarcerated adolescents (Grigorenko et al., 2010). While Grigorenko (2010) observed no significant differences in single genetic variation, this study found significant differences in major allele frequency for two MAO markers between inmates and controls (rs1799836 and rs909525; $p<0.00002$). Haplotype analysis also revealed different significant haplotypes compared to the findings reported by Grigorenko. Two MAOA haploytpes (including rs3788862, rs909525, and rs979605) were found to be significantly different between the inmate and control population. More specifically, the frequencies of haplotypes GAT and GGT were significantly different ($p=0.0012$ and 0.000036 respectively). This research provides the forensic community with novel findings on the etiology of violent crime. The

results contribute to the limited number of studies that have access to inmate samples. Furthermore, these results are more specific to the United States population and include adult incarcerated individuals rather than adolescents.

Anxiety disorders also have a negative impact on society as they are the most prevelant type of psychiatric disorders. Approximately one third of people in the United States experience some sort of anxiety disorder in their lifetime (National Institute of Mental Health). Not only do they hinder many aspects of an individual's daily life, they also burden the healthcare system. For example, anxiety patients experience impairment in quality of life, work productivity, and social life (Wittchen, 2002; Rapaport, 2005; Revicki *et al.*, 2012). Individuals with anxiety are also more likely to drop out of school early, which leads to increased risk of substance abuse among other problems (Van Ameringen, Mancini, & Farvolden, 2003). Anxiety disorder patients are at least 3 times more likely to visit a physician and 6 times more likely to be hospitalized (National Institute of Mental Health). Specifically, GAD patients had higher median medical costs and lower patient functioning compared to other anxiety disorders (Revicki *et al.*, 2012).

Since GAD has a major impact on society, it is important to determine if structural differences in the brain play a role in the development of GAD. This study explored the specific frontal regions potentially involved in GAD. Moreover, this study also compared GAD patients to both healthy controls and psychiatric controls. Using fMRI, thickness and surface area differences were assessed for 11 bilateral frontal regions. No differences in surface area were observed; however, lower cortical thickness was observed in GAD patients. Specifically, significant differences in cortical thickness were found for the pars triangularis, caudal middle frontal region, and superior frontal region ($p<0.0006$) between

GAD patients and healthy controls. These findings indicate that thickness differences rather surface area may be more important in GAD. Moreover, reduced thickness in the specific areas within the prefrontal cortex may make individuals more prone to anxiety. Altered thickness in these areas may be used as a diagnostic tool for GAD. Additionally, these differences may be used in conjunction with the DSM to confirm diagnosis of GAD. It may also be used to determine the success of antidepressant treatment, as it has been found to cause downstream neurogenesis, potentially combating the brain regions with reduced thickness.

In summary, the results of these studies may help provide insight to the underlying mechanisms involved in these behaviors. These types of behaviors have a negative impact on society, especially on the criminal justice system and medical field. These findings may also lead to better opportunities for early intervention and prevention. Moreover, it may be useful in developing treatments for addiction, depression, anxiety, and several other behaviors.

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APPENDIX

Table A.1

Amplification conditions for multiplex 1.

Step	Temp. (°C)	Time
1	95	5 min
2	95	30 s
3	64.5	90 s
4	72	30 s
5	95	30 s
6	61.8	90 s
7	72	30 s
8	95	30 s
9	56.5	90 s
10	72	30 s
11	Go to step 3	Repeat 30 times
12	72	15 min
13	4	∞

This multiplex included 3 SNPs: rs25531, rs877172, and rs4813625

Table A.2

Amplification conditions for multiplex 2.

Step	Temp. (°C)	Time
1	95	5 min
2	95	30 s
3	67	90 s
4	72	30 s
5	95	30 s
6	64.5	90 s
7	72	30 s
8	95	30 s
9	61.8	90 s
10	72	30 s
11	95	30 s
12	59.1	90 s
13	72	30 s
14	Go to step 11	Repeat 29 times
15	60	30 min
16	4	∞

This multiplex included 5 SNPs: rs979605, rs909525, rs3788862, rs2283729, and rs1799836.

Table A.3

Custom BED file created for data analysis.

chrX	43517363	43517364	rs3788862	0	+	REF=A;OBS=G;ANCHOR=A	AMPL7160468061
chrX	43553201	43553202	rs909525	0	+	REF=C;OBS=T;ANCHOR=T	AMPL7160468062
chrX	43601362	43601363	rs979605	0	+	REF=A;OBS=G;ANCHOR=A	AMPL7160468063
chrX	43627998	43627999	rs1799836	0	+	REF=T;OBS=C;ANCHOR=G	AMPL7156824384
chrX	43678041	43678042	rs2283729	0	+	REF=G;OBS=A;ANCHOR=A	AMPL7160468064
chr20	3049719	3049720	rs4813625	0	+	REF=G;OBS=C;ANCHOR=G	AMPL7159929816
chr20	3049889	3049890	rs877172	0	+	REF=T;OBS=G;ANCHOR=T	AMPL7160468060
chr3	8794544	8794545	rs1042778	0	+	REF=G;OBS=C,T;ANCHOR=G	AMPL7160468057
chr3	8788197	8788198	rs11476	0	+	REF=A;OBS=T;ANCHOR=A	AMPL7160468055
chr3	8809183	8809184	rs237902	0	+	REF=G;OBS=A;ANCHOR=A	AMPL7160468058
chr3	8804370	8804371	rs53576	0	+	REF=A;OBS=G;ANCHOR=G	AMPL7154408757
chr3	8793723	8793724	rs6770632	0	+	REF=C;OBS=A;ANCHOR=C	AMPL7160468056
chr22	19956780	19956781	rs165599	0	+	REF=G;OBS=A;ANCHOR=C	AMPL7156994993
chr22	19951270	19951271	rs4680	0	+	REF=G;OBS=A;ANCHOR=C	AMPL7154408863
chr22	19930120	19930121	rs737865	0	+	REF=A;OBS=G;ANCHOR=G	AMPL7156897210
chr22	19945176	19945177	rs740603	0	+	REF=A;OBS=G;ANCHOR=C	AMPL7158454272
chr9	136523668	136523669	rs129882	0	+	REF=C;OBS=T;ANCHOR=A	AMPL7160468050
chr9	136500514	136500515	rs1611115	0	+	REF=T;OBS=C;ANCHOR=G	AMPL7154408802
chr9	136516569	136516570	rs739398	0	+	REF=C;OBS=A;ANCHOR=C	AMPL7160468059
chr11	113283687	113283688	rs1076560	0	+	REF=C;OBS=A;ANCHOR=C	AMPL7160357703
chr11	113346250	113346252	rs1799732	0	+	REF=-;OBS=G;ANCHOR=A	AMPL7155292282
chr11	113270827	113270828	rs1800497	0	+	REF=G;OBS=A;ANCHOR=C	AMPL7156994968
chr11	636783	636784	rs1800955	0	+	REF=T;OBS=C;ANCHOR=G	AMPL7156509455
chr11	18047815	18047816	rs1800532	0	+	REF=G;OBS=T;ANCHOR=A	AMPL7154408809
chr13	47471477	47471478	rs6311	0	+	REF=C;OBS=T;ANCHOR=C	AMPL7155292284
chr13	47409033	47409034	rs6314	0	+	REF=G;OBS=T;ANCHOR=T	AMPL7158544166
chr1	46870760	46870761	rs324420	0	+	REF=C;OBS=A;ANCHOR=C	AMPL7156823369
chr11	27679915	27679916	rs6265	0	+	REF=C;OBS=T;ANCHOR=A	AMPL7154408810
chr6	154360796	154360797	rs1799971	0	+	REF=A;OBS=G;ANCHOR=C	AMPL7155292277
chr12	112241765	112241766	rs671	0	+	REF=G;OBS=A;ANCHOR=T	AMPL7153213811
chr4	100239318	100239319	rs1229984	0	+	REF=T;OBS=C;ANCHOR=G	AMPL7153318229
chr4	46334208	46334209	rs279826	0	+	REF=A;OBS=G;ANCHOR=A	AMPL7153991396
chr4	46339069	46339070	rs279836	0	+	REF=T;OBS=A;ANCHOR=A	AMPL7153991395
chr4	46329654	46329655	rs279844	0	+	REF=A;OBS=T;ANCHOR=A	AMPL7156707299
chr4	46329722	46329723	rs279845	0	+	REF=T;OBS=A;ANCHOR=G	AMPL7156707299
chr4	46314592	46314593	rs279858	0	+	REF=T;OBS=C;ANCHOR=T	AMPL7160357690
chr4	46308302	46308303	rs279867	0	+	REF=A;OBS=C;ANCHOR=A	AMPL7160357689
chr4	46305732	46305733	rs279871	0	+	REF=T;OBS=C;ANCHOR=A	AMPL7160357687
chr4	46250676	46250677	rs497068	0	+	REF=G;OBS=A;ANCHOR=T	AMPL7160357683
chr4	46241768	46241769	rs567926	0	+	REF=G;OBS=A;ANCHOR=C	AMPL7160357682
chr4	46371832	46371833	rs9291283	0	+	REF=G;OBS=A;ANCHOR=C	AMPL7160357694
chr6	88853634	88853635	rs1049353	0	+	REF=C;OBS=T;ANCHOR=C	AMPL7159420221
chr6	88861207	88861208	rs1535255	0	+	REF=T;OBS=G;ANCHOR=A	AMPL7154245447
chr6	88860481	88860482	rs2023239	0	+	REF=T;OBS=C;ANCHOR=A	AMPL7159420223
chr6	88872929	88872930	rs6454674	0	+	REF=T;OBS=G;ANCHOR=T	AMPL7159420225
chr6	88850099	88850100	rs806368	0	+	REF=T;OBS=C;ANCHOR=A	AMPL7160357696
chr6	88861266	88861267	rs806379	0	+	REF=A;OBS=T;ANCHOR=A	AMPL7154245447
chr6	88864652	88864653	rs806380	0	+	REF=A;OBS=G;ANCHOR=A	AMPL7159420224

Table A.4

Percentage ion sphere particle loading, final library percentage, and number of reads for each chip.

Chip #	ISP Loading	Final Library	Number of Reads
1	79%	86%	3,477,977
2	76%	85%	3,200,510
3	88%	92%	3,751,862
4	79%	77%	2,894,485

Table A.5:

Amplification and SBE primer sequences.

SNP	Primer	Sequence
rs740603	Forward	CTAGCTCTGCAGCAGACTGCTG
	Reverse	TAGAGGGCAGGCATGATCGTG
	SBE-Reverse	ACGCCACATGCAGATGCACG
rs737865	Forward	AAATCAGCATGGAGCCAGC
	Reverse	ACCACGTGGGAATGTTAGAG
	SBE-Reverse	GGATTTTTCCAGCCAGGG
rs739398	Forward	CGCTGCTCAGCTTGGTGGCTTTG
	Reverse	GCAGTTTGCTTCCCTGGAACACTTGC
	SBE-Reverse	CACGGGGAAGAGCGAGG
rs1611115	Forward	AGCGTAGAGCTCAGAGCTGAAG
	Reverse	GAGGGTCAGTCTCACCACG
	SBE-Reverse	CTCCCTCCTGTCCTCTCCC
rs165599	Forward	CTTGACGGACGCTAACGC
	Reverse	AGCACTGCATCCTCACTCATG
	SBE-Reverse	CTCCTCTTCGTTTCCCAGGC
rs4680	Forward	TGCACAGGCAAGATCGTGGACG
	Reverse	CTGGTGCCACCTTGGCAGTTTAC
	SBE-Reverse	GCATGCACACCTTGTCCTTCA
rs129882	Forward	TCACACCGGCACTGTGCAC
	Reverse	TCCCTGCACTGAGTCAGCC
	SBE-Forward	ATCCCCATGGAACAGCCCTGCA
rs1800532	Forward	CCAGAGCCGTAAGTACTT
	Reverse	CTCCATGGGACTCAACAC
	SBE-Forward	CTATGCTCAGAATAGCAGCTA
rs2283729	Forward	AAGCGCAAGCTATGAAACAGGC
	Reverse	AGCTATGAAGCCAGCCATATGC
	SBE-Reverse	GCCTGGAACTATGTCTTATTTAATTTCCG
rs1799836	Forward	TGGAGTGTTCTGGCCTTTAC
	Reverse	ACATAGCCTACCACAGACTCTG
	SBE-Forward	GGAGCAGATTAGAAGAAAGATGGTGTC
rs3788862	Forward	AGCATCAGAGGAAAGCAGC
	Reverse	CAGATGGTATGGAGATGGGAG
	SBE-Forward	GTCCCACTAGGCAAGCCTCCTAAAAGCA

rs909525	Forward	TAGGCTGCAATGTCAGATGG
	Reverse	CTACAGGCAATCCCTGAGC
	SBE-Forward	GTGAAGGCCAGGTACAGAGGAAAT
rs979605	Forward	ATGTCAAGTTGAGCTCACG
	Reverse	AAGAACTGGTGTGAGGAGC
	SBE-Forward	GACAACTATTTCTAGAATTTGCA
rs25531	Forward	CCTAGGATCGCTCCTGCATC
	Reverse	GGAGATCCTGGGAGAGGTG
	SBE-Forward	GCATCCCCCCTGCACCCCC
rs877172	Forward	CAGACTCTCCTGCCCTCTTG
	Reverse	CTCATGCCAGTGACTCATGC
	SBE-Reverse	GATGAGCTCTGTGACCTGCT
rs4813625	Forward	GAGGGGTTGTTGAACAGGTG
	Reverse	CTGCCCTCTTGTTGAGGAAG
	SBE-Forward	TCTCTGGGCCACTGCTG
rs6770632	Forward	TGGATATTCTGGGTCCCTTG
	Reverse	AGCAAGTTCCGCAAGGTTTC
	SBE-Reverse	GTGCAAGACTGAAAACTACAAAATT
rs11476	Forward	CACTGCCCTGAAAAACAGAC
	Reverse	GTTAGCCAAAGGGGAGGTTC
	SBE-Reverse	TCCTCCATTGGTGCCCATT
rs1042778	Forward	TGGCTGAGTCCCCTATCATC
	Reverse	CTCCTTTGTCCTGAGCCATC
	SBE-Forward	TGAAGCCACCCCAAGGAG
rs237902	Forward	GCCTTGGAGATGAGCTTGAC
	Reverse	GGCCTACATCACATGGATCAC
	SBE-Forward	GCAGCGGTCTTGAGCCGCAA
rs53576	Forward	TCTCCACATCACTGGGTCAC
	Reverse	GCCTGGTTTGAACTGTTTCC
	SBE-Forward	GTGTACGGGACATGCCCGAGG

Table A.6:

Multiplex 1 PCR parameters.

