Aspects of the genetics of human aggressive behaviour

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To my friends and family who showed their unwavering support during this time of my life, I am deeply grateful. To my sisters, thank you for believing in me.

Finally, to my Heavenly Father, all the glory goes to Him.

LIST OF ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
5-HTTLPR	5-Hydroxytryptamine transporter-linked polymorphic region
µl	Microliter
ADHD	Attention Deficit Hyperactivity Disorder
APS	Ammonium persulphate
AQ	Aggression Questionnaire
ASPD	Anti-Social Personality Disorder
ATQ	Adult Temperament Questionnaire
BAS	Behavioural Activation System
bp	Base pair
BIS	Behavioural Inhibition System
BSA	Bovine Serum Albumin
CNS	central nervous system
CSF	Cerebrospinal fluid
dH_2O	Distilled water
DMSO	Dimethyl sulfoxide
EATQ	Early Adult Temperament Questionnaire
EDTA	Ethylene diamine tetra-acetic acid
Ethanol	Ethyl alcohol
fMRI	Functional Magnetic Resonance Imaging
g	Gravitational force
GABA	Gamma Amino butyric acid
GAM	General Aggression Model
HTR1A	5-Hydroxytryptamine receptor 1A
HTR1B	5-Hydroxytryptamine receptor 1B
HTR2A	5-Hydroxytryptamine receptor 2A
MAO-A	Monoamine oxidase A
Mg^{2+}	Magnesium ion
$MgCl_2$	Magnesium chloride
mg/ml	Milligram per millilitre
ml	Millilitre
mM	Millimolar
n	Number of individuals
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
ng	Nanogram
ng/ul	Nanogram per microliter
NPP	Negative predictive power
OCD	Obsessive Compulsive Disorder
P	Probability

PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PPP	Positive predictive power
PRQ	Physiological reactions questionnaire
QTL	Quantitative Trait Loci
R	Repeat
R	Pearson product-moment correlation coefficient
R^2	Coefficient of determination
SDS	Sodium dodecyl sulphate
SLC6A4	Solute carrier family 6 (serotonin transporter), member 4
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
TBE	Tris-borate EDTA buffer
TCI	Temperament and Character Inventory
TEMED	N,N,N',N'-tetramethylethylenediamine ($C_6H_{16}N_2$)
Tris	2-Amino-2-(hydromethyl)-1,3-propanediol
U	Unit
UTR	Un-translated region
V	Volt
VNTR	Variable number of tandem repeats

CHAPTER 1

General introduction, aim and dissertation outline

Abstract

The most influential components of an individual's personality are the genes, environment and psychological development. An individual's personality will determine how he or she experiences their environment, and how they will react within that environment. The genetic component of personality is actually embedded in the temperament, also known as the predecessor to personality. The temperament can already be investigated at a very early age. Through experience it develops into personality. Of all these influential forces, the psychological component is the most variable. Parenting styles will influence the child's temperament development into personality, influencing the resulting behavioural phenotype that will be observed in certain situations. Problem behaviour, like aggression, may be the result of an inability to suppress behaviour. Individuals who can control their behaviour should show lower levels of aggressive behaviour. This will be regardless of surveillance, and according to social norms (as learned through experience and socialization). This chapter serves as a general introduction to and brief outline of this research project on temperament, aggressive behaviour and the genes from the serotonergic pathway that may influence both these behavioural constructs.

Keywords: Aggression, Effortful Control, Personality, Serotonin, Temperament

Even before an individual has gained experience of social interactions, he or she will react to certain environments in individually unique ways. This is called temperament. It is governed by neural circuits in the brain, modulating behaviour (Evans & Rothbart, 2007). Genes influence the brain, thus also influencing temperament. Genes set boundaries wherein temperament will develop. Personality is then the development of temperament through experience (Rothbart & Ahadi, 1994). This includes the child's thoughts about themselves, others, and the world they live in (physical and social). Later in life, personality will be influenced by the individual's values, attitudes and psychological development. Thus, personality is more flexible than temperament (Buss & Plomin, 1984; Reber & Reber, 2002; Rutter, 2006).

A more complete explanation of temperament can be defined as an individual's reactions and the differences in emotional, motor and attentional reactivity. It can be measured based on the latency, intensity, response recovery and processes of self-regulation (Lamb, 1981). All these responses are genetically influenced (Posner *et al.*, 2007).

Temperament can be divided into three groups, namely Effortful control, Negative affectivity and Surgency/Extraversion. Effortful control is seen as the individual's ability to control his or her behaviour. Later in development, social norms will also influence Effortful control (Kochanska *et al.*, 2000; Damon & Lerner, 2006). Effortful control, with its own four contributing constructs (Table 1.1), is the most important in modulating behaviour. It also has a strong genetic contribution (Posner *et al.*, 2007). It controls an individual's ability to regulate behaviour by considering past experiences, current situation and possible future outcomes. Negative affectivity can be compared to Neuroticism, one of the Big Five Personality factors (Evans & Rothbart, 2007). Individuals with Negative affectivity experience frustration, distress caused by fear, a higher intensity of discomfort and sadness. Returning to normal after an episode of distress is also difficult for these individuals (Rothbart, 2007). The last temperament construct, Extraversion or Surgency, can also be compared to the Big Five Personality factor of Extraversion. Higher activity levels, higher self-confidence, lessened timidity, a more intensive feeling of pleasure and overall a more positive anticipation from life can be seen in these individuals (Damon & Lerner, 2006).

Effortful control will receive special focus as it has already been suggested to modulate aggressive behaviour (Milich & Kramer, 1984; Kochanska *et al.*, 2000; Ormel *et al.*, 2005; Damon & Lerner, 2006; Posner *et al.*, 2007). It works in relation to the fight-and-flight response of the sympathetic nervous system. The reactive dimension of fear causes an individual to feel discomfort, relayed by internal cues. This indicates a relation to the Negative affect temperamental construct as well. A more fearful individual will exhibit behavioural inhibition by retreating from a threatening situation, thereby inhibiting aggression (Rothbart & Sheese, 2007). The conscience is, when linked to temperament and development, the inhibitory system that serves as an internal foundation of the child's conduct norms (Kochanska *et al.*, 1997).

Table 1.1: The four constructs of Effortful Control (Rothbart & Bates, 2006)

Construct	Definition
Attention Control	The ability to direct attention at will
Inhibitory Control	The ability to foresee the potential outcome of a situation, and suppression of inappropriate behaviour
Perceptual Sensitivity	The ability to detect even low-intensity environmental stimulation
Low-Intensity Pleasure	Experiencing pleasure from stimuli with low-intensity, complexity, or a novelty

The development of a conscience, which is the modulation of behaviour by internal cues, not external reward or persuasion, is also greatly affected by the parenting styles of the parents (Rothbart, 2007). When a child shows a tendency to violate the rules while not supervised or watched, the child has low Effortful control (Milich & Kramer, 1984), as there is no consideration for possible repercussions. This development of conscience (high Effortful control) can possibly also help the child develop empathy and guilt, thereby lowering aggression levels (Kochanska *et al.*, 2000). It provides the individual with the ability to regulate behaviour by considering past experiences, current situation and possible future outcomes while also taking into account how their behaviour would affect others. Low Effortful control can be related to the inability to control behaviour, leading to externalizing problems and possibly also aggressive behaviour (Ormel *et al.*, 2005; Damon & Lerner, 2006). Effortful control then reflects the ability to inhibit

behaviour, especially if a personally pleasurable outcome can be anticipated, by assessing social norms within the situation (Kochanska *et al.*, 2000).

Extensive studies were done on the development of problem behaviour (Patterson *et al.*, 1989, 1991, 1992; Dishion *et al.*, 1991). Other researchers (McCord *et al.*, 1961; West & Farrington, 1973; Farrington, 1978; Wadsworth, 1979; Olweus, 1980; Loeber & Dishion, 1983) supported their conclusion that parental management, specifically with regard to harsh and inconsistent discipline, poor supervision, family conflict and poor family involvement, are the largest contributors to the development of problem behaviour. This may manifest as adolescent delinquency, anti-social behaviour, high risk for sexual promiscuity, academic failure and substance use and abuse (Ary *et al.*, 1999). Behaviourists focussing on psychology, have come to the same conclusion that the environment as well as the individual is important entities in the developmental pathway. They found that reinforcement, punishment, practice and imitation lay the basis of learned behaviour (Skinner, 1938; Bandura & Walters, 1963; Reese & Lipsitt, 1970; Catania, 1973, 1978; Herrnstein, 1977). These theories discuss how an individual will learn behaviour through what they see. The response obtained by the behaviour will then encourage or discourage the use of the behaviour.

Development is a process of differentiation, reorganization and adaptation (Nigg, 2006), During this time an individual will inevitably undergo change. As mentioned earlier, the personality is plastic and changeable. Temperament remains more stable. This is known as normative behaviour. Temperament will be stable at specific points during development. Temperament thus gives us the ranges in which a specific behavioural trait may vary (Kagan & Snidman, 2004). It will not exceed these ranges. Therefore, temperament is not influenced by the incentive response system, like personality. It is through experiences, like rewarding behaviour, that temperament changes into personality. This also explains why the study of temperament and personality is important. It considers individual differences shaped by unique environments as motivation for behaviour. Temperament leads to personality through experience, shaping an individual's framework of consciousness and how the individual uses cognitive adaptations within the social world, as coping mechanisms (Rothbart *et al.*, 2000).

The study of heritability of personality shows a significant genetic contribution with only a small influence from the shared environment (Loehlin, 1992). The shared environment implies the family environment an individual grew up in. This only implies the family, as they mostly share the same house, same environment and mostly the same rearing and experiences. This can also apply to individuals that are not related but still share the same environment. The focus merely falls on the outcome of this environmental influence. It is considered a shared environment when the same environmental influence results in the same behavioural outcome (Plomin *et al.*, 2008). Psychologists agree that it seems as though the non-shared environment, which is the experiences unique to each individual within the family, will have a greater effect on the development of personality (Plomin & Daniels, 1987). This then explains how individuals who share a genotype and the same environmental influences may still have different behavioural phenotypes.

Longitudinal studies demonstrated that life events can also cause personality change (Agronick & Duncan, 1998). Both physical and psychological trauma can cause physical alterations to the brain (Bremner, 1998; Nelson, 1999). Physical trauma may be in the form of brain injuries. The most prominent form of psychological trauma is stress, where the result is reduction in the hippocampus. In this area, the neurotransmitters responsible for behavioural modulation will be affected (Bremner, 1999).

Piaget (1952, 1953) studied behaviour through a multidisciplinary approach. He focussed his studies on the individual, leading the research in the then still highly underdeveloped field of genetic contributions to behavioural development. The first behavioural studies considering the physiological influences to motivation found two major pathways, influenced by neurotransmitters in the brain that modulates behaviour (Gray, 1978). The first pathway is called the Behavioural Activation System (BAS). The second pathway is called the Behavioural Inhibition System (BIS).

All behaviour is associated with the brain and neural pathways, as this is how the individual senses his or her environment. This is also where the serotonergic system is actively modulating behaviour. The genetic contribution focusses on the aspect of the manifestation of behaviour that is related to the tendency, ability, frequency and intensity of the behaviour. The

serotonergic pathway in the brain is commonly called the pleasure pathway as it regulates pleasurable feelings. It aims to regulate and reduce the feeling of anxiety and discomfort. The psychologists' approach focusses more on the "How" of the behaviour, as opposed to the "Why" (Thomas, 1963; Thomas & Chess, 1977; Strelau, 1998). These studies investigated the mechanisms causing the behaviour, along with the intensity and frequency of occurrence. The serotonin pathway helps us understand the "Why" behind behaviour. It focusses on the emotional-motivational aspects of temperament and behaviour. From the definition and explanation of Effortful control, it seems that BIS will be most influential in modulating problem behaviour.

Aggressive behaviour can be considered as problem behaviour. The first distinction made by psychologists is between offensive and defensive aggression (Adams, 1979). Offensive aggression is seen as an attack (Blanchard *et al.*, 1977), whereas defensive aggression is driven by self-preservation as an act of self-defence (Adams, 1979; Anderson & Bushman, 2002). The behaviourists preferred their own distinction of proactive and reactive aggression, as it focussed more on the motivation behind the aggressive act. Reactive aggression is usually the result of provocation and seen as more instinctual. Proactive aggression, on the other hand, is a form of premeditated aggressive behaviour and is seen as more calculating and cold (Berkowitz, 1993a; Conner *et al.*, 2010).

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This research project has several components. The first component is a quantitative analysis of aggressive behaviour. This will be done using the Aggression Questionnaire (AQ) (Buss & Warren, 2000). The AQ divides aggressive behaviour into five identifiable manifestations, namely anger, physical and verbal aggression, hostility and indirect aggression. As there are various influencing factors to a specific behaviour, a stable person-factor is necessary. Temperament has been chosen as it remains most stable throughout life. It also predicts how an individual will react to and within his or her environment. For this, the Adult Temperament Questionnaire (ATQ) (Evans & Rothbart, 2007) will be used. Individuals participating in this research project will be based on convenience sampling, with snowball effects. They will also be voluntary participants (all this will be fully explained in Chapter 4).

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An in-depth discussion on the functionality of serotonin also follows in Chapter 2. In short, it seems that the main behavioural functionality of serotonin is as a modulator (Jacobs & Fornal, 1997). It regulates anxiety (Jacobs *et al.*, 1984; Jacobs & Fornal, 1995). A high co-morbidity rate exists between anxiety and other mood disorders (Gorman, 1996; Bakish D. *et al.*, 1998; Kessler, 1998, 2001). There is also an overlap in common symptoms of anxiety, aggression, depressed mood and impulsivity (Apter *et al.*, 1990). Where anxiety and aggression co-occurs with mood disorders, like depression, the directionality is of importance. When directed inwards it may result in suicide. When directed outwards it may result in irritability, shortness of temper, impatience and anger outbursts (Botsis, 1997; Van Praag, 2001). Focussing on anxiety and aggression two hypotheses arise: either anxiety and aggression are independently influenced by serotonin activity; or serotonin influences anxiety, and aggression is derived from anxiety (Van Praag, 1991). Just based on what one can observe, five forms of aggressive behaviour can be distinguished, namely anger, hostility, physical, verbal and indirect forms of aggression (Buss & Perry, 1992a; Buss & Warren, 2000). As will be discussed in Chapter 4, these are also the subscales that will be used in this study to measure aggressive behaviour.

Statistical analysis will be done on the data collected by the completed questionnaires. These will include the basic descriptive statistics, such as the mean and standard deviation (based on both questionnaires). Further data sets will be constructed based on age groups and gender. This will indicate whether age and gender may influence aggression. This will also be discussed in Chapter 4.

DNA will be collected from participating individuals in the form of saliva samples. As previously mentioned, the serotonergic system plays an important role in modulating behaviour. Specific genes from the serotonergic system have been selected. These genes include three receptor genes, namely *HTR1A* (5-hydroxytryptamite receptor 1A), *HTR1B* (5-hydroxytryptamite receptor 1B) and *HTR2A* (5-hydroxytryptamite receptor 2A). Specific single nucleotide polymorphisms (SNPs) that have been linked to anxiety, aggression and impulsivity (Oliver *et al.*, 1997; Lappalainen, 1998; Ramboz *et al.*, 1998; Heisler *et al.*, 1998; New *et al.*, 2001; Bjork *et al.*, 2002; Sanders *et al.*, 2002; Strobel *et al.*, 2003; Huang *et al.*, 2003; Harvey *et al.*, 2003; Lemonde *et al.*, 2003; Lesch & Gutknecht, 2004; Meira-Lima *et al.*, 2004; Abdolmaleky *et al.*, 2004; Khait *et al.*, 2005; Norton & Owen, 2005) will be investigated within specific regions of these genes. One

transporter gene, the *SLC6A4* (solute carrier family 6, member 4) and the gene encoding the enzyme responsible for the breakdown of serotonin, monoamine oxidase A (MAO-A) will also be investigated. These two both have a variable number of tandem repeats (VNTR) in the promoter regions of these genes that influence gene expression (Brunner *et al.*, 1993; Cases *et al.*, 1995; Heils *et al.*, 1996; Lesch *et al.*, 1996; Sabol *et al.*, 1998; Shih & Thompson, 1999; Sher *et al.*, 2000; Greenberg *et al.*, 2000; Osher *et al.*, 2000; Du *et al.*, 2000; Melke *et al.*, 2001; Jang *et al.*, 2001; Lotrich & Pollock, 2004; Feinn *et al.*, 2005; Hu *et al.*, 2006; Alia-Klein *et al.*, 2008). This will be discussed in Chapter 5.

Gene variants will also be discussed in Chapter 5. Further possible correlations between gene variants and specific behavioural patterns (specifically aggression) will also be investigated. The importance of first accurately quantifying behaviour will be of utmost importance for this chapter.

The outline of the study is based on the aims of the study. This chapter serves as a general introduction to the entire dissertation. As briefly mentioned in this chapter, Chapter 2 is a complete literature review of aggression, psychological development and the serotonergic pathway. This is followed by a literature review of a stable person-factor, seen as temperament and personality in Chapter 3. Chapter 4 focus on the statistical analysis of the data obtained by the AQ and ATQ questionnaires. Here, a stable person-factor will also be identified. The molecular analysis of the previously mentioned genes will be discussed in detail in Chapter 5 with a comparison to quantitative data obtained in Chapter 4.

1.2. Research aim

The most important aim of this project is to determine whether any of these genes, has an influence on different aspects of aggressive behaviour. A selection of individuals from the central South African region will be approached for participation in the study. Behaviour will be quantified by using the AQ (Buss & Warren, 2000) and ATQ (Evans & Rothbart, 2007). Statistical analysis will be done on the quantitative data to determine possible correlations between variables. From this, individuals will be selected to contribute DNA to the molecular analysis. Five genes involved in the serotonergic system will be investigated.

CHAPTER 2

The psychobiology of aggression in humans, focussing on the serotonergic pathway

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Abstract

Aggressive behaviour in humans has been classified as a complex behavioural trait. It has genetic influences interacting in an additive way with environmental stimuli. It is also very important to consider the psychological development of the individual studied. Various psychologists have theories of how children will develop behavioural patterns based on what they see. These learned behavioural patterns will also interact with the environment. Predicting certain individuals' behaviour based on the situation or provocation can also be done. New techniques to study genetic influences on neural biology have given deeper insights into influential mechanisms underlying this complex behaviour. Neurotransmitters are studied foremost in behavioural research. Of these the serotonergic system, also known as the pleasure system, is linked to anxiety disorders and aggression. The main focus of this article falls on the serotonergic pathways in the brain, and the influences of its different genetic components on the manifestation of aggressive behaviour.

Keywords: Aggressive behaviour, Anxiety, General Aggression Model (GAM), Serotonin, Social learning

Introduction

Behaviour can be classified as an organism's actions with and within its environment. It is a stimulus and response system (Moyer, 1967; Anderson & Bushman, 2002). The stimulus instigating the response can be internal or external, conscious or subconscious (Cosmides & Tooby, 1994; Buss, 1995). The response can be overt or covert, voluntary or involuntary. Scientists studying behaviour attempt to describe, explain, predict and influence a specific behavioural trait. They endeavour to do this in an objective and systematic way, for reproducibility of the results obtained. Initial behavioural studies focused only on observing the organism in its environment (Skinner, 1938, 1965, 1981; Lorenz, 1956; Bandura *et al.*, 1961; Bandura & Walters, 1963; Bandura, 1977; Anderson & Bushman, 2002). After the observation, they also attempted to manipulate the behavioural trait (Skinner, 1938; Bandura *et al.*, 1961; Kalikow, 1983; Anderson & Bushman, 2002; Brigandt, 2005; Anholt & Mackay, 2009). Through this experimental design however, only the environmental influence could be studied. As science developed, a better understanding of the intentions and motivations for specific behavioural traits also developed.

Currently behavioural research consists of several components. Observation of the behaviour is the first step. The psychological development of a child will have a significant influence on the behaviour throughout life (Rothbart *et al.*, 2000). An individual's psyche includes components like the temperament and personality. These components have boundaries within which an individual's behaviour may vary (Lamb, 1981; Kagan & Snidman, 2004). In the study of development within the family environment, genetics also plays a major role. Similar genes occur in families. This gives way to the third contributing component: the human physiology.

The brain communicates with the external and internal environment through neurons and neurotransmitters. The neurotransmitters act as chemical messengers. They influence cognition, conscious thought, the decision making processes, our perception of experiences and even motivation for behaviour, to name only a few (Rothbart *et al.*, 2000). Genetics determine the physiology of neurotransmitters. A specific gene influencing the expression of a specific neurotransmitter results in a specific behavioural pattern (Posner *et al.*, 2007).

This review will focus on the behavioural pattern of aggression, commencing with defining and categorising the trait. An investigation of the psychological development of the individual, with a special focus on how this may influence aggressive behaviour, will follow. Finally, serotonin will be discussed as one of the most influential neurotransmitters to aggressive behaviour.

2.1. Psychological aspects of behaviour

Before genetics emerged as a component of behaviour, research on behaviour was done based purely on observation. Psychologists, as the leaders of that age, formulated several models to describe how behaviour is learned. The mechanisms of these models include:

- a few basic learning mechanisms (Skinner, 1981);
- a large number of mechanisms, with aggression among them (Lorenz, 1956).

The basic function of these mechanisms is to receive input, process it, and produce a specific output or result. The input can be from the external or internal environment of the organism (Buss, 1995; Barkow *et al.*, 1995).

2.1.1. Human aggressive behaviour

Charles Darwin first discussed survival of the fittest in his book *On the Origins of Species by Means of Natural Selection: or, The Preservation of Favoured Races in the Struggle for Life* (Darwin, 1869). He stated that certain individuals within a population had the felicitous predisposition to be more adaptable to their environment, thus ensuring their future existence (Darwin, 1869). Aggression has been necessary for the survival of a species since the beginning of time (Hamilton, 1964; Cosmides & Tooby, 1994; Buss & Shackelford, 1997). It is also referred to as agonistic behaviour, occurring both in humans and animals (Olivier & Young, 2002). Skeletal remains from the early hominids show evidence of aggressive behaviour. Blunt force trauma can clearly be identified in the broken bones, indicating that the individual succumbed to his injuries (Trinkaus & Zimmerman, 1982). This shows that even though behavioural patterns were very primitive, aggressive behaviour was already prevalent. Darwin's theory was proven repeatedly to be the most fitting annotation to the survival of a species.

Different schools of thought are in constant conflict about the motivational force behind

aggressive behaviour. From evolutionary psychology an *Interactionist Model* was developed (Huesmann & Eron, 1989), providing two basic motivations for all human behaviour:

- it is driven by internal mechanisms, propelled into action by an environmental trigger or input (Huesmann & Eron, 1989) – the psychological viewpoint;
- it has gone through a process of evolution, remaining as a trait selected for (Cosmides & Tooby, 1994; Barkow *et al.*, 1995) – the genetic viewpoint.

Any basic definition of aggression state that it is behaviour that has the intent to harm. Humans have a higher brain function and can decide on and motivate behaviour. Therefore, in humans two further rules are applied to the definition of aggression (Berkowitz, 1993a; Bushman & Anderson, 2001; Anderson & Bushman, 2002; Baron & Richardson, 2004):

- the individual causing the harm (the offender) should know that harm is done, as intended;
- the individual being harmed (the target) should know that harm is coming, and try to avoid it.

When harm is not the intent, the behaviour is not seen as aggressive. The target didn't anticipate the harm, thus didn't avoid it (Anderson & Bushman, 2002). The difference then between violence and aggression is the amount of harm intended. With violence extreme harm, such as to cause death, is intended. All types of aggression are not violent, but the basis of violence is aggression (Anderson & Bushman, 2002).

Small specifications can be added to the definition in order to better understand the specific parameters included. This is done specifically in behavioural research and will be further discussed when other influencing factors are mentioned.

2.1.2. Types of aggressive behaviour

Internal and external stimuli are important factors in causing aggression. There are seven stimulus situations in animals (Moyer, 1967):

- predatory aggression, caused by being in the presence of natural prey;
- inter-male aggression, caused by the presence of an unfamiliar male in the natural

habitat;

- fear-induced aggression, caused by threats and characterized by following a failure at an escape attempt;
- irritable aggression, caused by an environmental stressor, like isolation, electric shock or sleep deprivation;
- territorial aggression, caused by an intruder in the home territory;
- maternal aggression, caused by a threat to the mother's young;
- instrumental aggression, caused by any of the above mentioned stimulus, but enhanced by learning (enforced by receiving a reward if the learned behaviour is exhibited).

Based on the stimulus leading to the aggressive response in humans the first distinction can be made between Offensive and Defensive aggression (Adams, 1979). Offensive aggression is seen as attacking and intending to do harm (Blanchard *et al.*, 1977). Defensive aggression is more about protection and self-preservation (Adams, 1979; Anderson & Bushman, 2002).

Based on the internal mechanisms of the individual, a further distinction can be made between hostile and instrumental aggression. Impulsive aggression is mostly driven by anger. Anger is an emotional state of arousal, also associated with the presence of irritability and frustration. This anger may be the result of provocation. Such behaviour can be defined as hostile or reactive aggression (Berkowitz, 1993a; b; Conner *et al.*, 2010). When the aggressive act is premeditated and calculated, it is defined as instrumental or proactive aggression (Berkowitz, 1993b). These distinctions are based on the psychological processing of a stimulus.

2.1.3. Psychological development

Variation in aggressive behaviour can be categorised for better understanding and quantification (Anderson & Bushman, 2002). Psychologists formulated different theories concerning the motivation of aggressive behaviour.

2.1.3.1. Instinct Theory

The *Instinct theory* was developed by Lorenz (1956), who was of the first scientists to study animal behaviour. Lorenz became famous for his idea of fixed action patterns of instinctive behaviours. He suggested that a specific environmental stimulus is followed by a specific

behavioural pattern (Brigandt, 2005), becoming stereotyped for specific situations (Kalikow, 1983; Anholt & Mackay, 2009).

2.1.3.2. Domain Specific Theories of aggression

The *Domain Specific Theories* focus on the specific situation leading to the aggressive act (Anderson & Bushman, 2002). Past experience will determine what the individual thinks should happen. If the only coping mechanism is aggressive behaviour, the individual will be more prone to aggression. Five theories can be classified under the *Domain Specific Theories*, namely the *Cognitive Neoassociation Theory*, the *Social Learning Theory*, the *Script Theory*, the *Excitation Transfer Theory* and the *Social Interaction Theory.* All these theories discuss how we handle specific situations. It is based on what we learned, with the focus on whom or where we learned it from.

The *Cognitive Neoassociation Theory* (Berkowitz, 1989, 1990, 1993a) relates to the process of automatic connotation between thoughts, memories and physiological responses to unpleasant situations. Physically uncomfortable situations or environments (such as noisy areas, very hot temperatures or unpleasant smells) can produce negative effects. The resulting behaviour is then connected to that specific stimulus. This stimulus can then be repeated, with a similar response. For individuals predisposed to aggression, the negative effect first presents as frustration or anger (Berkowitz, 1989, 1990, 1993b). When this pathway is accessed repeatedly, it becomes stronger, connecting different emotions to the same stimulus and memory. This conditions the behaviour. When anger then becomes more heated it can be turned into aggressive behaviour much easier (Collins & Loftus, 1975).

Albert Bandura is known for his *Social Learning Theory* (Bandura *et al.*, 1961; Bandura & Walters, 1963; Bandura, 1977). The four basic elements identified by Bandura for social learning involves:

- the ability of the individual to pay attention to all the aspects of another's behaviour,
- the ability then to remember the behaviour,
- putting it into action as the motor reproductive process,
- finally identifying and reacting to the social cues motivating the specific behaviour (Bandura, 1977; Louw & Edwards, 1998).

This is another way to condition behaviour. Bandura's *Social Learning Theory* explains how individuals can see behaviour, model it and adopt it (Bandura *et al.*, 1961). Positive reinforcement will enforce the prevalence of the behaviour. Negative enforcement will cause inhibition of the behaviour (Skinner, 1938, 1991; Bandura *et al.*, 1961). Learning can however also happen without the positive or negative reinforcement. The *Social Learning Theory* states that all behaviour can be learned in this way, even aggressive responses and other complex behavioural patterns (Mischel, 1973, 1999; Bandura, 1983, 2001; Mischel & Shoda, 1995). A better understanding is gained of the individual's beliefs about social behaviour and the expectations they have of social settings. The foundation for these believes and expectations are laid largely during the developmental years within the family setting (Patterson, 1982; Patterson *et al.*, 1989). Children react aggressively within the family situation in response to aversive behaviour from another family member. If there is a withdrawal by the other family member from the aggressive response, it serves as reinforcement. The aggressor will learn to counteract aversive behaviour with aggression (Patterson, 1982).

Apart from the recognition of his *Social Learning Theory*, Bandura also used this theory to deduce experimental designs. His Bobo doll experiments are used to observe toddlers' interaction with a life-sized doll. They are used to study and explain the importance of environmental influences on the development of appropriate and inappropriate (especially aggressive) behaviour (Bandura, 1977). Children from unstable homes, where they witness aggressive behaviour, have a higher prevalence of aggressive behaviour (Patterson, 1982; Anderson & Bushman, 2002).

The *Script Theory* is based on the concept of acting and role-play. Children learn behaviour from what they perceive (as in the *Social Learning Theory*). They connect the behaviour to a specific social setting (Schank & Abelson, 1977; Abelson, 1981). This behaviour will then always be used in this social setting. Thus, the script acts as a guide whereby social behaviour will be determined (Huesmann, 1983, 1986). The difference between the *Social Learning Theory* and the *Script Theory* is the individual being modelled. The *Social Learning Theory* focuses on the family environment and learning from the family. In the *Script Theory*, any individual respected by the observer can be regarded as the "role model". When this script is rehearsed repeatedly, the links between the social cues and the behavioural responses becomes stronger. This will

make the individual's behaviour constant. Another way to make this behaviour more constant is to link it to other social cues. This increases the number of situations that can cause a specific behaviour (Anderson, 1983; Anderson & Godfrey, 1987; Marsh *et al.*, 1998; Anderson & Bushman, 2002). An example of this is media violence where children watch numerous movies depicting situations where guns are used to force people into submission. This child might use this script to get his or her way also.

The *Excitation Transfer Theory* efficiently explains why some people seem to overreact in certain situations. Once an individual becomes physiologically aroused it takes time to return to a normal calm state. According to Zillmann (1983) if more than one of the provocative episodes occurs close to each other in time, the anger from the first episode can linger. The residual anger will influence the behaviour in the second episode. This can cause the behaviour to seem as an overreaction.

The *Social Interaction Theory* sees aggressive behaviour as the result of social influence. Tedeschi and Felston (1994) wrote that an individual with strong influence can use this to coerce his target into aggressive behaviour. This is made possible by the target's feelings of inferiority to the influencer. The reward for the aggressive behaviour is something of value (e.g. information, money, safety), to settle an injustice (felt by the target) or to gain social status (e.g. respect, toughness, competence). This explains gang behaviour where there is constantly a power-play. An individual with an over inflated self-esteem (bordering on narcissism) will react aggressively to protect his or her superiority (Baumeister *et al.*, 1996; Bushman & Baumeister, 1998). This theory shouldn't be confused with the script theory. With the *Social Interaction Theory* the observed individual has an active role in forcing and enforcing the observer's behaviour.

2.1.3.3. The General Aggression Model

The *General Aggression Model* (GAM) was devised by Anderson and Bushman (2002) by integrating the previously explained theories from the *Domain Specific Theories* (the *Cognitive Neoassociation Theory*, the *Social Learning Theory*, the *Script Theory*, the *Excitation Transfer Theory* and the *Social Interaction Theory*).

The GAM acts as a structure to guide how aggression is perceived and interpreted. It also explains the decision making process (Bushman & Anderson, 2001; Anderson & Bushman, 2002). The key features of these information structures are (Collins & Loftus, 1975; Fiske & Taylor, 1991; Bargh, 1996; Wegner & Bargh, 1998):

- experience helps to formulate and develop them;
- they have a multilevel influence – basic to complex;
- they are sometimes linked to beliefs about behaviour;
- they are used as a guide whereby people's social environments are interpreted, so they can respond to it.

The GAM's main focus is the episode or immediate situation. This episode happens in one cycle consisting of:

Input. Biological, environmental and psychological factors can have an influence on the manifestation of aggression. By knowing how these factors work, the trait they influence can be manipulated. Two major factors playing a role here are person-factors and situational-factors.

Person-factors include everything that makes a person unique – personality traits, beliefs, attitudes and genetic makeup (described later). Most person-factors are stable, meaning that the stay constant over time and for different situations. Personality influences how a person sees the world. It also influences the situations a person will be drawn to and feel comfortable in (Mischel & Shoda, 1995; Mischel, 1999; Anderson & Bushman, 2002).

Certain traits influence aggressive behaviour more. An example of this is a person with high self-esteem who is more prone to higher levels of aggression. As mentioned earlier, narcissists can become highly aggressive in a situation compromising or threatening to their abnormally high self-esteem and self-image (Kernis *et al.*, 1989; Baumeister *et al.*, 1996; Bushman & Baumeister, 1998).

