The Association Between the Serotonin Transporter Promoter Region Polymorphism and Aggressive Behavior

Daniel S. Linford, Matthew T. Schmolesky, Barbara C. Trask

Department of Psychology, Department of Zoology, Neuroscience Program,

Weber State University, Ogden, UT 84408.
5-HTTLPR GENOTYPE AND AGGRESSIVE BEHAVIOR

Abstract

Studies have shown a correlation between neurotic behavior and a functional polymorphism in the promoter region of the gene that codes for the serotonin transporter (5-HTTLPR). The short form of the 5-HTTLPR polymorphism has been linked with anxiety and depression (Takahashi et al. 2011). There is also a well-documented relationship between the short allele and Harm Avoidance (Kazantseva et al., 2008). Our study seeks to examine the relationships between this gene and Aggressive Behavior, and the Harm Avoidance personality trait. This is the first study of its kind in a non-pathogenic and mixed sex group of humans. The study was conducted by administering the Buss-Perry Aggression Questionnaire and Cloninger’s Temperament and Character Inventory (TCI) to university students (n=111), and genotyping subjects for the 5-HTTLPR polymorphism. Our results showed a significant two-tailed correlation between total aggression and total harm avoidance (r=.25, n=111, p=.008). Males were lower in total harm avoidance (M=10.6 ± 8.0) than females (M=18.35 ± 7.017); t(109)=-5.152, p < .001. Total aggression was not significantly affected by sex, but males scored higher than females in both physical aggression (M=20.5 ± 9.3) vs. (M=15.4 ± 7.3); t(109)=3.15, p =.002 and verbal aggression (M=17.6 ± 7.7) vs. (M=14.2 ± 5.6); t(109)=2.5, p =.012. In the sample of participants that have been successfully genotyped to date (n=16), no significant relationship with the aggression and TCI variables was found. Genotyping will be completed in the near future and will yield greater statistical power to evaluate the role of the 5-HTTLPR polymorphism in personality traits and aggression.

Keywords: 5-HTTLPR, Serotonin, Buss-Perry Aggression, TCI, Harm Avoidance
The Association Between the Serotonin Transporter Promoter Region Polymorphism and Aggressive Behavior

There are no less than five modern major psychological theories of aggression that guide most of the research on the subject in this field: Cognitive Neoassociation Theory, Social Learning Theory, Script Theory, Excitation Transfer Theory, and Social Interaction Theory. There is a great deal of overlap between these theories (Anderson & Bushman, 2002), but none of them addresses what is physically happening within the brain of a person displaying aggressive behavior. These theories focus entirely on what that person is thinking, their perception of the situation, or their past experiences. Under these major theories, there is no discussion of the interaction of neurons and the effect these interactions have on aggression.

David Buss, a professor of psychology at University of Texas at Austin, has proposed a model of aggression based in evolutionary psychology. His theory is a new approach, different from these other modern theories that argue aggressive behavior is developed purely through operational learning, or that our socialization of children is inherently violent (e.g. violent television, videogames, and toy weapons). He argues that these other models fail to explain the large-scale violence that historically existed long before the development of mass media and television. He claims that while operational learning does play a large role in aggressive behavior, it is an incomplete analysis, and there are other forces at work to produce aggression. Buss states that evolutionary psychology hinges on two principals: first, that all behavior is the product of mechanisms within the person, and that these mechanisms require input; second, these mechanisms exist through the process of selection (Buss, 1997). This essentially states that these
mechanisms are biologically related, and that the genetics, experiences, and memories of the person, whatever they may be, are all involved in their behavior.

Of the models discussed, this is the only model of aggression that allows for incorporation of the concept that behavior is the direct product of neural activity, but Buss’s theory is also a limited model. It merely applies the theory of evolution to the field of behavioral psychology. This model leaves unexplained and essentially unaddressed what system or circuit within the brain could actually produce aggressive behavior. The products of such a model of aggression still bring us no closer to the actual neurological source of the aggressive behavior.

