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Neurobiological Correlates of Personality Traits: A Study on Harm Avoidance and Neuroticism

by

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To my wife and best friend, Laura

ABSTRACT

Lauri Tuominen

Neurobiological Correlates of Personality Traits: A Study on Harm Avoidance and Neuroticism

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Harm Avoidance and Neuroticism are traits that predispose to mental illnesses. Studying them provides a unique way to study predisposition of mental illnesses. Understanding the biological mechanisms that mediate vulnerability could lead to improvement in treatment and ultimately to pre-emptive psychiatry. These personality traits describe a tendency to feel negative emotions such as fear, shyness and worry. Previous studies suggest these traits are regulated by serotonin and opiate pathways.

The aim of this thesis was to test the following hypotheses using personality trait measures and positron emission tomography (PET): 1) Brain serotonin transporter density in vivo is associated with Harm Avoidance and Neuroticism traits. 2) μ–opioid receptor binding is associated with Harm Avoidance. In addition, we developed a methodology for studying neurotransmitter interactions in the brain using the opiate and serotonin pathways.

32 healthy subjects who were consistently in either the highest or lowest quartile of the Harm Avoidance trait were recruited from a population-based cohort. Each subject underwent two PET scans, serotonin transporter binding was measured with [^{11}C] MADAM and μ-opioid receptor binding with [^{11}C]carfentanil.

We found that the serotonin transporter is not associated with anxious personality traits. However, Harm Avoidance positively correlated with μ-opioid receptor availability. Particularly the tendency to feel shy and the inability to cope with stress were associated μ-opioid receptor availability. We also demonstrated that serotonin transporter binding correlates with μ-opioid receptor binding, suggesting interplay between the two systems. These findings shed light on the neurobiological correlates of personality and have an impact on etiological considerations of affective disorders.

Key words: Harm Avoidance, Neuroticism, serotonin transporter, μ-opioid receptor, positron emission tomography

TIIVISTELMÄ

Lauri Tuominen

Persoonallisuuden neurobiologiset taustatekijät

Valtakunnallinen PET-keskus ja psykiatrian oppiaine, Turun Yliopisto

Turvallisuushakuisuus ja neuroottisuus ovat persoonallisuuden piirteitä, joihin liittyy ahdistustaipumus ja joiden on osoitettu altistavan mielenterveyshäiriöille. Tutkimalla näitä persoonallisuuspiirteitä on mahdollisuus saada ainutlaatuista tietoa myös alttiudesta sairastua mielenterveyshäiriöön. Tällaista tietoa voitaisiin käyttää hyväksi psykiatrisen hoidon ja sairauksien ennaltaehkäisyn kehittämiseen. Turvallisuushakuisuus ja neuroottisuus kuvaavat taipumusta kokea negatiivisia tunteita kuten pelkoa, ujoutta ja huolta. Aikaisempien tutkimusten perusteella aivojen serotoniini ja opioidijärjestelmien ajatellaan olevan yhteydessä näihin persoonallisuuden piirteisiin.

Tässä väitöskirjatyössä käytettiin positroniemissiotomografia (PET) –tekniikkaa aivojen välittäjäainejärjestelmien toiminnan mittaamiseen ja persoonallisuuskyselyjä (TCI, NEO) persoonallisuuspiirteiden määrittelyyn. Tutkimuksessa testattiin seuraavia hypoteeseja: serotoniinin takaisinottajaproteiini on yhteydessä turvallisuushakuisuuteen, serotoniinin takaisinottajaproteiini on yhteydessä neuroottisuuteen ja μ-opioidireseptori on yhteydessä turvallisuushakuisuuteen. Lisäksi tutkimuksessa kehitettiin menetelmä välittäjäaineverkoston tutkimiseen PET menetelmällä.

Tutkimukseen värvättiin laajasta väestöpohjaisesta kohorttitutkimuksesta 32 tervettä koehenkilöä, jotka olivat toistettujen mittausten perusteella turvallisuushakuisuuden suhteen joko ylimmässä tai alimmassa kvartiilissa. Kaikille koehenkilöille tehtiin kaksi PET-kuvausta saman päivän aikana. Ensimmäisessä kuvauksessa käytettiin [^{11}C]MADAM–merkkiainetta mittaamaan serotoniinin takaisinottajaproteiinisitoutumista. Toisessa kuvauksessa käytettiin [^{11}C]karfentaniili–merkkiainetta mittaamaan μ-opioidireseptorisitoutumista.

Tämän tutkimuksen perusteella serotoniinin takaisinottajaproteiini ei ollut yhteydessä turvallisuushakuisuuteen eikä neuroottisuuteen. Tutkimuksessa havaittiin kuitenkin positiivinen korrelaatio turvallisuushakuisuuden ja μ-opioidireseptorin välillä. Erityisesti ujous ja taipumus tuntea itsensä turvattomaksi vieraiden ihmisten seurassa sekä kyky selvitä stressistä olivat yhteydessä μ-opioidireseptoriin. Lisäksi serotoniinin takaisinottajaproteiinin havaittiin olevan yhteydessä μ-opioidireseptoriin tietyillä aivoalueilla, jotka liittyvät mieliala- ja ahdistuneisuushäiriöihin. Näitä löydöksiä voidaan tulevaisuudessa hyödyntää mielenterveyshäiriöiden etiologisessa ja mahdollisesti ennaltaehkäisevässä tutkimuksessa.

Avainsanat: turvallisuushakuisuus, neuroottisuus, serotoniinin takaisinottajaproteiini, μ-opioidireseptori, positroniemissiotomografia

TABLE OF CONTENTS

ABBREVIATIONS

5-HT	5-hydroxytryptamine
5-HTTLPR	5-hydroxytryptamine transporter linked polymorphic region
5-HIAA	5-hydroxyindoleacetic acid
ANOVA	Analysis of variance
BP	Binding potential
BP_{ND}	Non-displacable binding potential
DSM-IV	Diagnostic and statistical manual, fourth edition
ECAT	Emission computer aided tomography
EPQ	Eysenck personality questionnaire
FDR	False discovery rate
FOV	Field of view
FWE	Family-wise error
GABA	Gamma aminobutric acid
HRRT	High resolution research tomograph
KSP	Karolinska scales of personality
LogP	partition coefficient
LOR	Line of response
LSO	lutetium oxyorthosilicate
LYSO	Lutetium yttrium orthosilicate
MADAM	N,N-Dimethyl-2-(2-amino-4 methylphenylthio)benzylamine
MDMA	3,4-methylenedioxy-N-methylamphetamine
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
NEO-FFI	Neuroticism extraversion openness five-factor inventory
NEO-PI	Neuroticism extraversion openness personality inventory
ORPM1	Opioid receptor mu 1
OSEM	Ordinary Poisson-ordered subset expectation maximization
PET	Positron emission tomography
ROI	Region of interest
rTMS	repetitive transcranial magnetic stimulation
SCID-I	Structured clinical interview for DSM-IV axis I disorders
SPECT	Single photon emission computed tomography
SPM	Statistical parametric mapping
SRTM	Simplified reference tissue model
TCI	Temperament and character inventory
TPQ	Tridimensional personality questionnaire

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to by the Roman numerals **I-IV**:

I. Tuominen L, Salo J, Hirvonen J, Någren K, Laine P, Melartin T, Isometsä E, Viikari J, Cloninger CR, Raitakari O, Hietala J, Keltikangas-Järvinen L (2012) Temperament, character and serotonin activity in the human brain: a positron emission tomography study based on a general population cohort. Psychol Med 43:881-894.

II. Tuominen L, Salo J, Hirvonen J, Någren K, Laine P, Melartin T, Isometsä E, Viikari J, Raitakari O, Keltikangas-Järvinen L, Hietala J (2012) Temperament trait Harm Avoidance associates with μ-opioid receptor availability in frontal cortex: a PET study using [(11)C]carfentanil. Neuroimage 61:670-676.

III. Tuominen L, Nummenmaa L, Keltikangas-Järvinen L, Raitakari O, Hietala J. (2013) Mapping Neurotransmitter Networks with PET: an Example on Serotonin and Opioid Systems. Hum Brain Mapp. [epub ahead of print]

IV. Tuominen L, Jokela M, Hirvonen J, Helin S, Laine P, Melartin T, Isometsä E, Viikari J, Pulkki-Råback L, Viikari J, Raitakari O, Hietala J, Keltikangas-Järvinen L, Neuroticism and Brain Serotonin Transporter: a PET Study with [(11)C] MADAM. Submitted manuscript

1. INTRODUCTION

Personality refers to the characteristic ways how individuals feel and think and explains why people may behave very differently in similar situations. For instance, even healthy people vary vastly in the way they typically respond to threats; some remain calm even in the most stressful situations whereas others may feel insecure and alarmed already in everyday life situations. Diversity in personality and behavior ensures the survival of the species in diverse and changing environments. It is advantageous to be easily alarmed when the threats are real. Yet, excessive worrying and anxiousness may be harmful and progress into psychiatric disorders and functional impairment. In fact, anxious personality is one of the established risk factors for affective disorders such as major depression.

Various models for personality are numerous in the history of psychiatry and psychology including the psychodynamic theories of personality structure. Academic research on human personality traits began in the 1930s and since then, various theories and definitions have been proposed. One prominent research tradition has emphasized traits as the basic constituents of personality. All major trait models of personality describe an anxious personality trait, which can be understood as the tendency to feel easily negative emotions such as fear, shyness, insecurity, worry, and distress. The exact definition and measure of the anxious trait, however, differs from model to model.

Linking personality traits with neurobiology has been one of the long-standing goals of personality research (Eysenck, 2006). Finding the neurobiological correlates of anxious personality traits is also of paramount clinical interest, as it would increase understanding of the mechanisms that mediate vulnerability to affective disorders. Such knowledge could hopefully be used in the future to improve the treatment and pre-emptive care of these mental illnesses. Based on animal and human studies brain serotonin and opioid systems have been hypothesized to be important biological correlates of anxious personality traits.

The studies presented in this thesis seek to test hypotheses on the relationship between anxious personality traits and the function of serotonin as well as opiate pathways in vivo.

2. REVIEW OF THE LITERATURE

2.1. Personality

2.1.1. Definition of Personality

Personality, temperament and character can be defined, measured, and studied in various ways. Gordon Allport (1897-1967) was one of the founders of the academic study of personality. He defined personality (1961) as "the dynamic organization within the individual of those psychophysical systems that determine his characteristic behavior and thought". Allport also introduced the concept of trait as the fundamental unit of personality. He defined traits as "generalized and personalized determining tendencies—consistent and stable modes of an individual's adjustment to his environment" (Allport and Odbert, 1936). Since his time, several traits models and questionnaires to measure these traits have been developed, among them the Psychobiological model of personality and the Five-factor model of personality.

2.1.2. Psychobiological Model of Personality

Psychobiological model of personality is a seven-dimension trait model of personality (Cloninger et al., 1994). The model is unique in the explicit way it makes a division between temperament and character. The model describes four temperament traits and three character traits that together are thought to account for all differences in human personality. Temperament refers to congenital, biologically determined, independently and moderately heritable, automatic emotional responses. Temperament is assumed to remain stable throughout life. Character traits describe the part of personality that is acquired through learning and cultural influences, and they develop as the personality 'maturates'. These traits encompass goals, beliefs and values that an individual holds. Character traits are putatively less heritable than temperament traits.

2.1.2.1. Development of the Psychobiological Model of Personality

The development of the Psychobiological model of personality began in 1986 when American psychiatrist Robert Cloninger published an article (Cloninger, 1986) describing three temperament dimensions: Harm Avoidance, Novelty Seeking and Reward Dependence. Each of these temperament traits were described as an autonomic response to a given stimuli; Harm Avoidance referred to the tendency to respond intensely to aversive stimuli. Novelty Seeking referred to the tendency to respond intensely to novel stimuli and Reward Dependency referred to the tendency to respond intensely to rewarding stimuli (Cloninger, 1987). A high scorer in the Harm Avoidance dimension was described as fearful, inhibited, anticipating harm and

pessimistic, whereas low scorer was described as calm, uninhibited, carefree, energetic and optimistic (Cloninger, 1987). The original model was called the Biosocial theory of personality.

A model of three basic temperament traits stemmed from Cloninger's own clinical observations, neurobiological research of the time, and earlier personality theory. Especially influential was the previous work of Henrik Sjöbring, Jeffrey Gray and Hans Eysenck (Cloninger, 1986). Contrary to Eysenck, however, Cloninger was explicitly critical of the use of factor analysis as a means to extract the structure of human personality dimensions (Cloninger, 1987).

Central to the theory were assumptions on the phylogenetically conserved neurobiological factors that regulate or determine temperament. Cloninger hypothesized that specific monoamine pathways correspond to the three original temperament traits (Cloninger, 1986). According to this hypothesis, Harm Avoidance is positively correlated with serotonin turnover. That is, highly harm avoidant subjects are suggested to have high brain serotonergic activity. Evidence that supported this hypothesis came from behavioral studies investigating serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (Cloninger et al., 1994). 5-HIAA was found to correlate negatively with suicidal behavior, an example of low Harm Avoidance (Agren, 1983). 5-HIAA was also found to correlate negatively with Sjöbrings validity dimension, a trait that Harm Avoidance was partly based on (Cloninger, 1986). Cloninger's hypothesis also relied on the previous work by Gray, who had speculated the role of serotonin, noradrenaline and γ-amino butric acid (GABA) in the behavioral inhibition system (1982). In a similar manner, Cloninger also hypothesized that high Novelty Seeking associates with low dopamine activity and Reward Dependence with low noradrenalin activity.

Clinically, it was well established that anxiety symptoms could be divided into cognitive and somatic dimensions. Cognitive factors include symptoms such as worry, pronounced anticipatory reaction, prolonged post-stress reactions, and lack of concentration whereas somatic factors include symptoms such as hyperventilation, trembling, sweating and difficulties in breathing. Efforts to develop questionnaires disentangling these factors had already been made (Schwartz et al., 1978). However, the Biosocial theory of personality was an attempt to create a comprehensive etiological model to explain the development of somatic anxiety and cognitive anxiety states. According to the theory, high Harm Avoidance is associated with cognitive anxiety, whereas high Novelty Seeking is associated with somatic anxiety. The content of temperament dimensions Harm Avoidance and Novelty Seeking were therefore partly based on symptoms and cognitive biases that distinguish somatic anxiety from cognitive anxiety (Cloninger, 1986). The Biosocial theory of personality was operationalized as Tridimensional Personality Questionnaire (TPQ) containing 80 self-report 'true or false'-type questions (Cloninger, 1987). However, subsequent factor analyses showed that one of the subscales of Reward Dependence, Persistence, showed low loading

on Reward Dependence and it was separated into its own temperament dimension (Cloninger et al., 1994).