Bandura (1977) focussed more on beliefs and their role on aggression. He argued that a person first has to believe that he can commit the specific aggressive act (an indication of self-efficacy). Then they must also believe that doing this will have the desired effect (an indication of outcome efficacy). Possessing both these beliefs will be motivation for aggression. These

beliefs can then also be used to predict an individual's future aggressive episodes (Huesmann & Guerra, 1997).

Attitudes are closely related to beliefs. Attitudes are guides used by an individual to measure and evaluate themselves, others and issues (Petty & Cacioppo, 1986). An individual with a specific belief about something will develop an attitude towards it. In the case of racism, it is beliefs and attitudes towards other races. It becomes a problem when the attitude is positive towards aggression and violence. Because of the specific belief and attitude, the aggressive behaviour will only be directed at a specific race (Malamuth *et al.*, 1995; Anderson, 1996).

Values are also closely related to beliefs. Values are our beliefs of what we should and shouldn't do. It explains why certain populations find it acceptable to handle interpersonal conflict in a violent and aggressive way (Nisbett & Cohen, 1996). Similar to this, values also link up with social scripts, as it is a motivation for how we should behave.

Situational factors concerns the specific situation in which the aggressive behaviour occurred. It can be the presence of provocation, influenced by cognition, affect and arousal (Anderson & Bushman, 2002). Aggressive cues stimulate aggression related memories. An example is an individual prone to "Road Rage", who finds himself in rush hour traffic (Carlson *et al.*, 1990). Seeing violence – like watching violent movies or playing violent video games – can also act as a cognitive aggressive cue (Bushman, 1998; Anderson & Dill, 2000; Bluemke *et al.*, 2010; Coyne *et al.*, 2011). The *Cognitive Neoassociation Theory* explains how these cues are formed and enforced.

Provocation is the most important social cue that can lead to aggression (Berkowitz, 1993b). It can range from verbal provocation (like insults) to physical provocation (pushing or shoving). The aim is to provoke a reaction, specifically aggressive behaviour.

The abovementioned is related to how the individual experiences the immediate internal and external environment leading to an aggressive act. The next section in the aggressive response is then the channel by which this interpretation of the environment is then relayed.

Routes. This is the second step in the aggressive episode. Neurotransmitters modulating

and regulating behaviour will be active during this component of the GAM. This stimulation can now access aggressive thoughts (Anderson, 1997) resulting in aggressive behaviour (Bushman, 1995). These routes are the theories – which make the GAM – in essence. They consist of three important components (Anderson & Bushman, 2002):

- Cognition: Hostile thoughts and Scripts are most influential to this component. Cognitive Neoassociation, as explained earlier, is a way of accessing behavioural patterns through thought processes. By having hostile thoughts, more pathways to aggressive behaviour is learned and conditioned. Scripts on the other hand, are a guide to indicate the appropriate behaviour in situations. By repetitive aggression as a coping mechanism, adopts it as an appropriate behavioural response (Bushman, 1998; Anderson & Dill, 2000).

- Affect: It is seen as influences in the environment to affect the individual. This may include the specific mood and emotional state the individual is in, as well as the temperature or humidity for example. Expressive motor responses, which include automatic reactions (like facial expressions) (Izard, 1991) are also included. The neurotransmitter serotonin is responsible for suppressing the feeling of anxiety. A lack of suppression can give rise to frustration, fear or anger. A less anxious individual will be more prone to impulsive behaviour leading to aggressive behaviour, as will be explained later in the review.

- Arousal: This aspect has to do with the level of aggression felt right after the stimulus. It can affect the behaviour to follow in two ways, both applying to the Excitation Transfer Theory.

The first has to do with the stimulus of focus. If it follows an initial stimulus irrelevant to the specific situation, a heightened level of aggression will be observed (Geen & O'Neal, 1969). When an individual is already frustrated, anger and aggression will follow more easily the closer it is to the frustration stimulant.

The second way is when the aggression is seen in situations that require another form of aggressive behaviour. The aggressive behaviour is then mislabelled. An example of this is when the players in a rugby match exhibit violence while playing a tough game (Zillmann, 1983).

Outcomes. The outcome is maybe the most important component of the GAM. It is the behavioural manifestation (where interventions can be implemented to prevent negative

behaviour, like aggression). The first step involved in this process is called Immediate Appraisal. It is an automatic action (already discussed as the Inputs and Routes). It is effortless and spontaneous. What this action entails is merely an assessment of the situation and which action should follow. If the individual was thinking about unpleasant and upsetting things, his action will reflect that. The action furthermore also depends on the individual's personality (social learning history) and present state of mind (which action is mostly accessed). Before actually acting, the mind goes through reappraisal. It plans alternative pathways to respond, until a decision is reached. If the individual believe that the cue was intentionally harmful, anger will follow, then possibly aggression (Anderson & Bushman, 2002).

The GAM also takes into account the past and future experiences of the individual. The future experiences are the individual's goals, plans and expectations. They will have an influence in the decision making process. Past experiences shape a person's beliefs and attitudes.

2.1.4. Environmental influence

Researchers agree that aggression and anti-social behaviour have familial tendencies (Gottesman, 1963, 1966; Scarr, 1966; Reznikoff & Honeyman, 1967; Owen & Sines, 1970; O'Connor *et al.*, 1980; Rowe, 1983; Loehlin *et al.*, 1985, 1987; Rushton *et al.*, 1986; Tellegen *et al.*, 1988; Lytton *et al.*, 1988; Stevenson & Graham, 1988; Rende *et al.*, 1992; Miles & Carey, 1997; Plomin *et al.*, 2008). Researchers of the psychology, genetic and behavioural fields also agree that the developmental environment of humans is of utmost importance in shaping behaviour. Environmental stressors in the form of maltreatment and neglect during the childhood years results in an increased risk for the development of aggressive behaviour, violent tendencies and even abusive parenting styles (Craig, 2007). This is also known as the "cycle of violence" (Kessler *et al.*, 1997; Johnson, 1999; Caspi *et al.*, 2002). Longitudinal studies also showed that aggression remains constant (Farrington, 1986, 1989). A distinction between juvenile aggression and aggression in adults is necessary (Rowe & Rodgers, 1989; Carey, 1993; Gottesman & Goldsmith, 1994) as it seems that the environment plays the bigger role in juvenile aggression, as they are still developing physically and psychologically. The genetic contribution has a higher influence on adult aggression.

The "S*morgasbord*" model of gene-environment interplay (Plomin *et al.*, 1977; Eaves *et al.*,

1977; Scarr & McCartney, 1983) gives an interesting twist to genetic and environmental contributions. It suggests that the genotype will try different environments, and finally choose the environment that is most compatible with the genotype. Thus, the genotype actually determines the environment.

It may thus be concluded that aggression is a complex behaviour, with psychological, environmental and genetic factors. So far, emphasis has fallen on the importance of a stable developmental environment. The different theories explain how children may learn inappropriate behaviour. The *Social Learning theory*, *Script theory* and *Social Interaction theory* emphasise the importance of good role models. It explains from whom the child learns behaviour. All the different theories are integrated with a focus on aggression, to produce the *GAM*. The *Cognitive Neoassociation theory* and the *Excitation Transfer theory* have stronger physiological input. The *Cognitive Neoassociation theory* links the psychological aspect to the physiological aspect. The next section covers the physiological processes involved in aggressive behaviour.

2.2. Neurophysiology

The brain senses changes in the organism's environment by means of specialized sensory organs in the peripheral nervous system (Anholt & Mackay, 2009). The message from the environment is then relayed to the brain for interpretation. The pathway this message has to travel consists of several components and processes. These underlying mediating processes within the brain can be the result of different genetic components. There will be differences between the components (or then the pathway followed) of normal aggressive responses (primary aggressive behaviour), and aggressive behaviour as a symptom of another disorder (secondary aggressive behaviour).

Of all the different neurotransmitters, the most extensive and impressive in vertebrates is the serotonergic system. It targets primary, secondary and tertiary motor areas in the brain and spinal column (Steinbusch, 1981; Jacobs & Azmitia, 1992). Serotonin is responsible for mediating the organism's behaviour. Aggressive behaviour, the theme of this review, is a very complex form of human behaviour. As the serotonin pathway forms an integral component of human behaviour, it has an effect on aggression also. But to understand this system better, a basic

explanation of neurophysiology is necessary.

2.2.1. Neurotransmitters

Behaviour is the physical manifestation of a very complex and integrated messenger system embedded in the CNS (central nervous system). Neurons act as pathways between the environment and the brain (Anholt & Mackay, 2009).

An important component of neuronal behaviour is the action potential. The rate and pattern of the electrical impulse is responsible for the relay of the correct information. Each stimulus has a unique rate and pattern (Jacobs, 1991; Jacobs & Azmitia, 1992). The impulse then travels along the axon of the neuron until it reaches the axon terminal. Two adjacent neurons physically never touch. They are connected chemically in the form of neurotransmitters (Crossman & Neary, 2005; Sherwood, 2007). Neurotransmitters are chemical messengers stored in vesicles in the axon terminal of every neuron within the human body. It is thought that every neuron throughout the human body carries all the different neurotransmitters. Only the specific neurotransmitter for the specific stimulus is released at a time.

After a neurotransmitter is released it binds to the specific receptor in a lock-and-key formation (Sherwood, 2007). Thereafter, the neurotransmitter's function has been completed and it has to be removed from the synaptic cleft by one of two ways. The first way is by reuptake by a transporter. The other way is removal by means of metabolism (Jacobs, 1994). The latter is done by an enzyme active in the synaptic cleft. If any one of these components – the receptor, transporter or enzyme – is not functioning correctly, it would have serious effects on the transmission of signals to and from the brain (Crossman & Neary, 2005). Focus shift towards these components in the CNS when trying to identify specific genes influencing specific behavioural patterns (Våge & Lingaas, 2008). Changes in the genes regulating enzymes, transporters and receptors in the brain are associated with altered behaviour (Brunner *et al.*, 1993; Manuck *et al.*, 1999; Savitz & Ramesar, 2004).

Evidence has been found of the contribution of several neurotransmitters to behaviour. These neurotransmitters are acetylcholine, GABA (Gamma Aminobutyric acid), biogenic amines (like dopamine, norepinephrine and serotonin), and neuropeptides (like opioid peptides,

substance P, cholecystokinin, vasopressin) (Siegel *et al.*, 1997, 1999; Gregg & Siegel, 2001; Siegel, 2004). Some have excitatory and other inhibitory functions on behaviour. A few examples are summed up in Table 2.1. Here we see some neurotransmitters can be either inhibitory or excitatory in function. Also, certain neurotransmitters can have both inhibitory and excitatory functionality depending on which receptor it binds to. An example of this is serotonin, a neurotransmitter associated with behavioural modulation in animals and humans.

Table 2.1: Listing of excitatory and inhibitory neuro-transmitters and their receptors.

	Receptor	
Neurotransmitter	Inhibitory function	Excitatory function
Acetylcholine		Muscarinic
Cholecystokinin		CCK8
Dopamine		D2
GABA	GABA A	
Glutamate		NMDA
Interleukin-1		IL-I Type I
Interleukin-2	IL-3R alpha	IL-3R alpha
Norepinephrine		Alpha 2
Opioid peptides	μ	
Serotonin	5-HT1A	5-HT2A
Substance P	NK1	NK1
Vasopressin		V14

2.2.2. The Serotonergic Pathway

Serotonin {abbreviated as 5-HT (5-hydroxytryptamine)} activity is mostly located in an area of the brain called the raphe nuclei. This is a primitive brain region located in the brain stem. From there it projects nerve fibres to nearly all areas of the central nervous system. This primitive region has remained relatively constant throughout evolution (Jacobs, 1994). The serotonin binding and transport mechanisms have been studied as contributing factors to aggression in animals (Soubrié, 1986; Coccaro, 1989; Higley *et al.*, 1996; Sánchez & Meier, 1997; Edwards & Kravitz, 1997) as well as human aggression and anxiety (Gingrich & Hen, 2001).

Neurons responsible for serotonin activity represents about a millionth of the total number of neurons in the human body. Regardless, they have a far reaching effect, reaching around 500

000 targets (other neurons, glands or muscle cells). They also have a very distinguishing action potential pattern. The serotonin neurons have a constant rate of firing of around three spikes per second. This increases during emotional arousal, causing an increase in serotonin release (Jacobs, 1994).

Attempts at increasing synaptic activity by administrating serotonin resulted in the "serotonin syndrome" in all species tested. It is characterised by hyperactivity, shakes, tremors and certain repetitive movements of the head and limbs (Sternback, 1991). The response is limited to motor signs, with very little to no impact on the sensory system (Jacobs & Fornal, 1995). Serotonin appears primarily to have modulator functionality with muscle tone and motor activity. It also has a secondary role that inhibits the processes related to sensory-information processing (Jacobs & Fornal, 1997).

In 1984, Jacobs and his colleagues conducted a study on cats, where serotonin secretion rate and pattern of electrical impulses were monitored. They noted that when a cat was "surprised" by a novel or provocative stimulus (like a loud noise), the neuron's activity would seize (inhibiting motor function). This allows the cat to focus on the sensory stimulus for several seconds and then return to normal activity. Furthermore, it was found that serotonin neurons also go "silent" during REM (Rapid Eye Movement) sleep phase, when the muscles are completely paralysed. Dreaming also occurs during this phase, which is also sensory information usually inhibited by serotonin release (Jacobs *et al.*, 1984). Serotonin is responsible for initiating motor function, bringing the motor neurons to firing threshold (Jacobs & Fornal, 1995). It is also responsible for inhibiting sensory information input that might prevent or terminate the motor output. This is absent in impulsive behaviour and can be connected to the *Excitation Transfer theory* explained earlier. An individual with abnormal serotonin function will experience aggressive arousal easily. It will lead to aggressive behaviour if the individual does not have ample time to cool back down.

From previous studies done on the serotonergic pathway (Shih, 1991; Brunner *et al.*, 1993; Cases *et al.*, 1995; Lesch *et al.*, 1996; Oliver *et al.*, 1997; Greenberg *et al.*, 2000; New *et al.*, 2001; Bjork *et al.*, 2002; Hariri *et al.*, 2002; Sanders *et al.*, 2002; Anguelova *et al.*, 2003; Huang *et al.*, 2003; Abdolmaleky *et al.*, 2004; Khait *et al.*, 2005; Alia-Klein *et al.*, 2008) specific genes of interest is the serotonin receptor genes (*HTR1A*, *HTR1B* and *HTR2A*), and the serotonin

transporter (*SLC6A4*) along with the enzyme responsible for metabolising serotonin specifically, called Monoamine Oxidase (*MAO-A*). This pathway has been investigated in studies on animals and humans. In humans it has been linked to pathological aggression and anxiety (Gingrich & Hen, 2001). The influence of serotonin, the interaction with MAO-A, plus inappropriate social development will result in an individual who tries to manage the anxiety (created by a deficiency in serotonin) by means of aggressive behavioural responses. This individual will develop a level of tolerance, where progressively aggressive behaviour will be necessary to diminish anxiety.

2.2.3. Serotonin receptors

Receptor genes play a role in serotonergic neurotransmission. They are very good candidate genes for aggressive behaviour (Van Den Berg *et al.*, 2008). They function by means of negative feedback, where they initiate the inhibition of serotonin release as soon as they reach a specific firing rate (threshold). Below that firing rate, the presynaptic neuron will continue to release serotonin until negative feedback is initiated. An inadequate serotonin release causes anxiety in individuals. This is treated with selective serotonin reuptake inhibitors (SSRI's). It is prescription medication that blocks the reuptake of serotonin by the transporters, causing the synapse to be flooded with serotonin. This constant stimulation of the serotonin receptors causes desensitization, resulting in a lessened suppression of the serotonin release (Jacobs, 1994). An excess of serotonin receptors present will result in reaching the firing rate too soon thus inhibiting serotonin release. When SSRI's are present at the synaptic cleft, it will inhibit serotonin reuptake, thereby keeping the serotonin in the synaptic cleft.

Repetitive motor movement stimulate serotonin secretion. In the case of an individual experiencing anxiety (untreated by SSRI's), the individual will continue to jiggle his or her foot to achieve this. It is an attempt to lessen the anxiety, because negative feedback was initiated too soon. Individuals suffering from Obsessive Compulsive Disorder (OCD) activates the serotonin release by performing a repetitive motor behaviour or compulsion (like washing hands), motivated by a specific obsession (like fear of germs) in order to lessen anxiety. This repeated stimulation causes an increase in serotonin release, thereby reducing anxiety. This also explains how repetitive movement through exercise has a positive influence on most psychological disorders like depression and anxiety.

2.2.3.1. *HTR1A*

The 5-Hydroxytryptamine (serotonin) receptor 1A, or *HTR1A*, regulates anxiety, stress and aggression (Oliver *et al.*, 1997). The *HTR1A* gene consists of 1 270 bases in humans. This gene forms one exon in which several missense polymorphisms have been identified. The -1019C>G SNP has successfully been linked to anxiety and depression (Strobel *et al.*, 2003; Lemonde *et al.*, 2003). Other correlations found to this SNP are Schizophrenia and substance abuse disorders (Lesch & Gutknecht, 2004). This specific SNP over express the gene, because it regulates the transcription of the gene (Wasserman *et al.*, 2006).

Animal studies have also shown similarities to what have been found in humans. Knockout mice have shown heightened anxiety and stress phenotypes (Ramboz *et al.*, 1998; Heisler *et al.*, 1998). Studies on mice with this gene knocked out:

- The gene was disrupted by an insertion of a vector downstream of the sequence coding for the third transmembrane domain. This resulted in the lack of functional receptors. It caused increased anxiety and aggression (occurring more in males than in females) and reduced behavioural despair (where the homozygous mutant mice were more persistent on survival than the wild type) (Ramboz *et al.*, 1998).

- The gene was disrupted by inserting a vector and thereby deleting the 3' portion of the coding region. This caused the gene to be undetected, resulting in increased anxiety (by means of avoidance of novelty) (Heisler *et al.*, 1998).

- An insertion, deleting the start codon and 123 bp (base pair) of coding sequence, resulted in increased anxiety. This manifested as novelty avoidance and vigorous attempts to escape stressful situations. Also, hyperactivity for the homozygous mutant form and intermediate levels for the heterozygous form was seen (Parks *et al.*, 1998; Sibille *et al.*, 2000).

2.2.3.2. *HTR1B*

Abnormal functioning of the serotonin receptor 1B, or *HTR1B,* gene in humans, have been associated with alcoholism, suicidal behaviour and obsessive-compulsive behaviour (Sanders *et*

al., 2002; Huang *et al.*, 2003). The *HTR1B* gene has also been linked to ASPD, intermittent explosive disorder and alcoholism (Lappalainen, 1998).

The *HTR1B* gene in humans consists of an exon of 1179 bases in length. The 861G>C polymorphism (Sidenberg *et al.*, 1993) is associated with anti-social behaviour (Lappalainen, 1998) especially impulsivity. The G allele shows a decrease in receptor count, whereas the C then shows higher numbers of receptors. The G allele is associated with higher levels of self-directed aggression, as seen in suicidal individuals (New *et al.*, 2001).

Studies on mice with this gene knocked out was based on the disruption of the gene due to an insertion into the coding region via homologous recombination (Saudou *et al.*, 1994; Bouwknecht *et al.*, 2001) resulted in:

- Homozygous mutant form:
  - Increased drinking (Bouwknecht *et al.*, 2001).
  - Decreased anxiety related response (López-Rubalcava *et al.*, 2000).
  - Increased aggression towards males, also with increased frequency and intensity (Saudou *et al.*, 1994).
  - Decreased startle reflex (Dirks *et al.*, 2001).
  - Abnormal locomotor activation, for example limb movement speed increase (Buhot *et al.*, 2003).
  - Increased body weight (Dirks *et al.*, 2001; Bouwknecht *et al.*, 2001).
- Heterozygous
  - Abnormal operant conditional behaviour, meaning that “acquired auto-shaping” occurs faster (Pattij *et al.*, 2003).

As also with the *HTR1A* gene, the *HTR1B* gene causes aggression in the homozygous mutant form. Thus the functional *HTR1B* gene prevents aggression and anxiety.

2.2.3.3. *HTR2A*

The serotonin receptor 2A, or *HTR2A* gene is associated with schizophrenia, suicidal behaviour, problematic impulse control and aggression (Bjork *et al.*, 2002; Abdolmaleky *et al.*, 2004; Khait *et al.*, 2005). This gene has two introns and three exons, spanning 63 000 bases,

containing 230 SNPs of which a few has been associated with psychological disorders (Harvey *et al.*, 2003; Norton & Owen, 2005).

A correlation has been found between the -1438A SNP and an increase in gene expression in humans (Parsons *et al.*, 2004). Individuals with the 102C allele showed reduced expression, in comparison to the 102T allele (Polesskaya & Sokolov, 2002). Individuals suffering from OCD also showed an association with the 516C>T SNP variant along with the -1438A>G variant (Meira-Lima *et al.*, 2004). Other less prominent regions influencing gene expression are the -1420C>T and -738A>G polymorphisms (Myers *et al.*, 2005).

Knockout mice studies done:

Gene-stop was inserted into the 5' un-translated region (UTR) to block transcription. This caused reduced inhibition in a conflict situation, without affecting fear-conditioned behaviour. Also seen was acceptance to novelty in the homozygous mutant form, and abnormal anxiety related response in the heterozygous form. A conditional form was perceived when *HTR2A* heterozygotes also had the *SLC6A4* heterozygous form. This caused reduced inhibition and impulse control (Weisstaub *et al.*, 2006).

2.2.4. Serotonin transporters

The transporter is responsible for the reuptake of serotonin from brain synapses. It is also the target for most anti-depressant medication (SSRIs mentioned earlier) in humans (Barker & Blakely, 1996; Feldman *et al.*, 1997).

2.2.4.1. *SLC6A4*

Solute carrier family 6 (serotonin transporter), member 4, or *SLC6A4*, is a member of the sodium- and chloride-dependent transporters. It is associated with mental instability (Lesch *et al.*, 1996; Hariri *et al.*, 2002; Anguelova *et al.*, 2003). The *SLC6A4* gene identified a polymorphism in the gene related to anxiety in humans (Lesch *et al.*, 1996). Replications of this study have proven it to be correct (Greenberg *et al.*, 2000; Sher *et al.*, 2000; Osher *et al.*, 2000; Du *et al.*, 2000; Jang *et al.*, 2001; Melke *et al.*, 2001). Furthermore it was also linked to depression, neuroticism, affective disorders and suicidal behaviour (Lesch *et al.*, 1996; Greenberg *et al.*,

2000; Du *et al.*, 2000). The *SLC6A4* gene's main influence is related to sensitivity to stress. Chaouloff *et al.* (1999) investigated several neurons where this gene is expressed and have terminals. In the brain these are the neuroendocrine regions related to stress (in the hypothalamus and brainstem) as well as the behavioural regions (in the amygdala, where the previously named serotonin receptors are also active).

This gene consists of several polymorphic loci influencing its expression. Over expression is caused by different variants:

- the rare Ile425Val mutation (Ozaki *et al.*, 2003),
- the rare Gly56Ala mutation (Ozaki *et al.*, 2003),
- *5-HTTLPR* (5-hydroxytryptamite transporter-linked polymorphic region) long (L) allele, with the absence of the 44 bp deletion (Heils *et al.*, 1996), located in the promoter region of the gene.

Vulnerability to environmental stressors resulting in substance abuse, anxiety and aggression was linked to the S allele of the *5-HTTLPR* region (Lotrich & Pollock, 2004; Feinn *et al.*, 2005). The L allele has a specific polymorphism rs25531 (-544-1392A>G) that reduces its functionality to that of the S allele. This polymorphism thus also creates two variations namely the L_A and L_G alleles, of which the latter is equivalent to the S allele (Hu *et al.*, 2006). The L allele is formed by 16 repeat of the VNTR. The S allele is created by a deletion between the sixth and eighth repeat of these 16 repeats (Heils *et al.*, 1996). The under expressed variants of this gene have an influence on stress sensitivity and higher levels of anxiety. An excess of serotonin in the synaptic cleft (due to reduced reuptake because of under expression) would lead to the negative feedback system (controlled by the serotonin receptors) being activated prematurely.

Studies on mice with this gene knocked out:

- The insertion of a gene-trap vector into the intron region resulted in decreased exploratory behaviour in novel surroundings and abnormal response to novel objects (Zhao *et al.*, 2006).
- The partial deletion of the first coding exon of the transporting coding region caused abnormal fear (remained immobile in novel situation) and anxiety-related (increased indecision about what to do in novel situation) behaviour and abnormal avoidance

behaviour (high rate of escape failures with low rate of acceptance of failures) (Lira *et al.*, 2003).

- Replacement of a DNA segment containing exon 2 caused the functional protein not to be expressed (Bengel *et al.*, 1998). This resulted in the homozygous phenotype:
  - Decreased exploration of an unknown environment (Zhao *et al.*, 2006).
  - Abnormal response to novel objects (Zhao *et al.*, 2006)
  - Cocaine preference (Sora *et al.*, 1998).
  - Abnormal sleep patterns (Wisor *et al.*, 2003).
- As mentioned, *SLC6A4* heterozygous gene coupled with heterozygous *HTR2A* gene caused the conditional genotype resulting in decreased impulse control and inhibition (Weisstaub *et al.*, 2006).

2.2.5. Monoamine Oxidase A

Monoamine Oxidase is an enzyme (product of the gene *MAO-A*) responsible for the metabolising of norepinephrine, serotonin and dopamine in the brain. It has a greater affinity towards serotonin (Shih, 1991). It is mainly active in the breakdown of serotonin to its metabolite 5-HIAA (Jacobs, 1994). The nucleotide sequence of this gene and also its partner *MAO-B*, has very little differences between species. These genes have passed through mammalian evolution without changing very much (Hashizume *et al.*, 2003). When this gene is absent or non-functional the result is aggression (Cases *et al.*, 1995; Shih & Thompson, 1999; Alia-Klein *et al.*, 2008). Aggression is caused by a point mutation of the *MAO-A* gene in humans. This was positively linked to the manifestation of impulsive aggression (Brunner *et al.*, 1993).

The *MAO-A* gene is a very long gene of around 88 170 bases, comprising of 15 exons located on the X chromosome (Shih *et al.*, 1999). It is positioned on the presynaptic terminal of neurons with monoamine oxidase activity (Westlund *et al.*, 1993). It governs the release and metabolising of serotonin. Impulsive-aggressive behaviour as well as reactive aggression in males is linked to the inactivity of this gene (Buckholtz & Meyer-Lindenberg, 2008).

A rare missense mutation (936C>T) producing a stop codon causes the gene to appear knocked out. This causes a significant increase in violent aggression (Brunner *et al.*, 1993). Another variant within the gene is a functional variable-number tandem repeat (*MAO-A* pVNTR)

polymorphism in the upstream region. It consists of 30 bp repeats influencing transcription of the gene. The 3.5 and 4 repeat variants (or *MAO*-A-H alleles) produce higher expression levels of the gene, whereas the 3 and 5 repeats (or the *MAO-A*-L alleles) produce lower levels of expression (Sabol *et al.*, 1998). The influence on aggression would be from the *MAO-A*-L allele.

2.2.6. Serotonin and Behaviour

Controversy exists around the functionality of serotonin as either behavioural instigator or modulator (Coccaro, 1992; Spoont, 1992; Brown *et al.*, 1994). It seems as though the latter is the actual role of serotonin when it comes to aggressive behaviour (Vergnes *et al.*, 1977; Marks *et al.*, 1977). A variety of abnormal behaviours, such as aggression, suicide and alcoholism, can be seen in individuals who have a defective serotonergic system (Coccaro, 1992; Linnoila & Virkkunen, 1992). The reasons for the continued uncertainty towards the specific role of serotonin in behaviour (and specifically aggressive behaviour) have to do with the experimental design when studying the behaviour. The most prominent problem is that most behavioural studies focus on the measures of emotions or personality traits, rather than investigating the aggressive response itself (Coccaro, 1992; Linnoila & Virkkunen, 1992). Also, there is very little consideration for the influential psychological development of the individual and his or her family. The psychological development and learning of appropriate behaviour is of significant importance, since it separates humans from animals. It shows that we have the ability to evaluate our situational environment and then decide on an appropriate behavioural response.

Conclusion

Aggressive behaviour is a very complex response and can have several contributing factors. The environmental influence is as important as the genetic influence, where both can act in an additive or interactive way when influencing aggressive behaviour. Identifying the phenotype is not necessarily a reflection of the underlying genotype. Epistasis can have misleading effects. Positively identified genes can also have pleiotropic effects on different phenotypes.

Eugenic movements scared researchers away from behavioural genetics for some time, arguing that research is done in abnormal behavioural patterns in an attempt to “cure” the human race of it. This is exactly the case with research on aggression, though it should not be

classified as Eugenics. Finding genetic and environmental influences to aggressive behaviour can not only provide us with a greater understanding of one of the most primitive forms of human behaviour, but also help us to know how to prevent this behaviour from becoming out of control. As seen throughout this review, there are a great number of influences to aggressive behaviour, with psychological influences being prominent throughout. To shy away from the Eugenics movement, behaviour genetic research doesn't necessarily focus only on the genetic component, but also how the psychological and environmental influence can be manipulated to produce a different phenotype. As seen in this review, the psychological development is as important as the immediate environmental influence. The genetic component, creating the physiology of the individual, remains the most important and least understood of all.

CHAPTER 3

Temperament and Personality as contributing factors to aggressive behaviour

Abstract

Temperament is seen as a collection of characteristic traits of an individual. It will determine how this individual will react to his or her environment. Personality is in effect the same basic principle. The difference is that it is further matured through experience. During the childhood years, temperamental traits can already easily be observed. Because temperament can be observed as early as the age of two months, a definite genetic component should be present. The serotonergic and dopaminergic neurochemical systems in the brain have received most attention in research aiming to identify correlations between specific behavioural patterns and genes. The temperamental constitution of an individual will have a great influence on the environmental exposure, and thus also the development of personality. As temperamental traits remain relatively stable over the developmental years, it can be utilised as a stable person-factor when quantifying behaviour.

Keywords: Behaviour, OCEAN, Stable person-factor

3.1. Temperament

Each individual has a unique collection of characteristics, called their temperament. These characteristics remain relatively stable for emotional reactions, changes in mood and sensitivity to environmental stimuli (Reber & Reber, 2002). Particular patterns of emotional reactions, mood shifts and sensitivity to stimulation may be the result of a biological component to temperament (Goldsmith *et al.*, 1987; Reber & Reber, 2002; Rutter, 2006). Environmental stimuli include loud noises, bright lights, sudden movements, touching and psychical contact. Manifestation of temperamental traits in infants may be due to genetic composition. In infants, these traits include smiling and laughter, fear, distress, frustration, soothability and activity level (Rothbart, 1981). Temperamental traits are seen to emerge during infancy and stay constant throughout life. In adults, the traits are assessed according to how an individual will react instinctively and without thinking (autonomic reactivity). The traits include motor tension, motor activation, cortical reactivity, susceptibility to discomfort, fear, frustration, sadness, high-intensity pleasures and low-intensity pleasures, relief, attentional shifting and focussing and behavioural inhibition (Derryberry & Rothbart, 1988). In short, temperament encompasses reactivity (in the form of emotional responsiveness, activation and arousal) and self-regulation (as seen as approach, avoidance and attention) contributing to individual differences (Rothbart & Derryberry, 1982; Rothbart & Ahadi, 1994).

Temperamental traits act within specific boundaries, called the temperamental dimensions (to be discussed later). They can be measured for a specific individual, for a specific time, in various situations. Another important characteristic of temperament is that it is not static. Environmental influences may cause change within the trait (Rothbart & Ahadi, 1994). Over time development of an individual will however remain within the boundaries of that temperamental dimension. The result is the development of personality. The way we see ourselves and others, for example, may influence adaptations such as coping skills and defences. These are components of personality that develop through the interaction of experience and temperament (Rothbart & Ahadi, 1994). Personality (to be discussed in more detail further on) is the human factor that influences an individual's patterns of behaviour, thoughts and feelings (Buss & Plomin, 1984; Reber & Reber, 2002; Rutter, 2006).

Temperament is studied by disciplines like developmental psychology (Rothbart & Derryberry, 1982), personality theory (Eysenck, 1981; Zuckerman, 1984), psychophysiology and psychosomatic medicine (Panksepp, 1981; Gray, 1988), clinical psychiatry, behavioural genetics (Fuller & Thompson, 1978) and education research (Campos, 1983). Even though each discipline has criteria of their own on the development of temperament, they do agree on the following points (Goldsmith *et al.*, 1987):

- The dimensions of temperament can be related to behavioural tendencies. They cannot predict specific behavioural acts.
- Temperament is an interpretation of individual differences. It doesn't specify species-general characteristics.
- Temperamental traits exist in continuity. The expression of temperament can be modified just as gene expression can vary throughout life.
- Temperament can be considered as a component of personality. As a child matures, experience can shape the behaviour of the child, giving rise to personality. The correlation between temperament and personality thus becomes unclear as the child ages.