The field of neuroscience offers a large body of research on aggression, which we discuss at length later, but this research is rooted almost entirely in the wiring of the few circuits that are associated with aggression in animal models, and does not fully address the cognitive and affective components of aggressive behavior. To give a true description of what aggression is and why it is being employed, a model of aggression must include an explanation of the explicit neurobiological systems that are employed in creating the behavior as well as an explanation of the motivations and emotions involved. With the aim of developing a more comprehensive understanding of these underlying systems, we have examined the relationships between a measure of aggression, a facet of temperament, and a component of the serotonergic system.

**The Aggression Questionnaire**

Arnold Buss, who was also a professor of psychology at the University of Texas at Austin and began his tenure several decades before David Buss, is one of the leading sources on the psychology of aggression. His hostility inventory, developed in 1957 with
5-HTTLPR GENOTYPE AND AGGRESSIVE BEHAVIOR

Ann Durkee is still one of the most popular and widely used measures of aggression (Buss & Perry, 1992). It is an extensive and time-intensive questionnaire delivered in a true-false format. In his doctoral thesis, Mark Perry worked with Arnold Buss, and developed a shorter form of the Hostility Inventory using a factor analysis of the original questionnaire. This new questionnaire uses a 5-point scale, and produces better test-retest validity (Buss & Perry, 1992). This questionnaire has been translated into many different languages, and administered across many cultures. While showing some cultural differences, it maintains validity across these vastly different populations (Abd-El-Fattah, 2013; Uz Bas, & Yurdabakan, 2012).

The Buss-Perry Aggression Questionnaire (BAPQ) returns scores on aggressive behavior in four dimensions: physical aggression, verbal aggression, anger, and hostility. Physical and verbal aggression subscales represent the instrumental or motor component of hurting or harming others. Anger represents the affective component of aggressive behavior, encompassing the physiological arousal in preparation for action. Hostility is the cognitive component of aggressive behavior. It encompasses a feeling of ill will for others, and sense of personal injustice. Each of these dimensions is evaluated on a 5-point scale, and allows for subjects to be grouped in to high and low scoring populations by each variable. This test shows well-established sex differences in which males score higher in all variables excluding Anger (Buss & Perry 1992). We chose to use this short form of the hostility inventory for the comparatively short amount of time required to complete the questionnaire, the similarity of format to the other measures used in this study, the well-established validity, and subject grouping capabilities.
Harm Avoidance