The Biosocial theory of personality was further developed into a Psychobiological model of personality as two pieces of evidence suggested that even the four dimensional model of personality is inadequate. Firstly, comparative studies suggested that other personality models cover aspects of human personality that were not described by the Biosocial theory of personality. Secondly, the Biosocial theory of personality was unable to differentiate subjects that had a personality disorder from those who did not (Cloninger et al., 1993). Therefore three character dimensions: Self-Directedness, Cooperativeness and Self-Transcendence were added to the model. Instead of different dimensions of anxiety, the ability to differentiate between different personality disorders as well as between healthy subjects and those who have any personality disorder was emphasized in the development and evaluation of the Psychobiological model of personality (Svrakic et al., 1993).

Self-directedness refers to the ability to adapt behavior according to chosen goals and values. It is the source of feelings of personal integrity and self-esteem. Cooperativeness is defined as the ability to accept and identify with other people. Self-transcendence refers to spiritual experiences and feelings of unity with the universe. A questionnaire called Temperament and Character Inventory (TCI) was developed to measure these seven dimensions of human personality (Cloninger et al., 1994).

2.1.2.2. General Construct of the Temperament and Character Inventory

TCI (9th version) measures temperament dimensions Harm Avoidance, Novelty Seeking, Reward Dependence, and Persistence with 35, 40, 24 and 8 items, respectively. Character dimensions Self-directedness, Cooperativeness, and Self-transcendence are measured with 44, 42, and 33 items, respectively. In total TCI has 226 items. Personality dimensions except for Persistence in TCI are further divided into subscales. These subscales provide further information on each dimension and allow more individualized assessment of personality. TCI scales, subscales and examples of items for each dimension are presented in **table 2.1**

Table 2.1. Temperament and character dimensions, their subscales, examples of items in each dimension and hypothesized neurotransmitter that governs the trait.

Temperament	Subscales	Examples of Items	Hypothesized neurotransmitter
Harm Avoidance	Worry & Pessimism Fear of Uncertainty Shyness with Strangers Fatigability & Asthenia	I often feel tense and worried in unfamiliar situations, even when others feel there is little to worry about	Serotonin
Novelty Seeking	Exploratory Excitability Impulsiveness Extravagance Disorderliness	When nothing new is happening, I usually start looking for something that is thrilling or exciting	Dopamine
Reward Dependence	Sentimentality Attachment Dependence	I like to please other people as much as I can	Noradrenaline
Persistence	-	I am more hard-working than most people	-
Character			
Self-Directedness	Responsibility Purposefulness Resourcefulness Self-Acceptance Congruent Second Nature	My behavior is strongly guided by certain goals that I have set for my life	-
Cooperativeness	Social Acceptance Empathy Helpfulness Compassion vs. Revenge Integrated Conscience	I can usually accept other people as they are, even when they are very different from me.	-
Self-Transcendence	Self-Forgetfulness Transpersonal Identity Spiritual Acceptance	I have had moments of great joy in which I suddenly had a clear, deep feeling of oneness with all that exists.	-

2.1.2.3. Psychometric Properties of TCI

Internal consistency of TCI personality dimensions has been examined in numerous studies (Cloninger et al., 1994, Brändström et al., 1998). In the Cardiovascular Risk in Young Finns Study the Cronbach α values for each dimension were as follows: Harm Avoidance α = 0.92, Novelty seeking α = 0.85, Reward dependence α = 0.80, Persistence α = 0.64, Self-directedness α = 0.91, Cooperativeness α = 0.91, and Self-transcendence α = 0.91 (study **I**). Internal consistency of subscales is significantly lower (Brändström et al., 1998). Cloninger et al., (1994) reported six month test-retest correlation for Harm Avoidance (r=0.79), Novelty Seeking (r=0.76) and for Reward Dependency (r=0.70). Two week test-retest reliability of the TCI-R dimensions was reported by Hansenne et al. (2005). Interclass correlations for all dimensions were between 0.81 and 0.94. Josefsson et al. (2013) investigated the stability of TCI dimensions over long-term in the Cardiovascular Risk in Young Finns Study. The study found that the four-year test-retest correlation for TCI personality traits was between 0.71 and 0.81 for all age groups

except for persistence, which had a test-retest correlation of only 0.63. The stability of temperament traits over six and ten years was equally good.

Heuristic validity of TCI among psychiatric patients has been demonstrated in many studies. For example, people who have personality disorders score differently in TCI than those who have no personality disorder (Svrakic et al., 1993). Likewise, people with other psychiatric disorders tend to score differently in TCI compared to healthy individuals (Miettunen and Raevuori, 2012). The Harm Avoidance score has been prospectively associated with future incidence of psychiatric disorders (see below). Moreover, as expected, Harm Avoidance associates with different measures of stress (Heponiemi et al., 2005, Puttonen et al., 2005) and the correlation between TCI Harm Avoidance and Five-factor model Neuroticism is high (Jokela and Keltikangas-Järvinen, 2011).

The above-mentioned findings support the validity of TCI. In contrast, the claim that TCI measures the underlying 'true' structure of personality is more controversial. First of all, numerous independent studies have had difficulties producing the seven dimensions of personality using either exploratory or confirmatory factor analysis (Ball et al., 1999, Gana and Trouillet, 2003, Maitland et al., 2009). Critics have also pointed out that the distinction between temperament and character may not be valid as character traits have been shown to be equally heritable as temperament traits (Isen et al., 2009). Likewise, the claim that the model maps the underlying neurobiology has been strongly criticized, as the evidence is inconsistent (Paris, 2005). Finally, Cloninger's personality model has been revised several times. As a result, the model has become more complex and the associated concepts more loosely defined. Indeed, Cloninger's theory has been criticized for non-falsifiability and the theoretical assumptions have been criticized for not being based on empirical evidence (Farmer and Goldberg, 2008).

2.1.3. Five-Factor Model of Personality

The Five-factor model of personality describes five broad domains of personality, which are: Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness (Costa and McCrae, 1992). Each of these domains is further divided into six facets. The Five-factor model of personality is based on the lexical hypothesis, which states that all relevant differences in human behavior and character become encoded into everyday natural language (Goldberg, 1990). Therefore, it is assumed that all important personality domains can be dissected from the language based on how adjectives are used by laypersons to describe other people. The five domains have been derived using factor analysis on large sets of trait adjectives in English language (Goldberg, 1990). The five domains have also emerged in a similar manner when other languages have been analyzed, suggesting that they are universally encoded into human language (McCrae and Costa, 1997). It is important to note that these domains are purely descriptive terms and there are no etiological, developmental or neurobiological assumptions attached to them. Furthermore, the domains are not divided into

temperament and character dimensions. The Lexical Hypothesis has been criticized for its reliance on factor analysis and a theoretical approach to human personality. Furthermore, critics have argued that the layperson's description of personality may be an ill-advised starting point for an attempt to scientifically determine the basic constituents of human personality. It has also been questioned whether all significant aspects of personality can be covered with single word adjectives (Block, 1995).

Neuroticism refers to the general emotional stability of an individual. A highly neurotic person has a disposition to feel negative emotions such as fear, sadness embarrassment, anger and guilt (Costa and McCrae, 1992). On the other hand, someone who is low in Neuroticism is calm, relaxed and is usually capable of coping in stressful situations. The six facets of Neuroticism are Anxiety, Angry Hostility, Depression, Self-consciousness, Impulsiveness, and Vulnerability. Similarly to Harm Avoidance, Neuroticism has been speculated to be associated with the serotonin system and the serotonin transporter in particular. For example, the serotonin transporter gene knock-out mice exhibit anxious behavior (Holmes et al., 2003) and initial reports on humans suggested that promoter region allele leading to lower expression of serotonin transporter is associated with Neuroticism (Lesch et al., 1996).

The five factors and their facets can be measured with NEO-PI (Neuroticism Extraversion Openness Personality Inventory), a questionnaire developed by Paul Costa and Robert McCrea in 1985 and revised in 1991 (Costa and McCrae, 1992). The questionnaire consists of 240 items in a five-point likert scale.

2.1.3.1. NEO Five-Factor Inventory

NEO five-factor inventory (NEO-FFI) is a shorter version of the full NEO personality inventory. NEO-FFI was constructed choosing a subset of items from the full NEO-PI that had either the highest positive or negative loading on the corresponding factor (Costa and McCrae, 1992). NEO-FFI is comprised of 60 items and each factor contains 12 items. It allows for the measurement of the five domain scales of the five-factor model with acceptable reliability, but all of the 30 subscales cannot be calculated. Convergent Pearson correlations between NEO-PI-R and NEO-FFI factors vary between 0.62 for Neuroticism and 0.56 for Openness and divergent correlation, i.e. the correlation between two different factors, do not exceed 0.2 (Costa and McCrae, 1992). Cronbach's α's vary from 0.87 for Neuroticism to 0.75 for Agreeableness. 30-month test-retest correlations vary between 0.73 and 0.86. 6-month, 18-month and 30-month test-retest correlations for Neuroticism are 0.80, 0.79 and 0.75, respectively (Murray et al., 2003). It is acknowledged that NEO-FFI trades precision for convenience and speed (Costa and McCrae, 1992).

2.1.4. Comparison of TCI and NEO-PI

Even though the Psychobiological model of personality and the Five-factor model stem from different theoretical stances, the TCI and NEO-PI questionnaires have considerable overlap (MacDonald and Holland, 2002). That is, neither model describes dimensions of personality that are not at least in some part covered by the other model. Moreover, Five-factor model personality traits can be derived from the TCI questionnaire with factor analysis (Ramanaiah et al., 2002). Anxious personality traits in the models resemble each other to a great extent. A person who has a high Harm Avoidance score is very likely to have high a Neuroticism score as evidenced by the high correlation (r=0.75) between these two traits (Jokela and Keltikangas-Järvinen, 2011). Possibly the most significant difference between the two traits is that impulsivity is included into Neuroticism whereas in the Psychobiological model of personality impulsivity is part of Novelty Seeking not Harm Avoidance. There is evidence that higher impulsivity per se is associated with higher serotonin transporter binding (Rylands et al., 2012), which may be relevant when an association between the serotonin system and anxious personality traits are sought.

2.1.5. Personality and Psychiatric Disorders

Anxious personality is thought to be a risk factor for mood and anxiety disorders. As the threshold for tolerating stress differs among individuals, some people are more prone to develop a psychiatric disorder than others. Both Harm Avoidance and Neuroticism have subscales that are designed to measure how the subject can cope and recover from stress.

Numerous studies have shown that subjects with current mood or anxiety disorder typically have high scores in the Harm Avoidance scale. These findings have been summarized in a recent meta-analysis (Miettunen and Raevuori, 2012). The meta-analysis found that subjects with major depressive disorder, panic disorder, obsessive-compulsive disorder, social phobia as well as schizophrenia have higher Harm Avoidance scores in comparison to the psychiatrically healthy population.

So far the most compelling evidence on the associations of certain personality traits and psychiatric disorders is from a very large Swedish prospective twin study. Kendler and colleagues gathered Neuroticism scores from 20 692 same-sex twin pairs and interviewed the same subjects 25 years later to assess the incidence of major depressive disorder (Kendler et al., 2006). In this sample, Neuroticism was found to predict future major depressive disorder episodes with a hazard ratio of 1.31. Similarly, Gil and Caspi (2006) showed that Harm Avoidance assessed before a terrorist attack predicted development of post-traumatic stress disorder with an odd ratio of 1.32 in a group of 185 Haifa university students.

In addition to rendering subjects more prone to develop psychiatric disorders, certain personality traits may also protect individuals from psychiatric disorders. Farmer and colleagues (2003) found that higher Self-directedness was associated with lower incidences of depression. This finding was later replicated by Cloninger et al. (2006). They

suggested that higher Self-directedness scores indicate increased coherence of personality or 'maturity', which the authors suggest to protect individuals from depression.

2.2. *In Vivo* Imaging of Neurotransmitter Systems: Principles of Positron Emission Tomography

Positron emission tomography (PET) is an imaging method that enables quantification of biochemical and physiological processes in the brain. PET imaging is based on the use of short-lived radioactive isotopes (such as ^{15}O, ^{11}C, ^{18}F) that undergo positron decay and a scanner system that is able to measure the radioactivity. Radioactive isotopes are used to label molecules that are biologically interesting. This enables measuring the accumulation, binding, and turnover of the labeled molecule in the brain. [^{11}C]MADAM and [^{11}C] carfentanil are examples of intravenously administered PET tracers (**Figure 2.1.**). MADAM or N,N-Dimethyl-2-(2-amino-4-methylphenylthio)benzylamine is a serotonin transporter antagonist that is labeled with carbon-11, an isotope of carbon. Similarly, carfentanil or 4-carbomethoxy-fentanyl is a μ-opioid receptor agonist labeled with carbon-11.

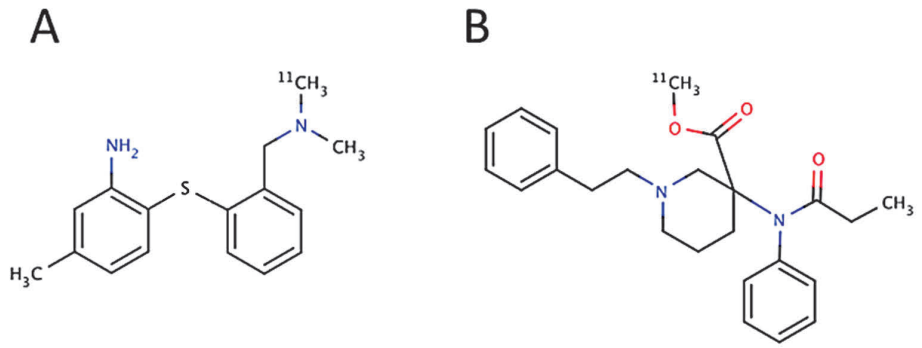

Figure 2.1. Chemical structure of [^{11}C]MADAM (panel A) and [^{11}C]carfentanil (panel B).

2.2.1. Production and Decay of Carbon-11

Carbon-11 is not a naturally occurring isotope of carbon and therefore it must be produced in cyclotron. Cyclotron is used to accelerate charged particles from an ion source into a spiral trajectory by means of a strong electromagnetic field and a rapidly alternating electrical current. The high-energy beam produced by cyclotron is used to bombard targeted stabile atoms to induce nuclear reactions that result in a radioactive isotope. Carbon-11 is produced by bombarding gaseous nitrogen-14 with protons, which induces an alpha decay of nitrogen:

(1) $^{14}N(p,\alpha)^{11}C$

Half-life of carbon-11 is 20.38 minutes. Carbon-11 decays into boron-11 by emitting a positron (e^+) and a neutrino (ν):

(2) $^{11}C \rightarrow {}^{11}B + e^+ + \nu$

Positron emitted by carbon-11 travels on average 0.85 mm in water until it has lost enough kinetic energy to be able to interact with an electron in a process called annihilation (Brown and Yasillo, 1997). Annihilation produces two 511 keV photons that are emitted in approximately (~180.25°) opposite directions:

(3) $e^+ + e^- \rightarrow \gamma + \gamma$

The basis of PET imaging lies in the simultaneous detection of the two 511 keV photons (**Figure 2.2.**). Annihilation is inferred to have taken place somewhere along the strait line connecting the two detections, called the line of response (LOR). Each LOR is characterized by its distance from the center of the field of view and the angle of orientation. PET image is reconstructed using these data from all simultaneous detections of photons.