Temperament provides us with dimensions or rather categories that individuals can be grouped in based on similarities (Kagan *et al.*, 1987). These categories then provide the ranges within which a trait may vary (Kagan & Snidman, 2004). For a comprehensive understanding of all the possible influential contribution to temperament (and thus individual differences), it should also be studied through a multi-disciplinary approach. The relevant disciplines are behavioural (e.g. inhibition or excitation of aggressive behaviour), psychological (e.g. the influence of anxiety), neural (e.g. activity and reactivity of the limbic system in the brain), physiological (e.g. autonomic arousal) and genetic (e.g. genes involved in the serotonergic system). All these disciplines should be integrated and the interaction between the different components should be investigated to understand the resultant behavioural manifestation (Calkins & Fox, 2002; Kagan *et al.*, 2002; Canli, 2004; Zuckerman, 2005; Rothbart & Posner, 2006). Only then can calculated deductions be made.

3.1.1. Measuring temperament

As previously mentioned, temperament should be regarded as a rubric, with specific trait (within the temperamental dimensions) measured in intensity and frequency. This is more preferential than the binominal presence or absence of a specific behaviour (Evans *et al.*, 2007). Temperament should be separated from motivations and abilities, components that may also influence behaviour. An example of this is a child kicking or hitting another child. The behaviour may be the result of provocation (motivation), or this child may lack appropriate socialization enabling play (abilities), or the behaviour may be the result of the child's inability to control his behaviour (temperament) (Rothbart, 1981; Goldsmith *et al.*, 1987; Evans & Rothbart, 2007).

Temperament is a very complex concept. Thus it is grouped first into three basic constructs. These constructs each have specific sub-constructs or contributing scales, also known as temperamental dimensions (Rothbart & Derryberry, 1982; Evans & Rothbart, 2007). As temperament undergoes changes over time, infant, adolescent and adult temperamental dimensions may vary (Rothbart & Derryberry, 1982; Rothbart & Ahadi, 1994).

3.1.1.1. Infant Temperament Dimensions

Various researchers have investigated infant behaviour to devise the six basic infant temperament dimensions (Shirley, 1933; Thomas, 1963; Escalona, 1968; Thomas *et al.*, 1968; Rothbart, 1981). These dimensions are based on response characteristics for different sensory receptors and response channels, and restricted response activation. During normal development, certain dimensions should be easily identifiable (Table 3.1).

- Rhythmicity: This dimension forms part of the characteristics of response. Its measurement is related to the maintenance level of the child, as it relates to sleep and hunger cycles (Thomas, 1963; Thomas *et al.*, 1968). An "easy child" will adapt to a rhythm more quickly.

- Threshold, intensity and adaptability of response: This dimension forms part of the characteristics of response, and can be assessed through the sensory channels on the scales of intensity, threshold, adaptability and mood (Thomas, 1963). Consistency in irritability, sensitivity and soothability can be established within the first four months (Birns *et al.*,

1969). In longitudinal studies, an individual's response showed consistency in the intensity along with positive correlations with other temperamental scales (Thomas *et al.*, 1968).

Table 3.1: Temperamental dimensions and components as identified at specific times during development, from birth to eight years of age.

Age	Dimensions	Components	References
0 – 2 years		Distress-proneness Activity level Attention orienting Alertness	(Rothbart, 1981)
2 – 3 years	Negative reactivity	Fearfulness Irritability / anger Frustration	(Thomas, 1963; Buss & Plomin, 1975, 1984; Thomas & Chess, 1977; Rothbart, 1981; Gray, 1988; Rothbart & Ahadi, 1994)
	Positive affect variable	Approach / positive affect Activity level Emotional persistence Rhythmicity	
3 – 8 years	Surgency / Extraversion	Approach High intensity pleasure Activity level Impulsivity Shyness	(Rothbart *et al.*, 1992; Ahadi *et al.*, 1993; Kochanska *et al.*, 1994)
	Negative Affectivity	Discomfort Fear Anger / frustration Sadness Soothability	
	Effortful Control	Inhibitory control Attentional focussing Low intensity pleasure Perceptional sensitivity	

- Distress to limitations: This dimension forms part of the activation of restricted response. When a child attempts to reach a goal and is unsuccessful, a resulting frustration may possibly be observed (Kramer & Rosenblum, 1970). Distress, resulting in "temper frequency" was a consistent behavioural pattern that showed evidence of a genetic component (Wilson *et al.*, 1971; Torgersen & Kringlen, 1978; Goldsmith & Gottesman, 1981). Studies on animal temperament showed a link between this trait and aggressive behaviour, as a response to frustration (Hall & Klein, 1942). This may be the case with human

temperament also.

- Fear: This dimension forms part of the activation of restricted response. It is related to the infant's acceptance or avoidance of intense or novel stimuli (Rothbart, 1981). Infants showed significant fearful behaviour (Gordon W. Bronson, 1968; Scarr & Salapatek, 1970). Measurements for anxiety showed correspondence between siblings and twin pairs (Gottesman, 1963; Scarr, 1966). Animal studies indicated a definite genetic component to fearful behaviour (Yerkes & Yerkes, 1936; Hall, 1951; Fuller & Thompson, 1978).

- Duration of orienting and distractibility: This dimension forms part of the activation of restricted response. It shows slight variation for different ages, as tested in several studies (McCall & Kagan, 1970; Paden, 1974; Self, 1974; Cohen, 1975). The most significant results indicated a negative correlation between attention span and the frequency and intensity of temper (as seen in the Distress to Limitations dimension). This occurs especially in ages six to 18 months (Wilson *et al.*, 1971).

- Activity level: This dimension forms part of the activation of restricted response. It is a measurement of motor activity. It is the most prominent childhood behaviour observed, and also the most studied (Richards & Newbery, 1938; Fries & Woolf, 1953; Thomas, 1963; Schaffer, 1966; Escalona, 1968). Several studies on heritability indicated a genetic contribution (Scarr, 1966; Willerman, 1973; Willerman & Plomin, 1973; Matheny *et al.*, 1976). It also has relative consistency in the trait for the first four months (Birns *et al.*, 1969) as well as later in life (Buss & Plomin, 1975).

- Smiling and laughter: This dimension forms part of the activation of restricted response, and serves as indicators that the infant feels safe (Rothbart, 1973; Srofe & Waters, 1976). Twin studies showed that monozygotic (MZ) twins had greater concordance in smiling and laughter than dizygotic (DZ) twins (Freedman, 1972). This is indicative of genetic loading.

3.1.1.2. Temperamental dimensions during development

Rothbart and Derryberry (1982) said that "temperament is defined as individual differences in emotional, motor, and attentional reactivity measured by latency, intensity, and recovery of response, and self-regulation processes such as effortful control that modulate reactivity". This

names the three most common dimensions of temperament as Effortful control, Negative affectivity and Extraversion/Surgency (Rothbart, 2007) (Table 3.2).

Table 3.2: The four temperamental dimensions, with contributing scales, as seen and classified in early adolescence (Rothbart, 2007).

Dimensions	Scales	Definition
Effortful control	Attention control	Focus and shift attention by will
	Inhibitory control	Plan future action; suppress inappropriate action
	Perceptual sensitivity	Perceptual awareness or detection
	Low-intensity pleasure	Stimuli: low intensity, rate, complexity, novelty and incongruity
Negative Affectivity	Frustration	Interruption of tasks or goal blocking
	Fear	Anticipation of distress
	Discomfort	Sensory quality: intensity, rate, complexity (light, movement, sound or texture)
	Sadness	Lowered mood and energy due to suffering, disappointment and object loss
	Soothability	Recovery rate from distress, excitement or arousal
Extraversion / Surgency	Activity	Level of motor activity; rate and locomotion
	Low shyness	Behavioural inhibition; social novelty and change
	High-intensity pleasure	Pleasure form activities; high intensity or novelty
	Smiling and laughter	Response to stimulus changes; intensity, rate, complexity and incongruity
	Impulsivity	Velocity of response inception
	Positive anticipation	Expected pleasure from activities
	Affiliation	Desired affection and intimacy with others

Of these dimensions (Table 3.2), Effortful control is mostly related to normal behavioural development. It seems to be involved in the development of conscience (responsibility for one's own actions) and beliefs of appropriate behaviour (Rothbart, 2007). Thus it seems as though Effortful control will be most influential to aggressive behaviour.

Fear in infancy however, may also be a predictor of fearfulness in adolescence and adulthood, causing lower levels of aggression. During infancy, frustration resulting in anger is then a predictor of aggression in adolescence and adulthood (Rothbart & Bates, 2006). This

shows that Negative affectivity will also have an influence on aggressive behaviour. Lowest amount of aggression will then be expected in individuals showing high levels of fear and Effortful control, along with a low level in frustration.

Impulsivity has been closely related to aggressive and violent behaviour (Wakai & Trestman, 2008). This is an indication that Extraversion/Surgency can be related to the externalizing behaviour of "acting out" (Rothbart, 2007). Aggressive behaviour can be internalized. Fear, sadness and low self-esteem will be most prominent characteristics. Externalizing aggressive behaviour is most prominently influenced by anger, frustration and low Effortful control. Individuals with high Negative affectivity as well as high Effortful control will be less likely to act out (Rothbart & Posner, 2006; Rothbart & Bates, 2006).

Negative affectivity can be linked to the neuroticism personality trait (Eysenck, 1967, 1981). Neurotics experience higher levels of sympathetic arousal producing the variable emotions (Derryberry & Rothbart, 1988). Likewise, most temperamental traits have clear affiliation to personality traits. Related to personality traits, Effortful control becomes Conscientiousness, Negative affectivity becomes Neuroticism and Extraversion remains (Evans & Rothbart, 2007) along with their contributing scales (Table 3.2), as reviewed from Rothbart's Early Adolescent Temperament Questionnaire (EATQ) (2007).

3.1.1.3. Temperamental constructs

According to Derryberry and Rothbart (1988), temperament can be grouped into three constructs. These constructs act as a measurement tool for the temperamental dimensions. Each of the constructs has distinct contributors, or subconstructs:

- Arousal: This implies the behavioural excitability. It is embedded in the cortical areas (Hobson & Scheibel, 1980), endocrine (Mason, 1975), autonomic (Lacey, 1967) and central nervous system's arousal. It is assessed by intensity, latency, rise time, recovery time and threshold parameters (Rothbart, 1981). The assessment of arousal is more significant when looking at specific patterns, as opposed to levels, of arousal (Zuckerman, 1979; Tucker & Williamson, 1984; Derryberry & Rothbart, 1988).

The reticular formation in the brain is responsible for regulating important survival processes, like arousal and sleep-wake patterns (Crossman & Neary, 2005). Differences in the functioning of this area have been linked to the temperamental construct of arousal. It has also been linked to extraversion and introversion personality traits (Eysenck, 1967, 1981). Introverts display a higher level of reactivity, enabling them to experience arousal even from milder levels of stimulation (Hebb, 1955). Autonomic arousal can also be linked to temperamental arousal.

- Emotion: Various researchers, like Eysenck (1967, 1981) believe that this temperamental construct should be grouped with Arousal. They believe that arousal leads to emotion. Gray (1981, 1988) proposed that emotion can also lead to arousal. The limbic system modulates emotion through a variety of circuits (Panksepp, 1981). Specific circuits modulate specific emotions like fear, frustration, pleasure or relief. These circuits are so complex. Individuals may well exhibit different patterns (intensity and frequency) for each emotion (Derryberry & Rothbart, 1988). Tellegen (1985; Watson & Tellegen, 1985) formulated the Positive emotionality and Negative emotionality as related to emotion, leading to arousal, and can be related to Extraversion and Introversion respectively (Table 3.3) (Gray, 1981, 1988; Derryberry & Rothbart, 1988).

- Self-regulation: This construct relates to the ability of an individual to consciously and actively control emotion and arousal, thereby controlling behaviour (Mischel, 1983; Derryberry & Rothbart, 1988). This is the ability to move attention toward a positive stimulus, resulting in arousal and emotion. When removing attention from a negative stimulus, the result is containment of arousal and emotion. By maintaining attentional control, optimal arousal and emotion can be achieved (Rothbart & Derryberry, 1982; Derryberry & Rothbart, 1988).

Table 3.3: Positive and Negative emotionality as related to five temperamental traits according to Tellegen (1985)

Temperament trait	Positive emotionality	Negative emotionality
Personality trait	Extraversion	Introversion
Reactive system	Approach	Behavioural inhibition
Sensitivity	Reward and non-punishment	Non-reward and punishment
Characteristic emotion	Hope and relief	Fear and frustration
Characteristic behaviour	Impulsivity	Anxiety

3.1.2. Linking Temperament and Personality

Temperament is defined as traits that can be distinguished in infants and appear to have a strong genetic influence (Goldsmith *et al.*, 1987). If differs from personality traits, in that personality is the result of environmental interaction with temperament (Buss & Plomin, 1984; Goldsmith *et al.*, 1987; Mayer, 2005). Temperament is characterised as a normative behaviour, meaning that it remains constant at specific points during development.

Development is a process of differentiation, reorganization and adaptation (Nigg, 2006). Personality develops from temperament through experience. These experiences will contribute to the individual's concept of self, others, the physical and social world. These concepts of self are related to his or her values, attitudes and coping mechanisms. Further personality traits will develop over time as the child matures and experience different environmental influences (Rothbart, 1981; Goldsmith *et al.*, 1987). Unlike temperament, personality is thus a reward dependant response system. The role of temperament in the development of individual personality is (Molfese & Molfese, 1993; Rothbart & Bates, 2006):

- Temperament sets the ranges within which a specific trait may vary without exceeding the ranges (Kagan & Snidman, 2004),
- Temperament determines social learning (Eysenck, 1967; Escalona, 1968; Gray, 1981; Wachs & Gandour, 1983),
- Temperament determines reactivity and adaptation to situations (Bell, 1974; Strelau, 1983)
- Temperament in itself also follows a developmental course. Certain traits will only manifest at certain stages of developmental age. This will influence the individual's personality development during that period (Rothbart & Derryberry, 1982; Rothbart, 1989; Rothbart *et al.*, 1992).

With both temperament and personality, identification of the trait can be made very early on. These traits also show similar patterns of inheritance and genetic influences. They also display consistency across different situations and timeframes. The most important similarity between temperament and personality is that they both have significant emotional and motivational aspects as related to behaviour (Nigg, 2006). An integration between temperament

and personality is thus of utmost importance when studying individual differences in behaviour (Strelau, 1972; McCrae *et al.*, 2000; Zuckerman, 2001; Shiner & Caspi, 2003; Watson *et al.*, 2005).

3.2. Personality

As the child develops a sense of self over the first two years of life, cognitive changes related to feelings of embarrassment or shame for one's actions also develops (Kagan, 1989). This enables an individual to make judgements on thoughts and actions. A child exhibiting healthy social development will form attachment to thoughts and actions that reflect positively on themselves. This child will attempt to avoid blame for negative thoughts and actions (Snyder *et al.*, 2005). A child with unhealthy social development may have had difficulty forming attachments, modelling appropriate behaviour, or finding appropriate models, positive or negative reinforcement (as seen with social learning) or the ability to self-regulate. Most often, it is a combination of these factors (Rothbart & Ahadi, 1994).

Unlike temperament, which is constant over time, personality is ever changing during childhood, even continuing into adulthood (Rutter, 2006), where after it becomes more stable. Differences observed between individuals, for the same personality trait can be attributed to different cultures, religious views or even moral beliefs. Similarities, then, may be attributed as a consequence of living together in groups, thinking in abstract and unique ways, or being aware of our own mortality (McCrae & Costa, 1997).

Studies on the evolution of psychology (Buss, 1995; Tooby & Cosmides, 2005) focus on complex psychological traits such as personality. Plomin *et al.* (2001) attested to the heritability of nearly all personality traits. This was supported by studies done by Turkheimer (Turkheimer & Gottesman, 1991; Turkheimer, 2000). Personality is seen as an individual's behavioural tendencies within specific situations. Motivations, abilities, standards, values, defence mechanisms and temperament are all traits included in personality. Similar temperaments may give rise to the same personality type, just as different personality types may have the same underlying temperament. Personality is greatly influenced and shaped by the environment (Thomas & Chess, 1977; Goldsmith *et al.*, 1987). Personality includes thought patterns and consideration. This influences the way a person perceives life, and reacts to it. Personality can

thus be seen as the description of lasting qualities in an adult individual, which lends consistency in behaviour when this individual is in a social environment (Livesley & Jang, 2005).

3.2.1. The Big Five Model of personality

The dimensions of personality traits can be divided into high level traits and low level traits. The high level traits are definite categories, and the traits seem to be completely independent of each other. The low level traits on the other hand are traits that overlap somewhat and show definite correlation (Hepple, 2002). In genetic research, focus will fall on the high level traits. The low level traits will have a greater environmental influence, making finding the genetic component difficult.

The high level personality consists of five traits, also called the "Big Five" model of personality (also known as the OCEAN model) consisting of (McCrae & Costa, 1987; Digman, 1990; McCrae & John, 1992; Hepple, 2002):

- Openness to Experience: Individuals scoring high in this trait are imaginative, behaviourally flexible and curious. These individuals are creative, intellectual and perceive developing and learning new things as rewarding (Hepple, 2002). They admire other individuals like themselves. They dislike being restricted and find it difficult to adhere to controlled or rigid situations. Characteristics associated with these high scoring individuals make them more philosophical and sensitive to interpersonal social cues (Hepple, 2002; McCrae & Sutin, 2009). These individuals also show great imagination, an appreciation for art (also music or literature). They value emotional experiences driven by the inclination to experience novel things due to their curiosity. This personality construct can be related to the Orienting sensitivity temperamental construct.

  Individuals not in possession of this personality trait tend to be set in their ways and are commonly called traditionalists. They show a preference to familiar routines and generally have fewer interests (McCrae & Sutin, 2009).

- Conscientiousness: Individuals possessing this personality trait are very dependable, consistent and rational. Because of this they are also very productive and have high

aspiration levels, being goal oriented and focussed (McCrae & Costa, 1997). They are also thoughtful of others and their own situations. A very strong impulse control helps them with their need for keeping rules, avoiding conflict and disorder (Hepple, 2002). This personality construct has a strong resemblance with the Effortful control construct of temperament.

On the opposite end of this spectrum, individuals will have a higher score in the Openness to Experience and Neuroticism aspects. Related to the temperamental traits, most correspondence can be seen with attentional persistence. It displays an overlap in the dimensions of control, which is a component of the self-regulatory system (Rothbart & Ahadi, 1994; Rothbart & Posner, 2006).

- Extraversion-Introversion: The distinction made between introverts and extraverts depend on the social situation each seek (Eysenck, 1967). The extravert seeks situations of high stimulation with social interaction and activities. The introvert prefers reservation and solidarity. Introverts may appear as extraverts to their intimate friends. Sensation seeking, impulsivity, aggressive tendencies and unreliability are characteristics of the extraverts (Hepple, 2002). There are some discrepancy about the spectrum of this trait, and whether extraversion and introversion are on opposite ends of the same spectrum. The general consensus, however, is that all individuals have both extraversion and introversion, though one tends to be more dominant over the other. It thus follows a bimodal distribution rather than a normal distribution. The social situation will also influence the manifestation of either. The obvious temperamental construct related to this personality construct is Extraversion/Surgency.

Temperamental dimensions of positive affect and activity level also correspond with the adult personality trait of extraversion. Characteristics of both these temperamental traits as well as extraversion are approach, stimulus-seeking and reward orientation (Gray, 1981; Zuckerman, 2005).

- Agreeableness: This trait has its foundation in the inter-individual variation. The characteristics include trust, compliance, empathy, altruism, friendliness, modesty and sensitivity (Digman & Takemoto-Chock, 1981). These individuals are very straight-

forward and tender-minded. They tend to be more yielding, shying away from conflict and aggressive behaviour. They tend to see others as being just as honest and trustworthy as themselves (Jensen-Campbell & Graziano, 2001; Meier & Robinson, 2004). This personality construct is the most difficult construct to relate to temperamental constructs as it relies mostly on socialization. The temperamental sub-construct that can be indicative of agreeableness is Sociability (component of the Extraversion/Surgency construct). This is however a weak association.

The opposite pole of Agreeableness is Antagonism. These individuals tend to be hostile and irritable. They have a constant need to oppose others, sometimes leading to attacking or punishing. This mostly stems from the constant mistrust felt towards individuals they dislike or feel are inferior to them. Emotional expression is lacking. These individuals will be perceived as cold and calculating (Costa *et al.*, 1991).

- Neuroticism: The temperamental dimensions most related to neuroticism are fearfulness and irritability (Rothbart & Ahadi, 1994). Individuals with this personality trait are characterised by anxiety, hostility, depression, self-consciousness and impulsivity. This causes them to be more sensitive to social conflict. They experience situations to be more stressful and respond in a distressed way (Suls *et al.*, 1998; Gunthert *et al.*, 1999). Therefore they isolate themselves even more from possible support. Because of this, these individuals tend to be very tense, agitated and moody as well as have a low self-esteem. They have trouble controlling urges (and delaying any gratification). Feelings experienced includes suspicion (or paranoia) and guilt (Hepple, 2002). They also fail to cope with stress and usually succumb to irrational thoughts. The temperamental construct mostly related to this personality construct is Negative affect.

On the opposite pole of this spectrum, individuals will be calm and emotionally more stable. Conscientiousness and Extraversion are found in individuals on this side of the spectrum.

3.2.2. Personality disorders

Personality can be seen as the individual's perception of the environment and situations, and how they function within this environment and these situations. Thus it can be deduced that Personality Disorders has to do with how the individual is unable to function within the environment. These individuals find their environment to be maladaptive, impairing themselves and causing them distress (American Psychiatric Association, 2000). It is furthermore a chronic state. It commences in childhood and stays constant throughout life. It therefore influences every aspect of the individual's life (Barlow & Durand, 2011).

Abnormal personality, like aggressive behaviour, can be the result of physical brain damage (Freedman & Hemenway, 2000). Lesions in the brain will result in offensive aggression. Brain tumours will result in defensive aggression (Hawkins & Trobst, 2000). Another pathway of abnormal aggression is when it is occurring co-morbidly alongside other disorders, like Personality Disorders. Anti-social Personality Disorder (ASPD) and Borderline Personality Disorder are mostly associated with aggressive behaviour (Eronen *et al.*, 1998; Moran, 1999; Ekselius *et al.*, 2001). It is one of the presenting symptoms of these Personality Disorders.

With aggressive behaviour, a further distinction based on age is necessary for ASPD. This disorder is only diagnosed after the age of 18. Preceding the age of 18, it is known as Conduct disorder. This may be the result of fluctuating hormonal levels and thus fade after the age of 18. However, if it persists and become ASPD, a genetic influence is prominent (Plomin *et al.*, 2001). Conduct disorder must however precede ASPD (Haller & Kruk, 2006). Individuals suffering from ASPD and showing signs of aggressive behaviour usually show a reduced sensitivity to stress and thus have reduced responses and emotional expressiveness (Raine, 1996; Brennan *et al.*, 1997; Herpertz *et al.*, 2001). This phenomenon is known as flat affect (Woodworth & Porter, 2002). It can be associated with anxiety, social fears (Sareen *et al.*, 2004) and reduced serotonin neurotransmission (Telegdy & Vermes, 1975; Van Loon *et al.*, 1982).

3.3. Environmental contributions to temperament and personality

The difficulty with studying personality is the environmental component. Families generally show similarity for traits. This can be either attributed to influential genes running within

families, or the relative constant environment experienced by individuals living together. Environmental influences can be divided into shared (similar influences resulting in similarity) and non-shared (similar influences resulting in difference) environmental influences (Hepple, 2002). Quantitative studies found that the greater environmental influence on personality seem to come from non-shared environmental influence (Riemann *et al.*, 1997). The same applies to temperamental heritability. Non-shared environment seems to contribute significantly. Shared environment seems to have very little to no contribution (Saudino, 2005). As temperament is equivalent to personality without the environmental influence, it is a more trustworthy assessment of the stable person-factor to use in behavioural genetic research.

3.3.1. Environmental influence to problem behaviour

A dysfunctional environment is defined as an environment that presents stressors that the individual are unable or inadequately adapted to cope with (Dempsey, 2002). Possible influences contributing to a dysfunctional environment are social status, economic status, family history and parental mental health problems. The influential power of these contributors is directly related to the amount of disruption it causes in the daily socialization and development of the child (Ary *et al.*, 1999). Initially these influences are greater from the family environment. As socialization with peers increases and expands, the influence from peers likewise increases. This is another motivation of the importance of parental monitoring and discipline in the prevention of problem behaviour.

During early childhood development, the family management is most influential in the development of problem behaviour (Patterson *et al.*, 1989, 1991, 1992; Patterson & Bank, 1989; Dishion *et al.*, 1991). Inconsistent and harsh discipline results in oppositional behaviour that may lead to early aggression. This usually leads to a coercive parent-child interaction. Here, the parent will attempt to regain control leading to increasingly inconsistent disciplining of the child. The child becomes more oppositional toward the parents. When the child enters school, this learned behaviour may lead to rejection by peers.

Patterson and co-workers found in numerous studies that delinquency and Conduct disorder follows when this child enters the adolescent years. This child then associates themselves with similar peers (Patterson *et al.*, 1989, 1991, 1992; Patterson & Bank, 1989; Dishion *et al.*, 1991).

These results were enforced by similar studies (McCord *et al.*, 1961; West & Farrington, 1973; Farrington, 1978; Wadsworth, 1979; Olweus, 1980; Loeber & Dishion, 1983).

Problem behaviour, especially in adolescence, is regarded as anti-social behaviour (Patterson *et al.*, 1989, 1991, 1992; Patterson & Bank, 1989; Metzler *et al.*, 1994). As previously mentioned, it is initially diagnosed as Conduct disorder. It is characterized by aggression, possible drug usage (Dishion *et al.*, 1991) and sexual promiscuity (Metzler *et al.*, 1994). Ary *et al.* (1999) investigated the influence of inconsistent parenting to eventually produce all above mentioned forms of problem behaviour. They found that intense problem behaviour stems from constant conflict within the family environment, minimal involvement in the family environment by the other family members and inconsistent and inadequate parental monitoring and discipline (Ary *et al.*, 1999) These findings agree with a study done by Loeber *et al.* (2005), concluding that aggressive adults most likely came from a conflict loaded childhood family environment.

3.4. The biological basis of temperament and personality

As complex traits, temperament and personality are influenced by more than one gene as well as an interaction with the environment (Plomin *et al.*, 2001). When studying a behavioural trait in a quantitative manner, comparisons are drawn between commonalities between individuals based on genetic (e.g. monozygotic vs. dizygotic twins) and environmental (e.g. biological vs. adopted children) contribution. Heritability estimates are very low during the neonatal period (Matheny, 1989; Riese, 1990). This is not unexpected, as most temperamental traits become truly evident after two months (Thomas, 1963; Buss & Plomin, 1975, 1984; Thomas & Chess, 1977; Gray, 1981; Rothbart, 1981; Rothbart & Ahadi, 1994; Zuckerman, 2001). Substantial heritability can be seen during the toddler years (Matheny, 1989; Saudino & Cherny, 2001). It rises to moderate heritability during primary school periods (Braungart *et al.*, 1992) into the adult personality (Bouchard & Loehlin, 2001). Between these, it seems that temperament is the stable, biologically based component in individual differences. Personality is then a reflection of the behavioural traits that started out in temperament and matured through experience (Kagan & Snidman, 1999, 2004). Just as temperament can be related to personality, both can also be related to physiological systems.

3.4.1. Neurochemical pathways

The "why" of behavioural manifestation can be explained through studying the behavioural context. This is only one of the complications when investigating individual differences in behavioural traits (Gunnar, 1994; Kagan, 1998; Angleitner, 2001; Pihl & Nantel-Vivier, 2005; Zuckerman, 2005). One gene influencing several traits (pleiotropy) can create confusion and difficulty when investigating the "how" of behaviour. Limbic networks, mostly linked to emotionality, can be linked to either temperament. It can also be linked to cognitive processing (which can then be linked to temperament indirectly). Specific neurochemical systems, such as the serotonergic and dopaminergic systems, may be related to several temperamental traits. It can also be related to other cognitive and even motor and learning functions (Nigg, 2006). Thus linking one system to one trait is highly unlikely (Canli, 2004).

Investigations into the genetic components of temperament and personality have yielded very little satisfying results (Strelau, 1972; Gale & Edwards, 1986). This may be because most investigators focus on only one component of behaviour. This is usually outside of the CNS in a psychological versus biological manner. Temperament has however a great biological component, equally as important as the psychological development of the traits (Eysenck, 1955; Strelau, 1972; Gray, 1988; Zuckerman, 2001, 2005; Watson *et al.*, 2005; Rothbart & Bates, 2006). The association of behaviour and components within the CNS is relevant. First it focuses on the physical formation of the brain associated with specific behaviour (mostly abnormal behaviour). Secondly it focuses on processing mechanisms associated with cognition, within the brain. And finally, it focuses on the response tendency, style or intensity associated with specific behaviour (e.g. the pleasure system, also known as the serotonergic system). Of these different systems, the ability to regulate all of them in unison is also investigated (Nigg, 2006).

Cloninger *et al.* (1993) formulated his theory of the psychobiology of temperament and biology, focussing on the serotonergic and dopaminergic systems as most influential. The dopaminergic system is known as the Reward System, and thus most influential in traits driven by reward seeking and dependence (Comings & Blum, 2000). The serotonergic system is known as the Pleasure system, and thus most influential in traits like Novelty seeking. The genes encoding for the serotonin transporters and receptors have been linked to anxiety and

impulsivity in humans (Lesch *et al.*, 1996; Lappalainen, 1998; Greenberg *et al.*, 2000; Du *et al.*, 2000; Bjork *et al.*, 2002; Hariri *et al.*, 2002; Sanders *et al.*, 2002; Anguelova *et al.*, 2003; Strobel *et al.*, 2003; Huang *et al.*, 2003; Lemonde *et al.*, 2003; Abdolmaleky *et al.*, 2004; Khait *et al.*, 2005). This influence of both the Dopaminergic and Serotonergic systems on Novelty Seeking in humans is a clear indication of the unlikelihood of correlating any one system to any one temperamental or personality trait.

Conclusion

Genetic studies on temperament and personality have failed to identify specific contributing genes to these traits. Most of these studies focus on the heritability and have found that all aspects of temperament and personality are heritable, to varying degrees (Heath *et al.*, 1994; Plomin *et al.*, 1994; Ebstein *et al.*, 1996; Lesch *et al.*, 1996; Turkheimer, 1998; Gelernter *et al.*, 1998; McCrae *et al.*, 2000; Jang *et al.*, 2001; Jang, 2005; Yamagata *et al.*, 2006). It can be concluded that temperament and personality are influenced by the pleiotropic genes with epistatic effects.

The only hope in identifying several possible influential genes to behaviour is by focussing on a specific behavioural trait, using temperament (rather than personality) as the stable person-factor and then looking at several neurochemical systems (as opposed to either the serotonergic or dopaminergic system alone).

CHAPTER 4

Quantitative measurement of behaviour by using the Aggression Questionnaire and Adult Temperament Questionnaire

Abstract

When studying behaviour, it is of utmost importance to accurately describe the specific behaviour. This enables the researcher to measure all possible influences of the behaviour. For this research project, the Aggression Questionnaire (AQ) was used to measure the amount of aggressive behaviour for a sample of 188 individuals from the central part of South Africa. This proved to be an excellent measurement battery as internal consistency values, as well as the mean and standard deviation are comparative to American populations, where the questionnaire was standardized. Since behaviour can be influenced by environmental effects, a stable person-factor measurement was also included, i.e. the Adult Temperament Questionnaire (ATQ). It proved to be an adequate measurement battery, considering that temperament shows varying plasticity within the traits. From the data gathered by both questionnaires, Anger and Physical aggression showed significant correlations in the Male and Female data sets as well as the entire sample. As the stable person-factor Frustration has correlated most significantly with Anger in all three mentioned data sets. Individuals scoring high in each of Anger, Physical aggression and Frustration; or scoring low in all three traits should show most genetic similarity respectively. Similarities in phenotype should provide most similarity in influential underlying genotypes.

Keywords: AQ, ATQ, personality, psychometric measurement, stable person-factor

Introduction

Before the human genome project (Collins *et al.*, 1998), studies on human behaviour relied largely on psychological analyses. As molecular genetic techniques developed, a reductionist approach to behaviour followed. This approach believed in one gene, one disorder. With the development of whole genome sequencing techniques, it became possible to not only focus on a specific gene, but investigate similarities and differences between individuals' entire genomes. This enriched Quantitative Trait Loci (QTL) mapping (Plomin *et al.*, 1994; Darvasi & Soller, 1997; Lynch & Walsh, 1998; Korstanje & Paigen, 2002). Through this approach, multiple DNA regions of influence can be identified, where each genetic contribution may vary in effect size, and may act in an additive or interactive way to bring about the phenotype (Plomin *et al.*, 1994).