The Temperament and Character Inventory (TCI) developed by Cloninger et al. (1993) is a widely used test in the field of psychology. It also has been translated into many other languages, and used internationally while maintaining validity (Dzamonja-Ignjatovic et al., 2010). The TCI is used to describe personality, and it does this categorically through seven traits, which are subcategorized into four higher order temperament traits and three higher order character traits. The temperament traits are Harm Avoidance (HA), Novelty Seeking (NS), Reward Dependence (RD), and Persistence (P). The character traits are Self-Directedness, Cooperativeness, and Self-Transcendence. Each of these traits has several facets, for a total of 25 facets within the TCI. Each facet is made up of a series of questions or statements that is evaluated on a 5-point scale. This allows for researchers to group populations by these variables into “high scoring” and “low scoring” groups. In this study, we focused on HA because it has been shown to display a relationship with aggression (Phan, et al., 2011). Individuals who score high in HA typically display excessive worrying, pessimism, shyness, fearful and doubtful behavior, and are easily fatigued. They are timid, careful individuals who are insecure, negativistic and pessimistic even in situations where most people would not be. They show a low energy level, and are usually quiet and shy in social situations. They require more social support and encouragement than average people. People who score low on the harm avoidance scale require little social support, and tend to be more outgoing in social situations. They are brasher than the average person and overly optimistic, even to the point of a decreased reaction to real danger (Cloninger et al., 1993).
As described earlier, HA scores are further broken down into subscales. The first of these is Anticipatory Worry and Pessimism vs. Uninhibited Optimism (HA1). Individuals who score high on this scale are pessimistic. They anticipate harm and failure outcomes in most situations, particularly in unfamiliar or difficult ones. This group also has a difficult time recovering from an embarrassing or humiliating situation, and tends to focus on the negative aspects of the experience for a long time. The individuals that score low on this subscale are more carefree and optimistic in the face of uncertainty and unfamiliarity than the average person, and they recover from embarrassment and humiliation easily. The second subscale is Fear and Uncertainty (HA2). High scorers in this category do not tolerate potentially dangerous situations with uncertain outcomes. They feel anxiety and discomfort even when there is little cause for concern. Low scorers are confident and calm in dangerous and uncertain situations as well as when exposed to commonplace stressors. This leads to these low-scoring individuals taking more risks. Shyness with strangers (HA3) is the third subscale within HA. People who score high in this category are shy and avoid meeting new people unless given a strong guarantee of acceptance. In contrast, low scorers seek out new people, speak their mind in social situations, and are uninhibited by unfamiliar people. Finally, the Fatigability vs. Vigor (HA4) subscale has high-scoring individuals that are lethargic, have less energy, and need more rest than most people. They also recover slowly from stress. People who score low in this subscale are enthusiastic, energetic, and are able to push themselves farther than most people. They recover more quickly from stress, and don’t find many tasks too difficult or tiring (Cloninger et al, 1993).
Cloninger had theorized that each sub-facet of behavior and personality was directly related to a particular neurotransmitter, and had linked HA and serotonin, which has been shown to be an oversimplification (Carver & Miller, 2006). Phan, Lee, and Coccaro (2011) showed that HA predicted Overt Aggression Scale-Modified (OAS-M) scores in patients with intermittent explosive disorder. Using HA in our study as a descriptor of personality gave us a more complete picture of the underpinnings of aggressive behavior and personality.

**Neurobiology of Aggression**

In his 2012 article, Jozsef Haller supports David Buss’s evolutionary psychology model of aggression with the description of two specific areas in the hypothalamus that are described as “attack areas.” Although certain differences exist across species, these two brain areas produce very similar effects when activated in mice, rats, and humans. These areas of the hypothalamus are topographically and functionally separate, and have been described to function in two separate types of aggression. The first, more medial area is located ventral to the fornix, and the second is positioned laterally to the first. The ventromedial hypothalamus is involved in what is described as “defensive rage” or “affective aggression”. Electrical stimulation of this area provokes displays of threatening postures and vocalizations. The subject becomes territorial, and if provoked, they will attack violently. The second described area, the ventral lateral hypothalamus, is involved in what is described as “silent biting” in cats, or also termed “predatory aggression.” This behavior consists of an immediate focused attack with the intent to kill, such as a cat biting the back of the neck of a mouse, and lacks threatening vocalizations and posturing (Haller, 2013 and Siegel, et al., 2008).
There are some inter-species differences in the experimental ability to elicit these behaviors. In mice, the researches were only able to elicit these behaviors through photogenetic stimulation. In hamsters, the areas for predatory aggression were not clearly identified. However, it is believed that the anterior lateral hypothalamus is involved in conspecific attacks. Human data on this subject is extremely rare, and has been primarily constructed from fMRI data showing activation of these hypothalamic areas (Haller, 2013).

According to Siegel et al. (1999), the areas within the hypothalamus involved in defensive rage and quiet biting have corresponding sites within the periaqueductal-grey (PAG). In the defensive rage pathway, the primary neurons originate in the anterior hypothalamus and in the PAG. These neurons run rostrally, and terminate on the preoptic zone and in the PAG. The primary pathway for eliciting defensive rage is comprised of descending neurons from the medial hypothalamus to the PAG. The pathway from the hypothalamus to the PAG is the primary pathway for defensive rage in the cat. The predatory aggression pathway has cells in the perifoncal region of the lateral hypothalamus, which give rise to ascending and descending fiber systems. The descending neurons project from the lateral hypothalamus to the trigeminal motor nucleus, locus ceruleus, and the ventral half of the PAG. The efferent neurons in the PAG involved in predatory attacks project back to the lateral hypothalamus, to the lateral tegmental region, and the pontine raphe complex. These structures within the PAG also produce predatory attack when electrically stimulated (Siegel et. al, 1999). The primary pathways involved in these two distinct behaviors rely on the PAG for input and output. This area is one of the primary serotonergic systems within the brain. Thus, serotonin
5-HTTLPR GENOTYPE AND AGGRESSIVE BEHAVIOR