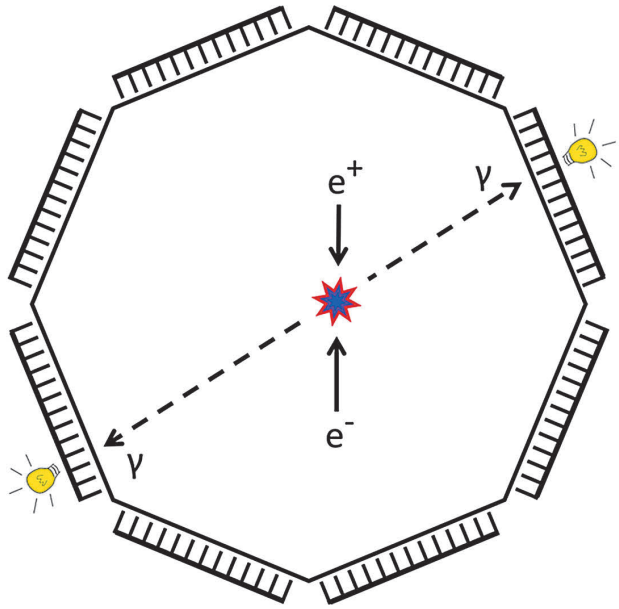

Figure 2.2. A schematic illustration of coincidental detection of two photons (γ) emitted in annihilation of positron (e^+) and electron (e^-) with a PET scanner. Photons are emitted approximately (180 ± 0.25°) in opposite directions.

2.2.2. ECAT HRRT PET Scanner

ECAT HRRT (Emission Computer Aided Tomography High-Resolution Research Tomograph) is a brain dedicated PET scanner manufactured by CTI-Siemens (**Figure 2.3.**). The axial resolution of the ECAT HRRT scanner is 2.5 mm full-width half maximum at the center of the field of view (FOV) (de Jong et al., 2007), which is the highest resolution reported so far for any PET scanner. This is achieved by innovative design of the scanner. Scanner gantry is only 35 cm in diameter; the FOV is 31.2 cm in diameter and 25 cm in axial length. The small size of the FOV reduces the error caused by the fact that photons produced in annihilation are not emitted in exactly opposite directions.

The ECAT HRRT scanner gantry is surrounded by eight detector heads (**Figure 2.2.**). Each detector head contains a layer of 117 lutetium-oxy-orthosilicate (LSO) and a layer of 117 lutetium yttrium orthosilicate (LYSO) crystals, which are used as scintillators. Photons are detected as a result of scintillation, which is a process where the scintillator absorbs high-energy radiation and emits light as a result. LSO crystal is a sensitive scintillator material permitting the small size of the crystals. Data in ECAT HRRT scanner is gathered in three dimensions meaning that all possible LORs between two detector heads are included, leading to increased sensitivity of the scanner. Moreover, together with small crystal size, three dimensional data gathering leads to large a number (4.5×10^9) of possible LOR's and increased resolution. Furthermore, LSO crystals have a very short recovery time (40 ns) after scintillation, which increases the systems sensitivity for radiation (Wienhard et al., 2002). Behind each LSO/LYSO layer are photomultiplier tubes that transform scintillations into electrical pulses that are recorded and stored by the scanner system. In order to measure and correct for attenuation of radiation caused by tissue, the ECAT HRRT scanner has a rotating Cesium-137 point source, which is used to gather tissue attenuation maps before each PET scan. Data gathered by the ECAT HRRT scanner is reconstructed using speed-optimized ordinary Poisson-ordered subset expectation maximization (OSEM) in full 3D reconstruction, an iterative reconstruction algorithm (Comtat et al., 2004). The reconstructed image is comprised of 1.22 x 1.22 x 1.22 mm^3 image elements (voxels) in a $200 \times 200 \times 150$ data matrix.

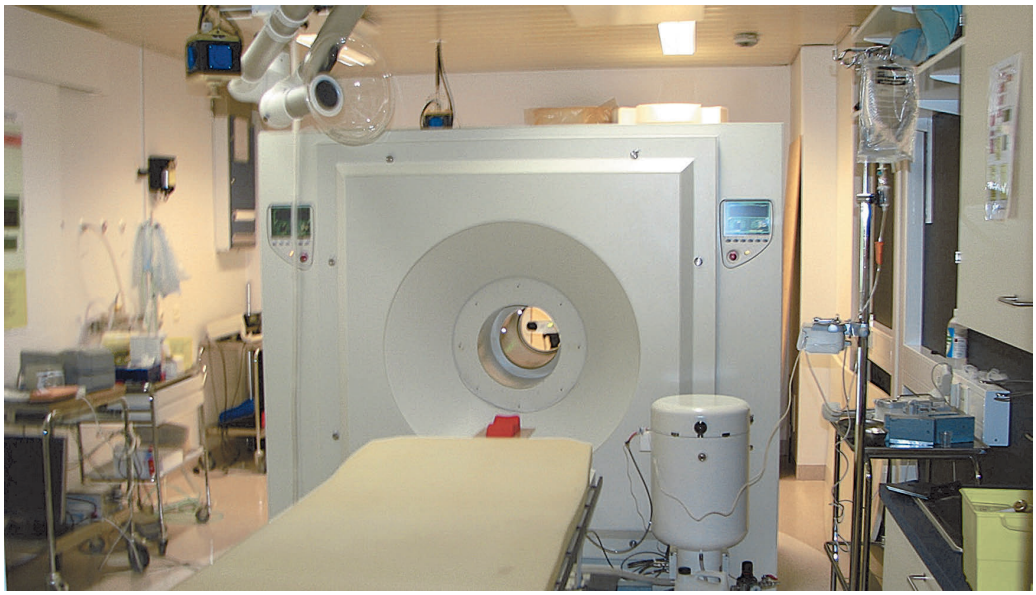

Figure 2.3. Picture of the ECAT HRRT scanner located at the Turku PET Centre.

2.2.3. Simplified Reference Tissue Model

A reconstructed PET image depicts spatial distribution of radioactivity concentration in time (e.g. kBq/cm^3). Multi-compartment models that describe tracer kinetics are used

to transform detected changes in radioactivity concentration into biologically relevant, pharmacokinetic information such as accumulation or binding of the tracer in the brain (Gunn et al., 2001, Heiss and Herholz, 2006). In the models, compartments represent physiological divisions into which the tracer is distributed after being administered intravenously and rate constants describe the rate of concentration change between the compartments. The studies presented here, use two-tissue compartment models called the simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996) to obtain regional non-displaceable binding potential (BP_{ND}) (**Figure 2.4.**). The main reason for using the simplified reference tissue model is that it allows the estimation of binding potential without arterial cannulation.

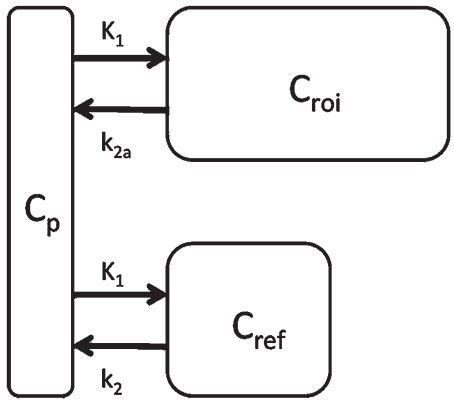

Figure 2.4. A schematic drawing of the two compartments and the rate constants governing the transfer of tracer in the simplified reference tissue model. C_p = tracer concentration in the arterial plasma; C_{roi} = tracer concentration in the region of interest, comprised of specifically bound ligand, nonspecifically bound ligand, and free ligand in the tissue; C_{ref} = tracer concentration in the reference region, comprised of non-displaceable uptake i.e. nonspecifically bound ligand and free ligand in the tissue. K_1 = Rate constant for transfer from arterial plasma to tissue; k_2 = rate constant for transfer from tissue to plasma and k_{2a} = apparent rate constant for transfer from tissue to plasma.

In *in vitro* studies, binding potential (*BP*) denotes the product of tracer affinity and density of available receptors (Mintun et al., 1984). However, with the simplified reference tissue model, free and nonspecifically bound ligand in non-displaceable compartment cannot be measured separately and thus BP_{ND} refers to the product of free fraction of non-displaceable compartment (f_{ND}), tracer affinity and density of available receptors (Innis et al., 2007).

$$(4) \quad BP_{ND} = f_{ND} \times \text{affinity} \times \text{density}$$

PET pharmacokinetic models make several assumptions on the modeled biological system as well as radioligand properties. Firstly, it is assumed that ligands are reversibly bound to their target and that they are delivered in tracer quantities and as such do not affect the density of the target molecules. Furthermore, radioactive metabolites should not cross the blood-brain barrier.

Usage of the simplified reference tissue model requires that there is a reference tissue i.e. a region in the brain virtually devoid of the target molecules that can be used to estimate non-displaceable uptake of the ligand. Simplified reference tissue does not differentiate between nonspecifically bound ligand and free ligand in the tissue and exchange between these compartments is assumed to be fast enough so that they can be described as one compartment. It is further assumed that non-displaceable uptake is unrelated to the studied property such as personality trait or disease state. Finally, volume of distribution of the free compartment is assumed to be same for region of interest and reference region (Lammertsma and Hume, 1996).

2.2.4. Region-of-Interest and Voxel-Wise Approaches

Conventionally, PET data is analyzed delineating a priori decided region-of-interests (ROIs). ROIs are comprised of voxels whose time-activity curves are averaged to obtain reliable statistics. In addition, single voxel-level analysis is often used to confirm the ROI-based finding and sometimes as the only approach method as it allows a fast, operator independent, and hypothesis free method for the data analysis. Since a nonlinear least squares approach is too sensitive to noise, specific methods applying basis-functions have been developed to model the time-activity of single voxels (Gunn et al., 1997). Furthermore, as the number of voxels in the brain is large (approximately 1.5×10^5 with 8 mm^3 isotropic voxels) specific statistical methods have been developed to deal with the problem of multiple comparisons (Worsley et al., 1992).

2.3. Neurobiology of Personality

2.3.1. The Serotonin System

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine that acts as a neurotransmitter in the human central and peripheral nervous system (Twarog and Page, 1953).

2.3.1.1. Serotonergic Pathways in the Brain

The serotonin system is an example of a classical ascending monoamine system in the mammalian brain (Dahlström and Fuxe, 1964). Somata of serotonergic neurons are located near the midline of the brain stem ventral to the aqueduct and 4th ventricle extending rostro-caudally all the way from the tegmentum to the level of the spinal cord. These somata are organized into bilateral nuclei together referred to as the raphe nuclei and they are further divided into superior and inferior nuclei (Jacobs and Azmitia, 1992). Superior nuclei are comprised of dorsal raphe nucleus, nucleus linearis, medial raphe nucleus and raphe pontis and these nuclei give rise to the ascending serotonergic projections. Inferior nuclei are comprised of nucleus raphe magnus, raphe pallidus and raphe obscurus and these nuclei give rise to the descending projections to the spinal cord.

The majority of the efferent serotonergic projections to the brain originate from the dorsal raphe nucleus and medial raphe nucleus. The projection areas of these two nuclei are to a large extent overlapping, but axons from the dorsal raphe nucleus utilize volume transmission whereas axons from medial raphe nucleus form true chemical synapses (Hornung, 2003). The highest serotonergic innervation is seen in the hippocampus, amygdala, thalamus, hypothalamus and striatum. In the cortical regions, innervation is lower and confined mainly to the layer IV granule cells (Jacobs and Azmitia, 1992).

Serotonergic neurons discharge at a typically slow frequency of 1-2 Hz with regular intervals (Aghajanian et al., 1968). Discharge of these neurons is controlled by afferent connections. The largest source of afferent input to raphe nuclei originate from within raphe nuclei themselves. Serotonin released by neighboring neurons or axon collaterals has a major role in controlling the neuronal activity of the serotonergic neurons by negative feedback (Aghajanian, 1972). Additional inhibition comes from GABAergic interneurons from within the raphe nuclei. Major inputs outside raphe nuclei include those from other brain stem nuclei, hypothalamus and lateral habenula (Peyron et al., 1998, Hornung, 2003). These afferent neurons utilize various neurotransmitters such as glutamine, GABA, other monoamines and neuropeptides such as enkephalines (Jacobs and Azmitia, 1992).

2.3.1.2. Serotonin Synthesis and Metabolism

Serotonin is produced in two steps within the serotonergic neuron from the essential amino acid tryptophan. Serotonin is produced both at the level of soma as well as in the axon terminal (Chugani et al., 1998). First L-tryptophan is hydroxylated to L-5-hydroxytryptophan by tryptophan hydroxylase. Then L-5-hydroxytryptophan is converted to serotonin by the enzyme called aromatic L-amino acid decarboxylase (Filip and Bader, 2009). After production, serotonin is stored into synaptic vesicles by the vesicular monoamine transporter. From these vesicles, serotonin is released into the synaptic cleft as a result of depolarization of the axon by a Ca^{+}-dependent mechanism. Serotonin is catabolized by mitochondria bound monoamine oxidases A and B, which degrade serotonin into 5-hydroxyindoleacetaldehyde, which is further degraded into 5-hydroxyindoleacetic acid (5-HIAA) by aldehydedehydrogenase (Filip and Bader, 2009).

2.3.1.3. The Serotonin Transporter

The serotonin transporter transports serotonin from the extracellular space into intracellular space. The serotonin transporter functions to terminate the serotonergic neurotransmission and control the serotonergic tone in the brain. This function is dependent on extracellular concentrations of both NA^{+}- and Cl^{-}-ions and reuptake of serotonin is accompanied by the uptake of these ions (Torres et al., 2011). The serotonin transporter is the target of commonly used antidepressants such as fluoxetine (Wong et al., 1974) as well as drugs of abuse such as MDMA (Rudnick and Wall, 1992).

The serotonin transporter is a transmembrane protein comprised of 630 amino acids that form 12 transmembrane helices connected with intra- and extracellular loops. Both N- and C-termini are located in the cytoplasm. The serotonin transporter belongs to the soluble carrier 6 gene family along with the dopamine transporter and the norepinephrine transporter. It is coded by the SLC6A4 gene located in the chromosome 17q11.1-17q12 (Ramamoorthy et al., 1993). An important regulator of transcription is an upstream promoter region (5-HTTLPR), where allelic variation is known to affect expression of the gene (Heils et al., 1995). Interestingly, there is some evidence that variation in 5-HTTLPR is reflected in [^{11}C]DASB binding in humans (Praschak-Rieder et al., 2007, Kobiella et al., 2011).