Multivariate genetic analysis has amplified the importance of investigating not only the variance of one specific trait, but rather the covariance of multiple traits (DeFries *et al.*, 1994). Through this, linkage between specific traits may be found as pleiotropic and/or epistatic effects of specific genes (as discussed in Chapter 2). Understanding this complex interaction between genes will have an enormous influence on the understanding of comorbidity between disorders. Further complications are the additive effects of certain genes as well as environmental influences. As related to the analysis of extremes, it may explain how certain influences may cause the development of a disorder, where the normal and abnormal behaviour can be depicted as a continuum (as seen on a normal distribution) (Plomin *et al.*, 1994).

With the development of technology, the environmentalist view of behavioural development fell away. An understanding of the balance between nature (genetics) and nurture (environment) as contributing influences ensued. This has caused a fusion between psychology, believing in the environmental influence, and genetics (Plomin & McClearn, 1993). The first of these collaborated research used different quantitative genetic approaches to studying behaviour. Of these, twin, adoption and family designs were used in human behavioural research. Though familial patterns could be detected in most human behaviour, these study designs gave no implication of a specific genetic influence (Plomin *et al.*, 1980, 1994; Plomin & McClearn, 1993).

Regardless of this, quantitative study design has aided in clarifying genetic and environmentally influences traits. Adoption and twin studies have also shown that behavioural disorders, like Schizophrenia, Autism, Alzheimers disease and even Affective disorder (aggression) have strong genetic influences (Plomin *et al.*, 1977, 1980; DeFries *et al.*, 1994). Plomin *et al.* (1994) suggested that quantitative genetic studies can indicate the genetic and environmental influences that will both contribute to the variance seen in a population. They continue to emphasize that differences observed between children from the same family is created by the non-shared environmental influence. They end of by implicating that an individual's genotype will largely determine the environment and experiences that the individual will seek out (Plomin *et al.*, 1994). This is a popular concept also known as the "*Smorgasbord*" model (Plomin *et al.*, 1977, 1994; Eaves *et al.*, 1977; Scarr & McCartney, 1983). Plomin *et al.* (1994) end this article by emphasising the importance of a synergy between quantitative study designs and molecular genetics, for a holistic and comprehensive investigation of human traits.

4.1. Quantification of behaviour

When attempting to identify underlying genotypic contributions to behaviour, it is important to find an accurate measurement of the behaviour studied. Inaccurate measurement may lead to false positives or negatives. As human behaviour is a very complex trait, it is also very difficult to measure. The main reason for this is that human behaviour is no longer governed solely by instinct. We, as humans, can motivate and manipulate behaviour. Social norms may also govern what we determine to be socially appropriate and inappropriate behaviour. Individuals may try to hide inappropriate behaviour from scrutiny (Power & Ikeda, 1996).

The most obvious measurement of behaviour is through observation, where the investigator observes an individual's behaviour for a specific timeframe. This is however not very trustworthy, as the investigator's judgement may change over time. Also, it is time consuming and requires constant comparison with situational absent normative behaviour (Power & Ikeda, 1996; DuPaul & Stoner, 2003).

A more trustworthy measurement is by using self-report. This measurement tool is far from perfect and can be manipulated (Reid & Maag, 1994; Power & Ikeda, 1996; Waschbusch & Willoughby, 1998; Rojahn *et al.*, 2001; Jachimowicz & Geiselman, 2004). Certain preventative

measures against this can be built into the questionnaire. A good psychometric test will have good construct validity, internal consistency and test re-test reliability. The validity is an indication that the test is measuring what it is supposed to measure, or the specificity. The reliability shows that the test will have consistency when repeated, also known as the sensitivity. These statistical values should be calculated for each questionnaire, within each sample or population.

When measuring behaviour, it is important to also consider the positive predictive power (PPP) and negative predictive power (NPP) of the questionnaire. These measures may even be more important than the sensitivity and specificity of the questionnaire (Chen *et al.*, 1994; Laurent *et al.*, 1994; Power & Ikeda, 1996). These predictive power scores provide confidence that the score obtained by the questionnaire can be generalized to the behaviour it measures (Power & Ikeda, 1996).

The focus of this chapter is to give an explanation and overview of the chosen questionnaires, as well as the statistical analyses of these questionnaires within a sampled central South-African population.

4.1.1. Questionnaires

This research project focuses on the genetic influences on aggressive behaviour. Thus, the first questionnaire selected for the measurement battery is the Aggression Questionnaire (AQ) (Buss & Warren, 2000) (Appendix A). In an attempt at eliminating possible variables, the Adult Temperament Questionnaire (ATQ) (Evans & Rothbart, 2007) (Appendix A) was chosen to measure the stable person-factor, temperament.

4.1.1.1. The Aggression Questionnaire (AQ)

Aggression is seen as behaviour where the intended outcome is harm (Berkowitz, 1993a; Bushman & Anderson, 2001; Anderson & Bushman, 2002; Baron & Richardson, 2004). For a complete review of aggressive behaviour see Chapter 2 of this thesis. Several widely used questionnaires are available to measure maladaptive and aggressive behaviour (reviewed by Rojahn *et al.*, 2001). Still more frequently used is the AQ (Buss & Perry, 1992b). This original AQ tested for four subcategories of aggressive behaviour, namely anger, hostility, verbal and

physical aggression. It is also an updated version of the Buss-Durkee Hostility Inventory (Buss & Durkee, 1957).

The AQ used in this study is the latest version of the AQ, a further adaptation from the Buss & Perry AQ (1992). It consists of 34 questions measuring five subscales of aggressive behaviour, namely anger, hostility, physical, verbal and indirect aggression. Each question is answered according to the Likert scale, with 1 representing "Not at all like me", and 5, "Completely like me". The questionnaire was standardized on a sample of 2 138 individuals, ages 9-88 (Buss & Warren, 2000).

The AQ measures aggressive behaviour in a random population. The authors of the AQ manual noted that validity of the questions remain dependant of certain factors. With any self-report measure inaccuracies may be obtained due to a dissociative self-image of the individual, or also from biased responses, termed "faking bad" by the authors of the AQ manual (Buss & Warren, 2000). In order to identify these individuals, 12 of the questions have been constructed to indicate consistency. Their scores contribute to the Inconsistent Responding Index (Buss & Warren, 2000). An Inconsistent Responding Index score of five or higher is an indication of deceitful or inaccurate responses.

4.1.1.1.1. Subscales

The AQ measures five subscales. Physical aggression (measured by questions 8, 10, 11, 17, 23, 24, 25 & 27) measures the individual's inclination to have a physical aggressive response possibly leading to violence. An example of one of these questions is number 23: "At times I can't control the urge to hit someone". A high score in physical aggression can be indicative of sadistic or anti-social personality characteristics, as well as alcohol abuse (Buss & Warren, 2000).

Verbal aggression (measured by questions 1, 4, 6, 20 & 26) is indicative of an individual's inclination to quarrelsome and hostile speech. An example of a question is number 26: "I tell my friends openly when I disagree with them". Individuals scoring high in verbal aggression characteristically externalise their behaviour. They are commonly aroused by anger, finding the specific situation unfair. As with physical aggression, individuals scoring high in verbal aggression tend to have anti-social personality characteristics. Neurological injury or degeneration may also

be reflected in high verbal aggression. Unlike physical aggression, verbal aggression is seen more commonly with abuse of substances other than alcohol. Individuals who perceive themselves of not being litigious will score low on this subscale. This has however been proven to be one of the subscales sensitive to dissociative self-image (Buss & Warren, 2000).

Anger (measured by questions 3, 7, 12, 19, 22, 29 & 32) is related to an individual's sense of control. An example of a question is number 29: "At times I feel like a bomb ready to explode". These individuals are characteristically irritable, frustrated and emotionally unstable. Anti-social personality disorder (ASPD) are characterised by individuals with high anger scores. Similarly to physical aggression, anger can also be associated with substance use disorders. Low anger scores are indicative of individuals with effective and acceptable coping strategies. Low scores are also associated with narcissistic or histrionic personality characteristics (though these aren't associated with effective and acceptable coping). The difference lies in that individuals with effective coping skills know what makes them angry (Buss & Warren, 2000).

Hostility (measured by questions 2, 5, 9, 16, 21, 28, 31 & 33) is a measure closely associated with maladaptive social adjustment and psychopathology, like depression, social phobias and anxiety disorders. These individuals tend to have feelings of social isolation, bitterness and paranoia. An example of a question is number 16: "I wonder what people want when they are nice to me". An elevated hostility score should be coupled with elevation in all other subscales. These individuals characteristically internalize, and have angry thoughts. Same as anger, low scoring individuals tend to have effective and acceptable coping strategies. They tend to be comfortable in social settings (Buss & Warren, 2000).

Indirect aggression (measured by questions 13, 14, 15, 18, 30 & 34) is the latest addition to the AQ. This subscale measures an individual's tendency to react to provocation in an indirect or avoiding manner. An example of such a question is number 13: "If I'm angry enough, I may mess up someone's work". Because of the avoidance seen in individuals scoring high in indirect aggression, heightened levels of frustration may also be observed. These individuals furthermore also have disrupted relationships. High indirect aggression scores can be correlated to ASPD and substance abuse (Buss & Warren, 2000).

All of these scores can be added together to obtain the total aggression score. It is a good

indication of aggressive behaviour, with a close correlation between physical aggression and anger. A high score may be obtained from individuals with either relatively few, but very intense aggressive responses; or individuals who experience chronic, but low intensity aggressive emotions. The reason may be obtained by examining the subscale scores. By correlating certain subscales, further assumptions may be made. As already mentioned, ASPD are characterised by high hostility, verbal and indirect aggression scores. "Externalizers" are individuals who characterise themselves as being angry. They tend to have either elevated physical and verbal aggression, or elevated hostility, verbal and indirect aggression. High verbal aggression and hostility are seen in individuals with anxiety disorders. Phobias and other anxiety avoidance disorders are also correlated with high hostility and indirect aggression. Individuals with high hostility scores are reluctant to disclose information and more likely to provide deceitful responses (Buss & Warren, 2000).

4.1.1.2. The Adult Temperament Questionnaire (ATQ)

Behaviour can be classified as the way in which an organism reacts to and within its environment. It is based on a stimuli-response system (Anderson & Bushman, 2002). Temperament can then be considered a compilation of characteristics for an individual. These characteristics will remain constant for emotional reactions, mood changes and environmental stimuli (Reber & Reber, 2002). Thus, a specific temperamental characteristic can be seen as a predictor of behaviour for an individual. Individuals sharing similar temperamental traits, may consequently exhibit similar behaviour in similar situations – also explaining the stable person-factor. Temperament can therefore be used as the stable person-factor. For a complete review of temperament and its association with personality and aggressive behaviour, refer to Chapter 3 of this thesis.

The measurement of temperament is somewhat more difficult than the measurement of aggressive behaviour. Temperament is seen as the biological basis to personality. Temperament determines and develops into personality through environmental influence and experience (Rothbart, 1981; Goldsmith *et al.*, 1987; Evans & Rothbart, 2007). Designing a measurement for temperament thus has to exclude any possible measurements that might be tainted by environmental influences. The Adult Temperament Questionnaire (ATQ) (Evans & Rothbart,

2007) is such a measurement tool, consisting of 77 questions in the short form (Table 4.1). It can be related to the Cloninger's Temperament and Character Inventory (TCI) and the Five Factor and Multi-Language Seven models of personality traits. It is an adaptation of the Physiological Reactions Questionnaire (PRQ) (Derryberry & Rothbart, 1988), and deduced from several studies' results (Derryberry & Rothbart, 1988; Rothbart *et al.*, 2000; Evans & Rothbart, 2007).

Table 4.1: Sub-constructs, contributing questions and reverse scoring questions (in bold) of the ATQ short form.

Construct	Sub-constructs	Questions
Negative affect	Fear	1, 12, 22, 51, 61, **68**, **75**
	Frustration	**6**, 17, 31, **38**, 48, **58**
	Sadness	**9**, **20**, 25, **34**, 45, 56, 65
	Discomfort	4, 32, 36, 42, 54, 59
Effortful control	Activation control	**2**, **8**, 15, 27, 47, 55, **72**
	Attention control	**5**, **29**, 35, **40**, **50**
	Inhibitory control	11, 26, 43, **53**, **60**, **63**, 76
Extraversion / Surgency	Sociability	**14**, 19, 37, **46**, 67
	High intensity pleasure	**7**, 23, 30, **44**, 64, 73, **77**
	Positive affect	3, **16**, 28, 49, **70**
Orienting sensitivity	Neutral perceptual sensitivity	**10**, 21, **33**, 52, **71**
	Affective perceptual sensitivity	13, 18, 57, **66**, 69
	Associative sensitivity	24, 39, 41, 62, 74

The ATQ is a measure of temperament. In this research project, temperament will be used as a stable person factor. As temperament is related to how an individual reacts within his or her environment. Temperamental constructs will be correlated to aggression constructs. It can thus be deduced that individuals with similar temperamental constructs will react in a similar way within a specific environment to produce a specific behaviour, in this case aggression.

4.2. The sample studied

For this research project, 188 individuals participated, generally located in central South Africa. The sample was based on convenience from voluntary participation from randomly selected individuals. This sample was composed of 60 males (31.9%) and 128 females (68.1%). Ages ranged from 18 to 88, though the sample is greatly represented by individuals between the ages of 20 and 30 (n=162; 82.4%) (Figure 4.1). South Africa is comprised of many ethnic groups, making this sample extremely unique. Apart from the great ethnic diversity, there also exists a great cultural diversity. This creates a great variation in environmental contributions that might

influence behaviour. For this reason temperament was selected to be the stable person-factor as it isn't influenced by beliefs or attitudes about behaviour (Buss & Plomin, 1975; Reber & Reber, 2002; Rutter, 2006).

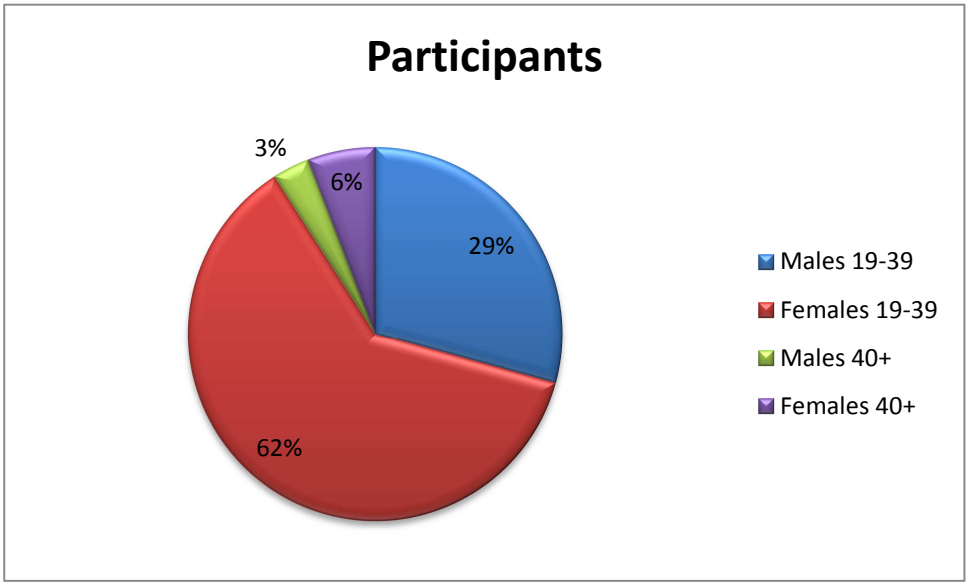


Figure 4.1: Participants divided according to ages 19 to 39 and 40 and above, as well as gender.

The sample comprised of individuals from the Afrikaans (n=68; 72.3%) and English (n=26; 27.7%) speaking Caucasian sample (n=94; 48.8%), individuals with African ancestry (n=91; 48.4%) and Asian individuals (n=3; 1.6%). The most fragmented group is the African sample (n=83; 44.1%), consisting of individuals who speak Xhosa (n=12; 14.5%), Zulu (n=6; 7.2%), South Sotho (n=38; 45.8%), Tswana (n=16; 19.3%), Swati (n=3; 3.6%), Venda (n=2; 2.4%), Tsonga (n=2; 2.4%) and other African languages (n=4; 4.8%). The questionnaires used were only constructed in English, and then translated to Afrikaans as well. All participants were fully English and/or Afrikaans capable. The research protocol (ethically approved by the University of the Free State Ethics Committee {ECUFS152/2011}) was fully explained to the participants. They were asked to give consent for voluntary participation, which all individuals mentioned (188) provided.

All statistical analyses were conducted using Microsoft® Office Excel 2010 and the Data Analysis Tools Add-In.

4.2.1. Cronbach's Alpha

The internal consistency and reliability of a questionnaire mainly comes to question when a questionnaire is used in a greatly varying sample (in relation to the sample it was standardised in) (Cronbach, 1951, 1988; Rajaratnam *et al.*, 1965). This also happens when a questionnaire has been translated. For this reason, Cronbach's Alpha, an indication of the internal consistency and reliability, was calculated for both questionnaires (AQ and ATQ) and both languages (Table 4.2). As previously mentioned, all the participating individuals were either English and/or Afrikaans literate.

Table 4.2: Cronbach's Alpha values for the AQ and ATQ, with reflection on original values and calculated values for participant languages.

AQ (Buss & Warren, 2000)	Original	Entire sample	English	Afrikaans	Other mother-languages
Total aggression score	0.94	0.92	0.93	0.92	0.92
Physical aggression	0.88	0.96	0.91	0.86	0.85
Verbal aggression	0.76	0.71	0.80	0.68	0.70
Anger	0.78	0.82	0.89	0.84	0.78
Hostility	0.82	0.81	0.82	0.84	0.78
Indirect aggression	0.71	0.56	0.58	0.71	0.45
ATQ (Evans & Rothbart, 2007)	Original	Entire sample	English	Afrikaans	Other mother-languages
Negative effect (NA)	0.81	0.80	0.86	0.86	0.73
Fear	0.64	0.65	0.74	0.76	0.54
Sadness	0.62	0.48	0.43	0.61	0.47
Discomfort	0.69	0.71	0.80	0.78	0.61
Frustration	0.72	0.70	0.83	0.83	0.47
Effortful control (EC)	0.78	0.80	0.83	0.85	0.51
Inhibition control	0.60	0.57	0.53	0.66	0.53
Activation control	0.69	0.77	0.82	0.78	0.74
Attentional control	0.73	0.57	0.43	0.66	0.53
Extraversion / Surgency	0.75	0.76	0.84	0.81	0.66
Sociability	0.71	0.73	0.83	0.50	0.71
High intensity pleasure	0.68	0.66	0.72	0.76	0.53
Positive affect	0.62	0.54	0.62	0.58	0.52
Orienting sensitivity (OS)	0.85	0.93	0.84	0.85	0.79
Neutral perceptual sensitivity	0.64	0.61	0.69	0.60	0.63
Affective perceptual sensitivity	0.79	0.63	0.70	0.67	0.60
Associative sensitivity	0.67	0.71	0.66	0.81	0.62

A Cronbach's Alpha value of 0.60 and higher is considered to be acceptable (Nunnally & Bernstein, 1994). This has not been attained for all of the data sets, though when considering the entire sample, it has mostly been attained. The reason for the discrepancies in values can be due to the language divide; especially for the "others" grouping of individuals whose mother language is neither Afrikaans nor English. It is seen that the internal consistency for the ATQ is not as high as for the AQ. This is even true for the original standardization sample. This may be due to the plasticity of the trait. Also, when investigating the questionnaires, it can be seen that the AQ has shorter questions and has been standardized to be comprehensible to individuals as young as 9 years. The ATQ is specifically designed for adult individuals and may thus consist of higher difficulty level questions. The five-point versus seven-point Likert scales as seen in the AQ and ATQ respectively may also influence the ease of answering the questionnaires (Dawes, 2012).

4.2.2. Standard deviation

For the AQ, the standardization sample gave a mean value of 73.3 with a standard deviation of 24.9 (Buss & Warren, 2000). For this South African sample a mean of 73.1 with a standard deviation of 19.6 were obtained. The mean indicates that the sample is not biased and can be compared to the Western sample in which the questionnaire was standardized. The difference in standard deviation can be due to the considerable difference in sample sizes. The standardization sample consisted of 2 138 individuals, whereas this sample consist of 188 individuals (Table 4.3). The comparisons between the sub-constructs show similar trends (Figure 4.2).

Table 4.3: The mean and standard deviation of the AQ as a comparison between the original sample (2 138 individuals) (Buss & Warren, 2000) and the current sample (188 individuals).

	2 138 individuals		188 individuals	
Trait	Mean	Standard deviation	Mean	Standard deviation
Total aggression score	73.3	24.9	73.1	19.6
Physical aggression	15.8	7.7	13.4	6.0
Verbal aggression	11.8	4.3	13.2	3.9
Anger	15.1	5.7	15.0	5.6
Hostility	17.1	6.6	18.2	5.9
Indirect aggression	13.5	4.8	13.3	3.7

Throughout the sample, Hostility consistently had a higher mean than the comparative sample (Figure 4.2). This may be due to the unique South African environment where competition for resources is comparatively high (Durham, 1976). An interesting trend was also observed for Hostility when comparing male and female samples. This is the only AQ construct and sub-construct where females consistently scored slightly higher than males. This however, may be due to the higher number of female participants and should not be considered as a significant occurrence.

The mean and standard deviation will not be discussed for the stable person-factor temperament. An individual will have a collection of different temperamental traits. This will be unique and show relative plasticity for all individuals (Goldsmith *et al.*, 1987). Because of this, high, low and medium scores are irrelevant. As temperament is used as the stable person-factor, correlations to specific aggressive traits are of greater importance.

4.2.3. Correlations and regressions

As should be the case, the constructs correlate most highly with their individual sub-constructs. This is true for both the AQ and ATQ. This is thus counted as expected, though irrelevant. The correlations between sub-constructs were also disregarded, though a persistent correlation was found between Anger and Physical aggression for the entire population (R=0.62) as well as male (R=0.63) and female data sets (R=0.72) (Table 4.4). This shows that individuals scoring high in the Anger sub-construct did so also in the Physical aggression sub-construct, and *vice versa*. The same trend was found for the standardization sample as well.

Correlations were drawn for the entire sample (188 individuals) as well as male (60 individuals) and female (128 individuals) data sets (Appendix B). Though other data sets (such as age groups) were also considered, it was disregarded due to the large decrease in sample sizes (Figure 4.1), providing unreliable data (Appendix B). Of all the correlations, the ones listed in Table 4.4 showed the highest correlation values. These correlations are also found to be significant, at a 99% level. Three correlations showed significance in all three samples (entire sample, males and females): Anger and Physical aggression, Anger and Frustration (R=0.51; R=0.52; R=48), and High intensity pleasure and Discomfort (R=-054; R=-0.48; R=-0.56).

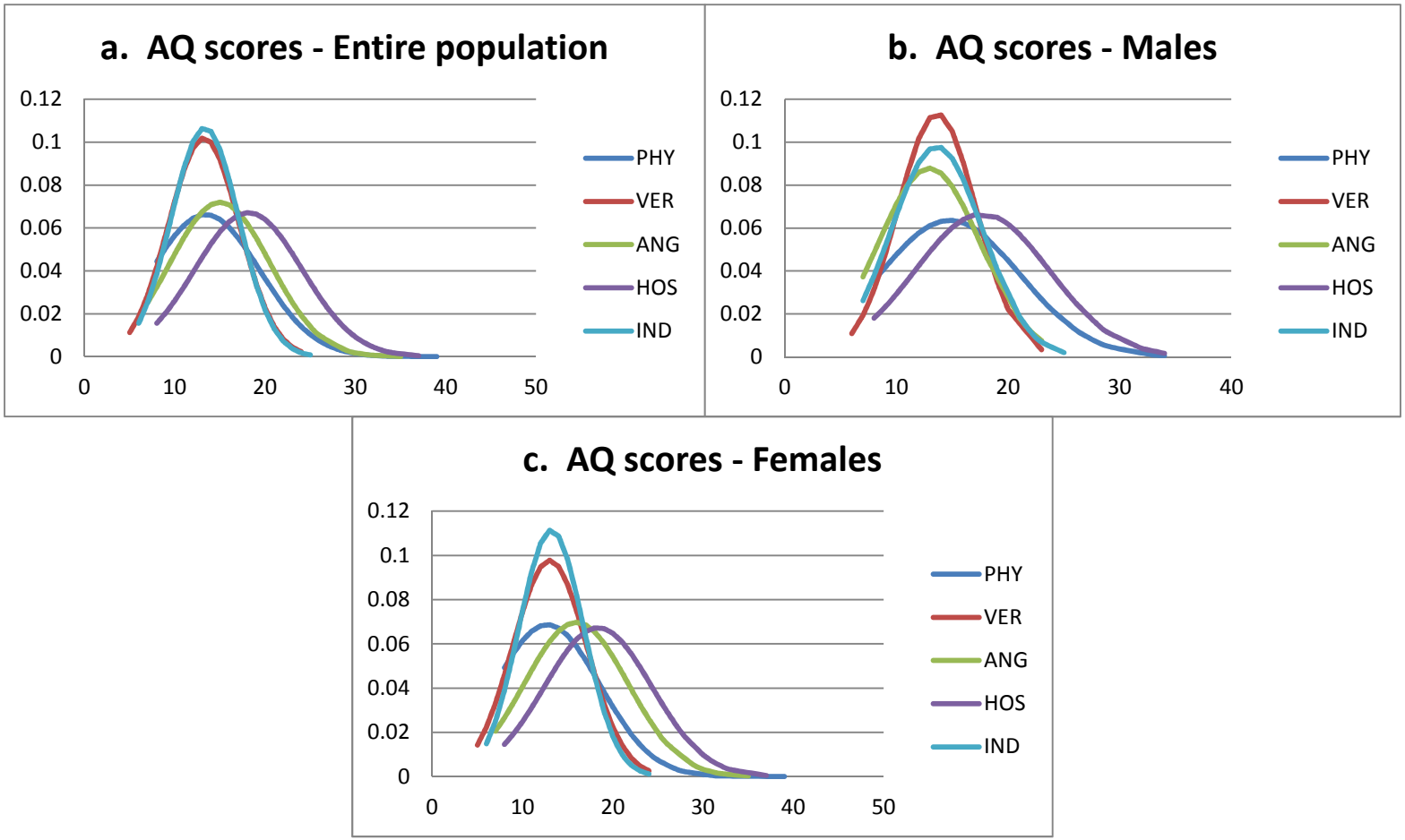


Figure 4.2:

a. Comparative normal distributions of the AQ sub-constructs for the entire population.
b. Comparative normal distributions of the AQ sub-constructs for males.
c. Comparative normal distributions of the AQ sub-constructs for females.

In all of the figures, abbreviations were used: PHY (Physical aggression), VER (Verbal aggression), ANG (Anger), HOS (Hostility) and IND (Indirect aggression). These are all measures of the AQ (Aggression Questionnaire).

The R^2 value gives you an estimation of how two correlated traits vary together. For example, in the entire sample, Anger and Frustration shows 26% directional similarity (correlation) in measured scores. This estimation takes the size of the sample selected into account also. Therefore, larger samples will give a more accurate estimation of how much two traits vary together. If we compare the R^2 values of the entire sample for Anger and Frustration correlations to that of Males (27%) and Females (23%) we can see that Anger and Frustration has constant correlations (approximately 25%).

Table 4.4: Correlation coefficients which were significantly different to 0 at the 99% level, within the entire sample, males and females.

Traits		*R*	R^2	*P*-value
Entire sample				
Anger	Physical aggression	0.62	0.38	$2.69x10^{-21}$
Anger	Frustration	0.51	0.26	$1.29x10^{-13}$
High intensity pleasure	Discomfort	-0.54	0.29	$1.11x10^{-15}$
Discomfort	Extraversion	-0.42	0.17	$2.41x10^{-9}$
Anger	Negative affect	0.42	0.18	$1.44x10^{-9}$
Males				
Anger	Physical aggression	0.63	0.40	$5.44x10^{-8}$
Anger	Frustration	0.52	0.27	$2.04x10^{-5}$
High intensity pleasure	Discomfort	-0.50	0.25	$4.21x10^{-5}$
Anger	Negative affect	0.49	0.24	$8.55x10^{-5}$
Verbal aggression	Frustration	0.44	0.19	$5x10^{-4}$
Negative affect	Hostility	0.51	0.26	$3.04x10^{-5}$
Negative affect	Total aggression score	0.46	0.21	$2x10^{-4}$
Frustration	Inhibitory control	-0.42	0.18	$7x10^{-4}$
Frustration	Effortful control	-0.46	0.22	$1x10^{-4}$
Females				
Anger	Physical aggression	0.72	0.52	$7.57x10^{-22}$
Anger	Frustration	0.48	0.23	$1.05x10^{-8}$
High intensity pleasure	Discomfort	-0.56	0.31	$6.19x10^{-12}$
Discomfort	Extraversion	-0.43	0.18	$5.7x10^{-7}$
Physical aggression	Indirect aggression	0.60	0.37	$3.37x10^{-14}$
Frustration	Total aggression	0.42	0.18	$8.48x10^{-7}$

Literature suggested a strong negative correlation should exist in individuals with low Effortful control and high aggressive levels (Milich & Kramer, 1984; Kochanska *et al.*, 1997, 2000; Ormel *et al.*, 2005; Damon & Lerner, 2006; Rothbart & Sheese, 2007). Effortful control is the temperamental construct related to the ability of an individual to control his or her behaviour. Thus, an individual with the inability to control their behaviour should be more prone to acting

out aggressively. This was however not found in this research study. As seen in Table 4.4, aggressive behaviour (specifically anger) correlates most highly with constructs of Negative affect in this sample (R=0.42) . As this sub-construct is anger, aggressive behaviour can logically be considered as a correlate.

Table 4.5: Correlations coefficients which were significantly different to 0 at the 95% level, within the entire sample, males and females.

Traits		R	R^2	P-value
Entire sample				
Total aggression	Effortful control	-0.23	0.05	1.39×10^{-3}
Total aggression	Inhibition control	-0.24	0.06	8.82×10^{-4}
Males				
Total aggression	Effortful control	-0.31	0.10	1.47×10^{-2}
Total aggression	Inhibition control	-0.29	0.09	2.31×10^{-2}
Females				
Total aggression	Effortful control	-0.20	0.04	2.71×10^{-2}
Total aggression	Inhibition control	-0.22	0.05	1.37×10^{-2}

Correlations drawn between the AQ and the ATQ construct of Effortful control showed only slight correlations for the entire sample (R=-0.23), male (R=-0.31) and female (R=-0.20) data sets (Table 4.5). These correlations only proved significant at the 95% significance level.

Conclusion

In order to study a behavioural trait, you need to define the trait in specific detail. This enables you to accurately measure the investigated trait. For this purpose, the AQ (Buss & Warren, 2000) has proven to be an excellent measurement battery. The questions posed as statements are short and easy to understand. Even translated, the internal consistency of the AQ scored on average above 0.9. The ATQ (Evans & Rothbart, 2007) proved to be more challenging and complex. This measurement battery was designed for adults, unlike the AQ, which are suitable for ages nine and up. Regardless, the complexity of temperament and its sub-constructs as traits are even difficult to define by itself. With internal consistency values averaging around 0.65, the ATQ is found to be an adequate measurement battery.

For the purpose of this research project, the AQ is the main measurement tool, with the ATQ serving only to measure a stable person-factor. As previously mentioned in Chapter 1, research suggested that Effortful control or one of its sub-constructs will make the best stable person-factor (Milich & Kramer, 1984; Kochanska *et al.*, 1997, 2000; Ormel *et al.*, 2005; Damon & Lerner, 2006; Rothbart & Sheese, 2007). From the data obtained in this research study, it has been decided that Frustration (a sub-construct of Negative affect) will be used as the stable person-factor. These findings are supported by research initially positively correlating Anger (Buss & Perry, 1992) to the Behavioural Approach System (BAS) (Carver & White, 1994), and then negatively correlating the BAS to Negative affect (Harmon-Jones, 2003).

For the molecular aspects of this research study, special focus will fall on individuals with high and low Anger and Physical aggression scores, as measured by the AQ (Buss & Warren, 2000). These individuals will then be grouped based on the stable person-factor of high and low Frustration scores, as measured by the ATQ (Evans & Rothbart, 2007). Taking this into consideration, these individuals with similar phenotypes, should have similar underlying genotypes influencing the manifestation of aggressive behaviour.