Step	Temp. (°C)	Time
1	95	5 min
2	95	30 s
3	64.5	90 s
4	72	30 s
5	95	30 s
6	61.8	90 s
7	72	30 s
8	95	30 s
9	56.5	90 s
10	72	30 s
11	Go to step 3	Repeat 30 times
12	72	15 min
13	4	∞

Table A.7:

Multiplex 2 PCR parameters.

Step	Temp. (°C)	Time
1	95	5 min
2	95	30 s
3	67	90 s
4	72	30 s
5	95	30 s
6	64.5	90 s
7	72	30 s
8	95	30 s
9	61.8	90 s
10	72	30 s
11	95	30 s
12	59.1	90 s
13	72	30 s
14	Go to step 11	Repeat 29 times
15	60	30 min
16	4	∞

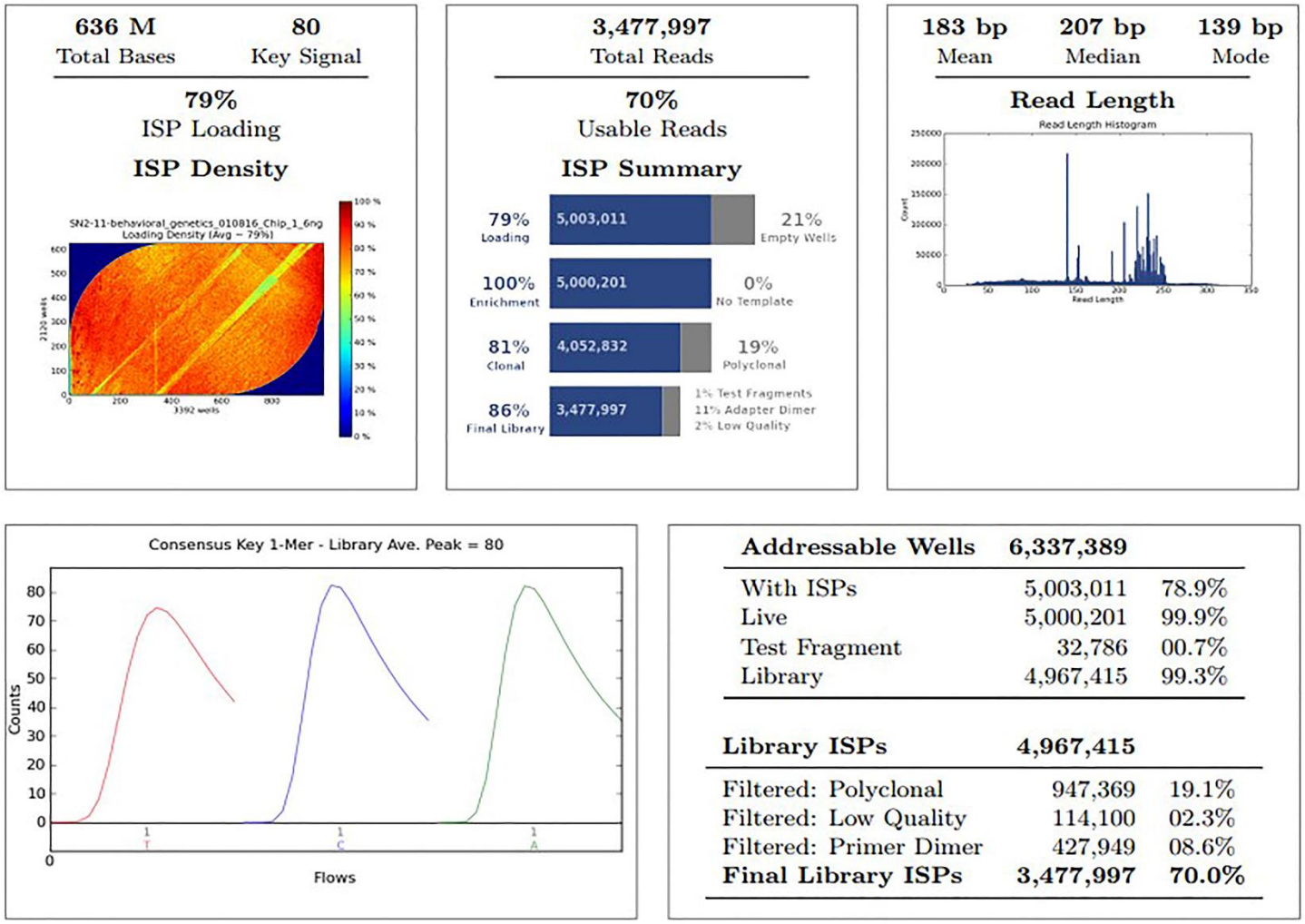
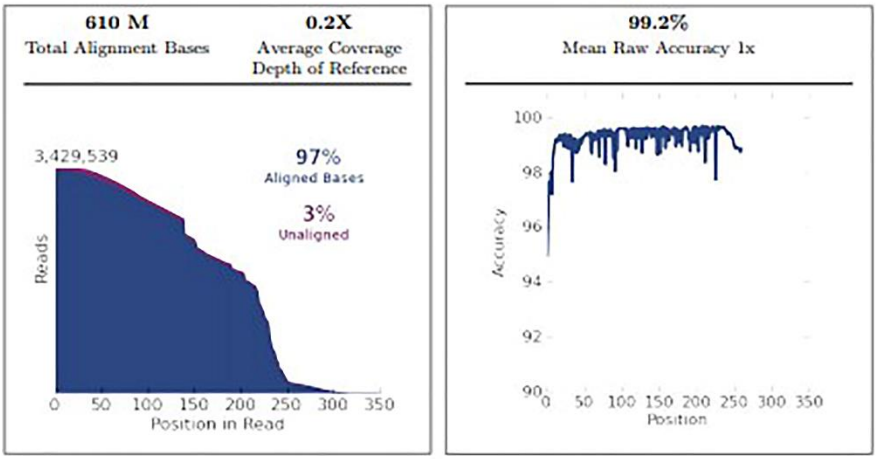


Figure A.1: Run summary for chip #1.



	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	575 M	532 M	377 M
Mean Length [bp]	184	179	137
Longest Alignment [bp]	372	361	345
Mean Coverage Depth	0.2	0.2	0.1

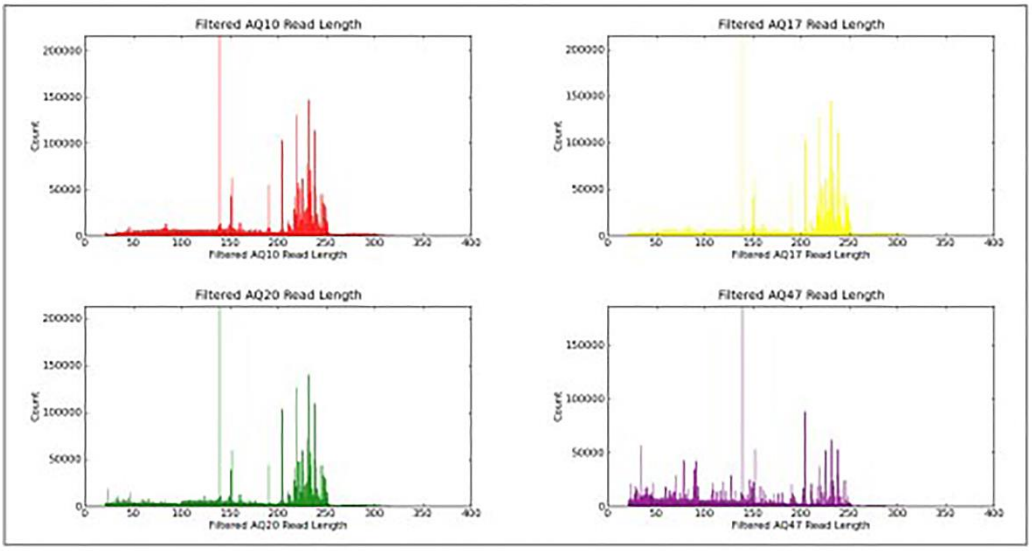


Figure A.2: Alignment summary for chip #1.

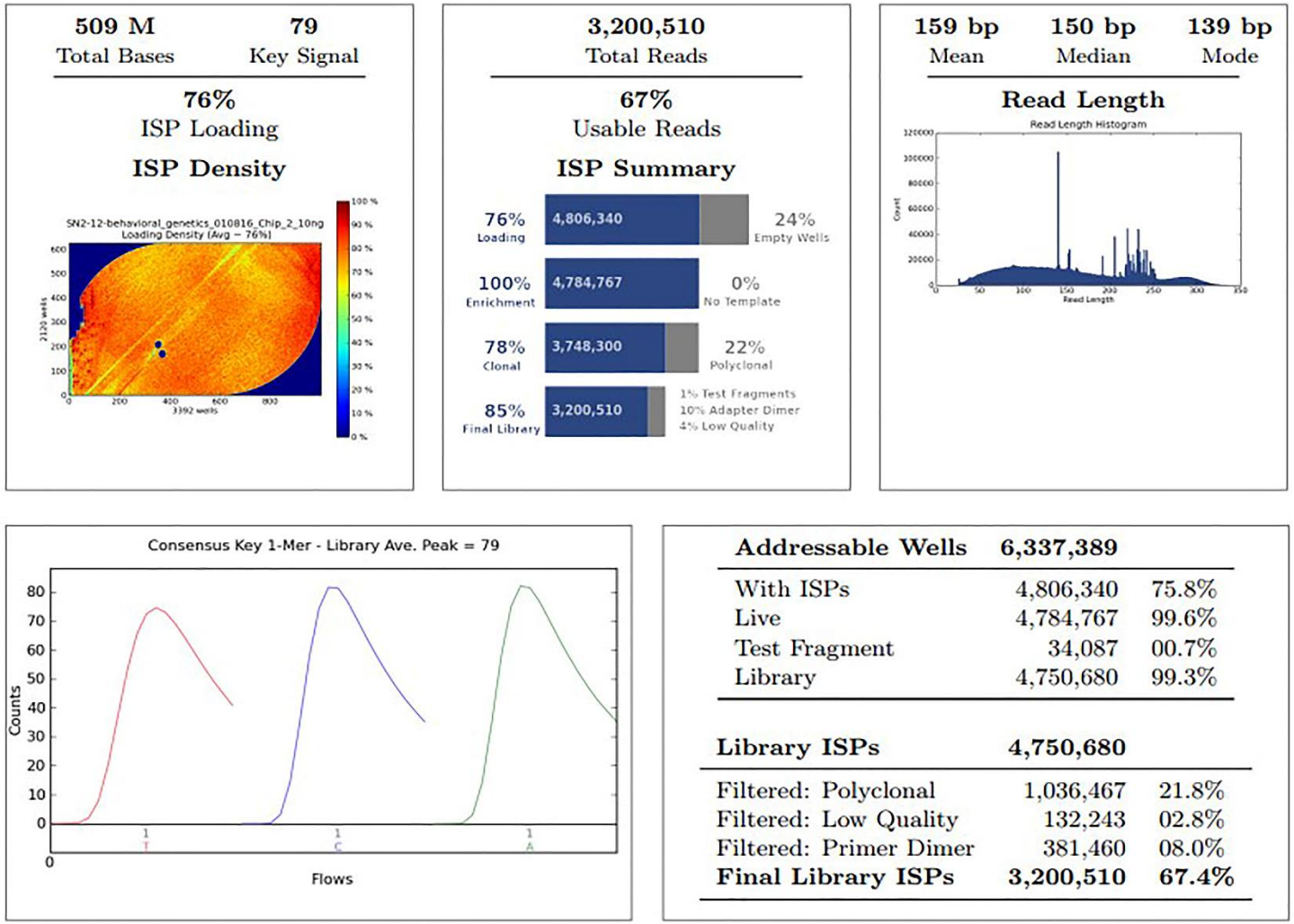
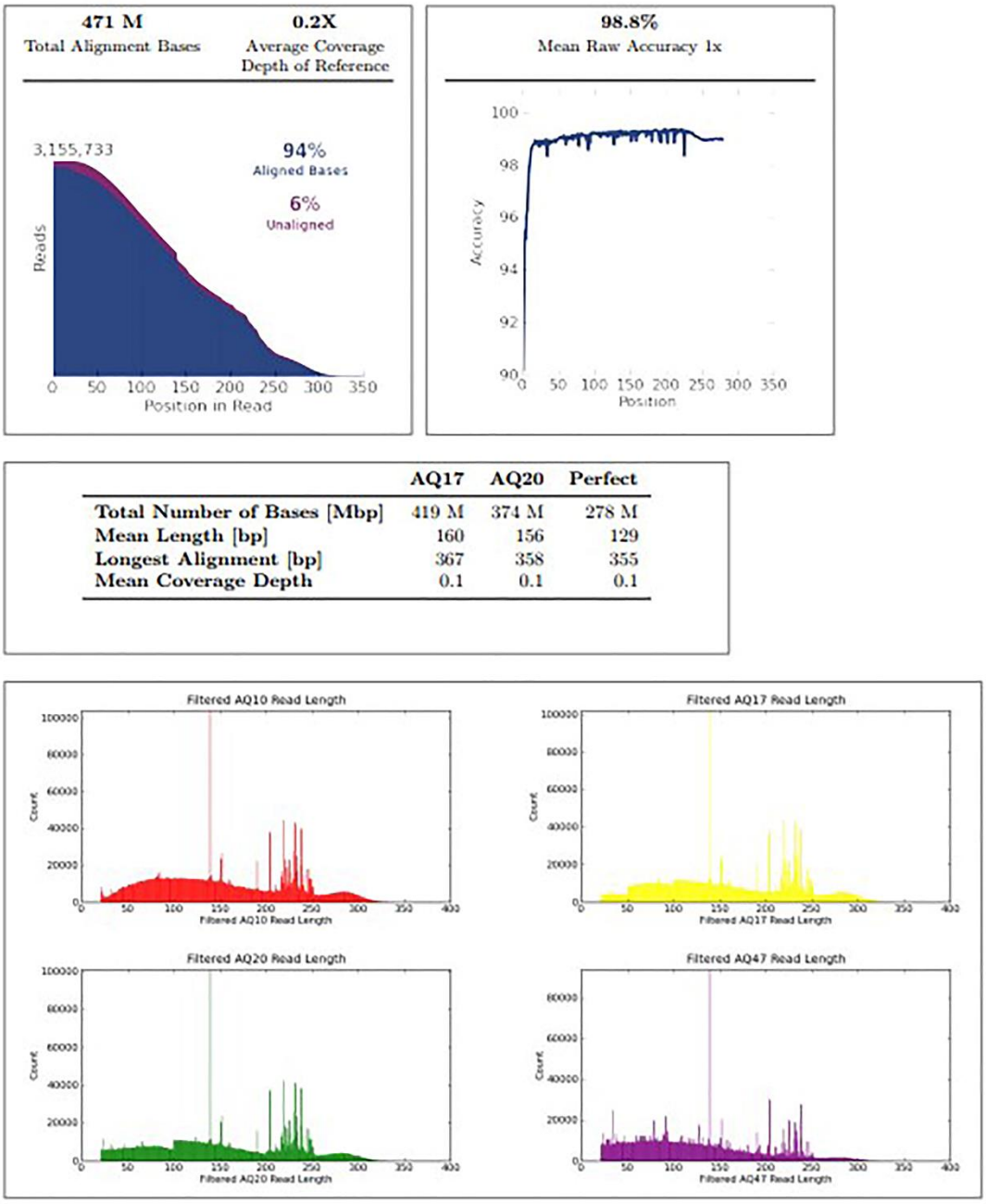