The serotonin transporter is widely distributed in the brain (see uptake of [^{11}C]MADAM in **figure 4.1.).** The rank order of concentration is raphe nuclei > thalamus > striatum > hippocampus and cingulate cortex > cerebral cortex > cerebellar cortex. The serotonin transporter is expressed by serotonergic neurons and to some extent also by glia cells in the brain (Dave and Kimelberg, 1994). In the serotonergic neurons, the serotonin transporter is located in the soma and axon terminal boutons (Sur et al., 1996).

2.3.1.4. In Vivo PET Imaging of Serotonin Transporter

Currently there are four PET radioligands that have been used to quantify serotonin transporters in humans: [^{11}C](+)McN5652, [^{11}C]DASB, [^{11}C]MADAM and [^{18}F](+)FMe-McN5652. [^{11}C](+)McN5652 was the first PET radioligand developed for serotonin transporter imaging in humans (Szabo et al., 1995). However, it was soon discovered to have too low a specific-to-non-specific binding ratio, a very low plasma free fraction hampering its measurement, and only a modest reversibility (Meyer, 2007). A ^{11}C labeled ADAM analog [^{11}C]DASB was developed a few years later and it demonstrated significantly faster kinetics and a higher specific-to-nonspecific binding ratio in comparison to [^{11}C](+)McN5652 (Houle et al., 2000, Frankle et al., 2004). [^{11}C]DASB has become the most widely used ligand for serotonin transporter quantification.

Another ADAM analog, [^{11}C]MADAM was developed in Karolinska Institute in 2005 (Lundberg et al., 2005). MADAM or N,N-Dimethyl-2-(2-amino-4-methylphenylthio) benzylamine is a serotonin transporter antagonist. MADAM is highly selective for the serotonin transporter as the affinity (K_i) value for serotonin transporter is 1.65 nM, for norepinephrine transporter 325 nM and for dopamine transporter over 1000 nM (Emond et al., 2002). Measure of lipophilicity the octanol/water partition coefficient (LogP) for MADAM is 3.12 (Halldin et al., 2005). [^{11}C]MADAM has two radioactive metabolites which are more polar than the parent compound and do not penetrate the blood-brain barrier (Lundberg et al., 2005). [^{11}C]MADAM binds reversibly to the serotonin transporter and it is displaceable by other selective serotonin reuptake inhibitors such as citalopram (Lundberg et al., 2007). The density of the serotonin transporter is very low in the cerebellar cortex and it can be used as a measure of free and non-specifically bound ligand in the tissue. Interestingly, the uptake of [^{11}C]

MADAM in cerebellar gray matter is not decreased after the citalopram administration as may be the case for [^{11}C]DASB (Parsey et al., 2006). Binding of [^{11}C]MADAM can be modeled with the simplified reference tissue model allowing estimation of specific binding without the need for an arterial input function (Lundberg et al., 2005). The rank order of [^{11}C]MADAM binding is similar to what has been shown for [^{3}H]MADAM in post mortem samples suggesting that [^{11}C]MADAM can be used to reliably measure serotonin transporters *in vivo* (Chalon et al., 2003).

2.3.1.5. Serotonin Receptors and Their *In Vivo* PET Imaging

The effects of serotonin on postsynaptic cells are transmitted via serotonin receptors. To date, 14 separate serotonin receptors subtypes have been discovered (Filip and Bader, 2009). Serotonin receptors are a G-protein coupled receptors except for serotonin $5HT_3$ receptor, which is a ligand gated ion channel. PET radioligands have been developed for $5HT_{1A}$, $5HT_{1B}$, $5HT_{2A}$, $5HT_4$ and $5HT_6$ receptors [for an excellent review see (Saulin et al., 2012)], but most of the imaging studies on humans have used either $5HT_{1A}$ receptor or $5HT_{2A}$ receptor ligands.

$5HT_{1A}$ receptor is the main inhibitory receptor in the serotonin system exerting its effects via activation of G-protein coupled K^+ -channels causing hyperpolarization of the neuron (Filip and Bader, 2009). $5HT_{1A}$ autoreceptors located in the somata of serotonergic neurons in superior raphe nuclei have an important autoregulatory role within the serotonin system. Stimulation of these autoreceptors inhibits the firing of serotonergic neurons. Postsynaptic $5HT_{1A}$ receptors are found in high concentrations in the hippocampus, insula and frontal cerebral cortex (Pike et al., 1995) and low concentrations in the basal ganglia and cerebellar cortex. The most widely used PET ligand for quantifying $5HT_{1A}$ receptors in humans is an antagonist [^{11}C-*carbonyl*]WAY-100635. Other ligands developed more recently include [^{18}F]MefWAY and [^{11}C]CUMI-101 (Saulin et al., 2012).

The $5HT_{2A}$ receptor is an important excitatory receptor in the serotonin system conveying its effects via phosholipase C mediated accumulation of inositol phosphates and Ca^{++} in the intracellular space causing depolarization of the postsynaptic neuron (Barnes and Sharp, 1999). $5HT_{2A}$ receptor is distributed widely in the brain with high concentrations in the cerebral cortex and hippocampus and low concentrations in the basal ganglia and cerebellar cortex (Biver et al., 1994). Most popular PET radioligand in $5HT_{2A}$ receptor imaging has been [^{18}F]altanserin. Other ligands include [^{18}F]FESB, [^{18}F] setoperone, [^{11}C]CIMBI-36 and [^{18}F]MH.MZ (Saulin et al., 2012).

2.3.2. Harm Avoidance and Neuroticism - Imaging Studies on the Serotonin System

Studies **I** and **IV** investigate the association of serotonin transporter binding potential and anxious personality traits. The association of anxious personality traits with the serotonin system has been investigated in eleven previous PET studies and in one SPECT study on healthy subjects. Findings that examine this association in psychiatric

populations have not been included in this short review. Reconciling the findings in these studies is complicated by significant methodological differences. Firstly, anxious personality traits have been measured with different questionnaires such as the TCI, NEO-PI, or Eysenck Personality Questionnaire. Differences in various personality measures have been discussed above. Secondly, the serotonin transporter and receptors have been measured with different imaging methods and ligands. Studies have also used a variable number of ROI's that have been delineated with differing criteria. Finally, the number of subjects in most of the studies is relatively small increasing the risk for type I and type II errors in statistical inference.

2.3.2.1. Neurobiological Correlates of Neuroticism and Harm Avoidance - the Serotonin Transporter

The serotonin transporter and anxious personality traits have been investigated by four previous studies summarized in **table 2.1.** Takano and colleagues (2007) found positive correlation between the serotonin transporter and NEO-PI Neuroticism in the thalamus. However, Kalbitzer and colleagues (2009) were unable to replicate this finding in their larger study with the same ligand and personality measure. Instead, Kalbitzer and colleagues found a negative association between the openness trait and the serotonin transporter. In line with Kalbitzer and colleagues, Reimold and colleagues (2008) found no association between TCI Harm Avoidance and serotonin transporter binding potential. In a single-photon emission computed tomography (SPECT) study Wu and colleagues (2010) found that TPQ Harm Avoidance correlates negatively with serotonin transporter availability in the midbrain. A major methodological question concerning all molecular imaging using reference tissue models is whether the reference region is indeed devoid of specific binding. All studies in **table 2.1.** have used cerebellar gray matter as the reference region for non-specific binding. Yet, both post mortem autoradiography studies and PET imaging studies suggest that the cerebellum has a measurable level of serotonin transporter (Cortés et al., 1988, Szabo et al., 2002). Moreover, approximately 30% of the binding of [^{11}C]DASB in cerebellar cortex is displaceable by selective serotonin reuptake inhibitor sertraline (Parsey et al., 2006). This fact has led some groups to abandon reference tissue models and return to the use of the arterial input function for modeling [^{11}C]DASB binding (Miller et al., 2013). Whether this is a true confounding factor in serotonin transporter imaging remains to be seen.

In the only SPECT study of human anxious personality traits, Wu and colleagues used [^{123}I]ADAM tracer and modeled its kinetics with a ratio method. This method tends to give overestimated values in high binding regions such as the midbrain and the test-retest variability is high (Frokjaer et al., 2008). Furthermore, due to lower resolution, SPECT imaging is more vulnerable to partial volume effects especially when measuring radioactivity from small midbrain structures. The evidence gathered by previous studies is diverging and does not allow one to draw a definitive conclusion on the association of the serotonin transporter and anxious personality traits. Therefore, more studies on this subject are warranted.

Table 2.1. Summary of in vivo imaging studies of serotonin transporter and anxious personality traits.

Reference	Trait	Method	N of healthy subjects*	Results
Takano et al. (2007)	NEO-PI Neuroticism	PET/[^{11}C]DASB	31 / 0	↑**
Reimold et al. (2008)	TCI Harm Avoidance	PET/[^{11}C]DASB	11/8	–
Kalbitzer et al. (2009)	NEO-PI Neuroticism	PET/[^{11}C]DASB	35/15	–
Wu et al. (2010)	TPQ Harm Avoidance	SPECT/[^{123}I]ADAM	21/19	↓***

* Males/Females
**Statistically significant positive Pearson correlation coefficient in thalamus
*** Statistically significant negative Pearson correlation coefficient in midbrain
Abbreviations: NEO-PI = Neuroticism Extraversion Openness Personality Inventory, TCI = Temperament and Character Inventory, TPQ = Tridimensional Personality Questionnaire, PET = positron emission tomography, SPECT = single-photon emission computed tomography

2.3.2.2. Neuroticism, Harm Avoidance, and the Serotonin 5HT$_{1A}$ Receptor

Studies investigating the association of the serotonin 5HT$_{1A}$ receptor and anxious personality traits are summarized in **table 2.2.** Studies by Tauscher and colleagues and our group (Hirvonen et al. unpublished manuscript) suggest a negative correlation between the serotonin 5HT$_{1A}$ receptor and anxious personality traits. These findings are contrasted by negative findings in three other studies. Even though all the studies have used [^{11}C-*carbonyl*]WAY-100635 there are still significant differences in the imaging methods. Three studies (Tauscher et al., 2001, Rabiner et al., 2002, Borg et al., 2003) used the simplified reference tissue model with the cerebellum as the reference region to calculate BP_{ND}. As discussed above, this method may pose problems when there is specific binding in the reference region. For [^{11}C-*carbonyl*]WAY-100635 this is unfortunately the case. Matters are complicated further, as the ligand enters the cerebellum insufficiently to be reliably measurable in later time points whereas radioactive metabolites probably enter the cerebellum disproportionately contaminating the signal (Shrestha et al., 2012). The remaining two studies (Hirvonen et al., unpublished manuscript, Karlsson et al., 2011) used arterial total plasma [^{11}C-*carbonyl*]WAY-100635 concentration as the input function. Even though this method is an improvement to simplified reference tissue modeling of [^{11}C-*carbonyl*] WAY-100635 binding, it may also be biased. Total plasma concentration refers to the concentration of both protein bound and free ligand in plasma but only free ligand in plasma may enter the brain. Therefore, changes in the concentration of protein bound ligand may affect the binding metrics in the brain. Similar methodological problems have caused discrepancies in studies investigating the role of the 5HT$_{1A}$ receptor in major depressive disorder (Shrestha et al., 2012). As with the serotonin transporter, the evidence gathered on the association of the serotonin 5HT$_{1A}$ receptor and anxious personality traits is lacking and further studies are needed.

Table 2.2. Summary of *in vivo* imaging studies of serotonin 5-HT_{1A} receptor and anxious personality traits.

Reference	Trait	Method	N of healthy subjects*	Results
Tauscher et al. (2001)	NEO-PI Anxiety subscale	PET/[carbonyl-^{11}C]WAY-100635	11/8	↓**
Rabiner et al. (2002)***	EPQ Neuroticism	PET/[carbonyl-^{11}C]WAY-100635	44/0	–
Rabiner et al. (2002)***	TPQ Harm Avoidance	PET/[carbonyl-^{11}C]WAY-100635	49/0	–
Borg et al. (2003)	TCI Harm Avoidance	PET/[carbonyl-^{11}C]WAY-100635	15/0	–
Karlsson et al. (2009)	TCI Harm Avoidance	PET/[carbonyl-^{11}C]WAY-100635	11/9	–
Hirvonen et al. (unpublished)	KSP Neuroticism	PET/[carbonyl-^{11}C]WAY-100635	18/16	↓****

* Males/Females

** Statistically significant negative Pearson correlation coefficient with NEO-PI Neuroticism Anxiety subscale in dorsolateral prefrontal cortex, anterior cingulate cortex, parietal cortex, and in occipital cortex.

*** Rabiner et al. presents correlations with two different questionnaires in the same population in the same article.

**** Statistically significant negative Pearson correlation coefficients in several cortical, subcortical regions as well as in the midbrain.

Abbreviations: NEO-PI = Neuroticism Extraversion Openness Personality Inventory, EPQ = Eysenck Personality Questionnaire, TPQ = Tridimensional Personality Questionnaire, TCI = Temperament and Character Inventory, KSP = Karolinska Scales of Personality, PET = positron emission tomography

2.3.2.3. Neuroticism, Harm Avoidance and the Serotonin $5HT_{2A}$ Receptor

Three studies that have examined the association of the serotonin $5HT_{2A}$ receptor and anxious personality traits are summarized in **table 2.3.** In the largest imaging study on the serotonin system and personality so far, Frokjaer and colleagues (2008) showed that NEO-PI Neuroticism is positively associated with the serotonin $5HT_{2A}$ receptor in several prefrontal regions. Two other studies with substantially fewer participants have found either no correlation (Soloff et al., 2010) or a negative correlation between serotonin $5HT_{2A}$ receptor and Harm Avoidance (Moresco et al., 2002). The diverging results in the two studies that have used [^{18}F]Altanserin may simply be due to insufficient power in the study by Soloff and colleagues. [^{18}F]Altanserin has two radioactive metabolites that cross the blood-brain barrier. Although the binding can still be fairly reliably measured (Price et al., 2001, Pinborg et al., 2003), these metabolites do contribute to the overall noise level in the scans. Therefore, substantial numbers of subjects may be required to reliably detect an effect. In addition to a very small group of participants, Moresco and colleagues used [^{18}F]FESB to quantify serotonin $5HT_{2A}$ receptors in the brain. Unfortunately, this radioligand has a comparable affinity for both the serotonin $5HT_{2A}$ receptor and the dopamine D_2 receptor, making it suboptimal tool for studying serotonin $5HT_{2A}$ receptor binding.