plays a central role in the function of these two separate aggression behaviors. Carver & Miller (2006) theorize that this serotonergic input is responsible for restraining impulses and therefore plays a depressive regulatory role over aggression. This theory is based in the action of selective serotonin reuptake inhibitor (SSRI) drugs in pathogenic populations. They failed to describe any particular pathway that actually executes this function, and his theory is discredited by the observations of Siegel et al. (1998) showing that stimulation of the PAG elicits these aggressive behaviors. Thus activation of a serotonergic system or an increase in serotonin should have the exact opposite effect, and incite aggressive behavior. This evidence, along with the link to Harm Avoidance, leads to our focus of examining the serotonin system as an underpinning of aggression behavior.

The Serotonin System

Serotonin is an excitatory biogenic amine neurotransmitter, produced inside the presynaptic neuron from the precursor molecule tryptophan using the enzyme tryptophan hydroxylase (Purves et al., 2008). The serotonin system is extremely complex, and involved in many diffuse circuits throughout the entire brain. It is the subject of study in a vast collection of literature and affects many different types of behavior. In their review Carver & Miller (2006) discussed a long list of studies linking serotonin and personality, but they concluded that the serotonin system is not simplistic enough to be attributed to one type of behavior or one personality trait. They argued that researchers should search a wide variety of traits and behaviors to allow a pattern of association to emerge.

There are thousands of genes that could have an effect on serotonin levels directly or indirectly. Here, we focus on the protein involved in reuptake of serotonin
back into the cell; one of the two primary ways serotonin is actively modulated once released into the synaptic cleft. The other major mechanism of modulation is breakdown within the synaptic cleft by monoamine oxidase (Purves, et. al, 2008). The gene SL6A4 codes for the membrane-bound serotonin transporter protein (SLC6A4 Solute Carrier Family 6, 2014). This protein moves whole serotonin molecules from the extracellular space, across the membrane, and into the cell, thereby reducing the amount of serotonin within the cleft and allowing the cell to re-use these serotonin molecules. The highest concentration of this gene is located in the PAG and raphe nuclei (see figure 1), and analysis based purely on the concentrations of this gene within the brain would suggest that it should have a strong impact on the serotonergic component of the attack pathways described earlier.

5-HTTLPR Genotype

Recent studies have shown a correlational relationship between the serotonin linked polymorphic region (5-HTTLPR) of the promoter region of the serotonin transporter gene and maladaptive behavior (Takahashi & Quadros, 2011). Two functional polymorphisms of this 20-23 base pair repeat have been described: the short form, which has 14 copies and is less transcriptionally efficient, and the long form that contains 16 copies (Whisman, et al., 2011). An individual has two copies of each gene, so it is possible for a person to have one of three possible combinations of the long and short alleles: Long-Long (LL), Short-Short (SS), and Short-Long (SL). The short allele is generally considered the maladaptive form in Caucasian populations (Long et al., 2013) and has been linked with anxiety and depression, as well as with some facets of personality such as neuroticism (Gonda et al. 2008). The exact effect that it has on
serotonin levels is not well understood. The effect of possessing the short form of the gene is the serotonin transporter protein will be produced at a slower rate, resulting in reduced reuptake of serotonin. Having the short allele does not equate to the same result as taking an SSRI drug, which blocks the serotonin transporter and leaves more serotonin in the synaptic cleft. This is likely due to postsynaptic neuromodulation of serotonin receptors and the pre-synaptic neuron’s decreased ability to reuse serotonin that has already been released. Additional copies of the short allele are expected to increase the severity of neurotic tendencies.