Figure A.3: Run summary for chip #2.



	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	419 M	374 M	278 M
Mean Length [bp]	160	156	129
Longest Alignment [bp]	367	358	355
Mean Coverage Depth	0.1	0.1	0.1

Figure A.4: Alignment summary for chip #2.

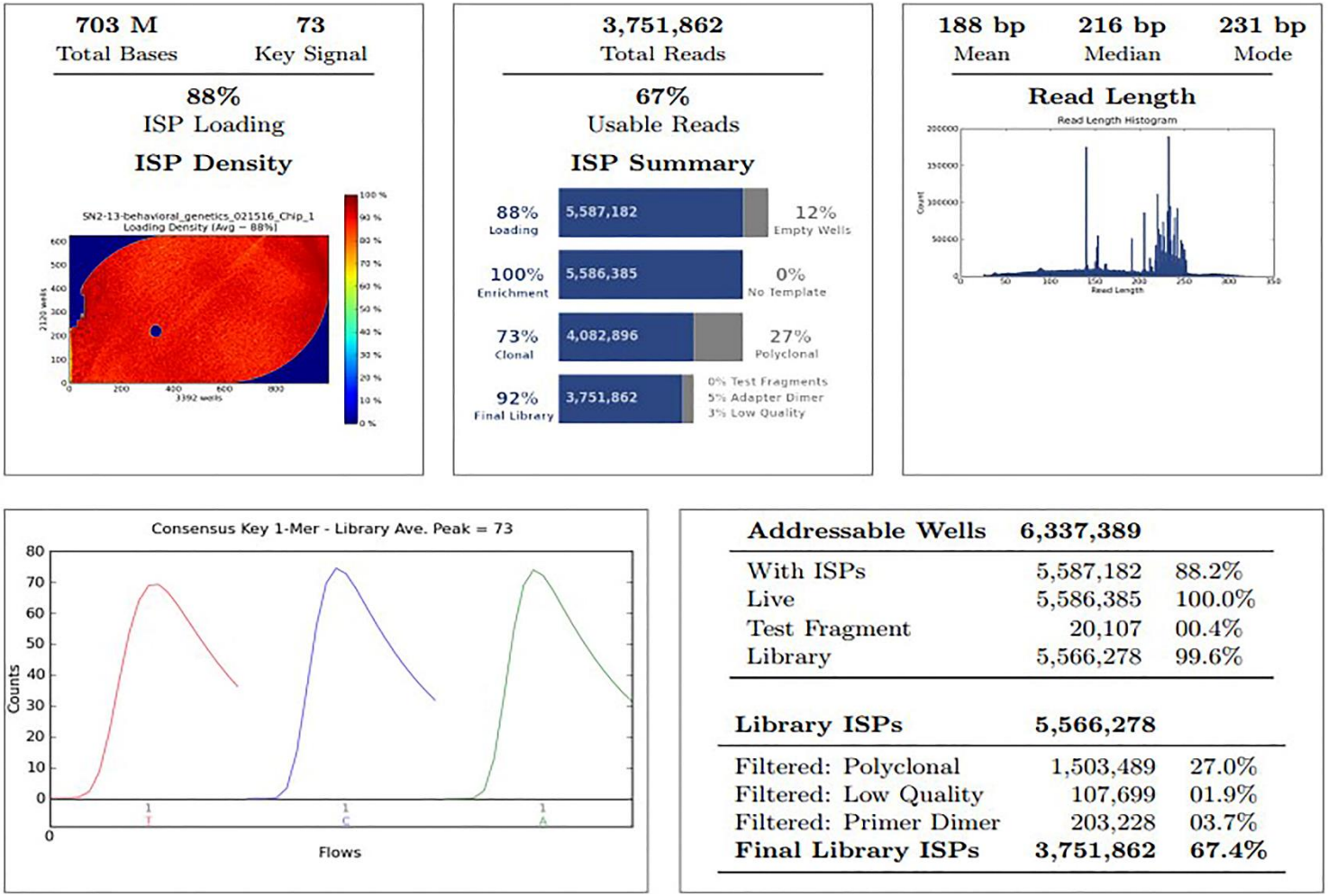
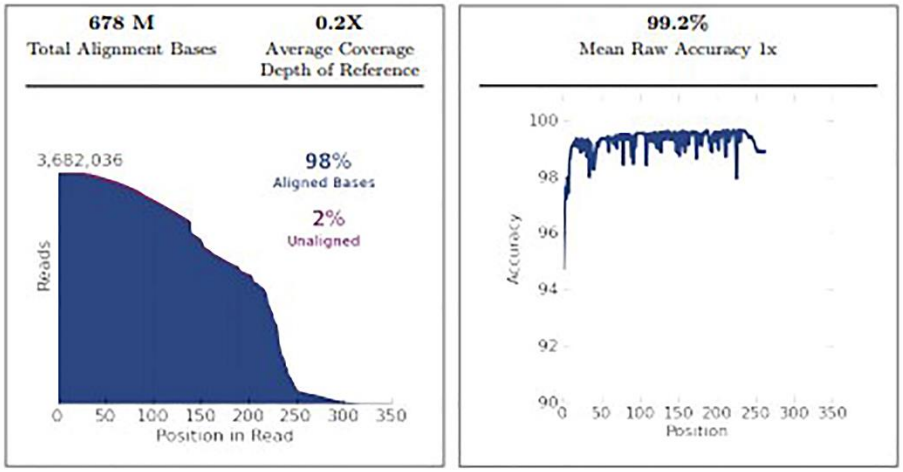


Figure A.5: Run summary for chip #3.



	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	639 M	594 M	423 M
Mean Length [bp]	188	184	142
Longest Alignment [bp]	359	359	350
Mean Coverage Depth	0.2	0.2	0.1

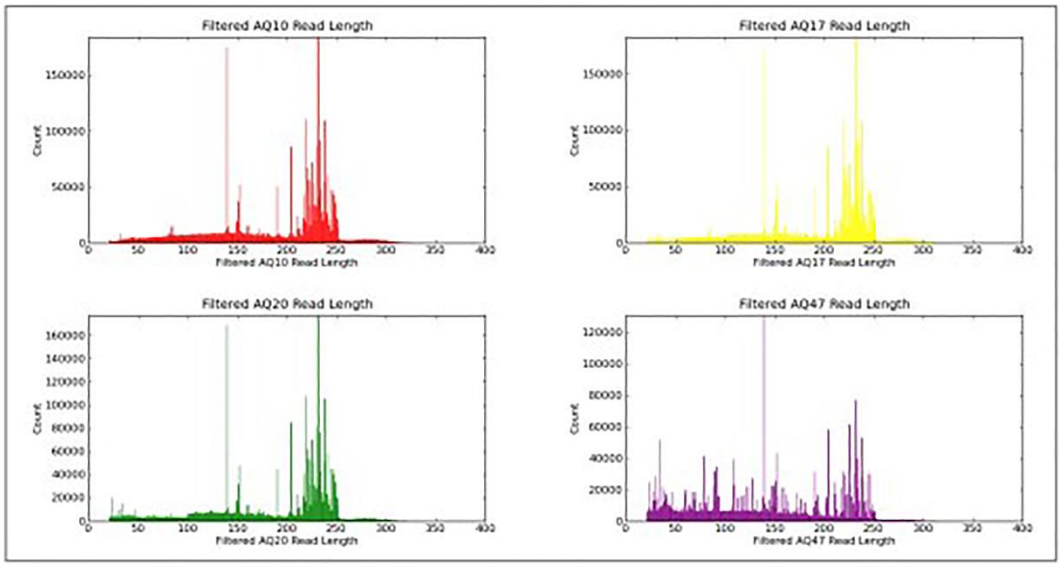
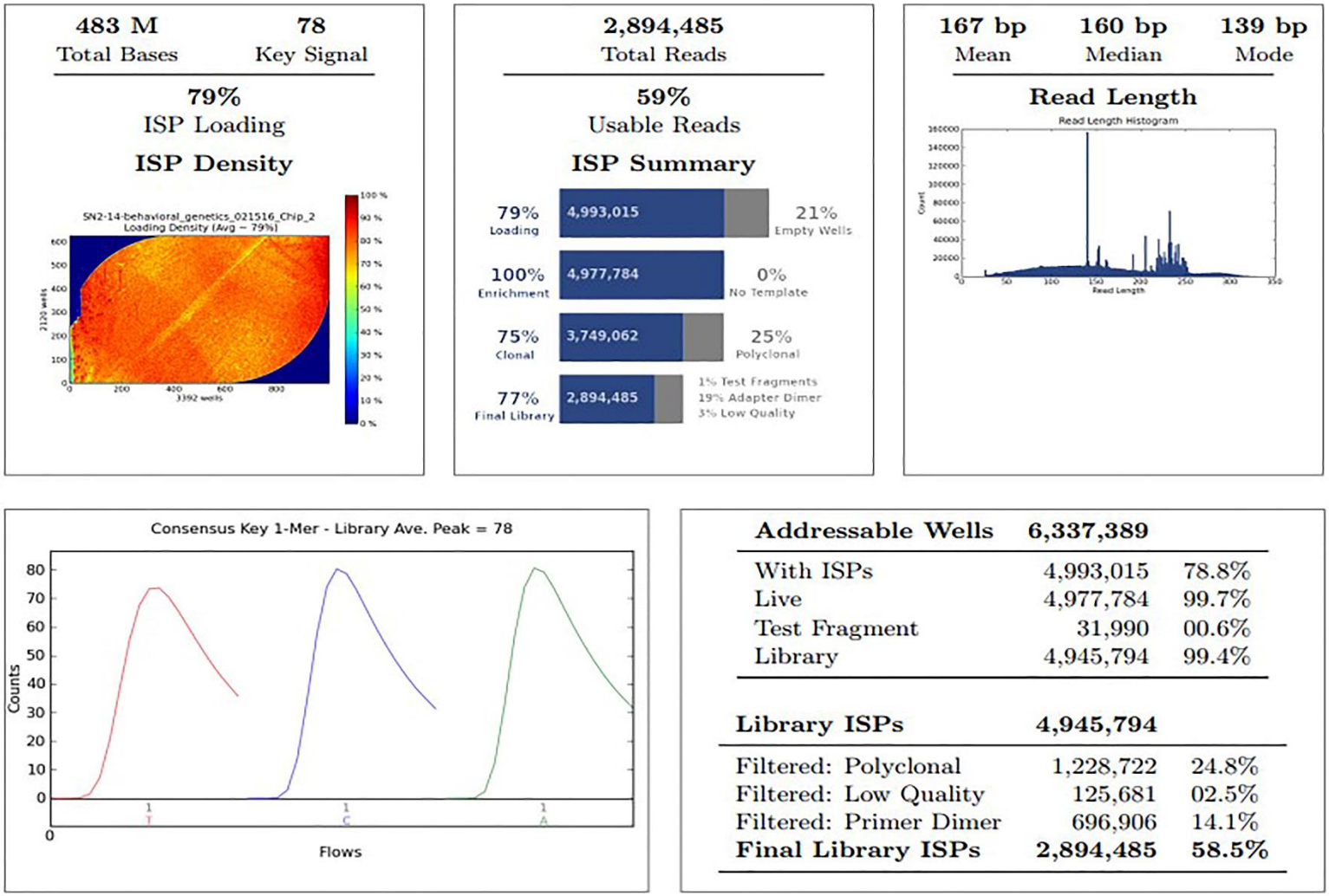
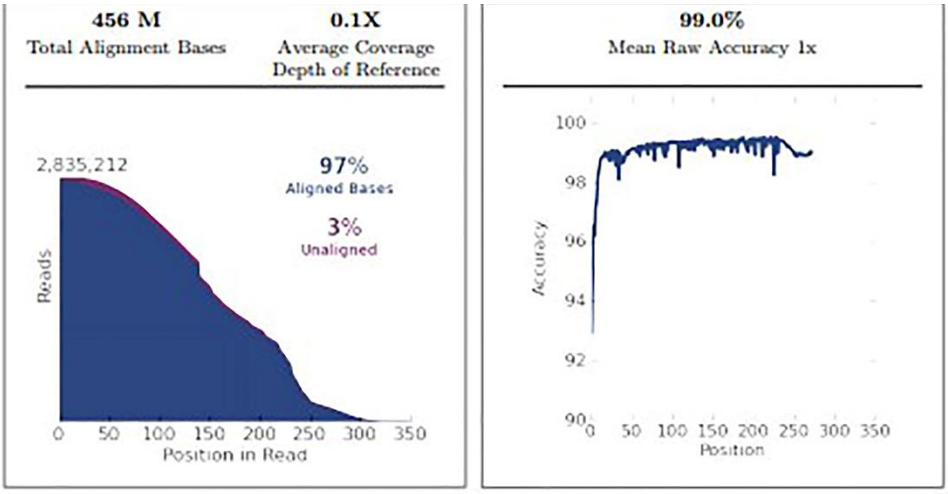


Figure A.6: Alignment summary for chip #3.