CHAPTER 5

The molecular analysis of the *HTR1A, HTR1B, HTR2A, SLC6A4* and *MAO-A* genes on aggression and anxiety

Abstract

The serotonergic system has been found to influence behaviour to a great extent. Expressed in the deeper more primitive brain regions, the *HTR1A, HTR1B, HTR2A, SLC6A4* and *MAO-A* genes can be assumed to influence primitive behavioural patterns such as aggression. Different forms of these genes have been associated with various psychological disorders and problem behaviour. Specific regions within each of these genes were investigated in a small South African sample. The three receptor genes (*HTR1A, HTR1B, HTR2A*) all had specific SNPs of interests. Restriction enzymes were used to identify these SNPs. Sequencing of these genes revealed that using restriction enzymes is actually a trustworthy technique, as it digested accurately and without fail. The *SLC6A4* and *MAO-A* genes both have repeat regions that influence gene expression. Agarose gel electrophoresis proved to have very little specificity. The more sensitive PAGE showed accurate results. Both these genes however proved difficult to sequence. Regardless of this, the PAGE results can be counted to be accurate. Thus in both instances, the more expensive sequencing of individuals can be discarded for other equally reliable techniques.

Keywords: Aggression, Effortful control, monoamine oxidase A, Negative affect, serotonin receptor, serotonin transporter

Introduction

Aggressive behaviour can be seen as the self-preservation or promotion through mindfully hurting others. The individual causing the harm should know this (as this is the intent), while the harm receiver should know that it is coming and try to avoid it (Berkowitz, 1993a; Bushman & Anderson, 2001; Anderson & Bushman, 2002; Baron & Richardson, 2004). Based on the ontogenic development, more primitive regions lies deeper within the brain (Isaacson, 2001; Toga *et al.*, 2006). This is where the serotonergic system can be found (Törk, 1990; Hensler, 2011). Functional Magnetic Resonance Imaging (fMRI) can identify areas active during certain emotional responses (Berman *et al.*, 1997; Davidson *et al.*, 2000; Horn *et al.*, 2003; Keele, 2005; Seo *et al.*, 2008; Nordquist & Oreland, 2010). This has revealed the possible influence of the serotonin receptors HTR1A, HTR1B and HTR2A as well as the serotonin transporter SLC6A4. These receptors and transporters regulate transmission of serotonin. The enzyme monoamine oxidase A (MAO-A) is another important component in this pathway and is responsible for the breakdown of serotonin. This enzyme is a crucial component of the serotonergic system and assists in maintaining the serotonin balance in the CNS. Monoamine oxidase A is expressed by the gene *MAO-A*. A functional VNTR upstream in the promoter region of this gene will be investigated. The *MAO-A* pVNTR regulates the expression of the gene.

The HTR1A receptor is known as an autoreceptor. Located on the pre- and post-synaptic neurons, this receptor is inhibitory by function and mainly responsible for the negative feedback that shuts down the constant serotonin release. The *HTR1A* gene, encoding the HTR1A receptor, is expressed in the limbic system (i.e. the hippocampus, entorhinal cortex, septum, amygdala and frontal cortex). Within this gene, the G allele of the -1019C>G (rs6295) polymorphism is of specific interest as it has been linked to anxiety disorders (Strobel *et al.*, 2003; Lemonde *et al.*, 2003). The C allele is ancestral, occurring almost equally to the G allele in most populations (according to sequences stored on Genbank, NCBI, 2011 [www.ncbi.nml.nih.gov]).

The HTR1B receptor is a presynaptic autoreceptor and a postsynaptic heteroreceptor. Presynaptically, this receptor modulates serotonin release. The postsynaptic function of this

receptor is the release of other neurochemicals (such as acetylcholine and dopamine). This receptor is also located on cerebral arteries where it plays a role in vasodilation and constriction. Anatomically, it is located primarily in the globus pallidus and substantia nigra of the basal ganglia. This localization, as well as the regulation of dopamine release, hints at a possible involvement in movement disorders like Parkinson's disease (Hensler, 2011). The *HTR1B* gene, encoding the HTR1B receptor has a 861G>C (rs6296) SNP. The C allele of this SNP has been linked to substance dependence (Lappalainen, 1998; Hasegawa *et al.*, 2002; Cao *et al.*, 2011), ASPD and impulsive behaviour (Noskova *et al.*, 2009).

The *HTR2A* gene encodes for a postsynaptic heteroreceptor that is found (amongst other regions) condensed in the prefrontal cortex. This receptor regulates body temperature and smooth muscle contractions in the peripheral nervous system (Hensler, 2011). The -1438G>A (rs6311) SNP in the promoter region influences the functional expression of this gene. The G allele has been associated with depression and anxiety disorders. It is also the ancestral allele (Nakamura *et al.*, 1999; Noskova *et al.*, 2009).

The serotonin transporter SLC6A4 is located presynaptically, and is responsible for the reuptake of serotonin back into the presynaptic neuron (Hensler, 2011). The *SLC6A4* gene encodes the SLC6A4 transporter. Approximately 1416 bp upstream in the promoter region of this gene is a VNTR region called the *5-HTTLPR* region. This repeat region consists of a GC-rich 20 to 23 bp repeat elements. Previous research indicated that this region is repeated 16 times (Table 5.1).

The short allele is the result of a deletion of 44 bp within the six to eight repeat region, which causes a reduction in the expression of the *SLC6A4* serotonin transporter gene (Heils *et al.*, 1996). Reduced expression of the gene leads to anxiety related behaviours and aggression (Heils *et al.*, 1996; Zhang *et al.*, 2009). This may be due to the build-up of serotonin in the synapse, causing the desensitized of the presynaptic autoreceptors (Chapter 2).

The long allele may, however, contain a SNP (rs25531) that causes a reduction in expression, equivalent to that of the short allele (Heils *et al.*, 1996).

Table 5.1: Nucleotide sequence (position -1376 to -1048) of the *5-HTTLPR* region within the *SLC6A4* gene promoter. The deleted region resulting in the short allele is indicated in red. No deletion of the indicated region results in the long allele. Table adapted from a figure (Heils *et al.*, 1996).

Repeat number	**Sequence of repeat elements**			
1	CCCTAC	TGCA	GCCTCCC	AGCAT
2	CCCCCC	TGCA	ACCTCC	AGCA
3	ACTCCC	TGTA	CCCCTCCT	AGGAT
4	CCCCCC	TTCA	TCCCCC	ATTATC
5	CCCCCC	TTCA	CTCCTCGC	GGCAT
6	CCCCCC	TGCA	**CCCCCC**	**AGCAT**
7	**CCCCCC**	**TGCA**	**GCCCCCCC**	**AGCAT**
8	**CTCCCC**	**TGCA**	CCCCC	AGCAT
9	CCCCCC	TGCA	GCCCTTCC	AGCA
10	TCCCCC	TGCA	GCCCTTCC	AGCA
11	CTCCCC	TGCA	ACCCCC	ATTAT
12	CCCCCC	TGCA	CCCCTCGC	AGTAT
13	CCCCCC	TGCA	CCCCCC	AGCATC
14	CCCCCA	TGCA	CCCCC	GGCAT
15	CCCCCC	TGCA	CCCCTCC	AGCAT
16	TCTCCT	TGCA	CCCTACC	AGTAT

After the transporter has removed the serotonin from the synapse, the enzyme monoamine oxidase, encoded by the *MAO-A* gene, is responsible for catalysing it. The location of MAO-A is on the mitochondrial membrane within the neuron terminal (Hensler, 2011). The *MAO-A* gene is located on the X-chromosome. Approximately 1200 bp upstream of the gene is a VNTR region consisting of 30 bp repeat regions (Sabol *et al.*, 1998). Lower to no expression levels of this gene has been associated with aggression and impulsivity (Brunner *et al.*, 1993; Sabol *et al.*, 1998; Jorm *et al.*, 2000; Das *et al.*, 2006; Guo *et al.*, 2008).

The first aim of this study is to determine the allele constitutions of the *HTR1A* (rs6295), *HTR1B* (rs6296), *HTR2A* (rs6311), *SLC6A4* (*5-HTTLPR*) and *MAO-A* (pVNTR) gene regions in a specific selection of individuals from the study group. The second aim of this study is to compare these genotypes to the quantitative phenotypes suggested in Chapter 4.

5.1. Materials and methods

Saliva samples were collected from 30 individuals who also participated[1] in the quantitative analysis (Chapter 4). Six additional family members of the selected individuals were included in the molecular analysis. The research protocol (ethically approved by the University of the Free State Ethics Committee {ECUFS152/2011}) was fully explained to the participants. They were asked to give consent for voluntary participation and DNA collection in the form of saliva. All the individuals mentioned (36) consented.

5.1.1. DNA extraction

All the participants were requested to dispense 1 ml of saliva into 1 ml of lysis buffer containing 50 mM trishydroxymethylaminomethane (Tris) (pH 8.0), 50 mM ethylenediaminetetraacetic acid (EDTA), 50 mM sucrose, 100 mM sodium chloride (NaCl) and 1% sodium dodecyl sulphate (SDS). Samples were extracted through a salting out method by Quinque *et al.* (2006). After DNA was quantified using the NanoDrop Lite Spectrophotometer (Thermo Scientific), the DNA was diluted to 100 ng/µl, as necessary for the PCR reactions.

5.1.2. Polymerase chain reaction (PCR) amplification

The *HTR1A* promoter region was amplified using the following reaction protocol in 20 µl volume PCR reaction mix: 200 ng of DNA, 10 µl of DreamTaq™ Master Mix by Fermentas (2X DreamTaq buffer, 4 mM magnesium chloride {$MgCl_2$} and 0.4 mM of each dATP, dCTP, dTTP and dGTP) and 0.5 mM of each primer (Table 5.1).

Both the 861G>C SNP (rs6296) within the *HTR1B* gene as well as the -1438G>A SNP (rs7939) region in the promoter of the *HTR2A* gene was amplified using the following reaction protocol in 20 µl volume PCR reaction mix: 200 ng of DNA, 10 µl of DreamTaq™ Master Mix by Fermentas (2X DreamTaq buffer, 4 mM $MgCl_2$ and 0.4 mM of each dATP, dCTP, dTTP and dGTP) and 0.75 mM of each primer (Table 5.2).

[1] Participants were selected based on levels of anxiety (indicated by the temperamental construct of Negative affect, and it's subconstructs) and aggressive tendencies. Individuals were initially selected based on relatively high and low scores in all of these constructs. No distinctions were made between age groups, race or gender. From individuals selected, a further selection was done on willingness to participate voluntarily.

The *5-HTTLPR* region in the promoter of the *SLC6A4* gene was amplified using 20 µl volume PCR reaction mix: 300 ng of DNA, 10 µl of KAPATaq™ HotStart DNA Polymerase from KAPABiosystems (5 U/µl Wild-type Taq with HotStart antibody, 5X KAPA Taq HotStart Buffer {Mg^{2+} free} and $MgCl_2$ {25 mM}), 0.35 mM of each primer (Table 5.1) and 1.25 ml Dimethyl sulfoxide (DMSO).

Table 5.2: Primers used to amplify *HTR1A* promoter region (Strobel *et al.*, 2003), *HTR1B* gene region (Lappalainen, 1998), *HTR2A* promoter region (Nakamura *et al.*, 1999), *SLC6A4* promoter region (Heils *et al.*, 1996) and *MAO-A* promoter region (Sabol *et al.*, 1998). The nucleotide in bold lettering is indicative of the induced polymorphism, creating a restriction site.

Gene region	Primer name	Primer sequence
HTR1A	SNPR1A-nor	5’ -GGC TGG ACT GTT AGA TGA TAA CG -3’
	SNPR1A-mod	5’ -GGA AGA AGA CCG AGT GTG TC**A** T -3’
HTR1B	5HT1B5	5’ -GAA ACA GAC GCC CAA CAG GAC -3’
	5HT1B6	5’ -CCA GAA ACC GCG AAA GAA GAT -3’
HTR2A	Forward	5’ -AAG CTG CAA GGT AGC AAC AGC -3’
	Reverse	5’ -AAC CAA CTT ATT TCC TAC CAC -3’
SLC6A4	stpr5	5’ -GGC GTT GCC GCT CTG AAT TGC -3’
	stpr3	5’ -GAG GGA CTG AGC TGG ACA ACC CAC -3’
MAO-A	MAOaPT1	5’ -ACA GCC TGA CCG TGG AGA AG -3’
	MAOaBP1	5’ -GAA CGG ACG CTC CAT TCG GA -3’

The *MAO-A*-pVNTR in the promoter region of the *MAO-A* gene was amplified in 20 µl volume PCR reaction mix: 200 ng of DNA, 10 µl of DreamTaq™ Master Mix by Fermentas (2X DreamTaq buffer, 4 mM $MgCl_2$ and 0.4 mM of each dATP, dCTP, dTTP and dGTP) and 0.75 mM of each primer (Table 5.2).

Each of these genes had a specific PCR regime that was used to amplify it (Table 5.3). After PCR amplification, all the amplicons were visualised on a 1% (m/v) agarose gel (Appendix D).

5.1.3. Restriction enzyme digestion

The amplified PCR product of *HTR1A, HTR1B* and *HTR2A* were digested with BseGI (Figure 5.1a), HincII (Figure 5.1b) and MspI (Figure 5.1c) restriction enzymes (Thermo

Scientific) respectively. Each 32 µl reaction contained 18 µl nuclease free water, 2.8 µl of 10X Buffer Tango™ (33 mM Tris-acestate {pH 7.9 at 37°C}, 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA {Bovine Serum Albumin}), 0.2 µl (10 U) of the restriction enzyme (Thermo Scientific) and 10 µl of the undiluted PCR product. The *HTR1A* reaction was digested for 16 hours at 55°C in an incubator. Both *HTR1B* and *HTR2A* were digested for 16 hours at 37°C in an incubator.

Table 5.3: PCR regimes used to amplify *HTR1A*, *HTR1B*, *HTR2A*, *SLC6A4* and *MAO-A* regions.

Gene region		Step	Temperature	Duration	Cycles
a.	*HTR1A*	Initial denaturation	95°C	5 min.	1
		Denaturation	95°C	30 s.	
		Annealing	59.5°C	40 s.	35
		Extention	72°C	50 s.	
		Final extention	72°C	10 min.	1
b.	*HTR1B*	Initial denaturation	95°C	5 min.	1
		Denaturation	95°C	20 s.	
		Annealing	61°C	45 s.	31
		Extention	72°C	30 s.	
		Final extention	72°C	10min.	1
c.	*HTR2A*	Initial denaturation	95°C	5 min.	1
		Denaturation	95°C	20 s.	
		Annealing	60°C	1 min.	30
		Extention	72°C	30 s.	
		Final extention	72°C	10 min.	1
d.	*SLC6A4*	Initial denaturation	95°C	7 min.	1
		Denaturation	98°C	30 s.	
		Annealing	63°C	30 s.	3
		Extention	72°C	1 min.	
		Denaturation	98°C	30 s.	
		Annealing	61°C	30 s.	25
		Extention	72°C	1 min.	
		Final extention	72°C	10 min.	1
e.	*MAO-A*	Initial denaturation	95°C	3 min.	1
		Denaturation	95°C	1 min.	
		Annealing	66°C	1 min.	30
		Extention	72°C	1½ min.	
		Final extention	72°C	10 min.	1

The digested *HTR1A* PCR product was visualised on a 12% polyacrylamide electrophoresis (PAGE) gel (4.9 ml dH_2O, 2.0 ml 5X TBE {Tris, Borate, EDTA}, 3.0 ml of 40% acrylamide, 100 µl of 10% APS {ammonium persulfate} and 10 µl tetramethylethylene-diamine {TEMED}), running at 150 V/cm for 90 minutes. The digested *HTR1B* and *HTR2A* PCR products were both visualised on an 8% PAGE gel, both running at 150 V for 90 minutes. The amplicons for both *SLC6A4* and *MAO-A* were also visualised on an 8% PAGE gel (5.9 ml dH_2O, 2.0ml 5X TBE {Tris, Borate, EDTA}, 2.0 ml of 40% acrylamide, 100 µl of 10% APS and 10 µl TEMED), running at 150 V/cm for 100 minutes. The DNA was stained by post gel staining with GelRed™ Nucleic Acid Gel Stain (Biotium). The gels were placed in a solution containing 45 ml distilled water and 15 µl of the 10 000X GelRed™ Nucleic Acid Gel Stain. The reaction was setup and kept away from light due to the light sensitivity of the GelRed™ Nucleic Acid Gel Stain. The solution was placed on an orbital shaker for 30 minutes. The gels were visualised under fluorescent light.

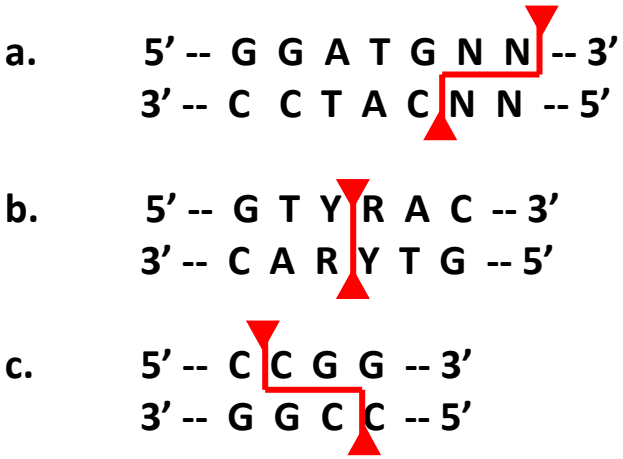


Figure 5.1: Restriction enzymes (Fermentas): a. Digestion site of BseGI. It will digest the *HTR1A* SNP (rs6295) in the presence of the variant G allele. b. Digestion site of HincII. It will digest the *HTR1B* SNP (rs6296) in the presence of the variant C allele. c. Digestion site of MspI. It will digest the *HTR2A* SNP (rs6311) in the presence of the ancestral G allele.

5.1.4. Sequencing

The amplified regions for all 36 samples were sequenced.

The BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®) was used. Sequencing reactions were set up in a 96 well plate, as 10 µl reactions. This consisted of 3.9

µl nuclease free water, 0.5 µl BigDye® premix, 1 µl of 5X Sequencing Buffer, 3.2 mM primer, 4% DMSO and 1 µl undiluted PCR product. The sequencing PCR regime consisted of denaturation for 10 seconds at 96°C, annealing for 5 seconds at 50°C and extension for 4 minutes at 60°C. These steps were repeated for 25 cycles.

The sequencing products were cleaned with EtOH/EDTA precipitation (Applied Biosystems, 2010). Precipitation of the DNA was accomplished by adding 2.5 µl of 125 mM EDTA to each well, followed by adding 25 µl of absolute ethanol to each well. This was incubated for 15 minutes at room temperature. Centrifuging at 4°C at 1650 *g* for 45 minutes followed. After discarding the supernatant, the sequencing product was washed with 30 µl of 70% Ethanol. This was again centrifuged for 15 minutes at 4°C and 1650 *g*. Thereafter the supernatant was discarded. After complete evaporation of the Ethanol, the sequencing product was resuspended in injection buffer. Sequences were analysed using the ABI3130 Genetic Analyser (Applied Biosystems). Sequences were assembled and edited with the Geneious 5.8 Software (Biomatters Limited).

5.1.5. Statistical analysis

All statistical analyses were done using Microsoft® Office Excel 2010 and the Data Analysis Tools Add-In.

5.2. Results and discussion

Of the 36 participants, 32 individuals are Caucasian, two individuals are African, one individual is of Eastern ancestry and one individual is from mixed ancestry. The ages ranged from nine to 69 years old. All these individuals provided informed consent for participation in this project.

Quantification of extracted DNA yielded an average of 250 ng/µl concentrations. This was diluted to 100 ng/µl concentrations.

5.2.1. *HTR1A*

The primers used to amplify this region has been modified to contain a -1016A>T polymorphism. A restriction enzyme recognizes and digests a specific nucleotide sequence.

The -1019C>G SNP is not recognized by any conventional restriction enzyme. With the substitution of an A for a T nucleotide at position -1016 (three positions from the SNP) within the primer annealing site, a restriction enzyme will be able to digest this particular DNA sequence in the presence of the G allele (Strobel *et al.*, 2003; Noskova *et al.*, 2009). The undigested *HTR1A* amplicon is 163 bp in length. This is indicative of the presence of the C (ancestral) allele. In the case of digestion, where the G allele is present, a 146 bp and 17 bp fragments can be seen. Three fragments should be visible for the heterozygous individuals with the CG genotype will show three fragments (163 bp, 146 bp and 17 bp) (Strobel *et al.*, 2003) (Figure 5.2).

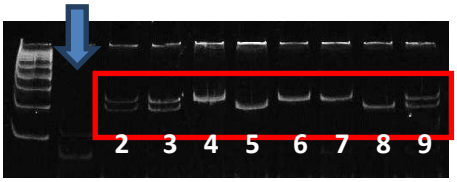


Figure 5.2: 12% PAGE gel of the digested *HTR1A* promoter region for samples 1 to 9 (as indicated by the numbers in the lanes), with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane. Primer dimers can be seen below the red box. A possible over-digested DNA sample is indicated by the blue arrow.

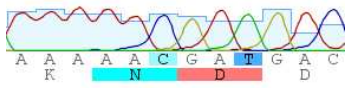
Successful PCR amplification and successive restriction enzyme digestion was obtained for 32 participants. In Figure 5.2 the visualized digested PCR product of individuals are indicated in the red block. Individuals 4, 6 and 7 (indicated by the numbers in the lanes) are homozygous for the C allele. Individuals 5 and 8 are homozygous for the G allele. Individuals 2, 3 and 9 are heterozygous. As previously mentioned, due to the small size of the 17 bp fragment, this fragment could not be visualised on the gel. It can be identified indirectly by the remaining fragment.

Successful sequences were obtained for 22 participants (Figure 5.3 d). The modified primer (with the T nucleotide instead of the A) creating the restriction site, can be observed in all the sequences (Figure 5.3).

Genotyping based on the PAGE results are consistent with the genotyping based on the sequencing results (Figure 5.3). The modified nucleotide due to the modified primer was observed in sequences of all the individuals. Five participants were genotyped as

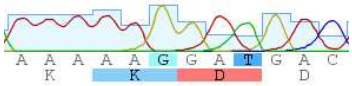
homozygous for the ancestral C allele. Four participants were genotyped as homozygous for the variant G allele. Heterozygous genotypes were observed in 13 individuals.

a.



b.

c.



d.

rs6295 refseq	T	T	T	T	A	A	A	A	A	S	G	A	A	G	A	C	A	C	A	C	T	C
CC12	T	T	T	T	A	A	A	A	A	C	G	A	T	G	A	C	A	C	A	C	T	C
CC14	T	T	T	T	A	A	A	A	A	C	G	A	T	G	A	C	A	C	A	C	T	C
CC29	T	T	T	T	A	A	A	A	A	C	G	A	T	G	A	C	A	C	A	C	T	C
CC6	T	T	T	T	A	A	A	A	A	C	G	A	T	G	A	C	A	C	A	C	T	C
CC7	T	T	T	T	A	A	A	A	A	C	G	A	T	G	A	C	A	C	A	C	T	C
CG10	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG16	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG19	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG2	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG22	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG24	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG27	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG28	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CG9	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CGE	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CGH	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CGJ	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
CGM	T	T	T	T	A	A	A	A	A	S	G	A	T	G	A	C	A	C	A	C	T	C
GG17	T	T	T	T	A	A	A	A	A	G	G	A	T	G	A	C	A	C	A	C	T	C
GG25	T	T	T	T	A	A	A	A	A	G	G	A	T	G	A	C	A	C	A	C	T	C
GG31	T	T	T	T	A	A	A	A	A	G	G	A	T	G	A	C	A	C	A	C	T	C
GG8	T	T	T	T	A	A	A	A	A	G	G	A	T	G	A	C	A	C	A	C	T	C

Figure 5.3: Sequences of *HTR1A* (rs6295): a. Electropherogram of a homozygous C individual (participant 6 in Figure 5.2); b. Electropherogram of a heterozygous individual (participant 9 in Figure 5.2); c. Electropherogram of a homozygous G individual (participant 5 in Figure 5.2). The modified -1016A>T SNP (indicated by the blue arrow) can be seen three positions right of the -1019C>G SNP (indicated by the red arrow); d. All successful nucleotide sequences indicating zygosity, as compared to the reference sequence (NCBI, 2011). The rs6295 SNP is indicated by the S. This is an indication of a C/G possibility. Individuals are number to reflect their individual number preceded by their genotype for this specific SNP.

5.2.2. *HTR1B*

PCR amplification was successful in 33 individuals. All of the amplicons were successfully digested. The undigested amplicon of *HTR1B* is 548 bp in length. In the presence of the ancestral G allele, two fragments of 452 bp and 96 bp are present. In the presence of the C allele, three fragments of 310 bp, 142 bp and 96 bp can be seen. Heterozygous individuals will show four fragments of 452 bp, 310 bp, 142 bp and 96 bp each (Figure 5.4) (Hasegawa *et al.*, 2002).

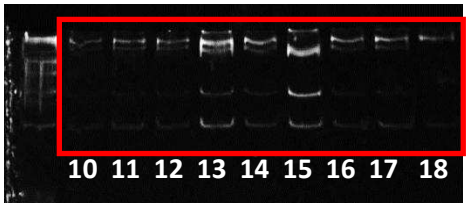
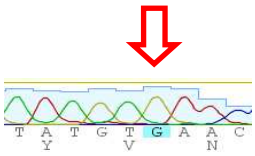


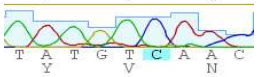
Figure 5.4: 12% PAGE gel of the digested *HTR1B* gene region for samples 10 to 18, with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in lane one. Individual 18 is homozygous for the G allele. Individuals 10, 11, 12, 13, 14, 16, 17 are all heterozygous. Individual 15 is homozygous for the C allele.

a.



b.

c.



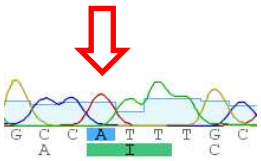
d.

rs6296 refseq	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
CC15	T	C	C	T	G	T	G	T	A	T	G	T	C	A	A	C	C	A	A	G	T
CC6	T	C	C	T	G	T	G	T	A	T	G	T	C	A	A	C	C	A	A	G	T
GC10	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC11	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC12	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC13	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC14	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC16	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC17	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC21	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC24	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC25	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC29	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC31	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GC4	T	C	C	T	G	T	G	T	A	T	G	T	S	A	A	C	C	A	A	G	T
GG1	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG18	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG19	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG2	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG20	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG22	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG23	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG26	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG27	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG28	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG3	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG5	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG7	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG8	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GG9	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T
GGE	T	C	C	T	G	T	G	T	A	T	G	T	G	A	A	C	C	A	A	G	T

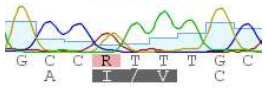
Figure 5.5: Sequences of *HTR1B* (rs6296): a. Electropherogram of a homozygous G individual (participant 18 in Figure 5.4); b. Electropherogram of a heterozygous individual (participant 15 in Figure 5.4); c. Electropherogram of a homozygous C individual (participant 10 in Figure 5.4); d. All successful nucleotide sequences were compared to a reference sequence (NCBI, 2011) to determine the type of zygosity in the individuals. The rs6296 SNP is indicated by the S. This is an indication of a C/G possibility. Individuals are number to reflect their individual number preceded by their genotype for this specific SNP.

In Figure 5.4 the visualization of the digested PCR product of individuals 10 to 18 are indicated in the red block. Individual 18 is homozygous for the G allele. Individuals 10, 11, 12, 13, 14, 16, 17 are all heterozygous. Individual 15 is homozygous for the C allele. Successful sequences were obtained for 31 individuals (Figure 5.5 d). Sequencing of this gene for individuals 10, 15 and 18 was consistent with the results obtained from the digested PCR (Figure 5.5 d). Sixteen individuals were genotyped as homozygous for the ancestral G allele, whereas only two individuals were genotyped as homozygous for the variant C allele. Thirteen individuals are heterozygous for this SNP.

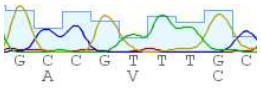
a.



b.



c.



d.

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AA10	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA11	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA13	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA14	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA15	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA16	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA17	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA18	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA19	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA20	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA21	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA22	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA23	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA24	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA25	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA27	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA28	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA29	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA31	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA6	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA7	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA8	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AA9	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
AAE	T	T	G	A	C	T	T	G	C	C	A	T	T	T	G	C	A	G	T	G	G	G
GA12	T	T	G	A	C	T	T	G	C	C	R	T	T	T	G	C	A	G	T	G	G	G
GA2	T	T	G	A	C	T	T	G	C	C	R	T	T	T	G	C	A	G	T	G	G	G
GA26	T	T	G	A	C	T	T	G	C	C	R	T	T	T	G	C	A	G	T	G	G	G
GA3	T	T	G	A	C	T	T	G	C	C	R	T	T	T	G	C	A	G	T	G	G	G
GA4	T	T	G	A	C	T	T	G	C	C	R	T	T	T	G	C	A	G	T	G	G	G
GG5	T	T	G	A	C	T	T	G	C	C	G	T	T	T	G	C	A	G	T	G	G	G

Figure 5.6: Sequences of *HTR1B* (rs6297): a. Electropherogram of a homozygous A individual (participant AA6); b. Electropherogram of a heterozygous individual (participant GA4); c. Electropherogram of a homozygous G individual (participant GG5); d. All successful nucleotide sequences were compared to the reference sequence (NCBI, 2011) to determine zygosity. Individuals are number to reflect their individual number preceded by their genotype for this specific SNP.

Further investigation of this amplified region revealed another SNP (Figure 5.6). This 1180A>G SNP (rs6297) has been associated with ADHD (Smoller *et al.*, 2006; Ickowicz *et al.*, 2007), depression (Kõks *et al.*, 2006), SSRI response (Villafuerte *et al.*, 2009) and self-reported anger and aggression (Conner *et al.*, 2010). This SNP will thus also be included in further analyses.

For this SNP, the A allele is the ancestral form (NCBI, 2011), present in the homozygous form for 25 of the participants. The very rare G allele was present in homozygous form, only in one individual. Five individuals were genotyped to be heterozygous.

5.2.3. *HTR2A*

Successful PCR amplification was obtained for 33 of the participants. All the amplicons were successfully digested. The undigested amplicon for *HTR2A* is 468 bp in length. This fragment is indicative of the presence of the A allele. In the presence of the G allele, two fragments with 224 bp and 244 bp lengths can be seen. Heterozygous individuals will show three fragments of 468 bp, 244 bp and 224 bp each (Figure 5.7) (Nakamura *et al.*, 1999).

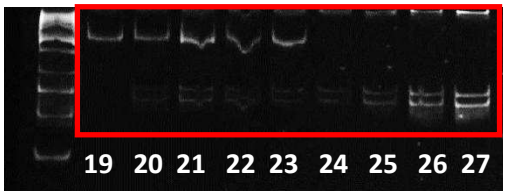


Figure 5.7: 12% PAGE of the digested *HTR2A* promoter region for samples 19 to 27, with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in first lane. Individual 19 is homozygous for the A allele. Individuals 24, 25, 26 and 27 are homozygous for the G allele. Individuals 20, 21, 22 and 23 are heterozygous.

In Figure 5.7 the visualization of the PCR product digestion of individuals 19 to 27 are indicated in the red block. Individual 19 is homozygous for the A allele. Individuals 24, 25, 26 and 27 are homozygous for the G allele. Individuals 20, 21, 22 and 23 are heterozygous. This corresponded to the sequencing. The only inconsistency lies in the labelling of the alleles. According to the NCBI website regarding this SNP details, the observed alleles are

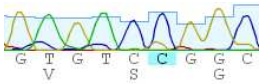
G/A, with the ancestral being the G allele (NCBI, 2011). However, the reference sequence obtained for this SNP (rs6311) indicates C/T alleles (Figure 5.8).

rs6311 *[Homo sapiens]*

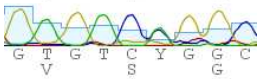
TATGTCCTCGGAGTGCTGTGAGTGTC[C/T]GGCACTTCCATCCAAAGCCAACAGT

Figure 5.8: Reference sequence obtained for *HTR2A* (rs6311) indicating a C/T allele (NCBI, 2011).

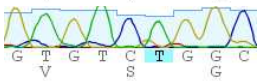
a.



b.



c.



d.

rs6311 refseq	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CC13	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CC14	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CC24	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CC25	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CC27	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CC29	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CC6	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CC8	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CCT	T	G	T	G	A	G	T	G	T	C	C	G	G	C	A	C	T	T	C	C	A	T
CT1	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT10	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT11	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT12	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT15	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT17	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT18	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT2	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT21	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT22	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT23	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT31	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT5	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT7	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT8	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
CT9	T	G	T	G	A	G	T	G	T	C	Y	G	G	C	A	C	T	T	C	C	A	T
TT19	T	G	T	G	A	G	T	G	T	C	T	G	G	C	A	C	T	T	C	C	A	T
TTE	T	G	T	G	A	G	T	G	T	C	T	G	G	C	A	C	T	T	C	C	A	T
TTH	T	G	T	G	A	G	T	G	T	C	T	G	G	C	A	C	T	T	C	C	A	T
TTM	T	G	T	G	A	G	T	G	T	C	T	G	G	C	A	C	T	T	C	C	A	T

Figure 5.9: Sequences of *HTR2A* (rs6311): a. Electropherogram of a homozygous C individual (participant 24 in Figure 5.9); b. Electropherogram of a heterozygous individual (participant 21 in Figure 5.9); c. Electropherogram of a homozygous T individual (participant 19 in Figure 5.9); d. All successful nucleotide sequences were compared to the reference sequence (NCBI, 2011) to determine the zygosity. The rs6311 SNP is indicated by the Y. This is an indication of a C/T possibility. Individuals are number to reflect their individual number preceded by their genotype for this specific SNP.