In conclusion, the most convincing evidence so far for an association between the serotonin system and anxious personality traits comes from the study by Frokjaer and colleagues, who found a positive correlation between the 5-HT_{2A} receptor and Neuroticism. Based on a single study, it is still too early to conclude that anxious personality traits are truly related to the serotonin system.

Table 2.3. Summary of *in vivo* imaging studies of serotonin 5-HT_{2A} receptor and anxious personality traits.

Reference	Trait	Method	N of healthy subjects*	Results
Moresco et al. (2002)	TPQ Harm Avoidance	PET/[^{18}F]FESP	8/3	↓**
Frokjaer et al. (2008)	NEO-PI Neuroticism	PET/[^{18}F]altanserin	52/31	↑***
Soloff et al. (2010)	TCI Harm Avoidance	PET/[^{18}F]altanserin	10/11	–

* Males/Females
** Statistically significant negative Pearson correlation coefficients in prefrontal and in left parietal cortices.
*** Statistically significant positive Pearson correlation coefficients in several prefrontal regions.
Abbreviations: TPQ = Tridimensional Personality Questionnaire, NEO-PI = Neuroticism Extraversion Openness Personality Inventory, TCI = Temperament and Character Inventory, PET = positron emission tomography

2.3.3. The Opioid System

Four G-protein coupled opioid receptors have been identified in the human brain, μ-, δ-, κ- and NOP receptors (Henriksen and Willoch, 2008). These receptors mediate the effects of several different endogenous peptides, which have different affinity profiles for different opioid receptors. Endomorphine-1 and endomorphine-2 are selective high affinity μ-opioid receptor agonists (Fichna et al., 2007), whereas β-endorphine, β-neoendorphin, and also enkephalin are non-selective μ-opioid receptor agonists, with affinities for δ-receptors as well (Waldhoer et al., 2004). Dynorphin A, Dynorphin B and α-neoendorphin are selective κ-receptor agonists (Henriksen and Willoch, 2008).

Endomorphins are structurally unrelated to other endogenous opioids, their precursor has not been identified, and it is currently unknown how they are synthesized in the brain (Fichna et al., 2007). In contrast, the synthesis of β-endorphine is well understood (Khachaturian et al., 1985). β-endorphine is produced by alternative splicing of pro-opiomelanocortin. Pro-opiomelanocortin is also the precursor of adrenocorticotropin and it is therefore not surprising that the highest concentrations of pro-opiomelanocortin are found in the pituitary gland and hypothalamus (Khachaturian et al., 1985). After release into synapse, endogenous opioids are degraded in the extracellular space by various peptidases (Fichna et al., 2007). There is no reuptake mechanism for endogenous opioids.

2.3.3.1. The μ-opioid Receptor

The first opioid receptor, the μ-opioid receptor, was found in 1973 (Pert and Snyder, 1973). It was named after the Greek god of dreams, Morpheus, as it mediates effects of morphine as well as effects of many endogenous opioids. In the brain, the μ-opioid receptor is expressed widely in both cortical and subcortical regions (see uptake of [^{11}C]carfentanil in **figure 4.1.**). μ-opioid receptor, like all typical G-protein coupled receptors, has seven transmembrane domains, extracellular N-terminal and intracellular C-terminal. μ-opioid receptor is coded by opioid receptor mu 1 (ORPM1) gene located at 6q24-q25 (Wang et al., 1993). Common allelic variation in ORPM1-gene, A118G, has been described. It leads to a three-fold increase in the μ-opioid receptor's affinity for β-endorphine (Bond et al., 1998) and possibly affects the binding of [^{11}C] carfetanil to μ-opioid receptor (Ray et al., 2011).

There are also several alternative splice variants of the μ-opioid receptor that are expressed differentially in the brain (Abbadie et al., 2004). Whether these variants also display differing pharmacodynamics in humans is currently unknown as well as their significance for [^{11}C]carfentanil binding (Henriksen and Willoch, 2008). μ-opioid receptors also form functionally relevant homo- or heterodimers with other opioid receptors (Waldhoer et al., 2004). The relevance of dimerization for [^{11}C]carfentanil binding is likewise presently unknown.

The μ-opioid receptors are located in both pre- and postsynaptic neurons. In general, endogenous opioids are thought to act as co-transmitter modulating the effects of fast-acting neurotransmitters such as GABA (Wagner et al., 1993). Similarly, the stimulation of the μ-opioid receptor affects both the release and post-synaptic effects of serotonin (Marek and Aghajanian, 1998, Sbrenna et al., 2000, Rutz et al., 2007). Yet, it is important to note that the μ-opioid receptor is a G-protein coupled receptor with an abundance of possible intracellular effects. When ligand is bound to the μ-opioid receptor, conformational changes in the intracellular part of the receptor lead to dissociation of G-protein α subunit from βγ subunit, which in turn leads to activation of second-messenger system (Venkatakrishnan et al., 2013). Subsequent activation of second-messenger system may modify functions of numerous intracellular proteins e.g. adenylate cyclases, phospholipases, or potassium- and calcium channels. As a result, changes in a multitude of intracellular functions such as transcription or phosphorylation of proteins, metabolism and excitability of the neuron may take place.

Following ligand binding, μ-opioid receptor may be deactivated in various ways. μ-opioid receptors may become desensitized to subsequent ligands e.g. via phosphorylation. μ-opioid receptors may also become ineffective by decoupling from G-proteins or it may be translocated back into the neuron by endocytosis (Waldhoer et al., 2004).

2.3.3.2. In Vivo PET Imaging of the Opioid System

Several radioligands have been developed to quantify opioid system *in vivo*. Most commonly used radioligands are opioid receptor subtype non-selective antagonists [^{11}C]diprenorphine and [^{18}F]fluoroethyldiprenorphine and μ-opioid receptor selective agonist [^{11}C]carfentanil. Recently developed radioligands include selective δ-opioid receptor antagonist [^{11}C-*methyl*] naltrindole and selective κ-opioid receptor antagonist [^{11}C]GR103545.

[^{11}C]carfentanil is a fentanyl analog developed at Johns Hopkins University (Frost et al., 1985). It is a highly selective ligand for the μ-opioid receptor with affinity (K_i) values of 0.024nM, 3.28nM and 43.1nM for μ-opioid, δ-opioid and κ-opioid receptors, respectively (Cometta-Morini et al., 1992). The binding of carfentanil to μ-opioid receptor is reversible and it can be blocked with subtype non-selective opioid receptor antagonists such as nalmefene (Ingman et al., 2005), but not with δ-opioid receptor or κ-opioid receptor antagonists (Titeler et al., 1989). As [^{11}C]carfentanil is a highly potent μ-opioid receptor agonist, it has physiological effects already at very low doses. In order to meet the assumption that ligand is administered at tracer quantities the injected mass of [^{11}C]carfentanil must be very low (<2μg) and subsequently specific activity must be high. Another important consequence of agonism is that [^{11}C]carfentanil binds preferably to μ-opioid receptors in high affinity-state (Henriksen and Willoch, 2008).

Octanol/water partition coefficient (LogP) of carfentanil is 3.89 (Finegan, 1984) indicating lipid-solubility which allows carfentanil to cross blood-brain barrier efficiently. The two radioactive metabolites of [^{11}C]carfentanil, do not cross the blood-brain barrier (Endres et al., 2003) and do not interfere with quantification. The occipital cortex has a negligible amount of μ-opioid receptors and this region can therefore be used as a reference region for non-specific binding. The simplified reference tissue model has been shown to produce comparable estimates of BP_{ND} as more complicated compartmental models using arterial samples for input function (Endres et al., 2003). Moreover, the regional BP_{ND} values are highly reproducible with the simplified reference tissue model (Hirvonen et al., 2009). [^{11}C]Carfetanil binding may be sensitive to endogenous opioids. This is evidenced for instance by a decrease in [^{11}C]carfetanil BP_{ND} during stimuli that putatively increases the endogenous opioid tone in the brain (Scott et al., 2007).

2.3.4. Imaging Studies on the Opioid System and Personality

In rodents, the endogenous opioid system regulates many important biological processes such as pain, reward and food intake as well as depressive states (Fichna et al., 2007). Correspondingly, PET-imaging studies in humans using [^{11}C]carfentanil have demonstrated the role of the μ-opioid system in pain, addictions, eating disorders, and affective states (Henriksen and Willoch, 2008).

The role of the μ-opioid system in anxious personality traits is especially intriguing as opioids have well demonstrated anxiolytic effects (Kalin et al., 1988, Fichna et al., 2007, Bilkei-Gorzo et al., 2008).

So far, only a few PET studies have investigated how personality traits are related to the opioid system. In 2008, Schreckenberger and colleagues reported a positive correlation between [^{18}F]fluoroethyldiprenorphine binding and temperament trait reward dependence in 23 healthy male subjects bilaterally in the ventral striatum (Schreckenberger et al., 2008). None of the other temperament traits correlated with [^{18}F]fluoroethyldiprenorphine binding. [^{18}F]fluoroethyldiprenorphine is an opioid receptor subtype non-selective ligand and therefore it cannot be distinguished by which of the three opioid receptor subtypes is associated with reward dependence.

The second study on human personality and the opioid system was conducted by Love and colleagues (Love et al., 2009). They found that baseline [^{11}C]carfetanil binding was associated with higher impulsivity in 19 healthy male subjects. Higher impulsivity also predicted a higher release of endogenous opioids in response to pain as implicated by lower [^{11}C]carfetanil binding during painful stimulus. Recently, the same group (Peciña et al., 2013) demonstrated that higher score in Five-factor model Neuroticism, and particularly its subscale Angry hostility, predicted a lower release of endogenous opioids in response to placebo treatment of pain.

2.3.5. Neurotransmitter Networks: Interaction of the Serotonin and the Opioid Systems

Neurons form an interconnected network and communicate using different neurotransmitters to produce complex behaviors as well as human consciousness. Interplay between different neurotransmitter systems is clinically highly relevant, as many drugs affect more than one neurotransmitter system directly or indirectly. Moreover, most neuropsychiatric disorders are associated with dysfunction of more than one neurotransmitter system. For example, functions of dopamine, serotonin and glutamate systems are disrupted in schizophrenia (Hirvonen and Hietala, 2011, Marsman et al., 2013). PET is the most suitable method to map quantitatively neurotransmitter interactions in the living human brain. Indeed, PET imaging has been used to correlate different constituents within a single neurotransmitter system. For example, five PET studies have investigated the interaction between the serotonin transporter and 5-HT$_{1A}$ -receptor (Lundberg et al., 2007, Jovanovic et al., 2008, Takano et al., 2010, Bose et al., 2011) or 5-HT$_{2A}$ receptor (Erritzoe et al., 2010). Still, there is a remarkable lack of PET imaging studies which simultaneously investigate more than one neurotransmitter system.

The possible *in vivo* correlation between serotonin and opioid systems is intriguing as the two neurotransmitters are involved in the modulation of similar functions in humans. For instance, both μ-opioid receptor agonists and serotonin transporter antagonists are used to treat pain. PET imaging has also linked the binding of these two molecules with pain regulation (Bencherif et al., 2002, Martikainen et al., 2007, Hagelberg et al., 2012). The association of both these neurotransmitters in modulation of affective states is likewise evident based on PET studies e.g. (Liberzon et al., 2002,

Savitz and Drevets, 2013). Similarity of function as well as similar spatial localization of serotonergic and μ-opioidergic innervation suggest that interaction of these systems may be important.

Animal studies have unraveled the possible mechanisms of this interaction. Tao and Auerbach (1995) demonstrated using rats and microdialysis methods that the systemic injections of morphine releases serotonin into the dorsal nucleus raphe and regions innervated by dorsal nucleus raphe such as amygdala, thalamus, prefrontal cortex and ventral striatum. Furthermore, injections of μ-opioid receptor agonists into dorsal raphe nucleus increases serotonin levels in nucleus accumbens (Tao and Auerbach, 2002a, b). The authors propose that μ-opioids inhibit both glutamatergic and GABAergic afferents to somata of serotonergic neurons in dorsal raphe nucleus, with the net effect of disinhibition of serotonergic neurons. On the other hand, in the cortex presynaptic μ-opioid receptors inhibit serotonin efflux into synapses (Sbrenna et al., 2000, Rutz et al., 2007), putatively directly via μ-opioid receptors located in serotonergic axon terminals. In addition, μ-opioids inhibit the effects of serotonin on the postsynaptic neuron (Marek and Aghajanian, 1998).

2.4. Summary of the Literature

There are several different models of human personality, which have evolved from different scientific traditions. The Psychobiological model of personality and the five-factor model of personality are two widely used trait models of personality. Despite the many differences, both models include an anxious personality trait; the Psychobiological model of personality calls it Harm Avoidance whereas the Five-factor model of personality has named it Neuroticism. Intercorrelation between these traits is high and they also both predict the incidence of mood and anxiety disorders.

Attempts have been made to associate these traits with neurotransmitter systems in the brain, especially with the serotonin system. However, the findings in imaging studies are contradictory. Possible explanations for this contradiction may be the fact that the previous imaging studies have not been designed to directly test the hypothesized association or some other methodological shortcoming. As the attempts to associate the serotonin system with anxious personality traits have not succeeded, it may also be plausible to search for new neurotransmitter systems that could explain inter-individual differences in these traits. One such neurotransmitter system may be the μ-opioid system, which has been investigated relatively little.

Finally, all neurotransmitter functions are intertwined with functions of other neurotransmitters. Serotonin and μ-opioid systems modulate similar functions in humans and also interact at the cellular level. It is important to better understand how and where these interactions occur.

3. OBJECTIVES OF THE STUDY

Studies **I**, **II** and **IV** are based on the assumption that differences in personality traits are explained by differences in the functioning of a single neurotransmitter system. These studies also test specific hypotheses based on this assumption. Furthermore, these studies took advantage of an oversampling method in order to increase sensitivity of detecting the association between neurobiological measures and personality. It is, however, evident that different neurotransmitter systems interact in the human brain to produce complex behaviors. In order to get a further understanding of such interaction, study **III** attempts to describe an interaction between regional BP_{ND} of the serotonin transporter and μ-opioid receptor in the human brain.

The specific objectives of the studies were:

I. To test the hypothesis that differences in serotonin transporter density explain the variability in Harm Avoidance scores in healthy volunteers.

II. To test the hypothesis that differences in μ-opioid receptor availability explain the variability in Harm Avoidance scores.

III. To develop a methodology for exploring the interaction of the serotonin transporter and the μ-opioid receptor at the voxel-level in the brain

IV. To test the hypothesis that differences in serotonin transporter density explain the variability in Neuroticism scores in healthy volunteers.

4. METHODS

These studies were conducted in accordance with the ethical principles of the Declaration of Helsinki. Study protocols were approved by the joint ethical committee of the University of Turku and Turku University Hospital. Before entering the study all subjects gave written informed consent.