Addressable Wells	**6,337,389**	
With ISPs	4,993,015	78.8%
Live	4,977,784	99.7%
Test Fragment	31,990	00.6%
Library	4,945,794	99.4%
Library ISPs	**4,945,794**	
Filtered: Polyclonal	1,228,722	24.8%
Filtered: Low Quality	125,681	02.5%
Filtered: Primer Dimer	696,906	14.1%
Final Library ISPs	**2,894,485**	**58.5%**

Figure A.7: Run summary for chip #4.



	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	417 M	382 M	282 M
Mean Length [bp]	167	164	133
Longest Alignment [bp]	364	364	363
Mean Coverage Depth	0.1	0.1	0.1

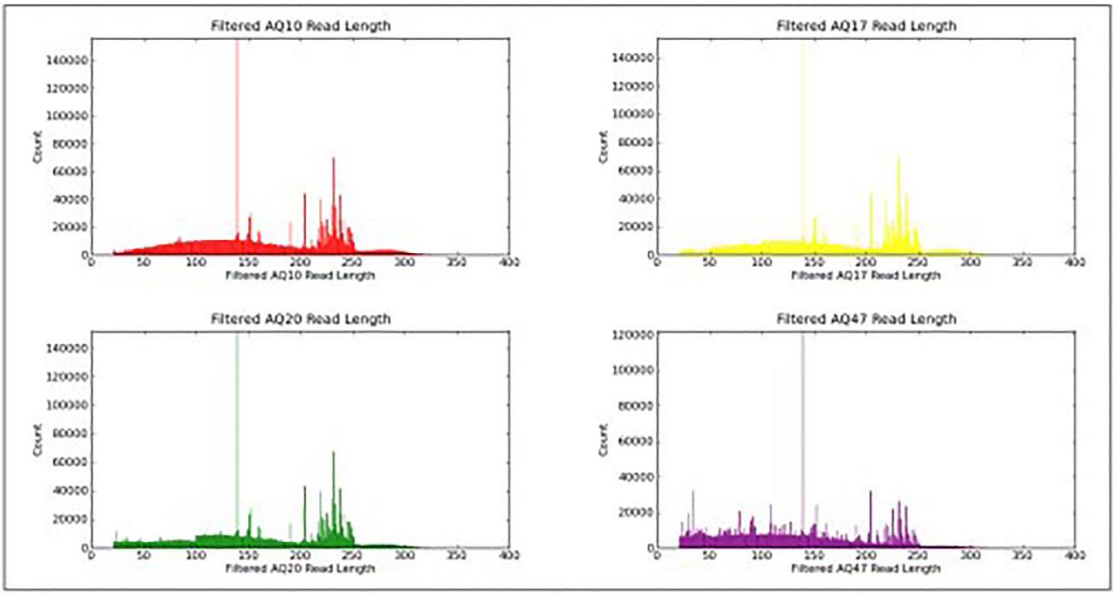


Figure A.8: Alignment summary for chip #4.

Survey A.1: Survey given to inmate participants.

Resting heart rate: _____

Survey

1. What is your birth date? _____ (month, day, year)

2. How tall are you? _____

3. What is your weight? _____

4. Which of the following best describes your race/ethnicity? (If more than one applies, check each one).

☐	African American	☐	Hawaiian or Pacific Islander
☐	Asian	☐	Hispanic
☐	Caucasian	☐	Other

5. What is the highest level of education that you have completed?

☐ Less than high school
☐ High school or GED
☐ Some college or vocational training
☐ Associates degree
☐ Bachelors degree
☐ Graduate degree

6. If you did not finish High school, what is the highest grade that you completed? _____

7. Are you now or have you ever been a member of the United States Armed Forces?

☐ Yes☐ No

8. Have you ever been diagnosed with post traumatic stress disorder?

☐ Yes☐ No

9. Were you working full time at the time of your arrest? ☐ Yes☐ No

10. I'm now going to ask you some questions about how you were treated as a child. Please respond yes or no, whichever is the best answer to the question.

As a child (from birth to 18 yrs.), were you ever abused physically?	☐☐Yes	☐☐No
As a child, did you ever witness or come to know of anyone in your family being abused physically?	☐☐Yes	☐☐No
As a child, were you ever abused mentally or emotionally?	☐☐Yes	☐☐No
As a child, did you ever witness or come to know of anyone in your family being abused mentally or emotionally?	☐☐Yes	☐☐No

11. Next, I'm going to ask you some questions about how you approach certain situations. Remember that all your answers are confidential. On a scale of 1 to 4 with 1 being strongly disagree and 4 being strongly agree, how much do you agree with the following statements.

Grasmick and Bursik self-control scale*

	STRONGLY DISAGREE	**DISAGREE SOMEWHAT**	**AGREE SOMEWHAT**	**STRONGLY AGREE**
I often act on the spur of the moment without stopping to think	☐	☐	☐	☐
I don't devote much thought and effort to preparing for the future	☐	☐	☐	☐
I often do whatever brings me pleasure here and now, even at the cost of some distant goal	☐	☐	☐	☐
I'm more concerned with what happens to me in the short run than in the long run	☐	☐	☐	☐
I frequently try to avoid projects that I know will be difficult	☐	☐	☐	☐
When things get complicated, I tend to quit or withdraw	☐	☐	☐	☐
The things in life that are easiest to do bring me the most pleasure	☐	☐	☐	☐
I dislike really hard tasks that stretch my abilities to the limit	☐	☐	☐	☐

I like to test myself every now and then by doing something a little risky	☐	☐	☐	☐
Sometimes I will take a risk just for the fun of it	☐	☐	☐	☐
I sometimes find it exciting to do things for which I might get in trouble	☐	☐	☐	☐
Excitement and adventure are more important to me than security	☐	☐	☐	☐
If I had a choice, I would almost always rather do something physical than something mental	☐	☐	☐	☐
I almost always feel better when I am on the move than when I am sitting and thinking	☐	☐	☐	☐
I like to get out and do things more than I like to read or contemplate ideas	☐	☐	☐	☐
I seem to have more energy and a greater need for activity than most other people my age	☐	☐	☐	☐
I try to look out for myself first, even if it means making things difficult for other people	☐	☐	☐	☐
I'm not very sympathetic to other people when they are having problems.	☐	☐	☐	☐
If things I do upset people, it's their problem not mine	☐	☐	☐	☐
I will try to get the things I want even when I know it's causing problems for other people	☐	☐	☐	☐
I lose my temper pretty easily	☐	☐	☐	☐
Often, when I'm angry at people I feel more like hurting them than talking to them about why I am angry	☐	☐	☐	☐

When I'm really angry, other people better stay away from me	☐	☐	☐	☐
When I have a serious disagreement with someone, it's usually hard for me to talk calmly about it without getting upset	☐	☐	☐	☐

Now I'm going to ask you some questions about how wrong you think certain actions are. Remember that your answers cannot be linked to you. Please think carefully and provide the best possible answer. Remember you should answer truthfully.

12. On a scale of 1 to 4 with 1 being not wrong at all and 4 being very wrong, how wrong do you think it is for someone your age to ....

	NOT WRONG AT ALL	**A LITTLE BIT WRONG**	**WRONG**	**VERY WRONG**
Destroy property	☐	☐	☐	☐
Use marijuana	☐	☐	☐	☐
Steal something	☐	☐	☐	☐
Hit someone	☐	☐	☐	☐
Break into a vehicle	☐	☐	☐	☐
Sell drugs	☐	☐	☐	☐
Get drunk	☐	☐	☐	☐
Use force or threat of force to get money or things from other people	☐	☐	☐	☐
Deliberately injure spouse/partner	☐	☐	☐	☐
Purposely damaged or destroy property that does not belong to you	☐	☐	☐	☐

Next, I'm going to ask you some question about how you react in certain situations.

13. On a scale of 1 to 4 with 1 meaning that you strongly disagree and 4 meaning that you strongly agree, how much do you agree with the following statements.

*****original fearlessness scale*****

	STRONGLY DISAGREE	DISAGREE SOMEWHAT	AGREE SOMEWHAT	STRONGLY AGREE
Scary events make my heart beat faster	☐	☐	☐	☐
When I'm afraid my thoughts are disorganized	☐	☐	☐	☐
I don't get startled easily	☐	☐	☐	☐
I get nervous in stressful situations	☐	☐	☐	☐
Even when most people become tense I remain calm	☐	☐	☐	☐
Even in scary situations I would be unafraid	☐	☐	☐	☐
I tremble when I'm stressed out	☐	☐	☐	☐
I'm rarely tense	☐	☐	☐	☐
A scary situation would leave me out of breath	☐	☐	☐	☐
I wouldn't get flustered because of a fearful situation	☐	☐	☐	☐
When most people panic I remain in control	☐	☐	☐	☐
I get lightheaded in scary situations	☐	☐	☐	☐
Other people might physically shake when things are alarming but I don't	☐	☐	☐	☐
Even when things are scary I don't get disoriented	☐	☐	☐	☐
My stomach gets upset in stressful situations	☐	☐	☐	☐

Now I'm going to ask you some questions about how you feel in certain situations. Remember all answers are confidential. Please answer truthfully.

14. On a scale of 1 to 4 with 1 meaning that you strongly disagree and 4 meaning that you strongly agree, how much do you agree with the following statements.

****Brief sensation seeking scale -4 (Stephenson et al. 2003 and Vallone et al., 2007)***

	STRONGLY DISAGREE	DISAGREE SOMEWHAT	AGREE SOMEWHAT	STRONGLY AGREE
I would like to explore strange places.	☐	☐	☐	☐
I like to do frightening things	☐	☐	☐	☐
I like new and exciting experiences even if I have to break the rules	☐	☐	☐	☐
I prefer friends who are exciting and unpredictable.	☐	☐	☐	☐

15. Please answer the following questions on a scale of 1 to 4 with 1 meaning not at all and 4 meaning very often.

Sensation Seeking 2 – Stephenson et al. 2003)*

	NOT AT ALL	SOMETIMES	OFTEN	VERY OFTEN
How often do you do dangerous things for fun?	☐	☐	☐	☐
How often do you do exciting things even if they are dangerous?	☐	☐	☐	☐

Next I'm going to ask you a few questions about your relationship with your parents. Please give the best answer possible

16. In the year before your arrest, how many months did you live with your family (meaning your parents, brothers and sisters): _____

17. How much warmth and affection have you received from your parents?

Mother	☐ Very little	☐ Not too much	☐ Some	☐ Quite a bit	☐ A great deal
Father	☐ Very little	☐ Not too much	☐ Some	☐ Quite a bit	☐ A great deal

18. How much support and encouragement have you received from your parents?

Mother	☐ Very little	☐ Not too much	☐ Some	☐ Quite a bit	☐ A great deal
Father	☐ Very little	☐ Not too much	☐ Some	☐ Quite a bit	☐ A great deal

19. Overall, how satisfied have you been with your relationship with your parents?

Mother	☐ Very dissatisfied	☐ Somewhat dissatisfied	☐ Neither	☐ Somewhat satisfied	☐ Very satisfied
Father	☐ Very dissatisfied	☐ Somewhat dissatisfied	☐ Neither	☐ Somewhat satisfied	☐ Very satisfied

20. How many times have your parents been arrested for committing a crime?

Mother	☐ Never	☐ Once	☐ Twice	☐ Three times	☐ Four or more times
Father	☐ Never	☐ Once	☐ Twice	☐ Three times	☐ Four or more times

21. How many times have your parents been convicted for committing a crime?

Mother	☐ Never	☐ Once	☐ Twice	☐ Three times	☐ Four or more times
Father	☐ Never	☐ Once	☐ Twice	☐ Three times	☐ Four or more times

The next set of questions is about your relationships. Please give the best answer possible.