The short allele has been associated with the emergence of suicidality, HA, and Neuroticism (Gonda et al., 2011). Some association with aggression and this gene has been shown in animals. In one study, they found that male rhesus macaques carrying the s allele was significantly more likely to respond with high-risk aggression when exposed to an unfamiliar conspecific in the form of an intruder challenge test (Schwandt, et al., 2010), their findings were also based on factor analysis of the animal’s reaction, and contingent upon rearing condition. Using the Buss-Durkee hostility inventory, in a group of human female subjects, Gonda, et al (2009) showed a significant interaction with global aggression and hostility index, but they did not show a significant relationship with HA. Other studies such as Kazantseva, et al. (2008) have established a well documented a relationship between the short allele and HA in a healthy population.

No current study has shown the relationship between harm avoidance, aggressive behaviors, and polymorphism of the serotonin receptor in a non-pathogenic and mixed-sex group in humans. This study will add a deeper understanding of the interactions of the serotonin system and neurotic and aggressive behaviors. This understanding could
5-HTTLPR GENOTYPE AND AGGRESSIVE BEHAVIOR

contribute to the production of therapeutic measures that would ultimately improve the quality of life for affected individuals and prevent the loss of life in the case of suicide.

Methods

The Pilot Study

Male and female Weber State University students (n=173) participated in a pilot study conducted in 2011. These participants completed digital versions of the TCI. In this first attempt, genotyping was entirely unsuccessful due to a faulty set of DNA primers. The data and samples from this experiment were saved and added to the pool of data for the second study. With the start of this second experiment, these subjects were re-genotyped with the new DNA primers. The data obtained from the pilot study was added to the new sample to increase statistical power for the TCI-Genotype relationship, but these subjects did not complete the aggression questionnaire, and were not used in the computation of the data relating Aggression and Genotype.

The Current Study

This second study consisted of 111 Participants, 33 male and 76 female, all above the age of 18. Participants were recruited from introductory psychology courses requiring research credit as part of the course. Each participant received five credits within the Weber State University Psychology Program for participating in the study. Data was collected in small groups, in a student computer lab that seated 26 participants. We used the BuccalAmp DNA Extraction kit from Epicentre to collect DNA for 5-HTLPR genotyping, which contained the QuickExtract DNA extraction solution 1.0 and Catch-All Sample collection swabs. Each participant was provided with a buccal swab and two disposable plastic cups. The first contained water for rinsing. The second cup was empty,
and was used to place the swab in to allow it to air-dry while minimizing contamination. Each participant was assigned a three-digit subject number automatically before being able to start the questionnaires to allow pairing of the Genotype, Aggression and TCI variable data. A script of instructions was read to maintain uniformity of the instructions given between collection groups. Participants were instructed to write their number on the label on the case of the buccal swab they were using, then to rinse with water for a few seconds, and to spit back into the first cup. After rinsing, they were to roll the brush of the DNA collection swab on the inside of their cheek a minimum of twenty times. Once finished, they were instructed to place the swab into the second cup and allow it to air-dry for 10-15 minutes, and began the electronic questionnaires. The swabs were recapped and collected once the drying time had passed, placed in a zip-lock bag, and frozen until DNA extraction could be conducted. All participants completed electronic versions of the Buss-Perry Aggression Questionnaire, and the Temperament and Character Inventory (TCI). We had elected to use an extended-scale form of the aggression questionnaire that is identical to the original questionnaire, using a 7-point scale to evaluate each question instead of a 5-point scale. This decision was based on access to the questionnaire format in our short time frame.

DNA extraction entailed placing the collection swab into a tube of DNA extraction solution, and rotating the brush five-to-ten times. The brush was pressed and rolled against the side of the tube during removal to retain the most liquid in the tube possible. After the buccal cells had been removed from the brush, a vortex was used to stir the mix for 10 seconds. A hot plate was used to incubate the sample at 65°C for 1 minute, followed by a second vortex for 15 seconds, and incubation at 98°C for two
minutes. The final steps were to vortex for another 15 seconds, and store in a freezer until PCR amplification could be conducted. Subjects were genotyped as SS, SL, or LL by examining DNA fragment bands that were XX base pairs and XX base pairs.