Successful sequences were obtained for 29 of the participants. Sequencing concurred with the C/T alleles (Figure 5.9 d). It might be that a reversed compliment was submitted to the NCBI website, and consecutive researchers referred to the reversed compliment SNP (G/A), when in fact the correct reference to this SNP should be C/T (NCBI, 2011). From sequences obtained during this research project, the alleles will be labelled C/T, with C being the complement of the ancestral allele. This can easily be compared with the restriction mapping, as the CCGG restriction site will remain the same. The only difference is that the original thought of CC**A**G change will now be a C**T**GG change. Nevertheless, this makes no difference to the digestion.

5.2.4. *SLC6A4*

The *SLC6A4* gene contains the *5-HTTLPR* region within its promoter region. It consists of a repeat region creating a long (528 bp) or a short (484 bp) allele. The short allele is created by a deletion between the six and eight repeat regions (Table 5.1). Successful PCR amplification was obtained for 31 of the participants. Sequencing for this gene proved to be troublesome. Due to the short repetitive repeat regions and high CG content (Heils *et al.*, 1996), poor quality sequences were obtained. Genotyping for this gene will have to rely solely on visualisation on the PAGE gel (Figure 5.10).

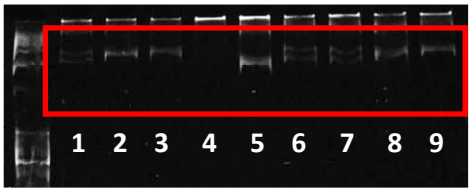


Figure 5.10: 12% PAGE gel of the *SLC6A4* promoter region for samples 1 to 9 (indicated with the numbers in the lanes), with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane. Individuals 2, 3, 8 and 9 are homozygous for the long allele. Individual 5 is homozygous for the short allele. Individuals 1, 6 and 7 are heterozygous. Amplification for individual 4 was unsuccessful.

To overcome the difficulties with sequencing, fragment analysis can be used to determine the number of repeats, thereby obtaining the genotype. This is the same as obtaining a genotype with the PAGE. The shortcoming of both the PAGE gel and fragment

analysis is that it will not identify the deletion site in the long allele that causes the same phenotype as the short allele (Heils *et al.*, 1996).

5.2.5. *MAO-A*

The *MAO-A* pVNTR occurs in the promoter region of the gene. It is a tandem repeat of 30 bp sometimes followed by a half repeat sequence of the first 15 bp of this sequence. Four alleles are most prevalent and expected, with either three and a half repeats (3.5R), four and a half repeats (4.5R), five repeats (5R) and five and a half repeats (5.5R) (Figure 5.11). This is contradictory to the results published by Sabol *et al.* (1998), who mislabelled the alleles as 3R, 4R, 4.5R and 5R (Laubscher, 2012).

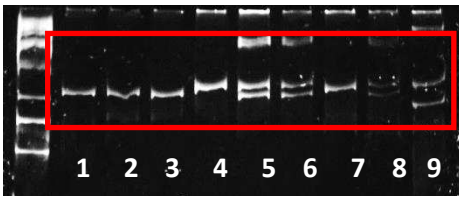


Figure 5.11: 12% PAGE gel of the *MAO-A* pVNTR promoter region for samples 1 to 9, with the 50bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane. Individuals 1, 2 and 3 are homozygous for the 3.5R. Individuals 4 and 7 are homozygous for the 4.5R. Individuals 5, 6 and 8 are heterozygous for the 3.5R and 4.5R alleles. Individual 9 has a 4.5R and the rare 2.5R.

Successful PCR amplification was obtained for 34 of the participants. Only male participants were selected to be sequenced. Since this gene is located on the X chromosome, hemizygous males should yield best sequences (Lu *et al.*, 2002; Contini *et al.*, 2006). This gene, like the *5-HTTLPR* however proved to be somewhat difficult to sequence. The reason for this may also be due to the short CG-rich repeats.

Sequences were successfully obtained from two of the 15 male participants (Figure 5.12), representing an individual with 3.5 repeats as well as an individual with 4.5 repeats (or 3R and 4R respectively according to Sabol *et al.* (1998)). This study, as well as other studies (Jorm *et al.*, 2000; Lu *et al.*, 2002; Das *et al.*, 2006; Laubscher, 2012) confirm that the expected alleles in a population will be 2.5R, 3.5R, 4R, 4.5R and 5.5R (and not 3R, 4R, 4.5R and 5R). It can thus be assumed that half a repeat (0.5R) should be added to all previously

published alleles (Jorm *et al.*, 2000; Lu *et al.*, 2002; Das *et al.*, 2006). The 4R and 4.5R produce high levels of expression for this gene, whereas the 3.5R and 5.5R show low levels of expression leading to aggressive behaviour. The 2.5R allele is mostly associated with delinquent behaviour in adolescents (Guo *et al.*, 2008).

Genotyping the rest of the sample will be done by comparing the fragment lengths from individuals 1 and 7 (the sequenced individuals) to that of the rest of the sample. The other individuals' genotype will be relative to that of individuals 1 and 7 (Table 5.4). The further assumption that 0.5R should be added to all previously published alleles will also be followed (Jorm *et al.*, 2000; Lu *et al.*, 2002; Das *et al.*, 2006).

a.

b.

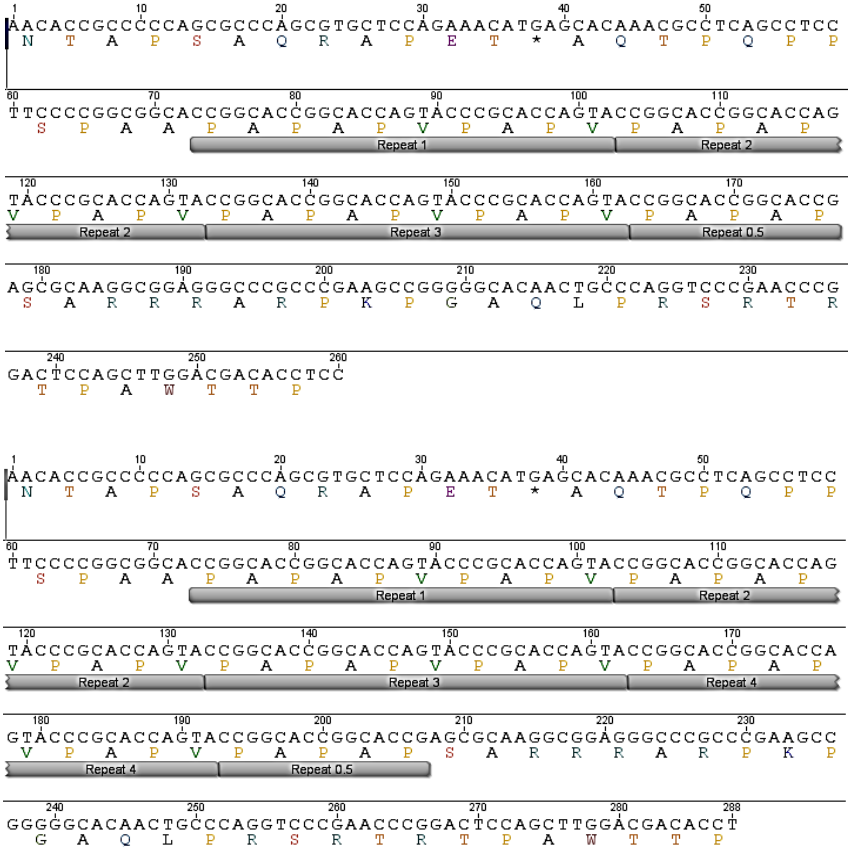


Figure 5.12: Sequences of *MAO-A* for an individual with a. 3.5R; and an individual with b. 4.5R.

5.3. Statistical analysis

Certain individuals could not be genotyped (Table 5.4). This may be due to different factors, such as degraded DNA. It is however interesting to note that the individuals that

Table 5.4: Genotyping of the individual for the five investigated serotonergic gene regions.

Individual	*HTR1A* (rs6295)	*HTR1B* (rs6296)	*HTR1B* (rs6297)	*HTR2A* (rs6311)	*SLC6A4*	*MAO-A*
1M	--	GG	AA	TC	SL	3.5
2F	CG	GG	GA	TC	LL	3.5/3.5
3M	CG	GG	GA	TT	LL	3.5
4M	CC	GC	GA	CC	--	4.5
5F	GG	GG	GG	TC	SS	3.5/4.5
6F	CC	CC	AA	CC	LS	3.5/4.5
7M	CC	GG	AA	TC	LS	4.5
8F	GG	GG	AA	TC	LL	3.5/4.5
9F	CG	GG	AA	TC	LL	2.5/4.5
10M	CG	GC	AA	TC	LL	5.5
11F	CG	GC	AA	TC	LS	3.5/3.5
12F	CC	GC	GA	TC	LS	3.5/3.5
13M	GG	GC	AA	CC	LS	3.5
14M	CC	GC	AA	CC	LS	3.5
15M	CG	CC	AA	TC	LS	4
16F	CG	GC	AA	TC	SS	3.5/4.5
17F	GG	GC	AA	TC	SS	3.5/4.5
18F	CC	GG	AA	TC	LS	3.5/4.5
19F	CG	GG	AA	TT	L-	3.5/4.5
20F	CC	GG	AA	TC	LL	3.5/4.5
21M	CG	GC	AA	TC	LS	4.5
22F	CG	GG	AA	TC	LS	3.5/4.5
23F	--	GG	AA	TC	LL	3.5/4.5
24F	CG	GC	AA	CC	--	4.5/4.5
25F	GG	GC	AA	CC	LL	3.5/4.5
26M	CG	GG	GA	CC	LS	3.5
27F	CG	GG	AA	CC	LS	3.5/3.5
28F	CG	GG	AA	TC	LS	3.5/3.5
29F	CC	GC	AA	CC	SS	3.5/4.5
30M	--	--	--	--	L-	3.5
31M	GG	GC	AA	TC	LS	3.5
EF	CG	GG	AA	TT	LS	4.5/4.5
HM	CG	GC	--	TT	--	4.5
MM	CG	GC	--	TT	LS	--
JM	CG	--	--	--	LS	--
WF	--	--	--	--	LS	4.5/4.5

produced the poorest results are also part of the advanced age group. There might be an environmental component that causes more degradation of epithelial cells (as would be the case in collection of saliva samples). Individuals whose DNA did not yield a satisfactory genotype for a specific gene were excluded for statistical analysis.

As previously mentioned, individuals were also chosen based on relatedness. Because of this, it was possible to deduce some of the unresolved genotypes. Figure 5.13 is a representation of the family included in this study. Since only some of the family members participated in the quantitative analysis, no pattern of inheritance can be discerned for the four indicated behavioural traits.

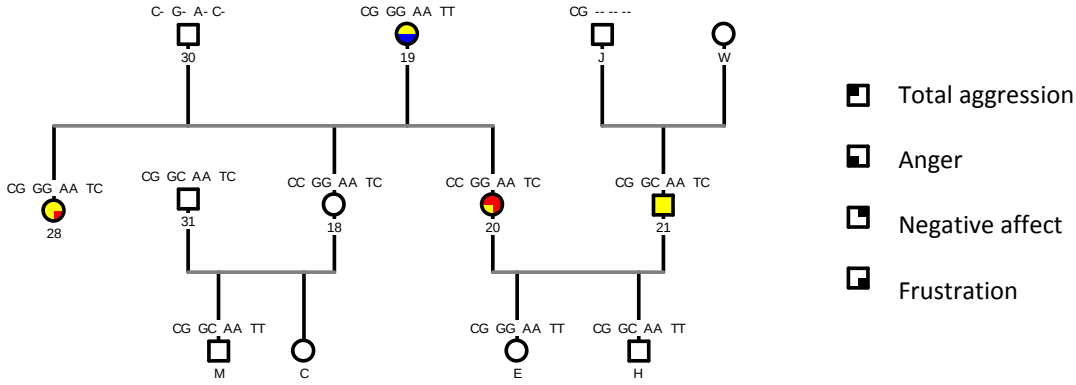


Figure 5.13: Pedigree of a family participating in the study. Each family member's genotype for the *HTR1A*, *HTR1B* (both SNPs) and *HTR2A* is indicated. Blue is an indication of a low score. Red is an indication of a high score. Yellow is an indication of an intermediate score.

The allelic frequency of each gene region's respective alleles was calculated. This is an indication of allelic prevalence within this sample. The phenotypic quantification done in Chapter 4 was added to the genotypes. From the results obtained, specific focus will fall on the Anger and Indirect aggression phenotypes (obtained from the Aggression Questionnaire) and Frustration (a subcontract of Negative Affect, obtained from the Adult Temperament Questionnaire).

Based on the genotypic summary, allele frequencies were determined within this sample (Table 5.4). It shows that even though it has a very small size, it is still representative of the general population. All possible alleles for the different gene regions were observed within this sample.

Allele Frequency for each gene region corresponds with that provided by the NCBI website. For *HTR1A* (rs6295) allele frequency within European populations was 50%. Heterozygosity was predominantly observed (NCBI, 2011). Slight deviation from this, as seen in our sample, can be due to the small sample size. It may also be due to the geographic location. Homozygosity for the C allele was observed in Sub-Saharan African allelic counts. It is the same as in the Bushman populations. Other studies done on populations also found similar results in a Japanese population (Kishi *et al.*, 2009), German and Italian populations (Serretti *et al.*, 2007).

Table 5.4: Allelic distribution within the studied sample.

Gene region	**Alleles**	**Sample (%)**
HTR1A (rs6295)	C	53.13
	G	46.87
	CC (n=8)	25
	GG (n=6)	18.75
	CG (n=18)	56.25
HTR1B (rs6296)	G	71.21
	C	28.79
	GG (n=16)	48.49
	CC (n=2)	6.06
	GC (n=15)	45.45
HTR1B (rs6297)	A	88.71
	G	11.29
	AA (n=25)	80.65
	GG (n=1)	3.22
	AG (n=5)	16.13
HTR2A (rs6311)	T	43.94
	C	56.06
	TT (n=5)	15.15
	CC (n=9)	27.27
	TC (n=19)	57.58
SLC6A4 (*5-HTTLPR*)	L	56.45
	S	43.55
	LL (n=8)	25.81
	SS (n=4)	12.9
	LS (n=19)	61.29
MAO-A (pVNTR)	2.5R	1.89
	3.5R	54.71
	4.5R	39.62
	4R	1.89
	5.5R	1.89

For *HTR1B* (rs6296), allele frequency for European populations was 70% for the G allele, favouring homozygosity for this allele. Compared to the Bushman population, the G allele is also more frequent (80%), though heterozygosity is more prevalent than the homozygous G allele (NCBI, 2011). Allelic count within this sample is similar to the European population, though intermediate between the Bushman and European populations for the zygosity. Similar results were obtained in a Caucasian population (Conner *et al.*, 2010), a Finnish population (Hakulinen *et al.*, 2012) and Han Chinese population (Gao *et al.*, 2011).

The unexpected *HTR1B* SNP (rs6297) has an allele frequency that greatly favours the A allele. According to the NCBI data, the occurrence of the A is 85%, with A homozygosity greatly prevalent (NCBI, 2011). This has been seen in the current sample as well. Only one individual in this sample is homozygous for the G allele. This allele has been associated with addiction (Proudnikov *et al.*, 2006; Conner *et al.*, 2010). Similar allele frequencies has been found in a Caucasian population (Conner *et al.*, 2010), a Japanese population (Ujike *et al.*, 2011) and a mixed ancestry population (Ickowicz *et al.*, 2007).

The allele frequency for the *HTR2A* SNP (rs6311) was also 50% for both SNPs, with both zygosity equally prevalent in the European population (NCBI, 2011). Values between different populations vary very little from that of the European population values. The current sample also follows this trend. Similar results were also found in a Turkish population (Boke *et al.*, 2007), a Swedish population (Kling *et al.*, 2008) and an Indian population (Guhathakurta *et al.*, 2009).

For the *5-HTTLPR* region upstream from the *SLC6A4* gene, individuals in this sample were mostly heterozygous. Similar results were found in a Columbian population (Ospina-Duque *et al.*, 2000), a Caucasian population and another Afrikaner population (Kinnear *et al.*, 2000). Neither the long nor the short alleles are more prevalent within a randomly selected population. The rs25531 SNP occurring in the long allele rendering it phenotypically equivalent to the short allele has not been investigated as sequencing of the *5-HTTLPR* region was unsuccessful. It has, however, been noted that the transition from an A to a G causes the under expressed long allele (Hu *et al.*, 2005; Wendland *et al.*, 2006).

Very few studies have been done on this polymorphism in a random population. According to the NCBI website, homozygosity for the A allele is more prevalent than for the G allele.

As previously discussed, there have been some controversial results regarding the repeat lengths of the pVNTR within the promoter region of the *MAO-A* gene. Within this sample, the 3.5R and 4.5R were relatively more prevalent than the other alleles. The rare 2.5R and 5.5R were also present within this sample. Compared to other population groups the same alleles (adapted to add the 0.5R) were also most prevalent in a Han Chinese male population (Lu *et al.*, 2003), a Brazilian population (Contini *et al.*, 2006), a Chinese population (Qiu *et al.*, 2009) and another South African sample (Laubscher, 2012). All of these studies focussed on individuals with either anxiety or aggressive behaviour disorders.

Allele frequencies were also calculated for specific phenotypes. This was first done for aggressive behaviour, Negative affect (because of the quantitative correlation found in Chapter 4) and Effortful control (as suggested by literature). This was done to determine the possible influence of specific alleles on all of the traits measured by the AQ (Buss & Warren, 2000) (Table 5.5) and the Negative affect and Effortful control constructs and subconstructs as measured by the ATQ (Evans & Rothbart, 2007) (Table 5.6).

Interesting influences were found for most of the alleles investigated. The *HTR1A* C allele (rs6295), especially in the homozygous form, showed a predisposing influence on hostility, indirect and total aggression scores. Merjonen *et al.* (2011) also found a weak positive correlation between this allele and hostility. More supportive research was done on the heterozygous (rs6296) influence of *HTR1B* on hostility and aggression (Conner *et al.*, 2010; Hakulinen *et al.*, 2012). This study found the heterozygous form of this polymorphism to also produce higher level of hostility as well as total aggression. For *HTR1B* G allele (rs6297), this study found the most consistent predisposing influence on almost all forms of aggression. It seems that this allele predisposes an individual in ineffective coping. This was also found by Conner *et al.* (2010) in a young male sample. In the current sample, only one individual was homozygous for this allele, reducing its significance. This deviation in scores can, however, not completely be disregarded.

Table 5.5: Allele frequencies of each gene compared to the average score as measured by the AQ, obtained by the specified allele in this sample.								
Gene	Genotype	# (%)	Physical	Verbal	Anger	Hostility	Indirect	Total
HTR1A	CC	7 (26.9)	13	12	16	23	16	80
(rs6295)	CG	14 (53.9)	14	14	16	18	12	74
	GG	5 (19.2)	13	9.6	15	19	13	70
	C	28 (53.8)	14	13	16	20	14	76
	G	24 (46.2)	14	13	16	19	13	73
HTR1B	GG	12 (46.15)	13	13	17	20	14	76
(rs6296)	GC	12 (46.15)	15	12	15	26	14	81
	CC	2 (7.7)	14	12	15	19	13	73
	G	36 (69.2)	13	12	16	19	14	75
	C	16 (30.8)	14	12	15	20	13	74
HTR1B	GG	1 (3.8)	14	12	26	25	20	97
(rs6297)	GA	4 (15.4)	13	13	15	19	12	73
	AA	21 (80.7)	14	12	16	19	14	74
	G	6 (11.5)	13	13	17	20	14	78
	A	46 (88.5)	14	12	16	19	13	74
HTR2A	TT	1 (3.8)	8	13	8	10	11	50
(rs6311)	TC	16 (61.6)	17	13	17	21	14	81
	CC	9 (34.6)	12	12	16	20	13	73
	T	18 (34.6)	12	12	16	19	13	72
	C	34 (65.4)	14	12	16	20	14	76
SLC6A4	LL	6 (23.1)	13	12	17	19	12	74
(*5-HTTLPR*)	LS	16 (61.5)	14	12	19	21	17	83
	SS	4 (15.4)	14	13	15	19	13	74
	L	28 (53.8)	14	12	15	19	13	74
	S	24 (45.2)	14	13	16	20	14	76
MAO-A	2.5	1 (2.3)	25	13	25	22	15	100
(pVNTR)	3.5	24 (55.9)	13	12	16	19	13	73
	4	1 (2.3)	12	10	9	19	11	61
	4.5	17 (39.5)	13	12	17	20	15	77

Where the previous three SNPs predisposed to aggressive behavioural traits, the *HTR2A* T allele (rs6311) seems to inhibit aggression. The role of the C allele is less clear. In previous research, weak associations have been found between this allele and aggressive traits (Serretti *et al.*, 2007; Merjonen *et al.*, 2011; Helmstaedter *et al.*, 2012; Yang *et al.*, 2012). It seems to be more of a predisposition to vulnerability when coping with stress.

For both VNTRs less reliable deductions can be made. For the *5-HTTLPR* VNTR in the promoter region of the *SLC6A4* gene, sequencing was unsuccessful. The effect of this was that only repeat length could be identified. The SNP in the long allele resulting in the phenotypical expression equivalent to that of the short allele could not be identified. Thus the genotype is not reliable. For the *MAO-A* pVNTR only the 3.5R and 4.5R occurred in a sufficient number in the sample. The rare 2.5R are only carried by one individual in this sample. This individual had relatively higher scores in all subconstructs of aggressive behaviour, as was expected (Guo *et al.*, 2008). However, the low number of individuals with this form, like with *HTR1B,* makes statistical comparisons unreliable. Another problem with the *MAO-A* pVNTR is the fact that it is located on the X chromosome. In females, X-inactivation (Lyon, 1961) of one of these alleles occur (Hendriks *et al.*, 1992; Kim-Cohen *et al.*, 2006). Most of the females in this sample had the 3.5R/4.5R genotype. Since X-inactivation was not tested within this sample, it would be unreliable to make assumptions based on the quantitative score. Thus, the *MAO-A* genotypes are also considered unreliable.

Temperament is considered to be the way an individual reacts to environmental stimuli (Rothbart & Derryberry, 1982; Rothbart & Ahadi, 1994; Reber & Reber, 2002). It is also biologically driven and stable for different environmental stimuli (Goldsmith *et al.*, 1987; Reber & Reber, 2002; Rutter, 2006). Because of this, a definite genetic component should be identifiable.

Very inconsistent results were found for the *HTR1A* rs6295 SNP. This SNP results had little to no influence on Negative affect and it's subconstructs. The G allele results indicated some influence on Action control, with the heterozygous GC genotype scoring the highest in both Action control, Inhibition control the total Effortful control. Previous research by

Table. 5.6: Allele frequencies of each gene compared to the average score as measured by the ATQ, obtained by the specified allele in this sample.

Gene	Genotype	# (%)	Fear	Frustration	Sadness	Discomfort	Negative affect	Action control	Attention control	Inhibition control	Effortful control
HTR1A	CC	7 (26.9)	30	25	30	26	111	27	23	25	76
(rs6295)	CG	14 (53.9)	30	22	27	30	109	34	21	31	86
	GG	5 (19.2)	28	24	29	30	111	33	16	27	76
	C	28 (53.8)	31	24	28	29	112	31	22	29	82
	G	24 (46.2)	30	23	28	30	110	34	20	30	84
HTR1B	GG	12 (46.15)	33	26	30	32	120	31	19	30	80
(rs6296)	GC	12 (46.15)	26	20	25	26	96	34	22	28	84
	CC	2 (7.7)	32	29	35	27	122	28	24	27	79
	G	36 (69.2)	30	23	28	29	109	32	21	29	82
	C	16 (30.8)	27	21	26	26	100	33	23	28	83
HTR1B	GG	1 (3.8)	39	39	30	41	149	20	16	27	63
(rs6297)	GA	4 (15.4)	34	27	32	29	122	32	23	31	86
	AA	21 (80.7)	29	22	27	28	106	32	21	28	82
	G	6 (11.5)	35	30	31	31	127	30	21	30	81
	A	46 (88.5)	29	23	28	28	109	32	21	29	82
HTR2A	TT	1 (3.8)	27	12	18	28	85	30	24	39	93
(rs6311)	TC	16 (61.6)	31	24	29	29	113	31	20	28	79
	CC	9 (34.6)	29	23	27	28	108	34	23	29	86
	T	18 (34.6)	30	23	29	29	111	31	20	29	79
	C	34 (65.4)	30	24	29	29	111	32	21	28	81
SLC6A4	LL	6 (23.1)	33	27	31	28	117	34	20	28	81
(*5-*	LS	16 (61.5)	29	21	27	28	106	32	21	29	82
HTTLPR)	SS	4 (15.4)	29	28	28	33	117	30	23	28	81
	L	28 (53.8)	30	22	28	28	109	32	21	29	82
	S	24 (45.2)	29	22	28	29	108	31	21	29	82
MAO-A	2.5	1 (2.3)	24	23	20	18	85	23	22	16	61
(pVNTR)	3.5	24 (55.9)	29	24	28	30	111	32	20	30	82
	4	1 (2.3)	32	26	35	18	111	23	23	22	64
	4.5	17 (39.5)	31	23	29	30	113	30	30	28	80

Strobel *et al.* (2003) concurs with this finding, stating further that it is also correlated to the personality trait of harm avoidance. Benko *et al.* (2010) found contradictory results to this. They found that the G allele specifically predisposes to impulsivity. Impulsive behaviour is considered to be characteristic of individuals scoring low in Effortful control and it's subconstructs.

Both the *HTR1B* SNPs (rs6296 and rs6297) showed interesting and consistent results. The heterozygous form of the rs6296 SNP seems to negatively influence Negative affect and its subconstructs. Homozygous G individuals are more prone to fear and discomfort, whereas homozygous C individuals appear to be predisposed to frustration and sadness. Only the latter assumption can be supported by previous research done by Mekli *et al.* (2011). They found a correlation between the C allele and stress susceptibility and depression. For the rs6297 SNP, the very rare G allele indicated a predisposition to Negative affect and it's subconstructs of fear, frustration and discomfort. It furthermore appears that the alleles act in an additive way. The G allele contributes to frustration and discomfort, producing a higher score in the homozygous form than the heterozygous form. The C allele relates more with the lower scores. No previous research was found that supports this assumption. Further indicative influence was found between the heterozygous genotype and high Effortful control and its subconstructs Attention and Inhibition control. This finding was supported by previous research on Attention Deficit Hyperactivity Disorder (ADHD) (Smoller *et al.*, 2006) and impulsivity (Grant & Potenza, 2011). Because of the rarity of the G allele, future research should aim to include more individuals with this allele. This study revealed indication that the G allele contributes additively to aggressive tendencies.

The *HTR2A* rs6311 SNP's heterozygous genotype indicated a predisposition to Negative affect and its subconstructs. The homozygous T genotype can be associated with high Effortful control. Research by Tencomnao *et al.* (2010) found that no association can be made between this SNP and depression related phenotypes. Nakamura *et al.* (1999) described an association between the heterozygous genotype and substance abuse and addiction, both related to anxiety disorders. Since the homozygous T genotype is again only present in one individual, a larger sample group is necessary to make significant deductions.

For both VNTR polymorphisms, less reliable results were obtained. The reasons remain the same as discussed previously. It is just worth noting that for *5-HTTLPR* the heterozygous form was found to correlate negatively to Negative affect and frustration. It seems the heterozygous form correlates more to effective coping and lower levels of aggression. Research by Lesch *et al.* (1996) found that the short allele are dominant over the long allele, with similar phenotypes for both SS and LS genotypes. Contrary to this, the results from the current research project found that the heterozygous form differed mostly from both homozygous forms. Again, it is not certain how many of the long alleles actually contain the expression restricting SNP previously discussed.

The *MAO-A* results indicated a positive influence in Attention control. The 3.5R genotype showed lower Attention control scores, whereas the 4.5R showed higher scores. Studies by (Khan & Faraone, 2006; Malmberg *et al.*, 2008) found an association between the short repeat form (3.5R) and ADHD. As Attention control should be low in individuals with ADHD, this may be an interesting result, regardless of previously discussed shortcomings.

Conclusion

With human genetic studies, a comparison between the genotype and phenotype is crucial in understanding how the human body functions based on the genetic composition and environmental influence. Very little research has been done on South African population groups. From the molecular data obtained during this study, it is clear that all described genotypes are present within the South African population, even though the sample studied was very small.

The C allele of rs6295 seems to predispose to aggressive behaviour. The G allele resulting in high Effortful control were confirmed during this study. The contradictive results obtained by Benko *et al.* (2010) can thus be disregarded.

The *HTR1B* gene, and both SNPs investigated in this research project, has proven to have a significant contributing influence on both aggression and temperamental traits. Further research should be done on the correlation between the heterozygous form of the rs6296 SNP and effective coping. This correlates negatively with depression (as related to Negative affect). For future research on the rs6297 SNP, more individuals with G alleles (preferable homozygous for the allele) should be included in the study. It seems that this allele has a definite influence on susceptibility to stress and negative coping. From the results obtained in this study, a possible

dominance of the G allele can be seen. This allele also acts additively, producing extreme phenotypes in the homozygous form. From this can be deduced that both alleles influence traits on the same continuum. A larger sample size might yield more significant results.

Literature (Chapter 2) suggested that the C allele of the rs6311 SNP of *HTR2A* should predispose to poor coping and was supported during this study. The C allele appears to be dominant over the T allele, predisposing individuals to poor coping and susceptibility to Negative affect, depression and addiction (Nakamura *et al.*, 1999). The recessive T allele inhibits aggression in the homozygous form.

Both of the VNTR polymorphisms in this study did not provide conclusive results. For future research on the *5-HTTLPR* VNTR of the *SLC6A4* measures should be taken to identify the SNP reducing the expression of the long allele to that of the short allele. This can be done through sequencing. It has, however, proven to be a troublesome process. An easier method will be to identify a restriction enzyme that will digest the fragments in the presence or absence of the SNP. Similarly, special measures when studying the *MAO-A* pVNTR can include only male participants. This will remove the interpretation problems associated with heterozygosity and X-chromosome inactivation.

Satisfactory results were however obtained for the low expression (3.5R) and high expression (4.5R) alleles of the *MAO-A* pVNTR. Low attention control was found for individuals with the low expression allele. This was also found by other researchers (Khan & Faraone, 2006; Malmberg *et al.*, 2008) to be a predisposing allele to ADHD. This warrants the need for more research on this predisposing effect.

Savitz *et al.* (2006) commented that "…as is the case with most complex traits, success tends to plateau at a point where good candidate genes are identified but conclusive causal inferences remain elusive because of replication failure…"

The results obtained from this project suggest more research to be done on specific variants of the serotonergic genes. It seems as though the questionnaires used in this research project was adequate for quantitative measurements. For future research, possible comparisons between different quantitative test batteries should be implemented in order to gain a more

holistic description of each individual's situation. Also, families should be included in order to ensure relatively stable environmental influences. This is supported by the behavioural genetic point of view that behaviour should be influenced by several genes as well as the environment (Plomin *et al.*, 1977, 1980, 1994; Plomin & Rende, 1991; Plomin & McClearn, 1993).

CHAPTER 6

Summary

Behaviour is the way an organisms responds and interacts with its environment. Human behaviour can be influenced by the environment and hereditary components. The serotonin system modulates many behavioural traits observed in humans. Genes influencing components within this system may thus alter behaviour.

This project focussed on aggression and the influence of the serotonergic system on the brain and behaviour. Both behaviour and individual temperament were quantified. A sample consisting of 188 individuals participated in the quantitative analysis. The basic descriptive statistics corresponded with the standardization populations, indicating an unbiased population. Correlations between the aggression and temperamental constructs scores were calculated. Literature suggested a negative correlation between aggression and Effortful control whereas this study suggested a stronger correlation between anger (aggression) and Negative affect.

Thirty individuals were selected, based on their cumulative high, low and intermediate anger, aggression and Negative affect scores, to participate in the molecular analysis. Three genes coding for serotonergic receptors (*HTR1A*, *HTR1B* and *HTR2A*), one gene coding for a transporter (*SLC6A4*) and one coding for an enzyme (*MAO-A*) were investigated. The receptor genes were genotyped using restriction enzymes to detect specific SNPs. Both the transporter and enzyme coding genes contained VNTRs within the promoter regions.

The rs6295 SNP (-1019C>G) in the *HTR1A* gene occur in the promoter region. Results indicated that the C allele has a predisposing influence on hostility, indirect and total aggression. Heterozygous individuals scored higher in Effortful control and it's subconstructs.