22. Are you currently married? ☐ Yes ☐ No

If yes, how long? _____

22. (If not married) Prior to your arrest, were you in a relationship (for example had a girlfriend)? ☐ Yes ☐ No

If no skip to question 31

24. In the year before your arrest, how many months did you live with the person that you were in your relationship with? _____

25. How important is your relationship with your _____ (spouse/girlfriend/boyfriend) to you?

☐ Not important at all ☐ Not too important ☐ Somewhat important ☐ Pretty important ☐ Very important

26. How much warmth and affection do you get from your _____ (spouse/boyfriend/girlfriend)?

☐ Very little ☐ Not too much ☐ Some ☐ Quite a bit ☐ A great deal

27. How satisfied have you been with your relationship with your? (spouse/girlfriend/boyfriend)

☐ Very dissatisfied ☐ Somewhat dissatisfied ☐ Neither ☐ Somewhat satisfied ☐ Very satisfied

28. How many time has your (spouse/boyfriend/girlfriend) been arrested for committing a crime?

☐ Never ☐ Once ☐ Twice ☐ Three times ☐ Four or more times

29. How many time has your (spouse/boyfriend/girlfriend) been convicted for committing a crime?

☐ Never ☐ Once ☐ Twice ☐ Three times ☐ Four or more times

30. In the year before your arrest how many times did your (spouse/boyfriend/girlfriend) use illegal drugs?

☐ Never ☐ Once or twice ☐ Monthly ☐ Weekly ☐ A couple of times a week ☐ Daily

The next set of questions asks about your friends. Please answer as truthfully as possible. Keep in mind that your answers are confidential and will never be linked to you as an individual.

31. Think of your friends. On a scale from 1 to 5, with 1 being none of your friends, 2 being very few of your friends, 3 some of your friends, 4 most of your friends and 5 being all of your friends, in the year before your arrest, how many of your friends:

	NONE OF THEM	**VERY FEW OF THEM**	**SOME OF THEM**	**MOST OF THEM**	**ALL OF THEM**
Were arrested	☐	☐	☐	☐	☐
Were convicted	☐	☐	☐	☐	☐
Used marijuana	☐	☐	☐	☐	☐
Stole something (less than $5)	☐	☐	☐	☐	☐
Got drunk	☐	☐	☐	☐	☐
Hit someone	☐	☐	☐	☐	☐
Broken into a vehicle	☐	☐	☐	☐	☐
Stole something (more than $50)	☐	☐	☐	☐	☐
Used hard drugs	☐	☐	☐	☐	☐
Used prescription drugs	☐	☐	☐	☐	☐
Pressured someone sexually	☐	☐	☐	☐	☐
Sold drugs	☐	☐	☐	☐	☐

The next set of questions asks about head injuries that you may have had.

32. How many times have you had a head injury resulting in one of the following symptoms?

- Loss of consciousness for more than twenty minutes,
- skull fracture
- excessive bleeding from puncture to the skull
- brain matter protruding from injury location
- non visible brain matter damage from injury (confirmed by professional)]

Enter the number here _____ (if never please enter zero)

33. If the respondent indicates a head injury with one or more of the symptoms described above please check the box below that corresponds to the region of the head where the injury occurred. Also indicate how old the respondent was when the injury occurred. Please select all that apply. If they were injured more than once in a particular region, select a box for each injury and indicate age at each injury)

Forehead	**Top of Skull**	**Left Side**	**Right Side**	**Back**
☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____
☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____
☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____
☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____
☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____	☐☐Age____

34. This next section asks questions about drug use. Please remember that all information is confidential and will not be linked to you in any way. In year prior to your arrest, how often did you use (*)

	Never	Once or Twice a Year	Once a Month	Once Every 2-3 Weeks	2-3 Times a Week	Once a Day	2-3 Times a Day	When did you first use? (age)
Alcohol	☐	☐	☐	☐	☐	☐	☐	☐
Marijuana or Hashish	☐	☐	☐	☐	☐	☐	☐	☐
Hallucinogens	☐	☐	☐	☐	☐	☐	☐	☐
Amphetamines	☐	☐	☐	☐	☐	☐	☐	☐
Cocaine	☐	☐	☐	☐	☐	☐	☐	☐
Heroin	☐	☐	☐	☐	☐	☐	☐	☐
Prescription drugs w/o prescription	☐	☐	☐	☐	☐	☐	☐	☐

Alcoholic beverages: beer, wine and hard liquor
Marijuana or hashish: pot, grass
Hallucinogens: LSD, mushrooms, mescaline,
Cocaine: crack or powder
Amphetamines: including methamphetamines, speed
Heroin: horse, smack

35. Next I'm going to ask you about different types of criminal and antisocial behaviors. Please remember that this information is confidential and will not be linked to you individually in any way. Truthful answers to these questions are very important. Please give your best estimate of the number of times per year you've done each of the following behaviors. During the year before your arrest how many times did you…? (convert number of times per month into correct category, if necessary read categories for clarification)

	Never	Once or Twice	Once every 2-3 Months	Once a Month	Once every 2-3 weeks	Once a week	2-3 times a week	Once a day	2-3 times a day
Purposely damaged or destroyed property that does not belong to you	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used marijuana	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used hard drugs (such as methamphetamines, heroin, cocaine, LSD)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Got into a verbal altercation with a stranger	☐	☐	☐	☐	☐	☐	☐	☐	☐
Stole something worth less than $50	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hit or threatened to hit someone without any reason	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used alcohol	☐	☐	☐	☐	☐	☐	☐	☐	☐
Broken into a vehicle or building to steal something	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gotten into a fight	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sold illegal drugs	☐	☐	☐	☐	☐	☐	☐	☐	☐
Stole something worth more than $50	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gotten drunk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sold or given alcohol to kids under 18	☐	☐	☐	☐	☐	☐	☐	☐	☐

Pressured or forced someone to do more sexually than he/she wanted to do	☐	☐	☐	☐	☐	☐	☐	☐	☐
Attacked someone with the idea of seriously hurting or killing them	☐	☐	☐	☐	☐	☐	☐	☐	☐
Exceeded the speed limit by 10-20 mph	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used force to get money or things from other people	☐	☐	☐	☐	☐	☐	☐	☐	☐
Deliberately injured spouse/partner	☐	☐	☐	☐	☐	☐	☐	☐	☐

In this section you are asked questions about how often you exercise.

36. Prior to your arrest, during a typical week, how many times did you do the following exercises for more than 25 minutes:

	Never	1 – 3	4 – 6	7 – 9	10 or more
Mild exercise that requires minimal effort where **you could easily sing while doing the activity**: yoga, fishing, bowling, golf, leisure walking etc.	□	□	□	□	□
Moderate exercise that is not exhausting where you could easily carry on a conversation while doing the activity: jogging, non-competitive sports, leisure dancing, leisure swimming, etc	□	□	□	□	□
Vigorous exercise where you become winded or too out of breath to carry on a conversation while doing the activity: running, competitive sports games (soccer, football, basketball, etc), energetic dancing swimming laps, etc.	□	□	□	□	□

This last set of questions asks about what you will do when you are released

37. Do you know where you will be staying when you are released?

□ Yes
□ No

If, no please skip to question 39

38. Who will you be staying with when you are released? (you can check more than one box)

□ Roommates/Friends
□ Mother and/or Father (including step-parents)
□ Spouse
□ Girlfriend/boyfriend
□ Children
□ Other relatives
□ Alone
□ Military
□ Half-way house
Other: _____

39. How confident are you in your housing arrangements?

- Very confident
- Confident
- Not very confident
- No confidence at all
- I haven't even thought about it

40. How prepared are you to join the workforce?

- Not prepared at all
- A little prepared
- Somewhat prepared
- Well prepared

41. How confident are you that you will be able to get a job that is satisfying?

- Very confident
- Confident
- Not very confident
- No confidence at all

42. About how many times a month did you talk on the phone with your family (parents, brothers and sisters) while you were in jail?

- I did not talk with them
- Less than once a month
- Once a month
- Twice a month
- Three or more times a month

43. About how many times a month did your significant other (girlfriend/boyfriend, spouse) visit you while you were in jail?

- They did not visit
- Less than once a month
- Once a month
- Twice a month
- Three or more times a month

44. About how many times a month did you talk on the phone with your significant other (girlfriend/boyfriend, spouse) while you were in jail?

- ☐ I did not talk with them
- ☐ Less than once a month
- ☐ Once a month
- ☐ Twice a month
- ☐ Three or more times a month

45. Do you plan on participating in drug treatment after you are released?

- ☐ Yes
- ☐ No

46. Have you already made arrangements to enter drug treatment when you are released?

- ☐ Yes, I have an appointment when I get out
- ☐ Yes, I have made contact with a treatment provider
- ☐ Yes, I have some information about treatment in my area
- ☐ No

47. Do you plan on participating in mental health treatment after you are released?

- ☐ Yes
- ☐ No

48. Have you already made arrangements to participate in mental health treatment when you are released?

- ☐ Yes, I have an appointment when I get out
- ☐ Yes, I have made contact with a treatment provider
- ☐ Yes, I have some information about treatment in my area
- ☐ No

49. What are your chances of going straight and not getting arrested again?

- I will definitely be successful
- I will probably be successful
- There is a small chance I will be successful
- There is no chance I will be successful

50. What are your chances of not using drugs after you are released?

- Good, I will not do drugs again
- Okay, I might start to do drugs again
- Poor, I probably do drugs after I am released

Please answer the following questions. Your responses will be used to gather information about the neighborhood you are returning to. It will not be used to identify you in anyway.

51. What is the zip code of the neighborhood that you will be returning to? _____

52. What are 2 major cross streets near the place that you will be staying?_____

Survey A.2: Survey given to student participants.

Student Survey

Thanks for agreeing to participate in this survey. Remember that all of your answers will be kept confidential. Please answer each of the questions as truthfully as possible.

Section One

1. What is your birth date? _____ (month, day, year)

2. How tall are you? _____

3. What is your weight? _____

4. What is your ☐ Male ☐ Female gender?

5. Which of the following best describes your race/ethnicity? If more than one applies, check each one.

☐	African American	☐	Hawaiian or Pacific Islander
☐	Asian	☐	Hispanic
☐	Caucasian	☐	Other

6. When you were young, who did you live with most of the time (select all that apply)?

☐	One parent	☐	Both parents
☐	Legal guardian(s)	☐	Other

7. What is the highest level of education obtained by your parent or guardian?

☐	Elementary	☐	High School
☐	Junior High	☐	College or beyond

8. What is the average income of your parent's or guardian's household?

☐ Less than $20,000
☐ $20,000-$29,999
☐ $30,000-$39,999
☐ $40,000-$49,999
☐ $50,000-$69,999
☐ $70,000-$99,999
☐ Over $100,000

Section Two - Interpersonal Reactivity Index

9. The next section asks about how you feel in certain situations. Remember all answers are confidential. Please answer truthfully.

	STRONGLY AGREE	AGREE SOMEWHAT	DISAGREE SOMEWHAT	STRONGLY DISAGREE
I often have tender, concerned feelings for people less fortunate than me.	☐	☐	☐	☐
I sometimes find it difficult to see things from the "other guy's" point of view.	☐	☐	☐	☐
I try to look at everybody's side of a disagreement before I make a decision.	☐	☐	☐	☐
When I see someone being taken advantage of, I feel kind of protective towards them.	☐	☐	☐	☐
I sometimes try to understand my friends better by imagining how things look from their perspective.	☐	☐	☐	☐
Other people's misfortunes do not usually disturb me a great deal.	☐	☐	☐	☐
If I'm sure I'm right about something, I don't waste much time listening to other people's arguments.	☐	☐	☐	☐
When I see someone being treated unfairly, I sometimes don't feel much pity for them.	☐	☐	☐	☐
I am often quite touched by the things that I see happen.	☐	☐	☐	☐
I believe that there are two sides to every question and try to look at them both.	☐	☐	☐	☐
I would describe myself as a pretty soft-hearted person.	☐	☐	☐	☐
When I'm upset at someone, I usually try to "put myself in his shoes" for a while.	☐	☐	☐	☐
Before criticizing somebody, I try to imagine how I would feel if I were in their place.	☐	☐	☐	☐

Section Two continued

	STRONGLY AGREE	AGREE SOMEWHAT	DISAGREE SOMEWHAT	STRONGLY DISAGREE
I daydream and fantasize, with some regularity, about things that might happen to me.	☐	☐	☐	☐
I sometimes find it difficult to see things from the "other guy's" point of view.	☐	☐	☐	☐
I really get involved with the feelings of the characters in a novel.	☐	☐	☐	☐
I am usually objective when I watch a movie or play, and I don't often get completely caught up in it.	☐	☐	☐	☐
I try to look at everybody's side of a disagreement before I make a decision.	☐	☐	☐	☐
I sometimes try to understand my friends better by imagining how things look from their perspective.	☐	☐	☐	☐
Becoming extremely involved in a good book or movie is somewhat rare for me.	☐	☐	☐	☐
If I'm sure I'm right about something, I don't waste much time listening to other people's arguments.	☐	☐	☐	☐
After seeing a play or movie, I have felt as though I were one of the characters.	☐	☐	☐	☐
I believe that there are two sides to every question and try to look at them both.	☐	☐	☐	☐
When I watch a good movie, I can very easily put myself in the place of a leading character.	☐	☐	☐	☐
When I'm upset at someone, I usually try to "put myself in his shoes" for a while.	☐	☐	☐	☐
When I am reading an interesting story or novel, I imagine how I would feel if the	☐	☐	☐	☐

events in the story were happening to me.				
Before criticizing somebody, I try to imagine how I would feel if I were in their place.	☐	☐	☐	☐

Section Three

10. How important has your relationship with your parents been to you?

☐ Very Important ☐ Pretty Important ☐ Somewhat Important ☐ Not too Important ☐ Not at all Important
☐ Not applicable

11. How much warmth and affection do you get from your parents?

☐ A Great Deal ☐ Quite a Bit ☐ Some ☐ Not too Much ☐ Very Little
☐ Not applicable

12. Think over your relationship with your parents in the past year. How much stress or pressure had there been in your relationship?