4.1. Study Design

4.1.1. Subjects

4.1.1.1. The Cardiovascular Risk in Young Finns Study

Subjects for these studies were recruited from a population based prospective cohort study called "The Cardiovascular Risk in Young Finns Study" (Åkerblom et al., 1985). This cohort was initiated in 1980 when 3,596 children and adolescents (baseline age 3-18) were recruited from five Finnish cities and their rural vicinities by university hospitals (Turku, Helsinki, Tampere, Kuopio and Oulu).

Since the beginning of the cohort study, the participants have been examined every 3-9 years (Juonala et al., 2013). In addition to extensive somatic assessments, various psychological parameters including TCI have also been measured. The number of subjects lost to follow-up has been remarkably low. For instance, 2,283 subjects (63 %) were still participating in the study in 2001.

4.1.1.2. Recruitment of Subjects for the Studies – an Oversampling Strategy

These studies included altogether 32 subjects that were recruited based on their Harm Avoidance scores from the Cardiovascular Risk in Young Finns Study. All subjects in the cohort who had a Harm Avoidance score either in the highest or the lowest quartile in two consecutive measures, in the year 1997 and in the year 2001 were first selected. This preselection created two groups: high and low Harm Avoidance groups. Subjects in these two groups were then matched for sex, age, and education. From the matched group, subjects were contacted and recruited for the study. However, for logistical reasons only subjects living in Turku, Helsinki or in the rural vicinity were contacted.

Exlusion criteria were as follows: Evidence of clinically significant and chronic somatic disorder, Evidence of earlier or current DSM-IV axis-I disorder including alcohol, drug or nicotine dependency or abuse, chronic medication excluding contraceptive medications, pregnancy in females, prior participation in PET or SPECT study. Data gathered along the follow-up was used to exclude known chronic somatic disorders and psychiatric disorders. Subjects were also interviewed by phone before further examinations.

Somatic health was also assessed prior to the PET scan by clinical examination and laboratory screening that included: electrocardiogram, blood samples for determination of erythrocyte sedimentation rate and blood cell counts, plasma sodium and potassium levels, serum calcium and fasting glucose levels, serum creatinine, alcaline phosphatase, gamma glutamyl transferase, alanine aminotransferase and thyroid stimulating hormone levels, antibodies for hepatitis C and hepatitis B surface antigens, and antibodies for human immunodeficiency virus. A urine drug analysis was also performed in order to exclude drug abuse.

Finally, possible earlier or current DSM-IV axis-I disorders were assessed with a structured clinical interview for DSM-IV axis-I disorders (SCID-I). Altogether 12 subjects were excluded for fulfilling the criteria for one or more earlier or current psychiatric disorder. All subjects that were excluded based on SCID-I were in the high Harm Avoidance group. In order to exclude any structural brain abnormalities, all subjects underwent a clinical MRI scan at 1,5T.

The final study sample in study **II** included 22 subjects, 10 high Harm Avoidance subjects and 12 low Harm Avoidance subjects. Studies **I** and **III** had 21 subjects as one [^{11}C] MADAM scan was unusable due to extensive movement during the scanning. Recruitment of subjects was continued using the same inclusion and exclusion criteria and the **IV** study had 31 subjects (see study **IV** for detailed description of the study population). Demographic characteristics of the subjects in studies **I-III** are presented in **table 4.1.**

Table 4.1. Demographics of subjects in studies **I-III**.

	High HA (N=10)	**Low HA (N=12)**	**p-value**
Males/Females	5/5	6/6	1.000
Age	38.0 ± 5.2	37.6 ± 5.1	0.868
Education (High/Low)	5/5	8/4	0.429
Body Mass Index	23.1 ± 3.3	25.4 ± 1.9	0.064
Harm Avoidance Score (PET)	2.90 ± 0.29	2.04 ± 0.33	<0.001
Harm Avoidance Score (2001)	3.32 ± 0.24	1.95 ± 0.24	<0.001
Harm Avoidance Score (1997)	3.28 ± 0.18	2.00 ± 0.17	<0.001

Mean values ± standard deviations are shown for age, body mass index and Harm Avoidance scores obtained at different time points. HA = Harm Avoidance. High education refers to a university degree.

4.2. Assessment of Personality

4.2.1. Temperament and Character Inventory

Temperament and Character traits were assessed with the Finnish translation of TCI from the ninth version of TCI (Puttonen, 1998). In the translation, answers to the items are rated in a 5-point Likert scale (1 = definitely false, 5 = definitely true) instead of the original dichotomous true or false response.

4.2.2. Neuroticism Extraversion Openness Five-Factor Inventory

Five personality domains described by a Five-factor model were assessed with a Finnish translation of NEO-FFI containing 60 items in a five-point Likert scale.

4.3. Positron Emission Tomography Methods

4.3.1. Radiochemistry of [^{11}C]MADAM and [^{11}C]carfentanil

Production and labeling of [^{11}C]MADAM is described in detail in study **I**. Whereas the production and labeling of [^{11}C]carfentanil is reported in detail in study **II**. There were no statistically significant differences between the high and low Harm Avoidance groups in any radiochemical parameters such as the injected dose, specific activity or chemical purity of the compound.

4.3.2. PET Image Acquisition

All subjects underwent two PET scans during the same day. [^{11}C]MADAM scan was carried out in the morning and [^{11}C]carfentanil scan was carried out in the afternoon. There was at least 3 hours between the two scans to allow carbon-11 to decay. All scans were performed with an ECAT HRRT scanner. In order to minimize the movement during the scanning, the subject's head was fixated with a thermoplastic mask. A thermoplastic mask was individually molded for each subject prior to the first PET scan. Despite the mask, the subject's head may move during the scanning. This movement was measured with an external Polaris Vicra movement detector. Information of the head movement was later used for movement correction.

The subject's antecubital vein was cannulated and the radioligand was injected rapidly at the beginning of the scan as a bolus injection and flushed with saline. The target injected dose for both radioligands was 500 MBq. In the 32 PET scans the injected dose (mean ± standard deviation) for [^{11}C]MADAM was 483.5 ± 44,58 MBq and for [11C] carfentanil 438.5 ± 71.86 MBq. Radioactivity of [^{11}C]MADAM and [^{11}C]carfetanil were measured for 75 and 69 minutes, respectively. [^{11}C]MADAM scan was reconstructed into 17 frames of increasing length (3x1 minutes, 4 x 4 minutes and 10 x 6 minutes). [^{11}C]carfentanil scan was reconstructed into 16 frames (3x1 minutes, 4 x 4 minutes and 9 x 6 minutes). Examples of [^{11}C]MADAM and [^{11}C]carfetanil uptake images are shown in **figure 4.1.**

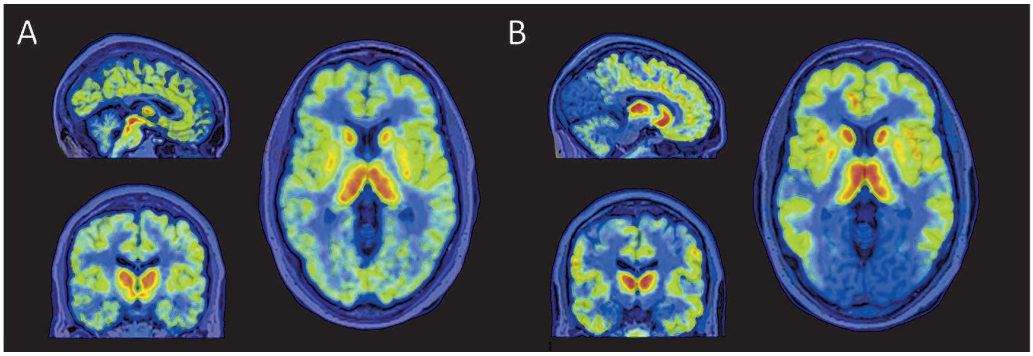

Figure 4.1. Uptake of [^{11}C]MADAM (panel A) and [^{11}C]carfentanil (panel B). Note the low uptake of [^{11}C]MADAM into cerebellum and the low uptake of [^{11}C]carfentanil into occipital cortex.

4.3.3. Preprocessing and Modeling of the PET Data

To correct for head motion during the scanning, frames in each scan were realigned. For this purpose the frames of least motion in each scan were first determined using the eternal motion data. In order to increase spatial information these frames were then averaged. Finally, all the frames in the scan were coregistered to this average image. In order to align [^{11}C]MADAM, [^{11}C]carfentanil images and the anatomical T1-weighted MR-image, the mean of all the [11C]MADAM frames was coregistered to the mean of all the [^{11}C]carfentanil frames. The mean of all the [^{11}C]carfentanil frames and coregistered [^{11}C]MADAM frames were then computed and all frames in both scans as well as T1-weighted MR-image were coregistered to this mean. All alignment and coregisteration steps were performed using rigid body transformations and mutual information algorithm with the 2nd version of Statistical Parametric mapping software (http://www.fil.ion.ucl.ac.uk/spm/) running on Matlab 7.2 (The Mathworks Inc., Sherborn, Massachusetts).

Regions of interest were delineated with Imadeus software (Version 1.4, Forima Inc., Turku, Finland) on coregistered T1-weighted MR-image. In bilateral structures the left and right hemispheres were averaged in order to increase the signal to noise ratio. In study **I** 22 regions, in study **II** 25 regions and in study **IV** 17 regions were delineated. Time activity curves were obtained from each region and the simplified reference tissue model was applied to the time activity curves to obtain regional BP_{ND} values (Lammertsma and Hume, 1996). Cerebellar gray matter and occipital cortex were used as reference regions for [^{11}C]MADAM and [^{11}C]carfentanil, respectively.

In addition to regional BP_{ND} values, parametric BP_{ND} images were also calculated. In a parametric BP_{ND} an image time activity curve is modeled in each voxel allowing voxel-wise statistical testing and visualization of the findings. These images were calculated using in-house software (http://www.turkupetcentre.net/software/show.php?program=imgbfbp) that applies the simplified reference tissue model using basis function method to estimate voxel-wise BP_{ND}.

4.4. Magnetic Resonance Imaging

Each subject was scanned with a clinical protocol using a Philips Gyroscan Intera 1.5 T CV Nova Dual MRI scanner to exclude structural abnormalities. T1-weighted image with 1 x 1 x 1 mm^3 voxel size was used as an anatomical reference in the preprocessing of the PET images.

4.5. Statistical Analysis

4.5.1. Region-of-Interest -Based Approach

Statistical analyses of region of interest data in studies **I** & **II** were performed with SPSS 17.0 for Windows (Release 17.0.0, SPSS Inc., Chicago, Illinois), whereas statistical analyses in study **IV** were performed with IBM SPSS Statistics for MAC version 19 (IBM Corp., Armonk, New York). Normality of the distributions of regional BP_{ND}'s was confirmed by observing the histograms. P-value less than 0.05 was considered statistically significant.

The objective in study **I** was to test the hypothesis that serotonin transporter binding in the brain is different in high and low Harm Avoidance groups. This was statistically tested with repeated measures ANOVA using regional [^{11}C]MADAM BP_{ND} as the within subject factor and group as the between-subjects factor. ANCOVA was used to control the effect of other personality dimensions on the hypothesized association between the serotonin transporter and Harm Avoidance. The association between sex, age, and [^{11}C]MADAM BP_{ND} was examined using the Student's t-test and the Pearson correlation, respectively.

In study **II,** the aim was to test whether differences in the μ-opioid receptor binding are associated with Harm Avoidance. This was statistically tested using a partial correlation while controlling for age. The association between sex, age, and [^{11}C] carfentanil BP_{ND} was examined using the Pearson correlation and the Student's t-test, respectively.

The objective in study **IV** was to replicate the finding that serotonin transporter binding is linked with Neuroticism. The association between Neuroticism and [^{11}C]MADAM BP_{ND} was tested with the Pearson correlation coefficients.

4.5.2. Voxel-Based Approach

4.5.2.1. Preprocessing of the Parametric BP_{ND} Images

Parametric BP_{ND} images were normalized into the Montreal Neurological Institute (MNI) space using an in-house ligand specific template. The mean of all the frames in a single scan was first normalized to the template using both linear and nonlinear transformations and these parameters were subsequently applied to the corresponding

parametric BP_{ND} image. In studies **I** and **II** parametric BP_{ND} images were resliced into a 2 x 2 x 2 mm^3 voxel size in the normalization step, whereas in study **III** parametric BP_{ND} images were resliced into a 4 x 4 x 4 mm^3 voxel size. After normalization parametric BP_{ND} images in all studies were smoothed using an 8 mm Gaussian filter. All normalization operations were performed using the 5th version of Statistical Parametric Mapping software (SPM5).

4.5.2.2. Voxel-Wise Statistical Analyses of the Parametric BP_{ND} Images

In studies **I** and **II**, voxel-wise statistical analyses were conducted with SPM5. In study **I**, differences in voxel-wise [^{11}C]MADAM BP_{ND} between high Harm Avoidance and low Harm Avoidance groups were tested using an independent two-sample t-test. Associations between other TCI traits and [^{11}C]MADAM BP_{ND} were tested with a multiple regression analysis with age and gender as nuisance variables. No correction for voxel-level multiple comparisons was applied. P-values less than 0.05 corrected for multiple comparisons at cluster level were considered statistically significant. In study **I**, the midbrain was analysed separately from other parts of the brain due to its complex anatomy. In this analysis an inclusion mask for the midbrain, pons and medulla was created using Wake Forest University PickAtlas software (http://fmri.wfubmc.edu/software/PickAtlas) and a separate statistical analysis was performed for voxels within the mask. In this analysis, a voxel-level false discovery rate correction for multiple comparisons as well as a cluster level correction for multiple comparisons were applied. P-values less than 0.05 were considered statistically significant.

In study **II**, the association between Harm Avoidance and [^{11}C]carfentanil BP_{ND} was tested using a multiple regression analysis with the Harm Avoidance score as the regressor and age as a nuisance variable. The associations of other TCI traits and [^{11}C]carfentanil BP_{ND} were also tested with a multiple regression analysis using age as a nuisance variable. Sex was not entered into the model as it did not have an effect on [^{11}C]carfentanil BP_{ND} in this sample. P-values less than 0.05 corrected for multiple comparisons at the cluster level were considered statistically significant.

In study **III**, voxels with [^{11}C]MADAM BP_{ND} or [^{11}C]carfentanil BP_{ND} values below 0.2 were excluded from the analyses. The Pearson correlation between [^{11}C]MADAM BP_{ND} and [^{11}C]carfentanil BP_{ND} was computed for every voxel.