Polymerase chain Reaction amplification of DNA was conducted by first combining all of the required constituent ingredients in a 500µL test tube prior to adding DNA, and then keeping it on ice until adding it to the DNA samples. This master mix contained 15µL of water, 10µL of buffer, 2µL of dNTPs, 1µL each of the forward and reverse DNA primer, and 1µL of TAQ polymerase per sample. These mix volumes were multiplied by the number of subjects being genotyped at a time. Typically batches of 19 samples were processed because the gel box for electrophoresis had 20 wells and one well was left empty for the 50 base pair hyper ladder. Samples were spun for five minutes in a centrifuge, and 20µL of extracted DNA was added to 30µL of the master mix. After adding extracted DNA, the sample was mixed with a vortex and spun in a centrifuge briefly to ensure a homologous sample. In a thermocycler, the samples were incubated at 95° C for two minutes, followed by 40 cycles of: 95° C for 30 seconds, 62° C for thirty seconds, and 72° C for thirty seconds. 2% agarose gel was used in electrophoresis, made by boiling granulated agarose in TAE buffer, with 7µL of ethidium bromide added per 100mL of gel. TAE buffer was diluted from a 50x concentrated solution to 1x using de-ionized water in 500mL batches. After electrophoresis, a picture of each gel was taken. Subjects were genotyped as SS, SL, or LL by examining the DNA fragment bands. The short bands were between 350 and 400 base pairs, and the long bands were between 400 and 450 base pairs. After genotyping, the gel was disposed of.
Responses from the questionnaires along with genotype and demographic information were recorded in an excel spreadsheet, arranged by subject number, and formatted into the following variables: Genotype, Gender, HA 1-4, Total Harm Avoidance (HAtot), Physical Aggression (Phys Agg), Verbal Aggression (Verb Agg), Anger, Hostility, and Total Aggression (AggTot). The Buss-Perry variables were calculated by adding the responses to all the questions of for each subscale, and HAtot was calculated by adding all of the subscales together for a sum of all raw scores. Data was then exported to SPSS 21 through Weber State University’s Online Virtual Lab. All correlational data was established using Pearson’s correlation. Sex differences were examined using a series of t-tests. All tables were produced by exporting data from SPSS into Microsoft Excel, and all graphs were produced using Microsoft Excel.

Results

HA and Aggression Data

The Buss-Perry and TCI questionnaire data indicated a significant two-tailed correlation between Total Aggression and Total HA ($r=.25$, $n=111$, $p=.008$), (see Figure 2). This is contradictory to what Cloninger and Zuckerman showed in their 1995 article, which reported no correlation between Zuckerman’s Aggression-Hostility Scale and HA. However, it does support what was reported in a fluoxetine study where HA independently predicted aggression scores in patients with intermittent explosive disorder (Phan, Lee, & Coccaro, 2011). To better understand this result, the relationships between the subscales must be examined (see table 1). HA1 showed a significant relationship with all of the BAPQ subscales. The weakest of these relationships was with verbal aggression with a significance of 0.049 (2-tailed). Hostility showed significant relationships with all
of the HA subscales, and had the strongest relationships of all of the Aggression variables. HA4 showed strong relationships with all of the aggression subscales, excluding physical aggression. HA2 and HA3 showed a significant relationship only with Hostility.

**Sex Differences**

Males were lower in total harm avoidance (M=10.62, SD=8.011) than females (M=18.35, SD=7.017); \( t(109)=-5.152, p < .001 \); (see table 2). Fifty percent of males scored less than 10 on HA, and 50 percent of females scored less than 17, whereas only 17% of males scored more than 20, 68% of females scored more than 20 (see table 2, figure 4). Females also displayed significantly higher scores in all of the subscales of HA. This pattern is in agreement with what was reported by Kose, et al. (2009) on a Turkish population and their findings were congruent with what Cloninger, et al. originally reported (Kose, et al., 2009).