☐ A Great Deal ☐ Quite a Bit ☐ Some ☐ Not too Much ☐ Very Little
☐ Not applicable

13. How much support and encouragement have you received from your parents?

☐ A Great Deal ☐ Quite a Bit ☐ Some ☐ Not too Much ☐ Very Little
☐ Not applicable

14. Overall, how satisfied have you been with your relationship with your parents?

☐ Very Satisfied ☐ Somewhat Satisfied ☐ Neither Satisfied or Dissatisfied ☐ Somewhat Dissatisfied ☐ Very Dissatisfied
☐ Not applicable

Section Four - Levenson Self-report Psychopathy Scale

15. Listed below are a number of statements. Each represents a commonly held opinion and there are no right or wrong answers. You will probably disagree with some items and agree with others. Please read each statement carefully and circle the number which best describes the extent to which you agree or disagree with each statement, or the extent to which each statement applies to you.

	STRONGLY AGREE	AGREE SOMEWHAT	DISAGREE SOMEWHAT	STRONGLY DISAGREE
I am often bored.	☐	☐	☐	☐
In today's world, I feel justified in doing anything I can get away with to succeed.	☐	☐	☐	☐
Before I do anything, I carefully consider the possible consequences	☐	☐	☐	☐
My main purpose in life is getting as many goodies as I can.	☐	☐	☐	☐
I quickly lose interest in tasks I start.	☐	☐	☐	☐
I have been in a lot of shouting matches with other people.	☐	☐	☐	☐
Even if I were trying very hard to sell something, I wouldn't lie about it.	☐	☐	☐	☐
I find myself in the same kinds of trouble, time after time.	☐	☐	☐	☐
I enjoy manipulating other people's feelings.	☐	☐	☐	☐
I find that I am able to pursue one goal for a long time.	☐	☐	☐	☐
Looking out for myself is my top priority.	☐	☐	☐	☐
I tell other people what they want to hear so that they will do what I want them to do.	☐	☐	☐	☐

Cheating is not justifiable because it is unfair to others.	☐	☐	☐	☐
Love is overrated.	☐	☐	☐	☐

Section Four continued

	STRONGLY AGREE	AGREE SOMEWHAT	DISAGREE SOMEWHAT	STRONGLY DISAGREE
I would be upset if my success came at someone else's expense.	☐	☐	☐	☐
When I get frustrated, I often "let off steam" by blowing my top.	☐	☐	☐	☐
For me, what's right is whatever I can get away with.	☐	☐	☐	☐
Most of my problems are due to the fact that other people just don't understand me.	☐	☐	☐	☐
Success is based on survival of the fittest; I am not concerned about the losers.	☐	☐	☐	☐
I don't plan anything very far in advance.	☐	☐	☐	☐
I feel bad if my words or actions cause someone else to feel emotional pain.	☐	☐	☐	☐
Making a lot of money is my most important goal.	☐	☐	☐	☐
I let others worry about higher values; my main concern is with the bottom line.	☐	☐	☐	☐
I often admire a really clever scam.	☐	☐	☐	☐
People who are stupid enough to get ripped off usually deserve it.	☐	☐	☐	☐

I make of point of trying not to hurt others in pursuit of my goals.	☐	☐	☐	☐

Section Five

Please read the following short story carefully. After reading the story, respond to the questions that follow it by answering what you think **would** happen to you or what you **would** do in the situation, not what you think **should** happen or what you think you **should** do. Please continue.

Short story one

Chris is in a very crowded bar on a Friday night. While he is talking with his girlfriend, another guy tries to squeeze by and spills some beer out of a pitcher onto Chris's girlfriend. The guy that spilt the beer laughs and pretends to wipe beer off Chris's girlfriend's arms and chest. She is obviously distressed and does not like what is happening. The guy that spilt the beer then turns to Chris and says "what are you looking at?". Chris pushes him and a fight breaks out.

Below circle the number that corresponds to what you would do in the situation described above.

16. What is the likelihood that you would do what Chris did in the situation described above?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

17. What is the chance you would be arrested by the police if you did what Chris did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

18. How much of a problem would it create in your life if you were arrested for doing what Chris did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

19. What is the chance you would be convicted if you did what Chris did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

20. How much of a problem would it create in your life if you were convicted for doing what Chris did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

21. Would you feel a sense of guilt or shame if you did what Chris did under the same circumstances?

☐ No ☐ Yes

22. How much of a problem would guilt or shame be if you did what Chris did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

23. How much fun would it be to do what Chris did in the situation described above?

0	1	2	3	4	5	6	7	8	9	10
No fun at all										A great deal of fun

Short story two

William lives in an apartment building. One afternoon he is helping with the laundry. He walks down to the laundry room, where there is a group of washers and dryers in one room and a separate room with a table to fold clothes. William notices that there is a pile of clothes on the table. As he starts to put his laundry in the washer, he notices $40 sticking out of the pocket of a pair of pants in the pile of clothes on the table. There is no one else in the laundry room. When he is done loading his laundry in the washer, William takes the $40 and leaves immediately.

24. What is the likelihood that you would do what William did in the situation described above?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

25. What is the chance you would be arrested by the police if you did what William did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

26. How much of a problem would it create in your life if you were arrested for doing what William did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

27. What is the chance you would be convicted if you did what William did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

28. How much of a problem would it create in your life if you were convicted for doing what William did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

29. Would you feel a sense of guilt or shame if you did what William did under the same circumstances?

□No □Yes

30. How much of a problem would guilt or shame be if you did what William did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

31. How much fun would it be to do what William did in the situation described above?

0	1	2	3	4	5	6	7	8	9	10
No fun at all										A great deal of fun

Short story three

Michael is out with some friends at a local bar. By the end of the evening, he is pretty sure his blood alcohol level is above the legal limit. He lives about 10 miles from the bar and has to be at work early the next morning. If he leaves his car at the bar, he will have to find some way to return early the next morning to pick it up. Michael decides to drive home.

32. What is the likelihood that you would do what Michael did in the situation described above?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

33. What is the chance you would be arrested by the police if you did what Michael did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

34. How much of a problem would it create in your life if you were arrested for doing what Michael did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

35. What is the chance you would be convicted if you did what Michael did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No chance at all										100 percent chance

36. How much of a problem would it create in your life if you were convicted for doing what Michael did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

37. Would you feel a sense of guilt or shame if you did what Michael did under the same circumstances?

□No □Yes

38. How much of a problem would guilt or shame be if you did what Michael did under the same circumstances?

0	1	2	3	4	5	6	7	8	9	10
No problem at all										Very big problem

39. How much fun would it be to do what Michael did in the situation described above?

0	1	2	3	4	5	6	7	8	9	10
No fun at all										A great deal of fun

Section Six

40. Has either of your parents ever been arrested?

□ Yes □ No

41. If yes, how many times?

□ Once □ Twice □ Three Times □ Four or More Times

42. Has either of your parents ever been convicted?

☐ Yes ☐ No

43. If yes, how many times?

☐ Once ☐ Twice ☐ Three Times ☐ Four or More Times

44. In the past year, have your parents used illegal drugs?

☐ Yes ☐ No

45. If yes how many times?

☐ Once or Twice ☐ Monthly ☐ Weekly ☐ A couple of times a week ☐ Daily

Section Seven

Below you are asked some questions about your friends' behavior over the last year. Please think carefully and provide the best answer for each question.

46. Think of your friends. In the past year, how many of your friends:

	ALL OF THEM	MOST OF THEM	SOME OF THEM	VERY FEW OF THEM	NONE OF THEM
Were arrested	☐	☐	☐	☐	☐
Were convicted	☐	☐	☐	☐	☐
Used drugs	☐	☐	☐	☐	☐
Stole something (less than $20)	☐	☐	☐	☐	☐
Got drunk	☐	☐	☐	☐	☐
Hit someone	☐	☐	☐	☐	☐
Broke into a vehicle	☐	☐	☐	☐	☐
Stole something (more than $20)	☐	☐	☐	☐	☐
Pressured someone sexually	☐	☐	☐	☐	☐
Sold drugs	☐	☐	☐	☐	☐
Used force or threat of force to get money or things from other people	☐	☐	☐	☐	☐
Purposely damaged or destroyed property that did not belong to them	☐	☐	☐	☐	☐

Section Eight

47. How much time do you spend studying?

☐ A great deal ☐ Quite a bit ☐ Some ☐ Not too much ☐ Very little

48. How important is schoolwork to you?

☐ Very Important ☐ Pretty Important ☐ Somewhat Important ☐ Not too Important ☐ Not Important at all

49. What is your grade point average?

☐ Mostly A's Excellent ☐ Mostly B's ☐ Mostly C's Satisfactory/ Passing ☐ Mostly D's ☐ Mostly F's Not Satisfactory/ Failing

50. How important is it to you to do well in hard subjects?

☐ Very Important ☐ Pretty Important ☐ Somewhat Important ☐ Not too Important ☐ Not Important at all

Section Nine - Reactive –Proactive Aggression Questionnaire

There are times when most of us feel angry, or have done things we should not have done. Don't spend a lot of time thinking about the items – just give your first response.

How often have you…	NEVER	HARDLY EVER	SOMETIMES	OFTEN	ALWAYS OR ALMOST ALWAYS
Yelled at others when they have annoyed you	☐	☐	☐	☐	☐
Had fights with others to show who was on top	☐	☐	☐	☐	☐
Reacted angrily when provoked by others	☐	☐	☐	☐	☐
Taken things from other students	☐	☐	☐	☐	☐
Had temper tantrums	☐	☐	☐	☐	☐
Vandalized something for fun	☐	☐	☐	☐	☐
Damaged things because you felt mad	☐	☐	☐	☐	☐
Had a gang fight to be cool	☐	☐	☐	☐	☐
Gotten angry when frustrated	☐	☐	☐	☐	☐
Hurt others to win a game	☐	☐	☐	☐	☐

Become angry or mad when you lost a game	☐	☐	☐	☐	☐
Used physical force to get others to do what you want	☐	☐	☐	☐	☐
Threatened and bullied someone	☐	☐	☐	☐	☐
Gotten angry when others threatened you	☐	☐	☐	☐	☐
Used force to obtain money or things from others	☐	☐	☐	☐	☐
Damaged things because you felt angry	☐	☐	☐	☐	☐
Made obscene phone calls for fun	☐	☐	☐	☐	☐
Felt better after hitting or yelling at someone	☐	☐	☐	☐	☐
Threatened or forced someone to have sex	☐	☐	☐	☐	☐
Gotten angry or mad when you lost a game	☐	☐	☐	☐	☐
Hit others to defend yourself	☐	☐	☐	☐	☐
Carried a weapon to use in a fight	☐	☐	☐	☐	☐
Gotten angry or mad or hit others when teased	☐	☐	☐	☐	☐

Section Nine continued

How often have you…	**NEVER**	**HARDLY EVER**	**SOMETIMES**	**OFTEN**	**ALWAYS OR ALMOST ALWAYS**
Gotten others to gang up on someone else	☐	☐	☐	☐	☐
Set fire to things because you felt angry	☐	☐	☐	☐	☐
Yelled at others so they would do things for you	☐	☐	☐	☐	☐

Section Ten

51. Are you currently married? ☐ Yes ☐ No
52. In the past year, were you in a relationship? ☐ Yes ☐ No

If you were not in a relationship, please proceed to the next section.

53. In the past year, how many months did you live with your (spouse/girlfriend/boyfriend)?

_____

54. How important is your relationship with your (spouse/girlfriend/boyfriend) to you?

☐ Not important at all ☐ Not too important ☐ Somewhat important ☐ Pretty important ☐ Very important

55. How much warmth and affection do you get from your _____ (spouse/boyfriend/girlfriend)?

☐ Very little ☐ Not too much ☐ Some ☐ Quite a bit ☐ A great deal

Section Eleven

56. Below you are asked to indicate how many times in the past year you have done a number of different things. Remember that all your answers are confidential. Please give your best estimate of the exact number of times you've done each thing during the last year.

In the past year, how many times have you…

	Never	Once or Twice	Once every 2-3 Months	Once a Month	Once every 2-3 weeks	Once a week	2-3 times a week	Once a day	2-3 times a day
Purposely damaged or destroyed property that does not belong to you	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used marijuana	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used hard drugs (such as methamphetamines, heroin, cocaine, LSD)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Got into a verbal altercation with a stranger	☐	☐	☐	☐	☐	☐	☐	☐	☐
Stole something worth less than $50	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hit or threatened to hit someone without any reason	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used alcohol	☐	☐	☐	☐	☐	☐	☐	☐	☐
Broken into a vehicle or building to steal something	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gotten into a fight	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sold illegal drugs	☐	☐	☐	☐	☐	☐	☐	☐	☐
Stole something worth more than $50	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gotten drunk	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sold or given alcohol to kids under 18	☐	☐	☐	☐	☐	☐	☐	☐	☐

Pressured or forced someone to do more sexually than he/she wanted to do	☐	☐	☐	☐	☐	☐	☐	☐	☐

Section Eleven continued

In the past year, how many times have you…

	Never	Once or Twice	Once every 2-3 Months	Once a Month	Once every 2-3 weeks	Once a week	2-3 times a week	Once a day	2-3 times a day
Attacked someone with the idea of seriously hurting or killing them	☐	☐	☐	☐	☐	☐	☐	☐	☐
Exceeded the speed limit by 10-20 mph	☐	☐	☐	☐	☐	☐	☐	☐	☐
Used force to get money or things from other people	☐	☐	☐	☐	☐	☐	☐	☐	☐
Deliberately injured spouse/partner	☐	☐	☐	☐	☐	☐	☐	☐	☐

Section Twelve - List of Threatening Experiences Questionnaire

57. During the past 12 months, have any of the following events occurred:

	YES	NO
You suffered a serious illness, injury or an assault	☐	☐
A serious illness, injury or assault happened to a close relative, partner/spouse, or friend	☐	☐
Your parent, child or partner/spouse died	☐	☐
A close friend or other relative (aunt, cousin, grandparent) died	☐	☐
You had serious relationship difficulties with your partner/spouse	☐	☐
You broke off a steady relationship	☐	☐
You had a serious problem with a close friend, neighbor, or relative	☐	☐
You had serious difficulties at work	☐	☐
You were fired from your job	☐	☐
You became unemployed or you were seeking work unsuccessfully for more than one month	☐	☐
You had a major financial crisis	☐	☐
You had problems with the police or a court appearance	☐	☐
Something you valued was lost or stolen	☐	☐
You were living in unpleasant surroundings	☐	☐
You were informed of having a sexually transmitted disease	☐	☐

Section Thirteen

The next section asks some questions about tobacco. If you have not smoked or used other tobacco products in the last 30 days please proceed to the next section.