Individuals who were heterozygous for the rs6296 SNP (861G>C) in the *HTR1B* gene, scored consistently higher in hostility and aggression, and low in Negative affect. This supported a possible correlation between aggression and the Negative affect. Another SNP (rs6297 – 1180A>G) occur in the gene region. The very rare G allele was observed and genotyping revealed that this G allele is dominant over the C allele, influencing all forms of aggressive behaviour and contributing to high frustration scores.

In the *HTR2A* gene the rs6311 SNP (-1438G>A) occur in the promoter region and genotyping indicated that the recessive T allele inhibits aggressive tendencies in the homozygous form. The role of the C allele is less clear, though it seems that it predisposes to vulnerability to stress. This was confirmed by the high Negative affect score observed in individuals with a C allele at this position.

For the *MAO-A* pVNTR in the promoter region of the gene (located on the X chromosome), male participants were sequenced. The rare 2.5R was observed in one individual. This individual also scored relatively high in all aspects of aggressive behaviour, as was expected. No other aggressive tendencies were revealed by the other repeats. Significantly lower Effortful control scores were observed in individuals with a 3.5R. Since the 3.5R is indicative of lower expression levels and higher aggression, this can confirm the literary hypothesis of a negative correlation between these constructs.

This study indicates that that all of the receptor gene polymorphisms investigated has a direct or indirect influence on aggressive behaviour and vulnerability to stress. The roles of both VNTR polymorphisms remain unclear.

Keywords: Aggression, Anger, Anxiety, Effortful Control, Monoamine Oxidase, Negative Affect, Serotonin, Temperament

Gedrag is die manier waarop ‘n organisme binne sy omgewing reageer. Menslike gedrag kan deur die omgewing en oorerwing beïnvloed word. Die serotonien stelsel reguleer verskeie menslike gedragspatrone. Gene wat dele van hierdie stelsel beïnvloed kan dus ook gedrag verander.

Die fokus van hierdie projek was aggressie en die invloed van die serotonien stelsel op die brein en gedrag. Individuele temperament en gedrag is gekwantifiseer. ‘n Steekproef van 188 individue het aan die kwantitatiewe analise deelgeneem. Basiese beskrywende statistiek het ooreengestem met die oorspronklike populasie waarop die vraelys gestandaardiseer is. Dit is beduidend van ‘n onbevooroordeelde steekproef. Korrelasies is uitgewerk tussen die aggressie en temperament konstrukte. Literatuur het ‘n negatiewe korrelasie tussen aggressie en Inspannende beheer (“Effortful control”) gevind. Hierdie studie het ‘n groter verband tussen woede (aggressie) en Negatiewe affek (“Negative affect”) gevind.

Dertig individue is op grond van hul kumulatiewe hoog, laag en gemiddelde woede, aggressie en Negatiewe affek tellings gekies om aan die molekulêre analise deel te neem. Drie gene wat vir serotonien reseptore (*HTR1A*, *HTR1B* and *HTR2A*), kodeer; een geen wat vir ‘n transporter (*SLC6A4*) kodeer; en een geen wat vir ‘n ensiem (*MOA-A*) kodeer, is in hierdie studie bestudeer. Genotipering van die reseptor gene was gedoen deur middel van beperkingsensiem snyding wat spesifieke SNPs identifiseer. Beide die gene wat vir die transporter en die ensiem kodeer het Varieërende Aantal Tandem Herhalings (VATHs) binne die promotor areas.

Die rs6295SNP (-1019C>G) kom in die promotor area van die *HTR1A* geen voor. Resultate het getoon dat die C alleel ‘n vatbaarheid tot vyandigheid, indirekte en totale aggressie veroorsaak. Heterosigotiese individue het hoër tellings vir Inspannende beheer en sy subkonstrukte gehad.

Individue wat heterosigoties vir die rs6296 Enkel Nukleotied Polimorfisme (ENP) (861G>C) in die *HTR1B* geen is het steekhoudend hoër tellings in vyandigheid en aggressie, en lae tellings in Negatiewe affek, gehad. Dit ondersteun ‘n moontlike korrelasie tussen aggressie en Negatiewe affek. ‘n Ander ENP (rs6297, -1180A>G) is ook in hierdie area waargeneem. Die seldsame G allele is in die steekproef populasie waargeneem. Genotipering het aangedui dat die G alleel dominant is oor die C alleel. Die G alleel beïnvloed ook alle vorme van aggressiewe gedrag asook hoë frustrasie tellings.

De rs6311 SNP (-1438G>A) kom in die promotor area van die *HTR2A* geen voor. Genotipering dui daarop dat die homosigoties resessiewe T alleel aggressiewe neigings inhibeer. Die rol van die C alleel is steeds onduidelik. Dit lyk egter of die C alleel bydra tot kwesbaarheid vir spanning. Die hoë Negatiewe affek tellings waargeneem by individue wat die C allele bevat, het dit bevestig.

Nukleotied volgorde bepaling van die *MAO-A* VATH (wat op die X chromosoom voorkom) is vir manlike deelnemers gedoen. Die seldsame 2.5R is by een individu waargeneem. Soos verwag, het hierdie individu ook relatief hoë tellings in alle aspekte van aggressie gehad. Geen ander aggressiewe tendense is deur ander allele aangedui nie. Beduidend laer Inspannende beheer tellings is by individue met die 3.5R alleel waargeneem. Die 3.5R alleel lewer laer uitdrukking van hierdie geen, dus ook hoër vlakke van aggressie, en dus kan die literêre hipotese van 'n negatiewe korrelasie tussen hierdie twee konstrukte aanvaar word.

Resultate van hierdie studie dui op 'n direkte of indirekte invloed deur die reseptor geen polimorfismes op aggressiewe gedrag en spannings vatbaarheid. Die effek van beide VATH polimorfismes bly onduidelik.

Sleutelwoorde: Aggressie, Angs, Inspannende beheer, Mono-amien Oksidase, Negatiewe affek, Serotonien, Temperament, Woede

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Appendices

Appendix A (1): The Aggression Questionnaire (AQ), as designed by Buss & Warren (2000), used to measure aggressive behaviour.

The Aggression Questionnaire (AQ)

Scale:
1 = Not at all like me.
2 = A little like me.
3 = Somewhat like me.
4 = Very much like me.
5 = Completely like me.

Questions:

1. My friends say that I argue a lot.

1	2	3	4	5

2. Other people always seem tog et the breaks.

1	2	3	4	5

3. I flare up quickly, but get over it quickly.

1	2	3	4	5

4. I often find myself disagreeing with people.

1	2	3	4	5

5. At times I feel I have gotten a raw deal out of life.

1	2	3	4	5

6. I can't help getting into arguments when people disagree with me.

1	2	3	4	5

7. At times I get very angry for no good reason.

1	2	3	4	5

8. I may hit someone if he or she provokes me.

1	2	3	4	5

9. I wonder why sometimes I feel so bitter about things.

1	2	3	4	5

10. I have threatened people I know.

1	2	3	4	5

11. Someone has pushed me so far that I hit him or her.

1	2	3	4	5

12. I have trouble controlling my temper.

1	2	3	4	5

13. If I'm angry enough, I may mess up someone's work.

1	2	3	4	5

14. I have been angry enough to slam a door when leaving someone behind in the room.

1	2	3	4	5

15. When people are bossy, I take my time doing what they want, just to show them.

1	2	3	4	5

16. I wonder what people want when they are nice to me.

1	2	3	4	5

17. I have become so mad that I have broken things.

1	2	3	4	5

18. I sometimes spread gossip about people I don't like.

1	2	3	4	5

19. I am a calm person.

1	2	3	4	5

20. When people annoy me, I may tell them what I think of them.

1	2	3	4	5

Scale:
1 = Not at all like me.
2 = A little like me.
3 = Somewhat like me.
4 = Very much like me.
5 = Completely like me.

21. I sometimes feel that people are laughing at me behind my back.

1	2	3	4	5

22. I let my anger show when I do not get what I want.

1	2	3	4	5

23. At times I can't control the urge to hit someone.

1	2	3	4	5

24. I get into fights more than most people.

1	2	3	4	5

25. If somebody hits me, I hit back.

1	2	3	4	5

26. I tell my friends openly when I disagree with them.

1	2	3	4	5

27. If I have to resort to violence to protect my rights, I will.

1	2	3	4	5

28. I do not trust strangers who are too friendly.

1	2	3	4	5

29. At times I feel like a bomb ready to explode.

1	2	3	4	5

30. When someone really irritates me, I might give him or her the silent treatment.

1	2	3	4	5

31. I know that "friends" talk about me behind my back.

1	2	3	4	5

32. Some of my friends think I am a hothead.

1	2	3	4	5

33. At times I am so jealous I can't think of anything else.

1	2	3	4	5

34. I like to play practical jokes.

1	2	3	4	5

Appendix A (2): The Adult Temperament Questionnaire (ATQ) short form, as designed by Evans & Rothbart (2007), used to determine temperamental constructs.

The Adult Temperament Questionnaire (ATQ)

1 extremely untrue of you
2 quite untrue of you
3 slightly untrue of you
4 neither true nor false of you
5 slightly true of you
6 quite true of you
7 extremely true of you

1. I become easily frightened.
   1 2 3 4 5 6 7 X
2. I am often late for appointments.
   1 2 3 4 5 6 7 X
3. Sometimes minor events cause me to feel intense happiness.
   1 2 3 4 5 6 7 X
4. I find loud noises to be very irritating.
   1 2 3 4 5 6 7 X
5. It's often hard for me to alternate between two different tasks.
   1 2 3 4 5 6 7 X
6. I rarely become annoyed when I have to wait in a slow moving line.
   1 2 3 4 5 6 7 X
7. I would not enjoy the sensation of listening to loud music with a laser light show.
   1 2 3 4 5 6 7 X
8. I often make plans that I do not follow through with.
   1 2 3 4 5 6 7 X
9. I rarely feel sad after saying goodbye to friends or relatives.
   1 2 3 4 5 6 7 X
10. Barely noticeable visual details rarely catch my attention.
    1 2 3 4 5 6 7 X
11. Even when I feel energized, I can usually sit still without much trouble if it's necessary.
    1 2 3 4 5 6 7 X
12. Looking down at the ground from an extremely high place would make me feel uneasy.
    1 2 3 4 5 6 7 X
13. When I am listening to music, I am usually aware of subtle emotional tones.
    1 2 3 4 5 6 7 X
14. I would not enjoy a job that involves socializing with the public.
    1 2 3 4 5 6 7 X
15. I can keep performing a task even when I would rather not do it.
    1 2 3 4 5 6 7 X
16. I sometimes seem to be unable to feel pleasure from events and activities that I should enjoy.
    1 2 3 4 5 6 7 X
17. I find it very annoying when a store does not stock an item that I wish to buy.
    1 2 3 4 5 6 7 X
18. I tend to notice emotional aspects of paintings and pictures.
    1 2 3 4 5 6 7 X
19. I usually like to talk a lot.
    1 2 3 4 5 6 7 X

1 extremely untrue of you
2 quite untrue of you
3 slightly untrue of you
4 neither true nor false of you
5 slightly true of you
6 quite true of you
7 extremely true of you

20. I seldom become sad when I watch a sad movie.
1 2 3 4 5 6 7 X
21. I'm often aware of the sounds of birds in my vicinity.
1 2 3 4 5 6 7 X
22. When I am enclosed in small places such as an elevator, I feel uneasy.
1 2 3 4 5 6 7 X
23. When listening to music, I usually like to turn up the volume more than other people.
1 2 3 4 5 6 7 X
24. I sometimes seem to understand things intuitively.
1 2 3 4 5 6 7 X
25. Sometimes minor events cause me to feel intense sadness.
1 2 3 4 5 6 7 X
26. It is easy for me to hold back my laughter in a situation when laughter wouldn't be appropriate.
1 2 3 4 5 6 7 X
27. I can make myself work on a difficult task even when I don't feel like trying.
1 2 3 4 5 6 7 X
28. I rarely ever have days where I don't at least experience brief moments of intense happiness.
1 2 3 4 5 6 7 X
29. When I am trying to focus my attention, I am easily distracted.
1 2 3 4 5 6 7 X
30. I would probably enjoy playing a challenging and fast paced video-game that makes lots of noise and has lots of flashing, bright lights.
1 2 3 4 5 6 7 X
31. Whenever I have to sit and wait for something (e.g., a waiting room), I become agitated.
1 2 3 4 5 6 7 X
32. I'm often bothered by light that is too bright.
1 2 3 4 5 6 7 X
33. I rarely notice the color of people's eyes.
1 2 3 4 5 6 7 X
34. I seldom become sad when I hear of an unhappy event.
1 2 3 4 5 6 7 X
35. When interrupted or distracted, I usually can easily shift my attention back to whatever I was doing before.
1 2 3 4 5 6 7 X
36. I find certain scratchy sounds very irritating.
1 2 3 4 5 6 7 X
37. I like conversations that include several people.
1 2 3 4 5 6 7 X
38. I am usually a patient person.
1 2 3 4 5 6 7 X

1 extremely untrue of you
2 quite untrue of you
3 slightly untrue of you
4 neither true nor false of you
5 slightly true of you
6 quite true of you
7 extremely true of you

39. When I am resting with my eyes closed, I sometimes see visual images.
1 2 3 4 5 6 7 X

40. It is very hard for me to focus my attention when I am distressed.
1 2 3 4 5 6 7 X

41. Sometimes my mind is full of a diverse array of loosely connected thoughts and images.
1 2 3 4 5 6 7 X

42. Very bright colors sometimes bother me.
1 2 3 4 5 6 7 X

43. I can easily resist talking out of turn, even when I'm excited and want to express an idea.
1 2 3 4 5 6 7 X

44. I would probably not enjoy a fast, wild carnival ride.
1 2 3 4 5 6 7 X

45. I sometimes feel sad for longer than an hour.
1 2 3 4 5 6 7 X

46. I rarely enjoy socializing with large groups of people.
1 2 3 4 5 6 7 X

47. If I think of something that needs to be done, I usually get right to work on it.
1 2 3 4 5 6 7 X

48. It doesn't take very much to make me feel frustrated or irritated.
1 2 3 4 5 6 7 X

49. It doesn't take much to evoke a happy response in me.
1 2 3 4 5 6 7 X

50. When I am happy and excited about an upcoming event, I have a hard time focusing my attention on tasks that require concentration.
1 2 3 4 5 6 7 X

51. Sometimes, I feel a sense of panic or terror for no apparent reason.
1 2 3 4 5 6 7 X

52. I often notice mild odors and fragrances.
1 2 3 4 5 6 7 X

53. I often have trouble resisting my cravings for food or drink, etc.
1 2 3 4 5 6 7 X

54. Colorful flashing lights bother me.
1 2 3 4 5 6 7 X

55. I usually finish doing things before they are actually due (for example, paying bills, finishing homework, etc.).
1 2 3 4 5 6 7 X

56. I often feel sad.
1 2 3 4 5 6 7 X

57. I am often aware how the color and lighting of a room affects my mood.
1 2 3 4 5 6 7 X

58. I usually remain calm without getting frustrated when things are not going smoothly for me.
1 2 3 4 5 6 7 X

1 extremely untrue of you
2 quite untrue of you
3 slightly untrue of you
4 neither true nor false of you
5 slightly true of you
6 quite true of you
7 extremely true of you

59. Loud music is unpleasant to me.
1 2 3 4 5 6 7 X

60. When I'm excited about something, it's usually hard for me to resist jumping right into it before I've considered the possible consequences.
1 2 3 4 5 6 7 X

61. Loud noises sometimes scare me.
1 2 3 4 5 6 7 X

62. I sometimes dream of vivid, detailed settings that are unlike anything that I have experienced when awake.
1 2 3 4 5 6 7 X

63. When I see an attractive item in a store, it's usually very hard for me to resist buying it.
1 2 3 4 5 6 7 X

64. I would enjoy watching a laser show with lots of bright, colorful flashing lights.
1 2 3 4 5 6 7 X

65. When I hear of an unhappy event, I immediately feel sad.
1 2 3 4 5 6 7 X

66. When I watch a movie, I usually don't notice how the setting is used to convey the mood of the characters.
1 2 3 4 5 6 7 X

67. I usually like to spend my free time with people.
1 2 3 4 5 6 7 X

68. It does not frighten me if I think that I am alone and suddenly discover someone close by.
1 2 3 4 5 6 7 X

69. I am often consciously aware of how the weather seems to affect my mood.
1 2 3 4 5 6 7 X

70. It takes a lot to make me feel truly happy.
1 2 3 4 5 6 7 X

71. I am rarely aware of the texture of things that I hold.
1 2 3 4 5 6 7 X

72. When I am afraid of how a situation might turn out, I usually avoid dealing with it.
1 2 3 4 5 6 7 X

73. I especially enjoy conversations where I am able to say things without thinking first.
1 2 3 4 5 6 7 X

74. Without applying effort, creative ideas sometimes present themselves to me.
1 2 3 4 5 6 7 X

75. When I try something new, I am rarely concerned about the possibility of failing.
1 2 3 4 5 6 7 X

76. It is easy for me to inhibit fun behavior that would be inappropriate.
1 2 3 4 5 6 7 X

77. I would not enjoy the feeling that comes from yelling as loud as I can.
1 2 3 4 5 6 7 X

Appendix B: Correlations calculated between the constructs and subconstructs as measured by the AQ (Buss & Warren, 2000) and ATQ (Evans & Rothbart, 2007). All statistical analyses were conducted using Microsoft® Office Excel 2010 and the Data Analysis Tools Add-In.

a. Calculated correlation values for the entire sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - Entire population																							
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	0.394	1																					
ANG	0.619	0.501	1																				
HOS	0.454	0.349	0.544	1																			
IND	0.593	0.41	0.54	0.594	1																		
TOT	0.81	0.645	0.84	0.778	0.786	1																	
FEAR	-0.021	0.038	0.28	0.286	0.085	0.183	1																
FRU	0.313	0.271	0.506	0.3	0.233	0.428	0.41	1															
SAD	0.103	0.152	0.263	0.26	0.121	0.238	0.426	0.406	1														
DISC	0.044	0.096	0.15	0.163	0.139	0.151	0.339	0.241	0.174	1													
NA	0.15	0.193	0.423	0.357	0.202	0.351	0.788	0.725	0.694	0.62	1												
ACT	-0.074	0.027	-0.152	-0.186	-0.12	-0.139	-0.163	-0.138	-0.131	0.119	-0.113	1											
ATT	-0.151	0.025	-0.198	-0.199	-0.108	-0.178	-0.179	-0.197	-0.22	-0.01	-0.213	0.514	1										
INH	-0.124	-0.194	-0.303	-0.121	-0.22	-0.241	-0.121	-0.259	-0.163	0.072	-0.165	0.438	0.324	1									
EC	-0.14	-0.06	-0.27	-0.211	-0.19	-0.231	-0.193	-0.244	-0.207	0.089	-0.197	0.866	0.724	0.755	1								
SOC	-0.082	0.094	-0.076	-0.132	0.006	-0.067	-0.147	-0.024	0.057	-0.221	-0.123	0.175	0.029	-0.225	0.004	1							
HIP	0.151	0.15	0.126	0.029	0.08	0.135	-0.196	0.06	0.053	-0.541	-0.225	-0.004	-0.118	-0.281	-0.16	0.387	1						
POS	-0.115	-0.07	-0.126	-0.322	-0.09	-0.199	-0.159	-0.102	-0.045	-0.063	-0.133	0.194	0.046	-0.041	0.097	0.421	0.131	1					
EXT	0.006	0.1	-0.009	-0.156	0.015	-0.025	-0.229	-0.013	0.039	-0.418	-0.225	0.144	-0.035	-0.27	-0.052	0.812	0.768	0.609	1				
NPS	0.085	0.071	0.02	-3E-04	0.027	0.051	0.049	0.119	0.092	0.179	0.152	0.258	0.078	0.22	0.251	0.089	0.041	0.156	0.117	1			
APS	0.061	0.243	0.116	0.138	0.133	0.167	0.176	0.115	0.265	0.337	0.312	0.073	-0.034	-2E-04	0.027	0.143	0.037	0.052	0.103	0.494	1		
AS	0.129	0.188	0.087	0.183	0.133	0.183	0.031	0.106	0.247	0.244	0.214	0.108	-0.042	0.04	0.059	0.183	0.145	0.134	0.208	0.444	0.515	1	
OS	0.114	0.208	0.093	0.134	0.122	0.167	0.104	0.14	0.25	0.313	0.28	0.179	-4E-04	0.104	0.136	0.172	0.093	0.14	0.178	0.79	0.826	0.814	1

b. Calculated correlation values for the 19-39 age group sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - 19-39 age group																							
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	0.383	1																					
ANG	0.622	0.493	1																				
HOS	0.429	0.327	0.536	1																			
IND	0.578	0.398	0.538	0.561	1																		
TOT	0.809	0.638	0.845	0.759	0.775	1																	
FEAR	-0.03	0.029	0.249	0.292	0.075	0.169	1																
FRU	0.295	0.257	0.478	0.265	0.202	0.4	0.408	1															
SAD	0.084	0.161	0.238	0.231	0.089	0.213	0.417	0.372	1														
DISC	0.071	0.117	0.141	0.198	0.176	0.179	0.325	0.222	0.151	1													
NA	0.144	0.195	0.394	0.354	0.191	0.34	0.792	0.712	0.681	0.605	1												
ACT	-0.06	0.035	-0.14	-0.14	-0.1	-0.11	-0.13	-0.18	-0.12	0.105	-0.12	1											
ATT	-0.15	0.005	-0.2	-0.19	-0.1	-0.18	-0.13	-0.2	-0.19	0.018	-0.18	0.488	1										
INH	-0.1	-0.18	-0.27	-0.06	-0.19	-0.2	-0.07	-0.23	-0.13	0.056	-0.13	0.425	0.297	1									
EC	-0.12	-0.06	-0.25	-0.16	-0.16	-0.2	-0.14	-0.25	-0.18	0.084	-0.17	0.858	0.707	0.753	1								
SOC	-0.1	0.087	-0.07	-0.16	-0.01	-0.08	-0.15	-0.01	0.074	-0.17	-0.1	0.231	0.04	-0.24	0.027	1							
HIP	0.104	0.115	0.104	-0.07	-0.01	0.064	-0.21	0.051	0.071	-0.52	-0.22	0.073	-0.13	-0.24	-0.11	0.388	1						
POS	-0.1	-0.06	-0.12	-0.32	-0.08	-0.19	-0.18	-0.09	-0.05	-0.05	-0.14	0.202	0.03	-0.05	0.093	0.431	0.179	1					
EXT	-0.03	0.081	-0.02	-0.22	-0.04	-0.07	-0.24	-0.01	0.055	-0.37	-0.21	0.215	-0.04	-0.25	-0.01	0.813	0.767	0.64	1				
NPS	0.087	0.083	0.025	0.008	0.021	0.058	0.07	0.125	0.107	0.182	0.17	0.249	0.059	0.202	0.236	0.111	0.061	0.156	0.137	1			
APS	0.063	0.262	0.122	0.133	0.123	0.171	0.177	0.122	0.278	0.313	0.314	0.098	-0	-0.04	0.033	0.174	0.07	0.09	0.148	0.506	1		
AS	0.107	0.171	0.07	0.147	0.085	0.148	0.014	0.08	0.241	0.268	0.207	0.174	-0	0.065	0.117	0.166	0.128	0.159	0.199	0.487	0.515	1	
OS	0.105	0.211	0.088	0.118	0.094	0.154	0.105	0.133	0.256	0.311	0.282	0.212	0.021	0.093	0.156	0.184	0.106	0.165	0.198	0.809	0.823	0.822	1

c. Calculated correlation values for the 40+ age group sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - 40+ age group																							
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	0.254	1																					
ANG	0.49	0.502	1																				
HOS	0.613	0.339	0.557	1																			
IND	0.564	0.246	0.395	0.697	1																		
TOT	0.723	0.6	0.823	0.877	0.728	1																	
FEAR	0.173	0.18	0.706	0.326	0.262	0.489	1																
FRU	0.547	0.29	0.756	0.378	0.311	0.618	0.454	1															
SAD	0.484	-0.1	0.534	0.536	0.479	0.54	0.524	0.684	1														
DISC	0.071	0.126	0.472	0.317	0.222	0.367	0.521	0.606	0.49	1													
NA	0.386	0.165	0.764	0.472	0.384	0.619	0.772	0.847	0.815	0.814	1												
ACT	-0.03	0.167	-0.14	-0.39	-0.11	-0.19	-0.44	0.214	-0.14	0.112	-0.07	1											
ATT	0.108	0.506	-0.09	-0.09	0.103	0.074	-0.6	-0.09	-0.42	-0.33	-0.44	0.631	1										
INH	-0.23	-0.06	-0.59	-0.29	-0.22	-0.41	-0.66	-0.36	-0.41	0.008	-0.43	0.457	0.452	1									
EC	-0.06	0.227	-0.3	-0.34	-0.1	-0.22	-0.65	-0.03	-0.35	-0.03	-0.32	0.905	0.809	0.732	1								
SOC	-0.25	-0.03	-0.3	-0.31	-0.18	-0.3	-0.12	-0.36	-0.2	-0.58	-0.39	-0.11	0.043	0.066	-0.03	1							
HIP	0.138	0.109	-0	0.082	0.24	0.117	-0.16	-0.3	-0.35	-0.65	-0.45	-0.25	0.258	-0.3	-0.16	0.221	1						
POS	-0.03	-0.02	-0.04	-0.16	0.251	-0.04	0.035	-0.01	0.04	-0.33	-0.09	0.048	0.072	-0.22	-0.02	0.525	0.147	1					
EXT	-0.05	0.04	-0.16	-0.16	0.14	-0.09	-0.13	-0.33	-0.27	-0.74	-0.46	-0.17	0.188	-0.21	-0.11	0.777	0.706	0.688	1				
NPS	0.186	-0.1	-0.02	-0.07	0.238	0.013	-0.21	0.097	-0.08	0.151	-0.01	0.368	0.265	0.448	0.438	-0.17	-0.13	0.146	-0.1	1			
APS	-0.16	-0.09	2E-04	0.125	0.218	0.042	0.16	0.01	0.108	0.635	0.286	-0.05	-0.24	0.435	0.046	-0.23	-0.43	-0.28	-0.45	0.374	1		
AS	-0.23	0.049	0.006	-0.02	0.156	-0.01	0.209	0.071	0.227	0.398	0.278	-0.16	-0.2	0.223	-0.07	0.162	-0.41	0.191	-0.08	0.008	0.556	1	
OS	-0.11	-0.07	-0.01	0.022	0.273	0.024	0.084	0.075	0.121	0.552	0.26	0.055	-0.1	0.496	0.169	-0.11	-0.45	0.002	-0.3	0.596	0.894	0.716	1

d. Calculated correlation values for the entire male sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - Males																							
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	0.365	1																					
ANG	0.634	0.468	1																				
HOS	0.309	0.313	0.481	1																			
IND	0.569	0.433	0.561	0.6	1																		
TOT	0.785	0.622	0.824	0.734	0.823	1																	
FEAR	-0.08	0.135	0.285	0.447	0.17	0.249	1																
FRU	0.315	0.436	0.52	0.336	0.168	0.461	0.415	1															
SAD	0.06	0.115	0.235	0.315	0.183	0.241	0.598	0.201	1														
DISC	0.217	0.27	0.364	0.371	0.215	0.38	0.312	0.322	0.344	1													
NA	0.172	0.329	0.485	0.511	0.255	0.459	0.808	0.667	0.723	0.695	1												
ACT	-0.1	-0.24	-0.15	-0.19	-0.16	-0.21	-0.36	-0.34	-0.29	0.027	-0.33	1											
ATT	-0.14	-0.12	-0.21	-0.35	-0.14	-0.26	-0.39	-0.36	-0.31	-0.29	-0.47	0.412	1										
INH	-0.25	-0.27	-0.22	-0.11	-0.3	-0.29	-0.13	-0.42	-0.19	-0	-0.25	0.542	0.419	1									
EC	-0.2	-0.27	-0.24	-0.25	-0.25	-0.31	-0.35	-0.46	-0.32	-0.08	-0.41	0.861	0.69	0.831	1								
SOC	-0.07	0.086	-0.09	-0.15	0.002	-0.08	-0.32	-0.19	-0.03	-0.24	-0.28	0.131	0.014	-0.16	-0	1							
HIP	-0.05	-0.14	-0.11	-0.17	-0.04	-0.13	-0.3	-0.1	-0.15	-0.5	-0.38	-0.04	-0.11	-0.27	-0.17	0.537	1						
POS	0.004	-0.16	-0.11	-0.34	-0.17	-0.2	-0.41	-0.21	-0.2	-0.09	-0.31	0.15	0.077	-0.13	0.043	0.37	0.186	1					
EXT	-0.05	-0.09	-0.13	-0.26	-0.07	-0.17	-0.43	-0.2	-0.16	-0.41	-0.43	0.078	-0.03	-0.26	-0.08	0.835	0.845	0.567	1				
NPS	-0.09	-0.06	-0.11	0.026	-0.12	-0.08	-0.11	-0.11	0.017	0.058	-0.05	0.331	0.095	0.268	0.311	0.125	0.098	0.162	0.157	1			
APS	0.036	0.248	0.164	0.317	0.204	0.246	0.237	0.068	0.397	0.361	0.365	-0.15	-0.33	-0.07	-0.21	0.133	0.012	-0.1	0.033	0.475	1		
AS	0.022	0.088	-0.12	0.094	0.031	0.032	-0.17	-0.13	0.132	0.29	0.04	0.085	-0.24	0.076	0.004	0.327	0.127	0.123	0.246	0.36	0.469	1	
OS	-0.01	0.109	-0.03	0.178	0.043	0.075	-0.03	-0.08	0.222	0.295	0.139	0.121	-0.19	0.122	0.055	0.251	0.103	0.086	0.19	0.779	0.806	0.782	1

e. Calculated correlation values for the entire female sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - Females																							
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	0.4	1																					
ANG	0.721	0.562	1																				
HOS	0.55	0.373	0.579	1																			
IND	0.606	0.401	0.589	0.599	1																		
TOT	0.844	0.659	0.881	0.799	0.777	1																	
FEAR	0.098	0.04	0.188	0.205	0.076	0.163	1																
FRU	0.362	0.23	0.479	0.277	0.285	0.419	0.369	1															
SAD	0.173	0.19	0.227	0.228	0.107	0.239	0.296	0.472	1														
DISC	-0.01	0.039	0.008	0.042	0.111	0.039	0.301	0.169	0.048	1													
NA	0.232	0.183	0.337	0.28	0.215	0.32	0.739	0.748	0.663	0.553	1												
ACT	-0.08	0.124	-0.13	-0.18	-0.11	-0.11	-0.04	-0.03	-0.04	0.192	0.024	1											
ATT	-0.19	0.069	-0.17	-0.13	-0.1	-0.14	-0.04	-0.11	-0.16	0.159	-0.06	0.553	1										
INH	-0.09	-0.18	-0.31	-0.12	-0.19	-0.22	-0.07	-0.16	-0.12	0.146	-0.08	0.38	0.269	1									
EC	-0.14	0.014	-0.26	-0.19	-0.17	-0.2	-0.06	-0.12	-0.13	0.215	-0.04	0.868	0.735	0.711	1								
SOC	-0.08	0.103	-0.09	-0.13	0.012	-0.06	-0.1	0.046	0.088	-0.23	-0.07	0.203	0.043	-0.25	0.015	1							
HIP	0.261	0.293	0.28	0.157	0.153	0.288	-0.1	0.178	0.199	-0.56	-0.1	0.007	-0.14	-0.31	-0.17	0.305	1						
POS	-0.18	-0.03	-0.14	-0.32	-0.05	-0.2	-0.05	-0.06	0.021	-0.05	-0.05	0.215	0.033	0.002	0.124	0.447	0.101	1					
EXT	0.036	0.194	0.054	-0.09	0.069	0.05	-0.12	0.096	0.156	-0.43	-0.11	0.18	-0.04	-0.29	-0.04	0.804	0.716	0.637	1				
NPS	0.234	0.16	0.031	-0.03	0.138	0.125	0.076	0.231	0.102	0.233	0.235	0.238	0.093	0.221	0.249	0.058	0.018	0.156	0.095	1			
APS	0.111	0.261	0.056	0.04	0.107	0.131	0.087	0.109	0.175	0.299	0.243	0.201	0.124	0.061	0.173	0.14	0.072	0.126	0.152	0.496	1		
AS	0.217	0.249	0.153	0.227	0.205	0.26	0.105	0.218	0.295	0.2	0.298	0.132	0.072	0.035	0.108	0.094	0.17	0.141	0.187	0.496	0.535	1	
OS	0.227	0.275	0.1	0.102	0.184	0.212	0.11	0.225	0.236	0.298	0.316	0.23	0.117	0.123	0.212	0.12	0.109	0.171	0.179	0.794	0.832	0.834	1