Males were higher in Total Aggression \( t(109)=1.96, p=.05 \). This is in line with what is reported in the literature (Buss & Perry, 1992). Although this result was only marginally significant, our sample was predominantly female, had our sample contained a more even distribution of males and females, this relationship might have been stronger (see table 3, and figure 5). 50 percent of males scored less than 65 on total aggression, and 50 percent of females scored less than 50, whereas 20% of females scored 80 or higher and 35% of males scored above 80.

Males scored higher than females in Physical Aggression (M=20.57, SD=9.39) vs. (M=15.41, SD=7.32); \( t(53.75)=2.874, p =.006 \), which was to be expected from what Buss & Perry reported (1992). Interestingly, there was no significant difference between
males and females on Verbal aggression, Anger, or Hostility. This data contradicts the findings of Buss & Perry. Some of this effect may be a consequence of the majority of our subjects being female. Also, the social and cultural influences of our sample are to be taken into account. One such influence, which is primarily related to the geographic location our sample was collected from, is the predominance of religion in this western region of the United States. The family-centered values of this subgroup of individuals may influence the expression of aggressive behavior. From a biological viewpoint, the immigration of settlers into this region and their propensity to stay here may also have selected for a particular collection of genotypes producing some of this effect. There is a surprising lack of literature on Aggression and Mormonism, and an examination of the psychology and genetics involved would provide fascinating results.

Genotype Data

Genotyping failed or has not been attempted on the majority of our test subjects from the second study. Only 16 of the original 111 were successfully genotyped. These 16, along with 76 of the participants from the pilot study, were successfully genotyped before our PCR amplification started to become very faint and eventually failed entirely. In an attempt to rectify the issue, we changed primers, used several different combinations of DNA, and made several subsequent attempts to complete genotyping, ensuring each protocol had been followed explicitly. Eventually, time limitations ended our efforts to complete the genotyping of new subjects at present.

Although it is not entirely certain, there are two likely explanations for this genotyping failure. The first is that something has again gone wrong with the DNA primers as occurred in the first study. If this is the case, the solution is simple. Once the
primers have been replaced genotyping will continue normally. The second possible explanation is that the BuccalAmp DNA extraction kits, particularly the DNA extraction solution, were intended to be stored at -20°C. When the kits were first received they were stored in a fridge until the extraction protocol was examined in detail. They were then placed in the freezer. This was done after the DNA from a small selection of the new subjects had been isolated. The DNA from the pilot study had all been isolated prior to the start of the new study. The DNA that had been isolated before the extraction kits were re-frozen was successfully genotyped. The samples that were extracted after refreezing the extraction fluid produced bands on the electrophoresis gels that were so faint that they could not be properly visualized, or the gel was blank entirely. The extraction protocol warns against freezing and thawing the extraction liquid, and it is possible that this freeze-thaw cycle damaged the extraction solution. This would explain the misleading combination of results we received. Examining the DNA yield using fluorimetry could substantiate this theory. If correct, it would be more productive to collect new data than to attempt to salvage the existing DNA samples.

Of the participants that have been successfully genotyped to date, in both the pilot study and the second study (n=92), no significant relationship to the TCI variables was found. The very limited sample of successfully genotyped subjects from the second study (n=16) showed no significant relationship with any of the aggression variables. The small size of the sample aside, this result is particularly insignificant due to the fact that only one of the successfully genotyped subjects was male. Males have higher scores in Total, Physical, and Verbal Aggression, and the relationship between Genotype and the Buss-Parry subscales could have been strengthened drastically had this sample contained more
males. It is our hope to complete the genotyping process in the near future, to add great statistical power to our findings, and establish a relationship between the Harm Avoidance, Aggression, and 5-HTTLPR genotype.