58. In the last 30 days about how many days did you smoke cigarettes?

☐ Did not smoke cigarettes ☐ 1 – 2 days ☐ 3 – 6 days ☐ 7 – 11 days ☐ 12 – 21 days ☐ 21 + days

59. On average, about how many cigarettes do you usually smoke on these days?

☐ 1 to 10 ☐ 11 – 20 ☐ 21 – 40 (1-2 packs) ☐ More than 2 packs a day

Not Applic

60. How old were you when you first started smoking cigarettes fairly regularly?_____

Not Applica

61. In the last 30 days about how many days did you use tobacco products other than cigarettes?

☐ Did not use other tobacco products ☐ 1 – 2 days ☐ 3 – 6 days ☐ 7 – 11 days ☐ 12 – 21 days ☐ 21 + days

62. On average, how many times a day do you use this tobacco product on these days?

☐ 1 – 10 ☐ 11 – 20 ☐ 21 – 40 ☐ More than 41

Not Applic

63. How old were you when you first started using this tobacco product fairly regularly?_____

Not Applica

Section Fourteen

64. For each of the statements below, choose the response that best describes your parents or primary guardians when you were growing up. Try to think about your parents or guardians as a whole and choose the statement that best describes them together.

	DESCRIBES THEM WELL	SOMEWHAT DESCRIBES THEM	DOES NOT REALLY DESCRIBE THEM	DOES NOT DESCRIBE THEM AT ALL
My parents were affectionate	☐	☐	☐	☐
My parents were verbally abusive	☐	☐	☐	☐
My parents had clear rules for my behavior	☐	☐	☐	☐
My parents were emotionally distant	☐	☐	☐	☐
My parents praised me when I did something right	☐	☐	☐	☐
My parents loved me	☐	☐	☐	☐
My parents never knew who I was hanging out with	☐	☐	☐	☐
My parents consistently enforced the rules	☐	☐	☐	☐
My parents were physically abusive	☐	☐	☐	☐

Section Fifteen- Center for Epidemiologic Studies—Depression inventory

65. These questions will ask about how you feel emotionally and about how you feel in general. How often was each of the following things true during the past week?

	NEVER OR RARELY	SOMETIMES	A LOT OF THE TIME	MOST/ALL OF THE TIME
You were bothered by things that don't usually bother you	☐	☐	☐	☐
You felt that you could not shake off the blues even with help from your family and your friends	☐	☐	☐	☐

You felt that you were just as good as other people	☐	☐	☐	☐
You had trouble keeping your mind on what you were doing	☐	☐	☐	☐

Section Fifteen continued

How often was each of the following things true during the past week?

	NEVER OR RARELY	SOMETIMES	A LOT OF THE TIME	MOST/ALL OF THE TIME
You felt depressed	☐	☐	☐	☐
You felt that you were too tired to do things	☐	☐	☐	☐
You felt sad	☐	☐	☐	☐
You enjoyed life	☐	☐	☐	☐
You felt that people disliked you	☐	☐	☐	☐
You didn't feel like eating, your appetite was poor	☐	☐	☐	☐
You felt hopeful about the future	☐	☐	☐	☐
You thought your life had been a failure	☐	☐	☐	☐
You were fearful	☐	☐	☐	☐
You talked less than usual	☐	☐	☐	☐
You felt lonely	☐	☐	☐	☐
People were unfriendly to you	☐	☐	☐	☐
You were happy	☐	☐	☐	☐
It was hard to get started doing things	☐	☐	☐	☐

You felt life was not worth living	☐	☐	☐	☐

Section Sixteen

In the section below you are asked about how morally wrong certain actions are. Remember that your answers cannot be linked to you. Please think carefully and provide the best possible answer.

66. How wrong is it for someone your age to ....

	VERY WRONG	WRONG	A LITTLE BIT WRONG	NOT WRONG AT ALL
Destroy property	☐	☐	☐	☐
Use marijuana	☐	☐	☐	☐
Steal something	☐	☐	☐	☐
Hit someone	☐	☐	☐	☐
Break into a vehicle	☐	☐	☐	☐
Sell drugs	☐	☐	☐	☐
Get drunk	☐	☐	☐	☐
Use force or threat of force to get money or things from other people	☐	☐	☐	☐
Deliberately injure spouse/partner	☐	☐	☐	☐
Purposely damaged or destroyed property that did not belong to you	☐	☐	☐	☐

Section Seventeen – Grasmick et al self control

67. In this section you are asked some questions about how you approach certain situations. Remember that all your answers are confidential. Please check the box that most accurately describes your feelings regarding each statement.

	STRONGLY AGREE	AGREE SOMEWHAT	DISAGREE SOMEWHAT	STRONGLY DISAGREE
I often act on the spur of the moment without stopping to think	☐	☐	☐	☐
I don't devote much thought and effort to preparing for the future	☐	☐	☐	☐

I often do whatever brings me pleasure here and now, even at the cost of some distant goal	☐	☐	☐	☐
I'm more concerned with what happens to me in the short run than in the long run	☐	☐	☐	☐
I frequently try to avoid projects that I know will be difficult	☐	☐	☐	☐
When things get complicated, I tend to quit or withdraw	☐	☐	☐	☐
The things in life that are easiest to do bring me the most pleasure	☐	☐	☐	☐

Section Seventeen Continued

	STRONGLY AGREE	**AGREE SOMEWHAT**	**DISAGREE SOMEWHAT**	**STRONGLY DISAGREE**
I dislike really hard tasks that stretch my abilities to the limit	☐	☐	☐	☐
I like to test myself every now and then by doing something a little risky	☐	☐	☐	☐
Sometimes I will take a risk just for the fun of it	☐	☐	☐	☐
I sometimes find it exciting to do things for which I might get in trouble	☐	☐	☐	☐
Excitement and adventure are more important to me than security	☐	☐	☐	☐
If I had a choice, I would almost always rather do something physical than something mental	☐	☐	☐	☐
I almost always feel better when I am on the move than when I am sitting and thinking	☐	☐	☐	☐
I like to get out and do things more than I like	☐	☐	☐	☐

to read or contemplate ideas				
I seem to have more energy and a greater need for activity than most other people my age	☐	☐	☐	☐
I try to look out for myself first, even if it means making things difficult or other people	☐	☐	☐	☐
I'm not very sympathetic to other people when they are having problems.	☐	☐	☐	☐
If things I do upset people, it's their problem not mine	☐	☐	☐	☐
I will try to get the things I want even when I know it's causing problems for other people	☐	☐	☐	☐
I lose my temper pretty easily	☐	☐	☐	☐
Often, when I'm angry at people I feel more like hurting them than talking to them about why I am angry	☐	☐	☐	☐
When I'm really angry, other people better stay away from me	☐	☐	☐	☐
When I have a serious disagreement with someone, it's usually hard for me to talk calmly about it without getting upset	☐	☐	☐	☐

Section Eighteen – Adapted from Social Support Questionnaire (SSQ) (Sarason, Levine, Basham, & Sarason, 1983)

68. Below please indicate how satisfied are you with the amount of:

	1	**2**	**3**	**4**	**5 COMPLETELY**

	COMPLETELY DISSATISFIED		NEITHER SATISFIED OR DISSATISFIED		SATISFIED
Practical support you receive from your spouse/partner	☐	☐	☐	☐	☐
Practical support you receive your family (besides spouse/partner)	☐	☐	☐	☐	☐
Practical support you receive from your friends	☐	☐	☐	☐	☐
Emotional support you receive from your spouse/partner	☐	☐	☐	☐	☐
Emotional support you receive from your family (besides spouse/partner)	☐	☐	☐	☐	☐
Emotional support you receive from your friends	☐	☐	☐	☐	☐

Section Nineteen

69. In this section you are asked questions about experiences in intimate relationships.

In the past, has a dating partner or spouse EVER …	**No**	**Yes**	**If yes, how old were you?**
Physically abused you (including kicking, slapping, hitting, punching, pushing, shoving, choking, throwing an object at you, etc.).	☐	☐	
I did this to my dating partner	☐	☐	
Psychologically / emotionally / mentally abused you (including threatening to hurt you, insulting you, cursing, preventing you from seeing friends/family, monitoring whereabouts or phone calls, shouting, yelling, etc.).	☐	☐	
I did this to my dating partner	☐	☐	

Sexually abused me (including using force to make you have vaginal, anal, and/or oral sex, forcing you to have sex without a condom, insisting on sex when you did not want it without using force, etc.).	□	□	
I did this to my dating partner	□	□	

Section Twenty

70. In this section you are asked questions about victimization experiences in intimate relationships.
1.
2.The following section asks you about **frightening or harassing things** someone may have done to you. Not including bill collectors, telephone solicitors, or other sales people, please indicate if anyone, male or female, has ever done any of these things to you. You may have experienced the following behaviors from strangers, friends, former boyfriends/girlfriends, or acquaintances, etc.
3.

Has ANYONE EVER… (select all that apply)	**No**	**Yes**	**Yes, more than once**
Followed, watched, or spied on you	□	□	□
Sent you unsolicited letters or written correspondence	□	□	□
Made unwanted phone calls to you	□	□	□
Left unwanted messages for you (including text messages)	□	□	□
Stood outside your home, school, or workplace	□	□	□
Showed up at places uninvited	□	□	□
Left unwanted items for you to find	□	□	□
Tried to communicate with you in other ways against your will	□	□	□
Vandalized your property or destroyed something you loved	□	□	□
Sent unwanted messages electronically (via email, instant messaging, etc.)	□	□	□
Posted unwanted messages/pictures to internet websites (such as Facebook, Myspace, etc.)	□	□	□

71. What was your relationship with the person who did these things to you? (Select only one at this time, circle the number that corresponds to the appropriate response)

1. Your current boyfriend
2. Your current girlfriend
3. An ex boyfriend

4. An ex girlfriend
5. Someone I've dated but not had a relationship with
6. A spouse
7. A friend
8. A family member
9. An acquaintance
10. A stranger
11. A co-worker
12. A classmate
13. Don't know/other

Not Applica

72. How old were you when these incidents began? _____ years old

Not Applica

73. Approximately how long did these incidents last? ____ months

Not Applica

74. Did you ask this person to stop these behaviors? (please circle the appropriate response)

1 Yes 2 No

Not Applica

75. Did you ever report any of these above behaviors committed by this person to any of the following: (circle the numbers, mark all that apply)

0 Did not report to anyone
1 Police
2 Victim services
3 Local Police Department Victim Services
4 Friend(s)
5 Family member(s)
6 Other official
7 Attorney
8 Someone else

Not Applica

Section Twenty One

76. In this section you are asked questions about <u>your own behavior</u>.
4.

5.The following section asks you about **frightening or harassing things** that you may have done to someone else. Not including bill collectors, telephone solicitors, or other sales people, please indicate if have done any of these things to another person, including strangers, current or former boyfriends/girlfriends, or acquaintances.

Have you ever… (Select all that apply)	**No**	**Yes**	**Yes, more than once**
Followed, watched, or spied on someone	☐	☐	☐
Sent someone unsolicited letters or written correspondence	☐	☐	☐
Made unwanted phone calls to someone	☐	☐	☐
Left unwanted messages for someone (including text messages)	☐	☐	☐
Stood outside someone's home, school, or workplace	☐	☐	☐
Showed up at places uninvited	☐	☐	☐
Left unwanted items for someone to find	☐	☐	☐
Tried to communicate with someone in other ways against their will	☐	☐	☐
Vandalized someone's property or destroyed something they loved	☐	☐	☐
Sent unwanted messages electronically (via email, instant messaging, etc.)	☐	☐	☐
Posted unwanted messages/pictures to internet websites (such as Facebook, Myspace, etc.)	☐	☐	☐

77. What was your relationship with the person who you did these things to? (Select only one at this time)

1 Your current boyfriend

2 Your current girlfriend

3 An ex boyfriend

4 An ex girlfriend

5 Someone I've dated but not had a relationship with

6 A spouse

7 A friend

8 A family member

9 An acquaintance

10 A stranger

11 A co-worker

12 A classmate

13 Don't know/other

Not Applica

78. How old were you when these incidents began? _____ years old

Not Applica

79. How long did these incidents last? ____ months

Not Applica

80. Did the person ever ask you to stop these behaviors? (please circle the appropriate response)

1 Yes 2 No

81. Did you stop these behaviors? (please circle the appropriate response)

Not Applica

1 Yes 2 No

Not Applica

Section Twenty-Two

Below there are some general questions about your use of social media, your feelings, and the structure of your family.