f. Calculated correlation values for the male 19-39 age group sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - Males 19-39																							
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	0.343	1																					
ANG	0.617	0.463	1																				
HOS	0.244	0.283	0.442	1																			
IND	0.539	0.425	0.543	0.566	1																		
TOT	0.769	0.619	0.821	0.697	0.811	1																	
FEAR	-0.13	0.131	0.255	0.444	0.152	0.222	1																
FRU	0.275	0.441	0.49	0.269	0.116	0.418	0.365	1															
SAD	0.027	0.102	0.204	0.289	0.171	0.211	0.569	0.129	1														
DISC	0.217	0.286	0.347	0.383	0.234	0.394	0.245	0.265	0.294	1													
NA	0.136	0.343	0.464	0.504	0.243	0.447	0.792	0.626	0.702	0.659	1												
ACT	-0.02	-0.21	-0.1	-0.08	-0.06	-0.11	-0.34	-0.28	-0.26	0.06	-0.29	1											
ATT	-0.06	-0.08	-0.14	-0.26	-0.06	-0.17	-0.32	-0.25	-0.23	-0.23	-0.37	0.321	1										
INH	-0.2	-0.27	-0.17	-0.02	-0.24	-0.23	-0.09	-0.38	-0.16	0.033	-0.21	0.507	0.37	1									
EC	-0.12	-0.25	-0.17	-0.13	-0.16	-0.21	-0.31	-0.4	-0.28	-0.02	-0.36	0.841	0.632	0.829	1								
SOC	-0.07	0.071	-0.05	-0.16	0.011	-0.07	-0.28	-0.15	0.014	-0.16	-0.22	0.168	-0.05	-0.22	-0.03	1							
HIP	-0.12	-0.18	-0.14	-0.28	-0.15	-0.24	-0.31	-0.13	-0.15	-0.49	-0.39	0.063	-0.1	-0.23	-0.1	0.554	1						
POS	0.068	-0.12	-0.07	-0.29	-0.13	-0.14	-0.39	-0.14	-0.15	-0.04	-0.27	0.085	-0.03	-0.19	-0.05	0.408	0.214	1					
EXT	-0.07	-0.11	-0.12	-0.31	-0.12	-0.2	-0.41	-0.18	-0.12	-0.35	-0.39	0.131	-0.08	-0.28	-0.08	0.846	0.844	0.597	1				
NPS	-0.07	-0.03	-0.1	0.072	-0.12	-0.06	-0.1	-0.1	0.047	0.067	-0.03	0.322	0.056	0.283	0.311	0.194	0.113	0.12	0.18	1			
APS	-0.02	0.218	0.126	0.28	0.153	0.19	0.216	0.008	0.391	0.365	0.352	-0.1	-0.29	-0.02	-0.15	0.173	-0.03	-0.03	0.046	0.527	1		
AS	-0.03	0.035	-0.17	0.026	-0.03	-0.05	-0.23	-0.2	0.105	0.306	-0.01	0.209	-0.18	0.126	0.112	0.34	0.101	0.219	0.265	0.445	0.449	1	
OS	-0.05	0.087	-0.07	0.15	-0.01	0.029	-0.06	-0.12	0.216	0.3	0.118	0.19	-0.16	0.166	0.125	0.295	0.081	0.132	0.208	0.822	0.803	0.791	1

g. Calculated correlation values for the female 19-39 age group sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - Females 19-39																						
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	0.393	1																					
ANG	0.73	0.551	1																				
HOS	0.54	0.351	0.584	1																			
IND	0.597	0.385	0.597	0.567	1																		
TOT	0.847	0.648	0.889	0.787	0.766	1																	
FEAR	0.104	0.025	0.164	0.223	0.076	0.16	1																
FRU	0.355	0.205	0.454	0.26	0.268	0.399	0.396	1															
SAD	0.16	0.208	0.207	0.201	0.068	0.219	0.3	0.457	1														
DISC	0.028	0.058	0.016	0.095	0.161	0.081	0.331	0.176	0.045	1													
NA	0.238	0.179	0.31	0.287	0.209	0.315	0.761	0.747	0.654	0.56	1												
ACT	-0.08	0.132	-0.16	-0.16	-0.12	-0.11	-0.02	-0.13	-0.06	0.136	-0.03	1											
ATT	-0.21	0.031	-0.21	-0.16	-0.13	-0.18	-0.03	-0.17	-0.16	0.146	-0.08	0.557	1										
INH	-0.07	-0.16	-0.29	-0.08	-0.17	-0.19	-0.01	-0.13	-0.09	0.093	-0.05	0.384	0.261	1									
EC	-0.14	0.009	-0.27	-0.17	-0.18	-0.2	-0.03	-0.18	-0.12	0.159	-0.06	0.867	0.738	0.712	1								
SOC	-0.11	0.1	-0.1	-0.16	-0.02	-0.09	-0.13	0.049	0.089	-0.19	-0.07	0.264	0.082	-0.24	0.06	1							
HIP	0.223	0.262	0.263	0.062	0.08	0.227	-0.13	0.177	0.225	-0.53	-0.09	0.077	-0.16	-0.26	-0.13	0.302	1						
POS	-0.18	-0.03	-0.15	-0.33	-0.06	-0.21	-0.1	-0.08	-0.01	-0.06	-0.09	0.258	0.058	0.029	0.165	0.441	0.165	1					
EXT	-0	0.171	0.027	-0.17	0.011	-0	-0.16	0.085	0.156	-0.39	-0.11	0.262	-0.02	-0.24	0.025	0.801	0.717	0.667	1				
NPS	0.236	0.166	0.03	-0.04	0.135	0.125	0.102	0.228	0.104	0.233	0.242	0.219	0.079	0.182	0.217	0.049	0.046	0.178	0.113	1			
APS	0.136	0.297	0.094	0.056	0.118	0.165	0.122	0.159	0.206	0.269	0.273	0.2	0.127	-0.03	0.134	0.168	0.148	0.148	0.211	0.485	1		
AS	0.209	0.247	0.157	0.211	0.166	0.248	0.123	0.22	0.306	0.236	0.32	0.158	0.089	0.038	0.127	0.063	0.158	0.126	0.157	0.515	0.552	1	
OS	0.233	0.29	0.116	0.096	0.17	0.219	0.141	0.245	0.253	0.299	0.339	0.232	0.12	0.073	0.191	0.115	0.145	0.181	0.197	0.796	0.832	0.842	1

h. Calculated correlation values for the males of the 40+ age group sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

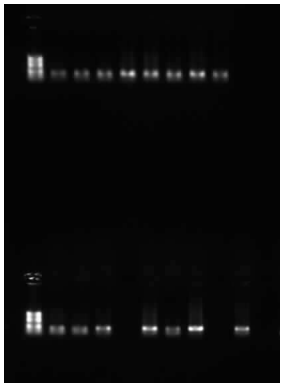
Correlations - Males 40+																							
	PHY	*VER*	*ANG*	*HOS*	*IND*	*TOT*	*FEAR*	*FRU*	*SAD*	*DISC*	*NA*	*ACT*	*ATT*	*INH*	*EC*	*SOC*	*HIP*	*POS*	*EXT*	*NPS*	*APS*	*AS*	*OS*
PHY	1																						
VER	-0.05	1																					
ANG	0.865	-0.14	1																				
HOS	0.788	-0.14	0.862	1																			
IND	0.508	-0.7	0.439	0.197	1																		
TOT	0.952	-0.08	0.965	0.902	0.438	1																	
FEAR	0.52	-0.11	0.725	0.513	0.181	0.595	1																
FRU	0.788	-0.09	0.83	0.77	0.227	0.812	0.891	1															
SAD	0.543	0.065	0.668	0.605	-0.06	0.599	0.936	0.935	1														
DISC	0.628	0.125	0.831	0.626	0.091	0.727	0.943	0.886	0.912	1													
NA	0.642	0.003	0.798	0.647	0.122	0.711	0.975	0.954	0.971	0.973	1												
ACT	0.024	0.587	-0.15	-0.14	-0.14	-0.02	-0.64	-0.49	-0.59	-0.36	-0.52	1											
ATT	-0.55	0.143	-0.61	-0.52	-0.14	-0.55	-0.93	-0.93	-0.96	-0.83	-0.93	0.718	1										
INH	-0.72	0.706	-0.76	-0.7	-0.76	-0.75	-0.56	-0.7	-0.46	-0.47	-0.56	0.484	0.584	1									
EC	-0.49	0.515	-0.59	-0.53	-0.37	-0.51	-0.85	-0.84	-0.81	-0.67	-0.81	0.859	0.921	0.78	1								
SOC	-0.77	0.211	-0.92	-0.6	-0.62	-0.83	-0.77	-0.74	-0.6	-0.82	-0.77	0.205	0.601	0.73	0.597	1							
HIP	-0.28	-0.59	-0.53	-0.44	0.451	-0.44	-0.62	-0.54	-0.69	-0.8	-0.69	0	0.461	-0.08	0.189	0.422	1						
POS	-0.38	-0.76	-0.38	-0.31	0.47	-0.38	-0.55	-0.56	-0.7	-0.71	-0.65	-0.07	0.524	-0.17	0.16	0.297	0.87	1					
EXT	-0.58	-0.38	-0.79	-0.58	0.055	-0.7	-0.8	-0.75	-0.79	-0.95	-0.86	0.08	0.631	0.26	0.409	0.748	0.911	0.809	1				
NPS	0.305	-0.61	0.335	0.041	0.927	0.292	0.01	-0.04	-0.28	-0.03	-0.07	0.064	0.142	-0.56	-0.1	-0.55	0.423	0.554	0.09	1			
APS	0.522	0.388	0.332	-0.04	0.294	0.357	0.382	0.368	0.309	0.455	0.401	0.202	-0.34	-0.07	-0.11	-0.53	-0.27	-0.55	-0.5	0.214	1		
AS	-0.51	0.503	-0.31	-0.53	-0.56	-0.47	0.262	-0.1	0.224	0.229	0.164	-0.17	-0.13	0.608	0.079	0.139	-0.48	-0.48	-0.29	-0.45	0.231	1	
OS	0.183	0.094	0.217	-0.3	0.447	0.11	0.361	0.112	0.105	0.355	0.26	0.053	-0.16	-0.05	-0.07	-0.56	-0.14	-0.2	-0.37	0.515	0.805	0.401	1

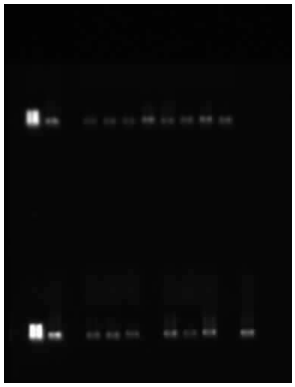
i. Calculated correlation values for the females of the 40+ age group sample population. Red indicates slight correlation. Yellow indicates moderate correlation. Green indicates strong correlation.

Correlations - Females 40+																						
	PHY	VER	ANG	HOS	IND	TOT	FEAR	FRU	SAD	DISC	NA	ACT	ATT	INH	EC	SOC	HIP	POS	EXT	NPS	APS	AS	OS
PHY	1																						
VER	0.311	1																					
ANG	0.535	0.688	1																				
HOS	0.634	0.443	0.463	1																			
IND	0.604	0.42	0.369	0.79	1																		
TOT	0.743	0.731	0.8	0.864	0.779	1																	
FEAR	0.15	0.394	0.638	0.159	0.256	0.423	1																
FRU	0.525	0.402	0.737	0.247	0.307	0.557	0.184	1															
SAD	0.523	-0.1	0.45	0.485	0.6	0.496	0.279	0.584	1														
DISC	-0.16	0.231	0.213	0.11	0.246	0.18	-0.1	0.439	0.203	1													
NA	0.422	0.343	0.768	0.374	0.518	0.625	0.479	0.857	0.781	0.555	1												
ACT	-0.09	0.108	0.08	-0.35	-0.04	-0.1	-0.22	0.502	0.045	0.659	0.377	1											
ATT	0.343	0.65	0.324	0.188	0.298	0.422	-0.22	0.394	-0.13	0.32	0.158	0.56	1										
INH	-0.17	-0.18	-0.52	-0.17	-0.08	-0.32	-0.71	-0.24	-0.35	0.405	-0.34	0.388	0.354	1									
EC	-0.02	0.172	-0.06	-0.22	0.023	-0.07	-0.46	0.317	-0.14	0.633	0.142	0.896	0.739	0.704	1								
SOC	0.056	-0.12	-0.13	-0.23	0.04	-0.14	0.451	-0.16	0.014	-0.42	-0.05	-0.27	-0.43	-0.21	-0.36	1							
HIP	0.403	0.384	0.315	0.393	0.19	0.431	0.372	-0.15	-0.15	-0.52	-0.16	-0.53	0.04	-0.53	-0.5	-0.04	1						
POS	0.039	0.072	0.151	-0.08	0.249	0.085	0.489	0.204	0.309	-0.07	0.342	-0.06	-0.22	-0.32	-0.21	0.725	-0.26	1					
EXT	0.276	0.194	0.191	0.073	0.253	0.218	0.677	-0.06	0.08	-0.54	0.054	-0.46	-0.3	-0.56	-0.57	0.831	0.42	0.708	1				
NPS	0.124	-0.01	-0.02	-0.06	0.043	-0	-0.24	0.214	0.053	0.464	0.183	0.41	0.257	0.703	0.579	0.097	-0.61	-0.03	-0.32	1			
APS	-0.31	-0.13	-0.39	0.006	0.15	-0.18	-0.38	-0.27	-0.12	0.616	-0.09	0.223	0.09	0.82	0.466	-0.13	-0.57	-0.12	-0.45	0.657	1		
AS	-0.13	-0	-0.1	-0.01	0.308	-0.01	-0.03	0.03	0.143	0.379	0.179	-0.02	-0.07	0.271	0.073	0.221	-0.38	0.473	0.128	0.258	0.565	1	
OS	-0.14	-0.06	-0.22	-0.02	0.209	-0.08	-0.27	-0.03	0.026	0.6	0.102	0.244	0.105	0.733	0.45	0.07	-0.64	0.131	-0.26	0.766	0.917	0.756	1

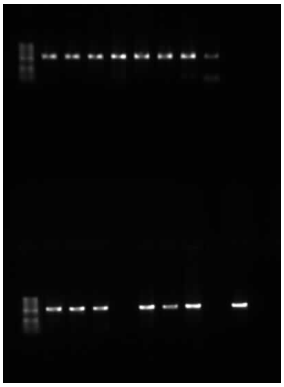
Appendix C: Agarose Gel Electrophoresis (1%) of each amplified gene region.

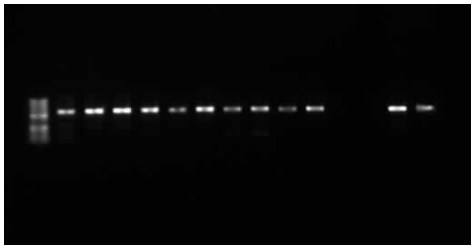
Amplified ***HTR1A*** promoter region, of approximately 163 bp. In each first lane is the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific).



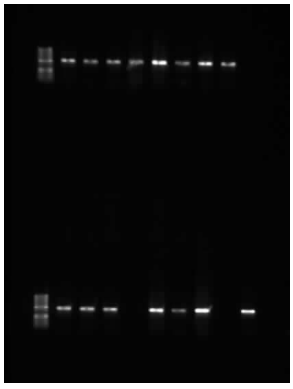


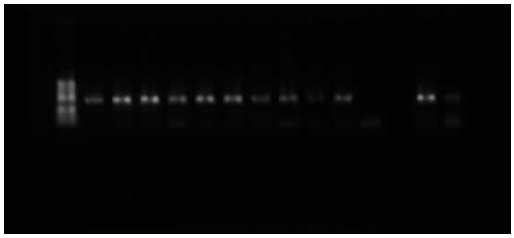
Amplified ***HTR1B*** gene region, of approximately 548 bp. In each first lane is the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific).





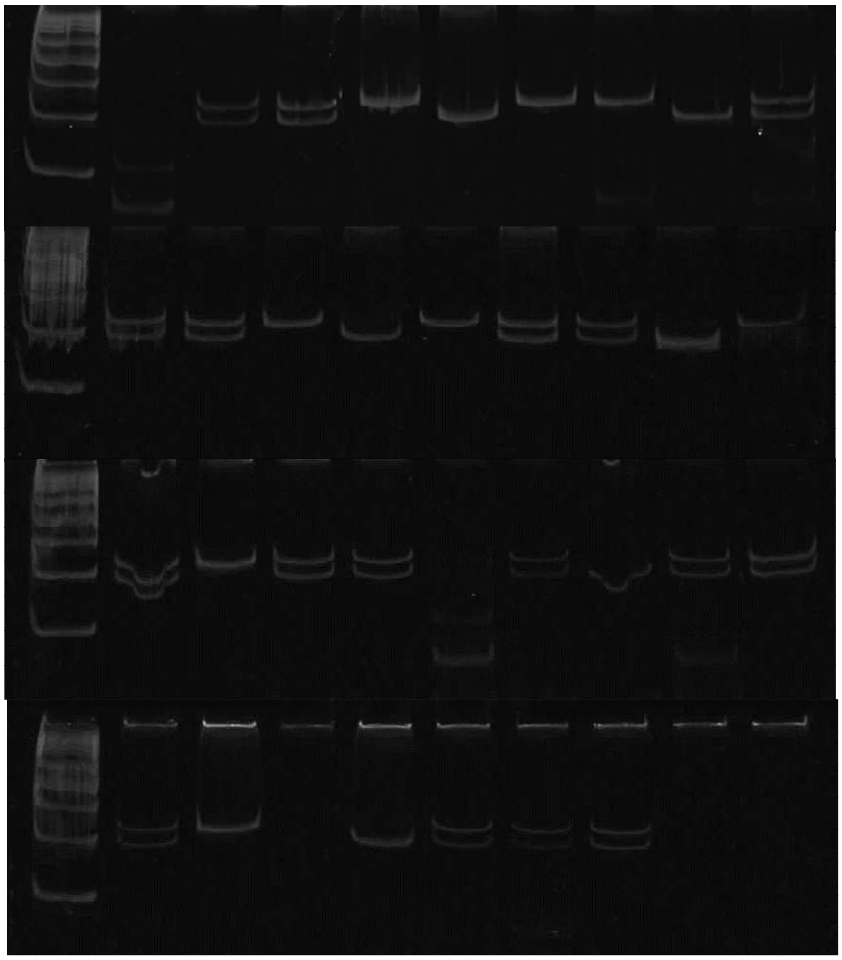
Amplified ***HTR2A*** promoter region, of approximately 468 bp. In each first lane is the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific).



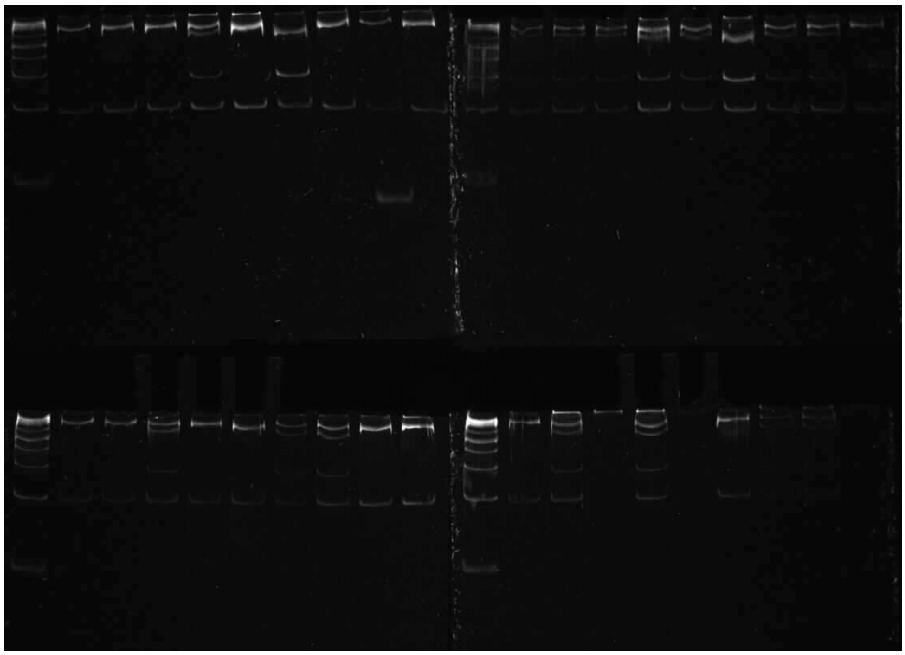


Appendix D: Polyacrylamide Gel Electrophoresis (PAGE) of each digested amplified gene region.

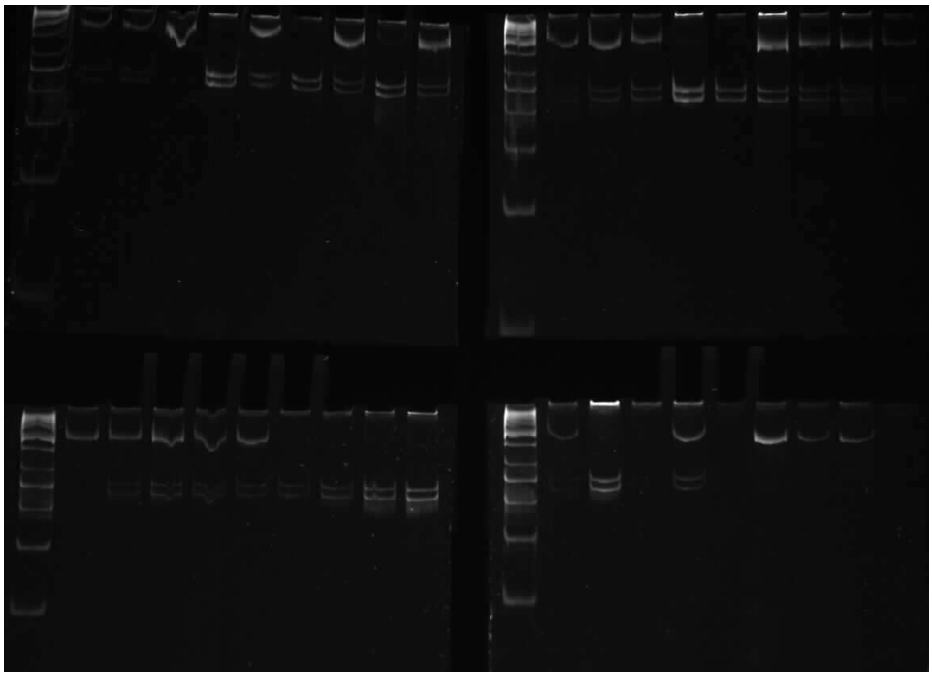
12% PAGE gel of the digested ***HTR1A*** gene region, with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane. Possible fragment lengths are 163 bp (indicating the C allele) and 146 bp and 17 bp (indicating the G allele).



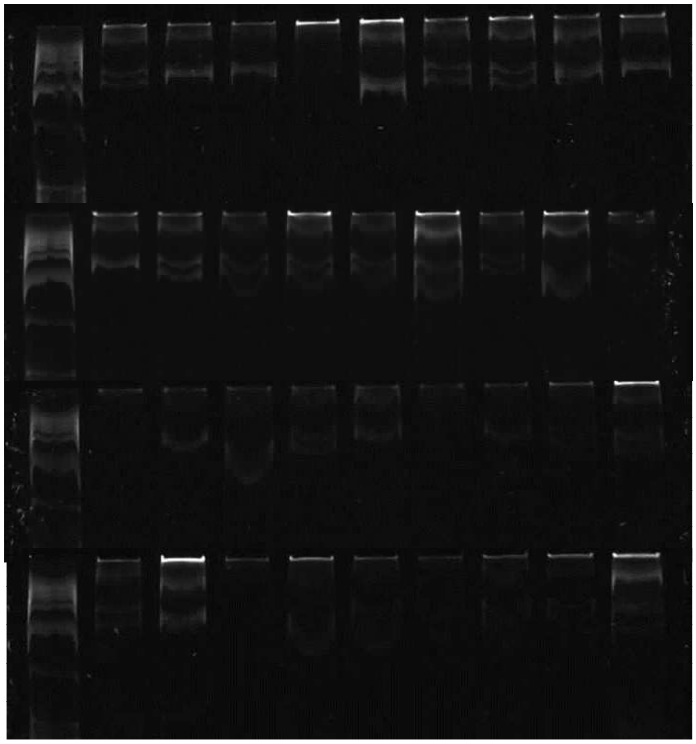
8% PAGE gel of the digested ***HTR1B*** promoter region, with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane. Possible fragment lengths are 452 bp and 96 bp (indicating the G allele) and 142 bp, 310 bp and 17 bp (indicating the C allele).



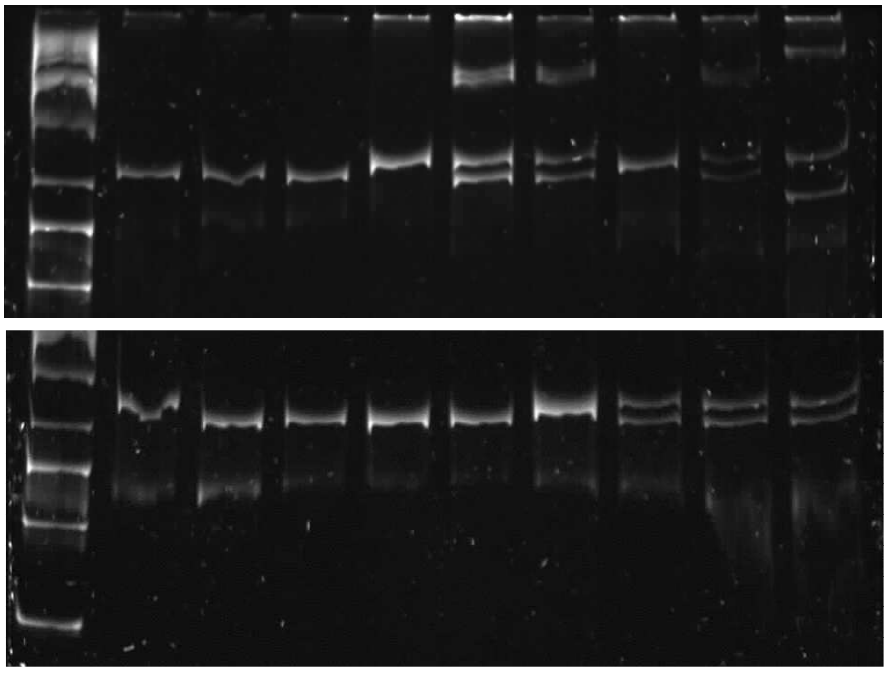
8% PAGE gel of the digested ***HTR2A*** promoter region, with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane. Possible fragment lengths are 468 bp (indicating the T allele) and 224 bp and 244 bp (indicating the C allele).

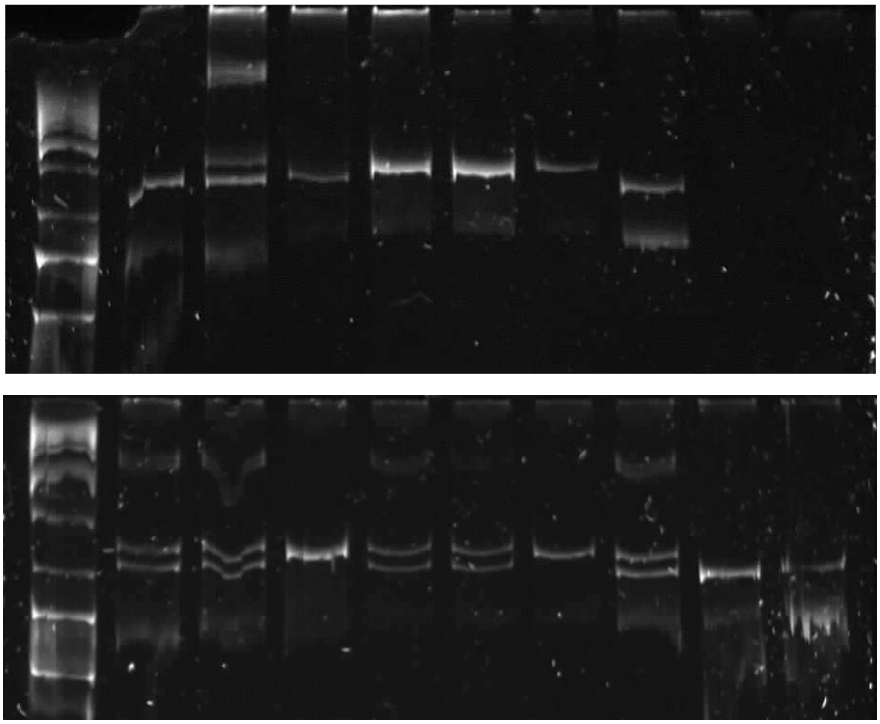


8% PAGE gel of the ***SLC6A4*** promoter region, with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane. Possible fragment lengths are 480 bp (indicating the short allele) and 520 bp (indicating the long allele).



8% PAGE gel of the digested ***MAO-A*** promoter region, with the 50 bp O'GeneRuler DNA ladder (Fermentas, Thermo Scientific) in the first lane





Appendix F (1): Sequences obtained for all the participants, for the amplified *HTR1A* promoter region.

>htr1a_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGAAGACACACTCGGTCTTCTTCC
>CG__2_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CC__6_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAACGATGACACACTCGGTCTTCTTCC
>CC__7_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAACGATGACACACTCGGTCTTCTTCC
>GG__8_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAAGGATGACACACTCGGTCTTCTTCC
>CG__9_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CG_10_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CC_12_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAACGATGACACACTCGGTCTTCTTCC
>CC_14_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAACGATGACACACTCGGTCTTCTTCC
>CG_16_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>GG_17_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAAGGATGACACACTCGGTCTTCTTCC
>CG_19_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CG_22_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CG_24_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>GG_25_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAAGGATGACACACTCGGTCTTCTTCC
>CG_27_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CG_28_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CC_29_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAACGATGACACACTCGGTCTTCTTCC
>GG_31_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAAGGATGACACACTCGGTCTTCTTCC
>CG__E_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CG__H_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CG__M_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC
>CG__J_AGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGCAATGGCGCGAGAACGGAGGTAGCTTTTTAAAAASGATGACACACTCGGTCTTCTTCC

Appendix F (2): Sequences obtained for all the participants, for the amplified *HTR1B* gene region.

>HTR1B____ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_1_AA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_2_GA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_3_GA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_4_GA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_5_GG__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>CC_6_AA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTCAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_7_AA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_8_AA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_9_AA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_10_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_11_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_12_GA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_13_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_14_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>CC_15_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTCAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_16_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_17_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_18_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_19_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_20_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_21_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_22_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_23_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_24_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_25_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_26_GA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_27_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_28_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_29_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GC_31_AA_ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTSAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT
>GG_E_AA__ACCTCTATTAACTCGCGGGTTCCCGACGTGCCCAGCGAATCCGGATCTCCTGTGTATGTGAACCAAGTCAAAGTGCGAGTCTCCGACGCCCTGCT

>HTR1B____GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GG_1_AA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GG_2_GA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GG_3_GA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GC_4_GA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GG_5_GG__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>CC_6_AA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GG_7_AA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GG_8_AA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GG_9_AA__GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GC_10_AA_GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GC_11_AA_GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
>GC_12_GA_GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
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>GC_14_AA_GGAAAAGAAGAAACTCATGGCCGCTAGGGAGCGCAAAGCCACCAAGACCCTAGGGATCATTTTGGGAGCCTTTATTGTGTGTTGGCTACCCTTCT
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>GG_2_GA__CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCRT
>GG_3_GA__CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCRT
>GC_4_GA__CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCRT
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>CC_15_AA_CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCAT
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>GG_23_AA_CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCAT
>GC_24_AA_CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCAT
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>GG_26_GA_CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCRT
>GG_27_AA_CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCAT
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>GC_31_AA_CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCAT
>GG_E_AA__CTCATCAACCCCATAATCTATACCATGTCCAATGAGGACTTTAAACAAGCATTCCATAAACTGATACGTTTTAAGTGCACAAGTTGACTTGCCAT

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>GG_7_AA__TTGCAGTGGGGTCGC
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Appendix F (3): Sequences obtained for all the participants, for the amplified *HTR2A* promoter region.

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>CT_2__TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CC_4__TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_5__TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CC_6__TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_7__TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CC_8__TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_9__TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_10_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_11_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_12_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CC_13_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CC_14_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_15_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_17_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_18_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>TT_19_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_21_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_22_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_23_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
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>CC_27_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CT_28_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
>CC_29_TGAGAGAAACTGGAGAAATGGCCTTTTGTGCAGATTCCCATTAAGGTAGGTAAGTGGCACTGTGGTAATTTTTTAGGCTGAAGGGTGAAGAGAGAACA
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>CT_5__CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CC_6__CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CT_7__CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CC_8__CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CT_9__CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CT_10_CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CT_11_CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CT_12_CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
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>CT_17_CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
>CT_18_CATTGGCCACATAGGCTCAGGGTGGCTAGGCACGTCTGTGGTGATAACTCTGATAAACTATTAGCACTATTTTTATTTAATAGATACACCA
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