**Discussion**

Our data showed that HA could predict Aggression scores (see figure 2), though the association is weak. Particularly, high scores in Anticipatory Worry and Pessimism (HA1) and Fatigability (HA4) are directly linked with Anger and Hostility. This indicates that an individual who actively engages in excessive worrying about a particular situation and expects a negative outcome is much more likely to engage in aggressive behavior or vice versa. It also suggests that this internal dialogue between their expectations of the situation and what they think other people are thinking of them colors their perception of the events that are taking place, thereby predisposing them toward aggressive behavior.

Large differences of character and personality are displayed from one individual to the next, and it is these personality differences that can affect the different types of aggressive behavior a person engages in. An analysis of the relationships between the HA subscales and the majority of the Buss-Perry subscales begins to describe the personality of the high-scoring populations in each type of aggression. Although the Buss-Perry subscales are correlated with each other, an individual who scores high on one kind of aggression may have a very different personality than another who scores high on a different subscale. Based on our data, a person who is very physically aggressive will be very pessimistic, and expect the worst outcomes from situations where an average person would not. This aggressive individual will be calm and confident in uncertain situations, and they will not be extremely shy with strangers or easily fatigued. A verbally
aggressive person will be less pessimistic than a high-scorer in physical aggression, and will not be afraid of changes in routine or uncertain situations. They will be easily fatigued, but not shy with strangers. A person scoring high in hostility is likely to have high scores in harm avoidance in general. They will be pessimistic about the outcomes of many social situations and show an inordinate amount of fearfulness. They will have an extremely negative view of other people, and be very averse to interacting with strangers.

Understanding the interaction between personality and aggression brings us back to the issue of the biological mechanisms at work behind this internal dialogue. It is unfortunate that the genotyping was unsuccessful in this study. Based on the constellation of evidence presented here, including the multifaceted relationship between harm avoidance and aggression scores, and the findings that the short allele of the 5-HTTLPR genotype is associated with maladaptive behavior and suicide (Gonda, et al., 2009), we predict that a person displaying an extreme personality, would more likely possess the SS genotype. It also follows that their genotype would be influencing their inner dialogue and the way they perceive the world. Establishing this link between aggression and the 5-HTTLPR genotype, and developing a better understanding of the other polymorphisms that are involved in the traits of Aggression and Harm Avoidance would provide a target for studies seeking to decrease aggression in an adversely affected population. This could also lead to the ability to develop medications capable of modulating harm avoidance, which could be therapeutically beneficial in a variety of applications. There is also the possibility that increasing aggression could lead to benefits in certain situations. If it were possible to transiently and preferentially decrease the activation threshold for the predatory aggression pathways, there could be applications to benefit soldiers in combat.
situations. Also, some aggression can be beneficial in interpersonal relationships. If it were possible to increase Verbal Aggression it might be possible to improve an individual’s performance in business. It would also be of benefit to have the ability to boosting confidence and suppress Harm Avoidance traits.

Future studies should focus on not only the 5-HTTLPR genotype as a underpinning of aggressive behavior, but also polymorphisms in the other genes coding for the biological mechanisms that affect serotonin function and other neurotransmitter systems, such as monoamine oxidase and tryptophan hydroxylase. Elucidating the inner-workings of the serotonergic system is not only at the forefront of today’s behavioral research, but will also produce many benefits for the scientific community in the future. A greater understanding of these underpinnings of behavior will produce better techniques in psychological counseling, better psychoactive medications, and indirectly improve the quality of life for many people in the future, as well as providing a greater understanding of what it is to be human.
References


Kazantseva, A., et al. (2008) Polymorphisms of the serotonin transporter gene (5-
5-HTTLPR GENOTYPE AND AGGRESSIVE BEHAVIOR

The long rather than the short allele of 5-HTTLPR predisposes Han Chinese to anxiety and reduced connectivity between prefrontal cortex and amygdala. *Neuroscience Bulletin*, 29(1), 4-15.


5-HTTLPR GENOTYPE AND AGGRESSIVE BEHAVIOR