83. Do you have an account on a social networking site like Facebook or Myspace?

YesNo

84. If you answered yes to the above question, how many times a day do you check social networking cites? (Circle the answer that is the best choice).

Noneabout 2 or 34 or 5 timesbetween 6 and 10between 10 and 20more than 20
times a daya daytimes a daytimes a daytimes a day

85. How important are social networking sites to you?

Not important SomewhatImportantVery
at allimportantimportant

86. If you use Facebook, about how many 'Facebook' friends do you have. _____

87. On a scale of one to ten with ten being extremely happy and one being very unhappy, please rate how happy you are: _____

88. Please Indicate the extent to which you agree with the following statements (check the appropriate box):

	I DON'T AGREE AT ALL	I AGREE A LITTLE	I AGREE SOMEWHAT	I COMPLETELY AGREE
I like interacting with others.	☐	☐	☐	☐
When I don't have a lot of information I'm good at making 'instinctive' decisions	☐	☐	☐	☐
I am the kind of person that gets things done.	☐	☐	☐	☐
My happiness is tied to the happiness of those around me.	☐	☐	☐	☐
I worry about what others think of me.	☐	☐	☐	☐
If I have something to do, it bothers me if I can't get it done.	☐	☐	☐	☐

I am a social person.	□	□	□	□
I'm good a seeing 'the big picture'.	□	□	□	□
I have good insight into what motivates people.	□	□	□	□

89. Overall, how content are you with the way that your life is going?
Not content
A little Content
Very content
at allcontent

90. If someone asked your friends about your own personal happiness, how happy would your friends say you are? Please indicate what your friends would say on a scale of one to ten, with ten being extremely happy and one being very unhappy. _____

91. How often do you delay going to the restroom in order to finish a task that you are working on?
Never
Rarely
Sometimes
Often

Table A.8:

Surface area differences in the left hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
GAD	0.027198	0.031762	0.021862	0.016176	0.018717	0.008139	0.015484	0.057597	0.066969	0.087627	0.002935
HC	0.026967	0.031478	0.022344	0.016157	0.018657	0.008127	0.015542	0.058083	0.066246	0.085993	0.003012
PC	0.026901	0.031729	0.022662	0.016266	0.018749	0.008249	0.015710	0.057513	0.067604	0.086448	0.002933

Table A.9:

P-values for surface area differences in left hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
HC v GAD	0.6208	0.3202	0.0721	0.9094	0.7755	0.8580	0.8739	0.4558	0.4384	0.0470	0.0880
PC v GAD	0.5339	0.9211	0.0053	0.7051	0.9326	0.3952	0.4410	0.8857	0.4689	0.1459	0.9639
PC v HC	0.8872	0.4121	0.2652	0.6187	0.7228	0.2807	0.5672	0.3568	0.1172	0.5755	0.0920

Table A.10:

Surface area differences in the right hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
GAD	0.026844	0.031478	0.022817	0.018512	0.015893	0.009680	0.017790	0.058205	0.070650	0.086301	0.003589
HC	0.025832	0.031626	0.023060	0.018403	0.015751	0.009911	0.017784	0.058711	0.070174	0.084611	0.003544
PC	0.026584	0.031994	0.023281	0.018472	0.015744	0.009897	0.017777	0.057281	0.071547	0.084800	0.003579

Table A.11:

P-values for surface area differences in right hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
HC v GAD	0.0260	0.8475	0.3809	0.6199	0.5323	0.1374	0.9868	0.3747	0.5967	0.0647	0.4178
PC v GAD	0.6213	0.1513	0.0508	0.8839	0.6067	0.1308	0.9699	0.1346	0.3458	0.0855	0.8522
PC v HC	0.1056	0.2021	0.3153	0.7546	0.9596	0.9899	0.9822	0.0166	0.1257	0.8500	0.5415

Table A.12:

Thickness differences in left hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
GAD	1.03662	1.07020	1.01343	0.93230	1.06054	1.11861	1.02292	0.99599	0.99322	1.10419	1.10466
HC	1.04929	1.07519	1.02472	0.92756	1.07428	1.12700	1.04708	0.99056	1.00722	1.12611	1.12920
PC	1.04042	1.07592	1.00756	0.92378	1.06519	1.11678	1.03283	0.98952	1.00066	1.10706	1.11167

Table A.13:

P-values for thickness differences in left hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
HC v GAD	0.0326	0.3923	0.1126	0.5064	0.0127	0.2941	0.0001*	0.3558	0.0013	0.0000*	0.0713
PC v GAD	0.4756	0.3236	0.3791	0.2127	0.3710	0.8297	0.0771	0.2779	0.1077	0.5211	0.5730
PC v HC	0.1098	0.8928	0.0059	0.5794	0.0830	0.2213	0.0207	0.8644	0.1373	0.0000*	0.1887

* indicates significance after correction for multiple comparisons is applied

Table A.14: Thickness differences in the right hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
GAD	1.01895	1.06673	1.00872	0.94353	1.05437	1.10825	1.02668	0.98266	0.97861	1.08822	1.08278
HC	1.03627	1.06663	1.01529	0.94328	1.06190	1.12190	1.03461	0.97788	0.99098	1.11160	1.10339
PC	1.02950	1.05983	1.00183	0.93464	1.05615	1.10752	1.02989	0.98149	0.98167	1.09897	1.09920

Table A.15:

P-values for thickness differences in right hemisphere.

	Caudal middle frontal	Lateral orbitofrontal	Medial orbitofrontal	Paracentral	Pars opercularis	Pars orbitalis	Pars triangularis	Precentral	Rostral middle frontal	Superior frontal	Frontal pole
HC v GAD	0.0006*	0.9853	0.2978	0.9681	0.1822	0.0773	0.1952	0.3726	0.0061	0.0000*	0.1484
PC v GAD	0.0424	0.2187	0.2804	0.1825	0.7294	0.9203	0.5813	0.8235	0.5187	0.0275	0.2561
PC v HC	0.1870	0.2603	0.0156	0.1929	0.2763	0.0776	0.4417	0.5191	0.0397	0.0099	0.7558

*indicates significance after correction for multiple comparisons is applied.

VITA

Elizabeth Chesna

Education

Sam Houston State University, Huntsville, TX *(2014-2019)*
- PhD in Forensic Science

Austin Community College, Austin, TX *(2013-Present)*
- EMT-B, National certification

Concordia University Texas, Austin, TX *(2009-2013)*
- B.S. in Biology, Minor in Behavioral Sciences
- Graduated Cum Laude: May 2013

Work Experience

Animal Annex, Sam Houston State University *(2016-Present)*
- Lead graduate student in development of mice behavior unit for the university
- Experience with ANY-maze tracking software
- Handling of mice, temporary anesthetization with isoflurane, and oxytocin delivery via nasal inhalation
- Administration of behavioral trials including open field assessment, resident intruder test, and three-chambered sociability apparatus

Doctoral Teaching Fellowship, Sam Houston State University *(2017-Present)*
- Taught undergraduate course FORS3366 Introduction to Forensic Science

Baylor College of Medicine *(2017-Present)*
- Collaborative research with Department of Psychiatry

Graduate Assistant, Sam Houston State University, Huntsville, TX *(2016-Present)*
- Aided in laboratory preparation, inventory, administrative duties, and troubleshooting instruments
- Teaching Assistant for Forensic Biology Lab

Internship- Austin Police Department, DNA Section *(2015)*
- Validation of Quantifiler® Trio, PowerPlex Fusion, Genemapper® IDX, and STRmix
- Extractions on QIAcube (lysis and purification), quantitation on the 7500 with Quantifiler® Trio, amplification on the 9700 with PowerPlex® Fusion, and sample runs on 3130

- Set-up for quantification, amplification, and capillary electrophoresis using the QIAgility
- New QIAgility scripts plus normalization on the instrument
- Experience with Genemapper® IDX software

Academic Coach/Tutor, Concordia University Texas, Austin, TX *(2012-2013)*
- Provided writing assistance for many of the English and research classes
- Tutored in mathematics, psychology, communications, physics, and biological sciences

Teacher Assistant, Concordia University T2012-May 2013
- Graded papers and organized classwork

Training

- IACUC Training (2016)
- Critical Incident Management Training (2013)
- PHI Air Medical Landing Zone Safety Course (2013)
- OSHA Bloodborne Pathogen Training (2014)
- The Ethics of Stewardship and the Stewardship of Ethics (2015)
- American Heart Association CPR for Healthcare Providers (2013-Present)

Grants

- Co-Investigator for Enhancement Research Grant (Sam Houston State University)
  - *"Influence of Regulation of Oxytocin on Social Behavior."* David Gangitano, PhD (Principal Investigator), Elizabeth Chesna, BS (Co-Principal Investigator)

Publications

- Submitted to Science & Justice:
  - Elizabeth Chesna,[1] B.S. and Charity Beherec,[1] M.S.; Rachel Houston,[1] PhD; Jessica Wells,[2] PhD; Danielle Boisvert,[2] PhD; Todd Armstrong,[2] PhD; and David Gangitano,[1] PhD. *"Development of a Behavioral Genetics Panel Using Massively Parallel Sequencing."*

- Drafted for Aggressive Behavior:
  - Elizabeth Chesna, B.S.; Todd Armstrong, PhD; Jessica Roberts, M.S.; Peyton Howell, M.S.; Shawn Keller, M.S; Charity Beherec, M.S; Ryan Gutierrez, B.S.; Stephen White, PhD; Bobby LaRue, PhD; Rachel Houston, PhD; Danielle Boisvert, PhD; Ramiro Salas, PhD; and David Gangitano, PhD. *"Sequence variation in genes affecting dopamine turnover and oxytocin in a sample of male inmates."*

Presentations

- Elizabeth Chesna, BS1, Todd Armstrong, PhD2, Peyton Howell, MS1, Shawn Keller, MS1, Charity Beherec, MS1, Danielle Boisvert, PhD2, Ramiro Salas, PhD3, and David Gangitano, PhD1, "Sequence Variation in Genes Affecting Dopamine Turnover and Oxytocin in a Sample of Male Inmates." Association of Forensic DNA Analysts and Administrators, Houston, Texas, July 2018.

- Elizabeth Chesna, BS, Charity Beherec, MS, Gabriella Cansino, MS, Peyton Gandy, MS, Jessica Wells, MS, Danielle Boisvert, PhD, Todd Armstrong, PhD, Sheree Hughes-Stamm, PhD, and David Gangitano, PhD. "Variation in Genes Affecting Dopamine Turnover, Oxytocin, and Serotonin in Inmate and Student Populations." American Academy of Forensic Sciences, Seattle, Washington, February 2018.

- Elizabeth Chesna, BS, Charity Beherec, MS, Gabriella Cansino, MS, Peyton Gandy, MS, Jessica Wells, MS, Danielle Boisvert, PhD, Todd Armstrong, PhD, Sheree Hughes-Stamm, PhD, and David Gangitano, PhD. "Relationship of Oxytocin (OXT) and the Serotonin Transporter (5-HTT) Single Nucleotide Polymorphisms and Antisocial Behavior." Association of Forensic DNA Analysts and Administrators, Austin, Texas, July 2017.

- Elizabeth Chesna, BS, Charity Beherec, MS, Gabriella Cansino, MS, Peyton Gandy, MS, Jessica Wells, MS, Danielle Boisvert, PhD, Todd Armstrong, PhD, Sheree Hughes-Stamm, PhD, and David Gangitano, PhD. "Relationship of Oxytocin (OXT) and the Serotonin Transporter (5-HTT) Single Nucleotide Polymorphisms and Antisocial Behavior." American Academy of Forensic Sciences, New Orleans, Louisiana, February 2017

Posters

- Elizabeth Chesna, BS, Gabriella Cansino, MS, Peyton Gandy, MS, Jessica Wells, MS, Danielle Boisvert, PhD, Todd Armstrong, PhD, Sheree Hughes-Stamm, PhD, and David Gangitano, PhD. "Application of Massive Parallel Sequencing in Forensic Psychiatry and Behavioral Science Using Custom Panels including Markers Linked to Human Behavioral Traits," International Symposium on Human Identification. Minneapolis, Minnesota, September 2016

- Elizabeth Chesna, BS, Gabriella Cansino, MS, Peyton Gandy, MS, Jessica Wells, MS, Danielle Boisvert, PhD, Todd Armstrong, PhD, Sheree Hughes-Stamm, PhD, and David Gangitano, PhD. "Genetic Study of Single Nucleotide Polymorphisms (SNPs) in the Oxytocin Receptor (OXTR)," American Academy of Forensic Science, Las Vegas, Nevada, February 2016

- Elizabeth Chesna, Bao Tran Nguyen, Whitney Holdbrook, Donna Janes. "Effects of low level hand sanitizer exposure on bacterial biofilm resistance," Texas Undergraduate Research at the Capitol, Austin, Texas, April 2013

Honors/Activities

Sam Houston State University Previous President and Vice President of the Society of Forensic Science Graduate School Organization

- Previous student affiliate of the American Academy of Forensic Sciences
- Volunteer for Saturdays at Sam

Concordia University Texas Volunteered to help rebuild a house damaged by the Texas fires on the show *Extreme Makeover: Home Edition*

- Served on a Disciplinary Hearing Committee and a Disciplinary Appeals Committee
- Senator of Commuters and for the College of Science in Student Government and Leadership Association
- Observed medical procedures under an oral and maxillofacial surgeon at Kasper, Heaton, Wright, Pagni and Associates
- Attended the Get Motivated Leadership Conference
- Served on a focus group with Noel Levitz National Higher Education Consulting Firm
- Volunteered at the Volunteer Health Clinic and Austin Humane Society
- Served as Public Relations Officer for both the Biology Club and Recycling Club
- Volunteered for the University's Field Day proctoring exams for young aspiring students
- Wrapped and donated gifts for the Operation Christmas Child Charity