In order to explore how strongly each voxel-wise BP_{ND} value is connected with BP_{ND} values of other voxels, an average Pearson correlation coefficient was calculated for every voxel x_i:

$$(5) \qquad x_i = \frac{\sum_{j=1}^{N-1} corr(\boldsymbol{v}_i, \boldsymbol{v}_j)}{N-1}$$

where $i = (1, \dots, N)$ is the number of voxels in the image and vectors $\boldsymbol{v}_i = [v_i^1, v_i^2, v_i^3, \dots, v_i^{21}]$ and $\boldsymbol{v}_j = [v_j^1, v_j^2, v_j^3, \dots, v_j^{21}]$contains BP_{ND} values from voxels and of all subjects. Statistically significant connectivity was considered to be voxel-wise FWE corrected

$p<0.05$. Regions that had statistically significant connectivity with other brain regions were then used as seeds. That is, mean BP_{ND} values from those regions were extracted with a WFU pickatlas toolbox (Maldjian et al., 2003) and used to predict BP_{ND} elsewhere in the brain using the General Linear Model in SPM2. A seed analysis was performed only for [^{11}C]MADAM images as an analysis of connectivity strength showed that [^{11}C] carfetanil BP_{ND} in all brain regions was equally connected.

5. RESULTS

Results of studies **I**, **II** and **IV** exploring the associations between the serotonin transporter, μ-opioid receptor and personality traits are summarized in **table 5.1**.

Table 5.1. Summary of results in studies **I**, **II** and **IV**.

Personality trait	Serotonin transporter	μ-opioid receptor
Harm Avoidance	no association	↑ in prefrontal cortex
Self-directedness	↑in midbrain, ↓in cortex	no association
Cooperativeness	↓in cortex and putamen	no association
Neuroticism	no association	not studied
Openness	no association	not studied

↑ indicates positive correlation, ↓ indicates negative correlation

5.1. Serotonin Transporter and Harm Avoidance (study I)

We did not find group × regional [^{11}C]MADAM BP_{ND} interaction in the repeated measures analysis of variance (N=21, F=0.961, p=0.408). That is, high and low Harm Avoidance groups had similar [^{11}C]MADAM BP_{ND} values in all ROI's. This finding was confirmed by a voxel-wise analysis using a t-test (cluster-level FDR-corrected p >0.05).

5.2. Serotonin Transporter and Character Traits (study I)

In a post hoc analysis of ROI-based data, we found statistically significant negative associations between character traits Self-directedness, Cooperativeness, and [^{11}C] MADAM BP_{ND} (N=21). A partial correlation adjusting for both age and sex between the Self-directedness score and [^{11}C]MADAM BP_{ND} was statistically significant in the posterior cingulate cortex (r=-0.66, p=0.002), anterior insular cortex (r=-0.61, p=0.005) and medial prefrontal cortex (r=-0.51, p=0.026). A partial correlation adjusting for both age and sex between the Cooperativeness score and [^{11}C]MADAM BP_{ND} was statistically significant in the putamen (r=-0.59, p=0.008), anterior insular cortex (r=-0.55, p=0.015), medial prefrontal cortex (r=-0.52, p=0.023), hippocampus (r=-0.41, p=0.041) and medial temporal gyrus (r=-0.47, p=0.044). ROI-based findings did not survive conservative Bonferroni correction for multiple comparisons. Voxel-wise analysis revealed a statistically significant positive association between the Self-directedness score and [^{11}C]MADAM BP_{ND} in the dorsal raphe nucleus (N=21, cluster-level FDR corrected p=0.004, cluster size 199 voxel) (**Figure 5.1.**). That is, higher Self-directedness tended to associate with higher [^{11}C]MADAM BP_{ND} in the dorsal raphe nucleus.

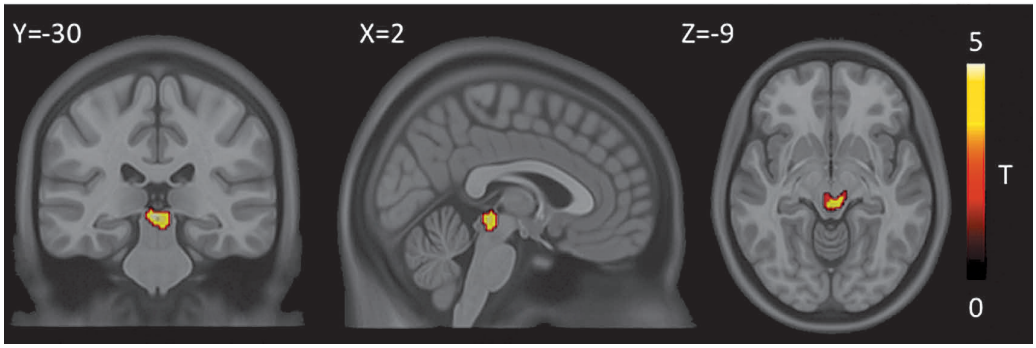

Figure 5.1. Self-directedness is associated positively with [^{11}C]MADAM BP_{ND} in dorsal raphe nucleus (cluster level FDR-corrected p=0.004, cluster size 199 voxels). MNI coordinates are shown above images. Color bar on the right indicates T-score.

5.3. Serotonin Transporter and Neuroticism (study IV)

The Neuroticism score did not correlate with [^{11}C]MADAM BP_{ND} in any brain region (N=31, p>0.05). Adjusting for age, sex or body-mass index did not affect the association. Other personality traits in the Five-factor model were not associated with [^{11}C]MADAM BP_{ND}.

5.4. μ-opioid Receptor and Harm Avoidance (study II)

In a whole-brain voxel-wise regression analysis we found that the Harm Avoidance score is positively associated with [^{11}C]carfentanil BP_{ND} in a large region comprising the anterior cingulate cortex, lateral and medial prefrontal cortices and anterior insular cortex (N=22, cluster-level FDR-corrected p=0.009, cluster size 15078 voxels, adjusted for age)(**Figure 5.2.**). That is, subjects with a higher Harm Avoidance score tended to have higher [^{11}C]carfentanil BP_{ND} values in these regions. In an ROI-based analysis statistically significant partial correlations when adjusting for age were found in the pregenual anterior cingulate cortex (r=0.44, p=0.049), dorsal anterior cingulate cortex (r=0.44, p=0.049) and posterior cingulate cortex (r=0.45, p=0.040). A positive trend was found in the medial prefrontal cortex (r=0.43, p=0.050), dorsolateral prefrontal cortex (r=0.41, p=0.064), lateral orbitofrontal cortex (r=0.43, p=0.055), putamen (r=0.39, p=0.081) and caudatus (r=0.37, p=0.098).

The association between Harm Avoidance and [^{11}C]carfentanil BP_{ND} was driven by two subscales, Shyness with strangers and Fatigability & asthenia. That is, these two subscales were statistically significantly associated with [^{11}C]carfentanil BP_{ND} whereas Anticipatory worry and Fear of uncertainty were not.

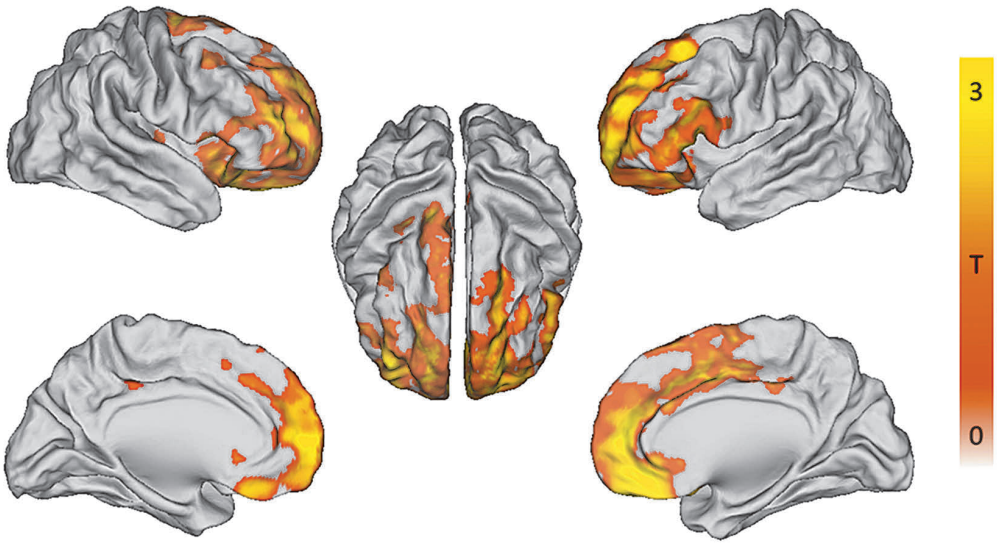

Figure 5.2. Harm Avoidance score is positively associated with [^{11}C]carfentanil BP_{ND} in frontal cortex, when adjusted for age. Color bar on the left indicates T-score. N=22, cluster-level FDR-corrected p=0.009.

5.5. Demographical Variables, the Serotonin Transporter and the μ-opioid Receptor (studies II & IV)

Males and females had similar [^{11}C]MADAM BP_{ND} (N=31, p>0.05)(study **IV**) and [^{11}C] carfentanil BP_{ND} values (N=22, p>0.05)(study **II**) in all brain regions. Similarly, body-mass index had no effect on [^{11}C]MADAM BP_{ND} values (N=31, p>0.05) or [^{11}C]carfentanil BP_{ND} values (N=22, p>0.05) in any brain region.

Age was not associated with [^{11}C]MADAM BP_{ND} but higher age was associated with lower [^{11}C]carfentanil BP_{ND} in periaqueductal gray matter (r= -0.568, p=0.006) and supramarginal gyrus (r= -0.434, p= 0.044). Although the age effect did not survive conservative Bonferroni correction for multiple comparisons, it was used as a covariate in study **II** when investigating the association between Harm Avoidance and [^{11}C]carfentanil BP_{ND}.

5.6. Brain Networks Within and Between Serotonin Transporter and μ-opioid Systems

5.6.1. Regional Correlation Between the Serotonin Transporter and the μ-opioid Receptor Binding (study III)

Correlating voxel-wise [^{11}C]MADAM BP_{ND} with voxel-wise [^{11}C]carfentanil BP_{ND} revealed statistically significant associations between the two tracers bilaterally in the amygdala, dorsolateral prefrontal cortex, dorsal anterior cingulate cortex and anteromedial thalamus, and unilaterally in the left parietal and medial temporal cortices (N=21, voxel-wise uncorrected p<0.05)(**Figure 5.3**).

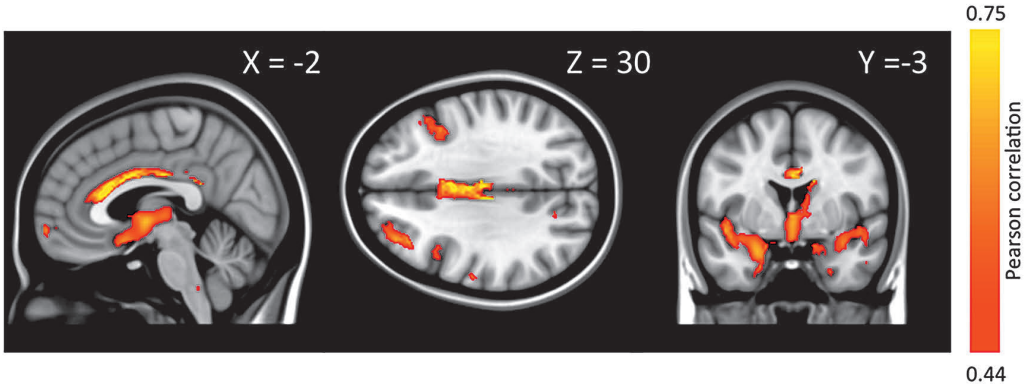

Figure 5.3. Voxel-wise Pearson correlation coefficient between [^{11}C]MADAM BP_{ND} and [^{11}C]carfentanil BP_{ND} shown in sagittal, axial and coronal sections. Significant correlations were found in the amygdala, dorsolateral prefrontal cortex, dorsal anterior cingulate cortex and anteromedial thalamus and unilaterally in the left parietal and medial temporal cortices. Numbers above sections indicate the Montreal Neurological Institute coordinates. The figure has been previously published in the original publication **IV**.

5.6.2. Local Connectivity and Seed Analysis of Binding within the Serotonin and the μ-opioid Systems

Hub analysis showed that [^{11}C]MADAM BP_{ND}'s in the midbrain, thalamus, caudatus, putamen and amygdala are interrelated (voxel-wise FWE corrected $p<0.05$, $r > 0.44$). Using [^{11}C]MADAM BP_{ND} from these regions as seeds to predict [^{11}C]MADAM BP_{ND} in every voxel of the brain demonstrated that [^{11}C]MADAM BP_{ND}'s in these five regions are independent from BP_{ND}'s in other regions of the brain. A Hub analysis also showed that [^{11}C]carfentanil BP_{ND}'s in all brain regions were interrelated with each other (**Figure 5.4.**).

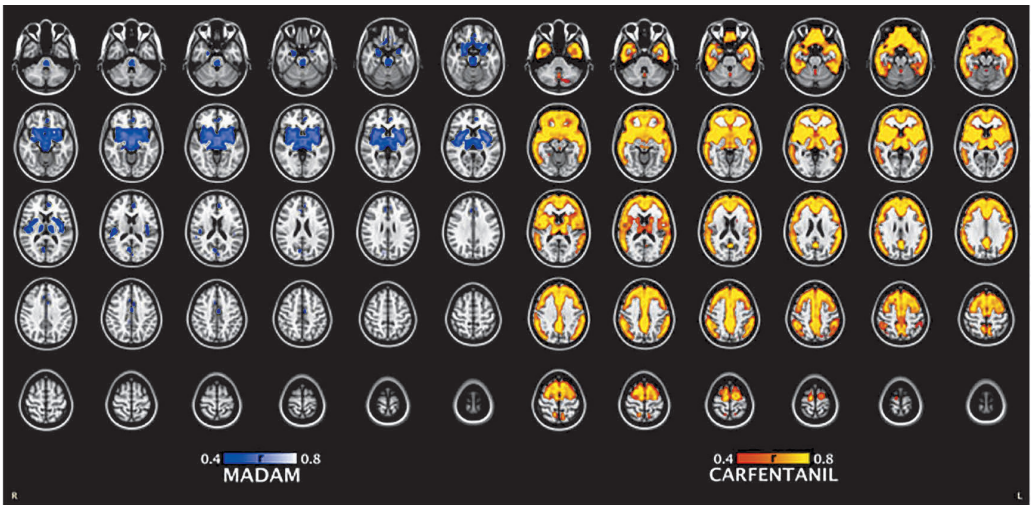

Figure 5.4. Axial sections of the brain showing voxel-wise strength of connectivity (average Pearson correlation coefficient). Connectivity of [^{11}C]MADAM (blue to white) is shown on the left panel and connectivity of [^{11}C]carfentanil (red to yellow) on the right panel. The data are thresholded at $p < .05$ FWE corrected. The figure has been previously published in the original publication **IV**.

6. DISCUSSION

6.1. Methodological Considerations

6.1.1. Study Design

To our knowledge, studies **I** and **II** are the first studies that have been specifically designed to test hypotheses concerning the neurobiology of personality traits. These studies take advantage of the personality measurements performed during the follow-up of The Cardiovascular Risk in Young Finns Study. These measurements allowed us to recruit subjects that had Harm Avoidance scores repeatedly in either the highest or lowest quartile, that is, in either of the two extremes of the Harm Avoidance dimension. This gave us three advantages. Firstly, even though temperament traits are considered to be stable in adulthood, measurement error causes variability in the assessment. Therefore, repeated measures of personality further increases the likelihood that subjects truly belonged to the extremes in the Harm Avoidance trait. Secondly, recruiting subjects from the two extremes increased the sensitivity to detect any linear association between Harm Avoidance and [^{11}C]MADAM or [^{11}C]carfentanil BP_{ND}. Finally, using the two extremes from a population based cohort assures that the sample captures the whole variability range in a given trait. This reduces the risk of Simpson's paradox, which refers to a situation where a trend appears in a subset of data but disappears when the whole dataset is analyzed (Simpson, 1951).

A major limitation in these studies is the relatively small sample size. Despite increased sensitivity due to study design, studies **I** and **IV** were unable to detect the hypothesized association between [^{11}C]MADAM BP_{ND} and anxious personality traits. [^{11}C]MADAM BP_{ND} in healthy subjects is highly variable, which led to, for example, large 95 % confidence intervals of Pearson correlation in study **IV**. The possibility that the negative findings in these studies are caused by a lack of statistical power leading to a type 2 error rather than by a true lack of association cannot therefore be fully excluded.

6.1.2. Personality Measures

Studies **I**, **II** and **IV** used personality measurements performed during the follow-up of The Cardiovascular Risk in Young Finns Study in the years 1997 and 2001. Subjects did not complete the TCI questionnaire during the PET scanning day. This led to an average interval of 6.1 ± 0.4 years between TCI measurement and the PET scans in studies **I** & **II**. This may not be a major issue as the test-retest correlation of Harm Avoidance over 6 year period (2001-2007) in The Cardiovascular Risk in Young Finns Study was 0.80 (Josefsson et al., 2013). However, as subjects in these studies were chosen from the two extremes there may have been some regression towards the mean.

6.1.3. Positron Emission Tomography

One of the strengths in these studies is the state-of-the-art PET methodology. The HRRT scanner used in all of the studies has the highest resolution so far reported for any PET scanner. Both tracers used in these studies are well established and highly specific for their targets and the tracers were modeled with validated pharmacokinetic models. The scanning time of [^{11}C]MADAM in these studies was only 75 minutes in comparison to earlier studies, which have used scanning times over 90 minutes (Lundberg et al., 2006). Scanning time is always a trade-off between reliability of the binding estimates and participant comfort. The time to peak uptake of [^{11}C]MADAM in raphe nuclei is 70.4 minutes. As a consequence of shorter scanning time used in these studies, estimates of [^{11}C]MADAM BP_{ND} in raphe nuclei are noisier than would have been obtained had the scanning time been longer.

There is some evidence that single nucleotide polymorphisms may affect binding of [^{11}C]MADAM and [^{11}C]carfentanil (Praschak-Rieder et al., 2007). In these studies, however, gene polymorphisms were not measured and it can be speculated whether controlling these variants could have affected the results.

6.2. Personality and the *In Vivo* Serotonin Transporter Binding (studies I & IV)

We found that the serotonin transporter is not associated with anxious personality traits. These findings do not support the original hypothesis by Cloninger (1986). First (study **I**), we carefully selected two matched groups from the two extremes of the Harm Avoidance scale. Comparison of these groups demonstrated that serotonin transporter binding anywhere in the brain does not explain differences in the Harm Avoidance score. We then extended this sample by recruiting more subjects from the two extremes of the Harm Avoidance scale. Instead of comparing the two groups again, we used another measure of anxious personality, i.e. Neuroticism described by the Five-factor model. Study **IV** demonstrated that serotonin transporter binding is not associated with scores in the Neuroticism scale anywhere in the brain. In other words, serotonin transporter binding does not explain the differences in the Neuroticism score either.

Findings in previous studies on the association of anxious personality traits and serotonin transporter are contradictory. Studies have shown either positive association (Takano et al., 2007), negative association (Wu et al., 2010) or no association at all (Reimold et al., 2008, Kalbitzer et al., 2009) (see **table 2.1**). Moreover, PET/SPECT studies investigating associations between serotonin receptors and anxious personality traits are contradictory (see section 2.1 and 2.2 and 2.3). It is worth noting that genetic linkage studies, studies investigating single nucleotide polymorphisms or genome wide association studies have not been able to show consistent association between genes affecting the serotonergic system and anxious personality traits (Munafo et al.,

2009, Munafò and Flint, 2011, de Moor et al., 2012, Service et al., 2012). Taken together, our findings, previous imaging studies, and converging evidence from genetic studies suggest that if there is any association between anxious personality traits and the serotonergic system in healthy subjects it is likely to be much smaller than previously anticipated.

Both studies **I** & **II** had subjects specially selected for absence of any psychiatric disorder, which limits the generalizability of the results to the healthy population. There are two interrelated questions that cannot be answered with studies excluding psychiatric disorders. Despite the fact that it is still controversial whether mood and anxiety disorders per se are associated with changes in serotonin transporter binding [for a review see (Savitz and Drevets, 2013)], psychiatric disorder by personality interaction may affect serotonin transporter binding. Secondly, subjects who have high scores in anxious personality scales but are free of psychiatric disorders may have some yet unknown protective factor that also affects serotonin transporter binding.

Cloninger et al., (2006) suggested that a high Self-directedness trait would protect against mood and anxiety disorders. Interestingly, in study **I** we found that both Self-directedness and Cooperativeness correlate negatively with serotonin transporter binding in cortical regions and Self-directedness positively in the midbrain. To our knowledge, this is the only study that has reported an association between *in vivo* serotonin transporter binding and TCI character traits. The finding is interesting as both Self-directedness and serotonin transporter binding in the midbrain have been associated with depression (Farmer et al., 2003, Reimold et al., 2008). However, Reimold and colleagues also measured TCI Self-directedness, but they were unable to show an association between the character trait and serotonin transporter binding. This finding, if reliably replicated, would question the validity of a strict division between temperament and character and their underlying neural correlates. Earlier studies have produced some circumferential evidence of the link between Self-directedness and the serotonin transporter. For example, Hamer et al., (1999) found an association between the serotonin transporter promoter region polymorphism and Self-directedness in a sample of 634 volunteers. In addition, successful treatment of depression with selective serotonin reuptake inhibitors seems to also increase Self-directedness (Allgulander et al., 1998, Hirano et al., 2002). It must be kept in mind that the study by Hamer and colleagues suffers from the same shortcomings as other single nucleotide polymorphisms studies, most importantly from the small number of participants (Munafò and Flint, 2011). The studies by Allgulander and colleagues and Hirano and colleagues may just indicate the state-dependency of the Self-directedness trait. Finally, it is important to note that this is a post-hoc finding in a highly selected and small sample and that the shortening of the scanning time has increased noise in the [^{11}C]MADAM BP_{ND} in raphe nuclei.

6.3. Harm Avoidance and the *In Vivo* μ-opioid Receptor Binding (study II)

We found that higher scores in the Harm Avoidance scale associate with higher μ-opioid receptor binding in the prefrontal cortex, the insular cortex, and particularly in the cingulate cortex. These results were evident in both ROI-based analyses as well as the voxel-wise analysis and the results remained statistically significant after adjusting for age.

The findings agree with and add to what is known about the brain circuitry that associates with Harm Avoidance. Harm Avoidance is associated with a higher limbic reactivity to aversive stimuli and a decreased ability of prefrontal cortex and anterior cingulate cortex to exert control over the limbic system. For example, highly harm avoidant subjects have decreased connections between the anterior cingulate cortex and amygdala (Westlye et al., 2011) and they tend to have a smaller size of the left amygdala but an increase in its response to negatively valenced images (Iidaka et al., 2006, Baeken et al., 2009). We speculate that the inability to activate μ-opioid system in prefrontal cortex may further explain the lack of control over the limbic system.

Our finding suggests that subjects at the higher end of the Harm Avoidance trait tend to have less endogenous opioids in the extracellular space and synapses of the prefrontal cortex. Here, extrapolating from a study with [^{11}C]raclopride (Hietala et al., 1999), we assume that baseline [^{11}C]carfentanil BP_{ND} reflects changes in density rather than affinity of μ-opioid receptors. Based on the evidence that the number of μ-opioid receptors increases in response to long-term blockade of endogenous opioids and vice versa (McConalogue et al., 1999, Lesscher et al., 2003), higher baseline BP_{ND} is typically interpreted to be the result of a lower long-term opioid tone (Prossin et al., 2010). However, it is possible that higher baseline BP_{ND} is a result of a short-term lower endogenous opioid tone i.e. deactivation of endogenous opioid system or simply lesser activation of the endogenous opioid system during the scan in comparison to subjects with lower Harm Avoidance scores. Unfortunately, whether Harm Avoidance associates with a long- or short-term lower opioid tone cannot be disentangled with a single baseline PET scan. The idea that Harm Avoidance associates with the inability to activate the opioid system in the short-term agrees with the recent study by Peciña and colleagues (2013) showing that the Five-factor model Neuroticism and particularly its subscale Angry hostility associate with lower release of endogenous opioids in response to placebo treatment of pain.

Decomposing Harm Avoidance into four subscales, revealed that Shyness with strangers and Fatigability & Asthenia correlated statistically significantly with [^{11}C] carfentanil BP_{ND} whereas the other two subscales did not. Higher scores in Shyness with strangers subscale is unassertive and shy in social situations (Cloninger et al., 1994). Other studies have suggested that that the activity of the endogenous opioid system is especially important in social interaction as the opioid system is linked with motivated behaviors (Van Ree et al., 2000) and particularly social reward (Trezza et al., 2011).

Perhaps endogenous opioids function to reassure in a socially distressing situation or maybe these subjects simply lack the incentive to engage in social interaction which with time could lead to withdrawal and shyness. Fatigability & Asthenia measures the ability to recover after stress. The result implies that this ability may also be associated with μ-opioid receptor binding in the brain.

6.4. Interaction of the Serotonin and the μ-opioid Systems and the Intraligand Correlations (study III)

We found that in the anteromedial thalamus, dorsal anterior cingulate cortex, dorsolateral prefrontal cortex and amygdala [^{11}C]MADAM BP_{ND} correlates with [^{11}C] carfentanil BP_{ND}. To our knowledge, there are no previous PET studies that have investigated the intercorrelation of two different neurotransmitter systems in healthy humans. We speculate that the correlation of these two neurotransmitters is due to a regional coexpression of the serotonin transporters and μ-opioid receptors. The finding suggests that these two molecules are in a homeostasis or there is a system that regulates the expression of both serotonin transporters and μ-opioid receptors. Based on the fact that the endogenous opioid system is a potent modulator of serotonergic neurotransmission (Tao and Auerbach, 1995, Sbrenna et al., 2000, Tao and Auerbach, 2002a), we hypothesize that the release of serotonin into these regions is under μ-opioid receptor control.

Our results from hub and seed analyses suggest that the relative level of the μ-opioid receptor expression is uniform in the brain. That is, if someone has high levels of μ-opioid receptor binding in some part of her brain, most probably she also has high levels of μ-opioid receptor binding in other parts of her brain as well. In contrast, the serotonin transporter seems to behave differently in the brain. We found that serotonin transporter binding was only interrelated within five brain regions. These regions were the midbrain, thalamus, putamen, caudatus, and amygdala. To our surprise, serotonin transporter binding in the midbrain was not related to serotonin transporter binding in any brain region outside these five regions. We speculate that genetic mechanisms controlling serotonin transporter expression may be regionally dependent unlike genetic mechanisms controlling μ-opioid receptor expression.

7. FUTURE DIRECTIONS

As discussed above, the studies on the serotonin system, Harm Avoidance and Neuroticism conducted so far are inconclusive. As the association is likely to be small future studies should either recruit substantially more subjects or use meta-analytical techniques to unravel the possible link between these measures.

Based on the findings in study **II**, we speculated that the ability to activate the endogenous opioid system in the prefrontal cortex is related to the ability to exert control over the amygdala. This could be tested using functional MRI to reveal the amygdala response to masked emotional faces (Sheline et al., 2001) under exogenous opioid blockade or opioid stimulation. Our result and results from previous studies imply that the activity of the prefrontal cortex has a paramount role in harm avoidant behavior. The role of the prefrontal cortex could be further studied in an rTMS experiment. If some parts of the prefrontal cortex are important in controlling the limbic system, their deactivation with rTMS should lead to an increase in response to masked emotional faces, which could be measured with EEG or [^{15}O]H_2O PET. We also speculate that the inability to activate endogenous opioid system impedes being at ease in social situations and that endogenous opioids may be released in response to social reward. Since [^{11}C] carfentanil may be sensitive to endogenous opioids (Scott et al., 2007) this hypothesis could be directly tested. Furthermore, the role of opioid system in neuronal responses to social cues could be investigated under exogenous modulation of opioid system using functional MRI.

Finally, findings in study **III** lead us to speculate that the release of serotonin in the anteromedial thalamus, dorsal anterior cingulate cortex, dorsolateral prefrontal cortex and amygdala is under μ-opioid receptor control. This hypothesis could be tested in a PET study using μ-opioid receptor agonist as a pharmacological challenge and a ligand sensitive to endogenous serotonin levels. The methodology introduced in the study could be extended to other interactions of two different neurotransmitter systems e.g. opioid system and dopamine system. Furthermore, it is important to understand how disease processes affect the interplay between neurotransmitters, for instance whether there is a decoupling of the regional coexpression of two neurotransmitters in some disorders. Finally, polypharmacy is commonplace in clinical practice and many drugs that are used have affinities to more than one neurotransmitter system. Future studies could use this methodology to target regional neurotransmitter balances and networks in etiological psychiatric research as well as in drug development.

8. CONCLUSIONS

The major findings in this work are as follows:

- Variability in Harm Avoidance or Neuroticism in healthy volunteers cannot be explained by differences in serotonin transporter density in the brain (**I** & **IV**).
- Harm Avoidance score correlates positively with μ-opioid receptor availability in the frontolimbic cortex. This suggests that higher Harm Avoidance associates with a lower endogenous opioid tone. Correlating μ-opioid receptor binding with the subscales of Harm Avoidance showed that μ-opioid receptor binding associates especially with a tendency to feel shy and insecure in social situations and an inability to cope with stress. The result is relevant in the research of etiology of affective disorders (**II**).
- Serotonin transporter binding correlates with μ-opioid receptor binding in a brain region-specific manner, suggesting interplay between the two systems. Neurotransmitter interaction and regional balance may be a useful target for etiological research as well as pharmacotherapeutic studies in psychiatry (**III**).

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